Some aspects of Gastrointestinal Adaptation to obstruction of the Small Intestine: modulating role of Diet.

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DECLARATION

The experimental design of the work presented in this thesis was that of the author. All the operative surgery for the induction of chronic small bowel obstruction was done by the author. The estimation of protein and DNA concentration was performed by the author with the help of Mr E Campbell at the University Department of Surgery, Western Infirmary, Glasgow and Mr J Hearns at the Gastrointestinal Centre, Southern General Hospital, Glasgow respectively. Histological preparation of the tissues was carried out in the Department of Pathology, Western Infirmary, Glasgow under the supervision of the technical staff. Finally the hormonal assays were carried out partly by the author and partly by the staff at the University Department of Medicine, Queen's University, Belfast.

Part of the Results have been published:

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SUMMARY

The "well being" of the gastrointestinal tract is maintained by the interplay of several factors, notably diet, pancreatic and biliary secretions and gastrointestinal hormones. These same factors may be responsible for enabling the organ to adapt to changing circumstances.

To date, most of what is known about gastrointestinal adaptation has been derived from animal experimentation and the progress in this field has, to some extent, been limited by the suitability of the animal models. One such example is the adaptive response of the gastrointestinal tract to small intestinal obstruction. Whereas a wealth of information exists regarding adaptation to acute obstruction, knowledge of the changes to the chronic event is limited.

The first aim of the thesis was therefore to further develop and modify a reproducible model of chronic small bowel obstruction in the rat.

The study was then extended to investigate the changes in both the proximal (i.e. oesophagus, stomach and proximal small intestine) as well as the distal (i.e. distal small intestine) bowel to obstruction. In addition, the presence and absence of food bulk on these changes was also

investigated. The gastrointestinal hormone profiles under each experimental condition were also identified.

It was found that:

1. An increase in oesophageal weight occurred following a high small bowel obstruction. Other levels of small bowel obstruction had no noticeable effect on the oesophagus.
2. The response of the stomach to obstruction varied with the site of the small bowel obstruction. A high obstruction produced a marked dilatation of the stomach. A mid small intestinal obstruction generated marked gastric muscle hypertrophy.
3. The jejunum and the ileum behaved in a similar fashion in that both showed an increase in weight and and in luminal circumference in response to obstruction. However the magnitude of the ileal response was far greater.
4. The administration of a low residue diet did not result, during the time period of the experiment, in a reduction of the mucosal weight of the gastrointestinal tract. However a reduction in both the DNA and protein concentration was observed. When this low residue diet was administered to rats subjected to a small bowel obstruction, the hypertrophic

response noted in chow-fed obstructed animals was abolished.

5. Disuse atrophy of the mucosa was seen in the ileum of rats with a mid small bowel obstruction fed on chow. Although some degree of atrophy did occur in similarly obstructed rats fed on a low residue diet, the degree of atrophy was significantly less than that in chow fed obstructed animals.
6. Hormonal assays showed that:
   a. Serum gastrin was raised following a high obstruction and following the administration of a low residue diet.
   b. Serum N-glucagon levels showed significant increases following mid and distal small bowel obstruction.
   c. Vasoactive intestinal peptide levels were increased following a distal obstruction.

ABBREVIATIONS

Bk	Blank
C	Control/s
CCPR	Crypt Cell Production Rate
d	Day/s
DA	Agouti breed of rat
DJ	Duodeno-jejunal flexure
EDTA	Ehtylene Diamine Tetra-acetic Acid
E coli	Escherichia coli
EGF	Epidermal Growth Factor
GIP	Gastric Inhibitory Polypeptide
GI	Gastrointestinal
gm	Gram
hr	Hour
i.u.	International Unit/s
kcal	Kilo Calories
L_1	Level 1 (10 cm from DJ flexure)
L_2	Level 2 (mid small bowel)
L_3	Level 3 (ileo caecal junction)
LRD	Low Residue Diet
mg	Milligram
ml	Millilitre
ug	Microgram
nm	Nanometer
CGLI	N-Glucagon like Immunoreactivity
O	Obstructed

O.D.	Optical Density
p	Levels of Significance
P_1	Period 1 (7 days)
P_2	Period 2 (11 days)
P_3	Period 3 (15 days)
SA	Surface Area
SI	Small Intestine
TCA	Trichloro Acetic Acid
TPN	Total Parenteral Nutrition
VIP	Vasoactive Intestinal Peptide
vit	Vitamin
vs	Versus
wt	Weight

PART ONE

REVIEW OF THE LITERATURE

1. Factors affecting Intestinal Adaptation

1. Introduction

The Gastrointestinal tract is a very dynamic organ with the highest cell turnover occuring in the small intestine. It should perhaps be stated at the outset that most of the investigative work on adaptation has been directed towards the small intestine. Consequently most of the work discussed in this introductory review will, of necessity, deal mostly with the small intestine.

A continuous cell movement within the gastrointestinal tract was first suggested in 1948[1] and demonstrated experimentally in the small intestine a few years later[2]. This movement consists of a rapid cell production in the crypts, rapid migration of cells from the crypts to the villi and exfoliation of the epithelial cells at the tips of the villi (Fig 1). In addition there is cellular differentiation from a proliferative to a non-proliferative (secretion/absorption) state during transit from the progenetic to the functional compartment along the villus[3-8]. It has

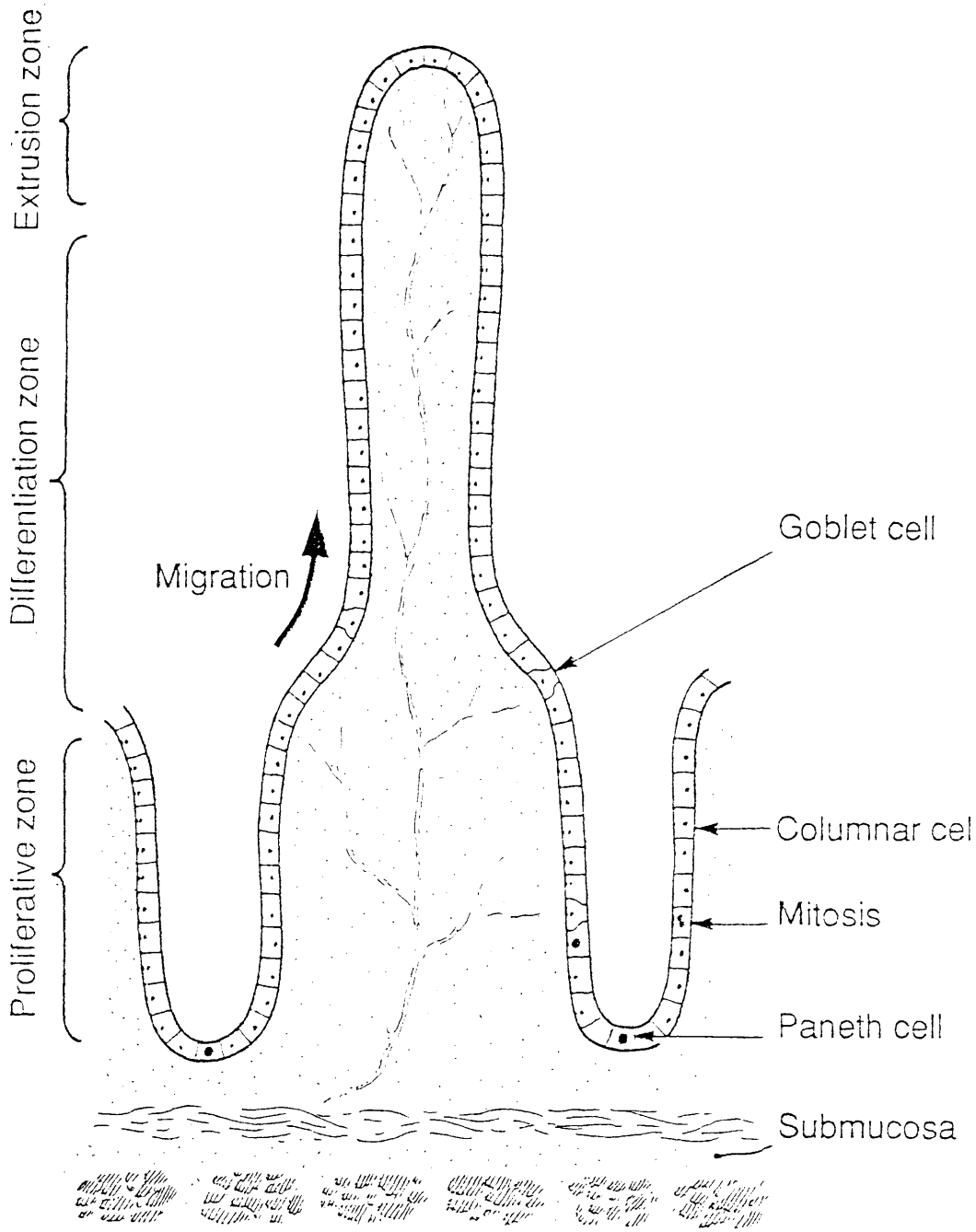


FIGURE 1

Schematic representation of the functional components of the intestinal villus

also been noted that the size of the villi as well as the number and lifespan of the cells composing the absorptive epithelium decreases gradually from the duodenum towards the ileum with the result that the villus size and the cell life span in the ileum are about half the values of the duodenum[9,10].

One can postulate that the structural integrity of the gut is under the influence of "central" (hormonal, neural) and "peripheral" (local) control. Whilst it can be appreciated that overall changes occuring in the bowel are under central control the "villus size gradient" that exists between the proximal and distal small intestine (SI), in spite of the regionally similar cell production rate, is presumably the result of adaptive changes in response to prevailing local influences.

To date, several factors have been identified as being important modulators of the intestinal response to adaptation, the most important being diet, pancreatic and biliary secretions and the gastrointestinal hormones. The evidence to support these factors will now be discussed.

11. Diet

It is thought that the main, although not exclusive, stimulus to the maintenance of the structural integrity of the SI is the diet[11]. Borgstrom[12] and later other workers[13], had realised that in the normal intestine, because of jejunal absorption, relatively little nutrient or unabsorbed food remains in the chyme when it reaches the ileum. Increased food intake, as in states of hyperphagia[14], exagerates the normal response, in that there is a general increase in intestinal mass but with the bulk of the changes occuring in the upper SI. On these findings, Borgstrom concluded that intraluminal nutrition is important in regulating and maintaining the differential growth and absorptive capacity of the SI.

Supportive evidence for this hypothesis comes from the observation that following the resection of part of the SI, the residual intestine develops both structural[13,15-22] and functional[13,23-26] adaptive changes. The adaptation is always greater after proximal than after distal small bowel

resection[13,16,27,28] and the intensity of the adaptive response is directly proportional to the length of the resected intestine[20,29]. These changes include dilatation of the bowel, muscle hypertrophy, villus enlargement and more rapid cell migration. Indeed, extensive resections of the SI led to a significant hyperplasia[29-32] as expressed in terms of an increase in mucosal weight, DNA and protein concentration in the remaining segment of bowel[20,32-34]. Later a differential rise in protein concentration relative to DNA and mucosal weight occurs, suggesting hypertrophy of epithelial cells[16]. This finding has yet to be confirmed. The net result is that the hyperplastic segment absorbs larger quantities of nutrient normally absorbed in that segment[13,35-37] e.g. increased B_{12} and bile acid absorption in the ileum following jejunectomy, or absorption of nutrients normally absorbed elsewhere in the intestine e.g. bile salt uptake by the jejunum following ilectomy[37,38]. Similarly, the transposed ileum adopts jejunal features following the operation of jejuno-ileal transposition (Fig 2). This was translated in marked dilatation, gross villus hyperplasia and

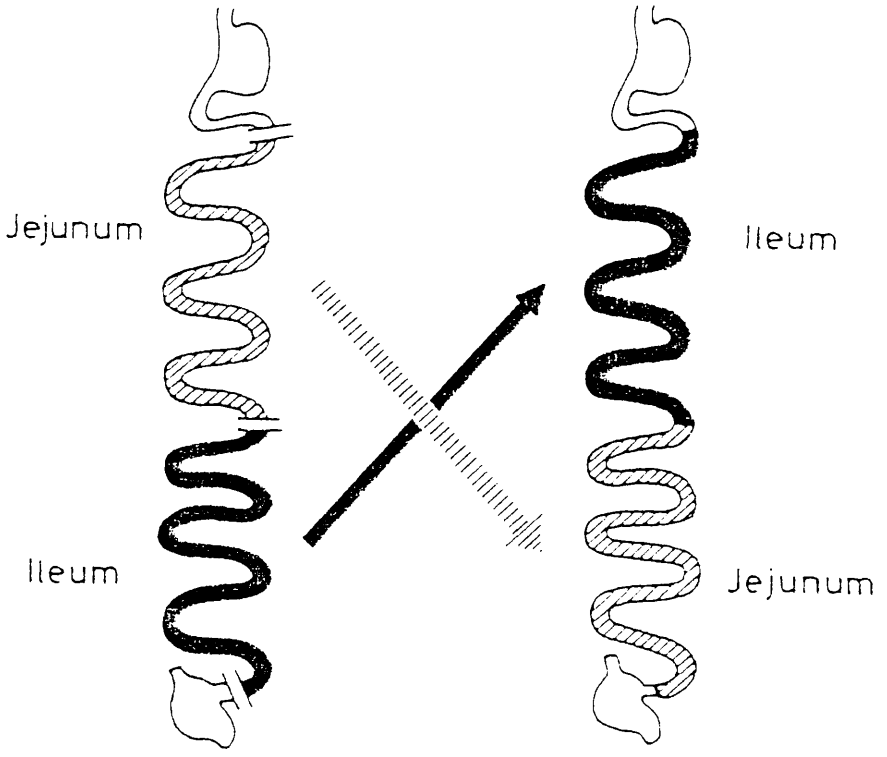


FIGURE 2

Diagrammatic illustration of the operation of jejuno-ileal transposition

greatly increased glucose absorption/unit length[10,13].

The above mentioned findings tend to support the concept that increased luminal nutrition stimulates mucosal growth. It should therefore follow that the intestine deprived of luminal nutrition should be rendered hypoplastic. This has been borne out from several studies which showed that:

1) Fasting[11,39-44] and protein deprivation[45,46] results in decreased epithelial cell renewal[11,45,46], decreased intestinal mass, villus size, mitotic index[11,39,41,47] and disaccharidase activity[48,49].

2) The transposed jejunum, following the operation of jejuno-ileal transposition, similarly showed both morphological as well as functional changes[13,24] to resemble the normal ileum.

3) Complete deprivation of luminal nutrition using the Thiry-Vella fistula (Fig 3) results in the bypassed intestine becoming narrow and contracted. The villi became shorter and more tongue like (similar to the weaning rat). In addition, there was a reduction in the mucosal wet

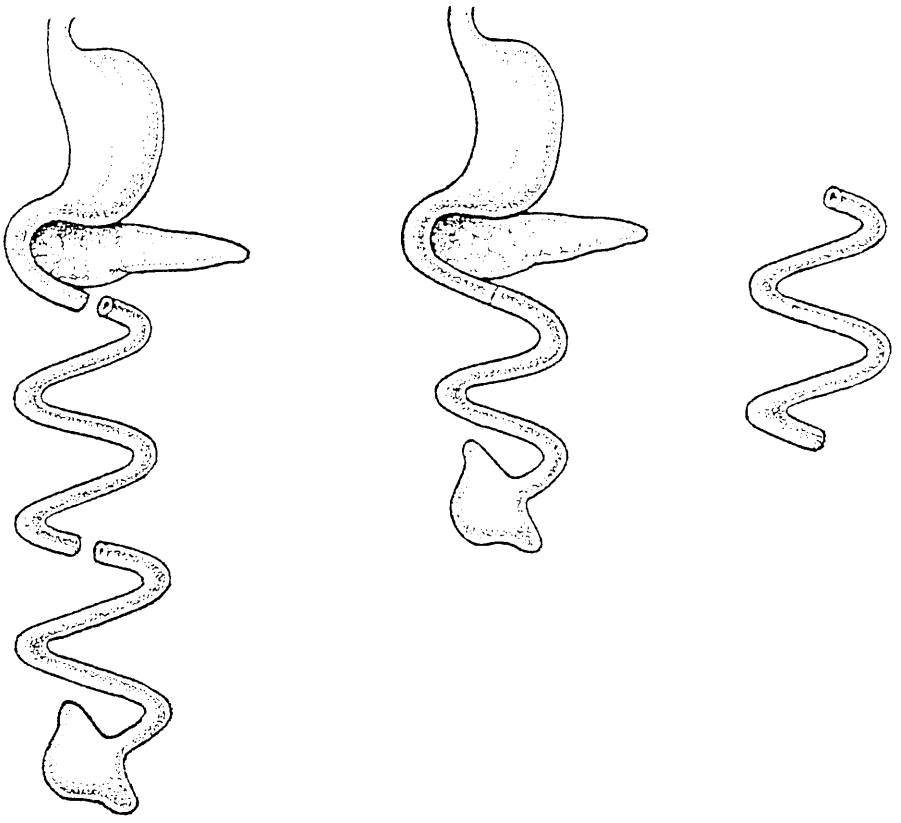


FIGURE 3

Thiry-Vella fistula

weight, protein and DNA concentration[24].

Although inanition or starvation of the animal may be partly responsible for these changes in small intestinal morphology, they cannot be considered to be major contributary factors for this situation, as a similar picture of mucosal atrophy is also seen in rats fed parenterally[50-55].

Interestingly enough although similar atrophic changes occur in both the bypassed jejunum and ileum, absorptive function of the jejunum falls commensurately with the degree of atrophy, whereas no such change occurs in the ileum. Although a great deal of data in the literature indicates that a positive correlation exists between villus height, mucosal surface and nutrient absorption[13,56,57] no absolute proof exists that alteration in villus height results in a similar change in the total absorptive capacity[58-60].

It would seem from the above evidence that luminal nutrition does play a major role in the maintenance of the gastrointestinal tract. The mere presence of nutrients is however not sufficient to prevent atrophy. Solutions used for parenteral

nutrition fed orally[61-63] and administration of elemental diets[59] result not only in some degree of atrophy as compared to chow fed controls but prevent the morphological and functional adaptation seen in the small bowel of rats and dogs after jejunectomy[15,64]. The addition of bulk to the elemental diet[65] did not prevent the reduction of the villus size, but it did cause a small increase in cell renewal which was still lower than that in chow-fed rats.

On this evidence, one can conclude that the nature of food itself or a stimulus produced by it may be responsible for some modulation of villus adaptation.

111. Pancreatic and Biliary Secretions

The nature of the food provided to the animal, as distinct from elemental nutrition or bulk, plays a major role in the maintenance of the small intestine. Several reasons may be put forward to explain this occurrence. One can postulate a food factor or a breakdown product of food digestion, but as can be seen from the above, atrophy of the mucosa still occurs after the ingestion of elemental diets or parenteral nutrition fluids. Digestive secretions or hormonal stimuli caused as an integral part of digestion may be implicated. The evidence for both these factors is strong and will therefore be considered.

Altmann[10] showed that following the transposition of a jejunal segment into the ileum, the size of the villi in the transposed segment become reduced to the size of the adjacent ileal villi. The converse of this also holds true so that when a segment of ileum is transposed into the jejunum, the villi in the transposed segment increase to the same size of the jejunal villi. These experiments indicate that local extrinsic factors influence villus size, because with tissue intrinsic factors alone, the villus size would not

adapt in the transposed segments. These extrinsic factors are able at least to 'enlarge' or 'decrease' villus size and thus maintain the "villus size gradient". It seems unlikely that the wall of the jejunum or ileum elaborate these factors, because if they did, the initial villus size in the transposed segments would be maintained. The most probable source for this factor is the intestinal chyme. This suggestion was confirmed when it was shown that when a duodenal segment carrying bile and pancreatic secretions was transposed into the ileum, the villi of the transposed segment remained unchanged. In addition the distal ileal villi adjacent to the anastomosis increased markedly. Similar and lasting effects were seen in the ileum when a blind sac of duodenum (containing the duodenal papilla) or the duodenal papilla itself[29,66,67] was anastomosed to the ileum or when an entero-entero anastomosis was created between the duodenum and the ileum. Preliminary data suggest that chronic stimulation of pancreato-biliary secretion by cholecystokinin and secretin may prevent intestinal atrophy found in dogs maintained exclusively by intravenous alimentation[68]. Since the duodenal papilla transmits both pancreatic and biliary secretions, it would seem that one or both of these substances

are involved as the villus enlarging influence.

When the bile and pancreatic ducts were anastomosed separately to ileal segments, those segments receiving the pancreatic secretions alone exhibited the same morphological changes as those seen after ampullary-ileal anastomosis. On the other hand, the ileal segment receiving bile only showed no change[66].

Administration of fresh hog bile into isolated ileal loops resulted in no significant morphological change, again confirming that bile exerts no effect on gastrointestinal morphology. On the other hand, infusion of hog pancreatic extracts or 'pancreatin' (commercial pancreatic extract preparation) into isolated ileal segments resulted in marked mucosal hyperplasia[69].

Regional variation in the villus size may therefore arise from one of two factors. This may be the sole result of the villus enlargement factor and its effect is maximal in the duodenum where it is released. Its effect wanes as one proceeds in an aboral direction with little or no effect being exerted in the distal ileum. On the other hand, a separate villus reducing factor, possibly arising in the ileum, may be implicated. Following the formation of isolated loops of ileal and duodenal segments, the villi in the jejunal segments were

reduced and the ileal villi were increased to the size of the villi normally found in the upper ileum (medium sized villi)[10], thereby suggesting that under functional conditions, mere lack of the enlargement factor does not explain the reduced size of the ileal villi.

The main difference between ileal and jejunal contents is the abundance of fat, bacteria, bile acids and pigments and the slower peristaltic activity in the former[70,71]. In addition a large amount of exfoliated cell material is found in the ileum[9]. Bacteria and their degrading action on food seem to play no part as the infusion of bacteria cultured from ileal and caecal contents into jejunal segments produced no reduction in villus size. Furthermore, germ free reared animals show the same villus size gradient as controls[72].

The nature of the intestinal contents again have no effect on villus size but the presence of a large quantity of bile, as produced by duodenal obstruction, resulted in a progressive decrease in villus size towards the obstruction, with the villi adjacent to the obstruction being reduced to ileal sized villi. The same effect on villi is noted when bile is instilled into ligated intestinal loops[69].

The mechanism of villus reduction by bile can only be speculated upon. Direct damage to the villi

can be excluded because villus tip damage characteristically produced by unconjugated bile salts was not seen[73]. A more probable mechanism is the possible lowering of the rate of protein synthesis. Cholesterol and fat, together with bile salts are absorbed through the ileum[74-76]. Fat transport, which requires protein synthesis[75,77] is closely related to the endoplasmic reticulum[78]. It would therefore seem that increased formation of chylomicrons and fat transport, which are facilitated by bile salts, would exhaust the capacity of the epithelial cells for protein synthesis. Lowering the rate of this synthesis would be expected to shorten the lifespan of the epithelial cells thereby leading to shortened villi in the ileum.

iv. Hormonal Influence

As already discussed, depriving the small intestine of food as in the course of parenteral nutrition[35,50,52-55] or total starvation[11,41-44] causes mucosal atrophy. Even when the solution for parenteral nutrition was fed orally, mucosal atrophy occurred[41-43,79]. However when Thiry Vella fistulae were created from the proximal small intestine of rat, oral as opposed to intravenous feeding maintained the total gut weight, mucosal weight and DNA content of the bypassed gut[50]. The maintenance of gut mass within the bypassed fistulae is clearly not due to direct contact of intraluminal nutrients or pancreatic secretions with the epithelial cells but rather to factors, possibly hormonal, initiated by the interaction of the diet with the small intestine in continuity. The idea of a humoral involvement has been further substantiated by vascular parabiosis techniques in pigs[80] and mice[32].

Several gastrointestinal hormones may be involved. Much attention has been focussed on gastrin as it was noted that unlike the Thiry Vella fistula experiment, where normal serum gastrin levels are maintained, the other above-mentioned

experiments (i.e. starvation and intravenous feeding) share a common decrease in the levels of antral and serum gastrin[54,79,81].

Gastrin was originally isolated by Gregory et al[82] from porcine antral mucosa and tentative evidence for its trophic action was initially suggested when an association was made between the Zollinger-Ellison syndrome and hyperplasia of the gastric and duodenal mucosa[83]. Further supportive clinical evidence came from the work of Lees and Grandjean[84]. They described complete mucosal atrophy in the gastric remnant of healthy post-antrectomy patients. A similar picture was apparent in patients who underwent partial gastrectomy for duodenal ulceration[85]. These results cannot be explained on the basis of disuse atrophy, for vagotomy and antrectomy both decrease acid by about 60%, yet only minor degrees of mucosal atrophy occur after vagotomy[86].

In 1969, Johnson's observation that single injections of the analog pentagastrin resulted in stimulation of protein synthesis in the gastric and duodenal mucosa[87], laid further support to the trophic action of gastrin. Since then, this trophic activity on most areas of the gastrointestinal tract have been well documented[88,89].

It has been demonstrated the pentagastrin

stimulates both in vivo[90] and in vitro[87] incorporation of amino acids into duodenal and gastric mucosal protein. DNA[91] and RNA[92] synthesis was increased. Gastrin has also been shown to stimulate the in vivo uptake of ^{3}H thymidine into canine gastric mucosa[93] and to increase the total amount of DNA present in the gastric and duodenal mucosa of antrectomised rats[94]. In contrast, antrectomy, which removes the major endogenous source of gastrin, results in a loss of DNA and RNA from the gastric mucosal remnant and the duodenal mucosa[94] resulting in massive mucosal atrophy[95]. Short term pentagastrin administration restores both the RNA and DNA content of these tissues to normal or near normal levels[94] and can maintain normal acid secretion in patients who have undergone antrectomy[96].

The trophic effects of gastrin are related mainly to the gastroduodenal area. However the rest of the gastrointestinal tract may also be affected[97] although the present evidence is eqivocal[31,98-102]. Notable exceptions to the trophic effect of gastrin are the oesophagus and the gastric antrum[89,100,102,103]. Regulation of antral growth by gastrin would be in opposition to the general concepts of endocrine physiology, for this tissue is the origin of most physiologically

released gastrin and this may account for the relative resistance of the antrum to atrophy during experimental deprivation of the gastrointestinal tract of food. No effect of gastrin has been found in liver[87,91,102,104], skeletal muscle[87], kidneys[102,105], spleen[102,105] and testes[105].

The trophic effects of gastrin noted above are apparently restricted to the mucosal layer, at least in the stomach and duodenum[90]. Gastrin did not increase ^{14}C leucine incorporation into the muscle of the oxyntic gland area[90] nor did it stimulate DNA synthesis in the smooth muscle layers of stomach or duodenum[88].

Food in the antrum is the primary stimulus to gastrin release[106-110]. The degree of G cell stimulation (and therefore of gastrin release) is therefore related to the presence and type of food and the so called "luminal effects" of food could be explained in terms of their capacity to stimulate the G cell. Hence during states of hyperphagia when gastrin levels are high, hypertrophy and hyperplasia of the mucosa occurs. Conversely in states of food deprivation, feeding of elemental diets or total parenteral nutrition (TPN), when gastrin levels are low, mucosal atrophy occurs. The addition of non-absorbable bulk to an elemental diet does not influence atrophy as bulk

is a poor stimulant to the antral production of gastrin[63].

The experimental administration of large doses of pentagastrin over a two week period resulted in pancreatic acinar cell hypertrophy[102,111] and hyperplasia (post hypophysectomy)[112]. Gastrin would therefore seem to be involved in the maintenance of pancreatic mass whose integrity is required, as described earlier, to maintain intestinal cell mass. This would suggest that gastrin has not only a direct trophic effect on the gastrointestinal tract but also has an indirect role by maintaining the bulk and integrity of the pancreas whose secretions in turn have also been shown to be involved in gut maintenance.

The evidence for the role of other hormones is less well defined. Circumstantial evidence that enteroglucagon and/or pancreatic glucagon may be responsible, comes from the report that high circulating levels of enteroglucagon (kidney tumour origin) were associated with small bowel enlargement and massive villus hyperplasia, which disappeared when the tumour was removed[113]. Enteroglucagon also seems to influence liver regeneration after partial hepatectomy[114]. Exogenous administration of glucagon has however resulted in a reduction in villus height and cell

migration in the enteric mucosa[115]. This effect is possibly mediated by the inhibition of gastric secretion and gastrin release[116], an action shared with the other gastrointestinal hormones of the same family.

Cholecystokinin, although structurally and functionally related to gastrin, is an important regulator of growth of the exocrine pancreas[111,117,118] but has no known trophic influence on the gut[119].

Secretin administration, in single doses, has been shown to inhibit the gastrin stimulation of DNA synthesis and protein accumulation in both the oxyntic gland region of the stomach and in the duodenum[98,103]. Chronic administration of this hormone results in a reduction of the parietal cell mass and acid secretion caused by pentagastrin alone[120,121]. Secretin alone causes a slight reduction in the parietal cell population[103] but has no effect on the weight, DNA, protein content or enzymic activity of the stomach[122]. This would therefore suggest that secretin has no inherent antitrophic effect in the stomach. These effects reside solely in its ability to counter the activity of simultaneously administered pentagastrin. Panser[123] observed a contrasting effect of secretin on the jejunum where an

antitrophic effect (reduction in cell proliferation in crypts) can be demonstrated.

Vasoactive intestinal polypeptide (V.I.P.), a peptide of the secretin family, has been shown to inhibit pentagastrin stimulated DNA synthesis in the rat colon[103] and may have a similar antitrophic effect on the upper GI tract.

Other non GI hormones may be implicated. A reduction in cell proliferation in the jejunal crypts has been noted following adrenalectomy and this reduction may account for the malabsorption[124] and reduced mucosal alkaline phosphatase[125] noted in this situation. An acceleration in crypt proliferation is seen in the small intestine after treatment with high levels of glucocorticoids[126].

In the intact intestine, the reduced food intake of both pair fed and hypophysectomised animals led to a comparable reduction in mucosal mass / cm length. Following resection, however, the magnitude of these changes (ileum >> jejunum) was significantly less after hypophysectomy than after pair feeding and this in turn was less than in normally fed controls. It therefore seems that the pituitary does influence intestinal adaptation after small bowel resection since the effect of hypophysectomy on mucosal regeneration cannot be

explained by reduced food alone. It may well be that it exerts its effect by a secondary mechanism, via its role as a regulator of other endocrine organs, since it is known that thyroxine[127,128] and testosterone[129] also influence crypt cell proliferation. The pituitary gland has also been implicated in the regulation of gastric mucosal growth[130].

Epidermal growth factor (EGF), a polypeptide containing 53 amino acids[131], was identified in the course of studying the nerve growth factor of the submaxillary gland of mice[132]. It has well defined mitogenic effects in that it causes marked proliferation of epidermal and epithelial tissues[133,134] and enhancement of DNA synthesis in various segments of the gastrointestinal tract[135,136]. In the stomach, it is a potent inhibitor of acid secretion[137,138] and is capable of stimulating growth of the oxyntic gland mucosa in mature rats[135]. It produces the same degree of growth stimulation as maximal effective doses of pentagastrin. However, on a molar basis, it is a more powerful stimulant than pentagastrin. The trophic effect on the stomach seems to be direct and not the result of acid inhibition leading to a raised serum gastrin. This is supported by the fact that no changes in the duodenal or colonic mucosa

is observed as in the case of gastrin; in addition, in contrast to gastrin, the trophic effect on the stomach is not inhibited by the concomitant administration of secretin[78].

v. Conclusion

Luminal nutrition is clearly one of the most important factors in the adaptation of the gastrointestinal tract. Precisely how luminal nutrition initiates these adaptive changes, is quite unknown. Which component of the diet, and whether the nutrients influence crypt cell proliferation directly, whether they release local or systemic hormones with trophic effects on the intestine, whether they stimulate the secretion of trophic factors from the pancreas, or whether they produce changes in intestinal blood flow are facts which have to be established.

2. Intestinal Obstruction

1. Introduction

The ability of the organism or its component parts to adapt in normal circumstances, has been the hallmark of evolutionary success. Adaptation is not an acute response. The stimulus effective to produce it must be intense and persistent but not such as to cause acute organ failure.

The concept of adaptation can be clearly exemplified from our existing understanding of intestinal obstruction. Intestinal obstruction may occur both as an acute or chronic incident. In both situations there is an attempt by the bowel to adaptation. In the acute phase, rapid decompensation of the physiological responses leads to organ failure and subsequently death; chronic obstruction allows a slower and therefore a better adaptive response until such time as:

1. the obstruction is relieved and normal function is returned or
2. a steady state is achieved such that the new functional demands are met by the new structural alterations or

3. continued obstruction finally leads to eventual organ failure.

It is the second course of events that this thesis is concerned with. However, most of the experimental observations to date have centered on morphological changes associated with the acute type of intestinal obstruction and these will now be reviewed.

11. Acute Intestinal Obstruction

It has long been established that following the onset of acute intestinal obstruction of whatever aetiology, but without vascular compromise, the intestinal contents accumulate at a rapid rate raising the intraluminal pressure[140]; this in turn leads to vascular stasis with concommitant oedema and ischaemic change in the intestinal mucosa which if left uncorrected leads to destruction of the mucosa.

This simplified version of intestinal obstruction is perhaps inaccurate and requires closer scrutiny. Bowel distension is an essential factor in the pathophysiology of intestinal obstruction. Some studies have suggested that the effects of mechanical obstruction in bowel distention and the level of sustained intestinal pressure are not impressive[140,141]. Most recorded pressures in these situations are low (6cm H_2O in rats; 8cm in cats)[142,143]. These pressures are however close to portal outflow and mean capillary pressure and they may even exceed these pressures during bowel activity. Ohmar therefore argues that the combined mechanisms of distention and increased intraluminal pressure may exert a detrimental effect on the microcirculation. The literature

affords some support to this concept[141,142]. On the other hand Mirkovitch[143] not only observed low intraluminal pressure but microcirculatory investigations in the rat revealed normal passage of dye to the tips of the villi, thereby suggesting that blood flow through loops of occluded intestine was unchanged.

In the denervated feline small bowel, the microcirculation was virtually normal 30 mins after decompression of simple bowel obstruction[140]. It can therefore be assumed from the above evidence that, whatever the initial effect, it seems to be rapidly ameliorated after decompression, possibly owing to rapid and complete recovery of the capillaries.

The collection of fluid in the intestinal lumen after occlusion does not solely result from the accumulation of gastric, pancreatic and intestinal secretions that cannot be reabsorbed. Water and electrolytes are concommitantly secreted by the intestinal mucosa into the lumen[142,144,145]. Increased intestinal pressure would be expected to favour absorption and thus any secretory mechanism would tend to be neutralized, or at least reduced[146]. The regulation of water and ions crossing the intestinal mucosa is under the influence of two separate pumps. An absorptive

pump, which is mediated by a Na^+-K^+dependent adenosine triphospatase, removes water and ions from the lumen. A secretory pump, which is cAMP dependent, counteracts the action of the first pump[147] and Shields[144] has shown, by unidirectional measurements, that the loss of water and electrolytes is due to increased efflux rather than decreased influx. This hypothesis is supported by the finding of near normal non-electrolyte transport in vitro and glucose absorption in vivo[142]. Certain bacterial toxins (notably cholera enterotoxin) are known to stimulate the intestinal secretory pump through their action on adenylate cyclase. Pathogenic strains of Escherichia coli are known to produce a similar effect[148-150]. It is well documented that a vast number of E coli organisms are present in the intestinal fluid during intestinal obstruction and it may therefore be reasonable to conclude that a toxin derived from these organisms may stimulate the secretion of water and electrolytes across the mucosa in the same way as enterotoxins act[139,142]. This effect may be mediated via serotonin[151].

There is some disagreement in the literature concerning the effects of acute obstruction on the intestinal mucosa. It has been suggested that, after acute obstruction, the villus height and

width in the intestine proximal to the obstruction was either decreased[142] or increased[152]. Crypt depth was increased[142,153] and this was associated with increased cell renewal[152].

On a microscopical basis, none of the investigators[142,152-154] found evidence of ulceration and the epithelial lining remained intact. There was, however, a moderate leucocytic infiltrate of the lamina propria and dilatation of the blood capillaries, but no evidence of oedema. Brush border acid phosphatase was increased[154].

Distal to the occlusion, villus size and crypt depth were noted to be either increased[152] or decreased[142]. The number of villi per unit length was reduced. Epithelial cell size may also be altered[152].

Kinetic studies indicated minimal change in the labelled index at the crypts proximal to the occlusion. No alteration in cell renewal between obstructed bowel and controls could be found distal to the occlusion.

Information as to the effect of acute obstruction on muscle tissue is rather scant. The initial functional response is a marked distention of the bowel wall to prevent any great rise in intraluminal pressure[140], thus obviating or reducing the possibility of pressure necrosis of

the mucosa. As a consequence of this distention, the gut wall tension may rise to very high values[155].

It has been suggested[139] that in the initial period following acute obstruction (i.e. within 12 hr), oedema of the submucosa and muscle coats occur. Interstitial bleeding follows and soon disruption and necrosis of the muscularis mucosa can be seen. By 18hr, there is an intense polymorphonuclear cell infiltrate throughout the whole of the muscle coats. Eventually, if the obstruction is not relieved, patchy necrosis of the muscle takes place leading to bowel perforation and subsequent peritonitis.

111. Chronic Intestinal Obstruction

Most muscular organs react to incomplete obstruction by dilatation and hypertrophy[156]. Indeed in children exhibiting malformations of the cloacal derivatives, such organs may be grossly enlarged and either thin or thick walled. Little is known about the nature of the increased muscle mass. It has been suggested that hypertrophy represents compensation of the gut and this type of hypertrophy is associated with an increase in the number of ganglion cells; dilatation on the other hand may represent decompensation and may be related to a reduction in the ganglion cell population[157].

An increase in the number of ganglion cells has been observed in both ulcerative colitis[158] and Crohn's disease[159], when the gut is overactive. Experimentally, a similar increase has been observed proximal to a stenosis[160,161]. It was initially thought that this increase represented postnatal hyperplasia of the neuronal cells and would consequently have great biological significance. However this observation has been disputed and whilst ganglion cells may increase in size as the muscle bulk increases[157,160-165], the

general conclusion is that no actual hyperplasia occurs[157,165].

The response of muscle in different organs to increased activity varies, and it is still not clear whether the hypertrophy that occurs after chronic obstruction is the result of increased cell size and/or cell number. The increased muscle bulk in diverticular disease, for instance, is due to contraction of the bowel wall and hyperplasia of the muscle cells, with no evidence of hypertrophy[166]. Hypertrophy only was involved in stretch induced myometrial hypertrophy in rabbits[167] and cardiac hypertrophy in rats[168]. However both hypertrophy and hyperplasia of smooth muscle cells resulted from incomplete obstruction of the canine ureter[169], rabbit colon[156], guinea pig small intestine[170,171] and rat stomach[171]. The apparently contradictory results of different investigations may be due to a number of variables including the methods used, the organ involved, the type of stress applied and the age of the animal.

The mucosal adaptive changes seen in the gut in chronic intestinal obstruction are limited.

Pyloric stenosis leads to marked hyperplasia of the gastric mucosa, with an increase of both the parietal and peptic cell population. Although the size of the stomach was noted to be increased, the

antrum:fundus ratio of the stomach was not significantly altered[173].

Intestinal obstruction resulted in the crypts, proximal to the obstruction, showing an increase in the total cell count in association with an increased mitotic index. Distal to the obstruction, the villi were reduced in number, the crypts became wider and diagonally displaced. No change was noted in the mitotic index[153].

3. Conclusion to Review

Maintenance of the integrity of the gut mucosa is a complex phenomenon. The literature details the multiple factors involved in its maintenance in normal circumstances. A considerable body of information, albeit not entirely in agreement, exists regarding the response of the small bowel to acute obstruction. As has been argued earlier, the acute event limits any adaptive changes. Therefore these reported findings can best be regarded as a sudden alteration in physiology as a result of the insult, rather than a remodelling of the physiological processes to function as harmoniously as possible in the new enviroment.

Analogous situations in clinical practice demands early surgical intervention to relieve the obstruction and reverse the morphological and physiological events occuring as an acute response. In the chronic form of obstruction, such as occurs following radiation induced strictures or Crohn's disease, the adaptive changes that do occur are not necessarily beneficial to the individual. Proximal dilatation may produce a larger surface area resulting in increased nutrient absorption. Distal atrophy, on the other hand, deprives that part of

the intestine of its function in whole or in part. This atrophy and subsequent malabsorption, may be partly responsable for the profuse diarrhoea occasionally seen in patients after the release of intestinal strictures.

The theme of this thesis is an investigation into the adaptive changes by the gastrointestinal tract in response to chronic subacute obstruction. Unlike previous reported studies, attention is given to the morphological changes in contiguous structures proximal to the small intestine. This investigation is extended also to identify the modulating effect of the diet on the adaptive response to obstruction.

Accordingly the following facets will be investigated:

1. the relationship between the adaptive response of the gut to obstruction with time.

2. the effect of small bowel stenosis on proximal structures.

3. the effect of the diet on the development and maintenance of the adaptive response to obstruction.

4. the effect of the diet on post stricture atrophy.

5. the gastrointestinal hormone profiles in these situations.

PART TWO

EXPERIMENTAL WORK

4. Statement of Aims

The aims of the work described in this thesis were to study the effects of subacute obstruction of the small intestine on the small intestine itself, as well as the oesophagus and stomach in the rat and to determine the effect of diet on such a response.

The response was defined in terms of:

a. morphological changes
    1. morphometry
    2. histology

b. functional changes
    1. DNA synthesis
    2. protein synthesis

c. hormonal changes

5. Evolution of the animal model

Chronic or subacute obstruction in humans is not an uncommon problem. Common causes include tumours, Crohn's disease, radiation strictures and adhesions. The translation of the clinical picture into an animal model is fraught with difficulty. Interest in the development of such a model has resulted in the description of several techniques. However these must be strictly defined for the site of obstruction and in particular the type of animal being used.

The techniques of obstruction can be categorized as:

1. methods which produce an immediate effect;
2. methods which rely on gradual fibrosis to induce the obstructive effect.

The use of a ligature to partially obstruct bowel was first suggested by Ivy[173] and later employed by Crean et al[174]. This method was employed primarily to study the effects of gastroduodenal obstruction. A bougie of known diameter was placed adjacent to the duodenum and a ligature placed around both structures and tightened (Fig 4). On withdrawing the bougie, a standard degree of obstruction could be produced and using the same bougie, it was assumed that a

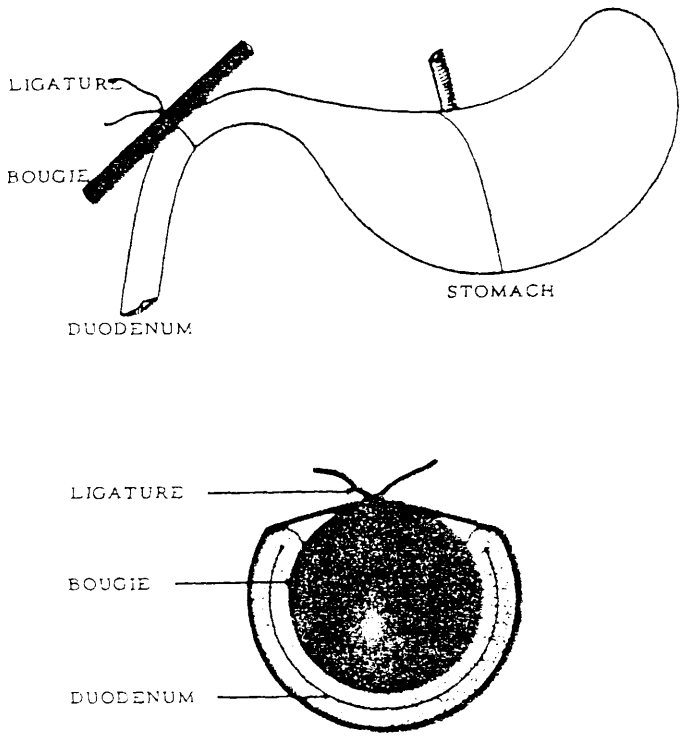


FIGURE 4

Diagrammatic representation of the method used to produce partial obstruction to gastric outflow

similar degree of obstruction would be achieved in different animals. Williams et al[153] utilized the same procedure for jejunal and ileal obstruction. In their hands, it produced good results in that marked dilatation of the proximal bowel was obtained. This procedure, however, was associated with a high mortality occuring within two days.

Earlam[157] used the Teflon mesh method to produce experimental stenosis in the small intestine of dogs (Fig 5). In this method, the mesh cuff was placed around the small intestine between 15 and 30 cm proximal to the ileo-caecal valve. The ends of the cuff were stitched together on the mesenteric border without interrupting the blood supply. A stenotic lesion, 5cm long was established. The degree of stenosis could be controlled by folding the mesh upon itself.

Another technique emloyed was that described by Brent[156], when studying the colonic response to anal stenosis. The size of the anal lumen was calibrated with catheters of known diameter introduced into the anus without undue force. The diameter of the orifice was then reduced to a known size with a non-absorbable circumanal suture. It was found to be an effective method and was associated with a low mortality.

In 1975, Gabella[171] showed that it was

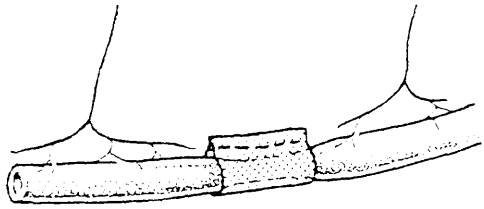


FIGURE 5

"Teflon mesh" method for producing intestinal obstruction in the dog

possible to induce a gradual and progressive stenosis of the small intestine by a different method. The small bowel was exposed and a small window is cut in the mesentery close to the bowel. A strip of cellophane was then passed through the window and rolled around the bowel so as to form a ring. This ring was then fixed with one or two sutures. The ring was free to move being only restrained by the mesentery . For a few days after the operation, there is no impairment to the free movement of ingesta. Subsequently the serosa of the gut under the acetate film reacts and proliferates, first anchoring the acetate strip to the gut and then slowly and progressively reducing the lumen of the intestine inside the ring (Fig 6). Impaired transit of ingesta and stasis within the loop proximal to the stenosis ensues. A slow development of partial obstruction is essential for the growth of the musculature to keep pace with distention, so that oral to the stenosis the gut is expanded and the wall thickened. The process develops within three to five weeks in the guinea pig.

The animals utilized for the work described in this thesis were female DA rats. The choice of the animal was made on the grounds of easy housing and avaliability in the large numbers required for the experiment. The disadvantages of using the rat

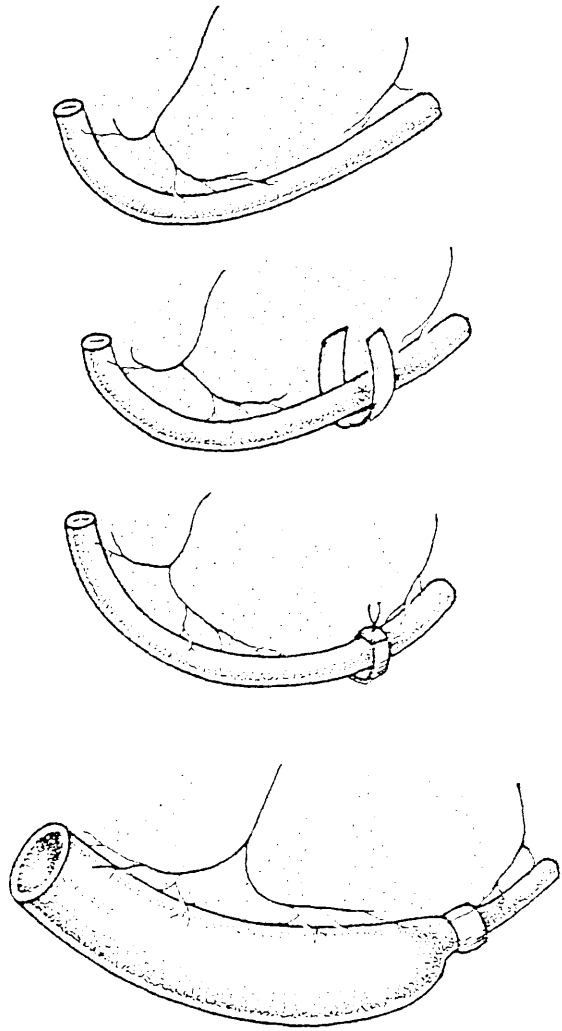


FIGURE 6

"Cellophane strip" method for inducing gradual fibrosis leading to chronic intestinal obstruction

include its size and its inability to vomit making it very vulnerable, in the immediate post-operative period, to acute gastric dilatation and death.

Of the techniques described above, the Teflon mesh method is inapplicable due to the small size of the rat bowel and the long segment of bowel stenosed. Ivy's method is simple and easily reproducible. However the small intestine, unlike the stomach, is unable to generate sufficient pressure to propel digesta through a partially occluded lumen. This appears to be the reason for the high mortality described by Williams[159] using this method. Modification of Brent's technique, i.e. through and through sutures placed into the bowel wall to reduce luminal size resulted in a high mortality due to necrosis of the bowel wall resulting in peritonitis.

The procedure using the acetate film, as described by Gabella was found to be suitable with some modification (see under Study Design). By a process of trial and error, it was found that using DA rats, the maximum stenosis occurred at about two weeks. Beyond that time, mortality rose rapidly.

6. Study Design

The experimental animals used throughout the project were inbred female DA rats. These were obtained from an established colony within the University Department of Surgery, Western Infirmary, Glasgow.

The animals were anaesthetised using Nembutal and subsequently maintained on ether. The abdomen was opened via a midline incision. The site of proposed obstruction in the small bowel was identified. A window was created in the mesentery adjacent to the bowel.

In the animals randomised to be obstructed, an acetate tape, measuring 3X17mm, was passed through this hiatus. The free ends of the tape were overlapped to form a ring and secured in that position using two sutures of 3/0 Prolene. In order to prevent a 'milking-down' effect on the ring by the peristaltic action of the bowel, resulting in trauma to the mesenteric vessels, the ring was anchored to the bowel both proximally and distally, by two seromuscular sutures of 6/0 Prolene.

The ring thus created, was fashioned in such a way that the overlap of the two ends was not more than 2mm. This resulted in an effective ring

diameter of 4.6 - 4.8mm. This ring circumference is comparatively much larger than the bowel circumference. As indicated earlier, however, obstruction is not produced by the direct mechanical pressure of the ring, but rather by a gradual process of fibrosis that occurs in the bowel wall in response to the foreign material.

Site of obstruction

In the first series of experiments i.e. those evaluating the effect of obstruction with time, three sites for obstruction in the small intestine were utilized. These were 1) upper small intestine, i.e. 10cm from the duodenojejunal flexure; 2) mid small intestine; 3) ileocaecal junction.

In the second experiment, where the effect of an elemental diet on the obstructed bowel was being investigated, one site of obstruction, namely mid small bowel, was chosen.

Duration

It has been previously reported[166] that that the maximum distention of the small bowel in guinea pigs occurs at about three weeks. Using the DA rat, maximum dilatation had occurred by two weeks. Prolonging the duration of the obstruction beyond two weeks resulted in a very rapid rise in

mortality with little alteration in bowel morphometry. Accordingly, a maximum limit of fifteen days was therefore set for the duration of each experiment. Animals were therefore sacrificed at 7, 11 and 15 day intervals for the first experiment and at 7 and 15 days for the second series.

Animal Management

At the start of each experiment, the animals were 8 weeks old and weighed 168.6±2.1gms. Throughout the whole of the experimental period, body weight was recorded on alternate days. The controls and the obstructed animals were housed separately, four to a cage. The cages were made of moulded polypropylene with a stainless steel wire mesh lid. Cages with wire mesh floors were employed for the animals maintained on an elemental diet to minimise the possibility of coprophagia. The animals were kept in a temperature controlled animal research unit in which 12 hourly light and dark cycles were employed.

Group Size

The number of animals in each of the treated and control groups for each experiment is shown in Tables 1,2. More animals were used for the longer

TABLE 1

Category	Total No	Final No	Acceptable Stenosis
L_1P_1	10	10	10
L_1P_2	15	14	10
L_1P_3	15	9	9
L_2P_1	14	11	10
L_2P_2	14	14	10
L_2P_3	16	11	10
L_3P_1	19	12	10
L_3P_2	17	14	10
L_3P_3	14	10	10

Number of CONTROLS for each category: n = 10.

Table illustrating the total number of animals used during the investigation of the site and duration of obstruction with time.

duration phase of the experiments in order to allow for treatment related mortality.

TABLE 2

Category	Total No	Final No	Acceptable Stenosis
7 d	16	12	10
15 d	17	14	10

No of CONTROLS for each category: n = 10

Table illustrating the total number of animals used during the investigation of the effect of diet on the obstructed bowel.

Randomisation

Randomisation, both for the site and the duration of the obstruction, was carried out using computer generated random numbers. For each randomised test animal a matched control was selected thus forming a "matched pair". If any animal of the "matched pair" did not survive, the remaining animal was sacrificed and a further matched pair was used as a substitute.

Feeding and Sampling Procedures

Diet

The animals for the first set of experiments were fed on a chow diet (CRM, Labsure UK) ad libitum. This provided them with 65 kcal of energy per day. The low residue diet employed was Trisorbon prepared in a concentration of 28 gm/100ml giving the animals 70 kcal/day. The appropriate analysis of each diet is illustrated in the Appendix Section (111).

Sampling Procedures

At the completion of the experimental period, the rats were anaesthetised with ether after a 12hr fast. A left paramedian approach was made to the abdomen. This ensured that any bowel caught up with adhesions to the previous midline scar, was not inadvertently damaged.

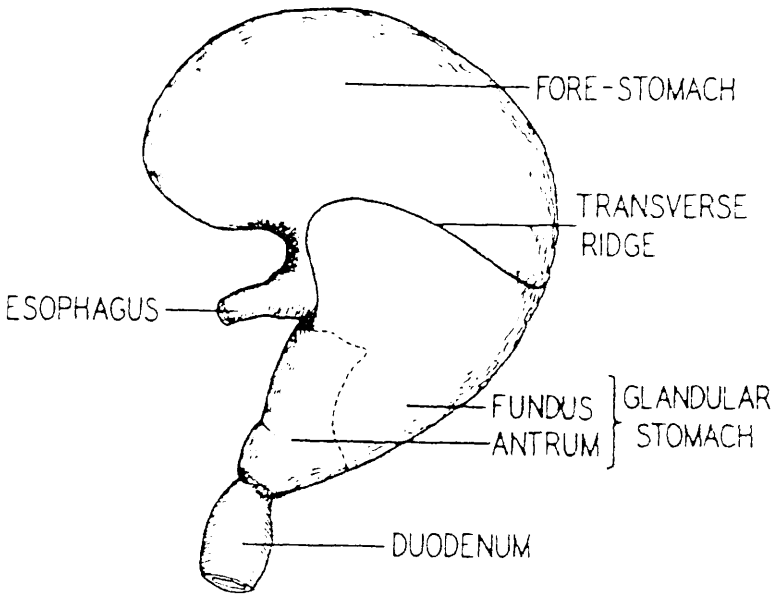
Blood Samples

Blood was withdrawn from the inferior vena cava by needle puncture. About 2mls of blood were withdrawm from each animal and placed in iced heparinised bottles. The samples were then centrifuged for 10mims at 4°C at 2500 revs min^{-1}. The serum was then separated and stored at -20°C until the hormone assays could be performed.

Tissue Samples

Oesophagus: A thoracotomy was performed via a midline incision. The incision was extended through the diaphragm down to the oesophageal hiatus. The oesophagus was identified in its subdiaphragmatic position and traced upwards by a process of blunt and sharp dissection to the region of the trachea. The oesophagus was then transected proximally, immediately posterior to the trachea at the level of the cricopharyngeus and distally, at the gastro-oesophageal junction. The weight of the resected segment was recorded and then opened and pinned to a board and its length and width noted. In the specimens where histological examination was required, a 0.5cm sample was taken from the intact mid-oesophageal region and placed in formol saline.

Stomach: The greater omentum was excised. The lesser omentum and the gastrosplenic ligaments were then divided. The stomach was then lifted and divided at the pyloroduodenal junction. It was then opened along the greater curvature aspect, washed in ice cold saline and weighed. The opened specimen was pinned out onto graph paper using sufficient tension to obliterate the mucosal folds. The



"Anatomy of the stomach of the rat, from Baker and Bridgeman 1954."

FIGURE 7

Diagramatic representation of the stomach anatomy illustrating the relative sizes of the rumen, fundus and antrum

perimeter of the organ was then traced onto the graph paper. This allowed subsequent measurement of the surface area.

The gastric mucosa was then removed by gentle scraping using the edge of a microscope slide. Complete removal of the mucosa was confirmed by random histological examination of the residual tissue. After placing in a preweighed Potter homogenising glass tube, the weight of the mucosa was determined.

Small Intestine: The first 10cm of jejunum and the last 10cm of ileum were removed and the attached mesentery was carefully excised. The intestinal length was measured under a fixed tension of 15gms. The samples were then rinsed with iced saline to remove intestinal contents, and the specimen weights determined. The samples were then opened along the anti-mesenteric border, pinned to a board and the width of the segment determined at 3, 6 and 9cm from the proximal end; a mean value was then obtained. The entire mucosa was then scraped off in an identical fashion as described for the stomach and the weight determined.

Mucosal scrapings: To the mucosal scrapings obtained above, 5mls of ice cold saline containing

EDTA were added. Homogenization was carried out at 4°C using 4X6 passes with 30 seconds between each series of passes as described by Potter and Elvehjem[175]. The homogenates were then stored at -20°C until the protein and DNA assays were performed.

Measurement of the Stenosis

The segment of the bowel containing the stenotic area was removed. The final degree of stenosis was estimated by passing bougies through the obstructed segment. This was done to ensure that a comparable degree of stenosis was being obtained at each site for each period of study.

Bougies of increasing diameter (Table 3) were passed until a size was reached which was too large

TABLE 3

Bougie Size	Diameter (mm)	Circumference (mm)
F 6	1.9	6
F 8	2.5	8
F 10	3.2	10
F 12	3.8	12
F 14	4.5	14

Bougie Sizes

to be admitted through the obstructed segment. The stenosis was therefore considered to be equivalent to the diameter of the largest bougie admitted (Table 4).

TABLE 4

Duration	Bougie Size
Period 1	F 12 - F 10
Period 2	F 8
Period 3	F 6

Acceptable degree of stenosis in relation to the duration of obstruction.

7. Materials and Methods

1. Introduction

This chapter is concerned with a description of the various laboratory methods and techniques used in the analysis of the various samples outlined in the previous chapter.

An account is given of the methods used in the preparation of the tissues for standard histology and subsequent morphometric analysis. Brief details are given of the biochemical methods used for DNA and protein analysis and the radioimmunoassay methods for hormone assays. Finally the statistical methods which were employed are described.

11. Morphometric Assessments

a. Morphometry

Morphometry is a useful method of examining the morphological response of the gastrointestinal tract to obstruction. The methods used, however, are indirect and essentially probabilistic ways of quantifying biological structures. Therefore the methods chosen must take into account the size and the anatomical nature of the tissue in question and the state of the tissue at the time of measurement.

Several methods are avaliable for measuring surface area both macroscopically and microscopically. The macroscopic methods all entail tracing the outlines of the sections onto paper (plain, graph or photographic). They differ in the actual process of establishing the area enclosed by the outlines. If the outlines are traced onto graph paper with squares of an appropriate size, the areas enclosed may be estimated by counting the number of squares encompassed. Alternatively the outlines may be drawn on plain paper or an X-ray exposure of the tissue obtained and provided the outlines are not tortuous and the area in question not more than $3cm^2$ a planimeter may be employed[176]. The graph paper method and planimetry, though tedious, are time honoured and

trustworthy. Because of the area and the tortuosity of the tissues, the above methods were thought to be unsuitable.

A more appicable morphometric method is the point counting technique. It can be utilized for both microscopic and macroscopic measurements. The principle employs a lattice of points in a square, triangular or hexagonal array. The "test grid" is of a known area uniformly covered with points. This is superimposed on each tracing and the number of points falling within each outline is counted. The area is then obtained by employing the equation: $A=P/N$; where A is the total area of the grid, P - the number of points falling within the outline and N - the total number of points in the grid.

In general, the more points superimposed on any given outline, the more accurate the estimate of the area. In order to improve the degree of accuracy of area measurement, a progressive mean was calculated as follows: the grid was superimposed on the outline and the proportion of the points falling within it was recorded. The procedure was repeated after a change of the grid position and the proportion of the hits again recorded. An average was taken of the two values. After a number of repetitions, the proportion of hits on the outline in question and hence the

estimate of its area, will settle down to a value whose variation lies within the derived limit.

Throughout the experiments, estimation of the surface area of the stomach was done on fresh tissue as described in the previous chapter. Oesophageal cross-sectional areas were done on fixed mounted tissues.

Histological processing introduces tissue derangements and care is required to standardize the procedures to ensure uniformity throughout all the tissues studied. One has to recognize errors introduced due to fixation, shrinkage during processing and compression during the cutting of sections, However, as much of the morphometric work carried out is comparative, provided all the material is treated in an identical fashion, any alteration brought about by the above mentioned factors becomes less important.

b. Histology

Histological processing was carried out purely for oesophageal work.

The oesophageal samples were fixed in buffered 10% formol saline. Tissue dehydration and blocking in paraffin wax was carried out in a Histokine automatic tissue processing machine. The cycle which lasted 22 hours was as follows. The sample

was initially transferred to 50% alcohol for 1 hr followed by a further hour in 80% alcohol. It was then transferred into the first of three beakers containing 8% phenol in methylated spirit. This was followed by immersion in 2 changes of absolute alcohol for 90 mins each. It then spent 30 mins in absolute alcohol and chloroform followed by 2 changes of xylol each lasting 45 mins. This was followed by 2 changes of melted paraffin wax lasting 3-4 hours respectively before the tissue is blocked in fresh paraffin wax and mounted in the microtome chuck.

The staining was carried out using a regressive haemalum and eosin technique. The 5um sections were dewaxed in xylol followed by alcohol, then stained with haemalum which was differentiated using 1% acid alcohol. After rinsing, eosin was used and differentiated in 30% alcohol. The tissue was again dehydrated with alcohol followed by xylol and mounted permanently using DPX prior to examination.

111. Biochemical Methods

a. Introduction

The gastrointestinal tract has a complex 3-dimensional configuration. Consequently it presents many problems in the study of quantitative morphology. The functional and therefore the most important part of the intestine is the villus and its size is a difficult parameter to measure with accuracy and precision.

The present methods, of which there are two, are indirect and unproven. The first assumes measurements, made in sections, of the height and width of the villus termed the "villus row count". Implicit in such an assumption is the uniformity of the villi. However, villi vary in shape, exhibit a proximo-distal gradient in size and are markedly distorted during technical preparation and counting. Even absolute measurement of the villus cell population size (Feulgen staining followed by microdissection of the villi then counting the squashed villi and crypts) although more accurate, cannot fully compensate for distortion caused by abnormally shaped villi. This method, therefore, in these circumstances is not recommended[177].

The second measurement is that of epithelial DNA concentration per unit length of mucosa. This

crude estimate however, will include the not inconsiderable amount of DNA present in the lymphoid cells in the lamina propria. Therefore it is assumed that the lymphoid cell populations are not different in the experimental and control groups. However it is well known that disordered bowel function and/or flora initiated by experimental manoeuvres could well alter the population of the lymphoid cells[177]. Accuracy,however, may be improved by measuring the total DNA and the epithelial DNA is estimated by multiplying the DNA content by the factor of the tissue sections occupied by epithelial cells[178]. This assumes that uniform DNA density in the epithelium, lamina propria and plasma cells. Consequently any DNA concentration in the epithelial mass will be necessarily 'diluted'.

In the tissues studied, plasma cell infiltrate formed <5% of the total surface area. Experimental manoeuvres failed to demonstrate any significant increase in the percentage surface area of the plasma cell infiltrate. For these reasons and in line with most other workers, the plasma cell infiltrate factor has therefore been ignored.

b. Deoxyribonucleic Acid (DNA) estimation

The estimation of tissue DNA gives an accurate

reflection of the total cell population of the tissue under study. Its concentration relative to that of protein indicates cellular size and proliferative activity.

The original method of DNA estimation was described by Dische[179] in 1930. This was subsequently modified by Slater and Lovell[180] and later by Croft[181]. This latter method was used for all estimations.

Reagents: see Appendix

Method: The rat homogenate was thawed out in a waterbath at 37°C. Two and a half mls of this thawed material was placed into a stoppered tube to which was added 6mls of 6.5% Trichloroacetic acid (TCA). The volumes were chosen to ensure that each solution contained at least 20-25 atoms DNA-P/ml. The contents were then hydrolysed at 80°C for 15mins. At the end of that period, the tubes were cooled rapidly to room temperature and centrifuged at 2300 revs min^{-1} for 30 mins. The supernatent was aspirated and placed into another tube.

A second hydrolysis was performed on the residue by adding 5mls of 5% TCA and heating at 80°C for 15mins. The mixture was again cooled, centrifuged at 2300 revs min^{-1} and the supernatant thus obtained, aspirated and added to the hydrolysate obtained from the first extraction. Two

extractions were sufficient as the total DNA extracted was 99% (i.e. 90% per extraction).

All estimations were carried out in duplicate.

One ml of the supernatant hydrolysate was placed in a glass stoppered tube and to this was added 0.1ml of 60% Perchloric acid. Similarly to 1ml of each of the 4 working standards and control (see reagents), 0.1ml of Perchloric acid was added.

To each resulting mixture (test hydrolysates, DNA standards and control) was added 1ml of 2% Diphenylene reagent. The containers were stoppered and the specimens placed in a refrigerator at 4°C for 48hrs to allow colour development. At the end of this period, the coloured solutions were removed from the refrigerator, transferred into cuvettes and extinctions were read against the reagent blank at 600nm.

The optical density was plotted vs DNA-P/ml(megatoms) for the 4 working standards (fig 8) and the slope of the graph thus obtained was used to determine the concentration of the unknown values.

The DNA working standards and the controls were freshly prepared for each batch of homogenates estimated. The was a close correlation (r=94%) between the slopes used, making the method highly reproducible.

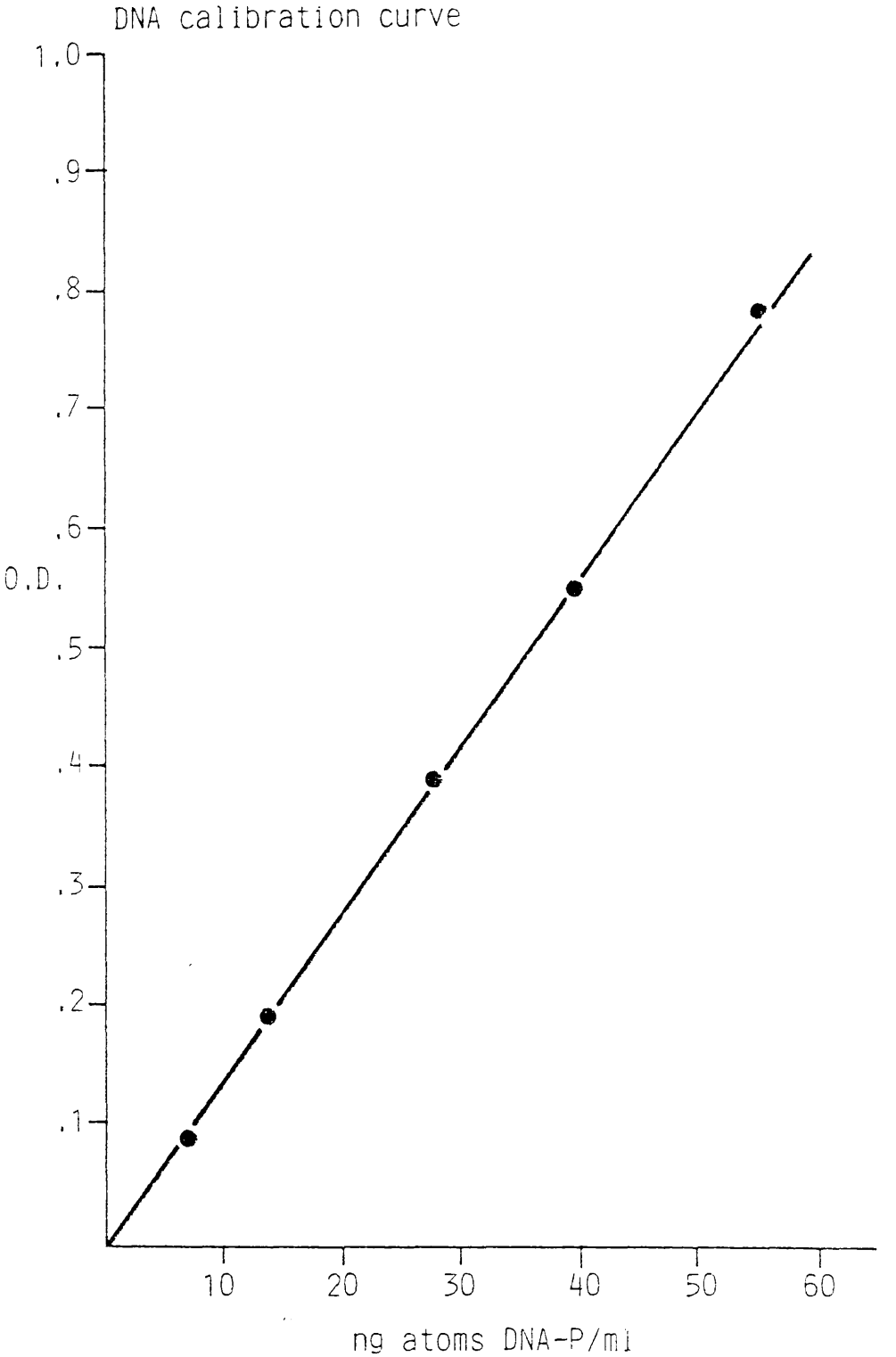


FIGURE 8

Graph illustrating Optical Density (O.D.) vs ng atoms DNA-P/ml for the working standards

c. Protein Estimation

Protein estimation was carried out using the Folin-phenol reagent method as described by Lowry[182]. This method is based on the production of a colour by the protein present and the density of the colour is read off sphectrophotometrically. The colour reaction occurs in two distinct steps: a) reduction with copper in alkali and b) reduction of the phosphomolybdic - phosphotungstinic reagent by the copper treated protein.

This method is very sensitive, simple and easy to adapt for small scale analysis. Its two main disadvantages are that the amount of colour varies with different proteins and that the colour is not strictly proportional to the protein concentration. In spite of the latter, however, it is a reasonable method for the measurements of mixed tissue proteins particularly when absolute values are not required.

Method: The mucosal homogenate was allowed to thaw. Particulate matter in the homogenate was removed by centrifugation at 2200 revs min^{-1} for 10 mins at 4°C. The resulting supernatant was then diluted with saline to give the equivalent of about 1mg of mucosa/ml.

Reagents: see appendix.

Fresh reagents and working standards were used for each batch of homogenates analysed.

To 1ml of each of the samples being analysed (homogenates, control and working standards), 5ml of the alkaline copper reagent was added. This was mixed well and allowed to stand for at least 10min at room temperature. One half ml of the Folin Ciocalteau phenol reagent (1:3 dilution) was added and mixed very rapidly. The samples are then allowed to stand for 30 mins away from direct sunlight. Following that period, the samples are transferred into a cuvette, placed into the sphectrophotometer and the optical density measured at 625nm.

Using the working standards, the optical density was plotted vs protein concentration (Fig 9) and the slope obtained was used to determine the protein concentration of the test samples. The standard slopes for each run of estimates showed a close correlation, suggesting high reproducibility of the method.

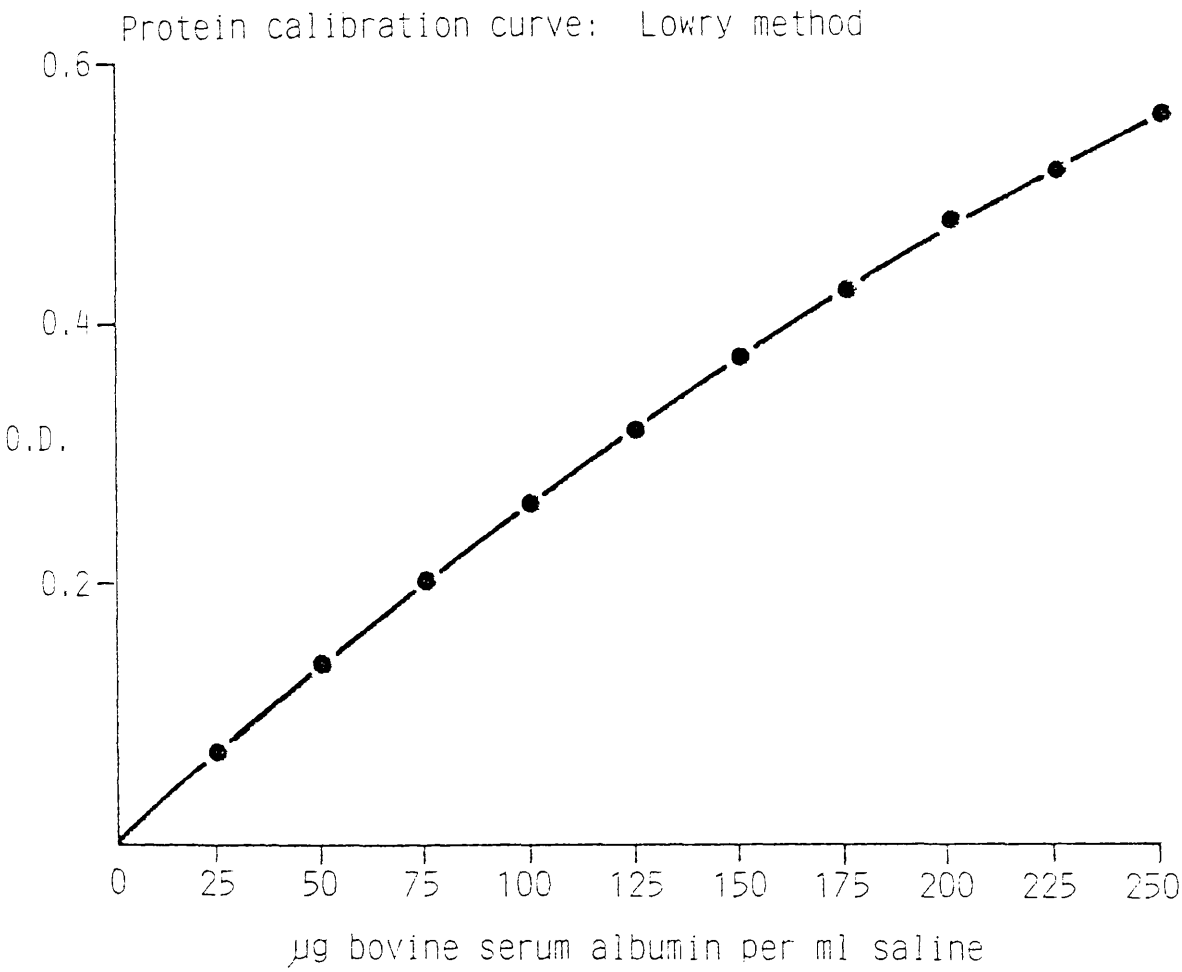


FIGURE 9

Graph illustrating the O.D. vs protein concentration for the working standards

1v. Gastrointestinal Hormone Assays

a.Introduction

The endocrine-paracrine cells scattered throughout the GI mucosa are now regarded as a diffuse modulatory system of intestinal motor and secretory function. They respond to luminal stimuli by releasing "extracellular mediators"[183].

The hormonal changes occuring in intestinal obstruction are as yet undefined and although 25 regulatory peptides have been shown to be present in the GI tract[184], it was decided to investigate only three particular peptides in this thesis, i.e. Gastrin, N-Glucagon and VIP.

Gastrin and N-Glucagon have been investigated because of the role they are alleged to play in gastrointestinal adaptation. In particular, N-Glucagon has been shown to exhibit a close correlation with crypt cell production rate[185]. VIP, on the other hand, is a neuro peptide and plays a direct role in gut motility[186,187] and blood flow[188-192] and is a potent stimulant of exocrine gut secretion[188,193,194].

b. Gastrin Assay:

As described earlier, blood samples for gastrin assay were taken in heparinized tubes and

centrifuged at 4°C. The plasma was separated and stored at -20°C pending assay.

This method is described in detail elsewhere[195]. The antibody was raised in rabbits against synthetic human gastrin I (ICI). The antibody (0098) used in the assay was at an approximate dilution of 1:10,000. This antibody does not cross react with any of the known gastrointestinal peptides (except for cholecystokinin-pancreozymin where a 10,000-fold concentration of this material is required to produce a similar fall in bound counts as standard gastrin).

Human synthetic gastrin is iodinated by chloramine T method. Plasma, rendered hormone free by charcoal, is added to the standards. A charcoal separation technique was then used[196]. The sensitivity of the assay is 5-10 picograms/ml gastrin.

c. N-Glucagon Assay:

Blood samples for glucagon assay were taken into chilled heparinized tubes, centrifuged at 4°C and the plasma extracted by the method of Heding[197]. The extracts were reconstituted in a 0.04M phosphate buffer solution to pH 7.4.

Glucagon ^{125}I was prepared according to

Jorgensen's method[195]. The separation method used was serum and dextran-coated charcoal[196]. Antibody YY57 was raised to pancreatic glucagon and was used at a final dilution of 1:22,500. This antibody reacts with the N-terminal portion of glucagon and the material measured by it is referred to as N-terminal glucagon-like immunoreactivity (NGLI). NGLI represents all known GLI's including those from the pancreas and gut.

The antibody can detect 5.9 ng/l of glucagon.

d. V.I.P. Assay:

Plasma extraction for V.I.P. assay was carried out by Heding's method[197]. Prior to assay, the extracts were reconstituted to pH 7.4 in a 0.04M phosphate buffer.

Natural porcine V.I.P. was used for standards, immunization and iodination. V.I.P. (2 micrograms) was iodinated by the chloramine T method. The labelled hormone was then purified by absorption to silica and eluted into acidified ethanol. The labelled hormone was then stored at -20°C in acidified ethanol.

Antibodies to V.I.P. were raised in New Zealand white rabbits, the rabbits being immunized with a conjugate of V.I.P. to ova albumin. Separation of free from bound hormone

radioimmunoassay was accomplished by serum and dextran-coated charcoal[196]. The standards used were natural porcine V.I.P. and were prepared in an alcohol extract of plasma.

The assay can detect 5 ng/l V.I.P. with 95% certainty and is sensitive over the ranges 0-300 ng/l. There is no cross reactivity in the assay with glucagon, secretin or GIP and the antibody is predominantly C-terminal reactive.

v. Statistical Analysis

For comparing normally distributed data, Student's t test was used. For data which was not normally distributed, the Mann Whitney U test or Wilcoxon matched pair signed ranks test was used.

For comparing proportions, Fisher's exact test was used[199]. A p value of 0.05 or less (two-tailed) was considered significant.

PART THREE

RESULTS

8. Introduction

This section details the experimental results. These are broadly subdivided into two chapters.

The first chapter, which is divided into four subsections, deals with:

1. the normal response of the tissues and organs to trauma and
2. the effects of obstruction of the small bowel on the oesophagus, stomach, jejunum and ileum. Small bowel obstruction was considered at three levels, mamely: high (L_1), mid (L_2) and distal (L_3) sites.

The second chapter investigates:

1. the effects of a low residue diet on the oesophagus, stomach, jejunum and ileum of normal rats and
2. the effect of a low residue diet on the adaptive response of the stomach, jejunum and ileum to obstruction.

The hormonal profiles of gastrin, N-glucagon and V.I.P. exhibited under the varying experimental conditions described above are also included in each section as appropriate.

Reference to animal weights will be made as appropriate in the text.

All results are expressed as mean ± 1SD unless stated otherwise.

The gross, muscle and mucosal weights of the small intestine are illustrated as mg/10cm bowel lenght.

DNA is expressed as ng atoms DNA-P/mg mucosa and protein as mg protein/mg mucosa.

Surface area is referred to in sq mm.

9. The effect of obstruction on the Gastrointestinal Tract.

The response of the gastrointestinal tract to operative trauma and to varying levels of partial small bowel obstruction will be divided into the respective anatomical sections, namely: oesophagus, stomach, and the small intestine (jejunum and ileum)

Each section of results will be followed by a summary of the main findings. These findings will then be discussed.

1. The Normal Response to trauma

The mean age of the rats at the time of commencing the experiment was 8 weeks with a body weight of 168.6±2.1gm. Following the initial response to the trauma of operation (sham) there was a fall in body weight (Table 5). The fall, which was maximal at P_1 (7 days), represented a

TABLE 5

Animal Weights

	Sham Operated	Unoperated Animals
Starting Weight	168±2.1	168±1.8
P_1 (7 days)	161±2.8*	172.3±2.4
P_2 (11 days)	167±3.1	173.9±3
P_3 (15 days)	172±4.5	174.5±3.1

*p=0.05

The effect of operative trauma on somatic growth: sham operated animals compared with unoperated matched controls.

4 % reduction from the starting weight prior to operation and a 7% (p=0.05) reduction compared with the expected weight of age matched unoperated controls. This reduction in weight persisted until P_2. Weight gain rapidly accelerated although it was still reduced at P_3 as compared with

unoperated controls.

Oesophagus:

As might be expected, the weight of the oesophagus from control (sham operated) animals showed a slight but progressive increase in weight from 33.5 mg/cm at P_1 to 38 mg/cm at P_3 (p=NS). This occurred despite a reduction in body weight. There was no obvious change in either the length or the outer circumference of the organ but this may partly reflect the "crude" measurements used to quantify change. Microscopical measurements of mucosal and muscle cross section surface area showed no change either.

Stomach:

The gross weight of the stomach in control animals behaved in a similar fashion to oesophageal weights during the three periods of study: i.e. a gradual increase in weight with time. These changes (Table 6) were accurately reflected in slow but progressive increase in both muscle and mucosal mass. Surface area was constant throughout the period of study. DNA concentration per mg of mucosa also remained unaltered. Protein concentration showed a slight (but not significant) drop between P_1 (0.087±0.004 mg protein/mg mucosa) and P_3

(0.078±0.004 mg protein/mg mucosa).

TABLE 6

Stomach Measurements

	P_1	P_2	P_3
Gross Wt(mg)	950±40	965±58	979±55
Muscle "	823±60	830±45	834±65
Mucosal "	128±8	135±15	145±14
DNA	5.45±0.8	5±0.35	5.7±1
Protein	0.087±0.04	0.089±0.06	0.078±0.04
Gross SA (mm)	1450±95	1570±135	1610±105
Rumen "	270±32	290±35	295±35
Fundus "	720±45	765±25	795±50
Antrum "	460±50	515±40	520±30

Changes in weight and surface area (SA) in the stomach from control (sham operated) animals over the three periods of study.

Jejunum:

The jejunum from control animals (Table 7) showed a linear increase in weight with time despite a reduction in body weight. This was reflected in an increase in muscle weight. No gross mucosal changes were noted. Bowel circumference did not alter appreciably. Mucosal DNA and protein concentration were constant throughout the period studied suggesting no gross alteration in cell kinetics.

TABLE 7

Jejunal Measurements

	P_1	P_2	P_3
Gross Wt (mg)	546±60	596±75	654±70
Muscle "	356±60	431±77	468±92
Mucosal "	191±38	165±10	186±34.6
DNA	4.6±0.9	4.7±0.4	4.7±0.9
Protein	0.63±0.007	0.062±0.005	0.063±0.01
Circumference (mm)	7.8±0.6	8.4±0.4	7.75±1.3

Table illustrating jejunal weights and mucosal DNA and protein concentration in control (sham operated) animals over the three periods of study

Ileum:

The ileum in controls behaved in an identical fashion to the jejunum in that there was a linear increase in the gross weight with time (Table 8). Alteration in the muscle mass between P_1 and P_3 was minimal. Changes in mucosal weights were, however, more pronounced. DNA concentration, unlike protein which remained essentially constant, showed a tendency to increase over the study period.

TABLE 8

Ileal Measurements

	P_1	P_2	P_3
Gross Wt (mg)	381.3±38	444.7±61	500.5±80
Muscle "	290.1±20.3	298.9±41.5	325±51.3
Mucosal "	91.2±28.7	145.8±37	175.5±78.8
DNA	4.34±0.8	4.62±0.32	5.1±0.7
Protein	0.058±0.004	0.56±0.001	0.52±0.019
Circumference (mm)	7.9±0.4	6.75±0.4	7.75±1.3

Changes in ileal weight and mucosal DNA and protein concentration during the three periods of study.

Hormonal Response (Table 9):

1. Gastrin: The serum gastrin level in the normal rat was found to be 207±32 ng/l, 180±14 ng/l and 168±15 ng/l at 7, 11 and 15 days respectively. These values are similar to those previously described.
2. N-Glucagon: The levels of N-terminal Glucagon showed a significant stepwise decrease for the phases of the study (P_1: 196±20ng/l - P_3: 165±15ng/l).
3. VIP: the serum VIP concentration remained at a similar level throughout the 3 periods of study, mean values being 52±14 ng/l.

TABLE 9

Hormone (ng/l)	P_1	P_2	P_3
Gastrin	207±32	180±14	168±15
N-Glucagon	196±20	180±20	165±15
V.I.P.	40±14	60±12	45±15

Serum levels of gastrin, N-glucagon and V.I.P. in control (sham operated) animals.

Summary of Results:

1. Body weight showed an initial drop after operation (maximum at P_1) and rapidly increased thereafter.
2. The various segments of the gut behaved in a similar fashion showing a linear increase in gross weight despite an initial reduction in body weight. Both muscle and mucosal moieties reflected the increase.
3. The DNA:protein ratio remained fairly constant throughout, except in the case of the ileum where the ratio showed an increase.
4. The hormone profile for gastrin and glucagon suggested a slight drop over the three periods of study. VIP profiles were unchanged.

Discussion.

These findings clearly illustrate the body response to the trauma of operation. A change in the energy source results in the intracellular protoplasm being converted to glucose via the carbohydrate oxidative pathway[200]. The net effect is the initial weight loss and the tissue predominantly involved is skeletal muscle.

Transient immobilisation and starvation (caused by post operative pain and nausea) add to this impost on skeletal muscle. The results of this experiment support the contention[201] that this type of post-traumatic catabolism has no impact on the total cell mass of the gastrointestinal tract. Indeed, as illustrated here, this transient post-traumatic draft on muscle has no adverse effect on visceral growth. Within this range of severity, the post traumatic cellular catabolism appears to be a species adaptation: a new set of metabolic conditions imposed by new demands placed upon the organism.

Whilst detailed knowledge exists of the endocrine response to trauma[202,203], little is known about the paracrine organ response. Alteration in serum gastrin and N-terminal glucagon is dependent on luminal nutrition. Any expected

post-operative drop in the serum levels consequent upon starvation is likely to occur early. It is therefore perhaps not surprising that during the 3 periods of study, during which period the animal has fully recovered, no significant change is noted. Vasoactive Intestinal peptide, a neuro peptide, mostly involved in gastrointestinal motility and vasodilatation, similarly remained constant throughout the period of study.

11. The effect of a high level obstruction (L_1) on the oesophagus, stomach, jejunum and ileum

Oesophagus:

The introduction of a high small bowel obstruction produces measureable changes in the oesophagus. The overall bulk was increased compared with controls. This change expressed as wt/unit area, was noticeable at P_1 (controls (C): 33.5±5mg/sq cm vs obstructed (O): 37±5 mg/sq cm) and peaked at P_2 (C:38.5±3.5 mg/sq cm vs O: 51.5±4.5 mg/sq cm:p=0.02). These changes were not sustained beyond this point and had returned to control values by P_3 (C: 38±4.5mg/sq cm vs O: 35±9.5mg/sq cm). The change was purely due to an increase in the muscle mass. Mucosal surface area and mass did not significantly change.

Stomach:

The response of the stomach to partial intestinal obstruction depends on the primary site of obstruction. Following a high (L_1) obstruction, the stomach, at 7 days,(P_1 period) responded with a rapid and profound increase in surface area with the main increase occurring in the rumen portion of the stomach. This represented a 53% increase in the

TABLE 10

Stomach

	P_1	P_2	P_3
Gross Wt (mg)			
O	1220±110*	1200±85*	1175±81*
C	946±40	954±54	968±62
Muscle Wt			
O	1065±120	1028±132	1052±93
C	818±63	819±42	829±82.4
Mucosal Wt			
O	160±32	179±29.2#	122±37.5
C	127±8	135±11	139±49
DNA			
O	8.3±0.8"	7.0±0.5*	5.79±0.66
C	5.5±0.9	5.09±0.17	5.78±0.9
Protein			
O	0.075±0.02	0.066±0.01	0.072±0.02
C	0.087±0.004	0.089±0.007	0.078±0.004
Stomach SA (sq mm)			
O	1952±139"	1652±150	1425±62.7
C	1451±77.8	1570±62	1580±97
Rumen SA			
O	424±22	299±15	270±29.4
C	276.3±60	289.3±30.9	235±72
Fundus SA			
O	909±48.6	702.5±82.7	666.5±36.2
C	715.3±40	769.6±34.4	717.9±61
Antrum SA			
O	614.6±30.5	650.8±42.1	488±27.7
C	459.7±81	511.4±36	401±69

*p=0.02 #p=0.05 "p=0.001

Changes in the stomach measurements in L_1 obstructed (O) animals as compared with controls (C).

rumen as compared with a 27% increase in the fundus and a 23% change in the antrum. In spite of continuing obstruction, the stomach surface area was gradually reduced in size so that by P_3 the stomach surface area had returned to control values. The three anatomical subdivisions of the stomach (i.e. the rumen, fundus and antrum) returned to the same relative sizes as controls.

During the initial dilatation period, the overall gross weight of the stomach (Fig 18) increased (O:1225±124 mg vs C: 986±62 mg; p=0.02) and the increased weight is maintained for the duration of the experiment (P_3). The relative weights of the muscle and mucosa reflect the different response. During the phase of dilatation, there was a rapid increase in mucosal weight both in the P_1 and P_2 periods but maximal at P_2. This was reflected in a significant increase in DNA concentration which peaked at P_1 (O: 8.3±0.9 nd DNA-P/mg mucosa vs C: 5.5±0.8 ng DNA-P; p=0.001) and was still raised at P_2 (O: 7±0.5 vs C:5±0.4 ng DNA-P atoms/mg mucosa p=0.02). There was also a slight increase in muscle mass. By P_3, the dilatational effect was lost and this was followed by a rapid reduction in the mucosal mass to control values (O:121.8±25 mg vs C: 138.8±28mg: p=ns).

Jejunum:

The magnitude of the jejunal response to partial obstruction depends on the location and duration of the stenosis. The introduction of a high small bowel obstruction (L_1) resulted in a linear increase in weight (Table 11) over the three periods studied, with significant values being achieved at P_2 and P_3. This increase in weight was mainly due to an increase in muscle mass with significantly increased mass being similarly attained at P_2 and P_3.

Increase in gross weight was associated with a rapid increase in bowel circumference with the peak value being achieved at P_1. This increase was maintained at the same level at P_2 and P_3. Increase in mucosal mass lags behind changes in bowel circumference with a maximal response occurring at P_2 and returning to control values thereafter.

There was no alteration in the DNA concentration during the periods of study in the obstructed animals as compared with controls. There was however a significant reduction in protein concentration in the obstructed animals at P_1 and P_2 but this returned to control values by P_3.

TABLE 11

Jejunum

	P_1	P_2	P_3
Gross Wt (mg)			
O	680±115	917±295^	1260±176"
C	550±55	590±78	650±74
Muscle Wt			
O	505.2±182	599±105#	996±246"
C	356±50	431±77	468±92
Mucosal Wt			
O	178.3±60	318±67#	264±79
C	191±38	165±10	186±34.6
Circumference(mm)			
O	9.75±1.7	9.5±1.5	9.7±2.7
C	7.8±0.6	8.4±0.4	7.8±1.3
DNA			
O	4.39±0.7	4.03±0.2	4.9±1.7
C	4.6±0.9	3.88±0.2	4.7±0.9
Protein			
O	0.048±0.01#	0.038±0.01*	0.063±0.02
C	0.063±0.007	0.062±0.005	0.063±0.01

^p=0.01, *p=0.02, #p=0.05, "p=0.001

Changes in the measured variables of the jejunum at the three periods of study as compared with controls.

Ileum:

It is not possible to compare changes in ileal morphology or morphometry with the site of obstruction. An L_3 type of obstruction induces hypertrophic changes. L_1 and L_2 types of obstruction, by virtue of their being proximal to the ileum, might be expected to induce disuse atrophy.

An L_1 obstruction had no appreciable effect on the distal ileum for the duration of the study.

Hormonal Response:

1. Gastrin: The introduction of a high (L_1) small intestinal obstruction resulted in a significant increase in the antral area at P_1 and P_2. By P_3, antral size had returned to control values. Serum gastrin levels rose significantly by P_1 and remained thus raised throughout the period of the study.
2. N-Glucagon: these levels were not significantly different from controls and showed a similar stepwise reduction with time.
3. VIP: there was no significant change in VIP levels during the course of the L_1 obstruction experiment.

TABLE 12

GI Hormones

Hormone	P_1	P_2	P_3
Gastrin			
O	320±64#	290±47*	307±69#
C	194±37	191±18	182±15
N-Glucagon			
O	208±17	195±12	191±13
C	187±24	194±18	178±18
V.I.P.			
O	40±13	65±9	40±14
C	38±12	61±16	44±16

*p=0.02, #p=0.05

Hormone profiles in animals with a high jejunal obstruction as compared with controls

Summary of results:

1. Oesophageal muscle showed a transient increase in weight at 7 and 11 days.
2. Compared with controls, the stomach showed an initial profound dilatation affecting mainly the rumen (53% increase). Gross weight increased rapidly and plateaued thereafter. This was closely paralleled by changes in muscle weight. Mucosal weight showed a peak increase at P_2 (11 days). DNA concentration reflected these mucosal changes.
3. Jejunal weight increased rapidly with the duration of obstruction. The main increase occurred in the muscle mass. Dilatation was not pronounced. Mucosal mass changes were not significant, reflecting minimal alterations in DNA concentration.
4. No ileal changes were noted with a high jejunal obstruction.
5. The serum gastrin level was significantly raised at 7, 11 and 15 days after an L_1 obstruction. N-glucagon and VIP levels were unaltered.

The effect of a small bowel obstruction on oesophageal tissue has hitherto not been investigated. The experimental results obtained suggest that, inkeeping with most muscular tubular organs elsewhere, the oesophagus reacts to a high small intestinal partial obstruction by a significant hypertrophy of the muscular layer. There was no evidence of associated dilatation of the organ and this would, according to Brent's concept[156], suggest complete organ compensation.

Closer analysis of the results suggest that the response is hyperbolic with the maximum effect noted at P_2 (11 days) and the values returning to their control equivalent by P_3 (15 days). As there was no evidence of dilatation and the mucosal surface remained constant, one would assume that alteration in the muscle bulk accounted for these changes. Both alteration in size (hypertrophy) as well as in the number (hyperplasia) of muscle cells can account for an increase in the muscle mass. Indeed, it has been demonstrated[171] that such changes do occur within the gastrointestinal tract over such short periods of study, in response to obstruction. It seems plausible, therefore, to extrapolate such changes to the oesophagus.

For such a response to occur, an increased workload on the oesophageal muscle is required to generate this 'demand' hypertrophy. Whilst jejunal dilatation and hypertrophy may influence oesophageal behaviour, the gastric response may have a key role in the behaviour of the oesophageal tissues. Superimposition of the oesophageal changes on the dilatational response of the stomach (Fig 10) indicates a "lag response" between the hypertrophic oesophageal changes and the stomach dilatational response. This would therefore suggest that in the initial phase following obstruction when stomach dilatation occurs, an increase in the intragastric pressure or perhaps increasing reflux into the oesophagus occurs, resulting in difficulty in clearance of the oesophageal contents. It may be assumed that increased peristaltic activity occurs leading eventually to an increase in the muscle bulk.

The rapid reduction in the oesophageal bulk over the P_2 - P_3 period is more difficult to interpret. Although atrophy may be responsible, such an adaptive change would be expected to take place over a "long term" period. Therefore the hypothesis of simple hypertrophy - atrophy cannot wholly explain such rapid changes. Two other factors may be considered relevant. The first may

FIGURE 10

Histograms illustrating oesophageal hypertrophy secondary to stomach dilatation. As stomach dilatation reverts to normal, a reduction in oesophageal mass (atrophy) occurs.

LAG RESPONSE BETWEEN STOMACH DILATATION AND OESOPHAGEAL HYPERTROPHY

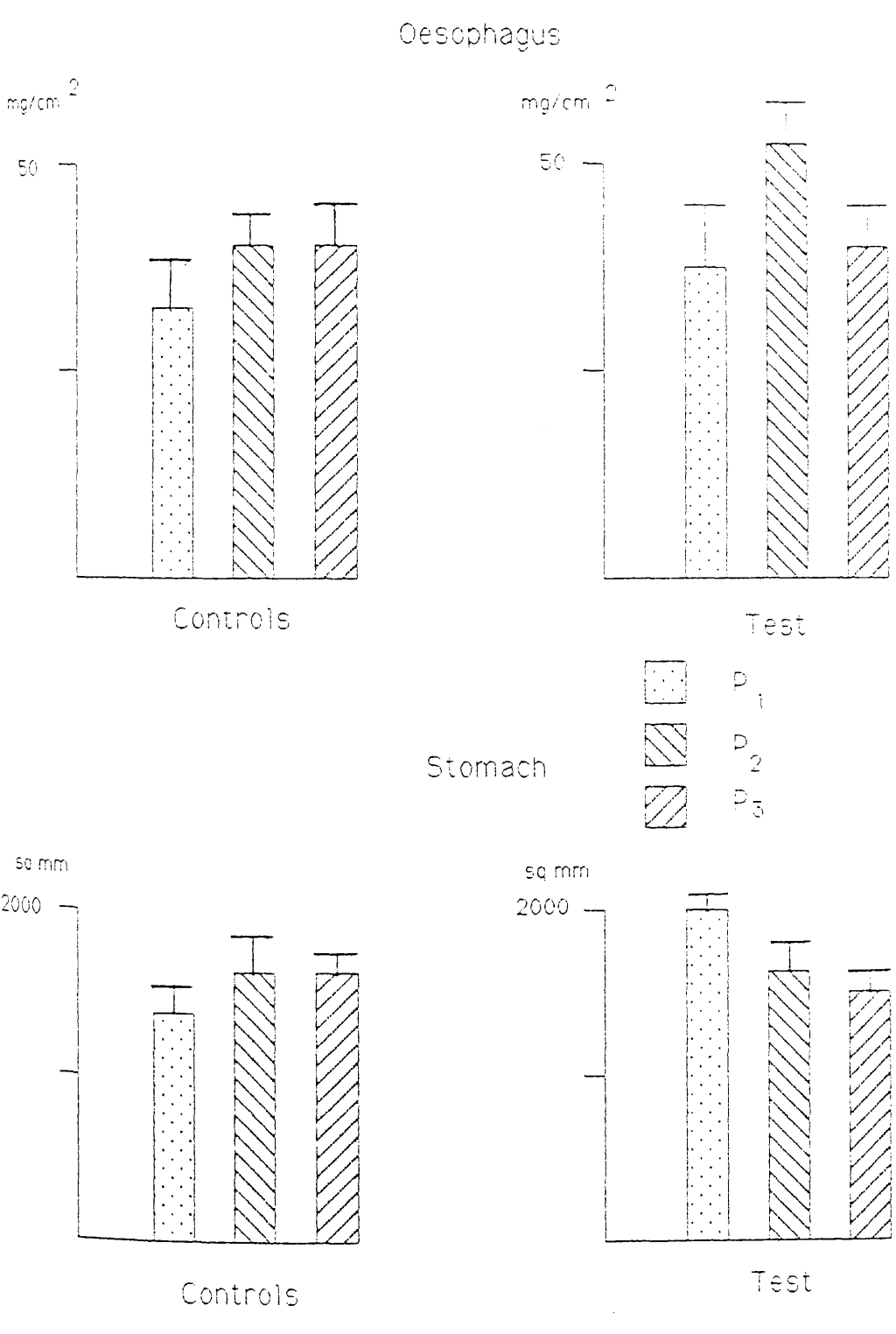


FIGURE 10

be an increase cellular infiltrate which occurs early following obstruction. Histological scanning of oesophageal sections fail to support such a contention and the change that occurs in the white cell population is less than 5%. The second factor may well be increased interstitial oedema[202]. This may be the result of either a simple mechanical effect due to the obstruction or the effect of vasoactive substances released by the increased plasma cell infiltrate. Changes in the oedema fluid can occur rapidly and it is therefore not unlikely that this factor may, in part, account for the changes noted.

It is most likely that the features observed are a combination of hypertrophy / hyperplasia of the muscle cells and oedema with a combination of both factors accounting for the observed peak in weight at P_2. As the oedema subsides, the hypertrophy and/or hyperplasia element is left behind. The findings at P_3 would therefore lead to the conclusion that it is unlikely that any significant morphological changes occurred during the period of study.

The rate and the nature of the response of the stomach to partial intestinal obstruction depends on the primary site of the stenosis. Following a high small bowel obstruction, the stomach responds

by an initial increase in the surface area (phase 1 - dilatational effect ? decompensation) and this represents the first step of the adaptational response to the insult. The main alteration occurs in the rumen, a finding not unexpected as this area of the stomach is highly elastic[204] and is therefore capable of considerable stretch. Simple stretching or dilatation, however, is not the only mechanism responsible for the change in the stomach size, because if so, one would have expected a drop in the weight per unit area. The results indicate that there is an increase in both the muscle and mucosal moieties and the increase in mucosal weight is accompanied by an early rise in DNA concentration per mg mucosa, hence suggesting hyperplasia as the reason for the increased mucosal mass. As the insult is not overwhelming and compensation occurs, increasing tone and peristaltic activity return the flaccid stomach to a normal size.

Marked dilatation does not seem to be a feature of the jejunal response to obstruction. There was only a slight but non-significant increase in the mean bowel circumference as compared with chow fed controls. The main response was an increase in the muscle mass. These findings would therefore suggest two possibilities: Firstly,

"storage capacity" of the upper jejunum is limited unlike the stomach where dilatation is very marked. This may simply be due to either an inherent inability of the jejunum to dilate or as the stomach seems to be "accomodating the insult", no physiological demand is being placed on the jejunum. Secondly, as the degree of stenosis increases, increasing isometric contractions are required to propel the semi-liquid food through the stenosis. This increased muscular activity ultimately results in an increase in muscle mass.

Ileal changes are not in evidence in this phase of the experiment. This may be related to the "long" column of food distal to the stenosis which is providing the "luminal nutrition" to the ileal mucosa and the mechanical stimulus to the bowel musculature. In addition, the semi-liquid food present in the jejunum could be more easily expressed through the stenosis, thereby providing the more distal bowel with adequate nutrition for a more prolonged period.

In this experiment, the main changes have occurred in the upper gastrointestinal tract. It is therefore perhaps not surprising that no alteration in the serum levels of N-Glucagon and VIP have been noted. As has been intimated earlier, these hormones are of mid and distal small bowel origin.

The only change noted was a persistent and significant rise in the serum gastrin level. Gastrin is released as a result of antral distention. In this type of experiment gastric emptying is markedly delayed. The resulting gastric distention and hence antral stretching, acts as a powerful stimulus to gastrin release resulting in the changes noted above.

111. Effects of a mid (L_2) small bowel obstruction on the oesophagus, stomach, jejunum and ileum.

Oesophagus:

Mid small bowel obstruction failed to produce any measureable response within the oesophagus.

Stomach:

Advancing the site of obstruction to the mid small bowel produced a different response from an L_1 type of obstruction (Table 13). There was a slow but progressive increase in the surface area of the stomach, with significant differences occurring by P_3. The main increase in the surface area occurred in the antrum showing a 35% ($p=0.02$), 34% ($p=0.05$) and a 97% ($p=0.001$) increase at P_1, P_2 and P_3 respectively as compared with controls. There was surprisingly no alteration in the gross weight of the stomach or in the muscle or mucosal portions. There was no alteration in the DNA concentration as compared with controls although a trend to increase with time was demonstrable. Protein concentration showed a slight but insignificant reduction.

TABLE 13

Stomach

	P_1	P_2	P_3
Gross Wt (mg)			
O	960±35.2	827.4±75.4	934.7±70.2
C	930±34	969±71.1	974±31.1
Muscle Wt			
O	836.9±62.4	715.2±31.6	786±54.2
C	789±74	801±34.7	834±86.6
Mucosal Wt			
O	123.1±33.4	112.2±45.4	147.9±23.5
C	141±15.7	168±23.3	140±18.3
DNA			
O	5.49±0.8	5.66±0.83	5.79±0.7
C	5.57±0.5	5.43±0.31	5.69±0.87
Protein			
O	0.066±0.006	0.72±0.01	0.07±0.006
C	0.083±0.007	0.09±0.006	0.08±0.005
Stomach SA (sq mm)			
O	1642.5±59.3	1700±59	1925.5±68*
C	1437.5±67	1489±86	1531±94
Rumen SA			
O	320.2±23	282.4±35	322.3±77.9
C	245.6±34.3	261.7±39.6	281.7±49
Fundus SA			
O	654.2±33	647.4±59.2	660.1±64.5
C	700±25	754.7±51.3	770.1±54.3
Antrum SA			
O	668±70.2*	633.6±34#	943.1±66.8"
C	491.6±33.7	472.6±29.2	480±44.8

#p=0.05, *p=0.02, "p=0.001

Table illustrating the changes in the stomach parameters following a mid small bowel obstruction.

Jejunum:

A mid small bowel obstruction (L_2) produces a pronounced increase in gross, muscle and mucosal weights as compared with controls (Table 14). Significant increases in gross weight was achieved by P_1 and mucosal and muscle weights by P_3. DNA concentration showed no change with time as compared with controls. Reduction in protein concentration occurred but this reduction was delayed until P_2 and achieved significant values at P_3. The bowel circumference showed a gradual but pregressive increase with the maximum values obtained being similar to those achieved during L_1 obstruction.

Ileum:

An L_2 obstruction resulted in a significant reduction in ileal weight at P_3 as compared with controls but this was mainly due to a reduction in mucosal mass (Table 15). Gross muscle weight was not appreciably reduced. Ileal circumference, though slightly reduced as compared with controls, was not significant. The mucosal DNA concentration was slightly increased over the three time periods as compared with controls. Protein concentration, on the other hand, showed a slight tendency to reduce.

TABLE 14

Jejunum

	P_1	P_2	P_3
Gross Wt (mg)			
O	716.9±34*	822.8±67.3^	1100.7±140.3"
C	568±71	612.6±75.7	655.4±83.1
Muscle "			
O	479.9±69.6	575.7±74.8	820.3±101.1#
C	370±48	431.3±77.6	460.1±49
Mucosal "			
O	237±61	246.9±53.1	280.4±53
C	198±43	181.3±27	195.3±51
Circumference (mm)			
O	7.65±1	8.75±1.1	9.85±1.5
C	7.6±0.5	7.8±0.8	7.7±0.76
DNA			
O	4.7±0.8	4.4±0.6	4.07±0.91
C	4.05±0.8	4.1±0.7	4.6±0.84
Protein			
O	0.062±0.02	0.053±0.016	0.045±0.04#
C	0.065±0.004	0.068±0.005	0.067±0.001

#p=0.05, *p=0.02, ^p=0.01, "p=0.001

Table illustrating the changing jejunal parameters following a mid small bowel obstruction

TABLE 15

Ileum

	P_1	P_2	P_3
Gross Wt (mg)			
O	437±53.6	400±45.8	369±61.3
C	375±71	426±39.7	489±56.1
Muscle "			
O	325±43.6	300±51.4	300±46.1
C	280±35.3	290.3±44.3	331.6±41.5
Mucosal "			
O	112±37.3	100±19.7	69±28.3#
C	95±27.5	136±21.3	158±33.6
Circumference (mm)			
O	6.3±0.3	6.15±0.7	5.86±0.6
C	7.7±0.3	7.6±0.5	7.78±0.7
DNA			
O	5.4±0.6	5.5±0.6	5.6±0.7
C	4.2±0.7	4.7±0/8	5.0±0.8
Protein			
O	0.051±0.006	0.047±0.006	0.045±0.003
C	0.055±0.005	0.054±0.003	0.052±0.004

#p=0.05

Changes in ileal weight, DNA and protein concentration and circumference following a mid small bowel obstruction.

Hormonal Response (Table 16):

1. A mid small intestinal obstruction failed to produce any change in the serum gastrin levels compared with controls, despite an appreciable increase in the antral surface area. Indeed early serum levels (at P_1) were significantly reduced.
2. N-Glucagon levels showed a significant and progressive increase during the period of study. The maximum values obtained were 250+30ng/l as compared with controls 165+15ng/l at period P_3.
3. VIP showed a significant rise in the serum levels at P_3. No changes were recorded at P_1 or P_2 periods.

TABLE 16

Gastrointestinal hormones

Hormone (ng/l)	P_1	P_2	P_3
Gastrin			
O	78±12.9#	140±38.8	166±45.3
C	185±41.3	197±33.1	198±17.8
N-Glucagon			
O	235±14.3#	245±17.7#	250±31.3#
C	170±37	179±23	183±19
V.I.P.			
O	62±10.3	65±17.3	112±23*
C	43+9	57+14	59+16

#p=0.05, *p=0.02

Changes in the hormonal profiles following a mid small bowel obstruction.

Summary of Results:

1. No oesophageal changes were noted following an L_2 obstruction.
2. The stomach response was one of a slow and gradual increase in surface area with no change in weight. The main increase in the surface area occured in the antrum. A rising trend in mucosal DNA concentration was noted. Protein concentration was unaltered.
3. The jejunum responded by an increase in weight. This change was mainly in the muscle although some increase in the mucosal mass did occur. Bowel dilatation did occur by day 11 (P_2) but the degree of dilatation was not in excess of that seen in L_1. DNA concentration was unaltered but the protein concentration showed a slight reduction with time.
4. Signs of atrophy (i.e. a reduction in the mucosal mass) were apparent in the ileum. DNA showed a slight increase whilst protein concentration was reduced.
5. Gastrin levels showed an initial early fall but the values had reverted to normal by P_3. Glucagon levels were significantly raised throughout, whereas VIP values showed only a significant rise at P_3.

Discussion

As the site of obstruction is moved more distally, the intensity and the duration of the initial dilatational response of the stomach is proportionately reduced as discussed earlier (vide supra). The stomach, after an L_2 obstruction, behaves in a way rather similar to the later periods of the L_1 obstruction. A slow and progressive increase in the surface area is evident as the gastric emptying becomes progressively more delayed. The maximal increase in the surface area occurs in the antrum which increased by 97% by P_3.

As this was a slow process of accomodation, no significant alteration in muscle or mucosal weight was noted. However, it is perhaps important to realise that the slight increase in DNA in the obstructed animals suggest an increase in the cell population to account for the slow increase in the surface area. Although no measureable change occurred in the muscle mass, this does not preclude the expected response of gradual muscle hypertrophy and hence increase in bulk. This may simply reflect the measurement of the early part of the response and it may well be that if survival of the animal could be extented, sufficient time would elapse to

allow a measurable increase in muscle bulk.

The jejunal response, rather mirrors the adaptive features exhibited by the stomach. As the obstruction was produced at the mid small bowel, dilatation of the upper jejunum was not an early feature. Indeed, when it did occur (at P_2 and P_3) it was not in excess of the dilatation achieved after a high (L_1) obstruction. Thereby, perhaps, enphasizing the concept that dilatation in this part of the bowel has its own morphological limitations.

As part of the normal response to obstruction, jejunal muscle mass did increase and this increase was identical to the muscle mass increase following the L_1 obstruction. One would have expected that the degree of hypertrophy / hyperplasia is maximal closest to the site of the obstruction and therefore the proximal jejunal response to L_2 obstruction should have been less marked. The changing nature of the food itself (fluid proximally , semi-solid distally) would support the latter concept; therefore, this is clearly not the mechanism involved. It is more feasible to assume that the stimulus to an increase in the muscle bulk originates from the peristaltic activity which is uniformly accentuated in the bowel proximal to the obstruction.

A slight increase in the mucosal mass together with a slight reduction in DNA concentration was observed, but this was not significant. This effect may well be spurious. However the fact that an increase in weight had occurred cannot be dismissed.

iv. The effect of a distal (L_3) small bowel obstruction on the oesophagus, stomach, jejunum and ileum

Oesophagus, Stomach and Jejunum:

The induction of a distal small bowel obstruction produces no appreciable change in either the morphology or morphometry of the oesophagus, stomach or jejunum.

Ileum:

Following an L_3 obstruction, there was a significantly rapid and massive increase in the gross weight of the ileum as compared with controls (Table 17). The alteration in gross weight was associated with with an increase in bowel circumference with significant changes occurring at day 7 (P_1). Increases in gross weight and bowel diameter were reflected in an increase in both muscle and mucosal weights with significant values being noted by P_1. DNA and protein concentration showed a gradual reduction with time, both being significantly reduced at P_3.

Hormonal Response

1. Distal small intestinal obstruction failed to produce any change in the serum gastrin levels

TABLE 17

Ileum

	P_1	P_2	P_3
Gross Wt (mg)			
O	1154±103"	1893.7±179!	2487±303!
C	320±31	383.9±39.6	429±47.3
Muscle "			
O	870.4±110"	1493±263"	1904±315!
C	240±39	258±31	271±26
Mucosal "			
O	283.6±57^	400.9±98^	582±156.5"
C	80±17.3	125±31	158±23.6
Circumference (mm)			
O	11±1.1#	16.9±2.8"	21.7±3.16"
C	6.8±0.9	7.3±0.7	7.5±1.0
DNA			
O	4.87±0.26	3.84±0.25	3.96±0.34#
C	4.1±0.37	4.5±0.41	4.8±0.34
Protein			
O	0.047±0.005	0.032±0.009	0.039±0.01#
C	0.056±0.004	0.055±0.003	0.051±0.007

#p=0.05, ^p=0.01, "p=0.001, !p>0.001

Changes illustrating the dilatation and hypertrophy occurring in the ileum following a distal small bowel obstruction.

compared with controls. In fact, plasma gastrin levels at day 7 were significantly reduced.

2. Plasma N-glucagon levels were found to be significantly ($p=0.001$) raised for all time periods as compared with controls. Maximum values obtained were 320±34ng/l (control values being 195±20ng/l).

TABLE 18

Gastrointestinal Hormones

Hormone (ng/l)	P_1	P_2	P_3
Gastrin			
O	74±14	152±35	124±21
C	196±27	199±31	180±21
N-Glucagon			
O	292±35"	320±45"	285±29"
C	200±41	193±34	179±27
V.I.P.			
O	115±11"	97±13	71±14
C	49±12	58±17	53±16

"$p=0.001$

Gactrointestinal hormone profiles in animals undergoing a distal small bowel obstruction.

3. VIP values showed a significant increase at P_1 (O:115±11ng/l vs C: 40±14ng/l; p=0.001). thereater the levels declined in a stepwise fashion to control values.

Summary of Results:

1. No morphological changes were identified in the oesophagus, stomach and jejunum.
2. The ileum responded by a gross increase in weight and circumference, suggesting dilatation of the bowel, hypertrophy of the muscle coats and an increase in mucosal weight. DNA concentration was increased whereas protein concentration showed a gradual reduction with time.
3. Gastrin levels were unchanged. VIP exhibited an early rise which returned to control values by P_{30}. N-glucagon levels showed a significant and persistently raised level.

Discussion.

The jejunum and ileum, although part of the small intestine exhibit fundamental differences both in structure and function. As has been argued earlier, some of the anatomical and physiological differences are the result of their location in the gastrointestinal tract[35-37] and although their weight / unit area is different, reflecting the larger bulk in the jejunum, the mucosa : muscle ratio is identical. Operations of jejuno - ileal transposition confirm that some anatomical adaptation does occur[10,13]. However this does not seem applicable in the response of the two segments of bowel to obstruction.

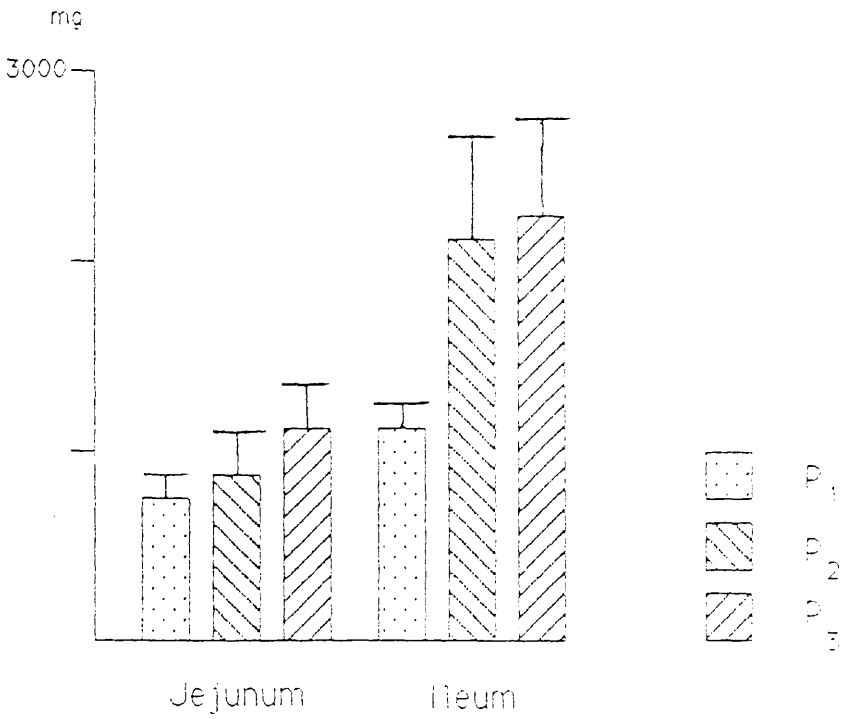
Although both structures respond with an increase in their gross weight, two fundamental differences emerge on closer inspection (Fig 11). The first is that the ileal response is more dramatic and is a combination of both muscle and mucosal increase in bulk. The jejunal response is more 'subdued' and the changes in weight are primarily an alteration in the muscle moiety. The second is that dilatation is not a pronounced feature of the jejunum.

Increase in muscle mass has been previously shown to be the result of both hypertrophy and

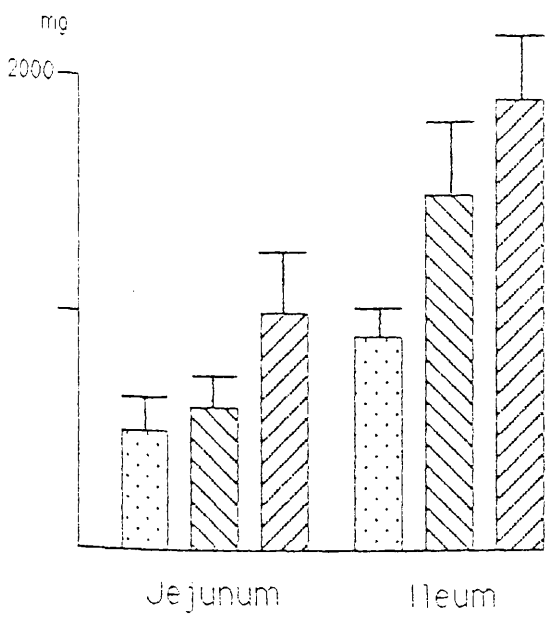
FIGURE 11

A comparison between the jejunal and ileal response, as measured by the gross weight and the muscle and mucosal mass, to obstruction.

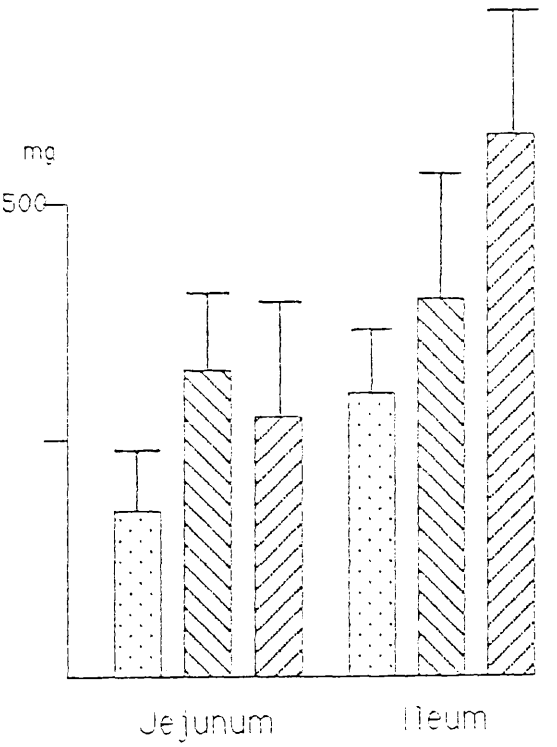
COMPARISON OF THE JEJUNAL AND ILEAL RESPONSES



Gross Weight



Muscle Weight



Mucosal Weight

FIGURE 11

hyperplasia and as there is no reason to suggest that ileal muscle differs from jejunal muscle, other factors must be responsible for this discrepancy. Blood-borne factors would affect both sections of the gastrointestinal tract. In addition, GI hormones are known to have little effect on muscle. It is therefore unlikely that systemic influences play any modulating role.

A plausible suggestion is that a mechanical factor is involved and this could operate in two ways. Increasing obstruction results in increased peristaltic activity leading to work hypertrophy. As the degree of stenosis was similar for both the jejunum and ileum, it is unlikely that this factor is operative for the noted discrepancy. The second reason may be related to the nature of the intestinal chyme. Ileal chyme is semisolid as opposed to the jejunal contents which are liquid. There would therefore be required a greater mechanical effort in the ileum to deliver this semisolid material through the stenotic lesion.

Gross dilatation does not seem to be a feature of the jejunum and this again may be related to the fact that the more liquid intestinal contents are more easily expressed through the stenosis. It is envisaged, that as the ileal contents are more difficult to express, a buildup of contents,

leading to dilatation, occurs.

As jejunal dilatation is limited, little change occurs in the mucosa. DNA concentration is unchanged and therefore hyperplasia is not a feature. Any alteration in the mucosa must therefore relate to a change in either cell size or cell thickness (stretch effect). The initial reduction in the protein concentration would favour the latter mechanism. The ileum, on the other hand, shows a marked increase in the circumference and this is accompanied by a marked cellular hyperplasia. Whilst these differences suggest different cellular responses between the jejunum and the ileum, this may simply represent the response of the cell to different degrees of stress with 'stretching out' or thinning of the cell and hyperplasia being at the extremes of the spectrum of response.

Increasing levels of both N-Glucagon and VIP have been noted with distal small bowel obstruction. As mentioned earlier, the neuropeptide VIP, is associated with vasodilatation of the mesenteric vessels[188-192].This may account for the hyperaemia noted. On the other hand, hyperaemia, may be the result of intestinal distention and dilatation and therefore, the VIP rise may simply be an incidental finding. The latter suggestion is

substantiated by the fact that although hyperaemia was noted at all periods of study (i.e. at 7, 11 and 15 days), VIP levels were indeed reduced to control values by P_3.

N-Glucagon, on the contrary, showed a stepwise increase with increasing dilatation of the distal small bowel. It has been demonstrated that this hormone bears a direct relationship to CCPR[ies]. it may therefore be argued that increasing levels may be required to sustain the increasing "mucosal bulk" noted in this situation. It may also be suggested, perhaps more plausibly, that it may simply be a passive response to increasing bowel dilatation or increasing intraluminal contents. Further work will be required to determine the "cause and effect" relationship.

10. Dietary Effect

1. The effect of a normal and low residue diet (LRD) on the normal gastrointestinal morphology.

Oesophagus:

There was no detectable difference in the morphology of the oesophagus in the LRD fed animals as compared with chow fed rats.

Stomach:

The feeding of a low residue diet (LRD) to normal rats led to a slight reduction in body weight (Chow fed vs LRD fed: 161±2.8gm vs 150±8gm at 7d; 172±4.5gm vs 141±7gm at 15d). Despite this change in body weight, stomach measurements in LRD animals were not dissimilar from chow fed controls (Table 19). Overall weight at 7 and 15 days post operatively showed a slight increase in the weight of the stomach as compared with chow fed controls. This was applicable to both muscle and mucosal weights.

The effect on the surface area was however different. Overall, the LRD group had a significantly larger surface area. The relative size of the rumen, fundus and antral areas was also

TABLE 19

Stomach

	day 7	day 15
Gross Wt (mg)		
Chow	870±103	930±140
LRD	950±35	1060±59
Muscle Wt		
Chow	744±85	792±90
LRD	832±73	898±67
Mucosal Wt		
Chow	126±16	138±22
LRD	118±10	162±15
Stomach SA (sq mm)		
Chow	1488±117#	1448±103
LRD	1817±150	1708±138
Rumen SA		
Chow	305±25	360±37
LRD	290±28	255±41
Fundus SA		
Chow	720±43	748±63
LRD	727±51	715±58
Antrum SA		
Chow	463±88	400±81
LRD	800±52	738±110
DNA		
Chow	5.3±0.63	5.1±0.43
LRD	4.6±0.45	4.7±0.38
Protein		
Chow	0.086±0.006"	0.088±0.008^
LRD	0.042±0.004	0.045±0.007

#p=0.05,

^p=0.01, "p=0.001

Comparison of the changes in the weight, surface area (SA), DNA and protein concentration of stomach from animals fed either chow or a low residue diet.

dissimilar. Whilst the fundus formed 1/2 (48%) of the total area of the stomach in the chow fed animals, with the antrum and the rumen forming 31% and 20% respectively, LRD fed animals developed an absolute and proportionately larger antral surface area (44%) at the expense of the rumen (11%). The surface area of the fundus was not significantly reduced (40%). Histological assessment of the thickness of the stomach layers suggested that the LRD group had a reduced thickness of both muscle and mucosal components compared with chow controls.

The DNA mucosal concentration of LRD animals was not dissimilar to the chow group either at 7 or 15 days. Protein concentration was however significantly lower in the LRD animals at both time intervals.

Small intestine.

Jejunum:

Despite a reduction in the total body weight when feeding on a LRD, the jejunal gross weight was not significantly different from that of chow fed controls (Table 20). In addition there was a similar graded increase at days 7 and 15 in the LRD group as in the chow fed animals. This would suggest that despite a reduction in body weight, jejunal mass increased. The mucosal to muscle ratio

remained constant. Unlike protein concentration which was slightly reduced, DNA concentration was unaltered at 7 days. However there was a significant reduction in both indices in the LRD group at 15 days.

Ileum:

The overall weight of the ileal samples obtained from animals fed on a LRD was not different from the chow group at day 7 (P_1). By day 15 (P_3, there was a slight overall reduction which was not significant (Table 21). There was no noticeable change in the muscle mass either at 7 or 15 days. Mucosal mass in the LRD group was similar to chow fed controls at 7 days but was significantly lower at 15 days. There was an initial significant increase in DNA concentration in the LRD group versus chow fed controls; this difference was however not in evidence at 15 days. Protein concentration was reduced both at 7 and 15 days in the LRD group.

TABLE 20

Jejunum

	day 7	day 15
Gross Wt (mg)		
Chow	552±61	646±73
LRD	735±73	758±51
Muscle Wt		
Chow	362±61	461±73
LRD	430±28	530±39
Mucosal Wt		
Chow	190±43	185±39
LRD	205±33	278±41
Circumference (mm)		
Chow	7.8±0.6	8.1±0.8
LRD	7.5±0.6	7.9±0.4
DNA		
Chow	4.6±0.8	4.7±0.5#
LRD	4.5±0.3	3.3±0.4
Protein		
Chow	0.0635±0.007	0.064±0.008#
LRD	0.055±0.003	0.047±0.004

#p=0.05

Table illustrating the jejunal parameters following feeding with chow and a low residue diet,

TABLE 21

Ileum

	day 7	day 15
Gross Wt (mg)		
Chow	479±64	506±45
LRD	448±71	431±53
Muscle Wt		
Chow	289±31	331±55
LRD	260±23	335±41
Mucosal Wt		
Chow	190±47	175±42#
LRD	188±21	96±25
Circumference (mm)		
Chow	7.6±0.3	7.8±0.5
LRD	7.5±0.4	7.9±0.6
DNA		
Chow	4.3±0.9	5.2±0.7
LRD	6.8±0.4	4.9±0.4
Protein		
Chow	0.058±0.003#	0.052±0.009*
LRD	0.046±0.003	0.035±0.002

#p=0.05, *p=0.02

Ileal changes following the ingestion of a low residue diet as compared with chow fed controls.

Hormonal Response:

Introducing normal rats to a low residue diet (Table 22), results in a significant and sustained drop in serum gastrin levels compared with chow fed animals; the values obtained being 72±8ng/l at 7 days and 42±8ng/l at 15 days. The differences noted between the two time periods were also significant, suggesting a continuing fall in the serum gastrin. It is interesting to note, that in spite of an increase in the antral area of the stomach in the LRD fed animals, this was not sufficient to maintain the normal serum gastrin levels.

N-glucagon levels in animals fed on an LRD were found to be at 183±17ng/l and 200±15ng/l at 7 and 15 days respectively. These values were not dissimilar to those obtained in chow fed controls (196±17ng/l at 7 days and 165± 21ng/l at 15 days).

Like N-glucagon, serum VIP levels in the LRD group were similar to their chow fed controls (7days: 40±12.6ng/l, 15days: 67±17ng/l).

TABLE 22

Gastrointestinal Hormones

Hormone	day 7	day 15
Gastrin		
Chow	186±31!	181±43"
LRD	73±10	41±12
N-Glucagon		
Chow	184±35	178±41
LRD	183±17	200±37
V.I.P.		
Chow	47±16	44±14
LRD	40±13	60±19

"p=0.001, !p>0.001

Gastrointestinal hormone profiles following the ingestion of chow and a low residue diet.

Summary of results

1. The rats on a low residue diet exhibited a reduction in body weight when maintained on a low residue diet.
2. No morphological changes in the oesophagus were noted.

3a. The gross weight of the stomach showed an increase at both 7 and 15 days. The increase was reflected in both the muscle and mucosal portions.

3b. Overall surface area increased, with the main increase occuring in the antrum.

3c. DNA concentration was similar to control animals, whilst protein concentation was significantly lower.

4a. Jejunal gross weight was maintained with a constant muscle : mucosal ratio.

4b. Both DNA and protein concentration were reduced at 15 days.

5a. There was a slight but insignificant reduction in ileal gross weight at 15 days.

5b. Muscle mass was constant. Mucosal weight was diminished at 15 days.

5c. DNA concentration showed an initial rise. Protein concentration was reduced at both 7 and 15 days.

6. Serum gastrin showed a significant drop both at 7 and 15 days as compared with controls. N-glucagon

and VIP levels were unchanged.

Discussion.

Diet plays a crucial role in gut maintenance both in a direct and an indirect manner. This indirect role is related to the effect diet has on the general nutrition and wellbeing of the animal and in therefore peripheral to the discussion. The direct effect, on the other hand, can be considered as being two pronged: firstly, it has a local effect on the mucosal integrity (local nutrition, hormonal stimulation) and secondly, a mechanical one; the presence of food bulk acting as a stimulus to peristaltic activity with the resulting maintenance of bulk and tone of the muscularis externa.

Reduction in the bulk of the diet should therefore, by the reduction of the mechanical stimulus to the bowel, result primarily in reduction of muscle mass (disuse atrophy). In addition, the reduced stimulus to gastrin secretion, as noted here, may result in mucosal atrophy.

The oesophagus exhibited no overall change. Muscular activity, consequent to peristalsis, is similar both during ingestion of liquids as well as

solids and therefore as shown, no change in muscle bulk occurs. The mucosa, being stratified squamous in nature, is completely independent of gastrin secretion and is therefore not liable to undergo atrophic change.

The results of the gastric changes in response to a low residue diet are at variance with previously reported work in animals[52-59] where it was noted that no increase in stomach surface area was noted after feeding with an LRD. This present work showed an initial significant increase in the surface area of the stomach as compared with chow fed controls. This increase in gastric surface area may well be due to an initial dilatational effect in response to the presence of a hyperosmolar solution. Such an effect is supported by the finding of a slightly reduced thickness of the stomach muscularis externa on light microscopy. In addition, mucosal DNA concentration remained constant whilst the protein moiety was reduced. This finding would suggest that mucosal change accompanying this dilatational effect is a purely passive one, in that there is no increased cell production (no change in DNA concentration) and the protein concentration is reduced, suggesting a 'stretch' effect. Surprisingly however, both the muscle and the mucosal moieties of the LRD fed

animals showed a slight increase in weight. This finding is obviously in conflict with the suggestion of gastric dilatation where no increase in weight would be expected. This spurious finding is therefore most probably due to an increase in the interstitial fluid in the stomach wall (oedema) as a result of the hyperosmolar feed.

The antral surface area exhibits an almost 100% increase in the surface area as compared with the chow fed controls and this increase, unlike the fundus and rumen changes, persists throughout the period of the study. This disproportionate increase in antral area is difficult to explain. One suggestion might be related to the presence of the hyperosmolar feed. It is known that administration of such liquids result in diminished gastric emptying. This might eventually lead to gastric dilatation where the brunt of the effect is borne by the antrum.

The features of dilatation noted in the stomach were not in evidence in the upper small intestine (jejunum). Indeed the response of the jejunum was very similar to the ileum where both segments of the small bowel had gross weights comparable to chow fed animals. Inkeeping with the features noted for the gastric muscle, no substantial weight differences were noted for the

small intestine, inspite of the significant reduction in the dietary bulk. This is particularly more surprising in the ileum as very little of the dietary intake is avaliable to the ileum. Several reasons may account for this discrepancy. It may perhaps be argued that the intestinal secretions present in the ileum may be sufficiently viscid to provide a effective stimulus to peristaltic activity thus preventing disuse atrophy. Alternatively perhaps, it may be the fact that the time interval of the study was not sufficiently long to register disuse atrophy.

The bulk of the small intestinal mucosa was well maintained even in the ileum. However, inkeeping with other studies[53-54], both the DNA and the protein concentration showed a marked reduction suggesting atrophy and one would suspect that if the experiment had been prolonged sufficiently, as described previously, the cellular atrophy noted would eventually be sufficient to result in the reduction of the gross mucosal weight.

11. The effect of a low residue diet on the gastrointestinal response to obstruction.

This section deals primarily with the effect of stenosis at the mid small bowel level (L_2) on the gastrointestinal tract in those animals fed on a low residue diet.

Oesophagus:

There was no obvious change in the oesophagus in rats with a mid small bowel obstruction fed on a low residue diet.

Stomach:

The introduction of a mid small bowel obstruction on animals fed on a LRD results in a decrease in the gross weight of the stomach as compared with LRD fed controls. This occurs at both the 7 and 15 day intervals (Table 23). The alteration in gross weight is purely a reflection in the reduction of the muscle moiety of the stomach. Mucosal bulk, at both the intervals studied remained unaltered. DNA concentration was not significantly altered in either the obstructed or control groups at both periods of study. Protein concentration/mg mucosa was however significantly

TABLE 23

Stomach

	day 7	day 15
Gross Wt (mg)		
O	850±63	875±89#
C	950±41	1064±43
Muscle Wt		
O	730±45	760±65#
C	817±39	910±31
Mucosal Wt		
O	120±25	115±28
C	133±15	154±29
DNA		
O	4.6±0.35	4.8±0.75
C	5.5±0.4	4.7±0.35
Protein		
O	0.073±0.005*	0.049±0.006
C	0.065±0.007#	0.045±0.006
Stomach SA (sq mm)		
O	2302±225^	1439±126
C	1799±190	1695±192
Rumen SA		
O	330±25	216±26
C	290±23	252±21
Fundus SA		
O	810±44	685±47
C	715±61	705±53
Antrum SA		
O	1163±186^	538±74
C	794±56	738±100

#p=0.05, *p=0.02, ^p=0.01

Animals fed on a low residue diet: Comparison of stomach parameters between obstructed (mid small bowel) and control animals.

increased in the obstructed animals at both 7 and 15 days.

Compared with obstructed animals (L_2) fed on chow, the LRD obstructed group showed an initially lower stomach weight; the main difference being, once again, due to a lower gross muscle weight. DNA and protein concentration per mg mucosa were unchanged.

The stomach surface area in chow fed obstructed animals showed a slow but progressive increase with time. On the other hand, obstructed animals fed on a LRD showed a rapid increase in the surface area at 7 days. This increase was however not sustained and had dropped to below control values at 15 days.

Alteration in the size of the antrum was the component responsible for the changes in the stomach surface area noted above. Antral increase persisted in chow fed obstructed animals at 15 days; this increase was however not maintained in the LRD fed group. Indeed antral size had reduced to below control values at 15 days. The relative proportions of the rumen and fundus remained unaltered.

Small Intestine

Jejunum:

Following 7 days obstruction, chow fed animals exhibited an increase in the gross weight, muscle and mucosal weights of the jejunum as compared with chow fed controls. At 15 days, the increase was significant for the parameters studied. Protein concentration was reduced at 15 days. DNA concentration showed no appreciable change at either 7 or 15 days.

After feeding the obstructed animals with a LRD for 7 and 15 days, no significant changes occurred in the gross weight, muscle or mucosal moieties as compared with controls fed either a LRD (Table 24) or chow. DNA and protein concentration were however significantly reduced at 7 days but returned to LRD fed control values at 15 days.

Ileum:

Seven days following obstruction, chow fed animals showed no significant change in the gross weight of the ileum. Muscle as well as mucosal moieties equally remained unaltered. Feeding the obstructed animals a LRD, did not result in any

TABLE 24

Jejunum

	day 7	day 15
Gross Wt (mg)		
O	675.9±25.5	729±49.4
C	633.3±55.7	728±68
Muscle Wt		
O	466±47.2	507±46.4
C	426.8±24.4	518±20.7
Mucosal Wt		
O	210.3±28.9	222±62.5
C	206.5±24.4	204±39
Circumference (mm)		
O	8.04±1.05	7.93±0.93
C	7.95±0.98	7.87±0.75
DNA		
O	2.71±0.44^	4.13±0.62
C	4.5±0.1	4.2±0.55
Protein		
O	0.039±0.006#	0.053±0.006
C	0.055±0.009	0.056±0.004

#p=0.05, ^p=0.01

Comparison of jejunal measurements from obstructed and control rats fed on a low residue diet.

alteration the ileal muscle or mucosal weight when compared with LRD (Table 25) or chow fed controls. DNA as well as protein concentration were reduced suggesting atrophy.

Intestinal obstruction leads to a significant atrophy in the gross weight of the ileum after 15 days, in the chow fed animals, with the main reduction in bulk occurring in the mucosa. Muscle bulk remained largely unaltered. The mucosal DNA/protein concentration was also unaffected.

Following 15 days obstruction in LRD fed rats, all the parameters measured (gross weight, muscle and mucosal weights) were maintained as compared with LRD fed controls. Compared with chow fed control rats, gross and muscle weights were largely unchanged. Mucosal weight was however significantly reduced. However when this group of animals was compared with the chow fed obstructed animals, the LRD group maintained their mucosal weight significantly better (Fig 12).

Ileal DNA concentration in the LRD fed group was not different from the chow controls. Obstruction in this group (LRD) did not alter the

TABLE 25

Ileum

	day 7	day 15
Gross Wt		
O	322±46.5	430±72
C	357±61.3	427±96
Muscle Wt		
O	221±43	315±74
C	262±36	328±33.5
Mucosal Wt		
O	101.3±19.5	114±33
C	94.6±23.1	98.8±14
Circumference (mm)		
O	7.2±0.4	7.3±0.5
C	7.3±0.6	7.0±0.9
DNA		
O	3.99±0.9#	4.36±0.56
C	6.73±0.1	4.4±0.69
Protein		
O	0.043±0.007#	0.043±0.005
C	0.066±0.008	0.035±0.002

#p=0.05

Ileal changes noted in obstructed and control rats fed on a low residue diet.

FIGURE 12

Histograms illustrating the different degrees of ileal mucosal atrophy occurring in rats: 1.undergoing a mid small bowel obstruction, fed a) either on chow or b) on a low residue diet, and 2. control rats fed on a) a low residue diet and b) normal chow.

MUCOSAL WEIGHTS

OBSTRUCTED VERSUS CONTROLS

CHOW VERSUS LOW RESIDUE DIET

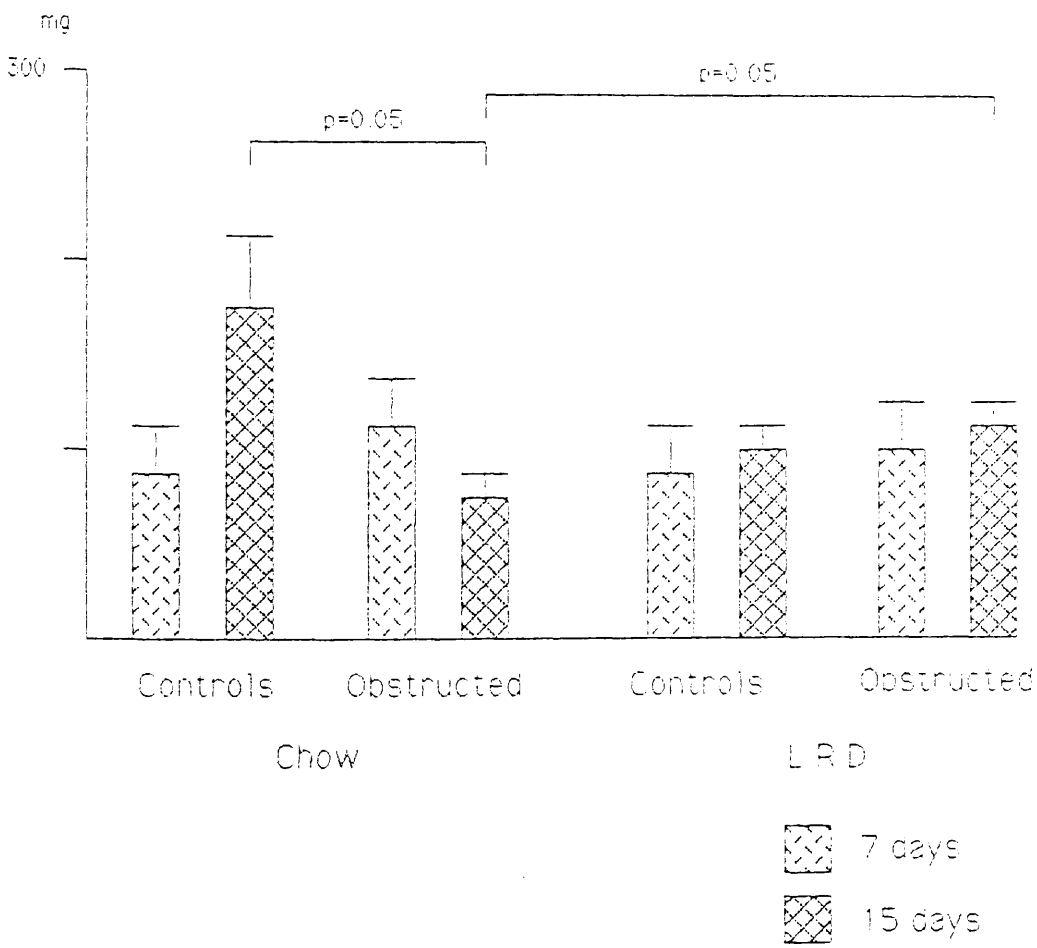


FIGURE 12

DNA concentration. Protein content in LRD controls was, on the other hand, markedly reduced and this reflected the overall reduction in mucosal weight. The LRD obstructed group had a reduced protein content compared to chow fed animals but maintained a higher protein concentration as compared with their own controls.

Hormonal Response:

Obstructed animals fed on an LRD produced serum gastrin levels in excess of 200ng/l at day 7. This was significantly greater than LRD fed controls and animals with a comparable obstruction fed on a chow diet. These levels however were no higher than chow fed controls.

The antral surface area of obstructed LRD animals was also significantly increased as compared with LRD controls and chow fed obstructed animals. At 15 days, serum gastrin levels returned to values similar to LRD fed controls. There was no concommitant reduction in the antral surface area.

No significant alteration was noted in the serum levels of either N-glucagon or VIP in LRD fed obstructed animals as compared with LRD and chow fed controls. There was however a significant difference in the N-glucagon levels between the LRD and chow fed obstructed groups at both 7 and 15

days.

TABLE 26

Gastrointestinal Hormones

Hormone	day 7	day 15
Gastrin		
O	266±23.1"	115±17
C	96±21	94±33
N-Glucagon		
O	165±27	183±40
C	183±17	200±37
V.I.P.		
O	53±12	41±17
C	40±13	60±19

"p=0.001

Gastrointestinal Hormone profiles in animals fed on a low residue diet: a comparison between obstructed and control animals.

Summary of results

1. No oesophageal response was identified.

2a. Gross weight of the stomach was reduced as compared with LRD fed controls. This was mainly due to a reduction in muscle weight. Mucosal weight and hence DNA concentrations were unaltered. Protein concentration was significantly increased. Surface area showed an intial significant increase but then returned to below control values at 15 days; the main change occurring in the antrum.

2b. Compared with chow fed obstructed (L_2) animals stomach weight was lower due to a reduction in muscle weight. DNA and protein concentrations were unchanged. Surface area changes were similar to (2a).

3a. There was no change in the muscle or mucosal weight of the jejunum in obstructed LRD fed animals as compared with LRD fed controls. DNA and protein concentration were reduced at 7 days but returned to control values at 15 days.

3b. As compared with chow fed obstructed animals, gross, muscle and mucosal weights were significantly lower. DNA and protein concentration were also significantly reduced.

4. Ileal weight remained constant in comparison with both LRD and chow fed controls. Muscle and mucosal

mass were maintained. DNA and protein concentration were reduced.

5. Serum gastrin showed an initial (7day) rise but then returned to normal levels by 15 days. N-glucagon and VIP levels were similar to LRD fed controls.

As highlighted earlier[65], the presence of food bulk is important for the maintenance of gut morphology, in particular, the muscle mass. Hence feeding the animal on a low residue diet completely abolishes the hypertrophic response of the muscle of both the stomach and small intestine to obstruction. This would therefore suggest that the increase peristaltic activity noted in small bowel obstruction is not, per se, the dominant factor in producing hypertrophy. It is the nature and in particular, the bulk of the food which seems to determine the hypertrophic response.

Food deprivation, either absolute as in starvation and bypass procedures or relative, as in the feeding of a low residue diet, result in the development of varying degrees of mucosal atrophy. Muscle mass is never appreciably reduced as the periods of study are relatively too short to allow gross evidence of muscle atrophy to appear. The mucosal features of disuse atrophy, i.e. reduced DNA and protein concentration / mg mucosa, could be demonstrated in the ileum progressively deprived of luminal nutrition following obstruction. Obstructed animals fed on a low residue diet did exhibit some degree of mucosal atrophy when compared with chow

fed controls. However the slight atrophy noted was not any more pronounced than the LRD fed controls. Indeed it was significantly less obvious than in comparably obstructed animals fed on chow. This observation, once again, suggests that it is the local factors rather than systemic ones that must be responsible for maintenance of the ileal mucosa. It is postulated that the bulk of a normal diet is too thick to be expressed through the strictured bowel. This is confirmed by the finding of progressively reduced intestinal contents with the progress of the experiment (gradually increasing stenosis). This would result in gradual deprivation of nutrients to the ileal mucosa leading to atrophy. The watery nature of the LRD, on the other hand, ensures that sufficient quantities of nutrients reach the ileal segment.

It would therefore appear thet this type of diet may be beneficial in not only preventing the adaptive features noted following a small bowel obstruction but it may also be beneficial in preventing the post-stenotic atrophy.

PART FOUR

GENERAL SUMMARY OF RESULTS AND DISCUSSION

11. Overall Summary of Results

Body Weight

Chow fed animals exhibited an intial post operative drop in weight. This was maximal at 3 days then accelerated to normal values thereafter.

Animals fed on a low residue diet exhibited the same post operative mean percentage drop in body weight. An increase in weight was evident after the 6th day but the incremental values were below those of their chow fed counterparts.

All animals undergoing a small bowel obstruction developed an early post operative reduction in weight. Their weight regain was below that of either chow or LRD fed controls and never returned to normal values during the course of the experiment; the chow fed animals with the high obstruction (L_1) exhibited the least deficit in weight and the LRD fed obstructed animals the most.

Oesophagus

The type of diet had no noticeable effect on oesophageal morphology in control animals.

Whilst mid and distal small bowel obstruction, in chow fed animals, had no effect on the oesophagus, a high obstruction does cause an

alteration in muscle weight. There was a gradual increase in muscle mass with significant levels being achieved at P_2. The muscle weight declined thereafter.

No changes were noted in obstructed animals fed on a LRD.

Stomach

The gross weight of the stomach from control animals fed on a LRD was not different from that of chow fed controls. Muscle and mucosal weights increased in a parallel fashion. The surface area and the relative size of the rumen, fundus and antrum were considerably altered and are illustrated in Table 27.

The adaptive response of the stomach appears to be related to the site of obstruction. An L_3 obstruction, fails, in the time period of the experiment, to produce any gastric reaction.

A mid and high small bowel obstruction produce two different responses. The main findings are shown in tabular form (Table 28). The different features of note are:

a. dilatation is an early and short lived phenomenon following a high obstruction. With a lower (L_2) obstruction, dilatation is gradual and occurs later.

TABLE 27

STOMACH CONTROLS

LRD vs Chow

	7 days (P_1)	15 days (P_2)
Gross surface area	++	+
Rumen "	--	--
Fundus "	0	0
Antrum "	++	++
DNA	0	0
Protein	--	--

Measurements of stomach parameters from control rats fed on chow and a low residue diet.

Key to tables 27 - 32

0 no change

+/- slight increase or decrease

++/-- significant increase or decrease

TABLE 28

STOMACH

Chow diet

L_1 Obstruction vs controls

	P_1	P_2	P_3
Gross surface area	++	+	0
Rumen "	++		0
Fundus "	+		0
Antrum "	+		0
Weight - gross	++	++	++
" muscle	++	++	++
" mucosa	+	++	+
DNA	++	+	0
Protein	0	0	0

L_2 Obstruction vs controls

	P_1	P_2	P_3
Gross surface area	0	+	++
Rumen	0		+
Fundus	0		+
Antrum	++	++	+++
Weight - gross	0	0	0
" muscle	0	0	0
" mucosa	0	0	0
DNA	0	0	+
Protein	0	0	?-

Stomach response in relation to a high and mid small bowel obstruction.

TABLE 29

STOMACH

LRD Obstruction (L_2) vs LRD controls

	7 days (P_1)	15 days (P_3)
Gross weight	--	--
Muscle "	--	--
mucosal "	0	0
Gross surface area	++	0
Rumen "	0	0
Fundus "	0	0
Antrum "	++	--
DNA	-	0
Protein	++	++

Comparison of stomach parameters of obstructed and control rats fed on a low residue diet.

TABLE 30

STOMACH

L_2 obstructed animals

LRD vs Chow diets

	7 days (P_1)	15 days (P_3)
Gross weight	--	-
" muscle	--	0
" mucosa	0	0
Gross surface area	++	--
Rumen "	0	-
Fundus "	++	-
Antrum "	++	--
DNA	0	0
Protein	0	0

The response of the stomach to obstruction: a comparison between chow and low residue diet fed rats.

b. an increase in gross weight accompanies dilatation in an L_1 obstruction. Delayed dilatation in an L_2 obstruction is not associated with a weight change.

c. DNA concentration mirrors the mucosal changes in both types of obstruction: hyperbolic in L_1, no change in L_2.

Introducing an LRD to obstructed animals (mid small bowel) results in a reduction in gross and muscle weight of the stomach for both time periods (7, 15 days) as compared with LRD fed controls. Surface area showed an early transient increase, the main changes occuring in the antrum (Table 29).

Feeding obstructed animals with an LRD rather than chow (Table 30) resulted in an initial reduction in gross stomach weight, but the weight returned to that of chow fed obstructed animals by 15 days. The initial dilatation seen early was completely reversed and by P_3, the surface area was significantly less than that of chow fed obstructed animals.

Jejunum

The jejunal gross weight of chow fed control animals was not different from that of LRD fed animals. In both instances there was a gradual increase in weight with time. This increase applied to both the muscle and mucosal sections. No

TABLE 31

JEJUNUM

Chow diet

L_1 obstruction vs controls

	P_1	P_2	P_3
Gross weight	++	+++	++++
Muscle "	+	+	++
Mucosal "	0	++	+
Circumference	+	+	+
DNA	0	0	0
Protein	-	--	0

L_2 obstruction vs Controls

	P_1	P_2	P_3
Gross weight	+	++	+++
Muscle "	+	+	++
Mucosal "	0	+	++
Circumference	+	+	+
DNA	0	0	0
Protein	0	-	--

Jejunal changes following a high and mid small bowel obstruction.

alteration in bowel circumference was noted. DNA and protein concentrations were however both reduced at 15 days in LRD fed animals.

The changes associated with L_1 and L_2 types of obstruction are illustrated in Table 31. The essential features illustrated are:

a. jejunal muscle weight increases rapidly in response to obstruction.

b. the bowel circumference does not show a significant change suggesting that jejunal dilatation is limited.

An L_3 type of obstruction produced no visible changes in the upper jejunum of animals fed on chow.

TABLE 32

JEJUNUM

L_2 obstructed animals

LRD vs Chow

	7 days (P_1)	15 days (P_3)
Gross weight	-	--
Muscle "	-	--
Mucosal "	0	0
Circumference	-	-
DNA	0	-
Protein	0	0

Jejunal response to obstruction: a comparison between chow and low residue diet fed rats.

No significant changes were seen in the gross, muscle and mucosal weights of obstructed animals fed on a LRD as compared with controls fed either on a LRD or chow. Bowel circumference was equally unaltered. DNA and protein concentration showed an initial drop but returned to control values by 15 days.

LRD fed obstructed animals failed to produce the hypertrophic jejunal response seen in obstructed (L_2) animals fed on chow (Table 32).

Ileum

The ileum of animals fed on a LRD showed a slight reduction in weight as compared with chow fed animals. The main reduction occurred in the mucosal mass (significant by P_3) and this was accompanied by both a reduction in DNA and protein concentration.

An L_1 obstruction had no effect on the ileum.

An L_2 obstruction resulted in a significantly reduced ileal weight with the main reduction occuring in the mucosa. This was associated with a slight increase in DNA and a reduced protein concentration.

L_3 obstruction produced a significant increase in muscle and mucosal weight of the ileum. The bowel circumference was also significantly

increased. DNA and protein concentration showed a gradual reduction with time.

LRD fed obstructed animals maintained their mucosal weight significantly better as compared with chow fed obstructed animals. This was reflected in a rise in DNA and protein concentration.

Hormones

Feeding of a low residue diet results in a significant drop in circulating gastrin. VIP and N-glucagon were unaffected.

Gastrin levels showed a significant rise following an L_1 obstruction and a slight drop at L_3.

Serum N-glucagon levels showed a significant rise at both L_2 and L_3 obstruction with the maximum values being obtained at L_3.

VIP values showed a significant elevation in L_3. A late rise in L_2 was also noted.

LRD fed obstructed animals showed an early rise in serum gastrin but these values returned to control (LRD) levels at 15 days. VIP and N-glucagon levels in this situation were unaltered.

12. Final Discussion

This study has been out to try to identify the gastrointestinal adaptation to obstruction and the modulating effect of the diet on these adaptive processes.

One important feature that emerged from the results is that adaptation in the alimentary system is not an event localised to the site of the stimulus. It has been shown that if the stimulus applied is intense and persistent, these adaptive changes can be seen to involve the more proximal segments of the bowel. Clear adaptational changes are seen, for example, in the oesophagus following a high jejunal obstruction.

In general terms, the response exhibited has been a spectrum of both dilatation (a passive response) and hypertrophy / hyperplasia (active response).

Dilatation is the acute phase response to an obstruction. It occurred early and was always found in close proximity to the obstruction. With the notable exception of the oesophagus, dilatation was a feature common to all segments of the gastrointestinal tract investigated with different segments responding in different ways. It would

therefore seem that any alterations that do occur are not only determined by the nature and duration of the stimulus (as discussed earlier) but are limited by the structural components of the organ (such as the relative thickness of the muscularis externa, wall:lumen ratio, the spatial arrangement of the muscle fibres and the strength of the lamina propria) and its predetermined functional capacity.

These are elegantly exemplified by the stomach which is a 'storage' organ, and therefore with the smallest wall:lumen ratio, showing maximum dilatation; the thicker walled jejunal (thick lamina propria) showing only modest dilatation compared with the ileum; the oesophagus, with a thick lamina propria and the largest wall to lumen ratio exhibiting no detectable response.

There was no evidence to suggest that either hypertrophy or hyperplasia of the mucosal cells occurred during dilatation. The DNA and protein concentration per mg mucosa (with the sole exception of the gastric mucosa in the early phases of L_1 obstruction) were unaltered. However when expressed as concentration per unit area, both DNA and protein concentration were markedly reduced. This would suggest that the mucosal response was simply a 'stretch' effect.

The muscle coat exhibited a different response

in that an increase in the bulk and weight was registered in all the tissues studied. The increase in weight, which was achieved early was maintained (with the exception of oesophageal muscle). As previous quantitative methods, utilizing ileal muscle, have shown[171], the muscular response is one of both hypertrophy and hyperplasia and it may not be inaccurate to assume that these changes occur not only in ileum but also in the more proximal portions of the gastrointestinal tract.

One cannot overlook the hyperbolic changes observed in the oesophagus. As discussed earlier, the sudden rise and fall in weight cannot be simply the result of simple hypertrophy and atrophy. Oedema of the bowel wall is thought to be a factor[140] and it is very probable that it also played a minor role in the weight gain noted in the stomach and small intestine.

As illustrated earlier, a normal diet failed to produce any active mucosal response in the obstructed bowel. It is thought however, to be the main stimulus for the production of hypertrophy and hyperplasia of the muscle coat. This concept is endorsed by the finding that the administration of a low residue diet, not only reduced the degree of dilatation in both the stomach and small bowel but also completely abolished the hypertrophic response

of the bowel to obstruction.

This finding lends support to the theoretical concept that the administration of a low residue diet to patients with intestinal strictures reduces the hyper-peristaltic activity which manifests itself as abdominal colic symptomatically and muscle hypertrophy morphologically.

In addition, it may be argued that some dilatation (and therefore increase in surface area) proximal to a strictured segment of small bowel might be considered advantageous. This is particularly so in conditions where normal small bowel is at a premium (e.g. Crohn's disease). Apart from allowing larger volumes of chyme to be accomodated in the intestine, these findings have shown that dilatation simply causes the mucosa to be 'stretched' out, further compromising the surface:volume ratio. This makes this adaptational response positively detrimental.

One disadvantage of the administration of a low residue diet is, as already stated, mucosal atrophy. It is usually mild and occurs early. Indeed, in these experiments using Trisorbon, gastric and small intestinal mucosal atrophy was in evidence by 7 days. Other trials have illustrated the same principle although the degree of atrophy was shown to vary with the individual diet[59].

Disuse atrophy in the post-stenotic segment of the bowel was shown to occur later and in parallel experiments using defunctioned bowel, was quite marked. Both dietary related and post-stenotic atrophy are natural physiological consequences. Whilst the former is inevitable, these present results have indicated that the severity of the post-stenotic atrophy can be reduced. Indeed, it has been shown that mucosal atrophy in obstructed animals fed on a low residue diet was significantly less than that of the chow fed counterparts.

Discrepancies in the size of the stenoses leading to unequal quantities of food being expressed, cannot be implicated as all the lumina were "standardised". This finding must therefore be due to either the liquid diet and/or intestinal secretions reaching the lower ileum, or the indirect result of some stimulus (?hormonal) generated by the diet.

The two hormones which have been most often implicated in the control of intestinal epithelial cell proliferation are gastrin and N-glucagon.

Gastrin was found to be persistently elevated in one situation, namely in chow fed animals with a high jejunal obstruction. A transient increase in the levels of this hormone was also seen in obstructed animals fed on a low residue diet. These

elevations simply represent the antral response to stretch in the former case and the presence of a hyperosmolar solution in the latter. Whilst no post-stenotic ileal mucosal atrophy was seen in L_1 obstructed animals, the trophic role of gastrin is doubtful and it is more likely that the "food column" present distal to the stenosis is responsible for the maintenance of the ileal mucosa. More pertinent is the fact that serum gastrin levels were more depressed in obstructed animals fed on a low residue diet as compared with chow fed counterparts.

The other trophic hormone N-glucagon and the neuropeptide VIP showed significant elevation in situations of mechanical stress to the bowel i.e. during dilatation (similar to the antral stretch effect leading to a rise in serum gastrin). No alterations in serum levels were seen during the administration of a low residue diet. One can therefore conclude that no evidence exists to implicate a hormonal role.

The direct role of the diet seems therefore to be primarily responsible. These results require further corroboration but if substantiated, this might suggest a possible way or reducing post-stenotic atrophy thereby leaving a more normal absorptive intestinal surface.

PART FIVE

APPENDIX AND REFERENCES

13. Appendix

1. Reagents used for Deoxyribonucleic Acid (DNA) estimation.

1. Stock DNA:

   Highly polymerised calf thymus DNA (sigma Ltd.) 40 mg DNA/100 ml 5mN NaOH. Equivalent to (=) .1836ug atom DNA-P/ml.

2. Working Standards:

   0.2ml Stock + 9.8ml 5%TCA = 7.005ng atoms DNA-P/ml.

   0.4ml " + 9.6ml 5%TCA = 14.01ng " " .

   0.8ml " + 9.2ml 5%TCA = 28.02ng " " .

   1.6ml " + 8.4ml 5%TCA = 56.04ng " " .

3. 6.5% Trichloroacetic Acid (TCA).

4. 5% " " .

5. 60% Perchloric Acid.

6. 2% Aqueous Acetaldehyde.

7. Diphenylamine Reagent:

5ml Diphenylamine dissolved in 240ml Glacial Acetic Acid;

add 5ml concentrated Sulphuric Acid;

make up to 250ml with Glacial Acetic Acid;

add 1.25ml of 2% aqueous Acetaldehyde.

11. Reagents used for Protein estimation

1. Stock protein:

   Bovine serum albumin (Sigma Ltd.) dissolved in saline.

2. Working Standards:

No	Saline ml	Standard ml	ug Protein/ ml	O.D. 625nm
Bk	1.0	0	0	0.022
1	0.9	0.1	25	0.071
2	0.8	0.2	50	0.140
3	0.7	0.3	75	0.202
4	0.6	0.4	100	0.262
5	0.5	0.5	125	0.318
6	0.4	0.6	150	0.374
7	0.3	0.7	175	0.426
8	0.2	0.8	200	0.479
9	0.1	0.9	225	0.521
10	0	1.0	250	0.558

3. Alkaline Copper Reagent:

Reagent A: 2% Na_2CO_3 in 0.10N NaOH.

Reagent B: 0.5% $CuSO_4.5H_2O$ in 1% sodium tartarate.

Mix 50ml of Reagent A to 1ml of Reagent B.

4. Folin Ciocalteu phenol reagent:

Titrate Folin-Ciocalteu phenol reagent (Sigma Ltd.) with NaOH to a phenolphthalein end point. Dilute the reagent thus obtained to make it to 1 N in acid.

111. Average calculated composition

of the Diets used

(Analysis: gm per 100 gm)

	Chow	LRD
Protein	17.9	19.0
Carbohydrate	57.0	56.0
Fat	17.4	19.0
Calcium	0.82	0.63*
Phosphorus	0.73	0.62*
Sodium	0.32	0.46
Potassium	0.72	0.78
Magnesium	0.1	0.085
Chloride	0.5	0.9
Iron	0.01	0.007
Vit A (i.u.)	1500	1250
Vit D3 (i.u.)	240	250*
Folic Acid (mg)	0.031	0.047
Energy (kcal)	320	500

* supplemented

The rat average daily consumption is 20 gm of chow. This provides approximately 65 kcal of energy.

The average daily water intake of an adult rat is 35-50 ml.

The low residue diet feed was prepared by dissolving 1 sachet (85 gm) in 300 ml water.

An average daily intake of 50 ml of this solution would provide the rat with about 70 kcal/day.

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