Control mechanisms of postresectional hyperplasia in the small bowel mucosa

An experimental study in rats

PROEFSCHRIFT

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To Lydia, Sascha, Thijs
and my parents.

CONTENTS

INTRODUCTION

The small bowel mucosa is an example of a 'cell renewing system' and in the rat complete replacement occurs within 48 hours (Leblond and Stevens, 1948).
Other cell renewing systems in the adult organism are the epidermis and its derivates, the testis and the blood forming tissues. The size and the functional capacity of such a cell population is dependent on the precise balance between cell production and cell loss. On the other hand some flexibility is needed to meet with possible perturbations of the system.
It has become clear that crypt cell proliferation is influenced by neural, hormonal or luminal factors as well as by the number of villous cells and intestinal excretion (see for reviews Dowling and Riecken, 1974; Rijke 1977). The exact mechanisms responsible for the regulation of epithelial cell proliferation, cell migration and cell loss are, however, largely unknown.
Experimental and clinical observations at the end of the 19th century proved that in man survival was possible after the loss of at least half the small bowel and that postoperative diarrhoea and malabsorption tended to improve with time.
It was concluded that small bowel, like liver and kidney, possesses a considerable functional reserve and a capacity to regenerate. For many years the problem was studied mainly by function tests and histological methods.
Althausen et al. (1950) suggested a number of mechanisms by which the body might compensate for a partial loss of small bowel. Firstly, a loss of total body weight will inevitably reduce nutrient requirements.
This process would continue until structural and functional adaptation of the remaining intestine had developed to the point at which both factors would sustain the diminished demand for food. Subsequently, continuing adaptation might increase the total body weight towards original levels, depending upon the extent of bowel loss and the individual capacity for regeneration. The final factor in this recovery was suggested to be the capacity of the rest of the gut (stomach and colon) to take over some of the absorptive functions of the missing small bowel. For many years it was not clear whether individual cells were able to increase their functional capacity or whether improved absorption depended entirely upon the production of more cells. It is now known that the major response to resection appears to be increased proliferation and migration of functionally immature cells, as was shown by Dowling and Gleeson, 1973, using autoradiography after ^{3}H-thymidine incorporation.
Since the principle of postresectional hyperplasia has been accepted, the attention has been centred upon the mechanisms controlling it. Most of the studies concerning compensatory hyperplasia have been carried out at least two weeks and often several months after intestinal surgery.

Therefore, Malt and co-workers in the surgical services at the Massachusetts General Hospital began to study the adaptation of small bowel and colon in the early period after operation. In these studies the overall proliferative activity was measured by biochemical assays of ^{3}H-thymidine into D.N.A. rather than by morphological and autoradiographic studies. This enabled us to perform quite a large number of experiments on early changes in bowel adaptation in a reliable way. This facilitated the search for possible factors governing the phenomenon 'compensatory hyperplasia'.

The objective of the studies, described in the present thesis was to get an answer to the following questions:

1. What is the effect of jejunal transection and jejunal resection on intestinal hyperplasia early after operation – i.e. 48 hours – and at 1 week and 1 month after operation?
2. Is there a difference in effect on intestinal hyperplasia between jejunal resection and jejunal bypass?
3. What is the contribution of intraluminal factors to postresectional hyperplasia?
4. What is the influence of bile and pancreatic juice separately as well as combined on the postresectional hyperplasia?
5. Are humoral factors involved in the control of postresectional adaptation?

Chapter I

SURVEY OF THE LITERATURE

1.1. Changes after small bowel resection

The precedent that the remaining part of an organ may grow to compensate for tissue loss produced by partial resection is well established from studies of the liver and the kidney. Following partial hepatectomy or after unilateral nephrectomy, the residual liver and the contralateral kidney respectively undergo 'compensatory hypertrophy', and the 'hypertrophied'* remaining organ may take over the function of the portion which has been removed. There is thus both a structural and a functional component to the compensation.
After a small bowel resection one can discern the same structural and functional adaptation in the residual small intestine.

* The term 'hypertrophy' is used in this and the following paragraphs in the gross sense and to describe villous enlargement, but at a cellular level the term hyperplasia is more correct. A special chapter is devoted to this subject (chapter 1.3).

1.1.1. Structural adaptation

In 1888 Nicholas Senn described in his article 'an experimental contribution to intestinal surgery with special reference to the treatment of intestinal obstruction' a series of experiments in dogs in which he performed extensive small bowel resections. At autopsy he found, that the remaining portions of the small bowel had undergone 'compensatory hypertrophy' as the coats were much thickened and exceedingly vascular'. Eight years later, in Bologna, Monari (1896) confirmed these findings also in dogs. He reported thickening of the muscular coats and especially of the mucosal coats of the intestine in a series of graded enterectomies. He stated that there was an increased number of villi in a given area of the residual bowel and that the villi were larger than in the control group.
Evans and Brenizer (1907) resected 33, 37, 41 and 50 per cent of the combined jejunum and ileum. They found a more localized 'hypertrophy', particularly marked in the neighbourhood of the anastomosis.
In a long article in the Bulletin of the Johns Hopkins Hospital (1912) Flint describes the effect of extensive resections of the small intestine in dogs. He observed that there was no increase in the lenght of the remaining part, but a marked increase of the transverse dimensions.
All intestinal coats contributed to this 'hypertrophy' occurring in the region of the crypts

as well as in the villi. In comparing the epithelial cells of the 'hypertrophied' villus with the control specimen he observed that the cells were distinctly higher and that even the nucleus took part in this process with a slight increase in size. Unlike Monari he did not find increased numbers of villi in a given area of bowel.
Only much later in 1958 Loran and Althausen described a comparable study in rats after resection of 10 per cent of the small intestine and they determined the wet and dry weights of the remaining small intestine. When compared to controls, an increase in weight of the intestine was noted. The authors also reported thickening with compensatory 'hypertrophy' due to a tissue-restoring mechanism which was initiated by resection.
Although all these reports have confirmed the 'compensatory hypertrophy', there have been expressions of dissent.
Trzebicky (1894) and Wildegans (1925), on gross examination of bowel remnants, could only find intestinal dilatation and no compensatory 'hypertrophy' of the small bowel after large small bowel resections. Jensenius (1945) came to the same conclusion, he found no evidence of intestinal 'hypertrophy' following either proximal or distal small bowel resection in the dog. In rats v.d. Meer (1973) did not find any change in crypt cell-kinetics after 10% or 40% resection of the small intestine.
Studies in man gave the same conflicting evidence. West et al. (1938) noted marked 'hypertrophy' of the remaining small intestine at laparotomy two years after a serie of extensive resections in a patient with regional enteritis.
In 1965 Porus studied mucosal biopsy specimens of two patients who had undergone resection of 75-80 per cent of the small intestine two years previously. He noted a 22 per cent increase in the mean number of cells per unit length of villous surface, compared to normal patients. Porus concluded that 'hyperplasia' occurs after massive small intestinal resection in humans. No evidence or lengthening or 'hypertrophy' of the villi was noted.
Some clinical observations on survivors of extensive bowel resection also denied the presence of any 'hypertrophy' in the remaining small intestine (Denk, 1907; Shonyo and Jackson, 1950).
Nevertheless it has become established beyond refute that the small bowel, like the liver and the kidney, undergoes a profound regeneration after partial excision.

1.1.2. Functional adaptation

One of the earliest studies on functional adaptation after resection was made by Stassoff (1914) who collected the chyme from a jejunal fistula following distal resection in the dog and found that with time there was a progressive decrease in the amount of fat, starch and soluble nitrogen recovered from the chyme. This was interpreted as an evidence of functional compensation by the remnant of the small bowel.
Stassoff (1914) and Sarnoff (1923) postulated that gastric stasis and hypersecretion might assist intestinal digestion and absorption.
Wildegans (1925) found that the amount of steatorrhoea and excess faecal nitrogen, present shortly after small bowel resection, gradually diminished with time. He interpreted this as evidence of compensation by the residual small intestine.

Althausen (1950) observed two patients with extensive small bowel resections. The total absorptive capacity of the intestine increased markedly in both patients, the increase appeared earlier and to a greater extent for some dietary constituents than for others.
The absorption of glucose and of water started to increase first and became normalized. The absorption of galactose and the amino-acids, methionine and glycine was fully restored at a later stage. Only the absorption of fats remained low as estimated by the vitamin A absorption test. The author stressed the possible role of the proximal colon in the process by which the gastro-intestinal tract adapts itself to the loss of the absorptive function of the small intestine.
Older work of Short and Bywaters (1939) already had shown that mixtures of amino-acids are readily absorbed from the colon.
Delayed intestinal transit-time might also contribute to functional compensation by prolonging the exposure of the remaining villi to the nutrient stream. Several studies have demonstrated a slowing down of passage of food through small bowel remnants, particularly after proximal resections, and this may contribute to the greater compensation found after jejunal resections compared to ileal resections (Clatworthy et al., 1952; Reynell and Spray, 1956; Nygaard, 1967).
Studies by Dowling and co-workers, have suggested that there is an adaptive increase in glucose, water and electrolyte absorption per unit length of intestine after resection both in rat (Dowling and Booth, 1967) and in man (Dowling and Booth, 1966).
Although the uptake of actively transported monosaccharides and amino-acids per cell is either unchanged (Wilmore et al., 1971) or diminished (Weser and Hernandez, 1971) after resection, the postresectional 'hyperplasia' produces an increased absorptive surface formed by an increased number of functionally immature cells.
The advantage of the increased surface outweighs the disadvantages of the functional immaturity of the cells. Consequently the net absorption per unit length of intestine is increased after resection (Dowling and Gleeson, 1973). In vivo transport studies following intestinal resection in the rat have shown enhanced uptake both of substances absorbed diffusely through the small bowel, such as mono- and di-saccharides, amino-acids, water and calcium (Dowling and Booth, 1967; Bury, 1972; Urban and Pena, 1974), and of those with localised transport mechanisms, such as bile acids and vitamin B12 (Perry, 1975).
After distal small bowel resection jejunal bile acid absorption increases presumably due to augmentated diffusion of both free and conjugated bile acids. After jejunectomy the active transport of conjugated bile acids becomes supranormal (MacKinnon, 1973; Perry et al., 1974), leading to an increasing size of the bile salt pool (McCarthy and Kim, 1972).
The first direct evidence of functional compensation in man was given by Dowling and Booth (1966). They studied 8 patients who had undergone previous bowel resection, by means of a segmental perfusion technique, using double lumen intubation of the jejunum and a polyethylene glycol marker. They showed an increased glucose absorption in the resected patients compared to controls.
Weinstein (1969) subsequently demonstrated in 4 survivors of massive intestinal resection an enhanced sodium and water uptake by the bowel remnant and related this to an increased villous cellularity measured in histological specimens.

1.2. Cell kinetics

1.2.1. Intestinal Cell Renewal

Small Bowel : The rapid renewal of cells lining the small bowel – a concept that is now clearly established – was first proposed by Bizzozero of Turin in 1888 to explain the numerous mitoses he observed within the crypts of small bowel mucosa. He postulated that cells migrated upwards from these areas of regeneration to cover the villi, where they differentiated into goblet and columnar cells (Bizzozero, 1892). This idea was consistent with earlier histological and embryological studies showing that epithelial cells lining intestinal crypts and villi shared a common morphological appearance (Heidenhain, 1888) and embryological origin (Paneth, 1888). There was no further advance in the knowledge of intestinal cytokinetics until Friedman (1945) demonstrated by using X-ray irradiation that goblet cells migrate from the crypt to the villus and subsequently to the villus top. Many later developments in this field have derived from the work of Leblond and his associates in Montreal (1948). Estimates of the mitotic index were obtained by counting the number of dividing and non dividing cells in histological sections, with the additional use of colchicine to block mitoses in the metaphase (Leblond and Stevens, 1948).
The duration of the mitosis was calculated by observation of cells in tissue culture or by the use of irradiation on the assumption that x-ray treatment prevents cells from entering prophase (Knowlton and Widner, 1950). The turnover time of a cell population is defined as the time taken for the replacement of a number of cells equal to that in the whole population. Under steady state conditions turnover time is equal to the duration of mitosis divided by the mitotic index (Leblond and Walker, 1956). The development of two new research techniques revolutionised the study of cell kinetics, i.e. isotopic labelling of cells and autoradiography. Radioactive phosphorus (^{32}P) was first used to label proliferating cells; with this method Leblond (1948) could follow newly formed epithelial cells from the crypt up to the top of the villus by means of autoradiography at different time intervals after labelling.
In 1951 Howard and Pelc described that DNA synthesis occurs during a specific part of the generative cell cycle (S-phase) separated timewise from the mitotic phase by 'gaps' which they later designated as G1–(pre-DNA synthesis) and G2–phase (post DNA synthesis) of the generative cell cycle. After several attempts to find specific radioactive labelled DNA-precursors, the introduction of tritiated thymidine (^{3}H-thymidine) was an important step forward (Taylor et al., 1957).
The use of microautoradiography to observe the movement of thymidine-labelled cells allowed the determination of rates of cell migration and renewal with much greater precision (Quastler and Sherman, 1959; Messier and Leblond, 1960).
More detailed cell kinetic studies were subsequently performed, and models were proposed for the cell renewing system of the intestinal epithelium (Cairnie et al., 1965 a,b). In the crypts of the rat small intestine cell proliferation is confined to the lower half of the crypt (Cairnie et al., 1965 a; Quastler and Sherman, 1959).
In the lower part of this proliferative cell compartment each dividing cell gives two proliferating cells, whereas in the upper part each dividing cell yields two non-proliferating cells (Cairnie et al., 1965 b).

The mean duration of the generative cell cycle is approximately 12 hours; 14-16 hours in the lowest crypt cell positions and 10-11 hours in the upper part of the proliferative cell compartments. After having completed 2-3 cell divisions, the crypt cell enters the 'critical decision zone' (Cairnie et al., 1965 b), halfway the crypt where the cell normally stops cell proliferation. After migrating through the upper half of the crypt, which takes 9-12 hours, the cell enters the functional villous compartment.
On the villus the epithelial cell performs its function, while migrating from the base to the top of the villus, and 36-42 hours after its last cell division the cell is extruded into the intestinal lumen (Galjaard et al., 1972).
Unfortunately the radiation risk plus the long half life of thymidine has prevented the application of these techniques to patients. Therefore the knowledge of epithelial cell kinetics in man remains relatively fragmentary. Estimates of the turnover time of the intestinal epithelial cell range from 1 to 2 days in rodents, 2 to 3 days in the cat and 2 to 6 days in man (Creamer, 1967).
There are four different types of cells in the small bowel epithelium of the rat, probably arising from a common precursor cell (Cheng and Leblond, 1974 c).
The majority of the epithelial cells, more than 85 per cent, consist of *columnar absorptive cells* or chief cells. These cells originate at the base of the crypts as immature proliferative cells and subsequently migrate along the upper half of the crypts. During their course through the crypt a gradual development of the ultrastructural cell components takes place (Cheng, 1974; v. Dongen, 1976). At the same time the activity of a number of enzymes shows a gradual increase in the crypt (de Both et al., 1974). At the crypt-villus junction a second period of ultrastructural and enzym activity changes takes place. On the villus the columnar absorptive cells progress steadily along the villus to the top. This migration along the villus takes about 30 hours in the rat (Galjaard et al., 1972). At the top of the villus the cells are extruded into the lumen.
The second cell type, *the goblet cell*, arises by proliferation of oligomucous cells in the lower half of the crypt. The oligomucous cell itself arises from early stages of the columnar absorptive cell. The goblet cell also migrates along the villus and is extruded into the lumen (Cairnie, 1970; Cheng, 1974a).
The Paneth cell is the third cell type in the intestinal epithelium. This cell is characterized by specific granulae and probably also originates from early stages of the columnar absorptive cell. The Paneth cell remains located at the bottom of the crypt, degenerates after a relatively long life-time and is eventually phagocytosed by an adjacent columnar absorptive cell (Cheng, 1974b).
The last and least frequent cell type is *the entero-endocrine* (argentaffin) *cell.* This cell is also thought to be originating from early stages of columnar absorptive cells (Cheng and Leblond, 1974b). This cell also migrates to the villus and is extruded from the villus top.

Colon : The architectural plan of the colon, with its closely spared crypts and flat surface, is simple compared to the small bowel. Furthermore the proliferative zone in the large intestine is less restricted than in the small bowel. Cells migrate from the proliferative zone toward the lumen and are extruded from the surface of the mucose (Clarke, 1973). In man colonic proliferative cells occupy the lower two-thirds of the colonic crypt columns. DNA synthesis occurs in about 15 to 20 per cent of cells in this zone showed by

labelling with ^{3}H thymidine. The mean duration of the proliferative cell cycle is about 2 days (Lipkin and Deschner, 1976). In healthy rodents, epithelial cell proliferation occurs in the lower portion of the colonic crypts. These cells, however, show a more rapid renewal rate and a shorter lifetime than those of humans. Abnormalities of colonic cell proliferation and differentiation have been shown in patients with polyposis coli (Deschner and Lipkin, 1975). The earliest detectable abnormality is persistent DNA synthesis in cells on the mucosal surface (Deschner et al., 1963). In areas of colonic epithelium that contain these cells, the normal transitional and maturational zone that normally separates proliferative cells from non proliferative mature surface cells is absent. Instead some cells move through the zone and retain the capacity to divide. The cells also produce greater amounts of RNA and protein than normal mature colonic cells.
As in the small intestine, the three types of colonic epithelial cells, columnar, goblet and entero-endocrine, probably arise from common stem cells in the base of the crypts (Chang and Leblond, 1971).

1.2.2. Post resectional cell kinetics

Cellular proliferation of intestinal epithelia after resection was first studied by Loran and Althausen (1960). They subjected rats to a partial resection (10 per cent) of the ileum. Two months later the cellular proliferation was studied by means of autoradiography with tritiated thymidine in the remaining parts of the small intestine. They reported a three fold increase in the migration rate of the cells on the villus. Increased cell migration rates after resection have since been widely confirmed (Knudson et al., 1962; Hanson and Osborne, 1971; Gleeson et al., 1972). Although cells migrate faster after resection, the accompanying increase in villous height means that the cells must travel a greater distance from the crypts to the tip of the villi. Therefore a more rapid cell migration does not necessarily indicate a shorter cell turnover time (Dowling and Gleeson, 1973).
By studying autoradiographs at different times after ^{3}H thymidine incorporation however Loran and Althausen (1960) showed a more rapid cell migration and an increased rate of cell turnover after a limited distal small bowel resection. In contrast Gleeson et al., 1972, could not confirm the increased cell turnover.
Rijke et al., 1974 even found that the life span of epithelial cells in rat small intestine is independent of the villous length. Possibly in the early recovery from resection crypt cell proliferation is increased, but thereafter a steady state is regained wehereby augmented villous height is maintained by more rapid cell migration by a greater number of cells (Dowling and Gleeson, 1973). Studies in rats by Hanson et al. (1971) in the shortened intestine 60 days after a partial resection have shown an increase in the number of villus cells and crypt cells throughout the small intestine whereas no change was observed in the percentage labelled crypt cells after ^{3}H thymidine labelling (Hanson and Osborne, 1971) indicating that a steady state was reached.
McDermott and Roudnew (1976) reported essentially the same changes in the ileum after 40 per cent proximal small bowel resection in rats.
There are a few reports of cell kinetic studies immediately after resection. Poulakos (1972) showed that after an 80 per cent resection, the intestinal compensation began

within the first postoperative week. Livstone and Tilson (1975) reported an increased labelling index 3 days postoperatively at a time when crypt depth or villous height had not changed.
However, Van der Meer-Fieggen (1973) reported no change in intestinal cellularity after 40 per cent resection. Her observations were made at the 2^{nd}, 6^{th}, 30^{st} and 60^{st} postoperative day, using autoradiography with ^{3}H thymidine. Obertop (1977) was the first to show that jejunectomy stimulates a continuing hyperplasia in the rest of the small bowel measured already within two days after operation. All these reports present different results concerning the structural changes after intestinal resection. However, there are differences in techniques, as various amounts of intestinal tissue have been resected, and the observations have been made at various postoperative times. Because of the in consistency in the literature about the relation between the extensiveness of the resection and the degree of adaptation, Hanson (1977) performed a study in rats to determine the effects of increasing size of intestinal resection on the residual intestine. His study indicated that the degree of hyperplastic change which occurred after resection is dependent on the amount of tissue removed.

1.2.3. Intestinal bypass

1.2.3.1. Effects on the small bowel remaining in continuity

In his original report on experimental enterectomy Senn (1888) included the results of seven dogs in which varying length of small bowel had been excluded from continuity, but were left in situ. In the four survivors he noted that 'the remaining distal portion of the small bowel did not undergo the same degree of compensatory hypertrophy as in the excision experiment'. Senn suggested two possible explanations for this finding: resection, by interrupting a number of mesenteric arteries, might increase blood flow in the vessels supplying the residual bowel, or intestinal contents might enter the lower end of the excluded segment and thus partially vitiate the short-circuiting procedure. The excluded small bowel he observed was atrophic, contracted and only sparsely supplied with blood vessels. Flint (1912) reported marked 'hypertrophy' distal to an intestinal short-circuit in dogs. Few subsequent studies have compared the effects of excision and exclusion on bowel remaining in direct continuity. Nygaard (1967) compared a 75 per cent proximal resection with a similar bypass (with end to side anastomosis) by measuring the external circumference and the thickness of the muscular coat of the remaining functioning part of the small bowel. The same pattern and degree of 'hypertrophy' were present in both groups.
Dowling and associates came to a similar conclusion but their studies on resection and bypass were seperated by a 5 year interval (Dowling and Booth, 1967; Gleeson et al., 1972a). Moreover they used a Thiry-Vella fistula (an isolated segment of bowel with both ends implanted seperately in the skin) as bypass, that may not be comparable to the classic bypass.
Nygaard (1968) found more steatorrhoea after bypass than after an equivalent resection, a fact which he attributed to bacterial colonisation of the partially excluded loop.

1.2.3.2. Effects on the bypassed small bowel

Senn (1888) had noted a striking contrast between the atrophic excluded segment and the hypertrophic bowel remaining in continuity.
Cunningham (1898) studied excluded canine segments of bowel four weeks after operation; he confirmed the atrophic appearance and deduced that fat absorption was impaired by inspection of the lacteals after instillation of neutral cottonseed oil into the defunctioned loop. By contrast, Plant (1908) found no evidence of atrophy and a normal fat absorption in similar loops 2½ years after operation.
Flint (1912) stated that bypassed bowel showed no histological changes at all. This controversy has largely been resolved by more recent detailed studies on the morphology, histochemistry and function of segments of rat jejunum and ileum completely excluded from intestinal continuity as in a Thiry-Vella fistula (Gleeson et al., 1972a; Gleeson et al., 1972b). Findings included luminal narrowing, mucosal atrophy, altered villous morphology, decreased villous height, diminished cell migration and turnover and decreased mucosal enzyme activity.
The number of cells per villous column, and to a lesser degree the number of cells per crypt column, decreases in the bypassed jejunum within 7 to 14 days, and subsequently remained constant up to one year.
The total proliferative activity per crypt, as determined by scintillation counting of isolated crypts after labelling with ^{3}H-thymidine, was found to be markedly reduced in bypassed jejunum (Rijke et al., 1977). This decrease is largely due to a reduction in the total crypt cell population. The same reduction in the total crypt cell population was also observed by Clarke (1974).
In contrast to Gleeson et al. (1972a), Rijke et al. (1977) observed that the migration rate of epithelial cells on the villus was somewhat larger in the Thiry-Vella fistula than in control jejunum.
In addition, in bypassed jejunal, but not in ileal segments, glucose absorption progressively diminished, although values reached significance after 10 weeks of exclusion. Similar changes were shown in self emptying blind loops of rat jejunum (Menge et al., 1970).
It is interesting that the processes can be completely reversed by restoration of normal intestinal continuity (Menge et al., 1973).

1.2.3.3. Intestinal bypass surgery in man

Bypass of the small intestine has traditionally been reserved for situations in which resection might prove difficult. Indications included the relief of obstruction due to multiple adhesions or local obstructing malignancy. In more recent years two new indications have widened the scope of this procedure and an increasing number of patients are being submitted to intestinal shortcircuits of varying length.
Buchwald and Varro (1966) namely introduced ileal bypass for the treatment of hyperlipidaemia associated with progressive coronary atherosclerosis. The bowel is transected 200 cm from the ileocaecal valve, the upper end of the bypassed distal bowel is closed

and the proximal segment of the small bowel is anastomosed as an ileoneocaecostomy to restore the continuity.

By decreasing the distal small intestinal absorptive surface and by decreasing transit time, the partial ileal bypass operation interferes with both the cholesterol and the bile enterohepatic cycles, causing a direct and an indirect drain on the cholesterol pool. By plotting the circulating cholesterol radioactivity for some days following oral administration of a C14 tracer, Buchwald and Varro (1966) were able to calculate an index of cholesterol absorptive capacity.

The second indication for ileal bypass surgery was initiated by Payne (1956). He started the first clinical program for massive obesitas by a small intestinal bypass of nearly the entire small intestine, the right colon and half of the transverse colon.

Bowel continuity was restored by end-to-side anastomosis of the proximal 15 inch of jejunum to the midtransverse colon. Weight loss from this procedure was dramatic, but the morbidity (uncontrolled diarrhoea, electrolyte imbalance and liver failure) was prohibitive, even a death was reported.

Payne and De Wind (1969) discovered empirically that excellent weight reduction and an acceptably low complication rate (see above) could be achieved by end to side jejunioleostomy. This is the so-called '14 + 4' operation: the proximal 14 inch of jejunum is anastomosed end to side to the terminal ileum 4 inch proximal to the ileocoecal valve. After 2 to 3 years of steady but diminishing decline, the body weight usually reaches a new plateau which is still above ideal weight. Dissatisfied with this meager weight reduction, Scott (1972) introduced an alternative method of end to end jejunoileal bypass, with drainage of the blind loop into the transverse or sigmoid colon. He reported a more complete return to normal physical appearance following this operation, with a closer approach to ideal body weight and a lower tendency to regain weight.

The end-to-end anastomosis is superior because of the decreased reflux of intestinal contents into the bypassed ileum. It has been pointed out that after end-to-side anastomosis reflux is demonstrable which would permit additional caloric absorption (Scott et al., 1975). A number of interesting findings about intestinal adaptation in man has resulted from the increasing interest in the intestinal bypass in the clinical situation.

Barium studies have confirmed Senn's (1888) speculation that food might reflux into blind loops defuctioned by end-to-side anastomosis. Such reflux of barium has been observed for distances of up to five feet after jejunoileostomy in man (Scott et al., 1970). Dudrick et al. (1977) demonstrated with histological techniques in his patients increased villous length and mucosal cell hyperplasia in the functioning incontinuity jejunum when compared with the patients own normal jejunum before the bypass operation.

Biopsy specimens from the bypassed jejunum showed mucosal villous atrophy and decreased crypt depth. Concerning functional adaption Nygaard and co-workers (1970) noted a significant recovery in B12 absorption between 3 and 12 months after partial ileal bypass, indicating an adaptation of absorption of vitamin B12 presumably by the remaining ileum.

These observations seems to indicate, that comparable alterations take place after bypass surgery in animals and in man as will be discussed in the experiments in the following chapters.

1.3. Hypertrophy or hyperplasia

A useful morphological differentiation of the enlargement of organs was introduced by Virchow, who speaks of *hypertrophy* in the sense of an enlargement of the cells, and of *hyperplasia* as indicating an increase of the number of cells of which the tissue or organ is composed. By observing a large number of karyokinetic (Mitotic) figures in bowel remnants after resection, Flint (1912) concluded that the process of structural adaptation involved both hyperthrophy and hyperplasia and introduced the notion of compensatory enlargement. It took 50 years before Bochov (1958) showed in dog experiments that after resection, the number of cells per uniform area of the epithelial layer of the villi was unchanged. Since the villi had become enlarged, his conclusion was that an overall increase in epithelial cell population (hyperplasia) must have been responsible for the associated lengthening of the villi and the crypts. Weser and Hernandez (1971) also found hyperplasia in the residual intestine following small bowel resection in the rat. In fact, rather than being hypertrophic, the cells were apparently smaller than normal. Autoradiographic studies with isotopic labelling (Hanson and Osborne, 1971) showed an increased crypt cell population following 70 per cent enterectomy in the rat.

In more recent biochemical studies of the small bowel mucosa from the functioning segment of rat intestine which remained in continuity following bypass, Gleeson et al. (1972b) showed that the amount of DNA/cm of intestine markedly increased, again indicating that hyperplasia must have occurred.

While in the past, the term 'hypertrophy' has been used to describe villous enlargement as seen after resection, current literature shows that increase in villous size is due to hyperplasia rather than to increase in the size of individual cells. This is certainly true in animals. Evidence in man has inevitably been more fragmentary. Porus (1965) found more epithelial cells per unit length of villus in peroral jejunal biopsies obtained from two patients with extensive distal small bowel resections. In two other patients with smaller resections this was not found.

In another study of 11 patients with distal small bowel resection, a significant increase in villous height with hyperplasia was observed in the jejunal mucosa (Dowling, 1968). Weinstein et al. (1969) also noted hyperplasia in peroral biopsies from the jejunum of 2 patients with small bowel resection. The number of cells lining the sides of the villi were increased approximately twofold the normal.

In conclusion one may say, that the small bowel is capable of a compensatory response following resection or bypass operation. The result of the adaptive compensatory changes is usually referred to as 'hypertrophy' in the literature. This term has been used for visible changes in the intestine, but it is now clear that the changes are due to an increase in the number of the epithelial cells i.e. hyperplasia.

1.4. Theories on the control of postresectional hyperplasia.

The hyperplastic response of the small bowel to partial resection or bypass is now established, but still there is not much certainty about the mechanisms controlling this adaptive process. Evidence exists to implicate both intraluminal, systemic, local or other

factors in this control. Since the present studies are focussed upon this controversy, current theories concerning the control of hyperplasia will be described detailed in this chapter.

1.4.1. Intraluminal factors

1.4.1.1. Exogenous nutrients

Under normal circumstances the absorptive functions of the ileum are largely limited to the active transport of vitamin B12, bile salts and cholesterol. Ileal mucosa is seldom exposed to high intraluminal concentrations of carbohydrate, fat or protein, for absorption of these nutrients is virtually complete within the jejunum. After proximal small bowel resection the ileum receives an increased supply of chyme, which might directly stimulate its hyperplastic response (Dowling, 1967). Experimental hyperphagia without resection causes villous enlargement, and epithelial cells lining the intestinal canal can probably use intraluminal glucose and amino-acids for their own nutrition (Smyth, 1962; Kinter and Wilson, 1965). This theory of luminal or 'topical' nutrition rests largely upon the work of Dowling and his associates (1970). It demands the presence of nutrient material within the intestinal lumen without necessarily implying that these nutrients must be absorbed to exert their effect (Dowling, 1974). The theory provides an attractive explanation for the fact that morphological and functional adaptation of residual small bowel is always much greater after proximal than distal resection, and that it is maximal near the anastomosis, tapering off distally (Booth et al., 1959). Confirmatory evidence is provided by transposition experiments which separate the effects of environmental change from those of resection. Dowling and Booth (1967) found increments in mucosal thickness and villous height in ileum following ileojejunal transposition, similar to those obtained after jejunectomy. In the transposed jejunum, partially deprived of luminal nutrition, there were no histological changes but an eventual reduction in glucose absorption 3 to 8 months after operation (Gleeson et al., 1972a).

Altmann and Leblond (1970) reported a diminishing aboral gradient of villous height in rats throughout the small intestine from pylorus to ileocaecal valve. In ileal segments transposed to the jejunum villi enlarged to the size of local jejunal villi. Unlike Gleeson et al. (1972) however, they found that villi in jejunal segments transposed to the ileum did undergo a reduction in size. Gleeson et al. (1972a, 1972b) described structural and functional atrophy in Thiry-Vella fistulae in rats, which contrast strikingly with the hyperplasia in the remaining intestine in continuity. After confirming this atrophy in selfemptying blind loops of jejunum, Menge et al. (1970, 1973) restored the anatomical continuity of the bowel; four weeks later values for mucosal thickness and glucose absorption were significantly higher than controls, although this may have been an 'overshoot' phenomenon.

Altmann and Leblond (1970) also studied adaptation in intestine deprived of nutrition by the construction of blind sacs of jejunum and ileum draining into the ascending colon. Whereas jejunal villi decreased in size, ileal villi actually increased.

The possible influence of luminal nutrition gets additional support from the intestinal changes seen after starvation. Jackson (1915) first showed that acute and chronic starvation nearly halved the weight of the stomach and intestines of the rat when expressed as a

percentage of total body weight. Starvation progressively lowers the cell population and the proliferative activity of the intestinal epithelium (Altmann, 1972). Furthermore, rats deprived of oral food but nourished parenterally to maintain positive nitrogen balance still undergo profound mucosal atrophy throughout the small intestine (Levine et al., 1974). This is again an argument for the hypothesis that the presence of nutrients in the bowel lumen is essential for the maintenance of the intestinal epithelium.
A recent study has compared the structural and functional adaptation of the small bowel after 50 per cent proximal enterectomy in dogs receiving either oral or total parenteral nutrition (Feldman et al., 1976). Biopsies were taken for measurement of histological parameters and in vivo studies of glucose absorption were performed at the time of resection and six weeks later. Although there was no significant difference in body weight between the two groups, dogs fed by mouth displayed obvious dilatation and enlargement of the residual ileum, which was confirmed by histological measurements and was accompanied by increased glucose absorption per unit length of bowel. Parenterally-nourished dogs however showed a significant reduction in villous height compared with original values, and showed no functional improvement. Similar absence of postresectional hyperplasia had also been shown in rats maintained for one week on total parenteral nutrition (Levine et al., 1976).
The presence of intraluminal nutrients seems therefore essential both for the maintenance of normal mucosal integrity and for the structural and functional response to partial intestinal resection. Nonetheless it is unlikely to be the only factor involved. Altered chyme could scarcely explain a modest jejunal hyperplasia which follows distal small bowel resection (Booth et al., 1959), nor the ileal response to colectomy seen both in the rat (Wright et al., 1969a) and in man (Wright et al., 1969b).
Bochkov (1959) even reported a greater increase in villous height in the jejunum than the ileum after mid-enterectomy.
Altered chyme does not account for villous hyperplasia encountered distal to an atretic segment of intestine in neonates; this was first reported by Tilson in 1972 and has since been confirmed in several other cases of neonatal atresia (Touloukian and Wright, 1973).
Finally, although exposure of the ileum to a richer nutrient load might cause mucosal hyperplasia, it would be less likely to explain the thickening of the muscular coat, which is also part of the adaptive process.

1.4.1.2. Endogenous secretions

The idea that villous height might be regulated by various gastrointestal secretions has been elaborated through a series of ingenious experiments by Altmann (1971). Having shown a progressive decrease in villous height in rats from the pylorus to the ileocaecal valve, which could be altered by transposition of intestinal segments, Altmann and Leblond (1970) proposed the existence of villous-enlarging factors in duodenojejunal chyme and of villous-reducing factors in ileal chyme. Transposition of a segment of duodenum into the ileum caused an increase in villous size in the ileum, distally to the transposed segment, while the villi of the transposed segment remained unchanged. It was assumed that a villous enlarging factor was secreted by the duodenal tissue and when propelled

distally by peristalsis, it did affect the ileum. Similar effects were seen in the ileum when a blind sac of duodenum (containing the duodenal papilla) was joined to the ileum. But these duodenal secretions could not prevent villous hypoplasia in a blind duodenal segment, when the papilla was not retained in that segment. Since the duodenal papilla transmits both pancreatic juice and bile, it seemed that one or perhaps both of these substances were involved as the villous enlarging factor.
In a further serie of experiments Altmann (1971) was able to separate these two secretions and he was able to show that pancreatic juice was more effective than bile alone in causing ileal hyperplasia. Subsequently he obtained marked mucosal hyperplasia by infusing pancreatic extracts but not bile into the lumen of conscious unrestrained rats (Altmann, 1974). Preliminary findings of Weser and Tawil (1975) are showing that the transposition of the duodenal papilla into the ileal remnant after jejunectomy significantly augment mucosal hyperplasia. These data supported Altmann's ideas. Since this augmentation was unaffected by feeding the rats on elementary diet, it cannot depend merely upon enhanced protein digestion and liberation of amino-acids for nutrition of the luminal cells.
Roy et al. (1975) investigated epithelial cell kinetics in rats with external biliary fistulae; they showed that after two days' absence of bile from the gut there was diminished cellular proliferation, particularly in the ileum, which could be partially restored by infusion of sodium taurocholate.
Evidence therefore exists that both bile and pancreatic juice play a role in modulating mucosal cell proliferation.

1.4.2. Humoral factors

Loran and co-workers postulated the existence of an 'intestinal growth hormone' to explain the generalised hyperplasia seen throughout the small intestine two months after a limited ileal resection in rats of only 10 per cent of the small bowel (Loran and Althausen, 1960; Loran and Crocker, 1963). They sought to transmit this factor between rats linked in cutaneous parabiosis, involving 'anastomosis' of the skin overlying the shoulders and the abdominal wall (Loran and Carbone, 1968). After 30 days one parabiont was subjected to 10 per cent ileal resection and after a further 30 days both animals were injected with tritiated thymidine and sacrificed. Autoradiography showed that half an hour after thymidine injection both members of the pair had about 66 per cent labelled cells, while in control pairs the average was about 38 per cent. Nine hours after injection both members showed a greater synchrony and uniformity in the period of DNA synthesis. In another experiment in which resection preceded parabiosis, they found persistence of the humoral stimulus for 30 days. Finally they studied animals maintained in parabiosis following prior resection of one member, then separated after one month and sacrificed a week later. Although increased cell proliferation was still seen in the unoperated partner, the greater synchrony and uniformity in the duration of DNA synthesis which followed resection was no longer shown. Loran and Carbone (1968) concluded that two systemic factors were involved in the response to resection, one

affecting the rate of cell proliferation and the other the degree of mitotic synchrony; only the first was irreversible and self-perpetuating.
Tilson and associates provided only partial corroboration of these findings in preliminary reports using similar techniques of cutaneous parabiosis (Tilson and Wright, 1971c; Tilson et al, 1975). They described to have found that villous cellularity was increased in unoperated partners of animals undergoing resection, but villous length was unaltered. When the unoperated parabionts were starved they showed no response at all to humoral stimuli.
Cross-circulation studies carried out in our laboratory (Moolten and Bucher, 1967; van Vroonhoven et al., 1972; Dijkhuis et al., 1975) have shown that humoral factors almost certainly are involved in renal and hepatic regeneration.
Using a technique introduced by Rowinski and Kaminski (1973), Tilson and Livstone (1975) tested the humoral theory by autoradiographic studies of ileal mucosal fragments transplanted beneath the renal capsule in rats after partial enterectomy and sham enterectomy. Experimental explants showed increased diameter, cellularity and percentage cell labelling of crypts as compared with shams.
These heterotopic autografts were denervated and out of continuity with the intestinal tract.
Tilson and Wright (1970) demonstrated increased villous height, cell count and migration rate in both defunctioned and functioning segments of ileum two weeks after jejunectomy. Hyperplasia was more pronounced in the ileal segments in continuity, suggesting that both local and systemic factors are involved in the regenerative process. Humoral agents might be either inhibitory or stimulatory.
Regulation of organ size by negative hormonal feedback related to mass is a well-established concept (Weiss and Kavanau, 1957) which might be applicable to the small bowel (Tilson and Wright, 1970). According to this theory each cell secretes its own growth inhibitor. Loss of mass would decrease circulating levels of this inhibitor until sufficient mass has regenerated to restore original concentrations. The main argument against this theory has been the hyperplastic response seen after intestinal bypass, where there is no loss of tissue mass. It is however possible to speculate that the inhibitory humoral factor might only be released by bowel mucosa in continuity with the nutrient stream.
Alternatively intestinal growth after resection might occur in response to a greater functional demand by the body, mediated either by stimulatory humoral agents or perhaps by diminished levels of circulating glucose or electrolytes. This concept has been elaborated in a series of papers by Tilson and Wright (Tilson and Wright, 1970; Tilson, 1972b; Wright and Tilson, 1974; Tilson et al., 1975), but remains difficult to prove. It would account for the villous hypertrophy shown by intermittently-starved rats (Fabry and Kujalova, 1960) and for the adaptation of the ileum to subtotal colectomy (Wright et al., 1969a, 1969b). Salt and water loss after colectomy would lead to mineralocorticoid secretion, and deoxycorticosterone acetate has been shown to stimulate ileal hyperplasia (Tilson et al., 1971). Intestinal remnants after mid-enterectomy show hypertrophy of the lateral cell membrane and increased activity of sodium-potassium-activated adenosine triphosphate, both of which may reflect increased sodium absorption by the cell (Tilson and Wright, 1971a, 1971b).
Hormones as possible candidates for the role as 'humoral factor' will be reviewed now.

Gastrin as a tropic hormone

Johnson (1976) strongly advocates gastrin as a tropic hormone for the entire gastro-intestinal tract including pancreas, but excluding gastric antrum and oesophagus. The evidence that there is an influence of gastrin on the mucosa of the gastric fundus and the proximal duodenum is strong but not completely convincing. Although mucosal hyperplasia of the stomach and duodenum are found in the presence of gastrin-secreting tumours, in pernicious anaemia high levels of circulating gastrin accompany gastric atrophy. The theory that gastrin is a tropic hormone of the gut rests heavily upon the actions of pentagastrin, which is a synthetic unphysiological drug. Tropic effects of pentagastrin may depend upon acute gastritis or dilatation of gastric submucosal arterioles (Guth and Smith, 1975; Hansen et al., 1976). The marked increase in cell proliferation caused by high doses of pentagastrin in fundic mucosal biopsies from healthy human volunteers cannot be reproduced by synthetic human gastrin at lower dosage (Hansen et al., 1976). An enterotropic role of gastrin is not supported by findings of Oscarson et al. (1977) that 20 fold fluctuations of endogenous gastrin within the physiological range neither prevent atrophic changes of short-term starvation on small bowel mucosa, nor alter the compensatory hyperplastic response to jejunectomy.

There is limited evidence to suggest that several other hormones may stimulate intestinal cell proliferation.

Mineralocorticoids, secreted perhaps in response to the fluid and electrolyte losses of massive enterectomy or colectomy, promote structural and functional adaptation in rat small bowel (Tilson et al., 1971). Enzymatic and ultrastructural changes suggesting enhanced sodium absorption in ileal mucosa after partial enterectomy could be mediated by aldosterone (Tilson and Wright, 1971a; 1971b).

Pituitary hormones may play a part in the postresectional response, for intestinal hypoplasia in hypophysectomised rats cannot simply be explained by reduced food intake (Taylor and Dowling, 1975). Although lactation causes hyperphagia, the associated growth and functional adaptation of the small bowel may result from humoral stimulants (Elias and Dowling, 1976) rather than increased luminal nutrition (Campbell and Fell, 1946).

Prolactin may mediate hepatic growth during lactation (Turkingston, 1972), but does not appear to have a definite enterotropic role (Dowling, 1976).

Cholecystokinin (CCK), known as a potent tropic hormone for the exocrine pancreas (Mainz et al., 1974). But CCK does not stimulate DNA synthesis in oxyntic gland mucosa (Johnson, 1976).

Secretin has been found to have no tropic activity of its own and is known to inhibit the tropic response to pentagastrin (Stanley et al., 1972; Johnson and Guthrie, 1974).

Pancreatic glucagon, like gastrin, did significantly increase the DNA content of oxyntic gland mucosa and of colonic mucosa, but the effect was less pronounced than that caused by gastrin (Johnson, 1977).

Enteroglucagon is probably the best single candidate for the role of enterotropic hormone. Gleeson et al. (1971) described a patient with an enteroglucagon secreting renal tumour. The existing villous hyperplasia disappeared following nephrectomy. Glucagon may influence hepatic regeneration alone (Price et al., 1972) or potentiated with insulin (Bucher and Swaffield, 1975). Chronic administration of glucagon to rats increases active

transport of amino-acids and sugars by small bowel epithelium (Rudo and Rosenberg, 1973). Moreover enhanced intestinal absorption in partial starvation (Rudo et al., 1973) and experimental diabetes (Rudo, 1973) is associated with elevated glucagon levels in plasma, and can be prevented by injections of glucagon binding antisera (Rudo et al., 1975).

1.4.3. Other factors

1.4.3.1. Mechanical factors

Nygaard (1967) found that local hypertrophy of the bowel on either side of an intestinal anastomosis extended for 5 cm proximal and distal of the anastomosis, confirming by Evans and Brenizer (1907). The changes probably result from a combination of mechanical factors and local alterations in motility or blood supply. Partial anastomotic obstruction has been suggested as the cause of postresectional adaptation (Lansky et al., 1968; Levine et al., 1976), but among many objections to this theory stands the fact that hyperplasia is always greater on the distal side. Although luminal dilatation is one of the structural changes seen in the adapted bowel, it is accompanied by increased thickness of muscular and mucosal coats. Furthermore it has been shown that addition of bulk to the diet doet not cause small bowel hyperplasia, though it does cause colonic muscular hypertrophy (Dowling et al., 1967). Dilatation and stasis may allow functional adaptation and it is possible that for example colonic hyperplasia after small bowel resection may result in part from an increased work load.

1.4.3.2. Bacterial factors

Conceivably some of the adaptive changes attributed to food and gastrointestinal secretions could be mediated by alterations in bacterial flora. This explanation was advanced by Gleeson et al. (1972a) to account for the reported differences in behaviour between ileal mucosa isolated from continuity and anastomosed to the skin or the colon. Bacterial contamination might contribute to post-colectomy ileal adaptation, but seems unlikely to play a significant role in the rapid response of the bowel to a small bowel resection. Altmann (1974) was unable to produce any mucosal changes by instilling bacterial suspensions into intestinal loops.
On the other hand animals raised in a germfree environment have thinner intestinal mucosa, shallower crypts, narrower vili than do conventional animals (see review article Eastwood, 1977).

1.4.3.3. Neurovascular factors

Increased blood supply to small bowel remnants was put forward as a possible cause of postresectional hyperplasia in Senn's original paper (1888), but there have been very few studies on this subject.

Nylander and Olerud (1962) reported normal microangiographic appearances in dilated bowel remnants. Touloukian and Spencer (1972) studied intestinal blood flow two days and two months after mid-bowel resection by the radiorubidium distribution technique. In the ileal remnant mucosal blood flow was markedly greater than that of controls after two days, but returned to normal at two months, by which time striking hypertrophy was apparent. The jejunal remnants showed no change in blood flow and no hypertrophy. This selective increase in blood flow in the remaining ileum only, was found to be associated with adrenergic denervation of the hypertrophied gut remnant, as shown by reduced catecholamine activity and histochemical fluorescence studies (Touloukian et al., 1972). Vagotomy has also been shown to depress villous blood flow and this effect can be reversed by splanchnicectomy (Padula, 1967).
It is impossible to tell at present whether alterations in blood flow are the cause or the effect of intestinal hyperplasia. In the remaining kidney after unilateral nehprectomy there is a similar increase in blood flow preceding hypertrophy (Malt, 1969), and hepatic regeneration can be reduced by diversion of blood from the liver (Bucher, 1967), and increased by arterialization of the distal portal vein after portocaval shunt (Fisher et al., 1954). Increased blood flow might conceivably have a 'wash out' effect and thus reduce the concentration of local inhibitor substances (Tilson and Wright, 1970).

1.4.3.4. Local factors

Local regulation of epithelial renewal involves the feed back control of crypt cell proliferation by the villous cell population. During recovery after irradiation in rats, the expansion of the proliferative zone in the crypts was accompanied by a decrease in the number of villous cells (Rijke et al., 1975).
Similarly germ free rats, whose villi contained more epithelial cells than conventional rats, had a delayed proliferative response during recovery from irradiation when compared to conventional controls (Galjaard et al., 1972).
These studies suggested that the number of cells on the villus might regulate the proliferation of cells in the crypt in an inverse fashion. This hypothesis of a feedback control mechanism is supported by studies of Rijke et al., 1976, who induced intestinal ischaemia by clamping the superior mesenterie artery and vein in rats for 1 hour. The ischaemia did sharply reduce the number of villous cells, whereas the number of crypt cells was not affected. After restoration of the bloodflow, autoradiography studies demonstrated an increase in the percentage of labelled cells per crypt column and in the relative size of the proliferative cell compartment compared to control values. The temporary ischaemia in a large part of the small intestine did not lead to changes in proliferative activity in adjacent normal intestine. This suggests that the response is of a local character.
Other local factors may be chalones. Chalones are substances which inhibit mitotic activity and are thought to be important in the control of many proliferative populations (Bullough, 1962, Thornly and Laurence, 1975).

1.5. Summary

There is general agreement that hyperplastic changes take place in the small bowel after surgical interventions like resection and bypass operations.
There is no consensus of opinion about the question if the effects after jejunal resection and jejunal bypass are quantitatively of the same order.
The earlier experiments studied the effect of small bowel adaptation after a relatively long time (weeks). More recent investigations were directed also towards the early stage (days). There are almost no longitudinal studies combining both the early and the later stadia.
In the literature several hypotheses about the regulating factors of the hyperplasia are mentioned. Probably it is a multifactorial process in which intraluminal, humoral and secretorial factors are playing a role.

Chapter II

THE EXPERIMENTAL DESIGN AND METHODS

2.1. Introduction

The aim of this work is the study of both the early and later stages of the intestinal hyperplasia after resection, transection and bypass-operations of the small bowel in the rat in one experimental set up.
It further aims at the question if there is a quantitative difference in response between the effects of jejunal resection and jejunal bypass on small bowel adaptation. It tries to evaluate the influence of a number of factors mentioned in the literature as possible mediators of postresectional hyperplasia i.e. intraluminal, humoral and secretorial factors.

2.2. Experimental animals

Male Holtzmann rats weighing 170–250 g. were used in the different experiments. The animals were kept in suspended cages with open wire-mesh bottoms under alternate 12-hour lighting cycles. Water and Purina rat pellets were allowed ad libitum, except where otherwise stated. The rats were allowed a minimum of 3 days acclimatisation before surgery.

2.3. Surgical procedures

All operations were performed under light ether anaesthesia + 0_2 between 8.00 and 13.00 hours to mitigate the effects of diurnal cycles. The operative techniques employed in the different experiments will be described under the relevant chapter headings, but certain general remarks are appropriate here.
All laparotomies were carried out through a midline incision. The length of the small intestine between the ligament of Treitz and the ileocaecal valve was measured by gently stretching the bowel against a ruler in 5 cm segments; the lenght varied between 80 and 100 cm. according to the age of the animal. All intestinal anastomoses were carried out using a single continuous layer of 6/0 silk or Tevdek sutures.

2.4. Radiochemicals

^{3}H-methyl-thymidine (6.7 Ci/mmole) was obtained from New England Nuclear Corpo-

ration. Samples were checked for purity by thin-layer chromatography at intervals. Specificity of incorporation into DNA was checked by occasional assays of radioactivity in RNA and protein. Doses of 50 μCi of tritiated thymidine in 0.5 cc saline were administered by subcutaneous injection one hour before death.

2.5. Mucosal specimens

Animals were sacrificed in the same time period as the operations were performed i.e. between 9.00 and 13.00 hours, either by cervical dislocation or by decapitation to allow collection of blood samples. The small bowel, excluding the duodenum, was stripped from its mesentery, removed, flushed gently with ice cold saline and then placed on a glass plate resting on crushed ice. Appropriate 5 cm. segments of intestine were cut, opened longitudinally and spread out on the plate with the mucosal surface uppermost. The circumference of the opened bowel was then measured to the nearest 0.5 mm. by a ruler. The segments for scraping were 5 cm. The measurement was done with a standard method along a slightly stretched bowel segment.
The mucosa was then scraped from these specimens between two slides and frozen in liquid nitrogen within 5–10 minutes of death. Specimens were stored at -70°C for subsequent biochemical analysis.
With practice excellent yield of mucosa was obtained and microscopic examination showed no residual crypts on random specimens of scraped intestinal wall. It was nearly always possible to avoid the inclusion of lymphoid follicles in the mucosal specimen. Adjacent 1 cm. segments of bowel were fixed in 10 per cent formalin for histological examination. In certain experiments the colon (excluding the caecum) was also removed, flushed and divided into ascending, transverse and descending portions from which scrapings were obtained in identical manner. The rat colon is just long enough to obtain three 5 cm. specimens plus intervening 1 cm. biopsies for histology studies, but the colonic mucosa is less easy to scrape cleanly from the underlying muscularis than the mucosa of the small bowel.

2.6. Biochemical determination of DNA and RNA

Nucleic acid contents in the mucosal scrapings were determined by the method of Scott, Fraccastoro and Taft (1956) as modified by Hinrichs, Petersen and Baserga (1964). The method is based upon the ultraviolet absorption of the purine and pyrimidine constituents of nucleic acids, using spectrophotometric quantitation.
The assay is described in detail in Appendix A, but the basic steps were as follows, mucosal specimens were thawed, homogenized in citric acid sucrose and treated with cold perchloraretic acid to precipitate the macro molecular fraction, including RNA, DNA and proteins. The radioactivity of the acid-soluble fraction was then measured, containing all the isotope not incorporated into DNA, mostly in the form of thymidine monophosphate (TMP), thymidine diphosphate (TDP) and thymidine triphosphate (TTP). After treatment with 80 per cent ethanol and cold alcohol-ether to extract the lipids the precipitate

underwent alkaline digestion for one hour in order to hydrolyse the RNA and render it acid soluble. Subsequently the optical density of the RNA was measured at two different wavelengths (260 mμ and 280 mμ) in order to minimize errors due to contaminants, notably protein, which might contribute to the ultraviolet absorption (Tsanev and Markov, 1960).
The DNA and protein fractions were then precipitated by strong acid and separated by incubation with hot acid to hydrolyze the DNA. The optical density of DNA was measured at one wavelength (260 mμ).
The residual pellet was available for estimations of the protein fraction.
Aliquots of the DNA fraction were counted by liquid scintillation spectrometry at 25 per cent efficiency using internal standardisation. The specific activity of the DNA was derived by dividing the total radioactivity (desintegrations per minute) incorporated into DNA by the DNA content (mg.). The amount of radioactivity in DNA expressed in terms of total DNA present (DNA specific activity) is an estimation of the proportion of cells synthesizing DNA (Baserga and Malamud, 1969).
Although rather a crude technique, the DNA specific activity is thought to be a good index of the proliferative cell pool of the tissue (Lea et al, 1966).
Autoradiography studies by Nundy et al. (1977) have shown that when mucosal contents of DNA are increased, active cell proliferation may coexist with low levels of DNA specific activity. Since cells contain a fixed amount of DNA which is constant for any given species (Boivin et al., 1948) and intestinal epithelial cells remain euploid during proliferation (Lipkin, 1973) the DNA content of the intestinal mucosa reflects the total number of cells. The RNA content of a cell however tends to increase with the size of the cell. The RNA/DNA ratio may therefore indicate the relative predominance of hyperplasia or hypertrophy in the enlargement of an organ. (Bucher and Malt, 1971).

2.7. Histological measurements

Specimens were coded to eliminate observers bias and histological sections (5) were prepared for staining with haematoxylin and eosin. Only sagitally transected villi and crypts were used for measurement. Ten determinations of villous height and crypt depth were made per slide by means of an ocular grid with stage micrometer; the readings were averaged for each section.

2.8. Statistics

Student's t-test for unpaired data was used throughout. When the term significant is used in the experiments, this means statistical significant.

2.9. Validity of the methods

While the turnover time of the villous epithelium gradually increases in the duodeno-ileal

direction (Altmann and Enesco, 1967), there is an aboral gradient of nucleic acid contents and of villous height throughout the small intestine. In addition there is a tenfold increase in renewal rate of the intestinal epithelium between weanling and adult rats.
For this reason care was taken to include rats from a relatively narrow weight (age) range in the different experiments and to obtain mucosal specimens from exact the same part of the small bowel in all groups as is customary in developmental biology. Still maturing rats, well after the infancy but before the adult inflection point were used to maximal changes in the chemical analysis.
Measurements of mucosal weight might be unreliable owing to the possible inclusion of intestinal contents, mucus or mesenteric fat in the specimen. Although such errors can be lessened by taking care to blot the bowel after flushing and to trim all extraneous tags of tissue, these procedures would delay the freezing of the specimen and thus risk the loss of nucleic acids through the action of specific nucleases. All biochemical results have therefore been expressed in terms of sample size, i.e. per 5 cm. of scraped bowel mucosa. The tendency of the bowel to undergo longitudinal shrinkage following removal was counteracted by gently stretching the segments prior to scraping, and great care was taken to obtain a consistent 5 cm. sample of mucosa.
Measurement of the circumference of the small bowel by a ruler had obvious limitations of accuracy but is rapid and simple and showed reasonably consistent results within groups of animals. Colonic measurements of the circumference were found to vary considerably and were therefore abandoned.
Certain precautions were taken to minimize errors in the nucleic acid assay. The notorious inclination of alkaline solutions to become contaminated with ultra-violet absorbing impurities was prevented by making a fresh solution of 1N NaOH and testing its absorbance against H_2O at the start of each set of determinations. Furthermore the stock solution was kept in a polyethylene bottle (Scott et al., 1956). The modification of Scott et al. (1956) in reducing both the temperature and the duration of the crucial stage of alkaline digestion is largely succesful in avoiding the main disadvantage of the Schmidt-Thannhauser technique, namely the release of significant quantities of protein into the RNA fraction as was shown by Fleck and Munro (1962). In the Schmidt-Thannhauser technique RNA and DNA are separated by a 15 hour digestion in alkali, which hydrolysis the RNA to mononucleotides and thus makes it acid soluble, whereas the DNA and most of the cell protein are precipitated on acidification of digest. Unfortunately, other substances become also soluble during this long period of alkaline digestion and thus contaminating the RNA fraction.
Hinrichs, Petersen and Baserga (1964) tested the accuracy of the method of Scott et al. by determining the amount of radioactivity recovered in the three acid-insoluble fractions (DNA, RNA and protein) after administration of their specific tritiated precursors (cytidine, thymidine and leucine). They concluded that the error in obtaining DNA values by the procedure of Scott et al. (1956) was less than 5 per cent, although there still was some contamination of the RNA fraction by protein. The adaptation of 'the two wave length technique' of Tsanev and Markov (1960) a method for spectrophotometric determination of nucleic acids, neutralizes the effect of this protein contamination. Since some nucleic acid must be lost during the many steps of the fractionation procedure, the probable error of the determinations has been expressed in the form of standard errors.

So far as the radioactive thymidine uptake and distribution is concerned, thymidine is incorporated exclusively into DNA (Reichard and Estborn, 1951; Amano et al., 1959). The process is virtually complete within 30 minutes of injection (Kisieleski et al., 1961). By this time unincorporated thymidine has been catabolized to products, including thymine, which the cells cannot utilize (Zajicek et al., 1963). Approximately half the thymidine incorporated into DNA is taken up by the small bowel (Hinrichs et al., 1964), the exact percentage depending upon the route of administration (Petersen and Baserga, 1964). Although diurnal variations do not affect intestinal uptake, they do affect the proportion of thymidine taken up by the rest of the body (Pilgrim et al., 1963). In the present study care was taken to minimize such variations by sacrificing all animals at the same time of day (9.00 – 13.00 hours). Radioactivity incorporated into DNA is preserved during the nucleic acid fractionation procedure although 30 per cent of the acid soluble radioactivity may be lost (Hinrichs et al., 1964). Comparison of the radioactivity into these two fractions is a useful internal control.

In order to test the accuracy of both the sampling technique and the biochemical assay in the present studies, 'duplicate' samples were obtained from all animals in the first experiment by taking mucosal specimens from contiguous 5 cm. segments of bowel and determining their nucleic acid contents. The apparent errors, expressed as a percentage of the mean value for each pair, were ± 3.4 per cent for RNA and ± 4.9 per cent for DNA. The true error in each case is almost certainly lower because of the aboral gradient of nucleic acid contents in the small bowel (Altmann and Enesco, 1967).

The result of the measurements by occular micrometry of villous height and crypt will of course be influenced by tissue shrinkage during fixation and staining, but in the present study it does not interfere with the proper comparison of the experimental groups. Although the biochemical changes develop more rapidly than the histological, in our studies the two techniques are complementary in the study of intestinal adaptation at later time points.

Chapter III, Experiment 1

JEJUNAL TRANSECTION AND RESECTION

3.1. Introduction

As already mentioned in chapter I, the small bowel reacts with hyperplasia in the residual intestine following resection of the small bowel in the rat. This adaptation is greater in the ileum after removal of the jejunum than vice versa. Although most of the observations concerning hyperplasia of the mucosal cells in residual small intestine came from studies several months after resection, stimulated cell proliferation can be identified within 5 to 6 weeks (Weser and Hernandez, 1971; Urban and Pena, 1974).
Recently Obertop et al. (1977) showed in our laboratory in Boston that already 48 hours after jejunectomy a compensatory hyperplasia was detectable in the ileal mucosa. In his studies jejunal transection, employed as a control operation, produced no significant difference from unoperated controls. Loran and Althausen however showed already in 1958 that mere division and reanastomosis of the bowel prevented the intestinal weight loss and mural thinning seen after laparotomy alone. So in our study again a group of rats with a jejunal transection was incorporated to compare with non operated, sham operated (simple laparotomy) and jejunal resected rats.

3.2. Material and methods

Intestinal cell proliferation was studied 48 hours after simple laparotomy, jejunal transection or resection in maturing male Sprague-Dawley rats (Holtzman Co., Madison, Wisconsin). Rats (N 55) weighing 200-250 g. were randomly assigned to one of the following 4 groups of rats.
Control animals had no operation.
Sham controls had a midline laparotomy with delivery and handling of the proximal third (ca. 30 cm.) of the small bowel.
Transection involved handling of the bowel, followed by division and reanastomosis 1 to 2 cm. distal to the ligament of Treitz.
Resection of the proximal 30 cm. of small bowel was followed by reanastomosis just distal to the ligament of Treitz. Intestinal anastomoses were performed with continuous 6-0 silk sutures under light ether anaesthesia between 8.00 and 13.00 hours.
Rats were fed until the time of operation and were allowed to drink, but not to eat, for 24 hours thereafter, when the food supply was restored.
One hour before death by cervical dislocation all animals received a subcutaneous injection of 50 μ Ci ^{3}H-methyl-thymidine (6.7 Ci/m mole, New England Nuclear Corp., Boston,

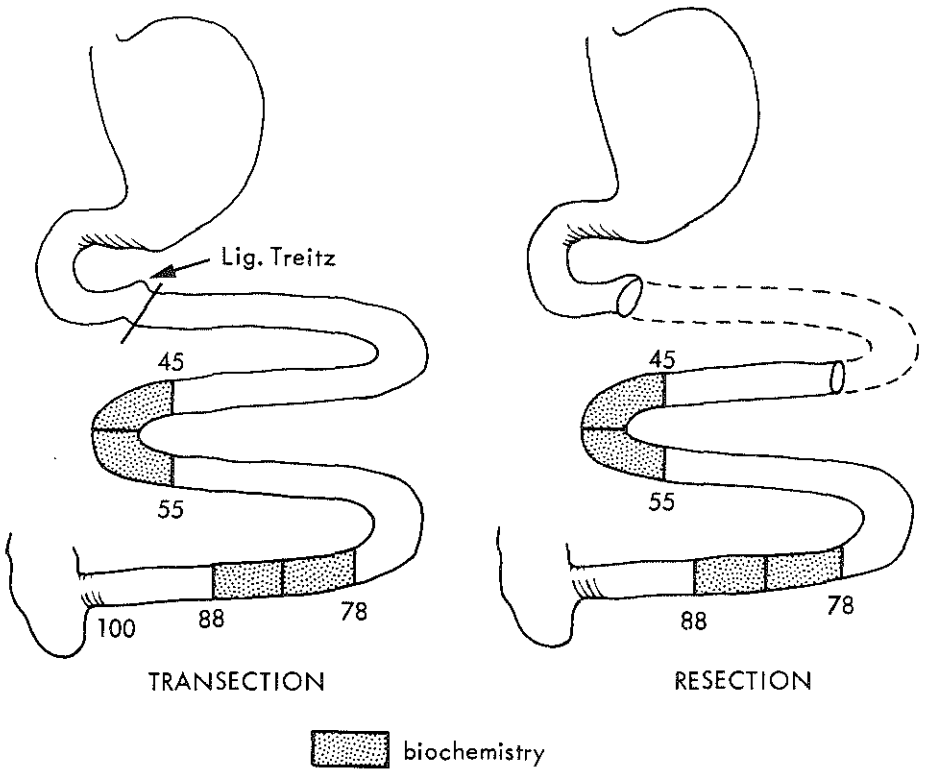

Figure 1
Jejunal transection and resection of 30 cm. of the proximal small bowel. Segments of bowel harvested for biochemical analysis are indicated.

MA). After death, the small bowel (excluding duodenum) was excised, gently flushed with cold isotonic saline solution and spread on a glass plate resting on crushed ice. 10 Cm. segments were cut from the centre of the middle and distal thirds of the intestine (fig. 1): i.e. 45 to 55 cm. (*mid small bowel*) and 78 to 88 cm. (*distal small bowel*) from the ligament of Treitz.
Longitudinal-opened segments were divided into two contiguous 5 cm. specimens, from which mucosal scrapings were obtained for estimation of RNA, DNA and radioactivity, as described under 'the experimental design and methods'. Mean values from each pair of contiguous specimens were employed for statistical analysis.

3.3. Results

Body weights: Although a 10 per cent loss was common to all groups after 24 hours of

fasting, during the next 24 hours when feeding was restored sham control rats virtually regained their initial weight, and control rats exceeded it (table I).

TABLE I

Weight change after 48 hours (means ± S.E.M.).

Group	N	Initial weight (g.)	Weight change (g.)
Control	12	225 ± 5	+ 8.4 ± 1.1[a]
Sham	13	221 ± 5	− 1.2 ± 1.5[b]
Transection	13	223 ± 2	− 3.6 ± 1.6
Resection	14	223 ± 4	− 7.4 ± 1.3

a. $P < 0.001$ vs sham, transection and resection
b. $P < 0.005$ vs resection

Nucleic acid contents

The reproducibility of the sampling technique and the biochemical assays was tested by comparing the nucleic acid contents of contiguous specimens. For RNA the variation was ± 3.4 per cent and for DNA 4.9 per cent of the mean value. The true analytic error must actually have been lower, because the specimens derived from contiguous 5 cm. segments of bowel, and animals tended to show a decreasing aboral gradient of nucleic acid content through the small bowel (fig. 2 and 3).

Nucleic acid contents in shams tended to be slightly higher and the RNA/DNA ratio slightly lower than control values (fig. 2, 3 and 4), but few of these differences were statistically significant when tested individually. The biochemical values obtained in this experiment are listed in full in Appendix B.

Although the weight of rats with enteric transections and resections remained below the baseline of the control group 48 hours later (table 1) and no significant change in RNA content was found, the DNA content rose 31 per cent in the mid small bowel and by 26 per cent in the distal small bowel mucosa after transection, as compared with control values (fig. 2 and 3). In rats undergoing resection, there was a striking rise in mid small bowel nucleic acid contents of 47 per cent for RNA and 51 per cent for DNA (fig. 2 and 3); in the distal small bowel there was a significant increase of 27 per cent for RNA and 28 per cent for DNA (fig. 2 and 3).

In the resected rats, when compared to the transected rats a significant rise in mid bowel nucleic acid contents of 38 per cent for RNA and 16 per cent for DNA was noted (fig. 2 and 3). In the distal small bowel there were significant increases over transection in RNA content (15 per cent), but not in DNA content. The RNA/DNA ratios after resection both in mid and distal small bowel were similar to values in control rats and were significantly higher than after transection (fig. 4). This means that no evidence for hypertrophy was found, because the RNA/DNA ratio gives an impression about the cell size.

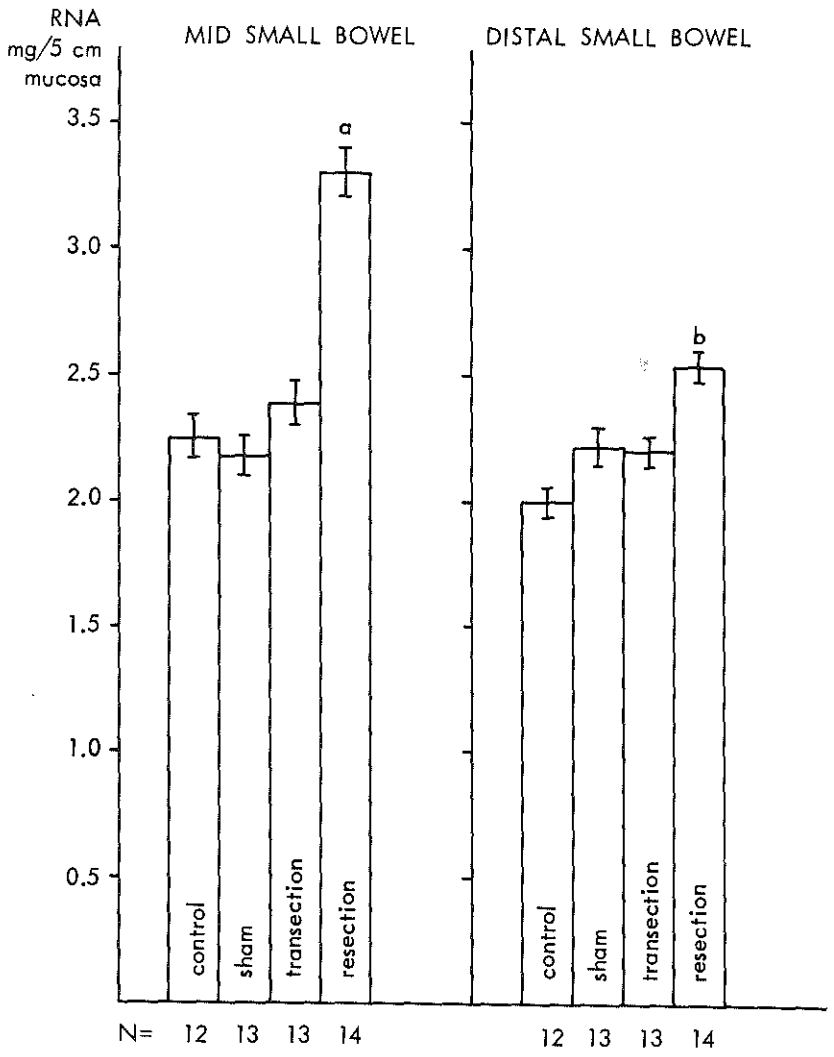

Figure 2
RNA content per 5 cm. mucosa of mid and distal small bowel (Means ± S.E.M.). The number of rats is shown under the bars.
Significance

versus control, sham and transection	a	$P < 0.001$
versus sham		$P < 0.01$
versus transection	b	$P < 0.005$
versus control		$P < 0.001$

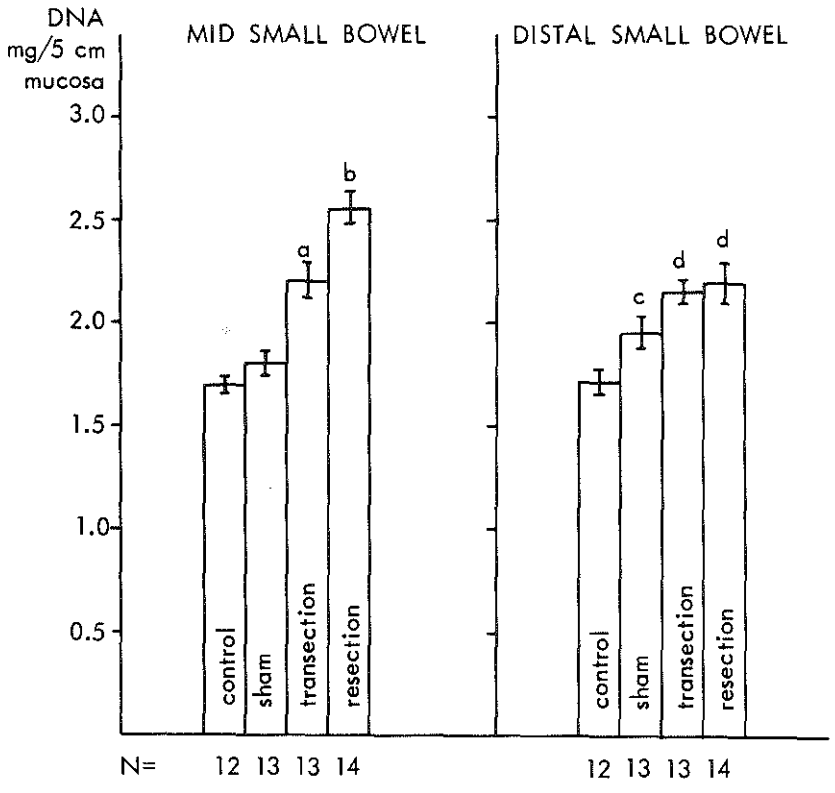

Figure 3
DNA content per 5 cm. mucosa of mid and distal small bowel (Means ± S.E.M.). The number of rats is shown under the bars.
Significance

versus sham	a	$P < 0.005$	versus control	c	$P < 0.05$
versus control		$P < 0.001$			
versus transection	b	$P < 0.01$	versus control	d	$P < 0.001$
versus sham and control		$P < 0.001$			

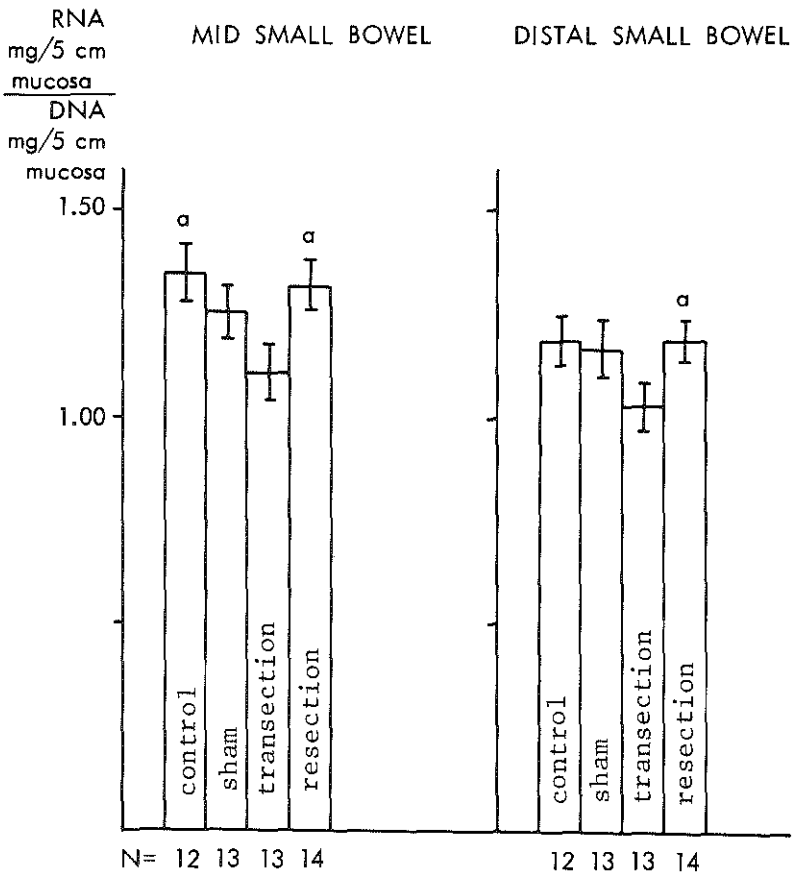

Figure 4
RNA/DNA ratios after resection in mid and distal small bowel (Means ± S.E.M.). The number of rats is shown under the bars.
Significance
versus transection a $P < 0.05$

Radioactivity in DNA per 5 cm. mucosa

Increased amounts of DNA radioactivity after injection of 50 μc ^{3}H thymidine were found only after jejunal resection: an increase of 102 per cent in the mid small bowel and 52 per cent in the distal small bowel compared to be transected rats (fig. 5). No significant differences in total radioactivity between control, sham and transected groups were noted. The amount of radioactivity in DNA expressed in terms of total DNA present (DNA specific activity) is an estimation of the proportion of proliferating cells (Baserga and Malamud, 1969).

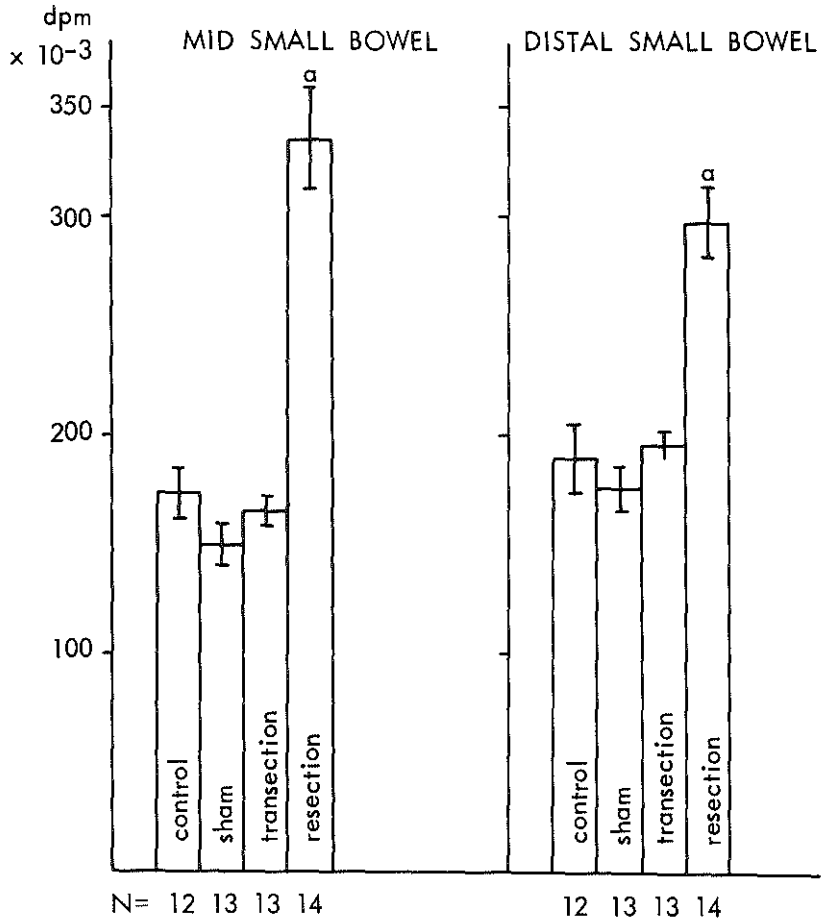

Figure 5
Total radioactivity in DNA per 5 cm. mucosa of mid and distal small bowel (Means ± S.E.M.)
Significance
versus control, sham and transection a $P < 0.001$

After jejunal resection the specific activity was significantly increased in mid and distal small bowel in comparison with specimens from rats with transected intestine: in the mid small bowel 68 per cent and in the distal small bowel 47 per cent (fig. 6).
Although there was no difference in total radioactivity between control, sham and transection, the DNA specific activity in the mid small bowel fell by 19 per cent in the sham group and by 25 per cent in the transected group (fig. 6). The DNA specific activity in the distal small bowel showed no significant differences between the 3 groups.

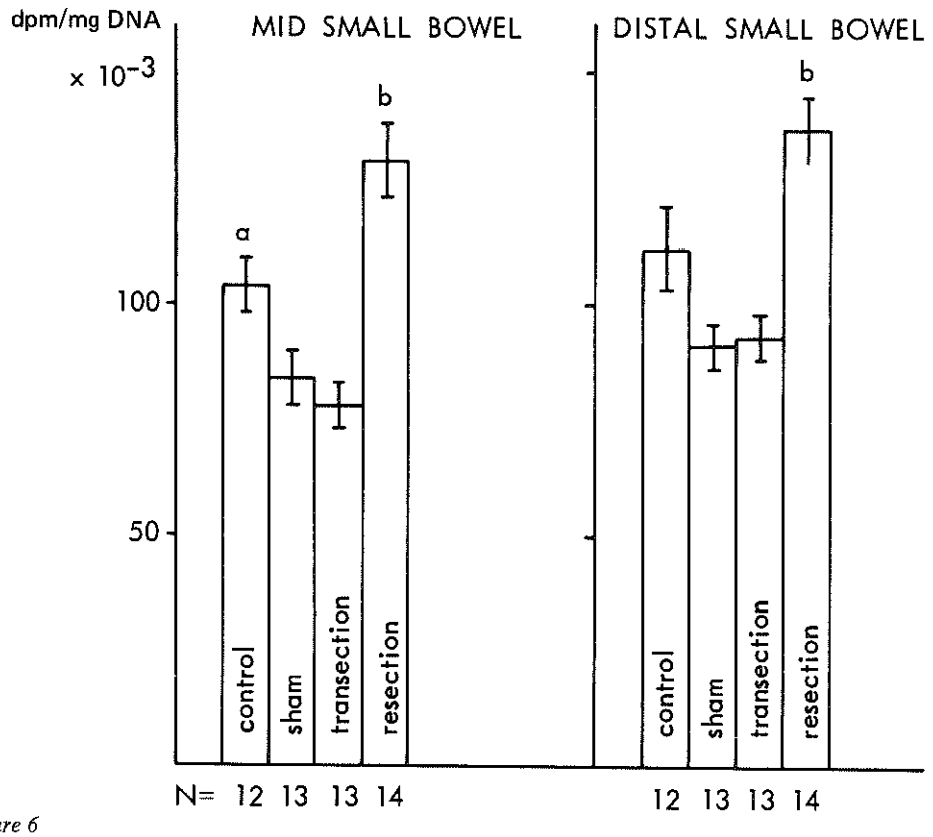

Figure 6
DNA specific activity of mid and distal small bowel mucosa (Means ± S.E.M.).
Significance
versus sham, versus transection: [a] $P < 0.05$, $P < 0.005$
versus control, versus sham and transection: [b] $P < 0.05$, $P < 0.001$

3.4. Discussion

These studies confirm an earlier report that jejunectomy stimulates prompt and continuing hyperplasia in the rest of the small bowel within two days (Obertop, 1977) and is in disagreement with a recent report of Hanson and Osborne (1977) who showed a significant hyperplasia proximal to the anastomosis (residual jejunum) within 2 days in rats who underwent a 70 per cent resection of combined jejunum and ileum. They could not detect any effect of transection itself on the small bowel.
The greater response to proximal resection found in mid bowel which lies closer to the anastomosis than distal bowel, is in agreement with previous work (Booth et al., 1959; Obertop et al., 1977).

The data of our experiment are consistent with the observations of Loran and Althausen (1958) on intestinal weight and thickness, in finding that the response to transection is intermediate between the response to simple laparotomy and resection. Unlike these investigators, whose study was performed 6 weeks after operation, we have shown no atrophic effects after 48 hours from laparotomy alone. The rats who underwent a laparotomy showed a slight tendency to increased nucleic acid levels. It is surprising that Loran and Althausen (1958) were able to detect lasting effects on the intestine from such a minor procedure.

The effects of transection depend upon actual division and reanastomosis of the bowel, rather than anaesthesia or non-specific surgical trauma. The effects after transection appear to be transient, after 48 hours there is no longer detectable a rise in DNA specific activity, whereas resection causes more persistent and intensive hyperplasia, with a high specific activity of DNA at 48 hours in spite of the increased cell count (indicated by DNA content).

Comparison of specific activity however, may not accurately reflect cell kinetics if DNA contents vary widely. The considerable increment in DNA content found 2 days after transection increases the denominator when calculating DNA specific activity. Thus this can explain the differences. Autoradiographic studies have shown that under these circumstances active proliferation may co-exist with low values for DNA specific activity during compensatory intestinal hyperplasia (Nundy et al., 1977).

In conclusion we can say that even a simple transection of the jejunum gives rise to a transient spurt of distal small bowel hyperplasia in comparison with a laparotomy followed by handling of the bowel. This in contrast with the greater and continuing response to jejunal resection measured 48 hours after the operation. Changes which might occur at later periods after operation will be discussed in the next chapter.

3.5. References

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PROXIMAL RESECTION OF THE SMALL BOWEL COMPARED TO PROXIMAL BYPASS AND PANCREATO-BILIARY DIVERSION TO THE MID SMALL BOWEL

4.1. Introduction

Intraluminal factors are essential for adaptive hyperplasia after partial loss of the small bowel in rats (Dowling and Gleeson, 1973; Lichtenberger et al., 1976; Feldman et al., 1976; Levine et al., 1976).
Possible essential enteric factors are gastroduodenal secretions, pancreato-biliary secretions, intestinal secretions and food. Food is essential for the maintenance of normal mucosal integrity (Steiner et al., 1968; Altmann, 1972; Levine et al., 1974) and for the development of postresectional hyperplasia in the dog and the rat (Feldman et al., 1976; Levine et al., 1976).
Pancreato-biliary effluent is a powerful determinant of villous height in the rat (Altmann and Leblond, 1970; Altmann, 1971) and transposition of the papilla into the ileum enhances ileal adaptation after jejunectomy (Weser, 1977). In the experiment presented here we will consider the role of pancreato-biliary secretions as intraluminal stimulators on compensatory hyperplasia. Furthermore we will compare in our experimental setup the effects of proximal resection and bypass on compensatory hyperplasia in the distal small bowel. Excision and exclusion of the jejunum expose the ileum to a richer supply of chyme (Dowling and Booth, 1967) and to a higher concentration of bile and pancreatic juice, because of greater proximity to the duodenal papilla (Roy et al., 1975).

4.2. Material and methods

Male Sprague-Dawley maturing rats (Holtzman Co., Madison, Wi.) weighing 170 to 210 g. (n = 181) were assigned to one of the following four groups:
Transection (fig. 1A) involved delivery and handling of the proximal half of the small bowel, followed by division and resuturing of the jejunum 1 to 2 cm distal to the ligament of Treitz.
Bypass (fig. 1B) of the proximal half of the small bowel was carried out by jejunal transection 1 to 2 cm. distal to the ligament of Treitz, followed by invagination of the distal cut end of the jejunum and insertion of the proximal jejunum into the mid-point of the small bowel with an end-to-side anastomosis.
Resection (fig. 1C) of the proximal half of the small bowel was followed by anastomosis just distal to the ligament of Treitz.
Pancreato-biliary diversion (PBD) (fig. 2) was based upon Altmann's method (1971). The combined pancreato-biliary duct was traced to its point of insertion into the duodenum

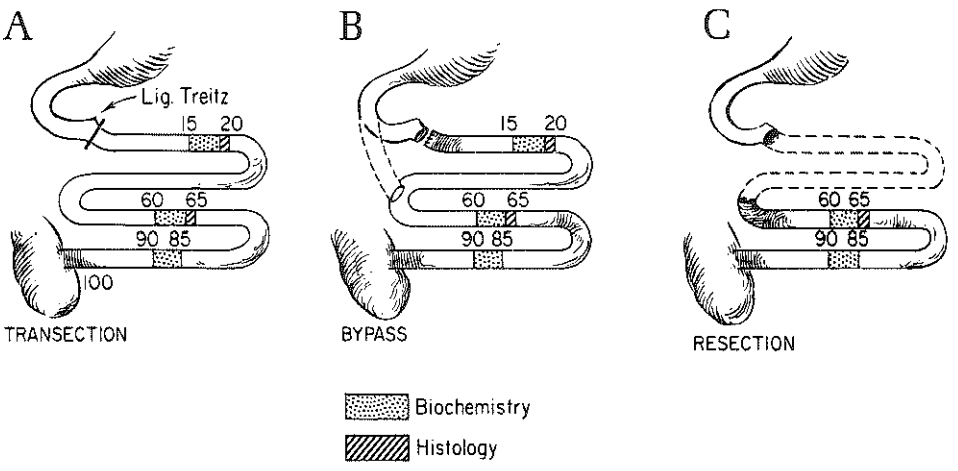

Figure 1
Jejunal transection, resection and bypass of 50 per cent of the proximal small bowel. Segments of bowel harvested for biochemical and histological analysis are indicated.

about 3 cm. beyond the pylorus; the duodenum was then divided 1 cm. above the papilla and 1 cm. below. The upper end of this isolated duodenal segment was closed and invaginated. After duodenal continuity was restored by end-to-end anastomosis, a retrocolic end-to-side anastomosis was constructed between the lower end of the papillary segment and the mid-point of the small bowel. This operation required 35 to 40 min. At the end of the operation all animals received a subcutaneous injection of 4 ml. 0.9 per cent NaCl solution. Animals were allowed 'Purina rat chow' until the time of operation and were permitted to drink, but not to eat, for 24 hours thereafter, when feeding was restored.

The four groups of rats were sacrificed by cervical dislocation resp. 48 hours, 1 week or 1 month after operation. One hour before death all animals received a subcutaneous injection of 50μ Ci ^{3}H-methyl-thymidine (6.7 Ci/mmole, New England Nuclear Corp., Boston, Ma.). After death the small bowel, excluding the duodenum, was removed, gently flushed with ice cold 0.9 per cent NaCl solution and spread on a glass plate resting on crushed ice. The colon was handled similarly. The internal circumference of the 3 small bowel segments was measured with a ruler to within 0.5 mm. after the specimens had been opened longitudinally. Mucosal scrapings were obtained from 5 cm. segments of small and large intestine according to the technique previously described in the chapter 'The experimental design and methods', which gives a reproducable yield of mucosa. As shown in figs. 1 and 2, segments were situated distal to the ligament of Treitz by 15-to-20 cm. (jejunum), 60-to-65 cm. (upper ileum) and 85-to-90 cm. (lower ileum); the colon segments were situated 0-to-5 cm. (ascending colon) and 5-to-10 cm. (transverse colon). In addition 1 cm. long segments of jejunum and upper ileum adjacent to these segments were fixed in 10 per cent formalin for histological examination. Mucosal scrapings were frozen in liquid nitrogen within 10 min. of death and were stored at -70° C for subsequent estimation of RNA and DNA by the method of Scott et al. (1956) modified by Hinrichs (1964),

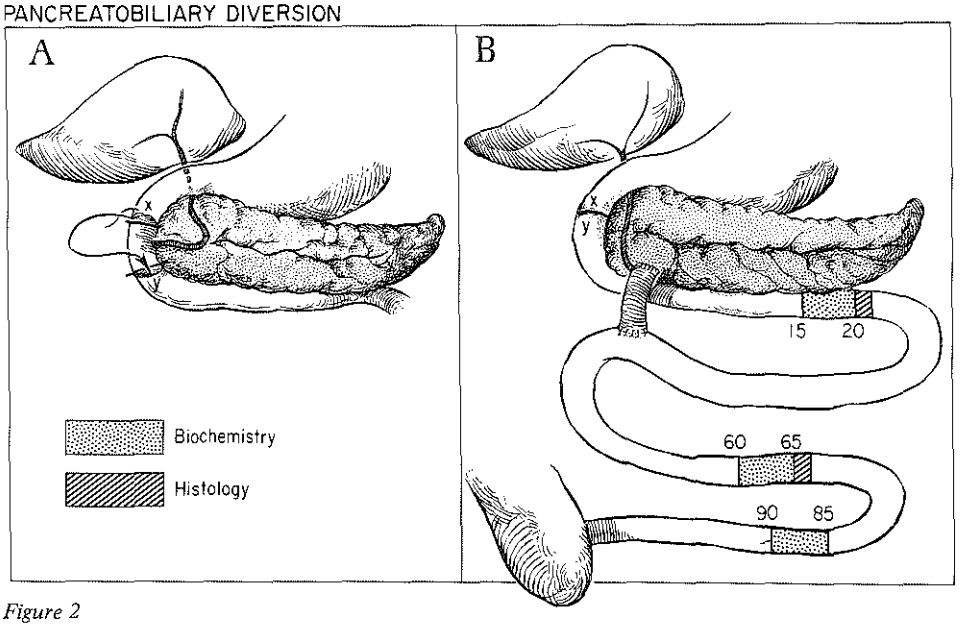

Figure 2
Pancreatobiliary diversion (PBD) to the mid-point of the small bowel. The end-to-side anastomosis between the isolated papillary segment and the mid small bowel was performed behind the transverse colon. Segments of bowel harvested for biochemical and histological analysis are indicated.

described in 'The experimental design and methods' and in detail in Appendix A.
Aliquots of the DNA fraction were counted by liquid scintillation spectrometry at 25 per cent efficiency, corrected by internal standardization. Ten sagitally transected villi and crypts from coded histological specimens were measured per slide by ocular micrometry.

4.3. Results

Weight loss
The yields of live healthy rats at the time of sacrifice were 36/40 after transection, 37/50 after PBD, 38/45 after bypass and 40/46 after resection. Rats regained their weight most rapidly after transection, throughout the experiment those rats remained the heaviest (fig. 3). Besides entailing the longest operation, PBD caused the highest mortality and the greatest initial weight loss. Though apparently healthy, rats with jejunal bypass weighed less than the 3 other groups at 1 month.

Effect of age
At all levels of the bowel there was an increase in amounts of RNA and DNA with age (figs. 4-to-8). This increase was accompanied by a reduction in DNA specific activity, as the proportion of proliferating cells decreased relative to the total number of cells. Higher

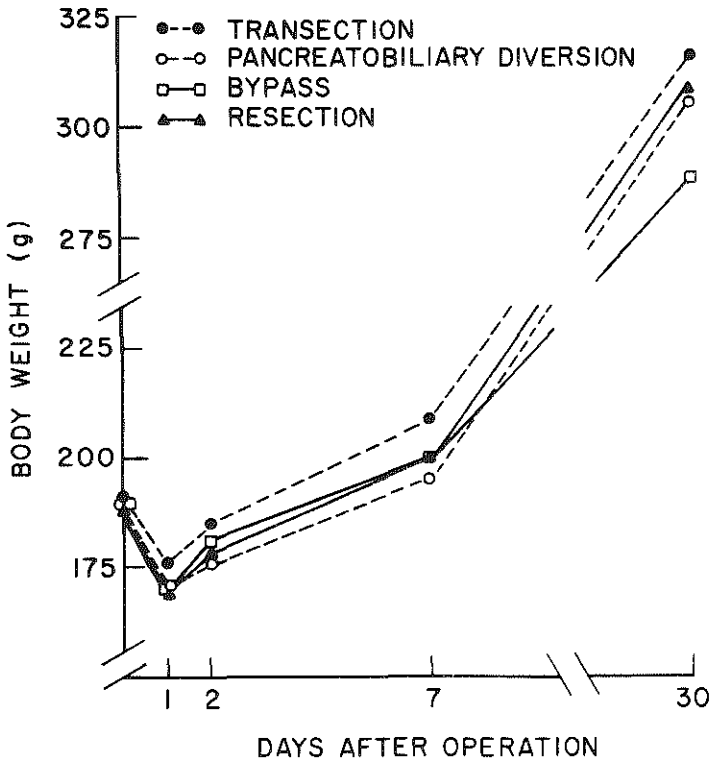

Figure 3
Postoperative weight changes. All animals lost 5-to-10 per cent of their total body weight during the first 24 hr after operation, when food was withheld.

villi and deeper crypts were consistent with the trend to greater nucleic acid contents (figs. 9, 10, see page 56). The mean values for RNA, DNA, RNA/DNA ratio, radioactivity and DNA specific activity in this experiment are tabulated in Appendix B.

Jejunum (fig. 4) Early reductions in mucosal contents of RNA and DNA seen after PBD and jejunal bypass persisted only in the blind jejunal loop of animals with bypass. Forty-eight hours after both PBD and bypass, RNA content decreased 22 per cent and DNA content decreased 17-to-18 per cent as compared with values from transected gut. In rats with PBD nucleic acid contents subsequently returned to control levels, but after bypass they did not. In the blind loop 1 month after operation RNA content was unchanged at any time, irrespective of the treatment.
Reductions in nucleic acid contents of the bypassed jejunum were associated with progressive narrowing of the lumen (table 1).

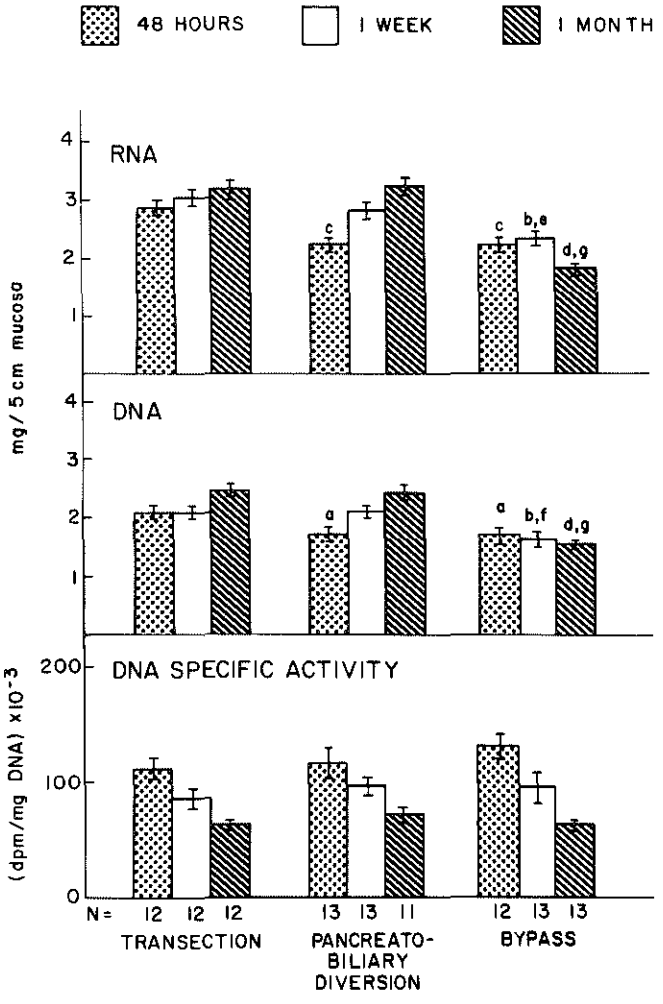

Figure 4
RNA and DNA contents and DNA specific activity in jejunum after operation (Means ± S.E.M.)

Significance

versus transection		versus pancreato-biliary diversion	
a	$P < 0.05$	e	$P < 0.05$
b	$P < 0.01$	f	$P < 0.01$
c	$P < 0.005$	g	$P < 0.001$
d	$P < 0.001$		

Table 1

Circumference of the bowel after operation (Means ± S.E.M.)

Operation	Time	N	Circumference (mm) Jejunum	Upper Ileum	Lower Ileum
Transection	1 wk.	12	11.9 ± 0.2	13.3 ± 0.2	12.9 ± 0.2
	1 mo.	12	14.1 ± 0.3	14.9 ± 0.2	14.2 ± 0.3
Pancreato-biliary Diversion	1 wk.	13	12.5 ± 0.3[g]	14.4 ± 0.3[b]	13.5 ± 0.3[e]
	1 mo.	11	15.0 ± 0.4[g]	16.0 ± 0.3[b,f]	15.2 ± 0.4[a]
Bypass	1 wk.	13	9.9 ± 0.2[c]	13.8 ± 0.2	13.3 ± 0.3
	1 mo.	13	8.5 ± 0.2[c]	17.5 ± 0.3[c]	14.8 ± 0.3
Resection	1 wk.	14		15.3 ± 0.2[c,d,g]	14.4 ± 0.2[c,d,f]
	1 mo.	13		17.1 ± 0.3[c,d]	15.8 ± 0.3[c,e]

Significance

versus transection: a $P < 0.05$; b $P < 0.005$; c $P < 0.001$

versus pancreatobiliary diversion: d $P < 0.05$

versus bypass: e $P < 0.05$; f $P < 0.005$; g $P < 0.001$

One month after operation the circumference of the excluded jejunum was 60 per cent that of the same segment of bowel following transection. One week and 1 month after PBD, intestinal circumference was unchanged from control values.
Villous height in bypassed jejunal loops declined only 14 per cent at 1 month (fig. 9), while crypt depth was unaltered. Histological measurements were unaffected by PBD.

Upper ileum (fig. 5)
Maximal increases in RNA and DNA contents were found in this region of the bowel, 10-to-15 cm. distal to the anastomosis. The response to resection, initially the greatest, was later on equal to the response in the PBD and bypass group. Within 48 hr. of operation both PBD and bypass increased RNA content (16-to-20 per cent) and DNA content (32-to-35 per cent) above control levels. But the response to resection was greater: elevations of 52 per cent for RNA and 40 per cent for DNA were found. DNA specific activity was increased by 33 per cent after bypass and by 46 per cent after resection as compared with transection.

One week postoperatively, elevated mucosal contents of nucleic acid were maintained and resection still had the greatest effect, with peak elevations of RNA (90 per cent) and DNA (73 per cent). Although specific activity after PBD and bypass had returned to

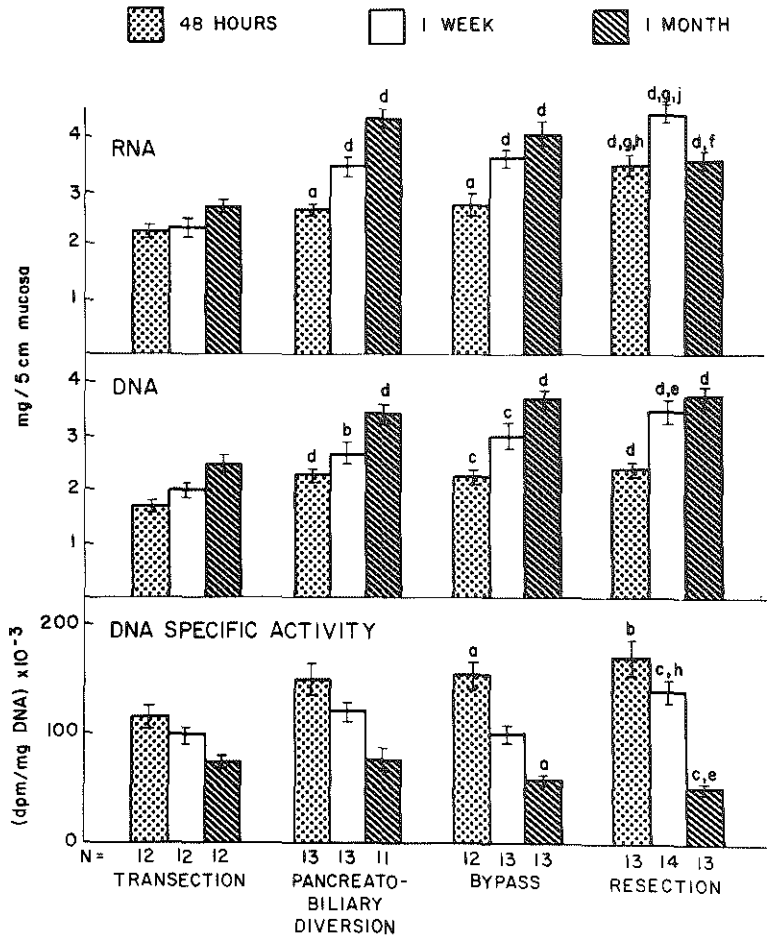

Figure 5
RNA and DNA contents and DNA specific activity in upper ileum after operation (Means ± S.E.M.)

Significance

versus transection
a $P < 0.05$
b $P < 0.01$
c $P < 0.005$
d $P < 0.001$

versus pancreato-biliary diversion
e $P < 0.05$
f $P < 0.005$
g $P < 0.001$

versus bypass
h $P < 0.01$
j $P < 0.005$

control levels, after resection it remained elevated by 43 per cent despite the substantial increase in total DNA.
By one month, however, mucosal contents of RNA and DNA after PBD and bypass equalled or surpassed those in rats with resection. The greatest elevation in RNA over control values was in rats with PBD (59 per cent). Increments in DNA in all 3 experimental groups ranged from 39 tot 51 per cent. DNA specific activity was actually reduced in the bypass and resection groups which showed the greatest increases in DNA content.
Biochemical changes in the upper ileum at 1 week had counter parts in luminal circumference, villous height and crypt depth. Both PBD and resection caused luminal dilation (table 1). All 3 experimental groups had taller villi (increase from 47 to 58 per cent) and deeper crypts (26 to 47 per cent) than transection controls (fig. 10 see page 56). Rats with jejunectomies had the greatest rise in every variable.
At 1 month marked luminal dilatation was present, especially in the bypass and resection groups (table 1). Villous height increased in the 3 experimental groups (37 to 48 per cent), with smaller increments in crypt depth (25 to 42 per cent) (fig. 10). The direction of these changes was similar to those in nucleic acid contents. Bypass and PBD caused the greatest change, rather than resection as at 1 week.

Lower ileum (fig. 6)
Results obtained from the terminal ileum generally reflected those in the upper ileal segment, but were less marked. After 48 hours the RNA content was 24-to-28 per cent higher after jejunectomy than in any other group. DNA content was elevated by 21-to-24 per cent over control values in all 3 experimental groups. Raised DNA specific activity was confined to animals with resection. By 1 week increments in RNA and DNA after resection were more than twice as great as those seen after PBD or bypass. DNA specific activity was similar in all 4 groups. Dilatation of the lower ileum was seen only after resection (table 1). One month following operation, mucosal RNA was highest after PBD, while rats with jejunal bypass also had higher RNA contents than those with transection or resection.
There was a striking decrease in RNA content after resection as compared with the level at 1 week; the difference between transection and resection was no longer significant for either RNA or DNA. By contrast, DNA contents after bypass and PBD remained elevated. As in the upper ileum, specific activity at 1 month was least in the resection group. Increased luminal circumference was found in the PBD and resection groups at 1 month (table 1).

Ascending colon (fig. 7)
After PBD and resection the trend of nucleic acid levels was somewhat higher than after transection at corresponding times. Higher RNA contents were found 1 month after PBD and 1 week after resection; higher DNA contents were seen in both groups at 48 hours after jejunal bypass nucleic acid levels were the same or less than controls. There was no consistent pattern in DNA specific activity.

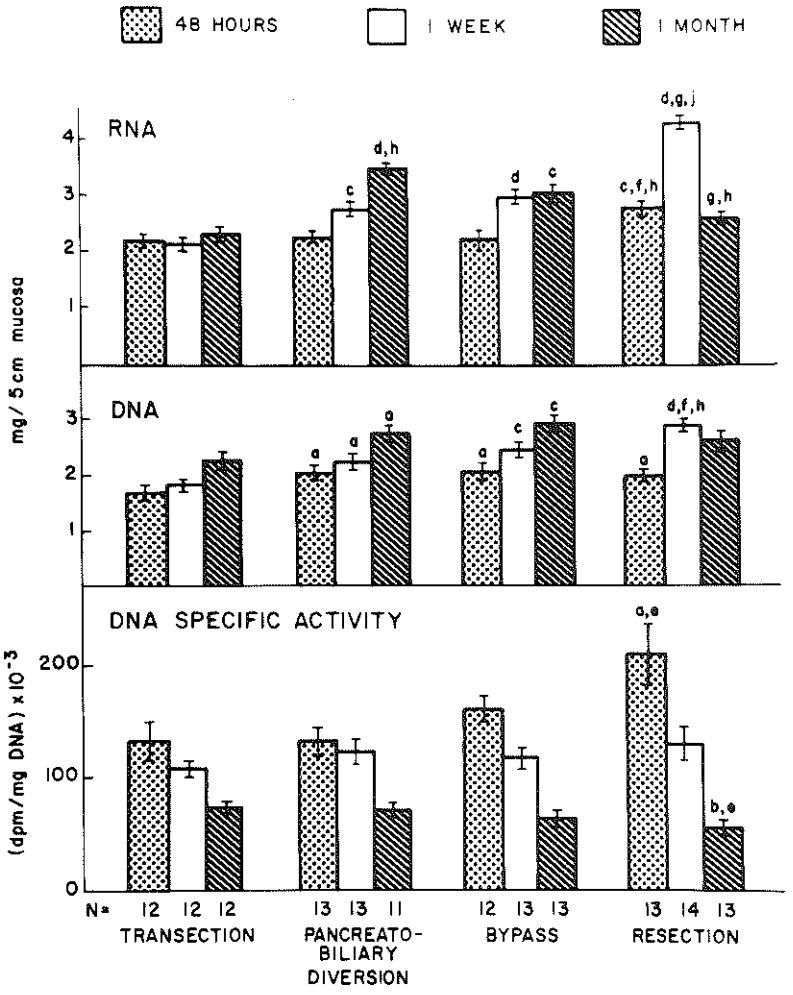

Figure 6
RNA and DNA contents and DNA specific activity in lower ileum after operation (Means ± S.E.M.).

Significance

versus transection
- a $P < 0.05$
- b $P < 0.01$
- c $P < 0.005$
- d $P < 0.001$

versus pancreato-biliary diversion
- e $P < 0.05$
- f $P < 0.01$
- g $P < 0.001$

versus bypass
- h $P < 0.05$
- j $P < 0.001$

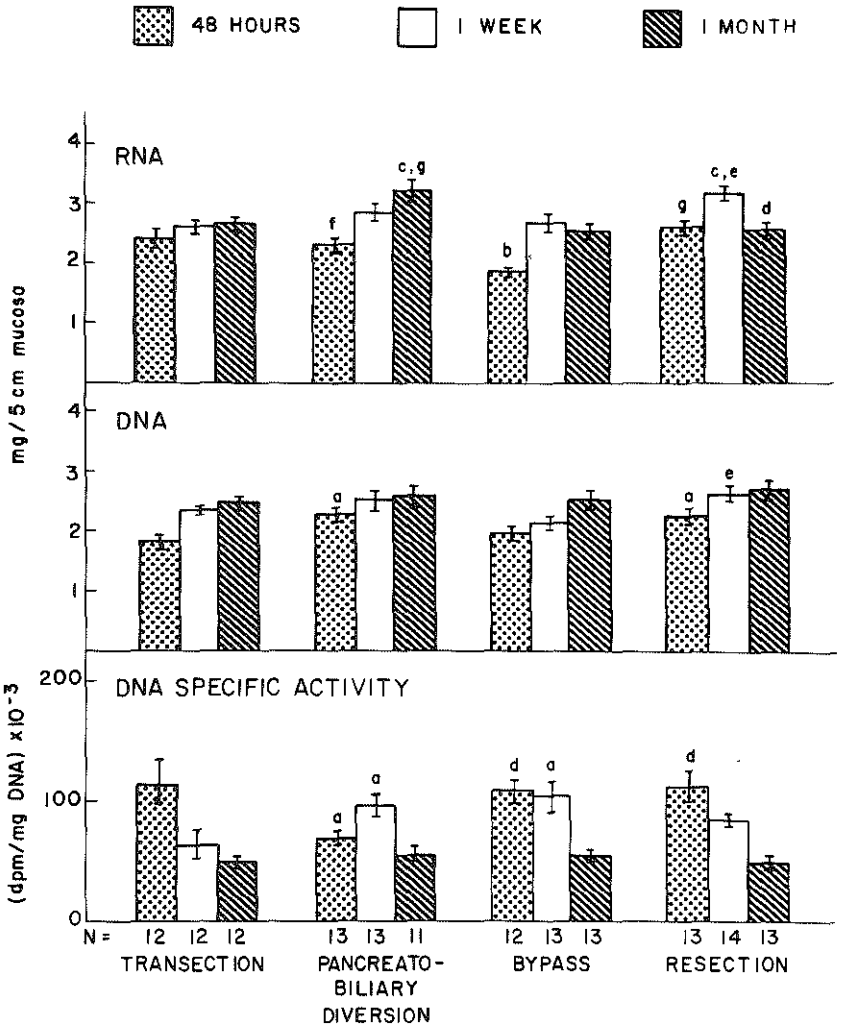

Figure 7
RNA and DNA contents and DNA specific activity in ascending colon after operation (Means ± S.E.M.).
Significance

versus transection	a	$P < 0.05$	versus bypass	e	$P < 0.05$
	b	$P < 0.01$		f	$P < 0.01$
	c	$P < 0.005$		g	$P < 0.001$
versus pancreato-biliary diversion	d	$P < 0.005$			

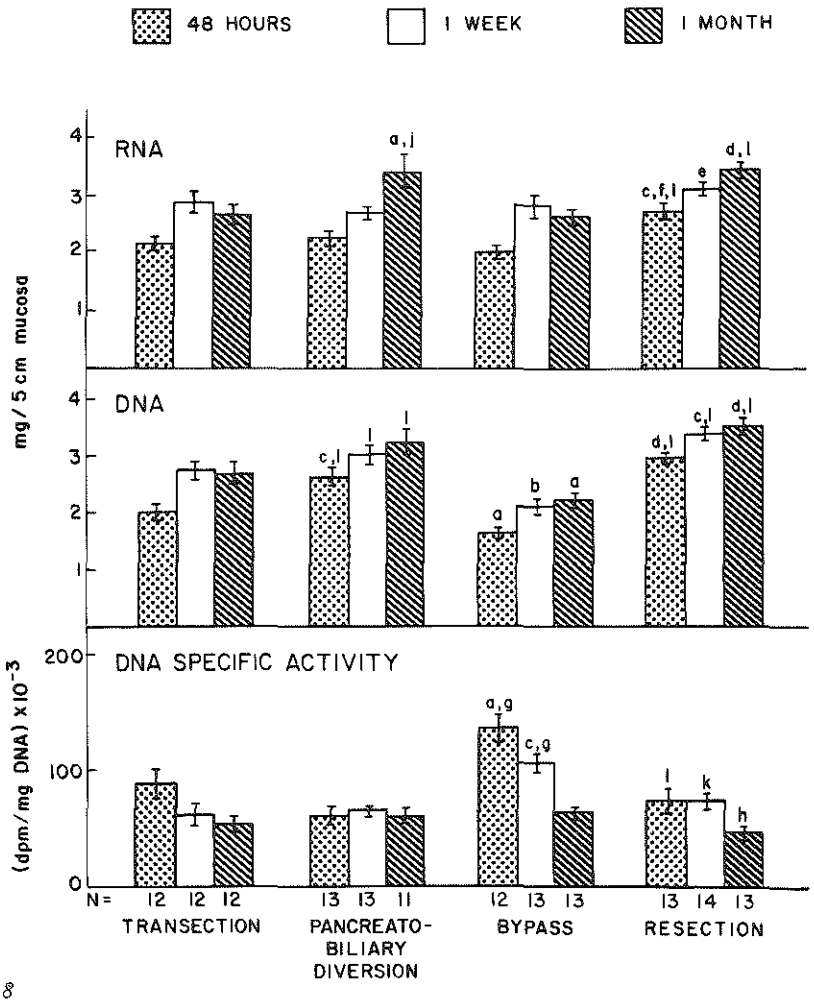

Figure 8

RNA and DNA contents and DNA specific activity in transverse colon after operation (Means ± S.E.M.).

Significance

versus transection

a $P < 0.05$
b $P < 0.01$
c $P < 0.005$
d $P < 0.001$

versus pancreato-biliary diversion

e $P < 0.05$
f $P < 0.01$
g $P < 0.001$

versus bypass

h $P < 0.05$
j $P < 0.01$
k $P < 0.005$
l $P < 0.001$

Transverse colon (fig. 8)
The response was similar to that in the ascending colon. After PBD nucleic acid contents were generally a little higher than after transection, but the increase was significant for RNA only at 1 month and for DNA only at 48 hours. Raised levels for RNA and DNA were found more consistently after resection; they ranged from 27 to 46 per cent of control values. After bypass RNA contents closely resembled values after transection, but DNA contents showed reductions of 18 to 23 per cent. There was a concomitant rise in DNA specific activity.

4.4. Discussion

Adaptation in distal small bowel
Proximal enterectomy produced the most intense ileal hyperplasia. Pancreato-biliary diversion to the mid small bowel and jejunal bypass both also caused ileal hyperplasia, but to a lesser degree. In all exp. groups the ileal hyperplasia could be detected already at 48 hours after operation.
Besides maximal increments in nucleic acid contents, the proportion of proliferating cells is also highest both 48 hours and 1 week after resection, indicating the persistence of intense hyperplasia beyond the first 7 days. Neither direct mucosal exposure to the pancreato-biliary effluent nor the provision of a richer chyme to the ileum reproduces the proliferative response to enterectomy. This phenomenon suggests that other factors beside pancreato-biliary secretions, duodenal chyme and food are required for adaptive hyperplasia. One such factor might be a humoral stimulator. The different responses to resection, PBD and bypass can not be explained by variations in body weight and by interference in food intake. Although immediately after operation rats with PBD weighed slightly less than the resection group, bypass and resection resulted in equivalent body weights at 48 hours and 1 week. One month after bypass, animals actually weighed less than after resection, possibly because of malabsorption resulting from the blind loop; yet the mucosal response equalled or surpassed that of resection. In general measurements of specific activity in DNA were consistent with variations in DNA synthesis as determined by assays of DNA content. Previous inability to distinguish between the responses of the distal small bowel to proximal excision and exclusion (Nygaard, 1967; Gleeson et al., 1972) can be attributed to performing these studies several weeks after operation. Biochemical and histological values 1 month after proximal resection are the same or lower than values at 1 week, while values after pancreato-biliary diversion, bypass and transection continue to increase with age. Consistent with clinical and experimental evidence suggesting persistence of postresectional hyperplasia for several months, we have found elevated mucosal contents of RNA and DNA in distal small bowel segments 4 months after jejunectomy and after pancreato-biliary diversion.

Adaptation in bypassed jejunum
The biochemical data confirm the presence of progressive mucosal atrophy in the bypas-

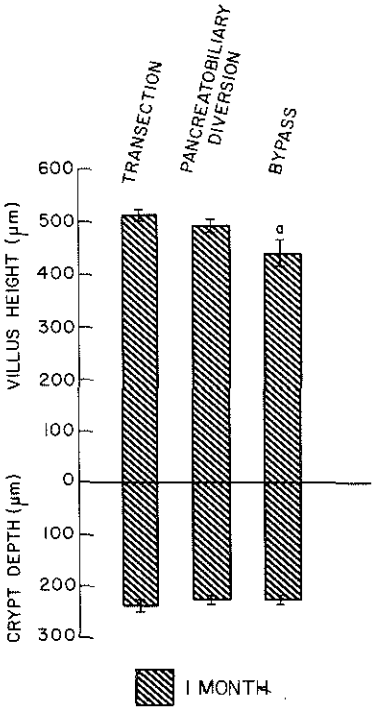

Figure 9
Villous height and crypt depth in jejunum (Means ± S.E.M.).
8 Rats were used for each determination.
Significance
versus transection a $P < 0.05$

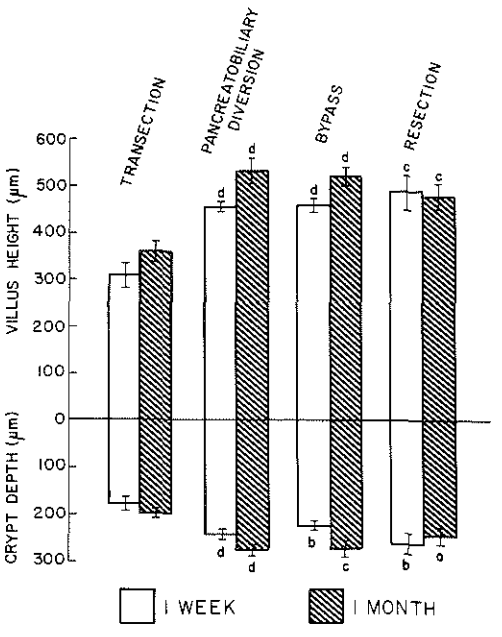

Figure 10
Villous height and crypt depth in upper ileum (Means ± S.E.M.).
6 Rats were used for each determination
Significance
versus transection
a $P < 0.05$
a $P < 0.01$
c $P < 0.005$
d $P < 0.001$

sed segment of jejunum as widely reported elsewhere (Senn, 1888; Cunningham, 1898; Menge et al., 1970; Gleeson et al., 1972a; Gleeson et al., 1972b; Keren et al, 1975; Fenyö and Hallberg, 1976).

Nucleic acid contents, already only 80 per cent of control values after 48 hours, decline to about 60 per cent at 1 month, and intestinal circumference diminishes by a similar amount. By contrast, histological measurements suggest that villous architecture is well preserved in the defunctionated bowel, with a relatively minor decrease in villous height and unchanged crypt depth. It could be that cellularity of the villus is a more sensitive index of hypoplasia than villous height, for the height of the villus in part may be maintained by its fibrovascular core (Rijke et al, 1977).

Unlike DNA content, DNA specific activity does not diminish in excluded segments of bowel even after 1 month, suggesting that jejunal mucosa retains its characteristic state of renewal in the absence of food. These findings are consistent with those of Oscarson's study (1977) that short-term starvation in the rat lowers mucosal content of nucleic acids, but does not alter DNA specific activity. The temporary depression of nucleic acid contents in the jejunum 48 hours after pancreato-biliary diversion might represent a transient hypoplasia caused by deprivation of bile and pancreatic juice, or may merely reflect the increased weight loss in these rats, as compared with transection controls.

Effect of age

The effect of age upon the small and large intestine is shown by the stepwise increase in nucleic acid contents of control animals with time. As in Experiment 1 there is an associated decrease in DNA specific activity probably due to dilution of the proliferating pool of crypt cells by the increased epithelial cell population, but possibly also a reflection of a larger thymidine pool. Histological measurements demonstrated taller villi and deeper crypts in all groups at 1 month, as compared with 1 week. Previous autoradiographic studies (Nundy et al., 1977) have shown that when mucosal contents of DNA are increased, active cell proliferation may co-exist with low levels of DNA specific activity.

Colonic adaptation

The results obtained from the large intestine are less consistent than those from the small bowel, possibly owing to greater difficulty in scraping the colonic mucosa from the underlying muscle. Nevertheless the data confirm a previous report (Obertop et al., 1977; Michaud et al., 1976) that 30 per cent proximal enterectomy in the rat provokes colonic hyperplasia within 2 days.

In the present study 50 per cent proximal resection caused a persistent response for 1 month in the transverse colon, but not in the ascending colon. In another experiment we have found that nucleic acid levels in colonic mucosa return to normal 4 months after proximal enterectomy.

Limited colonic hyperplasia following PBD may result from increased concentrations of bile salts within the colonic lumen. Fecal excretion of bile salts is increased by this operation (Chomchai et al., 1974), and bile salts stimulate cell proliferation in the small bowel of conventional and germfree rats (Ranken et al., 1971; Roy et al., 1975). Intestinal bypass, however, had no stimulatory effect upon the colon at all; indeed, mucosal levels of RNA and DNA showed even some tendency to diminish.

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COMPARATIVE EFFECTS OF BILE AND OF PANCREATIC JUICE ON CELL PROLIFERATION IN ILEAL MUCOSA

5.1. Introduction

Transposition of the duodenal papilla to the mid small bowel causes prompt and sustained ileal hyperplasia, as shown by increased mucosal contents of DNA, taller villi and deeper crypts (Chapter IV).
Papillary secretions may possibly constitute the villus-enlarging factors shown to be present in duodeno-jejunal chyme (Altmann and Leblond, 1970). Pancreatic juice has been reported to exert a greater tropic effect than bile upon intestinal mucosa (Altmann, 1971, 1974) possibly by increasing the supply of monosaccharides and aminoacids for utilisation by the luminal epithelium (Kinter and Wilson, 1965; Smyth, 1962). Bile has also a tropic effect on intestinal mucosa. Roy (1975) reported after biliary diversion an increased crypt depth and cell migration in the duodenum of the rat when sodium taurocholate was perfused, suggesting that bile alone has also a tropic effect on intestinal mucosa. The present experiment was devised to determinate the relative contributions of bile and pancreatic juice to postresectional hyperplasia.

5.2. Material and Methods

Male Spraque-Dawley maturing rats (Holtzman Co., Madison, Wi) weighing 170-210 g. (n = 141) were assigned to one of the following 3 experimental groups:
Transection (n = 36) (see Chapter IV) involved delivery and handling of the proximal half of the small bowel, followed by division and resuturing of the jejunum 1-to-2 cm. distal to the ligament of Treitz.
Pancreato-biliary diversion (PBD, n = 37) (see Chapter IV) was performed by a method similar to that described by Altmann (1971). The combined pancreato-biliary duct was traced to its point of insertion into the duodenum about 3 cm. beyond the pylorus; the duodenum was then divided 1 cm. above the papilla and 1 cm. below. The upper end of this isolated papillary segment was closed and invaginated. After duodenal continuity was restored by end-to-end anastomosis, a retrocolic end-to-side anastomosis was constructed between the lower end of the papillary segment and the mid point of the small bowel.
Biliary diversion (BD, n = 34) was designed to reroute the flow of bile, but to leave the pancreatic effluent draining normally through the duodenal papilla. The bile duct was ligated above its point of entry into the pancreas and was cannulated with polyethylene tubing (PE 10, internal diameter 0.011"). The choledochotomy was sited above the entry of the most proximal minor pancreatic duct, and the cannula was not inserted beyond the

confluence of the hepatic ducts. The lower end of the cannula draining pure bile was introduced through an enterotomy in the mid small bowel for a distance of 1-2 cm. and was fixed by a purse-string suture around the point of entry. Fixation of the cannuli and ligation of the bile duct was done with 5-0 silk. At the end of the operation all animals received a subcutaneous injection of 4 ml. 0.9 per cent NaCl solution. Animals were fed water and Purina Rat Chow as desired until the time of operation. After operation food but not water was withheld for 24 hours.

Collection of samples

Rats were sacrificed by cervical dislocation between 08.00 and 13.00 hours 48 hr., 1 week and 1 month after operation. One hour before death, each rat was given a subcutaneous injection of 50μ Ci ^{3}H methyl thymidine (6.7 Ci/mmole, New England Nuclear Corp., Boston. MA). Immediately after death of the animal, the small bowel was removed and flushed with ice cold 0.9% NAC1. Except the duodenum the rest of the small intestine was spread on a glassplate resting in crushed ice. The colon was handled in the same way. The circumference of the small bowel segments was measured with a ruler to within 0.5 mm. after the specimens had been opened longitudinally. Mucosal scrapings were obtained, as described in the Chapter 'Experimental design and methods', from 5 cm. segments of small and large intestine, situated 15-to-20 cm. (jejunum), 60-to-65 cm. (upper ileum) and 85-to-90 cm. (lower ileum) from distal to the ligament of Treitz.
The colon segments were situated 0-to-5 cm. distal to the coecum (ascending colon) and 5-to-10 cm. (transverse colon). In addition 1 cm. long segments of jejunum and upper ileum adjacent to these segments were fixed in 10 per cent formation for histological examinations. The mucosal scrapings were frozen in liquid nitrogen within ten minutes of death of the animal. Scrapings were preserved at -70° C until subsequent analysis. Representative specimens of intestinal wall when examined by light microscopy, revealed no remaining crypts after removal of the mucosa.

Analytical techniques

Content of RNA and DNA in mucosal samples was determined by the method of Scott et al. (1956), as modified by Hinrichs et al. (1964). Radioactivity in DNA was assayed from aliquots of DNA containing supernatants counted by liquid scintillation spectrometry with an efficiency of 25 per cent using internal standardization. Ten sagitally transected villi and crypts from coded histological specimens were measured per slide by ocular micrometry.

Statistical analysis

Data were evaluated by student's t-test for determination of statistical significance.

5.3. Results

Mortality: the yields of live healthy rats were 36/40 (transection), 37/50 (PBD) and

34/51 (BD). There were 8 early deaths after BD, mostly resulting from leaking of bile due to slipping of the cannula. Nine other rats in this group became jaundiced during the month after operation and were discarded. At autopsy these rats had gross distention of the biliary tree, usually with blocked cannulae; this complication was not encountered during the first postoperative week. The remaining 34 rats in this group were in good condition at autopsy, without obvious icterus or biliary distension. Weight loss at all times after BD slightly exceeded that of transection controls (table 1). Despite lower initial weights after PBD, rats in this group regained control levels by 1 month.

TABLE 1

Weight changes after operation (Means ± S.E.M.)

			48 hours		1 week		1 month
Operation	*N*	Initial Weight (g)	Weight Change (g)	*N*	Weight Change (g)	*N*	Weight Change (g)
Transection	36	191.0 ± 2.2	− 5.4 ± 1.0	24	+17.7 ± 2.1	12	+125.1 ± 8.9
Biliary Diversion	34	194.0 ± 1.7	−12.7 ± 1.3[b]	23	+ 6.7 ± 3.2[a]	11	+ 89.7 ± 8.2[a]
Pancreatobiliary Diversion	37	190.2 ± 1.9	−13.7 ± 1.1[b]	24	+ 5.3 ± 2.5[b]	11	+114.9 ± 10.8

Significance
versus transection
a $P < 0.01$
b $P < 0.001$

Nucleic acid synthesis
The mean values for RNA, DNA, RNA/DNA ratio, radioactivity and DNA specific activity in this experiment are tabulated in Appendix B.

Jejunum (fig. 1)
The transient early reduction in jejunal RNA and DNA (compared with controls) seen 48 hours after PBD was not found after BD, though the two operations caused comparable early loss of body weight (table 1). At 1 week and 1 month nucleic acid contents were similar in all 3 groups. Specific activity of DNA was lowest in rats with BD. Elimination of bile from the jejunum for 1 month, with or without concomitant pancreatic exclusion, did not alter villous height or crypt depth (fig. 2).

Upper ileum (fig. 3)
Diversion of bile to the ileum, alone or together with pancreatic juice, caused prompt mucosal hyperplasia. Forty-eight hours after either BD or PBD, similar elevations of RNA

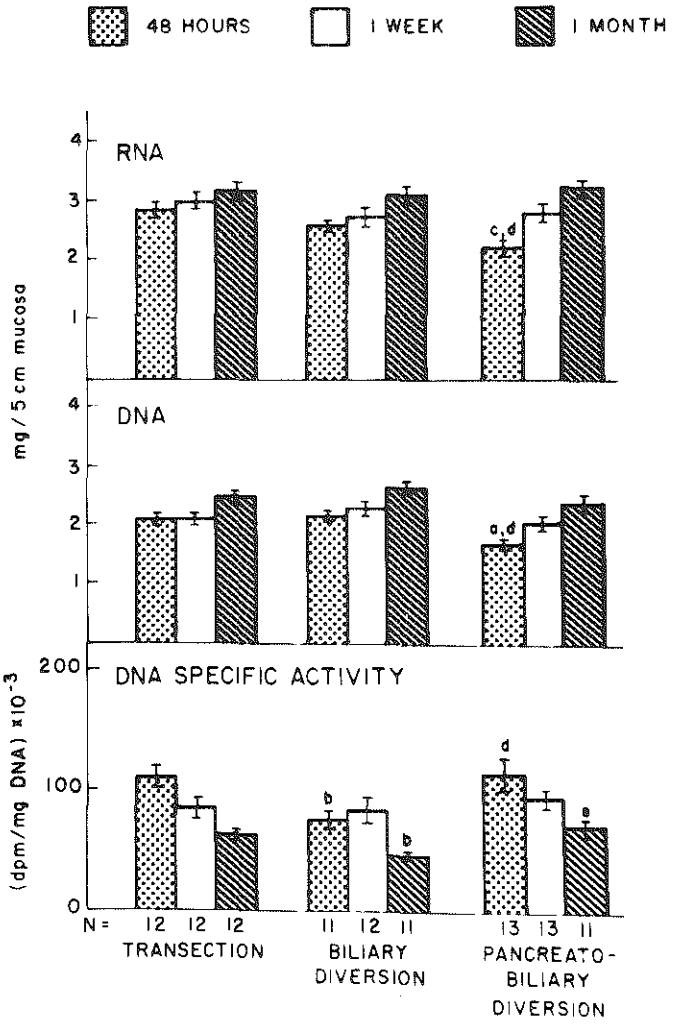

Figure 1
RNA and DNA contents and DNA specific activity in jejunum after operation (Means ± S.E.M.). Number of rats is shown below the bars.
Significance

versus transection		versus biliary diversion	
a	$P < 0.05$	d	$P < 0.05$
b	$P < 0.01$	e	$P < 0.005$
c	$P < 0.005$		

(16-22 per cent) and DNA (33-41 per cent) over control values were found. This hyperplasia was maintained 1 week postoperatively, when increments in RNA and DNA content after both procedures ranged from 20-50 per cent.
DNA specific activity was higher after PBD than after BD both at 48 hours and 1 week.

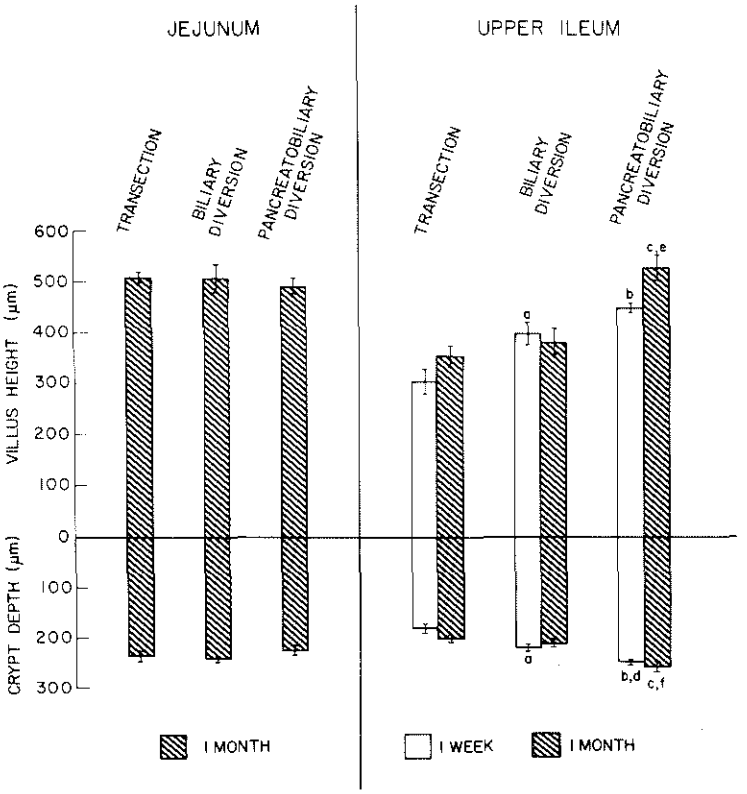

Figure 2
Villous height and crypt depth in jejunum and upper ileum after operation (Means ± S.E.M.). 6 – 8 Rats were used for each determination.
Significance

versus transection	a $P < 0.02$	versus biliary diversion	d $P < 0.02$
	b $P < 0.005$		e $P < 0.005$
	c $P < 0.001$		f $P < 0.001$

Although nucleic acid contents 1 month after BD had returned to control levels, persistent hyperplasia after PBD was manifested by higher RNA and DNA contents (37-59 per cent) than those in either of the other two groups.
Histological measurements (fig. 2) 1 week and 1 month after PBD confirmed the biochemical evidence of increased cell proliferation, with taller villi (increases of 32-47 per cent) and deeper crypts (37-42 per cent) than in control animals. Changes in nucleic acid content after BD were also mirrored by increases in villous height and crypt depth at 1 week (21-30 per cent), which reverted to normal at 1 month. Intestinal circumference followed a similar pattern (table 2); intestinal dilatation occurred 1 week after BD, and 1 week and 1 month after PBD.

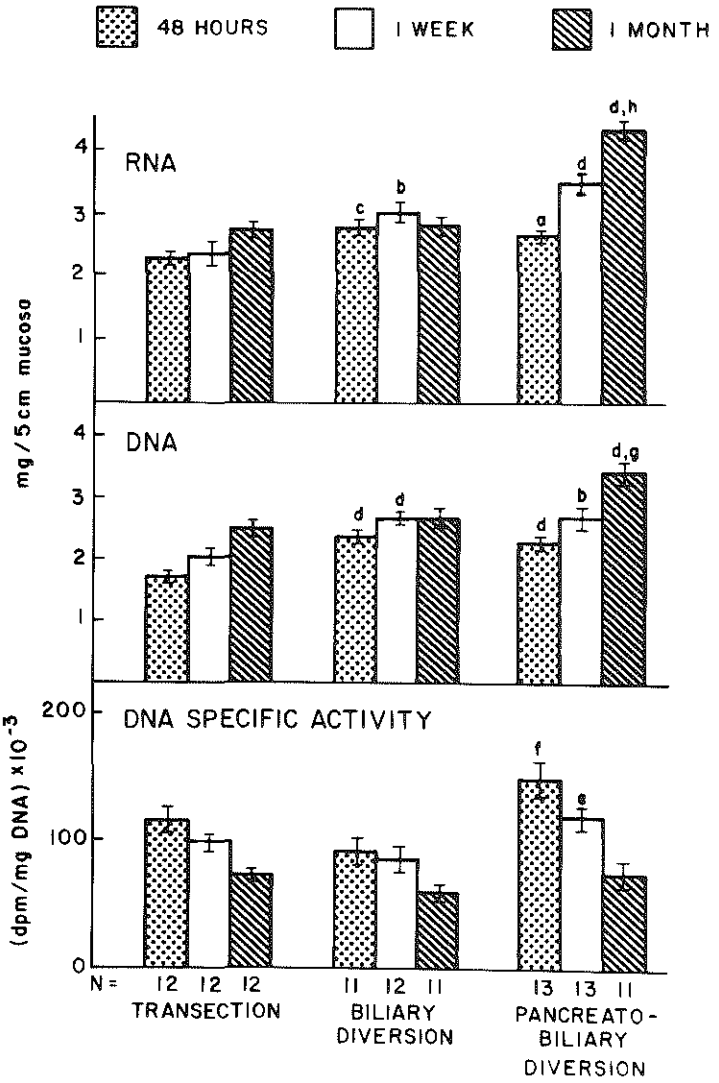

Figure 3

RNA and DNA contents and DNA specific activity in upper ileum after operation (Means ± S.E.M.). Number of rats is shown below the bars.

Significance

versus transection

a $P < 0.05$
b $P < 0.01$
c $P < 0.005$
d $P < 0.001$

versus biliary diversion

e $P < 0.05$
f $P < 0.01$
g $P < 0.005$
h $P < 0.001$

TABLE 2

Circumference of the bowel after operation (Means ± S.E.M.)

			CIRCUMFERENCE (mm)		
Operation	Time	N	Jejunum	Upper Ileum	Lower Ileum
Transection	1 wk.	12	11.9 ± 0.2	13.3 ± 0.2	12.9 ± 0.2
	1 mo.	12	14.1 ± 0.3	14.9 ± 0.2	14.2 ± 0.3
Biliary Diversion	1 wk.	12	12.4 ± 0.2	13.9 ± 0.2[a]	13.4 ± 0.2
	1 mo.	11	14.0 ± 0.2	15.0 ± 0.3	14.7 ± 0.2
Pancreatobiliary Diversion	1 wk.	13	12.5 ± 0.3	14.4 ± 0.3[b]	13.5 ± 0.3
	1 mo.	11	15.0 ± 0.4[c]	16.0 ± 0.3[b,c]	15.2 ± 0.4[a]

Significance
versus transection
a $P < 0.05$
b $P < 0.005$
versus biliary diversion
c $P < 0.05$

Lower ileum (fig. 4)

In the lower ileum biochemically smaller differences were observed than in the upper ileum. Both BD and PBD caused similar modest elevations (22-23 per cent) of mucosal RNA content at 1 week and of DNA content at 48 hours and 1 week. A persistent increase in cell proliferation beyond this time was only seen in the PBD group. Only in the PBD group at 1 month increments in RNA (53 per cent) and DNA (22 per cent) were found over controls, compared with the BD group only an increase in RNA alone (35 per cent) is found.

As in the proximal bowel DNA specific activity was generally lowest after BD. The only change in lower ileal circumference was a slight increase 1 month after PBD (table 2).

Colon (fig. 5 and 6)

RNA and DNA contents in the ascending colon (fig. 5) and transverse colon (fig. 6) were generally increased following BD and PBD. As in the small bowel, elevations in colonic RNA and DNA after BD, which ranged from 30-50 per cent, were confined to the first postoperative week. After an initial rise in DNA content at 48 hours, PBD resulted in higher RNA content only 1 month after operation, no consistent pattern in DNA specific activity was seen in the large intestine.

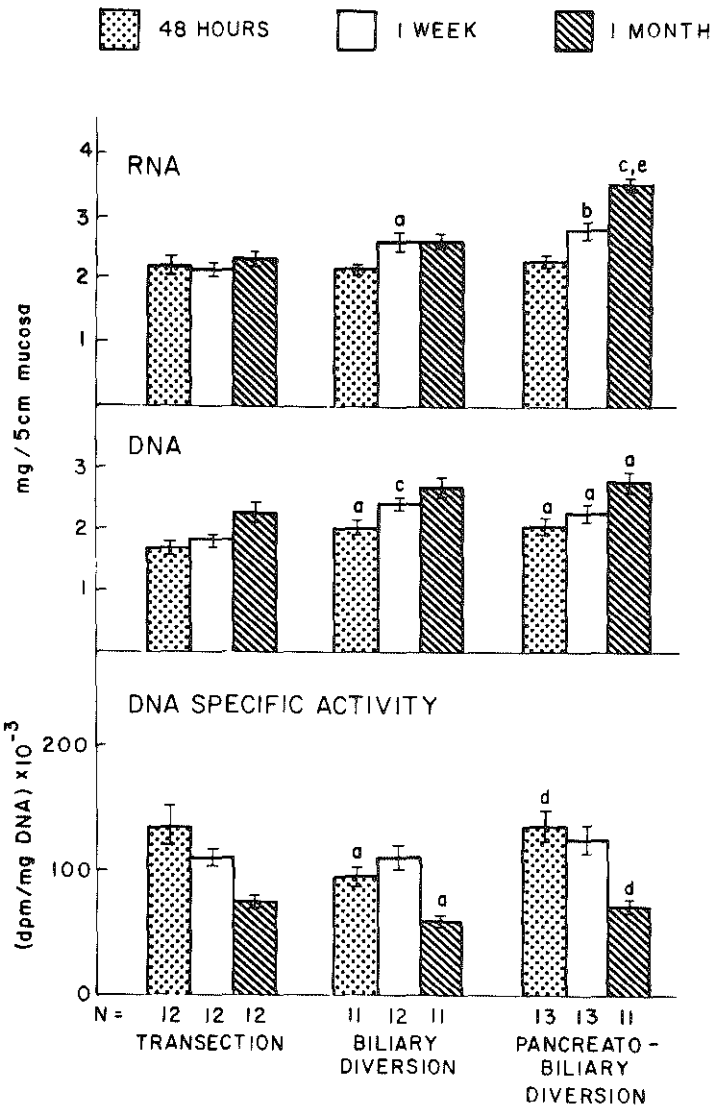

Figure 4
RNA and DNA contents and DNA specific activity in lower ileum after operation (Means ± S.E.M.). Number of rats is shown below the bars.
Significance
versus transection a $P < 0.05$; b $P < 0.005$; c $P < 0.001$
versus biliary diversion d $P < 0.05$; e $P < 0.001$

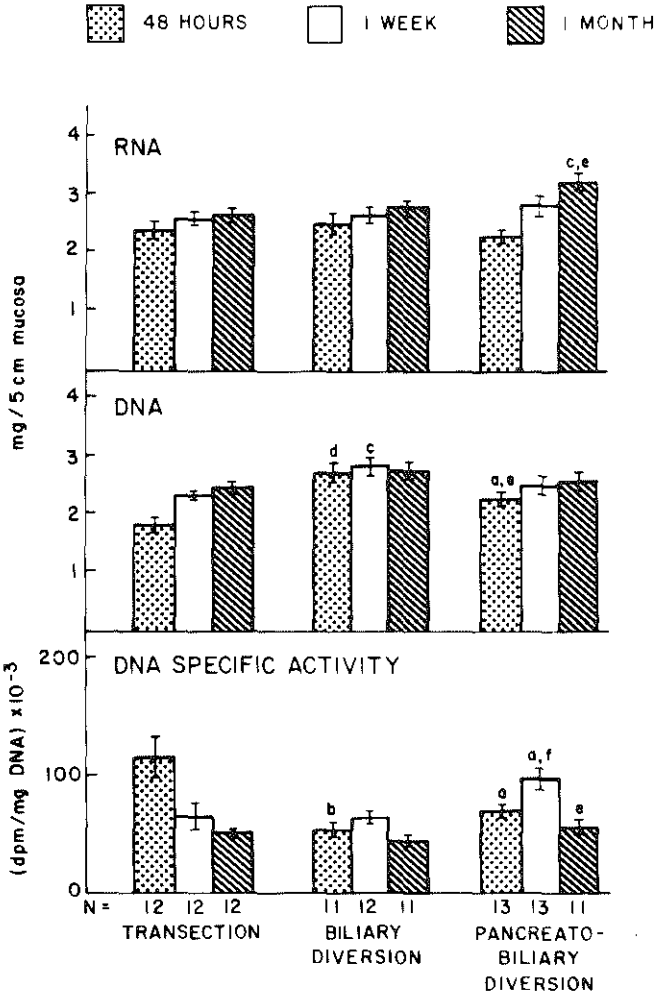

Figure 5
RNA and DNA contents and DNA specific activity in ascending colon after operation (Means ± S.E.M.). Number of rats is shown below the bars.
Significance
versus transection
a $P < 0.05$
b $P < 0.01$
c $P < 0.005$
d $P < 0.001$
versus biliary diversion
e $P < 0.05$
f $P < 0.01$

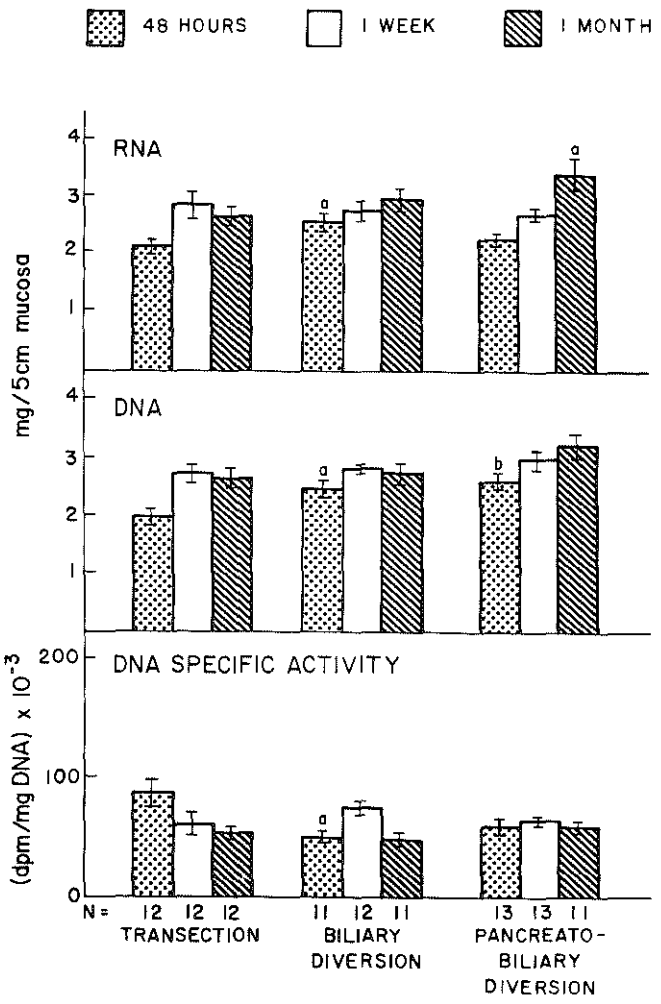

Figure 6
RNA and DNA contents and DNA specific activity in transverse colon after operation (Means ± S.E.M.). Number of rats is shown below the bars.
Significance
versus transection

a $P < 0.05$
b $P < 0.005$

5.4. Discussion

Bile pouring directly into the ileum with or without pancreatic juice promoted cell proliferation in the ileal mucosa. Higher specific activity of DNA both 48 hours and 1 week after PBD (as compared with BD), larger amounts of RNA and DNA, and persistence of deeper crypts and higher villi suggested progressive hyperplasia in the presence of both endogenous secretions. The limited tropic effect of bile upon intestinal mucosa is consistent with a previous report (Roy et al., 1975) that crypt depth and cell migration in rat duodenum are decreased by diversion of bile and restored by infusion of sodium taurocholate. Besides the persistence of early intestinal adaptation only shown after PBD in this experiment, we have found that mucosal hyperplasia is still present in rat ileum seven months after PBD.

Thus, although bile alone can initiate ileal cell proliferation the additional presence of pancreatic juice prolongs the adaptive response. Our finding that the tropic effect of the combined pancreato-biliary effluent exceeded that of bile alone agrees with the results of Altmann's work in 1971 comparing the effects of biliary diversion on isolated loops of rat ileum with or without pancreatic juice. In Altmann's study the number of surviving rats was small because of pancreatitis following transection of the bile duct and an end-to-side choledocho-enterostomy. Cannulation of the bile duct employed in our rats carried a lesser mortality, but was often complicated by blockage of the cannula after the first week, which impaired the flow of bile and reduced its tropic influence on the ileal mucosa.

However only healthy animals without evidence of biliary obstruction were included in the one-month BD group. The mean weight of this group was 90 per cent of that of controls, suggesting that malnutrition was not preventing hyperplasia. Although in the jejunum deprivation of both secretions caused transient hypoplasia of the mucosa, the mere absence of bile alone had no effect. This evidence confirms the greater tropic effect of the combined effluent and is not consistent with an other report (Fry et al., 1967), that short-term deprivation of bile does not affect the rate of cell renewal in the duodenum.

Prompt stimulation of colonic mucosa after BD may reflect greater quantities of bile salts reaching the large intestine in the early postoperative period. Adaptation could account for the transience of colonic hyperplasia after BD, although there is some evidence to suggest a more prolonged response in the colon as in the small bowel after PBD. Jejunectomy promotes supranormal ileal absorption of bile acids (Perry et al., 1974) and similar functional adaptation might also accompany the ileal hyperplasia that follows BD and PBD.

Pancreato-biliary secretions might stimulate epithelial hyperplasia in several ways. For example, hyperplasia might be mediated by hypergastrinaemia; if so, distal diversion of the papillary effluent, with or without duodenal transposition might produce hypergastrinaemia since it is associated with hypersecretion of acid from canine gastric pouches (Menguy and Mings, 1961; Storer et al., 1952).

However, despite assertions that gastrin exerts a tropic effect on intestinal mucosa (Johnson, 1976), Oscarson (1977) could not demonstrate any stimulatory role of gastrin in regulating intestinal cell proliferation.

Pancreatic digestive enzymes might also increase the concentration of glucose and amino-acids within the lumen of the gut, and epithelial cells lining the intestinal canal can probably use both these substances for their own nutrition (Kinter and Wilson, 1965; Smyth, 1962).

Any such action must represent only a part of the pancreatic influence. Altmann (1974) could not reproduce the full tropic influence of pancreatic secretions by infusion of aminoacid suspensions into isolated loops of bowel.

Weser and his associates (1977) have shown that in rats an elemental diet does not affect the enhancement of post-jejunectomy hyperplasia in ileal mucosa following transposition of the papilla to the ileal remnant. The complex physiological actions of bile may depend upon the degree of conjugation of bile salts and the composition of bile acid present in the intestinal lumen. For example, in germ-free mice, in which taurocholic acid is the only bile acid formed, the addition of cholic acid to the diet halves the turnover time of mucosal cells in the ileum (Ranken et al., 1971). Bile salts might stimulate epithelial proliferation by non-specific alteration of mucosal permeability (Dobbins and Binder, 1976), by stimulation of cyclic adenosine monophosphate (Coyne et al., 1976) or by simple mucosal irritation (Bloch et al., 1974; Menge et al., 1976).

In contrast to the marked hyperplasia caused by pancreatic extracts, fresh hog bile does not increase villous size when infused into isolated ileal loops in rats (Altmann, 1974). Perhaps the additional presence of food is needed for gastro-intestinal secretions to exert their tropic effect. Since oral intake of food stimulates the flow of bile and pancreatic juice, reduction in endogenous secretions in addition to the direct absence of intraluminal nutrients may contribute to the intestinal atrophy of starvation.

Besides intestinal hypoplasia, marked pancreatic atrophy occurs during total parenteral nutrition in rats (Johnson et al., 1975). Preliminary data suggest that cholecystokinin and secretin may prevent the intestinal hypoplasia otherwise found in dogs maintained exclusively by intravenous alimentation (Hughes et al., 1976).

In conclusion the results of this experiment indicate that increased luminal concentration of bile is sufficient for prompt ileal hyperplasia, but the additional presence of pancreatic juice prolongs this adaptive response.

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Chapter VI, Experiment 4

HUMORAL FACTORS AND POSTRESECTIONAL HYPERPLASIA

6.1. Introduction

Both intraluminal and humoral factors could mediate the adaptive hyperplasia of the remaining small bowel that occurs within 2 days of jejunectomy in the rat (experiment 1). Oral feeding is essential for the development of postresectional intestinal hyperplasia in the rat and the dog (Levine et al., 1976; Feldman et al., 1976), and pancreatobiliary secretions are contributing to the adaptation of the surgically shortened gut (experiment 3 and Roy et al., 1975). In chapter IV we have shown that local stimulating effects as food and enteric secretions can not explain all the features of intestinal adaptation after partial enterectomy.
To account for the generalized enteric effects of a limited (10 per cent) resection, Loran and co-workers proposed the existence of an 'intestinal epithelial growth hormone' (Loran and Althausen, 1960; Loran and Crocker, 1963) which could be transmitted between rats linked in cutaneous parabiosis (Loran and Carbone, 1968). Subsequent attempts to confirm these findings in rats have not been successful (Tilson and Wright, 1971; Tilson et al., 1975), possibly because of inadequate circulatory exchange. In this experiment we have tried to separate the effects of intraluminal nutrients and pancreatobiliary secretions from the other effects. The possibility that humoral factors might be involved in the control of postresectional adaptation was tested in individual rats. Therefore transected and jejunectomized rats, both groups with an excluded upper ileum were studied 48 hours after the operation.

6.2. Material and methods

Male Spraque-Dawley maturing rats (Holtzman Co., Madison, Wi), weighing 200-220 g., n = 39 were assigned to the following groups.
Control group (fig. 1): the rats underwent a *sham operation* with delivery and handling of the whole small bowel.
Transection and exclusion (fig. 2): This operation involved division at the mid small bowel (45 cm. from the ligament of Treitz) followed by excluding an loop of upper ileum (the next distal one quarter of the small bowel = 25 cm.) with invagination of the proximal end, the distal end ending as an ileostomy. The residual distal ileum was reanastomosed end-to-end with the mid point of the small bowel (6.0 silk).
Resection and exclusion (fig. 3): These rats underwent a 50 per cent proximal resection of the small bowel and the same exclusion of the upper ileum with invagination of the

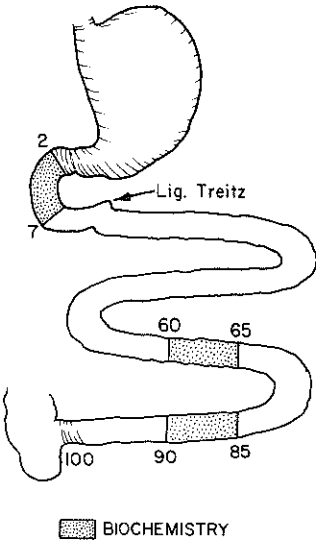

Figure 1
Sham operation as control group. Segments of bowel harvested for biochemical analysis are indicated.

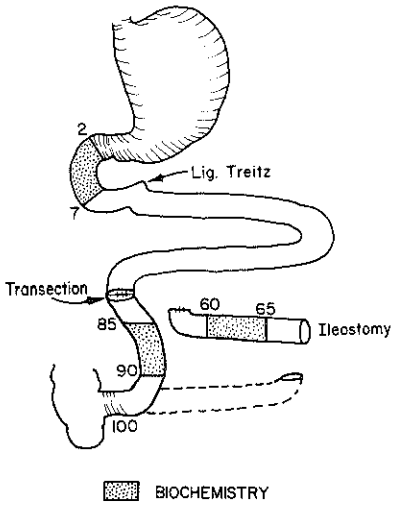

Figure 2
Transection and exclusion of the upper ileum. Segments of bowel harvested for biochemical analysis are indicated.

proximal part and the distal end ending as an ileostomy. The distal part of the ileum was reanastomosed to the remnant of the jejunum 3 cm. distal to the ligament of Treitz.

All operations were performed under light ether anesthesia between 08.00-13.00 hours. Intestinal anastomoses were performed with a single layer of continuous 6.0 silk. The rats were allowed to eat until the time of operation. Afterwards food was withheld for 24 hours; water was given throughout, ad libitum. The rats were sacrificed by cervical dislocation 48 hours after operation. One hour before death all animals received a subcutaneous injection of 50 μ Ci ^{3}H-methyl-thymidine (6.7 Ci/mmole, New England Nuclear, Boston, MA). After death both the whole small bowel in continuity as the excluded loop of upper ileum were removed, followed by gently flushing with 0.9 per cent NaCl solution to remove the intraluminal content in the excluded loop. The segments were spread on a glass plate resting on crushed ice. Mucosal scrapings were obtained from 5 cm. segments of the small intestine according to the technique previously described. As shown in fig. 1, 2 and 3, segments were situated 2-7 cm. distal to the pylorus (duodenum), 60-65 cm distal to the pylorus (upper ileum). For the experimental groups this means the middle part of the excluded loop and the third segments was situated 85-90 cm. distal to the pylorus (lower ileum). The mucosal scrapings were frozen in liquid nitrogen within 5 min. of death and were stored at -70° C for subsequent estimation of RNA and DNA contents by the method of Scott et al. (1956),

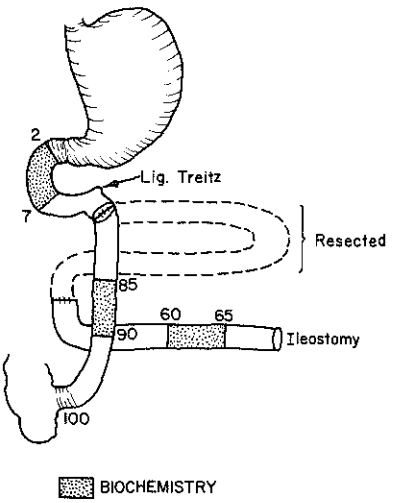

Figure 3
Resection and exclusion of the upper ileum. Segments of bowel harvested for biochemical analysis are indicated.

modified by Hinrichs et al. (1964). Aliquots of the DNA fraction were counted by liquid scintillation spectometry at 25 per cent efficiency, corrected by internal standardization. Statistical analysis: The student's t-test for unpaired data was employed for the analysis of the results.

6.3. Results

TABLE 1

Weight changes after operation (Means ± S.E.M.)

Operation	Initial weight (g)	24 hours Weight change (g)	N	48 hours Weight change (g)	N
Sham operation	208.4 ± 2.2	− 14.2 ± 0.9	13	− 0.1 ± 1.6	13
Transection + exclusion	205.1 ± 2.5	− 15.7 ± 1.4	13	− 15.5[a] ± 2.2	13
Resection + exclusion	207.2 ± 2.1	− 24.8[a,c] ± 1.2	13	− 23.1[a,b] ± 1.3	13

Significance

versus sham operation

a $P < 0.001$

versus transection

b $P < 0.01$

c $P < 0.001$

Weight loss: as is shown in table 1, 24 hours after the operation, the sham operated and the transected group had lost 7 per cent of their initial body weight but the resection group lost 12 per cent.
At 48 hours the control group had caught up their initial weight loss, but the transected were still 7.6 per cent down and the resection group 11.1 per cent, both significant from the control group and from each other.

Nucleic acid synthesis
The mean values for RNA, DNA, RNA/DNA ratio, radioactivity and DNA specific activity in this experiment are tabulated in Appendix B.

Duodenum (fig. 4) The transected group showed no significant rise in nucleic acid contents versus the control group. On the other hand the resected group was well up versus the control group with increased RNA and DNA values (15 per cent). The total radioactivity values showed a same increase for the resected group; 56 per cent as opposed to the control group (table 2).

Upper ileum (fig. 5) (in the experimental groups: the excluded segment). 48 Hours after operation the RNA content increased 18 per cent and the DNA content 17 per cent in the resected group versus the control group, both significant.
Although there was a tendency to increased values of RNA (4 per cent) and DNA (11

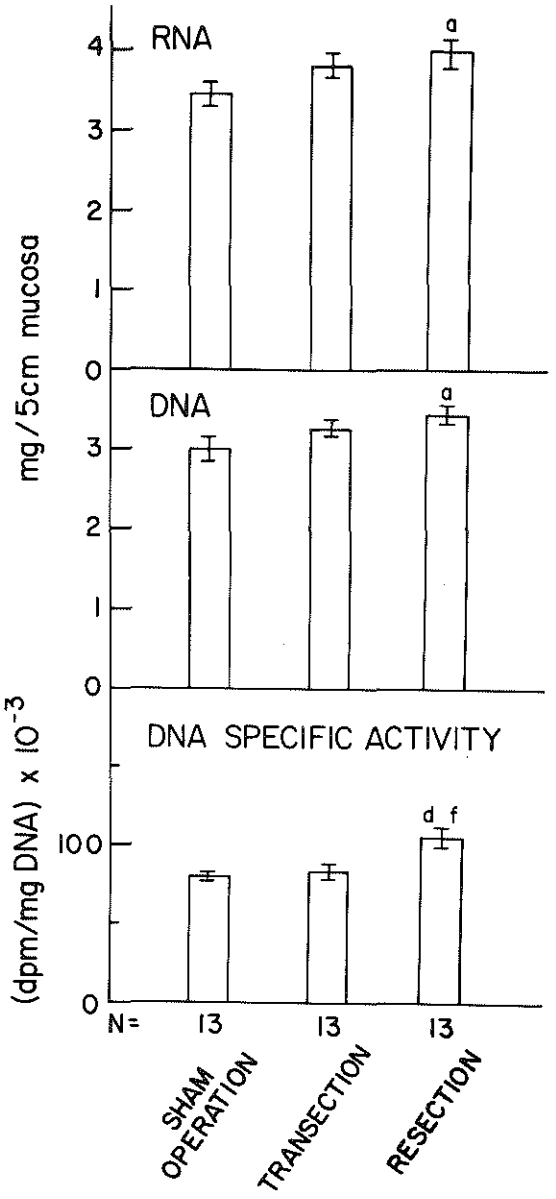

Figure 4
RNA and DNA contents and DNA specific activity in duodenum after operation (Means ± S.E.M.)
Significance
versus sham operation a $P < 0.05$ d $P < 0.001$ versus transection f $P < 0.01$

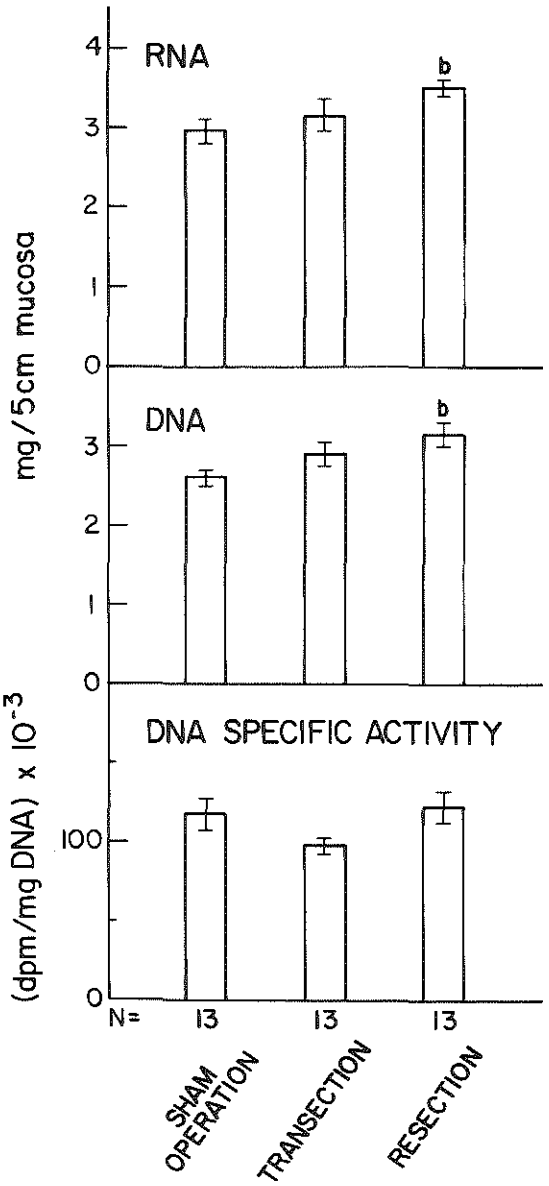

Figure 5
RNA and DNA contents and DNA specific activity in upper ileum (excluded loop) after operation (Means ± S.E.M.)
Significance
versus sham operation b $P < 0.01$

per cent) for the transected animals, these measurements were not significantly different in comparison with the control group. The RNA/DNA did not differ between the 3 groups. The total radioactivity values (table 2) showed an increase in the resected group of 23 per cent, but this was not significantly different in comparison with the control group, because of the wide standard error.
On the other hand a significant increase (36 per cent) in total radioactivity was found in comparison with the transected animals. The DNA specific activity showed no differences between the 3 groups. Although only the total radioactivity in the resected group was significantly different in comparison with the transected group, the significant increases in DNA and RNA content tells us that there must be hyperplasia in the excluded loop 48 hours after jejunal resection.

Lower ileum (fig. 6) At 48 hours in the lower ileum, increased RNA content (35 per cent) and DNA content (27 per cent) were found in the resected animals. Both values were significantly different compared to versus the controlgroup. There was a slight tendency to increased values for the transected group, 6 per cent RNA, 6 per cent DNA, but none of them were significant.
Total radioactivity: very marked increase in the resected group, 71 per cent versus the control group and 62 per cent versus the transected rats, both increases were significant (table 2).
The DNA specific activity showed a similar increase, 33 per cent in the resected group versus the control group (significant), 23 per cent versus the transected group (not significant).

TABLE 2

Total radioactivity in DNA per 5 cm mucosa (d.p.m. x 10^{-3})

	N	Duodenum	Upper ileum	Lower ileum
Sham operation	13	235 ± 10	309 ± 30	265 ± 15
Transection + exclusion	13	262 ± 15	279 ± 21	290 ± 27
Resection + exclusion	13	366 ± 28[a,c]	380 ± 35[b]	453 ± 35[a,c]

Total radioactivity in DNA per 5 cm mucosa of duodenum, upper ileum and lower ileum. (Means ± S.E.M.)
Significance
versus sham operation a $P < 0.001$
versus transection b $P < 0.025$
c $P < 0.005$

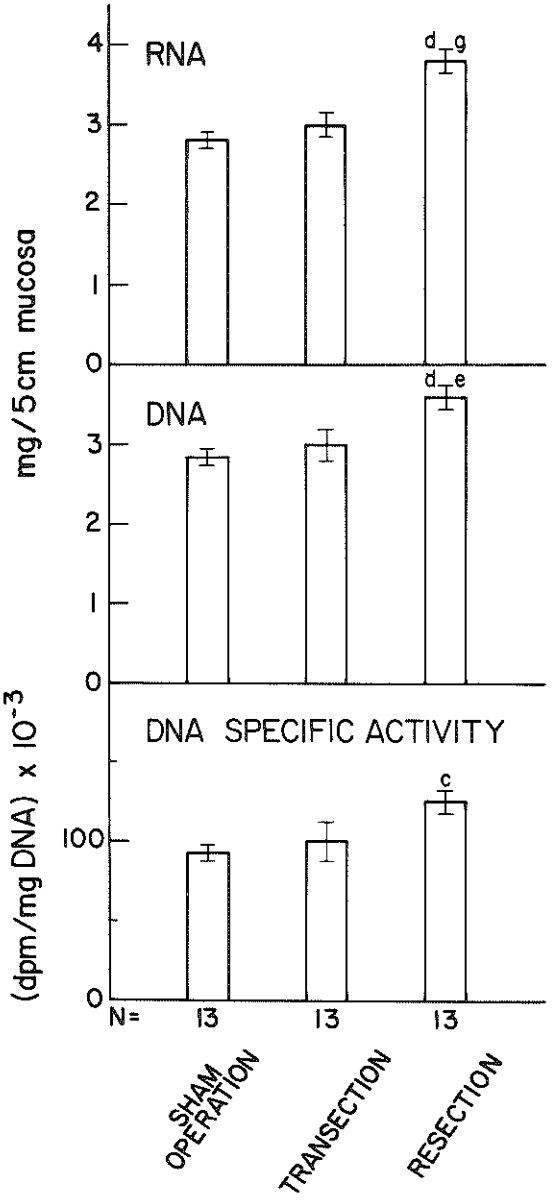

Figure 6
RNA and DNA contents and DNA specific activity in lower ileum after operation (Means ± S.E.M.)
Significance

versus sham operation	c $P < 0.005$	versus transection	e $P < 0.05$
	d $P < 0.001$		g $P < 0.005$

6.4. Discussion

This study demonstrates conclusively that dietary factors and pancreato-biliary secretions when in contact with intestinal epithelium do not exclusively account for the postresectional hyperplasia. In agreement with earlier findings by Dowling and Booth (1967) we found that the gut in continuity underwent hyperplasia, the distal segment of the small bowel more than the proximal. Besides there was marked hyperplasia in the excluded upper ileum 48 hours after resection compared to the control group. The same phenomenon was observed in a separate study 1 week after resection. This is in contrast to expectation, because the normal reaction in excluded or bypassed small bowel is as shown in experiment 2 (chapter IV), progressive mucosal atrophy already appearing 48 hours after the operation and determined by a decrease in nucleic acid contents and a diminished intestinal circumference. This was in agreement with earlier studies of Gleeson et al. (1972) who showed in rats using parts of small bowel as Thiry-Vella loops: luminal narrowing, decreased villous height, diminished cell migration and decreased mucosal enzyme activity.

The hyperplasia seen in the excluded upper ileum loop after resection of the proximal small bowel, as determined by significant increased RNA and DNA values as compared to the control group is difficult to explain.

There are two ways to explain this phenomenon: firstly and most promising 'humoral factors', secondly a physiologically altered intestinal circulatory system.

As to the latter argument Touloukian and Spencer (1971) determined the mucosal perfusion of rats with 50 per cent mid-intestinal resections, using the ^{86}Rb distribution technique (Sapirstein, 1958). In the ileal remnant the mucosal blood flow was markedly greater than that of the controls after two days, but returned to normal in two months, by which time striking 'hypertrophy' was apparent (Touloukian and Spencer, 1971). In these studies it seemed that the jejunal remnants showed no change in blood flow and no 'hypertrophy'. One could suggest, that alterations in the blood flow have caused the hyperplasia in the excluded loop, but sofar it is impossible to conclude whether the bloodflow alterations are the cause or the effect of intestinal hyperplasia.

A second more promising explanation of the hyperplasia found in the excluded upper ileum are the 'humoral factors' to explain this phenomenon. Loran and co-workers postulated the existence of an 'intestinal epithelial growth hormone' to explain the generalised hyperplasia seen throughout the small intestine two months after a limited ileal resection (Loran and Althausen, 1960; Loran and Crocker, 1963). This factor could be transmitted between rats linked in cutaneous parabiosis (Loran and Carbone, 1968). Tilson and associates provided only partial corroboration of these findings in preliminary reports using similar techniques of cutaneous parabiosis (Tilson and Wright, 1971; Tilson et al., 1975). They found that villous cellularity was increased in unoperated partners of animals undergoing resection, but the villous length was unaltered. When the unoperated parabiosis-animals were starved no response to humoral stimuli was noted at all.

In another contrasting report Tilson and Wright (1970) showed 'hypertrophy' in bypassed ileum after jejunectomy, which suggested a humoral factor similar to what Loran proposed. Wilmore and Dudrick (1969) observed villous 'hypertrophy' in partially enterectomized beagles maintained on intravenous hyperalimentation, which also suggested a

humoral factor, although a similar experiment done by Feldman et al. (1976) gave the impression that oral feeding is essential for the development of postresectional hyperplasia. Recent cross circulation studies in rats with 30% proximal resection in our laboratory done by Williamson et al., 1978 (unpublished) are suggestive for the existence of a transmittable humoral factor and as such support the previous studies mentioned. Possible candidates for the role of enterotropic hormones includes enteroglucagon, mineral corticoids (Tilson et al., 1971), pituitary hormones (Taylor and Dowling, 1975), but probably not gastrin in spite of assertions to the contrary (Johnson et al., 1975b; Johnson, 1976).

Gastrin. A lot of work on the 'tropic' effect of gastrin on the gastro-intestinal tract has been done by Johnson and co-workers. They studied the effect of pentagastrin on the incorporation of ^{14}C-leucine into protein of various tissues of the gastro-intestinal tract in rats. They concluded that gastrin exerts a 'tropic' effect on the gastro-duodenal mucosa (excluding the antrum) independent of its capacity for stimulating acid secretion (Johnson et al., 1969). Exogenous pentagastrin prevented atrophy of the gastro-duodenal mucosa in rats deprived of food but maintained by parenteral nutrition (Johnson et al., 1969; Johnson et al., 1975a).
More recently, Johnson (1977) studied the effect of pentagastrin on colonic DNA synthesis in the rat. A striking tropic effect on the colonic mucosa was found and he concluded that gastrin excerts a tropic effect on the mucosa lining the entire intestinal tract. But these assertions require substantiation, because a recent study by Oscarson et al. (1977) showed that a twenty fold variations of endogenous serum gastrin within the physiological range neither prevented atrophic changes of short term starvation on small bowel mucosa nor altered the compensatory hyperplasia response to jejunectomy. They postulated that the tropic effects distal to the duodenum produced by exogenous gastrin and pentagastrin may be results of supranormal levels, other reports are consistent with these findings (Hughes et al., 1976; Tilson and Axtmayer, 1976).
Other gastro-intestinal hormones studied in their effect on the gastro-intestinal tract are:
Cholecystokinin (CCK), known as a potent tropic hormone for the exocrine pancreas (Mainz et al., 1974). But CCK does not stimulate DNA synthesis in oxyntic gland mucosa (Johnson, 1976).
Pancreatic glucagon, like gastrin did significantly increase the DNA content of oxyntic gland mucosa and of colonic mucosa, but the effect was less pronounced than that caused by gastrin (Johnson, 1977).
Enteroglucagon, probably the best single candidate for the role of enterotropic hormone. In 1971 Gleeson et al. described an unique patient with high circulating enteroglucagon levels due to an enteroglucagon secreting tumour of the kidney. At the operation a marked intestinal enlargement with villous hyperplasia was found. The structural changes were apparently due to the raised enteroglucagon concentration since, when the tumour was resected, both the plasma enteroglucagon levels and the intestine promptly returned to normal.
Glucagon may influence hepatic regeneration, alone (Price et al., 1972) or potentiated with insulin (Bucher and Swaffield, 1975). Chronic administration of glucagon to rats increases active transport of amino-acids and sugars by small epithelium (Rudo and Rosen-

berg, 1973). Moreover, enhanced intestinal absorption of glucose and amino-acids is associated with elevated glucagon levels in partially starved rats (Rudo et al., 1973) and experimental diabetes (Rudo, 1973), and can be prevented by injections of glucagon binding antisera (Rudo et al., 1975).
To the contrary, administration of exogenous glucagon significantly reduces villous height and cell migration rate (Rudo et al., 1976).
The main objection against most of the above named studies is, that the effects studied are more pharmacological actions than physiological ones and that the physiological action of the different gastro-intestinal hormones are more complex than previously believed.
In conclusion we can say that there is strong evidence for a systemic factor involved in the control mechanism of the postresectional hyperplasia. Humoral factors are mentioned as possible systemic factor, but their role is still speculative.

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Chapter VII

GENERAL DISCUSSION

7.1. Aspects of enteric hyperplasia

The data of experiments 1, 2 and 4, described in this thesis (Chapter III, IV and VI) confirm a recent report from our laboratory (Obertop et al., 1977) that intestinal hyperplasia is manifest within 48 hours after partial enterectomy in the rat, rather than gradually developing over a period of several days. The rapidity of this intestinal compensation resembles that of renal and hepatic regeneration following loss of tissue mass (Bucher and Malt, 1971). Unlike the liver and kidney, however, intestinal adaptation does not involve cellular hypertrophy. Estimation of mucosal contents of RNA and DNA and uptake of labelled thymidine provide sensitive and reproducible indices of the early cellular response. Histological measurement of villous height and crypt depth (experiments 2 and 3, chapter IV and V) confirm the overall pattern of events, but the changes in villous height are more difficult to detect and the technique is less sensitive in the early period. Considerable changes in the rate of cell production may be associated with relatively minor changes in villous height (Clarke, 1974). It is interesting to speculate upon the course of events during the first 48 hours after intestinal resection. Most probably anaesthetic and surgical trauma, combined with the absence of food, cause initial hypoplasia, followed by a return to base line and subsequent intense cellular proliferation when oral feeding recommences. Since rats are disinclined to eat within 24 hours of intestinal surgery, food was withheld from all groups during this time. In studies of intestinal adaptation prior to 48 hours, it would be difficult to differentiate between the effects of temporary fasting and re-feeding and the effects of small bowel loss, without using total parenteral nutrition, which introduces non-physiological variables.

In all experiments the effects of resection (or bypass) were compared with those of jejunal transection, which itself causes transient distal hyperplasia (experiment 1, chapter III).

Besides failure to detect any increase in DNA specific activity after a 1-hour pulse label at 47 hours, the absence of any appreciable effect of transection at later moments in another study (Obertop et al., 1977) suggests that the response to transection is ephemeral. However it cannot be reproduced by laparotomy and handling of the bowel alone. Distal adaptation following 50 per cent proximal enterectomy (experiment 2, chapter IV) exceeds the reaction measured 48 hours after removal of only the first third of the small bowel (experiment 1, chapter III), though loss of total body weight is not dissimilar. Presumably the more bowel removed, the greater the response within the remnant, which is in agreement with Hanson et al. (1977). Nonetheless there must be a limit to this relationship (Flint, 1912), and subtotal enterectomy might be expected to delay the

development of adaptive changes in the remnant. Consistent with other reports (Booth et al., 1959), we have found a greater degree of hyperplasia in intestinal segments close to the anastomosis. At first sight proximal enterectomy appears to produce a peak response 1 week after operation, then cell proliferation declines up till values significantly above zero values and further shows a plateau (experiment 2, chapter IV). In a separate study (unpublished data) we have found elevated mucosal contents of RNA and DNA in rat intestinal remnants 4 months after jejunectomy, in agreement with most clinical and experimental evidence suggesting persistence of postresectional hyperplasia for at least several months. The rapidity and intensity of the early postresectional response might produce an 'overshoot' phenomenon at 7 days before declining to a persistent plateau.
Villous height is then maintained by an increased rate of cell migration, although turnover time is unchanged (Dowling and Gleeson, 1973). Alternatively, elongation of the remnant might partially restore the original length of the small bowel, whereby an increased cell population would be maintained by fewer cells per unit length of intestine. Elongation could explain the apparent decrease in nucleic acid contents between 1 week and 1 month after enterectomy. Nygaard (1967) reported an increase of one third in the length of the remaining small bowel 7 months after extensive proximal resection or bypass. Similar lengthening of functional bowel remnants has been described after massive resection in the dog (Wilmore et al., 1971) and after resection (McClenahan and Fisher, 1950) and bypass (Woodward et al., 1975; Fenyö et al., 1976) in man.

7.2. Colonic response to enterectomy

Just as ileal excision in the rat stimulates cell proliferation throughout the colon (Nundy et al., 1977), so does colectomy promote ileal hyperplasia in man and in rat (Wright et al., 1969a; 1969b, Bucholtz et al., 1976).
The results obtained from the large intestine in our experiments are less consistent than those in the small bowel, possibly due to difficulty in scraping the colonic mucosa clean off the underlying muscle. Nevertheless the data presented confirm a previous report (Obertop et al., 1977) in that 30 per cent proximal enterectomy in the rat provokes colonic hyperplasia within 2 days.
Although increased cell renewal has been reported 3 months after jejunectomy (Zufarov and Baibekov, 1972), in experiment 2 we showed only a persistent response for 1 month in the transverse colon but not in the ascending colon after 50 per cent proximal resection of the small intestine. We even found that nucleic acid levels in the colonic mucosa returned to normal 4 and 7 months after proximal enterectomy (unpublished data).
The limited colonic hyperplasia found following pancreato-biliary diversion may result from increased concentrations of bile salts within the colonic lumen. The fecal excretion of bile salts is increased by this operation (Chomchai et al., 1974).
Intestinal bypass however, had no stimulatory effect upon the colon at all; indeed mucosal levels of RNA and DNA showed even some tendency to diminish. Despite the possibility of colonic stimulation by 'overflow' of intestinal chyme after enteric shortening (Althausen et al., 1950), our finding that resection causes more colonic hyperplasia than pancreato-biliary diversion and bypass suggests the involvement of humoral factors.

7.3. Local control of hyperplasia

The progressive atrophy found in defunctioned jejunum after proximal enteric bypass (experiment 2, chapter IV) supports the implication of luminal nutrition in the maintenance of normal mucosal structure in the small bowel.
Nonetheless cell renewal continues in excluded loops even when there is gross mucosal atrophy after 1 month of diversion of the nutrient stream. Rijke (1977) found an increased migration rate of epithelial cells on the villus in a Thiry-Vella fistula (jejunum) compared to control jejunum. He indicates that this probably means some shortening of the life span of epithelial cells in bypassed jejunum, which also contributes to a decrease in the number of villous cells. Intestinal epithelium appears to possess an unexpected capacity for cell proliferation in the complete absence of both food and mechanical stimulation. Prompt and persistent hyperplasia of the ileal mucosa following distal transposition of the duodenal papilla is manifested by increments in nucleic acid contents, villous height, crypt depth and intestinal circumference (experiments 2 and 3, chapter IV and V). These findings confirm the tropic effect of pancreato-biliary secretions reported elsewhere (Altmann, 1971; Weser et al., 1977).
In another study we have found continuing ileal adaptation 7 months after pancreato-biliary diversion (unpublished data). Jacobs and Dowling (1975) report mucosal hyperplasia and increased absorptive function in jejunal segments transposed between the pylorus and the papilla. Assuming that pancreato-biliary secretions are excluded from these segments, they suppose that the absence of mucosal hypoplasia in the transposed jejunum contradicts the theory that pancreato-biliary secretions exert a tropic effect. But apart from the possibility that papillary secretions might reflux into the jejunal loop, cell proliferation in the transposed segment might be stimulated by proximity to tropic pyloric secretions (Altmann and Leblond, 1970), or by exposure to a nutrient concentration not yet diminished by duodenal absorption. Moreover, in normal rats duodenal villi are taller than jejunal villi (Altmann and Enesco, 1967), so that some degree of hyperplasia might be anticipated after transposition of the jejunum to a juxtapyloric position. Our finding that the tropic effect of the combined pancreato-biliary effluent exceeds that of bile alone (experiment 3, chapter V) is consistent with Altmann's work (1971) comparing the effect of biliary diversion, with or without pancreatic juice, to isolated rat ileal loops. In Altmann's study the number of surviving rats was very small, because of pancreatitis following transection of the bile duct and end-to-side choledocho-enterostomy. Cannulation of the bile duct causes less mortality but is complicated by an appreciable incidence of blocked cannulae. Experiment 3 shows that bile itself does appear to have a limited tropic effect on intestinal mucosa and can initiate ileal adaptation, but the additional presence of pancreatic juice prolongs the adaptive response. The mechanism by which pancreato-biliary secretions promote epithelial hyperplasia remains unclear. Distal diversion of the papillary effluent, with or without duodenal transposition, might produce hypergastrinaemia since it is associated with hypersecretion of acid from canine pouches (Menguy and Mings, 1961; Storer et al., 1952). However, despite assertions that gastrin exerts a tropic effect on intestinal mucosa (Johnson, 1976), Oscarson (1977) could not demonstrate any stimulatory role for gastrin in regulating intestinal cell proliferation. Pancreatic digestive enzymes will increase the supply of glucose and amino

acids available for direct nutrition of the villus epithelium, but this action may represent only part of the pancreatic influence (Altmann, 1974). The physiological actions of bile are complex and may depend upon the degree of conjugation of bile salts and the composition of bile acids present in the intestinal lumen. For example, in germ free mice, in which taurocholic acid is the only bile acid formed, the addition of cholic acid to the diet halves the turnover time of mucosal cells in the ileum (Ranken et al., 1971). Bile salts might stimulate epithelial proliferation by non-specific alteration of mucosal permeability (Dobbins and Binder, 1976), by stimulation of cyclic adenosine monophosphate (Coyne et al., 1976) or by simple mucosal irritation (Bloch et al., 1974; Menge et al., 1976). In contrast to pancreatic extracts, which cause marked hyperplasia, fresh hog bile does not increase villous size when infused into isolated ileal loops in conscious rats (Altmann, 1974). Quite possibly the concomitant presence of food is necessary for gastrointestinal secretions to exert a tropic effect. Since oral intake of food stimulates the flow of bile and pancreatic juice, it is difficult to separate the direct effect of the absence of nutrients from that of associated reduction in endogenous secretions in the pathogenesis of fasting atrophy. Besides intestinal hypoplasia, marked pancreatic atrophy occurs during total parenteral nutrition in rats (Johnson et al., 1975a). Preliminary data suggests that chronic stimulation of pancreato-biliary secretion by cholecystokinin and secretin may prevent the intestinal hypoplasia otherwise found in dogs fed exclusively by intravenous alimentation (Hughes et al., 1976).

7.4. Systemic control of hyperplasia

The accretion of DNA, but not of RNA, in ileal mucosa following jejunal transection (experiment 1, chapter III) has its parallel in isoproterenol stimulated cell proliferation in mouse kidney (Malamud and Malt, 1971). Just as this response is mediated by a humoral agent, the ability of the ileal mucosa to undergo rapid hyperplasia after transection performed 80 cm. proximal argues for the presence of a humoral stimulant, rather than a qualitative or quantitative change in enteric contents or secretions. The inability of proximal enteric bypass or pancreato-biliary diversion to match the rapidity and intensity of ileal adaptation after an equivalent resection (experiment 2, chapter IV) agrees with Senn's (1888) original observations and shows that the postresectional response is mediated in part by factors other than intraluminal nutrition or secretion.
Although bowel contents were observed in the lower end of defunctioned loops at autopsy, it is most unlikely that this degree of reflux could account for the discrepancy in response between resection and bypass, as suggested by Senn (1888). A selective increase in bloodflow to ileal remnants has been demonstrated within 2 days of mid-enterectomy (Touloukian and Spencer, 1972) and alterations in blood flow may affect renal and hepatic compensatory growth (Bucher and Malt, 1971). Nevertheless, insufficient evidence exists to show whether neurovascular changes are the cause or merely the effect of small bowel hyperplasia. Inhibitory hormones might regulate intestinal mass by negative feedback (Tilson and Wright, 1970). Besides lack of evidence, the main objection to this theory has been the reported similarity of response to resection and bypass (Nygaard, 1967; Gleeson et al., 1972a).

We have shown that the response is not entirely similar in the early postoperative phase. Progressive mucosal atrophy following intestinal exclusion might gradually deplete circulating levels of an inhibitor in contrast to a sudden drop produced by resection.
Loran and co-workers proposed the existence of a transmittable factor i.e. 'intestinal epithelial growth hormone' (Loran and Althausen, 1960; Loran and Crocker, 1963; Loran and Carbone, 1968).
Subsequent attempts to confirm these findings in rats have not been successful (Tilson and Wright, 1971c; Tilson et al., 1975).
Wilmore and Dudrick (1969) observed villous hypertrophy in partially enterectomized beagles maintained on intravenous hyperalimentation, which also suggested a humoral factor, although a similar experiment from Feldman et al., (1976) suggested that the presence of chyme was essential for intestinal adaptation.
Elias and Dowling (1974) also, showed hyperplasia in bypassed intestine of lactating rats, which suggested a possible hormonal influence on cell renewal. In agreement with the above mentioned studies we found in experiment 4 (chapter VI) strong evidence for humoral factors, by showing that excluded upper ileum loops develop postresectional hyperplasia. With this study we demonstrate that contact of dietary factors and pancreato-biliary secretions with the intestinal epithelium does not exclusively account for the postresectional hyperplasia.
The unpublished studies from our laboratory (Williamson et al., 1978) with a cross circulation model and a 30 per cent proximal resection also support a transmittable humoral factor.
Possible candidates for the role of enterotropic hormones are reviewed in the survey of literature and include mineralocorticoids (Tilson et al., 1971), pituitary hormones (Taylor and Dowling, 1975), pancreatic glucagon (Johnson, 1977), gastrin (Johnson et al., 1969) and enteroglucagon (Gleeson et al., 1971).
Enteroglucagon is probably the best single candidate for the role of enterotropic hormone, but there are a lot of objections against the above named studies.
Many 'physiological' actions attributed to enteric hormones may in fact be pharmalogical (Grossman, 1973).

7.5. Interdependence of tropic factors

Absence of the postresectional response in total parenteral nutrition (Feldman et al., 1976, Levine et al.,) and direct mucosal stimulation by nutrient perfusions in isolated bowel loops (Altmann, 1974; Jacobs et al., 1975; Menge et al., 1975) provide the strongest evidence of tropic factors present in food. As mentioned earlier, the output of pancreato-biliary secretions is also related to the presence or absence of food in the gut. The 'richer' chyme entering the ileum after jejunectomy or ileojejunal transposition may be accompanied by alterations in bacterial flora and in bowel motility, by increases in salivary and gastroduodenal secretions, by mechanical stimulation from increased bulk and conceivably by local hormones (Chalones) (Thornley and Laurence, 1975), all of which might contribute to adaptation of the mucosa (Dowling, 1976). Since the response to resection exceeds that following bypass and pancreato-biliary diversion (experiment 2,

chapter IV) systematic factors must also be involved, as was shown in experiment 4 (chapter VI). The rapidity and uniformity of enterocolic adaptation to small bowel resection, despite considerable loss of body weight, are also indicative of humoral control.

The presence of food in the duodenum causes secretin and pancreozymin release, which stimulates the output of enzyme rich juice from the exocrine pancreas and the output of glucagon from the endocrine pancreas (Buchanan et al., 1968). Digestion of food by pancreatic enzymes liberates glucose and amino acids into the bowel lumen, and glucagon enhances the uptake of these nutrients (Rudo and Rosenberg, 1973), both of which may be directly utilised by cells of the intestinal epithelium (Smyth, 1962; Kinter and Wilson, 1965).

In the postresectional period however we have now established beyond refute that the short bowel adaptation starts almost immediately after the experimental operations. Furthermore it seems clear that chyme is still a major factor in the mechanism of compensatory hyperplasia. Our experiments are also showing the important role of bile and pancreatic juice. As concerning the influence of humoral factors although not proven our experiments are showing that some systemic factor must be involved in the control mechanism of the postresectional hyperplasia. A lot of gastro-intestinal hormones have been investigated for their tropic role on the gastro-intestinal mucosa, sofar no conclusive evidence has been presented. Probably a complex mechanism of stimulatory and inhibatory hormones is involved.

Thus there is a close physiological link between the three major factors – food, secretions and gastro-intestinal hormones – which are thought to regulate postresectional adaptation. Since intestinal resection will alter the concentrations of all three agents, compensatory hyperplasia must be under multifactorial control. This of course does not exclude the possibility of the existence of local mediators which could be either stimulated or inhibited by the three above mentioned more general factors.

SUMMARY

For many years it was not clear whether individual villous cells were able to increase their functional capacity (hypertrophy) or whether improved absorption by the intestine after resection depended entirely on the increased production of more villous cells (hyperplasia). It is now known that the major response to small bowel resection is a compensatory hyperplasia of the (mucosal) remnant of the bowel.
In Chapter I a historical survey is given of the different studies on structural and functional adaptation of the small bowel after resection and bypass. At the same time theories concerning cell kinetics are mentioned. In the last part of the chapter the factors and theories connected with the control mechanism of the hyperplasia are discussed.
Chapter II 'Experimental design and methods' gives a general description about the experimental animals, the surgical operations, the histological measurements, the biochemical estimations and the statistical analysis used in the different experiments. The biochemical assay of DNA and RNA content and of ^{3}H thymidine in corporation is discussed. The last part of this chapter includes a section on the validity of the methods used.
Chapter III gives evidence that even a simple transection of the jejunum gives rise to a transient spurt of hyperplasia in the distal small bowel.
In Chapter IV, the contribution of intraluminal factors to postresectional intestinal hyperplasia, is discussed. The cell proliferation in the distal small bowel was studied after jejunum resection, jejunum bypass and after transposition of the bile duct to the mid small bowel, resulting in higher concentrations of bile and pancreatic juice into the ileum. Both secretions have been shown to modulate enterocyte proliferation. Nonetheless evidence is presented that neither pancreato-biliary diversion nor proximal enteric bypass produce the same rate of distal hyperplasia as proximal resection. This means that the effects of excision and exclusion of equivalent amounts of small bowel on the remnant of the bowel are not identical. This observation also points to other factors involved in the adaptive hyperplasia than food and pancreato-biliary secretion.
In Chapter V the relative contribution of bile and pancreatic juice to postresectional intestinal hyperplasia has been studied. The results indicate that increased luminal concentration of bile is sufficient for prompt ileal hyperplasia, but the additional presence of pancreatic juice prolongs this adaptive response.
In Chapter VI the possibility that humoral factors might be involved in the control of postresectional adaptation was tested in individual rats. Transected and jejunectomized rats with an excluded upper ileum were studied 48 hours after operation. In contrast to the mucosal atrophy found in a bypass without transection or jejunectomy, in this study a marked hyperplastic reaction was found in the excluded segment after a jejunectomy, suggestive for a systematic factor, i.e. 'a humoral factor'. The possible connection between gastrointestinal hormones and postresectional hyperplasia is mentioned. A lot of gastro-

intestinal hormones have been investigated for their tropic role on the gastro-intestinal mucosa, but until now the evidence is vague. In conclusion the data of the experiments described in this thesis and of the literature are pointing to a close link between three factors: food, bile and pancreatic juice and a humoral factor. Since intestinal resection will alter the concentrations of all three agents, compensatory hyperplasia is surely under multifactorial control. This of course does not exclude the possibility of the existence of local mediators which could be either stimulated or inhibited by the more general factors, which have been mentioned above.

SAMENVATTING

Gedurende lange tijd was het niet duidelijk of individuele villus cellen in staat waren na een resectie hun functionele capaciteit te vergroten (hypertrofie), of wel dat de waargenomen toename in absorptief vermogen van de resterende darm volledig afhankelijk was van de toegenomen productie van meer villus cellen (hyperplasie).
Het staat nu vast dat de belangrijkste reactie na een resectie bestaat uit een compensatoire hyperplasie van de resterende darmmucosa.
In Hoofdstuk I wordt een historisch overzicht gegeven van de verschillende in de literatuur vermelde studies aangaande de structurele en functionele adaptatie van de dunne darm na een resectie en een bypass-operatie. Tevens wordt melding gemaakt van verschillende celkinetische theorieën, die betrekking hebben op het controle-mechanisme van de hyperplasie.
Hoofdstuk II getiteld 'Proefopstelling en gebruikte technieken' houdt een algemene introductie in betreffende de dieren die gebruikt zijn in de verschillende eigen experimenten. De verschillende operatie-technieken, de histologische metingen, de biochemische bepalingen en de statistische bewerking worden beschreven. Voorts worden de achtergronden van de gebruikte biochemische onderzoekmethode voor de bepaling van DNA, RNA en de incorporatie van ^{3}H thymidine belicht.
Het hoofdstuk wordt besloten met een verantwoording over de gebruikte onderzoekmethoden.
In Hoofdstuk III wordt het bewijs geleverd dat zelfs een eenvoudige transectie van het jejunum een voorbijgaande hyperplasie van de distale dunne darm veroorzaakt.
Hoofdstuk IV. In dit hoofdstuk wordt de celproliferatie van het distale gedeelte van de dunne darm bestudeerd na resectie van het jejunum, na een jejunum bypass, alsmede na transpositie van de ductus choledochus naar het midden van de dunne darm. Eén van de gevolgen van een jejunumresectie is een hogere concentratie van gal en pancreassap in het ileum dan onder normale omstandigheden. Van zowel gal als pancreassap is bekend, dat zij een directe invloed uitoefenen op de proliferatie van de enterocyt.
Niettemin wordt in dit hoofdstuk het bewijs geleverd dat nòch transpositie van de ductus choledochus nòch een jejunumbypass een zelfde graad van hyperplasie in het distale gedeelte van de dunne darm kunnen bewerkstelligen als een jejunum resectie. Dit betekent, dat het effect van excisie of van exclusie van gelijke stukken dunne darm op de resterende darm niet identiek is. Tevens wijst deze waarneming op andere factoren dan de reeds eerder genoemde (voeding, gal en pancreassap).
In Hoofdstuk V wordt de bijdrage, die gal en pancreassap leveren aan de hyperplasie na een darmresectie besproken. De resultaten van het onderzoek geven aan dat een toegenomen concentratie van gal voldoende is voor het ontstaan van een snel optredende hyperplasie in het ileum, maar dat pancreassap nodig is voor een continuering van deze hyperplasie.

Dat humorale factoren mogelijk betrokken zijn bij het controle mechanisme van de post-resectionele adaptie wordt in Hoofdstuk VI besproken. 48 Uur na de operatie werden ratten bestudeerd, die een jejunum transectie en resectie hadden ondergaan, alsmede een uitsluiting van het proximale gedeelte van het ileum. In tegenstelling tot de mucosa atrophie, die normaal in een uitgesloten dunne darm segment wordt gevonden, werd in dit uitgesloten segment een duidelijke hyperplasie vastgesteld na een gelijktijdige jejunum-resectie.
Een en ander is suggestief voor een 'systemische' factor, in casu een 'humorale factor'. In dit hoofdstuk wordt verder de mogelijke samenhang van gastro-intestinale hormonen met de post-resectionele hyperplasie vermeld. Een groot aantal gastro-intestinale hormonen is met betrekking tot hun trofische invloed op de gastro-intestinale mucosa onderzocht, overtuigende conclusies zijn uit de verzamelde gegevens echter nog niet te trekken.
Concluderend kan gesteld worden, dat de gegevens van de experimenten, beschreven in dit proefschrift en de gegevens uit de literatuur, wijzen op een sterke samenhang van drie factoren, te weten: voeding, gal en pancreassap èn een humorale factor. Aangezien ten gevolge van een dunne darm resectie een verandering optreedt in al deze 3 factoren, zal de gevonden compensatoire hyperplasie na een resectie zeker onder invloed staan van meerdere gelijk optredende factoren.
De mogelijkheid, dat ook locale factoren een rol in dit proces spelen wordt niet uitgesloten geacht. Deze locale factoren zouden zowel in positieve als in negatieve zin beïnvloed kunnen worden door de 3 reeds vermelde algemene factoren.

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CURRICULUM VITAE

Schrijver van dit proefschrift werd in 1946 geboren te Amsterdam. Na het afleggen van het eindexamen Gymnasium in 1964 te Hilversum, studeerde hij medicijnen te Leiden, alwaar het artsexamen in 1971 werd afgelegd.
Na het vervullen van zijn militaire dienstplicht begon hij zijn opleiding tot chirurg in oktober 1973 in het Academisch Ziekenhuis Dijkzigt te Rotterdam. Als onderdeel van die opleiding was hij van januari 1976 – oktober 1976 werkzaam in het Massachusetts General Hospital te Boston (U.S.A.) als Clinical en Research fellow.

APPENDIX A

Procedure for nucleic acid extraction (Scott et al., 1956).

Reagents

a. 80 per cent ethanol*
b. alcohol ether (mix 3 volumes 95 per cent ethanol with 1 volume ethyl ether)**
c. 1N NaOH (4 gm. NaOH/100 cc glas distilled H_2O) in polyethylene bottle
d. 6N HCl (1 volume HCl + 1 volume H_2O).
e. 1N perchloric acid (PCA) (85,85 cc of 70 per cent PCA diluted with distilled H_2O to 1 liter)*
f. 0.3N perchloric acid (PCA) (25.75 of 70 per cent PCA diluted with distilled H_2O to 1 liter)*
g. 0.25 M Sucrose, containing 5 per cent citric acid (85.6 gm. sucrose + 50 gm. citric acid diluted with distilled H_2O to 1 liter)*
h. acid-alkali solution (20 ml 1N NaOH + 4 ml 6N HCl)

* refrigerated
** kept in freezer

Equipment

Pipettes, Pasteur pipettes stuffed with glass wool, racks and beachers, test tubes, graded centrifuge, vortex mixer, homogenizer, waterbath at 60° C and Beckman spectrophotometer.

Procedure

1. Homogenize thawed mucosal pellet in 2 ml. citric acid sucrose. Add 4 ml 0.3N PCA; vortex; centrifuge 5 min. Remove and collect supernatant.
2. Add 2 ml. 0.3N PCA to pellet; vortex, centrifuge 5 min. Collect supernatant and add to supernatant from step no. 1. The combined supernatants constitute the acid-soluble fraction.
3. Add 2 ml. 80 per cent ethanol to pellet, vortex, centrifuge 5 min. Discard supernatant.
4. Add 2 ml. alcohol ether to pellet; vortex; centrifuge 5 min. Discard supernatant. Repeat this step 1-2 times to obtain white pellet. Evaporate ether.
5. Add 2 ml. 1N NaOH; vortex. Allow to stand for 1 hour at room temperature. Vortex occasionally to break up the pellet.
6. Add 0.4 ml. 6N HCl; vortex, cool in ice bath for 2-5 minutes. Centrifuge until supernatant is clear. Filter supernatant through glass wool and collect.
7. Add 1 ml. acid-alkali solution; vortex; centrifuge 5 min. Remove, filter and collect supernatant. The combined supernatants from steps no. 6 and 7 constitute the RNA fraction.
8. Add 1 ml. 0.3N PCA to pellet; vortex, centrifuge 5 min. Discard supernatant.
9. Add 2 ml. 1N PCA to pellet; vortex. Heat at 60° C for 15 minutes. Cool for 2-5 minutes in ice bath; centrifuge 5 min. Filter supernatant through glass wool and collect.
10. Repeat step nr. 9 and add supernatant. The combined supernatants constitute the DNA fraction. Pellet available for determination of protein content.

Note: during steps no. 1-4 specimens are kept cold in ice bath.

Ultraviolet absorption

The RNA sample obtained by the fractionation procedure has a volume of ca. 3.0 ml. An aliquot of the sample is diluted x 10 (0.5 cc in 4.5 cc H_2O) and its optical density measured in the Beckman spectrophotometer against a blank of distilled water at both 260 and 280 mμ wave lengths. The reading at 280 mμ is subtracted from that at 260 mμ and the difference is divided by 0.00472 to obtain the concentration of RNA per ml. in micrograms. Purified mammalian RNA at a concentration of 100 μg per ml gives a difference between 260 and 280 mμ of 0.472 optical density units. A

concentration of one μg should then give a difference of 0.00472. This gives the following formula for the RNA per aliquot.

$$\text{RNA} = \frac{\text{reading at 260 m}\mu - \text{reading at 280 m}\mu}{0.00472} \times \text{dilution factor (10)} \times \text{volume } (\pm 3.0)$$

The DNA sample has a volume of ca. 4.0 ml. Once again it is diluted (6x) and its optical density measured against a similar blank at 260 mμ. A standard DNA sample gives a calibrating curve in which a concentration of 53 μg per ml. corresponds to one optical density unit. The DNA concentration of the sample in micrograms per ml. can therefore be calculated by multiplying its reading at 260 mμ by 53 x the dilution factor (6) and the volume (± 4.0 cc.).
This gives the following formula for DNA concentration in the sample:
DNA = reading at 260 mμ x 53 x 6 x 4

Radioactivity

The radioactivity of the acid-soluble and DNA fractions is determined by counting 0.5 ml. aliquots of each solution in 10 ml. PCS (scintillation fluid, Amersham/Searle Corp. Arlington Heights, Illinois) for 5 minutes in a scintillation counter and subtracting the background count. Suitable standards are also counted, containing tritiated toluene whose rate of decay in disintegrations per minute has previously been calculated. So gives 20λ ^{3}H toluene 46586 disintegrations per minute.
Thus the sample readings can be converted from counts to disintegrations (d.p.m.) by multiplying with

$$\text{the efficiency factor} = \frac{\text{disintegrations per minute for 20}\lambda\ ^3\text{H toluene}}{\text{counts per minute for 20}\lambda\ ^3\text{H toluene}}$$

The total radioactivity of the DNA fraction in disintegrations per minute = (experimental counts – background) x efficiency factor (± 4) x dilution factor (2) x volume (± 4).
The DNA specific activity is then calculated by dividing the radioactivity in d.p.m. by the DNA content in mg.

APPENDIX B

Experiment 1, Chapter III (Means ± S.E.M.)

MID SMALL BOWEL

	Control	Sham	Transection	Resection
N	12	13	13	14
RNA	2.25 ± 0.09	2.19 ± 0.08	2.39 ± 0.09	3.31 ± 0.10
DNA	1.69 ± 0.04	1.81 ± 0.07	2.21 ± 0.08	2.56 ± 0.09
RNA/DNA	1.35 ± 0.07	1.26 ± 0.07	1.11 ± 0.07	1.32 ± 0.06
Radioactivity	174 ± 11	150 ± 10	166 ± 7	336 ± 23
Sp. activity	103.9 ± 6.3	84.3 ± 5.9	78.3 ± 4.8	131.4 ± 8.0

DISTAL SMALL BOWEL

	Control	Sham	Transection	Resection
N	12	13	13	14
RNA	2.00 ± 0.07	2.22 ± 0.08	2.20 ± 0.07	2.54 ± 0.06
DNA	1.72 ± 0.06	1.96 ± 0.09	2.16 ± 0.07	2.21 ± 0.10
RNA/DNA	1.19 ± 0.06	1.17 ± 0.07	1.03 ± 0.06	1.19 ± 0.05
Radioactivity	191 ± 5	176 ± 10	196 ± 6	297 ± 16
Sp. activity	111.8 ± 8.8	91.1 ± 5.4	93.6 ± 4.6	137.5 ± 7.0

Experiment 2 and 3, chapter IV and V (Means ± S.E.M.)

TRANSECTION

		48 hours	1 week	1 month
	N	12	12	12
JEJUNUM	RNA	2.86 ± 0.13	3.00 ± 0.18	3.18 ± 0.15
	DNA	2.07 ± 0.11	2.09 ± 0.10	2.46 ± 0.10
	RNA/DNA	1.41 ± 0.08	1.44 ± 0.06	1.31 ± 0.07
	Radioactivity	230 ± 19	175 ± 15	153 ± 11
	Sp. Activity	111.3 ± 8.8	84.9 ± 7.4	62.2 ± 3.7
UPPER ILEUM	RNA	2.31 ± 0.10	2.35 ± 0.17	2.77 ± 0.10
	DNA	1.68 ± 0.08	1.99 ± 0.13	2.45 ± 0.14
	RNA/DNA	1.41 ± 0.09	1.19 ± 0.06	1.18 ± 0.09
	Radioactivity	192 ± 14	180 ± 8	174 ± 12
	Sp. Activity	116.7 ± 9.3	98.0 ± 7.8	72.4 ± 5.2
LOWER ILEUM	RNA	2.19 ± 0.13	2.15 ± 0.11	2.34 ± 0.12
	DNA	1.65 ± 0.10	1.80 ± 0.10	2.25 ± 0.13
	RNA/DNA	1.37 ± 0.09	1.22 ± 0.08	1.07 ± 0.07
	Radioactivity	211 ± 20	193 ± 12	164 ± 8
	Sp. Activity	134.5 ± 16.6	108.8 ± 6.4	74.6 ± 4.4
ASCENDING COLON	RNA	2.39 ± 0.15	2.60 ± 0.10	2.64 ± 0.11
	DNA	1.84 ± 0.12	2.37 ± 0.07	2.50 ± 0.11
	RNA/DNA	1.35 ± 0.09	1.11 ± 0.06	1.07 ± 0.04
	Radioactivity	204 ± 33	160 ± 28	136 ± 9
	Sp. Activity	117.2 ± 19.8	66.9 ± 11.1	52.1 ± 3.7
TRANSVERSE COLON	RNA	2.15 ± 0.12	2.86 ± 0.13	2.66 ± 0.14
	DNA	2.01 ± 0.15	2.74 ± 0.15	2.69 ± 0.17
	RNA/DNA	1.11 ± 0.08	1.06 ± 0.05	1.01 ± 0.05
	Radioactivity	163 ± 15	164 ± 23	141 ± 13
	Sp. Activity	88.1 ± 11.9	61.6 ± 9.4	53.3 ± 5.0

Experiment 2, chapter IV (Means ± S.E.M.)

RESECTION

		48 hours	1 week	1 month
	N	13	14	13
JEJUNUM	RNA			
	DNA			
	RNA/DNA			
	Radioactivity			
	Sp. Activity			
UPPER ILEUM	RNA	3.51 ± 0.17	4.46 ± 0.17	3.59 ± 0.16
	DNA	2.36 ± 0.12	3.44 ± 0.22	3.70 ± 0.15
	RNA/DNA	1.53 ± 0.11	1.34 ± 0.07	0.98 ± 0.04
	Radioactivity	400 ± 42	476 ± 45	192 ± 18
	Sp. Activity	170.2 ± 14.8	140.4 ± 10.3	51.6 ± 3.8
LOWER ILEUM	RNA	2.80 ± 0.14	3.85 ± 0.15	2.64 ± 0.09
	DNA	1.99 ± 0.10	2.86 ± 0.15	2.61 ± 0.17
	RNA/DNA	1.42 ± 0.05	1.38 ± 0.07	1.05 ± 0.05
	Radioactivity	432 ± 56	378 ± 51	155 ± 11
	Sp. Activity	212.5 ± 28.0	132.8 ± 15.7	58.5 ± 3.5
ASCENDING COLON	RNA	2.61 ± 0.10	3.19 ± 0.14	2.56 ± 0.12
	DNA	2.29 ± 0.14	2.66 ± 0.13	2.72 ± 0.15
	RNA/DNA	1.19 ± 0.07	1.25 ± 0.06	0.96 ± 0.06
	Radioactivity	255 ± 21	228 ± 13	145 ± 12
	Sp. Activity	116.4 ± 11.4	87.7 ± 6.1	53.3 ± 4.5
TRANSVERSE COLON	RNA	2.74 ± 0.12	3.11 ± 0.10	3.43 ± 0.12
	DNA	2.93 ± 0.09	3.37 ± 0.13	3.49 ± 0.13
	RNA/DNA	0.94 ± 0.03	0.93 ± 0.03	0.99 ± 0.02
	Radioactivity	210 ± 28	241 ± 18	161 ± 17
	Sp. Activity	73.1 ± 10.5	73.3 ± 7.0	46.7 ± 4.6

Experiment 2, chapter IV (Means ± S.E.M.)

BYPASS

		48 hours	1 week	1 month
	N	12	13	13
JEJUNUM	RNA	2.24 ± 0.12	2.32 ± 0.14	1.81 ± 0.11
	DNA	1.69 ± 0.12	1.63 ± 0.12	1.56 ± 0.05
	RNA/DNA	1.38 ± 0.11	1.46 ± 0.09	1.16 ± 0.07
	Radioactivity	216 ± 22	144 ± 17	95 ± 7
	Sp. Activity	129.0 ± 11.7	94.5 ± 13.5	61.2 ± 4.1
UPPER ILEUM	RNA	2.78 ± 0.19	3.67 ± 0.16	4.09 ± 0.21
	DNA	2.21 ± 0.13	2.97 ± 0.23	3.66 ± 0.16
	RNA/DNA	1.29 ± 0.10	1.32 ± 0.08	1.14 ± 0.07
	Radioactivity	335 ± 26	297 ± 25	209 ± 16
	Sp. Activity	154.7 ± 11.9	99.8 ± 8.0	57.8 ± 4.0
LOWER ILEUM	RNA	2.26 ± 0.18	3.03 ± 0.13	3.08 ± 0.16
	DNA	2.05 ± 0.15	2.43 ± 0.14	2.91 ± 0.13
	RNA/DNA	1.11 ± 0.06	1.30 ± 0.09	1.07 ± 0.06
	Radioactivity	323 ± 20	282 ± 24	191 ± 18
	Sp. Activity	163.4 ± 11.9	119.7 ± 11.9	66.2 ± 6.1
ASCENDING COLON	RNA	1.88 ± 0.08	2.69 ± 0.16	2.54 ± 0.11
	DNA	1.97 ± 0.12	2.16 ± 0.16	2.56 ± 0.13
	RNA/DNA	0.98 ± 0.05	1.29 ± 0.07	1.01 ± 0.05
	Radioactivity	210 ± 14	216 ± 21	142 ± 7
	Sp. Activity	110.8 ± 10.7	106.5 ± 12.2	57.0 ± 3.8
TRANSVERSE COLON	RNA	2.03 ± 0.11	2.84 ± 0.17	2.63 ± 0.12
	DNA	1.64 ± 0.08	2.10 ± 0.14	2.21 ± 0.13
	RNA/DNA	1.25 ± 0.06	1.37 ± 0.05	1.23 ± 0.07
	Radioactivity	217 ± 17	223 ± 21	137 ± 10
	Sp. Activity	135.1 ± 12.1	106.7 ± 7.8	62.9 ± 3.9

Experiment 2 and 3, chapter IV and V (Means ± S.E.M.)

PANCREATOBILIARY DIVERSION

		48 hours	1 week	1 month
	N	13	13	11
JEJUNUM	RNA	2.24 ± 0.12	2.82 ± 0.14	3.26 ± 0.14
	DNA	1.72 ± 0.13	2.10 ± 0.10	2.43 ± 0.13
	RNA/DNA	1.37 ± 0.07	1.39 ± 0.06	1.36 ± 0.07
	Radioactivity	189 ± 24	198 ± 17	173 ± 22
	Sp. Activity	115.1 ± 13.5	95.5 ± 8.0	70.8 ± 6.8
UPPER ILEUM	RNA	2.68 ± 0.10	3.52 ± 0.18	4.41 ± 0.15
	DNA	2.24 ± 0.10	2.65 ± 0.19	3.39 ± 0.16
	RNA/DNA	1.22 ± 0.06	1.37 ± 0.07	1.31 ± 0.04
	Radioactivity	335 ± 35	318 ± 24	247 ± 33
	Sp. Activity	150.0 ± 15.4	120.4 ± 9.1	75.1 ± 9.6
LOWER ILEUM	RNA	2.30 ± 0.10	2.80 ± 0.14	3.59 ± 0.11
	DNA	2.03 ± 0.13	2.24 ± 0.14	2.74 ± 0.12
	RNA/DNA	1.17 ± 0.07	1.28 ± 0.07	1.33 ± 0.06
	Radioactivity	265 ± 22	270 ± 23	199 ± 17
	Sp. Activity	133.9 ± 12.1	124.2 ± 11.8	73.1 ± 5.5
ASCENDING COLON	RNA	2.31 ± 0.12	2.84 ± 0.17	3.24 ± 0.14
	DNA	2.30 ± 0.12	2.53 ± 0.16	2.61 ± 0.14
	RNA/DNA	1.03 ± 0.05	1.15 ± 0.07	1.27 ± 0.06
	Radioactivity	168 ± 18	238 ± 21	149 ± 13
	Sp. Activity	71.9 ± 5.5	98.0 ± 9.2	58.7 ± 4.6
TRANSVERSE COLON	RNA	2.26 ± 0.12	2.71 ± 0.10	3.42 ± 0.26
	DNA	2.67 ± 0.15	2.99 ± 0.15	3.23 ± 0.22
	RNA/DNA	0.87 ± 0.06	0.94 ± 0.05	1.06 ± 0.04
	Radioactivity	151 ± 16	186 ± 9	191 ± 17
	Sp. Activity	60.3 ± 8.1	64.6 ± 3.7	59.5 ± 6.0

Experiment 3, chapter V (Means ± S.E.M.)

BILIARY DIVERSION

		48 hours	1 week	1 month
	N	11	12	11
JEJUNUM	RNA	2.62 ± 0.08	2.77 ± 0.17	3.16 ± 0.17
	DNA	2.19 ± 0.11	2.32 ± 0.12	2.66 ± 0.12
	RNA/DNA	1.23 ± 0.07	1.20 ± 0.07	1.20 ± 0.06
	Radioactivity	173 ± 14	200 ± 29	124 ± 7
	Sp. Activity	77.3 ± 7.0	85.7 ± 11.2	47.6 ± 3.5
UPPER ILEUM	RNA	2.82 ± 0.12	3.06 ± 0.16	2.85 ± 0.14
	DNA	2.37 ± 0.08	2.66 ± 0.09	2.66 ± 0.16
	RNA/DNA	1.20 ± 0.06	1.15 ± 0.06	1.09 ± 0.06
	Radioactivity	221 ± 29	232 ± 25	160 ± 17
	Sp. Activity	93.4 ± 11.0	87.9 ± 9.7	61.9 ± 7.1
LOWER ILEUM	RNA	2.18 ± 0.08	2.64 ± 0.16	2.65 ± 0.11
	DNA	2.01 ± 0.12	2.40 ± 0.10	2.67 ± 0.16
	RNA/DNA	1.13 ± 0.07	1.11 ± 0.06	1.02 ± 0.05
	Radioactivity	189 ± 22	261 ± 23	150 ± 9
	Sp. Activity	93.7 ± 9.2	109.4 ± 9.1	58.3 ± 4.1
ASCENDING COLON	RNA	2.57 ± 0.18	2.70 ± 0.16	2.85 ± 0.11
	DNA	2.76 ± 0.17	2.85 ± 0.14	2.81 ± 0.14
	RNA/DNA	0.94 ± 0.05	0.95 ± 0.03	1.04 ± 0.06
	Radioactivity	153 ± 21	185 ± 15	126 ± 5
	Sp. Activity	55.7 ± 5.8	66.0 ± 5.5	46.4 ± 3.7
TRANSVERSE COLON	RNA	2.59 ± 0.16	2.79 ± 0.16	3.01 ± 0.19
	DNA	2.46 ± 0.14	2.79 ± 0.07	2.75 ± 0.19
	RNA/DNA	1.06 ± 0.05	1.01 ± 0.07	1.13 ± 0.08
	Radioactivity	125 ± 18	214 ± 27	125 ± 14
	Sp. Activity	50.4 ± 6.2	75.8 ± 8.4	48.3 ± 5.8

Experiment 4, chapter VI (Means ± S.E.M.)

		48 hours	48 hours	48 hours
		Sham operation	**Transection + exclusion**	**Resection + exclusion**
	N	13	13	13
DUODENUM	RNA	3.45 ± 0.17	3.77 ± 0.15	3.96 ± 0.17
	DNA	3.00 ± 0.15	3.24 ± 0.10	3.44 ± 0.12
	RNA/DNA	1.16 ± 0.05	1.17 ± 0.03	1.15 ± 0.03
	Radioactivity	235 ± 10	262 ± 15	366 ± 28
	DNA-SA	79.7 ± 3.6	81.8 ± 5.4	106.1 ± 5.9
UPPER ILEUM (excluded)	RNA	2.95 ± 0.14	3.13 ± 0.20	3.48 ± 0.12
	DNA	2.62 ± 0.11	2.91 ± 0.17	3.16 ± 0.14
	RNA/DNA	1.13 ± 0.04	1.08 ± 0.04	1.11 ± 0.04
	Radioactivity	309 ± 30	279 ± 21	380 ± 35
	DNA-SA	118.7 ± 10.5	97.3 ± 6.7	121.8 ± 11.5
LOWER ILEUM	RNA	2.82 ± 0.11	2.99 ± 0.15	3.81 ± 0.17
	DNA	2.85 ± 0.13	3.01 ± 0.21	3.61 ± 0.15
	RNA/DNA	1.00 ± 0.03	1.02 ± 0.05	1.06 ± 0.04
	Radioactivity	265 ± 15	290 ± 27	453 ± 35
	DNA-SA	93.9 ± 5.1	102.5 ± 12.6	125.8 ± 8.9