STEATORRHOEA IN DOGS INDUCED BY GASTRIC HYPERSECRETION

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The occurrence of intractable peptic ulceration with a non-B-cell pancreatic adenoma in man (Zollinger and Ellison, 1955) has been associated in many cases with diarrhoea. In a few cases steatorrhoea has been reported to be present. It seems probable that this syndrome is due to elaboration of a gastrin-like material by the tumour capable of producing gross gastric hypersecretion (Gregory, Tracy, French and Sircus, 1960).

In an attempt to elucidate the mechanism behind the diarrhoea and steatorrhoea we have observed the effect in dogs of artificial gastric hypersecretion on the absorption of fat.

Methods

Eight healthy adult mongrel dogs were used, weighing between 10 - 15 kg. each. Three dogs were used with an intact gastrointestinal tract. Three dogs were prepared with small Heidenhain type pouches of gastric mucosa, drained by silver cannulae in the usual manner. On one dog a transthoracic total gastrectomy was performed; continuity was restored by an oesophago-duodenal anastomosis. A further dog, with a normal stomach, had a resection of 75 cm. of ileum with end-to-end anastomosis of the remaining small intestine (leaving the ileo-caecal valve and 5 cm. of terminal ileum intact). Each animal was /
was left for a minimal period of six weeks after the operative procedure before any attempt was made to assess the fat absorption.

Gastric hypersecretion was induced by injection of a histamine/beeswax mixture in a modification of the method of Hay, Varco, Code and Wangensteen (1942). A large batch of the mixture of histamine acid phosphate, beeswax and mineral oil was prepared for each experiment as described by these authors. The dose was injected into the muscles of the dog's back using a heated syringe. Hay et al. (1942) claimed that a single injection of this material was sufficient to produce a plateau of gastric secretion lasting for 24 hours. However, it was found that the gastric hypersecretion induced in Heidenhain pouch dogs by one injection tended to fall off after 12 hours (Fig. 1). This figure also shows the variability of the secretion obtained by the use of this mixture; presumably this is due to irregular absorption of histamine from the mass of beeswax injected into the animal. To maintain a high level of secretion throughout 24 hours, it was decided to give injections of the histamine/beeswax preparation every 12 hours.

Intramuscular promethazine hydrochloride was given at 12-hourly intervals to each animal to counteract the systemic effects of the histamine mixture.
The dogs were kept in metabolic cages and faeces were collected daily from each animal. For four days prior to each study period, and throughout the study periods, the animals were fed a standard meal at a fixed time daily consisting of 450 g. of a proprietary dog food with water as required. This gave a standard daily fat intake of $19.5 \pm 0.5$ g. of fat daily. If the dog refused food the study period was discontinued. In practice, the dogs ate avidly throughout the control and promethazine periods; we learned to recognise anorexia as the first sign of peptic ulceration during the administration of histamine.

The three dogs equipped with Heidenhain pouches had the pouch secretion collected by means of a rubber bladder which fitted onto the cannula.

The 24-hour output of faeces and pouch secretion was collected at a fixed time and measured daily. Faecal fat was determined on duplicate analysis of aliquots of each homogenised 24-hour faecal collection by the method of van de Kamer, Huinink and Weyers (1949): the results were expressed as total faecal fat in grams excreted per 24 hours. The gastric juice collected from the pouch dogs was titrated against $0.1 \text{ N}$ sodium hydroxide using phenolphthalein indicator, and the results recorded as total acid output per 24 hours in m.Eq. of hydrochloric acid.

Before /
Before the study periods each Heidenhain pouch dog had a one hour maximal histamine test according to the method of Kay (1953): the dose of histamine acid phosphate used was 0.6 mg./kg. body weight; the acid output in one hour after histamine (MAO) was multiplied by 24 to give a theoretical maximum 24-hour acid output for comparison with the 24-hour secretion after histamine/beeswax injections.

Procedure

A dog with a normal gastro-intestinal tract and a dog with a Heidenhain pouch were run concurrently. After a four-day period of stabilisation on the standard diet, faeces were collected from both dogs for a seven-day control period. Both dogs were then given a seven-day period on promethazine hydrochloride alone, 50 mg. intramuscularly at 12-hour intervals. They were then started on the histamine/beeswax mixture at a dose of 100 mg. 12-hourly, while the promethazine was continued at the same dosage. After seven days at this level the dose of histamine was increased to 200 mg. 12-hourly; this was continued until symptoms of peptic ulceration appeared. Each pair of dogs had a separate batch of the histamine/beeswax mixture prepared of sufficient quantity to last throughout the experiment. Throughout the periods of faecal collection, collection of secretion from the dog with the Heidenhain pouch enabled some estimate /
estimate to be made of the effect of the injections on gastric secretion. There was no interval between the control, promethazine, or promethazine and histamine/beeswax periods.

The dog with the total gastrectomy and the dog with the ileal resection were run concurrently with a pair of dogs as described above.

At the conclusion of the histamine periods, two dogs were subjected to laparotomy six hours after the last injection of histamine/beeswax; multiple biopsies were taken from the small intestine for histological study, and intestinal contents were sampled at various levels for pH measurements by aspiration with a large-bore needle.

**Results**

**Normal dogs.**—The mean daily faecal fat output of the normal animals has been tabulated in Table I. Only one dog (dog A) managed to complete a satisfactory period at a dose level of 400 mg. histamine daily. The other two dogs developed ulcer symptoms at a level of 200 mg. histamine daily, after six and four days respectively.

The mean daily faecal fat output of these dogs was 0.57 g. during the control period; there was no significant difference during the period on promethazine alone. Both dogs B and C showed an increase in fat excretion during the first histamine period.
period at 200 mg. daily. Dog C excreted no faeces for the third and fourth days of this period, and became anorexic and lethargic. Twelve hours after the histamine injections were discontinued this animal died of a large (1.5 cm.) perforated acute duodenal ulcer. The mean output of faecal fat of dog A during the period of 400 mg. histamine daily shows a highly significant increase over both control and promethazine periods. The results on this dog are shown in Fig. 2. After 12 days on a dosage of 400 mg. histamine daily injections were discontinued and the fat excretion followed as it returned to normal levels.

**Heidenhain pouch dogs.** - The mean faecal fat output of these animals is compared with the mean pouch output of gastric juice during the same period in Table II. Again two dogs (D and F) developed ulcer symptoms during administration of histamine and the injections were discontinued. The mean output of fat during the control and promethazine periods is unchanged; the output during these periods shows no significant difference when compared with the three normal dogs.

The mean faecal fat output was increased during the first histamine period at a dose of 200 mg. daily. The output during the second period at 400 mg. histamine daily was significantly increased when compared with control levels. The mean output during this period (1.51 g.) however is less than the mean output at /
at the same dose level observed in normal dogs (2.66 g.); the
difference between these means is also significant (p<0.01).

The mean daily output of acid from the small Heidenhain
pouches shows, as would be expected, a gross increase during the
histamine periods when compared with control levels. The
results from one dog are shown in Figure 3. It is interesting to
note that the mean output under the highest dose of histamine used
(400 mg. daily) approaches the theoretical 24-hour maximal acid
output for each pouch.

Ileal resection and total gastrectomy dogs.- These two dogs
were run concurrently with one of the Heidenhain pouch/normal dog
pairs, utilising the same batch of histamine/beeswax mixture.
The results are shown in Figure 4, and tabulated in Table III.

The total gastrectomy dog excreted a mean of 3.93 g. fat
daily during the control period; there was no significant change
during the administration of promethazine or histamine at a
dosage of 200 mg. daily for seven days and 400 mg. daily for
seven days.

The dog with the ileal resection showed moderate malabsorption
of fat during the control period. This was increased by the
administration of promethazine, but the difference between the two
means is not significant. The administration of 200 mg. histamine
daily produced gross steatorrhea, but doubling the dose of
histamine during the second period produced no further increase in
fat secretion. These results are discussed below.
Histology

The small bowel mucosa of two dogs taken six hours after the last injection of histamine showed no significant abnormalities, both under the dissecting microscope and conventional microscopy.

pH Measurements

Measurements in two dogs at laparotomy, six hours after the last dose of histamine, are tabulated in Table IV.

Discussion

The mean daily faecal fat output of normal dogs and Heidenhain pouch dogs has been found to be 0.57 g. and 0.59 g. respectively. This is similar to the results of Welbourn, Hallenbeck and Bollman (1953), who found that normal dogs on a diet containing 8.0 g. fat daily (as compared with our diet of 19.5 g. fat daily) excreted a mean of 0.20 g. per 24 hours.

The administration of promethazine hydrochloride alone produced no significant change in fat excretion in all the dogs studied. This antihistamine counteracts the systemic effects of histamine for a prolonged period, and activity is still present 12 to 16 hours after administration (Halpern, 1944). It has however no effect on histamine-induced gastric secretion (Sangster, Grossman and Ivy, 1946). There are no reports in the literature on the effects of histamine or the antihistamines on small bowel absorption, but the action of histamine on small bowel motility is completely antagonised by promethazine (Erdman and Henne, 1954).
In the normal dogs and the dogs with Heidenhain pouches the administration of histamine in a beeswax/mineral oil mixture produces an increase in faecal fat excretion. In the pouch dogs this effect roughly parallels the increase in acid secretion observed from the measurement of pouch secretion over each 24-hour period.

In one dog with a total gastrectomy faecal fat excretion on the standard diet was increased during the control period when compared with normal dogs; this has been well-documented in previous work (Everson, 1952). The histamine/beeswax mixture produced no further increase in fat excretion; the same batch of the histamine mixture was simultaneously shown to be effective in increasing gastric secretion from a Heidenhain pouch dog and to increase fat excretion in a dog with a normal stomach (Fig. 4). We have shown that the mean daily excretion of fat of normal dogs under histamine stimulation is significantly greater than the mean fat excretion of Heidenhain pouch dogs under the same conditions. These observations suggest that the interference with fat absorption produced by the histamine/beeswax mixture depends on the presence of secretory gastric mucosa in continuity with the gastro-intestinal tract. If the stomach is totally resected, yet the small bowel left intact (dog G), no increase in fat excretion is produced by histamine. In the Heidenhain pouch dogs construction of the isolated pouch probably removes 20 to 40 per cent. of the gastric body mucosa; this leaves proportionately/
proportionately less acid-secreting mucosa in continuity and is reflected in the smaller increase in fat excretion under histamine stimulation when compared with dogs with intact stomachs. It would appear logical to presume that the observed effect of chronic histamine administration on fat excretion in dogs is due to the induced hypersecretion of gastric juice.

Rawson, England, Gillain, French and Stammers (1960) describe a case of the Zollinger-Ellison syndrome which was cured by resection of a small pancreatic adenoma. This man had diarrhoea; investigations showed gastric hypersecretion and steatorrhoea. The basal 11-hour nocturnal secretion in this case was 246 m.Eq. of HCl; the maximal one-hour acid output by the histamine stimulation test of Kay (1953) was 60 m.Eq. Calculation based on these results indicates that this man had a basal secretion which was 37 per cent. of his stomach's maximal secretory ability. We have shown in dogs that prolonged histamine stimulation can produce basal secretion of this order, which results in impairment in fat absorption. As in the clinical situation of the Zollinger-Ellison syndrome, our main experimental difficulty was the severe peptic ulceration produced by the gastric hypersecretion.

Rawson et al. (1960) postulated that possible reasons for the steatorrhoea included:

(1) /
(1) The pancreas may be destroyed or rendered non-functional by the presence of the tumour. However, in their case the steatorrhoea disappeared after resection of the tumour.

(2) Hypersecretion may occur in the small and large bowels as well as in the stomach.

(3) The large volume of HCl passing into the small intestine may be incompletely neutralised by pancreatic bicarbonate, and the low pH impair emulsification and lipolysis. Not only is lipase inactivated at a low pH, but it may rapidly be destroyed in the presence of HCl and pepsin (Summerskill, 1959).

Telling and Smiddy (1961) report a case of an islet cell tumour associated with gross diarrhoea and steatorrhoea; repeated barium meals showed no evidence of peptic ulceration, although the patient had a haematemesis on two occasions. No measurement of gastric secretion was recorded. These authors collected 11 cases from the literature of islet cell tumours associated with diarrhoea or steatorrhoea. In only four cases was there evidence of peptic ulceration. They propose the attractive hypothesis that there may be two different types of non-insulin secreting islet cell tumour of the pancreas: the one may cause gastric over-secretion and peptic ulceration; the other intestinal over-secretion and diarrhoea. Our experimental data indicates that this hypothesis is unnecessary. In a small series of dogs with artificial gastric hypersecretion three animals out of seven (dogs A, E and H) showed significant steatorrhoea without evidence of peptic ulceration when they were finally sacrificed.
Summerskill (1959) describes a case of the Zollinger-Ellison syndrome where the pH in the upper small bowel was between 1.0 and 2.0 for most of the test period of 24 hours. In dogs with histamine-induced gastric hypersecretion, we have failed to demonstrate this finding. This however might merely indicate that the small intestine of the dog is more effective in coping with alteration in pH; in the dog the excess acid may be completely neutralised in the process of inactivating pancreatic and small bowel secretions.

Lawrie, Williamson and Hunt (1962) have suggested that there may be a relationship between the diarrhoea of the Zollinger-Ellison syndrome and the high rate of gastric emptying found in their case. It is possible that the effect of chronic histamine administration on the absorption of fat in dogs is mediated by changes in the gastric emptying rate. However, Hunt (1952) has shown that a single injection of histamine in humans produces no change in the rate of gastric emptying. In dogs equipped with gastric fistulas into the stomach, maximal doses of histamine have been shown to be without significant effect on gastric emptying times (Preshaw, 1963).

In the dog with 75 cm. of ileum resected (dog H), a moderate degree of steatorrhoea was found to be present during the control period. The administration of histamine in a dose of 200 mg. daily produced a significant degree of malabsorption of fat. Increasing the dose of histamine to 400 mg. daily produced no further increase in /
in fat excretion. This observation may be due to the possibility that the gastric mucosa in this animal responded to 200 mg. histamine daily with a maximum 24-hour acid output of acid. Increasing the dose of histamine therefore could not produce any further increase in acid output. This effect was not noted in the control dog with a Heidenhain pouch, and indeed all the pouch dogs studied showed an increase in acid output when the dose of histamine was doubled.

Recent work on pancreatic resection, duct ligation and pancreatic diversion in experimental animals has shown that these procedures are capable of inducing gastric hypersecretion (Elliot, Taft, Passaro and Zollinger, 1961). Although comparable results have not been found in any human pathological state, it is probable that pancreatic resection or diversion would induce similar gastric hypersecretion. This raises the question of whether gastric hypersecretion plays any part in the steatorrhoea found in pancreatic disease in humans.

Summary

(1) Chronic administration of histamine in a beeswax mineral oil mixture causes a significant interference with fat absorption in dogs.

(2) /
(2) Control studies indicate that this effect is due to the action of histamine in producing gastric hypersecretion.

(3) The results are discussed in relation to the steatorrhoea associated with the Zolliner-Ellison syndrome and steatorrhoea due to pancreatic disease.

Acknowledgement

We wish to thank Dr. A. Wynn Williams for his assistance with the histological studies.
LEGENDS TO FIGURES

Fig. 1.- Acid output from Heidenhain pouch dogs after one injection of histamine/beeswax: three-hour collections. Three-hour maximal acid output calculated from one-hour maximal histamine test.

Fig. 2.- Acid output from Dog E compared with faecal fat excretion from Dog A. Faecal fat expressed as a running three-day mean. Upper line above pouch output indicates theoretical 24-hour maximal acid output (one-hour MAO x 24). Both dogs run concurrently using the same batch of histamine beeswax. After histamine stimulation, Dog A was followed as fat excretion returned to normal levels.

Fig. 3.- Daily faecal fat excretion and Heidenhain pouch secretion of Dog E. Faecal fat expressed as a running three-day mean.

Fig. 4.- Faecal fat output of Dogs G and H compared. Both were run concurrently with a pouch dog whose output is indicated above; this animal however developed signs of peptic ulceration after four doses of histamine. Continuous administration /
administration was therefore discontinued until it recovered. The potency of the mixture was confirmed later by collection of pouch secretion after histamine injection at intervals.

Fig. 5.- Jejunal mucosa from Dog E six hours after last histamine injection.
Fig. 1.-

DOG D

HISTAMINE / BEESWAX 200 mg

PROMETHAZINE 50 mg

PROMETHAZINE 50 mg

3 HOUR MAO

ACID OUTPUT IN mEq/HCl PER 3 HOUR PERIOD

DOG E

16

14

12

10

8

6

4

2

0 3 6 9 12 15 18 21 HOURS

3 HOUR MAO
HEIDENHAIN POUCH DOG E

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Promethazine</td>
<td>100mg/24 hrs</td>
<td></td>
</tr>
<tr>
<td>Histamine/Beeswax</td>
<td>200mg/24 hrs</td>
<td>400mg/24 hrs</td>
</tr>
</tbody>
</table>

Pouch collections discontinued

NORMAL STOMACH AND SMALL INTESTINE

FAecal Fat Excretion in g/24 hrs.

Fig. 2.-
Fig. 3 -
Fig. 4.
<table>
<thead>
<tr>
<th>Dog</th>
<th>Weight</th>
<th>Control Period</th>
<th>Promethazine Period</th>
<th>Histamine/beeswax</th>
<th>200 mg. daily</th>
<th>400 mg. daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12 kg.</td>
<td>0.51 g. (7)</td>
<td>0.68 g. (7)</td>
<td>1.36 g. (7)</td>
<td>2.66 g. (10)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>11.5 kg.</td>
<td>0.67 g. (7)</td>
<td>0.68 g. (7)</td>
<td>1.23 g. (6)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>13 kg.</td>
<td>0.54 g. (14)</td>
<td>0.55 g. (7)</td>
<td>0.23 g. (4)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.57 g.</td>
<td>0.62 g.</td>
<td>0.93 g.</td>
<td>2.66 g.</td>
<td></td>
</tr>
</tbody>
</table>

1. Figures in brackets indicate number of days in each period.

Statistical analysis: difference between control mean and mean excretion on 400 mg. histamine daily, 1.89 g., statistically significant. ($t = 14.7; p < 0.001$).
TABLE II
MEAN DAILY FAT EXCRETION AND POUCH OUTPUT: HEIDENHAIN POUCH DOGS

<table>
<thead>
<tr>
<th>Weight</th>
<th>One-hour pouch MAO x 24</th>
<th>Control Period</th>
<th>Promethazine Period</th>
<th>Histamine/beeswax Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fat</td>
<td>Acid</td>
<td>Fat</td>
</tr>
<tr>
<td>Dog D</td>
<td>14 kg.</td>
<td>122 m.Eq.</td>
<td>0.58 g. 7.7 m.Eq.</td>
<td>0.54 g. 9.6 m.Eq.</td>
</tr>
<tr>
<td></td>
<td>(14)²</td>
<td>(7)</td>
<td>(7)</td>
<td>(7)</td>
</tr>
<tr>
<td>Dog E</td>
<td>15 kg.</td>
<td>107 m.Eq.</td>
<td>0.47 g. 0.61 m.Eq.</td>
<td>0.68 g. 0.98 m.Eq.</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(7)</td>
<td>(7)</td>
<td>(7)</td>
</tr>
<tr>
<td>Dog F</td>
<td>12 kg.</td>
<td>57 m.Eq.</td>
<td>0.61 g. 7.8 m.Eq.</td>
<td>0.52 g. 8.7 m.Eq.</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(7)</td>
<td>(7)</td>
<td>(3)</td>
</tr>
<tr>
<td>Mean excretion</td>
<td>0.59 g.</td>
<td>0.58 g.</td>
<td>0.90 g.</td>
<td>1.51 g.</td>
</tr>
</tbody>
</table>

1. Figures in brackets indicate number of days in each period.

Statistical analysis: difference between mean control output and output on 400 mg. histamine daily, 0.92 g., statistically significant ($t = 3.01$, $p < 0.01$).
### TABLE III

**MEAN FAT EXCRETION OF TOTAL GASTRECTOMY AND ILEAL RESECTION DOGS**

<table>
<thead>
<tr>
<th>Weight</th>
<th>Control Period</th>
<th>Promethazine Period</th>
<th>Histamine/beeswax 200 mg. daily</th>
<th>Histamine/beeswax 400 mg. daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog G</td>
<td>10 kg.</td>
<td>3.93 g.</td>
<td>3.74 g.</td>
<td>2.18 g.</td>
</tr>
<tr>
<td>Total Gastrectomy</td>
<td>(7)</td>
<td>(7)</td>
<td>(7)</td>
<td>(7)</td>
</tr>
<tr>
<td>Dog H</td>
<td>11 kg.</td>
<td>2.96 g.</td>
<td>5.65 g.</td>
<td>10.94 g.</td>
</tr>
<tr>
<td>Ileal Resection</td>
<td>(7)</td>
<td>(7)</td>
<td>(7)</td>
<td>(7)</td>
</tr>
</tbody>
</table>

1. Figures in brackets indicate number of days in each period.

Statistical analysis: difference between (a) mean control output and output during promethazine period (Dog H), 2.69 g., not statistically significant \( t = 1.84; p > 0.05 \).

(b) mean control output and output on 200 mg. histamine daily (Dog H), 7.98 g., statistically significant \( t = 4.41; p < 0.001 \).
<table>
<thead>
<tr>
<th>Site</th>
<th>Dog E</th>
<th>Dog H</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gastric Antrum</td>
<td>Whole Stomach</td>
</tr>
<tr>
<td>Heidenhain Pouch</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>2nd Part Duodenum</td>
<td>6.6</td>
<td>7.5</td>
</tr>
<tr>
<td>Proximal Jejunum</td>
<td>7.8</td>
<td>8.0</td>
</tr>
<tr>
<td>Mid-Jejunum</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Proximal Ileum</td>
<td>7.9</td>
<td>8.0</td>
</tr>
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</table>
REFERENCES


