DEVELOPMENT AND ADAPTATION TO RESECTION OF INFANT RAT GUT

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PROMOTOREN: PROF. DR. D.L. WESTBROEK

PROF. DR. J.C. MOLENAAR

CO-REFERENT: PROF. DR. H.K.A. VISSER

To my father

The work described in this thesis was performed at the Surgical Laboratory, Massachusetts General Hospital and the Department of Surgery, Harvard Medical School, Boston, Massachusetts, U.S.A.

CONTENTS

CHAPTER 1

INTRODUCTION

1.1 Motivation

Infants with malrotation of the gut easily develop midgut volvulus. If this volvulus is not treated immediately, ischemic necrosis of the small bowel may develop rapidly. The treatment of these children requires extensive small bowel resection. Infrequently, children are born with multiple jejunal or ileal atresias or with an 'apple peel' type of small bowel atresia. Both after massive small bowl resections and in severe atresias, the child is left with a very short length of bowel and great difficulties to thrive.

Fortunately, with time both functional and morphological adaptation of the remnant small bowel occurs. In the neonatal period it is not clear whether growth of the small bowel after a massive resection is part of normal growth or whether it is due to, or may be enhanced by, adaptational responses.

Neonates, especially in the recovery stage from intestinal surgery, often have to be fed parenterally, or later, with artificial milk formulas. It is known from experiments in adult animals, that oral feeding stimulates adaptation of small intestine after resections. In the newborn, colostrum seems to provide a special stimulus for gut growth, as has been suggested by animal studies and by experiments in vitro. Therefore, we thought it could be important to examine the effects of colostrum, of breast milk produced later in lactation, and of a commercial milk formula on normal development and on adaptation to resection of the intestine. We also studied the effect of the presence of food in the small bowel on development and adaptation.

Some of the components of breast milk which are not present in artificial formulas are macromolecules, especially γ-globulins, and viable macrophages. Breast milk provides passive immunity in some species and other host resistance factors, protecting the suckling in most species. Furthermore it has a regulatory effect on bacterial colonization in the bowel. Since necrotizing enterocolitis occurs with much lower frequency in breastfed human neonates, breastmilk may play a role in the prevention of this disease by maintaining the normal mucosal barrier to bacteria and

harmful macromolecules. Surgery of the small bowel also influences the uptake and transport of macromolecules. In some studies increased transport was noted due to the loss of mucosal integrity. From studies on adaptation after resection it can be concluded, however, that after surgery premature cessation of transport of macromolecules may occur. Since uncertainty still exists about the effects of surgery on macromolecular transport, this was also studied.

In addition to the stimulating effects of food on adaptation to resection of small intestine, hormones also play a role. Increased plasma levels of gastrin have been reported after intestinal resections. This may result in gastric hypersecretion and a very troublesome acidic diarrhea. On the other hand, many reports exist on the tropic effects of gastrin on the mucosa of the stomach, small intestine and colon. Some of these effects resemble the changes normally occurring in the intestinal mucosa around the time of weaning. During this period a rise in gastrin levels has been noted, and we thought it was important to establish the role of gastrin in the adaptation of intestinal mucosa to resection. Before this can be done however, its role in normal development must be defined.

To study the problems described above, we decided to perform laboratory investigations on the rat. It is a well studied animal and experiments requiring large samples are feasible. Furthermore, in the postnatal development of the rat intestine, periods of accelerated growth can be noted. These occur just after birth as the bowel adjusts to extrauterine life, and in the third week of life, when the rat starts to nibble chow in addition to its diet of breast milk. Afterwards, when suckling stops completely, changes to adulthood in the gastrointestinal tract are more gradual. Therefore, if one is to study the development and adaptation to resection of infant gut in the laboratory, the rat in the period between 10 and 28 days of age has many advantages.

1.2 Objectives

The objectives of the experiments described in this thesis are to find answers to the following questions:-

1. What is the influence of the presence of food in the lumen of the small intestine on development and adaptation to resection?
2. What is the influence of resection of the small intestine on the transport of a macromolecule across the mucosa of the remnant small bowel?
3. What is the influence of colostrum, of 'older' breast milk and of an artificial milk formula on development and adaptation to resection of infant rat gut?

4. What is the role of gastrin in the normal development of the intestine in the rat during the second half of the suckling and the weaning period?

Major relevant data from literature, which have been mentioned without references so far, can be found in the review articles listed at the end of this paragraph. In the second part of this chapter more detailed information on normal development of small and large bowel, and on intestinal adaptation, is given. A succinct review of short bowel syndrome follows.

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1.3 Survey of the literature

1.3.1 Early postnatal development of rat intestine

After birth, at the end of a gestation period of 22 days, the young rat spends some time in the nest with the mother, depending on her protection, body warmth, and breast milk. From day 15 on, it nibbles at other food and in the laboratory situation it is able to survive on rat chow from day 21 onwards. Normally it continues to suckle till day 28. In the following thesis the period from birth till day 15 will be regarded as the suckling period, from day 15 till day 28 as the weaning period (Hahn and Koldovský 1966). Thereafter, till adulthood, we will speak of the growing rat. The early weaning period is accompanied by many changes in intestinal kinetics, function and morphology and has received special interest. For better understanding of the experiments described in chapter 2-6 it is necessary to discuss the normal development of the small and large bowel taking place during the suckling and weaning period in the life of the rat.

Few observations of normal macroscopic growth of the gastrointestinal tract of neonatal rats have been performed, so some facts from other species have to be mentioned. In pigs a 23 percent increase in small intestinal length in the first 24 hours after birth has been noted, and during the next nine days growth with an average of 6 percent daily, is observed resulting after 10 days in a total length growth of 88 percent (Widdowson et al 1976). Length growth of the small intestine of pigs halts after 5 months (McCance 1974). In rats, serosal area does not increase after a body weight of 160 g has been reached (Clarke 1972). In rabbits the same fast growth in length and weight of the small bowel has been noted over the first 24 hours of life, decreasing over the next few days (Hall and Widdowson 1979).

Longitudinal growth and increases in diameter of fetal small intestine implanted subcutaneously in young syngeneic rats have been observed (Leapman et al 1974). Villi appear less than a week before birth in the duodenum and their formation then proceeds caudally reaching the ileum just before birth (Sunshine et al 1971). The total number of villi in the small intestine does not increase after birth (Clarke 1972, Forrester 1972). The number of crypts per villus shows a steady increase from 8-20 in rat ileum with time. A proximodistal gradient can be noted for the number of crypts per villus from duodenum to ileocecal valve (Clarke 1972). In rats of 17 days of age 78 per cent of newly formed crypt cells participate in growth, while at 37 days of age almost all cells (97%) participate in renewal. During this period the turnover time of the enterocytes decreases from 22 to 2.6 days

(Altmann and Enesco 1967, Herbst and Sunshine 1969).

The migration rate of cells formed in the depth of the crypts upwards along the villus is very slow at birth and in the ten-day-old suckling rat it is still 1/8th-2/8th of that in the adult (Koldovský et al 1966). Around day 15 deepening of the crypts with an increase of the labelling index of the crypt cells with 3H-thymidine occurs, together with an increase in small intestinal weight (Herbst and Sunshine 1969).

The RNA/DNA ratio in the mucosa is about 0.8 in suckling animals and, after a short decrease to 0.5, increases rapidly to adult values of 1.4 between day 18-20 (Koldovský et al 1970b). The ratio of RNA/DNA remains similar in all layers of intestinal mucosa (Sunshine et al 1971). A proximodistal gradient exists for gut weight, mucosal weight and mucosal DNA content (Buts and DeMeyer 1981). The concentrations of enzymes of digestion are very low in the crypts and increase as the cells migrate up the length of the villus (Nordstrom et al 1967, Herbst and Koldovský 1972, Boyle et al 1980b).

At the start of weaning the disaccharidases studied in this thesis show typical changes in activity. The levels of the glucosidases sucrase and maltase are undetectable or low during suckling and increase at the time of weaning at day 16-18 (Rubino et al 1964, Doell et al 1965). Diurnal changes are first seen after weaning is completed (Beam and Henning 1978).

The concentration of neutral β-galactosidase (Lactase), present in the brush border, is high in the immediate postnatal period, showing a jejuno-ileal gradient, and decreases to low adult levels at the time when sucrase appears (Doell and Kretchmer 1962, Koldovský and Sunshine 1970a). The decrease of lactase is the same in isografts as in normal suckling and weaning animals (Kendall et al 1979). In adult animals an increased lactase/sucrase ratio from crypt to villus can be observed (Boyle et al 1980b).

To receive full passive immunity, rodents are partly dependent on selective transport of intact homologous γ-globulins, mainly IgG, across the intestinal mucosa, especially in the jejunum, during the first days after birth (Rodewald 1973). Other macromolecules are transported non-selectively both in jejunum and ileum (Clark 1959, Walker et al 1972). Some workers, however, could only demonstrate uptake of macromolecules such as bovine serum albumin into the enterocyte and no transport into the blood. At the same time both uptake and transport of γ-globulins from a number of different species in mice of 1-17 days of age, was noted (Lecce 1972). In rats transport into the blood of both heterologous IgG and heterologous serum albumin occurs (Telemo et al 1982). Uptake occurs mainly by a pinocytic mechanism, with IgG selectively attached to Fc-receptor sites on the microvillous membrane, protecting it from lysosomal digestion (Rodewald 1976, Waldman and Jones 1976). Hereafter, significant quantities of γ-globulins and smaller amounts of other macromolecules are deposited by exocytosis into the baso-lateral intercellular space and then into the blood. This completes the transport of macromolecules across the intact intestinal mucosa. The cessation of both selective and unselective transport of macromolecules (briefly called mucosal closure) occurs in rats on day 18-20 accompanied by a disappearance of large vacuoles from the apical cytoplasm (Halliday 1955, Clark 1959,

Graney 1968, Abrahamson et al 1979).

Gastrin producing cells (G-cells) have been found in the pancreas of 18-day old rats and in the antral and duodenal mucosa of 4-day old rats (Larsson et al 1976). However, no gastrin activity was found in antral extracts till day 21, when it rapidly increased to adult levels (Zelenkova and Gregor 1971, Lichtenberger and Johnson 1974). Gastrin increases hydrochloric acid output by oxyntic cells in the gastric fundus and has been proven to be the humoral mediator released after mechanical stimulation of the antrum (Grossman et al 1948). Hydrochloric acid secretion is noted shortly after birth and increases sharply till day 6, whereafter a more gradual increase is noted (Zelenkova and Gregor 1971).

1.3.2 Influences of hormones on development of the small bowel

Corticosteriods, thyroxine and gastrin are the hormones which have been studied most in relation to the development of the gastrointestinal tract in the postnatal period.

Preceeding the changes in disaccharidase levels mentioned in paragraph 1.3.1, an upsurge in glucocorticosteroid titer in plasma can be noted, together with increased sensitivity in the intestinal mucosal cells to glucocorticosteroid stimulation (Herbst and Koldovský 1972, Daniels et al 1973). Hypophysectomy in 6-day-old rats results in lower sucrase and prolonged lactase activity at day 24, when compared to controls (Yeh and Moog 1974). Adrenalectomy slows the normal decrease in lactase activity between day 16 and 20 in rats (Koldovský et al 1964) and prevents precocious increase of sucrase in prematurely weaned rats (Boyle and Koldovský 1980a). It also delays the increase of sucrase activity in the gut in situ and in gut transplanted under the kidney capsule of isogeneic adult rats (Kendall et al 1979, Koldovský 1981). Injection of glucocorticosteriods into adrenalectomized or hypophysectomized rodents results in normalization of the development of sucrase activity (Yeh and Moog 1975). Injections of glucocorticosteriods into intact suckling rats increase relative weight, mitotic index and crypt depth in the jejunum (Herbst and Sunshine 1969) and can induce precocious increase of sucrase activity (Doell et al 1965, Herbst and Koldovský 1972, Lebenthal et al 1972). Part of this maturative effect is an increased RNA/DNA ratio (Koldovský et al 1970b). Injections of corticosteriods into adult mice hosts of fetal isografted intestine produce precocious sucrase activity in these grafts (Ferguson et al 1973). After glucocorticosteriod treatment sucrase appears first in the crypt-villus junction area and sucrase activity increases rapidly as cells migrate along the villus (Herbst and Koldovský 1972).

Although increased lactase activity has been found after glucocorticosteroid injections to suckling rats, neither adrenalectomy nor glucocorticosteroid injections to adrenalectomized suckling rats was found to affect lactase activity (Koldovský and Sunshine 1970a, Lebenthal et al 1972).

Precocious mucosal closure can be induced by injection of cortisone acetate from the fifth day while transport of macromolecules can be prolonged by adrenalectomy (Daniels et al 1973).

The decrease in lactase activity and increase in sucrase activity in early weaning is delayed by early thyroidectomy. Administration of thyroxine counteracts this effect (Yeh and Moog 1974, Koldovský et al 1975). Thyroxine injections into suckling rats produce a precocious increase of sucrase activity, independent of the presence of the hypophysis (Yeh and Moog 1975). Thyroxine effect is dose dependent and sensitivity towards this hormone increases during suckling (Koldovský 1981).

After hypophysectomy in adult rats, serum and antral gastrin levels are decreased, and can be restored by injections of growth hormone (Enochs and Johnson 1976). Injections of pentagastrin to suckling rats between day 14 and 24 has no effect on lactase activity. However, a slight effect on gut wet weight, RNA and protein per gram bodyweight is present in rats prevented from weaning (Lichtenberger and Johnson 1974). Gastrin also exerts a tropic action when given intraluminally in the ileum or after subcutaneous or intraperitoneal injections (Johnson et al 1978). Pentagastrin is the only hormone whose presence is mandatory in a chemically defined medium capable of sustaining development of fetal rat jejunum (DeRitis et al 1975).

1.3.3 Influence of nursing and starvation on intestinal development

The presence of food in the gastrointestinal tract for the first time in life seems to have strong effects on the gastrointestinal tract itself. Some researchers ascribed these effects specifically to colostrum. In piglets, after suckling for 24 hours, increased gut weight and DNA content was noted (Widdowson et al 1976). The authors ascribed this increase to colostrum, but it may also be noted after feeding breastmilk produced later in lactation, since controls were waterfed. In puppies raised on artificial bitch milk, jejunal mass, protein, RNA and DNA content reached the same levels after 120 hrs, as in suckling pups after 24 hrs. In this rapidly proliferating mucosa an immature villus cell population was noted as measured by a decreased lactase specific activity (Schwartz and Heird 1981). However, in an earlier report from the same laboratory increased sucrase and lactase content was noted after colostrum suckling when compared to formula feeding (Heird and Hansen 1977). Stressfeeding induces precocious mucosal closure, and starvation results in prolonged macromolecular transport (Lecce 1973). The presence of a 'mucosal growth factor' in breast milk was suggested (Walker 1979) after the finding of increased DNA synthesis in epithelial cells and fibroblasts in culture, due to addition of supernatant of human colostrum to the culture medium (Tapper et al 1979).

Sucrase activity can be induced in normal rats by sucrose feeding but not after adrenalectomy (Lebenthal et al 1972). After lactose feeding increased lactase activity is present for a prolonged period. After feeding a diet high in glucose after weaning, the same prolonged activity of lactase was found (Koldovský et al 1964, Bolin et al 1969).

In the literature, untimely removal of the lactating dam is usually referred to as premature weaning. In 16-day-old adrenalectomized rats premature weaning carries a 100 percent mortality after 3 days while intact animals all survive (Boyle and Koldovský 1980a). Undernutrition from week 3 to 6, after a period of rapid growth in the first weeks of life, results in lighter small intestines as compared with the small intestine of steadily growing animals of the same weight (Widdowson and McCance 1963). Undernutrition from birth till day 19 results in decreased body weight with decreased intestinal weight and total DNA content. DNA and protein per gram wet weight remain unaltered. Synthesis of brush border membrane lipids is increased by undernutrition from birth till day 21 (Pathak et al 1981). Under these circumstances sucrase specific activity is diminished while lactase specific activity is elevated (Hatch et al 1979). If rats are starved or prematurely weaned at day 16, however, increased sucrase specific activity results. This increase is mediated by the adrenals (Boyle and Koldovský 1980a). Intact, prematurely weaned or starved 16-day-old rats preserve their jejunal protein content in the presence of decreased body weight. Starvation of 10-day-old rats results in a high mortality and a 35 percent loss of both body weight and intestinal weight in survivors (Hahn and Koldovský 1966). At all ages the total number of villi is unchanged after undernourishment with a decreased number of crypts (Clarke 1972).

1.3.4. Features and mechanisms of intestinal adaptation

Adaptation of the small bowel to resection consists of structural, kinetic and functional changes, which have been well studied in adult rats. Where no species are mentioned in the following section, the experiments were performed on rats.

Increased villous height and diameter of remnant bowel in dogs was already noted in 1912 by Flint. It is accompanied by increased crypt depth and some lengthening of the bowel in rats (Nygaard 1967). Mucosal wet weight and dry weight increases with increased content of nucleic acids and other proteins and increased DNA synthesis (Obertop et al 1977, Oscarson et al 1977, Williamson et al 1978b, c). True hyperplasia results, with smaller cells (Weser and Hernandez 1971). The total number of villi may be increased (Nygaard 1967) or unchanged (Forrester 1972). Increases in RNA and DNA content and DNA synthesis have been shown in the mucosa of the ileum within 48 hours after jejunectomy and reach a peak after seven to twelve days (Obertop et al 1977, Williamson et al 1978b). Continuing adaptation thereafter may be achieved by elongation of residual bowel (Williamson) 1978a)

although our preliminary results failed to show adaptational elongation four weeks after resection in suckling rats (De Vries et al 1980). In the crypt both total number of cells and the number of proliferating cells increase, resulting in an unchanged mitotic index (McDermott and Roudnew 1976). These cells have a great migration velocity along the elongated villus, resulting in a stable turnover time and a new steady state (Gleeson et al 1972, Dowling and Gleeson 1973).

The structurally adapted ileum after proximal small bowel resection is capable of enhanced absorption of glucose and water per cm bowel (Dowling and Booth 1966, Bury 1972). However, disaccharidase specific activity is normal or decreased, suggesting comparative immaturity of enterocytes in the hyperplastic mucosa (Weser and Hernandez 1971, Bury 1972)

These changes are mainly caused by intraluminal and humoral factors (Bauer 1978). As absorption of nutrients is normally complete in the jejunum, abnormally rich chyme in the ileum after proximal enterectomy directly stimulates mucosal hyperplasia (Dowling and Booth 1967). Topical nutrition can maintain the normal deminishing aboral gradient of villous height and also explains the greater enteric adaptation after proximal than after distal resection (Altmann and Enesco 1967, Levine et al 1974). Dogs on parenteral nutrition show reduced villous height in the remaining ileum after 50 percent proximal small bowel resection as compared to values during enteral feeding (Feldman et al 1976).

Apart from exogenous nutrition, endogenous secretions from the first part of the alimentary tract also play an important role in mucosal adaptation. Gastric and duodenal juice both increase the size of ileal villi, with pancreatobiliary secretions exerting the strongest effect (Altmann and Leblond 1970, Williamson et al 1978b). Augmentation of post-resectional hyperplasia by concomitant diversion of the papilla of Vater, to the remnant ileum, still occurs if rats are fed an elemental diet instead of chow (Weser et al 1977). This shows that the trophic effect of pancreatic juice cannot entirely depend on digestion of protein and release of aminoacids for topical nutrition.

Altered chyme or enteric secretions cannot explain jejunal hyperplasia after distal enterectomy (Dowling and Booth 1967) although it may only develop in the presence of hyperphagia (Menge et al 1975). Midgut bypass stimulates growth of jejunoileal remnants in the absence of pancreatobiliary secretions (Fenyö 1977). Moreover, the rapidity and extent of the adaptive response, even to transection of the bowel in the operated animal and in its parabiotic partner, suggests involvement of a humoral stimulant (Williamson et al 1978c). Generalised hyperplasia observed after a 10 percent enteric resection has been attributed to an intestinal epithelial growth hormone (Loran and Crocker 1963). Humoral influences may account for increased proliferative activity in mucosal fragments implanted beneath the kidney capsule of animals subjected to a 50 percent midgut resection (Tilson and Livstone 1975).

After intestinal resections increased gastrin levels have been reported by some, but not all workers (Straus et al 1974, Niessen et al 1978). Gastrin exerts a tropic action on the gastrointestinal tract, as has been mentioned in the previous section

(MacGregor and Way 1976, Johnson 1977). A single intraperitoneal injection of pentagastrin increases 3H-thymidine uptake in duodenal and colonic crypts (Mak and Chang 1976). Continuous infusion of pentagastrin prevents the enteric hypoplasia of parenteral nutrition (Johnson et al 1975). Treatment with 3 daily s.c. injections of pentagastrin increases relative weight, protein and DNA content of the intestine in delayed weaning (Lichtenberger and Johnson 1974). Chronic endogenous hypergastrinemia increases intestinal weight (MacGregor and Way 1976). On the other hand, 20-fold variations in endogenous gastrin levels do neither prevent hypoplasia induced by starvation nor affect compensatory hyperplasia in the ileal remnant after jejunectomy (Ocarson et al 1977). Antrectomy does not influence post-resectional hyperplasia (Hughes et al 1976). Crypt cell production rate after 75 percent small bowel resection in rats on various diets shows no relationship with gastrin levels, but a correlation with levels of enteroglucagon has been found (Sagor et al 1982).

Glucocorticosteroids have been shown to increase intestinal cell proliferation, while adrenalectomy results in mucosal atrophy (Tutton 1973). Other factors acting on intestinal cell proliferation include neural and neurovascular changes and incrased blood flow to the ileum after midgut resection (Touloukian et al 1972a, Touloukian and Spencer 1972b, Tutton 1977). Total abdominal vagotomy results in reduction of mucosal thickness with an increase in cell turnover (Liavåg and Vaage 1972).

In conclusion, it has been shown that luminal influences play an important role but cannot explain all aspects of compensatory responses of the intestinal mucosa to partial resection. Most experiments have been performed in adult animals and the contribution of normal development to adaptation in fast growing neonatal animals has not yet been fully explored.

1.4 Some aspects of short bowel syndrome

In recent years, improved quality of surgical, anesthesiological and nutritional management has resulted in increased patient survival after extensive small bowel resections and after restoration of intestinal continuity in multiple small bowel atresias. Understanding of intestinal adaptation and accurate management of nutritional problems are necesary to obtain optimal results in the care of these patients. In neonates and infants interactions with normal growth make the clinical problem even more complicated.

The limiting factor in short bowel syndrome is the nutrient absorption from the short small bowel. This is influenced by, among other factors, extent and site of the resection, the presence of the ileocecal valve, the transit time of food through

the bowel and the adaptation of the remaining intestine.

If less than 75 cm of small bowel in addition to an intact duodenum is preserved, survival is greatly reduced (Rickham 1967). Survival after resection of all but 15 cm of the small intestine has been reported in children (Tepas et al 1978). In normal small bowel absorption of most nutrients and water soluble vitamins occurs primarily in the duodenum and jejunum. The ileum is the site of absorption of bile acids and of vitamin B12. Small resections of the distal ileum may result in a reduced enterohepatic circulation of bile acids and depletion of the bile salt pool. This may produce severe diarrhea (Mekhjian et al 1971, Ammon and Phillips 1973), while the water absorption in the colon is further reduced by the presence of unabsorbed fatty acids and bile acids (Hofmann and Poley 1972). The presence of the ileocecal valve may result in a prolonged transit time and protect the shortened bowel against bacterial overgrowth with coliform bacteria, capable of deconjugating bile salts (Gazet and Kopp 1964). In an adult man with only 140 cm proximal jejunum ending in a jejunostomy, best bile acid absorption was achieved with a high fat diet (Simko et al 1980).

Although in dogs the existence of the lower gut has no significant influence upon the regular periodic occurrance of the interdigestive migrating contractions in the stomach and on their migrating time, retrograde intestinal pacing has been shown to increase the absorption of water, glucose, sodium and potassium in segments of canine jejunum (Takeuchi et al 1980, Gladen and Kelly 1980). In rats, construction of intestinal pouches results in increased transit time after intestinal resection (Deliere 1980). Glucagon has an inhibitory effect on gastrointestinal motility (Necheles et al 1966). After 75 percent resection in rats elevated levels of enteroglucagon have been found (Sagor et al 1982).

In man, absorption improves with time after small bowel resection, but the changes in morphology are less striking than in animals. Dilatation of remnant bowel has been observed (Althausen et al 1950, Benson et al 1967, Sheldon 1979). Elongation of up to 185 percent of initial length has been reported in adults (Scheflan et al 1976) while Harrison and Booth (1960) did not note any increase in length growth. In infants Moe (1964) observed only normal length growth. Moreover, increases in villous height have not been reported, although the total number of enterocytes per segment of bowel seems to be increased (Porus 1965, Weinstein et al 1969). Nevertheless, enhanced absorption per segment of bowel of protein, carbohydrate, fat, water and electrolytes has been demonstrated after small bowel resections (Althausen et al 1950, Dowling and Booth 1966, Weinstein et al 1969, Scheflan et al 1976). Despite increased electrolyte absorption in patients after enteric resections, low plasma volume, total body sodium with high plasma renin activity and aldosterone level has been found (Ladefoged and Ølgaard 1979). Parenteral supply of calcium, magnesium and zinc seems indispensible (Ladefoged et al 1980). Initially, a reduction in lactase activity occurs, that may result in inadequate hydrolysis of lactose and osmotic diarrhea (Richards et al 1971).

Gastric hypersecretion may be a troublesome event after intestinal resection, usually improving with time (Windsor et al 1969,

Buxton 1974, Niessen et al 1978, Meyers and Jones 1979). Cimetidine has been shown to decrease both gastric acid output and gastric secretion volume, resulting in improved nutrient absorption (Cortot et al 1979, Murphy et al 1979). Increased levels of gastrin have been reported after small bowel resection in man and dogs, and may result from decreased gastrin catabolism by reduced small bowel mass (Becker et al 1973, Straus et al 1974, Wickbom et al 1975).

Most patients with massive small bowel resections have to be kept on parenteral alimentation initially (Sheldon 1979). With careful selection of oral feeding a gradual withdrawal of parenteral nutrition can usually be achieved (Tepas et al 1978). In infants breast milk from the own mother can be used successfully even after lactation has stopped for several months (Brink 1977).

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CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 Animals and timing of the experiments

For experiments with neonatal animals, pigs, rabbits, and dogs have been widely utilized in the past. One of the reasons why rats were not used, was that prior to 1967 it was impossible to make nursing rats accept offspring that had been operated upon or even simply handled by researchers (Rickham 1967). The mother rat shows a distressing tendency to cannibalism of her offspring if they are disturbed. Elaborate schemes have been proposed to produce habituation of the dams to the researcher by daily handling of the pregnant rats. After delivery several special measures have been proposed (Libbin and Person 1979). Recently, others communicated a similar finding to ours, that cannibalism does not occur if the operated newborn are allowed sufficient time, i.e. 1-2 hours after surgery, to recover from the effects of the operation and anesthesia (Reynolds 1981). All experiments were performed on 10-day-old suckling Wistar rats of both sexes, supplied by Charles River Breeding Laboratories, Wilmington, Massachusetts.

The animals were shipped on the 6th or 7th day of life to give the dam and her litter of 10-11 suckling rats sufficient time to accommodate to the plastic cages with wood chips as nesting material. The cages were placed in racks in the animal room of the Shriners Burns Institute, Boston Ma., with alternate 12 hour lighting cycles. Purina rat chow and water were provided ad libitum and weaning was not interfered with, unless stated otherwise.

As outlined before, the period in the life of the young rat of special interest to us, is a period of rapid changes in the development of the gastrointestinal tract, the weaning period. All animals were operated on at 10 days of age. In the short term experiments described in chapter 4 and 5, the animals were sacrificed at day 15, to enable us to note precocious changes of maturation, normally occurring later, in the third week of life. In the gastrin experiment described in Chapter 6, animals were sacrificed at day 15, 21, and 27, or at the beginning, in the middle or at the end of the weaning period. This made it possible to observe normal

development in this important period in the life of the rats, when they change from breast milk to other food. In the long term experiment described first, sacrifice was postponed till weaning was fully completed, four weeks after the initial operation.

2.2 Operative procedures

Before operation animals were not starved and a full stomach was usually noted at laparotomy. Prior to operation the animals received 2.4 mg kanamycin and ±2000 U penicillin G in Ringers lactate solution by subcutaneous injection.

All laparotomies, utilizing a simple operation microscope, were performed under light ether anesthesia through a midline incision. The abdomen was closed with a continuous 6.0 silk or Tevdek suture taking all layers of the abdominal wall. Gut length was measured along a silk thread held on the anti-mesenteric side without stretching of the intestine. Anastomoses of the small bowel were made with 7.0 or 8.0 interrupted silk sutures on an atraumatic needle taking all layers of the bowel wall. In gastric operations the stomach was emptied before reanastomosis, see also figure 6.1, page 64. Antrectomy included the first 0.5 cm duodenum. A new lesser curvature was constructed with a continuous 7.0 silk atraumatic suture. The end-to-end gastroduodenostomy was made with interrupted sutures of the same material taking the whole thickness of the bowel wall. After fundectomy continuity was restored by anastomosing with interrupted sutures, while after rumenectomy the fundus was closed with a single continuous suture of 6.0 silk. In these operations most of the gastrin producing cells, oxyntic cells and a neutral area are resected respectively producing low, high and normal gastrin levels (Håkanson and Liedberg 1970, Oscarson et al 1977). In all three types of operation the capacity of the stomach is reduced by one third.

2.3 Specimens for histology and biochemistry

At the time of sacrifice the abdominal cavity was quickly opened by transverse incision. By displacing the intestine to the left the inferior caval vein was exposed and then punctured to withdraw blood for gastrin measurement when necessary. The stomach, small and large bowel were then removed by cutting the esophagogastric junction and sigmoid and the hepatoduodenal ligament, and mesenteric radix. The postion of the ligament of Treitz was marked. The bowel was stripped of its mesentery in ice cold sa-

line. In most experiments the bowel length was measured suspended with a standard 2 g weight to straighten the gut without stretching. The location of histological and biochemial specimens was identified as described at the individual experiments. Samples of bowel for biochemistry were cut open, rinsed in ice cold saline and the mucosa was scraped off with a glass slide (Bauer 1978). Only in young sucklings of 10-15 days of age this was not always feasible due to fragility of the muscle coat and from these young animals full thickness specimens were taken. After weighing, the samples were then quickly frozen in dry ice and stored at -20° C till further processing. The bowel samples for histometry were also cut open, rinsed in ice cold saline and preserved in 10% buffered formalin.

2.4 Biochemical assays

2.4.1 Nucleic acids

Ribonucleic acids in mucosal scrapings and full thickness gut specimens were determined by the method of Scott et al (1956) Specimens were thawed, homogenized in citric acid sucrose and treated with cold perchloric acid to precipitate the macromolecular fraction. After treatment with 80% ethanol and cold alcohol-ether, to extract the lipids, the precipitate underwent alkaline digestion to hydrolyze the RNA and render it acid soluble. Subsequently, optical density was measured at 260 and 280 mu (Tsanev and Markov 1960). The residual pellets were used for DNA determination according to the method of Burton (1968). They were treated with perchloric acid, heated and filtered. The DNA in the supernatant was calculated from its optical density at 260 mu.

2.4.2 Albumin

Bovine serum albumin (BSA) was obtained from Sigma Chemical Company, St. Louis, Mo., in lyophilized crystalline form. Serum levels were determined by an immuno-electro-diffusion technique using rat serum with known amounts of BSA added as standards (Laurell 1966, Udall et al 1981).

2.4.3 Disaccharidases

Lactase and sucrase activity was assayed using a glucostat reagent (Statzyme, Worthington, Ma.,) made up in 1.0 molar Tris solution (Tsuboi et al 1979). Cytoplasmic and lysosomal lactase (acid β-galactosidase) activity was inhibited by p-chloro-mercuribenzoate (Asp and Dahlqvist 1972).

2.4.4 Gastrin

Serum samples were shipped frozen to Malmo, Sweden for duplicate radioimmunoassay measurements using rabbit antibody to synthetic human gastrin (2-17 SHG; ICI, Ltd., Wilmslow, England), (Rehfeld et al 1972).

2.5 Histological measurements

All specimens were embedded in paraffine, stained with hematoxylin and eosine and sectioned to 4 μ thick slices, longitudinally. Five complete villi and crypts were measured from each specimen. The mean value was used. All specimens were coded prior to sectioning to eliminate observers bias.

2.6 Statistical methods

Student's t-test for unpaired data was used in most experiments. When the word significant is used in this thesis, this means statistically significant ($p<0.05$ for the two sided test). Where other statistical methods had to be applied, this will be indicated.

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CHAPTER 3

THE INFLUENCE OF JEJUNAL RESECTION AND ILEAL BYPASS ON GROWTH OF THE SMALL INTESTINE

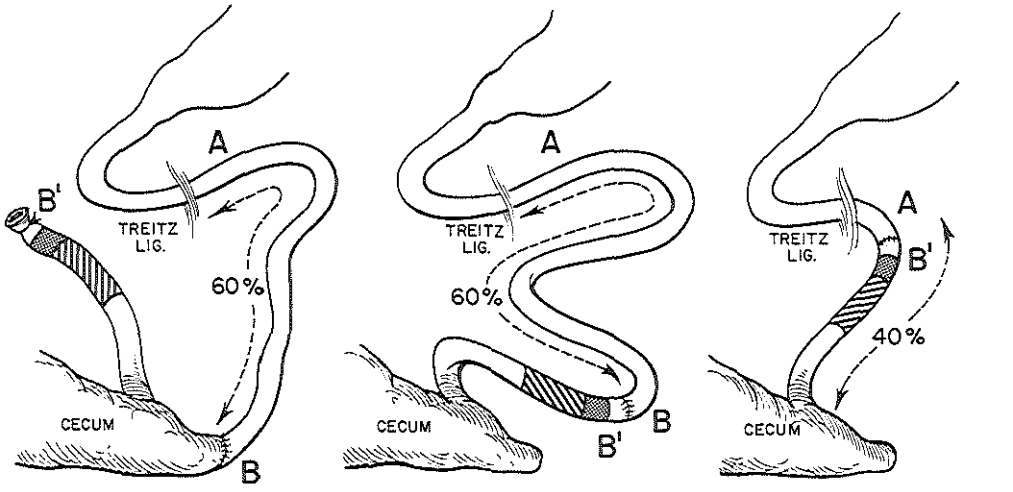
EXPERIMENT 1

3.1 INTRODUCTION

Compensatory longitudinal growth of the small intestine has been documented in adults after massive small bowel resection (Fenyö 1976, Scheflan et al 1976), but most reports note only small increases in length of residual small bowel after resections (eg McClanahan and Fisher 1950), even in children (Moe 1964, Rickham et al 1977). In one report, up to 70 percent length increase in residual gut has been noted in eighteen months following massive intestinal resection in human neonates (Rickham 1967). However, most reports are unclear about increases in length noted in children after intestinal resections (Benson et al 1967). To determine which part of longitudinal growth observed after resection is normal growth, and which part is additional, compensatory growth, is very difficult since figures of normal gut length vary.

The intestine grows rapidly after birth (Sunshine et al 1971, Grand et al 1976, Widdowson et al 1976, Hall and Widdowson 1979) and obligatory growth might be expected to occur even in the absence of functional demand. Some length growth has been noted in isografts of small intestine in rats in the absence of direct stimuli from food present inside the gut (Leapman et al 1974). In young dogs and rats placed on total parenteral nutrition after small resection, diminished longitudinal and mucosal growth occurs (Levine et al 1974, Feldman et al 1976). Both luminal bulk and feeding stimulates growth in adult and neonatal intestine (Widdowson et al 1976, Hall and Widdowson 1979, Ryan et al 1979). Removal of that stimulus by bypass, or increase of luminal stimuli to the ileum by proximal intestinal resection might be expected to produce changes in the subsequent growth and adaptation of the ileum.

To establish whether luminal nutrition influences longitudinal and mucosal growth and adaptation of the ileum of the suckling rat, we performed bypass of the ileum or resection of the proximal small bowel. Animals after transection with reanastomosis of the midgut acted as control, while normal animals were also studied, to document development in this period of rapid growth.



40% Ileal Bypass Transection 60% Proximal Resection

Figure 3.1. Dotted areas represent location of specimens for histology, hatched areas represent the sites used for biochemical analysis in experiment 1.

3.2 Materials and Methods

Ten-day-old rats were used. They were subjected to one of three operations: a 60 percent proximal small bowel resection, a transection with reanastomosis at 60 percent of bowel length or a bypass of the 40 percent distal small bowel (see fig. 3.1). All animals were killed four weeks after operation by exsanguination under light ether anesthesia between 09.00 and 14.00 hours. Unoperated animals of 10 to 38 days of age kept under identical circumstances were used as normal reference animals.

Immediately after sacrifice and measurement of the remnant bowel, from the mid point of the intestine in continuity proximal to the transection or bypass, one centimeter was removed for histological measurements, then a further five cm immediately distal to this was removed for biochemical analysis.

Similar specimens were taken from the ileum of all groups of animals from 1-7 cm distal to the anastomosis or the tied off blind end of bypassed ileum proximally, and from 1-7 cm before the ileocecal valve distally. Although only 80 percent of the ileum

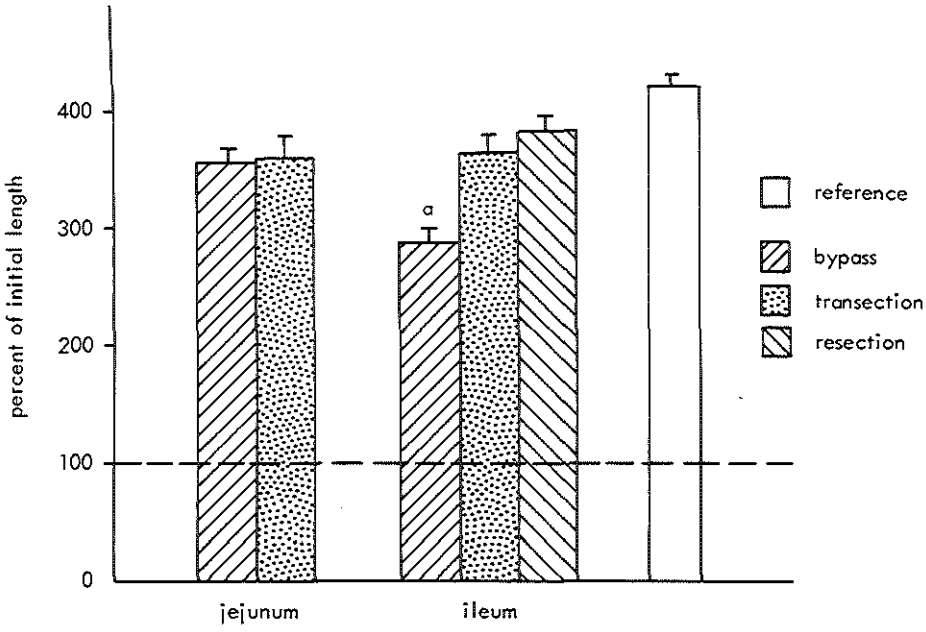


FIGURE 3.2 Final gut length. 100 percent represents initial length. Values are mean ± SEM.
a: $p<0.001$ versus transection and resection.

remains after 60 percent proximal small bowel resection, the proximal part of the remnant will be referred to as 'proximal ileum'. In the unoperated animals specimens from identical areas were taken. Circumference was measured at the longitudinally opened mid-jejunum or proximal ileum. Biochemical assays as described in section 2.4 and histological measurements as described in section 2.5 were performed.

Table 3.1 Weight of experimental animals and reference animals.

	n	Weight in grams (mean ± SEM)
10-day-old normal	10	21.0 ± 0.6
Resection	18	116.4 ± 1.8
Transection	13	118.5 ± 2.2
Bypass	14	116.9 ± 1.2
38-day-old normal	13	149.7 ± 2.1

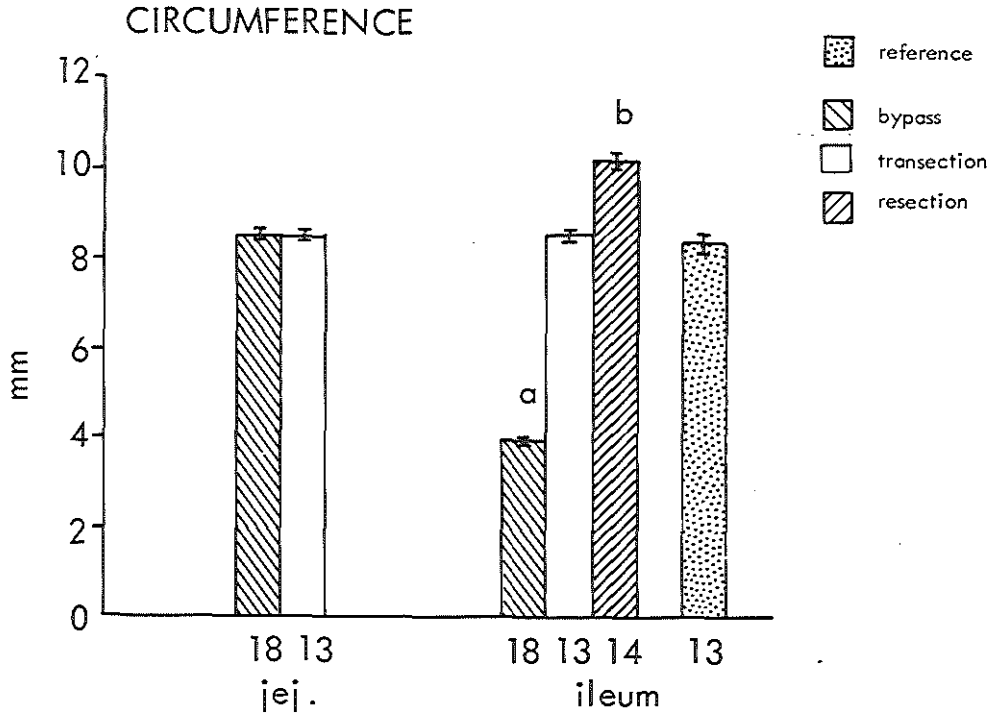


FIGURE 3.3 Circumference. Values are mean ± SEM.
a: $p<0.002$ versus transection and resection
b: $p<0.02$ versus transection

3.3 Results

Sixty of 87 animals survived operation, a mortality of 31 percent. Later animals were selected for weight to obtain comparable groups since with increasing weight, gut length increased (McCance 1974), resulting in 14 animals that had been subjected to resection, 13 that had had transection and 18 after bypass operation. Ten unoperated animals of 10 days-of-age and 13 of 38 days-of-age were used to obtain figures for normal development. The mean weights of all animals are shown in table 3.1

Four weeks after transection or bypass the jejunum had grown to a similar extent but the ileum when bypassed elongated less than after transection or proximal resection (see fig. 3.2). The exact means and SEM's of results given in graphical form can be found in the addendum at the end of this chapter.

The circumference of the bypassed ileum did not increase in the four weeks after surgery, and after proximal resection the ileum showed some dilatation (see fig. 3.3).

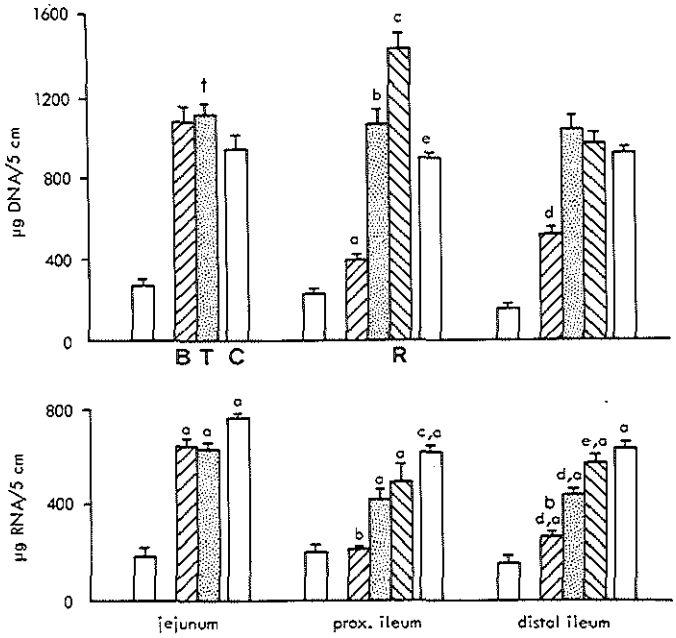


FIGURE 3.4: DNA and RNA content of specimens. White bars to the left of each location denote 10-day-old reference animals. Capital letters refer to bypass, transection, resection, and 38-day-old control animals. Values are mean ± SEM. All DNA values are significantly above 10-day levels.

DNA
a: $p<0.001$ vs transection
b: $p<0.001$ vs resection
c: $p<0.001$ vs bypass
d: $p<0.001$ vs transection, resection and reference
e: $p<0.001$ bypass and resection
$p<0.01$ vs transection
f: $p<0.05$ vs reference

RNA
a: $p<0.001$ vs 10-day controls
b: $p<0.001$ vs resection and reference
c: $p<0.001$ vs transection and bypass
d: $p<0.001$ vs reference
e: $p<0.05$ vs reference

The DNA content of the mucosa is shown in fig 3.4. After proximal enterectomy the DNA content of the proximal ileum was the highest, after transection less and after bypass least. In the distal ileum the DNA content was similar after both transection or resection but lower after bypass. The DNA content of the jejunum did not increase after 40 percent distal bypass. The RNA content of the mucosa showed a pattern similar to the DNA content.

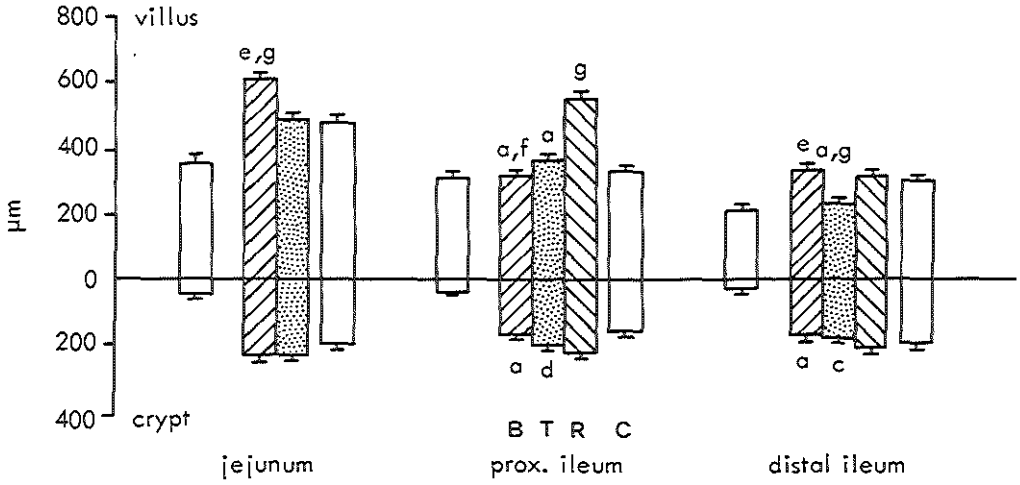


FIGURE 3.5 Villus and crypt. Values are mean ± SEM. Capital letters refer to bypass, transecton, resection and 38-day-old control animals. White bars at the left of each location denote 10-day-old reference animals.

a: $p<0.001$ vs resection
b: $p<0.01$ vs resection
c: $p<0.02$ vs resection
d: $p<0.05$ vs resection
e: $p<0.001$ vs transection
f: $p<0.02$ vs transection
g: $p<0.001$ vs reference

The villus height in the jejunum of animals undergoing ileal bypass was greater than after transection as seen in fig 3.5. The villi of the proximal ileum in the animals with bypass failed to grow whereas after proximal small bowel resection the villi were taller than after bypass or transection. There was no difference in crypt depths between animals undergoing transection or bypass in the jejunum, proximal or distal ileum, but the crypt depths of the proximal and distal ileum were greater after intestinal resection.

3.4 Discussion

After proximal jejunectomy the ileum is exposed to chyme rich in gastric and pancreatobiliary secretions and unabsorbed nutri-

ents. These factors may be the main stimulant for the observed increase in villous height, crypt depth and RNA content in the mucosa of the proximal and distal ileum, while in the proximal ileum also the number of intestinal cells as reflected by the DNA content is increased. However, length growth is not increased when compared with the transected animals.

In the bypassed ileum all mucosal parameters are lower than in the ileum after transection of the bowel. Moreover, length growth of the bypassed ileum lags behind that in the transected group. Although RNA and DNA content in the bypassed ileum are above the content of the 10-day-old animals, when compared with the reference animals of the same age, they are compatible with atrophy. The increased RNA content ($p<0.001$) and slightly higher DNA content and longer villi in the bypassed distal ileum versus proximal ileum may be due to reflux into the distal ileum despite the presence of the intact ileocecal valve.

From these facts, we may conclude that in the bypassed ileum some inherent length growth can be observed, together with some mucosal growth, as in perinatal bowel transplanted under the capsule of the kidney (Ferguson et al 1973, Leapman et al 1974, Kendall et al 1979).

In the transected control animal parameters for length and mucosal growth have approximately normal values. After proximal resection the mucosa shows hyperplasia, but length growth is not more that normal obligatory growth as seen in the transected controls. This may be due to the rather short observation period, since it could only occur after full mucosal adaptation has taken place (Williamson 1978).

Addendum

1. Increase in gut length. Percent of initial length. Mean ± SEM.

	Bypass	Transection	Resection	Reference
jejunum	255 ± 15	260 ± 18	----	320 ± 12
ileum	191 ± 11	265 ± 15	281 ± 16	320 ± 12

2. Gut circumference. MM. Mean ± SEM.

	Bypass	Transection	Resection	Reference
jejunum	8.9 ± 0.7	8.5 ± 0.2	----	8.3 ± 0.8
ileum	3.9 ± 0.2	8.3 ± 0.5	10.2 ± 0.5	8.3 ± 0.8

3. DNA content. μg DNA/5 cm. Mean ± SEM.

	Bypass	Transection	Resection	Reference
jeunum	1073 ± 70	1122 ± 50	----	957 ± 51
prox. ileum	399 ± 20	1067 ± 60	1418 ± 70	887 ± 40
dist. ileum	514 ± 30	1043 ± 50	959 ± 50	913 ± 50

Ten-day-old jejunum: 268 ± 20; prox ileum: 230 ± 20;
dist. ileum: 158 ± 10

4. RNA content. μg RNA/5 cm. Mean ± SEM.

	Bypass	Transection	Resection	Reference
jejunum	639 ± 30	631 ± 20		748 ± 20
prox. ileum	215 ± 10	412 ± 40	489 ± 70	621 ± 30
dist. ileum	267 ± 10	433 ± 20	585 ± 30	666 ± 30

Ten-day-old jejunum: 162 ± 10; prox ileum: 203 ± 20
dist. ileum; 158 ± 20

5. Villous height. μm. Mean ± SEM.

	Bypass	Transection	Resection	Reference
jejunum	610 ± 10	486 ± 22	----	482 ± 12
prox. ileum	316 ± 10	357 ± 12	543 ± 26	330 ± 6
dist. ileum	323 ± 15	225 ± 10	302 ± 15	196 ± 9

Ten-day-old jejunum: 354 ± 20; prox. ileum: 300 ± 10
dist. ileum: 210 ± 11.

6. Crypt depth. μm. Mean ± SEM

	Bypass	Transection	Resection	Reference
jejunum	237 ± 4	236 ± 11	----	188 ± 6
prox. ileum	176 ± 6	194 ± 10	227 ± 11	170 ± 9
dist. ileum	175 ± 6	180 ± 11	213 ± 9	197 ± 7

10-day-old: jejunum: 47 ± 2; prox. ileum: 45 ± 3;
distal ileum: 37 ± 3

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CHAPTER 4

THE INFLUENCE OF RESECTION ON MACROMOLECULAR TRANSPORT

EXPERIMENT 2

4.1 Introduction

One of the characteristics of human and animal infant gut is its ability to transport intact macromolecules across the mucosa into the blood. This transport enables ruminants to aquire full passive immunity after birth from absorbed immunoglobulins, mainly IgG, present in colostrum in the first days of life (Ogra 1979). In man however, passive immunity is acquired in utero by the transplacental route (Walker and Isselbacher 1974). Rodents take an intermediate position. In mice, γ-globulins (M.W. ± 150.000) are absorbed till day 17, but no transport of albumin, which has a molecular weight of 69.000 has been noted (Lecce 1972). In rats, cessation of macromolecular transport occurs abruptly on day 20 (Halliday 1955, Clark 1959, Daniels et al 1973, Rodewald 1973, Abrahamson et al 1979). This period of macromolecular transport can be extended by dietary manipulations (Worthington and Boatman 1974) or adrenalectomy, while precocious closure can be induced by the injection of cortisone acetate (Daniels et al 1973).

Shortly after surgical biopsy of the bowel wall in the presence of macromolecules in guinea pigs, the integrity of the mucosa is lost over 10 cm on both sides of the biopsy site resulting in increased macromolecular transport (Rhodes and Karnovsky 1971). However, after gastrostomy and sucrose feeding in neonatal rats, precocious maturation of mucosa is induced, resulting in early appearance of α-glucosidases, doubling of mitotic index in crypts, increased crypt depth and increased 3H-thymidine incorporation with DNA (Lebenthal et al 1972). The precocious maturation after surgical manipulation may be accompanied by premature mucosal closure, which is in itself another feature of maturation.

This experiment was performed to determine whether five days after a 60 percent mid small bowel resection the integrity of the mucosa is still lost, as would be expressed by increased transport of macromolecules, or cessation of macromolecular transport occurs as part of precocious maturation of mucosa.

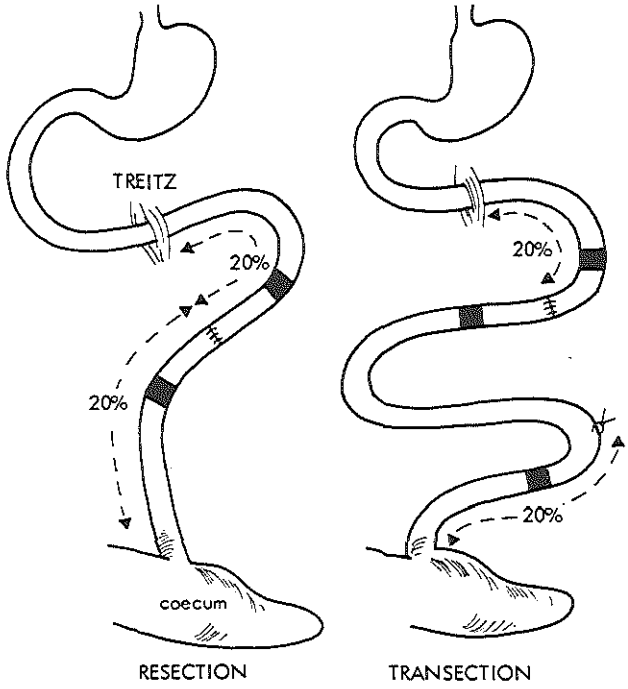

FIGURE 4.1: The black area's in the diagram indicate the location where specimens for biochemistry and histology were taken from.

4.2 Materials and methods

Ten day old rats were subjected to one of two operations (Fig 4.1): a 60 percent mid small bowel resection or a transection at 20 percent of the bowel length measured from the ligament of Treitz to the ileocecal valve. A marker suture was placed at 80 percent of this distance. Unoperated animals were used as reference for normal development and sacrificed on day 10 and 15.

To determine transit time of the gastrointestinal tract, animals that had undergone resection (n=6), transection (n=10) and unoperated animals (n=10), fifteen days old, were given 1 ml of a suspension of powdered charcoal (50% W/V in water) by gavage. Animals of each group were sacrificed at half hour intervals until charcoal, which can easily be seen through the bowel wall at laparotomy in these animals, was noticed at the ileocecal valve. The average transit time for charcoal in resected animals was 2.5 hrs. In normal animals and in those after transection, charcoal had reached the last part of the ileum at this time.

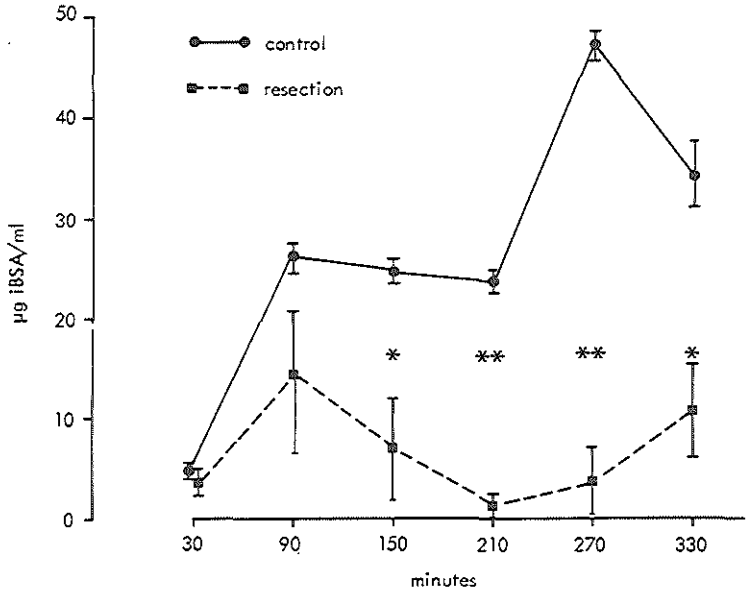

FIGURE 4.2. Serum levels of iBSA at various times after gavage. *: p 0.01; ** p 0.001

At day 15, the experimental and control animals were gavaged with 100 mg bovine serum albumin (BSA) after one night starvation. The first set of animals was sacrificed 30, 90, 150, 210, 270, and 330 minutes later. Blood was withdrawn and stored as serum at -20° C until electroimmunoassay was performed. A second set of animals was sacrificed 150 minutes after gavage, and after exsanguination, the whole of the small bowel was removed, washed and measured as described in section 2.3. One half cm of bowel, three cm proximal to the anastomosis and three cm distal of the anastomosis in the resected animals and distal of the marker suture in the transected group, was removed for histological examination. The remainder of the small bowel was divided and stored as separate sections till further processing: section A from the pylorus to the anastomosis, section B from the anastomosis to the marker suture (in transected animals only) and section C from the marker suture or anastomosis (in resected animals) to the ileocecal valve (see fig. 4.3) These sections were weighed, frozen, and stored. Later RNA, DNA, and sucrase assays were performed in samples from these sections after homogenizing. The small bowel of control animals was divided in equivalent sections. Specimens for histological examination were taken at similar points to those in animals after operation.

iBSA, RNA, DNA, lactase, and sucrase determinations were performed as described in section 2.4. Histological measurements were done as described in section 2.5. Appropriate statistical analysis was carried out as described in section 2.6.

4.3 Results

After operation, a mortality of 17 percent occurred. Serum iBSA levels as measured at various time points after gavage in the first set of animals are depicted in fig. 4.2. In unoperated animals an initial peak after 90 minutes can be noted, followed by a plateau and a second peak after 4.5 hrs. This is consistent with the maximal peak found in neonatal rabbits after 4 hrs with a gradual decrease thereafter (Udall et al 1981). The earlier peak in resected animals may be due to the shorter passage time in these animals. Therefore, a time interval after gavage of 2.5 hrs was selected for further comparative studies.

The weights of the suckling rats in the second set at 10 days of age and at 15 days of age are shown in table 4.1. There was no significant difference at sacrifice and all animals had gained weight.

The length of the remaining small bowel at sacrifice, five days after operation is shown in figure 4.3. Exact figures of results given in graphical form, are included in the addendum at the end of this chapter. The small intestine after transection grew significantly less in length than control (unoperated) animals and those undergoing resection.

TABLE 4.1 Weight of experimental and control animals in grams. Mean ± SEM.

	n	g
Ten-day-old	48	21.1 ± 0.2
Resection	18	27.1 ± 0.5
Transection	13	28.6 ± 0.8
Control (15-day-old)	17	29.2 ± 1.0

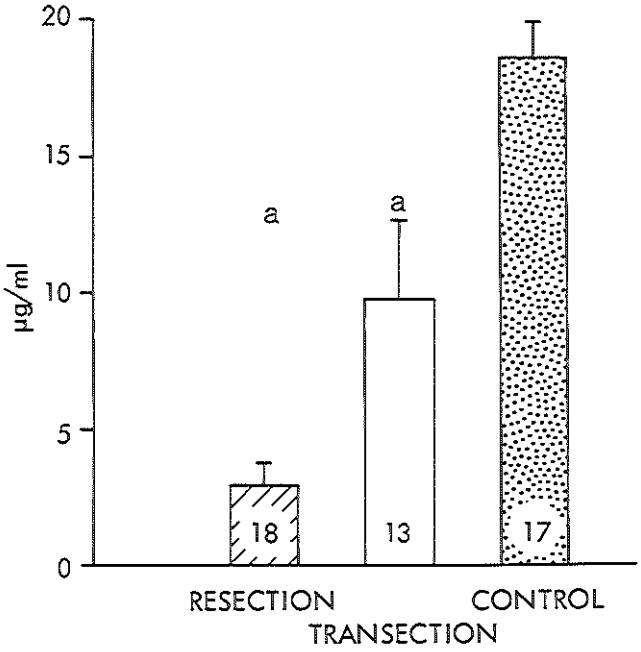

FIGURE 4.4 Concentration of iBSA in serum 150' after gavage. Values are Mean ± SEM.
a: $p<0.01$ versus controls (Wilcoxon rank test).

Serum levels of iBSA after 150 minutes are shown in fig. 4.4. If the serum levels of iBSA are compared to the length of the remaining bowel, then the contribution of each cm is shown in fig. 4.5.

FIGURE 4.3. (See next page). Length of the small bowel at sacrifice. The contribution of various sections (A, B, C) is indicated. R: resection, T: transection, C: control. a: $p < 0.01$ distal section versus transection, $p<0.05$ distal section versus control, b: $p<0.05$ proximal section versus control $p<0.001$ midgut versus control.

FIGURE 4.5. (See next page). Concentrations of iBSA expressed per cm remaining small intestine. Mean ± SEM. a: $p<0.01$ versus control.

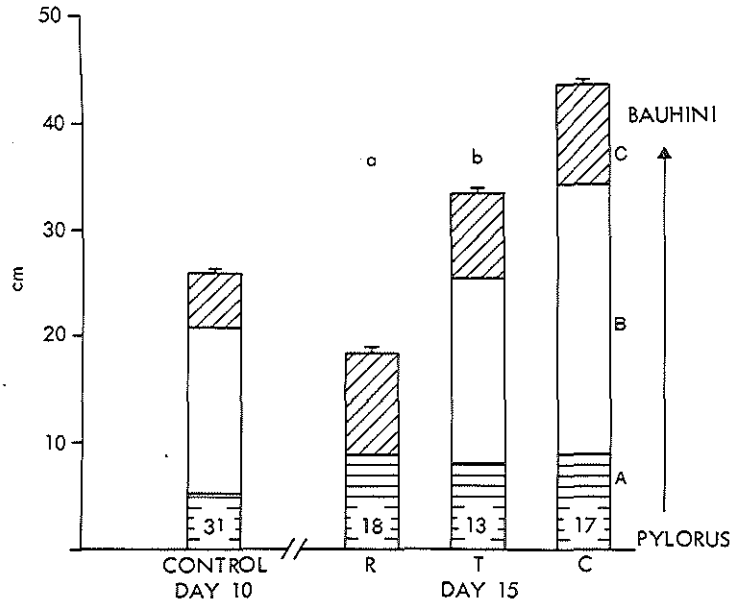

FIGURE 4.3. Length of the small bowel at sacrifice.

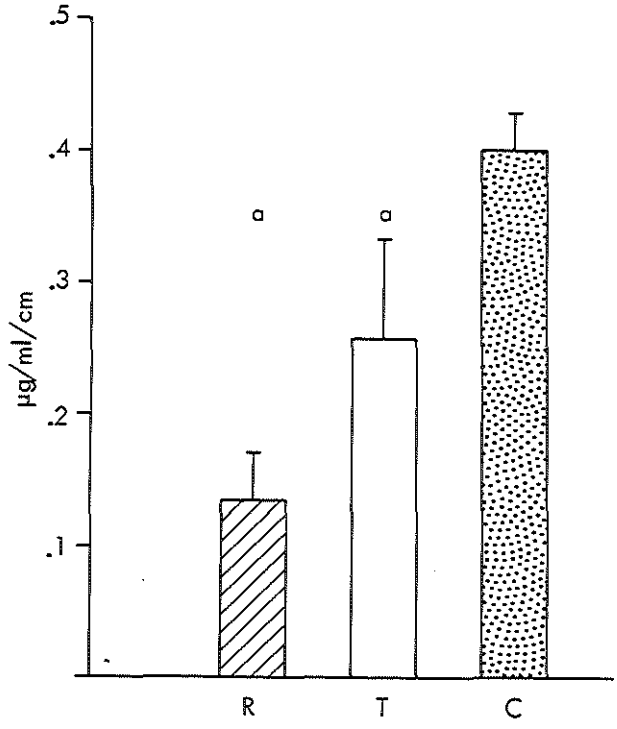

FIGURE 4.5. Concentrations of iBSA per cm remnant small bowel.

TABLE 4.3 RNA and DNA content and RNA/DNA ratio.
Values are μg/cm. Mean ± SEM.
a: p<0.001 versus control
b: p<0.02 versus transection
c: p<0.01 versus transection
d: p<0.05 versus control

	Resection	Transection	Control
RNA CONTENT			
proximal bowel	345 ± 25a, b	217 ± 23a	88 ± 10
midbowel	----	202 ± 18a	94 ± 8
distal bowel	164 ± 15a	115 ± 11a	55 ± 5
DNA CONTENT			
proximal bowel	242 ± 9a, c	200 ± 13	157 ± 11c
midbowel	----	177 ± 13a	131 ± 7
distal bowel	98 ± 5a,	80 ± 5a	53 ± 4
RNA/DNA CONTENT			
proximal bowel	1.42 ± 0.12a	1.09 ± 0.12a	0.56 ± 0.07
midbowel	----	1.14 ± 0.12a	0.72 ± 0.11
distal bowel	1.67 ± 0.23d	1.44 ± 0.21d	1.04 ± 0.11

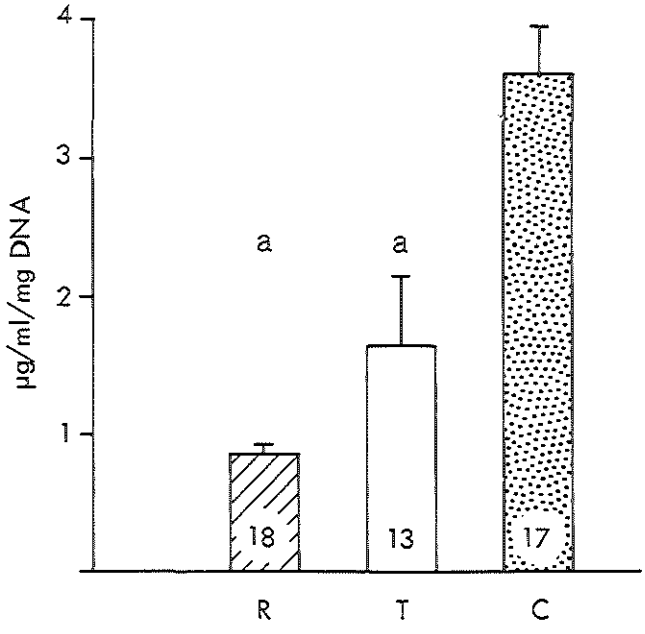

FIGURE 4.6 Concentrations of iBSA per mg DNA. Mean ± SEM
a: p<0.01 versus control (Wilcoxon Rank Test).

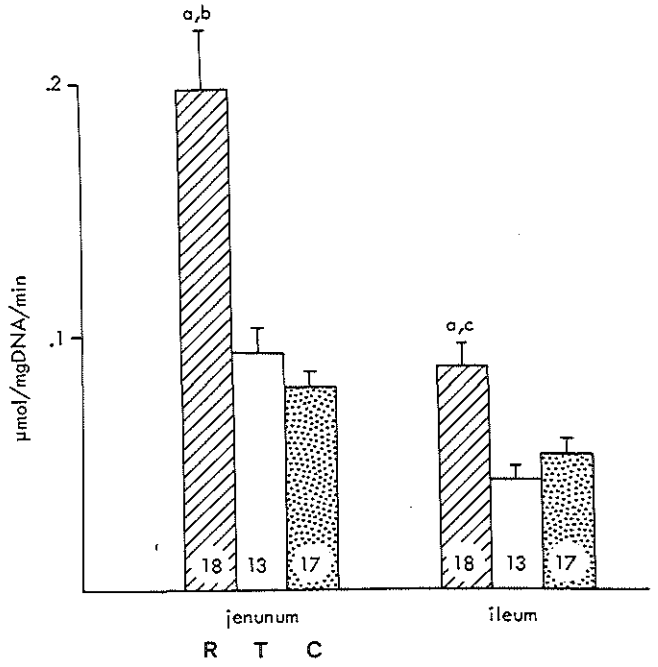

FIGURE 4.7. Sucrase specific activity in the proximal and distal section. Mean ± SEM.
a: p<0.01 versus transection
b: p<0.001 versus control
c: p<0.02 versus control

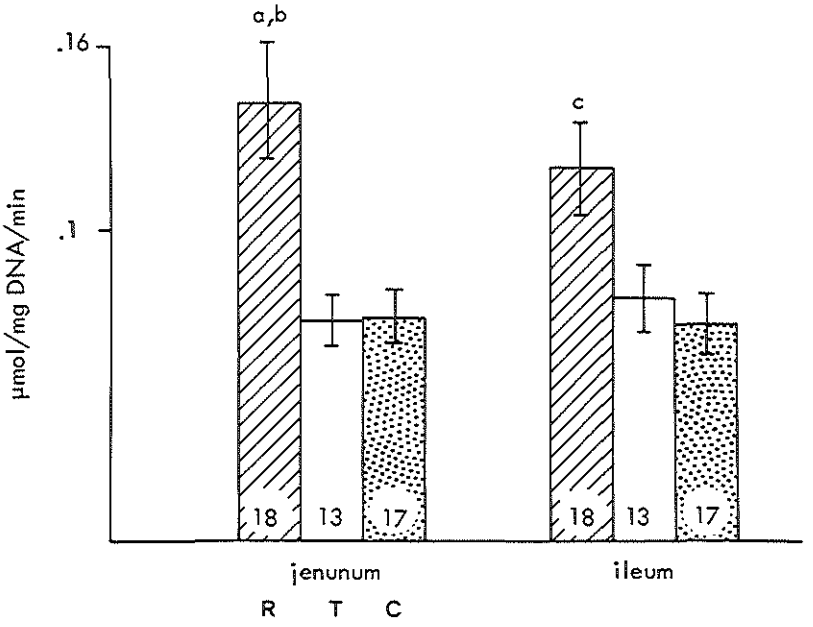

FIGURE 4.8 Lactase specific activity in proximal and distal sections of small intestine. Mean ± SEM.
a: p<0.005 versus transection
b: p<0.005 versus control
c: p<0.01 versus control

The DNA per cm bowel is shown in table 4.3 and if the serum levels of iBSA are related to the total DNA in the residual small bowel, then the contribution of each mg DNA to the serum level of BSA can be calculated, see figure 4.6. Villous height and crypt depth are shown in table 4.2.

RNA content and DNA content, as well as RNA/DNA ratio are increased throughout the small bowel after resection and transection, together with increases in villous height and crypt, showing hyperplasia.

Sucrase activity expressed as micromoles substrate liberated /min/mg DNA ('specific activity') increased in the animals undergoing intestinal resection in both jejunum and ileum. The lactase specific activity also increased after resection but less significantly than the increase in sucrase specific activity as shown in figures 4.7 and 4.8.

4.4 Discussion

After intestinal resection, the transit time was reduced and it is possible that this rapid transit has influenced the results. By choosing a sampling time of 2.5 hours, it is possible that the absorption of BSA has not been completed in normal animals, as the entire gut surface will not have been exposed to BSA. Total absorption might therefore be even greater, increasing the difference we found. It has been shown that macromolecules other than γ-globulins are transported across the mucosa of both jejunum and ileum (Sunshine et al 1971, Walker et al 1972). Differences in blood volumes between the groups could have influenced our results but it is unlikely that these differences would have been significant in animal groups with similar weights and no gross diarrhea. Furthermore, had there been any dehydration in the animals after resection, these rats would have had the lowest extracellular volume. As a result, any dilutional effects should have been noted in the control animals, with the highest serum concentration of BSA.

TABLE 4.2 Villous height and crypt depth. μm. Mean ± SEM.
a: $p<0.001$ versus control c: $p<0.01$ versus control
b: $p<0.02$ versus transection d: $p<0.001$ versus transection

	Resection	Transection	Control
VILLUS			
jejunum	503 ± 20a	472 ± 20c	384 ± 20
ileum	502 ± 25a, d	357 ± 23	333 ± 14
CRYPT			
jejunum	128 ± 6a,b	107 ± 5a	75 ± 4
ileum	112 ± 4a	99 ± 5a	72 ± 3

The decrease in transport of BSA recorded in our experiments after midbowel resection and transection is in marked contrast to the loss of mucosal barrier function seen as an acute effect of local or systemic stress in the adult animal. Surgical biopsy of the intestine in guinea pigs, enteric infusion of hyperosmolar solutions, local inflammation, haemorrhagic shock and protein malnutrition all produce increases of the uptake of macromolecules in adult rats (Rhodes and Karnovsky 1971, Rhodes et al 1973, Worthington and Boatman 1974, Cooper et al 1978, Bloch et al 1979). In all these situations however, the mucosa has suffered acute damage, whereas after five days, the mucosa in our model seems to have recovered from the acute stress.

The decrease of BSA transport after resection and transection is real and not an artifact brought about by the diminished gut length available for macromolecular absorption after resection of 60 percent of the mid small bowel. If the BSA concentration in serum is expressed per cm small intestine available for absorption after resection, transection or in the control animals, the noted decrease is still present. Although the residual gut, after both operations, shows marked increased cellularity, as expressed by increased DNA content per cm, the differences in serum BSA persist when calculated per mg DNA. These calculations support the validity of the observed decrease in BSA transport after resection and transection in suckling rats.

The increase of RNA/DNA ratio seen in the animals after resection, and, to a lesser extent after transection, is similar to the changes seen in the 18 to 25 day old animal developing normally (Koldovsky and Sunshine 1970). Together with the elevated sucrase specific activity after operations noted on day 15, normally occurring around day 16-18 (Tsuboi et al 1979) it suggests precocious maturation also including premature mucosal closure.

At sacrifice, five days after operation, we found a significant increase in length growth of the distal ileum after resection with 8 mm as compared to controls, and with 14 mm as compared to transected animals. The same distal ileum after transection stayed 6 mm shorter than in control animals. In the first experiment, after a 60 percent proximal resection, which produces more luminal stimulation to the distal ileum than the 60 percent midgut resection does, we found no increased length growth after 4 weeks. Apparently the gut is not able to maintain an initial length growth spurt found after five days. This may refute the speculation of Williamson (1978) that increased length growth occurs in a later stage of intestinal adaptation.

Addendum to chapter 4

1. iBSA levels in serum at various times after gavage. Value µg/ml. Mean ± SEM.

	n	Resection	n	Control
30'	5	3.54 ± 1.48	5	4.78 ± 0.39
90'	5	14.59 ± 6.20	5	26.05 ± 1.40
150'	3	7.08 ± 4.98	5	24.78 ± 1.15
210'	4	1.52 ± 0.84	5	23.88 ± 1.09
270'	5	3.80 ± 3.14	5	45.90 ± 1.65
330'	5	10.68 ± 4.43	5	34.52 ± 2.87

2. iBSA levels in serum at 150' after gavage for experimental groups. µg/ml. Mean ± SEM.

	Resection	Transection	Control
serum iBSA	2.96 ± 0.78	9.74 ± 2.94	18.62 ± 1.36

3. Intestinal length. Values in cm. Mean ± SEM.

	prox. intestine	midgut	distal intestine
10-day-old	5.2 ± 0.1	15.4 ± 0.2	5.2 ± 0.1
Resection	8.9 ± 0.3	----	9.7 ± 0.3
Transection	8.1 ± 0.3	17.0 ± 0.3	8.3 ± 0.2
Control	8.9 ± 0.2	22.6 ± 0.3	8.9 ± 0.2

4. iBSA concentration per cm small bowel. µg/ml/cm. Mean ± SEM

Resection	0.135 ± 0.034	a: $p<0.05$ vs control
Transection	0.257 ± 0.078	(Wilcoxon Rank Test)
Control	0.404 ± 0.030	

5. iBSA concentration expressed per mg DNA. µg/ml/mg DNA Mean ± SEM

Resection	0.73 ± 0.19	a: $p < 0.01$ versus control
Trasection	1.65 ± 0.50	(Wilcoxon Rank Test)
Control	3.62 ± 0.33	

6. Sucrase specific activity on μmoles/mg DNA/min. Mean ± SEM.

	Resection	Transection	Control
jejunum	0.198 ± 0.024[a,c]	0.094 ± 0.010	0.080 ± 0.006
ileum	0.088 ± 0.012[b,c]	0.043 ± 0.006	0.053 ± 0.006

a: $p<0.001$ vs Control
b: $p<0.02$ vs Control
c: $p<0.01$ vs Transection

7. Lactase specific activity in μmoles/mg DNA/min. Mean ± SEM.

	Resection	Transection	Control
jejunum	0.142 ± 0.019[a,b]	0.072 ± 0.009	0.073 ± 0.009
ileum	0.121 ± 0.016[c]	0.080 ± 0.011	0.071 ± 0.010

a: $p<0.02$ vs transection
b: $p<0.05$ vs control
c: $p<0.01$ vs control

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CHAPTER 5

THE INFLUENCE OF BREAST MILK OF DIFFERENT PERIODS IN LACTATION AND OF FORMULA FEEDING ON DEVELOPMENT AND ADAPTATION TO RESECTION OF INFANT RAT GUT

EXPERIMENT 3

5.1 Introduction

The mere presence of food in the lumen of the small intestine determines in part the extent of the adaptational process after resection or bypass in the ileum of suckling rats, as has been shown in chapter three, and by others (Levine et al 1974, Feldman et al 1976, Morin and Ling 1978).

Recently, mitogenic properties have been found in colostrum from cows, acting on epithelial cell cultures (Klagsbrun 1980) and in human colostrum acting on fibroblasts and epithelial cells in culture (Tapper et al 1979). This mitogenic action could not, or only to a lesser extent, be found in milk produced 8 days later in lactation. Epidermal growth factor, originally extracted from mouse salivary glands has been found in breast milk and has a strong mitogenic effect on DNA synthesis in small bowel of mice (Starkey and Orth 1977, Feldman et al 1978, Scheving et al 1980).

Breast milk has been noted to exert a protective effect against NEC in man (Santulli et al 1975, Hunt and Inwood 1980) and in rats (Barlow et al 1974). It contains several host resistance factors (factors that protect the recipient infant) important for the establishment of normal bacterial flora (György 1953, Goldman and Smith 1973 and for the preparation of the human infantile digestive system to weaning (Hemmings 1980).

Since after surgery neonates are usually fed exclusively with artificial milk formulas, we tried to establish whether colostrum, breast milk produced later in lactation, or a commerical formula for low birth weight neonates (Similac LBW 24, provided by Ross Lab. Columbus, Ohio) influenced adaptation after mid small bowel resection in suckling rats.

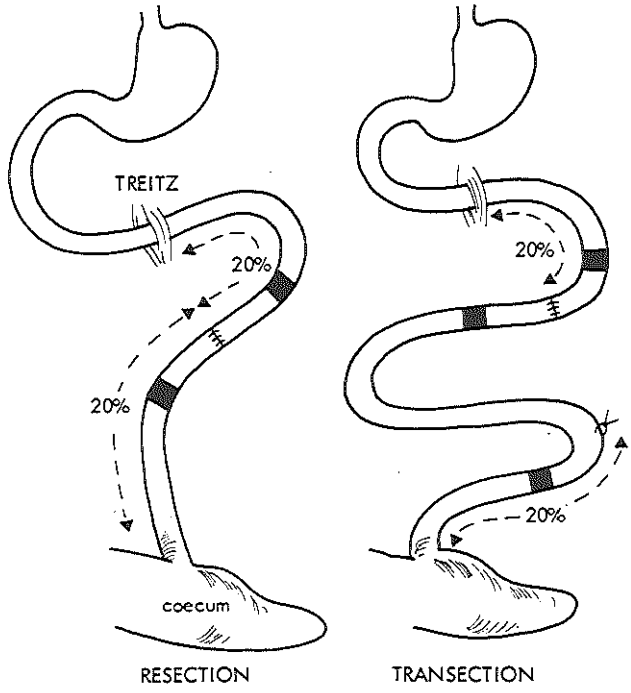

FIGURE 5.1: Diagram of performed operations. The black areas indicate the location of specimens for biochemistry and histometry.

5.2 Materials and Methods

One group of suckling rats 10 days-of-age were subjected to a 60 percent mid small bowel resection (n=69) and a second group to transection (n=71) at 20 percent of the bowel length from the ligament of Treitz to the ilecocecal valve (see figure 5.1). A third, control group underwent no operation. Animals of the three experimental groups were transferred to lactating females. Since female rats within 48 hours of parturition repeatedly cannibalized freshly operated 10-day-old sucklings, we could not provide one experimental set with real, immediate post partum, colostrum, but only with milk of foster mothers in the third day after birth. So, after recovery from the operation, the suckling rats were put with lactating dams on the third, tenth and twentieth day after giving birth. Another set of experimental and control animals was kept

in similar cages under a heat lamp and received three hourly feeding by gavage of increasing amounts of formula (for exact composition see point 5 of the Addendum at the end of this chapter) warmed to 30° C between 8.00 and 24.00 hrs. After every feeding the animals were made to empty their bladder and bowel by gently stroking the perineum, a task usually performed by the dam.

All animals were sacrificed on day 15 under light ether anaesthesia. The entire small bowel was removed, washed and measured as described before. Histological specimens were taken from 1-2 cm proximal to the anastomosis. Full thickness specimens were taken proximal to these for biochemical assays. From the ileum similar specimens were obtained. Control animals were treated in a similar fashion. All specimens were preserved in the usual way until processing as has been described in chapter 2, could be performed.

5.3 Results

At sacrifice five days after operation, the formula fed (FF) animals had started gaining weight. However, mortality was high. While overall mortality in this experiment was 12.3 percent, for the FF group it was 22 percent. The initial and final weight is given in table 5.1. To obtain comparable results final groups were weight matched.

Gut length measurements at sacrifice are shown in fig. 5.2. Average increase in gut length is 60 percent in jejunum and 68 percent in distal ileum in all groups. There are no differences after resection, transection or in control animals, and different types of milk produced no differences in length.

TABLE 5.1 Weight of animals in grams. Mean±SEM.

	n	3rd Day Milk	n	10th Day Milk	n	20th Day Milk	n	Formula
Resection	19	25 ± 0.75	16	25.7 ±0.8	14	23.5 ±1.2	15	22.7 ±0.8
Transection	17	24.7 ± 1.0	17	25.5 ±0.8	19	23.3 ±0.8	14	23.8 ±0.8
Control	12	24.7 ± 1.0	16	26.5 ±1.3	19	24.0 ±1.0	15	22.2 ±0.15

Day 10 starting weight: (n=202) 22.0 ±0.3

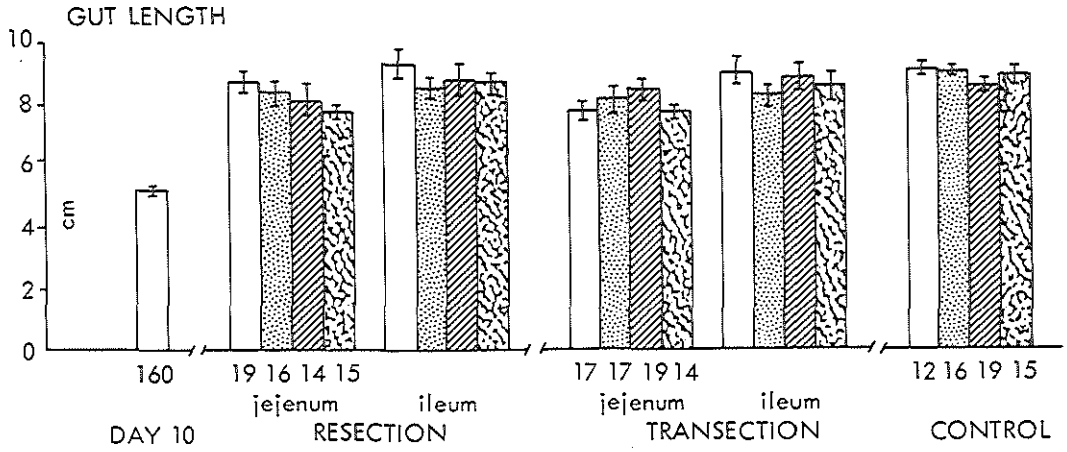

FIGURE 5.2: Gut length in cm. Mean ± SEM . Figure under each bar is the number of animals. No significant differences.

DNA content of the specimens is given in figure 5.3. Although several statistical significant differences can be found, it is difficult to discern consistent influences after different types of milk offered. The lower final weight of the FF animals is neither reflected in the DNA content of the small intestine nor in its length.

The results of measurement of villous height and crypt depth are given in figure 5.4, and measurements of sucrase specific activity are given in figure 5.5. After midbowel resection increased villous height and crypt depth are noted both in proximal and distal bowel without any influence of different types of milk. Both resection and formula feeding result in the early induction of sucrase.

Exact figures of results given only in graphical form here can be found in the Addendum at the end of this chapter.

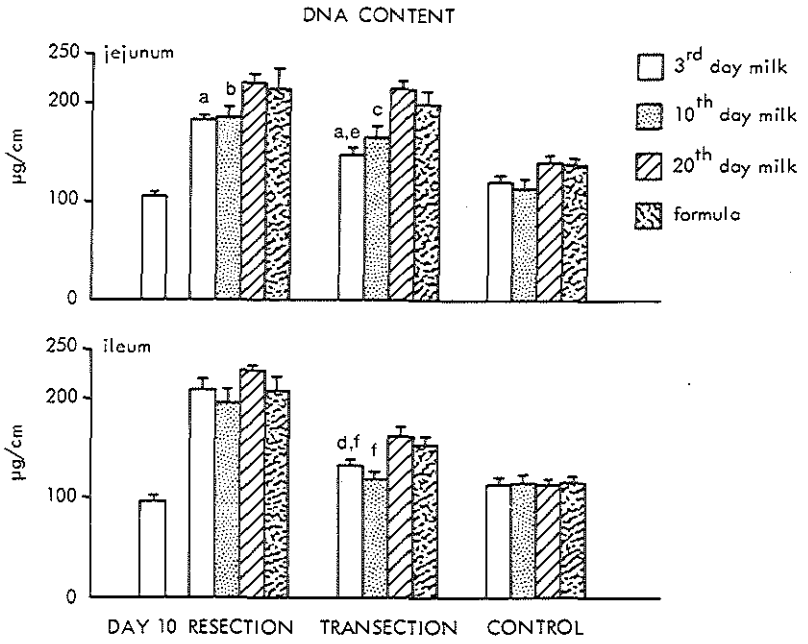

FIGURE 5.3: DNA content in μg/cm Mean ± SEM
a: p<0.001 vs 20th day milk
b: p<0.002 vs 20th day milk
c: p<0.01 vs 20th day milk
d: p<0.02 vs 20th day milk
e: p<0.001 vs Formula
f: p<0.05 vs Formula

FIGURE 5.4: (See next page) Villous height and crypt depth. Mean ± SEM. For statistical differences see the addendum at the end of this chapter.

FIGURE 5.5: (See next page) Sucrase specific activity. Mean ± SEM.
a: p<0.01 vs Formula
b: p<0.001 vs Formula
c: p<0.05 vs Formula

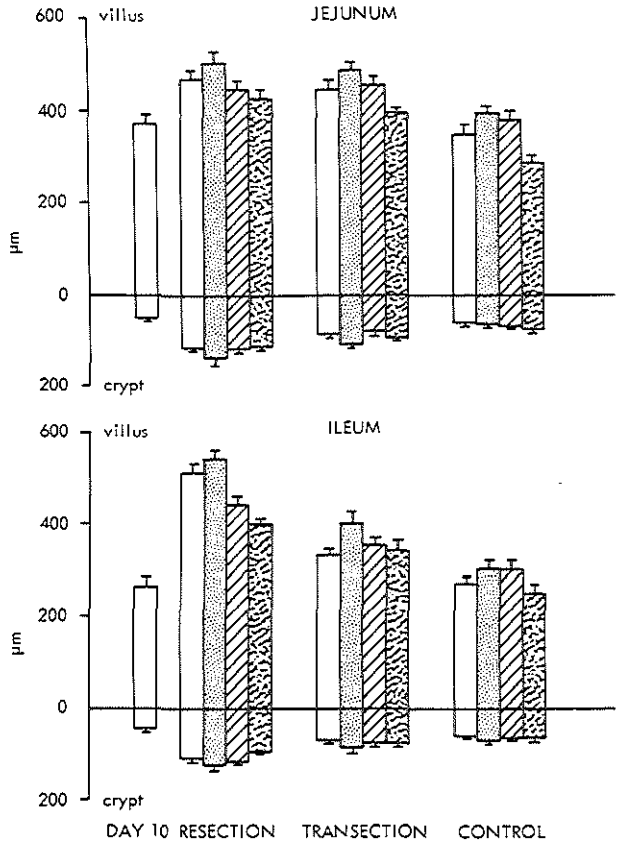

FIGURE 5.4

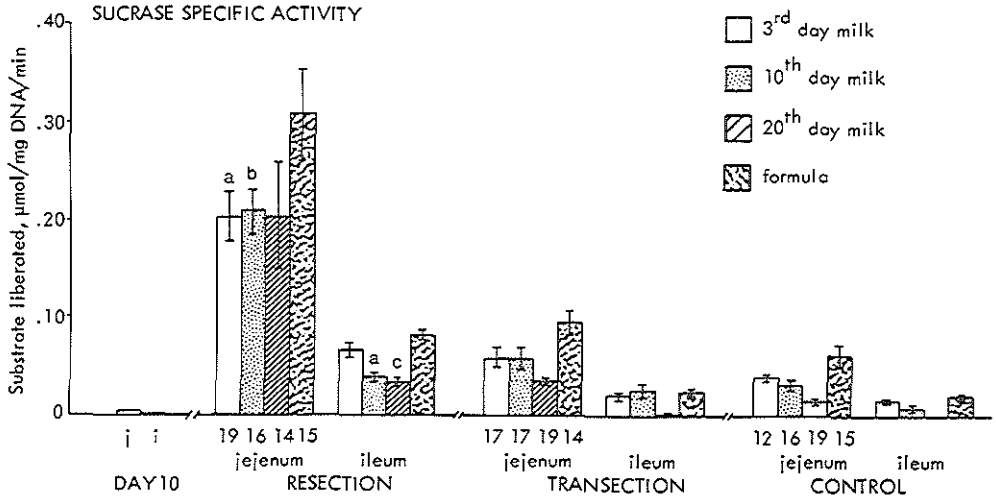

FIGURE 5.5

5.4 Discussion

Although Widdowson et al (1976) have shown colostrum to increase gut length in pigs, and others showed the same in rabbits (Hall and Widdowson 1979), their results were obtained comparing fed newborns to controls given water only. Others suggested that early mucosal mass increase was not an effect of feeding per se, but of colostrum specifically (Heird and Hansen 1977). We were not able to detect consistent effects of colostrum (or, in fact, breast milk from 3 days post partum rats) not induced by breast milk later in lactation, or not produced by a commercially prepared formula. The mitogenic factors detected in cow and human colostrum may be active in vivo, because they are acid-stable and accompanied by anti-trypsin activity (Tapper et al 1979). However, these mitogenic properties may be too weak to express their effect in the jejunum in the presence of strong systemic factors mediating adaptation (Loran and Crocker 1963, Tilson and Wright 1970) while in the ileum additional luminal stimulants may obscure the effects of colostrum. On the other hand, also in the absence of hyperplasia in the unoperated control animals, no effect was found of breast milk produced at different periods in lactation. Our failure to show any mitogenic effect of breast milk may also be due to the fact that these mitogenic factors may have disappeared from rat milk at day three of lactation.

In normal development, sucrase appears in the brush border of enterocytes around weaning (Herbst and Koldovský 1972). This aspect of maturation occurs independent of the presence of luminal stimuli (Kendall et al 1979) and can be induced prematurely by administration of corticosteriods (Herbst and Koldovský 1972) or sucrase feeding. The presence of the adrenal glands is necessary for precocious induction of sucrase (Lebenthal et al 1972). In unoperated animals that were suckled, we noted increased levels of sucrase specific activity in the jejunum and ileum, but the levels in FF animals in the jejunum was even higher. This may be an expression of the extra stress these rats must have experienced in the absence of a mother. Also the uniform increase in sucrase specific activity in the jejunum in animals after resection, regardless of diet, may be an expression of stress resulting in precocious maturation of mucosa, also noted by others (Herbst and Sunshine 1969). In all animals the normal jejunoileal gradient of sucrase activity is retained, as shown before after precocious maturation of intestinal mucosa induced by injections of corticosteroids (Herbst and Koldovský 1972).

ADDENDUM TO CHAPTER 5

1. Gut length at sacrifice in cm. Mean ± SEM

jejunum	3rd day milk	10th day milk	20th day milk	Formula
resection	8.63 ± 0.41	8.31 ± 0.49	8.04 ± 0.49	7.69 ± 0.20[a]
transection	7.74 ± 0.30[a]	8.41 ± 0.35	8.42 ± 0.33	7.68 ± 0.24[a]
control	9.05 ± 0.2	8.88 ± 0.16	8.54 ± 0.16	8.77 ± 0.26
ileum				
resection	9.32 ± 0.36	8.47 ± 0.29	8.68 ± 0.55	8.64 ± 0.30
transection	8.91 ± 0.45	8.19 ± 0.33	8.79 ± 0.33	8.46 ± 0.48

Ten day old: jejunum: 5.2 ± 0.05 cm; ileum: 5.2 ± 0.05 cm.

Figures of ileum of control animals are identical to those of the jejunum.

a: $p<0.01$ vs control on the same type of milk.
Horizontally no significant differences could be found.

2. DNA content in μg cm. Mean ± SEM

jejunum	3rd day milk	10th day milk	20th day milk	Formula
resection	183.0 ± 5.7[a,1]	185.3 ± 10.9[c,2]	220.9 ± 8.6[c]	214.4 ± 18.5[c]
transection	148.0 ± 6.4[b,1,3]	166.2 ± 12.9[d,4]	216.5 ± 9.0[c]	199.7 ± 13.1[c]
control	122.2 ± 7.6	117.7 ± 10.8	141.6 ± 7.0	140.7 ± 7.8
Ten day old	106 ± 3			
ileum				
resection	209.5 ± 11.0[a]	197.3 ± 12.7[a]	228.2 ± 6.3[a]	208.3 ± 14.7[a]
transection	133.9 ± 5.0[b,2,5]	118.8 ± 10.4[4,6]	163.6 ± 9.8[c]	155.7 ± 8.5[d]
control	114.2 ± 6.3	115.2 ± 8.7	114.1 ± 4.6	116.2 ± 7.9
Ten day old	96 ± 5			

Differences vertically: between difference operations in animals on the same type of milk:
a: $p<0.001$ vs transection and control
b: $p<0.02$ vs control
c: $p<0.001$ vs control
d: $p<0.01$ vs control

Differences horizontally: the effects of various types of food after the same operation.
1: $p<0.001$ vs 20th day milk
2: $p<0.002$ vs 20th day milk
3: $p<0.001$ vs formula
4: $p<0.01$ vs 20th day milk
5: $p<0.05$ vs formula
6: $p<0.002$ vs formula

3. Villous height and crypt depth in μm. Mean ± SEM.

VILLUS jejunum	3rd day milk	10th day milk	20th day milk	Formula
resection	475 ± 21[a]	512 ± 24[a,2]	448 ± 21[c]	431 ± 16[a]
transection	452 ± 24[b]	440 ± 26[c,3]	463 ± 14[a,2]	396 ± 14[a]
control	358 ± 16 [1]	395 ± 15 [1]	384 ± 10 [1]	284 ± 10
ileum				
resection	504 ± 21[a,d,1,4]	536 ± 21[a,d,1,5]	441 ± 15[a,e,6]	345 ± 16[a]
transection	338 ± 17[b]	395 ± 19[a]	352 ± 17	346 ± 23[c]
control	272 ± 7	302 ± 13	307 ± 12	253 ± 18

Ten day old: villus jejunum: 379 ± 20; ileum 258 ± 17

Vertically

a: p<0.001 versus Control
b: p<0.02 vs Control
c: p<0.01 vs Control
d: p<0.001 vs Transection
e: p<0.01 vs Transection

Horizontally

1: p<0.001 versus Formula
2: p<0.02 vs Formula
3: p<0.01 vs Formula
4: p<0.05 vs 20th day milk
5: p<0.01 vs 20th day milk
6: p<0.05 vs Formula

CRYPT jejunum	3rd day milk	10th day milk	20th day milk	Formula
resection	118 ± 6[a,e]	139 ± 10[a,1]	119 ± 8[a,d]	115 ± 4[a,f]
transection	86 ± 7[b]	106 ± 8[a,3]	82 ± 4[c]	97 ± 7[b]
control	62 ± 4 [1]	66 ± 3 [1]	69 ± 3	75 ± 4
ileum				
resection	106 ± 8[a,d]	122 ± 9 [a,e,2]	113 ± 10[a,d]	96 ± 6[a,e]
transection	67 ± 3	87 ± 7 [b,4]	75 ± 5	74 ± 4
control	61 ± 3	69 ± 2	64 ± 3	62 ± 3

Ten day old: crypt jejunum 55 ± 3, ileum 47 ± 1

Vertically

a: p<0.001 versus Control
b: p<0.02 vs Control
c: p<0.05 vs Control
d: p<0.001 vs Transection
e: p<0.01 vs Transection
f: p<0.05 vs Transection

Horizontally

1: p<0.05 versus Formula
2: p<0.02 vs Formula
3: p<0.01 vs 20th day milk
4: p<0.02 vs 3rd day milk

4. Sucrase Specific Activity in nmoles/mg DNA/min. Mean ± SEM

jejunum	3rd day milk	10th day milk	20th day milk	Formula
resection	204 ± 37[a]	210 ± 40[a]	204 ± 63[b]	309 ± 45[a]
transection	60 ± 10	59 ± 12	34 ± 9	93 ± 14
control	40 ± 6	33 ± 10	22 ± 5	73 ± 8
Ten day old	3 .6 ± 1.9			
ileum				
resection	68 ± 1[a]	40 ± 9[c]	37 ± 8[a]	53 ± 6[a]
transection	19 ± 3	25 ± 9	3 ± 1	27 ± 5
control	15 ± 2	9 ± 3	4 ± 2	19 ± 3

Ten day old : 0

Vertical differences between groups with the same type of milk:
a: p<0.001 vs control and transection
b: p<0.01 vs control and transection
c: p<0.001 vs control

5. Composition of rat milk and Similac 24 LBW®(2)

	rat milk (1)	Similac 24 LBW
Fat	12 - 20%	4.5 g/100 ml
Carbohydrates	3.3%	8.5 g/100 ml (3)
Protein	9.2%	2.2 g/100 ml
Water	73 - 65%	
Osmolality		316 mosmol/l

(1) From Barlow et al 1974, and Hahn and Koldovský 1966
(2) As provided by Ross Laboratories, Columbus Oh.
(3) 1:1 (wt/wt) mixture of lactose and Polycose
Polycose is a hydrolyzed corn starch, containing glucose polymers with α-1,4 glycosidic bonds.

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CHAPTER 6

THE ROLE OF GASTRIN IN INTESTINAL DEVELOPMENT

EXPERIMENT 4

6.1 Introduction

At the beginning of the weaning period in rats many changes occur, one of them being an abrupt rise of antral mucosal gastrin to adult levels (Zelenkova and Gregor 1971, Lichtenberger and Johnson 1974). In the same period of life marked maturation of the intestinal mucosa occurs: DNA synthesis, mitotic index, turnover time and disaccharidase concentration, and morphological parameters like villus size, change to adult values (Sunshine et al 1971).

Pentagastrin is capable of initiating changes in non-weaned rats imitating changes at normal weaning. Wet weight, RNA per body weight and protein per body weight of the small intestine are all higher in weaned rats than in rats kept on prolonged breast-feeding only. If these animals get pentagastrin injections daily, these parameters change to weaning levels (Lichtenberger and Johnson 1974). Pentagastrin injections in fasted rats promote ^{3}H-thymidine incorporation into DNA and produce higher DNA concentration in oxyntic glands, duodenal and colonic mucosa (Johnson 1977a). The same can be observed in duodenal and colonic mucosa after pentagastrin injections, after antrectomy in rats (Dembinsky and Johnson 1979). Both endogenous high serum gastrin levels and pentagastrin injections cause increased DNA synthesis and DNA content in induced colonic cancers (McGregor et al 1982).

Luminal infusion of pentagastrin into the ileum of rats on a liquid diet also produces increased ileal mucosal DNA content, DNA synthesis and RNA content (Johnson et al 1978). Intravenous pentagastrin infusion prevents atrophy of stomach and small intestinal mucosa of parenterally fed rats (Johnson et al 1975a).

Since systemic hormones play a role in the changes of the mucosa at weaning (Herbst and Koldovský 1972) an effect of a specific enteric hormone like gastrin seems likely. Therefore, we examined the effect of various levels of endogenous gastrin on the development of the small intestine at weaning.

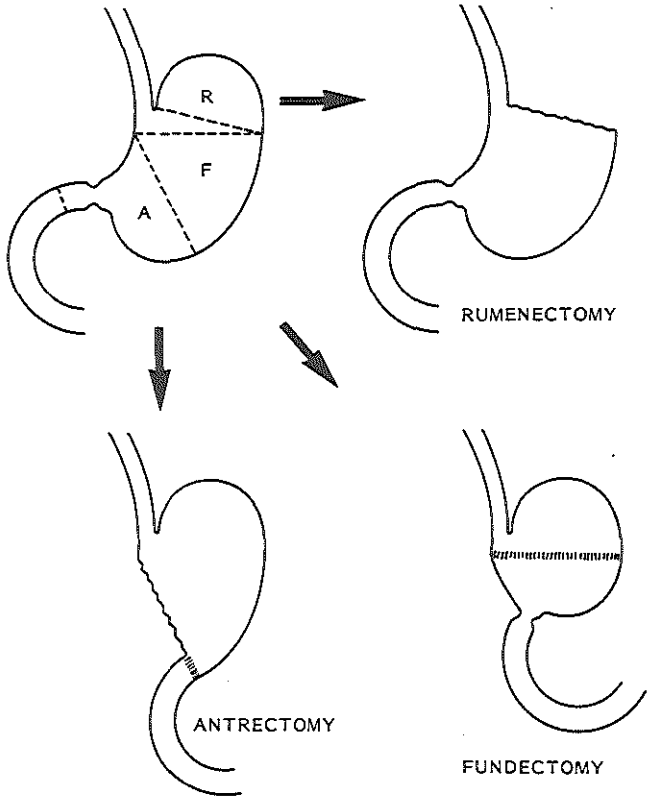

FIGURE 6.1: Resection lines and final situation after gastrin-modulating operations.

6.2 Materials and methods

To create different gastrin levels several gastric operations were performed (see figure 6.1) on non-starved 10-day-old suckling rats (Håkanson and Liedberg 1970, Oscarson et al 1977). One group was subjected to resection of the gastrin cells containing part of the stomach and duodenum, ie. antrectomy (Ax), and another group was subjected to resection of the main oxyntic gland mass of the stomach, ie. fundectomy (Fx), thus interrupting the feedback mechanism for gastrin. Animals that underwent resection of the rumen

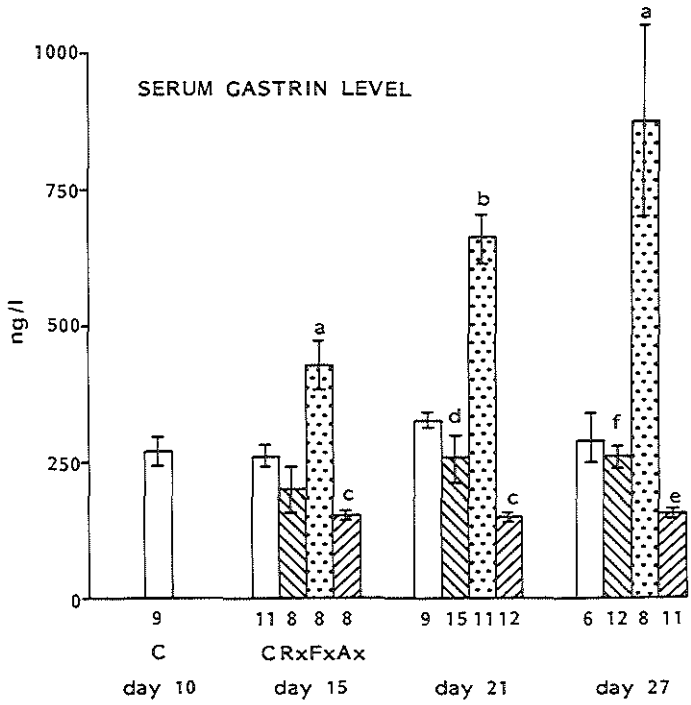

FIGURE 6.2: Serum gastrin level in ng/l. Mean ± SEM.
a: p<0.01 vs all other groups at day 15
b: p<0.001 vs all other groups at day 21 and 27
c: p<0.001 vs control
d: p<0.01 vs Antrectomy
e: p<0.02 vs controls
f: p<0.001 vs Antrectomy

(rumenectomy, Rx) served as controls. This operation was chosen because it results in a comparable loss of capacity of the stomach, without influencing the gastrin-system. In addition, unoperated reference animals were used. The animals were allowed to wean normally after these operations. They were sacrificed on the 15th, 21st, and 27th day of life; before, at the beginning, and at the end of the weaning period, by exsanguination from the inferior caval vein under light ether anaesthesia. The blood was kept on ice, centrifuged after clotting, and the serum was frozen in dry ice and stored at -20° C till gastrin assay was performed as has been described in section 2.4.4. The entire small intestine and colon was removed from the carcass and rinsed in ice-cold saline

solution and measured. Specimens were taken from 5-6 cm past the ligament of Treitz for histology and from 6-11 cm for biochemistry ('jejunum'). The colonic specimens from 1-2 cm past the dilated cecum for histology and from 2-7 for biochemistry.

Before further determinations were done, all animals that had gained weight poorly, or from whom a complete set of data was not available, were excluded. Thereafter results of 85.9 percent of the animals were suitable for statistical analysis, as described in section 2.6. In addition to the Student t-test for unpaired data, the Wilcoxon rank test was applied to the results of the determinations of sucrase specific activity at day 15, because these do not show a normal distribution. All results were also analysed by a two way analysis of variance, adjusted for the gastrin level as a covariate. This analysis shows results similar to those of the Student t-test.

6.3 Results

Gastrin levels in antrectomized animals were significantly lower than in animals after rumenectomy or in unoperated animals. After removal of the oxyntic cells in the fundectomized animals a significantly higher gastrin level was found when compared to the rumenectomized, and the control animals. The mean gastrin levels and the number of animals in each group are given in figure 6.2 and show a 4.5 fold difference between antrectomized and fundectomized animal groups.

After any operation, animals are lighter than unoperated controls at day 21 and 27. At the last time point, the difference between the rumenectomized group and the animals after fundectomy is also significant ($p<0.005$, Student t-test) (See table 6.1).

Gut length at sacrifice is given in Figure 6.3. Gut length in all operated groups is significantly below gut length of the control animals. There is no difference in gut length between the animals with high serum gastrin level (Fx) and the animals with low serum gastrin level (Ax).

GROUPS	day 15	day 21	day 27
Control	27.18 ± 2.55	51.0 ± 1.26a	65.38 ± 3.66b
Rumenectomy	27.38 ± 0.46	40.07 ± 1.35	61.50 ± 1.08c
Fundectomy	26.63 ± 1.07	41.18 ± 1.48	55.13 ± 1.26
Antrectomy	27.38 ± 1.22	37.83 ± 2.10	54.45 ± 3.47

TABLE 6.1: Weight at sacrifice in grams. Mean ± SEM.
a: $p<0.001$ vs operated groups
b: $p<0.02$ vs Fundectomy, p 0.05 vs Antrectomy
c: $p<0.005$ vs Fundectomy

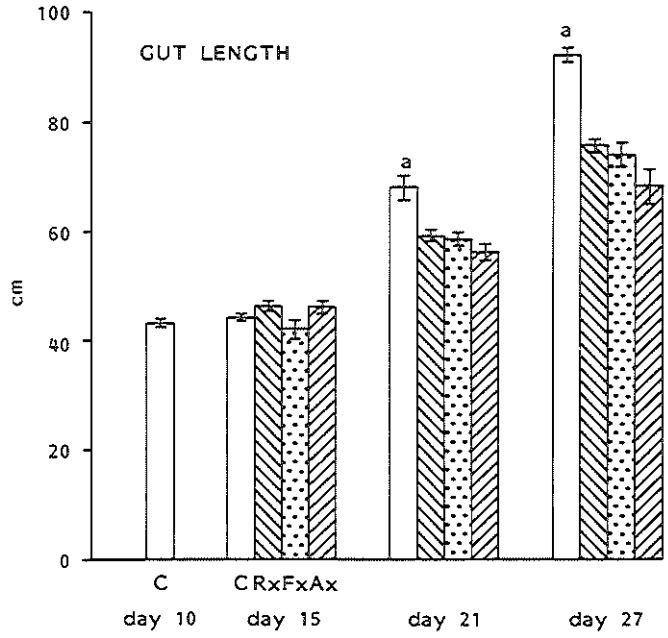

FIGURE 6.3: Gut length in cm. Mean ± SEM.
a:$p<0.001$ vs all other groups at the same time point.

For the DNA contents see figure 6.4. At day 21 the DNA content in the jejunal mucosa was below that in the other animals ($p<0.02$), while at day 27 it is above the content in the specimens from operated animals. In the colon this pattern is reversed. The DNA content of jejunal mucosa in Fx-animals with a high serum gastrin level, is below that in control animals ($p<0.02$ at day 27; for all time points at $\alpha = 0.05$ in the two way analysis of variance). This pattern is also visible in the results for the colonic mucosa, but does not reach significance.

Villous height increases in time in all groups without any effect of various gastrin levels, see figure 6.5.

Also crypt depth increases significantly in all groups with time from a mean level of 74.4 to 190.4 micron at day 27. In fundectomized animals the crypt depth is significantly lower than in the other three groups at $\alpha=0.05$ for all time points (two way analysis of variance; $p<0.005$ versus Rx at day 27 with the Student t-test). However, in the colon the crypt depth in the animals with the high gastrin level (Fx) is significantly higher than in the other groups ($p<0.001$ at day 21 with Student t-test and for all time points at $\alpha = 0.05$ with the covariance analysis).

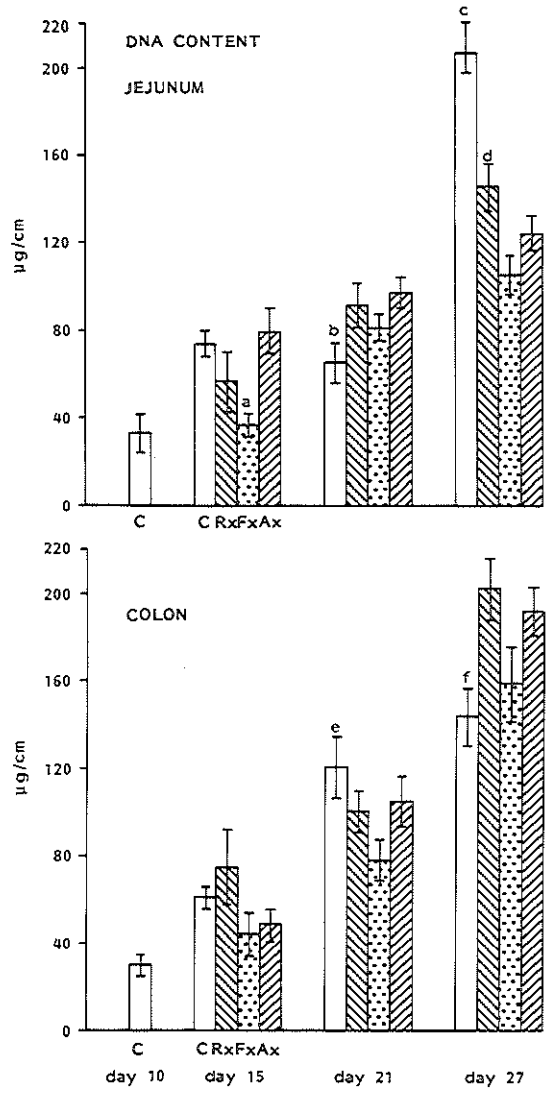

FIGURE 6.4: DNA content in μg/cm. Mean ± SEM.
a: $p<0.005$ vs control and Antrectomy
b: $p<0.02$ vs Antrectomy
c: $p<0.01$ vs all other groups at the same time point
d: $p<0.02$ vs Fundectomy
e: $p<0.05$ vs Fundectomy
f: $p<0.05$ vs Rumenectomy and Antrectomy

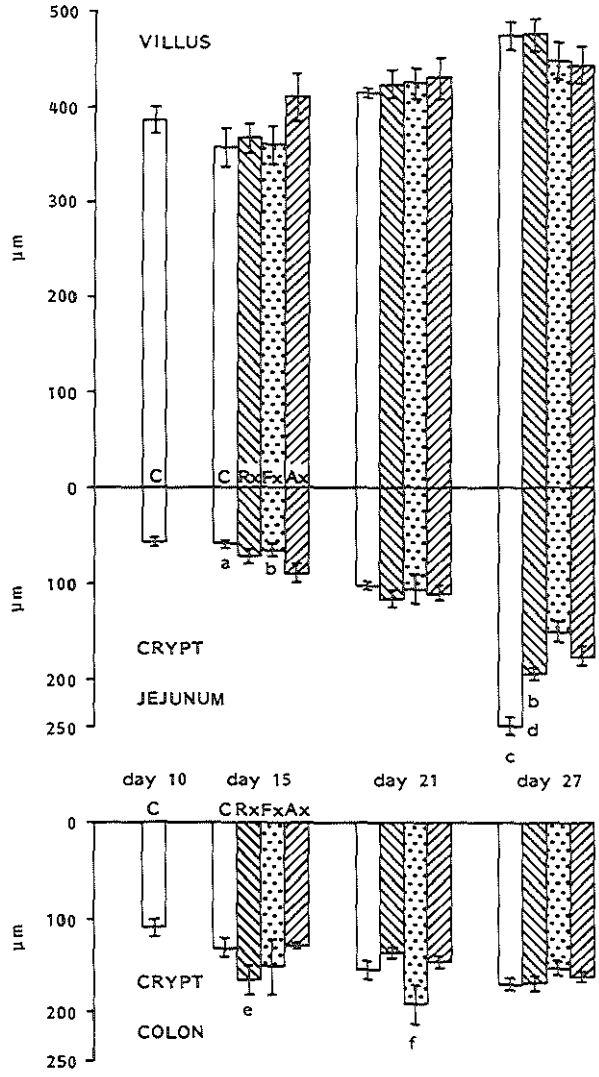

FIGURE 6.5: Villous height and crypt depth of jejunum and colon in μm. Mean ± SEM.

a: p<0.001 vs Antrectomy
b: p<0.02 vs Antrectomy
c: p<0.001 vs all other groups at the same time point
d: p<0.005 vs Fundectomy
e: p<0.05 vs Antrectomy
f: p<0.001 vs Rumenectomy and Antrectomy
p<0.01 vs Controls

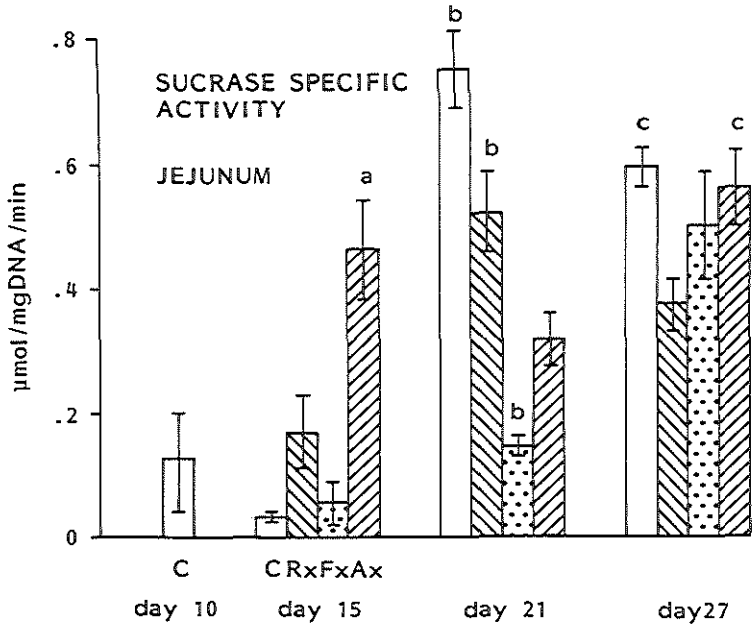

FIGURE 6.6: Sucrase specific activity in μmoles substrate liberated/mg DNA/min. Mean ± SEM.
a: $2\alpha < 0.02$ vs all other groups at the same time point
b: $p<0.02$ vs all other groups at the same time point
c: $p<0.05$ vs Rumenectomy
For the results at day 15 the Wilcoxon rank test was used, while for the other time points Students t-test was used.

The mean sucrase specific activity level increases from 0.18 at day 15 to 0.35 μmoles/mg DNA/min at day 27, and the levels at the three time points are significantly different from each other (see figure 6.6). The mean levels of sucrase specific activity in fundectomized and rumenectomized groups are below the levels of control animals and the antrectomized groups (see figure 6.6).

6.4 Discussion

As has been described before in adult rats, antrectomy and fundectomy are capable of producing abnormally low and abnormally high serum gartrin levels in infant rats. Although in our hands gastrin levels after antrectomy remained higher than in the adult rats, the levels after fundectomy reached comparable heights (Oscarson et al 1977). The abundant gastrin secreting cells in duodenum and pancreas, disappearing just before weaning (Larsson et al 1976) may be the source of serum gastrin found in antrectomized animals of this young age.

By eleven days after the operation, all animals groups after any operation had reached comparable weight, but at day 27 the animals after fundectomy and antrectomy were lighter. This is partly reflected in the decreased gut length after 11 and 17 days, although we did not record an influence of 4.5 fold differences in systemic gastrin levels on longitudinal growth.

After antrectomy, pentagastrin injections restore DNA synthesis and DNA content of duodenal and colonic mucosa to normal levels (Dembinsky and Johnson 1979). Pentagastrin stimulates DNA synthesis in the oxyntic gland area and duodenal mucosa in starved rats, who have low serum gastrin levels (Johnson 1977b). In animals with low serum gastrin levels induced by a liquid diet, the same was noted in colonic mucosa (Ryan et al 1979). In vitro the same effect of pentagastrin was found (Willems et al 1972). In the experiment described here, however, abnormally low endogenous gastrin levels after antrectomy, failed to induce the atrophic changes seen in other situations that produce low antral mucosal or serum gastrin (Johnson et al 1975b, Mak and Chang 1976, Johnson 1977a).

On the contrary, we found low mucosal DNA content in the jejunum of animals with a high endogenous gastrin level. In the colonic mucosa we found no influence of various operations producing various gastrin levels. Our results are in agreement with those of Weser (1978), and with recently published observations where no effect of pentagastrin could be noted on cell production rate or 3Hthymidine incorporation in small intestinal and colonic mucosa (Stevens et al 1981)

No variations in villous height could be seen after the gastrin modulating operations of this experiment. Fundectomy results in decreased crypt depths in the jejunum but in colonic mucosa the crypts after this operation are deeper than after all other operations. The low crypt depth in colonic mucosa after antrectomy is the only result of this study that it is in agreement with earlier claims on a tropic effect of gastrin on coconic mucosa.

In all operated groups the mucosa of the jejunum shows increase of sucrase specific activity with time. Antrectomy results in precocious sucrase activity at day 15 (α=0.05) while this is not significant fot the other operation groups. In earlier experiments in rats on intravenous alimentation sucrase specific activity did increase after intragastric administration of pentagastrin, but not after intravenous administration (Morin and Ling 1978).

In conclusion, we failed to note changes produced by various endogenous gastrin levels as suggested by the demonstration of the pleiotypical effect of (penta-)gastrin by others. The only effect we could find was a decreased jejunal mucosa crypt depth and DNA - content in the fundectomized animals.

Addendum to chapter 6

1. Gastrin levels at sacrifice in ng/l. Mean ± SEM.

	Day 15	Day 21	Day 27
Control	260 ± 23.0	327.8 ± 17.0	288.3 ± 47.1
Rumenectomy	200 ± 41.9	258 ± 40.2	260.8 ± 41.1
Fundectomy	425 ± 46.9	712.7 ± 45.6	877.5 ± 174.8
Antrectomy	150.6 ± 8.6	149.2 ± 7.2	153.6 ± 8.3

Control day 10: 270 ± 24.2

2. Gut length at sacrifice in cm. Mean ± SEM

	Day 15	Day 21	Day 27
Control	44.7 ± 0.78	68.44 ± 2.06	93.25 ± 1.50
Rumenectomy	46.5 ± 1.32	59.33 ± 1.12	75.92 ± 1.01
Fundectomy	42.38 ± 1.72	58.09 ± 1.07	74.13 ± 2.27
Antrectomy	46.38 ± 1.15	56.58 ± 1.30	68.55 ± 3.14

Control Day 10: 43.33 ± 0.85

3. DNA content of jejunal and colonic mucosa in µg DNA/cm Mean ± SEM.

JEJUNUM	Day 15	Day 21	Day 27
Control	74.1 ± 6.1	65.1 ± 9.9	206.4 ± 13.4
Rumenctomy	56.7 ± 14.1	91.1 ± 10.1	145.6 ± 11.5
Fundectomy	36.5 ± 6.5	81.0 ± 6.1	105.9 ± 9.0
Antrectomy	79.4 ± 10.1	97.1 ± 7.3	124.1 ± 8.1
COLON			
Control	61.5 ± 5.4	121.2 ± 14.1	144.3 ± 13.2
Rumenectomy	75.4 ± 17.4	101.9 ± 9.3	202.7 ± 14.4
Fundectomy	44.7 ± 9.9	78.8 ± 9.2	159.2 ± 17.4
Antrectomy	49.6 ± 7.2	105.9 ± 11.9	192.0 ± 11.0

Control day 10: Jejunum: 33.0 ± 8.1 ; Colon 30.5 ± 4.7

4. Sucrase specific activity in nmoles substrate liberated/mg DNA/min.

	Day 15	Day 21	Day 27
Control	32.5 ± 10.2	794.1 ± 66	595.9 ± 37.5
Rumenectomy	169.3 ± 60.3	522.6 ± 65.4	374.3 ± 42.5
Fundectomy	56.3 ± 35.8	148.4 ± 22.5	497.9 ± 87.9
Antrectomy	465.2 ± 88.1	318.9 ± 44.9	560.7 ± 66.7

Control day 10: 128.7 ± 81.3

5. Villous height and crypt depth in jejunum and colon in μm. Mean ± SEM.

JEJUNUM VILLUS	Day 15	Day 21	Day 27
Control	357.1 ± 20.3	416.4 ± 5.7	476.0 ± 13.5
Rumenectomy	367.0 ± 16.7	423.7 ± 15.0	477.0 ± 18.8
Fundectomy	360.5 ± 21.5	427.6 ± 16.7	449.5 ± 19.5
Andrectomy	411.5 ± 23.5	432.3 ± 21.9	444.4 ± 19.7
JEJUNUM CRYPT			
Control	58.05 ± 3.3	102.7 ± 3.3	249.0 ± 9.6
Rumenectomy	72.0 ± 7.9	117.3 ± 9.2	194.7 ± 7.5
Fundectomy	66.5 ± 6.7	106.7 ± 14.4	150.0 ± 11.7
Antrectomy	92.5 ± 7.4	110.0 ± 7.5	177.8 ± 7.3
COLON CRYPT			
Control	130.9 ± 10.5	153.3 ± 9.5	169.5 ± 6.0
Rumenectomy	164.6 ± 15.2	137.3 ± 6.2	168.7 ± 7.8
Fundectomy	149.7 ± 20.4	189.1 ± 21.7	151.0 ± 7.6
Antrectomy	128.0 ± 7.2	145.8 ± 6.1	161.5 ± 5.9

Control Day 10:	
Jejunum villus	372.4 ± 27.1
Jejunum crypt	567.8 ± 4.6
Colon crypt	109.3 ± 9.2

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CHAPTER 7

GENERAL DISCUSSION

7.1 Growth and adaptation

In the suckling rat over 78 percent of newly formed enterocytes participate in growth, but at day 37 only three percent is meant for growth and almost all cells participate in renewal of the population (Altmann and Enesco 1967). In other tissues increases in total DNA till 8-12 weeks after birth have been noted recently (Sands et al 1979). After small intestinal resection in the adult, the remaining mucosa shows marked hyperplasia, temporarily destining more crypt cells for adaptive growth. As shown in the experiments described in this thesis, already in the suckling rat the ability for mounting this adaptive response is present, despite the high proportion of enterocytes normally involved in growth at this age. However, this response does not include increased longitudinal growth over and above normal length growth occurring in the young rat. In the second experiment no overall increased length growth was noted. However, if the distal ileum is regarded separately, a slight but significant increase in length growth above normal length growth is noted five days after midbowel resection. This is in contrast to the results in animals after transection, where all segments stayed behind, as also noted in adult rats (Nygaard 1967). The absorptive surface is only increased by a slight dilatation of the bowel and increases in villous height, since the number of villi does not increase with age in rats (Clarke 1971, 1972). Others have found that the number of villi is unaltered after partial small bowel resection in adult rats (Forrester 1972). So the dilated bowel may not accommodate more villi, but merely broader, leafier ones, or their spacing may be increased.

Both in the first experiment and in experiments two and three, after resection mucosal hyperplasia can be noted in the ileum. Both at five days after 60 percent midbowel resection and at four weeks after 60 percent proximal small bowel resection, the ileal mucosa shows increased DNA content, crypt depth and villous height, overshadowing normal increases due to growth. So the

mucosa of these young suckling rats, is capable of mounting an adaptive response within five days over and above normal growth. This adaptation is partly expressed as precocious maturation, as indicated by an early rise in sucrase specific activity and mucosal closure for macromolecular transport. In the second experiment an increased RNA/DNA ratio after resection and transection was found in 15-day-old rats, comparable to a rise in RNA/DNA ratio normally occurring between day 15 and 18 from 0.4 to 1.1 in the jejunum (Sunshine et al 1971), again indicating precocious maturation.

Precocious maturation as part of adaptation can be noted five days after proximal small bowel resection and at the same time some hyperplastic indices are found, which can still be noted after four weeks. Although a commencement of increased length growth five days after midbowel resection was found, no residual compensatory increased length growth could be noted four weeks after a comparable operation. However, in adult rats increased length growth has been noted seven months after proximal resection (Nygaard 1967). Adrenergic denervation, which may play a role initiating the hyperplastic response, can still be noted two months after a 50 percent midbowel resection (Touloukian et al 1972), suggesting that increased length growth may only take place after our relatively short observation period has elapsed. The sequence of our observations seems to refute this speculation.

After proximal or midbowel resection the brush border enzymes sucrase and lactase show different responses. In experiment two, studying macromolecular transport, and three, studying the influence of different types of milk, we found precociously increased sucrase activity in the ileum at day 15, normally appearing between day 16-18 (Rubino et al 1964, Boyle and Koldovský 1979). In experiment four, studying the influence of gastrin in development, we found the same in the jejunum after antrectomy and rumenectomy but not after fundectomy. The presence of lactase in the jejunum and ileum at day 15 is in agreement with age (Doell and Kretchmer 1962) but the finding that it is higher after resection than in control animals and the increased RNA/DNA ratio, suggests the presence on the villus of a relatively old population of enterocytes. In adult rats lactase specific activity increases from the crypt to villus tip with maximal lactase activity only reached near the extrusion area (Boyle et al 1980). At the same time a new, more mature population of enterocytes with high sucrase activity may be present lower on the villus. On longer villi this would mean an increased life span of the enterocytes. However, shorter life span after resection in suckling animals with early decrease of lactase has been noted (Tsuboi et al 1981). This is in agreement with the 10-fold decline in turnover time occurring in the first five weeks of life (Altmann and Enesco 1967, Herbst and Sunshine 1969) with an increased migration rate (Koldovský et al 1966). Others found reduced migration rate in the repair phase after X-irradiation, dependent on production of epithelial cells in shorter crypts (Rijke et al 1974).

7.2 The role of surgery and hormonal influences

The insult of injury seems to affect suckling animals more than adult animals. Four weeks after a 60 percent proximal small bowel resection, or after transection in infancy, the animals are still at least 20 percent lighter than the unoperated controls. Also after partial gastrectomy all animals were still over 8 percent lighter at two and a half weeks, when compared to unoperated reference animals. In adult animals after small bowel resections comparable to the resections discussed in this thesis, postoperative weight is comparable to the weight of unoperated control animals (Menge et al 1975). Other stressful situations can produce premature sucrase activity not seen after adrenalectomy (Boyle and Koldovský 1979). After gastrostomy feeding with a sucrose rich diet increased mitotic indexes in the crypt, increased crypt depth and DNA synthesis and precocious appearance of sucrase have been noted. These changes did not occur after adrenalectomy (Lebenthal et al 1972). In pigs precocious mucosal closure can be induced by stress feeding or diarrhoea (Lecce 1973). Other changes noted in the described experiments can be explained by general systemic influences as well. In our second experiment, studying macromolecular transport after midgut resection, the jejunum proximal to the resection shows increased villus height and crypt depth, DNA-, RNA-content and RNA/DNA ratio and sucrase specific activity. Some of these changes are also present in the third experiment irrespective of type of milk consumed.

For changes of adaptation in adult rats systemic humoral causes have been suggested after demonstration of increased 3H-thymidine uptake in small intestine of intact parabionts after transection or resection of the partner (Williamson 1978). Increased proliferation is also noted in mucosal transplants placed under the kidney capsule of animals subjected to 50 percent midgut resection (Tilson and Livstone 1976). An intestinal epithelial growth hormone has been suggested to be the mediator of generalized hyperplasia after a limited small bowel resection (Loran and Crocker 1963). Injection of adrenocorticosteroid hormone into adult rats causes acceleration of crypt cell proliferation while adrenalectomy causes deceleration (Tutton 1973).

On the other hand, the relative atrophy of the mucosa in the bypassed ileum in our first study, indicates the importance of luminal nutrition. Limiting food intake after a 60 percent distal resection in growing rats abolished increases in villous height and crypt depth noted after feeding ad libitum (Menge et al 1975). The deprivation of our animals on formula feeding in the third experiment, was not serious enough to prohibit villous growth or increase of DNA content after a proximal resection, although we did observe stunted weight gain.

After massive small bowel resection in man and dogs, equivocal reports exist reporting hyperacidity with or without associated hypergastrinemia (Straus et al 1974, Wickbom et al 1975, Scully et al 1976, Niessen et al 1978, Bohane et al 1979). After 50 percent midgut resection in i.v. alimented rats, increased weight, mucosal

weight, DNA and protein content have been reported after additional pentagastrin infusion (Morin and Ling 1978). However, the results described in the fourth experiment as well as previous results from studies from the same laboratory (Oscarson et al 1977) suggest gastrin is neither a major mediator of adaptation nor necessary for normal development. After a 50 percent proximal small bowel resection additional gastrectomy does not result in diminished ileal hyperplasia (Hughes et al 1976). If any effect on the developing intestine exists, it may be the inverse relation between gastrin level and mucosal DNA content of the jejunum, also noted before (Lichtenberger 1978). In lactation also, no correlation between serum gastrin and crypt cell proliferation exists, (Lichtenberger and Trier 1979) and recently, even the tropic actions of pentagastrin were disputed (Stevens et al 1981).

7.3 Conclusions

After reviewing the objectives formulated in section 1.2 and the results of the described experiments, we can make the following conclusions:

a. The development of ileal mucosa is proportional to the amount and quality of chyme present in its lumen and capable of adaptation after proximal resection over and above normal changes of growth. The observed ileal length growth 4 weeks after proximal resection, however, only represents normal elongation for age, but is diminished in ileum excluded from gastrointestinal tract continuity.

b. Mucosal closure for the transport of bovine serum albumin is part of the mucosal maturation occurring precociously after midbowel resection in suckling rats.

c. We are not able to differentiate between effects of different rat milks produced from 3-25 days after parturition, or effects of gavage of an isoosmolar low birth weight formula, on adaptation after resection or transection. The tested types of milk did not influence normal development of the intestinal mucosa in suckling and weaning rats.

d. Normal development of the intestine of suckling and weaning rats is not influenced by over four-fold differences in endogenous serum gastrin levels.

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SUMMARY

In the first chapter, the motives and objectives for this study are given, and basic facts concerning the experiments detailed. In the treatment of midgut volvulus and some other diseases, massive small bowel resection sometimes have to be performed. After small bowel resection the remnant gut adapts functionally. Controversy still exists whether morphological changes noted after resections in infants are due to normal growth, intestinal adaptation or both.

One of the factors regulating both intestinal adaptation and development of small intestine is the presence of food in the gastrointestinal tract. Colostrum especially is supposed to contain a mucosal growth factor. It also contains several macromolecules and viable macrophages important in the defense system of the infant, that may protect it against certain diseases. The influence of surgery on the transport of intact macromolecules was not clear up till now.

Another factor that could clearly play a role after intestinal resection, is the enteric hormone gastrin since it has strong tropic actions on gastrointestinal tract mucosa. Its role in the ontogeny of rat gut is not yet clear.

Four experiments were designed. In chapter two the animals used, the period of development studied, and the techniques of the operations performed in these experiments are described. The biochemical assays are described as well as the way measurements are performed in histological sections of the bowel wall.

Chapter 3 describes the first experiment. Ten-day-old rats were subjected to a 60 percent proximal small bowel resection, a 40 percent distal bypass or a transection with reanastomosis of the proximal small bowel. This enabled us to study the ileum in the presence of stimulation by 'rich' chyme in the resected group, and in the absence of luminal stimulation after bypass. Four weeks later, the gut length and several mucosal parameters (DNA and RNA content, villous height and crypt depth) were studied. Ileal mucosa, four weeks after transection, bypass, or resection, shows adaptation proportional to the intra-luminal stimulation. After resection only normal length growth can be observed.

Chapter 4 gives the second experiment. This was designed to study the influence of small bowel resection on macromolecular transport across the mucosa of the jejunum and ileum. Ten-day-old rats were subjected to a 60 percent midbowel resection while animals after a transection served as controls. Normal animals were also studied. Five days after operation cessation of transport of bovine serum albumin, administered by gavage, is noticed to occur several days precociously. Increased RNA/DNA ration and premature appearance of jejunal sucrase also suggest precocious maturation

of mucosa after resection.

In the third experiment, (chapter 5), we studied the influence of colostrum, of breast milk produced later in lactation, and of a commercial low birth weight formula, on intestinal adaptation in suckling rats. The same operations as in the previously described experiment were performed. Again five days later, no important differences between groups nourished with different types of milk can be distinguished.

Finally in the last experiment, described in chapter 6, the effects of various levels of gastrin on the development of jejunal and colonic mucosa around the weaning period is studied. At ten days of age rats were subjected to antrectomy, to produce a low endogenous gastrin level, or to fundectomy, to produce a high endogenous gastrin level. Animals after excision of the rumen of the stomach served as controls. At the beginning, during, or after the completion of the weaning period, no effects of various gastrin levels can be recognised. In the jejunal mucosa, however, decreased DNA content and more shallow crypts are noted in the animals with high endogenous gastrin levels.

This thesis is concluded in chapter seven by the general discussion where results common to all experiments are compared to the facts from the literature.

SAMENVATTING

De motivatie tot en de doelstellingen van de experimenten beschreven in dit proefschrift worden in hoofdstuk 1 uiteengezet. In de behandeling van volvulus van de dunne darm moeten soms uitgebreide resecties worden verricht. Na dunne darm resecties treedt een functionele aanpassing op van het resterende deel. Het is nog steeds niet duidelijk of de morfologische veranderingen in de resterende darm, die na resecties bij zuigelingen kunnen worden waargenomen, het gevolg zijn van normale groei, aanpassing aan de korte darmlengte of beide.

Een van de factoren die, zowel de aanpassing als de ontwikkeling van de dunne darm reguleren, is de aanwezigheid van voedsel in het maagdarmkanaal. Er wordt verondersteld dat juist colostrum een "slijmvlies groei factor" bevat. Het bevat bovendien macrofagen en groot moleculaire stoffen die van belang zijn in het afweer mechanisme van de zuigeling, en die het zouden beschermen tegen het ontstaan van sommige ziekten. Tot nu toe was de invloed van darmresecties op de opname van deze grootmoleculaire stoffen niet duidelijk.

Een ander factor die een rol zou kunnen spelen na dunne darm resecties, is het hormoon gastrine, omdat het een sterk troop effect op het slijmvlies van het maagdarmkanaal heeft. De rol van gastrine in de ontwikkeling van de darm van de rat is nog onduidelijk.

Vanuit boven beschreven achtergrond, werden vier experimenten ontworpen. De dieren die in deze studies werden gebruikt, de periode in de ontwikkeling van deze proefdieren, die werd bestudeerd en de operaties die ze ondergingen worden beschreven in hoofdstuk 2. De biochemische analyses en de metingen in histologische secties die werden uitgevoerd worden beschreven.

Hoofdstuk 3 beschrijft het eerste experiment. Tien dagen jonge ratten ondergingen een resectie van 60% van het eerste deel van de dunne darm, een bypass van 40% van het laatste deel van het ileum of een doorsnijding met reanastomosering in het jejunum. Dit maakte het mogelijk het ileum te bestuderen onder invloed van stimulatie door "rijke" chymus in de groep dieren na resectie, en zonder enige stimulatie vanuit het darmlumen in de groep na bypass. Vier weken later werd de lengte van de darm gemeten samen met enkele parameters voor groei van het slijmvlies (DNA en RNA gehalte, hoogte van villus en diepte van de crypte). Vier weken na resectie, bypass of doorsnijding is het slijmvlies van het ileum evenredig gegroeid aan de stimulatie vanuit het lumen. Na een darmresectie kan slechts normale lengtegroei van de dunne darm gevonden worden.

Hoofdstuk 4 geeft het tweede experiment weer. Dit werd ontworpen om de invloed na te gaan van een dunne darm resectie op het

transport van macromoleculen door het slijmvlies van jejenum en ileum. Tien dagen oude ratten werden onderworpen aan een resectie van 60% van het middenste deel van de dunne darm. Dieren na een doorsnijding van de darm met reanastomosering, dienden als controle, terwijl ook dieren die niet werden geopereerd, bestudeerd werden. Vijf dagen na operatie blijkt de opname van bovien serum albumine, dat per maagsonde was toegediend voortijdig opgehouden te zijn. Een gestegen RNA/DNA verhouding en het voortijdig verschijnen van sucrase in het jejunum suggereren het optreden van voortijdige rijping van het dunne darm slijmvlies in de groepen na resectie.

In het derde experiment, hoofdstuk 5, werd de invloed bestudeerd van colostrum, van moedermelk later geproduceerd en van een flesvoeding voor kinderen met een laag geboorte gewicht, op de aanpassing van de darm van ratten in de zuigelingen leeftijd. Deze ratten ondergingen dezelfde operaties als in het voorgaande experiment. Weer na vijf dagen konden geen verschillen gevonden worden tussen groepen die verschillende soorten melk aangeboden hadden gekregen.

In het laatste experiment, beschreven in hoofdstuk 6, werd de invloed bestudeerd van verschillende gastrine spiegels op de ontwikkeling van het slijmvlies van het jejunum en het colon gedurende de periode waarin de rat gespeend wordt. Tien dagen oude ratten ondergingen een antrectomie om een lage gastrine-spiegel te produceren of een resectie van de maagfundus om een hoge gastrine-spiegel op te wekken. Controle dieren ondergingen een resectie van het rumen. Aan het begin, tijdens en aan het einde van de speen-periode konden geen invloeden van verschillende endogene gastrine-spiegels gevonen worden. Alleen in het slijmvlies van het jejunum bestond een verlaagd DNA gehalte en ondiepere crypten in de dieren met een hoge gastrine spiegel.

In hoofdstuk 7 worden de gemeenschappelijke resultaten van de vier beschreven experimenten getoetst aan de bestaande literatuur en de conclusies nog eens weergegeven.

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CURRICULUM VITAE

I was born in Hengelo, Overijssel, where I attended primary school and the Gemeentelijke HBS-B. Afterwards I studied medicine at the State University Groningen from 1965 till 1972. From 1973 till 1974, drs A.A.F. Asjes, J.A.I. van Prooije and J.G.F.M. Gras taught me the basic practice of surgery, gynecology and obstetrics. After this preparation, and attending the National course in Tropical Medicine and Hygiene, I worked in the service of the Ministry of Health of the Kingdom of Lesotho as a District Medical Officer for eighteen months.

I started my formal surgical training at the University Hospital Rotterdam - Dijkzigt in July 1976, under the late Prof dr H. Muller and Prof dr H. van Houten, and later Prof dr J. Jeekel. From August 1979 till July 1980 I worked in Boston, Massachusetts as a Research Fellow in Surgery at the Massachusetts General Hospital and Harvard Medical School. Hereafter, I resumed my residency in surgery in Rotterdam.

The drawing on the cover by De Bry (1617) illustrates the appearance of the first created light.