T H E H O R M O N A L M E C H A N I S M

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THE HORMONAL MECHANISM OF INTESTINAL ADAPTATION

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CHAPTER I

THE BACKGROUND

The gastrointestinal tract has a large functional reserve. This is particularly true of the small intestine, and early studies by Flint in 1912 (1), showed that dogs could withstand 50%-70% small intestinal resection, returning to normal health after an initial period of weight loss and malabsorption. No doubt, this reserve is in part due to the very high rate of epithelial proliferation in small bowel mucosa.

Intestinal adaptation is the result of morphological and functional changes, and while these parameters can be accurately appreciated, the mechanisms by which these changes take place, are still under active investigation.

This section summarises the changes, both structural and functional, in the adaptive process, and this is followed by a review of the background work done on the possible mechanism of adaptation. The normal anatomy of intestinal mucosa is however, considered first. Most of the work done to date in the field of intestinal adaptation, involves the small bowel, and this part of the gut will be discussed predominantly, but data available on colonic growth will be mentioned.

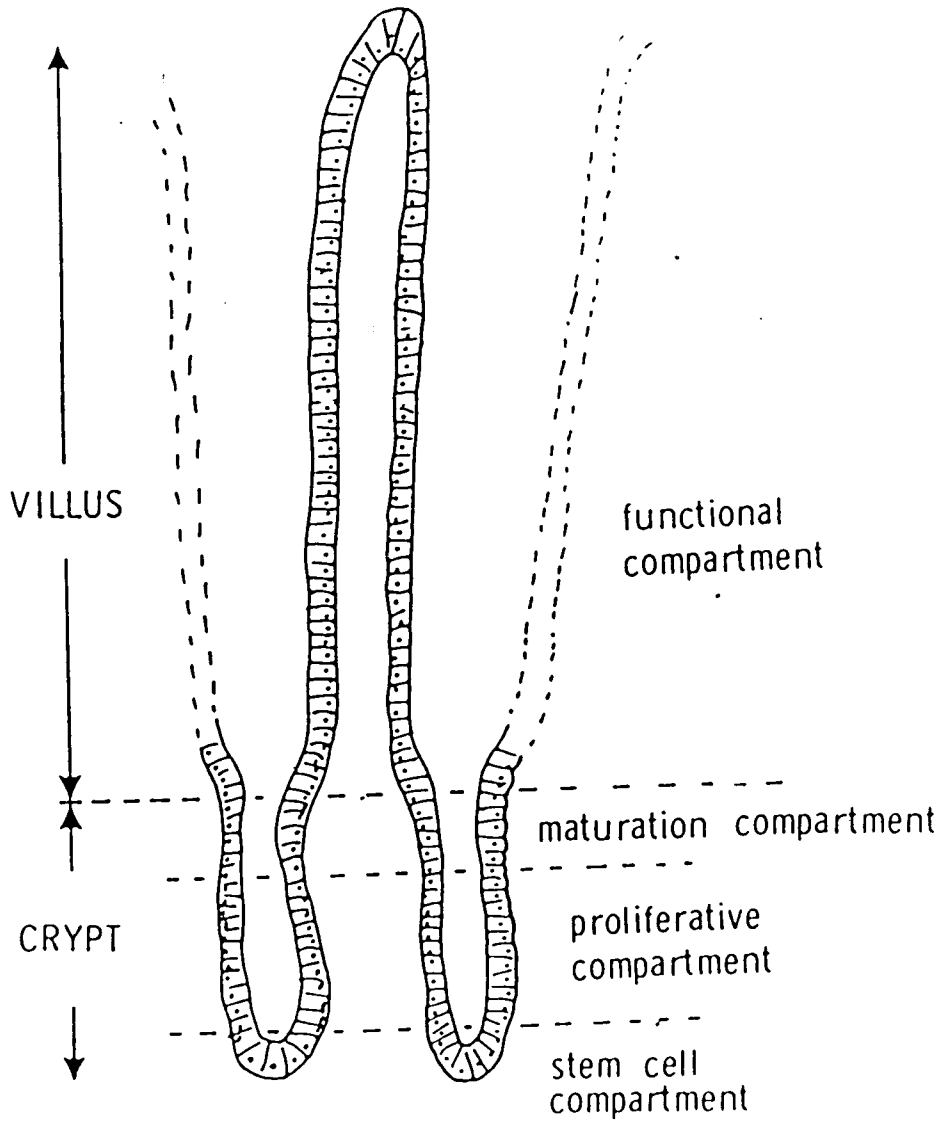


Fig 1.1: The compartments of the epithelium of intestinal mucosa.

CELL ORGANISATION AND RENEWAL IN INTESTINAL MUCOSA

The epithelial system is divided into functional and proliferative compartments. The functional compartment is the epithelium lining the villus, which is concerned with absorption, while the cells lining the crypts of Lieberkühn, form the proliferative compartment (2). Between these two areas, is the maturation compartment (Fig 1.1), where cells are losing their proliferative capacities and acquiring the characteristics of mature, functional cells. The boundaries of these compartments require kinetic measurements for their determination, as they are not structurally discrete. Two such measurements, the labelling index, using tritiated thymidine (^{3}HTdR) labelled cells, and mitotic index (metaphase arrest method), have been used for this purpose (3,4).

Cell division takes place in the base of the crypts of Lieberkühn (5). The complete cell cycle lasts for 24 hours in man (6) and 10 to 17 hours in rodents (5), and consists of the M, S and G phases. The epithelium is completely replaced in 2 to 3 days in rodents (7,8) and 3 to 6 days in man (6). After about two divisions in the crypts, the cells migrate up to the villus (9,10,) and become differentiated into mature columnar cells containing enzymes necessary for absorption (9,11). The cells are then extruded from the villus tip and under normal conditions, this cell loss from the villus tip equals cell birth, in the base of the crypt. Because of the decreased villus height in the distal compared with the proximal small bowel, cell migration is completed more rapidly in the ileum than jejunum (12).

Besides the columnar cells (enterocytes), which are the most numerous, endocrine cells, mucous (Goblet) cells and Paneth cells are also found in intestinal mucosa, and all four cell types are thought to arise from undifferentiated columnar stem cells at the base of the crypt (13). Mucous and endocrine cells also migrate up the villus, the latter having a longer turnover time than columnar cells (14).

In general, cell turnover is similiar in the large bowel although more prolonged (four to eight days) than in small bowel (15), and as in small bowel, the columnar, mucous and endocrine cells of the colon, develop from stem cells at the base of the crypts (16).

THE PHENOMENA OF INTESTINAL ADAPTATION

Just as the remaining kidney undergoes 'compensatory hypertrophy' after contralateral nephrectomy (17) and the residual liver compensates after partial hepatectomy (18), so the small bowel undergoes similiar adaptive changes after partial resection. This has been appreciated for some time, early studies noting these changes in the residual intestine after resection (1). The adaptive process is composed of a structural and functional component, and these are in turn, the result of complex cellular changes. In addition, motility changes occur, and these probably effect the absorptive capacity of the residual bowel.

Structural changes

At a macroscopic level, the residual bowel after extensive enterectomy, shows compensatory dilatation. This was shown to be due to villus enlargement, and was initially termed 'villus hypertrophy' (1). However, it is now known that there is an increase in enterocyte number, not size, and this process is thus a true hyperplasia. In man, 22% increase in the number of epithelial cells per unit length of mid-villus has been demonstrated after massive distal small bowel resection (19). This has been shown in dogs, where villus enlargement is due to hyperplasia after resection, manifest by an increase of cells/10 microscopic fields, these changes being more pronounced in ileum than jejunum (20). Other animal studies (21,22) have confirmed hyperplasia rather than hypertrophy as the cause for the villus enlargement

after intestinal resection, and this has been shown biochemically in bypassed rat intestine (23) where there is an increase in DNA/cm in the bowel in continuity, and after resection where there is an unaltered RNA/DNA ratio in the residual bowel (24), both indicating hyperplasia.

Hyperplasia, the cause of the increase in villus height, is itself a result of an increase in cell turnover. The term 'cell turnover', embraces all aspects of cell renewal, including replication in the crypts, migration up the villus, and extrusion at the tip of the villus. Thus 'turnover time' is the time taken to replace a number of epithelial cells, equal to that in the entire population (7,8). It has been shown by autogradiography, 2 months after small bowel resection in the rat, that there is an increased migration rate, a shorter crypt transit time, and a shorter cell turnover time in the residual intestine, and that this increase in the rate of cell renewal amounted to 141% in the ileum, 114% in the jejunum and 23% in the duodenum compared with control segments (25). The cell migration rate increased to 13 μm/hour in this study (normal = 9 μm/hour) and this has also been shown after intestinal bypass in rats (26). In the latter study, although an increase in migration rate of cells up the villus was shown, no change in cell turnover could be demonstrated. This would suggest that in the initial period of adaptation, although there is an increase in cell turnover, once an enlarged villus has been produced, a new steady state is achieved in which the duration of cell turnover reverts to its previous level, and villus height is maintained by accelerated cell migration (27). An increased rate of cell

division and a shorter cell cycle time has been demonstrated by autoradiography after intraperitoneal ^{3}HTdR, in residual bowel after 10% ileal resection in rats (28). The shorter cell cycle is brought about predominantly by a reduction in the S phase of the cycle (21).

Thus, as the migrating epithelial cells govern the size and shape of the villus (29), the end result of the observed changes in cell turnover in the residual intestine after intestinal resection, is villus enlargement (1,21,22,25,27,28,29). This is also true of the bowel in continuity after intestinal bypass (26,27,30,31), and by the same token, a decrease in villus height has been demonstrated in the bypassed segment of bowel (26).

Functional changes

The adaptation of the intestine in terms of the functional capacity of the absorptive epithelium, has been investigated measuring both mucosal enzymes and actual absorptive function.

While the lipid esterifying enzymes, fatty acid CoA lipase and acyl-CoA monoglyceride acyl transferase, in residual ileal mucosa after jejunectomy in rats is markedly increased per unit length of intestine (32), enzyme specific activity (enzyme levels per mg. of protein or per mg. of DNA) such as disaccheridase-specific activity, is unchanged, or reduced, as occurs with lactase activity (33). Similiarly, isolated epithelial cells from animals having undergone resection, showed an actual reduction in specific activity of disaccharidases (34). These findings suggest

immaturity of the cells within the hyperplastic mucosa, which is to be expected, due to the more rapid cell migration and turnover produced by partial resection. Similar findings have been found in enteric bypass in the rat (23) where enzymic activity per unit length of bowel is increased in the functional segment and reduced in the bypassed segment, while enzyme specific activity was diminished in the gut in-continuity and increased in the bypassed segment.

Studies on absorption in intestinal adaptation, follow a similiar pattern to mucosal enzyme measurements. Thus, while there is an increase in glucose, water and electrolyte absorption per unit length of intestine after resection in man (35) and the rat (36), per unit cell, the uptake of monosaccharides and amino acids is unchanged (37) or diminished (34). Similiarly, B12 absorption is increased in the guinea pig after jejunal resection, but when this is measured per unit cell, a decreased absorption is demonstrated (38). Similiar changes have been shown for bile acid absorption in the rat (39).

Thus, because of the more rapid cell migration and turnover in intestinal mucosa after resection or bypass, the villus is populated by functionally immature cells, and function per unit cell is consequently reduced. However, because of the hyperplasia which results, the net absorption per unit length is increased in the adapted bowel.

Changes in bowel motility

The increase in absorption per unit length of intestine during adaptation

is brought about by mucosal hyperplasia. However, an alternative mechanism of compensation might be a change of motility of the bowel, allowing a longer period of contact between the food and mucosa, thus enhancing absorption.

Using radioactive chromate as a marker, intestinal transit was measured in rats after 50% and 75% proximal and distal small bowel resections (40). A decrease in intestinal motility was found, and this was more pronounced after proximal than distal resection. There was also a delay in gastric emptying. These changes were maximal in the second post-operative week, and thereafter returned to normal.

THE MECHANISMS OF INTESTINAL ADAPTATION

While the changes, both structural and functional, which comprise the adaptive response have been fully illucidated, the mechanism by which this adaptation is initiated and maintained, are still under active investigation. No one single putative mechanism can account for all the changes seen in adaptation, and it seems likely that the pathogenesis is multifactorial. To date, three major and some minor influences have been proposed, and it is likely that a degree of interdependence exists between these. The major influences are luminal nutrition, pancreatico-biliary secretions and humoral factors, while changes in mucosal blood flow, bacterial content and neural factors have been investigated to a lesser extent, and may be regarded as minor influences in the promotion of adaptation.

A) LUMINAL NUTRITION

Under normal circumstances most of the ingested nutrients are absorbed in the proximal small bowel, and the chyme reaching the ileum contains little if any nutrition. Evidence for luminal nutrition being important in influencing cell turnover, villus morphology and absorptive capacity of the small bowel, is derived from experimental studies in which segments of bowel are exposed to increased nutrition by proximal resection, transposition of segments, or hyperphagia, or deprived of it by bypass operations or by starvation or parenteral nutrition.

After proximal small bowel resection, marked hyperplasia and a more rapid cell turnover is seen in the residual small bowel (21,36,41, 42,43). Here the ileum receives a relatively greater nutrient load than normal, and increases its absorptive capacity to jejunal levels. That this is not due to a shortened bowel per se, and thus loss of absorptive surface area, is shown by ileojejunal transposition experiments (36,44). When the positions of the ileum and jejunum are interchanged, the ileum again receives a greater amount of luminal nutrition than usual, and develops hyperplastic changes. Nor are these adaptive changes due to work hypertrophy secondary to a relative increase in luminal bulk, for when the bulk of the diet in rats was increased by 80% with added kaolin, no change in small bowel structure was found (45). Further support for luminal nutrition as a promotor of the adaptive hyperplasia, is derived from the finding that the intensity of the adaptive response is directly proportional to the length of intestine resected (42,43) and that proximal resection causes more pronounced adaptive changes than an equivalent distal resection (43). Furthermore, the amount of nutritional intake influences cell turnover, as hyperphagia produced by a number of experimental models (46,47,48, 49,50,51,52) results in villus hyperplasia, increased mucosal thickness and enhanced absorptive capacity.

Thus it seems that luminal nutrition in the above models, stimulates adaptive hyperplasia, by coming into contact with mucosa that normally is unaccustomed to chyme containing such high levels of nutrients.

Similar changes occur in the bowel in continuity after proximal small bowel bypass procedures (23,26), for the same reasons. Further support for luminal nutrition as a promotor of the adaptive response, is derived from experiments depriving the bowel of nutrients.

During starvation (53,54), mucosal hypoplastic changes occur, and these are reversed by refeeding (54). However, this model produces malnutrition and it is difficult to know whether this or the lack of luminal nutrition is responsible for the mucosal changes. It is thus important to maintain nutrition of the experimental animal, and this can be done by employing Thiry-Vella bypass loops of the jejunum (55,56,57) or self emptying blind loops of jejunum (26,58). In both these models, hypoplastic changes occur in the bowel excluded from the nutrient stream. In the ileo-jejunal transposition experiments (36,44), the ileum receives more luminal nutrition and develops hyperplastic changes, but the jejunum, now lying downstream and receiving chyme with less nutrition after ileal (proximal) absorption, undergoes hypoplasia. Total parenteral nutrition (TPN) provides another model for studying intestinal changes due to deprivation of luminal nutrients, without producing malnutrition. Parenterally fed rats with intact intestines (59,60) show a decrease in small bowel weight and loss of mucosal DNA, despite an increase in body weight. Similiar changes are found in the dog with an intact bowel (61) and after 50% proximal small bowel resection (62) where, in orally fed animals, the expected ileal adaptive hyperplasia was found, while in parenterally fed dogs, no such changes occured.

Additional evidence for luminal nutrition as a promotor of adaptation, is derived from experiments where direct stimulation by luminal nutrients occurs, of intestinal mucosa previously excluded from the nutrient stream. Thus refeeding after starvation (54) and restoration of intestinal continuity after intestinal bypass (58,63) completely reverses the hypoplastic changes caused by luminal nutrient deprivation. Direct perfusion of isolated Thiry-Vella fistulae in dogs (64) with saline prevented villus hypoplasia, while instillation of the elemental diet 'Vivonex', not only prevented the hypoplasia, but induced hyperplasia. Similarly, infusion of glucose and amino acids with blind loops (65,66) prevents the hypoplasia and hypofunction caused by the bypass. Fat seems to be more potent stimulant than carbohydrates and protein as demonstrated by intragastric infusion of these nutrients in jejunectomized rats (67).

There is therefore, much evidence to suggest that luminal nutrition plays a part in maintaining small intestinal mucosal structure and function, and also stimulates the adaptive response after resection or bypass procedures. The mechanism of action of luminal nutrition in this respect is at present hypothetical. A number of possibilities exist. These include direct action on the mucosa, stimulation of other enterotrophic factors such as pancreatico-biliary secretions or other regulators of tissue growth such as ornithine decarboxylase/diamine oxidase system (68). Neurovascular changes and alterations of luminal bacteria may occur secondary to alterations in luminal nutrition, and these in turn may have an affect on growth. Finally changes in gut

regulatory peptides are known to take place after small intestinal resection (69) and bypass (70), and it seems likely that some of these peptides may promote intestinal growth (see below). They are secreted by the APUD cells found in the intestinal mucosa. Some of these cells are of the 'open' variety, with microvilli connecting with the gut lumen and these microvilli act as chemoreceptors to luminal stimuli (71). It may thus be, that luminal nutrients may, in part, act by stimulating trophic hormones in the intestinal mucosa.

B) PANCREATICO-BILIARY SECRETIONS (PBS)

The various models discussed above viz: proximal small bowel resection, jejuno-ileal bypass, and ileojejunal transposition, not only expose the ileum to a richer nutrient stream, but also bring the ileum closer to higher concentrations of upper alimentary secretions, in particular bile and pancreatic juice (PBS). The oral intake of food stimulates the flow of bile and pancreatic juice. Thus in starvation and total parenteral nutrition, which causes pancreatic atrophy in rats (59) there are decreased amounts of these secretions. It has therefore been proposed that PBS may account for the intestinal hyper and hypoplasia seen in these models, and furthermore, that PBS are responsible for the fact that the villi in the duodenum are twice the size of the villi in the ileum, the so called 'villus size gradient' (44,72). Diverting the duodenal ampullary region into self-emptying loops of ileum (44,72) or into the middle part of the small bowel (73), produces distal hyperplasia, and furthermore, when the duodenal ampullary region is implanted into the mid-point of the intestinal remnant after jejunectomy (73,74), the ileal hyperplasia seen after proximal small

bowel resection, is significantly increased. Although differential implantation of biliary and pancreatic ducts suggests that pancreatic juice has the greater 'trophic' effect of the two (72), the maximal changes are found following exposure to combined PBS (73). In addition to the enhanced adaptive response that duodenal ampullary diversion has on jejunectomised rats (73,74) similar increased adaptive responses are found when this procedure is performed in cold-acclimated rats (75).

To differentiate between the effects of luminal nutrition and pancreatic juice, or lack of these, on the small bowel mucosa, Hughes et al (61), administered iv CCK and secretin together, to a group of dogs on TPN, and compared this with a second group of dogs on TPN alone. The CCK and secretin, which stimulate the pancreas and bile flow, prevented the villus hypoplasia seen in the TPN group without added hormone administration, and similar studies in rats (76) confirm the above findings. This supported the hypothesis that PBS are trophic to the intestine. However, an alternative explanation is that CCK and secretin may have a direct trophic effect on the gut. This was studied further (77,78) by administraton of CCK-octapeptide (CCK-OP) alone, and of secretin alone in orally and parenterally fed rats, and no direct tropic effect on the gut by these hormones was found although CCK-OP clearly was trophic to the pancreas. The discrepancy between the dog and rat experiments could be related to species specificity, differences in method of administration (bolus in the dog experiments and continuous infusion in the rats), differences in CCK preparations, or possibly the fact that the hormones have to be given together to exert their enterotrophic effect. Indeed a potentiating effect of CCK and secretin on pancreatic bicarbonate and water secretion is established (79).

On balance, neither CCK nor secretin alone seem to have any major direct effect on intestinal adaptation.

Further support for PBS having a trophic effect on small intestine, is derived from experiments where pooled, pre-harvested pancreatic juice was perfused into isolated intestinal loops, producing a modest degree of ileal adaptation (72,80).

Recent studies, however, using pancreatico-biliary diversion (PBD), have produced results contrary to those expected, assuming that PBS are trophic to the intestine. In this model (81), the proximal 50 cm of small bowel is transposed to lie between the stomach and duodenum, thus depriving the jejunum of PBS. Rats with PBD were nourished either orally or with TPN, and results in each of these groups, were compared with control animals having intestinal transection only either nourished orally or on TPN. Assuming that PBS are trophic to the small intestine and are responsible for the proximal to distal gradient in villus size (72) one would expect to find hypoplastic changes in the jejunum, now deprived of PBS after PBD. However, exactly the reverse occurred. While the ileum, now lying immediately downstream from the duodenal ampullary region after PBD, showed modest but significant increases in structure and segmental function, the jejunum, deprived of PBS after PBD, surprisingly showed hyperplasia with increase in villus height, mucosal mass and absorptive capacity. These experimental results do not support the hypothesis of PBS being trophic to the intestine, and indeed, the authors have suggested that they may inhibit intestinal mucosal growth,

the results seen in PBD being due to removal of this inhibitory effect, although this would not explain the modest hyperplastic changes seen in the ileum. Other explanations are that the changes in the jejunum after PBD may be due to other secretions such as gastric juice or epidermal growth factor (82,83) neurovascular changes or possibly humoral factors (see below). Indeed, plasma levels of enteroglucagon after PBD, are raised (84) and this peptide may in part, be responsible for the changes seen in PBD (see below).

C) HUMORAL/HORMONAL FACTORS

Luminal nutrition and PBS, cannot account for all the changes taking place in intestinal adaptation, and it is partly for this reason, that humoral or hormonal factors have been proposed as one of the putative mechanisms in the adaptation process. This partly negative circumstantial evidence is reinforced by more positive data, and in our present state of knowledge, it seems highly likely that humoral factors are important as a mechanism in intestinal adaptation.

The circumstantial evidence, which does not negate the luminal nutrition and PBS hypothesis, is derived from the following:-

i) Adaptive hyperplasia occurs rapidly (24 to 48 hrs) and extensively after resection and transection of small bowel (85) before the animals are eating normally, and this 'anticipatory' adaptation, suggests that factors other than luminal nutrition, possibly humoral, may be operative.

ii) Jejunal transection and re-anastamosis, causes transient hyperplasia

maximally around the anastomosis, but also extending to ileal mucosa (43), and this cannot be explained on the basis of luminal nutrition, or PBS.

iii) After total colectomy in man (86) and the rat (87), the ileum undergoes adaptive changes, despite the fact that there is no change in nutrition or PBS reaching this part of the intestine. Similarly, after ileal resection, jejunal adaptation takes place (36,42). In both these models, factors other than luminal nutrition and PBS must be operative.

iv) The fact that the adaptive changes after resection are diffuse, involving the bowel above and below the anastomosis (28,43), the muscle layer as well as the mucosa (43), and that gastro-intestinal motility and transit are altered (40), points to a systemic factor involved in this process.

v) After creation of defunctioned segments of ileum the normal hypoplastic changes in these isolated segments of bowel are reduced, with the production of biochemical and morphologic evidence of mucosal hyperplasia, by partial resection of the intestine in continuity, suggesting that both luminal and systemic factors account for these findings, with luminal nutrition perhaps being responsible for releasing an enterotropic factor from the bowel in continuity, which in turn, passes via the intact circulation to the isolated bowel, to bring about the limited hyperplasia seen in these loops devoid of luminal nutrition and PBS. In support of this, is the fact that atrophic changes develop in

isolated loops of bowel when food given by mouth is withdrawn (91). Similar findings in bypassed Thiry-Vella jejunal loops in lactating rats occur (92), where there are adaptive changes in the isolated loops, and it seems that an endocrine influence is operative in this model.

vi) As mentioned before, the finding of jejunal hyperplasia after pancreatico-biliary division (PBD) (81), certainly does not support the hypothesis of PBS as a trophic factor, and as similiar results were obtained after both oral and parenteral nutrition, luminal nutrition cannot be held responsible for these changes. Other intraluminal factors, such as epidermal growth factor (82,83) may be important in this model, but humoral factors must be considered, and the finding of raised circulating enteroglucagon levels in PBD (84), supports this assumption.

More direct evidence for the release of an enterotrophic hormone following intestinal surgery, is found in a number of cross-circulation experiments. Williamson et al (93) linked pairs of rats in free-running vascular parabiosis, with cannulae connecting carotid artery to jugular vein and vice versa. The dominant rat had either no operation, jejunal transection or jejunal resection, while the responding partner had no abdominal operation. Although the intact parabionts did not show an increase of RNA and DNA, as did the transected and resected partners, there was marked and similiar elevations (>60%) in total and specific radioactivity in both the dominant and responding members of transection and resection pairs, implicating a transmissible factor in the adaptive response, although the above findings suggest that the transmitted

response was weaker than the direct response, suggesting that both systemic and local factors appear to be involved. Two similiar studies using cutaneous parabiosis (94,95), support the above data, although this is not a uniform finding (96), and it may be that intermingling of blood in the latter model could have been inadequate to detect an enterotropic factor.

It seems likely that a systemic enterotropic factor or factors, contribute to the initiation and/or maintenance of the adaptive changes which occur in the shortened bowel. Furthermore, it seems likely that the other major factors, luminal nutrition and PBS, probably act in concert with hormonal factors, and that much of the data quoted above, suggsts a unifying concept for the several mechanisms that govern intestinal adaptation. The APUD cells in the intestinal mucosa which secrete the gut hormones, some of which have been proposed as candidates for an enterotropic hormone, may be of the 'open' variety (71) and are stimulated by luminal contents via their microvilli, which act as chemoreceptors. It seems likely, that luminal nutrition and secretions trigger the release of these enterotropic hormones by this mechanism. Thus, total parenteral nutrition prevents the development of post-resectional hyperplasia, (59,60,61,62) not only by loss of nutrition within the lumen, but probably also by reducing PBS and APUD cell stimulation and thus hormonal release. Similiar changes occur in starvation (53,54), probably for the same reasons. Indeed a positive relationship between the amount of food ingested, the degree of ileal

TABLE 1.1

CANDIDATES FOR THE ROLE OF 'ENTEROTROPHIN'

1. Gastrin.
2. Enteroglucagon.
3. Secretin.
4. Cholecystokinin (CCK).
5. Corticosteroids.
6. Prolactin and placental lactogen.
7. Epidermal growth factor (EGF) and ornithine decarboxylase.
8. Other hormones (pituitary hormones, testosterone, thyroxin, VIP).

hyperplasia, and the plasma level of one of the candidate hormones, enteroglucagon, after small bowel transection and resection has been demonstrated (97). While Thiry-Vella loops of bowel undergo limited hyperplasia following jejunectomy (89), when food is withheld by mouth, further atrophic changes develop in these isolated loops of bowel (91, 98). Similarly, in parabiotic rats (99), the hyperplastic changes in the unoperated parabiont, are prevented by starvation.

Thus it seems likely, that enterotropic factors influence cell turnover in the bowel, and these hormones are in turn, affected by changes in luminal nutrition and secretions. These hormones may act systemically or locally, in a paracrine fashion. The fact that the hyperplasia after resection is more intense in the bowel in continuity than in bypassed segments (89) suggest that the latter mode of action is likely.

A number of possible candidate humoral agents have been investigated. These are listed in Table 1:1.

1) Gastrin

Raised serum gastrin levels have been observed following intestinal resection in man (100), dog (101) and rat (102), and Johnson has claimed that this hormone may be important in intestinal mucosal growth (103). While the trophic effect of gastrin on parts of the gastrointestinal tract seems well established, its relationship to small intestinal mucosal growth, is controversial. Small bowel mucosal hyperplasia has not been recorded in other conditions where

hypergastrinaemia occurs, such as pernicious anaemia, the Zollinger-Ellison syndrome, and antral G-cell hyperplasia.

Studies, administering pentagastrin and observing its effect on small intestinal proliferation have produced conflicting results. While Pansu et al (104) showed that a single injection of pentagastrin caused proliferative activity of small intestinal mucosa to stay at nocturnal peaks for up to 24 hours, Mayston et al (105) found that pentagastrin injection into rats for 15 days, increased the weight and villus height only of the proximal duodenum, but not of the distal duodenum and jejunum. Furthermore, while atrophy of the small intestine during parenteral feeding could be prevented by pentagastrin infusion (106), this could not be confirmed when rats, having had 50% small intestinal resection, were fed parenterally, with or without pentagastrin infusion (107). Pentagastrin in this study (107) was able to cause a significant increase in mucosal weight, protein and DNA content, only in the proximal part of the small intestine. Additional evidence against gastrin as a trophic hormone to the small intestine, is derived from the fact that antrectomy has no effect on the growth of the middle region of the small bowel, nor on its adaptive response to partial intestinal resection (108). Furthermore, no correlation could be found between crypt-cell production rate in the terminal ileum and circulating gastrin levels, in a number of different models of intestinal adaptation (109). The effect of luminal gastrin has also been investigated (110). While gastrin infused into the ileum has trophic effects, no such changes on the ileum could be demonstrated when gastrin is infused into the stomach, its normal site of release.

Gastrin does appear to have a regulating effect on growth of the stomach, duodenum, colon and pancreas (103,111,112,113). The effect of gastrin on the stomach appears to be confined to the oxyntic gland area, and does not effect the gastric antrum (106). Thus, pentagastrin administered to rats undergdng parenteral nutrition, prevented the decrease in weight of the oxyntic gland area, but had not affect on the antrum. Similiarly, admimistration of tetragastrin to starving rats, prevented the loss of DNA from the oxyntic gland area (114). This trophic effect of gastrin on the oxyntic area of the stomach, has been shown to be independent of acid secretion (115), but can be blocked by simultaneous administration of secretion (115,116).

Gastrin has also been shown to have a trophic effect on fundic but not antral mucosa in the dog (117) and man (118). Furthermore, marked hyperplasia of gastric mucosa is found in cases of the Zollinger-Ellison syndrome (119).

Gastrin also has a trophic effect on duodenal mucosa. Thus, pentagastrin given for 15 days, increased the duodenal weight in rats (120) and also has an effect on the doubling time of duodenal mucosal cells in vitro (121). Gastrin is also thought to have a trophic effect on colonic mucosa. Thus, while rats having either resection of 50 cms of distal small bowel alone, or resection plus antrectomy, showed no difference in DNA content of the proximal and distal ileum between the two groups, there was a significant fall in DNA content in the colon, in the resection plus antrectomy group, compared to rats having resection

alone (110). This is supported by infusion studies of pentagastrin (111).

Thus, while it seems likely that gastrin has an established role as a trophic hormone to the mucosa of the oxyntic gland region of the stomach, duodenum, colon, and the exocrine pancreas (122), its possible trophic role on the small bowel, remains unlikely.

2) Enteroglucagon:

This gastrointestinal peptide has been investigated as a possible enterotropic hormone, and numerous reports have shown an association between plasma levels of immunoreactive enteroglucagon and situations, both naturally-occurring and experimental, in which intestinal adaptation is known to occur. Indeed, so close is this correlation, that enteroglucagon has been proposed as the 'growth hormone to the small intestine' (123).

In 1971, Gleeson et al (124) reported a patient with an enteroglucagon producing renal tumour. The patient had gross small bowel dilatation, mucosal thickening, with marked villus enlargement, which was visible naked eye. There was also a markedly raised plasma enterglucagon level. In addition to the structural changes, there was also delayed gastrointestinal transit, as suggested by constipation and delayed transit on barium studies. After removal of the tumour, the plasma enteroglucagon levels returned to normal. There was a marked change in the clinical picture, with the patient's constipation disappearing almost immediately. Barium studies now showed that there was rapid intestinal transit and

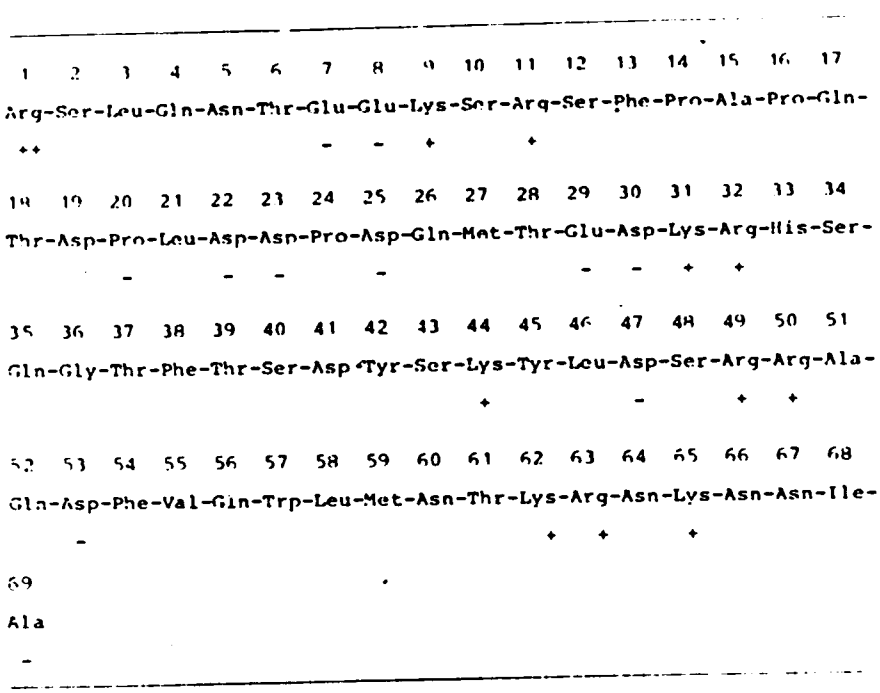


Fig 1.2: Porcine enteroglucagon (glicentin).

previous dilatation of the small bowel had returned to normal. Per-oral jejunal biopsy showed that the gross villus hyperplasia had reverted to normal. The immunoassay and bioassay characteristics of the tumour, showed that this was indeed producing enteroglucagon (125). Furthermore, when saline extracts of the tumour were injected intraperitoneally into mice, obvious enlargement of the small bowel occurred (126). The idea was thus born, that enteroglucagon may have been the trophic factor responsible.

In 1959, Unger et al, described a radioimmunoassay (RIA) for glucagon (127) and it soon became apparent that this RIA was not specific for pancreatic glucagon. The anti-glucagon sera available, was shown to cross react with extracts from the small intestine of many animals. However, when a more specific assay for pancreatic glucagon was devel-oped, this detected only the pancreatic but not the intestinal peptide. This demonstrated that the two peptides were not identical, and the name enteroglucagon was coined for the intestinal peptide. Although this name is still most frequently used, other terms such as gut glucagon cross-reacting material, gut glucagon, gut glucagon-like immunoreactivities, and glucagon-like immunoreactants (gut GLI), have also been proposed to describe this peptide. The complete amino acid sequence of porcine enteroglucagon (glicentin) has been published (128) (Fig. 1.2), and it is apparent that the reason for the cross reaction between the intestinal and pancreatic peptides, is that the sequence 33-61 of enteroglucagon is the complete sequence of pancreatic glucagon. Antisera directed towards the N-terminal portion of pancreatic glucagon

also detects enteroglucagon, while those directed towards the C terminal portion of the pancreatic glucagon molecule, do not, and it has been suggested that this can be explained by the tertiary folding of enteroglucagon which in some way covers the C terminal portion, but not the N terminal portion of the included pancreatic glucagon sequence (129). Furthermore, amino acids 62-69 in the enteroglucagon sequence, are virtually identical with the previous C terminal extension peptide, which was thought to be part of the sequence of pancreatic pro-glucagon, and it has been suggested that the gene coding for pancreatic glucagon and porcine glicentin, and thus the precursor peptide first synthesised, is identical, the two cell types differing only in their post-translational enzyme processing (71,130). Thus, enzymes in the pancreatic alpha cell, splits the parent molecule, to release the glucagon sequence. This hypothesis is supported by the facts that positions 31-32 and 62-63 of the enteroglucagon molecule, are double basic amino acids, which is a combination particularly favoured for attack by proteolytic enzymes of the trypsin class, and that the alpha cell of the pancreas, and enteroglucagon cell (EG cell) of the intestine, have very similar histological appearances (129). Furthermore, it has been reported that the core of the granule of the alpha cell contains pancreatic glucagon, while the less dense halo contains the N-terminal fragment of porcine glicentin (131,132). Additional morphologic support comes from studies in which sections of ileal mucosa were subjected to enzymatic digestion with trypsin and carboxypeptidase B, whereby the glicentin-storing EG-cells exposed the C-terminal glucagon immunodeterminant (133). Although chromatographic analysis shows enteroglucagon immunoreactivity to be present in several molecular-sized

peaks, it seems likely that this is a single hormone molecular family, and not several different cross-reacting hormonal systems, as was first suggested (71). There are, unfortunately considerable species differences, and antisera raised to porcine glicentin, does not react with human enteroglucagon. Furthermore, only minute amounts of porcine material have thus far been purified and although an inhibition of gastric acid secretion by this peptide has been demonstrated (134), studies related to its direct effect on enterocyte turnover are still awaited.

The distribution of the enteroglucagon (EG) cells in the bowel, and the factors controlling secretion of this hormone, are of importance in appreciating the changes in enteroglucagon seen in many pathological and experimental situations. The enteroglucagon (EG) producing cells have a distal distribution in the gut. The greatest concentrations are found in the distal ileum, with a lesser but still significant concentration found in the colon (135). These EG cells are long, flask-shaped cells, reaching from the basal membrane to the intestinal lumen, where microvilli protrude into the lumen (71,129,136), and it is thought that these microvilli act as chemoreceptors or cell 'sensors' to luminal stimuli. Indeed, the major stimuli for the production of enteroglucagon, are carbohydrates (eg glucose) and digested fat (eg long chain fatty acids) (137,138). In situations where luminal nutrients are not absorbed in the upper small bowel, either following surgery to this part of the gut, or in various malabsorption states, the enteroglucagon producing distal intestinal mucosa is exposed to abnormally rich chyme, which leads to an exaggerated secretion of enteroglucagon, and it has been postulated that

this peptide may be responsible, at least in part, for the adaptive hyperplasia and increased enterocyte turnover found in these situations (70,71). Although hypertonic glucose solutions provide a strong stimulus to enteroglucagon release, it seems to be the amount of glucose presented to the mucosa, rather than the tonicity which determines the strength of the stimulus (71). Indeed, this has been confirmed in experimental models of adaptation, where the greatest plasma levels of enteroglucagon and the greatest rates of crypt cell production, were found in hyperphagic animals, while the lowest hormone and cell production levels were found with parenteral nutrition (97). Furthermore, it has been shown that long chain triglycerides (LCT) given intragastrically, promote small bowel adaptation following resection, and in this respect, the quantity of LCT seems to be important (67). It has been speculated, that these effects may be due to the release of enteroglucagon (67). The EG cell is not responsive to hypoglycaemia (as is the pancreatic alpha cell (139)) and intravenous administration of nutrients, such as carbohydrates and lipids, is ineffective (137). Thus it seems that the luminal route of stimulation is the most important. However, bombesin and gastrin-releasing peptide (GRP) both stimulate enteroglucagon (140,141) while somatostatin inhibits luminally stimulated secretion (142). These findings suggest that local peptidergic control may play a part in enteroglucagon secretion.

Besides evidence derived from the case of the enteroglucagon producing tumour (124,125), enteroglucagon is found to be significantly elevated in the plasma, in many clinical situations where intestinal adaptation occurs. The underlying mechanism of this hypersecretion, appears to be

abnormal and excessive exposure of the distal intestinal mucosa (the predominant site of production of enteroglucagon) to unabsorbed nutrients. Thus in coeliac disease, which affects only the upper small intestine, enteroglucagon is found to be markedly elevated (143,144). This contrasts to normal enteroglucagon levels in patients with coeliac disease treated with a gluten-free diet (143). Enterocyte turnover is known to be increased in coeliac disease, but not in patients successfully treated with a gluten-free diet. Similiarly, enteroglucagon levels are raised in tropical malabsorption (145), and the degree of elevation of enteroglucagon correlates closely with breath hydrogen, which reflects the degree of functional malabsorption of carbohydrate in the small intestine, and its consequential metabolism by colonic bacteria. Enteroglucagon is also three-fold increased in acute infective diarrhoea (146). The same mechanism operates after various surgical procedures, giving rise to malabsorption in the upper small bowel. Elevated enteroglucagon levels, especially postprandially, are seen after total pancreatectomy (147). By the same token, patients with chronic pancreatitis and steattorrhoea, are found to have elevated enteroglucagon levels (148). After partial small bowel resection, which has the effect of exposing the distal intestinal mucosa to unabsorbed nutrients, enteroglucagon was approximately two-fold elevated in the basal state, and three-fold elevated after a meal (69). Jejunoileal bypass operations, done for morbid obesity, has the same effect on the distal intestinal mucosa, and despite significant weight loss, enteroglucagon levels are greatly increased (70,149). After both small intestinal resections and bypass procedures, marked adaptive changes occur in the residual small bowel in continuity. Interestingly, after

colonic resections, a fall in enteroglucagon occurs (69). However, when it is appreciated that although the colon has approximately an eighth of the enteroglucagon concentration compared to the ileum, because of the greater weight of this region, the total amount of enteroglucagon is considerably larger than the ileum (136), and the finding of reduced enteroglucagon levels after colonic resection, is thus not unexpected. After gastric resection, the dumping syndrome may occur. This is associated with rapid intestinal transit, and is accompanied by raised enteroglucagon levels (150,151,152,153). Indeed, a significant correlation between gastric emptying rate and enteroglucagon secretion has been found in duodenal ulcer subjects (154).

Thus in man, there is strong circumstantial evidence of an association of enteroglucagon and adaptive changes which occur in the situations discussed above. In addition to this, when healthy infants commence enteral feeding, there is a rise in most gastrointestinal hormones, including enteroglucagon (155), but no such rise is seen in infants who for various reasons, are kept on parenteral feeding (156). When infants commence oral feeding, there is rapid gut adaptation, and the organs increase in size significantly (136). Enteroglucagon, which shows the most dramatic change, may well be involved in this process.

As in man, there is also in the experimental animal, much circumstantial evidence to suggest that enteroglucagon may be one of the trophic humoral factors involved in the adaptation process. Thus, in one study, tissue levels and fasting plasma levels of enteroglucagon were measured in rats after small bowel resection, during lactation, and when hyper-

phagia was induced by hypothermia, all situations where intestinal adaptation is known to occur (157). After proximal two-thirds small bowel resection plasma and tissue enteroglucagon levels nearly doubled, during cold acclimation plasma levels tripled and tissue levels doubled, while on the 12th day of lactation, tissue enteroglucagon levels doubled, although plasma enteroglucagon did not change significantly. The number of enteroglucagon cells in this study were increased, while the cells also increased in size. The intensity of the adaptive hyperplastic response is known to be directly proportional to the length of small bowel resected (42,158), and the enteroglucagon concentrations have also been shown to be elevated in proportion to the amount of bowel resected (159). Changes in plasma enteroglucagon were also studied in rats undergoing 75% proximal small bowel resection, with the excluded bowel fashioned into a Thiry-Vella fistula (98). Half the animals were fed orally, while the remainder were nourished isocalorically by total parenteral nutrition. Plasma enteroglucagon was found to be much greater in oral than IV fed animals, while cell turnover in the terminal ileum in continuity, as measured by the crypt cell production rate (CCPR) was greater in the oral compared with IV fed rats. The CCPR in the excluded Thiry-Vella fistula, although less than the CCPR in the bowel in continuity, was significantly greater in the orally fed compared with the IV fed group. This study, therefore, suggested that there is indeed a humoral agent affecting the CCPR, and that enteroglucagon may well be a candidate for this role. Gastrin was also measured in this study, but the changes in this peptide did not correlate with the adaptive changes. Besides the humoral mechanism, this study also underlined the importance of the nutrient stream, and it is likely that food in

the lumen, was responsible for the changes in enteroglucagon in the orally fed group, which were much reduced in the IV fed group. Luminal nutrition, in particular carbohydrates and long chain fatty acids, are known to be the major stimuli of the EG cells, and thus of enteroglucagon release (137,138). There appears to be a relationship between the amount of ingested nutrients, the CCPR and plasma enteroglucagon. Forty-eight rats were studied after either 75% proximal small bowel resection or jejunal transection (as controls) and the animals were divided into 3 groups, the first of which was allowed food ad libitum, the second was kept hypothermic (resulting in hyperphagia) while the third group was nourished intravenously (97). The greatest plasma enteroglucagon and CCPR changes were found in the hyperphagic animals, while the lowest levels of these two parameters, were found in the TPN group. Gastrin was also measured in this study, and showed no significant changes.
Long chain triglycerides, administered intragastrically, promotes adaptive hyperplasia after small bowel resection, and the extent of this adaptation is proportional to the amount of fat given (67). It has been suggested that this effect may be mediated via enteroglucagon release, although this peptide was not measured in the study. Bombesin is known to stimulate (140,141) and somatostatin to inhibit (142) enteroglucagon release. These two peptides were administered over a 7 day period to rats having small bowel resection and transection (160), and plasma enteroglucagon and CCPR were measured. After administration of somatostatin, plasma enteroglucagon and CCPR fell in parallel, while bombesin had the effect of increasing these parameters after transection, although there was little effect in resected animal, where maximal adaptation had already taken

place. This further demonstrates that regulatory peptides can influence adaptation, but it is as yet not clear whether the effect of somatostatin and bombesin is a direct one, or whether the changes in cell proliferation occurred indirectly, via changes in enteroglucagon Studies where the duodenal ampullary region is implanted into the mid small bowel, or into the intestinal remnant after jejunectomy, produce intestinal hyperplasia distal to the site of implantation, and intensifies the hyperplasia seen after resection (73,74). These studies, and others (44,72), have promoted the suggestion that pancreatico-biliary secretions (PBS) may have a trophic influence on small bowel mucosa. Plasma enteroglucagon and CCPR were measured in rats having intestinal resection or transresection, half of these animals having their ampullary region re-implanted into the mid colon. While rats not having PBS diverted to the colon, had significantly greater hyperplastic changes in the ileum compared with animals having a diversion, plasma enteroglucagon levels were found to change in parallel to those of the CCPR (161). Likewise, after pancreatico-biliary diversion (PBD) in the rat (84), plasma enteroglucagon levels are high eight days after the procedure, and mucosal hyperplasia has become established. After three months, although intestinal mucosal hyperlasia was unchanged, plasma enteroglucagon levels had returned to normal levels, and it has been suggested that in this model, enteroglucagon may act as a 'trigger' mechanism, but may not necessarily be involved in maintaining the adaptive hyperplasia. Using various models of intestinal adaptation, a strong correlation was found between mucosal hyperplasia, as indicated by the crypt cell production rate, and plasma enteroglucagon levels (109). Studies on the enteroglucagon (EG)

cell, have shown marked changes in response to an increased demand for hormone synthesis and release (162). Ultrastructural studies demonstrate reduced hormone storage in the EG cells with a reduction in the number of secretory granules and immunocytochemistry shows an apparent decrease in enteroglucagon cell content in the mucosa of adapted animals after resection. These changes, coupled with high plasma enteroglucagon levels observed in these models, would indicate a functional hyperactivity of the enteroglucagon cells. Using a microdissection technique and immunostaining, EG cells were found to be more numerous in the crypt region after 80% proximal small bowel resection, compared with control animals (136).

Thus, there is strong circumstantial evidence, both in man and in the experimental situation, that enteroglucagon may be trophic to the intestinal mucosa. To date, a definite cause-and-effect relationship between enteroglucagon and intestinal mucosal growth has not been shown, although enteroglucagon remains a strong candidate for such a role. This has been re-inforced recently, by the finding that low dose, partially purified rat enteroglucagon produced a 50% increase in DNA synthesis in cultural guinea pig jejunal mucosal cells (163).

3) Secretin:

Secretin inhibits gastrin release, and may therefore be expected to have an antitrophic effect on parts of the stomach and duodenum. Indeed, the 90% increase in maximal acid output and 70% increase in the parietal cell population obtained when rats were injected daily with pentagastrin, was abolished when pentagastrin and secretin were injected together, and animals receiving only secretin, had slightly lower secretory capacities

and parietal cell counts, than saline injected controls (116). This effect of secretin is unlikely to be due to its acid inhibiting effect, as metiamide, a potent inhibitor of gastrin stimulated acid secretion has no effect on the trophic influence of pentagastrin on the stomach and duodenum (164), and is more likely to be due to inhibition of trophism by suppression of endogenous gastrin (165). Secretin is thus unlikely to have a direct antitrophic effect on the stomach and upper duodenum (166).

Conflicting evidence exists regarding the effect of secretin on the small intestine. Pharmacological doses of secretin administered to rats, increased the weight and DNA, sucrase and maltase content of the small intestine (167) suggesting that this peptide may have a trophic effect on this organ. Indeed, when mucosal structure and function was investigated in dogs, who had either total parenteral nutrition (TPN) alone, or TPN plus daily administration of CCK and secretin together, the CCK and secretin was found to prevent the villus hypoplasia of the group having TPN alone (61). The results of this study suggest that secretin and CCK produced their effect either directly on the gut, or via the stimulation of pancreatico-biliary secretions (PBS) which are thought to have a trophic influence. When, however, low and high doses of CCK and secretin were administered separately to orally and parenterally fed rats, no trophic effect on the gut was found (77,78).

Thus it seems that secretin probably does not have a major direct trophic effect on the small bowel.

4) Cholecystokinin (CCK):

CCK is structurally and functionally related to gastrin, with the active C-terminal tetrapeptide amide of gastrin, duplicated in CCK. The major structural difference is the position of the tyrosyl residue and whether or not it is sulphated. These differences appear to determine that gastrin has affinity for receptors stimulating acid secretion, while CCK has affinity for receptors lower down, ie the gall bladder, pancreas and possibly the small bowel (165). CCK has been shown to regulate pancreatic growth, as indicated by an increase in pancreatic weight (168), pancreatic DNA content (169), and an increase in pancreatic RNA, protein, DNA content with the stimulation of (14C) thymidine incorporation into DNA (170). These results have been interpreted as indicating that CCK is a trophic hormone to the pancreas.

Regarding the intestine, CCK has been shown to cause an increase in duodenal but not gastric mucosal DNA synthesis (171). As mentioned above, CCK and secretin administered together, prevented the small bowel villus hypoplasia of TPN in dogs (61). When, however, secretin and CCK were administered separately to orally and parenterally fed rats, no trophic effect of these hormones could be found (77,78).

Thus, although CCK is probably a physiologically important regulator of pancreatic growth, it is unlikely to have any major trophic influence on the gut.

5) Corticosteroids:

The effect of glucocorticoids on the intestinal mucosa seems to be two-fold, on the one hand stimulating enterocyte function, while on the other, reducing enterocyte production (172). Thus, prednisolone fed to rats for 7 days, had little effect on mucosal structure or cell kinetics, but enhanced the maximum absorptive capacities of the jejunum and ileum for galactose (173,174). This was due to an increase in carrier-mediated transport in the individual enterocytes and not to a change in cell population. Activities of brush border enzymes were elevated, and turnover studies indicated an increase in the rate of synthesis of brush border proteins, associated with an enhanced glycoprotein content of the microvillus membrane (175). These findings suggest a direct action of prednisolone on the enterocytes to increase their absorptive and digestive capacities by the induction of specific functional proteins. When, however, prednisolone and betamethasone-17-valerate, a locally active glucocorticoid, were given for 28 days (176) there was mucosal hypoplasia, with an inhibition of cell turnover, in the betamethasone-17-valerate group, but prednisolone had very little effect on jejunal mucosal structure. Both the steroids enhanced D-galactose absorption and the activities of brush border enzymes, although, as betamethasone-17-valerate caused a reduction in enterocyte population, there was no change in absorption per centimetre compared to controls. Thus it would seem that glucocorticoids have separate and opposing actions on intestinal mucosa, ie. stimulating enterocyte function, and reducing enterocyte turnover, and the predominant activity appears to be a function of each individual steroid.

In the adapted bowel after proximal intestinal resection, prednisolone is capable of enhancing further the hyperplasia of the residual bowel, although there is no increase in the degree of hyperplasia. Prednisolone increases the functional capacity by enhancing the enzyme activities of the residual enterocytes, resulting in a further increase in brush border enzyme activities per centimetre (177). Using mucosal explants, it has been shown in vitro, that dexamethasone induces sucrase and maltase enzyme activity (178).

Thus it may be that corticosteroids, secreted in response to major intestinal resections, could well play a part in promoting functional and structural adaptation in the residual intestine (179) and this is supported by the finding of mucosal atrophy found after adrenalectomy (180).

6) Lactation:

Small intestinal hyperplasia and hyperfunction occurs during lactation (47,48,92). While these changes may be related, in part, to the hyperphagia observed, these adaptive changes are not completely prevented by restricting food intake to normal levels. This points to the possibility of a humoral agent responsible for these changes, and indeed, by-passed Thiry-Vella jejunal loops fashioned in lactating rats, undergo comparable adaptive changes to those found in lactating animals with an intact intestine, despite being deprived of food and PBS, supporting this hypothesis (92). Prolactin seemed a strong candidate for such a role. However, when hyperprolactinaemia was induced experimentally in rats by perphenazine injections and pituitary transplantation (181), no changes of adaptation in small bowel were observed. It therefore seems unlikely

that prolactin can be held responsible for these changes in lactation. Furthermore, the duodenal mucosal proliferation seen in lactation, has been shown to be unrelated to changes in gastrin levels (182). While prolactin and gastrin have been excluded as trophic hormones to the small bowel during lactation, ileal tissue enteroglucagon levels have been shown to double in lactating rats, although plasma enteroglucagon levels in this study did not change (157). Thus, enteroglucagon remains a candidate for a trophic role on small bowel mucosa during lactation, although this requires further elucidation.

7) Epidermal Growth Factor (EGF), and Ornithine Decarboxylase:

EGF is a 53 amino acid polypeptide and has been shown to have biological activity on cell proliferation in the intestinal tract. It was isolated from mouse submandibular glands, and resembles urogastrone (183). It has also been shown to stimulate ornithine decarboxylase in the digestive tract (184). Ornithine is the starting substrate for the biosynthesis of the polyamines, putrescine, spermidine and spermine (68), and these polyamines in turn, have been shown in vitro, to facilitate nearly all aspects of DNA, RNA and protein synthesis (185,186).

It has been shown that specific receptors for EGF exist in isolated rat intestinal epithelial cells (187). EGF, given intraperitoneally or intraluminally, stimulates gastroduodenal mucosal DNA synthesis in the rat (82), DNA synthesis in mouse duodenal and jejunal mucosa (188) and duodenal, ileal and colonic crypt cell proliferation in rats and mice (189). Stimulation of salivary secretion by isoproterenol, produces an increase in nucleic-acid content of mucosal cells and villus height in mouse small

intestine (83), while anti-nerve growth factor (NGF), also contained in saliva, decreases villous epithelial cell renewal (190).

Increased intestinal mucosal ornithine decarboxylase activity and increased polyamine content of intestinal mucosa in rats, has been found in the period of intestinal maturation up to 40 days after birth, during mucosal recovery after injury with arabinosyl cytosine, after jejunectomy and during lactation (68), all recognised models of intestinal adaptation. The adaptive processes during mucosal maturation and mucosal recovery after injury, were markedly delayed by administration of the ornithine decarboxylase inhibitor, α-difluoromethyl ornithine (DFMO), as was the ornithine decarboxylase and polyamine content of the mucosa (68).

Thus, saliva, containing EGF, NGF or other trophic factors, may act via the intraluminal route directly, in a similiar fashion to luminal nutrition and PBS, or by stimulation of other known trophic agents, such as ornithine decarboxylase (184).

8) Other hormonal candidates:

After hypophysectomy, marked hypoplastic changes occur in the intestine, although the reduced food intake associated with this procedure, may account for these intestinal changes. When hypophysectomy is combined with jejunal or ileal resection, the degree of adaptation is less than in pair-fed controls (191), suggesting that pituitary hormones may have a trophic influence on small bowel mucosa. Testosterone (192), growth hormone and thyroxine (193) may all influence mucosal growth, although thyroxine appears to exert its effect by hyperphagia (194). Small doses

of histamine and seratonin stimulate intestinal cell proliferation, while large doses are inhibitory (195). Vasoactive intestinal polypeptide (VIP) inhibits the trophic effect of pentagastrin on the gastrin mucosa, while glucagon stimulates DNA synthesis in both oxyntic gland mucosa and colon (103). The influence of drugs effecting the autonomic nervous system, are discussed under mucosal blood flow and neural factors.

D) MUCOSAL BLOOD FLOW

After small bowel resection, there is an increase in ileal mucosal blood flow two days after the procedure, but this has returned to normal by two months (196). This initial increase in blood flow may be associated with adrenergic denervation of the residual small bowel (197). It is uncertain to what extent the altered blood flow contributes to the adaptive mechanism, but certainly manipulation of blood flow experimentally can effect cellular proliferation. Alpha-adrenergic stimulation is known to increase intestinal mitotic activity and epithelial cell migration rate, while beta-adrenergic stimuli have the opposite effect. Thus infusion of noradrenaline, which is a vasoconstrictor, produces increased cellular proliferation, while α-adrenergic blockade has the reverse effect (198). Inhibition of mitotic activity occurs with adrenaline, and this is reversed by β-adrenergic blockade (198). Deprivation of the blood supply to produce temporary intestinal ischaemia, results in hypoplasia, with subsequent hyperplasia during the recovery phase (199).

E) NEURAL FACTORS

Manipulation of the autonomic nerves to the gut, have been shown to have, an effect on mucosal proliferation, but whether this is a direct action or via changes in the blood flow or possibly by producing local hormonal changes, either related to paracrine secretions, circulating hormones, or possibly by its effect on neurotransmittors of the peptidergic system (135), is at present unclear. Thus, sympathectomy has an inhibitory effect on cell proliferation (198,200), and accentuates the mucosal hypoplasia in IV fed rats (201), although inhibition of proliferation was prevented by intragastric infusion of luminal nutrients. Electrical stimulation of mesenteric nerves results in an increase in the mitotic rate of intestinal crypts (202). The effect of changes of sympathetic tone on mucosal cell proliferation, produced either by chemical, surgical or immune means, is not surprising, as histochemical studies have revealed an abundant adrenergic innervation at the base of the crypts of Lieberkühn which may be involved in the regulation of crypt cell turnover (203).

Vagotomy causes mucosal hypoplasia throughout the small intestine (204, 205,206). When the afferent or sensory fibres of the vagus in the pig are divided, there is an inhibition of the adaptive response to jejunectomy (207), and it has been suggested that these sensory vagal nerves, may act as the afferent pathway for signals from the residual intestine after enterectomy, to the hypothalamo-hypophyseal axis, thus triggering the humoral response (208), in keeping with the hypothesis of functional demand

(209), to explain the adaptive changes.

F) GUT BACTERIAL FLORA

Although germ free rodents have reduced rates of cellular turnover in the intestinal mucosa (210,211), instillation of bacterial suspensions into isolated intestinal loops, fails to produce an increase in cellular proliferation (212). The extent to which changes in bacterial flora play a part in intestinal adaptation is uncertain, but these changes do not appear to be a major factor.

CHAPTER 2

THE PURPOSE, AIMS AND OUTLINE OF THE EXPERIMENTAL STUDIES

The mechanisms governing the adaptive process of the intestine during disease or after surgical manipulation, appear to be multifactorial and probably interrelated. Although luminal nutrition and pancreatico-biliary secretions (PBS) have been most extensively investigated and are important, they cannot account for all the aspects of the adaptive response, and systemic factors are also likely to be operative. Gastrin was proposed for such a role (103), and following the publication of a case report of a patient with an enteroglucagon producing tumour (124,125), and the findings of raised plasma enteroglucagon levels in a number of gastrointestinal conditions in which intestinal adaptation is known to occur (129), interest has been directed to this peptide, as a possible 'enterotrophin'.

The purpose of the work to be described, was to investigate further the role of humoral factors in the adaptive process of the small intestine, and as enteroglucagon and gastrin seemed to be the main candidates for a trophic role, these two peptides were studied.

The aims of the studies were to determine:

a) the extent to which luminal nutrition and pancreatico-biliary secretions (PBS) contribute to the mechanism of adaptation after small bowel resection, jejuno-ileal bypass and total colectomy.

b) whether systemic or hormonal factors participate in this process, and if so, their relationship to oral nutrition and PBS.

c) whether specific gastrointestinal hormones can affect cellular proliferation in small bowel.

d) the part played by the two main candidate trophic hormones, gastrin and enteroglucagon, in the adaptive process.

e) whether enteroglucagon is capable of initiating and maintaining these adaptive changes after intestinal resection.

f) the mode of release of enteroglucagon during adaptation, and the relationship between this peptide and luminal nutrition in the context of the adapted bowel.

Although the full amino acid sequence of enteroglucagon is known (71,129) there are considerable species differences, and only a small amount of porcine enteroglucagon, (glicentin) has thus far been produced. Thus, very little material has been available for direct infusional studies, and work related to enterocyte turnover has, as yet, not been done. For these reasons, the experimental approach in these studies, was to relate the hormonal changes in the various models, to the crypt cell production rate, which accurately reflects the proliferative status (213). The majority of the studies were undertaken in male Wistar rats, while the work relating to the mode of enteroglucagon release after intestinal surgery, was done in man.

CHAPTER 3

EVIDENCE FOR A HUMORAL MECHANISM IN INTESTINAL ADAPTATION, AND THE POSSIBLE RELATIONSHIP TO ENTEROGLUCAGON AND GASTRIN

INTRODUCTION

The role of luminal nutrition and PBS in promoting intestinal adaptation in the shortened bowel, is well established. Humoral factors may well play a role. It is, however, not yet established which humoral factors are involved, and what their possible relationship is to the luminal factors mentioned. Furthermore, it is not yet known whether humoral agents are released from the residual bowel, and if so, whether this is in response to luminal nutrition and PBS.

The purpose and aims of the following study, was firstly to determine if a humoral agent is involved after small intestinal resection, and to investigate whether enteroglucagon and gastrin qualify for the role of 'enterotrophin' to the small bowel. It was also intended to determine the relationship of the release of these peptides to intraluminal factors.

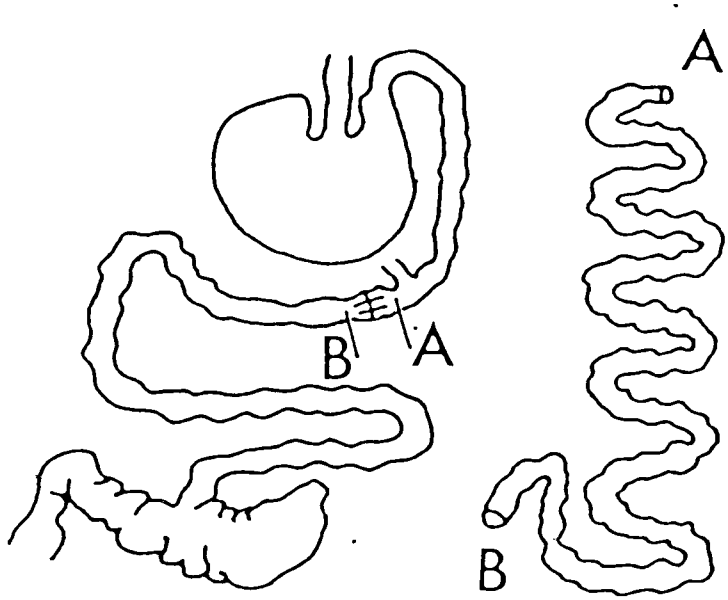


Fig 3.1: Thiry-Vella fistula, comprising 75% of proximal small bowel (AB).

MATERIAL AND METHODS

Sixteen male Wistar rats, weighing 220-250g at the time of surgery, were used for the study. On the day of surgery, the rats were anaesthetised with intramuscular Hypnorm (fentanyl and fluanisone) (Janssen Pharmaceutica. Inc. New Brunswick, NJ), and intraperitoneal Valium (diazepam, Roche Laboratories, Nutley, NJ). A midline abdominal incision was made, and each animal had a 75% proximal small bowel exclusion performed, measured from the ligament of Treitz. The excluded bowel was left attached to its mesenteric neurovascular supply, and the two open ends of this excluded segment were brought out via puncture wounds on the anterior abdominal wall (Fig 3.1) creating a Thiry-Vella mucus fistula (TVF) (214). Continuity of the residual bowel, was restored with an end-to-end anastomosis, using a single layer of continuous 6/0 black silk. Care was taken not to stenose the lumen at the anastomosis, and this was tested for by ensuring that bowel content could pass across the anastomosis. The Thiry-Vella fistula thus created, is excluded from luminal nutrition and PBS.

In eight rats, a length of fine vinyl tubing (Bradley miniature catheter set, Portex Ltd., Hythe, Kent, UK) was inserted into the superior vena cava via the internal jugular vein. The tube was brought out at the back of the neck, via a subcutaneous tunnel, and secured with a suture, for intravenous feeding purposes (215).

The first group of rats, without the catheter, were allowed food ad

libitum. This was in the form of Pelleted Rat Diet (Labsure Animal Foods, Poole, Dorset, UK). The diet, containing 2568 Kcal/kg metabolizable energy consists of 19.7% crude protein, 53.5% carbohydrate, 5.3% crude fibre, 2.7% crude oils, 0.6% calcium, 0.7% phosphorus, 1% NaCl, trace elements and added vitamins. The group with the venous cannulae were nourished exclusively by total parenteral nutrition (TPN) (215). The end of the catheter was attached to a perfusion pump. The catheter itself was protected by a wire coil spiral which was attached to a harness fitted around the chest of the animal. The feeding regime consisted of a mixture of 35% dextrose solution and synthamine amino acid solution with added electrolytes, trace elements and vitamins. (Baxter Division, Travenol Laboratories Ltd., Thetford, Norfolk, UK). An amount of 40ml/day for the TPN fluid was administered to each animal.

A further 16 animals had a laparotomy and transection just distal to the ligament of Treitz. The transected bowel was then reanastomosed with a single layer of 6/0 black silk, and these animals acted as controls. Eight animals of this transected control group were allowed food ad libitum, while the other eight in this group had Vinyl tubing inserted at the time of surgery, and were nourished exclusively by TPN as described above. A record was kept of the amount of food consumed by the orally fed rats, and animals were weighed at the time of surgery and before they were killed.

All rats were killed on the twelfth post operative day, after an overnight fast or discontinuation of TPN, depending on the group. At 9.30 hrs all animals were given vincristine (Oncovin, Eli Lilly & Co. Ltd.,

Basingstoke, UK) which was administered by intraperitoneal injection in a dose of 1mg/kg body weight, for stathmokinetic studies (see below). Animals were then killed at 20 minute intervals. Blood was taken by direct cardiac puncture for plasma gastrin and enteroglucagon radio-immunoassay. The blood was placed into heparinised tubes, containing 0.2ml of Aprotinin (trasylol 20,000 KIU/ml), centrifuged immediately, and the plasma stored at -20°C, to await radioimmunoassay.

PLASMA ENTEROGLUCAGON ASSAY (216):

Two assays were performed, one for total glucagon-like immunoreactivity, using an N-terminally reacting antiserum, R-59, which reacts fully with pure porcine enteroglucagon (glicentin) and one for pancreatic glucagon, using a relatively specific C-terminally reacting antiserum RCS-5. Plasma enteroglucagon levels were then obtained by subtracting the small concentration of pancreatic glucagon from total glucagon. Changes of 10pmol/litre plasma, could be detected with 95% confidence. The cross-reacting antibodies were raised in rabbits to porcine pancreatic glucagon. The antibody-bound and 'free' peptide was separated using dextran-coated charcoal.

PLASMA GASTRIN ASSAY (217):

Plasma immunoreactive gastrin was measured using a C terminal antisera to G-17. This fully detected G-34, but showed less than 5% cross-reaction to cholecystokinin. Changes of 2pmol/litre plasma could be detected with 95% confidence.

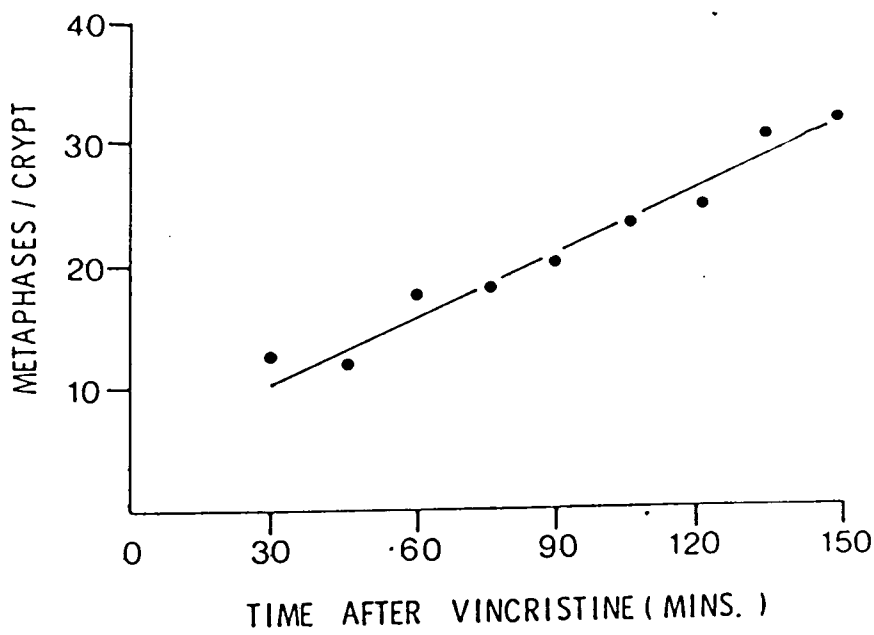


Fig 3.2: Example of arrested metaphases plotted against time after vincristine injection. The crypt cell production rate (CCPR) is represented by the slope of the line.

CRYPT CELL PRODUCTION RATE (CCPR) (213,218):

The CCPR was measured using the stathmokinetic method, involving a metaphase arrest technique (219,220,221) with vincristine (219,222). At 9.30 hrs on the twelfth post-operative day, each rat was given vincristine (Oncovin, Eli Lilly & Co. Ltd., Basingstoke, UK), 1 mg/kg body weight, by intraperitoneal injection. The first rat in each group was killed 30 mins after vincrisine injection and the remaining animals were killed at 20 minute intervals thereafter, for 3 hours. Five cm, segments of bowel were taken in each animal, from the terminal ileum, and mid point of the Thiry-Vella fistula. These tissues were fixed in Carnoy's fluid for 4 hours, and then transferred to 75% ethanol for storage, pending microdissection. On the day of examination, the tissues were rehydrated and hydrolysed for 6 minutes in N HCl at 60°C, and then stained with Feulgen reagent for 1 hour. The mucosa was stripped from the muscle coat, and the crypts were individually dissected out, using a dissecting microscope. The number of arrested metaphases in each crypt was counted, and the mean metaphase counts of ten crypts was taken as the reading for each individual rat. This was then plotted against time after vincristine injection, and the CCPR for the whole group is represented by the slope of the line that best fitted the eight individual points (Fig 3.2), produced by linear regression and least-squares curve fitting (222,223). This method also produces standard errors of the mean.

STATISTICAL METHODS:

The Student's t-test for unpaired data was used for the group analysis and results are given as mean and standard error of the mean.

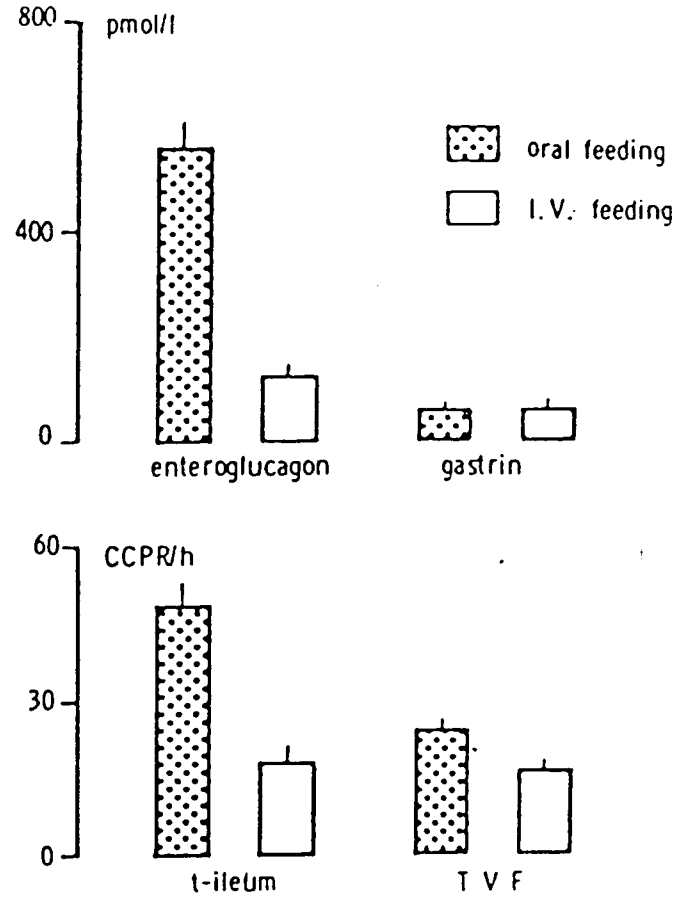


Fig 3.3: Mean and SEM values of plasma enteroglucagon and gastrin in the Thiry-Vella fistula (TVF) animals, shown in the upper panel. The lower panel shows the crypt cell production rate (CCPR) per hour, in the terminal ileum (t-ileum), and in the TVF.

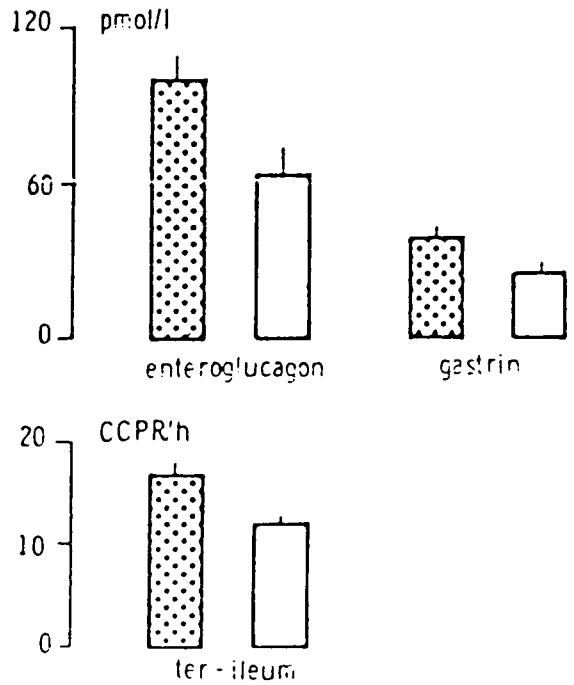


Fig 3.4: Mean and SEM values of plasma enteroglucagon and gastrin after jejunal transection, in the upper panel. The lower panel shows the CCPR/hour in the terminal ileum.

RESULTS

NUTRITIONAL INTAKE:

There was no significant difference in the daily caloric intake of the different groups. In the TPN rats, 40ml of the feeding fluid per day, provided 27.0Kcal. Measurement of food taken by rats on oral nutrition, revealed a caloric intake of 26.8 ± 4.1Kcal/day in Thiry-Vella fistula animals, and 27.7 ± 4.5Kcal/day in transection animals.

PLASMA ENTEROGLUCAGON AND GASTRIN:

In rats with the Thiry-Vella fistula (TVF) enteroglucagon was significantly greater in orally fed rats (566 ± 59pmol/l), compared with TPN animals (120 ± 42pmol/l) ($P<0.01$) (Fig 3.3). Plasma gastrin, however, did not differ in the two groups (56 ± 5.8 and 56.4 ± 15.2pmol/l respectively). In transected orally fed rats, plasma enteroglucagon (99.1 ± 9.6pmol/l) was greater than in transected TPN rats (63.1 ± 9.4) ($P<0.02$) (Fig 3.4). Similiarly, gastrin in orally fed transected animals (38.1 ± 4.4pmol/l) was greater than in transected TPN rats (25 ± 3.2pmol/l) ($P<0.05$) (Fig 3.4). Thiry-Vella fistula rats had greater concentrations of hormones, both enteroglucagon and gastrin, than transected rats in both groups. Thus TVF orally fed rats had greater enteroglucagon and gastrin levels than transected orally fed rats ($P<0.001$ and $P<0.025$ respectively), and TVF rats on TPN had greater enteroglucagon and gastrin levels compared to transected TPN rats ($P<0.001$ and $P<0.05$ respectively).

CRYPT CELL PRODUCTION RATE:

In the terminal ileum in continuity in the Thiry-Vella group, the CCPR

per hour in orally fed rats (52 ± 8) was greater than in the TPN group (18 ± 5) ($P<0.001$) (Fig 3.3). In the midpoint of the excluded TVF, the CCPR per hour in orally fed rats was 28.3 ± 2, and this was significantly greater than the CCPR per hour in the fistula in TPN animals (16 ± 1.5) ($P<0.01$). The CCPR per hour in the terminal ileum of orally fed transected animals (16.8 ± 0.9) was significantly greater than in transected TPN rats (12 ± 0.4) ($P<0.01$) (Fig 3.4). Orally fed TVF rats had a greater CCPR per hour in the terminal ileum than orally fed transected rats ($P<0.001$). In the TPN rats, although the terminal ileal CCPR per hour in TVF animals was greater than in the transected rats, this did not reach statistical significance.

DISCUSSION

A number of radioimmunoassays for enteroglucagon have been reported (224,225,226). These are all broadly similiar, and rely on the use of antisera raised to pancreatic glucagon, which cross react with enteric glucagon-like immunoreactivity. A small number of antibodies cross react completely with enteric and pancreatic glucagon, and can thus be used for measurement of total glucagon-like immunoreactivity. These antibodies, such as R59, are directed towards the middle and N-terminal portion of the glucagon sequence. Other antisera, such as RCS5, show minimal cross reactivity with enteroglucagon, and are directed towards the C-terminal region of the glucagon molecule, and are thus 'specific' for pancreatic glucagon. Since circulating pancreatic glucagon levels are usually small, subtraction of this level from total glucagon will result in the level of enteroglucagon concentration. It has been found, that all the cross-reacting glucagon antisera give the same molar values for enteroglucagon and provide reproducible results when a glicentin standard is assayed (227).

A multiplicity of methods for measuring the proliferative rate in the gut have been used (213), and these include: incorporation of tritiated thymidine (^{3}HTdR) into DNA, mitotic index, ^{3}HTdR labelling index, cell cycle time, growth fraction, birth rate measurement such as CCPR, cell migration rate, transit time, and rate of exfoliation of mucosal cells. The incorporation of ^{3}HTdR into tissues has been used as a measurement of DNA synthesis in the gut (228), although it is now recognised that it is not necessarily an accurate reflection of the DNA synthetic rate, or

indeed of the crypt cell production rate (229). ^{3}HTdR is only minimally incorporated into the DNA of intact cells, there are many steps and pathways for the incorporation of ^{3}HTdR into DNA, and this may be modified by thymidine incorporation enzymes, changes in TdR pool size and ^{3}HTdR transport through the cell membrane (230,231). Furthermore, by this method, the non-epithelial cell populations, especially the lymphoid cells, will be included (213). The proliferative indices, such as mitotic index and labelling index, are measured by autoradiography, and give the proportion of cells in DNA synthesis. Unlike ^{3}HTdR incorporation into DNA, these measurements are confined to the epithelial component of the intestine. However, they only reflect the fraction of cells in mitosis and DNA synthesis, and changes in the duration of these phases can cause an increase in mitotic index or labelling index (232,233) giving rise to limitations of these methods. Furthermore, changes in crypt population will effect proliferative indices. Thus, large crypts may contain the same fraction of labelled or mitotic cells as smaller crypts, but the cell production rate will be higher because of an increase in crypt cell number. For these and other reasons, crypt cell production rate has been used as a measurement of proliferative rate in these experiments. It reflects the rate of production of new cells, and is measured by the metaphase arrest, or stathmokinetic method (219,220,221). In this method, the ability of drugs such as vincristine, is used to arrest cells in metaphase, and thus to measure the rate of entry of cells into mitosis. This rate of entry into mitosis, reflects the birth rate of new cells, since each mitosis results in the net production of one cell. This method has been found to be cost effective in terms of time expenditure on analysis and in terms of animals. The experiments take a short time (up to 3 hours). The method is independent of any other

parameter, and gives information on changes in both cell cycle time and growth fraction. This technique has been regarded as a method of choice (221,234).

The finding in this study, of greater cellular proliferation (CCPR) in the terminal ileum of orally fed Thiry-Vella fistula rats compared to orally fed controls (jejunal transection), supports the findings of previous workers (1,21,24,25,26,27,28,29,34,35,41,43), that shortening the bowel, promotes adaptive hyperplasia in the residual intestine. These changes may well be promoted by luminal nutrition, and pancreatico-biliary secretions. Here the distal shortened intestine comes into contact with greater volumes of luminal content, and food in which nutrients have not been absorbed higher up. This abnormally rich chyme has the effect of stimulating hyperplasia in the terminal ileum of the shortened bowel, and this view is supported by jejuno-ileal transposition experiments (36,44) in which the transposed ileum is placed more proximally in the gastro-intestinal tract and undergoes hyperplasia after coming into contact with a greater volume and richer chyme. Equally, pancreatico-biliary secretions, which have been regarded as trophic agents (44,72, 73,74) may well be implicated in the changes in CCPR in the terminal ileum, seen in the orally fed Thiry-Vella fistula rats. Furthermore, the reduced CCPR, signifying hypoplasia, in the terminal ileum of intravenously fed (TPN) animals, both TVF and transection groups, is in keeping with previous work (59,60,61,62), and once again supports the view of luminal nutrition as an important promotor of intestinal mucosal growth. Total parenteral nutrition is known to cause pancreatic atrophy in rats (59), and the findings in the TPN rats in this study, may equally be in part, due to reduced pancreatic secretions and bile. Unlike the orally fed rats, there

was no significant difference in CCPR in the terminal ileum, between Thiry-Vella fistula and jejunal transected animals having TPN, once again supporting the role of luminal nutrition in adaptive changes in orally fed animals.

The changes taking place in cellular proliferation within the Thiry-Vella fistula, must be totally independent of the direct effect of luminal nutrition and pancreatico-biliary secretions. Crypt cell production rate within this isolated fistula, was significantly greater in orally fed compared to TPN rats. In this respect, it is not possible to incriminate food and PBS as being responsible for these changes, and the changes in CCPR in the fistula can only be explained on the basis of a systemic trophic factor released in the case of the orally fed TVF animals. This finding is in keeping with the adaptive changes seen in Thiry-Vella jejunal loops in lactating rats (91), and the biochemical and morphological adaptive changes in defunctioned segments of bowel, produced by partial resection of the bowel in continuity (57,88,89,90). Such studies all point to a systemic humoral 'enterotrophin'. This being the case, the results of the present study, indicate that a systemic trophic factor is released from the bowel in the orally fed TVF rats, which then passes via the circulation to the isolated fistula to produce the mucosal hyperplasia. Such a systemic factor is either not released or only minimally released in the case of the TPN Thiry-Vella fistula rats; hence the hypoplasia seen in the fistula in this group. As the only difference in the two groups was the mode of administration of nutrition, luminal nutrition in the orally fed rats is very likely to be responsible for releasing this humoral factor. PBS were probably reduced in the TPN rats (59), and these secretions may

also contribute to the release of a humoral factor. Furthermore, the data suggest that the direct effect of trophic agents on intestinal mucosa is stronger than the transmitted effect, as the hyperplastic changes in the fistula were less than the changes in the terminal ileum in continuity, in orally fed rats. This is in keeping with the findings in cross circulation parabiotic studies (93) where the dominant partner had an intestinal resection and transection, while the responding partner had no abdominal operation. The resected and transected partners only showed rises in RNA and DNA, while both partners showed marked and similiar elevations ($>60\%$) in total and specific radioactivity, implicating a transmissible factor, although the transmitted response was weaker than the direct response.

The two possible trophic hormones investigated in this study, enteroglucagon and gastrin, showed very different responses. In Thiry-Vella fistula animals, orally fed rats had significantly greater plasma enteroglucagon levels than TPN animals, while plasma gastrin showed no change between the groups. After jejunal transection, plasma enteroglucagon in orally fed rats was much greater than in TPN rats. Although orally fed transected rats had greater plasma gastrin levels than transected TPN rats, this difference was not marked ($P<0.025$). While gastrin may well have a regulating effect on growth of the oxyntic gland area of the stomach, the duodenum, colon and exocrine pancreas (103,111,112,113,122), the results in the Thiry-Vella fistula rats, supports the view (105,107, 108,110), that gastrin has little, if any trophic influence in the mucosa of small bowel.

In contrast, the changes in plasma enteroglucagon are in keeping with this

peptide being a strong candidate for a trophic role on small bowel mucosa. The markedly greater levels of enteroglucagon in orally fed compared with TPN rats in both Thiry-Vella fistula and transection groups, suggests that this peptide is released by food, and that in the shortened bowel, greater concentrations of richer chyme reaching the distally placed greatest concentration of EG cells (135), is the stimulus for the excessive secretion of enteroglucagon in this situation. The EG cells have microvilli protruding into the lumen, which act as sensors to changes in luminal content (71,129,136), and indeed, the major stimuli for the release of enteroglucagon has been shown to be carbohydrates and fats (137,138). In this study, the changes in plasma enteroglucagon and CCPR are in close agreement. While the changes in CCPR in the bowel in continuity may well be partly due to the direct stimulatory effect of food, the changes within the Thiry-Vella isolated fistula, must be independent of food and due to a systemic factor, and enteroglucagon, in this study, fulfils all the criteria for such a role. Assuming that the mechanisms of the adaptive response are uniform, it seems likely that food, and possible PBS, are the local stimuli for the release of a trophic humoral agent from the bowel in continuity, which may then act locally in a paracrine fashion, and systemically as a conventional hormone. This would explain the more marked rise in CCPR in the bowel in continuity, with a lesser effect on the fistula. The data in this study relating to enteroglucagon, while not producing a cause-and-effect situation, suggests that this peptide may well be trophic to small bowel mucosa, and is in agreement with the suggestion that this peptide should be regarded as 'growth hormone to the small intestine'. (123).

CONCLUSION

The results of this study, indicate that the mechanisms of the adaptive hyperplastic response in the shortened small intestine are multifactorial, with luminal nutrition being important. However, systemic trophic agents are almost certainly involved in this process. In this respect, although gastrin is unlikely to exert a trophic effect on small bowel, enteroglucagon should be regarded as a likely candidate.

CHAPTER 4

THE INFLUENCE OF THE AMOUNT OF INGESTED NUTRIENTS ON CELLULAR PROLIFERATION IN THE BOWEL AFTER INTESTINAL RESECTION, AND ITS EFFECT ON POSSIBLE 'ENTEROTROPHINS', ENTEROGLUCAGON AND GASTRIN.

INTRODUCTION

The mechanisms of intestinal adaptation are multifactorial, with luminal nutrition playing an important role. The previous study (Chapter 3) confirmed that humoral agents almost certainly participate in this process, and in this respect, enteroglucagon appears to be a likely candidate. The present study was undertaken, to investigate the interrelationship of luminal nutrition, humoral agents and cellular proliferation more closely. In particular, it was aimed to determine the effect of the amount of ingested food on cellular proliferation, in control states, and after small intestinal resection, and the influence on two of the candidate humoral trophic agents, enteroglucagon and gastrin.

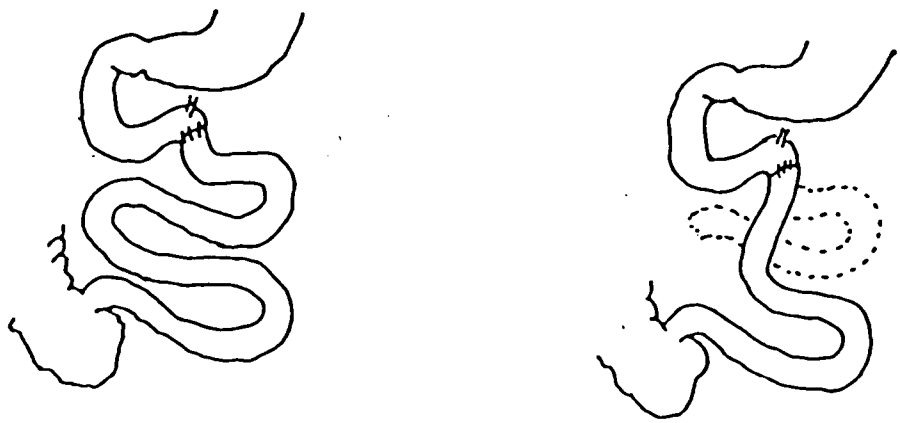


Fig 4.1: Jejunal transection (a), and 75% proximal small bowel resection (b).

MATERIAL AND METHODS

Fifty-six Wistar rats, weighing 220-250g at the time of surgery, were used for this study. Intramuscular Hypnorm (fentanyl and fluanisone - Janssen Pharmceutica Inc. New Brunswick, NJ) and intraperitoneal Valium (diazepam, Roche Laboratories, Nutley, NJ)were used as anaesthetic. Eight animals had a sham laparotomy, with handling of the bowel only. The remaining 48 rats, were allocated to one of two operation groups. Twenty-four animals had a transection of the jejunum just distal to the ligament of Treitz, with reanastomosis. The remaining 24 rats had a 75% proximal small bowel resection, as measured from the ligament of Treitz, with jejuno-ileal end-to-end anastomosis to restore continuity (Fig 4.1). All anastomoses were fashioned with a single layer of continuous 6/0 black silk, and care was taken that there was no stenosis at the site. This was checked for by confirming that bowel content could be 'milked' across the anastomosis. The 48 rats, having either transection or resection, were further subdivided into 3 groups, depending on their nutritional intake, as follows:

a) Eight transected rats and 8 resected rats were allowed food ad libitum. This was in the form of Pelleted Rat Diet (Labsure Animal Foods, Poole, Dorset).

b) Eight transected rats and 8 resected rats were allowed food ad libitum, but from the 5th post operative day, were kept under hypothermic conditions at 6°C, to encourage hyperphagia (235).

c) Eight rats having transection and 8 rats having resection, had fine vinyl catheters (Bradley miniature catheter set, Portex Ltd., Hythe, Kent) inserted into the superior vena cava, via the int.

jugular, and brought out at the back of the neck, via a sub-cutaneous tunnel (215). This group was nourished exclusively intravenously (TPN) as described in Chapter 3. The feeding regime consisted of a mixture of Synthamin amino acid solution with added electrolytes and trace elements and vitamins (Baxter Division, Travenol Laboratories Ltd., Thetford, Norfolk) and a 35% dextrose solution. The amount of TPN fluid thus administered, was 40ml/day.

All animals were weighed at the beginning and the end of the experiment the daily intake of food was calculated by subtracting the food weight at the end of the experiment from the starting amount. All animals were killed on the 12th post operative day. Blood was taken by direct cardiac puncture for gastrin and enteroglucagon assay, and was placed in heparinised tubes containing Trasylol, centrifuged immediately, and the plasma was stored at -20°C pending assay. Segments of intestine, 5cms in length were taken from the duodenum, above and below the anastomosis, the terminal ileum and the colon, for cell kinetic studies.

RADIOIMMUNOASSAY:

Assays for enteroglucagon (216) and gastrin (217), were performed as described in Chapter 3.

CRYPT CELL PRODUCTION RATE (CCPR) (213,218):

This was performed for the segments of bowel removed, as described in Chapter 3.

TABLE 4.1

WEIGHT LOSS AFTER THE DIFFERENT PROCEDURES, AND DAILY FOOD INTAKE.

Procedure	Weight loss (g)	Food intake (g/day)
Sham op.	10 ± 4	18.2 ± 3
Transection (n.f.)	14.2 ± 9	19.5 ± 3
Resection (n.f.)	18.7 ± 5.8	21.8 ± 4.8
Transection (iv)	19.2 ± 3	
Resection (iv)	20 ± 4	
Transection (h)	17.3 ± 4	22.4 ± 5
Resection (h)	20.2 ± 1.5	25 ± 3

(n.f. = Normal feeding; iv = intravenous feeding; h = hyperphagia)

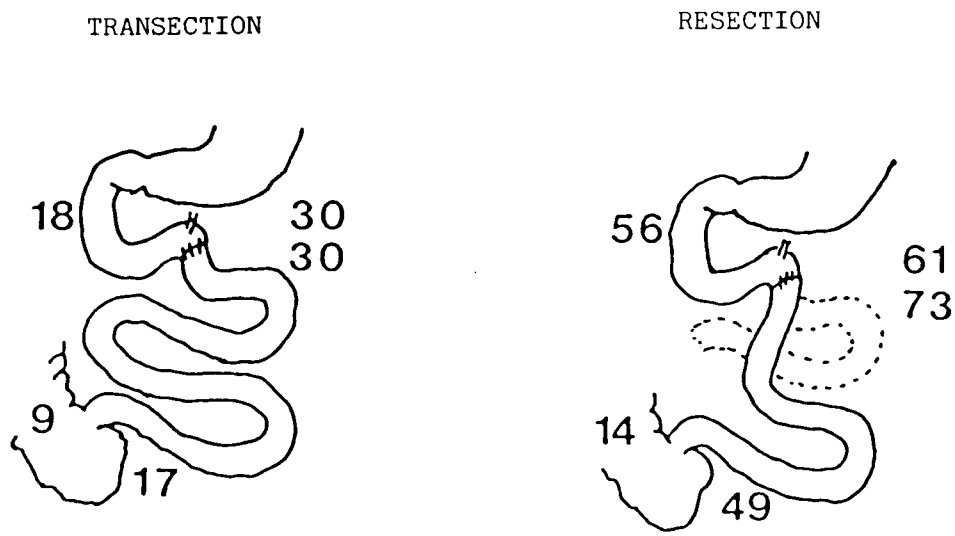


Fig 4.2: Mean crypt cell production rates per hour (CCPR/h) in the intestine, after jejunal transection and 75% proximal small bowel resection.

RESULTS

WEIGHT CHANGES AND NUTRITIONAL INTAKE:

Weight changes and nutritional intake for the groups, are shown in Table 4.1.

CRYPT CELL PRODUCTION RATE (CCPR):

The general distribution of CCPR in the rats taking food ad libitum, is shown in Fig 4.2 for transected and resected rats. The figures were highest just distal to the anastomosis and then tapered off distally. However increased cell production occurred above the anastomosis and in the duodenum, in resected rats. In all positions, resection produced higher cell production rates compared to transection ($P<0.001$). Similiar distribution of CCPR was found in TPN and hyperphagic rats, but for simplicity only the results in the terminal ileum will be given.

CCPR IN TERMINAL ILEUM, AND PLASMA ENTEROGLUCAGON AND GASTRIN:

a) Normal feeding (ad lib):

Fig 4.3 shows CCPR in the terminal ileum and plasma enteroglucagon and gastrin in sham operated, transected and resected animals. There was no significant difference between these results in the sham and transected groups. However, CCPR increased from 16.8 ± 0.9 cells per crypt per hour in transected to 49.2 ± 4.9 in resected rats ($P<0.001$). Enteroglucagon increased from 99.1 ± 96pmol/l in transected to 667.0 ± 70.1pmol/l in resected animals ($P<0.001$). Gastrin increased from 38.1 ± 4.3pmol/l in transected to 81.9 ± 13.3pmol/l in resected rats ($P<0.005$).

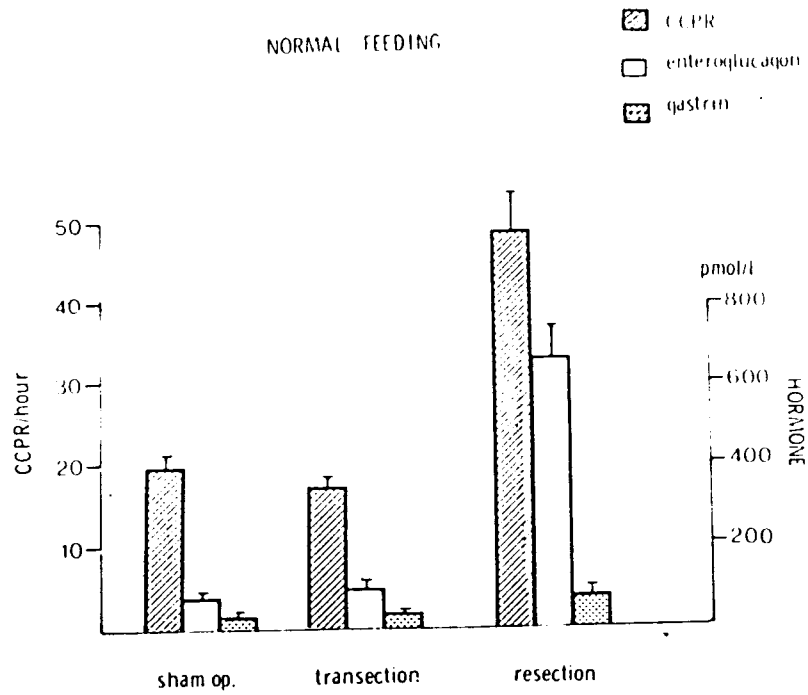


Fig 4.3: Changes in crypt cell production rate per hour (CCPR/h) the terminal ileum, and plasma enteroglucagon and gastrin, after sham operation, jejunal transection and 75% proximal small bowel resection, in animals on normal oral feeding.

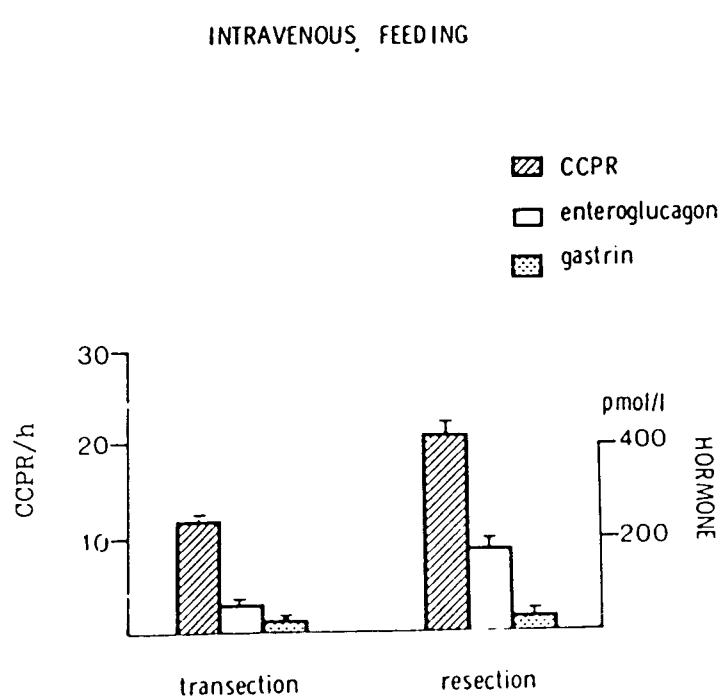


Fig 4.4: Crypt cell production rate per hour (CCPR/h) in the terminal ileum and plasma enteroglucagon and gastrin after jejunal transection and 75% small bowel resection, in rats on intravenous feeding.

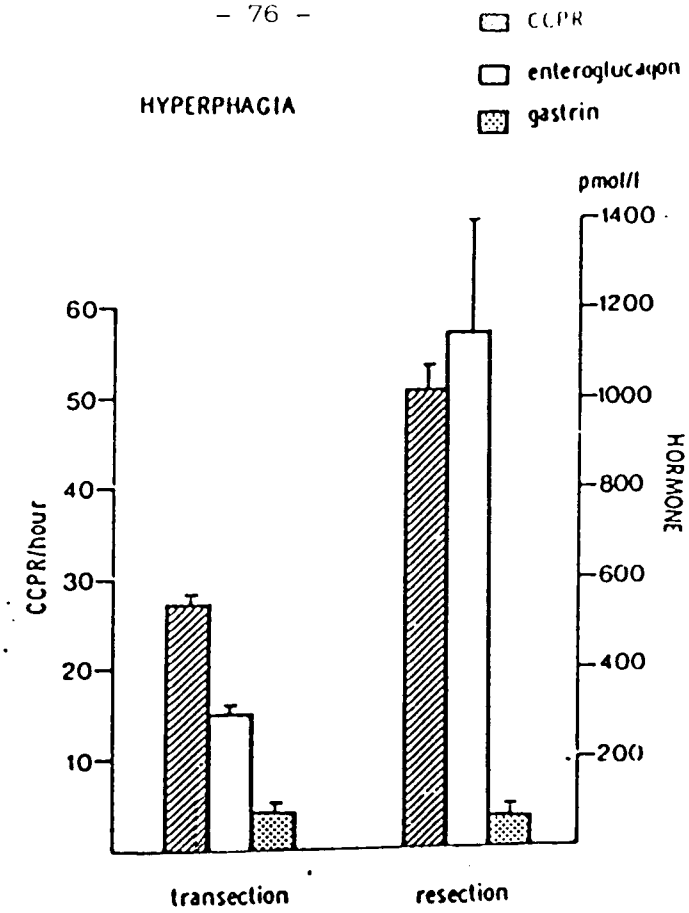


Fig 4.5: Crypt cell production rate per hour (CCPR/h), and plasma enteroglucagon and gastrin, after ileal transection and 75% proximal small bowel resection, in animals with hyperphagia, induced by hypothermia.

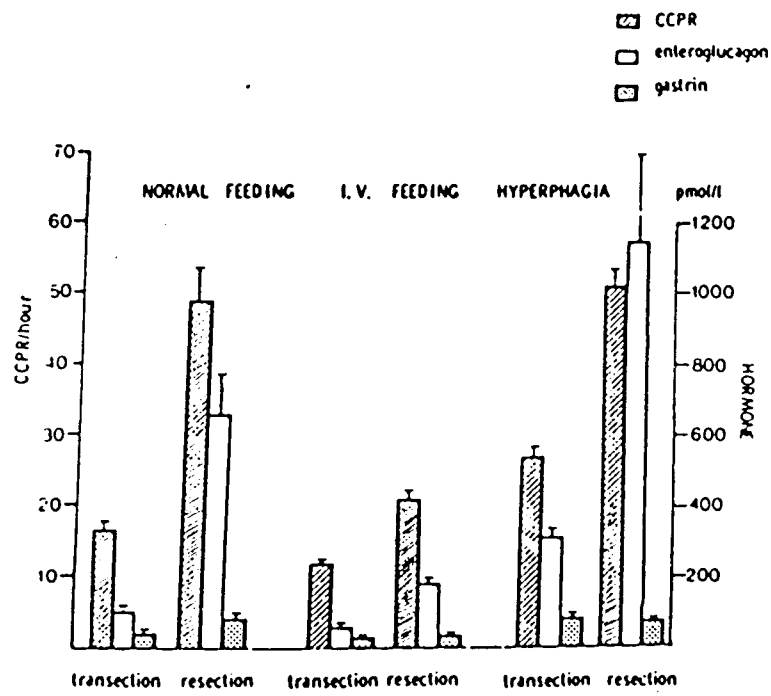


Fig 4.6: Crypt cell production rate per hour (CCPR/h) and plasma enteroglucagon and gastrin, after ileal transection and 75% proximal small bowel resection in the three groups (normal feeding, IV feeding and hyperphagia).

b) Intravenously fed rats (Fig 4.4):

The CCPR in the ileum increased from 12 ± 0.4 cells per crypt per hour in transected animals to 21.3 ± 1.3 cells per crypt per hour in resected animals ($P<0.001$). Enteroglucagon incresed from 63.1 ± 9.4 pmol/l in transected rats to 184.4 ± 15.8pmol/l in resected animals ($P<0.01$), although there was no significant change in plasma gastrin.

c) Hypothermic hyperphagic rats (Fig 4.5):

The CCPR in the ileum increased from 27.9 ± 1.2 cells per crypt per hour in transected, to 51.6 ± 2.5 in the resected rats ($P<0.001$). There was an increase of enteroglucagon from 311.9 ± 22.2pmol/l in transected rats, to 1152 ± 236.5pmol/l in the resected group ($P<0.005$). Gastrin, however, did not change in the two groups.

d) Group as a whole:

Fig 4.6 shows the CCPR and plasma enteroglucagon and gastrin, for transected and resected rats, undergoing the different forms of nutritional intake.

i) CCPR:

Fig 4.7 illustrates a cross sectional view of the terminal ileum in transected animals and animals having 75% proximal small bowel resection on normal feeding, IV feeding and hyperphagia induced by hypothermia. In transected rats the CCPR on normal feeding (16.8 ± 0.9) fell to 12 ± 0.4 after TPN($P<0.001$) but rose to 27.9 ± 1.2 with hyperphagia ($P<0.001$). In resected rats the CCPR on normal feeding (49.2 ± 4.9), fell to 21.3 ± 1.3 ($P<0.001$) in TPN rats. However, although the CCPR in hyper-

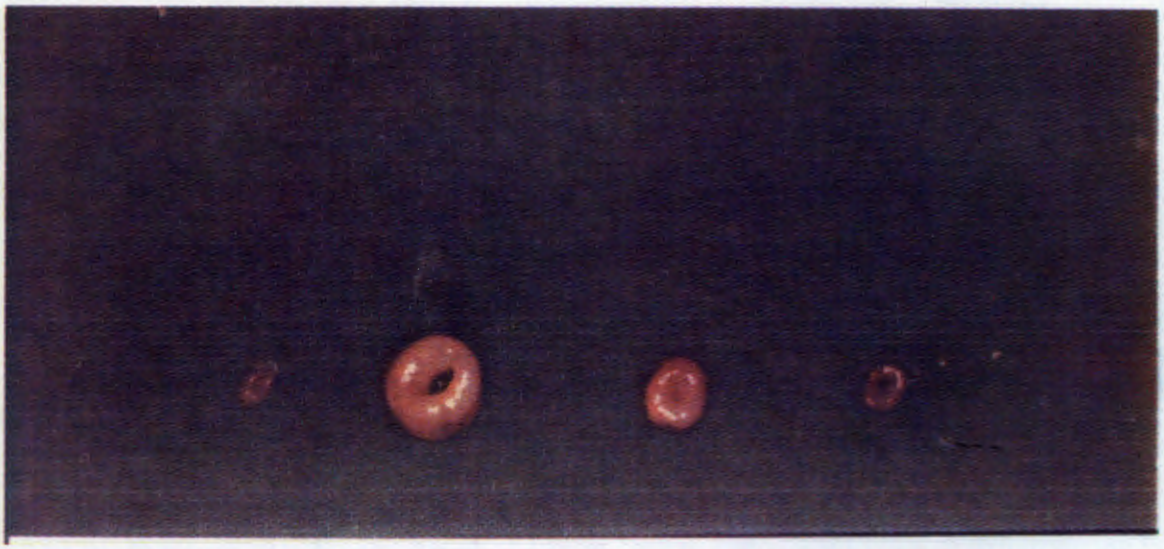


Fig 4.7: Cross section of ileum in animals with (from left) intestinal resection and IV feeding, intestinal resection and hypothermic hyperphagia, intestinal resection on normal feeding, and jejunal transection and normal feeding.

phagic resected rats did increase to 51.6 ± 2.5, this was not statistically different from resected rats on normal feeding.

ii) Plasma enteroglucagon and gastrin:

In transected rats, the plasma levels of enteroglucagon and gastrin in normally fed rats, was significantly higher than levels in TPN rats ($P<0.02$ and <0.001 respectively) while these levels rose significantly in hyperphagic transected rats ($P<0.001$). In resected rats, the levels of enteroglucagon and gastrin in animals feeding normally, fell significantly with TPN ($P<0.001$ and <0.02 respectively). In hyperphagic resected animals, although enteroglucagon rose from 667 ± 70.1 pmol/l in resected rats eating normally, to 1152 ± 236.5pmol/l this failed to reach statistical significance. Gastrin in this situation decreased slightly, although once again, this was not significant.

DISCUSSION

The marked adaptive hyperplasia seen in the terminal ileum after extensive proximal small bowel resection in this study, is in agreement with previous studies showing intestinal adaptation in the residual shortened intestine (1,21,24,25,26,27,28,29,34,35,41,43). There seems very little doubt, that luminal nutrition is important in promoting these adaptive changes, and this is confirmed by the data in this study, which furthermore, indicates that the amount of food presented to the residual bowel, is important. This is clearly seen in transected rats, where a CCPR of 12 in the TPN group indicates hypoplasia, this increasing to 17 in normally fed rats, and ultimately to 28 cells per crypt per hour in the hyperphagic group, all these changes being highly significant ($P<0.001$). The same trend is shown in the resected rats, where TPN animals had a CCPR of 21, which increased to 49 in normally fed animals ($P<0.001$). There was a further increase to 52 cells per crypt per hour in hyperphagic rats, although this did not show a statistically significant change from the normally fed group, and it may well be that a limit is reached, above which cell production rate is not able to increase. These findings confirm those of others, that the hyperphagia in pregnant and diabetic rats (47,48,49,50,51,52), results in villus hyperplasia in small bowel, although clearly, other endocrine factors may be operative in these models, and that TPN leads to hypoplastic changes (59, 60). Indeed, it has been shown that jejunal and ileal mucosa undergoes hypoplasia during TPN, as early as the third day(60).

The highly significant increases in CCPR from transected to resected

animals in the normally fed and hyperphagic groups, can certainly be explained on the bases of luminal nutrition, by the fact that after resection, the residual ileum comes into contact with a larger volume of food and unabsorbed nutrients, to which it is normally unaccustomed, which then results in the hyperplastic changes. This view is supported by ileojejunal transposition experiments (36,44), where the ileum is transported to a proximal position, and undergoes hyperplasia, although the total length of the gut is unaltered. Luminal nutrition, can however, not be held responsible for the significant increase in CCPR in resected compared with transected rats having TPN. While it is possible that pancreatico-biliary secretions, which have been regarded as having a trophic influence on small bowel (44,72,73,74), may be responsible for these changes, in view of the absent food in the gut, it seems likely that reduced amounts of PBS would be produced in these animals. Indeed, pancreatic atrophy has been observed in TPN rats (59). However, pooled pre-harvested pancreatic juice perfused into isolated intestinal loops, has produced modest adaptation (72,80).

An alternative explanation for the changes seen in TPN rats, would be a humoral factor. The two hormones investigated in this study, showed very different patterns of release. Although gastrin levels were greater in resected compared with transected normally fed rats, in keeping with the hypergastrinaemia noted after major small bowel resections (100,101,102), there was no such change in the TPN and hyperphagic groups of animals, despite very marked alterations in CCPR. It is unlikely, therefore, that gastrin has played a part in promoting hyperplasia in these models. The changes in plasma enteroglucagon, however, correlate closely to the changes in CCPR in all the different groups (Fig 4.6). In transected rats, there was a stepwise increase in plasma enteroglucagon, related to the amount

of luminal nutrition taken, and this corresponded to increases in CCPR. In resected rats, there was an increase from TPN to normally fed rats, similiar to changes in CCPR. Although enteroglucagon increased from 667 in normally fed resected rats, to 1152 in resected hyperphagic animals, as with CCPR in these modes, this did not reach statistical significance, and there may similiarly be a limiting level above which it is not possible for the EG cells to produce more peptide, no matter how great the stimulus.

Food is the major stimulus for the production of enteroglucagon, and in this respect, carbohydrates and long chain fatty acids are important (137,138). The EG cells are of the 'open' variety, with microvilli, which act as 'sensors' to changes in luminal content, protruding into the lumen (71,129,136), and these EG cells have a distal distribution, with the maximum concentration found in the distal ileum, with a lesser but still significant concentration found in the colon (135). Furthermore, although hypertonic glucose solutions provide a strong stimulus to enteroglucagon release, it appears to be the amount of glucose presented to the mucosa, rather than the tonicity which determines the strength of the stimulus (71). These facts explain the changes in plasma enteroglucagon seen after resection in orally fed animals in this study. After 75% proximal small bowel resection because of proximal malabsorption, the EG rich terminal ileum is effectively brought into contact with a greater volume of luminal nutrition, and furthermore, this contains nutrients which should have been absorbed more proximally in the gut, thus providing the EG cells with a richer mixture of chyme. The EG cells are thus stimulated to hypersecrete, and under these circumstances, a reduction in the number of secretory granules in the EG cell, denoting hyperfunction, has been observed (162). Clearly, hypersecretion

of enteroglucagon can occur by increasing the volume of food taken, without resection, as seen in the transected rats in this study.

As with CCPR, the increase in enteroglucagon in resected compared to transected TPN rats, must be independent of food. Other causes for the change in enteroglucagon, must be operative. It is possible that PBS may stimulate enteroglucagon, although this is speculative. If this is the cause, the amounts of PBS in TPN are probably small because of pancreatic atrophy (59). Whatever the other factors responsible for enteroglucagon release in TPN rats, the levels of this peptide correspond to changes in CCPR in these models.

CONCLUSION:

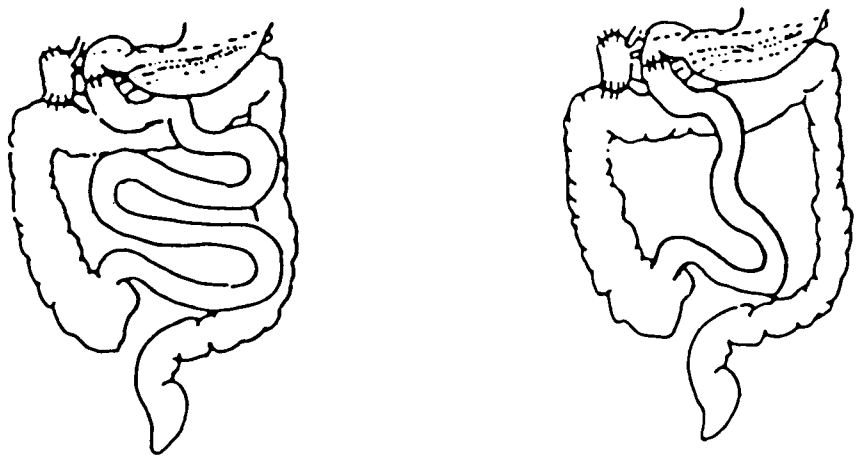
The findings in this study, confirm that intense adaptive hyperplasia is produced in the residual small bowel, after major proximal resections. The mechanisms of this adaptation are multifactorial, as although luminal nutrition is clearly important, it cannot explain the changes seen in TPN rats, and humoral factors may well be operative. Because of the close correlation to changes in CCPR, enteroglucagon seems a more likely candidate than gastrin in this respect. Because of the situation of the EG cells, and the stimulatory mechanisms, it seems likely that luminal nutrition may produce its effect on mucosal enterocyte turnover, through enteroglucagon, although there appears to be other mechanisms of enteroglucagon release and thus changes in cell proliferation. Furthermore, the amount of luminal nutrition is important in regulating enteroglucagon release, and thus cell turnover, although there is a limiting level, above which peptide secretion and cell production rate, cannot increase, despite increase in luminal nutrition.

CHAPTER 5

THE INFLUENCE OF PANCREATICO-BILIARY SECRETIONS ON CELLULAR PROLIFERATION IN SMALL BOWEL, AND THEIR POSSIBLE RELATIONSHIP TO HUMORAL AGENTS

INTRODUCTION

The recognition that the intestinal villi in the duodenum are twice the size of the villi in the ileum, the so called 'proximodistal gradient' of villus size (44,72,236) prompted the view, that pancreatico-biliary secretions (PBS) may be an important factor in promoting mucosal growth. This view has been supported by finding of enhanced hyperplasia distal to the reimplanted duodenal ampullary region (44,72,73). In order to investigate this further, and to determine the relationship of PBS to the release of enteroglucagon and gastrin, the following study was undertaken.



(a) (b)

Fig 5.1: Pancreatico-biliary diversion, (a) alone, and (b) with 75% proximal small bowel resection.

MATERIAL AND METHODS

Thirty-two male Wistar rats, weighing 200-250g at the time of surgery, were used. Intramuscular Hypnorm and intraperitoneal Valium were used for anaesthetic. Eight rats had a jejunal transection below the ligament of Treitz, with end-to-end anastomosis, while a further 8 animals had a 75% proximal small bowel resection, with an end-to-end jejuno-ileal anastomosis to restore continuity (Fig 4.1). The remaining 16 rats had a few cms of duodenum, containing the ampulla of Vater, excised. The proximal part of this segment was closed with a single layer of sutures, while the distal end was reanastomosed, end-to-side, to the mid-transverse colon. Eight rats in this group of pancreatico-biliary diversion (PBS), had an end-to-end duodeno-jejunal anastomosis to restore continuity, at the region of the ligament of Treitz. The remaining 8 rats in this group, in addition to the pancreatico-biliary diversion, had a 75% proximal small bowel resection, with a duodeno-ileal anastomosis to restore continuity (Fig 5.1). All anastomoses were performed with a continuous single layer of 6/0 black silk. All rats were allowed water and food ad libitum. Animals were weighed at the commencement of the experiment and at the time of killing, which was on the 12th post operative day. The amount of food consumed was calculated by measurements at the beginning and the conclusion of the experiment. On the 12th post-operative day, after an overnight fast, animals were killed by ether anaesthesia. Blood was taken by direct cardiac puncture and placed in heparinised tubes, containing Trasylol. The blood was immediately centrifuged and the serum was stored at -20°C to await assay. Five cm segments of bowel were taken from the duodenum, immediately below the anastomosis, the terminal ileum, and sigmoid colon for cell kinetic studies.

RADIOIMMUNOASSAY:

Assay of enteroglucagon (216) and gastrin (217) were performed as described in Chapter 3.

CRYPT CELL PRODUCTION RATE (CCPR) (213,218):

This was performed, to assess cellular proliferation, as described in Chapter 3.

STATISTICS:

The students t-test for unpaired data was used for the group analysis and results are given as mean and standard error of the mean.

TABLE 5.1

WEIGHT LOSS AFTER THE DIFFERENT PROCEDURES, AND DAILY FOOD INTAKE

Procedure	Weight loss (g)	Food intake (g/day)
Jejunal transection	14.2 ± 9.76	19.5 ± 3
75% small bowel resection	18.75 ± 5.8	21.8 ± 4.8
Jejunal transection + PBD	46.87 ± 7.04	22.5 ± 2
75% small bowel resection + PBD	54.14 ± 8.4	18.8 ± 1.2

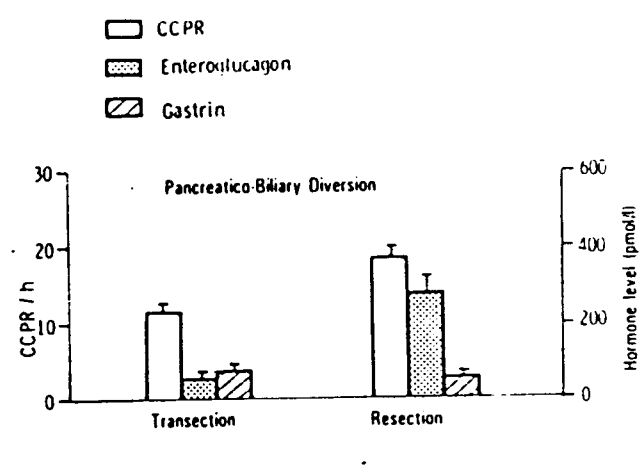


Fig 5.2: Plasma enteroglucagon and gastrin levels, and CCPR/h after pancreatico-biliary diversion, with and without intestinal resection.

TABLE 5.2

CCPR/h (±s.e.m.) AT DIFFERENT SITES OF THE SMALL INTESTINE WITH STATISTICAL SIGNIFICANCE.

Position	Experimental group			
	1. Jejunal transection (v. Group 3)	2. 75% small bowel resection (v. Group 1)	3. Transection with biliary diversion (v. Group 4)	4. 75% resection with biliary diversion (v. Group 2)
Duodenum	27.5±1.7¥	56.5±3.9¥	18.0±0.8#	21.7±0.9¥
Above anastomosis	34.02±2.0*	61.5±4.3¥	30.0±4.3*	38.6±1.0¥
Below anastomosis	30.0±1.3*	72.6±6.6¥	26.45±2.0#	35.8±2.8¥
Terminal ileum	16.8±0.9¥	49.2±4.9¥	12.0±0.6¥	18.8±0.9¥

* Not significant

\# $P<0.02$

¥ $P<0.001$

TABLE 5.3

PLASMA ENTEROGLUCAGON AND GASTRIN LEVELS (pmol/l $\pm$ s.e.m.) WITH STATISTICAL SIGNIFICANCE.

	1. Jejunal transection (v. Group 3)	2. 75% small bowel resection (v. Group 1)	3. Jejunal transection with biliary diversion (v. Group 4)	4. 75% small bowel resection with biliary diversion (v. Group 2)
Enteroglucagon	99.1 ± 9.6¥	$667.1 \pm 70.1$$	$55.7 \pm 7.4$$	$289.0 \pm 38.4$$
Gastrin	33.1 ± 4.3#	81.9 ± 13.3¥	72.5 ± 10.8*	55.7 ± 7.4*

* Not significant

\# $P<0.01$

¥ $P<0.005$

$ $P<0.001$

RESULTS

NUTRITIONAL INTAKE AND WEIGHT CHANGES (Table 5.1):

All animals lost weight post-operatively. Although this was significantly more in the group having PBD, despite similiar intake of food in the groups. The rats having PBD, had marked diarrhoea, and looked unwell.

CRYPT CELL PRODUCTION RATE (Fig 5.2 and Table 5.2):

In the terminal ileum of rats not having PBD, the CCPR in transected rats was 16.8 ± 0.9 cells per crypt per hour, and this increased to 49.2 ± 4.9 after 75% proximal small bowel resection ($P<0.001$). After PBD alone, the CCPR in the terminal ileum was 12 ± 0.6 cells per crypt per hour, and this increased to 18.8 ± 0.9 in rats having PBD plus 75% proximal resection ($P<0.001$). When comparing rats having PBD alone with those having jejunal transection, the CCPR in the PBD group was significantly lower ($P<0.001$). Similiarly, the PBD rats with 75% resection, had a significantly lower CCPR than those having resection alone ($P<0.001$).

PLASMA ENTEROGLUCAGON AND GASTRIN (Fig 5.2 and Table 5.3):

In rats not having PBD, the plasma enteroglucagon level after jejunal transection was 99.1 ± 9.6pmol/l and this increased to 667.0 ± 70.1pmol/l after 75% proximal resection ($P<0.001$). Plasma gastrin increased from 38.1 ± 4.3pmol/l in transected, to 81.9 ± 13.3pmol/l in resected rats ($P<0.005$).

Rats having PBD alone, had a plasma enteroglucagon level of 55.7 ± 7.4pmol/l,

and this increased to 400 ± 38.4pmol/l in rats having PBD plus 75% proximal resection ($P<0.001$). Plasma gastrin in the PBD group alone (72.5 ± 10.8pmol/l) did, however, not differ from the level in rats having PBD plus resection (55.7 ± 7.4pmol/l).

When comparing rats not having 75% proximal resection, plasma enteroglucagon was significantly lower after PBD ($P<0.005$), as was plasma gastrin ($P<0.01$), compared with jejunal transection. Similiarly, plasma enteroglucagon was significantly higher after 75% resection compared with resection plus PBD ($P<0.001$), while gastrin did not show any significant change.

DISCUSSION

The proximodistal gradient of villus size, has prompted workers in the field to suggest that intestinal secretions may be important in producing this size gradient, and pancreatico-biliary secretions have been regarded as being important in this respect (44,72,236). Indeed, diverting the ampullary region of the duodenum into the mid-small bowel (73) and into self emptying ileal loops (44,72), has produced distal hyperplasia, supporting the view that PBS may be trophic to small intestinal mucosa. Furthermore, perfusion of pre-harvested pancreatic juice into isolated intestinal loops, produced a modest degree of adaptation in these loops (72,80).

The cell kinetic data in this study, are in keeping with the view that PBS may well be trophic to small intestine. A significantly lower CCPR in the terminal ileum, was found in rats having their PBS diverted to the colon, compared with those not having this procedure. This result when comparing the PBD rats with non-PBD animals, is almost certainly due to the presence or lack of PBS on the terminal ileum. Groups of rats had similiar nutritional intake, and food can thus not be incriminated in these changes, when comparing these 2 broad groups (pancreatico-biliary diversion and non diversion). The marked loss of weight in the diverted group, can be accounted for by the marked diarrhoea and malabsorption. Luminal nutrition, on the other hand, was probably responsible for the increased CCPR in the terminal ileum of rats having PBD plus 75% proximal resection, compared with the groups having PBD alone.

The two hormones studied, gastrin and enteroglucagon, behave differently in this study. While there was an increase of gastrin in 75% resected rats compared with transected animals ($P<0.005$), the changes in plasma gastrin in the PBD rats and rats with PBD plus resection, were not significant, and gastrin can thus not be held responsible for the changes in CCPR in these models. Enteroglucagon, on the other hand, showed significant ($P<0.001$) changes in the four different groups, and these changes in enteroglucagon, corresponded closely to the changes in CCPR in these models. When comparing rats having jejunal transection and PBD alone, as the food intake was similiar, PBS must be regarded as being responsbile for the significant differences in plasma enteroglucagon and CCPR in these two groups. This must also be the case in rats having 75% proximal resection and those with PBD plus resection. Although pre-harvested pancreatic juice has a direct stimulatory effect on isolated segments of intestine (72,80), the direct effect of PBS on enteroglucagon release has as yet not been investigated. However, the data in this study suggests, that besides carbohydrates and fats (137,138), PBS may be a stimulatory factor for enteroglucagon release. In support of this hypothesis, is the work done by Miazza et al (81). A different type of pancreatico-diversion to the one in this study, was performed in rats. Here the proximal 50cm of small bowel was transposed to lie between the stomach and duodenum, thus depriving the jejunum of PBS. Animals were nourished either by TPN or orally, and PBD was compared with control animals having no PBD, but nourished either orally or by TPN. The ileum in the PBD animals, lying immediately downstream from the duodenal ampullary region, underwent adaptive changes, supporting the view that PBS are trophic to small bowel. Surprisingly, however, the jejunum in the PBD animals, now deprived of PBS, underwent hyperplasia

with increases in villus height, mucosal mass and absorptive capacity. This finding, at first sight, would be contrary to the theory of PBS being trophic to small bowel, with deprivation of the jejunum of PBS, this segment of bowel was expected to undergo hypoplasia. However, after measuring the plasma for enteroglucagon, it was found that animals undergoing PBD, had significantly raised levels of this peptide compared with controls (84). As there was no shortening of the gut in this model, it may well be, that PBS coming into contact with the enteroglucagon rich ileum, was the stimulatory factor in the PBD animals, and this peptide may well be responsible for the hyperplasic changes found in the jejunum in this model.

CONCLUSION

The data in this study, support the view that PBS are trophic to small bowel. Unlike gastrin, plasma enteroglucagon changes correlated well with changes in cell turnover, and this peptide may well be important in the adaptive response seen in these models. The results also suggest, that PBS may stimulate the release of enteroglucagon, and that trophic effect of PBS may be exerted via the release of this peptide.

CHAPTER 6

THE INFLUENCE OF JEJUNO-ILEAL BYPASS AND RESECTION, ON INTESTINAL ADAPTATION AND PLASMA CONCENTRATIONS OF ENTERO-GLUCAGON AND GASTRIN

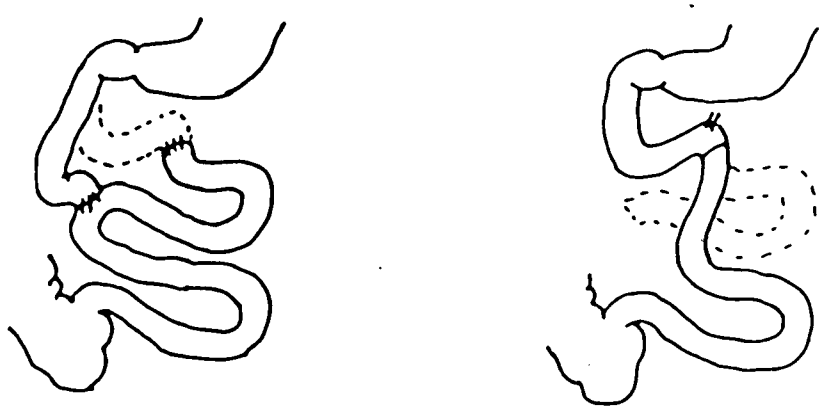
INTRODUCTION

As with proximal small bowel resection, the shortened segment of small bowel in continuity with the nutrient stream after proximal jejuno-ileal bypass operations in the experimental animal, undergoes compensatory hyperplasia and enhanced absorptive function (23,26,58,63,65). This has also been shown to be the case in man, where jejuno-ileal bypass procedures have been carried out in the management of morbid obesity (30,31,237,238, 239). Besides hyperplasia of the enterocytes, goblet (mucous) cell hyperplasia has also been shown to occur as part of the adaptive response in jejuno-ileal bypass (240). This is perhaps not surprising, as the stem cells in the base of the crypts of Lieberkühn, are regarded as pluripotential, giving rise to columnar, goblet, endocrine and Paneth cells (13).

The mechanisms by which this adaptation takes place, are probably similiar to intestinal resection. Thus the direct effect of luminal nutrition is probably important, and in support of this, is the fact that the bypassed jejunum, undergoes marked hypoplasia, has slower migration and epithelial cell turnover, with reduced mucosal content of enzymes, protein and nucleic acids (23,26,58,63,65). Furthermore, these structural and

functional changes in the defunctioned jejunum, are completely reversed by restoration of normal intestinal continuity (58,63). While this certainly supports the concept of luminal nutrition as a mechanism in the adaptive response after jejuno-ileal bypass, intestinal secretions, such as bile and pancreatic juice, may similiarly account for these changes.

However, as humoral mechanisms have been postulated to be important in intestinal adaptation (85,92,93,94,95,98), and hormonal changes have been shown to occur after jejuno-ileal bypass for morbid obesity (149), this study was undertaken to compare the adaptive changes after jejuno-ileal bypass with those taking place after an equivalent length of small bowel resection, and to determine the role of hormones in the mechanism of the adaptive response after bypass surgery.



(a) (b)

Fig 6.1: 75% proximal jejuno-ileal bypass (a), and 75% proximal small bowel resection (b).

MATERIAL AND METHODS

Twenty-four male Wistar rats were used, weighing 200-250gms at the time of surgery. Eight rats had a 75% proximal small bowel resection, measured from the ligament of Treitz, with an end-to-end jejuno-ileal anastomosis to restore continuity. Eight rats had a 75% proximal jejuno-ileal bypass. The bowel was divided distal to the ligament of Treitz, and the bowel distal to the division was closed, while the proximal bowel was anastomosed end-to-side to the ileum, 75% of the distance down the small bowel, thus excluding an equivalent amount of bowel to that resected in the first group (Fig 6.1). The last eight rats had transection of the bowel below the ligament of Treitz only, with reanastomosis. All anastomoses were constructed with a single layer of 6/0 black silk. Intramuscular Hypnorm (fentanyl and fluanisone) and intraperitoneal Valium (diazepam) were used for anaesthetic. Animals were allowed food (Pelleted Rat Diet - Labsure Animal Foods, Poole, Dorset) and water ad libitum. All animals were killed on the 12th post operative day. At 9.30 hrs, all animals were given vincristine (Oncovin, Eli Lilly & Co. Ltd., Basingstoke, UK), by intraperitoneal injection in a dose of 1mg/kg body weight, for measurement of cell kinetics. Animals were then killed at 20 minute intervals by ether anaesthesia and blood was taken immediately by direct cardiac puncture. The blood was placed in heparinised tubes containing 0.2ml of aprotinin (Trasylol 20,000KIU/ml), centrifuged immediately, and the plasma was stored at -20°C to await radioimmunoassay for plasma gastrin and entero-glucagon. A record was kept of the weight loss of the animals following surgery, and the amount of food consumed.

RADIOIMMUNOASSAY:

Assays for enteroglucagon (216) and gastrin (217) were performed as described in Chapter 3.

CRYPT CELL PRODUCTION RATE (CCPR) (213,218):

This was performed on the terminal ileum, as described in Chapter 3.

STATISTICS:

The students t-test for unpaired data was used for the group analysis and results are given as mean and standard error of the mean.

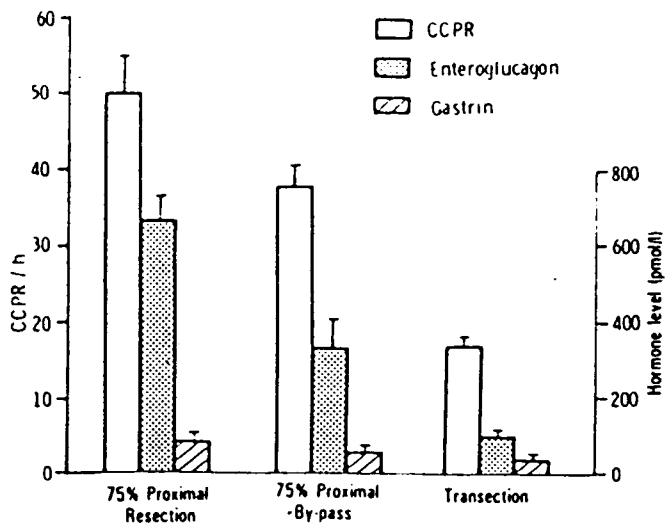


Fig 6.2: Plasma enteroglucagon and gastrin, and CCPR/h after intestinal resection, jejuno-ileal bypass and jejunal transection.

RESULTS

NUTRITIONAL INTAKE AND WEIGHT CHANGES:

There was no significant difference in the amount of food consumed per day in the resected animals, jejuno-ileal bypass group and transected animals (21.8 ± 5g, 23.5 ± 6g, 19.5 ± 3g respectively). The resected animals lost 18.7 ± 6g and transected animals lost 14.2 ± 9g following operation. However, the animals with jejuno-ileal bypass lost 42.6 ± 4g in body weight ($P<0.01$).

CRYPT CELL PRODUCTION RATE (CCPR) (Fig 6.2):

After 75% proximal jejuno-ileal bypass, the CCPR in the terminal ileum increased from 16.8 ± 0.9 cells per crypt per hour in the transected control animals, to 37.8 ± 2.2 cells per crypt per hour ($P<0.001$). The CCPR in jejuno-ileal bypass in the terminal ileum, was further increased to 49.2 ± 4.9 cells per crypt per hour after 75% proximal resection ($P<0.001$).

PLASMA ENTEROGLUCAGON (Fig 6.2):

Plasma enteroglucagon increased from 99.1 ± 9.6pmol/l in transected controls, to 340 ± 62.4pmol/l in the jejuno-ileal bypass animals ($P<0.001$). While this level increased still further to 667 ± 70.1pmol/l after 75% proximal resection ($P<0.001$).

PLASMA GASTRIN (Fig 6.2):

Although the increase in plasma gastrin from 38.1 ± 4.3pmol/l in transected

to $81.9^{+}_{-}13.3$pmol/l in resected rats, was significant ($P<0.005$), there was no significant increase from control levels to those in jejuno-ileal bypass ($52.9^{+}_{-}12.4$pmol/l), nor was the difference between resection and bypass significantly different.

DISCUSSION

The increased CCPR in the terminal ileum of the rats with jejuno-ileal bypass, compared with control transected animals in this study, is in keeping with previous reports of hyperplasia and increased absorptive capacity in the residual small bowel in continuity after bypass (23,26,58,63,65). The finding of increased CCPR in the terminal ileum after resection compared with bypass, despite an equal amount of residual ileum in both procedures, is in agreement with the work of others (158) in which jejunectomy caused an initial greater ileal hyperplasia than an equivalent end-to-side jejunal bypass. This difference, however, was not observed from four weeks onward, when no morphologic or biochemical difference was found between excision and exclusion (26,158,241). The present study was of short duration (12 days) so that the findings were in keeping with a more pronounced hyperplasia after resection compared with bypass, in the early post operative period. Of interest is the fact, that small bowel resection produces a higher azoxymethane induced colonic tumour yield compared with an equivalent small bowel bypass at 20 and 25 weeks, although the yields at 30 weeks were similiar (241). This may well be due to the earlier hyperplasia seen after resection, although the later adaptive effects of resection and bypass are similiar (241).

The mechanisms of adaptation in the residual ileum in continuity, are probably similiar to those operative after intestinal resection. In contrast to the hyperplasia seen in the distal ileum in continuity with

the nutrient stream, the finding of marked hypoplasia in the defunctioned jejunum after jejuno-ileal bypass, with reduced absorptive capacity (23,26,58,63,65) is in favour of a role for the direct effect of luminal nutrition and possibly pancreatico-biliary secretions. Furthermore, this concept is supported by the fact, that these changes in the defunctioned jejunum, are completely reversed by restoration of normal intestinal continuity (58,63). However, evidence is available from work with defunctioned segments of bowel, which supports the concept that humoral agents are involved in the adaptive process after bypass procedures, and that these humoral agents probably mediate part of the trophic effect of luminal nutrition. Thus, increased cellular proliferation has been found in heterotropic autografts of ileal mucosa transplanted beneath the renal capsule after partial enterectomy (242). Furthermore, the degree of mucosal hypoplasia found in excluded intestinal segments, is decreased by partial resection of the bowel in continuity (57,88,89) as witnessed by morphologic and biochemical evidence of mucosal hyperplasia in these bypassed segments. However, the greater hyperplasia found in the bowel in continuity, suggests a combination of local (such as luminal nutrition) and humoral (systemic) factors. When animals with isolated segments of bowel, are deprived of food by mouth (91) or nourished exclusively by TPN (98,243), further hypoplastic changes occur in the isolated segment of bowel, compared with similiar animals with isolated loops of bowel, feeding normally. This suggests that humoral agents probably mediate part of the trophic effect of luminal nutrition, a concept supported by the fact that ileal infusion of glucose increases epithelial mass in the jejunum (244). In the present study, serum gastrin levels did not change significantly in relation to the changes in CCPR, and it seems very unlikely that this peptide can be

responsible, even in part, for the kinetic changes observed in these models. In contrast, however, serum concentrations of enteroglucagon changed in parallel to the CCPR in the terminal ileum. These changes in enteroglucagon after jejuno-ileal bypass, are in keeping with those reported after this procedure performed in man in the management of morbid obesity (70,149,245,246). As with proximal intestinal resection, greater quantities of unabsorbed luminal nutrients are presented to the terminal ileum after jejuno-ileal bypass. This segment of bowel is particularly rich in EG cells (135) and these cells are stimulated to secrete enteroglucagon, mainly by luminal carbohydrates and long chain fatty acids (137,138). It is well known that after an initial period of weight loss, patients who have had jejuno-ileal bypass for obesity, may regain a considerable amount of weight. This is probably due to the adaptation which takes place in the ileum in continuity. When patients with bypassed bowel have required repeat laparotomy, besides the gross enlargement of the residual adapted bowel in continuity, light and electron microscopic evaluation of the bypassed segments has surprisingly revealed no obvious evidence of widespread mucosal atrophy, as would be expected, although the segments had been bypassed from the alimentary stream for a number of years (247,248), and it has been suggested that non luminal or systemic factors are sufficient to maintain small intestinal mucosal integrity for long periods in the absence of intestinal chyme. It has been suggested that enteroglucagon, which may be as much as 16-fold elevated in the plasma postprandially after jejuno-ileal bypass, is the trophic hormone responsible for this (136). The data from this study, support this concept.

CONCLUSION

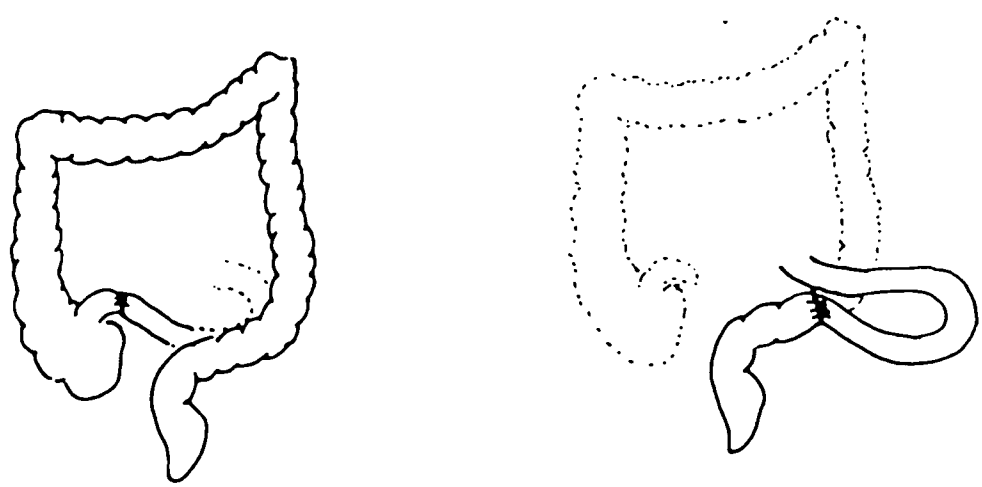
In the models studied, significant adaptive hyperplasia occurs in the terminal ileum in continuity after jejuno-ileal bypass compared with intestinal transection. However, the adaptation (at 12 days) after an equivalent intestinal resection, is significantly greater. As with intestinal resection, luminal nutrition is important, but it is likely that part of the effect of luminal nutrition is mediated via humoral agents. The results of this study indicate, that while gastrin does not fulfil the criteria for such a role, enteroglucagon must remain a favoured candidate.

CHAPTER 7

THE EFFECT OF COLECTOMY ON CELLULAR PROLIFERATION IN SMALL BOWEL, AND SERUM GASTRIN AND ENTEROGLUCAGON LEVELS

INTRODUCTION

After total colectomy or panproctocolectomy and ileostomy, the ileostomy effluent is initially watery and profuse, with electrolyte loss, and this may on occasion, be difficult to control. However, after a variable period of time, the effluent becomes more solid, less profuse, and very much easier to manage. This is due to ileal adaptation, and it has been shown by morphometric, cell proliferative and absorptive studies, that ileal adaptation after total/subtotal colectomy, takes place in rats (87), and man (86,249). Because of the unaltered length of small bowel after total colectomy it is unlikely that luminal nutrition or PBS could be implicated in bringing about these adaptive changes. The following study was undertaken to investigate further the adaptive changes in the terminal ileum after total colectomy, and the possible trophic role of gastrin and enteroglucagon on cellular proliferation in the terminal ileum.



(a) (b)

Fig 7.1: Ileal transection (a), and total colectomy and ileo-rectal anastomosis (b).

MATERIAL AND METHODS

Eight male Wistar rats had a total colectomy, and continuity was restored by an end-to-end ileo-rectal anastomosis. A further eight rats acted as controls, and had an ileal transection just proximal to the ileo-caecal valve, with an end-to-end anastomosis to restore continuity (Fig 7.1). All rats weighed 200-250g at the time of surgery. Anaesthetic was by intramuscular Hypnorm (fentanyl and fluanisone) and intraperitoneal Valium (diazepam). All anastomoses were constructed with a single layer of 6/0 black silk. The animals were killed on the 12th post operative day. A record was kept of the daily food intake of the animals, and weight changes of the time of the study. On the 12th post operative day, all animals were given vincristine (Oncavin, Eli Lilly and Co. Ltd., Basingstoke, UK) at 9.30hrs, by intraperitoneal injection, in a dose of 1mg/kg body weight, for cell kinetic measurements. Animals were then killed at 20 minutes intervals by ether anaesthesia, and blood was taken by direct cardiac puncture. The blood was placed in heparinised tubes containing 0.2ml of aprotinin (Trasylol 20,000KIU/ml), centrifuged immediately, and the plasma stored at -20°C to await radioimmunoassay for plasma gastrin and enteroglucagon.

RADIOIMMUNOASSAY:

Assays for enteroglucagon (216) and gastrin (217) were performed as described in Chapter 3.

CRYPT CELL PRODUCTION RATE (CCPR) (213,218):

This was performed on tissue obtained from the terminal ileum of rats in both groups, as described in Chapter 3.

STATISTICAL METHODS:

The students t-test for unpaired data was used for the group analysis, and results are given as mean and standard error of the mean.

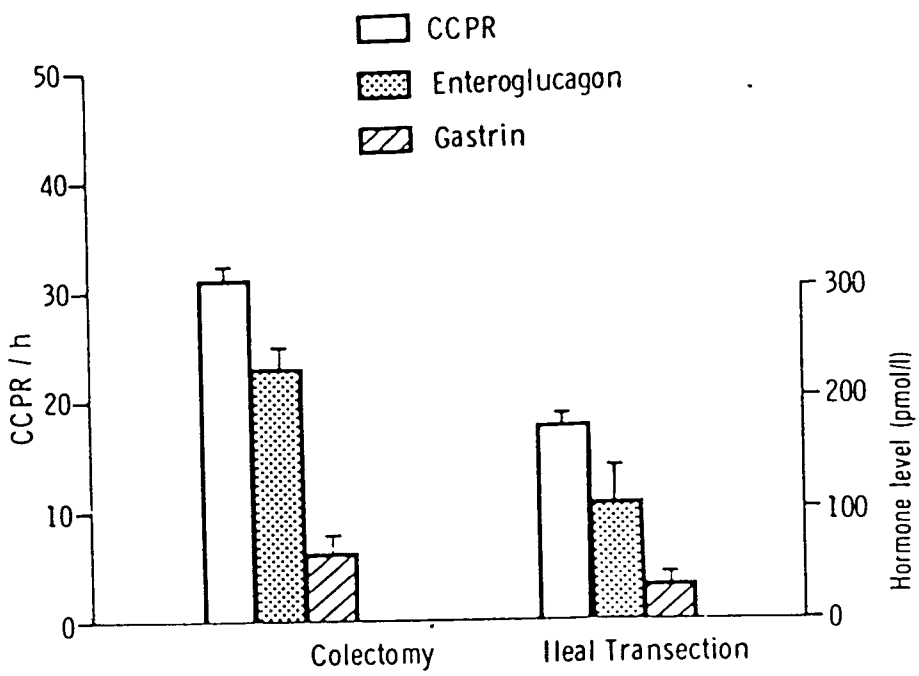


Fig 7.2: Crypt cell production rate per hour (CCPR/h) in the terminal ileum, and plasma enteroglucagon and gastrin, after colectomy and ileal transection.

RESULTS

WEIGHT CHANGES AND FOOD INTAKE:

Rats after total colectomy lost 38.4 ± 6g in weight, while those with ileal transection only lost 10.4 ± 7g ($P < 0.001$). There was no significant difference in food intake per day in the colectomy and transected groups (21.4 ± 3g and 20.2 ± 6g).

CRYPT CELL PRODUCTION RATE (CCPR) (Fig 7.2):

The CCPR in the terminal ileum after ileal transection was 18 ± 0.9 cells per crypt per hour. After total colectomy and ileo-rectal anastomosis, the CCPR in the terminal ileum increased to 31.2 ± 1.19 cells per crypt per hour ($P < 0.001$).

PLASMA ENTEROGLUCAGON AND GASTRIN (Fig 7.2):

The plasma enteroglucagon level after ileal transection was 108.8 ± 3.2pmol/l, and this increased to 234.5 ± 20.8pmol/l after total colectomy and ileo-rectal anastomosis ($P < 0.005$). Similiarly, plasma gastrin increased from 23.11 ± 3.8 pmol/l in ileal transected animal, to 62.5 ± 11pmol/l after total colectomy ($P < 0.005$).

DISCUSSION

The CCPR findings in this study are in agreement with previous reports (86, 87,249), that ileal adaptation takes place after total colectomy, and clinical impressions related to the reduction in ileostomy effluent and its change in character with time, support this. This being the case, in view of the unaltered length of small bowel in the two groups and the similiar food intake, it is unlikely that luminal nutrition can be held responsible for these changes.

It is possible, therefore, that humoral factors may account for these changes. In this study, both enteroglucagon and gastrin were significantly raised after total colectomy compared with controls, and both these peptides may account for the ileal changes seen. However, previous studies (110,111) and results of the data in the preceeding chapters, suggests that gastrin plays little if any role in small bowel adaptation.

Besides the effect of humoral agents, it is possible that motility changes may be involved. A decrease in intestinal motility and a delay in gastric emptying is known to occur after intestinal resections (40) and this is more pronounced after proximal than distal resections. In this respect, enteroglucagon may be involved. This peptide, together with another distally located peptide, neurotension, has been implicated in the 'ileal brake' mechanism whereby ileal fat perfusion results in an inhibition of jejunal motility and delays caudal transit of jejunal contens (250). Enteroglucagon is known to potently inhibit pentagastrin-stimulated gastric acid secretion in the rat (134), and thus almost certainly acts as an enterogastrone. The

major concentration of enteroglucagon (EG) cells in the terminal ileum (135) is in keeping with a role for this peptide in the ileal adaptation seen in this study. Indeed, this may account for the diminished adaptation after ileal resections compared with proximal small bowel resection (251). However, after total colectomy, the'ileal brake'(250) is still intact.

CONCLUSION:

This study confirms ileal adapation after total colectomy. In this respect, it is unlikely that luminal nutrition can be implicated in the pathogenesis of this adaptive response. The situation of the EG cells, and the role of this peptide as an enterogastrone and its involvement in the 'ileal brake' mechanism, makes enteroglucagon a favoured candidate for a humoral role in the ileal adaptation after total colectomy.

CHAPTER 8

THE RELATIONSHIP BETWEEN CELL PROLIFERATION AND ENDOGENOUS GUT HORMONES (GASTRIN AND ENTEROGLUCAGON) IN MODELS OF INTESTINAL ADAPTATION

INTRODUCTION

In the previous chapters (2-7), models of intestinal adaptation have been used to determine the cell proliferation (CCPR) and plasma gastrin and enteroglucagon levels, twelve days after the procedure. The results of these experiments, make it possible to determine the relationship of these two peptides to cellular proliferation in the models studied.

MATERIAL AND METHODS

The models used, have been described in detail in the previous chapters. These were:- 75% proximal small bowel Thiry-Vella fistula, with and without TPN (Chapter 3), 75% proximal small bowel resection, and jejunal transection, with normal feeding, hyperphagia or TPN (Chapter 4), pancreatico-biliary diversion into the colon, with and without 75% proximal small bowel resection (Chapter 5), 75% proximal small bowel jejuno-ileal bypass (Chapter 6), total colectomy or ileal transection (Chapter 7). One hundred and four male Wistar rats were used. The methods of anaesthesia and operative details have been described in the relevant chapters. All animals were killed on the twelfth post operative day. The crypt cell production rate (CCPR) in the terminal ileum (213,218) and plasma levels of gastrin (217) and enteroglucagon (216) were determined, as described in detail in Chapter 3. Taking the data from each of these 13 groups, the CCPR in the terminal ileum was correlated with plasma gastrin and enteroglucagon levels, using the Spearman rank method.

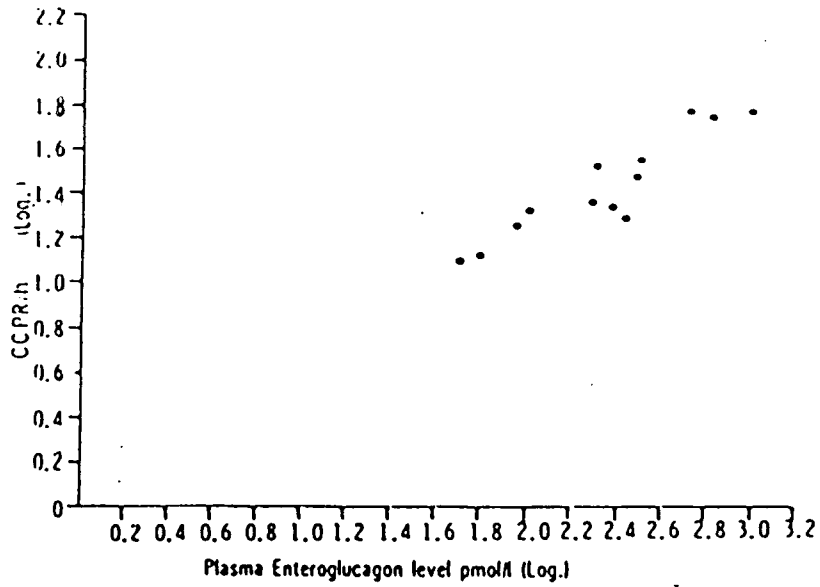


Fig 8.1: Correlation between crypt cell production rate per hour (CCPR/h) and plasma enteroglucagon in different models of adaptation.

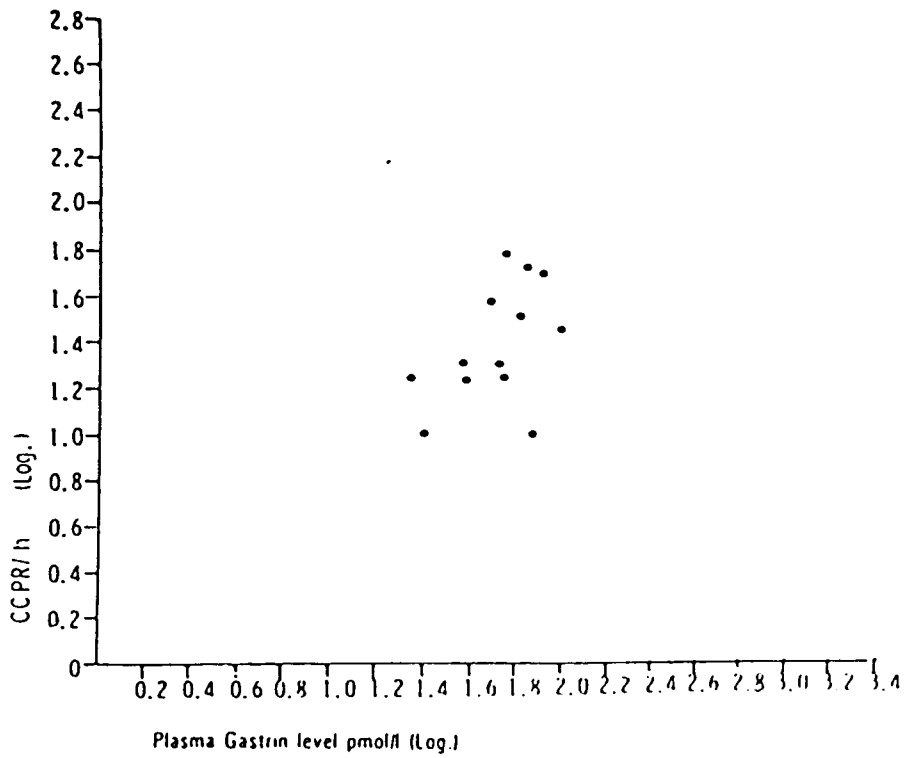


Fig 8.2: Poor correlation between CCPR/h and gastrin levels in the various models.

RESULTS

The relationship of CCPR and hormone levels is shown in Figures 8.1 and 8.2. There was a strong correlation between terminal ileal CCPR and plasma enteroglucagon levels in the different models; Spearman rank correlation coefficient, r_s=0.649 ($0.01<P<0.02$). However, a poor relationship between CCPR and gastrin was found r_s=0.439 ($0.2<P<0.3$).

DISCUSSION

The finding in this study, that there was a very poor correlation between plasma gastrin levels and the CCPR in the terminal ileum in the various models of adaptation suggests that gastrin probably has little, if any, trophic influence on the small bowel. Although the results of the two studies (104,106) employing the administration of pentagastrin, have suggested a trophic role for gastrin on small bowel, the vast majority of reports do not confirm this, and support the results of this study. Thus, pentagastrin administration for 15 days, had no influence on the distal duodenum and jejunum in rats, although there were increases in weight and height of the proximal duodenum (105). Furthermore, pentagastrin administration failed to abolish the hypoplasia in small bowel after resection with TPN; although there was an effect more proximally (107). Antrectomy has no effect on the growth of the small bowel, nor does it influence the adaptive response to partial enterectomy (108), and intragastric administration of gastrin has no trophic effect on the ileum (110). Thus, while gastrin almost certainly has a trophic influence on the stomach (with the exception of the antrum), duodenum, colon and pancreas (102,111,112,113,114,117,118), the results of the present study and of other studies (105,107,108,110) suggests that gastrin has little trophic influence on small bowel. In support of this is the fact that although marked hyperplasia of gastric mucosa is found in cases of the Zollinger-Ellison syndrome (119), small bowel mucosal hyperplasia has not been recorded in this condition, or other conditions with hypergastrinaemia, such as pernicious anaemia or antral G-cell hyperplasia.

In contrast to gastrin, there was a very strong correlation between

terminal ileal CCPR and plasma enteroglucagon in the models studied. Clearly, while these results do not demonstrate a cause-and-effect relationship, they suggest strongly that enteroglucagon may be involved in the adaptive process, either as the final factor in the pathway, having been released by luminal products such as food, or possibly by stimulating the release of some other, as yet unrecognised, trophic agent. At the present time, there is a lack of sufficient pure enteroglucagon available for direct infusion studies. Nevertheless, an enteroglucagon-enriched extract of rat intestine has recently been shown to produce a dose-dependent stimulation of DNA in guinea-pig jejunal mucosa over a concentration range similiar to that following intestinal resection (163), and this supports the results of the present study. Besides the trophic effects on small bowel noted in a patient with an enteroglucagon producing renal tumour (124,125), further support for the trophic effect of enteroglucagon on small bowel is derived from the findings of markedly raised enteroglucagon levels in clinical situations where intestinal adaptation is known to occur, such as untreated coeliac disease (143,144) tropical malabsorption (145) after small bowel resection (69) and jejuno-ileal bypass (70,149) and when healthy infants commence enteral feeding (155). Indeed, the distal situation in the gut of the EG cells (135), the anatomy of these cells (71,129,136) and their ability to respond to changes in intraluminal fats and carbohydrates (137,138) all fit in with the concept of a role for enteroglucagon in the regulation of mucosal growth.

CONCLUSION

The results of this study suggest that gastrin has little trophic influence on small bowel mucosa. Conversely, enteroglucagon displays a strong correlation with CCPR in the terminal ileum in various models of intestinal adapatation and must be regarded as a possible factor in the regulation of small bowel growth.

CHAPTER 9

THE ENTEROGLUCAGON RELEASE PATTERN AFTER SMALL BOWEL RESECTION, AND ITS RELATIONSHIP TO CELLULAR PROLIFERATION

INTRODUCTION

In the predeeding studies, the plasma enteroglucagon levels in models of adaptation, have been shown to be closely correlated with cell turnover in the small bowel. Both enteroglucagon and CCPR, have been measured at 12 days after the operation, and although a close relationship exists between these two parameters at this time, the following study was undertaken to determine whether enteroglucagon rises rapidly enough to be involved in the early cellular response to intestinal resection and whether its influence persists in the maintenance of adaptive changes in the long term.

MATERIAL AND METHODS

Male Wistar rats, weighing 250-350g at the time of surgery, were used for the study. The animals (n=96) were anaesthetised with intramuscular Hypnorm (fentanyl and fluanisone) and intraperitoneal diazepam (Valium). Half the animals had a 75% proximal small bowel resection, while the rest had an intestinal transection and re-anastomosis, just distal to the ligament of Treitz, as described in Chapter 4. All animals were allowed food ad libitum (pelleted rat diet). A record was kept of the food intake and weight changes over the experiment. Groups of rats (N=7-10) were killed at 1.5,3,6,12,24 and 48 days following surgery. On these days rats were given vincristine 1mg/kg body weight, by intraperitoneal injection at 9am. The first animal was killed 30 minutes after this, and the following animals in the group were killed serially at 20 minute intervals thereafter, by ether anaesthesia. Blood (5-10ml) was taken by direct cardiac puncture, and placed into heparinised tubes containing 0.2ml of aprotinin (Trasylol, Bayer), centrifuged, and the separated plasma stored at -20°C to await assay. At the same time, 5cm segments of terminal ileum were taken for cell kinetic studies and prepared as described in Chapter 3.

CRYPT CELL PRODUCTION RATE (213,218):

This was performed as described in Chapter 3, and results expressed as cells per crypt per hour.

PLASMA ENTEROGLUCAGON ASSAY (216):

Radioimmunoassay of plasma for enteroglucagon, was performed as described

in Chapter 3, and results are given as pmol/l.

STATISTICAL METHODS:

The students' t-test for unpaired data was used for inter-group analysis, and results are given as mean and standard error of the mean.

TABLE 9.1

AVERAGE CUMULATIVE FOOD INTAKE PER ANIMAL (g, MEAN ± SEM)

Days	Transection		Resection	
	food g	obser-vations	food g	obser-vations
1.5	6±1	8	8±2	10
3	23±2	8	23±2	7
6	48±7	7	98±14	8
12	108±12	8	143±24	8
24	280±14	10	501±23	8
48	926±72	7	1,059±45	7

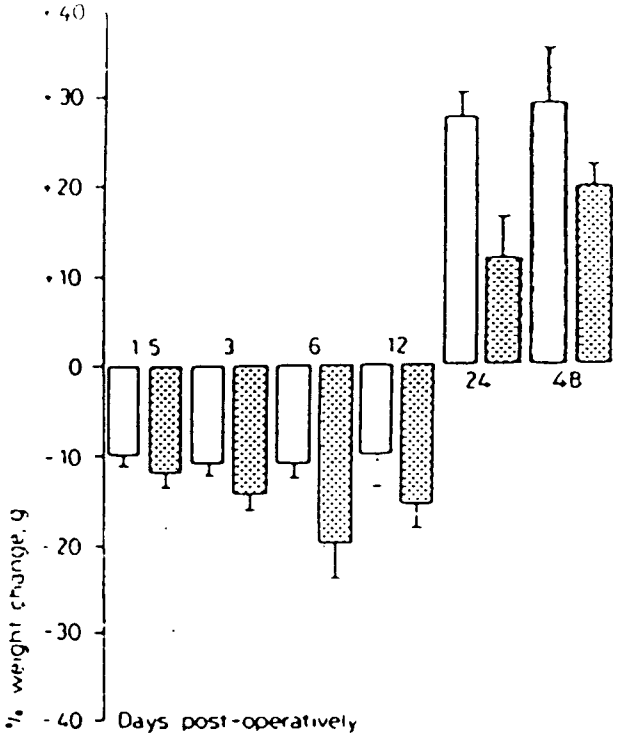


Fig 9.1: Percentage weight change in animals over the 48 day period.

□ = transection. ▨ = resection.

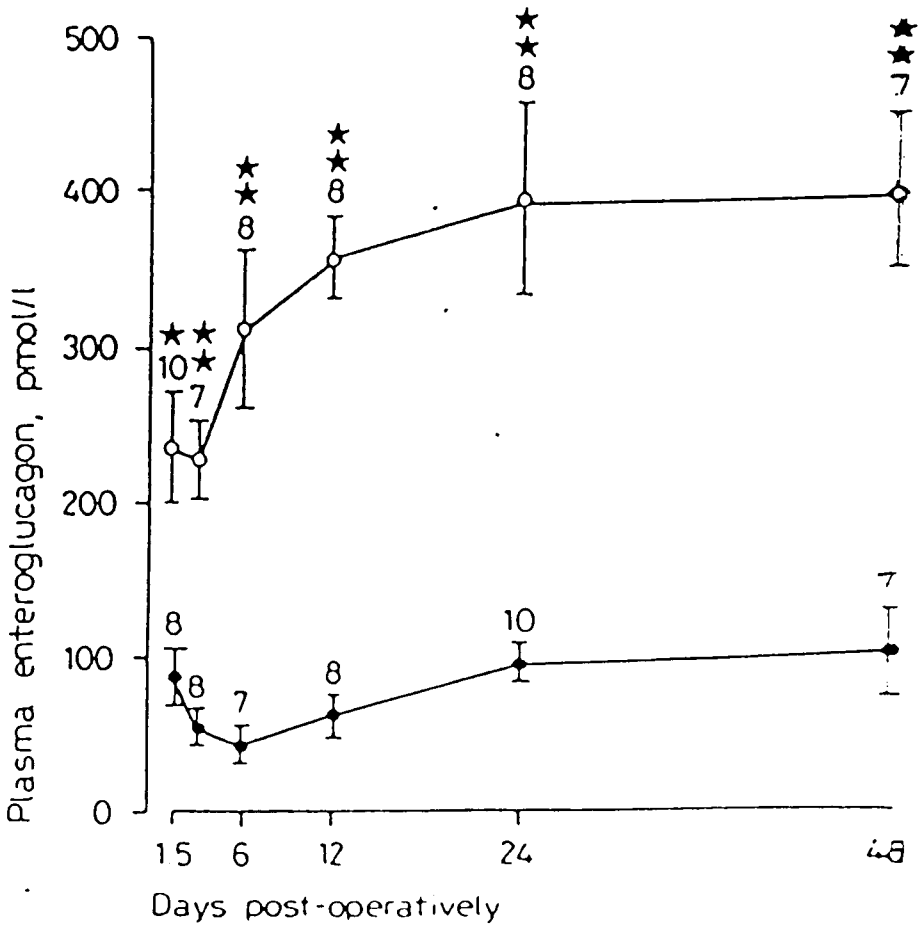


Fig 9.2: Plasma enteroglucagon levels (mean ± SEM) plotted against time after operation.

* = P<0.005; ** = P<0.001. numbers above each point denote n value.

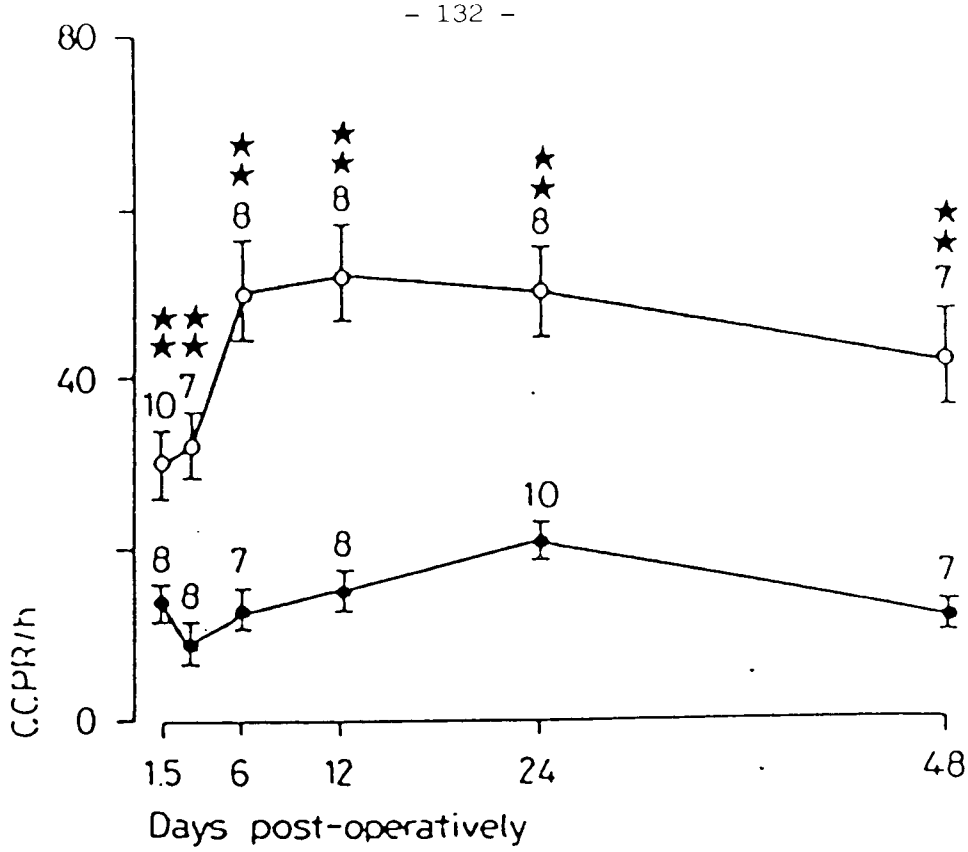


Fig 9.3: Crypt cell production rate per hour (CCPR/h; mean ± SEM) plotted against time after operation.
o = resection; ● = transection; ** = P<0.001.
numbers above each point denote n value.

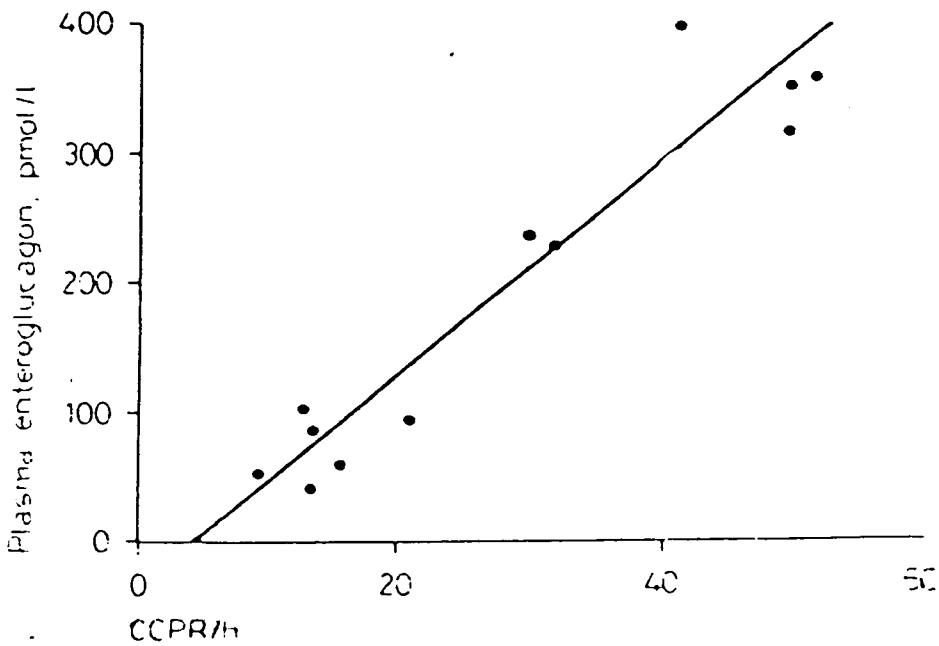


Fig 9.4: Correlation between CCPR/h and plasma enteroglucagon.

RESULTS

WEIGHT CHANGES AND FOOD INTAKE:

These are shown in Fig 9.1 and Table 9.1 respectively. Both resected and transected animals lost weight in the initial post operative period, but then began gaining weight, and this weight gain was well established by 24 days. Throughout the experiment, resected animals lost more weight than transected controls, despite a higher food intake.

PLASMA ENTEROGLUCAGON AND CCPR:

Plasma enteroglucagon levels over the 48 days in both resected and transected animals, is shown in Fig 9.2, while the corresponding changes in CCPR are shown in Fig 9.3. Resected animals had plasma enteroglucagon levels three times greater than controls while CCPR was doubled as early as 1.5 days. Thereafter, there was a steady rise in both enteroglucagon and CCPR, and once the highest mean values were reached (CCPR between 6-12 days and enteroglucagon at 24 days) the levels remained steady until 48 days. In transected animals, there was a small fall in entero-glucagon and CCPR levels early on, followed by a slow rise to levels found at 1.5 days. Taking all groups as a whole, there was a strong correlation ($r=0.95; P<0.001$) between plasma enteroglucagon concentrations and CCPR throughout the experiment (Fig 9.4).

DISCUSSION

Previous studies (85,86,108,158) have indicated, that intense hyperplasia of ileal mucosa is well established within 48 hours of proximal small bowel resection. This 'anticipatory' adaptation occurs at a time when food intake is not fully established, and mechanisms other than luminal nutrition must be operative. This is supported by the finding in this study, of a significantly greater CCPR in resected compared with transected rats at 1.5 days. The food intake at this stage was minimal once again supporting the concept that a systemic factor, other than luminal nutrition, might be operative. Enteroglucagon levels at this early stage (1.5 days) were significantly greater in resected, compared with transected animals. Although luminal contents, in particular, carbohydrates and triglycerides, are known to be the major stimulants of enteroglucagon release (137,138), it has been shown in rats nourished exclusively by TPN, that resected animals have significantly greater levels of plasma enteroglucagon and greater cell production rates in the terminal ileum, compared with transected animals (97), although these levels were well below those in rats taking food by mouth. Thus, other, albeit less potent stimuli to enteroglucagon release after intestinal resection, must exist, and may be particularly relevant to the enteroglucagon and CCPR levels after resection at 1.5 days, in this study.

Throughout the study, a close parallelism was seen between plasma enteroglucagon and CCPR levels, both in the resected and transected animals, with a very close correlation between these two parameters. This does not mean a cause-and-effect relationship, but provides further strong

circumstantial evidence for enteroglucagon as an 'enterotrophin'. Miazza et al (84), using the model of pancreatico-biliary diversion (PBD), have shown intestinal mucosal hyperplasia and high plasma levels of enteroglucagon, eight days after the procedure, but at three months after PBD, although the degree of mucosal hyperplasia was maintained, plasma enteroglucagon had returned to normal. This suggested that enteroglucagon may play a part in initiating the adaptive process, but does not contribute to its maintenance. This is in contrast to the findings in the present study, in which high levels of enteroglucagon were found in the plasma for the duration of the experiment up to 48 days, and is in agreement with the finding of raised plasma enteroglucagon in man after small bowel resection, both in the basal and postprandial states (69).

CONCLUSION

The findings in this study, not only confirm a close relationship between enteroglucagon levels in the plasma and mucosal enterocyte production rate at different times after small bowel resection, but also show that levels of this peptide do rise quickly enough to be involved in the initial rapid 'anticipatory' adaptation after resection, and are persistent enough to be involved in the maintenance of this adaptation. The finding of a very early rise in CCPR, before oral feeding is fully established, argues for a humoral factor in the mechanism of adaptation, and enteroglucagon appears to be a strong candidate for such a role.

CHAPTER 10

THE EFFECT OF EXOGENOUS HORMONES ON PLASMA ENTEROGLUCAGON AND CELL PROLIFERATION AFTER INTESTINAL RESECTION

INTRODUCTION

Enteroglucagon has emerged as a favoured candidate for a role as 'enterotrophin' to small bowel mucosa. Although luminal nutrients are important stimulants for the release of enteroglucagon (137,138), with alterations in the amount of orally ingested food effecting both plasma enteroglucagon levels and cell turnover after intestinal surgery (97), other factors may well contribute to the release of this peptide. and hence effect cell proliferation under certain conditions. Thus, the marked rise in CCPR and plasma enteroglucagon which occurs very early after intestinal resection (85,86,108,158,252) is not entirely related to luminal nutrition, as food intake at this stage is minimal. Furthermore, rats nourished solely on TPN, exhibit a modest but significant rise in enteroglucagon and CCPR after intestinal resection, compared with transected controls (97).

In the following study, somatostatin which suppresses (142), and bombesin which stimulates (140,141) the release of enteroglucagon, were administered to rats after intestinal resection and transection, and the effect of these peptides on plasma enteroglucagon and cell proliferation was observed.

MATERIAL AND METHODS

Forty-eight male Wistar rats, weighing 200-250g at the time of surgery, were used in the study. Half the rats had a 75% proximal small bowel resection, while the remainder had a jejunal transection only, as described in detail in Chapter 4. Diazepam and Hypnorm were used as anaesthetic. Animals were allowed water and food (pelleted rat diet), ad libitum. The 48 animals were divided into 3 groups, each group comprising 8 animals with resection, and 8 with transection. Group 1, was given long acting somatostatin (Des-AA^{1,2,4,5,12,13}(D-Trip^{8})-SS) (253), 100μg in 100μl rat plasma subcutaneously twice a day for the last seven days of the experiment. Group 2 animals were given bombesin for a similiar period, and this was administered via the Alzet osmotic minipump, model 2001 (Scientific Marketing Associates, London), which was implanted subcutaneously at the back of the animals neck. Each pump, which delivers a constant flow at a rate of 1μl/hour for one week, and has a capacity of 168μl, was filled with a solution of bombesin, 96μg in 100μl normal saline. Group 3 animals were given a minipump containing saline only, over the last 7 days of the experiment, and this group acted as control.

On the 12th post operative day, animals were given Vincristine, 1mg/kg body weight by intraperitoneal injection, at 9.00hrs. The first rat was killed at 9.30am, and the following animals serially at 20 minute intervals thereafter. Five cm segments of terminal ileum were taken for cell kinetic studies, as described in Chapter 3. At the same time, blood was taken by direct cardiac puncture, and placed in heparinised tubes containing 0.2ml of aprotinin (Trasylol, Bayer), centrifuged, and the separated plasma stored at -20°C to await assay.

A record was kept of the food intake and weight changes over the course of the experiment.

CRYPT CELL PRODUCTION RATE (213,218):

This was performed as described in Chapter 3, and results expressed as cells per crypt per hour.

PLASMA ENTEROGLUCAGON ASSAY (216):

This was performed as described in Chapter 3 and results expressed in pmol/l.

STATISTICAL METHODS:

The students t-test for unpaired data was used for group analysis, and results given as mean and standard error of the mean.

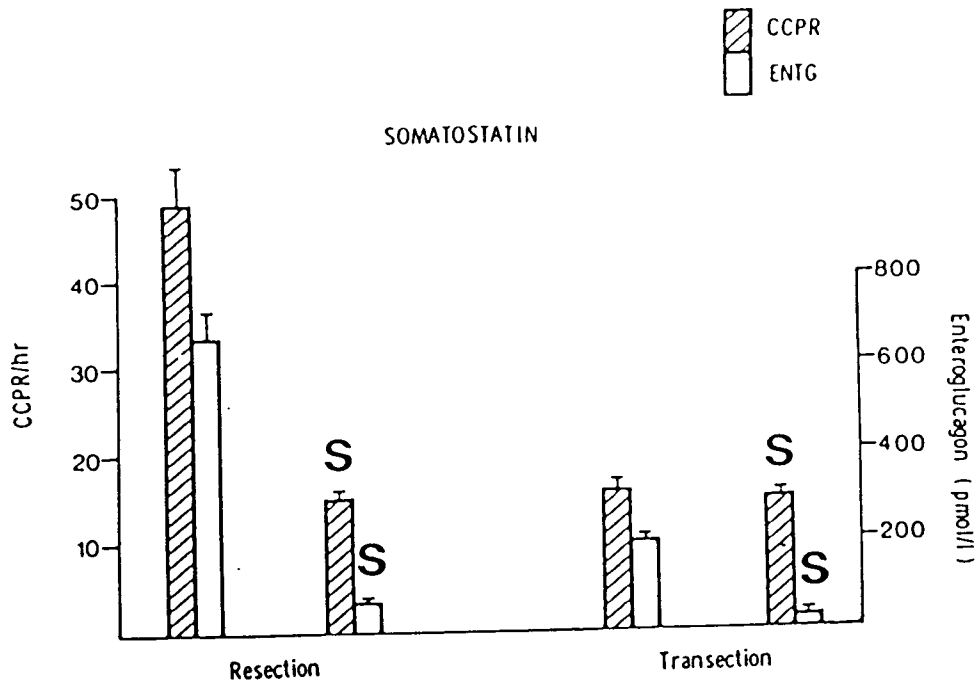


Fig 10.1: Plasma enteroglucagon and CCPR/h after 75% resection and jejunal transection, in animals administered somatostatin and controls having saline only. S = somatostatin.

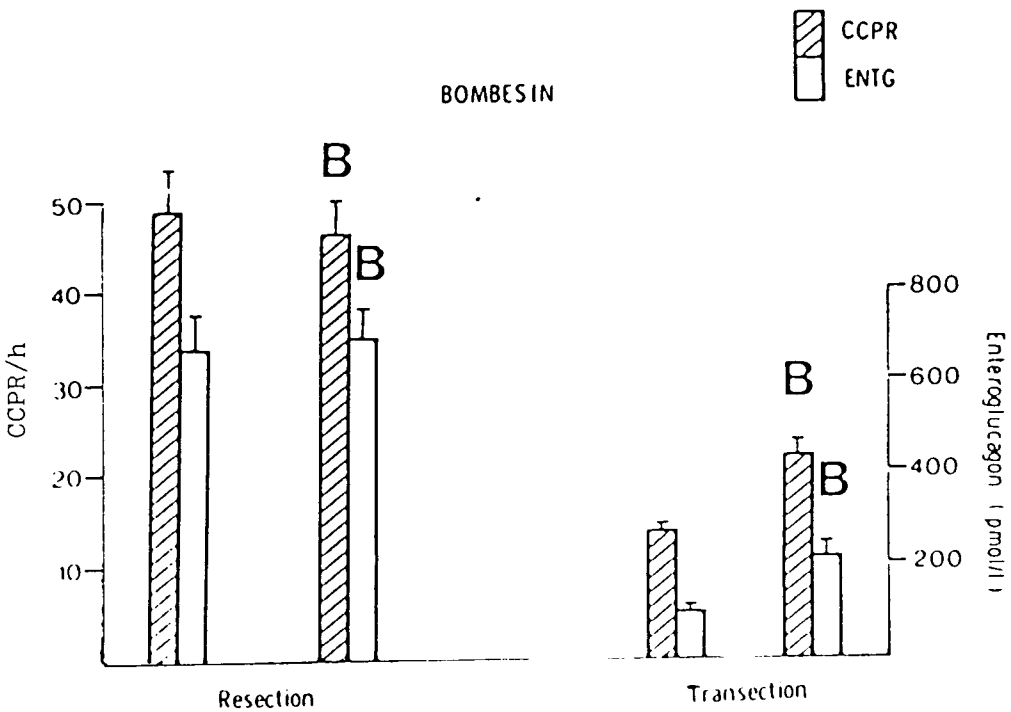


Fig 10.2: Plasma enteroglucagon and CCPR/h after 75% proximal small bowel resection and jejunal transection, in animals having bombesin administration, and controls with saline administration. B = bombesin.

RESULTS

FOOD INTAKE AND BODY WEIGHT:

There was no significant difference in food intake per day between the three groups (21.4±3g, 23.1±2g and 24±3g respectively), nor were the weight changes significantly different, with all animals approaching their pre-operative weights at the conclusion of the experiment.

CCPR AND PLASMA ENTEROGLUCAGON (Figs 10.1,10.2):

In the control animals (Group 3), there was an increase in CCPR/hr in the terminal ileum from 16.8±0.9 in transected, to 49.2±4.9 in resected animals ($P<0.001$) (Fig 10.1). Similiarly, plasma enteroglucagon increased from 99.1±9.6pmol/l in transected, to 667±70.1pmol/l in resected rats ($P<0.001$).

After administration of somatostatin (Group 1), rats with resection had a fall in CCPR to 15.4± compared with resected control rats (Group 3) ($P<0.001$) (Fig 10.1). There was also a fall in plasma enteroglucagon in resected Group 1 rats compared with resected controls (Group 3) ($P<0.001$). Although there was a fall in plasma enteroglucagon in transected rats administered somatostatin (26.3±8.9pmol/l) compared with control animals ($P<0.001$), there was no significant change in CCPR in the two groups.

After administration of bombesin (Group 2), transected rats showed a rise in CCPR to 24.5±1.9 ($P<0.005$) with a concommitant rise in enteroglucagon to 218±34pmol/l ($P<0.005$) compared to transected controls, (Fig. 10.2).

However, the resected bombesin treated rats showed no change in CCPR and plasma enteroglucagon compared with the resected control group.

DISCUSSION

The data in this study show that small intestinal cell proliferation can be influenced by gastro-intestinal hormones. There are, of course, a number of possible explanations for these results. It may be, that bombesin and somatostatin have a direct influence on cell proliferation. Bombesin administration has been shown to induce antral gastrin cell hyperplasia (254) and thus increase both antral and serum gastrin concentrations in rats (255). Its direct effect on small bowel mucosa is unknown. Similiarly, somatostatin, which has a wide range of inhibitory actions on the gut, may have a direct anti-trophic influence on cell proliferation. Secondly, these peptides might exert their influence on cell proliferation, by inducing alterations in luminal nutrition, intestinal secretions or other hormeostatic mechanisms. In this study, there was no difference between the groups in terms of food intake and weight changes, which makes this second possibility unlikely. This is supported by the results of other workers (256) who found that somatostatin infusion for 4 days in rats, had no effect on either plasma concentrations of glucose, insulin, glucagon, growth hormone and cyclic AMP, or on body weight gain, food consumption or water intake.

Finally, it is possible that both bombesin and somatostatin may have induced the effects seen, by influencing other trophic hormones. The influence of these peptides on gastrin is well established. Bombesin, a tetradecapeptide first isolated from amphibian skin, has been shown to have a powerful stimulatory effect on gastric acid secretion (257) and

the release of gastrin from the gastric antrum (255,258) and is consequently also called gastrin-releasing peptide (GRP). Somatostatin, on the other hand, suppresses gastric acid secretion (259,260) and the release of gastrin (260,261,262,263). Gastrin was not measured in this study, but as it is not thought to have any significant trophic influence on small bowel mucosa (105,107,108,109,110), it is unlikely that the changes in cell proliferation after administration of bombesin and somatostatin noted in this study, could have been mediated via this peptide. Somatostatin administration in this study, resulted in a fall in plasma enteroglucagon concentrations, in both transected and resected rats, while bombesin administration produced a rise in enteroglucagon only in transected animals, the resected group showing no change compared to controls. Changes in CCPR showed a similiar trend to changes in enteroglucagon, excepted that somatostatin administered transected animals did not have a fall in CCPR to follow the fall in enteroglucagon, and it may well be, that there is a threshold below which it is not possible to inhibit cellular proliferation by somatostatin administration. Similiarly, the inability of bombesin to increase both enteroglucagon and CCPR still further after intestinal resection, could represent a limiting factor over and above which it is not possible to increase cell proliferation and peptide secretion, from the already very high levels observed after 75% proximal small bowel resection. In view of the close relatioship between the changes in plasma enteroglucagon and CCPR observed in this study, it is possible that the effect of somatostatin and bombesin on cellular proliferation are mediated via enteroglucagon, although the data do not allow a distinction to be made between this, or other possible modes of action, such as a direct effect of these two peptides on cell turnover.

CONCLUSION

The results of this study indicate that hormones can influence cellular proliferation in small bowel mucosa. It is possible that these changes may be mediated via changes in enteroglucagon, although further investigation is required to elucidate the exact mechanism. The data, however, provide further circumstantial evidence, for the involvement of enteroglucagon in the mechanism of small bowel adaptation.

CHAPTER 11

MECHANISM OF RELEASE OF ENTEROGLUCAGON

INTRODUCTION

The major stimuli for the release of enteroglucagon after a meal are carbohydrates and long chain fatty acids (137,138). The enteroglucagon cells (EG cells) are found distally in the gut where the highest concentration is in the ileal mucosa, with lesser, but still significant concentrations found in the colon (135). The finding of raised concentrations of enteroglucagon in coeliac disease (143,144), tropical malabsorption (145), infective diarrhoea (146), after total pancreatectomy (147) and in patients with chronic pancreatitis and steattorrhoea (148), after small bowel resection (69) and jejuno-ileal bypass (70,149), all situations where there is rapid intestinal transit, has prompted the hypothesis that these raised levels of the peptide are brought about by abnormal and excessive exposure of the distal intestine, and thus the major concentrations of EG cells, to chyme which is rich in unabsorbed nutrients. These nutrients have not been absorbed higher in the gastrointestinal tract, either because of rapid intestinal transit, or due to disease of this part of the gut, or because of surgical removal or bypass of the upper small bowel. To test this hypothesis, plasma enteroglucagon changes were measured in patients after gastric surgery, who were given an oral glucose load. These patients could be expected to have rapid upper intestinal transit due to the changes in gastric emptying consequent on the surgery. Changes in haematocrit and blood glucose, as well as subjective and objective signs of dumping were sought and these changes were compared with control subjects.

MATERIAL AND METHODS

Twenty-one patients were used in this study, and they were divided into 3 groups. Group A was comprised of 7 patients with total or near total gastrectomy with oesophago - or gastroenterostomy. Groups B consisted of 7 patients after upper partial gastrectomy, but with an intact, unstenosed pylorus. All patients in Groups A and B had an associated truncal vagotomy, and were studied between one and 18 months after surgery. Seven patients who had not had previous surgery, and were awaiting routine operations, such as hernia repair, were used as controls, and comprised Group C.

All patients were studied after an overnight fast. An indwelling 'butterfly' cannula was placed into a large antecubital vein, and serial blood samples of 10ml were taken for enteroglucagon levels, haematocrit and blood sugar estimations. The blood for hormone assay, was placed into heparin tubes containing 200µl of aprotinin (Trasylol 20,000KIU/ml), was centrifuged immediately, and the plasma stored at -20°C to await assay.

Each patient was asked to drink 200ml 50% glucose solution over 2 minutes. Blood samples were taken 15 minutes before and at the time of drinking the solution, and then at 15,30,45,60 and 90 minutes after ingestion. Throughout the test the presence of symptoms and signs of the dumping syndrome, such as abdominal fullness and pain, sweating, diarrhoea, faintness and tachycardia, were sought.

PLASMA ENTEROGLUCAGON ASSAY (216):

This was performed as described in Chapter 3.

PACKED CELL VOLUME:

The PCV was determined using a Coulter Model S Counter. Changes in haematocrit were regarded as a good index of plasma volume change (264).

BLOOD GLUCOSE:

This was calculated by the glucose oxidase method (265).

STATISTICAL METHODS:

The students t-test for unpaired data was used for group analysis, and results are given as mean and standard error of the mean.

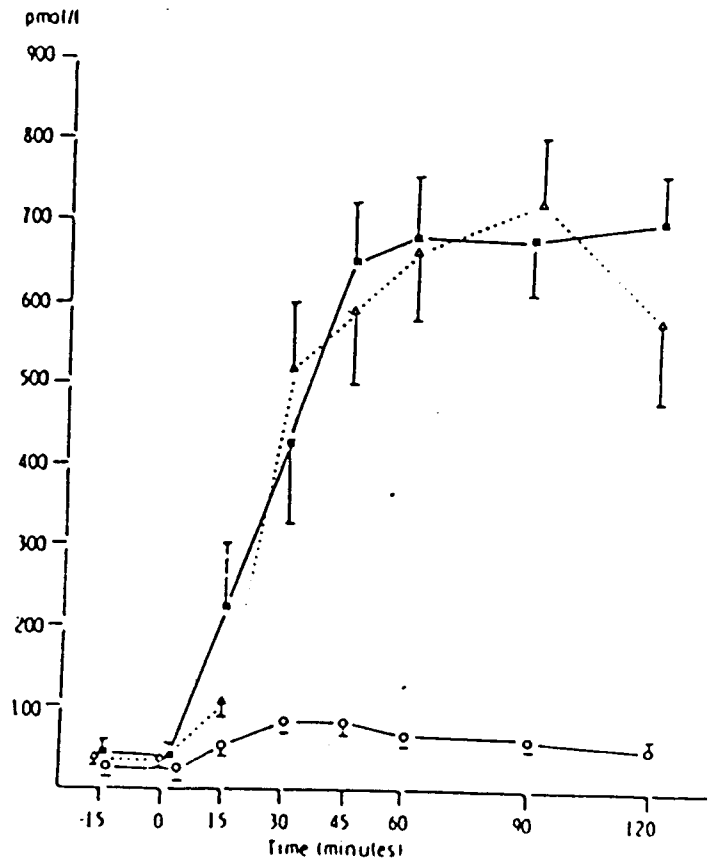


Fig 11.1: Plasma enteroglucagon levels after oral glucose. ■ = total/near total gastrectomy; Δ = upper partial gastrectomy; o = controls.

TABLE 11.1

CHANGES IN HAEMATOCRIT AFTER GLUCOSE INGESTION (mean and s.e.m. values).

	Total/near total gastrectomy (group A)	Upper partial gastrectomy (group B)	Controls (group C)
Haematocrit change (%) (basal to 45 min)	3.9 ± 0.5	3.7 ± 0.5	-0.6 ± 0.5
			$P<0.001$*
Rate of rise or fall of haematocrit (%/min) (basal to 45 min)	0.09 ± 0.01	0.08 ± 0.01	-0.01 ± 0.01
			$P<0.001$*

* P values relate to gastrectomy groups compared with controls.

RESULTS

PLASMA ENTEROGLUCAGON:

The enteroglucagon release pattern during the test in the three groups is shown in Fig 11.1. While no difference existed between Groups A and B in the level of enteroglucagon after glucose ingestion, these levels were significantly greater than in controls ($P<0.001$). Furthermore, although the mean rate of rise of enteroglucagon, measured from basal levels to 45 minutes after ingestion of glucose was similiar in Group A and B (13.3 ± 2.6pmol $min^{-1}l^{-1}$ and 12.2 ± 2.1pmol $min^{-1}l^{-1}$ respectively), this was significantly more rapid than in control subjects (1.2 ± 0.2pmol $min^{-1}l^{-1}$) ($P<0.001$).

HAEMATOCRIT CHANGES:

The change in haematocrit from basal levels to 45 min after glucose ingestion is shown in Table 11.1. While both gastrectomy groups showed a rise in haematocrit, signifying a fall in plasma volume, there was a drop in haematocrit in the control group, and the differences between the gastrectomy patients and controls, were highly significant.

BLOOD GLUCOSE:

The changes in blood glucose during the test is shown in Fig 11.2. There was a significantly greater rise of glucose in gastrectomy groups compared to the controls. Similiarly, while the mean rate of rise of glucose, measured from basal levels to 60min, was not significantly different in Groups A and B (0.1 ± 0.02mmol $min^{-1}l^{-1}$ and 0.14 ± 0.02mmol

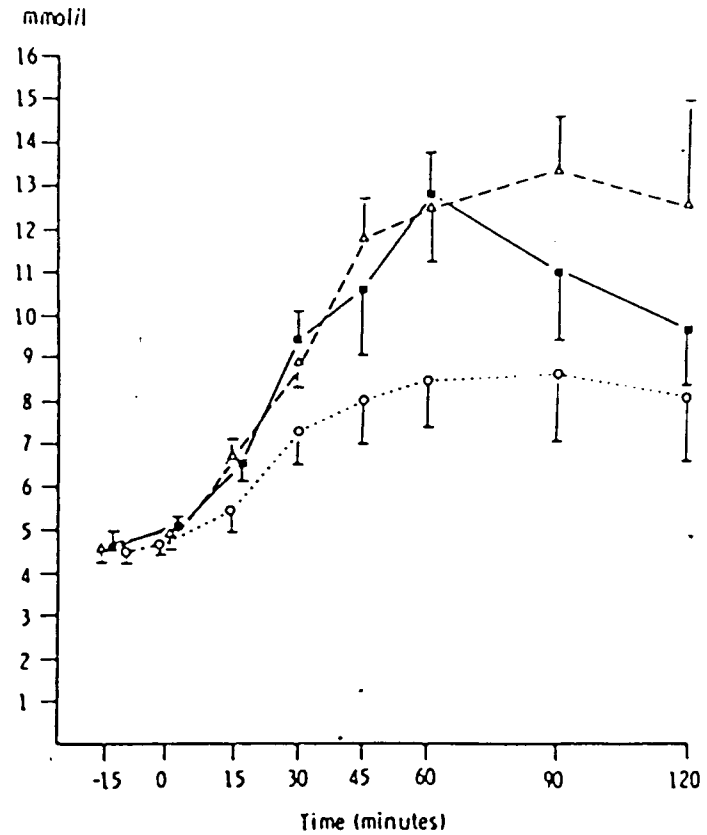


Fig 11.2: Blood glucose levels after oral glucose load. ■ = Total/near total gastrectomy; △ = upper partial gastrectomy; o = controls.

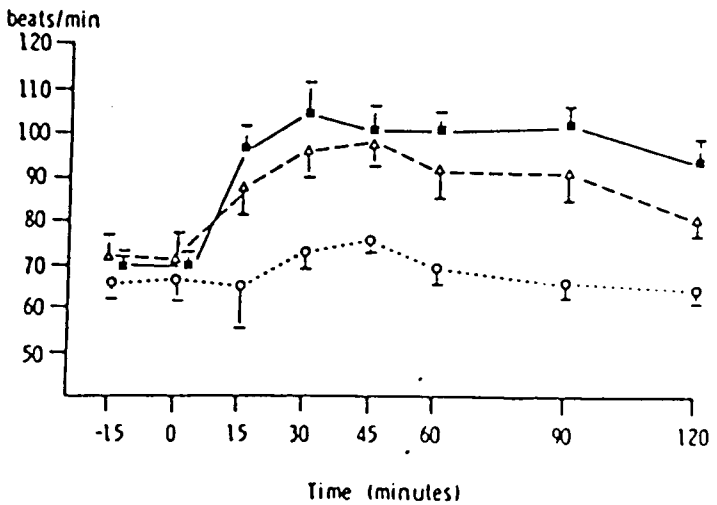


Fig 11.3: Changes in pulse rate after glucose ingestion. ■ = total/near total gastrectomy; △ = upper partial gastrectomy; o = controls.

min $^{-1}$l^{-1} respectively), these rates were significantly more rapid than in control subjects (0.06 ± 0.02mmol min^{-1}l^{-1}) ($P<0.025$ and $P<0.02$ respectively).

OCCURENCE OF DUMPING:

Six patients in each gastrectomy group experienced subjective symptoms of dumping during the test, while none of the control group were affected. Objectively, the changes in pulse rate in the three groups (Fig 11.3) were in keeping with the occurrence of dumping symptoms.

DISCUSSION

The dumping syndrome is thought to be due to rapid gastric emptying of hyperosmolar nutrients, which will attract water from the circulation, the intestinal epithelium acting as a semi-permeable membrane (266). This results in a fall in plasma volume (267), the magnitude of which is directly related to the rate of gastric emptying (268).

In the present study, a standard dumping provocation test was employed (269) to test the 3 groups. Group A patients, who had had a total gastrectomy, would be expected to exhibit dumping symptoms after the provocation test. Group B patients, had an intact pylorus, but upper partial gastrectomy has an unavoidable truncal vagotomy associated with it. Patients after vagotomy have impairment of receptive relaxation and accommodation to distension by the gastric smooth muscle with raised post prandial intragastric pressure, and this results in more rapid emptying of liquids from the vagotomised stomach (270). Group B patients thus also exhibited dumping after the provocation test, probably due to the rapid emptying of the glucose solution, while dumping did not occur in controls. The presence of the dumping syndrome in Groups A and B, was determined by subjective evidence of symptoms complained of by the patient, such as abdominal pain and fullness, nausea, faintness, sweating and diarrhoea, in addition to a significantly more rapid pulse rate in the two groups during the test, compared with controls. Furthermore, the findings of a rise in haematocrit, and a higher and more rapid increase in blood glucose in gastrectomised patients compared with controls, is in keeping with dumping in Groups A and B, but not in Group

C. Changes in haematocrit are regarded as a good index of plasma volume change (264), with a rise in haematocrit indicating a fall in plasma volume. As mentioned before, a fall in plasma volume is thought to be a major factor in the pathophysiology of the dumping syndrome (267). It is also well established that blood glucose levels after oral hypertonic glucose, are higher in patients with dumping symptoms than in asymptomatic postoperative duodenal ulcer patients (271). Thus, it seems that in the present study, the above mentioned findings in Groups A and B are due to rapid gastric emptying and increased transit down the small bowel. Plasma enteroglucagon levels were markedly raised after oral glucose ingestion, in Groups A and B, with a much greater rate of rise, compared with Group C, and it seems likely, that like the changes in haematocrit and blood glucose, the plasma enteroglucagon changes in gastrectomised patients, are also due to rapid gastric emptying and intestinal transit. In this situation, the distally placed EG cells in the ileum (135), would be subjected to a greater volume of nutrients which have not been absorbed higher up because of the rapid transit, thus releasing increased concentrations of enteroglucagon.

The findings in the above study, confirm the hypothesis, that in many situations where intestinal adaptation occurs, the raised levels of enteroglucagon found post-prandially, are due to the rapid transit of nutrients down the gut, thus bombarding the EG cells with unabsorbed nutrients. After proximal small intestinal resection or jejuno-ileal bypass, the upper small bowel is missing, and nutrients, pass from the stomach and duodenum, directly into the enteroglucagon rich ileum. The EG cells in the distal small bowel and colon (135) are thus ideally

located to respond in these situations, and the above findings confirm this, and provides further support for the role of enteroglucagon in the adaptive process.

CONCLUSION

The results of this study support the hypothesis that hypersecretion of enteroglucagon in situations of rapid intestinal transit, is due to the EG cells in the distal ileum and colon being stimulated by chyme rich in unabsorbed nutrients, which is presented rapidly to this part of the gut. Such a situation exists after proximal small bowel resection, and many of the situations where intestinal adaptation takes place, and argues in favour of a role for enteroglucagon in this process.

CHAPTER 12

SUMMARY AND FINAL CONCLUSION

The findings in the various studies outlined in the preceeding chapters, confirm that adaptive changes occur in the distal small bowel in continuity after either proximal small bowel resection or bypass, as indicated by the increase in crypt cell production rates in these models. Furthermore, there is confirmation that luminal nutrients (Chapter 4) and pancreatico-biliary secretions (PBS) (Chapter 5) are important as mechanisms in this adaptation. However, luminal nutrition and PBS cannot explain all the findings in these studies. Thus, the terminal ileal adaptive changes after total colectomy compared with ileal transection alone (Chapter 7) cannot be explained on the basis of luminal nutrition, as the length of small bowel is unaltered, and food intake in these animals was not different from controls. Similiarly, the very early adaptive changes after intestinal resection seen at 36 hours after resection, are well established and marked, despite a very much reduced food intake at this stage (Chapter 9). Furthermore, after intestinal resection, rats maintained on TPN have significantly greater cell proliferation than transected control animals on TPN (Chapter 4), once again indicating that factors other than luminal nutrition must be operative.

The findings of increased adaptive changes (or lack of hypoplasia) seen in isolated Thiry-Vella fistulae in orally fed rats, compared with similiar animals maintained on TPN (Chapter 3) argues that luminal nutrition releases a systemic factor, which then passes via the circulation, to bring about these changes in the isolated fistula. That humoral factors

or hormones can influence cell proliferation, is clearly shown in Chapter 10.

The data in these studies, provide strong circumstantial evidence that enteroglucagon should be regarded as a likely candidate for the role of humoral 'enterotrophin' in the mechanism of the adaptive process. In all the studies, enteroglucagon and CCPR changes showed a very close correlation (Chapter 8), while the lack of such a relationship between gastrin and cell proliferation, excludes this peptide as having any trophic influence on small bowel mucosa. Furthermore, the findings after intestinal resection over a prolonged period (Chapter 9), shows that enteroglucagon is released in sufficient quantities in the early stages of the adaptive process, and that the release of this peptide in high concentrations over a prolonged period would allow for the maintenance of cell proliferation, thus providing further support for enteroglucagon as a growth factor to small bowel. Enteroglucagon is released by carbohydrates and long chain triglycerides, and the findings in Chapter 11 indicated that the mechanism of hypersecretion of this peptide, is stimulation of the distally placed EG cells by luminal content which is rich in unabsorbed nutrients passing rapidly down the gut. This mechanism almost certainly operates after proximal small bowel resection and jejuno-ileal bypass, and the various other malabsorption states, such as coeliac disease, in which intestinal adaptation occurs. Furthermore, the amount of food ingested is important to the degree of enteroglucagon release, and the strength of the adaptive response (Chapter 4) once again indicating the inter relationship between these two factors and their influence on adaptation.

The process of intestinal adaptation is complex and almost certainly multifactorial, and it is likely that the various trophic mechanisms act in a co-ordinated fashion. Thus ingested nutrition and other luminal secretions, such as bile and pancreatic juice, are clearly important. They probably act in two ways, firstly by a direct trophic effect on the residual mucosa, support for this concept being that they are gradient-based, and secondly by indirect influences, the most important of these being the release of trophic hormone. The trophic hormones may in turn act locally, in a paracrine fashion, or have a more systemic (endocrine) effect. Of the hormones thus far investigated, enteroglucagon has emerged as the most likely candidate for a trophic role on small bowel mucosa. The results of the work presented, provide further strong support for this concept. Enteroglucagon has, however, not been purified in sufficient quantities for direct infusional studies to be undertaken, although recently, an enteroglucagon-enriched extract of rat intestine has been shown to produce a dose-dependent stimulation of DNA synthesis in guinea-pig jejunal mucosa over a concentration range similiar to that found following intestinal resection (164). Clearly, a pure preparation is needed for direct infusional studies to be carried out, and this promises to be an important and exciting avenue of investigation in the field of intestinal adaptation.

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