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Role of gastrointestinal hormones in the response to massive resection of the small bowel

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SUMMARY Hypersecretion of gastric acid and accelerated intestinal transit are largely unexplained consequences of massive resection of the small bowel; several postulated humoral mechanisms remain unsubstantiated. The purpose of the study was to investigate the effects of a 75% resection of the distal small bowel in dogs on circulating levels of a range of gastrointestinal hormones. Basal and meal-stimulated concentrations of insulin, secretin, gastrin, pancreatic glucagon, and total glucagon-like immunoreactivity (GLI) were measured by radioimmunoassay techniques. After resection, significant depletions of basal and stimulated total GLI ($P < 0.05 - P < 0.001$) and a significant rise of stimulated gastrin ($P < 0.05$) were discovered. These hormonal alterations may produce an important imbalance of humoral influences on gastrointestinal function. It is suggested that these changes may hold a key to the aetiology of the complications of massive resection of the small bowel.

An early observation (Stassoff, 1914) that hypersecretion of gastric acid followed massive resection of the small bowel in dogs was confirmed by more recent reports on the subject which are critically analysed in an excellent review article (Buxton, 1974). Despite the various humoral theories that have been advanced to explain the cause of hypersecretion of gastric acid, knowledge of the mechanism of its occurrence remains fragmentary.

Experimental and clinical observations of the marked reduction in intestinal transit time after extensive resection have been well documented (Kremen *et al.*, 1954; Spohn and Schreier, 1958; Barros D'Sa, 1975). The exact pathophysiological processes which cause more rapid transit after distal or ileal resections than after proximal bowel resections (Kremen *et al.*, 1954; Booth, 1961) have yet to be elucidated. In the past, regulation of gastrointestinal motility has been ascribed predominantly to nervous mechanisms. While there is little physiological evidence that hormones regulate intestinal motility, they certainly do alter it (Chey *et al.*, 1967; Fasth and Hultén, 1971; Grossman, 1974).

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With the acceptance of the concept of the small bowel as an endocrine organ, the place of circulating gastrointestinal hormones in the pathophysiology of massive resection of the small bowel deserves investigation. The aim of the study was to determine the effects of massive resection of the distal small bowel on basal and meal-stimulated levels of certain circulating gastrointestinal hormones which were measured by radioimmunoassay techniques.

Methods

Registered greyhounds weighing between 20 and 28 kg were employed. Seven dogs were studied in the group of preoperative or intact animals (subsequently termed 'controls'). Nine dogs, including the above seven, were studied after a 75% resection of the distal small bowel (subsequently termed 'resections').

PREPARATION OF 'RESECTION' MODEL

Under anaesthesia with pentobarbitone sodium (Nembutal) 26 mg/kg body weight, a midline incision was made. The small bowel, defined as that part between the ligament of Treitz and the ileocaecal junction, was carefully and gently measured along its antimesenteric border. The total length, the segment to be resected, and the remnant were measured immediately after opening the abdomen so that spasm caused by handling was reduced to a

minimum. A routine resection of 75% distal small bowel was performed, preserving the proximal 25% which was anastomosed end-to-end to a 3 cm stump of ileum, thus leaving the ileocaecal valve undisturbed. Postoperatively, the dogs were given parenteral fluids for five days, followed by a gradual resumption of oral feeding. Additional parenteral fluids and potassium supplements were given daily according to the general condition of the animal and to correct any fluid and electrolyte imbalances. Animals made an uneventful recovery from the operation itself. Diarrhoea was evident immediately postoperatively but diminished in rate and quantity of loss. All such losses were replaced parenterally and the diet provided was consumed normally. Animals were tested after a two to three week interval after operation.

PROTOCOL

All animals were fasted for 16 hours before testing. Peripheral venous samples were taken at −15 minutes for a basal level. A corn oil meal of 5 ml/kg body weight was given orally and was easily taken in less than a minute. Serial blood samples were collected from zero time for two hours for stimulated levels at zero, five, 10, 15, 30, 45, 60, 75, 90, 105, and 120 minutes.

Radioimmunoassay techniques were used to measure plasma levels of hormones: insulin assay (McCarroll, 1971), gastrin assay (Ardill, 1973), and secretin and glucagon assays (Buchanan, 1973). Blood samples were collected in 10 ml heparinised bottles, chilled, and immediately centrifuged at +4°C. Aliquots for insulin and gastrin estimation were frozen as plasma awaiting assay, while those for secretin and glucagon were first extracted in ethyl alcohol. Further details of the assays are as follows:

1. Insulin antibody was raised to pork insulin. Natural dog insulin (Novo) was used as a standard. The sensitivity of the assay was 1 μU/ml.

2. Gastrin antibodies were raised in rabbits to synthetic gastrin I(2-17). Synthetic human gastrin I (MRC) was used as a standard. The sensitivity of the assay was 5-10 pg/ml.

3. Secretin antibodies were raised in rabbits to natural secretin. Natural secretin (GIH Research Institute, Stockholm) was used as a standard. The sensitivity of the assay was 5-10 pg/ml.

4. Glucagon antibodies were raised in rabbits to porcine pancreatic glucagon. Two types of antibodies, YY 57 and YY 89, were obtained, and defined in terms of their ability to cross-react with crude gut glucagon-like immunoreactivity (GLI) extracted from small gut, being strongly cross-reactive and having a low cross-reactivity respectively. These antisera have been further characterised in that the gut cross-reactive antisera react with N-terminal fragments of pancreatic glucagon and the antisera of low gut cross-reactivity react with C-terminal fragments of pancreatic glucagon. To simplify this explanation, Table 1 gives the terminology used for antibodies (Buchanan, 1973).

Similarly, as depicted in Table 2, the GLI with which these methods react is referred to either as N-terminal reactive GLI (N-GLI) or C-terminal reactive GLI (C-GLI).

It is thought that N-GLI represents both GLI of gut origin and pancreatic glucagon, whereas C-GLI mainly represents GLI of pancreatic glucagon. As the knowledge concerning the chemistry and forms of GLI remains incomplete, it is appropriate to discuss the measurements as N-GLI and C-GLI respectively. The sensitivity of glucagon assay was 10-30 pg/ml.

Insulin, gastrin, and glucagon were iodinated using modifications of the chloramin-T method of Hunter and Greenwood (1962). Secretin was labelled using lacto-peroxidase (Holohan *et al.*, 1973). Purification of labelled gastrin was on Sephadex G-10, of labelled glucagon and secretin on ion-exchange chromatography, and of labelled insulin on silica. All were stored in acid alcohol at −20°C. Secretin and glucagon assays were performed in extracts reconstituted in phosphate buffer (0·04 M) at pH 7·4. Gastrin and insulin assays were performed directly on plasma samples. Separation of antibody bound from free hormone was achieved using dextran-coated charcoal in phosphate buffer.

Results

The results of stimulation could be analysed by simply computing total integrated responses over a two-hour period. However, such an approach would obscure the finer temporal alterations in the character and calibre of the response. The results are therefore presented as mean levels at each time of sampling.

INSULIN

Levels rose significantly at zero time in 'controls' ($P < 0·05$) but otherwise remained unaffected. Reciprocal glucose levels were found. Group comparisons were unremarkable.

SECRETIN

This was unaffected by stimulation and no significant differences were noted at any stage of sampling in either of the two groups.

GASTRIN (Fig. 1)

Significant rises occurred at zero time in both groups ($P < 0·05$) but the gastrin response was otherwise

Table 1 *Terminology of glucagon antibodies*

Antibody	*Classical terminology*	*Our terminology*
High cross-reactivity with gut GLI	Cross-reactive antibody	N-terminal reactive
Low cross-reactivity with gut GLI	Antibody specific for pancreatic glucagon (pancreas specific)	C-terminal reactive

Table 2 *Terminology of GLI reacting with glucagon antibodies*

Our terminology	*Classical terminology*
N-terminal reactive GLI (N-GLI)	Total GLI
C-terminal reactive GLI (C-GLI)	Pancreatic glucagon

uninfluenced by the stimulus. In comparing the results of 'resections' with those of 'controls' it was observed that mean stimulated concentrations of the hormone were raised in the former group ($P < 0.05$ at 60 minutes).

PANCREATIC GLUCAGON (C-GLI)

In both groups a triphasic response was noted. Significant alterations of this hormone were not found in the 'resection' group.

TOTAL GLI (N-GLI) (Fig. 2)

Stimulated levels were significantly higher than basal levels in both groups of animals ($P < 0.05$-$P < 0.01$). When circulating total GLI was compared in 'controls' and 'resections' significantly lower mean basal levels ($P < 0.05$) and significant suppression of stimulated levels from 15 to 105 minutes ($P < 0.05$-$P < 0.001$) were revealed in the latter group. The maximal response at 75 minutes of 525 ± 135 pg/ml in the 'control' group contrasted with that of 132 ± 18 pg/ml in the 'resection' group.

Briefly, the results of insulin, secretin, and pancreatic glucagon (C-GLI) were unremarkable. Two significant findings emerged from this study: firstly, that massive distal small bowel resection in dogs produced significant falls in basal and stimulated levels of total GLI (N-GLI) and, secondly, that such resections resulted in significant increment in the stimulated gastrin response.

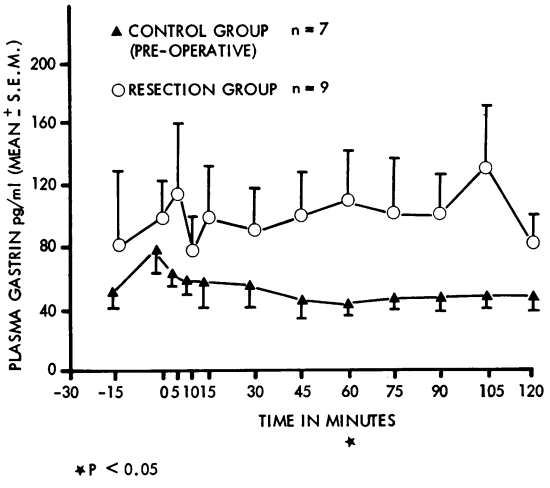

Fig. 1 *Comparison of basal and stimulated levels of circulating gastrin in the two groups of animals.*

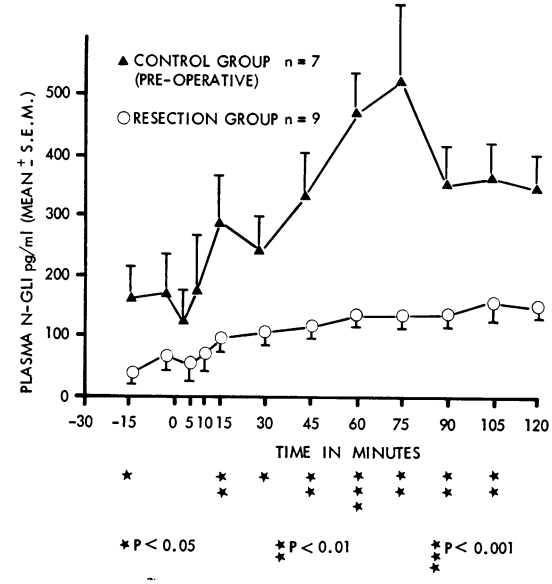

Fig. 2 *Comparison of basal and stimulated levels of circulating total GLI (N-GLI) in the two groups of animals.*

Discussion

Resections of the order of 75%, particularly when distal small bowel is removed, constitute a rigorous challenge likely to be associated with high morbidity and mortality rates (Chen Kai-Mo, 1969).

It must be stated first of all that the assays measure only immunoreactivity, which must not be confused with biological activity. With the gastrin assay, for example, where all species of gastrin have been isolated and appear to be biologically active and are measured by the radioimmunoassay, then there is probably a close correlation between gastrin biological activity and gastrin immunoreactivity. However, in the case of GLI where many of the immunoreactive

fractions have not been isolated and purified, then it is much more difficult to correlate immunoreactivity with biological activity.

The molecular structure, biological activity, and functions of GLI are unknown despite the efforts of many workers (Valverde *et al.*, 1970; Murphy *et al.*, 1973). The complexity of this family of polypeptides and the lack of characterisation of GLI makes it difficult to interpret the results. Nonetheless, the importance of the significant suppression of total GLI (N-GLI) in the 'resection' group cannot be minimised, particularly as pancreatic glucagon (C-GLI) levels in both groups were the same. In other words, the significant falls in total GLI are probably largely because of changes in GLI originating from the gut.

A discussion of the merits and possible relevance of the main results must be preceded by a brief analysis of other parallel observations. A mixed meal or a number of different meals apart from fat would have provided a more comprehensive picture of hormonal response. The results of these studies to some extent support and supplement the limited knowledge of the effects of fat in stimulating release of gastrointestinal hormones. It is noted that fat provided prompt and significant rises in levels of total GLI and pancreatic glucagon in intact animals and this is in keeping with recent work (Böttger *et al.*, 1973; Ohneda *et al.*, 1975). The response was more marked for total GLI indicating that gut GLI may share a role with its pancreatic counterpart in the metabolic regulation of fat. This evidence further supports the hypothesis that an enteric islet-cell axis for fat may exist (Böttger *et al.*, 1973), and pancreozymin or gastric inhibitory polypeptide may qualify to serve as such a signal.

The significant rise of insulin, gastrin, and pancreatic glucagon at zero time may suggest that some conscious or cephalic phase of hormone secretion is responsible. There is good evidence of neural control and stimulation of these hormones (Porte *et al.*, 1973; Mayer *et al.*, 1974). It is noteworthy that secretin was the only hormone not to be so affected.

What is the possible relationship of the alterations in gastrin and total GLI to the consequences of massive resection? With regard to gastric acid hypersecretion, the role played by innervation of the bowel is not clear. The interesting hypothesis that the remnant elaborates a gastric secretagogue remains unsubstantiated (Westerheide *et al.*, 1965). The consensus of opinion tends to favour the interruption of an inhibitory mechanism as the principal cause. Patients with terminal ileal resection for Crohn's disease have had increased gastric acid outputs (Fielding *et al.*, 1971), which supports the hypothesis that the terminal ileum in some way inhibits gastric secretion. These findings do not give an indication as to whether inhibition is achieved by inactivation of a secretagogue or by production of an inhibitor (Menguy, 1960). Maximal concentrations of GLI have been located in the ileum (Bloom *et al.*, 1973) and the extensive distal resections performed in this study would have severely depleted the potential gut GLI-secreting mass. It has been demonstrated that in both rats and dogs the small bowel is an important site of gastrin inactivation; if this were so in man it might explain reported observations (Fielding *et al.*, 1971), as well as account for the raised stimulated gastrin levels noted in our study.

Serotonin (Santillana *et al.*, 1969) and histaminase (Caridis *et al.*, 1969) have been nominated as missing inhibitory factors but there is no evidence that either is of physiological significance in the normal state. As gastric acid hypersecretion is more marked after proximal rather than distal small bowel resections it may be enlightening to measure gastric inhibitory polypeptide levels after proximal gut resection as this hormone bears a close resemblance to enterogastrone. This complication also results from distal resections and it has been demonstrated in the study that both basal total GLI levels as well as those after stimulation are significantly depleted. It is possible that total GLI or one of its components may be an inhibitor of gastric secretion.

With regard to transit, the crucial question posed is whether the significant alterations in gastrin and total GLI levels observed in the study actually affect motility. The other difficulty is that release of the endogenous hormone being studied simultaneously initiates other hormonal and neural events. It is conceivable that gut GLI or total GLI may be an agent which normally inhibits gut motility and that depletion of this hormone removes that restraint. It has certainly been shown that high levels of this hormone produce severe stasis (Bloom, 1972).

It seems reasonable to suggest that an imbalance between the stimulatory and inhibitory influences underlies the disorders which follow massive resection. The normal facilitatory effect of gastrin (Tracy and Gregory, 1964) and the inhibitory effect of glucagon (Fasth and Hultén, 1971; Bloom, 1972) on gastric acid hypersecretion and gut motility have been observed. If these two gastrointestinal hormones are considered in isolation, then the alterations in their levels after massive resection—namely, the rise in gastrin and the fall in total GLI concentrations—should quite logically explain the gastric acid hypersecretion and the accelerated transit which follow. Although this is a rather simplistic explanation, the experimental study furnishes hormonal evidence which may throw some light on these sequelae of extensive intestinal resections.

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