THERAPEUTIC MODALITIES
FOR THE SHORT BOWEL SYNDROME

improvement of adaptation and small-bowel transplantation

M.C.J. WOLVEKAMP

The author's research fellowship was financially supported by a grant of the Sophia Foundation for Medical Research.
Financial support for the publication of this thesis was generously provided by Nutricia, Sandoz, Pfizer BV, HopeFarms, and Harlan CPB.

Wolvekamp, Monica Christina Johanna

THERAPEUTIC MODALITIES
FOR THE SHORT BOWEL SYNDROME
improvement of adaptation and small-bowel transplantation

THERAPEUTISCHE BEHANDELINGSMODALITEITEN
VOOR HET KORTE DARM SYNDROOM
verbetering van de adaptatie en dunne darm transplantatie

PROEFSCHRIFT

Ter verkrijging van de graad van Doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof.dr. P.W.C. Akkermans M. Lit.
en volgens besluit van het College van Dekanen.
De openbare verdediging zal plaatsvinden op
15 juni 1994 te 11.45 uur

door

MONICA CHRISTINA JOHANNA WOLVEKAMP

geboren te Rotterdam

Promotiecommissie

Promotor:	Prof. dr. J.C. Molenaar
Co-promotor:	Dr. E. Heineman
Overige leden:	Prof. dr. J.H.P. Wilson
	Prof. dr. J. Jeekel
	Dr. H.R. de Jonge

The investigations presented in this thesis were performed at the Laboratory for Experimental Surgery, Erasmus University Rotterdam.

Be careful for what you want:
it might become true

Voor allen van wie ik heb mogen leren,
en vooral aan hen die mijn gids hebben willen zijn.

TABLE OF CONTENTS

GENERAL INTRODUCTION

This thesis deals with two therapeutic modalities for patients with an irreversible short bowel syndrome: improvement of adaptation and small-bowel transplantation. Thereby, emphasis is put on the role of these therapeutic modalities for children.

The irreversible short bowel syndrome may arise if, after massive small-bowel resection, the remaining intestine is not able to adapt sufficiently to the loss of bowel. The process of functional intestinal adaptation after small-bowel resection is a complex process which is poorly understood and therefore it is difficult to manipulate the outcome of the adaptation phase. The importance of oral intake as a positive stimulus to the adaptive stage of the remaining bowel has been recognized. Predigested food became the standard treatment of choice when starting oral refeeding but it is still disputed whether this is more effective than non-predigested food in stimulating adaptation, and thus further research is mandatory.

Although long-term total parenteral nutrition provided the first satisfactory treatment for the short bowel syndrome, its complications including metabolic, infectious, and psychologic aspects, form the main reason to envision this therapy as a temporary solution.

Recently, small-bowel transplantation in man has become feasible and therefore children with irreversible intestinal failure would also be suitable candidates for a small-bowel graft. However, before a clinical small-bowel transplantation program in children is justified, it must be demonstrated that a small-bowel transplant can give adequate nutritional support to a growing individual with the short bowel syndrome.

This thesis evaluates a method to monitor the process of functional intestinal adaptation in rats subjected to near total small-bowel resection and unravels the role of diet composition and/or complexity on adaptation.
Furthermore, it outlines the role of total and segmental orthotopic small-bowel transplantation in the treatment of short bowel syndrome in enterectomized young dogs, and presents experiments on the follow-up of a small-bowel graft including monitoring of rejection and the histologic, bacteriologic, and functional status of a graft in both rats and dogs.

CHAPTER 1

Short bowel syndrome in infancy

INTRODUCTION

A variety of pathophysiologic circumstances in childhood necessitate extensive small-bowel resection (1-4). Developmental abnormalities of the small bowel include intestinal atresia, gastroschisis, malrotation and volvulus. Major causes in the postnatal period are necrotizing enterocolitis, midgut volvulus and inflammatory bowel disease.

The clinical course after extensive small-bowel resection can be subdivided into three main stages (5). Directly after resection, severe diarrhea, steatorrhoea, malabsorption and fluid and weight loss is seen. During this stage, total parenteral nutrition is indispensable to provide sufficient calories. In the second stage, after a few months to over one year, the remaining intestine gradually adapts to the loss of bowel and oral intake is gradually increased until intravenous alimentation can be stopped. Unfortunately, some of these patients never reach this third stage, and develop the short bowel syndrome (6).

The short bowel syndrome is characterized by irreversible small-intestinal failure, which is defined as inability to maintain nutritional status and/or positive fluid and electrolyte balance without special measures.

1 TOTAL ADAPTATION AFTER MASSIVE SMALL-BOWEL RESECTION OR NOT ?

The likelihood of developing the short bowel syndrome depends on several established factors, including the length and anatomic identity of the enteric remnant and the presence of residual disease (7,8). The time it takes to reach a state of maximal adaptation in the remaining small bowel is influenced by both non-nutritive and nutritive stimuli.

In general, following small-bowel resection, there is an increase in both epithelial cell proliferation rate in the crypts and migration rate of the cells onto the villi, resulting in enlarged villi and deeper crypts (9). The villus hyperplasia is characterized by an increase in mucosal cell mass and in DNA, RNA and protein content. It has been reported that following jejunal resection, from a functional point of view, in ileum more effective adaptation takes place than in the reverse situation (10).

Non-nutritional factors, of importance in stimulating intestinal adaptation, are endogenous secretions, hormones, and neurovascular components (summarized in Table 1). There was

speculative, indirect evidence on the strong enterotropic capacity of enteroglucagon. In contrast to this, Gregor and associates (14) showed that immunoneutralization of circulating endogenous enteroglucagon had no effect on the adaptive response. The results concerning the role of gastrin and cholecystokinin are controversial and these hormones are not considered as key determinants of the adaptive response so far. In addition, the role of epidermal growth factor, insulin, vasoactive intestinal polypeptide, bombesin, corticosteroids, and pancreatic peptide remains to be elucidated. So there are still many candidates for the role of "enterotrophin-like hormone". Most probably the hormones involved will function in concert with the neurovascular effects responsible for refinement of the major adaptive changes. These non-nutritional factors however, are mostly under influence of luminal nutrition themselves. Therefore, the role of nutrients in intestinal adaptation, being major regulating factors, is discussed in detail below.

1.1 The role of nutrients in intestinal adaptation

The effect of nutrition on intestinal adaptation following resection has been widely recognized. By now, experimental approaches have been devoted to the relative contribution of three nutritional aspects: the presence of food in the gut lumen, the complexity of the diet, and the role of a specific nutritional component.

Presence of enteral nutrition

The importance of oral food intake in maintaining both gut structure and function is beyond dispute. Dietary manipulation such as hyperphagia increases the intestinal epithelial cell renewal and leads to an increase in small-bowel mass (24). In contrast, hypoplasia is found after fasting or protein depletion (25).

More than 25 years ago, with the advent of total parenteral nutrition, the importance of luminal nutrition in intestinal adaptation became a field of interest (26). The presence of luminal nutrients is considered as a prerequisite in achieving adaptive postresectional hyperplasia. In growing rats, subjected to small-bowel resection and receiving total parenteral nutrition for 8 days, a lower mucosal mass, providing lower absorptive capacity per centimeter of intestine, was found compared to rats receiving luminal nutrition (27). In contrast, increased specific transport activity of alanine was found in parenterally nourished rats. Ford et al (28) demonstrated in young rats that total parenteral nutrition inhibits adaptive hyperplasia following resection and even caused a decrease in ileal crypt depth and jejunal as well as ileal DNA and RNA contents compared to unoperated rats after 10 days of total parenteral nutrition. However, ileal sucrase activity was significantly elevated compared to nonoperative values at that time.

Table 1 **Non-nutritive factors affecting intestinal adaptation following small-bowel resection**

Factor	Model	Time	Response	References
Endogenous secretions				
Bile and pancreas secretions	* 50% jejunal resection + transplantation of duodenal papilla	* 4 weeks	* intensifies ileal hyperplasia	11
Gastric secretion	* 40% ileal resection	* 6-8 weeks	* increased gastric secretion precedes intestinal hyperplasia	12
Hormones				
PGE2 ↑	* 70% jejunoileal resection + aspirin administration 20 mg/kg/8 hr	* 12 days	* inhibition of mucosal weight, DNA, protein and maltase levels in distal ileum	13
Enteroglucagon↓	* 70% jejunal resection + immunoneutralisation of EG	* 2 weeks	* normal hyperplasia	14
Growth stimulating activity ↑	* 50% jejunal resection	* 4 days	* associated with proliferation of intestinal epithelial cells	15
Testosterone	* 50% jejunoileal resection + 0.35 mg/kg/wk testosterone	* 4 weeks	* enhanced weight gain, increased hyperplasia	16
Growth hormone ↑	* 70% jejunoileal resection + PGF treatment	* 2 weeks	* mucosal weight, DNA, protein and sucrase activity increased	17
Somatostatin	* 50% proximal jejuno-ileal resection + continuous infusion somatostatin 1667 ng/kg/min	* 96 hours	* inhibits post resectional hyperplasia	18
Neurovascular effects				
Nongut hormones				19
Blood flow	* 50% midbowel resection	* 2 days and 2 months	* increased ileal blood flow preceding hypertrophy	20
	* 80% midbowel resection	* 1,2, 3 and 5 days	* hyperplastic response not directly caused by hemodynamic changes	21
Adrenergic denervation	* 50% midenterectomy	* 2 days and 2 months	* 50% reduction endogenous catecholamine activity and corresponding decrease in density of adrenergic terminals	22
Mesenchymal regulations	* in vitro culturing of small intestine	* not reported	* mesenchyme required for maintaining mucosal architecture	23

Thus, although total parenteral nutrition leads to a state of morphologic atrophy, the specific transport activity of a component may increase during parenteral feeding, indicating that adaptation of transport function at the cellular level may be independent of oral feeding. Resumption of oral diet reverses the decrease in villus height, crypt depth, and mucosal DNA and RNA contents, and even results in augmented final body weight and intestinal length after 4 weeks, compared to rats fed rat chow from the start. These findings support previous work by Wilmore et al (29), who found that both long-term somatic growth and intestinal growth are improved after providing essential nutrients i.v. for 30 days compared to enteral feeding from the start, following massive intestinal resection in growing dogs.

The complexity of the diet

The belief that small-bowel hyperplasia occurs only if luminal nutrition is provided led to early introduction of enteral alimentation during the intestinal adaptation process following resection (30). The short bowel syndrome is a possible indication for using an elemental, predigested diet also known as "space diet" or chemically defined diet. The reasoning behind this suggestion is that an elemental diet requires less digestion and is absorbed over shorter lengths of bowel (31). Until now, only some uncontrolled clinical trials have been reported from which it is unclear whether an elemental diet has any effect different from that of whole food (32,33,34). On the top of that, there is no animal study showing benefit of an elemental diet used in this manner. Several investigators (35,36) have demonstrated that after small-bowel resection, both in adult and growing rats, adaptive changes take place with elemental diet, but less well than with normal rat chow. The validity of their conclusion that a complex diet is superior over an elemental diet may be questioned because the relative composition of the diets also differed.

The composition of the diet

Various studies show that the composition of the diet also influences intestinal adaptation in the rat (37-47). Investigators have studied the effects of bulk and/or fiber, lipid, carbohydrate, and amino acid supplemented diets in supporting the intestinal adaptation process in comparison with non-supplemented diets.

The addition of non-soluble fiber, which contributes to fecal bulk, had no influence on both small-intestinal morphology and cell renewal in the rat (48). In contrast, elemental diet supplementation with soluble non-cellulosic dietary fiber, which is completely fermented by colonic bacteria and does not contribute to fecal bulk, enhanced intestinal adaptation in both small bowel and colon, as was confirmed by significantly increased mucosal growth and improved maintenance of body weight (39). It has been shown that

intraluminal infusion of short chain fatty acids (SCFAs), the components mainly produced by fermentation of soluble fiber, stimulates colonic mucosal proliferation (49) and increases intestinal blood flow, which may facilitate small and large intestinal adaptation (50). In a recent rat study on short bowel syndrome in rats, the effect of an elemental diet supplemented with short chain triglycerides (SCTs), reduced to SCFAs by fermentation, was compared to medium chain triglycerides (MCTs) or non-supplementation. The results showed enhancement of colonic and jejunal adaptation, revealed by increased segmental weight and mucosal protein in the SCT group compared to the other diet groups (40).

For the treatment of the short bowel syndrome the use of MCTs as part of a restricted fat, high-carbohydrate diet was recommended (51). Various clinical studies were unable to confirm the advantages of replacing long chain triglycerides (LCTs) by MCTs or carbohydrates (52). In a rat study, the effect of an elemental diet containing 83% of the fat in the form of MCTs was compared with a diet containing 40% of the fat in the form of MCTs (45). The remaining fat in both diets consisted of LCTs. This study demonstrated greater morphologic mucosal adaptation, increased sucrase activity and leucine uptake, and improved weight gain after feeding the high-percentage LCT diet, in spite of more easy absorbance of MCTs. In addition, Grey et al. undertook a study of the relative efficacy of intragastrical supplementation of free fatty acids (FAs) or LCTs in parenterally nourished rats with a 50% small-bowel resection (37). They found that FAs were more effective in promoting adaptation by measuring DNA and protein contents in different small-bowel segments. Moreover, DNA and protein contents of the FA group equalled the values found in the orally fed animals. Hart et al (53) investigated the effect of essential fatty acids (EFA) on intestinal adaptation after small-bowel resection in the rat. Feeding the EFA-poor diet resulted in significantly lowered hyperplastic response in comparison with feeding the control diet. Reversal to the control diet for two weeks increased the mucosal adaptation process significantly. Morin et al (54) showed that LCTs given intragastrically enhance intestinal adaptation more than protein, polysaccharide or MCT. Intraluminal infusion of amino acids and dextrose in parenterally nourished rats showed that amino acids are more effective than dextrose in maintaining the small-bowel mass (46). Vanderhoof investigated the relative trophic effect of glutamine, glycine or glucose in rats subjected to 70% proximal jejuno-ileal resection (44). The different components were supplemented (5% of total amount of calories) to enterally given powdered rat chow. This study learned that large amounts of glutamine may produce negative effects on the adaptation, possibly mediated by excess of ammonia production. Other studies showed that glutamine-enriched diets prevent intestinal atrophy and thus optimal dosing of this enteral fuel is of utmost importance. Together, all these observations show the relative importance of fat in promoting intestinal adaptation and

indicate that more research is necessary to find the optimal combination of diet supplementation with substances like glutamine.

1.2 The interrelationship between histologic changes and function

In the literature, there are indications that there is a separation between the regulation of morphologic and functional mucosal growth. Urban et al (55) examined the transport of sodium, chloride, water and galactose in relation to mucosal growth, both 2 and 4 weeks after 70% small-bowel resection in rats. This study showed that maximal morphologic growth occurs 2 weeks after 70% small-bowel resection and precedes functional adaptation, which was demonstrated by unaltered transport capacities per amount intestine. In addition, functional adaptive mechanisms may be localized to specific regions of the remaining intestine as indicated by specific increases of electrolyte and water transport in the duodenum and of galactose transport in the ileum 4 weeks after resection. Bury et al (56) evaluated carbohydrate absorption 2, 6 and 12 weeks after an 80% resection in rats. A progressive rise, lagging behind the morphologic changes, was first seen by 6 weeks in both jejunal and ileal remnants.

1.3 Mechanisms responsible for intestinal adaptation

Unravelling the identity of both nutritive and non-nutritive signals capable of inducing adaptive changes of the intestine has been subject of descriptive studies. Recently, attention has turned to the mechanisms responsible for the adaptive morphologic and functional changes occurring in response to a signal. Mechanistically, changes may occur at the pretranslational, translational and posttranslational level. These changes may result in alterations in mucosal growth as well as in biochemical and biophysical properties.

Polyamines are essential substances for vital processes of cell proliferation, as phase-specific rises in polyamine amount are a prequisite when entering the G1 phase, at the time of initiation of DNA synthesis and prior to cell division. Although the cellular mechanisms controlling intestinal adaptation are poorly understood, it has been hypothesized that intestinal polyamine levels, regulated by key enzymes controlling their synthesis (ornithine decarboxylase; ODC) and degradation (diamine oxidase; DAO) play a major regulating role in intestinal mucosal growth. These key enzymes, in turn, may be up- and down regulated (in)directly by trophic factors like the nutritive and non-nutritive factors.

A recent rat study of Rountree et al (57) analyzed the proglucagon and ODC messenger RNAs after 80% jejunoileal resection before and after refeeding. In ileum both mRNAs increased before refeeding within 2 hours after resection, indicating nutrient-independent components of the adaptive bowel response. The ODC mRNA remained elevated for 24 hours after resection. This was in contrast to the elevation in proglucagon mRNA, which was sustained for up to 8 days. Taylor et al (58) observed a rapid increase in glucagon mRNA levels, reaching maximal levels 2 days after small-bowel resection in rats. In animals fasted after the resection, elevation of glucagon mRNA, but not of ODC mRNA, was significantly lower compared to fed animals 48 hours after the resection. Compared to glucagon, a similar less abundant pattern of changes has also been recognized for the expression of the Cholecystokinine (CCK) gene. In jejunum, however, no changes in either glucagon or CCK mRNA levels are found after resection, while morphologic changes are seen.

Rokkas et al (59) found that inhibition of DAO, by administering aminoguanidine subcutaneously (25 mg/kg/day), enhanced the adaptive intestinal mucosal growth after 80% proximal small-bowel resection. Blocking ODC, using difluoromethylornithine, markedly inhibited the adaptive hyperplasia normally found 4 days after 50% proximal small-bowel resection in the rat (60).

All these observations underline the hypothesis that a cascade of events, initiated by both nutritive and non-nutritive factors, result in increased polyamine synthesis which in turn upregulates DNA, RNA and protein synthesis, crypt cell proliferation and finally leads to villus hyperplasia (61).

1.4 Concluding remarks

This overview demonstrates that non-nutritive as well as nutritive factors are involved in stimulating the adaptation process of the remaining intestinal remnant following small-bowel resection. The importance of oral food intake is underlined and the indistinctness of the role of food complexity is expressed. Moreover, the importance of diet composition is pointed out.

By now, little information is available on the precise functioning of the complicated regulatory network operative in intestinal adaptation found after small-bowel resection. Although it is generally accepted that the factors discussed affect the adaptation of the residual small bowel, the mechanistic basis has until recently received relatively little

attention. Presently, we begin to unravel the cellular events that regulate the adaptive cell growth, recognizing the conducting role of the polyamines. It is realized that suppression of DAO may be helpful in stimulating hyperplasia after major resections. The precise patterns, and signals responsible for region-related functional alterations in intestinal nutrient uptake in response to bowel resection have yet to be established.

At this stage, it is impossible to answer questions like which diet is optimal, or whether the adaptation response can be maximized. Future studies in transgenic animals that overexpress or underexpress a specific factor will provide direct information about the effect on the adaptive response. In addition, it has to be realized that following intestinal resection not only adaptive changes occur at the level of the small intestine but that in fact the total organism tries to find a new homeostatic balance.

2 IRREVERSIBLE INTESTINAL FAILURE

If ultimately the remaining small intestine does not sufficiently adapt irreversible intestinal failure will develop leaving the patient totally dependent on total parenteral nutrition.

2.1 Limitations of total parenteral nutrition

Patients on home total parenteral nutrition are limited in their lifestyle and, more importantly, they have a considerable risk of serious complications such as sepsis, thrombosis, liver impairment and metabolic disorders (69,70). Especially in children the long-term outlook is less favorable (14-31% mortality rate) because compared to adults they have a higher risk of liver impairment, have limited adequate venous access, and need more specific nutrition to grow and develop normally (71).

Considering these long-term complications of total parenteral nutrition it will be clear that this form of therapy, albeit very effective and life-saving, should only be employed as a temporary solution. Therefore, research is undertaken to find other ways of treating irreversible intestinal failure (72,73). One such option is small-bowel transplantation.

2.2 Incidence in the Netherlands

In the Netherlands, contrary to the United Kingdom (67) and the United States (68), there is no Home Parenteral Nutrition registry in which all patients receiving home parenteral nutrition are listed. Therefore, the incidence of irreversible intestinal failure in the Netherlands can only be estimated. Extrapolating the number of patients recorded in the university hospital Rotterdam (Sophia Children's Hospital for children and Hospital Dijkzigt for adults), being about 3 patients/year, the estimated incidence of irreversible intestinal failure in the Netherlands is 2-3 cases per million of the population per year, leading to a total of 30-45 patients per year.

3 EXPERIMENTAL BASIS FOR SMALL-BOWEL TRANSPLANTATION

Although experimental small-bowel transplantation has already been introduced by Lillehei et al in 1959 (74), convincing evidence that the small bowel can be transplanted in humans has not been obtained until recently. After initial unsuccessful attempts with "classic" immunosuppression like azathioprine and methylprednisolone some thirty years ago (reviewed by Kirkman 75), interest in small-bowel transplantation dwindled. For years, major obstacles including technical complications, rejection, graft-versus-host disease and sepsis have hindered clinical examples of a functioning small-bowel graft. The introduction of cyclosporine A (CsA), which appeared capable of preventing rejection of small-bowel grafts in several animal studies, provided a rationale for further experimentation to determine the feasibility of clinical small-bowel transplantation.

The experimental basis for clinical trials of small-bowel transplantation includes the search for an optimal method and tools to monitor rejection and functional status of the graft. Table 2 illustrates the results obtained so far of experimental research for providing a valuable clinical model.

3.1 Towards an optimal method

Experimental research in small-bowel transplantation was carried out in many ways including a variety of experimental models, modalities of immunosuppression, and methods to alter the immunogenicity of the graft.

Table 2 Comparison of methodologic factors: summary of experimental research in small-bowel transplantation

Outcome (in terms of)	Experimental model A	Experimental model B	References
	Orthotopic SBT	**Heterotopic SBT**	**76,77,78**
Operative time	-	+	
Complications	-	+	
Graft removal	-	+	
Mucosal atrophy	+	-	
Graft permeability	+	-	
	Portoportal drainage	**Portocaval drainage**	**79,80,81**
Physiologic route	+	-	
Metabolic complications	+	-	
Technical complications	-	+	
Immunologically advantageous	+?	-	
	Isolated SBT	**Combined SBLT**	**82,83**
Technical complications	+	-	
Rejection incidence	-	+?	
	CsA	**FK 506**	**84**
Therapeutic window	+	-	
Toxicity	+	-	
Long-term survival	-	+	
Quality of life	-	+	
Effects on growth	+	-	
Synergistic capacity	+	-	
Chronic rejection	-	+	
	MHC matched	**Non-MHC matched**	**85,86**
Long-term survival	+	-	
Graft-versus-host disease	?	?	
	Jejunal graft	**Ileal graft**	**87,88**
Nutritional function	-	+	
Immune function	+	-	
Adaptive capacity	-	+	

Abbreviations used: small-bowel transplantation; SBT, small-bowel-liver transplantation; SBLT
+ = a more positive outcome (e.g shorter operative time, less complications) in comparison with -
- = a less positive outcome (e.g longer operative time, more complications) in comparison with +
+? = not conclusively documented

Position of the graft

Table 2 demonstrates that at first stage a heterotopic transplant is preferable. In this manner less complications will arise and it is possible to remove the graft with less severe morbidity and mortality in case of rejection (76,77,78). It has been reported that a heterotopically placed graft may induce increase in permeability and subsequent bacterial translocation although luminal nutrition is expected to minimize this. Later on, these experimental studies were combined in the so-called two-stage operative technique. The

first stage is the heterotopic small-bowel transplantation, in which the graft is transplanted alongside the recipient's own bowel. With respect to the ends of the intestinal graft there are several possibilities: 1) they both are sutured to the abdominal wall as an enterostoma; 2) the oral end is either ligated, or exteriorized with the aboral end anastomosed to the recipient's distal bowel; 3) the oral end is anastomosed end-to-end to the recipient's proximal bowel with the distal end either ligated or exteriorized. The recipient's own bowel is removed and the graft is interposed several weeks after heterotopic small-bowel transplantation.

Drainage of the graft

The graft's superior mesenteric vein can be anastomosed either to the portal vein (portal drainage) or to the inferior vena cava or iliac vein (systemic drainage). It has been reported that both techniques can be used, as only minor metabolic complications occur following systemic drainage (79,80,81). However, portal drainage, being the more physiologic route, is preferred.

Immunologic aspects

Several studies have demonstrated an immunologic advantage of combined small-bowel-liver transplantation over small-bowel transplantation alone (82,83). However, this topic deserves further study because not all patients on total parenteral nutrition have severe liver damage, and replacing a normal functioning liver by an allograft seems not the most ideal treatment.

In Paris, at the International Symposium on Organ Transplantation in 1992, Starzl compared FK 506 with CsA and concluded that both drugs should be used, as each has its own advantages and drawbacks (84). Major histocompatibility complex (MHC)-matching benefits the long-term graft survival in dogs and it may reduce the occurrence of graft-versus-host disease as this is most commonly caused by MHC antigen differences (85,86).

Composition of the graft

The use of a segmental graft seems feasible and it possesses sufficient adaptive absorptive capacity to give adequate nutritional support. Because of the specialized functional capacities of the ileum, for absorbing bile acids and vitamin B12, the ileum is preferable to a jejunal graft. Theoretically, an ileal graft, containing a greater amount of lymphoid tissue, is more immunogenic than a jejunal graft. Notwithstanding the potential risk of rejection, it seems prudent to use an ileal graft or, if the abdominal cavity has no spatial constraints, a jejunoileal graft in combination with potent immunosuppressive drugs (87,88).

In summary, from a methodologic point of view the experimental data suggest that a

MHC-matched jejunoileal graft, transplanted using the two-stage technique with portoportal drainage is the optimal starting point. The necessity of transplanting a liver as well is questionable, and the use of either CsA or FK 506 can be recommended.

Preservation

In the field of intestinal preservation, pioneering work was already started in 1959 by Lillehei et al (74), and although several factors were extensively studied, the results of small-intestinal preservation remained poor until recently. Research included the effect of several vascular perfusates, preservation temperatures, cryopreservation and flushing pressures (89,90,91). These studies learned that short-term preservation (up to 6 hours) can be obtained with various methods and solutions. It is felt at present that University of Wisconsin solution is best to preserve the bowel. Attention is now given to the role of free radical scavengers (92) and membrane stabilizers (93) to prolong the viability of the intestine.

Strategies to overcome postoperative problems

Frequent problems following small-bowel transplantation include rejection, infection, and graft-versus-host disease. Acute rejection, characterized both clinically and histologically, can be suppressed by using efficient immunosuppressive agents, by decreasing graft antigenicity, and preconditioning of the recipient (94,95).

The use of currently available non-specific immunosuppression has, however, specific side-effects. It was shown in a rat model that CsA treatment may be the critical factor that predisposes to sepsis following small-bowel transplantation (96). It is likely that bacterial translocation is an active process. This was investigated by comparing particles, from which it is known that they translocate passively, with bacterial translocation. It was then found that such particles do not translocate as bacteria do (97). In addition, Grant et al (98) showed that breakdown of the gut barrier during an episode of rejection is associated with bacterial translocation, possibly being the etiologic agent of infectious complications. Some investigations on the process of chronic rejection, becoming a predominant cause of graft loss in the long run, have been started recently (99). Graft-versus-host disease is the clinical manifestation of the immunologic reaction of graft T-lymphocytes against host antigens following small-bowel transplantation. This phenomenon has been well described in rat studies (100), and it is thought that a balance exists between rejection and graft-versus-host disease in such a way that prevention of graft-versus-host disease accelerates graft rejection (101). This implies that the occurrence of graft-versus-host disease should be manipulated, by means of donor pretreatment (102), to prevent the clinical symptoms and simultaneously maintaining the immunosuppressive capacities.

3.2 Monitoring for rejection and function

In Table 3, the tools for monitoring the rejection and/or function of the graft are summarized.

Table 3 **Studied tools to monitor rejection[1] and/or function[2]**

Tool	Disadvantages	Advantages	References
Biopsy based			
Histology[1,2]	* patchy character * perforation risk * presence stoma	* reliable	103,104,105
Intraepithelial lymphocytes[1]		* sensitive * increased during rejection	106
Immunohistology[1]	* presence stoma * perforation risk	* increased sensitivity compared to histology	107,108
Brush border enzyme activity[1,2]	* does not precede histology * presence stoma	* indicates carbohydrate absorptive capacity	109,110
Absorption based			
Transepithelial potential differences[1,2]	* presence stoma	* non-invasive * parallels histology * reflects graft function	111,112,113 114
Maltose absorption[1,2]	* oral administration test solution * bloodsampling	* controversy on its reliability	115,116
D-Xylose absorption[1,2]	* oral administration of test solution * blood sampling * sensitive to infection		117
Lactulose-Mannitol absorption[1]	* oral administration	* insensitive to infection * detection in urinary sample	Chapter 7
^{14}C-labeled Glucose absorption[1,2]	* non-specific	* parallels histology	118
Cyclosporine absorption[1,2]	* not useful under FK 506 * bloodsampling * fat soluble * unreliable		118
Fecal fat absorption[2]		* inexpensive * stool assay	119

Table 3 continued

Tool	Disadvantages	Advantages	References
alpha 1 - antitrypsine[1]		* stool assay * inexpensive * sensitive	119
Permeability based			
Polyethylene glycol leakage[1]	* oral administration test solution * non-specific	* detection in urine	120
^{51}Cr-EDTA leakage[1]	* oral administration test solution * expensive	* detection in urine * increasing during rejection * sensitive	121
Hyaluron leakage[1]	* luminal fluid sampling * non-specific		122
Peripheral blood based		* inexpensive	
Monocyte procoagulant activity[1]		* not conclusively reported	123
N-Acetylhexosaminidase[1]	* does not precede histology	* non-specific	124,125
IL1 and IL2 levels[1]	* do not increase		126
NO_2-/NO_3- levels[1]	* specificity questionable	* increased during rejection	127
Cytoimmunology[1]		* probably sensitive, reliable	128

This Table shows that histology is reliable for monitoring the rejection process and that its sensitivity can be improved using immunohistochemical staining. Considering the disadvantages of histology including the need of taking full thickness biopsies and the patchy character of the rejection process, functional tests have been examined for their use in recognizing rejection. With respect to the use of an absorption method, a test based on different absorptive substances is preferable in that it gives an increased specificity. These methods are unlikely to be useful in acute rejection because the patient has not returned to enteral feeding in that space of time. But an absorptive method may have some value in the search for chronic rejection. A permeability-based method, not useful for functional monitoring of the graft, seems to be reproducible and reliable because the permeability is increased during rejection. However, it is non-specific at the same time, also being altered during some infections. A reliable serum marker as early detector of rejection has not been found yet, although many substances including monocyte

procoagulant activity, N-Acetylhexosaminidase, cytokine, and NO_3-/NO_2-levels have been advocated.
Margreiter suggested that monitoring of neoptrin in serum is possibly less masked by infection than NO_3-/NO_2- levels. Recent work suggests that the use of cytoimmunologic monitoring of the peripheral blood is valuable for monitoring rejection and thus deserves further study.

It is important to realize that it is uncertain whether the sensitivity and/or specificity of the studied parameters are preserved in human small-bowel transplantation. Therefore, at this stage, the combined use of all available tools is probably the best to establish rejection with the highest sensitivity and specificity. It is not easy to distinguish between graft-versus-host disease and rejection on the basis of clinical signs, as their symptoms are often the same and both may be present at the same time or in each other's absence (87). A relatively non-invasive test for diagnosing graft-versus-host disease is still lacking. In late graft-versus-host disease, a skin biopsy that demonstrates lymphocytic infiltration at the dermal-epidermal junction together with basal-cell degeneration is helpful in making the distinction with rejection possible. In contrast, no histologic confirmation of graft-versus-host disease is possible in case of humoral graft-versus-host disease (with anti-host hemolytic anaemia).

Studies on graft function have addressed immunologic, nutritional, motor and hormonal aspects (129). The immunologic status of transplanted intestine has not been well defined yet. Normally, the small bowel has a significant role in the host immune defense. One component of this important function is the production of secretory IgA (sIgA), the predominant local antibody. Normal levels of total sIgA have been found following small-bowel transplantation, but allografts fail to respond to a new antigen with production of a specific sIgA (130).
However, priming of the recipient abrogates the inhibitory effect of CsA on specific sIgA production (131). T lymphocytes are thought to carry out important effector functions in the intestinal immune system and it is known that even in the absence of rejection donor-lymphocytes are replaced by recipient-lymphocytes, which implies that mucosal immune function may be mediated by host-derived cells (132).

Monitoring weight gives a sensitive indication of the functional competence of the graft following small-bowel transplantation and in addition a battery of absorption-based function tests is available (Table 3). Long-term studies in adult animals showed that the overall nutritional status can be maintained satisfactorily, although late effects on both fecal fat absorption and D-xylose absorption were noted (129,133). More studies are

needed, both in auto- and allotransplantation models, to determine the precise long-term consequences of small-bowel transplantation and how compromised functions can be restored. Grant et al (134) demonstrated in growing pigs that transplantation of the entire small bowel enabled normal weight gain. In contrast, Kimura demonstrated that transplanting a segmental jejunal allograft showed reduced weight gain (129,135). Grant et al, however, did not create short gut controls and Kimura did not include a normal healthy control group, which makes it impossible to conclude that a segmental graft is less able to increase weight. *Thus, so far, it has still not been established whether a segmental graft provides sufficient functional capacity to maintain a normal growth pattern and nutritional status.*

The interdigestive motility activity of the small bowel (termed the migrating motor complex; MMC) is a well-defined, spontaneous and recurring cyclic pattern. Sarr et al (136) demonstrated in a canine jejunoileal autotransplantation model that the characteristic MMC was present in both the innervated duodenum and the transected jejunoileum, but that coordination between these regions was lacking. The physiologic function of the MMC is to clear the small bowel of non-indigestible intraluminal debris and lacking of MMC coordination throughout the gastrointestinal tract, due to permanent extrinsic denervation, is thought to be responsible for eventual bacterial overgrowth (137). Moreover, extrinsic denervation may result in the loss of inhibitory and/or excitatory neural fibers present in the gut and having an extrinsic origin. Nelson et al (138) reported alterations in tissue neuropeptide concentrations that may indicate long-term changes of denervated transplanted bowel.

4 CLINICAL EXPERIENCE WITH SMALL-BOWEL TRANSPLANTATION

The reported small-bowel transplantations in pediatric recipients have been carried out in Paris and Pittsburgh between 1987 and 1992 (see Table 4). In all cases cadaveric donors of similar or smaller size were used.

4.1 Paris; transplantation under cyclosporin-based immunosuppression

Recipients

The Ethical Committee of the French Hôpital Nêcker Enfants Malades authorized the start of a clinical small-bowel transplantation program in 1987. The consent was partly based

Table 4 Clinical characteristics of transplanted pediatric patients

Patient	Age (yr)	Etiology	Remaining Anatomy	Duration Home total parenteral nutrition (months)
1 (male)[1]	0.75	neonatal total volvulus	10 cm duodenum entire colon	6 and 12 respectively
2 (female)[1]	9	secondary total volvulus after ileocecal intussusception at 3 years of age	15 cm jejunum, 5 cm ileum and entire colon	72
3 (male)[1]	5	total volvulus	10 cm jejunum, 5 cm ileum, entire colon and ileocecal valve	12
4 (female)[1]	0.5	total volvulus	duodenum and colon no ileocecal valve	6
5 (male)[1]	?	total volvulus	duodenum and colon no ileocecal valve	not reported and 12 respectively
6 (male)[1]	4	small-bowel atresia	20 cm jejunum	48
7 (male)[1]	0.8	volvulus	duodenum and colon no ileocecal valve	10
8 (female)[2]	2.3	necrotizing enterocolitis	duodenum and colon	38
9 (male)[2]	4.3	gastroschisis	10 cm jejunum and colon	52
10 (male)[2]	2.8	intestinal atresia	duodenum and colon	33
11 (female)[2]	0.6	intestinal atresia	not reported	6
12 (female)[2]	1.1	volvulus	not reported	12
13 (female)[2]	1.7	volvulus	not reported	18
14 (female)[2]	2.5	microvillus inclusion	not reported	29
15 (male)[2]	1.3	intestinal atresia	not reported	15
16 (female)[2]	10.2	chronic intestinal pseudo obstruction	not reported	132

[1] Data from Revillon et al, 1992 (141); [2] Data from Todo et al, 1992 (149)

on positive results obtained in piglets since 1975 (139,140).

The clinical characteristics of the patients are shown in Table 5. Nine isolated small-bowel transplantations were performed in 7 children. In all cases the patient was on long-term total parenteral nutrition before undergoing small-bowel transplantation and was considered to have irreversible small-intestinal failure.

Transplant protocol

In the performed transplantations, donor and recipient were ABO compatible in all

patients except in patient 5 in which donor was O^+ and recipient A^+. All grafts used were harvested from human leucocyte antigens (HLA)-mismatched donors ranging from neonates (n=3) to 17-year-old children (n=6) (141). Grafts were harvested during multiple-organ harvesting and prepared by in situ vascular flushing of the small bowel with Collin's or University of Wisconsin solution at 4°C. The small-bowel graft included a jejunoileal segment of 90 to 120 centimeters on a vascular pedicle of the superior mesenteric vessels. The graft was always placed heterotopically at first instance to facilitate eventual removal of the graft in case of complications. The vascular washing solution included CsA (100 mg/L) and anti-thymocyteglobulin (50 mg/L).

Postoperative immunosuppression included CsA, usually combined with methylprednisolone (2 mg/kg/d). In all except the first two cases antithymphocyteglobulin (5mg/kg/d over a 6 hr infusion) was given for 15 days and Azathioprine (1.5 mg/kg/d) was included from day 6 onwards.

Antibiotic treatment consisted of administration of systemic antibiotics, including ceftazimidime (100 mg/kg/d), phosphomycin (200 mg/kg/d) and ornidazole (30 mg/kg/d) and, total decontamination of the recipient bowel by using vancomycin (100 mg), colimycin (1 million units), tobramycin (100 mg) and nystatin (500 mg) four times a day.

Postoperative course

Rejection. The graft was monitored by regularly observing the stoma for early histologic signs of rejection (villus edema and mucosal sloughing) and alterations in immunohistochemically stained biopsy specimens (increased T-cell infiltrates and increased HLA-DR expression on crypt enterocytes) the graft was monitored.

Early acute rejection was observed in two patients (2 and 5) and was marked by increased ileostomy output. A prompt increase in immunosuppression (ATG 5 mg/kg/d) reversed the rejection process in patient 2. Patient 5 was treated unsuccessfully with OKT3 after which the graft had to be removed.

Delayed acute rejection was successfully treated with OKT3 in two cases (patient 3 and 4). No attempt to reverse rejection was undertaken in patients 1 and 2 because of the life-threatening condition of the patients at that time.

Graft-versus-host disease. Using CsA combined with methylprednisolone and azathioprine, rejection occurred in all cases whereas no signs of graft-versus-host disease were seen.

Infection. The infectious complications observed were opportunistic infections with

Pneumocystis carinii and Cytomegalovirus and can therefore be attributed to the immunocompromised condition of the recipient and not to rejection.

Graft function. Intestinal continuity was reestablished if a patient had good clinical condition without signs of rejection and tolerated enteral feeding. In patient 3, the graft was brought in orthotopic position in the 11th postoperative month. This infant was able to maintain a normal eating pattern for several months. Six months after the orthotopic placement of the graft, the patient developed chronic rejection necessitating removal of the graft.

In patient 4, better known as Virginie, intestinal continuity was reestablished at 8 months. Total parenteral nutrition was progressively diminished and discontinued at 10 months. A recent report (142) describes the first 26 months in detail. The results show normal growth and height curves. Intestinal transit time studies demonstrated normal barium through flow. The fat absorption rate was also in the normal range. The patient is now totally enterally fed and is receiving oral immunosuppression.

4.2 Pittsburgh; transplantation under FK 506-based immunosuppression

Recipients

In several rat small-bowel transplantation models (143,144,145) the usefulness of FK 506 was demonstrated to be superior to the immunosuppressive capacity of CsA. This finding prompted the Pittsburgh group to initiate a clinical trial of combined small-bowel-liver transplantation in May 1990.

The pretransplant clinical characteristics of the pediatric recipients are summarized in Table 5. All patients were on total parenteral nutrition for a certain period, varying from 1 to 132 months. Three patients received an isolated small-bowel graft. The other six patients had experienced severe liver damage (total bilirubin from 6.3 to 50 mg/DL) and therefore received a combined small-bowel-liver transplant (146).

Transplant protocol

The donors were ABO-identical with the respective recipients. As in Paris, HLA matching was random and resulted in two cases in which lymphocytotoxic cross-matching was positive. Graft harvesting and technical details of the construction of liver engraftment and heterotopic placement of the small bowel have been described in detail by Starzl et al (147). The small-bowel graft consisted of almost its entire length, from a

Table 5 Transplantation-related features of individual patients

Patient and date of transplantation	Composition of the graft	Postoperative complications	Graft Survival	TPN
1 01/09/87	small bowel	* graft necrosis and subsequent hemodynamic disorders	3 hours	
03/26/89	small bowel	* no early complications * week 7: development of sepsis, pneumonia and rejection	8 weeks	
2 03/21/87	small bowel	* no early complications * day 15: acute rejection * day 205: graft rejection and subsequent renal insufficiency, major liver disease and hematologic disorders	205 days	
3 02/18/88	small bowel	* no early complications * day 30: CMV infection * day 60-64: recurrent pancreatitis * day 150: subacute rejection * month 17: chronic rejection	17 months	
4 03/18/89	small bowel	* rejection episodes (at 3, 5 and 19 months) * pneumocystis infection	> 3 years	free
5 06/30/89	small bowel	* day 8: severe rejection	25 days	
?/?/90	small bowel	* hemodynamic disorders leading to death	none	
6 ?/?/89	small bowel	* no early complications * month 5: acute rejection * month 7: chronic rejection	7 months	
7 ?/?/90	small bowel	* no early complications * day 30: acute rejection	30 days	
8 07/24/90	small bowel and liver	* rejection episodes: 3 (SB), 1 (L) * infectious episodes: 3(b), 1 (v), 2 (t) * refused to eat	> 2 years	free
9 11/24/90	small bowel and liver	* spinal cord injury after spinal tip * rejection episodes: 1 (SB), 6 (L) * infectious episodes: 3 (b), 2 (v), 2 (t) * pylorospasm	> 2 years	free
10 03/24/91	small bowel and liver	* paralysis of the right hemidiaphragm * rejection episodes: 4 (SB), 6 (L) * infectious episodes: 3 (b), 2 (v), 2 (t)	> 1.5 years	free
11 08/09/91	small bowel and liver	* anastomotic bowel leak died of sepsis and possible graft-versus-host disease	23 days	
12 08/10/91	small bowel and liver	* no complications	> 1 year	free
13 08/12/91	small bowel and liver	* no complications	> 1 year	free
14 10/31/91	small bowel	* not reported	> 1 year	free
15 12/25/91	small bowel	* not reported	> 10 months	free
16 ?	small bowel	* not reported	?	partial

TPN= total parenteral nutrition, SB= small bowel, L= liver, b = bacterial , v = viral, t = translocation

few centimeters distal to the ligament of Treitz to a few centimeters proximal to the ileocecal valve. The graft was perfused in isolation with cold University of Wisconsin solution but no luminal washing was performed. No effort on donor pretreatment (immunomodulation) was made.

Administration of FK 506, the basic immunosuppressive therapy, was started immediately after graft revascularisation by continuous intravenous infusion (0.1 to 0.15 mg/kg/per day) (148). Intravenous administration of the drug was switched to enteral administration (twice daily with a total dose of 0.3 mg/kg/per day) a few days after enteral feeding was restarted. Low dose methylprednisolone, 200 mg at first dose tapered to a 20 mg/day maintenance level over 5 days, was given additionally. From patient 14 on, prostaglandin E1 infusion (0.6 to 0.8 g/kg/hour) was added to the immunosuppressive cocktail.

Selective intestinal decontamination was the same in donor and recipient and consisted of a mechanically given mixture of amphotericin B, tobramycin and polymyxin E given four times a day for a period of 4 to 6 weeks. Systemic antibiotics included ampicillin and claforan and was given the first five postoperative days.

Postoperative course

Rejection. Endoscopy was performed and biopsy material was obtained if clinical symptoms of intestinal rejection, including fever, malaise, dysmotility (ileus or diarrhea) of the graft or malabsorption were present. On base of these findings (clinical, endoscopic and biopsy) the degree of rejection was diagnosed (149), and treatment was adjusted if necessary. Treatment resulted in control of rejection in all cases. In Table 5, rejection episodes are shown for each patient for both intestine and liver.
The incidence of graft rejection appeared to be lower, after isolated small-bowel transplantation than after combined small-bowel-liver transplantation, at least in the early postoperative period (150).

Graft-versus-host disease. Patient 11 possibly died of graft-versus-host disease, because clinical findings of graft-versus-host disease, including apoptosis and infiltration of the skin with lymphocytes of donor phenotype, were found one day before death. The patient died 23 days after the operation after having developed sepsis and multiple organ failure. In the other patients no signs of graft-versus-host disease were encountered.

Infection. Four patients developed in total 6 infectious episodes in which microorganisms detected in the blood were proven the same as present in the stool. In half of these occasions, translocation could be associated with rejection. It appeared that isolated small-

bowel grafted recipients had an incidence of infectious complications comparable to small-bowel-liver grafted ones. Patient 10 developed an opportunistic adenovirus infection.

Graft function. Reestablishment of the intestinal continuity was established 8 to 16 weeks after heterotopic placement of the graft. All but one patient (16) had become independent of total parenteral nutrition (149). These updated results are more optimistic than those reported previously by the same group (151). At that time only two of nine patients were totally independent of total parenteral nutrition. Initially, all patients had aversion to food and had to be taught to eat. By now, patients 11 and 13 still refuse to eat normally. The graft's function was assessed by means of histology, intestinal graft transit time studies and several absorptive tests (fecal fat excretion, FK 506 kinetics and D-xylose absorption).

Fat absorption was abnormal in all patients, even as long as one year after transplantation (149). In the early postoperative period both accelerated and prolonged intestinal transit time were found which improved later on. The D-xylose absorption test showed slightly abnormal results in patient 12 and 13 on 111 and 73 days postoperatively respectively. The other patients had normal D-xylose absorption. The available weight data are summarized in Table 6, which shows that all patients gained weight after being fed enterally.

Table 6 **Results on start of oral feeding after heterotopic placement and subsequent weight gain after orthotopic placement of intestinal graft (56-112 days after heterotopic placement), updated to January 1992**

Patient	Starting enteral feeding (days)	Complete enteral feeding (days)	Preoperative weight (kg)	Current Weight (kg)	Postoperative survival (days)
8	11	60	12.8	14.8	> 557
9	23	210	19.6	20.2	> 434
10	37	200	14.8	19.3	> 314
12	16	60	10.8	12.7	> 175
13	30	45	10.8	12.7	> 175
14	9	49	12.4	14.6	> 93
15	5	14	12.6	12.8	> 38

4.3 Discussion

The experimental basis obtained by the Paris group was not very convincing for starting a clinical trial. Their average failure rate was 20 out of 44 operations and the clinical immunosuppressive protocol was based on only 4 long-term surviving animals. In the clinical cases described, no attention was given to HLA matching. No comment can be made on the benefits of tissue matching for small-bowel transplantation on the basis of clinical experience. However, concerning the results of HLA matching in case of other solid organ transplant programs and those obtained in large animals a potential effect of tissue matching on graft survival can certainly not be excluded (152,153). The bad results obtained in the nine performed transplantations led to a stop of the clinical transplantation programm in Paris.

The Pittsburgh transplant protocol included no efforts on immunomodulation of the graft. Such policy, regarding the immunogenicity of the graft, may be expected to result in a high incidence of graft-versus-host disease. However, their experimental work has routinely demonstrated prevention of graft-versus-host disease in rats treated with FK 506 (144). The one patient that developed clinical graft-versus-host disease was receiving decreased immunosuppression because of a technical complication.

The clinical results show that successful intestinal transplantation is now feasible from a technical point of view. The postoperative course was stormy in most patients, rejection still being a major barrier, even under FK 506 immunosuppression. Surveillance endoscopy is indispensable for early diagnosing rejection with the "official" tools as confirming instruments. Infectious complications, in some cases early indicators of graft rejection, were often encountered. These problems underline the complexity and difficulties in managing the early postoperative phase after small-bowel transplantation. The studied parameters on functioning of the transplanted intestine were satisfying, although the abnormal results on fecal fat excretion give reason for caution with respect to long-term functioning.

At this moment, the available data on weight gain do not indicate that a small-bowel transplant can provide for adequate nutritional support to a growing individual with short bowel syndrome. Besides, very recently it has been decided to stop the clinical small-bowel-liver transplantation programm in Pittsburg also as a consequence of the bad overall results in the long run.

REFERENCES

1. Affourtit MJ, Tibboel D, Hart AEH, Hazebroek FWJ and Molenaar JC. Bowel resection in the neonatal phase of life: Short-term and long-term consequences. *Z Kinderchir 1989; 44: 144.*
2. Schwartz MZ and Maeda K. Short Bowel syndrome in infants and children. *Pediatr Clin North Am 1985; 32: 1265.*
3. Ziegler MM. Short bowel syndrome in infancy: etiology and management. *Clin Perinatol 1986; 13: 163.*
4. Grosfeld JL, Rescorla FJ and West KW. Short bowel syndrome in infancy and childhood. *Am J Surg 1986; 51: 41.*
5. Jones BJM. Nutritional management of the short bowel syndrome. *Gastroenterology 1987; 2: 99.*
6. Zlotkin SH, Stallings VA and Pencharz PB. Total parenteral nutrition in children. *Pediatr Clin Am 1985; 32: 381.*
7. Weaver LT, Austin S and Cole TJ. Small intestinal length: a factor responsible for gut adaptation. *Gut 1991; 32: 1321.*
8. Wilmore DW. Factors correlating with a succesful outcome following extensive intensive resection in newborn infants. *J Pediatr 1972; 80: 88.*
9. Rijke RPC. Control mechanisms of cell proliferation in intestinal epithelium. *Thesis 1977, Erasmus University Rotterdam.*
10. Jeejeebhoy KN. Therapy of the short-gut syndrome *The Lancet 1983: 1427.*
11. Weser E, Heler R and Tawil P. Stimulation of mucosal growth in the rat ileum by bile and pancreatic secretions after jejunal resection. *Gastroenterology 1977; 73: 524.*
12. Goldmann LI, Dorn B, Weinberger M, Bralow P and Gruenstein M, The small intestine and gastric secretion in the rat. *Arch Surg 1972; 104: 73.*
13. Vanderhoof JA, Park JH and Grandjean CJ. Reduced mucosal prostaglandin synthesis after massive small-bowel resection. *Am J Physiol 1988; 254: G373.*
14. Gregor M, Menge H, Stossel R and Riecken EO. Effect of monoclonal antibodies to enteroglucagon on ileal adaptation after proximal small bowel resection. *Gut 1987; 28(Suppl 1): 9.*
15. Grey VL, Kenscoff JM and Morin CL. Growth stimulating activity associated with an altered cell renewal pattern in the small intestine. *Gut 1987; 28(Suppl): 15.*
16. Pul M, Yilmaz N, Gurses N, Orzgur KG and Turgutalp H. Enhancement by testosterone of adaptive growth after small bowel resection. *Isr J Med Sci 1991; 27: 339.*
17. Hart MH, Phares CK, Erdman SH, Grandjean CJ, Park JH and Vanderhoof JA. Augmentation of postresection mucosal hyperplasia by pierocercoid growth factor (PGF). Analog of human growth factor. *Dig Dis 1987; 32: 1275.*
18. Holmes SJK and Moosa AR. Somatostatin inhibits post-resectional ileal hyperplasia. *Br J Surg 1981; 68: 819.*
19. Williamson RCN. Intestinal adaptation. *N Engl J Med 1978; 298: 1393.*
20. Touloukian RJ and Spencer RP. Ileal blood flow preceding compensatory intestinal hypertrophy. *Ann Surg 1972; 175: 320.*
21. Ulrich-Baker MG, Hollwarth ME, Kvietys PR and Granger DN. Blood flow responses to small bowel resection. *Am J Physiol 1986; 251: G815.*
22. Touloukian RJ, Aghajanian GK and Roth RH. Adrenergic denervation of the hypertrophied gut remnant. *Ann Surg 1972; 176: 633.*
23. Hahn V, Shuppan D, Hahn EG, Merker HJ and Riecken EO. Intestinal cells produce basement membrane protein in vitro. *Gut 1987; 28(Suppl 1): 143.*
24. Tepperman J, Brobeck JR and Long CNH. The effects of hypothalamic hyperphagia and of alterations in feeding habits on the metabolism of the albino rat. *Yale J Biol 1973; 15: 855.*
25. Altmann GG. Influence of starvation and refeeding on mucosal size and epithelial renewal in the rat small intestine. *Am J Anat 1972; 133: 391.*
26. Heird WC and Winters RW. Total parenteral nutrition. The state of the art. *J Pediatr 1975; 86: 2.*
27. Morin CL, Ling V and Van Caillie M. Role of oral intake on intestinal adaptation after massive small bowel resection in growing rats. *Pediatr Res 1978; 12: 268.*
28. Ford WDA, Boelhouwer RU, King WWK, de Vries JE, Ross JS and Malt RA. Total parenteral nutrition inhibits intestinal adaptive hyperplasia in young rats: reversal by feeding. *Surgery 1984: 527.*
29. Wilmore DW, Dudrick SJ, Daly JM and Vars HM. The role of nutrition in the adaptation of the

small intestine after massive resection. *Surg Gynecol Obstet 1971: 673.*
30. Weser E. The management of patients after small bowel resection. *Gastroenterology 1976; 71: 146.*
31. Koretz RL and Meyer JL. Elemental diets-facts and phantasies. *Gastroenterology 1980; 78: 393.*
32. Kinney JM, Goldwijn RM, Barr JS and Moore FD. Loss of the entire jejunum and ileum, and the ascending colon. *JAMA 1962; 179: 529.*
33. Thompson WR, Stephen RV, Randall HT and Bowen JR. Use of the "space diet" in the management of a patient with extreme short bowel syndrome. *Am J Surg 1969; 117: 449.*
34. Voitk AJ, Echave V, Browne RA and Gurd FN. Use of elemental diet during the adaptive stage of the short gut syndrome. *Gastroenterology 1973; 65: 419.*
35. Voitk AJ and Crispen JS. The ability of an elemental diet to support nutrition and adaptation in the short gut syndrome. *Ann Surg 1975: 220.*
36. Touloukian RJ, Mitruka B and Hoyle C. Growth and nutrition of enterectomized rats fed an elemental liquid diet. *Surgery 1971; 69(4): 637.*
37. Grey VL, Carofalo C, Greenberg GR and Morin CL. The adaptation of the small intestine after resection in response to free fatty acids. *Am J Clin Nutr 1984; 40: 1235.*
38. Buts J, Morin CL and Ling C. Influence of dietary components on intestinal adaptation after small bowel resection in rats. *Clin Invest Med 1979; 2: 59.*
39. Koruda MJ, Rolandelli RH, Settle RG, Saul H and Rombeau JL. The effect of a pectin-supplemented elemental diet on intestinal adaptation to massive small bowel resection. *JPEN 1986; 10: 343.*
40. Kripke SA, De Paula JA, Berman JM, Fox AD, Rombeau JL and Settle RG. Experimental short-bowel syndrome: effect of an elemental diet supplemented with short-chain triglycerides. *Am J Clin 1991; 53: 954.*
41. Koruda MJ, Rolandelli RH, Settle RG, Zimmaro DM and Rombeau JL. Effect of parenteral nutrition supplemented with short-chain fatty acids on adaptation to massive small bowel resection. *Gastroenterology 1988; 95: 715.*
42. Weinberg LM, Pusateri JP and Levine GM. Comparison of different caloric substrates on intestinal adaptation in the rat. *Gastroenterology 1989; 96: 1514.*
43. Young EA. Short bowel syndrome: high-fat versus high carbohydrate diet. *Gastroenterology 1983; 84: 872.*
44. Vanderhoof JA, Blackwood DJ, Mohammadpour H and Park JHY. Effects of oral supplementation of glutamine on small intestinal mucosal mass following resection. *J Am Coll Nutr 1992; 11: 223.*
45. Vanderhoof JA, Grandjean CJ, Kaufman SS, Burkley KT and Antonson DL. Effect of high percentage medium-chain triglyceride diet on mucosal adaptation following massive small bowel resection in rats. *JPEN 1984; 8: 685.*
46. Spector MH, Levine GM and Deren JJ. Direct and indirect effects of dextrose and amino acids on gut mass. *Gastroenterology 1977; 72: 706.*
47. Morin CL, Grey VL and Garofalo C. Influence of lipids on intestinal adaptation after resection. *In: Robinson JL, Dowling RH, Riecken EO, eds. Mechanism of intestinal adaptation, proceedings of an international conference. MTP Press, Lancaster, England 1982: 175.*
48. Ecknauer R, Sircar B and Johnson LR. Effect of dietary bulk on small intestinal morphology and cell renewal in the rat. *Gastroenterology 1981: 781.*
49. Sakata T and Engelhardt WV. Stimulating effect of short chain fatty acids on the epithelial cell proliferation in the rat large intestine. *Comp Biochem Physiol 1983; 74A: 459.*
50. Dowling RH. Small bowel adaptation and its regulation. *Scan J Gastroenterol 1982; 17(suppl 74): 53.*
51. Zurier RB, Campbell RG, Hashim SA and Van Itallie TB. Use of medium chain triglyceride in management of patients with massive resection of the small intestine. *N Engl Med 1966; 274: 450.*
52. Simko V, Carroll AM, Goodman S, Weenser RE and Kelley RE. High-fat diet in a short bowel syndrome. Intestinal absorption and gastroenteropancreatic hormone responses. *Dig Dis 1980; 25: 333.*
53. Hart MH, Grantjean CJ, Park JHY, Erdman SH and Vanderhoof JA. Essential fatty acid deficiency and post resection mucosal adaptation in the rat. *Gastroenterology 1988; 94: 682.*
54. Morin CL, Grey VL and Garofalo C. Influence of lipids on intestinal adaptation after resection. In: Robinson JWL, Dowling RH and Riecken EO, eds. *Mechanisms in intestinal adaptation. MTP Press, Lancaster, England 1981.*
55. Urban E and Michel M. Separation of adaptive mucosal growth and transport after small bowel

resection. *Am J Physiol 1983; 244: G295.*
56. Bury K. Carbohydrate digestion and absorption after massive resection. *Surgery 1972; 135: 177.*
57. Rountree DB, Ulshen MH, Selub S, Fuller CR, Bloom SR, Ghatei MA and Lund PK. Nutrient-independent increases in proglucagon and ornithine decarboxylase messenger RNAs after jejunoileal resection. *Gastroenterology 1992; 103: 462.*
58. Taylor RG, Verity K and Fuller PJ. Ileal glucagon gene expression: ontogeny and response to massive small bowel resection. *Gastroenterology 1990; 99: 724.*
59. Rokkas T, Vaja S, Murphy GM and Dowling RH. Aminoguanidine blocks intestinal diamine oxidase activity and enhances the intestinal adaptive response to resection in the rat. *Digestion 1990; 46(suppl 2): 447.*
60. Bamba T, Vaja S, Murphy GM and Dowling RH. Role of polyamines in the early adaptive response to jejunectomy in the rat: effect of DFMO on the ileal villus: crypt axis. *Digestion 1990; 46(suppl 2): 410.*
61. Dowling RH, Hosomi M, Stace NH, Lirussi F, Miazza B, Levan H and Murphy GM. Hormones and polyamines in intestinal and pancreatic adaptation. *Scand J Gastroenterol 1985; 20(suppl 112): 84.*
62. Keelan M, Thomson AB, Wierzbicki AA, Wierzbicki E, Rajotte R and Clandinin MT. Isocaloric modification of dietary lipids influences intestinal brush border membrane composition in diabetic rats. *Diabetes Rev 1991; 16: 127.*
63. Fedorak PN, Thomson AB and Porter VM. Adaptation of intestinal glucose transport in rats with diabetes mellitus occurs independent of hyperphagia. *Can J Physiol Pharmacol 1991; 69: 1143.*
64. Keelan M, Thomson AB, Garg ML and Clandinin MT. Critical period programming of intestinal glucose transport via alteration in dietary fatty acid composition. *Can J Physiol Pharmacol 1990; 68: 642.*
65. Thomson AB, Mc Intyre Y, Macleod J and Keelan M. Dietary fat content influences uptake of hexoses and lipids into rabbit jejunum following ileal resection. *Digestion 1986; 35: 78.*
66. Thomson AB. Defined formula diets alter jejunal and colonic uptake of lipids in rabbits with intact intestinal tract and following ileal resection. *Res Exp Med (Berl) 1986; 186: 413.*
67. Ingham Clark CL, Lear PA, Wood S, Lennard-Jones JE and Wood FFM. Potential candidates for small bowel transplantation in the United Kingdom. *Br J Surg 1992; 79(7): 676.*
68. Wolman SL, Jeejeebhoy KN, Stewart S and Grieg PD. Experience in home parenteral nutrition and indications for small-bowel transplantation. *In: Deltz E, Thiede A and Hamelmann, eds. Small-bowel transplantation. Experimental and clinical fundamentals. Berlin: Springer-Verlag, 1986: 214.*
69. Steiger E and Srp F. Morbidity and mortality related to home parenteral nutrition in patients with gut failure. *Am J Surg 1983; 145: 102.*
70. Bowyer BA, Fleming CR, Ludwig J, Petz J and Mc Gill DB. Does long-term home parenteral nutrition in adult patients cause chronic liver disease. *JPEN 1985; 9: 111.*
71. Cooper A, Floyd TS, Ross AJ, Bishop HC, Templeton JM and Ziegler MM. Morbidity and mortality of short bowel syndrome acquired in infancy: an update. *J Pediatr Surg 1984; 19: 711.*
72. Bianchi A. Intestinal loop lengthening. A technique for increasing small intestinal length. *J Pediatr Surg 1980; 15: 145.*
73. Waag KL and Heller K. Surgical techniques in short bowel syndrome. *Prog Pediatr Surg 1990; 25: 81.*
74. Lillehei RC, Goott B and Miller FA. The physiological response of the small bowel of the dog to ischaemia including prolonged in vitro preservation of the bowel with succesful replacement and survival. *Ann Surg 1959; 150: 543.*
75. Kirkman RL. Small bowel transplantation. *Transplantation 1984; 37: 429.*
76. Lee KKW and Schraut WH. *In: Deltz E, Thiede A and Hamelmann, eds. Small-bowel transplantation. Experimental and clinical fundamentals. Berlin: Springer-Verlag, 1986: 7.*
77. Kort WJ, Westbroek DL, Mac Dicken I and Lameyer LDF. Orthotopic total small bowel transplantation in the rat. *Eur Surg Res 1973; 5: 81.*
78. Grant D, Zhong R, Hurlbut D, Garcia B, Chen H, Lamont D, Wang P, Stiller C and Duff J. A comparison of heterotopic and orthotopic intestinal transplantation in rats. *Transplantation 1991; 51: 948.*
79. Schraut WH, Abraham VS and Lee KKW. Portal versus systemic venous drainage for small bowel allografts. *Surgery 1985; 98: 579.*
80. Schaffer D, Diflo T, Love GHA, Clowes T, Maki T and Monaco AP. Metabolic effects of systemic versus portal drainage of orthotopic small bowel isografts. *Transplant Proc 1989; 21:*

2872.

81. Koltun WA, Madara JL, Smith RJ and Kirkman RL. Metabolic aspects of small bowel transplantation in inbred rats. *J Surg Res 1987; 42: 341.*
82. Kobayashi E, Kamada N, Enosawa S, Toyama N, Kai T, Kimura N and Miyata M. Liver transplantation regulates a graft-versus-host reaction in the rat. *Transplant Proc 1992; 24: 1204.*
83. Sarnacki S, Cerf-Bensussan N, Revillon Y, Calise D, Goulet O, Ricour C and Brousse N. Long-term small bowel graft survival induced by spontaneously tolerated liver allografts in inbred rat strains. *Transplant Proc 1992; 24: 1210.*
84. Starzl TE. Oral presentation at the 18th International Congress of the Transplantation Society, August 1992, Paris.
85. Meijssen MAC, Heineman E, de Bruin RWF, Wolvekamp MCJ, Marquet RL and Molenaar JC. Successful canine small bowel transplantation using major histocompatibility complex matched segmental ileal allografts. *Transplant Proc 1992; 24: 1141.*
86. Ingham Clark CL, Price BA, Malcolm P, Lear PA and Wood RFM. Graft versus host disease in small bowel transplantation. *Br J Surg 1991; 78: 1077.*
87. Schraut WH. Current status of small-bowel transplantation. *Gastroenterology 1988; 94: 524.*
88. Stangl MJ, Schraut WH, Moynihan HL and Lee T. Rejection of ileal versus jejunal allografts. *Transplantation 1989; 47: 424.*
89. Luther B, Wolff H, David H and Lehman CH. Results of perfusion and preservation of animal and human small-bowel transplant. *Transplant Proc 1990; 22: 2435.*
90. Guttman FM, Berdnikoff G, Sangbhundhu K and Braun P. Preservation of canine small bowel by freezing. *Arch Surg 1976; 111: 260.*
91. Van Oosterhout JMA, de Boer HHM and Jerusalem CR. Small bowel transplantation in the rat: the adverse effect of increased pressure during the flushing procedure of the graft. *J Surg Res 1984; 36: 140.*
92. Sun S, Greenstein SM, Schecher RS, Sablay LB, Veith FJ and Tellis VA. Improved small intestinal preservation with additional use of superoxide dismutase to University of Wisconsin solution. *Transplant Proc 1992; 24: 1092.*
93. Tomita K, Yagi M, Masutani H, Hashimoto T, Konishi K and Miyazaki I. Changes in mucosal glycoprotein at ischemia and reperfusion of the small bowel. *Transplant Proc 1992; 24: 1098.*
94. Fukuzawa M, Santiago S, Nakata S, Shirakuar R and Okada A. Effect of donor-specific transfusions and FK-506 on small intestine allotransplantation. *Transplant Proc 1991; 23: 3252.*
95. Grant D. Intestinal transplantation: current status. *Transplant Proc 1991; 21: 2869.*
96. Browne BJ, Johnson CP, Edmiston CE, Hlava MA, Moore GH, Roza AM, Telford GL and Adams MB. Small bowel transplantation promotes bacterial overgrowth and translocation. *J Surg Res 1991; 51: 512.*
97. Fabian MA and Bollinger RR. Rapid translocation of bacteria in small bowel transplantation. *Transplant Proc 1992; 24: 1103.*
98. Grant D, Hurlbut D, Zhong R, Wang P, Chen H, Garcia B, Behme R, Stiller C and Duff J. Intestinal permeability and bacterial translocation following small bowel transplantation in the rat. *Transplantation 1991; 52: 221.*
99. Langrehr JM, Muller AR, Banner B, Lee TK, Schraut WH and Lee KKW. Graft morphology and serum chemistry profile during chronic rejection of intestinal allografts in the rat. *Transplant Proc 1992; 24: 1068.*
100. Diflo T, Maki T, Balogh K and Monaco AP. Graft-versus-host-disease in fully allogeneic small bowel transplantation in the rat. *Transplantation 1989; 47: 7.*
101. Saat RE, de Bruin RWF, Heineman E, Jeekel J and Marquet RL. Total orthotopic allogeneic small bowel transplantation in rats: the effect of allograft irradiation combined with cyclosporine A therapy. *Gut 1991; 32: 654.*
102. de Bruin RWF, Saat RE, Heineman E, Jeekel J and Marquet RL. Effects of donor-pretreatment with antilymphocyte serum and cyclosporine on rejection and graft-versus-host disease after small bowel transplantation in immunosuppressed and nonimmunosuppressed rats. *Transplant Int 1992; 6: 22.*
103. Schmid TH, Oberhuber G, Korozsci G, Klima G and Margreiter R. Histologic pattern of small bowel allograft rejection in the rat. *Gastroenterology 1989; 96: 1529.*
104. Millard PR, Dennison A, Hughes DA, Collin J and Morris PJ. Morphology of intestinal allograft rejection and the inadequacy of mucosal biopsy in its recognition. *Br J Exp Path 1986; 67: 687.*
105. Rosemurgy AS and Schraut WH. Small bowel allografts. Sequence of histologic changes in acute

and chronic rejection. *Am J Surg 1986; 151: 470.*

106. Oberhuber G, Schmid T, Thaler W, Luze T, Klima G and Margreiter R. Increase in intraepithelial lymphocytes as an early marker of rejection in a fully allogeneic rat small bowel transplantation model.*Eur Surg Res 1993; 25: 310.*
107. Hurlbut D, Garcia B, Ohene-Fianko D, Duff J and Grant D. Immunohistochemical assessment of mucosal biopsies following human intestinal transplantation. *Transplant Proc 1992; 24: 1195.*
108. Borgstrom A, Wider M, Marks WH, and Vinik A. Porcine ileal peptide: a potential marker for rejection of small-intestine allografts. *In: Deltz E, Thiede A and Hamelmann H, eds. Small-bowel transplantation. Experimental and clinical fundamentals. Berlin: Springer-Verlag, 1986; 250.*
109. Meijssen MAC, Heineman E, de Bruin RWF, Marquet RL and Molenaar JC. Diminished functional capacity and compromized functional integrity in acute rejecting DLA-matched and mismatched canine small-bowel allografts. *Transplant proc 1992; 24: 1116.*
110. Schroeder P, Gundlach M, Quaroni A, Hansmann ML, Schweizer E and Deltz E. Follow-up of small bowel transplantation by monoclonal antibodies against brush border membranes. *Transplant Proc 1990; 22: 2476.*
111. Meijssen MAC, Heineman E, de Bruin RWF, Veeze HJ, Bijman J, de Jonge HR, ten Kate FWJ, Marquet RL and Molenaar JC. Value of in vivo electrophysiological measurements to evaluate canine small bowel autotransplants. *Gut 1991; 32: 1329.*
112. Meijssen MAC, Heineman E, de Bruin RWF, ten Kate FJW, Marquet RL and Molenaar JC. Detection of canine intestinal allograft rejection by an in vivo electrophysiologic monitoring. *Transplantation 1991; 51: 955.*
113. Lee MD, Smith SD, Yunis EJ and Rowe MI. In vivo transmural potential difference: an early monitor of rejection in small bowel transplantation. *J Pediatr Surg 1989; 24: 767.*
114. Madara JL and Kirkman RL. Structural and functional evolution of jejunal allograft rejection in rats and the ameliorating effects of cyclosporine therapy. *J Clin Invest 1985; 75: 502.*
115. Biliar TR, Carberoglio C and Schraut WH. Maltose absorption as an indicator of small intestinal allograft rejection. *J Surg Res 1984; 37: 75.*
116. Murase N, Demetris A, Matsuzaki T, Yagihashi A, Todo S, Fung J and Starzl TE. Long survival in rats after multivisceral versus isolated small-bowel allotransplantation under FK 506. *Surgery 1991; 110: 87.*
117. Ruiz JO, Uchida H, Schultz LS and Lillehei RC. Problems in absorption and immunosupression after entire intestinal allotransplantation. *Am J Surg 1972; 123: 297.*
118. Nordgren S, Cohen Z, Mackenzie R, Finkelstein D, Greenberg G and Langer B. Functional monitors of rejection in small intestinal transplants. *Am J Surg 1984; 147: 152.*
119. Kitagawa H, Ford EG, Sinatra F, Thomas D and Atkinson JB. Fecal fat, cyclosporine and alpha 1-antitrypsine for assessment of small bowel function following transplantation. *J Pediatr Surg 1991; 26: 1091.*
120. Teitelbaum DH, Dunaway DJ, Sonnino RE, Stellin G, Berend ME and Harmel R.P. Leakage of intraluminal low molecular weight polyethylene glycol as a marker of small bowel transplant rejection. *J Ped Surg 1989; 24: 64.*
121. Grant D, Hurlbut D, Zhong R, Wang P, Chen H, Garcia B, Behme R, Stiller C and Duff J. Intestinal permeability and bacterial translocation following small bowel transplantation in the rat. *Transplantation 1991; 52: 221.*
122. Wallander J, Johnson C, Hallgren R, Gerdin B and Tufveson G. Intestinal ditribution and leakage of hyaluronan in small bowel allografting in the rat. *Transplant Proc 1992; 24(3): 1100.*
123. Silverman R, Cohen Z, Levy G, Graig M, Cullen J and Langer B. Immune responses in small intestinal transplantation in the rat: correlation of histopathology and monocyte procoagulant activity. *Surgery 1987: 395.*
124. Maeda K, Schwartz MZ, Bamberger MH and Daniller A. A possible serum marker for rejection after small intestine transplantation. *Am J Surgery 1987: 68.*
125. Meijssen MAC, Heineman E, de Bruin RWF, Marquet RL and Molenaar JC. The value of N-acetyl hexosaminidase in detecting canine intestinal allograft rejection. *Transplant Proc 1991; 23: 615.*
126. Cohen Z, Silverman RE, Wassef R, Levy GA, Burnstein M, Cullen J, Makowka L, Langer B and Greenberg GR. Small intestinal transplantation using cyclosporine. *Transplantation 1986; 42: 613.*
127. Langrehr JM, Muller AR, Lee TK, Schraut WH, Simmons RL and Hoffman RA. Serum NO_2^- /NO_3^- from oxidative L-arginine metabolism: a possible marker for small bowel allograft rejection. *Transplant Proc 1992; 24(3): 1148.*

128. Graeb C, Stangl MJ, Mebert H, Fischer T and Hammer C. Cytoimmunologic monitoring of small bowel allograft recipients. *Transplant Proc 1992; 24: 1153.*
129. de Bruin RWF, Heineman E and Marquet RL. Small bowel transplantation; an overview. *Transplant Int 1993; in press.*
130. Xia W and Kirkman RL. Immune function in transplanted small intestine. Total secretory IgA production and response against cholera toxin. *Transplantation 1990; 49: 277.*
131. Xia W and Kirkman RL. Priming the recipient abrogates the inhibitory effect of cyclosporine on specific sIgA production against cholera toxin in small bowel transplantation. *Transplant Proc 1992; 24: 1139.*
132. Lear PA, Cunningham AJ, Crane PW and Wood RFM. Lymphocyte migration patterns in small bowel transplants. *Transplant Proc 1989; 21: 2881.*
133. Quigley EMM, Thomspon JS and Rose SG. The long-term function of canine jejunoileal autotransplants - insight into allograft physiology. *Transplant Proc 1992; 24: 1105.*
134. Grant D, Duff J, Zhong R, Garcia B, Lipohar C, Keown P and Stiller C. Successful intestinal transplantation in pigs treated with cyclosporine. *Transplantation 1988; 45: 279.*
135. Kimura K, La Rosa CA, Bland MA and Jaffe BM. Successful segmental allotransplantation in enterectomized pigs. *Ann Surg 1990; 211: 158.*
136. Sarr MG, Duenes JA and Tanaka M. A model of jejunoileal in vivo neural isolation of the entire jejunoileum: transplantation and the effects on intestinal motility. *J Surg Res 1989; 47: 266.*
137. Thompson JS, Rose SG, Spanta AD and Quigley EMM. The long-term effect of jejuoileal autotransplantation on intestinal function. *Surgery 1992; 111: 62.*
138. Nelson DK, Sarr MG and Go VLW. In vivo neural isolation of the canine jejunoileum: temporal adaptation of enteric neuropeptides. *Gut 1991; 32: 1336.*
139. Ricour C, Revillon Y, Arnaud-Battandier F, Ghnassia D, Weyne P, Laufenburger A, Jos J, Fontaine JL, Gallix P and Vaiman M. Succesful small bowel allografts in piglets using cyclosporine. *Transplant Proc 1983; 15: 3019.*
140. Arnaud-Battandier F, Salmon H, Aynaud JM, Bernard S, Revillon Y and Ricour C. In vitro and in vivo studies of the mucosal immune barrier after long-term small bowel allotransplantation in pigs using cyclosporine. *In Deltz E, Thiede A, Hamelmann H, (eds). Small-bowel transplantation. Experimental and clinical fundamentals. Berlin: Springer-Verlag 1986: 39.*
141. Revillon Y, Jan D, Sarnacki S, Goulet O, Brousse N and Ricour C. Intestinal transplantation in the child; experimental and clinical studies. *J Pediatr Surg 1993; 28: 292.*
142. Goulet O, Revillon Y, Brousse N, Jan D, Canion D, Rambaud C, Cerf-Bensussan N, Buisson C, Hubert D, de Potter S, Mougenot JF, Fisher A and Ricour C. Successful small bowel transplantation in an infant. *Transplantation 1992; 53: 940.*
143. Lee K, Stangl MJ, Todo S, Langrehr JM, Starzl TE and Schraut WH. Succesful orthotopic small bowel transplantation with short term FK506 immunosuppressive therapy. *Transplant Proc 1990; 22: 78.*
144. Murase N, Demetris AJ, Matsuzaki T, Yagihashi A, Todo S, Fung J and Starzl TE. Long survival in rats after multivisceral versus isolated small bowel allotransplantation under FK506. *Surgery 1991; 110: 87.*
145. Hoffman AL, Makowka L, Banner B, Cai X, Cramer DV, Pascualone A, Todo S and Starzl TE. The use of FK506 for small intestine allotransplantation: inhibition of acute rejection and prevention of fatal graft-versus-host disease. *Transplantation 1990; 49: 483.*
146. Todo S, Tzakis A, Reyes J, Abu-Elmagd K, Casavilla A, Nour BM, Selby R, Fung JJ, Van Thiel D and Starzl TE. Clinical small bowel or small bowel plus liver transplantation under FK506. *Transplant Proc 1991; 23: 3093.*
147. Starzl TE, Todo S, Tszakis A, Alessiani M, Casavilla A, Abu-Elmagd K and Fung JJ. The many faces of multivisceral transplantation. *Surg Gynecol Obstet 1991; 172: 335.*
148. Todo S, Tzakis AG, Abu-Elmagd K, Reyes J, Fung JJ, Casavilla A, Nakamura K, Yagihashi A, Iain A, Murase N, Iwaki Y, Demetris AJ, Van Thiel DH and Starzl TE. Cadaveric small bowel and small bowel-liver transplantation in humans. *Transplantation 1992; 53(2): 369.*
149. Todo S, Tzakis AG, Abu-Elmagd K, Reyes J, Nakamura K, Casavilla A, Selby R, Nour BM, Wright H, Fung JJ, Demetris AJ, Van Thiel DH and Starzl TE. Intestinal transplantation in composite visceral grafts or alone. *Ann Surg 1992; 216:223.*
150. Tzakis AG, Todo S, Reyes J, Nour B, Abu-Elmagd K, Selby R, Casavilla A, Kocoshis S, Demetris AJ, Yunis E, van Thiel DH and Starzl TE. Intestinal transplantation in children under K 506 immunosuppression. *J Pediatr Surg 1993; 28: 1040.*

151. *Tzakis AG, Todo S, Reyes J, Abu-Elmagd K, Casavilla A, Ohya T, Fung JJ, Yunis E, Demetris AJ, Kocoshis S, Van Thiel DH and Starzl TE. Clinical intestinal transplantation: focus on complications. Transplant Proc 1992; 24:1238.*

152. Pirsch JD, D'Alessandro AM, Sollinger HW, Hoffman RM, Roecker E, Voss BJ, Lorentzen D, Knechtle SJ, Reed A and Kalyoglu M. The effect of donor age, recipient age and HLA match on immunologic graft survival in cadaver renal transplant recipients. *Transplantation 1992; 53: 55.*

153. Karuppan SS, Lindholm A and Moller E. Fewer acute rejection episodes and improved outcome with selection criteria based on crossmatching. *Transplantation 1992; 53: 666.*

CHAPTER 2

Aims of the experimental work

A. SCOPE OF THE ADAPTATION-RELATED STUDIES

After extensive small-bowel resection, the remaining intestine will adapt to the loss of absorptive area. The adaptive changes include alterations in gut morphology as well as gut function. The overall process, which is very complex, is still not precisely understood. A tool for monitoring the functional status of the intestine is still missing. This is why it is still impossible to study the effect of factors that might affect the functioning of the intestine. There is evidence that enteral nutrition is a major stimulator of intestinal adaptation. However, the belief that an predigested diet is the optimal treatment of the short bowel syndrome is not based on any well-designed experimental study.

The adaptation-related experiments described in this thesis were performed to answer the following questions:

* *Is it possible to monitor the functional process of intestinal adaptation after massive small-bowel resection ?*
  This was investigated in rats that underwent near-total small-bowel resection; we developed a non-invasive method for in vivo measurement of transepithelial potential differences (Chapter 3).
* *What is the role of the complexity and composition of enteral nutrition on the morphologic and functional adaptation after massive small-bowel resection ?*
  This was investigated in rats that underwent small-bowel resection; the rats were fed either normal rat chow, a diet with intact or partial hydrolyzed proteins, or a non-identical polymeric diet (Chapter 4).

B. SCOPE OF THE TRANSPLANTATION-RELATED STUDIES

Small-bowel transplantation in man is now technically feasible. Before a clinical small-bowel transplantation program in children is justified, it must have been ascertained that a small-intestinal transplant can give adequate nutritional support to a growing individual with the short bowel syndrome. There are a number of aspects that are involved in the functioning of the small intestine. Firstly, the transplantation procedure itself may temporarily or permanently alter the small-intestinal function in such a way that intestinal transplantation is not a realistic treatment modality. Secondly, optimally MHC-matched persons could donate allogeneically identical segments of intestine to serve as grafts for persons with severe short bowel syndrome. In growing individuals this approach is only beneficial if a segmental graft adapts sufficiently to treat the short bowel syndrome and to

sustain growth and development.

Major problems in human small-bowel transplantation are the stormy postoperative course, requiring multidisciplinary management, and the lack of an early, simple rejection marker. A simple test indicating the functional capacity of the graft is also not available at present.

The transplantation-related studies were performed to answer the following questions:

* *Can normal growth and development be expected after performing total orthotopic small-intestinal autotransplantation in growing individuals ?*
  This was investigated in growing dogs by performing one-step total orthotopic small-intestinal autotransplantation (Paragraph 5.1).
* *What are the long-term effects of the small-bowel transplantation procedure on function, morphology, and bacteriology of the small intestine of a growing individual ?*
  This was evaluated by long-term analysis of the functional, morphologic, and bacterial status of the small intestine after one-step total orthotopic small-intestinal autotransplantation in growing dogs (Paragraph 5.2).
* *Is ultrasonography of value in the follow up of small-bowel transplantation ?*
  This was determined in growing dogs, by means of ultrasonography, to evaluate the postoperative course after a two-stage segmental small-intestinal allotransplantation (Chapter 6).
* *What is the role of a MHC-matched segmental small-intestinal allotransplantation model in the treatment of the short bowel syndrome in growing individuals ?*
  This was investigated in growing dogs by performing a two-stage MHC matched segmental intestinal allotransplantation (Chapter 7).
* *Is it possible to detect acute intestinal rejection at an early stage by use of a serum marker ?*
  The value of diamine oxidase as serum marker of acute rejection was studied in rats after performing fully allogeneic total orthotopic small-bowel transplantation (Paragraph 8.2).
* *Is it possible to evaluate intestinal graft function by use of a simple test ?*
  The value of assessing postheparin diamine oxidase release as indicator of graft function was determined after fully allogeneic total orthotopic small-bowel transplantation in rats (Paragraph 8.3).

PART A. ADAPTATION

CHAPTER 3

The value of in vivo electrophysiologic measurements for monitoring functional adaptation after massive small-bowel resection in the rat

Published in Gut 1993; 34: 637-642, supported by a research-fellowship from the European Society for Parenteral and Enteral Nutrition

THE VALUE OF IN VIVO ELECTROPHYSIOLOGIC MEASUREMENTS FOR MONITORING FUNCTIONAL ADAPTATION AFTER MASSIVE SMALL-BOWEL RESECTION IN THE RAT

Abstract

The process of functional adaptation after extensive small-bowel resection is complex and imprecisely understood. We set out to evaluate the value of in vivo electrophysiologic measurements for monitoring the functional adaptation process after massive small-bowel resection in Brown-Norway rats. Rats underwent either a sham operation (sham-operated rats) or a 90% small-bowel resection (small-bowel resected rats). Standard rat chow was fed ad libitum. At 3 or 10 weeks postoperatively, jejunal and ileal transepithelial potential differences (in mV) were determined. Electrogenic ion transport in villus and crypt was measured after glucose (sodium-coupled active glucose absorption) and theophylline infusion (theophylline-stimulated chloride secretion) respectively. Biopsies were obtained simultaneously. Each experimental group consisted of 3 to 5 animals. At 3 weeks the theophylline-stimulated chloride secretion and the sodium-coupled active glucose absorption in small-bowel resected rats were significantly lower than in sham-operated rats in both jejunal and ileal segments. At 10 weeks the theophylline-stimulated chloride secretion and the sodium-coupled active glucose absorption were significantly diminished in the jejunal segment of the small-bowel resected rats as compared to the sham-operated rats. However, the values of theophylline-stimulated chloride secretion and sodium-coupled active glucose absorption in the ileal segments were not different anymore between the two groups. Three and ten weeks postoperatively the villus length in the small-bowel resected group was increased significantly as compared to the sham-operated controls. These results indicate that in the early phase of adaptation in vivo electrophysiologic parameters do not correlate with histologic changes in the small-bowel resected rats. This might be due to cell immaturity resulting from an increased cell turnover rate and/or lack of intercellular tight junctions. This hypothesis is supported by a recovery of transepithelial potential differences, in response to stimulation, in the ileum 10 weeks after resection.

Introduction

Extensive small-bowel (SB) resection triggers complex adaptive changes (1,2). Initially, all resected patients need intravenous infusion to avoid the development of malnutrition while oral feeding is being attempted (3). Most negatively, the markedly reduced absorptive surface results in the short bowel syndrome, which is defined as incapability to thrive through oral nutrition. Most positively, the remaining bowel adapts sufficiently to provide essential nutritional support. Manipulating the outcome is difficult, as the process of functional adaptation after extensive SB resection is not fully understood.

Intestinal adaptation has been described as the proliferative, morphologic and functional response to a variety of internal and external stimuli to maintain equilibrium in the gastrointestinal tract (4). After intestinal resection, hyperplastic changes caused by increased cell turnover in the proliferative zone of the crypts, are generally seen in the enteric remnant (5). There is evidence that the unchanged number of cells per unit length may indicate immature enterocytes (5,6,7), but increased absorptive capacity per unit length has been reported as well (8,9). These divergent results may be ascribed to chronologic differences in the studies performed. Urban et al postulated that morphologic growth precedes functional components of intestinal adaptation after resection (10). However, the exact interrelationship between histologic changes and function, possibly regulated by different factors that may be sequentially interrelated, is unknown. The purpose of this study was to determine the value of in vivo electrophysiologic measurements for monitoring functional intestinal adaptation.

Electrophysiologic responses are reliable indicators of SB function (11). We developed an in vivo technique, measuring transepithelial potential differences (PD) evoked by sodium-coupled active glucose absorption, which is an index of villus function, and cAMP mediated chloride secretion, predominantly reflecting crypt cell function.
Moreover, histologic specimens were collected to explore the interrelationship between morphologic and functional changes during the adaptation phases studied in this investigation.

Material and Methods

Animals
Male rats of the inbred Brown-Norway (BN) strain (Harlan CPB, Zeist, The Netherlands) were used. The animals weighed 250-400 g and were bred under specific pathogen-free conditions. The animals underwent either a sham operation (SH), a 90% SB resection

(SBr), or were negative control animals (IS). During the experimental period, all animals were kept under standard laboratory conditions (12 hours light/12 hours dark) and were given water and standard rat chow (AM-II; Hope Farms, Woerden, The Netherlands) ad libitum.

Ethics

The experimental protocols adhered to the rules laid down in "The Dutch Animal Experimentation Act" (1977) and the published "Guidelines on the Protection of Experimental Animals" by the Council of the E.C. (1986). Specific protocols were approved by the Commitee on Animal Research of the Erasmus University, Rotterdam. Permission was given as the expected animal harm was considered minor to the expected social benefit.

Operation procedure

The rats were anesthetized with ether after which a midline laparotomy was performed. SBr rats were created by near total SB resection, i.e from 2.5 cm distally from the ligament of Treitz to 2.5 cm proximally from the ileocecal valve. A sham operation was performed by transection midway ileum and jejunum, without removal of bowel mass. After each procedure gastrointestinal continuity was restored in an end-to-end fashion, using Ethicon 7-0. After the operation, all animals were given 1 ml (10% v/v in PBS) Depomycin 20/20 (Gist-Brocades, Animal Health b.v., De Bilt) subcutaneously. Half of the animals in each group underwent electrophysiologic monitoring and were sacrificed 3 weeks postoperatively (SH_1, n=5 and SBr_1, n=7), the other animals (SH_2, n=5 and SBr_2, n=5) 10 weeks postoperatively. A third group of animals (IS, n=3) were not subjected to any operation. In these rats the superior mesenteric artery was clamped two hours before starting the electrophysiologic measurement in order to provoke ischemic intestinal injury. The intestinal segment selected for measuring was anatomically identical in all rats.

Techniques

Growth assessment. Postoperatively, the animals were weighed three times a week.

Electrophysiology. Rats were anaesthetized with ether, and a midline laparotomy was performed to visualize the bowel for the electrophysiologic measurement. This method is a modification of the technique developed by Meijssen et al to monitor function of the SB in dogs (12). A well-defined jejunal and ileal segment was chosen as measure-area in which a continuous flow of test solution was maintained using canulas. Several iso-osmolar test solutions were flushed through the segment to determine intraluminal transepithelial PD in reference to a subcutaneous $Ag/AgCl_2$ electrode (37°C, 8 ml/min).

The standard solution consisted of (in mM): Mannitol 50; NaCl 110; HEPES 5; KCl 4; Na_2SO_4 10. In the theophylline and glucose containing solutions part of the mannitol was iso-osmotically replaced by 5 mM Theophylline or 30 mM α-D-Glucose respectively. Before starting the measurement preperfusion was performed (6 minutes) to equilibrate luminal content with the standard perfusion solution. Subsequently the following solutions were infused for 5 minutes each: Standard-Theophylline-Standard-Glucose-Standard. The standard solution was used to assess the basal potential differences which reflect physiologic active ion transport. Infusion of Theophylline-solution (5mM) evoked chloride secretion, predominantly a crypt function, resulting in an intraluminal negative PD (PD-theo). A Glucose-solution (30mM) evoked sodium-coupled glucose absorption, reflecting villus function, also resulting in an intraluminal negative PD (PD-glu). An overview of the technique is depicted in Figure 1.

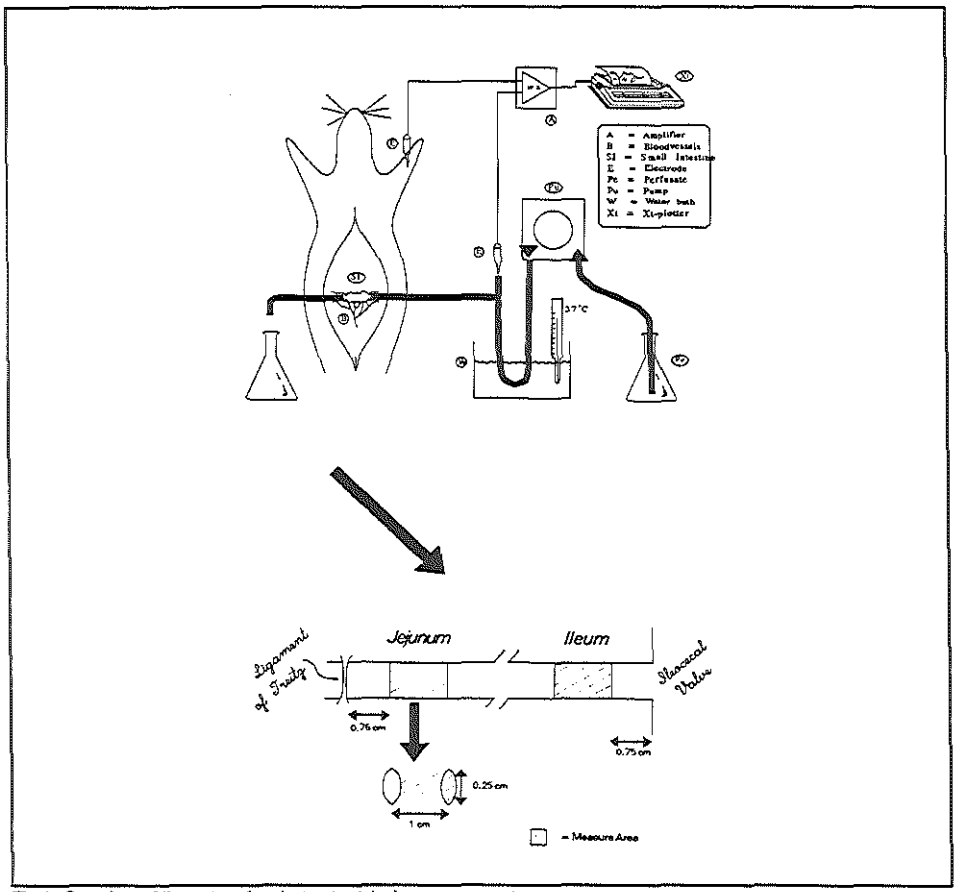


Fig.1. *Overview of the set up for electrophysiologic measurements.*

Histology. Full thickness biopsies were collected just before the electrophysiologic measurements and immediately fixed in 3.6% buffered formalin, then dehydrated and embedded in paraffin. Sections of 4-5 μm were stained with hematoxylin-azophloxin-safran. By standardized projection of sections on a screen through a light microscope, crypt and villus length could easily be measured.Villus height was measured by subtracting crypth length, i.e the shortest distance between the bottom of intestinal villi and the lamina muscularis mucosae, from mucosa height. For each rat, a total of 10 measurements was performed and the average was considered representative in the comparative study.

Metabolic parameters. During the sixth postoperative week, rats were kept for 4 days in metabolic cages provided with a system to collect faeces and urine, and to measure food and water consumption.

Statistical analysis

Statistical analysis of data between experimental groups was performed using the Student's t-test. We preferred to express our data as the difference between SH and SBr at each time point, because there was a tendency in the sham-operated group to a decreased PD response, apparently as a consequence of the operation. Differences with p-values lower than 0.05 were considered to be significant.

Results

Mean weight change curves during the postoperative period for resected and sham-operated animals are shown in Figure 2.

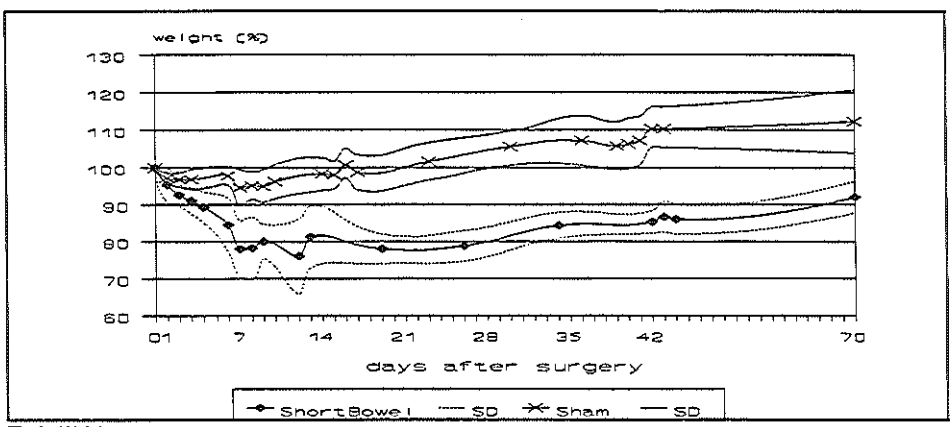


Fig.2. *Weight curves*

All electrophysiologic data obtained in SH and SBr rats are summarized in Figures 3 and 4.

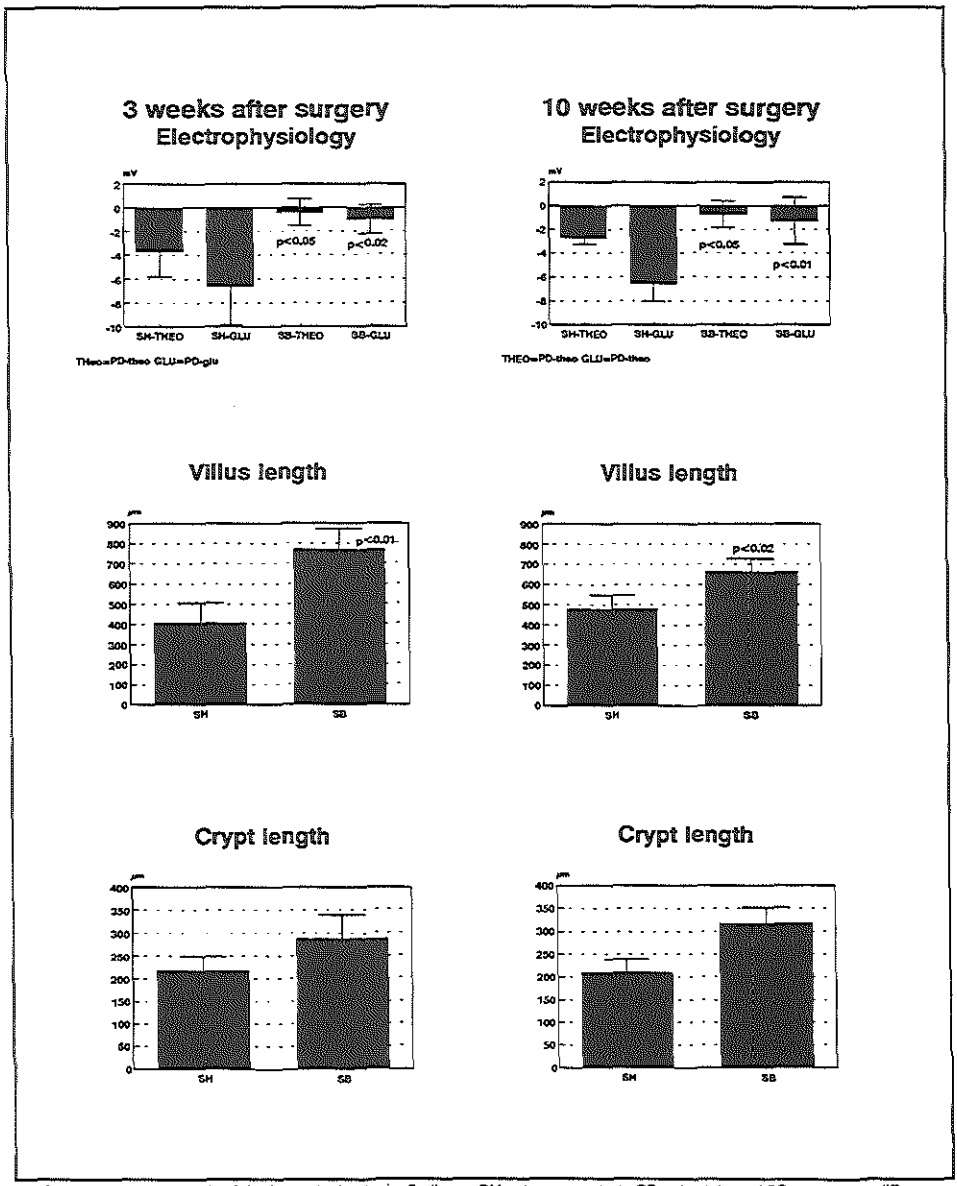


Fig.3. *Jejunum: electrophysiologic and histologic findings. SH, shamoperated; SB, short bowel;PD, potential difference; Glu, glucose; THEO, theophylline; values in mean (SD).*

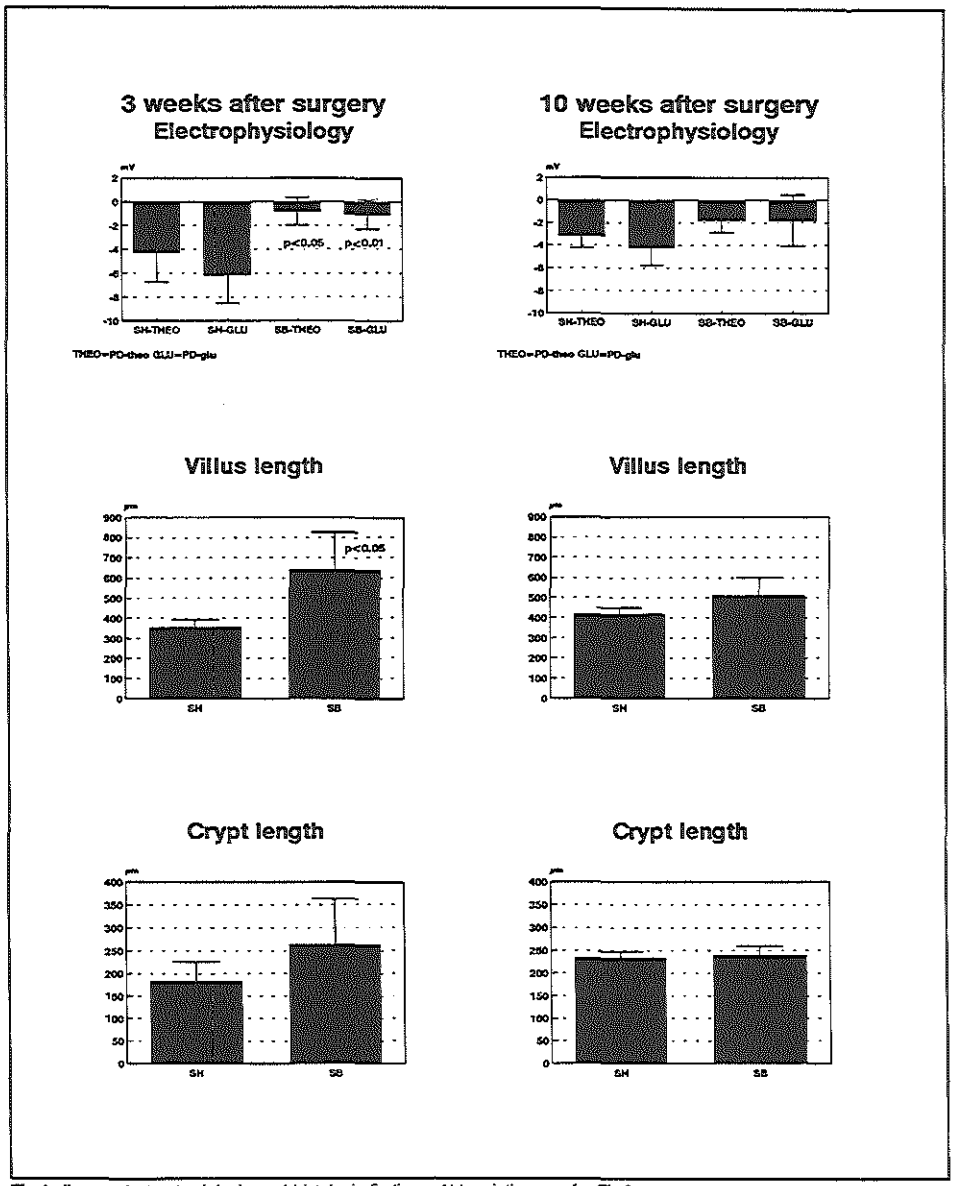


Fig.4. *Ileum: electrophysiologic and histologic findings. Abbreviations as for Fig.3.*

Weight

Sham-operated animals exhibited a small postoperative weight loss followed by weight gain to 110% of the preoperative weight. In the short bowel group the follow-up revealed postoperative weight loss to 70-80% followed by a gradual recovery to 90% of the preoperative weight. None of these rats gained preoperative body weight within 10 weeks. Two SBr_1 rats died during the experimental period due to clinical short bowel syndrome.

Electrophysiology

Jejunal electrophysiologic measurements. At 3 weeks, the glucose and theophylline stimulated PD responses in SBr_1 rats were significantly diminished compared to SH_1 rats ($p<0.02$ and $p< 0.05$ respectively). At 10 weeks the stimulated PD responses in SBr_2 rats were significantly lower in the jejunal segment as compared to SH_2 rats (PD-glu: $p< 0.01$; PD-theo: $p<0.05$). In IS rats, no response was found to either of the test solutions (results not shown).

Ileal electrophysiologic measurements. At 3 weeks, stimulated PD responses in rats from the SBr_1 group were significantly diminished for both glucose ($p<0.01$) and theophylline ($p<0.05$) compared to those in group SH_1. At 10 weeks no significant differences in PD responses were found in either of the groups. In IS rats, again, no response was found to either of the test solutions (results not shown).

Histology

Histologic data are depicted in Figures 3 and 4 respectively.

Jejunal findings. At 3 weeks postoperatively, villus length was significantly enlarged in SBr_1 rats compared to SH_1 rats. Also 10 weeks postoperatively, jejunal villus length was significantly enlarged in SBr_2 rats compared to SH_2 rats. In addition, no differences in crypt length were found in either of the animals.

Ileal findings. Three weeks postoperatively, villus length was significantly increased in SBr_1 animals compared to SH_1 animals ($p< 0.05$). Ten weeks postoperatively, no significant difference was found in ileal villus length between the experimental groups. Again, no differences in crypth length were found in either of the groups.

Metabolic parameters

Figure 5 shows that 6 weeks postoperatively there were no significant differences between SH and SBr operated animals for food intake, water consumption, and urine and faeces production.

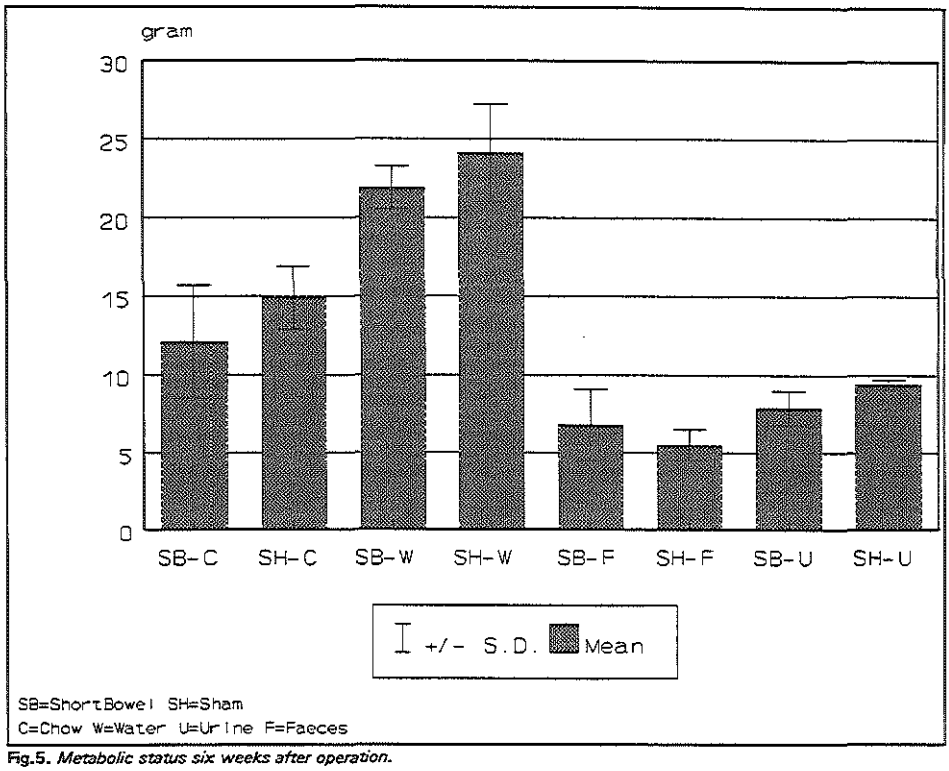


Fig.5. *Metabolic status six weeks after operation.*

Discussion

In our experiments we performed near total SB resection to obtain a sublethal SBS model. In our view the occurrence of malnutrition, which is a characteristic of our sublethal model, may be a major trigger for intestinal adaptation. We set out to evaluate the interrelationship between active electrolyte transport mechanisms and mucosal growth in jejunal as well as ileal segments in the follow-up of intestinal adaptation. The metabolic studies, performed six weeks postoperatively, demonstrated that at that time differences between sham-operated and resected animals were not caused by major metabolic changes. Electrophysiology, a reliable tool for functional assessment of active electrolyte transport mechanisms (12), might be useful to monitor the functional adaptation process. The electrophysiologic results show that the methodology developed is technically feasible in rats. It has been demonstrated that clamping the superior mesenteric artery for two hours results in ischemic intestinal injury in which active electrolyte transport mechanisms have been damaged (13). As expected, ischemic control animals did not develop a transepithelial PD in response to any of the solutions. In contrast, transepithelial PD

values obtained in sham-operated and resected animals show that active transepithelial transport of electrolytes by an isolated bowel segment can be evaluated quantitatively by in situ electrophysiologic monitoring. Previous studies have already shown that in vitro preparations of intestine can be used to study electrogenic Na^+ absorption and electrogenic Cl^- secretion (14). From both in vitro and in vivo studies, it is generally assumed that villus epithelial cells are responsible for electrolyte coupled absorptive processes (14,15). Crypt cells, on the contrary, are thought to be mainly responsible for secretion of ions and water (14,16). Barry et al demonstrated (17) that electrical potential differences are fundamentally similar comparing in vivo and in vitro results. The in vivo technique should be preferred to avoid ischemia, disruption of neural,lymphatic and blood supply and to have better sources of endogenous metabolites. However, the technique described in this study should be refined to develop a non-invasive measuring system, as recently developed for large animals (12). Such a refinement would have great value in the investigation of adaptation mechanisms by determining the effect of diet composition (18), hormonal supplementation (19,20), or manipulating the polyamine metabolism (21).

Previous experiments showed that reported timing of functional adaptation varies. These variations may be related to the amount of tissue resected, postoperative time points studied, the specific region of the enteric remnant explored and the nature of the transport activities tested. For example, Urban et al (10) found decreased duodenal and ileal transport of sodium, chloride, water and galactose in rats 2 weeks after a 70% SB resection. By 4 weeks postresection, increased duodenal sodium, chloride and water, and ileal galactose transport were found. There was increased morphologic growth both 2 and 4 weeks after resection. Another study (22) evaluated carbohydrate absorption 2, 6 and 12 weeks after an 80% resection. Both in jejunal and ileal remnants, a progressive rise, first seen by 6 weeks, was evident.

The present experiments show that 3 weeks after resection, both the glucose-induced (reflecting Na+-couple glucose transport by the villus epithelium) and the theophylline provoked PD response (reflecting active Cl- secretion by the crypt epithelium) in ileum were significantly diminished as compared to sham operation. At that time, a considerable increase in villus length was found in resected rats but no enlargement of the crypts was found. In jejunum, 3 weeks after resection, similar results were found. We assume that 3 weeks after resection the enteric remnant exhibits functional immaturity as a consequence of increased cell turnover. Such immaturity has already been postulated (5,6,7,10) and may readily explain the reduction in Na+-glucose cotransport, a characteristic feature of mature villus cells. However, the parallel reduction in the theophylline-induced PD is more difficult to interpret, considering the prominent role of immature intestinal crypt

cells in active Cl- secretion.This chromologous finding cannot be explained by a hyperactivation of the Cl- secretory poars by endogenous secretagogues in the SB group, because the basal PD was not significantly different between SH and SBr rats. An potential factor expected to result in impairment of the electrical response to both glucose and theophylline in the early adaptive phase after SB resection might be the absence, or a functional alteration, of intercellular tight junctions during this hyperplastic period (23).

In ileum, a different picture emerges 10 weeks after resection. Ileal PD measurements in resected rats were then no longer different from those in sham-operated rats. Besides, villus length was no more significantly increased in resected animals. In contrast, by 10 weeks after resection, evoked jejunal PD responses remained decreased as compared to values obtained 10 weeks after sham operation. This finding, coupled with still significantly enlarged jejunal villus length after resection, implies that, for the specific transport capacities tested, functional adaptation of ileum precedes functional adaptation of jejunum after a 90% SB resection in rats.

References

1. Urban E and Weser E. Intestinal adaptation to bowel resection. *In Stollerman GH, ed. Advances in Internal Medicine. Chicago: Year book Medical Publishers, Inc, 1980; 26: 265.*
2. Thomson ABR, Keelan M, Sigalet D, Fedorak R, Garg M and Clandinin MT. Patterns, Mechanisms and signals for intestinal adaptation. *Dig Dis 1990; 8: 99.*
3. Wilmore DW. Factors correlating with a succesful outcome following extensive intestinal resection in newborn infants. *J Pediatr 1972; 80: 88.*
4. Bristol JB and Williamson RCN. Mechanisms of intestinal adaptation. *Pediatr Surg Int 1988; 4: 233.*
5. Hanson WR, Osborne JW and Sharp JG. Compensation by the residual intestine after intestinal resection in the rat. II Influence of postoperative time interval. *Gastroenterology 1977; 72: 701.*
6. Loran MR and Althausen RL. Transport of vitamin A in vitro across normal isolated rat intestine and intestine subjected to "partial" resection. *Am J Physiol 1959; 197: 1333.*
7. Menge H and Robinson JWL. The relationship between functional and structural alterations in the rat small intestine following resection of varying extents. *Res Exp Med 1978; 173: 41.*
8. Chaves M, Smith MW and Williamson RCN. Increased activity of digestive enzymes in ileal enterocytes adapting to proximal small bowel resection. *Gut 1988; 88: 981.*
9. Dowling RH and Booth CC. Structural and functional changes following small intestinal resection in the rat. *Clin Sci 1967; 32: 139.*
10. Urban E and Michel AM. Separation of adaptive mucosal growth and transport after small bowel resection. *Gastrointest Liver Physiol 1983; 7: G295.*
11. Keast JR. Mucosal innervation and control of water and ion transport in the intestine. *Rev Physiol Biochem Pharmacol 1987; 109: 1.*
12. Meijssen MAC, Heineman E, de Bruin RWF, Veeze HJ, Bijman J, de Jonge HR, ten Kate FJW, Marquet RL and Molenaar JC. Value of in vivo electrophysiological measurements to evaluate canine small bowel autotransplants. *Gut 1991; 32: 1329.*
13. Robinson JWL, Anatoli JA and Minkovitch V. The intestinal response to ischemia; *Naunyn Schiedebergs Arch Pharmacol 1966; 255: 178.*
14. Sullivan SK and Field M. Ion transport across mammalian small intestine. *In Field M and Frizzell RA, eds. Handbook of Physiology 1991; 4: 287.*
15. Frömter E. Electrophysiological analysis of rat renal sugar and amino acid transport. I Basic Phenomena. *Pflugers Arch 1982; 393: 179.*

16. Welsh MJ, Smith PL, Fromm M and Frizzel RA. Crypts are the site of intestinal fluid and electrolyte secretion. *Science 1982; 218: 1219.*
17. Barry RJC, Dikstein S, Matthews J, Smyth DH and Wright EM. Electrical potentials associated with intestinal sugar transfer. *J Physiol 1964; 172: 316.*
18. Koruda MJ, Rolandelli RH, Settle RG, Saul SH and Rombeau JL. The effect of a pectin-supplemented elemental diet on intestinal adaptation to massive small bowel resection. *JPEN 1986; 10: 343.*
19. Bloom SR. Gut hormones in adaptation. *Gut 1987; 28 suppl: 31.*
20. Bass BL, Fischer BA, Richardson C and Harmon JW. Somatostatin analogue treatment inhibits postresectional adaptation of the small bowel in rats. *Am J Surg 1991; 161: 107.*
21. Erdman SH, Park JHY, Thompson JS, Grandjean CJ, Hart MH and Vanderhoof JA. Suppression of diamine oxidase activity enhances postresection ileal proliferation in the rat. *Gastroenterology 1989; 96: 1533.*
22. Bury K. Carbohydrate digestion and absorption after massive resection. *Surgery 1972; 135: 177.*
23. Madara JL. Loosening tight junctions. Lessons from the intestine. *J Clin Invest 1989; 83: 1089.*

CHAPTER 4

The effect of diets with intact or partial hydrolyzed protein on functional adaptation after massive small-bowel resection in the growing rat

Submitted for publication in J Pediatr Surg

THE EFFECT OF DIETS WITH INTACT OR PARTIAL HYDROLYZED PROTEIN ON FUNCTIONAL ADAPTATION AFTER MASSIVE SMALL-BOWEL RESECTION IN THE GROWING RAT

Abstract

The aim of this study was to examine the relative importance of protein complexity and protein composition in promoting adaptation after extensive small-bowel resection.
Ninety rats subjected to near-total small-bowel resection received either normal rat chow, a diet with partial hydrolyzed proteins, an identical diet but with intact proteins or a non-identical diet with intact proteins. During the experimental period survival was scored and weight was measured regularly.
Either 2 or 10 weeks after resection the adaptive state was evaluated by determining the metabolic status, serum enzyme activities, postheparin diamine oxidase activity, villus and crypt lengths, as well as cell proliferative activity. The results show that the diet with partial hydrolyzed protein is as effective as the diet with intact proteins in initiating and supporting adaptation. In the first two weeks, the non-identical diet with intact proteins was superior to these diets as evidenced by a lower short term mortality rate, increased postheparin diamine oxidase activity, increased crypt lengths and crypt cell proliferative activity. No long term differences were found among the experimental groups.
These results suggest that the role of protein composition is more important than the role of protein complexity in the early adaptive phase following small-bowel resection.

Introduction

Intestinal adaptation, the ability of the residual small bowel to compensate for the loss of intestinal mucosa which may occur after surgery, is critical to the succesful recovery of patients with short bowel syndrome (SBS). The management of SBS requires a period of TPN, at least until some bowel regeneration occurs, allowing the introduction of enteral nutrition. With respect to the well-established TPN, the importance of oral intake as a positive stimulus to the adaptive stage of the remaining bowel has been recognized. Lack of luminal content leads to absence of the hyperplastic response, occurring in the residual intestine (1). In segments exposed to enteral nutrients following resection there is an increase in both epithelial cell proliferation rate in the crypts and migration rate of the cells onto the villi, resulting in enlarged villi (2,3). The villus hyperplasia is characterized by an increase in mucosal cell mass and in DNA, RNA and protein content (4). Functionally, these segments exhibit increased absorptive capacity per unit length (4). Other factors stimulating intestinal adaptation to SB resection include bile and pancreatic secretions (5), circulating hormones (6), and polyamines (7).

Several findings indicate that complexity and composition of the proteins within the diet may play a role in the adaptation process of the bowel (8-14). Experimentally, the effect of complex versus chemically defined diets as well as the effect of individual nutrients on intestinal adaptation have been studied. Although not based on experimental justification, elemental diet became the standard treatment of choice when starting oral refeeding (15). The few data obtained from rat studies revealed no benefit from elemental diets over nonelemental food regimens and even suggest that a polymeric composition is better (16,17,18). In these studies, the inferiority of elemental diets could reflect differences in protein composition instead of complexity. However, a semi-elemental diet normally consists of partial hydrolyzed proteins, but with the same amino acid composition as the intact protein.

The present study, therefore, was designed to compare a diet with partial hydrolyzed proteins with an identical diet containing intact proteins. The diet with partial hydrolyzed proteins is used in children suffering from SBS in the age of 10 -16 year.

In addition, a macromolecular diet differing in composition from the diet with partial protein, was tested. In the clinical situation, this diet is prescribed to children of that age when there is no indication for a hydrolyzed diet and may reveal the relative importance of diet composition in comparison to diet complexity. Adaptation, after near-total small bowel resection, was evaluated by means of the following parameters: survival, weight,

serum parameters indicating the nutritional status, postheparin DAO assessment, histology and crypt cell proliferation.

Materials and methods

Animals

Ninety male WAG rats (Harlan CPB, Zeist, The Netherlands) weighing 200-225 gram (10-12 weeks: young adults) were used. Animals were housed in a wire-netting cage (to avoid saw-dust eating) and maintained under conventional conditions with 12-hour light and dark cycles in accordance with the guidelines of the National Research Council. The animals were given the specific diet and water ad libitum.

Operative procedure

The rat was anesthetized with ether. The abdomen was shaved and desinfected with alcohol 70%. Laparotomy was performed through a midline incision, whereafter the small intestine was exteriorized from the ligament of Treitz to the ileocecal valve. Ninety percent of the small intestine was then resected, from 2 cm distal to the ligament of Treitz to 2 cm proximal to the ileocecal valve. Intestinal continuity was restored in an end-to-end fashion with continuous suturing using micro sutures (7-0 silk, B. Braun-SSC AG, Switzerland). The abdomen was closed in two steps, first the muscle layer was closed and subsequently the skin layer using sutures (2-0 silk, B. Braun Melsungen, Germany).

Diet protocol

Before resection all rats were fed the standard laboratory diet (AM2, Hope Farms, Woerden, The Netherlands, normal rat chow). After resection, the rats were randomly assigned to one of the four experimental groups, differing in diet composition. All diets were given enterally in solid form. Group 1 (n=20) received AM2. Group 2 (n=21) was given a polymeric diet, based on casein protein (Nutrison, Nutricia-Zoetermeer, The Netherlands; non-identical polymeric diet). Group 3 (n=26) received a diet with partial hydrolyzed protein, based on whey protein (Pepti-2000, Nutricia-Zoetermeer, The Netherlands). Group 4 (n=23) was fed the same diet as group 3, but with intact protein (Pepti 2000 N.H., produced for experimental purposes, Nutricia-Zoetermeer, The Netherlands). The nutrient compositions of Nutrison, Pepti 2000 and Pepti 2000 N.H were optimalized for use as rat food, according to Table 1. All used diets were isocaloric. AM2 contained more nitrogen in contrast to the other isonitrogenous diets.

Table 1 **Composition of experimental diets (values given per gram of the specific diet)**

Group→	1	2	3	4
product name→ ↓ component	AM2	Nutrison	Pepti 2000	Pepti 2000 N.H
Protein (g)	0.24	0.19	0.18	0.18
* protein hydrolysate	0.19	0.18	-	
* casein	-	-	-	
* whey protein	-	-	0.18	
Fat (g)	0.06	0.18	0.15	0.15
* vegetable oil	0.18	0.08	0.08	
- linoleic acid	0.02	0.08	0.04	0.04
- alpha-linolenic acid	0.002	0.001	0.001	
* MCT	-	0.08	0.08	
Carbohydrates (g)	0.51	0.58	0.51	0.51
* glucose	0.02	0.01	0.01	
* maltose	0.14	-	-	
* polysaccharides	0.42	0.04	0.04	
* MD/glucose syrup	-	0.60	0.60	
Fiber (g)	0.05	-	-	-
* dicacell 2+4	0.05	-	-	-
Minerals (mg)				
* sodium	2.70	4.76	2.03	2.03
* potassium	7.91	6.30	6.19	6.19
* chloride	3.16	5.85	3.42	3.42
* calcium*	7.58	5.62	5.32	5.32
* phosphorus *	5.21	4.52	4.28	4.28
* magnesium	2.09	0.94	0.67	0.67
* iron	0.17	0.05	0.04	0.04
* zinc	0.01	0.04	0.03	0.03
* copper*	0.01	0.006	0.006	0.006
* manganese *	0.06	0.06	0.06	0.06
* iodine	0.0003	0.0003	0.0003	0.0003
Vitamins (mg)				
* vitamin A (IU)	15.75	11.65	10.99	10.99
* vitamin D3 (IU)*	1.80	1.13	1.07	1.07
* vitamin C	-	0.21	0.25	0.25
* vitamin E (IU)	0.10	0.07	0.04	0.04
* vitamin K	0.007	0.0001	0.0003	0.0003
* thiamin (B1)*	0.02	0.004	0.004	0.004
* riboflavin (B2)	0.02	0.004	0.004	0.004
* niacin	0.08	0.05	0.04	0.04
* pyridoxine (B6)*	0.02	0.007	0.006	0.006
* vitamin B12*	0.05	0.06	0.05	0.05
* folic acid*	0.004	0.001	0.001	0.001
* panthothenic acid	0.03	0.02	0.02	0.02
* biotin	0.003	0.007	0.007	0.007
* choline*	1.87	1.13	2.00*	2.00
* meso-inositol**	-	1.06	1.06	1.06
Energetic value(Cal)	4.65	4.51	4.76	4.51
* EN% protein	28.7	16	16	16
* EN% fat	7.6	35	29	29
* EN% carbohydrate	59.6	49	55	55
* EN% fiber	4.1			

* = nutrients in original diets were in shortage compared to values given by the National Research Council and therefore supplemented to given amounts.** = meso-inositol is not indicated in the NRC-table and therefore the amount in Nutrison has been adjusted to the amount available in Pepti diets.

In Table 2, the major differences in composition between the experimental diets are summarized.

Table 2 Major differences in diet composition

Group→	1	2	3	4
Product name→ ↓ Components	AM2	Nutrison	Pepti 2000	Pepti 2000 N.H
Fiber	+	-	-	-
Protein		casein	whey-hydrolysate	whey-intact protein
Fat		vegetable oil 100%	MCT 50% vegetable oil 50%	MCT 50% vegetable oil 50%
Carbohydrates		polysaccharides 73% glucose 2.6% maltose 24%	polysaccharides 91.8% glucose 2% maltose 6%	polysaccharides 91.8% glucose 2% maltose 24%

Experimental design

Each experimental group was divided into two sub-groups, "A animals" (Follow-up 14 days before sacrifice; group 1: n=7, group 2: n=8, group 3: n=8 and group 4: n=10) and "B animals" (Follow-up 70 days before sacrifice; group 1: n=13, group 2: n=13, group 3: n=18 and group 4: n=13)

Parameters obtained during lifetime:

Survival

Animals were sacrificed if they had a deteriorating condition or if weight decline was more than 30% of the preoperative weight.

Weight

Throughout the experimental period, the animals (A & B) were weighed three times a week, at 8.30 a.m.

Metabolic status

On postoperative days 10-14 (A), and 66-70 (B) respectively, animals were housed in separate metabolic cages to measure food and water consumption and urine and feces production.

Serum analysis

On the 14th (A) and 70th (B) postoperative day, blood was obtained by tail bleeding, under ether anesthesia, for determination of serum levels of alkaline phosphatase; (AF,

Units/l at 37°C), alanine aminotransferase; (ALT, Units at 37°C), aspartate aminotransferase; (AST, Units/l at 37°C), albumin; (ALB, g/l), cholesterol; (CHOL, mmol/l), triglyceride; (TRIG, mmol/l), total protein; (TP, g/l) and blood urea nitrogen; (BUN, mmol). Serum levels of AF, ALT and AST were determined by photometric methods using special pack kits (Merck Diagnostica, Germany) for a multi-test analyser system (ELAN). Using the ELAN, serum Alb levels were determined by means of the bromcresolgreen method, Chol levels using the enzymatic CHOD-PAP method, serum TP concentration by means of the biuret method and BUN using the GIDH method (special pack kits from Merck Diagnostica, Germany).

Postheparin DAO assessment

On the 14th (A); 21th, 42th and 70th (B) postoperative day, 100 international units (IU) of heparin was injected into the penile vein. Fifteen minutes after injection blood was obtained by tail bleeding to determine postheparin diamine oxidase activity in serum.

Diamine oxidase (DAO) activity, as a possible measure for the adaptive state of the remaining small intestine, was measured using a radioactivity- based modification of the method of Okyama and Kobayashi **(19,20)**. The method is based on the principle that DAO converts ^{14}C-putrescine to^{14}C-Δ^1-pyrroline. In a final concentration of 2.5 ml, the assay mixture consisted of (a) 200 μl test-sample solution; (b) 2100 μl 0.1 M sodium phosphate buffer, pH 7.0; (c) 100 μl chloral hydrate; 41.4 g/l and (d) 100 μl of substrate solution (a mixture of 1.25 mmol/l putrescine dihydrochloride including 0.1μl Ci ^{14}C putrescine dihydrochloride). The samples were incubated for 120 minutes at 37°C. The reaction was stopped by adding 200 μl of an aminoguanidine-containing solution (10mM aminoguanidine in 2% sodium carbonate). Thereafter, the labeled product, ^{14}C-Δ^1-pyrroline, was extracted into 4 ml of a toluene-based scintallation mixture (Packard Instrument Company Inc, reorder no 6013089, Downers Grove, USA) by centrifugation. The radioactivity present was measured using a liquid scintillation analyzer (Packard, Tricarb 2500 TR, Downers Grove, USA). Assay blanks consisted of 2300 μl sodium phosphate buffer, 100 μl chloral hydrate and 100 μl substrate solution.

DAO activity was expressed as Units/ml (1 Unit= nmol of putrescine dihydrochloride oxidated in 1 hr at 37°C, pH 7.0)

Parameters obtained after sacrifice:

Histology

Specimens of intestine just distal to the ligament of Treitz and just proximal to the

ileocecal valve were obtained for histology. The collected tissues were dehydrated and embedded in paraffin. Longitudinally cut sections of 4-5 μm were stained with hematoxylin-azophloxin-safran. Quantitative morphometric analysis of crypts and villi was performed on sections projected on a screen through a light microscope. In each section 10 villi and crypts were measured with the mean value representing one animal. The mean of all rats (data given in μm $\pm$ SD) was considered representative for an experimental group.

Cell proliferative activity

Cellular proliferation was assessed by means of an immunohistochemical method using 5-bromo-2'-deoxy-uridine (BrdU, Boehringer Mannheim, Germany), a thymidine analogue incorporated during the S phase of the cell cycle into reduplicating DNA (21). Animals were injected intravenously with 10 mg BrdU/kg bodyweight two hours before sacrifice. After death, paraffin sections were prepared and sections of 4-5 μm were stained using a BrdU monoclonal (Becton Dickinson Immunocytometry Systems, San Jose USA).

Identification of stained cells was by projecting a section on a screen through a light microscope. Labeled cells were present in a definable crypt area, consisting of the first twenty crypt cells from the bottom towards the top at each side, and therefore calculated as percentage positive cells in this area. In this way, differences in crypt length were excluded and absolute proliferative activity was assessed.

Statistical analysis

Differences in survival rate were analyzed using the chi-square test. For other test results, one way analysis of variance was used, followed by the Student-Newman-Keuls test. A p value $\leq$ 0.05 was considered statistically significant.

Results

Survival

Survival data are shown in Table 3. Statistical analysis showed that the short-term mortality rate in animals fed the non-identical polymeric diet (group 2) was significantly lower than in animals fed either the diet with partial hydrolyzed proteins (group 3) or the identical diet with intact proteins (group 4). Rats fed normal rat chow (group 1) had an intermediate position with no statistic significant mortality rate compared to the other diet groups. No differences were found in long term mortality and overall mortality.

Table 3 Survival data of experimental animals

	Short term mortality (<14 days)			Long term mortality (>14 days)
Animals	A+B	A	B	B
Group 1(n=20)	3	0	3	3
Group 2(n=26)	5	2	3	4
Group 3(n=21)	1	0	1	2
Group 4(n=23)	8	4	4	1

Statistical differences; ↔ = in comparison to, Short term A+B, 3↔4, 3↔2

Weight

The mean percentual weight changes (weight/preoperative weight) of all experimental animals (subgroups A & B) are shown in Figure 1. Initially the animals showed a weight decline of 20-25% of the preoperative weight, but regained their preoperative weight at 10 weeks postoperatively. Weights differed significantly on day 7 ($P<0.05$; group 1 > any other group).

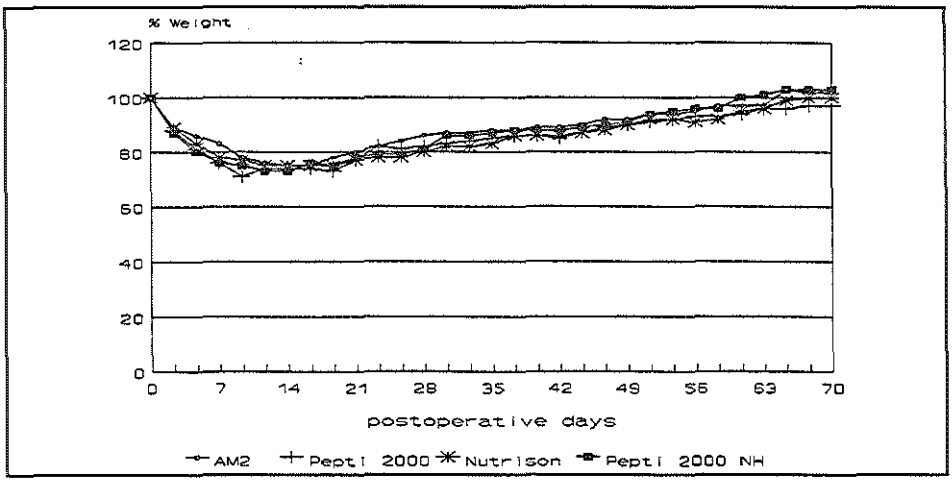


Fig.1. *Weight changes in the adaptive phase following 90% small bowel resection. AM2, normal rat chow (group 1); Nutrison, non-identical diet (group 2); Pepti 2000, diet with partial hydrolyzed proteins (group 3); Pepti 2000 N.H., identical diet with intact proteins (group 4).*

On day 10 less differences were found ($P<0.05$; group 3 < group 1 and group 3). From day 14 on, there were no longer any differences.

Results of subgroup A; visualized in Figure 2 and Table 4A

Metabolic status

Metabolic results showed a significantly higher food intake and fecal output in group 1 compared to group 4 values. In addition, group 2 had significantly more fecal output

compared to group 4.

Serum analysis

Serum analysis demonstrated significant decreases in cholesterol and protein concentrations in group 1 compared to the other groups. In this group, the blood urea level was significantly higher.

Postheparin DAO assessment

Postheparin DAO levels in animals fed the non-identical polymeric diet (group 2) were significantly higher than in the other groups.

Histology

Jejunal findings. With regard to villus length there were no differences among groups. Crypth depth, however, was significantly increased in group 1 and group 2 compared to group 3 and group 4.

Ileal findings. Villus length in group 3 was significantly enlarged compared to any other group. Crypt depth was significantly greater in group 1 compared to any other group and crypth depth in group 2 was significantly increased compared to group 3 and group 4.

Cell proliferative activity

Jejunal findings. The percentage labeled cells was significantly increased in group 2 compared to any other group.

Ileal findings. The percentage labeled cells was significantly increased in group 1 and group 2 compared to group 3 and group 4.

Table 4A Serum analysis of A animals

Group	1	2	3	4	P<0.05
AF (Units/l)*	185.9±119.5	227.0± 86.6	281.4±168.9	233.6± 53.6	NS
ALT (Units/l)*	93.3± 45.4	53.0± 16.3	78.1± 48.5	179.7±238.4	NS
AST (Units/l)*	181.3± 97.5	129.1± 63.6	132.7± 52.5	308.0±319.7	NS
ALB (g/l)	21.5± 5.2	23.2± 3.3	21.4± 2.0	20.3± 2.5	NS
CHOL (mmol/l)	0.19± 0.08	0.94± 0.25	0.71± 0.31	0.91± 0.33	2↔1, 4↔1,3↔1
TRIG (mmol/l)	0.16± 0.09	0.30± 0.17	0.37± 0.20	0.23± 0.12	NS
TP (g/l)	36.1± 8.5	48.0± 6.2	50.8± 5.6	44.3± 5.1	3↔1, 2↔1, 4↔1
BUN (mmol)	16.5± 0.09	8.8± 2.1	10.3± 3.8	8.5± 3.1	1↔4, 1↔2, 1↔3

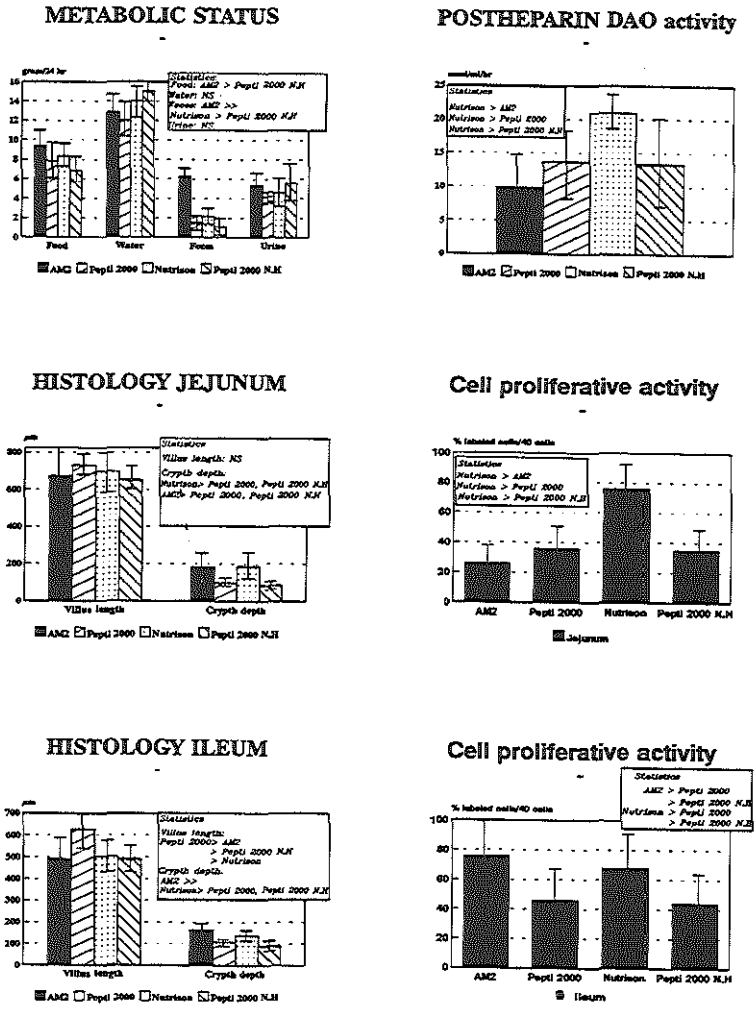


Fig.2. *Experimental findings 14 days after 90% small bowel resection. Abbreviations as for Fig.1.*

Results of B animals; visualized in Figure 3 + Table 4B

Metabolic status

Rats fed rat chow (group 1) had significantly increased food and water intake and faeces and urine output compared to rats fed either the non-identical polymeric diet (group 2), the diet with hydrolyzed proteins (group 3), or the identical diet with intact proteins (group 4).

Serum analysis

Serum alanine aminotransferase concentration in group 2 was significantly increased compared to the other groups. In group 1, serum urea level was still significantly elevated compared to the other groups.

Postheparin DAO assessment

At 3 weeks after the resection, postheparin DAO activity was still significantly increased in animals fed the non-identical polymeric diet (group 2) compared to the other animals. At 6 and 10 weeks after the resection, no significant differences were found among each group.

Histology

In jejunum as well as ileum there were no significant differences in villus length and crypth depth between the experimental groups.

Cell proliferative activity

The percentage labeled cells in jejunum nor ileum did not differ among each groups.

Table 4B **Serum analysis of B animals**

Group	1	2	3	4	P<0.05
AF (Units/l)*	135.3± 28.1	95.1± 35.3	99.8± 27.6	93.3±30.0	NS
ALT (Units/l)*	71.8± 63.3	63.6± 45.3	94.9± 88.4	16.7± 4.5	3↔4
AST (Units/l)*	175.6±110	204.9±105.9	322.4±251	120.8±96	NS
ALB (g/l)	24.1± 3.6	24.7± 1.9	25.0± 2.0	24.2± 2.5	NS
CHOL (mmol/l)	0.89± 0.30	0.64± 0.34	0.73± 0.40	0.53±0.20	NS
TRIG (mmol/l)	0.43± 0.28	0.37± 0.30	0.32± 0.16	0.25±0.11	NS
TP (g/l)	49.6± 10.1	55.8± 6.2	56.1± 3.5	56.0± 4.4	NS
BUN (mmol)	9.6± 1.8	7.5± 1.9	7.8± 1.1	7.3±0.8	1↔4,1↔2,1↔3

* = assayed at 37°C
↔ = in comparison to

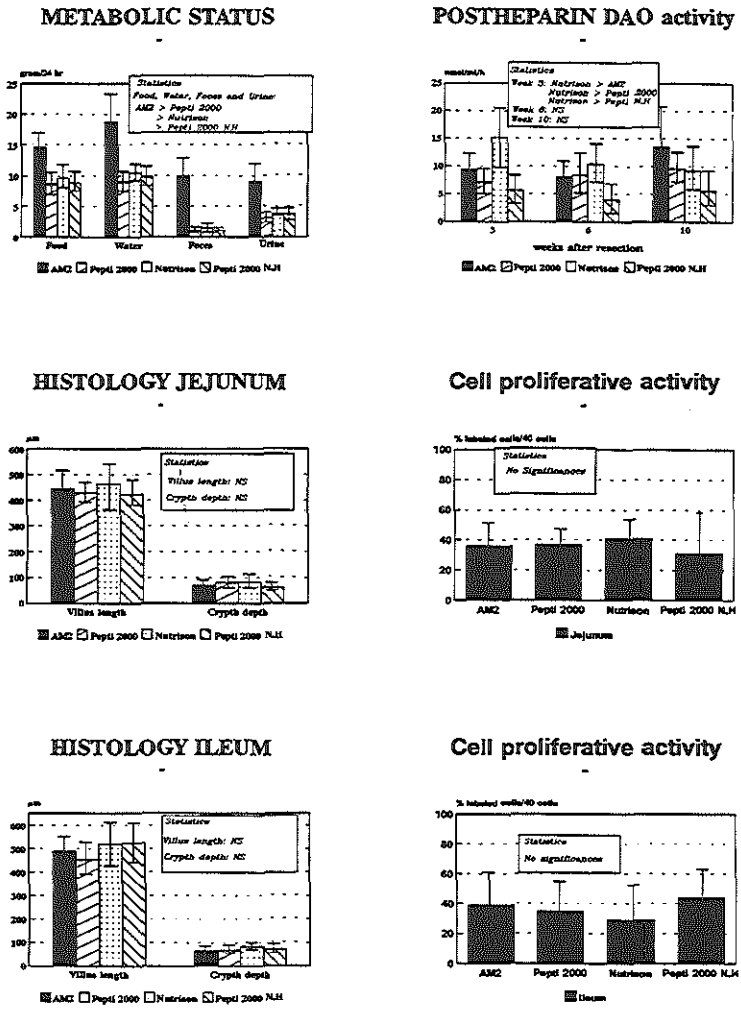


Fig.3. *Experimental findings 10 weeks after 90% small bowel resection. Abbreviations as for Fig.1.*

Discussion

Awareness of the importance of diet composition and/or complexity on intestinal adaptation after small bowel resection arose from a variety of studies (8-14). To exclude influences of non-isocaloric feeding, we studied diets containing similar isocaloric amounts per 1000 Kcal Bruto Energy. With respect to the nitrogenous composition, rat chow can be considered as a relative high-protein diet compared to the other tested diets, which itself can result in growth differences (22).

This study demonstrates that rat chow (group 1) is superior to other diets in enhancing the onset of the adaptive phase after near-total small bowel resection. Rats fed rat chow already had significantly less weight loss on the 7th postoperative day. The relatively high protein content in the normal rat chow may be responsible for the observed weight loss. Besides, this formula was the only diet containing dicacel, a partly soluble fiber. Non-fermentable fiber is known as a major regulator of bowel function, increasing stool weight and bowel frequency, and decreasing transit time. The importance of fermentable fiber is most probably the enhancement of colonic adaptation by fermentating fiber to short chain fatty acids (23). In a study of enterectomized rats, infusion of Triglycerides has been shown to improve jejunal and colonic adaptive growth (24). Because of the possible role of the non-isonitrogenous composition of rat chow and/or colonic adaptation in the rat chow group emphasis will be put on the differences between the diet with partial hydrolyzed proteins (group 3), the identical diet with intact proteins (group 4) and the non-identical polymeric diet (group 2) to facilitate interpretating the test parameters of the different groups.

The early adaptive response of animals fed the non-identical polymeric diet, as evidenced by mortality, postheparin DAO activity and histology, was superior to both the the diet with partial hydrolyzed proteins and the identical diet with intact proteins. The short term mortality rate in animals fed the non-identical polymeric diet was significantly lower than in animals fed either the partial hydrolyzed or identical nonhydrolyzed diet. In the SBS model used, the occurrence of malnutrition was a major trigger for intestinal adaptation. The additive effect of, for example non-identical polymeric feeding, may overcome the lethality of the massive resection. Our weight data indicate that the resected amount is indeed near total because some rats were unable to thrive and the remaining animals, although reaching their preoperative weight 10 weeks after resection, did not show further weight gain. This observation implies that in this model, manipulation with dietary components like glutamine(25,26), aminoguanidine treatment (7) and growth factors (27) should be evaluated in order to establish whether they are able to enhance adaptation.

The metabolic results show that animals fed the non-identical polymeric diet had more fecal output in the short term compared to animals fed the identical nonhydrolyzed diet. However, the intake/output percentage was similar among groups. These data, obtained at week 2 and 10 respectively indicate that influences of non-pair feeding are probably negligible.

Two weeks after resection, serum analysis revealed a similar results among animals fed partial hydrolyzed, identical nonhydrolyzed or non-identical polymeric diet. After 10 weeks, the activity of ALT in rats fed the identical nonhydrolyzed diet was significantly decreased for unknown reasons, compared to rats fed the non-identical polymeric diet. However, no differences in serum protein and albumin levels, sensitive parameters of the nutritive status, were found.

The activity of DAO, an enzyme primarily found in the villus tip cells of the small intestine has been shown to increase at least five fold after intestinal resection (7). Previous studies suggest that DAO acts as a negative feedback modulator in adaptive intestinal proliferation by producing undefined active metabolites and suppressing putrescine content (7,28,29,30). Therefore, one may reason that the DAO activity correlates with the adaptive state of the intestine after resection. Two weeks after resection, non-identical polymeric diet fed animals had significantly increased Postheparin DAO activity compared to partial hydrolyzed or identical nonhydrolyzed diet fed animals and they also showed increased jejunal and ileal crypt cell proliferation in this group. Thompson measured DAO activity throughout the gastrointestinal tract and demonstrated a decreasing proximodistal gradient (31). This may explain why no significant elevations in DAO activity were found in rat chow fed animals. These results suggest that colonic adaptation in some way takes over the role of DAO in regulating proliferative activity in the small intestine. Three weeks after resection, animals fed the non-identical polymeric diet still had significantly increased Postheparin DAO activity. However, 6 and 10 weeks after the resection, no more differences were revealed between the groups. Long-term analysis showed that DAO activity remains significantly elevated compared to non-resected rats (previous unpublished studies; control values 0.09 ± 0.06, $n=9$). This observation led us to speculate that massive resection leads to a state in which the remaining intestine is continuously triggered to nonfunctional hyperplasia, which is prevented by diamine oxidase. Previous work performed in the same rat model (32), had already shown that in the hyperplastic period three weeks after resection, impairment of the electrical response to both glucose and theophylline is present.

Two weeks after resection, histologic analysis showed markedly increased ileal villus

length in animals fed the partial hydrolyzed diet compared to the other groups. These findings support the hypothesis propounded by Touloukian (20) that compensatory changes found after feeding an elemental diet are the effect of a systemic stimulus, rather than the result of increased load of nutrient.

In the early adaptive phase the non-identical polymeric diet is superior to the partial hydrolyzed diet and the identical nonhydrolyzed diet in enhancing intestinal adaptation. No differences were found between the partial hydrolyzed diet and the identical nonhydrolyzed diet, which provides an experimental base for a clinical trial in SBS patients in which hydrolyzed are compared with nonhydrolyzed diets. Nonhydrolyzed diets lead to less complications compared to hydrolyzed diets. In addition, these results indicate that the role of diet composition is superior to the role of diet complexity on the adaptive process after small-bowel resection. More research is necessary to unravel which components in the non-identical polymeric diet that differ from the ones in the partial hydrolyzed and identical nonhydrolyzed diet (Table 2), are effective in promoting intestinal adaptation after resection.

In future studies it is important to address the question whether after near-total small bowel resection the observed adaptive capacity has already reached its limits or may even be further enhanced. In summary, the common belief that feeding an elemental diet is better than a complex diet seems a fairy tale that tends to underestimate the effect of diet-composition.

Acknowledgements

The authors are very grateful to Miss Lydia v Gastel and Mr Pim v Schalkwijk for performing the DAO assays and blood chemistry determinations, respectively and Drs Ko Hagoort for correcting the manuscript. In addition, the technical assistence of Mr Ron Briegoos and Mr John Mahabier is gratefully acknowledged. Miss Gemma Smulders (Hope Farms, The Netherlands) is gratefully acknowledged for her help in preparing diets optimalized for rat food.

References

1. Bristol JB and Williamson RCN. Mechanisms of intestinal adaptation. *Pediatr Surg Int 1988; 4: 233.*
2. Dowling RH and Booth CC. Structural and functional changes following small intestinal resection in the rat. *Clin Sci 1967; 32: 139.*
3. Tilson MD and Wright HK. Adaptation of functioning and bypassed segments of ileum during compensatory hypertrophy of the gut. *Surgery 1970; 67: 687.*
4. Williamson RCN. Intestinal adaptation. Structural, functional and cytokinetic changes. *N Engl J Med 1978; 298: 1393.*
5. Weser E, Heller R and Tawil T. Stimulation of mucosal growth in the rat ileum by bile and pancreatic secretions after jejunal resection. *Gastroenterology 1977; 73: 524.*
6. Enochs MR and Johnson LR. Trophic effects of gastrointestinal hormones: physiological implicati-

ons. *Fed Proc 1977; 36: 1942.*

7. Erdman SH, Park JHY, Thompson JS, Grandjean CJ, Hart MH and Vanderhoof JA. Suppression of diamine oxidase activity enhances postresection ileal proliferation in the rat. *Gastroenterology 1989; 96: 1533.*
8. Buts JP, Morin CL and Ling C. Influence of dietary components on intestinal adaptation after small bowel resection in rats. *Clin Invest Med 1979; 2: 59.*
9. Vanderhoof JA, Grandjean CJ, Burkley KT and Antonson DL. Effect of casein versus casein hydrolasate on mucosal adaptation following massive bowel resection in infants rats. *J Pediatr Gastroenterol Nutr 1984; 3: 262.*
10. Vanderhoof JA, Grandjean CJ, Kaufman SS, Burkley KT and Antonson DL. Effect of high percentage medium-chain triglyceride diet on mucosal adaptation following massive bowel resection in rats. *JPEN 1984; 8: 685.*
11. Grey VL, Garofalo C, Greenberg GR and Morin CL. The adaptation of the small intestine after resection in response to free fatty acids. *Am J Clin Nutr 1984; 40: 1235.*
12. Koruda MJ, Rolandelli RH, Settle G, Saul SH and Rombeau JL. The effect of a pectin-supplemented elemental diet on intestinal adaptation to massive small bowel resection. *JPEN 1986; 10: 343.*
13. Young EA. Short bowel syndrome: high-fat versus high carbohydrate diet. *Gastroenterology 1983; 84: 872.*
14. Russell RI. Intestinal adaptation to an elemental diet. *Proc Nutr Soc 1985; 44: 87.*
15. Koretz RL AND Meyer JH. Elemental diets-facts and phantasies. *Gastroenterology 1980; 78: 393.*
16. Voitk AJ and Crispin JS. The ability of an elemental diet to support nutrition and adaptation in the short gut syndrome. *Ann Surg 1975; 181: 220.*
17. Bury KD. Carbohydrate digestion and absorption after massive resection of the small intestine. *Surg Gynecol Obstet 1972; 135: 177.*
18. Touloukian RJ, Mitruka B and Hoyle C. Growth and nutrition of enterectomized rats fed an elemental diet. *Surgery 1971; 69: 637.*
19. Okyama T and Kobayashi Y. Determination of diamine oxidase activity by liquid scintillation counting. *Arch Biochem Biophys 1961; 95: 242.*
20. Kobayashi Y, Kupelian J and Maudsley DV. Release of diamine oxidase by heparin in the rat. *Biochem Pharmacol 1969; 18:1585.*
21. Yu CCW, Woods AL and Levison DA. *Histochem J 1992; 24: 121.*
22. Zaloga GP, Ward KA and Prielipp RC. Effect of enteral diets on whole body and gut growth in unstressed rats. *JPEN 1991; 15: 42.*
23. Hosoda N, Nishi M, Nakagawa M, Hiramatsu Y, Hioki K and Yamamoto M. Structural and functional alterations in the gut of parenterally or enterally fed rat. *J Surg Res 1989; 47: 129.*
24. Kripke SA, De Paula JA, Berman JM, Fox AD, Rombeau JL and Gregg Settle R. Experimental short-bowel syndrome: effect of an elemental diet supplemented with short-chain triglycerides. *Am J Clin Nutr 1991; 53: 954.*
25. Souba WW, Herskowitz K, Salloum RM and Copeland EM. Gut glutamine metabolism. *JPEN 1990; 14: 45s.*
26. Vanderhoof JA, Blackwood DJ, Mohmmadpour H and Park JHY. Effects of total supplementation of glutamine on small intestinal mucosal mass following resection. *J Am Coll Nutr 1992; 11(2): 223.*
27. Williamson RCN and Malt RA. Humoral modulation of compensatory intestinal hyperplasia. *In Robinson JWL, Dowling RH and Riecken EO, eds. Mechanisms of intestinal adaptation. Lancaster, England: MTP Press, 1982: 215.*
28. Rokkas T, Vaja S, Murphy GM and Dowling RH. Aminoguanidine blocks intestinal diamine oxidase (DAO) activity and enhances the intestinal adaptive response to resection in the rat. *Digestion 1990; 46: 447.*
29. Dowling RH. Polyamines in intestinal adaptation and disease. *Digestion 1990; 46: 331.*
30. Kusche J, Menninger R and Erpenback K. The intestinal diamine oxidase activity under the influence of adaptive proliferation of the intestinal mucosa- a proliferation terminating principle? *Agents Actions 1988; 23: 354.*
31. Thompson JS. Significance of the intestinal gradient of diamine oxidase activity. *Dig Dis 1990; 8: 163.*
32. Wolvekamp MCJ, Durante NMC, Meijssen MAC, Bijman J, de Jonge HR, Marquet RL and

Heineman E. The value of in vivo electrophysiological measurements for monitoring functional adaptation after massive small bowel resection in the rat. *Gut 1993; 34: 637.*

PART B.
SMALL-BOWEL TRANSPLANTATION

CHAPTER 5

Technical and functional analysis of total small-bowel autotransplantation

5.1 One-step total orthotopic small-bowel autotransplantation in growing dogs: one step too far ?

5.2 Long-term analysis of the functional, morphologic, and bacteriologic status of the small intestine following autotransplantation in growing dogs.

This chapter has been published as abstract in Acta Chirurgica Austriaca supplement 97, 1992: 3.

1. ONE-STEP TOTAL ORTHOTOPIC SMALL-BOWEL AUTOTRANSPLANTATION IN GROWING DOGS: ONE STEP TOO FAR ?

Perioperative events and functional evaluation

Abstract

Transplantation of a functioning small intestine might be the treatment of choice for children suffering from the short bowel syndrome. It is unclear, however, whether bowel graft function is sufficiently preserved to maintain growth and development. This study was undertaken to evaluate the role of total orthotopic small-bowel autotransplantation in the treatment of the short bowel syndrome in enterectomized Beagle puppies (16 weeks; 5-6 kg). Short bowel control animals (n=3) had a fast and dramatic weight loss and there was no adaptation of the remaining bowel. Sham-operated animals (n=3) grew normally, and in animals that underwent small-bowel autotransplantation (n=10) only a minor and temporary weight loss was observed. Unfortunately, the mortality rate in this group was extremely high (80%), so only two animals could be evaluated functionally. Studies on fecal fat excretion, in vivo electrophysiologic responses, D-xylose absorption and brush border enzyme activities were performed at several time points during three months postoperatively. In short bowel control animals, fecal fat excretion was severely increased, while transplanted animals had fecal fat excretion similar to that of sham-operated animals. Determination of D-xylose absorption, in vivo electrophysiologic responses and brush border enzyme activities confirmed physiologic functioning of the graft. These results suggest that the function of an autotransplanted bowel is not impaired on the short term.

Introduction

Intestinal adaptation occurs after massive small bowel loss from disease or surgery. Gradually, varying from a few months to over one year, the remaining intestine adapts to the loss of bowel and oral intake is gradually increased until intravenous alimentation can be stopped. Unfortunately, some of these patients never reach this stage and remain totally dependent on total parenteral nutrition (TPN) (1). Although long-term TPN provided the first satisfactory treatment for the short bowel syndrome (SBS) (2), its complications including metabolic, infectious and psychological aspects, form the main reason to envision this therapy as a temporary solution (3,4). Non-adapting children would be candidates for a SB graft. Although small-bowel transplantation (SBT) is now technically possible in man (5,6), physiologic analysis of the posttransplant gut is a neglected area. This lack of knowledge is even more obvious in view of the many immunobiologic studies performed, and therefore the importance of physiologic studies should be stressed (7). The intrinsic and extrinsic denervation and the lymphatic drainage disruption alter the bowel's functional capacity at least temporarily after transplantation. It is of utmost importance to assess whether the effects of the transplantation procedure itself bear a temporarily character or whether transplanted gut has definitive altered functional capacity, e.g. specific transport abnormalities. Our previous studies showed that adult dogs can survive long-term after a two-stage segmental allotransplantation. In these experiments MHC-matched segmental ileal allografts were used, to determine whether for living-related transplantation it has a potential role (8). The present study was initiated to investigate whether total orthotopic intestinal autografts are able to maintain growth and development in puppies with surgically created SBS. Several tools were used to assess the physiologic function of the graft. In vivo electrophysiologic measurements were performed to determine cAMP-mediated chloride secretion and sodium-dependent active transport of carbohydrates and amino acids. Furthermore, absorption of D-xylose and fat was monitored and brush border enzyme activities were determined.

Materials and Methods

Animals

Sixteen Beagle puppies, weighing about five kilogram, were used (Harlan CPB, Zeist, The Netherlands). To exclude differences in terms of growth only female dogs were used. The bitches were divided into three groups: group 1, short bowel controls (n=3); group 2, sham-operated animals (n=3); and group 3, autotransplanted animals (n=10). During the experimental period the animals were housed in separate cages.

Operative procedure

The premedication consisted of 2 ml thalamonal (0.05 mg fentanyl + 2.5 mg droperidol) and 0.5 mg atropine, given intramuscularly. Thereafter, general endotracheal anesthesia was introduced using nesdonal (20mg/kg) and a mixture of N_2O, O_2 and ethrane was used as intubation gas. During the operation, a Ringer-lactate infusion (30 ml/hr) and 0.1 mg fentanyl were administered. In case of autotransplantation, 25 ml rheomacrodex was given at the end of the ischemic time, simultaneously with 2 ml calcium and 15 ml bicarbonate solution. While finishing the operation, 0.04 mg narcan was administered and if necessary another 5 ml bicarbonate was given. After the operation, extubation took place and the dog awoke under a heating lamp.

Technique

Short bowel control group. Following midline incision, short bowel control animals were created by resection of the total SB i.e. from the ligament of Treitz to 5 cm proximally from the ileocecal valve. Thereafter the proximal jejunum was reanastomosed end-to-end to the distal terminal ileum (Figure 1).

Sham-operated group. A sham operation was defined as a control operation without interrupting vascular, neural and lymphatic supply. In these animals an antiperistaltic Roux-Y loop was created about 20 cm proximally to the ileocecal valve. In detail, a bowel part was heterotopically placed from approximately 20 to 45 cm from the ileocecal valve. The proximally transected part was exteriorized as a cutaneous ileostomy. Gastrointestinal continuity was restored in an end-to-side fashion (Figure 1B). the loop was created in order to collect biopsy specimens and to perform functional monitoring.

Autotransplant group. The small intestine was totally harvested from the ligament of Treitz to 5 cm proximally from the ileocecal valve. After removal, the graft was perfused with 40 cc heparinized saline (5 IU heparin/ml 0.9% saline) at 20°C until the venous effluent became clear and the graft white. We developed a transplantation technique to guarantee maximal bllod in- and ou-flow as well as sufficient tightness to prevent kinking and torsion at the anastomosis site. This technique consisted of anastomosing the superior mesenteric artery and vein in an end-to-side fashion to the abdominal aorta and caval vein respectively, and of fixation of nervous tissue and the lymphatic vessels to the periaortal and pericaval tissue. Mean warm ischemic time was about 40 minutes. In this group a distally situated Roux-Y loop was constructed as described above. Therafter, the graft was proximally anastomosed with the host's duodenum and distally with distal 5 cm of the remaining terminal ileum (Figure 1C).

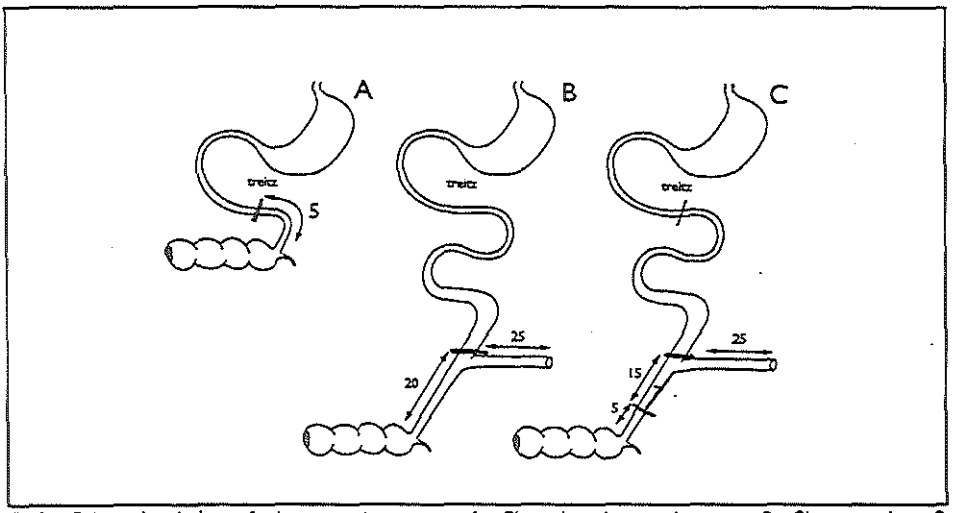


Fig.1. *Schematic design of the separate groups; A, Short bowel control group; B, Shamoperation; C, Autotransplantation.*

Perioperative care

The perioperative care consisted of standard care given after all major abdominal surgery. All animals received 250 mg neomycin twice daily on days -2 and -1 preoperatively. The first five postoperative days 1.5 ml depomycin was given subcutaneously. In addition, 2 X 500 IU heparin was given subcutaneously from day -2 until day 5 after the operation. The animals were supported with parenteral fluids for 2 days postoperatively and gradually restored to normal kennel diet (Puppipap, Hopefarms BV, Woerden, The Netherlands). Urinary sodium and potassium values were determined repeatedly during the first several weeks after operation to detect severe electrolyte loss that might be caused by the Roux-Y loop.

Evaluation

The general condition of the animals was observed daily.

Survival. After enterectomy animals were sacrificed if they lost more than 30% weight or had a deteriorating physical condition.

Weight. Measured twice a week, always at 8.00 a.m.

Blood analysis. Routine laboratory blood chemistry data (hemoglobin, leucocytes and serum total protein, albumin, cholesterol, and triglyceride) were recorded at days -8, 10, 34, 62 and 97 postoperatively. Between 5 and 7 months postoperatively, serum levels of NH_3, vitamin B12, folic acid, vitamin A, vitamin E, calcium, and iron were determined.

The D-xylose absorption test. The dogs were fasted overnight prior to the xylose challenge. D-xylose (0.5 gram/kg Body Weight) was dissolved in water and given orally at days 57 and 92. Blood samples (in heparinized tubes) were collected before, and half-an-hour, 1 and 2 hours after the ingestion of xylose. A simplified xylose assay procedure developed by Eberts et al (9) was used to obtain a xylose blood level time curve.

Fecal fat analysis. The method used, described in detail by Van de Kamer et al (10), gives the amount of fat in feces and the relative proportion of fatty acids. Fecal fat excretion was determined at day -8, 10, 34, 62 and 97 respectively.

Electrophysiologic measurements. The Roux-Y loop was used for electrophysiologic measurements in vivo on postoperative days 34, 62 and 97. The electrophysiologic test for intestinal transplant monitoring was carried out as described earlier (11). Briefly, a triple-lumen-double-balloon catheter was used to isolate a SB segment of 2.5 cm. A peristaltic roller pump (Gilson, minipuls 3) secured continuous flow of test solution. The segment was perfused with several iso-osmotic test solutions ([theophylline] = 5 mM, [glucose] = 30mM, [phenylalanine] = 30 mM) to determine transepithelial potential differences (PD) relative to a standard solution. The electrophysiologic responses to theophylline (evokes cAMP mediated chloride secretion), glucose (evokes sodium-coupled active carbohydrate absorption) and phenylalanine (evokes sodium coupled active amino acid absorption) are commonly accepted parameters to monitor SB function (12,13). The test was performed under general endotracheal anesthesia and atropin was added to obtain reproducible, accurate measurements.

Disaccharidase activities. The activity of lactase and maltase was determined during operation by open biopsy and at fixed moments postoperatively (days 10, 34, 62 and 97) by blind biopsies. The mucosal biopsies were snap-frozen in liquid nitrogen and stored at -70°C. The method used is a modification of the assay introduced by Dahlquist (14). Briefly, the mucosal biopsy was weighed and homogenized using 0.2% Triton X-100 (0.2 ml/mg mucosal weight) and see sand. The mixture was incubated for 30 minutes at 4°C and centrifuged at 10.000 g for 3 minutes. Thereafter, 16.7 μl homogenate (5 or 10 times diluted with 0.2% Triton for maltase activity) was added to 25 μl of a 0.025 M maltose solution or a 0.05 M lactose solution (substrate dissolved in 0.15 M citrate buffer pH 5.6) and incubated in a water bath at 37°C for different periods. The reaction was interrupted by adding 1 ml (1:20) glucose reagent (containing Tris, Merck 19700) and incubating for additional 10 minutes at room temperature. Reading was performed at 25°C in a spectrophotometer (Gilford 3500 computer directed analyzer, Oberlin, Ohio USA) at 340 nm. The protein content of the homogenate was measured, using the method described by

Watanabe et al (15). The disaccharidase activity was expressed in U/mg protein, in which a Unit was defined as the activity hydrolyzing 1 μmol of disaccharide per minute under conditions used.

Histology. Biopsies from the Roux-Y loop were taken at approximately 5 cm from the cutaneous stomas on days 0, 10, 34, 62 and 97 postoperatively. Tissue were fixed in 3.6% formaldehyde, dehydrated and embedded in paraffin. Subsequently, 4-5 μm sections were stained with hematoxylin-azophloxin-safran.

Statistics

Results are given as mean $\pm$ SD. Xylose absorption test data were calculated using area under curve analysis. For statistical analysis, the study population was too small.

Results

Survival

The survival times of all experimental dogs are listed in Table 1.

The mean survival after enterectomy (group 1) was 11.3 days. Animals with a sham operation (group 2) survived indefinitely. Dogs undergoing a one-step orthotopic SB autotransplantation in combination with a Roux-Y loop (group 3) showed a high mortality rate. The technical failure due to vascular thromboses was 20% (2/10). These dogs died from arterial thrombosis within 48 hours postoperatively. Two dogs were excluded from overall analysis because of an overdose heparin. These dogs did not contribute to the vascular-related failure rate as they survived beyond two days. The failure rate due to other causes was 50% (4/8). One dog in group 3 died from peritonitis of uncertain origin on day 2, another from invagination on day 5, a third from unknown causes on day 5 and a fourth on day 14 due to cachexia and pneumonia.

Weight

The weight data of the experimental animals are illustrated in Figures 2A and 2B. The animals of group 1 (SBS control) showed a fast and dramatic weight loss as a result of the near-total SB resection. The animals of group 2 (sham operation) grew normally and recovered uneventful when these animals are compared with none-operated historical controls (data supplied by Harlan CPB, Zeist). Surviving animals of group 3 (SBS + SB autotransplantation) initially lost little weight, but subsequently manifested a catch up growth.

Table 1 Postoperative survival time

Group	Survival in days	Cause of death
I, Short bowel control	10	SBS
	10	SBS
	14	SBS
II, Sham operation	>150	Alive and well
	>150	Alive and well
	>150	Alive and well
III, Autotransplantation	2	Peritonitis
	2	Arterial thrombosis
	2	Arterial thrombosis
	3	Hemorrhage (Overdose heparin)
	4	Hemorrhage (Overdose heparin)
	5	Unknown
	5	Invagination
	14	Cachexia/Pneumonia
	>150	Alive and well
	>150	Alive and well

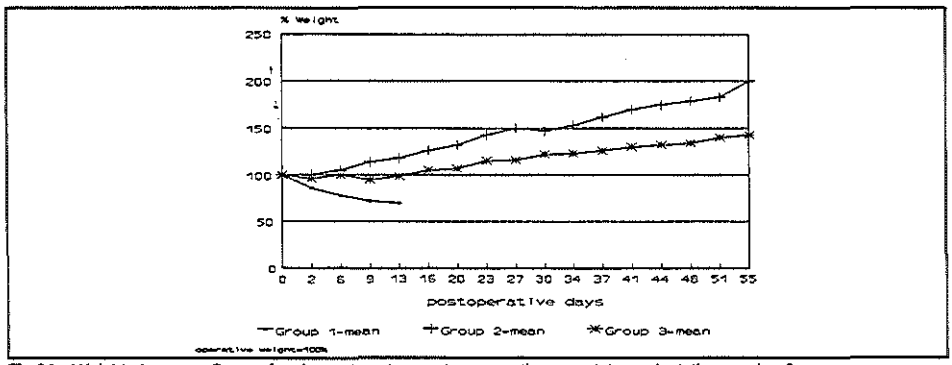


Fig.2A. *Weight changes after performing enterectomy, shamoperation or autotransplantation on day 0.*

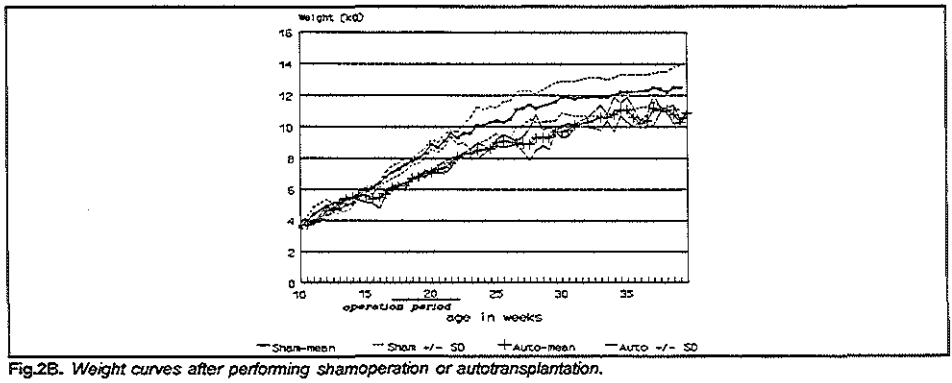


Fig.2B. *Weight curves after performing shamoperation or autotransplantation.*

Blood analysis

Biochemical parameters shown in Table 2A indicate that the general condition of the animals in group 2 is similar to that in group 3. Table 2B shows that serum ammonia levels differed: 53.0 ± 5.4 in the sham-operated animals, 78.2 ± 3.2 in the autotransplanted animals. Other serum values revealed no differences.

D-xylose absorption

Area-under-curve calculations revealed that total D-xylose absorption after sham operation was not remarkably different from D-xylose absorption after autotransplantation at 57 and 92 days posttransplant (Figure 3A). Also, no differences were found between the 57 and 92 days posttransplant values within each group.

Table 2 **Blood analysis, nutritional status (Mean values ± SD)**

2A: Values obtained at several time points

↓ Group	Day→	-8	0	34	62	97
2	Hemoglobin	6.9±0.4	7.1±0.5	7.8±0.1	7.4±0.5	8.7±1.1
3	(mmol/l)	6.7±0.2	8.1±0.3	7.9±0.4	7.7±0.3	9.0±0.6
2	Leucocytes	11.9±1.7	5.8±1.8	7.1±0.2	6.0±0.8	6.0±0.8
3	(%)	7.6±2.4	6.4±1.2	7.6±2.3	8.1±0.2	6.0±0.5
2	Total protein	54.3±2.1	53.0±1.4	54.9±3.4	53.8±1.2	57.8±2.9
3	(g/l)	56.2±2.0	56.2±1.3	54.9±0.4	54.4±3.5	53.4±3.0
2	Albumin	28.1±0.6	27.0±1.0	27.0±1.4	25.8±0.7	29.4±1.1
3	(g/l)	28.3±0.5	29.2±0.4	26.3±0.8	24.0±3.5	25.8±0.3
2	Cholesterol	3.23±0.09	3.13±0.57	3.76±0.84	3.61±0.93	4.02±0.7
3	(mmol/l)	3.26±0.13	4.07±0.20	3.34±0.06	4.01±0.28	3.60±0.1
2	Triglyceride	0.28±0.15	0.29±0.10	0.44±0.16	0.31±0.29	0.31±0.06
3	(mmol/l)	0.37±0.01	0.43±0.08	0.35±0.01	0.44±0.03	0.26±0.02

2B: Values obtained between 5 and 7 months

Group ↓	NH3 (μmol/l)	Vit B12 (pmol/l)	Folic acid (nmol/l)	Vit A (μmol)	Vit E (μmol)	Ca (mmol/l)	Fe (μmol/l)
2	53.0±5.4	241±137	21.3±0.6	3.20±0.73	30.43±2.93	2.82±0.08	25.13±6.01
3	78.2±3.2	223±50	23.2±2.6	2.23±0.71	32.15±5.30	2.94±0.03	24.9±8.49

Fecal fat analysis

Fecal fat excretion appeared to be a sensitive indicator of malabsorption as it was severely increased in short bowel control animals (Figure 3B). Following sham operation or auto-

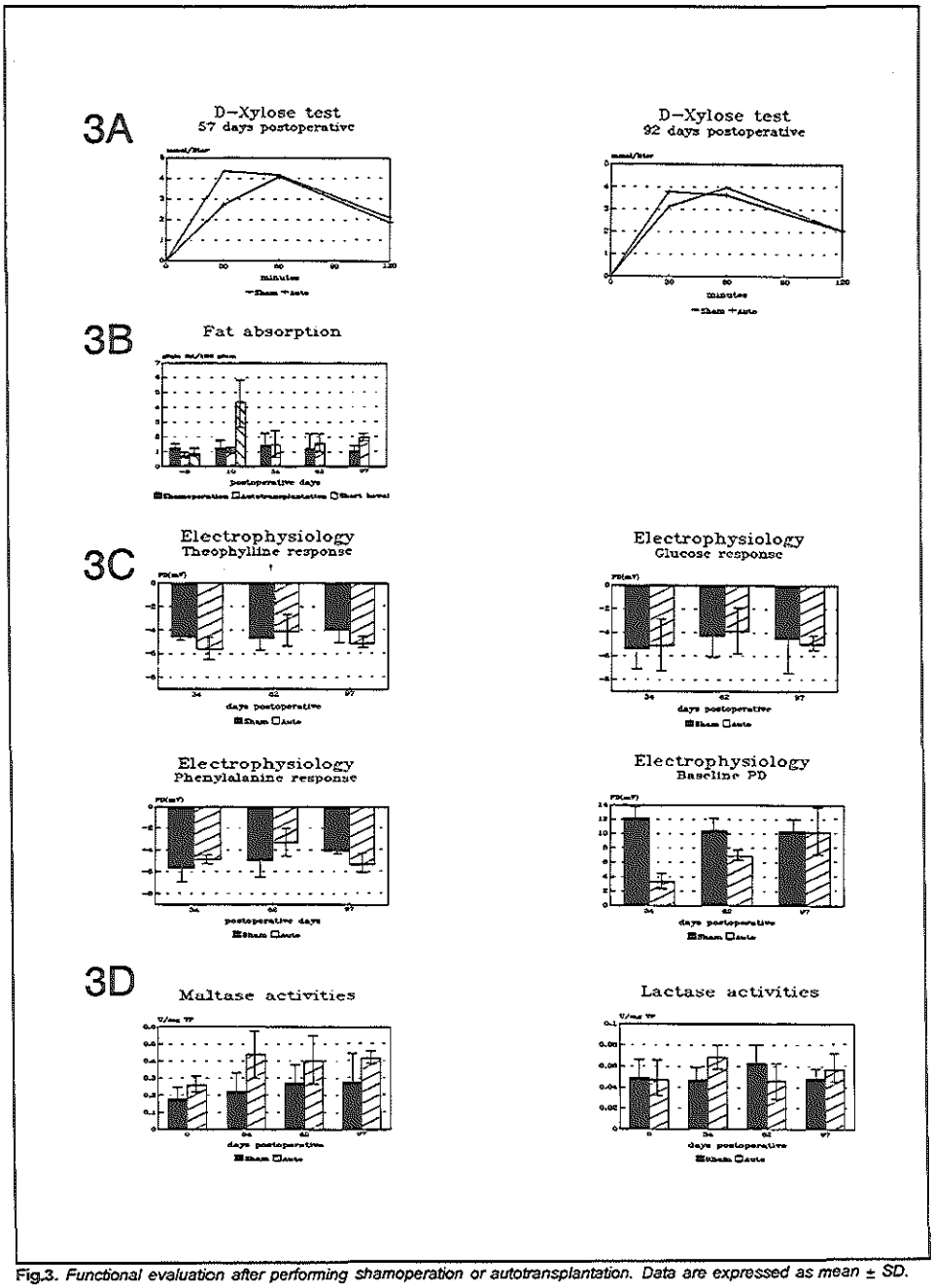


Fig.3. Functional evaluation after performing shamoperation or autotransplantation. Data are expressed as mean ± SD.

transplantation no decreased fat absorption was found at the typical time points.
However, one of the long-term surviving autotransplanted dogs developed several episodes of enteritis, attended with weight loss and impaired fat absorption.

Electrophysiology
The results of the electrophysiologic measurements are depicted in Figure 3C. No differences were found in stimulated PD responses (Glucose-evoked, Theophylline-evoked and Phenylalanine-evoked) throughout the test period for group 2 and group 3. The baseline PD measurements in group 2 were comparable at the several time points. Compared to this group the baseline PD response in group 3 were lower at 34 and 62 days postoperatively but were not different anymore at 97 days.

Disaccharidase activities
Brush border enzyme activities are shown in Figure 3D. In the sham-operated animals, activities of both maltase and lactase remained constant throughout the test period. In the autotransplant group, stable enzyme activities were found as well. Comparing enzyme activities between the groups showed no clear differences between group 2 and group 3.

Histology
The biopsies of both the sham-operated and autotransplanted animals showed intact villi with no evidence of inflammation in the mucosa and submucosa at the regular time points after grafting. Besides no signs of atrophy could be seen.

Discussion

For infants with SBS who are totally dependent on TPN, transplantation of a functioning small intestine might be the best treatment (16). There is little experimental proof that orthotopic bowel transplants may improve the morbidity and mortality rates of infants suffering from the SBS (17-20). It is suggested that after bowel transplantation sufficient functional capacity will remain after SBT to permit normal growth. However, until now, all experimental research concerning SBT physiology in large animals has been performed in adult recipients. These results cannot simply be translated to immature recipients and therefore supplementary research is necessary. The present study was undertaken to evaluate the technical and functional feasibility of a one-step total orthotopic transplantation in the treatment of the SBS in young animals.

It has been learned from clinical and experimental experience that the adaptation capacity following major bowel resection is enormous (21,22). To exclude the possibility that a

puppy after near total enterectomy thrives on its remaining bowel, we included a short bowel control group. Such a control group is often omitted [19]. From the results of group 1 (SBS control), it can be concluded that the model used was suitable for the aim of the study, as the animals had irreversible weight loss and persistent diarrhea, despite adequate intake of food and water. Group 2 (sham operation) was included to monitor the effect of creating a Roux-Y loop without interrupting the vascular, neural and lymphatic supply. The two surviving dogs in group 3 (SBS + SB autotransplantation) demonstrate that the SBS in a growing animal can be succesfully treated with a total orthotopic SB autotransplant.

Weight, being a "sensitive" marker of the nutritive status, was gained normally after autotransplantation.

Comparing blood parameters between group 2 and 3, only ammonia levels were markedly increased in autotransplanted animals. Several rat studies (23) and a piglet study (24) have already shown that venous drainage into the systemic circulation produces abnormalities in ammonia and amino acid levels. Our study underlines that ammonia levels will rise after altering the anatomy of the portal vascular system. However, no major argument to avoid systemic drainage can be given as dietary manipulations are likely able to correct metabolic disturbances found.

The D-xylose absorption test has been widely used as a pivotal test to evaluate small intestinal function in both adults and children (25). Although the pathway of D-xylose uptake has not yet been elucidated, it is clear that the assessment of D-xylose absorption reflects jejunal mucosal integrity. Our results showed unaltered D-xylose absorption after autotransplantation, indicating that the transplantation procedure itself did not influence its absorption, suggesting a normal jejunal function.

Another functional test, mainly challenging the ileum, is the determination of fat absorption. Schraut and colleagues reported that a high level of fecal fat excretion is generally found in adult recipients of allografts or isografts (19). This impaired fat absorption was assumed to be caused by denervation and interruption of the lymphatic system. Raju et al (26), using adult dogs, evaluated long-term nutritional function of orthotopic SB autotransplantation and found depressed D-xylose and fat absorption as late as 12 months after surgery. We also detected impaired fat absorption in the short bowel control group. Surprisingly, no impaired fat absorption was found in the puppies after autotransplantation during periods of clinical health. The fact that one of the long-term surviving autotransplanted dogs developed several episodes of enteritis, during which

increased fecal fat excretion was found, might pose a serious problem. This finding may suggest that, although the recipient can nutritionally be supported entirely by its intestinal graft, recurring periods of infection and dysfunction in the long-run cannot be excluded.

The electrophysiologic responses after autografting were comparable to those after sham operation at any time point. This finding suggests that the autotransplantation procedure did not disturb specific sodium-coupled active transport of carbohydrates and amino acids and cAMP mediated chloride secretion. Thus, the SB capacity of both absorption and secretion of fluid and electrolytes, which facilitates the solubilization and absorption of ingested macro- and micronutrients, remained unaffected. The lowered base-line PD values after autografting during the first three months may be related to the effects of denervation. Lear et al (27) demonstrated enhanced chloride secretion in both isografts and allografts, which was identified as active secretion of chloride from the crypt as a result of lost autonomic control. In our study, the reduced base-line PD values also may have been due to increased secretory activity of the crypts. In addition, theophylline-evoked responses were not any different following denervation. Therefore, it can be postulated that the chloride secretion caused by denervation is non cAMP linked.
Malmfors and associates (28) showed that peptidergic nerves, storing substance P, VIP, enkephalin and somatostatin, are intrinsic in a piglet model of jejunal autotransplantation, whereas adrenergic nerves were extrinsic. Other results indicate that denervation has no effect on serotonin secreting cells (29). We propose that the increased secretory flux found in the early postoperative period may be the resultant of an imbalance between endogenous secretagogues (e.g., acetylcholine, serotonin, substance P) and anti-secretagogues (e.g., somatostatin, enkephalin).

Our electrophysiologic results suggest that the functional condition of the heterotopically placed Roux-Y loop is not considerably diminished in the recorded time period and thus atrophy is unlikely. This finding was confirmed by determination of brush border enzyme activities and histologic monitoring of the graft.

The reasons for performing a one-stage total orthotopic autotransplantation were to evaluate whether this procedure was technically feasible and, if so, whether near normal growth and development could be established. Our results show that a one-step total orthotopic SB autotransplantation in puppies is technically feasible, although the early postoperative mortality rate is unacceptably high. Most canine studies report a high incidence of thrombotic complications, most probably caused by low blood flow due to graft edema (30-32). We found a vascular failure rate of 20% (2/10), which is acceptable. In young animals, disturbance of the water and electrolyte homeostasis, enhanced by

denervation, greatly contributes to the outcome. Other contributing factors include bacterial translocation, which results in sepsis, paralysis, invagination and intestinal obstruction. In our model, the mortality rate due to other causes is 50% (4/8). Therefore, we postulate that a one-step transplantation procedure, as described above, is fraught with an inordinate mortality of 75% (6/8) for a growing dog. In the near future, we will investigate the role of a two-stage segmental allotransplantation in achieving lower morbidity and mortality rates of enterectomized puppies.

Acknowledgements

We appreciate the technical assistance of the Laboratory for Experimental Surgery's staff in performing the experiments.

References

1. Zlotkin SH, Stallings VA and Pencharz PB. Total parenteral nutrition in children. *Pediatr Clin North Am 1985; 32: 381.*
2. Heird WC and Winters RW. Total parenteral nutrition. The state of the art. *J Pediatr 1975; 86: 2.*
3. Perl M, Hell RCW, Dudrick SJ, Englert DM, Stickney SK and Gardner ER. Psychological aspects of long-term home hyperalimentation. *JPEN 1980; 4: 554.*
4. Dudrick SJ, O'Donnell JJ, Englert DM, Matheny RG, Blume ER, Nutt RE, Hickey MS and Barroso AO. 100 Patient-years of ambulatory home total parenteral nutrition. *Ann Surg 1984; 199: 770.*
5. Schraut WH. Current status of small-bowel transplantation. *Gastroenterology 1988; 94: 525.*
6. Watson AJM and Lear PA. Current status of intestinal transplantation. *Gut 1989; 30: 1771.*
7. Sarr MG, Dueres JA and Walters AM. Jejunal and ileal absorptive function after a model of canine jejunoileal autotransplantation. *J Surg Res 1991; 51: 233.*
8. Meijssen MAC, Heineman E, de Bruin RWF, Wolvekamp MCJ, Marquet RL and Molenaar JC. Successful canine small-bowel transplantation using major histocompatibility complex matched segmental ileal allografts. *Transplant Proc 1992; 24: 1116.*
9. Eberts TJ, Sample RHB, Glick MR and Ellis GH. A simplified, colorimetric micromethod for xylose in serum or urine, with phloroglucinol. *Clin Chem 1979; 25: 1440.*
10. Van de Kamer JH, Ten Bokkel Huinink H and Weyers HA. Rapid method for the determination of fat in feces. *J Biol Chem 1949; 177: 347.*
11. Meijssen MAC, Heineman E, de Bruin RWF, Veeze HJ, Bijman J, de Jonge HR, ten Kate FJW, Marquet RL and Molenaar JC. In vivo electrophysiologic evaluation of intestinal grafts in dogs. *Transplant Proc 1990; 22: 2449.*
12. Donowitz M and Welsh MJ. Regulation of mammalian small intestinal electrolyte secretion. *In Johnson LR, ed. Physiology of the Gastrointestinal tract. New York: Raven Press, 1987: 1351.*
13. Fromter E. Electrophysiological analysis of rat renal sugar and amino acid transport. 1. Basic phenomena. *Pflugers Archiv 1982; 393: 179.*
14. Dahlquist A. Assay of intestinal disaccharidases. *Anal Biochem 1968; 22: 99.*
15. Watanabe N, Kamei S, Onkubo A, Yamanaka M, Ohsawa S, Makino K and Tokuda K. Urinary protein as measured with a pyrogallol red-molybdate complex, manually and in a hitachi 726 automated analyzer. *Clin. Chem 1986; 32: 1551.*
16. Goulet O, Revillon Y, Cerf-bensussan N, Nezelof C, Fischer A, Buisson C, Hubert P, Lokec P, Martelli H and Niandet P. Small intestinal transplantation in a child using cyclosporine. *Transplant Proc 1988; 20: 288.*
17. Grant D, Duff J, Zhong R, Garcia B, Lipohar C, Keown P and Stiller C. Successful intestinal transplantation in pigs treated with cyclosporine. *Transplantation 1988; 45: 279.*

18. Kimura K, LaRosa C, Blank MA and Jaffe BM. Successful segmental intestinal transplantation in enterectomized pigs. *Ann Surg 1990; 211: 158.*
19. Schraut WH, Lee KKW and Sitrin M. Recipient growth and nutritional status following transplantation of segmental small-bowel allografts. *J Surg Res 1987; 43: 1.*
20. Ricour C, Revillon Y, Arnaud-Battandier F, Ghnassia D, Weyne P, Lauffenburger A, Jos J and Fontaine P. Succesful small bowel allografts in piglets using cyclosporine. *Transplant Proc 1983; 15: 3019.*
21. Reid IS. The significance of the ileocecal valve in massive resection of the gut in puppies. *J. Pediatr Surg 1975; 10: 507.*
22. Urban E and Weser E. Intestinal adaptation to bowel resection. *In Stollerman GH, ed. Advances in Internal Medicine. Chicago: Year book Medical Publishers, Inc, 1980; 26: 265.*
23. Koltun WA and Kirkman RL. Recent results of small-bowel transplantation in the rat model. *In Deltz E, Thiede A and Hamelmann H, eds. Small-bowel transplantation-experimental and clinical fundamentals. Berlin: Springer-Verlag, 1986: 14.*
24. Kaneko H, Fischman MA, Buckley TM and Schweizer RT. A comparison of portal versus systemic drainage in the pig small-bowel allograft recipient. *Surgery 1991; 109: 663.*
25. Craig RM and Atkinson AJ. D-xylose testing: a review. *Gastroenterology 1988; 95: 223.*
26. Raju S, Fujiwara H, Grogan JB and Achord JL. Long-term nutritional function of orthotopic small bowel autotransplants. *J Surg Res 1989; 46: 142.*
27. Lear PA, Watson AJ, Crane PW, Farthing MJG and Wood RFM. Autonomic function limits mucosal transport in cyclosporine-treated small intestinal transplants. *Transplant Proc 1988; 20: 436.*
28. Malmfors G, Hakanson R, Okman L and Sandler F. Peptidergic nerves persist after jejunal autotransplantation: an experimental study in the piglet. *J Pediatr Surg 1980; 15: 53.*
29. La Rosa CA, Kimura K, Dresner LS, Birnbaum E and Jaffe BM. The effect of small intestinal transplantation on intraluminal levels of serotonin and substance P. *J Surg Res 1989; 46: 600.*
30. Kirkman RL. Small bowel transplantation. *Transplantation 1984; 37: 429.*
31. Ballinger WF, Christy MG and Ashby WB. Autotransplantation of the small intestine: the effect of denervation. *Surgery 1962; 52: 151.*
32. Reznick RK, Craddock GN, Langer B, Gilas T and Cullen JB. Structure and function of small bowel allografts in the dog: immunosuppression with cyclosporin A. *Can J Surg 1982; 25: 51.*

5.2 LONG-TERM ANALYSIS OF THE FUNCTIONAL, MORPHOLOGIC, AND BACTERIOLOGIC STATUS OF THE SMALL INTESTINE FOLLOWING AUTOTRANSPLANTATION IN GROWING DOGS

Abstract

Many candidates for small-bowel transplantation are children, who need enough functional intestine to grow and develop normally. Our aim, therefore, was to evaluate the long-term non-immunologic effects of the small intestine on function, morphology and quantitative bacterial counts in subsets of the gastrointestinal tract after one-step small-bowel autotransplantation (n=10, only 2 dogs survived long-term due to technical- and management related complications) or sham operation (n=3) in a growing animal. To monitor graft function animals were weighed and serum parameters were determined; we also performed D-xylose absorption testing, fecal fat analysis and intestinal transit time studies. After sacrifice (1 year after the operation) disaccharidase activities were determined. Morphologic and bacterial studies were also performed after death. Comparing data of sham-operated and autotransplanted animals, increased maltase activity in both jejunum and terminal ileum and increased villus height in both duodenum and ileum was found following autotransplantation. The bacterial counts (log_{10} CFU/gram tissue $\pm$ SD) showed aerobic overgrowth following autotransplantation in terminal ileum (7.00 ± 1.00 vs 6.00 ± 0.13) and ascending colon (7.72 ± 0.66 vs 5.33 ± 0.04) compared to sham operation. These results suggest that autotransplantation, in a growing animal, leads to adaptive functional and morphologic changes, and to elevated aerobic bacteria counts in distally situated parts. This overgrowth did not lead to secondary impaired function in these growing animals.

Introduction

Small-bowel transplantation (SBT) is considered an exciting new field in transplant surgery and immunology and the worldwide interest is still increasing. The current clinical experience demonstrates the inherent feasibility and practibility of SBT in humans (1,2,3). Among the functions of the intestinal graft that has to be preserved in the long-term after SBT are motor-, hormonal-, immunologic- and nutritional- components. Several studies have been performed to evaluate the long-term consequences of SBT on various aspects of graft physiology. Thompson et al reported long-term evaluation after SBT up to 20 months in adult dogs that had received an autotransplant (4). They showed that the effects of total extrinsic denervation are permanent and resulted in bacterial overgrowth and eventual disturbance of stool fat excretion. In addition, no intrinsic dependent gut functions were disturbed. Raju et al performed similar studies in adult autografted dogs (5). They found persistently increased fat excretion and D-xylose absorption for 12 months. This study learned that systemic venous drainage of the graft shows more severe functional abnormalities than physiologic portal venous drainage. In other investigations the transplantation procedure itself resulted in impaired motility, which may explain the bacterial overgrowth (6,7).

Although many studies have addressed the effect of SBT on gastrointestinal function, little work has been done to evaluate the effect on intestinal morphology. In adult dogs normal villus height was found in both jejunum and ileum 12 to 18 months after autotransplantation (4). Whether intestinal morphology is unaltered along the entire gastrointestinal tract is unknown. This issue is important because quantitative morphometric analysis may parallel the functional condition of the graft. Moreover, segmental allotransplantation most likely involves adaptive changes in both function and morphology to overcome the "shortened" gut.

Many candidates for SBT are children having irreversible small intestinal failure. SBT is widely considered as the best method to treat the short bowel syndrome. In our opinion it must be demonstrated that these grafts are functionally as well as histologically preserved before SBT can be proposed as a practical option. Our aim, therefore, was to evaluate the long-term effects on intestinal function as well as on morphology and quantitative bacterial counts in well-defined parts of the gastrointestinal tract in a growing animal after autotransplantation.

To establish graft function in the long run growth and serum parameters were studied; and we also performed in vivo D-xylose absorption testing, fecal fat analysis, and

intestinal transit time studies. One year after the operation the animals were sacrificed and disaccharidase activities were determined.

Materials and Methods

Animals

Female Beagle puppies, weighing 5-6 kilogram, were purchased from Harlan CPB, the Netherlands. They were maintained in separate cages under conventional conditions in accordance with the guidelines on care of laboratory animals.

Experimental design

Two groups of animals were studied: dogs that underwent a sham operation (Group 1; n=3) and dogs that underwent a one-step orthotopic small-bowel (SB) autotransplantation (Group 2; n=10). The surgical technique has been described in detail in paragraph 5.1. Enteritis during the experimental period could be successfully treated with 2.5 ml Depomycin subcutaneously for 5 days.

In group 2, only two animals survived the major operation and could be evaluated in the long run. During lifetime the following functional parameters were used to determine graft function: blood analysis, D-xylose absorption testing, fecal fat analysis and intestinal transit time.

The dogs were sacrificed one year after surgery with an overdose barbiturate. Just before sacrifice, the animals were heparinized with 5000 IU heparin for enabling blood collection. After death, samples for functional-, histologic-, and bacteriologic studies were collected.

Functional evaluation

Weight. Measured twice a week during the experimental period.

Between 11-12 months after the operation the following parameters were studied:
Blood analysis. The following routine laboratory blood chemistry data were determined: hemoglobin, leukocytes, serum total protein, albumin, cholesterol, triglyceride, alkaline phosphatase; AF, alanine aminotransferase; ALT, aspartate aminotransferase; AST, blood urea nitrogen; BUN, Creatinine; Crea and total bilirubin; TBIL. Additionally, serum levels of vitamin B_{12}, ammonia, folic acid, vitamin A, vitamin E, calcium, iron were determined.

D-xylose absorption test. Food was withheld 12 hours before performing the test. D-xylose (0.5 gram/kg body weight) dissolved in water was prepared as test solution. Blood samples (in heparinized tubes) were collected before, and 0.5, 1 and 2 hours after oral administration. A D-xylose blood level time curve was obtained using the method described by Eberts et al (8).

Fecal fat analysis . The method used has been described in detail by Van de Kamer et al (9).

Small-intestinal transit time study. Dogs were fasted overnight. Orogastric intubation was performed using a sterile 18CH levin stomach tube (Argyle, Sherwood/Ireland). After confirming that the trachea had not been intubated, using transillumination, 120 cc barium-sulfate containing solution (Micropaque mixed with water; 1: 1.5) was brought into the stomach. Lateral radiographs were then taken every fifteen minutes using a Sirescop 3 (Siemens), and the intestinal transit time was determined.

Harvesting for histologic, bacteriologic, and disaccharidase activity studies;
The exact sites of collection are schematically given in Figure 1A and Figure 1B.
In Figure 1A the sites of the gastrointestinal tract from which specimens were taken are defined: from the duodenum (25 cm proximal to the ligament of Treitz)[1], the jejunum (8 cm distal to the ligament of Treitz)[2], the middle small-bowel (50 cm distal to the ligament of Treitz)[3], the ileum (50 cm proximal to the ileocecal valve)[4], the terminal ileum (3 cm proximal to the ileocecal valve)[5] and the ascending colon (25 cm distal to the ileocecal valve)[6]. In Figure 1B the sites of the Roux-Y from which material was collected (*R-Y 1 to R-Y 4*) are defined.

Harvesting for disaccharidase activity assessment. After death, material for disaccharidase activity determination was collected from sites 2, 4 and 5 of the gastrointestinal tract and from *R-Y 1* to *R-Y 4*.

Harvesting for histologic assessment. For histologic purposes specimens of heart, lung, spleen, liver and mesenteric lymph node were harvested. From the gastrointestinal tract specimens were taken from sites 2, 3, 4, 5 and 6. From the Roux-Y specimens were taken from sites *R-Y 1 to R-Y 4*.

Harvesting for quantitative bacteriologic studies. After death, the thoracic cavity was opened to obtain 2.5 ml of blood by cardiac puncture and to harvest a piece of lung tissue. Subsequently, the abdominal cavity was opened and 2.5 ml of portal and inferior

caval vein blood was collected. Moreover, pieces of liver, spleen and mesenteric lymph node were harvested. From the gastrointestinal tract specimens were taken from sites 2, 3, 4, 5 and 6. From the Roux-Y a specimen was taken from *R-Y 3*.

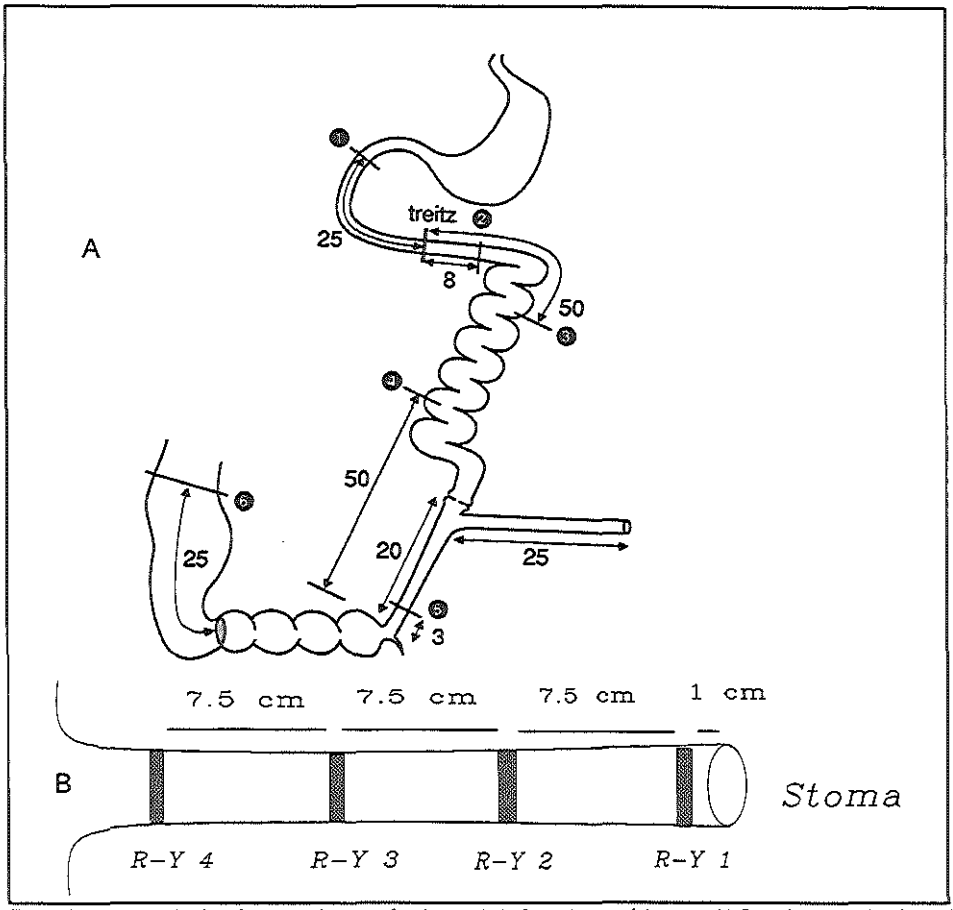


Fig.1. *Specimen collection for assessing functional, morphologic and bacterial status (defined in text): A, sites of gastrointestinal tract; B, sites of Roux-Y.*

Specimen processing

Disaccharidase activity. Disaccharidase activity was determined as described in detail in paragraph 5.1 (**10**).

Histology. The specimens that were collected for histology were fixed in 3.6% buffered formalin, dehydrated, and embedded in paraffin. Longitudinally cut sections of 4-5 μm were then stained with hematoxylin-azophloxin-safran. They were examined by standardized projection on a screen through a light microscope.

Bacteriology. Blood samples (1 ml) were cultured in Thioglycolate-Broth (10 ml) and

Brain-Heart Infusion Broth respectively. After an incubation time of 24-48 hr, 100 μl of broth was plated on blood agar, chocolate agar and fastidious anaerobe agar. The plates were examined after 24 and 48 h of incubation at 37°C. A blood sample was considered positive if any growth was found. Fecal samples (1 gram) were suspended in sterilized saline (9 ml), and subsequently diluted by a factor of 10, 10^2, 10^3, 10^4 and 10^5. Hundred μl was plated on blood agar and McConkey agar. Plates were inspected after 18-24 hr of incubation at 37°C and the CFUs were counted. Data were reported as CFU/gm feces $\pm$ standard deviation (SD).

Tissue specimens were weighed and diluted 10 times with sterilized saline. The suspension was thoroughly homogenized, whereafter 100 μl of the homogenate was plated on blood agar and McConkey agar. Following an incubation time of 18-24 h, the number of bacteria per tissue specimen was calculated after counting the number of viable colonies.

Selected colonies, obtained from blood-, fecal- and tissue samples, were subsequently identified using standard aerobic (BBL gaspak CO_2-, $AP_{20}E$-, Titertek Non-Fermenter- and Titertek-Enterobacteriaceae automated system) and anaerobic (BBL gaspak anaerobic system) identification methods.

Statistical analysis

Statistical analysis was not performed as the study population was too small.

Results

Weight

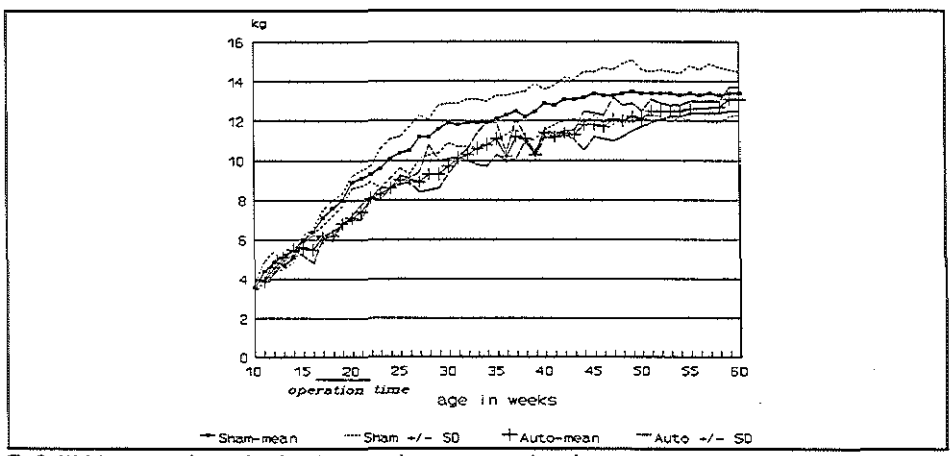


Fig.2. *Weight curves after performing shamoperation or autotransplantation.*

Figure 2 shows that dogs that underwent a sham operation grew normally during the observation period when compared with non-operated historic controls (data not shown). In the first postoperative weeks, dogs that underwent a one-step total orthotopic SB autotransplantation showed a minor and temporarily weight loss. Subsequently a catch up growth approaching the weight curve of sham operated animals was observed. In the first postoperative months, however, autotransplanted dogs developed several enteritis-like episodes attended by diarrhea and weight loss. Such episodes were no longer observed from six months postoperatively and in the long run the weights of autotransplanted animals were equal to data of sham-operated animals. Apart from the first postoperative weeks and the infectious periods, stool consistency was normal.

Blood analysis

Blood parameters are shown in Table 1. The nutritional parameters indicate that the general condition of the animals after sham operation is comparable to the condition of animals after autotransplantation. In autotransplanted animals serum ammonia levels were increased compared to sham-operated animals (75.8 ± 8.1 vs 37.9 ± 16.9) 12 months postoperatively.

Table 1 **Blood analysis, nutritional status (Mean values ± SD)**
Values obtained one year posttransplant

Parameter	Group 1	Group 2
Hemoglobin (mmol/l)	10.3 ± 0.3	10.3 ± 0.2
Leukocytes (10^9/l)	6.1 ± 1.4	7.4 ± 1.3
Total Protein (g/l)	57.4 ± 2.7	63.8 ± 1.0
Albumin (g/l)	28.2 ± 2.6	29.5 ± 0.5
Cholesterol (mmol/l)	4.5 ± 0.7	5.6 ± 1.4
Triglyceride (mmol/l)	0.24 ± 0.03	0.33 ± 0.01
AF (Units/l, 37°C)	31.7 ± 7.4	25.1 ± 2.0
ALT (Units/l, 37°C)	34.5 ±11.4	55.2 ± 41.6
AST (Units/l, 37°C)	14.4 ± 2.7	18.5 ± 1.3
BUN (mmol)	4.1 ± 0.7	5.1 ± 1.0
CREA (μmol/l)	72.0 ± 2.0	75.0 ± 4.0
TBIL (μmol/l)	3.2 ± 0.5	3.9 ± 0.8
Vit B12 (pmol/l)	233 ± 88	246 ± 135
NH3 (μmol/l)	37.9 ± 16.9	75.8 ± 8.1
Folic acid (nmol/l)	24.3 ± 2.1	26.2 ± 2.4
Vit A (μmol)	2.80 ± 0.74	2.60 ± 0.73
Vit E (μmol)	39.3 ± 4.22	47.5 ± 17.5
Ca (mmol/l)	2.77 ± 0.06	2.85 ± 0.04
Fe (μmol/l)	27.12 ± 4.02	30.1 ± 12.0

D-xylose absorption test

The D-xylose absorption test results, obtained one year posttransplant, are shown in Figure 3A. Area-under-curve calculations indicated that total D-xylose absorption after sham operation (350.6 ± 95.0) is similar to that after autotransplantation (394.3 ±

146.2). Moreover, comparing these results with values obtained on the short term (data given in paragraph 5.1), no alterations were found.

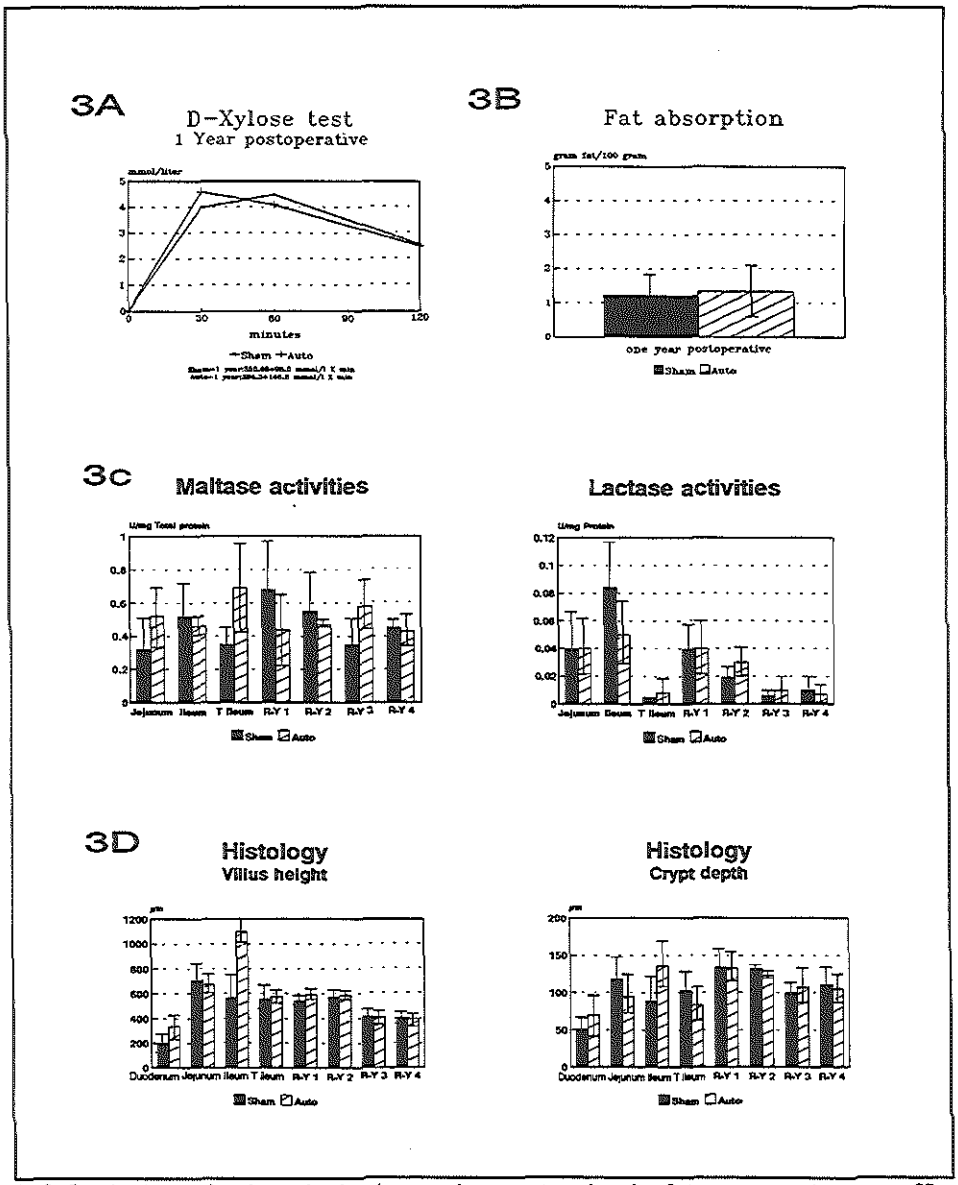


Fig.3. Functional evaluation after performing shamoperation or autotransplantation. Data are expressed as mean ± SD.

Fecal fat analysis

The results of fecal fat excretion are depicted in Figure 3B. One year after the operation no elevated fat excretion could be detected in either group.

Small-intestinal transit time study

The intestinal transit time was similar in both groups (visualized in Figure 4).

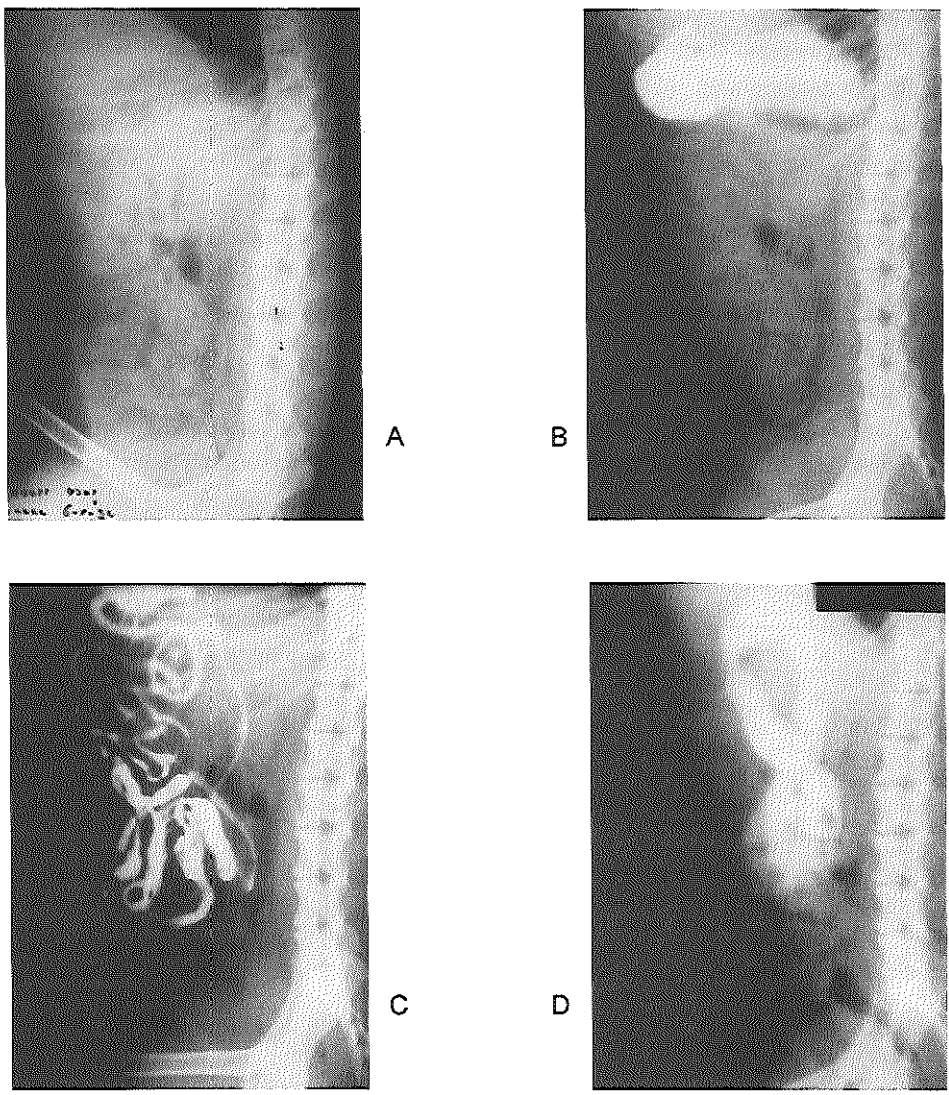


Fig.4. *Intestinal transit time study: 4A, before Barium-sulfate solution was brought into stomach; 4B, stomach filled with Barium-sulfate solution (t=0 hours); 4C, barium-sulfate solution present in the small intestine (t=15 minutes); 4D, Barium-sulfate solution present in colon (t=2 hours).*

Disaccharidase activities (Maltase and Lactase)

Data of maltase and lactase activities are shown in Figure 3C. In well-defined parts of the gastrointestinal tract lactase enzyme activities of autotransplanted animals were comparable to those in sham-operated animals. Maltase activities of autotransplanted animals were increased as compared to sham-operated animals, both in jejunum and terminal ileum.

Histology

At the macroscopic level, no obvious differences between dogs in group 1 and group 2 could be demonstrated. In autotransplanted animals, bowel-lengths measured from the ileocecal valve to the transection point and from the ligament of Treitz to the transection point were markedly increased as compared to preoperative lengths (from 5.0 $\pm$ 0 to 17 $\pm$ 4 and from 1.0 $\pm$ 0 to 19 $\pm$ 2 centimeters).

Microscopic analysis is depicted in Figure 3D. After autotransplantation obvious increases in villus size were seen in the duodenum as well as the ileum one year after the operation compared to sham operation. No other major changes in intestinal morphology were seen.

Quantitative bacteriologic studies

The bacteriologic data are shown in Table 2. The numbers of aerobic bacteria found in the ascending colon and the terminal ileum were markedly elevated. The bacteria most frequently identified were Micrococcus- , Pasteurella-, and hemolytic and other E coli species.

Table 2 **Bacterial counts; results given in $\log_{10}$ CFU/gram tissue $\pm$ SD**

Tissue	Group 1	Group 2
lung	none	none
liver	none	none
spleen	none	none
MLN	none	none
portal blood	none	none
caval blood	none	none
cardiac blood	none	none
jejunum	5.25 $\pm$ 2.33	6.24 $\pm$ 0.72
ileum	6.12 $\pm$ 0.71	5.59 $\pm$ 0.16
mid jejunum/ileum	5.30 $\pm$ 2.26	5.90 $\pm$ 0.23
terminal ileum	6.00 $\pm$ 0.13	7.00 $\pm$ 1.00
Roux-Y	none	6.49 $\pm$0.65
colon	5.33 $\pm$ 0.04	7.72 $\pm$0.66

Discussion

Although it is becoming increasingly clear that transplanting of the small intestine has shown its inherent feasibility the postoperative course is extremely difficult. It is often

complicated by problems such as graft-versus-host disease, rejection and sepsis (11). In the present animal model we circumvented rejection by performing a one-step total orthotopic SB autotransplantation and were thus able to study the long-term non-immunologic effects of SBT on function, morphology and bacteriology of the intestine in a growing animal.

Weight analysis, sensitively reflecting the overall nutritional status, showed that no significant effect on weight could be found in autotransplanted animals compared to sham-operated animals. This observation was supported by analysis of nutritional parameters one year postoperatively. Again, no significant alterations were demonstrated, indicating that a normal nutritional balance is maintained after autotransplantation.

In agreement with other studies elevated ammonia levels were present following systemic venous drainage of an intestinal graft (12,13). This physiologic consequence, already occurring within three months (Chapter 2), may have implications for the diet after transplantation.

We found that growing autotransplanted animals had normal D-xylose and fat absorption one year after the operation despite the presence of bacterial overgrowth. Others (4,5) have reported depressed D-xylose and fat absorption for the 12-month period after transplantation. A major difference with our study is their use of adult animals. The adaptive functional reserve of the intestine of a growing animal may well prevent the development of functional abnormalities following extrinsic denervation. This statement is supported by our data on morphology and disaccharidase activities of autotransplanted intestine. One year after autotransplantation, increased duodenal as well as ileal villus height was observed and the overall bowel length was markedly increased. In addition, increased maltase activity in jejunum as well as terminal ileum was detected.

One explanation is that denervation itself is able to exert trophic influences on the morphology and specific functional characteristics of the intestine. There is evidence that the myenteric plexus regulates cell proliferation and cell growth in the rat jejunum (14). Furthermore, the segments contained higher levels of sucrase activity (U/mg protein).

Another explanation for the morphologic and functional adaptive changes, at least in the distal part of the gastrointestinal tract, may be that it compensates for malabsorption related to bacterial overgrowth. This is in agreement with the results of Thompson et al (4), who found unaltered morphology in association with impaired absorptive function of the intestine in adult animals.

Moreover, an altered ecologic balance of the gut flora following SBT is one of the factors that might promote sepsis. The alteration in microflora may be caused by ischemic injury, lymphatic and neural disruption, systemic venous drainage, immunologic components, and use of antibiotics (15). Browne et al found that even SBT alone leads to Gram-negative aerobic overgrowth in the distal small-bowel as well as in the ascending colon in a rat model three weeks posttransplant (16). Moreover, it was found that addition of cyclosporine in isografts facilitated translocation of bacteria from the gut to the mesenteric lymph nodes. So, the addition of cyclosporine therapy may be the critical factor which predisposes patients to sepsis following SBT, even in the absence of rejection.

This study shows that infectious complications after SBT can be documented in the first postoperative months. Determining the number of fecal bacteria during such infectious episodes revealed "abnormal flora", which was defined as $>10^5$ organisms/gram feces. This phenomenon is most probably caused by alterations in bacterial gut homeostasis as a result of the surgical procedure itself. From six months on, there were no more clinical signs of bacterial overgrowth. At autopsy, however, we found elevated aerobic bacteria counts in the terminal ileum and ascending colon after autotransplantation. The hypothesis that the detected bacterial overgrowth was due to a transplant with discoördinated motor complexes is unlikely because a normal intestinal transit time was found. Our findings suggest that although bacterial overgrowth is present in the distal part of the gastrointestinal tract one year after autotransplantation in a growing animal, this has no clinical importance in the long run.

Acknowledgements

The authors wish to thank Miss Wiebeke v Leeuwen, Department of Radiology, Erasmus University, for help with the intestinal transit time studies, Mr John Kong A San and Mr Piet Kreeft for analyzing the bacteriologic samples and the staff of the Laboratory for Experimental Surgery for biotechnical assistance.

References

1. Mc Alister V, Wall W, Ghent C, Zhong R, Duff J and Grant D. Successful small intestine transplantation. *Transplant Proc 1992; 24: 1236.*
2. Goulet O, Revillon Y, Canioni D, Jan D, Brousse N, Sadoun Clomb V, Beringer A, Hubert P, De Potter S, Fischer A, Mougenot JF, Cerf-Benssussan N and Ricour C. Two and one-half-year follow-up after isolated cadaveric small bowel transplantation in an infant. *Transplant Proc 1992; 24: 1224.*
3. Starzl TE, Todo S, Tzakis A and Murase N. Multivisceral and intestinal transplantation. *Transplant Proc 1992; 24: 1217.*
4. Thompson JS, Rose SG, Spanta AD and Quigley EMM. The long-term effect of jejunoileal autotranplantation on intestinal function. *Surgery 1992; 111: 62.*
5. Raju S, Fujiwara H, Grogan JB and Achord JC. Long-term nutritional function of orthotopic small bowel autotransplantation. *J Surg Res 1992; 46: 142.*

6. Sarr MG, Duenes JA and Tanaka M. A model of jejunoileal in vivo neural isolation of the entire jejunoileum: transplantation and the effects on intestinal motility. *J Surg Res 1989; 47: 266.*
7. Vane DW, Grosfeld JL, Moore W, Abu-Dalu K and Hurwitz A. Impaired bowel motility following small intestinal transplantation. *J Surg Res 1989; 47: 288.*
8. Eberts JJ, Sample RHB, Glick MR and Ellis GH. A simplified, colorimetric method for xylose in serum or urine, with phloroglucinol. *Clin Chem 1979; 25: 1440.*
9. Van de Kamer JH, ten Bokkel Huinink H and Weyers HA. Rapid method for the determination of fat in feces. *J Biol Chem 1949; 177: 347.*
10. Dahlquist A. Assay of intestinal disaccharidases. *Anal Biochem 1968; 22: 99.*
11. Schraut WH. Current status of small bowel transplantation. *Gastroenterology 1988; 94: 525.*
12. Schraut WH, Abraham VS and Lee KKW. Portal versus caval venous drainage of small bowel allografts: technical and metabolic consequences. *Surgery 1986; 99: 193.*
13. Lee KKW and Schraut WH. Metabolic effects of systemic venous drainage in small-bowel transplantation. *In: Deltz E, Thiede A, and Hamelmann H, eds. Small-Bowel transplantation Experimental and clinical fundamentals. Berlin: Springer-Verlag, 1986: 19.*
14. See NA, Epstein ML, Dahl JL and Bass P. The myenteric plexus regulates cell growth in rat jejunum. *J Aut Nerv Sys 1990; 31: 219.*
15. Grant D, Hurlbut D, Zhong R, Wang P, Chen H, Garcia B, Behme R, Stiller C and Duff J. Intestinal permeability and bacterial translocation following small bowel transplantation in the rat. *Transplantation 1991; 52: 221.*
16. Browne BJ, Johnson CP, Edmiston CE, Hlava MA, Moore GH, Roza AM and Telford GA. Small bowel transplantation promotes bacterial overgrowth and translocation. *J Surg Res 1991; 51: 512.*

CHAPTER 6

Ultrasonography; a useful tool in the follow-up of small-bowel transplantation

Surgical Research Communications 1994; 15: 183-197.

ULTRASONOGRAPHY; A USEFUL TOOL IN THE FOLLOW-UP OF SMALL-BOWEL TRANSPLANTATION

Abstract

This study was undertaken to determine whether ultrasonography is a valuable tool for monitoring the postoperative course after performing small-bowel transplantation in growing dogs.

First, we report ultrasonographic features of normal canine bowel using a 7.5 MHz transducer. Secondly, in dogs that received an isoperistaltic Roux-Y loop, after sham operation or autotransplantation, ultrasound was used to assess signs of atrophy one year postoperatively. And thirdly, we performed sonography in seven growing dogs after two-stage segmental small-bowel allotransplantation to evaluate the postoperative course.

In normal canine bowel; it appeared technically feasible to distinguish mucosa and muscularis by ultrasound. Long-term ultrasound studies of a Roux-Y loop demonstrated mild to moderate atrophy in segments close to the intestinal continuity, but not in segments close to the ileostomy. Monitoring the postoperative course of two-stage segmental small-bowel allotransplantation, early complications, including intussusception, thrombosis, and postoperative ileus, were noted. Moreover, in one case rejection-related mucosal atrophy was clearly demonstrated by ultrasound.

We conclude that ultrasonography is useful in monitoring early complications of small-bowel transplantation and therefore we propagate using ultrasound as part of the multidisciplinary support.

Introduction

In patients with extensive small-bowel (SB) resection, transplantation of the small intestine would be the best treatment modality and is the only alternative to permanent Total Parenteral Nutrition (TPN) (1,2,3).

Technically, small-bowel transplantation (SBT) in human is now feasible (4,5), but rejection, graft-versus-host-disease (GVHD) and infectious complications are key problems that should be overcome in the postoperative period (4-7). In this rather stormy postoperative period, intensive multidisciplinary support and monitoring is necessary to identify and resolve postoperative complications. Bach recently described the radiologic findings in five patients after orthotopic SBT (8). He concluded that radiology had limited value in monitoring rejection, but helped to detect or exclude technical complications such as perforations and anastomotic leaks.

In this study, we investigated the value of ultrasonography in the follow-up of SB grafting in Beagle puppies.

Materials and methods

Animals and experimental groups
In total twenty healthy Beagle puppies (Harlan CPB, Zeist, The Netherlands), weighing 5-

A sham operation was performed in 3 animals and a one-step orthotopic SB autotransplantation in 10 animals as described in detail in paragraph 5.1.

A two-stage segmental SB allotransplantation was performed in another 7 animals. In the first stage, a heterotopic SBT was performed in which the recipient's own bowel was still intact. The transplant (1 meter) was harvested on a vascular pedicle, consisting of both superior mesenteric artery (with aortic cuff) and vein, from a fully Major Histocompatibility Complex (MHC)-matched donor. The graft was revascularized by anastomosis of the mesenteric vessels to the abdominal aorta and caval vein in an end-to-side fashion. The distal end of the graft was constructed as an isoperistaltic Roux-Y loop in continuity with the recipient's terminal ileum about 5 cm proximally to the ileocecal valve, and the proximal end was exteriorized as open ileostomy. After several weeks enterectomy took place and the transplant was placed in continuity in an end-to-end fashion (Figure 1). These animals received Cyclosporine (10 mg/kg/d i.m. at day -1 t/m +7 and 20 mg/kg/d orally afterwards) until the end of the experimental period.

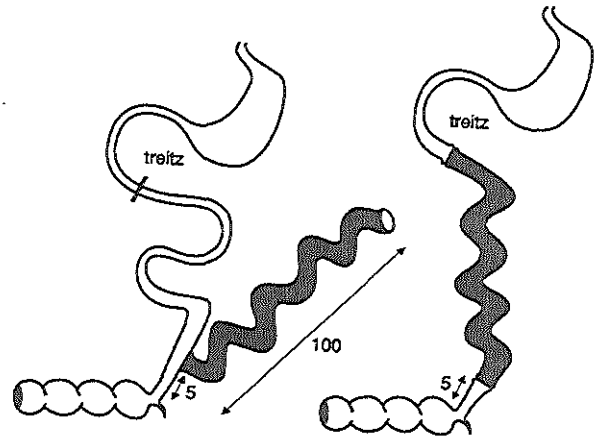


Fig.1. *Schematic design of two-stage segmental allotransplantation.*

Monitoring

For histologic and, or ultrasonographic evaluation the following material was collected:

- normal SB segments from dogs prior to autotransplantation to minimize number of used animals.
- at sacrifice, a defined segment of a Roux-Y loop, 16 centimeters distally from the enterostomy loop one year after its performance during shamoperation or one-step orthotopic SB autotransplantation (see paragraph 5.2, Figure 1B: *R-Y 3*).
- pieces of lung, liver, mesenterial lymph node, kidney, transplant and host bowel were obtained at sacrifice from animals that underwent a two-stage segmental SB allotransplantation.

Histology

The material was fixed in 3.6% buffered formaldehyde, and embedded in paraffin. Hematoxylin-azophloxin-safran staining was performed on 4-5 μm sections.

Ultrasound

All ultrasound examinations were performed by an experienced pediatric radiologist using a commercially available ultrasound unit (Aloka Model SSD-630, Aloka Co., Ltd) equipped with a 7.5 MHz linear transducer for in vivo studies. In sham-operated and autotransplanted animals, ultrasound was used to evaluate the condition of the constructed Roux-Y loop one year posttransplant. The loop was identified through water-filling using

an urinary catheter, inserted into the loop about 15 cm from the enterostomy. Optimal visualization of the loop was accomplished in all cases. Real-time images were obtained in both transverse and longitudinally planes in the supine position after sedation with 5 mg droperidol, injected intramuscularly. In this way diameter, muscle- and mucosal thickness could be measured. After segmental allotransplantation ultrasonographic studies were frequently performed, without any sedation or premedication, to evaluate the postoperative course.

In vitro ultrasound studies were performed with a commercially available 7.0 MHz linear array transducer (Acuson, Mountain View, CA). A small segment of bowel submerged in either saline or 3.6% buffered formaldehyde was used for in vitro examination.

Results

Survival

The 3 sham-operated dogs survived indefinitely. Unfortunately, 8 of 10 autotransplanted animals died due to complications related to management and technique in the first several weeks (**9, paragraph 5.1**). Thus, only in 2 autotransplanted dogs the condition of the Roux-Y loop could be evaluated in the long run.

The postoperative course after a two-stage segmental SB allotransplantation in 7 dogs is summarized in Table 1.

Table 1 The posttransplantation course; after a two-stage segmental allotransplantation

Dog	Stages	Postoperative complications	Survival and cause of death
1	1	venous thrombosis	1 day, venous thrombosis
2	1	recipient intussusception (day 1 and 7), ileus	53 days, pneumonia
3	1	transplant intussusception (day 10)	14 days, hemorrhagic transplant
4	1 & 2	none	48/19 days, functional bile congestion
5	1	none	24 days, rejection
6	1	heart failure	11 days, multiorgan failure
7	1 & 2	transient gastroenteritis and anemia	>>100/50 days

Notes:
* A/B days; A is number of days after first stage/ B is number of days after second stage.
* Dogs 2, 3, 4, 5 and 7 developed transient thrombopenia accompanied by a transient anemia.

Histologic- versus ultrasound appearance of normal canine intestine

In Figure 2A, the schematic characteristics of canine small intestine are given as anatomically identified.

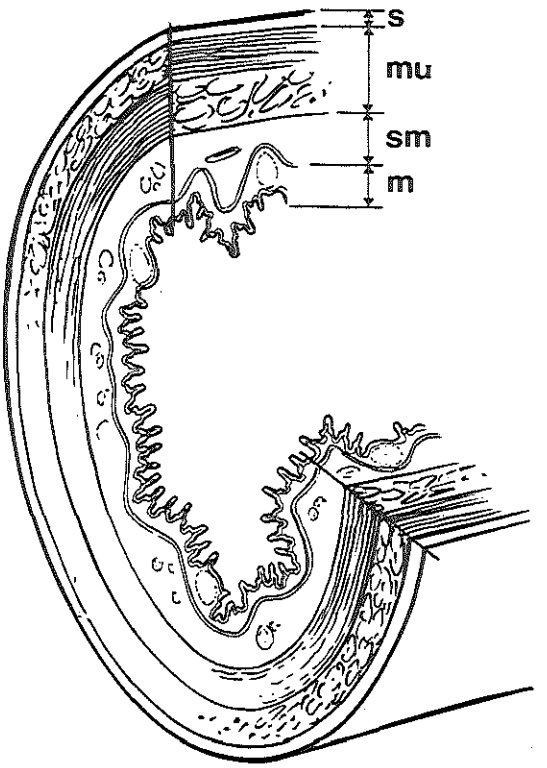


Fig.2.A. *Generalized cross section of vertebrate small bowel, consisting of four layers: an outermost layer of serosa and mesothelial (s), the muscularis (mu) composed of longitudinal and circular fibers, a relative thick layer of submucosa (sm) and the innermost mucosa (m).*

Figure 2B is a cross section of normal canine SB after histological preparation. The principal four layers; mucosa, submucosa, muscularis, and serosa, are easily identified on histologic sections.

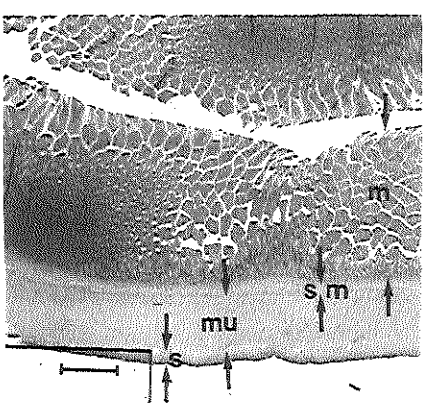


Fig.2.B. *Histologic cross section of normal canine bowel. The layers visualized represent serosa and mesothelial (s), muscularis (mu), submucosa (sm) and mucosa (m).*

Using 7.5 MHz transducers in vitro with fresh tissue immersed in saline, mucosa and muscularis can easily be distinguished as separate hypoechoic bands. The hyperechoic rings represent strong reflections from interfaces between mucosa and muscularis and between serosa and surrounding fluid (Figure 2C).
The submucosa and serosa are located at this position but do not cause these reflections. The thickness of these layers is overestimated on sonography by abnormally strong reflections. Therefore, the muscularis appears small compared to histologic prepared material. In vivo imaging of the canine bowel by ultrasonography is represented in Figure 2D.

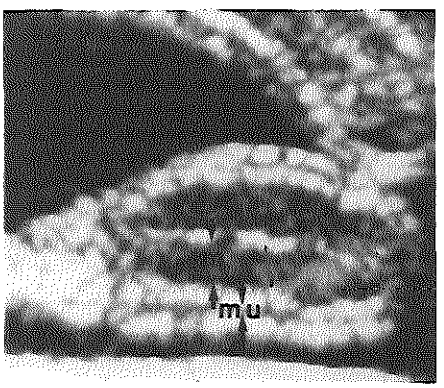


Fig.2.C. *Transverse ultrasound scan of canine bowel in vitro surrounded by saline; hypoechoic bands represent mucosa (m) and muscularis (mu).*

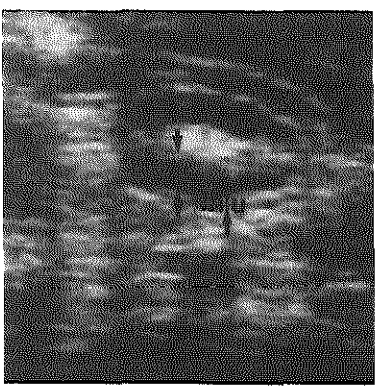


Fig.2.D. *Transverse ultrasound scan of canine bowel in vivo surrounded by formaldehyde; hypoechoic bands represent mucosa (m) and muscularis (mu).*

Comparing Figure 2C with 2D, it appears that the echogenic band between mucosa and muscularis is thicker in vitro. Simultaneous reduction of the muscularis band suggests that the echogenic band is part of the muscularis. Moreover, an echogenic band reflecting the outer layer is clearly visible in vitro but this layer can hardly be distinguished in vivo because the individual loops are packed closely together.

Histologic versus ultrasound appearance of Roux-Y loop, one year after its performance
The Roux-Y loop was constructed for indirect functional monitoring of the graft (in vivo electrophysiologic responses and disaccharidase activity; exact data given previously (9)). Figure 3A shows the histologic appearance of a Roux-Y loop one year after the performance. The histologic section showed altered mucosal architecture characterized by reduced crypt depth and diminished villus height. Additionally, in vivo ultrasonographic examination of the Roux-Y loop demonstrated reduced mucosal thickness as well (Figure 3B). No other deviations could be found.

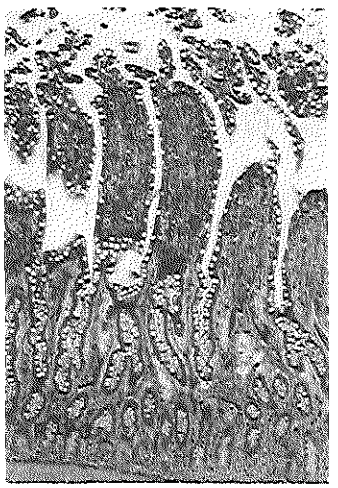


Fig.3.A. *Histologic cross section of Roux-Y loop (corresponding to R-Y 3, Fig 1, paragraph 5.2), showing reduced mucosal architecture.*

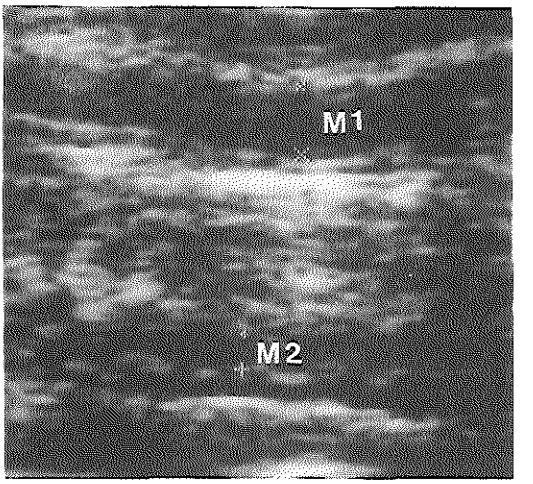
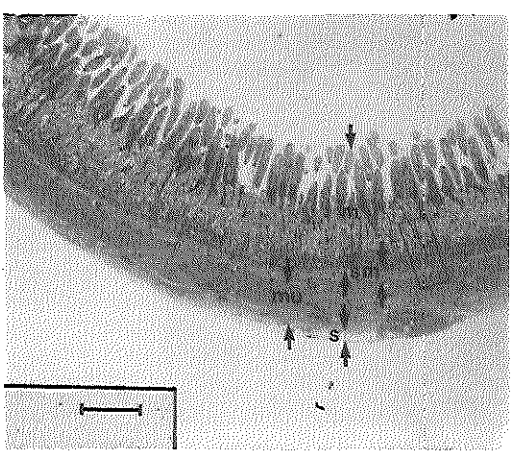


Fig.3.B. *Longitudinal sonogram of Roux-Y loop demonstrating reduction of mucosal layer (corresponding to Figure 1, Chapter 5, R-Y 3).*

M1 = mucosal layer of normal loop,
M2 = mucosal layer of Roux-Y loop.

Postoperative complications following segmental allotransplantation, visualized by ultrasound

Venous thrombosis. Figure 4A shows the histologic appearance of intestine following venous thrombosis. The mucosal architecture was intact but villi and crypts were completely desquamated.



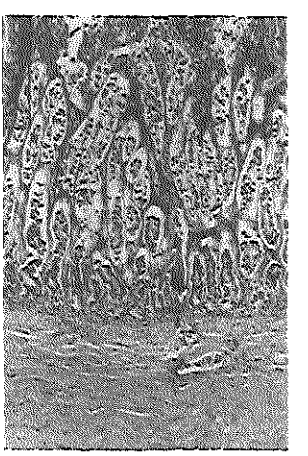


Fig.4.A. *Histologic section of intestine following venous thrombosis. The layers represent serosa (s), muscularis (mu), submucosa (sm) and destroyed mucosa (m).*

Figure 4B shows ultrasonographic features of intestine in saline following venous thrombosis.

In venous thrombosis, ultrasound studies showed wall thickening, resulting in distention of the bowel. In the bowel wall intramural air was seen. Moreover, the mucosa showed increased echogenicity compared to normal bowel.

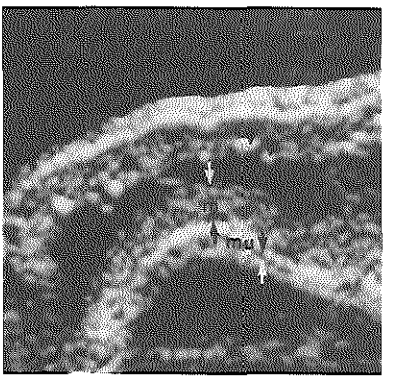


Fig.4.B. *Longitudinal in vitro ultrasound image of canine small intestine following venous thrombosis; arrows indicate mucosa (m) and muscularis (mu) layer respectively (surrounded by saline) Arrowhead: mucosal air.*

Intussusception. Intussusception was diagnosed twice in one dog and one time in another dog using ultrasound (Figure 5). One developed jejuno-jejunal intussusception of host bowel, and the other ileo-ileal intussusception of donor bowel. Only the dog that developed intussusception of the host bowel showed clinical signs (vomiting, abdominal mass, colicky pain). Sonographically, no differentiation could be made between intussusception in transplant or host bowel. The intussusceptions did not reduce spontaneously, therefore, successful surgical reduction was performed after 24 hours.

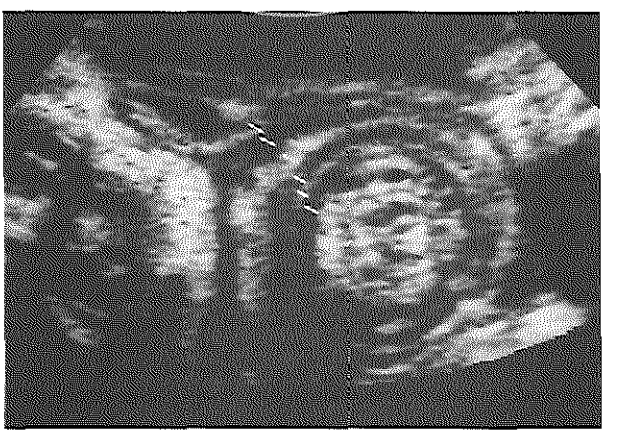


Fig.5. *Cross-sectional ultrasound image through the intussusception, demonstrating alternating hyperechoic and hypoechoic rings (arrows).*

Mesenterial lymph node enlargement. All dogs that received an allotransplant developed enlarged mesenterial lymph nodes, varying from 1.5-5 centimeters. Histologically, sinus histiocytosis and erythrophagocytosis were revealed. Moreover, lymphoid depletion was clearly demonstrated in the sections but lymph nodes still contained a few germinal centers. Sonographically, mesenterial lymph nodes had a homogenous hyperechoic appearance (Figure 6). In studies performed in autotransplanted animals, no such enlargement was detected.

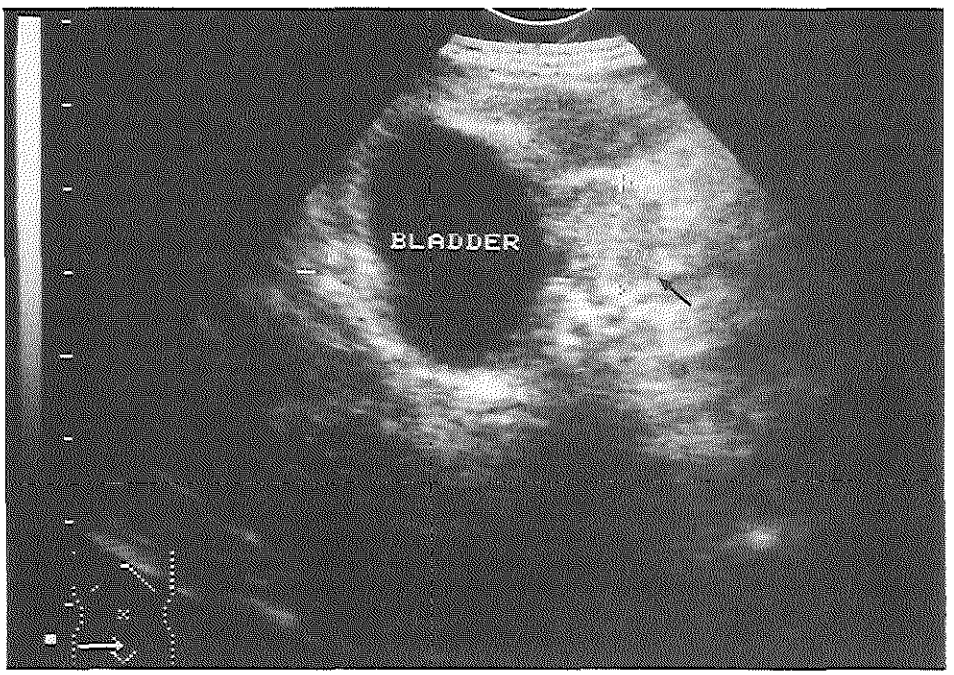


Fig.6. *Enlarged mesenterial lymph node (MLN) in transverse ultrasound section.*

Rejection. The histologic appearance of rejected transplant-intestine is shown in Figure 7A. There was a strongly altered intestinal architecture due to massive infiltration with inflammatory cells in the intestinal wall. In vitro ultrasound study of a rejected bowel showed slightly increased echogenicity and mucosal atrophy, reflected by a thin mucosal layer and thickening of muscularis with loss of strong reflections between muscularis and mucosa (Figure 7B).

Miscellaneous abnormalities. In the early postoperative period intraperitoneal effusions were identified that usually disappeared spontaneously. Inspissated bile in gallbladder was demonstrated in some dogs.

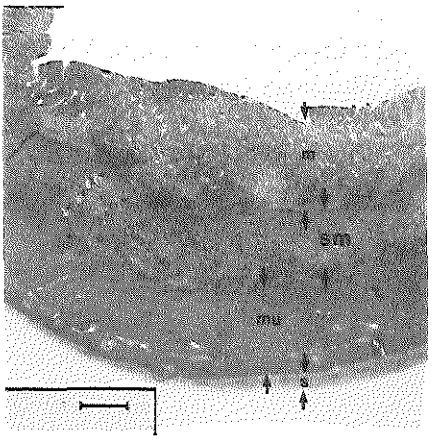


Fig.7.A. *Histologic cross section of acutely rejected small intestine, demonstrating serosa (s) and muscularis (mu) and edema of mucosa (m) and submucosa (sm).*

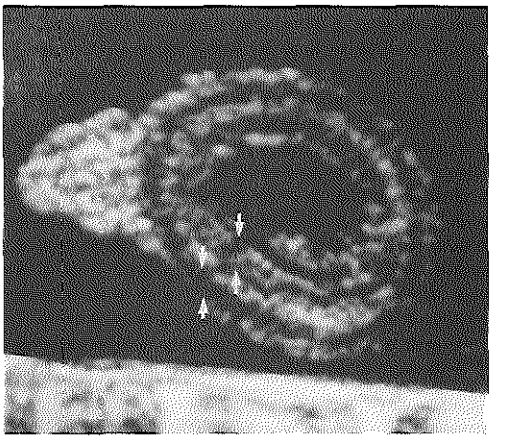


Fig.7.B. *In vitro ultrasound image in formalin of acutely rejected small intestine; bands represent sloughing mucosa (arrowhead) and a thickened muscular (mu) layer. Section demonstrates decreased echogenicity of interface between mucosa and muscularis.*

Discussion

The present findings demonstrate that using a 7.5 MHz transducer, exploratory research of canine bowel is feasible, as mucosa and muscularis can clearly be identified. It is known from the literature that, to distinguish all four layers of bowel, at least a non-commercially 20 MHz transducer should be used (10).

The histologic- and sonographic studies, performed in sham-operated and autotransplanted animals, showed that Roux-Y grafting resulted in mild mucosal atrophy depending on the position relative to the gastrointestinal tract. Comparing sham-operated and autotransplanted animals, no differences were found in the presence of atrophy, histologically or sonographically of the Roux-Y loop.

Seven dogs that underwent a two-stage segmental allotransplantation demonstrated postoperative complications including intussusception and infectious episodes. Previously, we performed the same transplantation procedure in adult dogs (11). In these experiments no such complications were encountered suggesting that young recipients of a bowel transplant are more susceptible to infectious complications and intussusception. In future experiments, we will try to circumvent these complications by introducing selective decontamination of the digestive tract (SDD) and early gastrostomy feeding (12,13).

We observed the sonographic abnormalities found after venous thrombosis. Clinically,

ultrasonography has already been demonstrated to be quite valuable in the area of vascular abnormalities (14). Therefore, ultrasound can be of help to detect or exclude suspected vascular complications after SBT. On ultrasound, thrombosis of the superior mesenteric vein appears as echogenic material filling a dilated structure (15). The described dilatation of the intestinal wall is a reflection of the presence of edema which is found after ischemic injury. Events that follow bowel ischemia include paralysis of the small intestine and subsequent formation of intramural air in the intestinal wall caused by bacterial entrance, which in its turn is caused by breakdown of mucosal integrity. These features are clearly detectable using ultrasonography.

Ultrasound is generally applied to diagnose quickly and accurately cases of human intussusception (16,17). It was shown that intussusception in dogs is quite similar to human intussusception. The appearance can be described as "bowel within bowel" and consist of alternating hypo- and hyperechogenic rings. Our study again demonstrated that intussusception is a postoperative complication occurring after SBT in large animals. This was already seen by Pritchard who found 22% intussusception in a one-step orthotopic allogeneic SBT model in young pigs (18).

The presence of enlarged mesenterial lymph nodes, as detected, may be the leading point for transplanted bowel to be trapped into intussusception. Other contributing factors may be neural nonregulation or episodes of bacteraemia (19), and it is even described as a complication of Roux-en-Y anastomosis (20). Enlargement of mesenterial lymph nodes following allogeneic SBT in rats has already been described by Grant et al (21). Mesenteric lymph nodes contain the main part of lymphoid components of intestine, so enlargement probably reflects an immunological process after allogeneic transplantation. We even speculate that, in our experiments, it is a subclinical feature of GVHD. Histologic examination of the lymph nodes several weeks after transplantation revealed lymphoid depletion, which may underline this hypothesis (22-24). In these dogs we found anemia, but no other evidence for GVHD could be revealed. The design of our experiments suggests that MHC-matching would not guarantee protection from GVHD (25). The suspected subclinical GVHD may occur because of minor histocompatibility antigen differences between graft and host (26).

Finally, in vitro ultrasonography could readily identify villous sloughing associated with rejection (27). This finding suggests that ultrasound may even be able to identify signs of rejection by regular quantitative ultrasound analysis of mucosal thickness of transplanted bowel. Recently, Cheung found that in vitro ultrasound studies can potentially differentiate, normal versus abnormal bowel wall after porcine SBT (28). We speculate

that ultrasound may be of great value in assessing relationships of mucosa and muscularis thickness.

In conclusion, ultrasound is a valuable tool to monitor the postoperative course of SBT. Routine investigations enable early detection of postoperative complications seen after SBT. In vitro sonography detects interesting findings of the bowel wall, including venous thrombosis and acute rejection, and therefore in vivo application seems promising. Ultrasound may also have some value in assessing GVHD and chronic rejection after SBT because altered bowel architecture is present in these situations.

Acknowledgements

The authors express their thanks to the staff of the Laboratory of Experimental Surgery.

References

1. Affourtit MJ, Tibboel D, Hart AEH, Hazebroek FWJ and Molenaar JC. Bowel resection in the neonatal phase of life: short-term and long-term consequences. *Z Kinderchir 1989; 44: 144.*
2. Mughal M and Irving M. Home parenteral nutrition in the United Kingdom and Ireland. *Lancet 1986; ii: 383.*
3. Wolman SL, Jeejeebhoy KN, Stewart S and Grieg PD. Experience in home parenteral nutrition and indications for small-bowel transplantation. *In: Deltz E, Thiede A and Hamelman H, eds. Small-bowel transplantation- experimental and clinical fundamentals. Berlin: Springer-Verlag, 1986: 214.*
4. Schroeder P, Goulet O and Lear PA. Small bowel transplantation: the European experience. *Lancet 1990; ii: 110.*
5. Mc Alister V, Wall W, Ghent C, Zhong R, Duff J and Grant D. Successful small intestine transplantation. *Transplant Proc 1992; 24: 1236.*
6. Reyes J, Albu-Elmagd K, Tzakis A, Nour B, Casavilla A, Kusne S, Green M, Alessiani M, Jain A, Fung JJ, Todo S and Starzl TE. Infectious complications after human small bowel transplantation. *Transplant Proc 1992; 24: 1249.*
7. Tzakis AG, Todo S, Reyes J, Abu-Elmagd K, Casavilla A, Ohya T, Fung JJ, Yunis E, Demetris AJ, Kocoshi S, Van Thiel DH and Starzl TE. Clinical intestinal transplantation: focus on complications. *Transplant Proc 1992; 24: 1238.*
8. Bach DB, Hurlbut DJ, Romano WM, Sutherland FR, Garcia BM, Wall WJ, Ghent CN and Grant DR. Human orthotopic small intestine transplantation: radiologic assessment. *Radiology 1991; 180: 37.*
9. Wolvekamp MCJ, Heineman E, Meijssen MAC, de Bruin RWF, de Jonge HR, Bijman J, Hogen Esch H, Marquet RL and Molenaar JC. One-step othotopic small-intestinal autotransplantation in growing dogs: one step too far? Perioperative events and functional evaluation. *Paragraph 5.1, this thesis.*
10. Pozniak MA, Scanlan KA, Yandow D and Mulligan G. Current status of small-bowel ultrasound. *Radiologe 1990; 30: 254.*
11. Meijssen MAC, Heineman E, de Bruin RWF, Wolvekamp MCJ, Marquet RL and Molenaar JC. Successful canine small-bowel transplantation using major histocompatibility complex matched segmental ileal allografts. *Transplant Proc 1992; 24: 1141.*
12. Alexander JW. Nutrition and infection. *Arch Surg 1986; 121: 966.*
13. Boom S and Ramsay G. Selective Decontamination of the Digestive Tract. Theoretical and practical treatment recommendations. *Drugs 1991; 42: 541.*
14. Wolf EL, Sprayregen S and Bakal CW. Radiology in intestinal ischemia. Plain film, contrast and other imaging studies. *Surg Clin North Am 1992; 72: 107.*

15. Franquet T, Bescos JM and Reparaz B. Noninvasive methods in the diagnosis of isolated superior mesenteric vein thrombosis: US and CT. *Gastrointest Radiol 1989; 14: 321.*
16. Beek FJA, Rovekamp MH, Bax NMA, Donckerwolcke RAMG, Feldberg MAM and Kramer PPG. Ultrasound findings in post-operative jejunojejunal intussusception. *Pediatr Radiol 1990; 20: 601.*
17. Myllyla V, Paivansalo M and Linna O. Intussusception in infancy and childhood. *Rontgen-Bl 1990; 43: 94.*
18. Pritchard TJ, Madara JL, Tapper D, Wilmore DW and Kirkman RL. Failure of cyclosporine to prevent small bowel allograft rejection in pigs. *J Surg Res 1985; 38: 553.*
19. Ein SH. Leading points in childhood intussusception. *J Pediatr Surg 1976; 11: 209.*
20. Gerst PH, Iyer S, Murthy RM and Albu E. Retrograde intussusception as a complication of Roux-en-Y anastomosis. *Surgery 1991; 110: 917.*
21. Grant D, Zhong R, Gunn H, Duff J, Garcia B and Keown P, Wijsman J and Stiller C. Graft-versus-host disease associated with intestinal transplantation in the rat. *Transplantation 1989; 48: 545.*
22. Ingham Clark CL, Price BA, Malcolm P, Lear PA and Wood RFM. Graft versus host disease in small bowel transplantation. *Br J Surg 1991; 78: 1077.*
23. Masaki Y, Shimazu R, Grogan JB, Subramony C and Raju S. Histology of host and graft lymphoid tissue in a chronic bowel allograft model in the rat. *Transplant Proc 1991; 23: 672.*
24. Pirenne J, Lardinois F, D'Silva M, Fridman V, Boniver J and Mahieu P. Relevance of mesenteric lymph nodes to graft-verus host disease following small bowel transplantation. *Transplantation 1990; 50: 711.*
25. Korngold R and Sprent J. Surface markers of T cells causing lethal graft-versus-host disease to class I versus class II H-2 differences. *J Immunol 1985; 135: 3004.*
26. Korngold R and Sprent J. Lethal graft-verus-host disease after bone marrow transplantation across minor histocompatibility barriers in mice. *J Exp Med 1978; 148: 1687.*
27. Holmes JT, Klein MS; Winawer SJ and Fortner JG. Morphological studies of rejection in canine jejunal allographs. *Gastroenterology 1971; 61: 693.*
28. Cheung AHS, Ferguson DC, Jiranek GC, Haggitt RC, Silverstein FE and Perkins JD. High-frequency ultrasound detects abnormal bowel wall after porcine small bowel transplantation. *Transplant Proc 1992; 24: 1069.*

CHAPTER 7

MHC-matched segmental small-bowel allotransplantation in enterectomized growing dogs: an adequate therapeutic modality

Submitted for publication in Pediatric Research

MHC-MATCHED SEGMENTAL SMALL-BOWEL ALLOTRANSPLANTATION IN ENTERECTOMIZED GROWING DOGS: AN ADEQUATE THERAPEUTIC MODALITY

Abstract

The present study was undertaken to investigate whether a two-stage segmental small-bowel allotransplantation can promote normal growth and development of young dogs (16 weeks, 5-6 kg) with a surgically created short bowel syndrome.

After near-total small-bowel resection (group 1, n=3) an irreversible weight loss was seen. After sham operation (group 2, n=3), no growth disturbances were found. Major histocompatibility-matched small-bowel transplantation with cyclosporine A as immunosuppressant was performed in two-stages; during the first stage one meter jejunoileum from an adult donor was placed heterotopically. Four weeks later, the native gut was removed whereafter the graft was placed orthotopically (group 3, n=7). In this group only one dog survived long-term, all other dogs died due to infectious complications. Addition of selective decontamination of the gut and early gastrostomy feeding resulted in long-term survival in 66% of the dogs (group 4, n=10). Follow-up (4 months) learned that growth of a young dog after transplantation of a major histocompatibility-matched segmental small-bowel allograft is compromised about 20% compared to sham-operated animals. Functional analysis showed normal D-xylose absorption, normal values of serum parameters, but increased lactulose/mannitol ratio, fecal fat excretion and postheparin DAO release. These results show that under these conditions a segmental small-bowel transplant functions sufficiently to treat the short bowel syndrome but functions suboptimally to maintain a normal growth pattern. Thus the value of segmental small-bowel allotransplantation under cyclosporine treatment in maintaining the normal growth and development in young dogs without specific nutritional interference is questionable.

Introduction

Small-bowel transplantation (SBT) will probably become the ultimate therapeutic modality for a variety of life-threatening gut-related diseases in early childhood.

In transplant medicine, matching donor and recipient for major histocompatibility (MHC) antigens is a successful modality to achieve prolonged graft survival (1,2). With respect to this, living relatives could donate segments of bowel if a segmental small-bowel (SB) transplant shows to be a reasonable alternative to total parenteral nutrition (TPN). It is still not clear, however, whether a segmental SB transplant functions sufficiently to maintain growth and development of a child.

In a previous study we showed that adult dogs are able to survive long-term after receiving a segmental MHC-matched SB graft (3). The present study was undertaken to investigate the value of a MHC-matched two-stage segmental SB allotransplantation in the treatment of the short bowel syndrome (SBS) in enterectomized puppies. We evaluated the functional capacity of a segmental graft, derived from an adult donor, by assessing the general condition of the animal (growth, serum parameters), absorptive capacity of the intestine (D-xylose absorption and fecal fat excretion), mucosal integrity (lactulose/mannitol excretion ratio), and the adaptive response (postheparin DAO release).

Materials and methods

Animals

Donors. In total 17 adult male and female Beagles, weighing 10 to 20 kg were used (Harlan CPB, Zeist, The Netherlands). Their ages ranged from one to three years.

Recipients. In total 23 healthy female Beagle puppies, 16 weeks of age, weighing 5 to 6 kg were used (Harlan CPB, Zeist, The Netherlands).

MHC matching

Matching for antigens of the canine MHC complex, DLA, consisted of typing for both class I and class II antigens, as described previously (4,5). Direct and indirect microcytotoxicity tests, based on a battery of about 60 alloantisera, were used to define the class I, serologically defined, antigens belonging either to DLA-A, DLA-B or DLA-C. Typing for class II antigens (DLA-D) was done by means of the unilateral mixed lymphocyte reaction using a culture period of 6 days. The selection of donor-recipient pairs was based on a two haplotype similarity.

Experimental groups

The dogs were subdivided into four experimental groups: group 1 (n=3), short bowel controls; group 2 (n=3), sham operated animals; group 3 (n=7), MHC matched segmental jejunoileal allografts and group 4 (n=10), MHC matched segmental jejunoileal allografts + selective decontamination of the digestive tract (SDD) + early gastrostomy feeding. In group 3 and 4 immunosuppression consisted of cyclosporine A (CsA).

Operative procedure

The anesthesia and the operative techniques of enterectomy and of the sham operation are described in detail in chapter 5. In chapter 6 this has been done for the two-stage segmental SB allotransplantation.

Perioperative treatment

The perioperative care of group 1,2, and 3 consisted of "standard" care described in detail in chapter 5. Animals in group 3 and group 4 received 10 mg/kg/day CsA in olive oil (Sandimmune, Sandoz, Basel, Switzerland) intramuscularly from one day prior to surgery up to and including the seventh postoperative day. From then on, CsA was given orally, in capsules, at a dose of 20 mg/kg/day. In addition, group 4 animals received SDD-treatment starting 1 week prior to surgery and continuing throughout the experimental period. After bowel engraftment in group 3, early posttransplant infections, associated with bacterial translocation into the blood, were caused by Enterobacteriaceae and staphylococcus areus species. This determined the chief ingredient of our daily SDD regimen in group 4: 6.5 mg/kg/d ciprofloxacin (Bayer Nederland BV, Mijdrecht, The Netherlands) to prevent bacteremia due to Enterobacteriaceae and sensitive Staphylococcus areus strains. Polymyxin B (10 mg/kg/d; Pfizer Chemicals, New York, USA) was included for providing cover against a wide range of potentially pathogenic Gram-negative aerobic bacteria, with multiresistant Proteus as a major candidate. The mixture of ciprofloxacin and Polymyxin B can give yeast, such as Candida, overgrowth and therefore Nystatin (2 X 60.000 Units/d; Labaz Sanofi-Winthrop, Maassluis, The Netherlands) was included. After food was withheld for 36 hours prior to surgery, an energy-containing solution was daily supplemented orally (Extran, Nutricia, Zoetermeer, The Netherlands). Group 4 animals were also supported with force feeding via the gastrostomy (Nutrison Pediatrics, Nutricia, Zoetermeer, The Netherlands) in the first postoperative week after both the first-stage and the second-stage operation in group 4 (6). Thereafter irradiated (0.9 megaRad) dog food (Puppap, Hope Farms, Woerden, The Netherlands) was given and water was freely available.

Evaluation

The general condition and the enterostomy outlook, if present, were observed daily.

Survival. Animals were sacrificed if their general condition deteriorated or if they lost more than 30% of their preoperative body weight.

Weight: measured twice a week, always at 8.00 a.m.

Detailed technical information about blood chemistry, D-xylose absorption testing and fecal fat analysis is given in paragraph 5.1 and 5.2.

Blood analysis. In long-term surviving animals of group 4 blood analysis was performed regularly after the first-stage and second-stage operation. In animals receiving CsA, plasma trough levels were determined using a radioimmunoassay for CsA (Cyclo-Trac SP-Serum/Plasma; Incstar Corporation-Stillwater, Minnesota, USA).

Assessment of the absorptive capacity, the integrity, and the adaptive status of the small-intestine was performed in animals of group 2 and in long-term surviving animals in group 4, always at the same time interval during five months after surgery. (See Table 1 for a schematic overview of the experimental design).

Table 1 Experimental design of functional assessment of the graft

Weeks after OK 2	Test related to specific functional component of the small intestine		
	Absorption	**Permeability**	**Adaptation**
-6		Lactulose/mannitol ratio	
-5	Fecal fat excretion		
-4 **OK 1***			
-2	Fecal fat excretion		
0 **OK 2****			
1	Fecal fat excretion		
2	Fecal fat excretion		
3	Fecal fat excretion		
4	D-xylose absorption		
5		Lactulose/mannitol ratio	
6			Postheparin DAO release
7			
8	Fecal fat excretion		
9			
10			
11	Fecal fat excretion		
12	D-xylose absorption		
13		Lactulose/mannitol ratio	
14			Postheparin DAO release

* = sham operation/heterotopic placement of allograft
** = orthotopic placement of allograft

D-xylose absorption test. The D-xylose absorption test was performed 4 and 12 weeks after orthotopic grafting.

Fecal fat excretion. Fat excretion in faeces was determined at 5 (preoperative) and 2 weeks before and 1, 2, 3, 8 and 11 weeks after orthotopic placement of the graft.

Lactulose/mannitol excretion test. The lactulose/mannitol excretion ratio assessment was performed preoperatively, and 5 and 13 weeks after the orthotopic placement of the graft. Dogs were fasted overnight and a specimen of urine (for background sugar concentrations) was taken immediately before starting the test. Per kg body weight, 400 mg D-lactulose and 100 mg D-mannitol were dissolved in 2 ml of water. Following oral administration, urine was collected for five hours. After two hours the dogs were allowed to drink water freely. The urine samples were stored at -20°C until analysis. Samples were analyzed for lactulose and mannitol content using a gas liquid chromatographic method (7). The test results were expressed as the lactulose: mannitol concentration ratio measured in the urine pool five hours after sugar ingestion.

Postheparin DAO assessment. Food was withheld 12 hours before performing the test. An amount of 1500 IU Heparin (Thromboliquine, Organon Technica B.V., Boxtel, The Netherlands) was dissolved in 5 ml sterile saline, and injected intravenously. Blood samples were collected at 0, 15, 30, 60 and 120 minutes after the injection. The DAO concentration in plasma was determined as described in detail in chapter 4. The test was performed 6 and 14 weeks after orthotopic placement of the graft.

Statistics

All data are expressed as means ± standard deviation (SD). Differences of the tested parameters between groups (weight, fecal fat excretion and area-under-curve calculated data of D-xylose absorption, postheparin DAO release, and lactulose/mannitol excretion ratios) were analyzed using Student's t test for two means. Comparisons within a group were performed using the paired Student's t test. Significance is defined as a p value < 0.05.

Results

Survival

Survival data of all animals are given in Table 2. The enterectomized dogs (group 1) had a mean survival time of 11.3 days. The sham-operated animals (group 2) survived the experimental period without complications. In Table 3, the postoperative course and cause

Table 2 **Postoperative survival time**

Group	Survival in days
I, Short bowel control	10
	10
	14
II, Sham operation	throughout experimental period
	throughout experimental period
	throughout experimental period
III, SBS + segmental	1
allotransplantation	53
+ CsA	14
	48
	24
	11
	throughout experimental period
IV, SBS + segmental	3
allotransplantation	21
+ CsA + SDD + early	51
gastrostomy feeding	62
	throughout experimental period
	throughout experimental period
	throughout experimental period
	throughout experimental period
	throughout experimental period
	throughout experimental period

Abbreviations: SBS: short bowel syndrome; CsA: Cyclosporine A
SDD: selective decontamination of the digestive tract

Table 3 **The posttransplantation course**

After a two-stage segmental allotransplantation without SDD (Group 3)

Dog	*Stages*	*Postoperative complications*	*Survival and cause of death*
1	1	venous thrombosis	1 day, venous thrombosis
2	1	recipient intussusception (day 1 and 7), ileus	53 days pneumonia
3	1	transplant intussusception (day 10)	14 days, hemorrhagic transplant
4	1 & 2	none	48 days, functional bile congestion
5	1	none	24 days, rejection
6	1	heart failure	11 days, multiorgan failure
7	1 & 2	transient gastroenteritis and anemia	throughout experimental period

After a two-stage segmental allotransplantation with SDD (Group 4)

Dog	*Stages*	*Postoperative complications*	*Survival and cause of death*
1	1	arterial thrombosis	3 days, arterial thrombosis
2	1	hypoalbuminemia	21 days, pulmonary thrombosis
3	1 & 2	none	51 days, rejection
4	1 & 2	intussusception (autopsy)	62 days, acute peritonitis
5	1 & 2	none	throughout experimental period
6	1 & 2	none	throughout experimental period
7	1 & 2	none	throughout experimental period
8	1 & 2	none	throughout experimental period
9	1 & 2	none	throughout experimental period
10	1 & 2	none	throughout experimental period

Notes:
* The survival data given in days after the first-stage operation, with the second-stage performed at day 28
* Dogs 2,3,4,5 and 7 in group 3 developed transient thrombopenia accompanied by a transient anemia
* Dogs 2,4 and 7 in group 4 developed transient thrombopenia accompanied by a severe anemia

of death in group 3 and group 4 (two-stage segmental allotransplantation without or with SDD) is given.

In group 3 only one of the seven dogs survived the experimental period. One dog died of venous thrombosis (technical complication; 14%). Five of the remaining six dogs had to be sacrificed as a result of the postoperative complications (67%). All these dogs had positive blood cultures, containing gut-derived bacteria like Hemolytic E Coli and Staphylococcus aureus, in the early postoperative period. One dog died of pneumonia at day 53, one due to transplant intussusception at day 14, one developed a deteriorating condition due to functional bile congestion and had to be sacrificed at day 48, and another dog developed multiorgan failure at day 10. In only one dog rejection was the causal factor of death at day 24.

In group 4, one dog had to be sacrificed due to arterial thrombosis (technical complication; 10%). The failure rate due to transplantation-related factors was 30%. In this group no episodes of bacteraemia were encountered. One dog was sacrificed at the 21th day due to a deteriorating condition. Autopsy revealed thrombus formation at the pulmonary artery. Only one of eight died of rejection 23 days after the orthotopic placement of the graft. A third died from acute peritonitis caused by intussusception at day 34 after OK2. The remaining six dogs had no postoperative complications and survived the experimental period.

Weight

Group 1 animals (SBS control) had a dramatic, irreversible weight loss. The animals in group 2 (sham operation) showed a normal growth pattern compared with non-operated historical controls (data supplied by Harlan CPB, Zeist). In Figure 1A, the mean weight curves and ages of the animals from group 2 and group 4 is given. Throughout the experimental period the growth pattern of group 4 animals was significantly compromised (-20%) compared to group 2 animals.

In Figure 1B the weightcurves of group 4 animals after CsA treatment was stopped, 200 days after OK2, are shown. At that time, the dogs did not show significant growth. However, after stopping CsA treatment a surprisingly catch up growth was found before rejection took place.

Blood analysis

The follow-up of blood parameters of group 4 is given in Table 4. Only ammonia levels were significantly increased in the long term compared to the preoperative values.

Compared to group 2 ammonia levels were also significantly increased in the long term (exact data given in chapter 5). Figure 2 gives the CsA plasma trough levels of group 4. The levels showed a considerable variation. However, compared to week 2, mean CsA levels were significantly increased at week 8 and week 12.

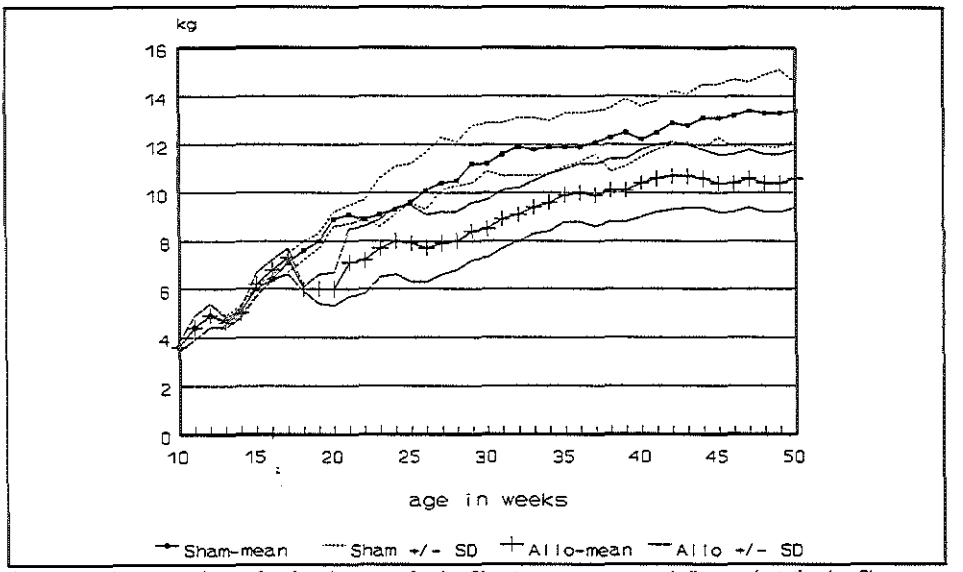


Fig.1A. *Weight curves after performing shamoperation (n=3) or two-stage segmental allotransplantation (n=6).*

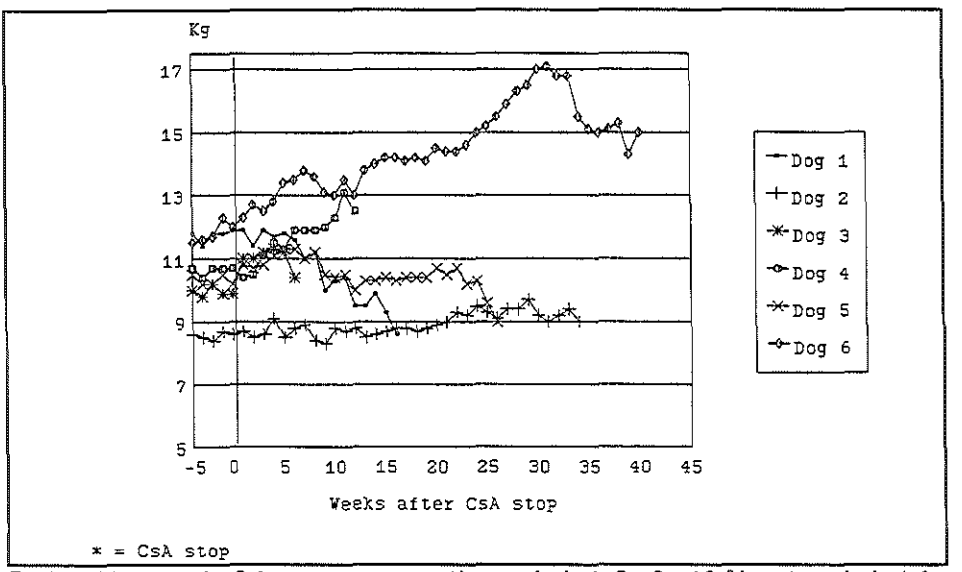


Fig.1B. *Weight curves after CsA treatment was stopped in group 4 animals. Dog 2 and 6 did not show rejection before sacrifice.*

Table 4 Blood analysis, nutritional status (mean values ± SD)

4A: values obtained following heterotopic placement of intestinal graft

days→	-7	0	28		-7	28
Hb(mmol/l)	7.7±0.5	8.6±1.0	7.6±2.3	Vit B12(pmol/l)	274±122	316±83
L(10^9/l)	7.3±1.2	5.7±1.5	9.3±1.7	Folic acid(nmol/l)	29.1±2.1	29.2±3.3
Na(mOsmol/l)	143.5±1.5	140.8±2.9	141.3±0.7	vit A(μmol)	3.45±0.65	3.01±0.63
K(mOsmol/l)	4.3±0.6	3.3±0.3	3.8±0.7	vit E(μmol)	42.9±8.2	44.6±7.6
Chol(mmol/l)	4.7±0.9	4.6±0.6	4.7±0.7	Ca(mmol/l)	2.81±0.34	2.78±0.11
TG(mmol/l)	0.43±0.11	0.50±0.28	0.43±0.08	Fe(μmol/l)	15.3±4.5	19.8±17.8
TP(g/l)	55.2±4.1	51.3±4.1	55.6±4.0	NH_3(μmol/l)	37.5±25.3	43.2±7.8
Alb(g/l)	27.6±1.5	27.0±4.3	25.4±1.3			
ALAT(Units/l)*	33.9±13.6	49.6±23.3	52.6±50.5			
ASAT(Units/l)*	26.7±7.1	36.0±9.8	29.5±6.8			
AF(Units/l)*	118.2±10.6	118.9±36.2	79.5±19.4			
Creat(μmol/l)	55±12	55±9	59±7			
BUN(mmol)	5.5±1.6	3.0±0.6	4.2±1.6			
TBIL(μmol/l)	2.2±1.1	1.3±1.3	2.0±1.5			
NAG (IU/l)	3.6±0.7	3.5±2.4	5.6±5.3			

4B: values obtained following orthotopic placement of intestinal graft

days→	6	34	69	112		112
Hb(mmol/l)	7.6±1.1	7.9±1.2	8.8±0.6	8.9±1.0	vit B 12(pmol/l)	233±33
L(10^9/l)	10.7±10.0	7.4±3.1	6.6±2.9	3.9±0.3	Folic acid(nmol/l)	28.7±3.8
Na(mOsmol/l)	141.3±1.9	143.3±2.2	142.9±2.1	143.4±1.2	vit A(μmol)	3.19±0.39
K(mOsmol/l)	3.95±0.26	4.20±0.19	4.22±0.18	4.21±0.22	vit E(μmol)	52.6±10.2
Chol(mmol/l)	4.55±0.66	4.73±0.56	4.75±0.96	5.13±0.96	Ca(mmol/l)	2.76±0.07
TG(mmol/l)	0.46±0.06	0.48±0.14	0.44±0.15	0.44±0.07	Fe(μmol/l)	13.8±3.7
TP(g/l)	54.8±7.9	61.4±3.9	61.5±3.7	61.1±3.0	NH_3(μmol/l)	123±19.1**
Alb(G/L)	22.3±2.4	25.7±2.7	25.7±3.2	25.3±2.7		
ALAT(Units/l)*	30.5±10.1	31.8±13.2	37.3±12.4	32.1±3.7		
ASAT(Units/l)*	24.5±8.2	30.7±5.9	31.3±10.7	28.8±5.8		
AF(units/l)*	68.4±16.8	61.7±10.3	54.1±12.7	51.5±9.5		
Creat(μmol/l)	51±8	60±8	77±10	70±11		
BUN(mmol)	4.4±0.8	5.7±0.9	6.6±0.6	5.9±0.7		
TBIL(μmol/l)	1.8±1.2	3.0±2.4	2.9±1.1	2.6±1.1		
NAG (IU/l)	6.7±4.5	7.7±8.2	13.9±5.7	13.6±4.9		

* assayed at 37°C

** $p<0.05$ versus preoperative values in the same group

Abbreviations used: Hb, hemoglobin; L, leukocytes; Na, sodium; K, potassium; Chol, cholesterol; TG, triglyceride; TP, total protein; Alb, albumin; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; AF, alkaline phosphatase; Creat, creatinine; BUN, blood urea nitrogen; TBIL, total biliribin; NAG, N-Acetyl Hexosaminidase

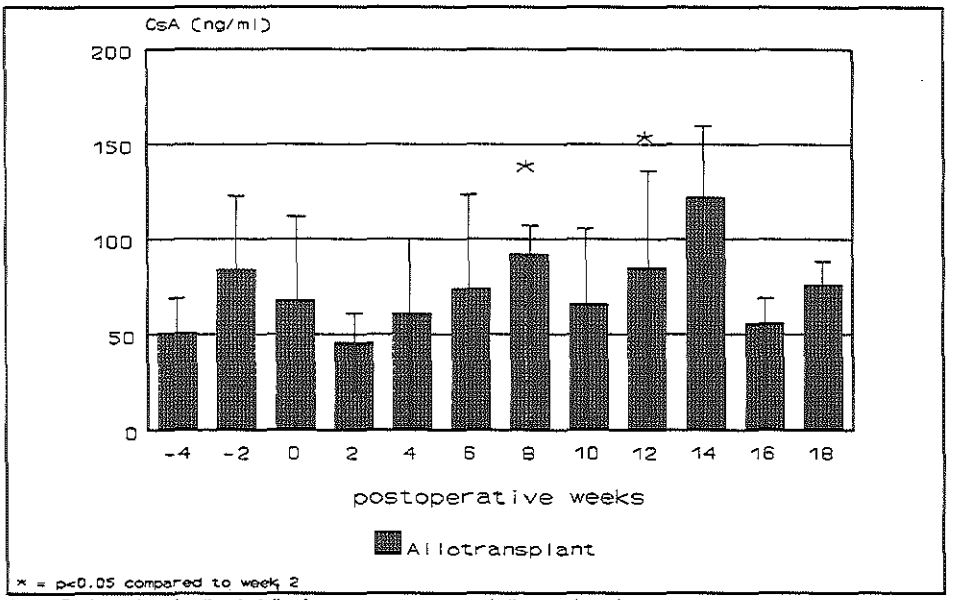


Fig.2. *Cyclosporine bloodlevels following two-stage segmental allotransplantation*

D-xylose absorption testing

D-xylose absorption test results of group 2 and group 4 are depicted in Figure 3A. Area-under-curve calculations revealed that D-xylose absorption is similar for both test groups at 4 and 12 weeks.

Fecal fat analysis

Fecal fat excretion was significantly higher in group 4 compared to group 2 from week 3 after the orthotopic positioning of the segmental graft (Figure 3B).

Lactulose/mannitol absorption testing

In the allotransplanted group, the mean lactulose/mannitol excretion ratio increased significantly from 0.04 ± 0.03 preoperatively up to 0.08 ± 0.06 and 0.11 ± 0.07 at week 5 and 13 after the orthotopic placement of the graft (Figure 3C).

Postheparin DAO assessment

Postheparin DAO release was significantly increased at week 6 after orthotopic positioning of the segmental graft compared with sham-operated dogs of the same age (Figure 3D). Fourteen weeks after orthotopic placement, postheparin DAO release did not differ significantly between the groups.

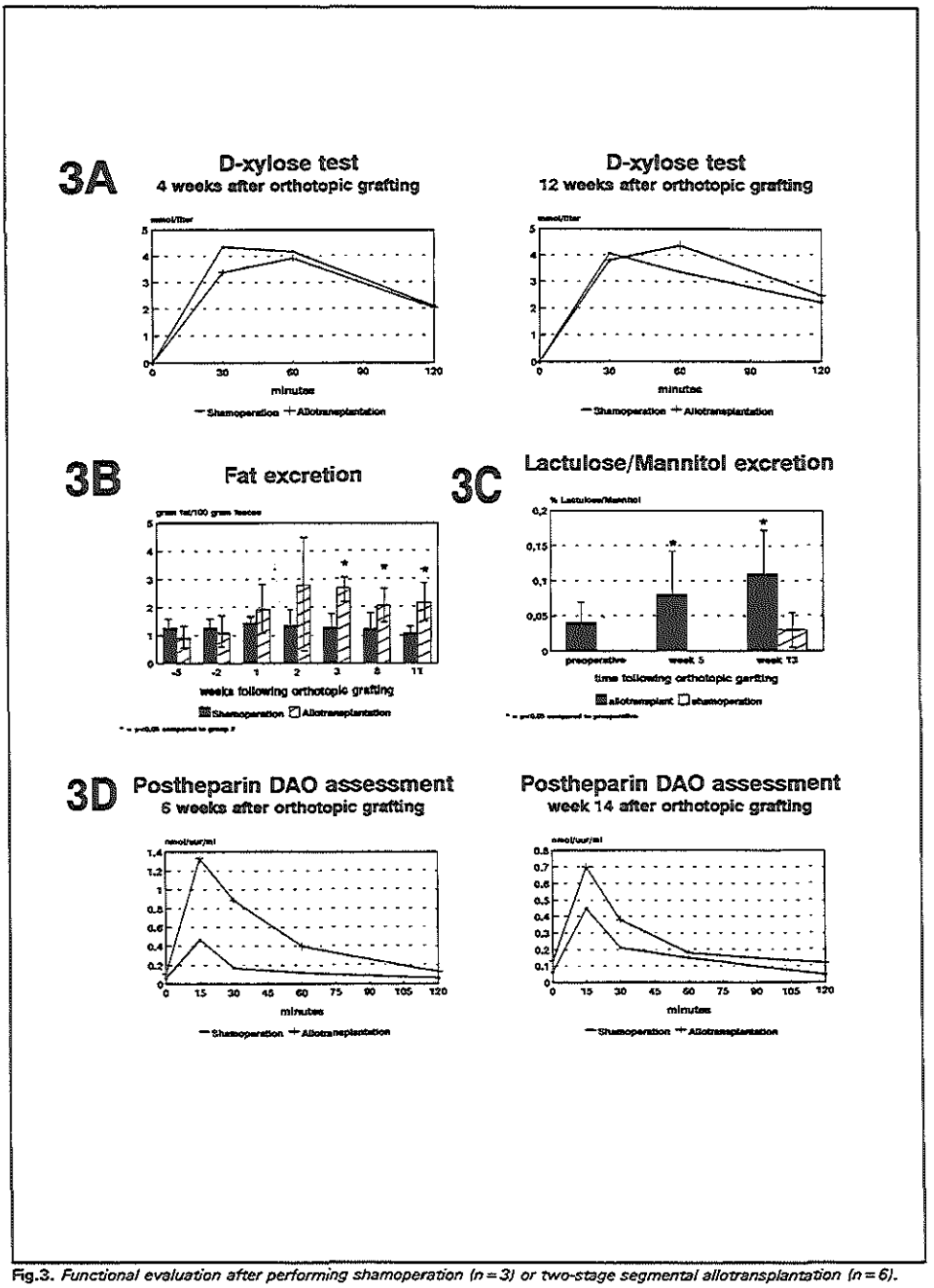


Fig.3. *Functional evaluation after performing shamoperation (n = 3) or two-stage segmental allotransplantation (n = 6).*

Discussion

The postoperative course of human SBT is often complicated by infectious episodes, which are associated with fungal and/or bacterial translocation (8). In immunocompromised patients, disturbance of the ecologic equilibrium in the gastrointestinal tract can lead to bacterial overgrowth, which in turn results in septicemia, a major postoperative complication (9,10). When performing intestinal transplantation, susceptibility to sepsis increases due to surgery-related changes in the small intestine, i.e. ischemic injury, lymphatic- and neural disruption, altered motility and systemic venous drainage (11,12). Compared to adults, children have an increased risk of developing infectious complications because they have still an immature immune system and often lack previous exposure to antigen.

Previous work in adult dogs showed that DLA-matched segmental allotransplantation resulted in long-term survivors without the need of SDD (3). In the present study, we found that young recipients have indeed increased susceptibility to develop posttransplant infections that were associated with bacterial translocation into the blood of enterobacteriaceae and staphylococcus aureus, species that translocate more easily through a leaky intestine. The institution of enteral feeding may influence the postoperative course positively since immediate postoperative enteral feeding is known to decrease the translocation of bacteria, to reduce the hypermetabolic response to stress, and to enhance the immunocompetence (13,14).

A comparison of the survival data of group 3 and group 4 revealed that SDD together with early institution of enteral nutrition led to a lower mortality rate without infectious complications and bacterial translocation. The low rejection rate is a surprising finding as it has been suggested that younger transplant-recipients are at higher risk for graft rejection (2), and it thus implicates that MHC-matching is also a promising avenue in young recipients of a SB graft. In group 3 as well as in group 4 transient thrombopenia was encountered in a number of dogs. We have speculated already (chapter 6) that the transient thrombopenia is a subclinical feature of GVHD, which may imply that MHC-matching does not protect from GVHD.

This study demonstrates that near-total SB resection in growing dogs leads to the short bowel syndrome, which becomes lethal after 10-14 days. Enterectomized dogs who had a segmental jejunal allograft were able to get away from the symptoms of the surgically created syndrome. Although others (15) had already demonstrated that weight gain after segmental allografting is possible, no comparison with sham-operated control animals has

been made so far. In our study, long-term surviving dogs increased in weight but their growth was compromised during the entire experimental period compared to sham-operated animals.
The weight curve shows that, following segmental allografting, the weight gain was only compromised in the early postoperative phase whereafter the growth remained stunted thoughout the experimental period as no catch up growth was found. In our opinion, the dogs were probably still suffering from under-nourishment in the early postoperative phase. This finding may imply that if TPN is administered in the critical postoperative phase, normal growth after segmental allografting under CsA is possible.
The weightdata after CsA treatment was stopped indicate that the CsA regimen is a major factor in the absence of catch up growth following segmental grafting.

Blood analysis showed that serum parameters of group 4 animals were comparable to group 2 animals, which indicates that a segmental SB graft is able to maintain an adequate conditional status of the host. The elevated serum ammonia levels in group 4 animals are most probably due to the caval shunting of the mesenteric vessels, a phenomenon already found in autotransplanted dogs (**chapter 5**).

Fujiwara et al (**16**) studied CsA absorption in chronically surviving allotransplanted mongrel dogs. They found that during the early postoperative period CsA absorption was unpredictable and highly variable. To assure adequate plasma CsA levels, the animals were given CsA intramuscularly during the first postoperative week. We found that an oral dose of 30 mg/kg/day CsA was sufficient to prevent rejection in adult DLA-matched dogs (**3**). However, most dogs suffered from commonly known CsA side-effects (**17,18**). This study shows that 20 mg/kg BW CsA given orally, resulting in relative low plasma CsA levels, in combination with DLA-matching was sufficient to prevent graft rejection without encountering CsA-related side-effects.

No impaired D-xylose absorption was noted in the allotransplanted dogs. Although the xylose absorption test has been widely used to study the absorptive capacity of the small intestine (**19**), it may have limited value owing to variation in renal function and gastrointestinal transit time, parameters that affect excretion of oral xylose (**20,21**). Moreover, subtle changes in intestinal permeability are not detected with D-xylose, as it is actively absorbed (**22**). Nevertheless, we included this test as a reference point of the literature with respect to functional evaluation of a SB graft.

Analysis of fecal fat excretion revealed that excretion of animals that had received an orthotopic allotransplant was significantly increased from day 21 on. These values,

however are still within the normal fat excretion range. Because our control dogs did not receive CsA, a drug that can induce decreased fat absorption by itself (23), we conclude that the transplanted segment of ileum functions sufficiently to maintain at least near normal fat absorption.

In addition, we included the lactulose-mannitol absorption test, a test being independent of the amount of urinary excretion. Lactulose, a disaccharide, is absorbed via the paracellular route and the amount excreted is a reliable index of mucosal leakiness. On the contrary, mannitol, a monosaccharide, is absorbed by the transcellular route and thus reflects mucosal surface area. The increased lactulose/mannitol ratio found in the allotransplanted dogs shows that subtle changes in intestinal permeability, not apparent with D-xylose, are present at both 29 and 85 days after orthotopic transplantation. Andre et al (24) made a direct comparison between the lactulose-mannitol ratio and ^{51}Cr EDTA test, another permeability test, in inflammatory bowel diseases. They concluded that the combination of these tests provides increased sensitivity and might therefore be useful to monitor SB transplant recipients on alterations in mucosal architecture.

DAO is an enzyme, being unique in that its blood levels correlate positively with the integrity of the intestinal mucosa (25). The intestinal content is known to be released into the blood upon stimulation by intravenously injected heparin (26). In case of intestinal mucosal damage, like SB atrophy, a lower plasma release of DAO was found (25,27). Rose et al (28) have shown that postheparin serum DAO is not significantly affected by autotransplantation and systemic drainage, suggesting that it may serve as a potential indicator of the condition of the transplanted intestine. In the present study, the area-under-curve values of the heparin stimulation curve of allotransplanted animals is significantly increased at 43 days following orthotopic grafting compared to sham-operated control animals. No such elevated value was found 99 days after orthotopic grafting. The temporary rise in intestinal DAO activity can be explained by hypothesizing that the segmental graft demonstrates an adaptive response to overcome the effects of shortening its length. This hypothesis is supported by results of Rokkas et al (29), suggesting that DAO is an important regulator of intestinal mucosal growth.

In conclusion, this study shows that a two-stage segmental allotransplantation in combination with SDD and early gastrostomy feeding is able to prevent the symptoms of the surgically created short bowel syndrome. However, although it is able to maintain an adequate nutritional status, a segmental graft under CsA regimen is unable to maintain the normal growth pattern without specific nutritional interference in the critical postoperative phase.

References

1. Karuppan SS, Lindholm A and Moller E. Fewer acute rejection episodes and improved outcome in kidney-transplanted patients with selection criteria based on crossmatching. *Transplantation 1992; 53: 666.*
2. Pirsch JD, D'Alessandro AM, Sollinger HW, Hoffmann RM, Roecker E, Voss BJ, Lorentzen D, Knechtle SJ, Reed A, Kalayoglu M and Belzer FO. The effect of donor age, recipient age, and HLA match on immunologic graft survival in cadaver renal transplant recipients. *Transplantation 1992; 53: 55.*
3. Meijssen MAC, Heineman E, de Bruin RWF, Wolvekamp MCJ, Marquet RL and Molenaar JC. Long-term survival of DLA-matched segmental intestinal allografts in dogs. *Transplantation (in press).*
4. Bijnen AB, Dekkers-Bijma AM, Vriesendorp HM and Westbroek DL. Value of the mixed lymphocyte reaction in dogs as genetic assay. *Immunogenetics 1979; 8: 287.*
5. Bull RW, Vriesendorp HM, Cech R, Grosse-Wilde H, Bijma AM, Ladiges WL, Krumbacher K, Doxiadis I, Ejima H, Templeton J, Albert ED, Storb R and Deeg HJ. Joint report of the third international workshop on canine immunogenetics II. Analysis of the serological typing of cells. *Transplantation 1987; 43: 154.*
6. Tube feeding. *In Lewis LD, Morris ML and Hands MS (eds). Small animal clinical nutrition. mark Morris Associates Topeka, Kansas 1986: chapter 5: 22.*
7. Jansen G, Muskiet FAJ, Schierbeek H, Berger R and van der Slik W. Capillary gaschromatographic profiling of urinary, plasma and erythrocyte sugars and polyols as their trimethylsilyl derivatives, preceded by a simple and rapid prepurification method. *Clin Chem Acta 1986; 157: 277.*
8. Tzakis AG, Todo S, Reyes J, Abu-Elmagd, Casavilla A, Ohya T, Fung JJ, Yunis E, Demetris AJ, Kocoshis S, Van Thiel DH and Starzl TE. Clinical intestinal transplantation: focus on complications. *Transplant Proc 1992; 24(3): 1238.*
9. Wilmore DW, Smith RJ, O'Dwyer ST, Jacobs DO, Ziegler TR and Wang X. The gut: a central organ after surgical stress. *Surgery 1992; 104: 917.*
10. Leonard EM, van Saene HKF, Shears P, Walker J and Tam PKH. Pathogenesis of colonisation and infection in a neonatal surgical unit. *Critical Care Medicine 1990; 18: 264.*
11. Browne BJ, Johnson CP, Edmiston CE, Hlava MA, Moore GH, Roza AM, Telford GL and Adams MB. Transplantation promotes bacterial overgrowth and translocation. *J Surg Res 1991; 51: 512.*
12. Grant D, Hurlbut D, Zhong R, Wang P, Chen H, Garcia B, Behme R, Stiller C and Duff J. Intestinal permeability and bacterial translocation following transplantation in the rat. *Transplantation 1991; 52(2): 221.*
13. Alexander JW. Nutrition and infection. *Arch Surg 1986; 121: 966.*
14. Moore EE and Jones TN. Benefits of immediate jejunostomy feeding after major abdominal trauma -a prospective, randomized study. *J Trauma 1986; 26(10): 874.*
15. Kimura K, Larosa CA, Blank MA and Jaffe BM. Successful segmental intestinal transplantation in enterectomized pigs. *Ann Surg 1990; 211: 158.*
16. Fujiwara H, Raju S and Grogan J. Cyclosporin absorption in total orthotopic transplantation in dogs. *Transplant Proc 1987; 19: 1125.*
17. Thomson AW. Immunobiology of cyclosporin A - a review. *Aust J Exp Biol Med Sci 1983; 61: 147.*
18. Crane PW, Ingham-Clark CL, Davies RL, Slavin G, Wood RFM, Lear PA. Cyclosporine toxicity in the small intestine. *Transplant Proc 1990; 22: 2432.*
19. Quigley EMM, Thompson JS and Rose SG. The long-term function of canine jejunoileal autotransplants-insights into allograft physiology. *Transplant Proc 1992; 24: 1105.*
20. Lambadusuriya SP, Packer S and Harries JT. Limitations of the xylose tolerance test as a screening procedure in childhood coeliac disease. *Arch Dis Child 1975; 50: 34.*
21. Behrens ME, Northrop C, Wraight P and Neale G. Evaluation of mannitol, lactulose and ^{51}Cr-labelled ethylenediaminetetra-acetate as markers of intestinal permeability in man. *Clin Sci 1987; 73: 197.*
22. Nathavitharana KA, LLoyd DR, Raafat F, Brown GA and Mc Neish AS. Urinary mannitol: lactulose excretion ratios and jejunal architecture. *Arch Dis Child 1988; 63: 1054.*
23. Wassef R, Cohen Z, Nordgren S, Langer B. Cyclosporine absorption in intestinal transplantation.

Transplantation 1985; 39: 496.

24. Andre C, Descos L and Minaire Y. Comparisons between lactulose-mannitol test and ^{51}Cr-labelled ethylene diamine tetra-acetate test in inflammatory bowel diseases. *Hepato Gastroenterol 1990; 37(suppl 2): 113.*
25. D'Agostino L, Ciacci C, Daniele B, Vittoria Barone M, Sollazzo R and Mazzacca G. Postheparin plasma diamine oxidase in subjects with small bowel atrophy. *Dig Dis Sci 1987; 32: 313.*
26. Rokkas T, Vaja S, Taylor P, Murphy GM and Dowling RH. Is the intestine the sole source of heparin-stimulated plasma diamine oxidase ? Acute effects of jejunectomy, ileoectomy and total enterectomy. *Digestion 1990; 46(suppl 2): 439.*
27. Luk GD, Bayless TM and Baylin SB. Plasma postheparin diamine oxidase. Sensitive provocative test for quantitating length of acute intestinal mucosa injury in the rat. *J Clin Invest 1983; 71: 1308.*
28. Rose SG, Thompson JS, Spanta AD and Quigley EMM. The effect of intestinal autotransplantation on serum diamine oxidase activity. *J Surg Res 1991; 50: 223.*
29. Rokkas T, Vaja S, Murphy GM and Dowling RH. Aminoguanidine blocks intestinal diamine oxidase (DAO) activity and enhances the intestinal adaptive response to resection in the rat. *Digestion 1990; 46(suppl 2): 447.*

CHAPTER 8

The value of the enzyme diamine oxidase in small-bowel transplantation

8.1 Diamine oxidase; an overview
Dig Dis 1994; 12: 2-14.

8.2 Serum diamine oxidase has no prognostic value in acute small-bowel rejection in rats
Accepted for publication in Transplant Proc

8.3 Postheparin diamine oxidase activity as monitoring tool for small-bowel graft function in rats
Accepted for publication in Transplant Proc

8.1 DIAMINE OXIDASE; AN OVERVIEW

INTRODUCTION

Diamine oxidase (DAO), catabolizing a variety of substrates including histamine and diamines, is the degradative enzyme of the catabolic pathway of polyamines. To understand the relevance of DAO it is necessary to have knowledge of polyamine metabolism and the physiologic functions in which polyamines are implicated. Until recently, polyamines were considered as metabolic end products, having no physiologic significance. The first account indicating that polyamine reputation should be changed dates back to some pioneering studies in the early sixties (1,2). By then, it was proposed that polyamines are a prerequisite for vital processes of cell proliferation. Consequently, interest in polyamine research increased and considerable progress has now been made in studying the mechanisms underlying the physiologic implications of polyamines.
Catabolism of polyamines is due to reactivity with Cu^{2+}-dependent amine oxidases, of which only diamine oxidase has been well defined. Altered DAO levels have been found in some pathologic states suggesting a role in either etiology or symptomatology.

1 FUNCTIONAL IMPLICATIONS OF POLYAMINE METABOLISM

1.1 Pathways of polyamine metabolism

Polyamines, such as putrescine, spermidine, spermine and homologues or analogues of these derivates tend to be conserved during the evolution from prokaryotes to vertebrates. Such conservation implies an important physiologic function, which is confirmed by a variety of recent reports (3,4,5). By now, the polyamine metabolism, depicted in Figure 1, has been elucidated and this has led to an accepted set of theories describing how this metabolic pathway is directly related to physiology (3,4,5), clinical medicine (6,7) and pharmacology. In vertebrates, polyamines are metabolized along two major pathways; the interconversion pathway and the so-called terminal polyamine catabolism. Arginine, an essential amino acid, is converted into ornithine, which in turn is converted into the polyamine putrescine by ornithine decarboxylase (ODC; EC 4.1.1.17). Polyamine turnover is sensitively regulated by the interconversion pathway; spermidine synthase forms spermidine from putrescine and spermine is synthesized from spermidine by spermine synthase. These reactions involve the donation of aminopropyl residues from decarboxy-S-adenosylmethionine, which is formed by a potential rate limiting enzyme S-adenosylmethionine decarboxylase (SAM-DC; EC 4.1.1.50), to the appropriate polyamines. Reversely, according to physiologic requirements, spermidine can be formed from spermine and putrescine from spermidine by an acetylation in the N^1 position, completed by acetyl CoA: spermidine/spermine N^1-acetyl transferase (cytosolic;

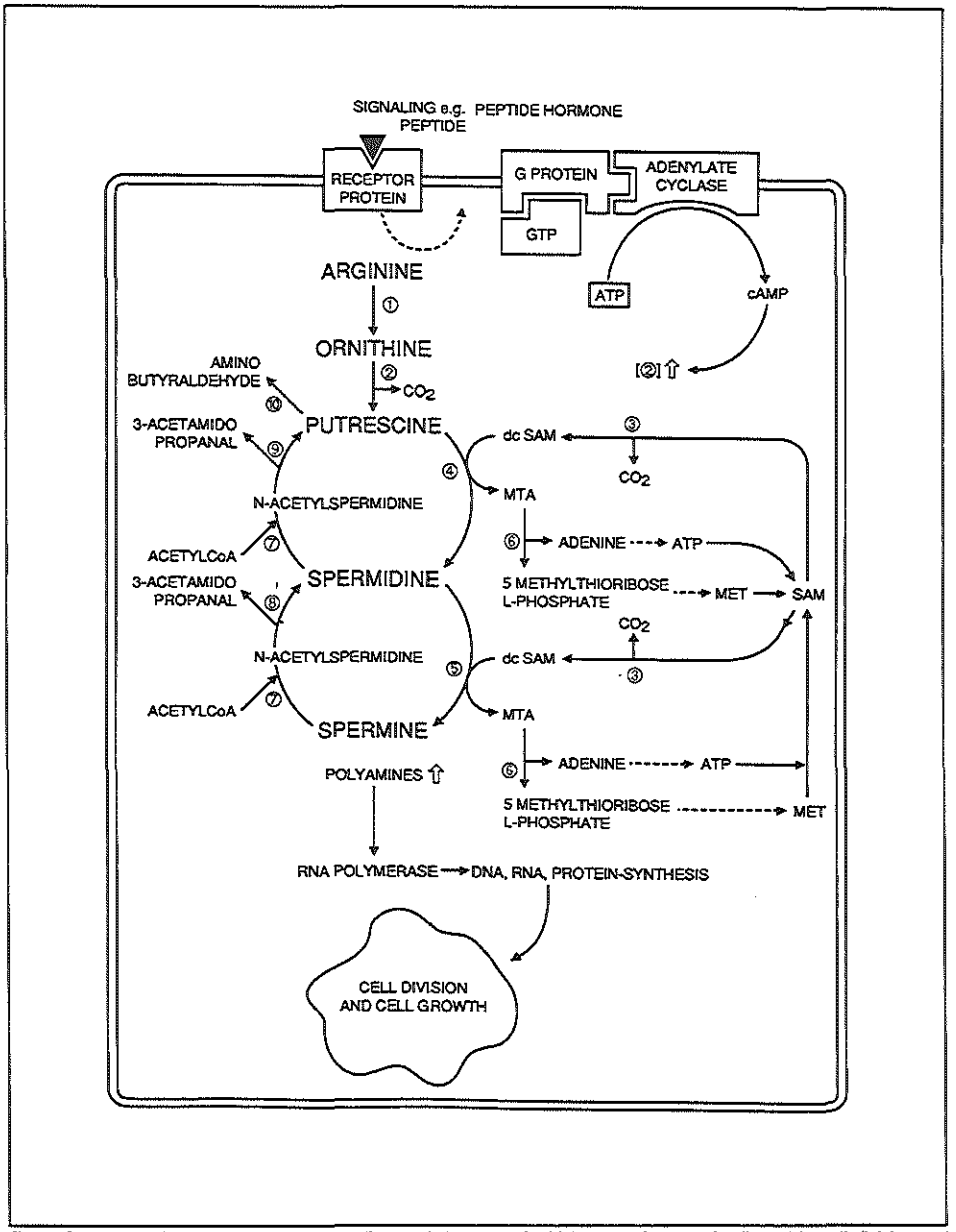


Fig.1. *Schematic view of polyamine metabolism and the way of which polyamines are implicated in cell division and growth.*

Enzymes: (1) arginase; (2) ornithine decarboxylase (ODC); (3) S-adenosylmethionine decarboxylase (SAM-DC); (4) spermidine synthase; (5) spermine synthase; (6) 5'-methylthioadenosine (MTA) phosphorylase; (7) acetyl CoA:polyamine N^1-acetyltransferase (cytosolic; cSAT); (8) acetyl CoA:spermidine N^8-acetyltransferase (nuclear; nSAT); (9) polyamine oxidase (FAD-dependent); (10) diamine oxidase (DAO).

cSAT) and subsequent oxidative splitting performed by FAD-dependent polyamine oxidases. Re-utilization of spermidine and putrescine makes this a cyclic process, which is

controlled by enzymes that provide sensitive metabolic regulation. Two basic requirements of the key enzymes, ODC, SAM-DC and cSAT are high inducibility and a short half-life time. Mechanisms that may improve sensitivity of an enzyme are known as multiplicity of regulators, co-operativity, substrate cycles and interconversion cycles (8).

In general, ODC is usually the rate limiting enzyme (9), having the shortest in vivo half-life of any enzyme yet studied, but under certain circumstances SAM-DC and cSAT may also become rate limiting. Each intermediate of the interconversion cycle is degraded into an aldehyde, if serving as substrate of a copper-containing amine oxidase, like serum amine oxidase and diamine oxidase (DAO; EC 1.4.3.6).

1.2 The role of polyamines in perireplicative events

Polyamines may play an essential role in a variety of biological processes.
Growth processes are mainly controlled by polyamine concentrations (10,11), as one of the prerequisites for cell proliferation is the enhanced synthesis of polyamines when entering the G1 phase, at the time of initiation of DNA synthesis and prior to cell division. This phase-specific rise in polyamine demand is reflected by an increase of ODC activity, usually the rate limiting enzyme in polyamine synthesis. More specific, it is evident from studies in cells partially depleted of polyamine (12,13) that DNA, RNA and protein synthesis are the prereplicative events regulated, at least in part, by these cations.

Many investigators have attempted to elucidate involvement of the polyamines in these processes. In several studies using polyamine depleted cells, it was found that the interference with DNA synthesis was the result of a decrease in DNA chain initiation rather than reduced DNA chain elongation (14,15). The results of Geiger and Morris (16,17), however, suggest that polyamine deficiency results in markedly reduced rate of DNA replication fork movement and not in a decreased initiation frequency. The relative importance of polyamines for RNA synthesis remains elusive although in vitro data suggest a role in the regulation of almost every step of the synthesis and degradation of RNA (18). Moreover, polyamines have been implicated in the stimulation of mRNA transcription and translation, some molecular events of protein synthesis (19). In general, DNA synthesis is the cell event mostly affected by polyamine deprivation. In cell division, the postreplicative event, physiologic concentrations of polyamines interact with polymerization and transformation of actin filaments (20).

1.3 The degradative enzyme of the polyamine metabolism

It is clear that rapidly proliferating cells, including bone marrow-, intestinal mucosal- and tumor cells have a high requirement for polyamines.

Normally, terminal polyamine catabolism does not participate in the regulation of cellular polyamine levels, although copper-containing amine oxidases are present in a wide variety of tissues. DAO starts functioning in a regulating manner in rapidly proliferating tissues, as it is produced in particularly high amounts. Moreover, under certain pathophysiologic circumstances elevated DAO levels are found, which can be either a causal factor or a disease-related effect. Apart from its role in the polyamine pathway, DAO also catalyzes the deamination of histamine and even plays a major role in histamine degradation in some animal species (21).

2 HISTORICAL BACKGROUND

Since its discovery in 1929 the histamine-inactivating capacity of diamine oxidase, by then called histaminase by Best (21), was known. It was renamed by Zeller in 1938 (22), because the enzyme also deaminated several diamines, including putrescine, cadaverine and agmatine. These two names were used synonymously, until the enzyme was proposed to be called DAO, as it was shown that putrescine and cadaverine are preferentially deaminated by the enzyme. So far, DAO has been purified and characterized from various sources (Table 1).

Table 1 Purification and characterization of DAO

Tissue	Source	Molecular mass (M)	References
Kidney	horse	not determined	23
	pig	185,000	24
	human	not determined	25
Placenta	human	90,000-120,000	26,27
Amniotic fluid	human	245,000 (DAO-A; dimer) 485,000 (DAO-B; tetramer)	28,29
Pregnancy plasma	human	245,000 (DAO-A; dimer) 485,000 (DAO-B; tetramer)	26,29

Several studies have been performed on the biochemical properties of DAO from different species. It appeared that multiple forms of DAO exist (29), probably varying in stability and in kinetic properties. The differences observed are probably species-dependent but storage and experimental conditions may also differently influence the biochemical properties of DAO obtained from various sources (30).

3 BIOCHEMIC ASPECTS

3.1 DAO assays

There is a variety of methods to detect DAO activity due to the multiplicity of substrates and reaction products. Table 2 gives a summation of used substrates and reaction products. The numerous assays described differ in specificity, sensitivity, rapidity and simplicity. As normal DAO activity in plasma is in the low detecting range, a method with a high sensitivity and simplicity is preferred. The most commonly used method for in vitro measurement of DAO activity is the ^{14}C-putrescine assay. This isotope assay is based on spontaneous nonenzymatic cyclization of γ-aminobutyraldehyde, the product of DAO catalyzed degradation, to Δ_1-pyrroline or its polymers.

Table 2 DAO detection methods

Substrate	Assay	Determination of	References
Histamine	biological	residual ileal histamine	31,32
		blood pressure changes	21
	fluorometric		33
	polarographic		33
	spectrophotometric	DNFB-residual histamine	34
	manometric	O_2 consumption	35
	spectrophotometric	O_2 consumption	36
Cadaverine	spectrophotometric	aldehyde formation	37
	vacuum distillation	ammonia formation	22
	diffusion analysis	ammonia formation	38
	colorimetric	decolorization time	39
	colorimetric	residual dye	40
	titration	colorization $KMnO_4$	41
	spectrophotometric	O-dianisidine complex	42
	spectrophotometric	ammonia liberated	43
Putrescine	spectrophotometric	aldehyde formation	37
	vacuum distillation	ammonia formation	22
	diffusion analysis	ammonia formation	38
	colorimetric	decolorization time	39
	colorimetric	residual dye	40
	titration	colorization $KMnO_4$	41
	spectrophotometric	O-dianisidine complex	42
	spectrophotometric	ammonia liberated	43
Hexamethylenediamine	spectrophotometric	aldehyde formation	37
	vacuum distillation	ammonia formation	22
	diffusion analysis	ammonia formation	38
	colorimetric	decolorization time	39
	colorimetric	residual dye	40
	titration	colorization $KMnO_4$	41
	spectrophotometric	O-dianisidine complex	42
	spectrophotometric	ammonia liberated	43
^{14}C Putrescine	radiochemical	Δ_1-pyrroline formation	44

Since putrescine and Δ_1-pyrroline have a differential solubility, DAO activity can be measured by counting radioactivity of Δ_1-pyrroline extracted from the aqueous into the organic phase.

3.2 DAO inhibitors

Some attention has been paid to the inhibition of diamine oxidase activity by several specific blockers. Sattler et al (45) tested over 300 drugs on inhibitory potencies with human and canine DAO in vitro. The underlying thought was that in intensive care units, in patients producing elevated histamine levels (e.g. in case of shock, polytrauma), unwanted clinical side-effects could be elicited by drugs inhibiting DAO. It was previously believed that blocking intestinal DAO would be swamped by the immense enzyme amounts present (46). However, recent studies showed that inhibition with aminoguanidine reduces the intestinal DAO activity to unmeasurable levels (47).

4 FUNCTIONAL ASPECTS

4.1 Role of DAO in mucosal growth regulation

Proliferation of cells in the gastrointestinal tract is controlled by multiple substances including hormones (glucocorticoids, thyroxine, insulin) and growth factors (epidermal growth factor). Additionally, it has been proposed that cell proliferation in gut mucosa depends on the supply of polyamines reaching the progenitor cells, regardless of the original trophic stimulus (48). It has been documented in several studies that ODC probably plays a key role in regulating mucosal growth by regulating polyamine levels.

To study the relative role of ODC and DAO in regulating mucosal growth, their activities were measured in epithelial cells after small bowel resection in rats. Dowling described that it was suspected that during the adaptive response the mucosal DAO activity would drop to permit and/or facilitate intestinal growth (49). However, results from several studies demonstrated that not only ODC activity rises during adaptive hyperplasia, but that DAO activity as well rises parallel to ODC (50). The speculative actions of ODC and DAO in the mucosal-growth-regulation process is outlined in Figure 2.

ODC reacts to a variety of trophic stimuli (for the gut mucosa) with immediately increased activity. The increased enzyme activity causes an increase in polyamine content and a subsequent increased cell proliferation activity. Proliferation-associated increases in DAO activity, mainly found in the villus tip of the mucosa, suggest that DAO acts as a

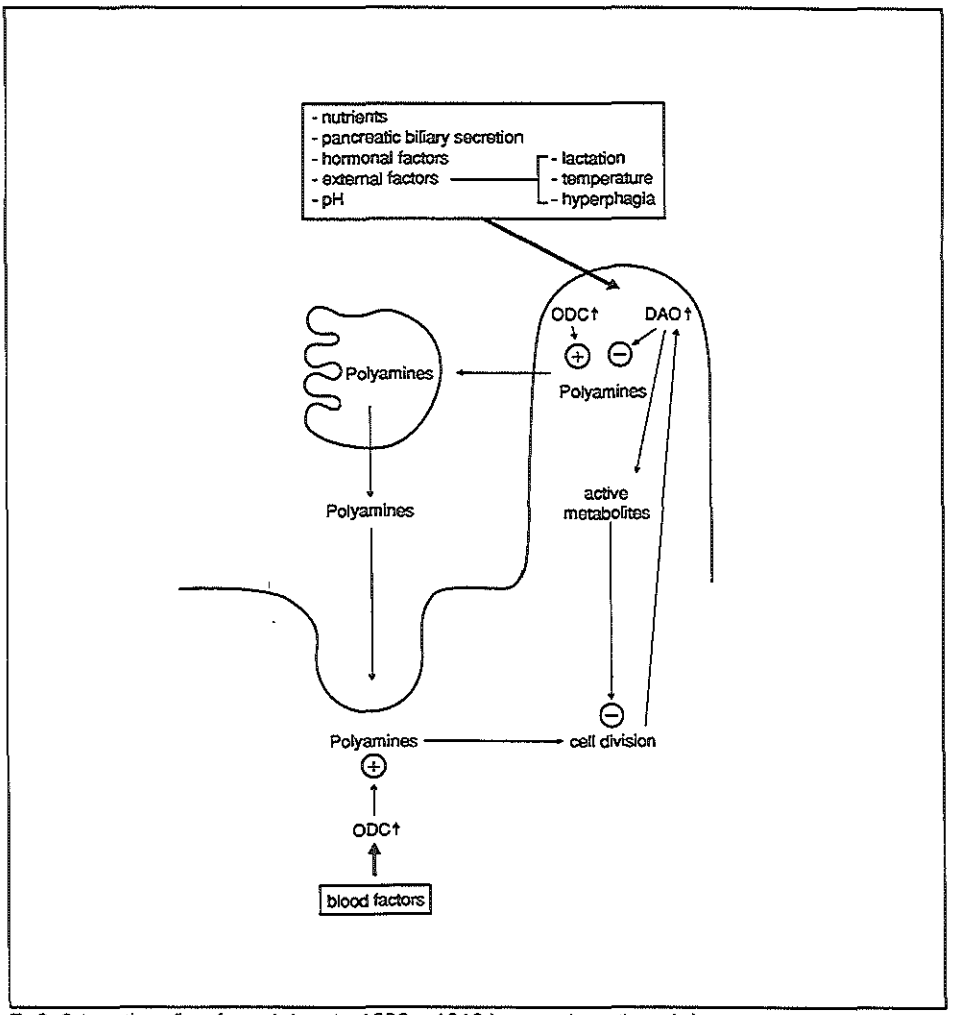


Fig.2. *Schematic outline of speculative role of ODC and DAO in mucosal growth regulation.*

brake inhibiting crypt cell production by regulating putrescine content or by the production of undefined active metabolites. Moreover, in resected rats treatment with aminoguanidine not only abolished DAO activity but in turn stimulated adaptive intestinal mucosal growth. This observation suggests that following extensive resection the structural and functional adaptive response of the remaining intestine may be enhanced by blocking of DAO activity. The stimulated response may lead to significant reduction of the resultant malabsorption seen in patients after massive small-bowel resection. The basic questions that remain to be answered are: is aminoguanidine induced intestinal adaptation transient or sustained ?; does the adaptive response after temporary aminoguanidine treatment revert to its non-enhanced state ?; is the effect of aminoguanidine dose-

dependent ? No clinical consequences have been described in healthy controls after administration of strong inhibitors of DAO no clinical consequences have been described (51). So the clinical use of efforts to block DAO in patients after massive bowel loss might become reality although negative side effects in unhealthy state should not be underestimated.

4.2 Postheparin DAO release

In healthy state very low basal values of plasma DAO are detected (52). The enzyme is unique in that its plasma levels appear to correlate positively with the maturity and integrity of the intestinal mucosa. For example, lowered enzymatic activity is found in plasma of patients with intestinal mucosal atrophy, indicating that normal plasma DAO is primarily intestinal-derived (53). At present, the potential value of DAO as an index of intestinal disease is widely investigated. Some recent studies suggest that measuring serum DAO activity is more suitable in the follow-up of enteropathies than as a screening test, due to the large biological variations in serum values obtained (54). DAO is mainly synthesized by mature villus cells, with about 60% present in the organelles and the rest free in the cytosolic compartment. In the majority of studies it has been hypothesized that after synthesis DAO is translocated to heparin-sensitive binding sites in the capillaries of the lamina propria (55). Such location may involve the prevention of polyamine crossing from the intestine into the circulation. Recent immunofluorescence studies revealed that the base of the villus cells, and not the endothelial binding sites, represents the primary storage compartment in the intestine (56). DAO is most probably released through the secretory pathway at the basolateral membrane, induced by heparin or other highly negatively charged molecules. DAO linked to organelles is released only when the cytosolic compartment has been depleted. Upon its release, DAO is translocated to endothelial binding sites, already depleted by heparin stimulation, and subsequently released into the circulation. It is generally assumed that, following heparin stimulation, plasma DAO levels rise markedly and that the intestine is the major (or sole) source of such increase (57). It is thought that this provocative plasma postheparin DAO measurement enhances the sensitivity of using DAO to monitor mucosal maturation and integrity. Further studies on the application of such a non-invasive test for determining its usefulness are now warranted.

4.3 Relevance of DAO in pathologic states

The association of DAO activity with a number of pathophysiologic states is often hypothesized upon. Although altered DAO activities have been found in a number of pathologic situations (Table 3), the precise role remains speculative. Continued exploration on the metabolic role of DAO might provide clues to the functioning of the enzyme in normal and diseased states. Moreover, the investigations on the diagnostic value of DAO in clinical evaluation of certain diseases may provide a sensitive tool for monitoring.

The placenta is proposed to be the source of elevated DAO activity in serum during pregnancy. Normally, DAO is thought to have a barrier function in the placenta by preventing harmful histamine/polyamine crossing into the maternal circulation. Experimental findings suggest a positive correlation between placental DAO and the vitamin B6 status in pregnant women. In addition, the value of measuring serum DAO activity in cases of pregnancy toxemia and threatened abortion, being markedly reduced by then, is identified by an increasing number of reports (58,59).

Elevated DAO activity in neoplastic disorders has been observed in cases of endometrial adenocarcinoma, granulosa cell carcinoma, myosarcoma of the uterus, medullary carcinoma of the thyroid gland, in small-cell carcinoma of the lung, and in stomach and colon carcinoma (60). Immunohistochemical studies have confirmed that some cell types in these tumors are the source of the DAO in the circulation. However, several studies have shown that circulating DAO activity is no reliable diagnostic marker, probably due to variability of its release from the tumor. Whether the increased DAO activity in neoplastic cells has a link to tumor cell development remains to be determined.

Luminal histamine-induced histaminosis is a general name for allergic-like reactions that may occur after food induced histaminosis under DAO blockade. DAO inhibition is known to accentuate allergic responses, suggesting a regulating role of DAO in the pathophysiologic reactions involving histamine release (61).

Consistently elevated polyamine levels have been described in cystic fibrosis. No abnormalities in polyamine catabolism have been revealed in this disease, although abnormally high circulating DAO activity is found in about 30% of the patients. More investigations are needed to resolve the role of DAO in some of the clinical manifestations seen in this disease (62).

Table 3 Pathophysiologic states associated with altered DAO activity

State	Alteration	postulated role in healthy	References
Pregnancy toxemia Abortion	↓ serum DAO levels	barrier function; preventing histamine/polyamine crossing	58,59
Neoplasia	↑ intracarcinoma DAO level subsequent ↑ circulating DAO	unclarified	60
Allergic response	DAO-blockade	regulator of histamine release	61
Cystic fibrosis	↑↑ serum DAO activity	unclarified	62
Infertility	↑↑ DAO activity in semen	unclarified	63
Uremia	↓↓ Kidney & Urine DAO ↑ plasma DAO	protecting from nephrotoxicity	63
Bowel ischemia	↑ plasma DAO levels following decreased tissue activity	protecting the bowel from toxic effects	60,64

Recent studies have shown a negative correlation between DAO activity and sperm motility, and thus fertility. Adding aminoguanidine to the ejaculate might offer new therapeutic modalities to improve fertility is some cases of male infertility (63).

In uremia, polyamines are retained and may play a role in certain secondary complications, including infection and anemia. In uremic patients elevated serum DAO levels have been detected in combination with decreased urinary and kidney DAO. Such DAO levels may implicate removal of toxic polyamines in plasma and inhibition of toxic aldehyde formation in the kidneys (63).

Several investigators have described interaction of DAO with ischemic states of the bowel. Mucosal DAO activity, being the major source of tissue DAO (>90%), shows an increasing proximal-to-distal gradient, decreasing to low levels in the colon. The enzyme is primarily synthesized and located in the villus absorptive cells of the gastrointestinal tract. Since villus tip cells are most sensitive to ischemia, decreased tissue DAO activity and subsequent increased circulating plasma levels of DAO have been found due to its luminal release and uptake in the blood. Measuring DAO activity could therefore provide a marker of intestinal injury. Moreover, it has been postulated that DAO has a role in protecting the bowel from toxic effects following increased histamine concentrations observed in intestinal ischemia (64).

REFERENCES

1. Tabor H, Tabor CW and Rosenthal SM. The biochemistry of the polyamines: spermidine and spermine. *Ann Rev Biochem 1961; 30: 579.*
2. Dijkstra WG and Herbst EJ. Spermidine in regenerating liver: relation to rapid synthesis of ribonucleic acid. *Science 1965; 149: 428.*
3. Seiler N. Polyamine Metabolism. *Digestion 1990; 46(suppl 2): 319.*
4. Tabor CW and Tabor H. Polyamines. *Ann Rev Biochem 1984; 53: 749.*
5. Smith TA. Polyamines. *Ann Rev Plant Physiol 1985; 36: 117.*
6. Russell DH. Clinical relevance of polyamines. *Crit Rev Clin Lab Sci 18: 261.*
7. Morris DR and Marton LJ. *In: Farber E, and Pitot HC, eds. Polyamines in Biology and Medicine, New York and Basel, Marcel Dekker Inc, 1981.*
8. Newsholme EA and Leech AR. Chapter 3 Kinetics and Metabolism. *In: Wiley J, and Sons Ltd, eds. Biochemistry for the medical sciences, 1986.*
9. Hayashi S. Ornithine decarboxylase: biology, enzymology and molecular genetics. *In: Sartorelli, ed. International encyclopedia of pharmacology and therapeutics. Pergamon Press.*
10. Pegg AE and McCann PP. Polyamine metabolism and function. *Am J Physiol 1982; 243: C212.*
11. Heby O. Role of polyamines in the control of cell proliferation and differentiation. *Differentiation 1981; 19: 1.*
12. Maas WK, Leifer Z and Pointdexter J. Studies with mutants blocked in the synthesis of polyamines. *Ann N Y Acad Sci 1970; 171: 957.*
13. Morris DR and Jorstad CM. Growth and macromolecular composition of a mutant of Escherichia coli during polyamine limitation. *J Bacteriol 1973; 113: 271.*
14. Krokan H and Eriksen A. DNA synthesis in HeLa cells and isolated nuclei after treatment with an inhibitor of spermidine synthesis, methyl glyoxal bis (guanylhydrazone). *Eur J Biochem 1977; 72: 501.*
15. Boynton AL, Whitfield JF and Isaacs RJ. A possible involvement of polyamines in the initiation of DNA synthesis by human WI-38 and mouse BALB/3T3 cells. *J Cell Physiol 1976; 89: 481.*
16. Geiger LE and Morris DR. Polyamine deficiency reduces the rate of DNA replication fork movement in Escherichia coli.
17. Geiger LE and Morris DR. Stimulation of deoxyribonucleic acid replication fork movement by spermidine analogs in polyamine-deficient Escherichia coli. *J Bacteriol 1980; 141: 1192.*
18. Karpetsky TP, Hieter PA, Frank JJ and Levy CC. Polyamines, ribonucleases, and the stability of RNA. *Mol Cell Biochem 1977; 17: 89.*
19. Algranati ID and Goldemberg SH. Polyamines and their role in protein synthesis. *Trends Biochem Sci 1977; 2: 272.*
20. Oriol-Audit C. Polyamine-induced actin polymerization. *Eur J Biochem 1978; 87: 371.*
21. Best CH. The disappearance of histamine from autolysing lung tissue. *J Physiol 1929; 67: 256.*
22. Zeller EA. Über den enzymatischen abbau von histamin und diaminen. *Helv chim Acta 1938; 21: 880.*
23. Corda M, Pellegrini M and Rinaldi A. Diamine oxidase from horse kidney: ionic strength dependence of stability and activity. *It J Biochem 1984; 33: 303.*
24. Bardsley WG, Hill CM and Lobley RW. A reinvestigation of the substrate specificity of pig kidney diamine oxidase. *Biochem J 1976; 117: 169.*
25. Shindler JS and Bardsley WG. Human kidney diamine oxidase: inhibition studies. *Biochem Pharmacol 1976; 25: 2689.*
26. Bardsley WG, Crabbe MJC and Scott IV. The amine oxidases of human placenta and pregnancy plasma. *Biochem J 1974; 139: 169.*
27. Lin CW, Kirley SD and St Pierre M. Tumor and placental histaminase. 1. Affinity chromatography purification and characterization of the placental enzyme. *Oncodev Biol Med 1981; 2: 267.*
28. Tufvesson G. Purification and properties of human amniotic fluid fluid diamine oxidase. *Scan J Clin Lab Invest 1978; 38: 463.*
29. Tufvesson G. A comparison between diamine oxidase from human post-heparin blood serum, pregnancy blood serum and amniotic fluid. *Scand J Clin Lab Invest 1978; 38: 473.*
30. Romijn JC, Verkoelen CF and Splinter TAW. Species-dependent differences of the biochemical

properties of diamine oxidase. *Int J Biochem 1986; 18: 835.*

31. Edlbacher S and Zeller EA. Über den enzymatischen abbau des histamins I. *Helv chim Acta 1937; 20: 717.*
32. Anrep GV, Ayacki MS, Barsoum GS, Smithu JR and Talaat NM. The excretion of histamine in urine. *J Physiol 1944; 103: 155.*
33. Uozumi K, Nakahara J, Higahin T and Sakamato Y. Purification and properties of histaminase from hog kidney. *J Biochem (Tokyo) 1964; 56: 601.*
34. Dodge CA. A test for pregnancy based on histaminolytic activity in serum. *Am J Obstet Gynecol 1952; 63: 1213.*
35. Best CH and McHenry EW. The inactivation of histamine. *J Physiol 1930; 70: 1349.*
36. Lawskowski M. Oxygen consumption during histamine-histaminase reaction. *J Biol Chem 1942; 145: 457.*
37. Holmstedt B and Tham R. A spectrophotometric method for determination of diamine oxidase (DAO) activity. *Acta Physiol Scandinav 1959; 45: 152.*
38. Kapeller-Adler R. Studies on histaminase. *Biochem J 1949; 44: 70.*
39. Zeller EA. Zur kenntnis der diamin-oxydase. 3. Mitteilung über den enzymatischen abbau von poly-aminen. *Helv Chim Acta 1939; 21: 1645.*
40. Zeller EA. Über die verwendung von indigo-disulfonaat in der fermentmethodik. *Helv Chim Acta 1940; 23: 1502.*
41. Kapeller-Adler R. The estimation of histaminase by a microvolumetric method involving a peroxidatic oxidation of indigo disulphonate. *Biochem Biophys Acta (Amsterdam) 1956; 22: 291.*
42. Aarsen PN and Kemp H. Rapid spectrophotometric micromethod for determination of histaminase activity. *Nature 1964; 204: 1195.*
43. Kirsten E, Gerez C and Kirsten R. Eine enzymatische mikrobestimmung des ammoniaks, geeignet fur extrakte tierischer gewebe und flussigkeiten. *Biochem Z 1963; 337: 312.*
44. Okuyama and Kobayashi Y. Determination of diamine oxidase by liquid scintillation counting. *Arch Biochem Biophys 1961; 95: 242.*
45. Sattler J, Hetserberg R, Lorenz W, Schmidt U, Crombach M and Stahlknecht CD. 2. Metabolism of Histamine. Inhibition of human and canine diamine oxidase by drugs used in an intensive care unit: relevance for clinical side effects? *Agents and Actions 1985: 16.*
46. Rokkas T, Vaja S, Murphy GM and Dowling RH. Aminoguanidine blocks intestinal diamine oxidase (DAO) activity and enhances the intestinal adaptive response to resection in the rat. *Digestion 1990; 46: 447.*
47. Erdman SH, Park JHY, Thompson JS, Grandjean CJ, Hart MH and Vanderhoof JA. Suppression of diamine oxidase activity enhances postresection ileal proliferation in the rat. *Gastroenterology 1989; 96: 1533.*
48. Mc Cormack SA and Johnson LR. Role of polyamines in gastrointestinal mucosal growth. *Am J Physiol 1991; 260: G795.*
49. Dowling RH. Polyamines in intestinal adaptation and disease. *Digestion 1990; 46(suppl 2): 331.*
50. Mennigen R, Bieganski T, Elbers A and Kusche J. The histamine-diamine oxidase system and mucosal proliferation under the influence of aminoguanidine and seventy percent resection of the rat small intestine. *Agents and Actions 1989; 27: 221.*
51. Sjaalstadt OV. Potentiation of aminoguanidine of the sensitivity of sheep to histamine given by mouth. Effect of aminoguanidine in urinary excretion of endogenous histamine. *Q J Exp Physiol 1967; 52: 319.*
52. Rokkas T, Vaja S, Murphy GM and Dowling RH. Postheparin plasma diamine oxidase in health and intestinal disease. *Gastroenterology 1990; 98: 1493.*
53. D'Agostino L, Ciacci C, Daniele B, Vittoria Barone M, Sollazzo R and Mazzacca G. Postheparin plasma diamine oxidase in subjects with small bowel atrophy. *Dig Dis Sci 1987; 32(3): 313.*
54. Corazza GR, Falsca A, Strocchi A, Rossi CA and Gasbarrini G, Decreased plasma postheparin diamine oxidase levels in celiac disease. *Dig Dis Sci 1988; 33(8): 956.*
55. Robinson-White A, Baylin SB, Olivecrona T and Beaven MA. Binding of diamine oxidase activity to rat and guinea pig microvascular endothelial cells. Comparisons with lipoprotein lipase binding. *J Clin Invest Inc 1985; 76: 93.*
56. Danielle B and Quaroni A. Polarized secretion of diamine oxidase by intestinal epithelial cells and its stimulation by heparin. *Gastroenterology 1990; 99: 1675.*
57. Rokkas T, Vaja S, Taylor P, Murphy GM and Dowling RH. is the intestine the sole source of heparin-stimulated plasma diamine oxidase? Acute effects of jejunectomy, ileectomy and total

enterectomy. *Digestion 1990; 46(suppl 2): 439.*

58. Kapeller-Adler R. Investigations on the activity of the histaminase in normal and toxaemic pregnancy. *Biochem J 1944; 38: 270.*
59. Ahlmark A. Studies on the histaminolytic power of plasma with special reference to pregnancy. *Acta physiol scand 1944; 9(suppl 28): 1.*
60. Baylin SB and Luk GD. Diamine oxidase (histaminase) activity in human disease. *In: Morris DR and Marton LJ, eds. Polyamines in Biology and Medicine. New York, Marcel Dekker 1981: 377.*
61. Sattler J, Hafner D, Klotter HJ, Lorenz W and Wagner PK. Food-induced histaminosis as an epidemiological problem: plasma histamine elevation and haemodynamic alterations after oral histamine administration and blockade of diamine oxidase. *Agents and Actions 1988; 23: 361.*
62. Baylin SB, Rosenstein BJ, Marton LJ and Lockwood DH. Age-related abnormalities of circulating polyamines and diamine oxidase activity in cystic fibrosis heterozygotes and homozygotes. *Pediatr Res 1980; 14: 921.*
63. Gahl WA and Pitot HC. Clinical aspects of polyamines in nonneoplastic conditions. *In: Morris DR and Marton LJ, eds. Polyamines in Biology and Medicine. New York, Marcel Dekker, 1981: chapter 18.*
64. Bragg LE, Thompson JS and West WW. Intestinal diamine oxidase levels reflect ischemic injury. *J Surg Res 1991; 50: 228.*

8.2 SERUM DIAMINE OXIDASE HAS NO PROGNOSTIC VALUE IN ACUTE SMALL-BOWEL REJECTION IN RATS

Abstract

Rejection of intestinal allografts is still a major problem hindering clinical transplantation. A serum marker that could detect rejection at an early stage would be of great help in adjusting immunosuppressive protocols. The enzyme diamine oxidase is mainly produced by mature enterocytes and a correlation exist between intestinal and serum diamine oxidase activity.

To study the value of diamine oxidase as marker of acute rejection fully allogeneic total orthotopic small-bowel transplantation was performed in rats using the WAG-to-BN donor host combination (group 2; daily serum diamine oxidase determination until rejection, n=9). Syngeneically transplanted WAG rats served as control (group 1; daily serum diamine oxidase determination until day 16, n=6). Animals in group 1 survived indefinitely and animals in group 2 died of rejection between 10 and 18 days. In both group 1 and 2 a fluctuating serum diamine oxidase pattern with time was found. Basal serum diamine oxidase level was significantly decreased in allogeneic animals compared to syngeneic controls at day 13, 14 and 16. At that time, however extensive mucosal sloughing had already taken place. Studying allotransplanted animals individually, no prognostic diamine oxidase change, indicating onset of rejection, could be revealed.

These data indicate that the small-bowel transplantation procedure itself is responsible for fluctuating serum diamine oxidase levels in the first two weeks postoperatively and that monitoring serum diamine oxidase has no value in early detection of acute small-bowel rejection. Whether the prognostic character of diamine oxidase is suppressed by early transplantation-related factors should be investigated in a chronic rejection model following small-bowel transplantation.

Introduction

A major obstacle to clinical small-bowel transplantation (SBT) is the vigorous rejection elicited by the graft (1-3). In order to treat this rejection optimally, monitoring of this process is of utmost importance. An early and reliable index of rejection would allow for institution of anti-rejection therapy before the graft is irreversibly damaged.

Histologic analysis is the "gold standard" for reliable detection of small-bowel (SB) allograft rejection. This method, however, has several shortcomings: biopsies require the presence of an enterostomy; full-thickness slides, which bear the risk of graft perforation, are mandatory to accurately predict early onset of rejection; and multiple biopsies must be taken to avoid sampling error because of the patchy distribution of the rejection process in the graft (4,5).

Another approach of monitoring of rejection is to study functional absorption of agents like cyclosporin and maltose (6,7). These methods suffer from considerable variability and have proven unreliable. With time more sensitive tests, based on absorption or permeability, have been developed. The use of such methods is still cumbersome in that they require oral administration of the test-solution and/or blood sampling at several time points (8,9). Recently, Meijssen et al developed a non-invasive method to assess electrophysiologic parameters in an enterostomy (10). It was found that electrophysiologic parameters correlate with histologic alterations of acute rejection but dismiss the disadvantages of histologic monitoring.

Contrary to some other types of organ transplantation (11) a simple serum marker, which reflects functional deterioration of the mucosa, is still unavailable. N-Acetylhexosaminadase (12,13), procoagulant activator (14) and nitric oxide (15) have recently been shown to have some value as markers of early SB rejection.

Another potential serum marker for early detection of SBT rejection is the enzyme diamine oxidase (DAO), which degrades polyamines (essential substances for cell growth) and is a key regulator of cell proliferation in the intestine (16). About 95% of tissue DAO activity is found in the intestine, primarily located in the mature mucosal cells of the villus tip (17). Cell division is a major regulator in up-regulating intestinal DAO activity, whereas DAO in turn down-regulates mucosal growth by polyamine degradation and suppresses cell division via active metabolites (18).

A positive correlation exists between DAO activity in mucosal extracts and serum, with

decreasing activities found in case of intestinal injury (19). Intestinal rejection is a form of intestinal injury, starting with crypt necrosis followed by mucosal sloughing, and could conceivably be associated with changes in basal DAO activity. The aim of the present study was therefore to determine the usefulness of monitoring basal serum DAO levels as a marker for early detection of acute SB allograft rejection in rats.

Materials and methods

Small-bowel transplantation

SBT was done as described previously (20). In brief, a one-step total orthotopic SBT was performed. The donor's SB was harvested from the ligament of Treitz to the terminal ileum, along with the attached vascular pedicles consisting of the superior mesenteric artery and the portal vein. End-to-side anastomoses were performed between the recipient aorta and donor superior mesenteric artery, and recipient inferior caval vein and donor portal vein, respectively, whereafter the recipient's own SB was resected. Gastrointestinal continuity was restored by end-to-end anastomosis of the graft, proximally with the host's duodenum and distally with the remaining 1-2 cm of terminal ileum.

Experimental groups

Group 1: Syngeneic SBT using WAG rats (n=6). Blood was collected daily from day 0 (preoperative value) until day 16 postoperatively.

Group 2: Allogeneic SBT using BN rats as donors and WAG rats as recipients (n=9). Blood was collected from the day of transplantation (preoperative value) until the day the animals were sacrificed because of their deteriorating condition. Time of rejection was defined as the day on which animals were sacrificed after macroscopic confirmation of end-stage rejection, which was proved by histologic examination.

For determination of basal DAO activity

One ml of blood was collected via the tail vein. Serum was stored at -70°C. DAO activity was measured as described by Romijn et al (21). In brief, 200 μl serum was incubated with 100 μl chloral hydrate, 2100 μl 0.1 M phosphate buffer (pH 7.0) and 100 μl of substrate solution (^{14}C-labeled putrescine) for 2 hours at 37°C. The reaction was stopped by addition of 200 μl of a 10 mM solution of aminoguanidine in 2% sodium-carbonate. The reaction product, ^{14}C-Δ^1-pyrroline was extracted into 4 ml toluene-based scintillation fluid and the radioactivity present was measured using a liquid scintillation analyzer (Packard, Tricarb 2500 TR, Packard Instrument Company Inc, Downers Grove, USA). DAO activity was expressed as Units/ml with one Unit defined as nmol of ^{14}C-Δ^1-pyrroline formed per hour.

Statistics

The results are expressed as means ± SD and the significance of differences among the groups on postoperative days was tested using Student's nonpaired t test after taking the logarithm of the individual data with $p \leq 0.05$ considered significant. The prognostic value of DAO for SBT rejection was evaluated using a non-parametric graphical representation of the relationship between rejection and the occurrence of DAO level changes (22) and a Basic computer program developed by Hop et al (23).

Results

Survival

All syngeneic rats survived indefinitely. Rats with allogeneic SB graft in group 2 had a mean survival time of 14.3 ± 3.1 days. Two rats (no 1 and 5 see Table 1B) showed transient grade 1 graft-versus-host disease characterized by light redness of ears, snout and paws (24).

Diamine oxidase activity

The individual basal DAO levels on postoperative days following syngeneic and allogeneic SBT are given in Table 1A and 1B respectively. Figure 1 shows the mean DAO serum level of DAO activity over time following the operation. Basal DAO level was significantly lowered in allogeneically transplanted animals compared to syngeneically transplanted animals on postoperative days 13, 14 and 16. Figure 2 shows a graph to visualize the possible prognostic character of DAO in rats 1 and 2, in which rejection took place on day 13. In both rats, DAO values were markedly lower on postoperative day 7 compared to the other rats given in the Figure, that rejected their grafts later. Although such a pattern in basal DAO level was clearly noted 6 days before rejection in rats 1, 2, 3, 8 and 9, statistical analysis could not establish a prognostic value of basal DAO level for SB rejection.

Table 1A Serum DAO values[1] after syngeneic small-bowel transplantation.

	WAG → WAG					
animal → ↓ day	1	2	3	4	5	6
0	0.17	nt	0.17	0.11	0.19	0.11
1	nt	nt	nt	0.03	0	nt
2	0.01	0	0.04	0.06	0.03	0.38
3	0.04	0.03	0.02	0.21	0.26	0.08
4	0.07	0.10	0.15	0.27	0.52	0.05
5	0.10	0.12	0.13	0.52	0.08	0.26
6	0.11	0.18	0.14	0.87	0.77	0.28
7	0.15	0.15	0.19	0.51	0.36	0.13
8	0.39	0.19	0.21	0.05	1.02	0
9	0.24	0.21	0.29	0.76	0.34	0.23
10	0.34	0.17	0	0.32	0	0.09
11	0.30	0.23	0.58	0	0.23	0.07
12	0	0.10	0.30	0.31	0.39	0
13	0.34	0.22	0.15	0.47	0.43	0.13
14	0.34	0.24	0.14	0.07	0.34	0.06
15				0.16	0.55	0.22
16				0.30	0.71	0.22

Table 1B Serum DAO values[1] after allogeneic small-bowel transplantation

	BN → WAG								
animal → ↓ day	1	2	3	4	5	6	7	8	9
0	0.15	0.13	0.07	nt	0.12	0.20	nt	0.25	nt
1	0	nt	nt	nt	0.06	0.14	nt	nt	0.09
2	0.07	0.02	0	nt	0.06	0.06	0.16	0.07	0.14
3	0.12	0.09	0.03	nt	0.20	0.14	0.29	0.06	0.22
4	0.30	0.13	0.02	0.12	0.30	0.16	0.06	0.21	0.15
5	0.36	0.21	0.01	nt	nt	0.28	0.20	0.42	0.25
6	0.22	0.26	0.04	0.64	0.55	0.20	0.22	0.62	0.48
7	0.04*	0.01	0.07	nt	nt	0.09	0.14	0.47	0.25
8	0.02	0.01	nt	0.02	0.23*	0.26	0.13	0.32	0.20
9	0	0.03	nt	0.73	nt*	nt	0.19	0.27	0.17
10	0	0	[a]	1.07	nt	[b]	0.03	0.17	0.14
11	0.14	0		0.19	[a]		0.08	0.25	0.15
12	0.15	0		nt			0.02	0.22	0.05
13	0.13[a]	0.04[a]		nt			0.02	0	0.08
14				0.10			0.01	[a]	0.03
15				nt			0.02		0.18
16				nt			0.03		0.08
17				nt			[a]		nt
18				[a]					[a]

[1]: DAO values are expressed as nmol ^{1}pyrroline formed/ml serum/hour
[a]: day of graft rejection
[b]: recipient died with functioning graft (ethernarcose)
nt = not tested
*: GVHD grade 1

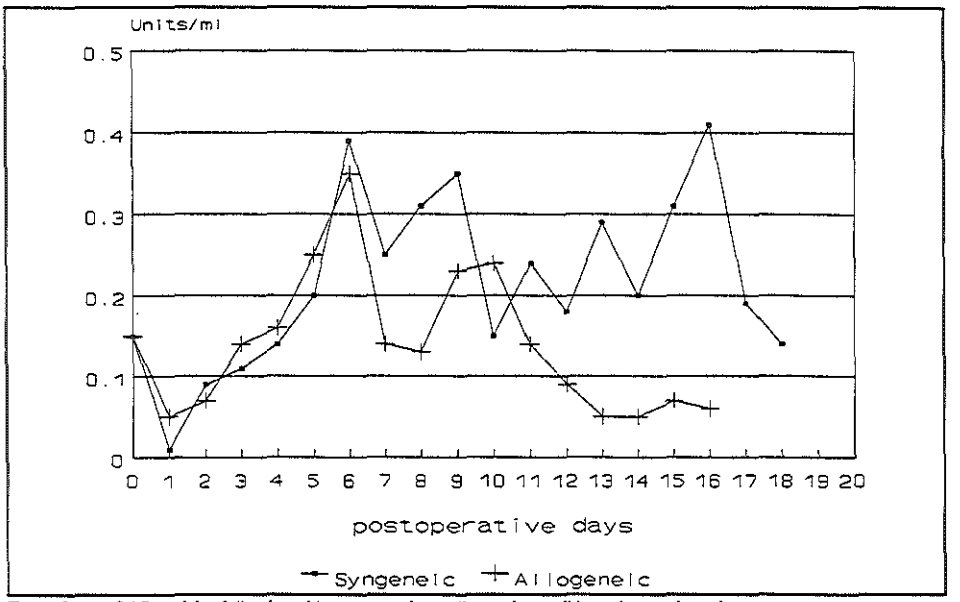


Fig.1. *Serum DAO activity following either syngeneic or allogeneic small-bowel transplantation.*

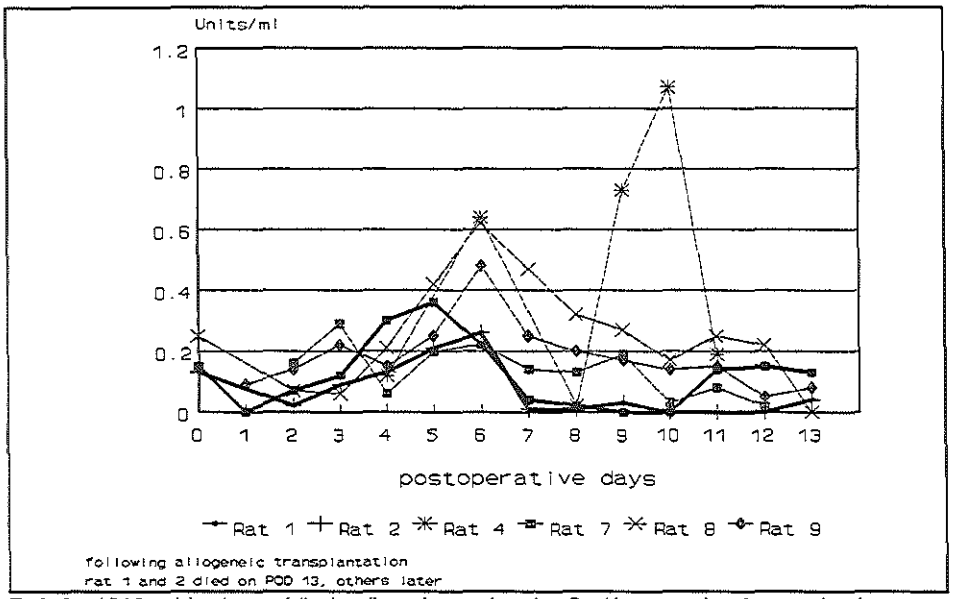


Fig.2. *Basal DAO activity changes following allogeneic transplantation. Graphic presentation of prognostic value.*

Discussion

Because acute rejection of the SB is associated with intestinal ischemia, it is valid to evaluate the prognostic significance of substances originating from gut during mucosal ischemia. In this respect, N-acetyl hexosaminidase, a lysosomal acid hydrolase which is known to be elevated in serum in association with intestinal ischemia is a potentially interesting candidate. Conflicting data on its usefulness as early marker to detect rejection have been reported (12,13), however, and this enzyme needs further study. Serum DAO levels are also known to be elevated during acute ischemia of the SB mucosa (25). Intestinal DAO is distributed relatively uniformly from jejunum to ileum, whereas in colon a lower activity is present (26,27). Differentiated enterocytes are the primary site of storage of DAO in the intestinal compartment. Upon stimulation DAO is secreted through the basolateral membrane, enters the microvasculature and is released into the circulation directly or through lymphatics. DAO release from the bowel can also be provoked by intravenous heparin administration which is associated with a rapid increase of plasma DAO. Postheparin DAO plasma levels are lowered in SB mucosal damage (28), Crohn's disease (29), celiac disease (30) and suspected mucosal atrophy during TPN feeding (29). This directly indicates that basal DAO levels may also reflect integrity of the mucosa. Ischemia leads to a decrease in intestinal DAO activity briefly followed by elevated serum DAO levels (31,32). It is unknown whether the causal factor of DAO release in ischemia is mucosal injury itself or that circulating factors capable to interact with microvascular receptors are responsible.

Transplantation of the small intestine is accompanied by neural and lymphatic disruption, and ischemia. Rose et al (33), demonstrated that basal serum DAO levels equalled preoperative values 1 month after intestinal autotransplantation in dogs and remained stable for more than 18 months. Basal DAO values showed significant rises on day 2 and 3 postoperative. This increased basal DAO level was interpreted as delayed appearance of DAO in serum due to disruption of lymphatics. Whether basal DAO activity remains elevated beyond three days has not been tested yet.

In the present study, decreased basal serum DAO levels were found 1 day following either syngeneic or allogeneic intestinal transplantation. We believe that this decrease is caused by luminal DAO release during the transplantation procedure, before unclamping. Thus, DAO lost during luminal perfusion of the intestinal graft represents ischemic injury of the mucosa.

In rats that received a syngeneic intestinal graft the DAO serum activity fluctuated with time, probably because of a temporary loss of the homeostatic mechanism that normally

regulates mucosal growth. The ischemic injury of the transplantation procedure has a damaging effect on intestinal mucosa. In a model of canine SBT normal crypt function, assessed by measuring cyclic-AMP-mediated chloride secretion, was already found by day 3 (34). This may explain the obvious increases in DAO serum levels within the first postoperative week found in both syngeneically and allogeneically transplanted animals. Following intestinal transplantation, the regulatory role of DAO in mucosal growth may become of more importance in maintaining mucosal integrity and is accompanied by fluctuating DAO levels.

A similar pattern of fluctuating DAO serum levels was recognized in rats following allogeneic intestinal transplantation. In allotransplanted animals, mild histologic injury including some crypt cell death was present from day 4 onwards (unpublished data). We hypothesize that crypt cell death results in diminished cell division and is followed by a decrease in intestinal DAO activity. Although a decline in DAO activity was found in some animals 6 days before rejection, its prognostic value could not be proved statistically. However, non-rejected related factors present in the first two weeks may alter DAO activity following allogeneic SBT and as such overrule a possible prognostic character of DAO. It was demonstrated that on day 13, 14, and 16 significant decreases in serum DAO activity were found in allogeneically transplanted animals compared to syngeneically transplanted animals. However, at that time, extensive mucosal sloughing had already taken place (unpublished data).

In summary, the results from this study suggest that changes in basal serum DAO level do not have a prognostic value for acute intestinal allograft rejection.

Further studies are needed to define the role of monitoring basal serum DAO in chronic rejection and the importance of performing the postheparin DAO test in the assessment of graft function.

On top of that suppression of DAO may be a means of stimulating SB adaptation after major resections, and it is hypothesized that this may consequently be used to stimulate adaptation of SB grafts.

Acknowledgements

We acknowledge the technical assistance of Miss L. v Gastel in performing the DAO assays.

References

1. Wood RFM. Introduction and overview. *Transplant Proc 1990; 22: 2423.*

2. Schraut WH. Current status of small bowel transplantation. *Gastroenterology 1988; 94: 525.*
3. Grant D. Intestinal transplantation: Current Status. *Transplant proc 1989; 21: 2869.*
4. Sonnino RE, Riddle JM and Pritchard TJ. Grading system for histologic changes in rat small bowel transplants. *Transplant Proc 1992; 24: 1201.*
5. Dennison AR, Collin J, Watkins RM, Millard PR and Morris PJ. Segmental small intestinal allografts in the dog. 1 Morphological and functional indices of rejection. *Transplantation 1987; 44: 474.*
6. Nordgren S, Cohen Z, Mackenzie R, Finkelstein D, Greenberg GR and Langer B. Functional monitors of rejection in small bowel transplants. *Am J Surg 1984; 147: 152.*
7. Billiar TR, Caberoglio C and Schraut WH. Maltose absorption as an indicator of small intestinal allograft rejection. *J Surg Res 184; 37: 75.*
8. Mc Alister V, Wall W, Ghent C, Zhong R, Duff J and Grant D. Successful small intestine transplantation. *Transplant Proc 1992; 24: 1236.*
9. Grant D, Lamont D, Zhong R, Garcia B, Wang P, Stiller C and Duff J. ^{51}Cr-EDTA: a marker of early intestinal rejection in the rat. *J Surg Res 1991; 46: 507.*
10. Meijssen MAC, Heineman E, de Bruin RWF, ten Kate FJW, Marquet RL and Molenaar JC. detection of canine intestinal allograft rejection by in vivo electrophysiologic monitoring. *Transplantation 1991; 51: 955.*
11. Macpherson NA, Moscarello MA, Goldberg DM, Ish-Shalom N and Arbus GS. Aminoaciduria as a marker of acute renal transplant rejection - a patient study. *Clin Invest Med 1991; 14(2): 111.*
12. Maeda K, Schwartz MZ, Bamberger MD and Daniller A. A possible serum marker for rejection after small intestine transplantation. *Am J Surg 1987; 153: 68.*
13. Meijssen MAC, Heineman E, de Bruin RWF, Marquet RL and Molenaar JC. The value of N-acetyl hexosaminidase in detecting canine intestinal allograft rejection. *Transplant Proc 1991; 23: 615.*
14. Kim PCW, Levy GA, Graig M, Cullen J and Cohen Z. Immune responses during small-intestinal allograft rejection: correlation between procoagulant activity and histopathology. *Transplant Proc 1990; 22: 2477.*
15. Langrehr JM, Muller AR, Lee TK, Schraut WH, Simmons RL and Hoffman RA. Serum NO_2^- /NO_3^- from oxidative L-arginine metabolism: a possible marker for small bowel allograft rejection. *Transplant Proc 1992; 24(3): 1148.*
16. Dowling RH. Polyamines in intestinal adaptation and disease. *Digestion 1990; 46(suppl 2): 331.*
17. Baylin SB, Stevens SA and Shakin KMM. Association of diamine oxidase and ornithine decarboxylase with maturing cells in rapidly proliferating epithelium. *Biochem Biophys Acta 1978; 541:415.*
18. Kusche J, Mennigen and Erpenbach K. The intestinal diamine oxidase activity under the influence of adaptive proliferation of the intestinal mucosa - a proliferation terminating principle. *Agents and Actions 1988; 23: 356.*
19. Thompson JS, Bragg LE and West WW. Serum enzyme levels during intestinal ischemia. *Ann Surg 1990; 211: 369.*
20. Saat RE, Heineman E, de Bruin RWF, Marquet RL and Jeekel J. Total orthotopic allogeneic small bowel transplantation in rats: attempts to ameliorate the graft-versus-host disease by irradiation and transfusions to the donor. *Transplantation 1989; 47: 451.*
21. Romijn JC, Verkoelen CF and Splinter TAW. Species-dependent differences of the biochemical properties of diamine oxidase. *Int J Biochem 1986; 18: 835.*
22. Simon R and Makuch RW. A non-parametric graphical representation of the relationship between survival and the occurence of an event: application to responder versus non-responder bias. *Statist Med 1984; 3: 35.*
23. Hop WCJ and Buuren HR. A method to evaluate changes in prognostic status during follow-up, illustrated by an assessment of the effect of a rebleed in patients with oesophageal variceal bleeding. *Comput Biol Med 1989; 19: 181.*
24. de Bruin RWF, Saat RE, Heineman E, Jeekel J and Marquet RL. Effects of donor pretreatment with antilymphocyte serum and cyclosporine on rejection and graft-versus-host disease after small bowel transplantation in immunosuppressed and nonimmunosuppressed rats. *Transplant Int 1993; 6: 22.*
25. Wollin A, Navert H and Bonous G. Effect of intestinal ischemia on diamine oxidase activity in rat intestinal tissue and blood. *Gastroenterology 1981; 80: 980.*
26. Thompson JS. Significance of the intestinal gradient of diamine oxidase activity. *Dig Dis 1990; 8:*

163.

27. Luk GD, Bayles TM and Baylin SB. Diamine oxidase: a circulating marker for rat intestinal mucosa maturation and integrity. *J Clin Invest 1980; 66: 66.*
28. Forget P, Saye Z, van Culsem JL and Dandrifosse G. Serum diamine oxidase activity in acute gastroenteritis in children. *Pediatr Res 1984; 19: 26.*
29. Rokkas T, Vaja S, Murphy GM and Dowling RH. Postheparin plasma diamine oxidase in health and disease. *Gastroenterology 1990; 98: 1493.*
30. Forget P, Grandfils C, van Cutsem JL and Dandrifosse G. Diamine oxidase in serum and small intestinal biopsy tissue in childhood celiac disease. *J Pediatr Gastr 1986; 5: 379.*
31. Bonous G, Echave V, Vobecky SJ, Navert H and Wollin A. Acute necrosis of the intestinal mucosa with high serum levels of diamine oxidase. *Dig Dis Sci 1984; 29: 872.*
32. Bragg LE, Thompson JS and West WW. Intestinal diamine oxidase levels reflect ischemic injury. *J Surg Res 1991; 50: 228.*
33. Rose SG, Thompson JS, Spanta AD and Quigley MM. The effect of intestinal autotransplantation on serum diamine oxidase activity. *J Surg Res 1991; 50: 223.*
34. Meijssen MAC, Heineman E, de Bruin RWF, Veeze HJ, Bijman J, de Jonge HR, ten Kate FWJ, Marquet RL and Molenaar JC. The value of in vivo electrophysiological measurements to evaluate canine small bowel autotransplants. *Gut 1991; 32: 1329.*

8.3 POSTHEPARIN DIAMINE OXIDASE ACTIVITY AS MONITORING TOOL FOR SMALL-BOWEL GRAFT FUNCTION IN RATS

Abstract

A biochemic marker that could provide information about the actual condition of an intestinal graft is still missing. The enzyme diamine oxidase, mainly found in villi tips, is a potential circulating marker of mucosal maturation and integrity.

The aim of this study was to investigate whether diamine oxidase has any value in monitoring the status of an intestinal graft in rats. Therefore we examined, up to 6 months postoperatively the differences with time in basal as well as postheparin diamine oxidase activity in resected WAG rats that had received either a syngeneic total small-bowel transplantation or a fully allogeneic small-bowel transplantation from a BN donor. Statistical analysis was performed using the paired Student's t test and the Student's t test for two means with a p value less than 0.05 considered significant.

The results show that the basal diamine oxidase activity measured preoperatively has a considerable variation, and that it has no validity in establishing alterations following small-bowel transplantation.

Animals with a syngeneic total small bowel had a significantly increased postheparin diamine oxidase activity during the experimental period compared to preoperative values. In animals with an allogeneic total small bowel, postheparin diamine oxidase was not increased up to the fourth month after operation. Postheparin diamine oxidase activity was significantly increased in syngeneic animals compared to allogeneic animals during the first 4 months postoperatively. Previous work showed that in these rats no differences could be found monitoring weight, nutritional serum parameters, and fecal fat excretion. Therefore, we conclude that measuring postheparin diamine oxidase activity has potential value in establishing the condition of the graft. The increased values in syngeneically transplanted animals indicate that the effects of the transplantation procedure induce an intestinal adaptive response that is compromized in allogeneically animals. This may have implications for the use of segmental small-bowel grafts.

Introduction

Recent clinical experiences in small-bowel transplantation (SBT) are promising and strengthen the belief that this will be the ultimate therapeutic treatment for patients with irreversible small-intestinal failure (1,2).

Following SBT, monitoring the graft is of utmost importance to have an indication of the momentary graft function (3) and to be able to early recognize rejection (4,5).

The long-term graft function of transplanted intestine can be influenced by both non-immune as well as immune mediated factors. The non-immune mediated factors are primarily caused by the transplantation procedure itself and include denervation, lymphatic disruption, and ischemic and reperfusion injury (6,7).

Conflicting data have been reported on the long-term consequences of the procedure on graft physiology. Steatorrhea and reduced D-xylose absorption were observed following autotransplantation in adult dogs, which was ascribed to permanent extrinsic denervation (8,9). However, in young dogs, recipients of a total autotransplant did not differ in growth and functional graft indices (Chapter 5) from sham-operated controls, indicating adequate graft function despite the presence of significant bacterial overgrowth. Among the immune mediated factors that may influence the long-term functioning of the graft are infiltrating cells and their products (10,11). Little attention has been paid to the long-term immune mediated consequences on graft function. Previous work (12) performed in our laboratory showed long-term survival in a fully allogeneic total orthotopic SBT model. During an immunologically quiescent phase in which no rejection was encountered, the functional capacity of the graft was studied by monitoring weight, determination of serum parameters, and fecal fat excretion. Up to one year after transplantation, no differences could be detected compared to syngeneically transplanted rats using these parameters.

Serum activity of the enzyme diamine oxidase (DAO), an enzyme found almost exclusively in the intestinal mucosa, serves as a sensitive circulating marker of mucosal maturation and integrity (13,14). Because basal levels of the enzyme are just within the detection limits, DAO is often measured after stimulation with heparin, which releases intestinal DAO from the gut into the peripheral blood (15). The postheparin DAO activity time curve is known to be reduced during intestinal atrophy and enhanced during an intestinal adaptive response (16,17).

We examined the differences with time in basal as well as postheparin plasma DAO

activity in resected rats that received either syngeneic or allogeneic SBT, in order to determine whether DAO is a more specific tool to monitor the condition of the graft than the previously studied tool (12).

Materials and methods

Animals

Male rats of 10 to 14 weeks old (200-250 gram), that were bred under specific pathogen free conditions, were obtained from Harlan-CPB (Austerlitz, The Netherlands). Inbred Wistar-Agouti (WAG-Rt1^u) and Brown Norway (BN-Rt1^n) strains were used.

Small-bowel transplantation

One-step total orthotopic SBT was performed as described in paragraph 8.2.

Experimental groups

Group 1: Syngeneic SBT using adult WAG rats (n=6).

Group 2: Fully allogeneic SBT using adult BN rats as donors and adult WAG rats as recipients (n=6). Rats in both groups received intramuscular CsA (Sandimmun, Sandoz, Basel, Switzerland) in a dose of 15 mg/kg at days 0, 1, 2, 4 and 6. (Untreated control rats reject their grafts in 12.8 $\pm$ 1.0 days (18).

Measurement of DAO activity

Basal and postheparin plasma DAO activity was determined preoperatively and then monthly until 6 months. A blood sample, consisting of 1 ml blood obtained from the tail vein, was collected in heparinized tubes prior to administration of 100 Units/kg heparin, and 15 minutes afterwards.

Diamine oxidase activity, expressed in Units/ml, was determined as described in paragraph 8.2.

Statistics

All data are expressed as means $\pm$ standard deviation (SD) and were analyzed using the paired Student's t test for comparisons between the same animals at different time points with the preoperative value, and the Student's t test for two means for comparisons between the various groups at the same time point. A p-value less than 0.05 was considered as the a priori level of significance.

Results

Basal DAO levels

In Figure 1, the basal plasma DAO activities of rats with a syngeneic (group 1) or allogeneic (group 2) small-bowel (SB) graft are shown monthly up to 6 months after surgery. Even the preoperative basal plasma DAO levels showed some variation. Basal DAO activity of syngeneically transplanted animals was significantly different from preoperative values 1, 3 and 5 months postoperative. For the allogeneically transplanted animals the basal DAO activity was statistically different from preoperative values at 3 months. There were no significant differences between the experimental groups.

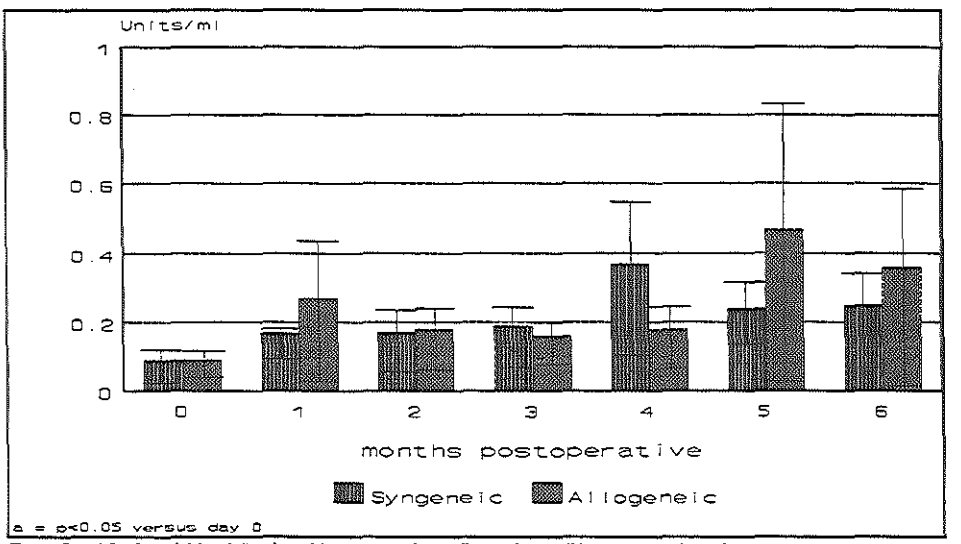


Fig.1. *Basal DAO activities following either syngeneic or allogeneic small-bowel transplantation.*

Postheparin DAO levels

Figure 2 shows the DAO plasma levels in syngeneic (group 1) and allogeneic (group 2) total SB transplanted animals 15 minutes after stimulation with heparin.

The animals with a syngeneic total SB graft had a significantly increased postheparin DAO activity during the total follow-up period compared to the preoperative value.

In animals with an allogeneic total SB graft the postheparin DAO activity did not increase up to the fifth month after operation as compared with preoperative values.

Between the experimental groups, significant differences in postheparin DAO activity could be found up to the fifth postoperative month, in that group 1 animals had higher

levels. Both 5 and 6 months after the transplantation, no differences between the groups were found.

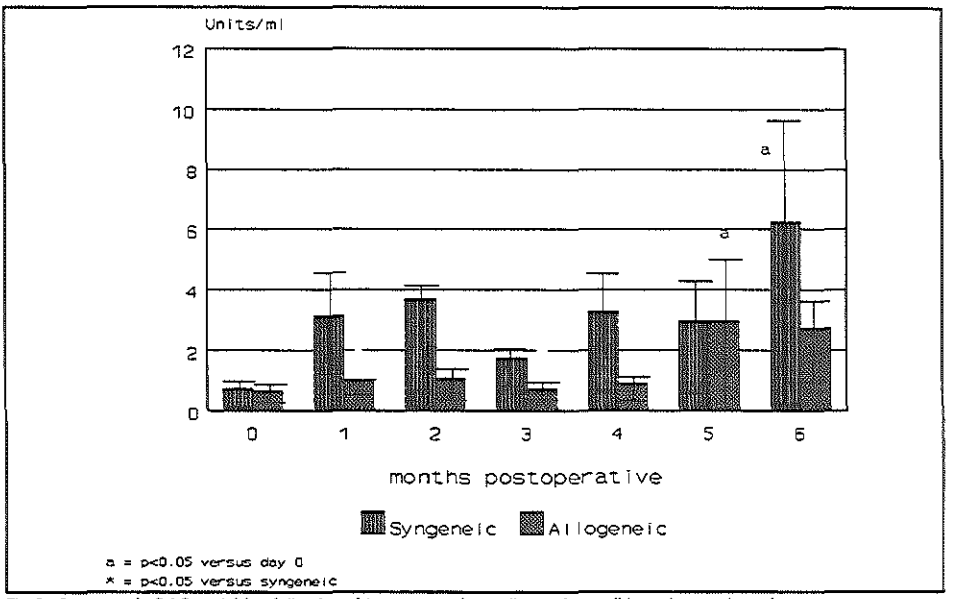


Fig.2. *Postheparin DAO activities following either syngeneic or allogeneic small-bowel transplantation.*

Discussion

We have previously shown that one year after surgery recipients of syngeneic-, and allogeneic total SB transplants were not different from age-matched untreated control rats, except for decreased plasma triglyceride levels (12). Between animals grafted with a syngeneic or allogeneic graft, no such difference was found. Extensive analysis showed that total serum protein and albumin concentrations, serum cholesterol values, fecal fat excretion, percentage of split fatty acids, and weight gain were equal.

DAO, located primarily in the villus tip, is involved in the regulation of mucosal growth (19). The enzyme seems to act as a negative feedback regulator in intestinal growth in that it inhibits crypt cell proliferation by degrading polyamines, essential components for cell division. Erdman et al (20) have shown that DAO activity increases significantly early after SB resection, a period characterized by adaptive enterocyte proliferation. Inhibiting DAO, using aminoguanidine, enhanced the morphologic adaptation response indicating that DAO downregulates nonfunctional proliferative responses of the intestine.

Several investigators have shown that following heparin administration, increase in peripheral blood DAO levels derives almost exclusively from the intestine and reflect the intestinal DAO content more sensitively than the basal plasma DAO levels (21,22). In chapter 4 of this thesis, it was demonstrated that postheparin DAO activity is a sensitive measure for the adaptive response following resection, because it enables to quantify the degree of adaptation of resected rats fed different diets.

In this study we showed that basal plasma DAO activities have a considerable variation and we could not find alterations within the experimental groups in comparison with the preoperative values. We further demonstrated that measuring basal plasma DAO activity reveals no differences between animals that received a syngeneic (group 1) or allogeneic (group 2) graft. However, it appeared that the use of postheparin DAO activity as marker for the actual condition of the graft enables to reveal differences between pre and postoperative values as well as between group 1 and group 2.

In group 1 the postheparin DAO activity was significantly higher during the follow-up period compared to the preoperative value. This rise can be seen as an adaptive response of the transplanted intestine to overcome the effects of the transplantation procedure. These non-immune mediated phenomena may disregulate the hormonal, neural, nutritional as well as the immunological function of the graft. The increase found in postheparin DAO activity can be seen as a sign that the repair-phase has been started.
No such rise was detected in allogeneic transplanted animals during the first 4 months after the operation. The heparin stimulated DAO activity was increased at 5 and 6 months following allotransplantation compared to values measured preoperatively.

This indicates that the addition of an immunologic component delays the adaptation process after intestinal transplantation of the total SB. This finding may have implications for the transplantation of a segmental bowel graft. In that case, the segment should adapt more rapidly to compensate for the shortened length. These findings suggest that segmental SBT may require enhancement of intestinal adaptation by means of specific stimuli. On the other hand, it cannot be excluded that the grafting of a segmental transplant may be the required trigger for the onset of the adaptive response of the intestine.

References

1. McAlister V, Wall W, Ghent R, Zhong R, Duff J and Grant D. Successful small intestine transplantation. *Transplant Proc 1992; 24: 1236.*
2. Todo S, Tzakis AG, Abu-Elmagd K, Reyes J, Fung JJ, Casavilla A, Nakamura K,Yagihashi A, Jain A, Murase N, Iwaki Y, Demetris AJ, Van Thiel D and Starzl TE. Cadaveric small bowel and small bowel-liver transplantation in humans. *Transplantation 1992; 2: 369.*

3. Meijssen MAC and Heineman E. Functional aspects of small bowel transplantation: Past, present, and future. *Gut (accepted for publication).*
4. Dennison AR, Collin J, Watkins RM, Millard PR and Morris PJ. Segmental small intestinal allografts in the dog. I. Morphological and functional indices of rejection. *Transplantation 1987; 44: 474.*
5. Meijssen MAC, Heineman E, de Bruin RWF, Ten Kate FJW, Marquet RL and Molenaar JC. Detection of canine intestinal allograft rejection by in vivo electrophysiologic monitoring. *Transplantation 1991; 51: 955.*
6. Schmid T, Korozi G, Oberhuber G, Klima G and Margreiter R. Lymphatic regeneration after small bowel transplantation. *Transplant Proc 1990; 22: 2446.*
7. Hamato I, Merchav H, Zhu Y, Suzuki M, Fujita S, Murase N, Todo S and Starzl TE. Lipid peroxidation, brush border, and neutrophil enzyme activity after small bowel preservation: A comparison of preservation solutions. *Transplant Proc 1992; 24: 1095.*
8. Raju S, Fujiwara H, Grogan JB and Achord JL. Long-term function of orthotopic small bowel autotransplants. *J Surg Res 1989; 46: 142.*
9. Thomson JS, Rose SG, Spanta AD and Quigley EMM. The long-term effect of jejunoileal autotransplantation on intestinal function. *Surgery 1992; 111: 62.*
10. Lear PA, Cunningham AJ, Crane PW and Wood RFM. Lymphocyte migration patterns in small bowel transplants. *Transplant Proc 1989; 21: 2881.*
11. Iwaki Y, Starzl TE, Yagihashi A, Taniwaki S, Abu-elmagd K, Tzaki A, Fung J and Todo S. Replacement of donor lymphoid tissue in small bowel transplants. *Lancet 1991; 337: 818.*
12. de Bruin RWF, Heineman E, Jeekel J, Meijssen MAC, Lindemans J, Bonthuis F and Marquet RL. Functional aspects of small bowel transplantation in rats. *Scand J Gastroent 1992; 27: 483.*
13. Mennigen R, Kusche J, Leisten L and Erpenbach K. Diamine oxidase (DAO) activity and intestinal mucosa integrity: influence of suture techniques. *Agents and actions 1987; 20: 277.*
14. Luk GD, Bayless TM and Baylin SB. Diamine oxidase (histaminase) a circulating marker for rat intestinal mucosal maturation and integrity. *J Clin Invest 1980; 66: 66.*
15. Hansson R, Holmberg CG, Tibbling S, Tryding N, Westling H and Wetterqvist H. Heparin-induced diamine oxidase increase in human blood plasma. *Acta Med Scand 1966; 180: 533.*
16. Rokkas T, Vaja S, Murphy GM and Dowling RH. Postheparin plasma diamine oxidase in health and intestinal disease. *Gastroenterology 1990; 98: 1493.*
17. D'Agostino L, Giacci C, Daniele B, Barone MV, Sollazo R, Mazzacca G. Post-heparin plasma diamine oxidase with small bowel mucosal atrophy. *Dig Dis Sci 1987; 32: 313.*
18. de Bruin RWF, HogenEsch H, Heineman E, Jeekel J and Marquet RL. Fulminant graft-versus-host disease after FK 506 allogeneic small bowel transplantation. *Transplant Proc 1991; 23: 3257.*
19. Dowling RH. Polyamines in intestinal adaptation and disease. *Digestion 1990; 46(suppl 2): 331.*
20. Erdman SH, Park JHY, Thompson JS, Grandjean CJ, Hart MH and Vanderhoof. Suppression of diamine oxidase activity enhances ileal proliferation in the rat. *Gastroenterology 1989; 96: 1533.*
21. Kobayashi Y, Kupelian J and Maudsley DV. Release of diamine oxidase by heparin in the rat. *Biochem Pharmacol 1969; 18: 1585.*
22. Rokkas T, Vaja S, Taylor P, Murphy GM and Dowling RH. Is the intestine the sole source of heparin-stimulated plasma diamine oxidase? *Digestion 1990; 46(suppl 2): 439.*

GENERAL DISCUSSION AND CONCLUSIONS

A number of conditions in children, notably small-bowel (SB) atresias, malrotation with midgut volvulus, gastroschisis, enterocolitis and extensive aganglionosis, may require resections that could result in the short bowel syndrome (SBS) (**1,2,3**). The common causes of the SBS in adults include Chrohn's disease and mesenteric infarction (**4**). Short bowel syndrome is characterized by irreversible small-intestinal failure, being defined as inability to maintain nutrition and/or positive fluid and electrolyte balance without special measures (**Chapter 1**).

This dissertation deals with "therapeutic modalities for the short bowel syndrome", whereby emphasis is put on the value of these options for children. The experimental work can be divided into two parts, each dealing with a different therapeutic option: (A); enhancement of adaptation of the remaining bowel and (B); small-bowel transplantation (**Chapter 2**).

Enhancement of adaptation

Extensive loss of intestine brings about functional as well as morphologic adaptive changes in the remaining intestine. Functional adaptation is determined by numerous factors, including the absorption per enterocyte, intestinal transit time, the presence of the ileocecal valve, intestinal contents and/or specific activities of brush border enzymes, and bacterial colonization of the remaining SB (**5,6,7**). Increased exposure to (specific) luminal nutrients, pancreatico-biliary secretions, trophic effects of enteric hormones, and neurovascular effects on the remaining intestine are major factors that stimulate mucosal growth (**8**). The overall process is still unknown, but some factors that regulate the morphology and function of the gut may be directly interrelated, the overall process is still unknown.

In an attempt to unravel the interrelationship between active electrolyte transport mechanisms and mucosal growth, we evaluated the value of in vivo electrophysiologic measurements for intestinal functional adaptation in rats following near-total SB resection (**Chapter 3**). Using in vitro techniques, other investigators (**9,10**) have already shown that morphologic growth precedes functional adaptation. We found that histologic changes precede functional adaptation of both sodium-coupled glucose absorption and theophylline-stimulated chloride secretion, and that changes in the ileum precede changes in the jejunum. At 3 weeks after resection the electrophysiologic responses to both glucose and theophylline in jejunum as well as ileum of resected animals were significantly lower than

in sham-operated control animals. These findings suggest that the complexity of the adaptive events is indicated by region-related differences in mechanisms controlling mucosal growth and function as well as by the existence of dissociation between mucosal growth and components of functional adaptation. One explanation for the impairment of the electrophysiologic response at this time point is an alteration in tight junction permeability. Madara (11) highlighted the important role of tight junctions in intestinal barrier function as well as the maintenance of epithelial cell polarity, as prerequisite for vectorial transcellular transport. The analysis of possible changes in occluding junction structure and function should be the subject of future experiments.

Studies on biochemic and biophysic aspects responsible for functional alterations following resection have received little attention so far. These aspects include alterations in brush border membrane composition (contents of cholesterol, total and individual phospholipids, and fatty acyl constituents should be assessed), transporter activity as well as protein and mRNA levels of the transporter. These aspects have been studied already in other types of intestinal adaptation like response to aging, variations in and composition of the diet, and models of disease such as diabetes and chronic ethanol digestion (12,13,14).

Increases in absorptive capacity of the residual gut in response to bowel resection have been found with regard to carbohydrate, lipid, and amino acid absorption, and alterations in absorption following dietary modifications have been recognized (15,16).
In chapter 4 of this thesis, a rat study is presented in which the effect of a diet with partial hydrolyzed proteins on intestinal adaptation is compared with an identical diet with intact proteins. This study indicates that a diet with partial hydrolyzed proteins has no clear superiority over a complex diet with regard to improvement of the adaptation process. The necessity of a predigested diet in the standard treatment of the SBS is probably one of the many fallacies of enteral feeding (17,18). The results give an experimental basis for a clinical trial in SBS patients in which diets with partial hydrolyzed proteins are compared with diets with intact proteins. The high costs and the possible side effects of specialized food products are additional reasons to start a clinical trial.

In future studies, the emphasis should be put on determining the potential factors leading to enhanced lipid uptake such as: alterations in the passive permeability properties of the brush border membrane, and the factors leading to enhanced glucose uptake (alterations in the activity, the protein or the mRNA level of the glucose transporter). These studies will give insight into the mechanisms responsible for upregulation of nutrient transport

following resection. Based on this knowledge, it may be possible to maximize the process of adaptation to resection by feeding nutrient components that have the ability to upregulate in a synergistic manner.

Small-bowel transplantation

Experimental basis

From the literature and previous work in our laboratory (**19,20,21**), it can be concluded that SB grafts can survive for at least one year in both rat and dog. Clinical efforts to undertake SBT in humans have started over the last few years although the optimal requirements are still unknown (**22,23**). Therefore, it is not very surprisingly that both in Paris and in Pittsburgh one has to decide to stop the clinical programs because of the bad overall results. Before starting again, it would be worthwile to have available an immunosuppressive protocol that achieves successful graft survival without rejection and toxic side-effects, as well as clarity about the long-term physiologic consequences of SBT.

In a pre-clinical animal model using adult Beagles, we demonstrated that MHC-matching had a beneficial influence on the survival of segmental SB grafts transplanted in a two-stage manner, and that it is possible to achieve long-term survival with an oral dose of 30 mg/kg/day of cyclosporine A (CsA) (**24,25**). Using this dose, we were confronted with the toxic side-effects of CsA like wart formation and infections. Furthermore, in children there was no experimental evidence that an intestinal graft is able to maintain growth, development and maturation. Thus, we felt that before making SBT a practical therapeutic option for children further research in a young growing animal was required.

Necessity of selective decontamination of the graft

In **chapter 5** of this thesis, we examined the role of one-stage total orthotopic small-intestinal autotransplantation in growing individuals. The one-stage transplantation procedure was fraught with inordinate mortality of 75%, due to postoperative complications including electrolyte problems, invagination and infections. A two-stage segmental allotransplantation procedure in young dogs (**chapter 6 and 7**) showed that SBT in a young individual gives a "stormy" postoperative course and that selective decontamination of the gut and early gastrostomy feeding are necessary to overcome the postoperative problems. This is in accordance with the reported high incidence of infectious episodes after human SBT (**26**). The few long-term surviving dogs that received an autotransplant showed a normal growth pattern and normal D-xylose and fat absorption despite the presence of bacterial overgrowth in the graft. The bacterial overgrowth was not caused by extremely discoordinated motor complexes as a normal transit time was

found. This study suggests that the non-immunologic effects of intestinal transplantation, although causing bacterial overgrowth, do not promote bacterial translocation in the long run. There is, however, some evidence indicating that the combination of intestinal bacterial overgrowth and host immunosuppression synergistically promotes translocation of gastrointestinal bacteria (27). This may imply that selective decontamination of the digestive tract is needed at least temporarily before and after allogeneic transplantation to avoid episodes of infection, and thus underscores the above- mentioned results following two-stage segmental allotransplantation.

MHC Matching

In **chapter 7**, we examined whether MHC matching paves the way for using a reduced immunosuppressive regimen of 20 mg/kg/day of CsA in growing Beagle puppies, weighing 5 - 6 kg, that underwent a two-stage segmental allotransplantation. The graft was 1 meter jejunoileum, MHC-matched for 2 haplotypes, deriving from an adult donor. It was shown that this modality effectively prolonged intestinal graft survival without the toxic effects of the higher CsA dose. It might well be that matching in human SBT, in adults as well as in children, also provides a basis for using an immunosuppressive protocol that leads to long-term graft survival without the complications of administering high doses of immunosuppressiva.

Functional assessment of the graft

Follow-up of graft function following transplantation of 1 meter jejunoileum showed normal D-xylose absorption, normal values of serum parameters, but increased fecal fat excretion and lactulose/mannitol excretion ratios as well as compromised growth. It was concluded that a segmental graft under CsA regimen is unable to maintain the normal growth pattern without specific nutritional interference in the critical postoperative phase. After CsA treatment was stopped, a catch up growth was found before rejection took place. This finding indicates that a segmental SB graft under CsA regimen functions suboptimally to allow normal growth and development and that the condition of the graft is more important than the length of transplanted bowel. However, it seems reasonable that if a segmental graft under CsA regimen functions and adapts suboptimally, as much bowel length as possible should be transplanted. The importance of this is further stressed by findings in rat models (20,28) in which rats transplanted with a segmental allograft developed impaired nutritional parameters, in contrast to rats that received a total SB graft.

Sigalet et al (29) found that CsA had a significant effect on bowel function in the normal rat. CsA administration, independent of route of administration, caused a reduction in

both active glucose transport and passive fatty acid absorption by the bowel.
No experimental data on the use of FK 506 in SBT in growing recipients are available. Future research should be aimed to a better understanding of the influence of FK 506 and CsA on the mechanisms of nutrient absorption and intestinal adaptation, so that strategies to minimize the effects on bowel function can be devised. Nutritional intervention by adding polyamines, short chain fatty acids or glutamine to improve the degree of adaptation of a segmental graft may be of value (30,31,32). However, one should not consider these components as trophic factors per se. A recent study reported that adding glutamine to TPN enhances the intestinal mucosal immune function by preserving the ability to produce sIgA-synthesizing plasma cells (33). In other studies it was noted that, for example, arginine supplementation increases thymic weight and enhances the responsiveness of T-lymphocytes to mitogens (34). This may have further implications for application in SBT in that so called immunonutrition could improve the overall immune function of a recipient and may therefore be associated with increased risk of graft rejection. Further studies are needed to select nutrients that preserve or enhance intestinal function but do not have a stimulating influence on the immune status of the transplant recipient.

Advanced graft monitoring
Now SBT in the near future is expected to become a widely applied technique in patients suffering from irreversible intestinal failure, it has been realized that monitoring the intestinal graft is important.

In chapter 6, we examined the value of ultrasonography for monitoring the postoperative course after SBT. Regular monitoring proved to be useful in detecting early complications, including intussusception, thrombosis and postoperative ileus. Therefore we do recommend ultrasonography as a useful tool in the multidisciplinairy support after SBT. Using in vitro ultrasonography, we identified villous sloughing associated with rejection in one case. A recent study of Cheung et al (35) confirms this finding by demonstrating that it is possible to differentiate between normal and abnormal changes in the bowel wall after SBT with acceptable sensitivity and specificity.

In addition, monitoring the graft during the postoperative course following SBT is necessary for early detection of acute rejection and the adjustment of treatment. The value of mucosal biopsies as a tool to monitor rejection has limitations because of its patchy character and invasiveness (36). Although intestinal permeability studies are now available to assess rejection (37), studies are still focussed on the search for a simple sensitive serum marker. We previously examined the value of monitoring the enzyme N-

acetylhexosaminidase in acute rejection and found that substantial ischemic damage occurred before enzyme levels rose (38). In **chapter 8.2**, we evaluated the prognostic value of serum diamine oxidase in acute SB rejection in rats. Monitoring this enzyme was of no value because the SBT procedure itself is responsible for fluctuating serum DAO levels in the early postoperative period in rats.

The long-term follow up of patients after orthotopic SB grafting should include a number of functional and permeability tests to study graft function and to diagnose chronic rejection in an early phase to rescue the graft. Routine absorption tests, including the lactulose-mannitol test and fecal fat analysis may indicate good functioning of the graft (39). In **chapter 8.3**, we examined whether plasma and postheparin DAO values could serve as a sensitive marker of the functional status of the graft after intestinal allografting in rats. We concluded that measuring postheparin DAO activity has potential value in assessing the condition of the graft. Whether a specific test alone provides sufficient information on the behavior of the graft or whether combined results of some of the above mentioned tests gives more specific and/or sensitive information is a question that will have to be clarified by further experiments.

Future prospects

There are a number of gaps in our fundamental knowledge. These include the optimal solution for intestinal preservation, the factors responsible for the severity of SB rejection, the immunologic mechanism responsible for reduced immunogenicity of a combined small bowel-liver graft, the value of an auxiliary liver graft in combination with a SB graft, the role of GVHD, the role of microchimerism (40), and the metabolic profile of transplanted intestine and the effect of nutritional intervention on it.

Despite these gaps, clinical experience takes up the running from experimental studies by now. It can be questioned whether it is justifiable to start a clinical SBT program before the basic requirements as optimal immunosuppression and knowledge about the long-term consequences of the transplantation procedure have been extensively studied in a pre-clinical animal model. It is questionable whether these requirements had been met at the time the clinical programs in Pittsburgh, Toronto and Paris were started. However, seeing that they have already begun, the insights that will be derived from the current clinical cases in SBT will be highly valuable because the patients are extensively monitored. These insights, together with the experimental studies that are now being performed, will give direction to the experimental need of SBT and to the way in which it can be achieved succesfully.

New avenues that deserve further experimental attention as potential alternatives to intestinal transplantation include the transplantation of selective enterocytes (41) or transplantation of the colon. Preliminary results suggest that colon transplantation produces a milder rejection response than transplantation of the small intestine (42). Moreover, from a physiologic point of view a large bowel would probably be a wonderful transplant, having enormous adaptive capacity, especially if supplemented with fiber.

References

1. Affourtit MJ, Tibboel D, Hart AEH, Hazebroek FWJ and Molenaar JC. Bowel resection in the neonatal phase of life: short-term and long-term consequences. *Z Kinderchir 1989; 44: 144.*
2. Schwartz MZ and Maeda K. Short bowel syndrome in infants and children. *Pediatr Clin North Am 1985; 32: 1265.*
3. Caniano DA, Starr J and Ginn-Pease ME. Extensive short bowel syndrome in neonates: outcome in the 1980s. *Surgery 1989; 105: 119.*
4. Weser E, Fletcher JT and Urban E. Short bowel syndrome. *Gastroenterology 1979; 77: 572.*
5. Wilmore DW. Facors correlating with a successful outcome following extensive intensive resection in newborn infants. *J Pediatr 1972; 80: 88.*
6. Urban E and Weser E. Intestinal adaptation to bowel resection. *In Stollerman GH, ed. Advances in Internal Medicine. Chicago: Year book Medical Publishers, Inc, 1980; 26: 265.*
7. Galea MH, Holliday H, Carachi R and Kapila L. Short-bowel syndrome: a collective review. *J Pediatr Surg 1992; 27: 592.*
8. Bristol JB and Williamson RCN. Mechanisms of intestinal adaptation. *Pediatr Surg Int 1988; 4: 233.*
9. Urban E and Michel AM. Separation of adaptive mucosal growth and transport after small bowel resection. *Gastrointest Liver Physiol 1983; 7: G295.*
10. Bury K. Carbohydrate digestion and absorption after massive resection. *Surgery 1972; 135: 177.*
11. Madara JL. Loosening tight junctions. Lessons from the intestine. *J Clin Invest 1989; 83: 1089.*
12. Keelan M, Thomson AB, Wierzbicki AA, Wierzbicki E, Rajotte R and Clandinin MT. Isocaloric modification of dietary lipids influences intestinal brush border membrane composition in diabetic rats. *Diabetes Rev 1991; 16: 127.*
13. Fedorak PN, Thomson AB and Porter VM. Adaptation of intestinal glucose transport in rats with diabetes mellitus occurs independent of hyperphagia. *Can J Physiol Pharmacol 1991; 69: 1143.*
14. Keelan M, Thomson AB, Carg ML and Clandinin MT. Critical period programming of intestinal glucose transport via alteration in dietary fatty acid composition. *Can J Physiol Pharmacol 1990; 68: 642.*
15. Thomson AB, Mc Intyre Y, Macleod J and Keelan M. Dietary fat content influences uptake of hexoses and lipids into rabbit jejunum following ileal resection. *Digestion 1986; 35: 78.*
16. Thomson AB. Defined formula diets alter jejunal and colonic uptake of lipids in rabbits with intact intestinal tract and following ileal resection. *Res Exp Med (Berl) 1986; 186: 413.*
17. Koretz RL and Meyer JL. Elemental diets-facts and phantasies. *Gastroenterology 1980; 78: 393.*
18. Thompson WR, Stephen RV, Randall HT and Bowen JR. Use of the "space diet" in the management of a patient with extreme short bowel syndrome. *Ann Surg 1969; 117: 449.*
19. Saat RE, de Bruin RWF, Heineman E, Jeekel J and Marquet RL. Irradiation of the donor combined with Cyclosporine A treatment of the recipeint prevents GVHD and leads to prolonged survival of small bowel grafts. *Transplant Proc 1990; 22: 2465.*
20. de Bruin RWF, Heineman E, Jeekel J, Meijssen MAC, Lindemans J, Bonthuis F and Marquet RL. Functional aspects of small-bowel transplantation in rats. *Scand J Gastroenterol 1992; 27: 483.*
21. Meijssen MAC, Heineman E, de Bruin RWF, Wolvekamp MCJ, Marquet RL and Molenaar JC. Successful canine small-bowel transplantation using major histocompatibility complex matched segmental ileal allografts. *Transplant Proc 1992; 24: 1141.*

22. McAlister V, Grant D, Wall W, Roy A, Ghent C, Zhong R and Duff J. Immunosuppressive requirements for small bowel/liver transplantation. *Transplant Proc 1993; 25: 1204.*
23. Reyes J, Tzakis AG, Todo S, Nour B, Casavilla A, Abu-Elmagd K, Fung JJ and Starzl TE. Nutritional management of intestinal transplant recipients. *Transplant Proc 1993; 25: 1200.*
24. Meijssen MAC, Heineman E, de Bruin RWF, Wolvekamp MCJ, Marquet RL and Molenaar JC. Long-term survival of DLA-matched segmental intestinal allografts in dogs. *Transplantation 1993; 56: 1062-1066.*
25. Meijssen MAC. Functional and immunological aspects of small bowel transplantation, an experimental study in dogs. *Thesis 1993, Erasmus University, Rotterdam.*
26. Reyes J, Abu-Elmagd K, Tzakis A, Nour B, Casavilla A, Kusne S, Green M, Alessiani M, Jain A, Fung JJ, Todo S and Starzl TE. Infectious complications after human small bowel transplantation. *Transplant Proc 1992; 24: 1249.*
27. Berg RD, Wommack E and Deitch EA. Immunosuppression and intestinal bacterial overgrowth synergistically promote bacterial translocation. *Ann Surg 1988; 123: 1359.*
28. Schraut WH, Lee KKW and Sitrin M. Recipient growth and nutritional status following transplantation of segmental small-bowel allografts. *J Surg Res 1987; 43: 1.*
29. Sigalet DL, Kneteman NM and Thomson AB. Reduction of nutrient absorption in normal rats by cyclosporine. *Transplantation 1992; 53: 1103.*
30. Bamba T, Vaja S, Murphy GM and Dowling RH. Role of polyamines in the early adaptive response to jejunectomy in the rat: effect of DFMO on the ileal villus: crypt axis. *Digestion 1990; 46(suppl 2): 410.*
31. Kripke SA, De Paula JA, Berman JM, Fox AD, Rombeau JL and Settle RG. Experimental short-bowel syndrome: effect of an elemental diet supplemented with short-chain triglycerides. *Am J Clin 1991; 53: 954.*
32. Grant JP and Snijder PJ. Use of L-glutamine in total parenteral nutrition. *J Surg Res 1990; 48: 1.*
33. Frankel WL, Zhang W, Afonso J, Klurfeld DM, Don SH, Laitin E, Deaton D, Furth EE, Pietra GG, Naji A and Rombeau JL. Glutamine enhancement of structure and function in transplanted small intestine in the rat. *JPEN 1993; 17: 47.*
34. Barbul A, Wasserkrug HL, Seifter E, Rettura G, Levenson SM and Efron G. Immunostimulatory effects of arginine in normal and injured rats. *J Surg Res 1980; 29: 228.*
35. Cheung AHS, Ferguson DC, Jiranek GC, Haggitt RC, Silverstein FE and Perkins JD. Experimental use of high-frequency ultrasound to image bowel wall after porcine intestinal transplantation.*J Pediatr Surg 1993; 28: 591.*
36. Millard PR, Dennison AR, Hughes DA, Collin J and Morris P. Morphology of intestinal allograft rejection and the inadequacy of mucosal biopsies in its recognition. *Br J Exp Path 1986; 67: 687.*
37. Grant D, Hurlbut D, Zhong R, Wang P, Chen H, Garcia B, Behme R, Stiller C and Duff J. Intestinal permeability and bacterial translocation following small bowel transplantation in the rat. *Transplantation 1991; 52: 221.*
38. Meijssen MAC, Heineman E, de Bruin RWF, Marquet RL and Molenaar JC. The value of serum N-acetyl hexosaminidase in detecting canine intestinal allograft rejection. *Transplant Proc 1991; 23: 615.*
39. Sigalet DL, Kneteman NM, Fedorak RN, Kiziski T and Thomson ABR. Intestinal function following allogeneic small intestinal transplantation in the rat. *Transplantation 1992; 53: 264.*
40. Starzl TE, Demetris AJ, Murase N, Thomson AW, Trucco M and Ricordi C. Donor cell chimerism permitted by immunosuppressive drugs: a new view of organ transplantation. *Immunol Today 1993; 14: 326.*
41. Nguyen BL, Thompson JS and Sharp JG. Comparison of techniques for harvesting enterocytes for transplantation. *J Surg Res 1993; 54: 157.*
42. Plapler H and Cohen Z. Colon transplantation: a new microvascular technique. *Microsurgery 1993; 14: 211.*

SUMMARY

Chapter 1

This chapter treats the role of non-nutritive and nutritive factors in promoting adaptation of the remaining small-bowel (SB) after massive SB resection, and summarizes the present state of experimental and clinical small-bowel transplantation (SBT) in growing individuals. Following extensive SB resection, total parenteral nutrition (TPN) is indispensable for a short or long time. The influence of enteral nutrition on the intestinal adaptation process has been widely recognized, although little information is available on the precise functioning of the complicated process of intestinal adaptation found after SB resection. It is unknown whether the adaptative response can still be optimalized and which diet is most suited in the adaptive phase. About 2-3 patients per million of population per year turn out to have irreversible intestinal failure and remain totally dependent on TPN. These patients have a considerable risk of serious complications, and the long-term outlook for children is even less favorable because they have a higher risk of liver impairment than adults. Moreover, children have even limited adequate venous access and need specific nutrition to grow and develop normally. One reasonable alternative to TPN is SBT. This technique is on the verge of becoming an established technique in transplant medicine, although there is still no experimental evidence that an intestinal graft is able to maintain growth, development, and maturation in juvenile recipients. Thus, for now, ethical decision making has to be on basis of full awareness that the transplantation procedure still has an experimental character.

Chapter 2

In this chapter, the aims of the experimental work are formulated. On the one hand, adaptation-related experiments are performed to get insight in the intestinal adaptation process and the contribution of predigested nutrition. On the other hand, transplantation-related experiments are performed.

Chapter 3

An in vivo electrophysiologic technique, by which transepithelial potential differences in response to theophylline- or glucose containing test solution are measured, was used to monitor intestinal adaptation both 3 and 10 weeks following 90% SB resection in rats. The experiments reveal that in the early phase of adaptation the histologic changes precede in vivo electrophysiologic parameters. This points at a separation between morphologic and functional adaptive changes, and indicates that different factors (both nutritive and non-nutritive) may regulate the morphologic and/or functional adaptation differently.

Chapter 4

This chapter examines the relative importance of food complexity and food composition in promoting intestinal adaptation after SB resection in rats. To this end the rats were fed either normal rat chow, an diet with partial hydrolzed proteins, an identical diet with intact proteins, or a non-identical diet with intact proteins. Two and 10 weeks after resection the adaptive state was evaluated by means of the following parameters: metabolic status, nutritional serum parameters, postheparin DAO activity, histology, and crypt cell proliferative activity. The results show that a diet with partail hydrolzed proteins is as effective as an identical diet with intact proteins in initiating and supporting adaptation. In the first two weeks, the non-identical diet with intact proteins was superior to these diets, suggesting that the influence of diet composition on the early adaptive phase following resection is superior to that of diet complexity.

Chapter 5

The non-immunologic effects of SBT in a growing individual were investigated by performing one-stage total orthotopic SB autotransplantation in dogs. This operation technique had an high mortality rate due to non-technical complications (80%). Analysis of the surviving animals showed that the function of an autotransplanted bowel is not impaired, neither in the short term (paragraph 5.1) nor in the long run (paragraph 5.2). Adaptive functional and morphologic changes were seen after one year together with bacterial overgrowth in terminal ileum and colon.

Chapter 6

The value of ultrasonography for monitoring the postoperative course after SBT was determined in growing dogs. It appeared that this technique is useful in monitoring early complications following SBT.

Chapter 7

In this chapter, the role of a two-stage segmental SB allotransplantation in the treatment of the short bowel syndrome in growing dogs was investigated. The dogs received cyclosporine A; CsA (20 mg/kg/d) as immunosuppressive agent. It appeared necessary to add selective decontamination and early gastrostomy feeding to the perioperative management protocol. Six months follow-up learned that a segmental intestinal transplant with CsA functions suboptimally in maintaining the normal growth and development. This means that without specific nutritional interference in the early postoperative phase, a segmental intestinal allotransplant, under CsA, has not the potential to maintain normal growth in a growing individual.

Chapter 8

Paragraph 8.1 presents a review of the historical background and the biochemic- and functional aspects of DAO. The value of this enzyme as serum marker of acute rejection following allogeneic total orthotopic SBT was studied in rats (paragraph 8.2). The results indicate that monitoring of serum DAO has no value in early detection of acute rejection of SB grafts. The significance of postheparin DAO assessment for the evaluation of graft function was investigated in paragraph 8.3. It appeared that postheparin DAO activity is a reliable indicator for graft function, showing that syngeneically transplanted rats had a higher activity during the first 4 months compared to allogeneic animals. This implies that immunologic factors have a negative effect on the adaptive response of the intestine following SBT.

SAMENVATTING

Hoofdstuk 1

In dit hoofdstuk wordt uiteengezet welke rol voeding en andere componenten spelen bij stimulatie van het adaptatieproces van de overgebleven dunne darm na uitgebreide dunne-darm resectie. Daarnaast wordt de huidige stand van zaken besproken van zowel de experimentele als de klinische dunne-darm transplantatie bij kinderen. Na uitgebreide dunne-darm resectie is totale parenterale voeding (TPV), voor kortere of langere tijd, onmisbaar. Dat enterale voeding van betekenis is voor het intestinale adaptatieproces is algemeen bekend, hoewel we weinig weten over hoe de adaptatie nu eigenlijk tot stand komt. Het is daarom onduidelijk of de adaptatierespons nog kan worden verbeterd en met welke voeding een optimaal resultaat behaald kan worden. Ongeveer 2-3 patiënten per miljoen inwoners per jaar blijken onomkeerbaar darmfalen te hebben, en derhalve levenslang afhankelijk te zijn van TPV. Deze patiënten lopen een groot risico om één van de vele complicaties te ontwikkelen die met TPV gepaard gaan. Vooral voor kinderen zijn hierdoor de uitzichten op de lange termijn beperkt. Het transplanteren van de dunne darm zou een alternatief kunnen zijn voor behandeling met TPV. Darmtransplantatie staat op het punt om een erkende techniek te worden in de transplantatiegeneeskunde, hoewel er momenteel nog geen experimenteel bewijs is dat een darmtransplantaat in staat is om de groei en ontwikkeling van een kind te waarborgen. Daarom zal heden ten dage een beslissing over het uitvoeren van een dunne darmtransplantatie bij een groeiend individu genomen moeten worden op basis van een ethische afweging.

Hoofdstuk 2

In dit hoofdstuk worden de doelstellingen van het experimentele werk geformuleerd. Enerzijds zijn er experimenten uitgevoerd om inzicht te verkrijgen in het intestinale adaptatieproces en de rol van voorverteerde voeding op dit proces. Anderzijds zijn er experimenten uitgevoerd om een aantal aspecten van dunne darmtransplantatie te bestuderen.

Hoofdstuk 3

Een in vivo electrofysiologische techniek, waarmee transepitheliale potentiaalverschillen als reactie op een theophylline of glucose bevattende testoplossing worden gemeten, werd gebruikt om zowel drie als tien weken na een 90% dunne-darmresectie het intestinale adaptatieproces in de rat te bestuderen. In een vroeg stadium van de adaptatie bleken de histologische veranderingen voor te lopen op de in vivo electrofysiologische parameters. Hiermee wordt aangetoond dat er een verschil is tussen de morfologische en functionele aanpassingen. Verschillende factoren (zowel nutritionele als andere) kunnen deze vormen van adaptatie op verschillende wijze beïnvloeden.

Hoofdstuk 4

In dit hoofdstuk wordt in de rat bestudeerd wat het relatieve belang van voorverteerde voeding is bij de bevordering van het adaptatieproces na dunne-darmresectie. Vier groepen ratten kregen respectievelijk normaal ratten-voer, een voeding met partieel gehydrolyseerde eiwitten, of een qua samenstelling hieraan identieke dan wel niet identieke voeding met intacte eiwitten. Twee en tien weken na de resectie vond evaluatie van de adaptatie plaats met behulp van de volgende parameters: het stofwisselingspatroon, nutritionele serum-parameters, de postheparine DAO activiteit, de histologie en de delingsactiviteit van de crypt cel. De resultaten tonen aan dat de voeding met partieel gehydrolyseerde eiwitten even effectief is als de hieraan identieke voeding met intacte eiwitten bij het initiëren en in stand houden van het adaptatieproces. In de eerste fase bleek de niet-identieke voeding met intact eiwitten superieur te zijn aan de andere geteste voedingen, hetgeen impliceert dat tijdens deze fase de samenstelling van de voeding een grotere rol speelt dan de mate van voorvertering.

Hoofdstuk 5

In dit hoofdstuk worden bij de Beagle pup de niet-immunologische gevolgen beschreven van een één-staps autotransplantatie van de gehele dunne darm, die orthotoop geplaatst is. Deze operatietechniek bleek gepaard te gaan met een hoge postoperatieve mortaliteit (80%). Uit een analyse van de funktie van het transplantaat bij de overlevende dieren bleek dat de funktie van een autotransplantaat zowel op de korte termijn (paragraaf 5.1) als op de lange termijn (paragraaf 5.2) gewaarborgd blijft. Een jaar na transplantatie werden functionele en ook morfologische aanpassingen gevonden naast een bacteriële overgroei in het terminaal ileum en colon.

Hoofdstuk 6

In dit hoofdstuk wordt de waarde van sonographie in de postoperatieve fase na een dunne-darmtransplantatie vastgesteld. Deze techniek is zeer bruikbaar om vroege complicaties vast te stellen.

Hoofdstuk 7

In dit hoofdstuk wordt onderzocht wat het effect is van een twee-staps, MHC getypeerde, allotransplantatie van een gedeelte van de dunne darm op het groeipatroon van de Beagle pup met het korte darmsyndroom. Cyclosporine A (20 mg/kg/d) werd gebruikt als immunosuppressivum. Duidelijk is geworden dat het noodzakelijk is selectieve decontaminatie van de tractus digestivus en, in een vroeg stadium, voeding via een gastrostomie toe te voegen aan het behandelingsprotocol. Observatie gedurende een half jaar wees uit dat tijdens de behandeling met cyclosporine A een suboptimaal groeipatroon

aanwezig is. Hieruit kan worden geconcludeerd dat zonder speciale voeding in de vroege postoperatieve fase een segmentaal darmtransplantaat onder cyclosporine geen normale groei kan waarborgen.

Hoofdstuk 8

In paragraaf 8.1 wordt de historische achtergrond en de biochemische en functionele aspecten van het enzym Diamine oxidase (DAO) besproken. De waarde van de serum DAO concentratie voor het aantonen van acute transplantaatafstoting werd onderzocht bij de rat na een allogene totale orthotope dunne darm transplantatie. Serum DAO blijkt geen goede indicator te zijn voor het aantonen van acute afstoting van een dunne darm transplantaat (paragraaf 8.2). In paragraaf 8.3 wordt de betekenis van de postheparine DAO aktiviteit als indicator voor het funktioneren van het transplantaat vastgesteld. Bij dit experiment werden syngeen getransplanteerde ratten vergeleken met allogeen getransplanteerde ratten. De postheparine DAO aktiviteit bleek bij syngeen getransplanteerde ratten gedurende de eerste 4 maanden significant hoger te zijn. Dit betekent dat de bijkomende immunologische factoren bij allogeen getransplanteerde ratten een vertragend effekt op de adaptatieresponse hebben.

APPENDICES

LIST OF ABBREVIATIONS

AF	alkaline phosphatase
ALB	albumin
ALT	alanine aminotransferase
ASAT	aspartate aminotransferase
BN	Brown Norway
BUN	blood urea nitrogen
c-AMP	cyclic adenosine monophosphate
CCK	Cholecystokinin
CFU	colony forming units
CHOL	cholesterol
CsA	cyclosporine A
DAO	diamine oxidase
DLA	dog leucocyte antigen
GVHD	graft-versus-host disease
HLA	human leucocyte antigen
i.m.	intramuscular
i.v.	intravenous
LCT	long chain triglycerides
MCT	medium chain triglycerides
MHC	major histocompatibility complex
MLC	mixed lymphocyte culture
MLN	mesenterial lymph node
MMC	migrating motor complex
ODC	ornithine decarboxylase
n	number of observations/animals
p	level of significance
PBS	phosphate buffered saline
PD	potential difference
SB	small bowel
SBT	small-bowel liver transplantation
SBS	short bowel syndrome
SBT	small-bowel transplantation
sc	subcutaneous
SCFA	short chain fatty acids
SCT	short chain triglycerides
SD	standard deviation
SDD	selective decontamination of the digestive tract
SH	sham operation
sIgA	secretory immunoglobulin A
TBIL	total bilirubin
TP	total protein
TPN	total parenteral nutrition
TRIG	triglyceride
U	unit
WAG	Wistar Albino Glaxo

LIST OF PUBLICATIONS

Bouwman E, Loonen EM, Wolvekamp M, de Bruin RWF, Jeekel J and Marquet RL. Hyperacute rejection in the guinea pig to rat heart xenograft model. Transplant Proc 1990; 22: 1063-1064.

Bouwman E, Wolvekamp MCJ, de Bruin RWF, Jeekel J and Marquet RL. Long-term survival of hamster heart and skin xenografts in the rat. Transplant Proc 1991; 23: 214-215.

Bouwman E, Wolvekamp MCJ, de Bruin RWF, Jeekel J and Marquet RL. Experimental xenotransplantation in rodents II: skin versus heart grafts. In: Xenotransplantation. Cooper DKC, Kemp E, Reemtsma K White DJG (eds) Springer Verlag Berlin-Heidelberg 1991; 323-329.

Bouwman E, Wolvekamp MCJ, Scheringa M, de Bruin RWF, Bonthuis F, Jeekel J and Marquet RL. Long-term survival of xenogeneic skin grafts in widely disparate combinations. Transplant Proc 1993; 25: 383-384.

Meijssen MAC, Heineman E, de Bruin RWF, Wolvekamp MCJ, Marquet RL and Molenaar JC. Successful canine small-bowel transplantation using major histocompatibility complex matched segmental allografts. Transplant Proc 1992; 24: 1141-1142.

Meijssen MAC, Heineman E, de Bruin RWF, Wolvekamp MCJ, Marquet RL and Molenaar JC. Long-term survival of DLA-matched segmental small-bowel allografts in dogs. Transplantation 1993; 56: 1062-1066.

Wolvekamp MCJ and de Bruin RWF. Diamine oxidase; an overview of historical, biochemical, and functional aspects. Dig Dis 1994; 12: 2-14.

Wolvekamp MCJ, de Bruin RWF, Marquet RL and Heineman E. For assessing small-bowel graft function in rats post-heparin DAO activity is a sensitive monitoring tool and basal plasma DAO activity is not. Accepted for publication in Transplant Proc.

Wolvekamp MCJ, de Bruin RWF, HogenEsch H, Marquet RL and Heineman E. Serum diamine oxidase has no prognostic value in acute small bowel rejection in rats. Accepted for publication in Transplant Proc.

Wolvekamp MCJ, Durante NMC, Heineman E, Meyssen MAC, Bijman J, de Jonge HR, Marquet RL and Molenaar JC. The value of in vivo electrophysiologic measurements in the follow-up of intestinal adaptation after massive small bowel resection in the rat. Gut 1993; 34: 637-642.

Wolvekamp MCJ, Heineman E, Marquet RL, de Bruin RWF and Molenaar JC. MHC-matched segmental small-bowel allotransplantation in enterectomized growing dogs: an adequate therapeutic modality. Submitted for Pediatric Research.

Wolvekamp MCJ, Marquet RL, de Bruin RWF, Geerling RA and Heineman E. The effect of diets with intact or partial hydrolyzed protein on functional adaptation after massive small bowel resection in the growing rat. Submitted for J Pediatr Surg.

Wolvekamp M, Heineman E, Meijssen M, de Bruin R, Marquet R and Molenaar J. Small bowel transplantation in a growing dog. Acta Chirurgica Austriaca 1992; suppl 97: 3.

Wolvekamp MCJ and Marquet RL. Minireview; Interleukin-6: Historical background, genetics and biological significance. Immunology Letters 1990; 24(1): 1-10.

Wolvekamp MCJ, Robben SGF, Marquet RL and Heineman E. Ultrasonography; a useful tool in the follow-up of small-bowel transplantation in growing dogs. Surgical Research Communications 1994; 15: 183-197.

NAWOORD

De totstandkoming van dit proefschrift is zeker niet het werk van de promovenda alleen en daarom wil ik hier de gelegenheid aangrijpen om een aantal van hen te noemen. Allereerst wil ik mijn promotor, Prof. J.C. Molenaar bedanken, uw steun was onontbeerlijk voor de totstandkoming van dit proefschrift.
Erik Heineman, voor de wijze waarop je ondanks de verhuizing naar Maastricht bij het onderzoek betrokken bleef en telefonisch consulten hield !! Richard Marquet, voor je sturende, maar zeker niet overheersende, aanwezigheid. Bedankt hiervoor. Het is daarom niet meer dan rechtvaardig dat jullie beiden mijn co-promotoren zijn.
Kas (Jaap Kasbergen), Amélie Bijma, Jannie de Kam, Erik Ridderhof, Enno Collij en Rob Meyer voor de manier waarop jullie ervoor zorgden dat een pasgeboren hondje uiteindelijk een dunne darmtransplantatie patiënt werd. Roy Spruyt, ik heb bewondering voor de manier waarop je altijd trouw onze "kinderen" verzorgde, zelfs toen je het zelf niet al te makkelijk had. Pim v Schalkwijk en Lydia v Gastel, voor het uitvoeren van de talloze bloedbepalingen. Pim, hoe staat het met de bloedjes ? Ron Briegoos, bedankt voor de verleende hulp bij de ratten-experimenten.
John Kong A San en Piet Kreeft, voor de tijd die ik bij jullie op de kamer heb mogen doorbrengen.
Simon Robben, voor je welwillendheid om vlak na zonsopgang het Echo-apparaat te komen bedienen en dat terwijl een Beagle toch echt geen varken is. Ko Haagoort, voor de constructieve verbeteringen van het Engels. Alle Marqueto's, want een prettige werksfeer is een *conditio sine qua non* voor het behalen van goede onderzoeksresultaten. Mijn Leidse collega's, Annemiek Töns en Marie-Therèse te Bulte, zij hebben zonder geklaag mijn afwezigheid opgevangen als ik weer eens een dagje thuis zat te schrijven.
Pa en Ma, behalve om de triviale reden dat zonder jullie dit boekje er nooit zou zijn geweest denk ik dat de manier waarop jullie mij "gegidst" hebben minstens even zwaar weegt.
Mijn paranimfen Ronald en Eelco, bedankt voor de morele steun bij de laatste loodjes.
En vooral Roon, want zonder jou had ik de finish in ieder geval niet op deze manier gehaald.
Tot slot Peter, bedankt dat je mijn uitlaatklep hebt willen zijn voor alle proefschriftperikelen maar nog meer voor je liefde en geloof in mij. Dankzij jou ben ik nu in staat de grenzen te verleggen voorbij mijn eigen horizon.

CURRICULUM VITAE

De auteur van dit proefschrift werd geboren op 30 maart 1968 te Rotterdam. Het VWO-diploma werd behaald in juni 1986 aan de Thorbecke Scholengemeenschap te Rotterdam. In september van datzelfde jaar werd de studie Medische Biologie begonnen aan de Medische Faculteit van de Rijksuniversiteit te Utrecht. De hoofdvakstage (10 maanden) werd bij de vakgroep Algemene Heelkunde van het Academisch Ziekenhuis Dijkzigt (hoofd: Prof.dr.J. Jeekel) te Rotterdam gelopen, alwaar xenotransplantatie onderzoek werd verricht. Een tweede stage (5 maanden) werd gelopen bij de vakgroep Medische Fysiologie van het Academisch Ziekenhuis Utrecht; hier werd kinematische en kinetische ganganalyse verricht bij kinderen met stoornissen in het looppatroon. Daarnaast werd een stage (3 maanden) gelopen bij de Raad voor Gezondheidsonderzoek, in het kader van het gevolgde bijvak "Algemene Gezondheidszorg en Epidemiologie". Het doctoraalexamen werd behaald in december 1990. In januari 1991 werd zij voor een periode van twee jaar aangesteld als wetenschappelijk onderzoeker bij het instituut Kinderheelkunde van het Sophia Kinderziekenhuis te Rotterdam (hoofd: Prof.dr.J.C. Molenaar) waar het promotieonderzoek begeleid werd door Dr. E. Heineman en Dr. R.L. Marquet. Vanaf 15 april 1993 is zij werkzaam als wetenschappelijk onderzoeker bij de vakgroep Algemene Heelkunde van het Academisch Ziekenhuis Leiden (hoofd: Prof.dr.O.T. Terpstra).
Per 1 juli 1994 zal zij een postdoc plaats gaan bekleden in het Prince Henry's Institute for Medical Research in Melbourne. Hier zal zij met behulp van moleculair biologische technieken het adaptatieproces van de dunne darm na uitgebreide resectie verder gaan onderzoeken.