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Production of Acute Duodenal Ulcers in Rats by Infusion of Gastric Secretogogues

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PRODUCTION OF ACUTE DUODENAL ULCERS IN RATS BY INFUSION OF GASTRIC SECRETOGOGUES

by

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CHAPTER I

THE PURPOSE, BACKGROUND AND GENERAL METHOD

The Purpose

Prior to this investigation no procedure for the production of acute duodenal ulcers in laboratory rats had been published. This study was therefore undertaken to: (1) develop a procedure of ulcer production in rats which would be rapid (48 hours or less) and give a 100% incidence of ulceration, and (2) determine, if possible, the way such a procedure acts to produce duodenal ulcers. The ability to produce acute duodenal ulcers in rats may facilitate further study and greater understanding of duodenal ulcers.

Previous Studies

In species of laboratory animals other than rats several methods have been used to produce duodenal ulcers. Injection of histamine in beeswax has been successful in producing duodenal ulcers in dogs, guinea pigs, and cats (Hay, Varco, Code and Wangensteen, 1942). Gastrin has been used successfully in the production of duodenal ulcers in dogs (Dragstedt, Oberhelman and Smith, 1951), cats (Emas and Grossman, 1967), and guinea pigs (Gobbels and Adkins, 1967). Burns have been used successfully in dogs and rabbits (Hartman, 1945). Finally, among the more extensively reported techniques, production of a biliary fistula in dogs has been used to produce duodenal ulcers (Berg and Jobling, 1930).

Rats apparently are resistant to the formation of duodenal ulcers. Only one method, deprivation of dietary pantothenic acid, has been shown

to produce duodenal ulcers in rats (Berg, Zucker and Zucker, 1949). This method posed three difficulties: first, thirteen weeks were required for ulcer formation; second, the maximum incidence of ulceration was 60%; and, third, specific strains of laboratory rats were required.

The General Method

In this investigation, one general method was used to produce and study duodenal ulcers in laboratory rats. This general method consisted of subcutaneous infusion of various secretogogues by means of continuous-flow variable-speed infusion machines designed and owned by The Upjohn Company. The term "gastric secretogogue" as used in this investigation refers to a pharmacological agent which increases the output of pre-existing gastric secretions.

Each infusion machine permitted continuous emptying of a maximum of twelve syringes during adjustable periods of time up to twenty-four hours. The agents used were forced out of the syringes, through plastic tubings, through needles at the ends of the tubings, and were delivered subcutaneously to the rats. The specific infusion techniques used to establish dose responses for the various agents, and used to study the mechanism of ulcer formation differ and are described in the methods sections of chapters two and three respectively.

CHAPTER II

PRODUCTION OF ULCERS

Introduction: The Pharmacological Agents

Three gastric secretogogues were used individually as experimental agents: histamine (histamine dihydrochloride); carbachol (carbamylcholine chloride); and pentagastrin (an analog of the C-terminal five amino acids of gastrin, N-5-butyloxycarbonyl-B-ala-try-met-asp-phe-NH_2). In addition, two combinations of agents were used: histamine and carbachol, and histamine and pentagastrin. In each case the original compound was a dry chemical which was put into solution in normal saline. The combinations of two compounds were put into solution together and injected through the same syringe. Normal saline was used as the control agent against which the effects of the experimental agents were compared. Uninfused rats were also used as controls against which the effects of the infusion process itself were compared.

Histamine is a decarboxylation product of histidine, and is a potent vasodilator (Grollman, 1962). Carbachol is a synthetic compound with parasympathomimetic activity (Grollman, 1962). Pentagastrin is produced synthetically, and has the properties of the secretion-stimulating hormone gastrin, but is less active on a per-molecule basis (Barrett, 1966).

The use of gastric secretogogues is based on the investigations of duodenal ulcer formation cited in Chapter I. Both histamine and gastrin have been used successfully in the production of duodenal ulcers in species other than rats. Carbachol had apparently not been used for duodenal ulcer production before this investigation. Like histamine (Shay, Rayport

and Fels, 1944) and pentagastrin (Konturek and Grossman, 1966), carbachol stimulates gastric secretion in the rat and was therefore included as an experimental agent.

Although all three experimental agents stimulate gastric secretion, they are dissimilar in other respects. Histamine is best known for its vasodilator function. It is usually released by cells in response to some stimulus and is localized in areas of trauma or inflammation (Grollman, 1962). Carbachol is primarily a parasympathomemetic agent (Grollman, 1962). Pentagastrin has been reported to stimulate exocrine pancreas secretion, bile secretion and intestinal motility (Gregory and Tracy, 1964), in addition to its primary function of gastric secretion stimulation.

Methods

Infusion technique

The general procedure for the production of ulcers consisted of 48 hours of continuous subcutaneous infusion of the experimental agent(s) at an infusion rate of .54 ml/min (13 ml/24 hrs). A 72 hour infusion of pentagastrin was the only experiment which involved a different procedure. Under the same experimental conditions and at the same time as the infusion of experimental agent, one control group of rats was infused with saline alone, while the other control group of rats was not infused..

Female Sprague-Dawley rats weighing between 200 and 225 grams were fasted overnight and weighed immediately prior to infusion. The average fasted weight for the rats was used as the basis for calculation of

doses to be administered. All doses were calculated as weight of compound per kilogram of rat per minute (mg/kg/min, or μg/kg/min). After calculation of the amount of experimental agent required, the compound was weighed on an analytical balance and put into solution with normal saline. After appropriate dilution, the solution was put into 12 ml syringes, which were then placed in the infusion machines. After the solutions had begun to flow from the tubings, the needles at the ends of the tubings were implanted subcutaneously into the rats' backs. The rats were then placed in stainless steel cages which were housed in plastic racks. After twenty-four hours of infusion, the empty syringes were replaced with syringes containing fresh solution. This replacement procedure involved little, if any disturbance of the rats. At the end of the second twenty-four period of infusion all rats were removed from their cages, the needles were removed, and the surviving rats were placed in killing jars containing chloroform.

Variables studied

All the rats were autopsied during which the appearance of the abdominal organs, the intestinal wall and the intestinal contents in the experimental rats was compared with that of the control rats. The stomach and 3-4 cm of duodenum were then removed and cut open. The stomach was cut open along the greater curvature, and the duodenum was cut open along the mesenteric attachment. After washing, the mucosa was examined with a 2X binocular magnifier for the presence of erosions and ulcerations.

An arbitrary rating scale was used for all observed unmeasured variables. This rating scale ranged by half-units from one-half-plus

for barely visible to three-plus for the most extensive. For ulcers, a one-half-plus rating of severity indicated a barely visible ulcer while a three-plus rating indicated a perforation.

Other variables which were observed included: site (stomach or duodenum) of ulceration, number of ulcers per site and distance from the pylorus to duodenal ulcers. In the stomach, three sites were considered separately: the forestomach, the corpus, and the antrum including the pylorus.

The data from this investigation are presented graphically, and several terms are used in their presentation. The incidence of ulceration, incidence of perforation and incidence of mortality are expressed as percentages. These percentages represent the proportion of rats which received a given treatment and which showed the specific response. The average number of duodenal ulcers per rat represents the total number of duodenal ulcers among rats which received a given treatment divided by the number of rats receiving that treatment. Duodenal ulcer severity is represented in two ways. First, the average severity units per rat is the total number of severity units among all rats which received a specific treatment divided by the total number of rats which received that treatment. Second, the average severity units per ulcer represents the average severity units per rat for a given treatment divided by the average number of duodenal ulcers per rat for that treatment. When a specific variable was not studied or if a specific variable was not produced in response to a given experimental agent, no graphs are given. Instead, the circumstances which caused such data to be excluded are described.

In cases where a given dose of an experimental agent was administered more than once, the data from these experiments have been pooled.

Results

Characteristics of ulcers by site

The appearance, and to some extent the severity, of ulcers varied according to the site of ulceration. However, except for some very severe ulcers all were roughly circular in outline.

Forestomach ulcers were generally 1 mm in diameter or smaller and blister-like in appearance. They were superficial, did not appear to be hemorrhagic and were usually multiple.

Corpus ulcers were generally .5 mm in diameter or smaller and looked like black specks or small round black craters. The corpus ulcer site was usually at the top of a fold of the mucosa. Some corpus ulcers located at the top of a mucosal fold were up to .3 mm in length. In 92.5% of rats with corpus ulcers, the most severe ulcer was 1.0 plus or less. However, these ulcers were frequently black and hemorrhagic. Two or more ulcers per corpus were observed in 91.5% of rats with corpus ulcers.

Antral ulcers were usually flat areas of erosion without any distinct craters. Antral ulcer size ranged from .5 mm to ulcers which covered the entire antral surface. Perforated antral ulcers were observed in 3.3% of rats with antral ulcers. The number of ulcers per antrum was three or less in 97.7% of rats with antral ulcers. Antral ulcers which extended to the pylorus were also seen.

Duodenal ulcers were variable in severity and typically appeared as prominent craters. Ulcers which had perforated were sometimes 3 mm in diameter or larger and showed no crater. In mild ulcers (.5 to 1.0 plus) the center of the crater was white, in moderately severe ulcers (1.5 to 2.0 plus) the center of the crater was red, while in very severe ulcers (2.5 to 3.0 plus) the center of the crater was black and hemorrhagic. All duodenal ulcers were located on the antimesenteric wall. Among all duodenal ulcers, 35.3% were located within 2 mm from the pylorus, 25.5% were located between 2 and 6 mm from the pylorus, 11.0% were located between 6 and 10 mm from the pylorus, and 28.2% were located further than 10 mm from the pylorus. The maximum distance from the pylorus for duodenal ulcer formation was 40 mm. More than one ulcer per duodenum was observed in 97.3% of instances of ulcer formation further than 10 mm from the pylorus. Sixty-eight and three-tenths per cent of rats with duodenal ulcers had one ulcer per duodenum, while the maximum number of ulcers per duodenum was six.

Associated changes

There were several observable changes associated with duodenal ulceration. Duodenal dilatation was seen both in the presence of duodenal ulcers and at doses of experimental agents too low for ulcers to develop. In the absence of duodenal ulcers the extent of dilatation appeared to correspond somewhat to the dose of experimental agent. However, in the presence of duodenal ulcers, the extent of dilatation was variable.

In 58% of mild ulcers (.5 to 1.5 plus) a red streak was observed on the serosal side of the duodenal wall. This red streak was perpen-

dicular to the long axis of the duodenum and was located at the site of mucosal ulceration.

With more severe ulcers (2.0 to 3.0 plus) it was possible to see the ulcer through the wall of the duodenum. As seen through the duodenal wall the ulcer had reddish-brown indistinct appearance.

Several changes were associated with ulcer perforation (antral and duodenal). Fluid in the abdominal cavity was associated with open perforated ulcers. Erosion of the mesenteric attachment, pancreas or liver was observed adjacent to sites of duodenal ulcer perforation. Fluid in the abdominal cavity was observed in 84.9% of instances of death among rats with perforated duodenal ulcers.

A semi-solid yellow material was observed in the duodenum and/or jejunum of 26.4% of rats without duodenal ulcers and 41.4% of rats with duodenal ulcers. The chemical nature of the yellow material was not determined.

Controls

Among 161 rats infused with saline for 24 hours there were no rats with duodenal ulcers, 31 rats (19%) with forestomach ulcers, one rat (.6%) with a corpus ulcer, and 13 rats (8%) with antral ulcers.

Twenty rats were infused with saline for 72 hours. Among these rats there were no duodenal ulcers, no forestomach ulcers, no corpus ulcers, and six rats (30%) with antral ulcers.

Twelve rats served as uninfused controls. Among these rats there were no duodenal ulcers, one rat (8%) with forestomach ulcers, no corpus ulcers, and two rats (16%) with antral ulcers.

Histamine alone

Histamine was administered alone at doses from .1 to 3.0 mg/kg/min. Histamine produced duodenal ulcers (Figure 1) at doses from .5 mg/kg/min to 3.0 mg/kg/min, with a maximum incidence of 73% at a dose of 2.0 mg/kg/min. Between doses of 2.0 mg/kg/min and 3.0 mg/kg/min there was a reduction in duodenal ulcer incidence. Coincident with this reduction in incidence there was an increase in mortality.

Mortality (Figure 2) was produced in response to histamine doses of 1.75 mg/kg/min and higher. At 3.0 mg/kg/min the mortality incidence reached a maximum of 95%. Duodenal ulcer perforations (Figure 3) were produced in response to histamine doses from 1.0 mg/kg/min to 2.0 mg/kg/min. The incidence of duodenal perforations reached a maximum of 14% at a dose of 2.0 mg/kg/min. The average number of duodenal ulcers per rat (Figure 4) increased in response to histamine doses from .5 mg/kg/min to 2.0 mg/kg/min and decreased in response to a dose of 3.0 mg/kg/min. The average number of duodenal ulcers per rat reached a maximum of 1.09 at a dose of 2.0 mg/kg/min and dropped to .36 at a dose of 3.0 mg/kg/min. The average severity units per rat (Figure 5) generally increased in response to doses up to 2.0 mg/kg/min and decreased thereafter. The average severity units per ulcer (Figure 6) was variable.

Only one dose of histamine, 1.0 mg/kg/min, produced forestomach ulcers (10% incidence), so no graph of forestomach ulcer incidence is given.

Corpus ulcers (Figure 7) were produced in response to every histamine dose except .75 mg/kg/min. Corpus ulcers incidence did not exceed 11% in response to histamine doses up to 1.5 mg/kg/min. Doses

Figure 1
Duodenal Ulcer Incidence

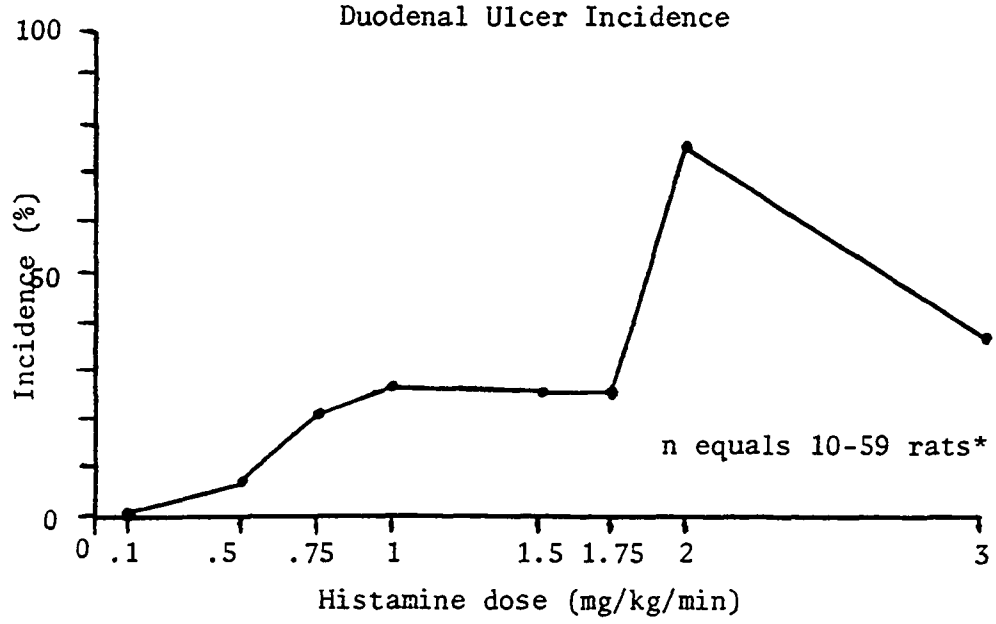
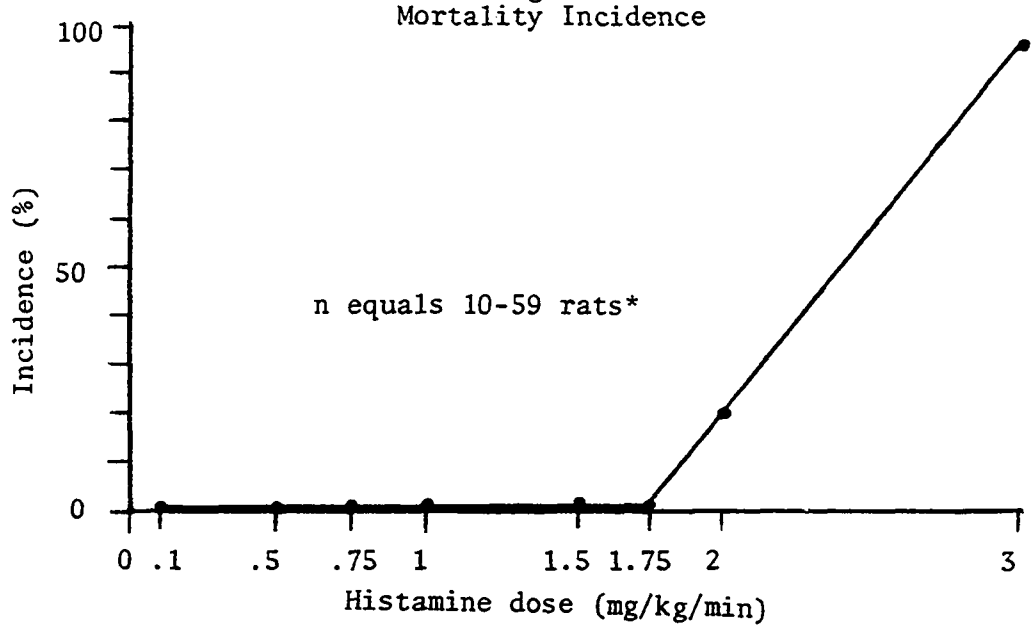


Figure 2
Mortality Incidence



* number of rats which received doses indicated

Figure 3
Perforation Incidence

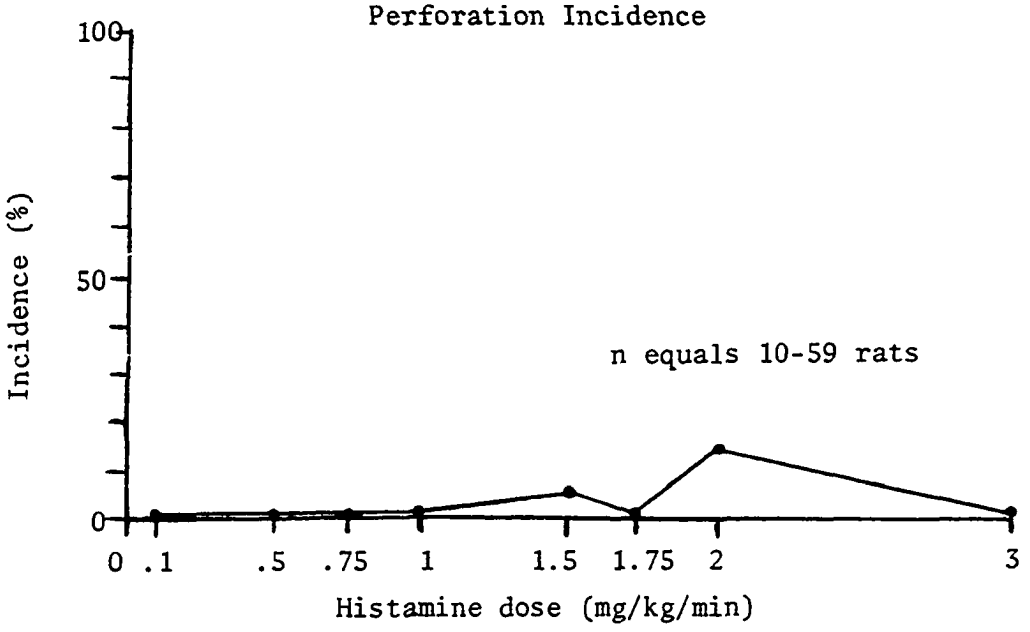


Figure 4
Duodenal Ulcers Per Rat

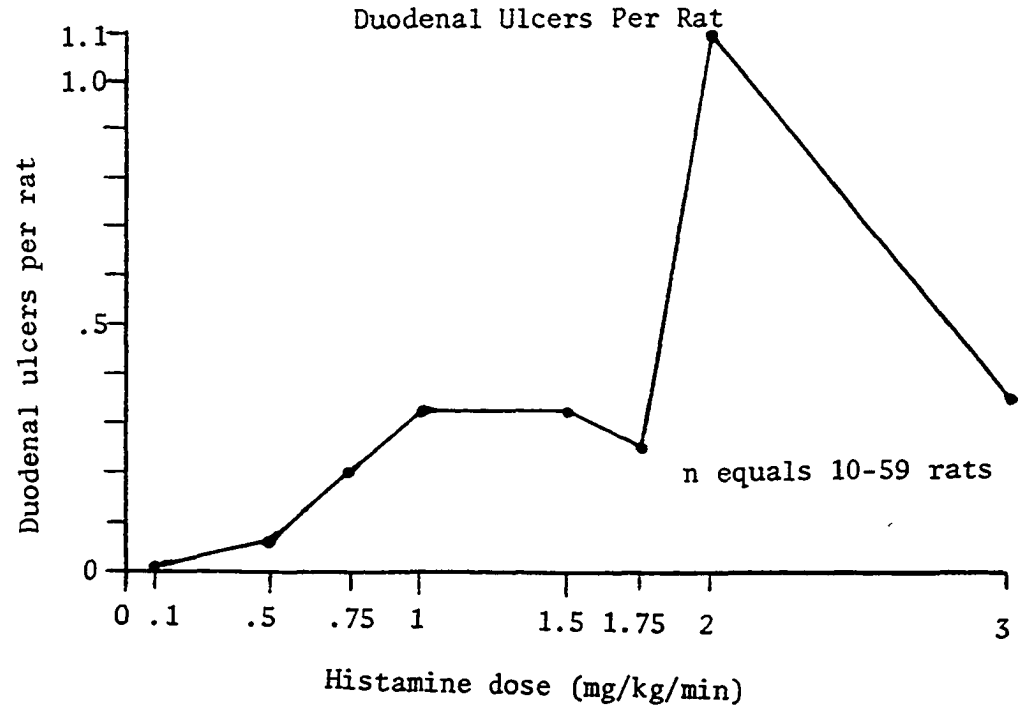


Figure 5
Severity Units Per Rat

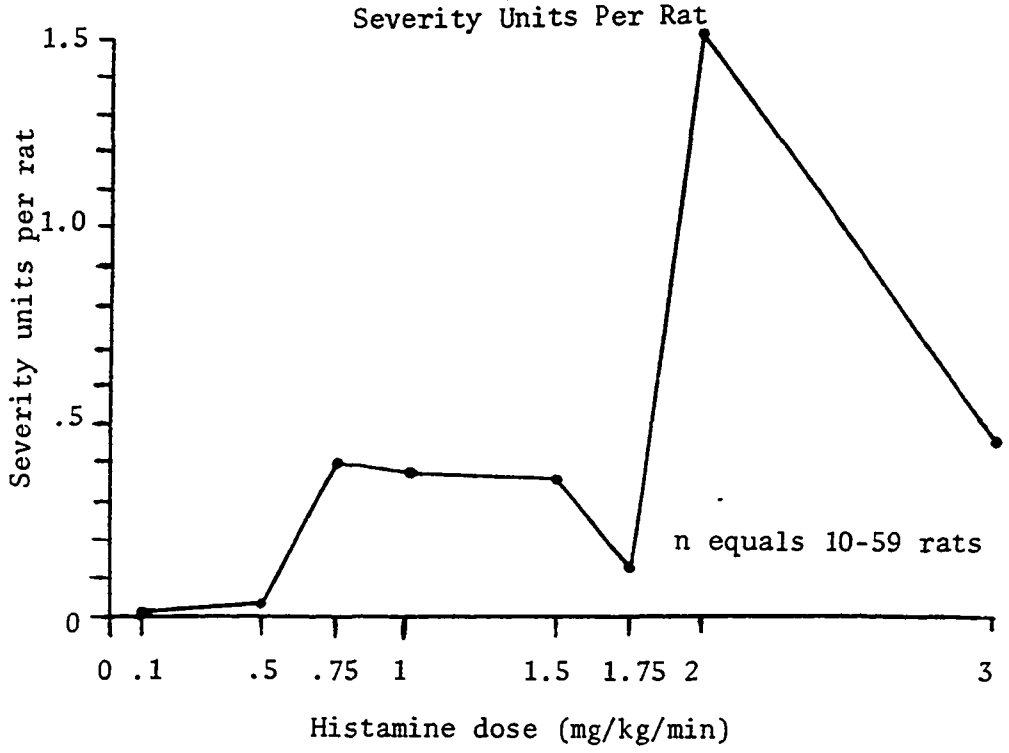


Figure 6
Severiry Units Per Ulcer

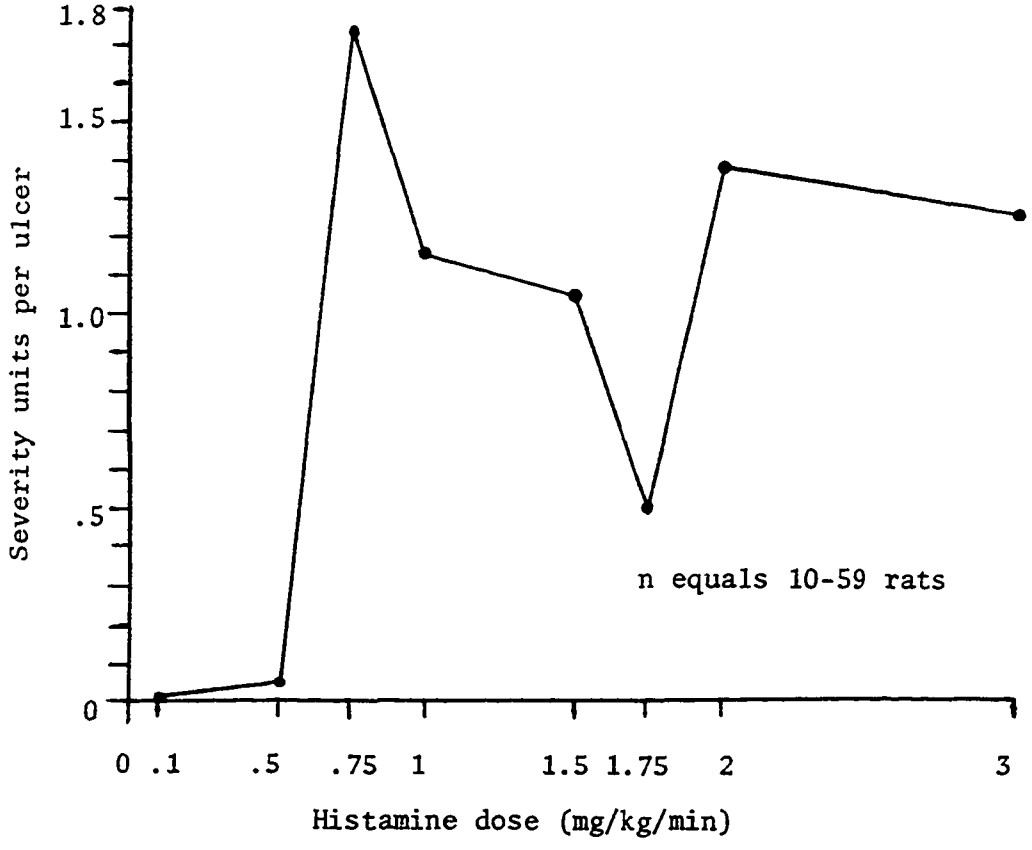


Figure 7
Corpus Ulcer Incidence

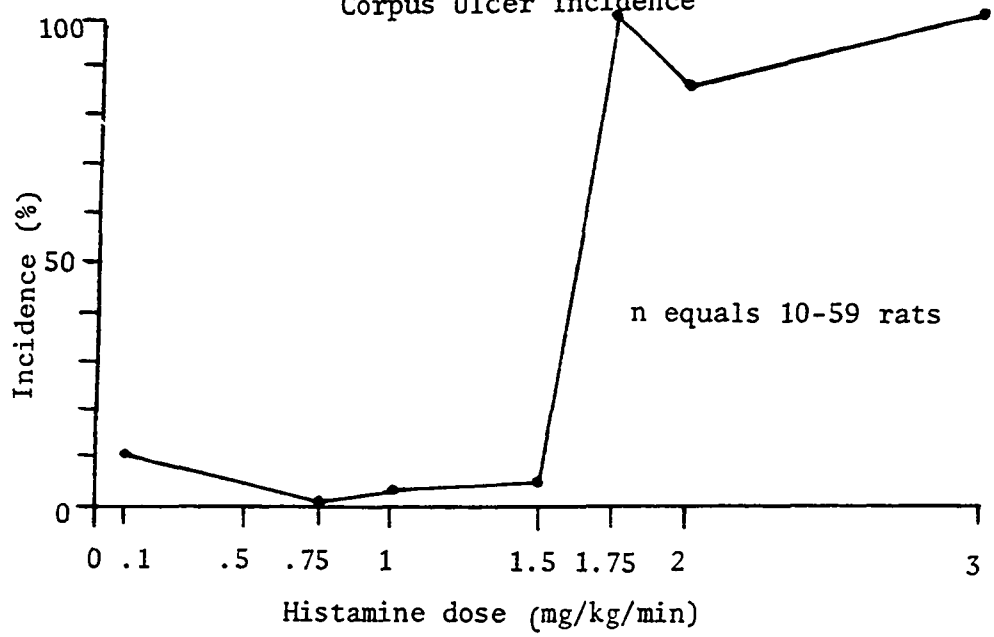
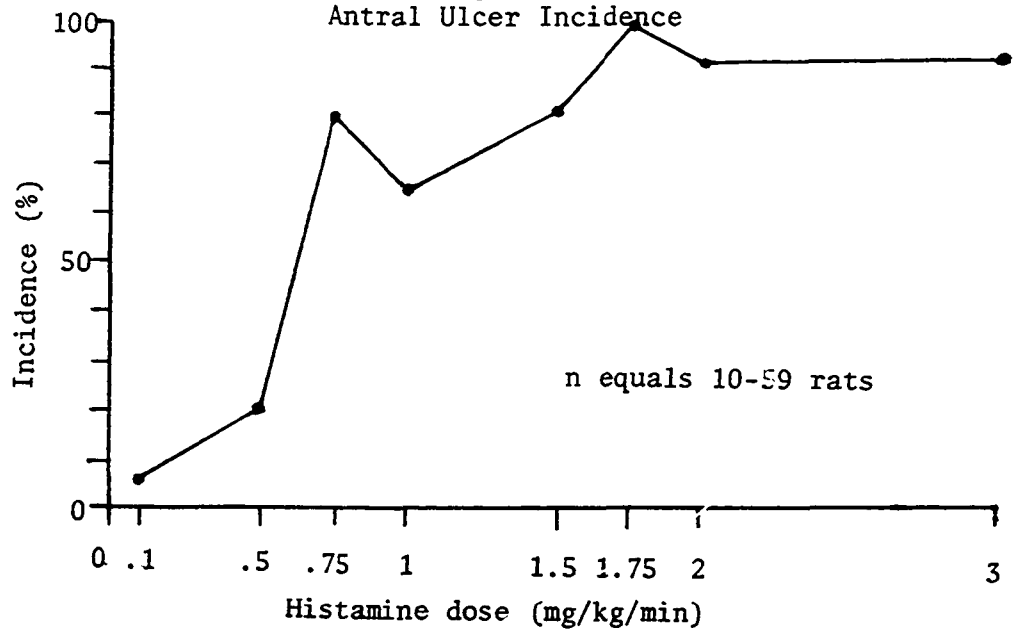


Figure 8
Antral Ulcer Incidence



of histamine from 1.75 mg/kg/min to 3.0 mg/kg/min produced corpus ulcer incidences of at least 86%. A maximum corpus ulcer incidence of 100% was reached at histamine doses of 1.75 and 3.0 mg/kg/min. Up to 100 or more small (.1 mm diameter or smaller corpus ulcers were produced in response to histamine doses from 1.75 to 3.0 mg/kg/min.

Antral ulcers (Figure 8) were produced in response to all histamine doses. Antral ulcers increased in incidence in response to doses up to 1.75 mg/kg/min. Antral ulcer incidence reached a maximum of 100% at a dose of 1.75 mg/kg/min, and decreased to an incidence of 91% at a dose of 2 mg/kg/min and an incidence of 92% at a dose of 3.0 mg/kg/min. Among rats with antral ulcers, 15.5% developed antral ulcers 2 mm in diameter or larger, while 6.2% developed perforated antral ulcers.

Among rats receiving histamine alone, yellow material was observed in the duodenum and/or jejunum of 52.9% of rats with duodenal ulcers and 10.3% of rats without duodenal ulcers.

Carbachol alone

Carbachol alone was administered at doses of .3 to 20 µg/kg/min. Carbachol alone produced duodenal ulcers (Figure 9) at a dose of .3 µg/kg/min and at doses of 1.0 µg/kg/min and higher. Duodenal ulcer incidence increased in response to carbachol doses of 1.0 to 2.0 µg/kg/min and reached a maximum of 70% at a dose of 2.0 µg/kg/min. At doses of 2.5 µg/kg/min and higher, the incidence decreased. Perforated duodenal ulcers (Figure 10) were produced in response to carbachol doses of 2.0 to 5.0 µg/kg/min. The incidence of perforated duodenal ulcers reached a maximum of 20% at a dose of 2.0 µg/kg/min. Deaths (Figure 11) were produced in response to carbachol doses of 2.0 µg/kg/min and higher.

Figure 9
Duodenal Ulcer Incidence

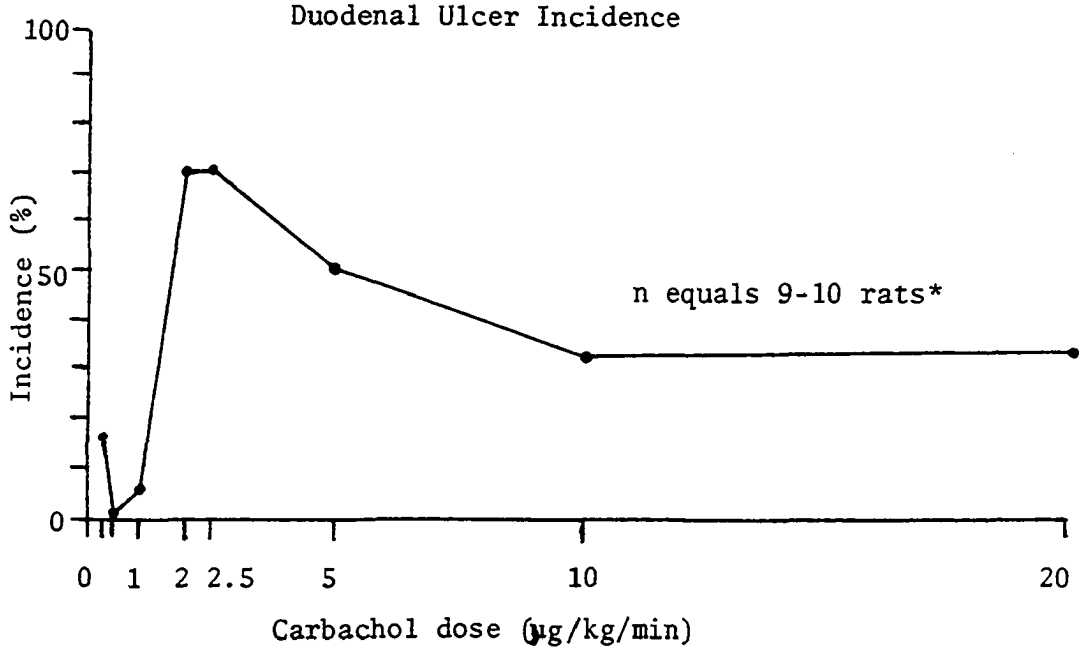
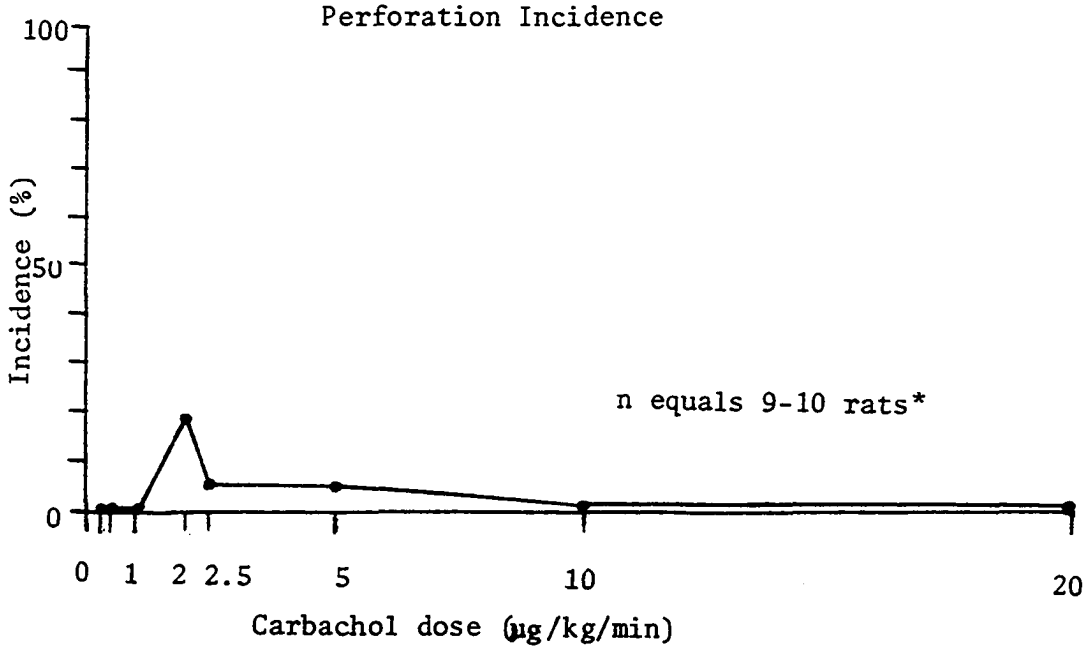


Figure 10
Perforation Incidence



* number of rats which received doses indicated

Figure 11
Mortality Incidence

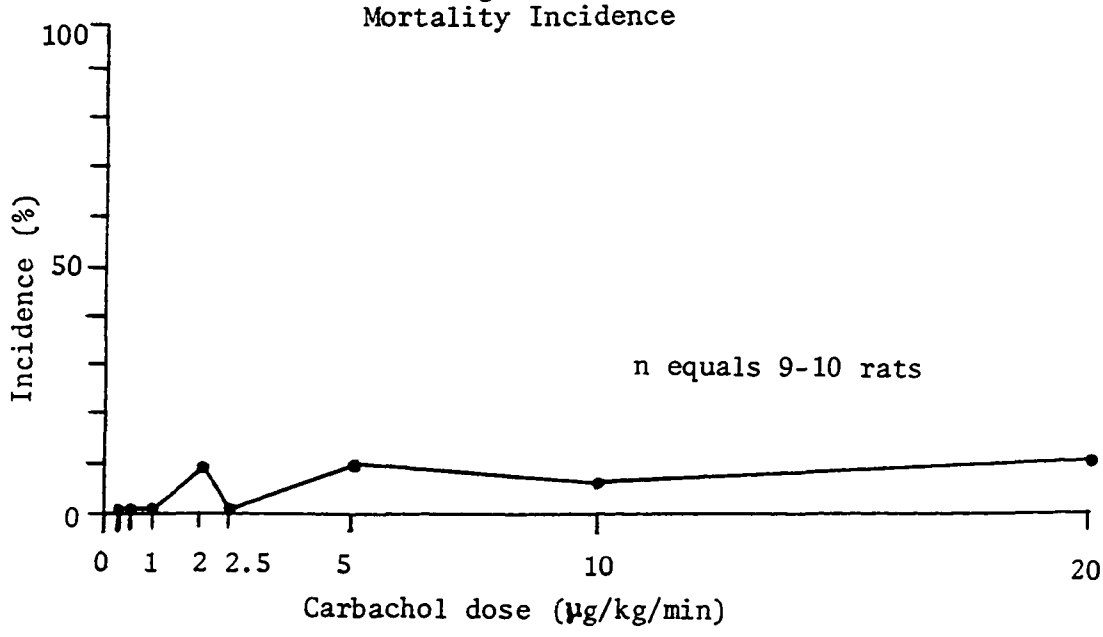


Figure 12
Duodenal Ulcers Per Rat

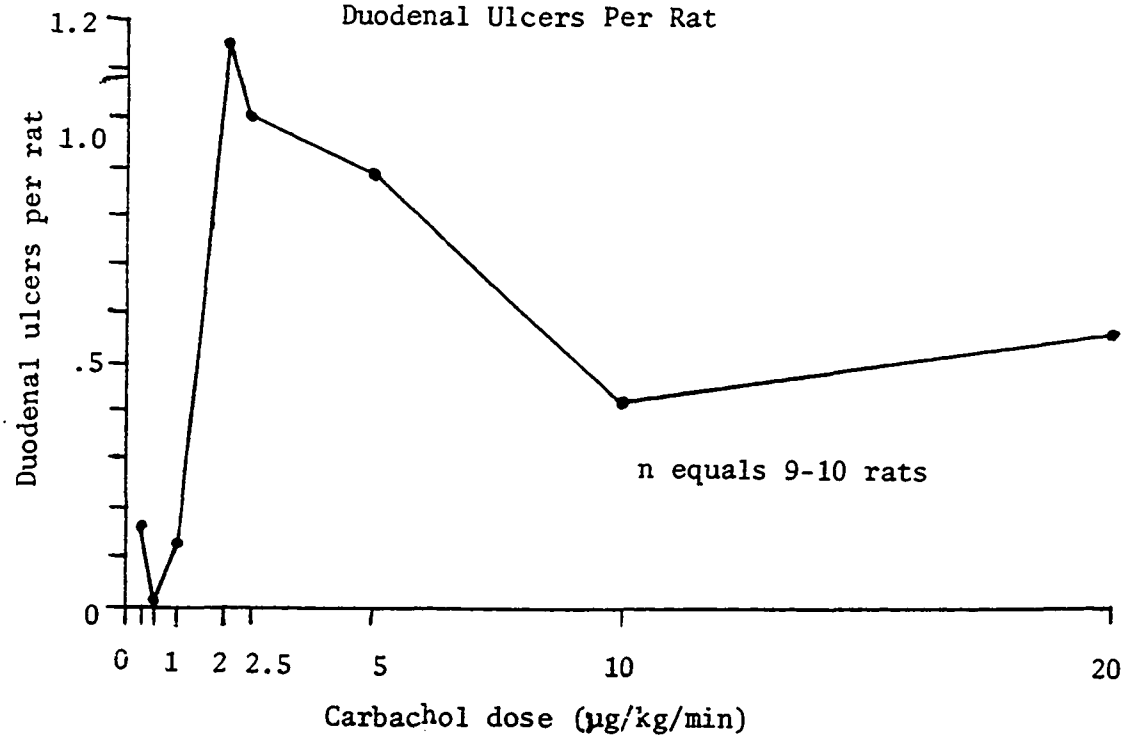


Figure 13
Severity Units Per Rat

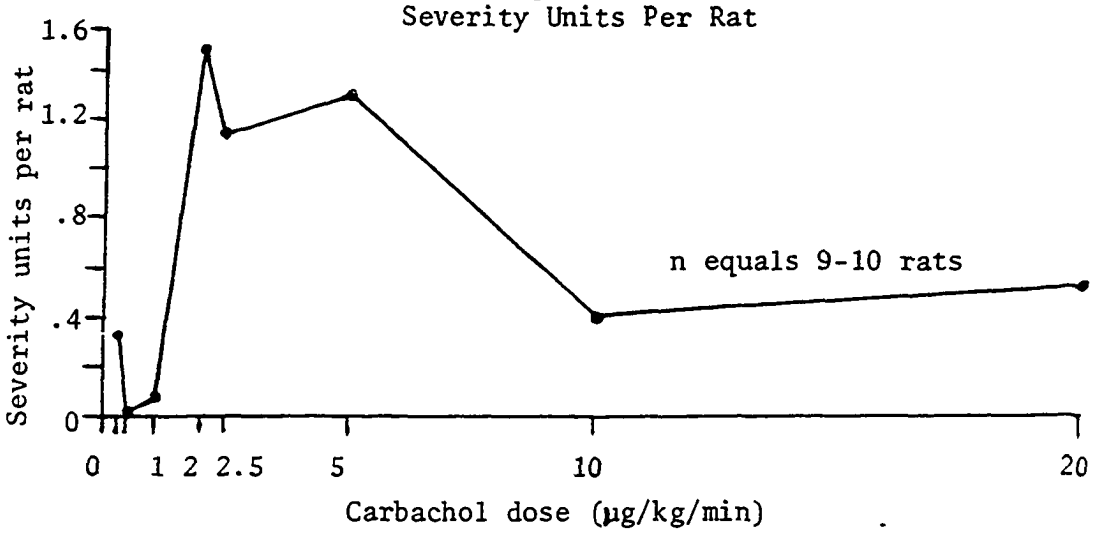


Figure 14
Severity Units Per Ulcer

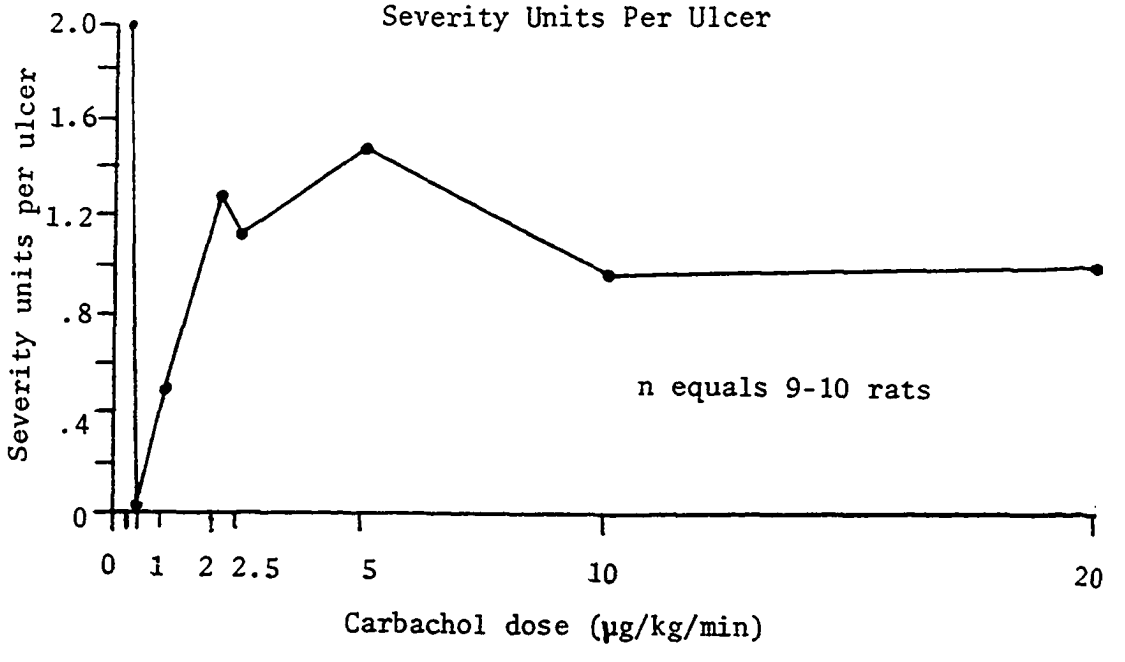


Figure 15
Forestomach Ulcer Incidence

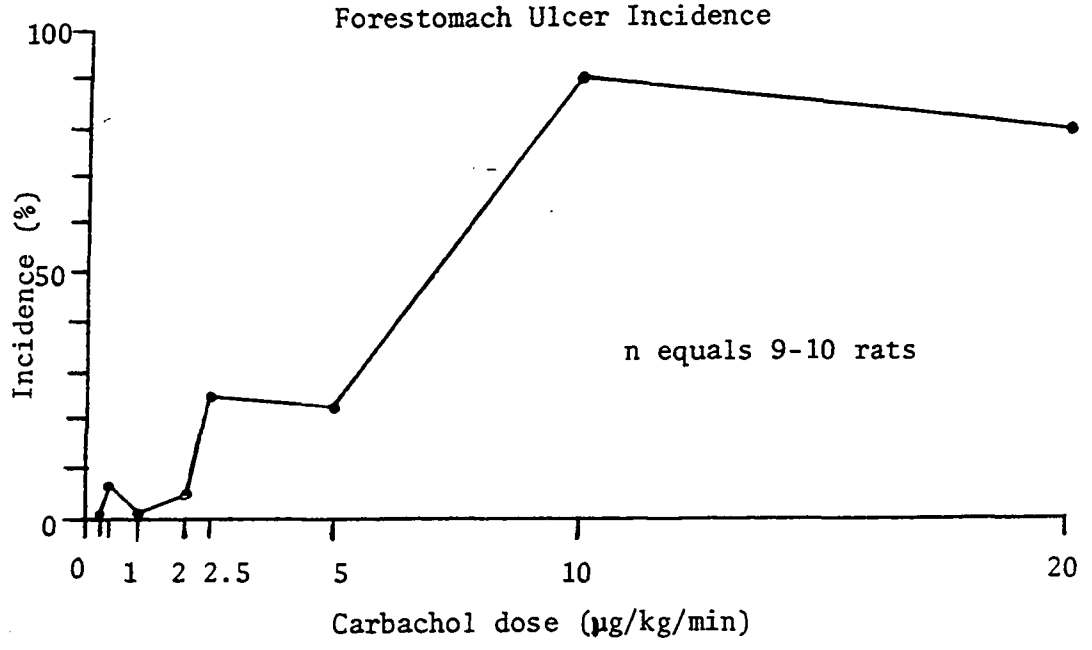


Figure 16
Corpus Ulcer Incidence

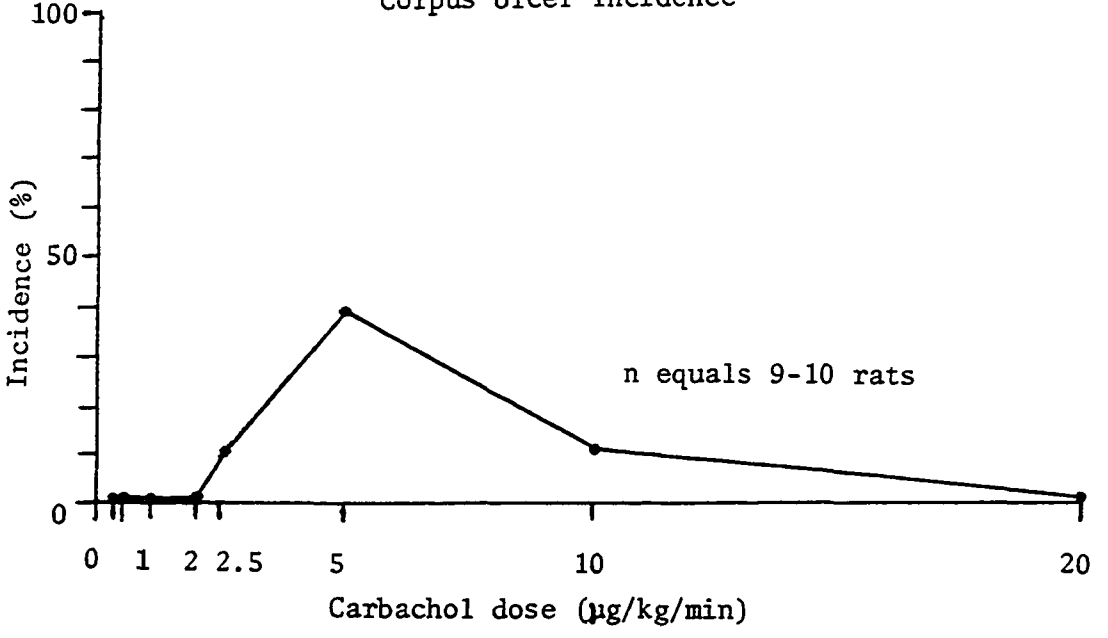
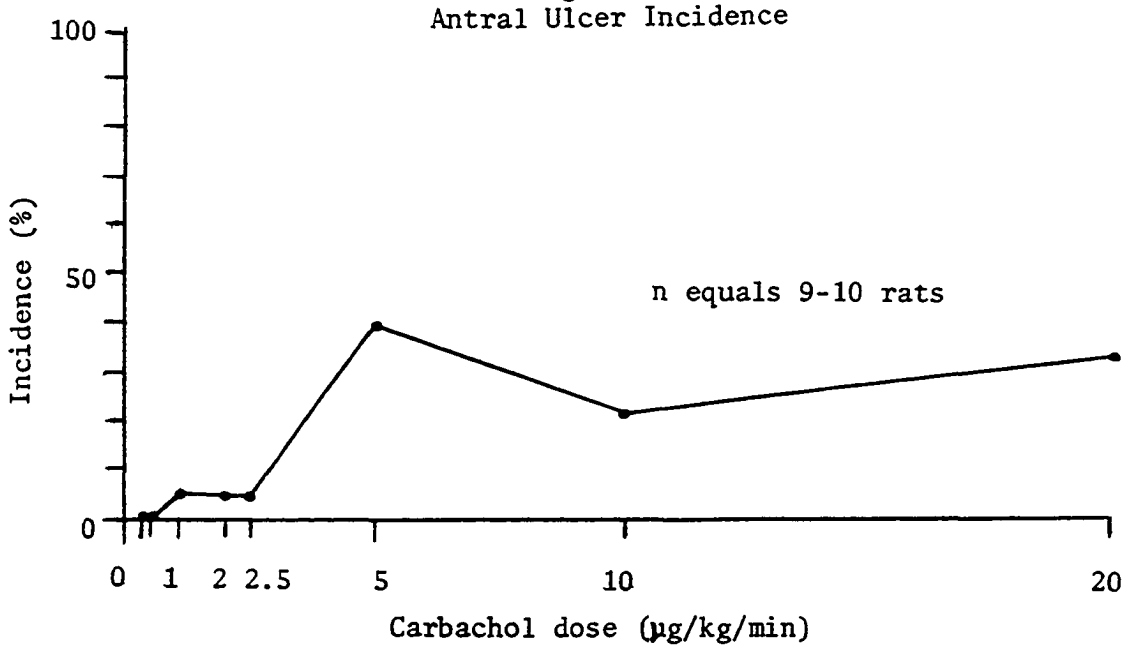


Figure 17
Antral Ulcer Incidence



The incidence of mortality never exceeded 10%. The average number of duodenal ulcers per rat (Figure 12) increased in response to carbachol doses of 1.0 to 2.0 µg/kg/min, decreased in response to doses of 2.0 to 10 µg/kg/min and increased again in response to a dose of 20 µg/kg/min. The average number of duodenal ulcers per rat reached a maximum of 1.15 at a dose of 2.0 µg/kg/min. The average severity units per rat (Figure 13) reached a maximum of 1.50 at a dose of 2.0 µg/kg/min. The average severity units per ulcer (Figure 14) reached a maximum of 2.00 at a dose of .3 µg/kg/min.

Forestomach ulcers (Figure 15) were produced in response to carbachol doses of .5 µg/kg/min and at doses of 2.0 µg/kg/min and higher. The maximum forestomach ulcer incidence was 89% at a dose of 10.0 µg/kg/min.

Corpus ulcers (Figure 16) were produced in response to carbachol doses of 2.5 to 10 µg/kg/min with a maximum incidence of 39% at a dose of 5.0 µg/kg/min.

Carbachol produced antral ulcers (Figure 17) at doses of 1.0 µg/kg/min and higher. The maximum incidence of antral ulcers was 39% at a dose of 5.0 µg/kg/min.

Among rats receiving carbachol alone, yellow material was observed in the duodenum and/or jejunum of 26.8% of rats with duodenal ulcers and 12.7% of rats without duodenal ulcers.

Pentagastrin alone

Pentagastrin alone was administered at doses of .5 to 5.0 µg/kg/min. Pentagastrin alone produced duodenal ulcers (Figure 18) at doses of 2.0 and 5.0 µg/kg/min. The maximum duodenal ulcer incidence was 38%

Figure 18
Duodenal Ulcer Incidence

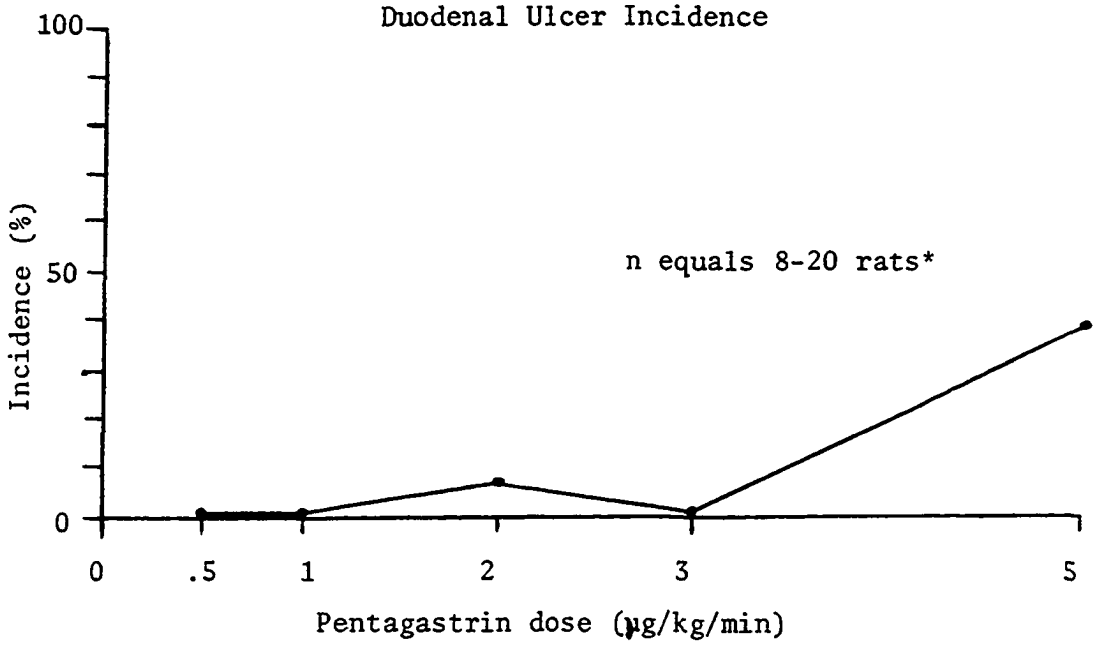
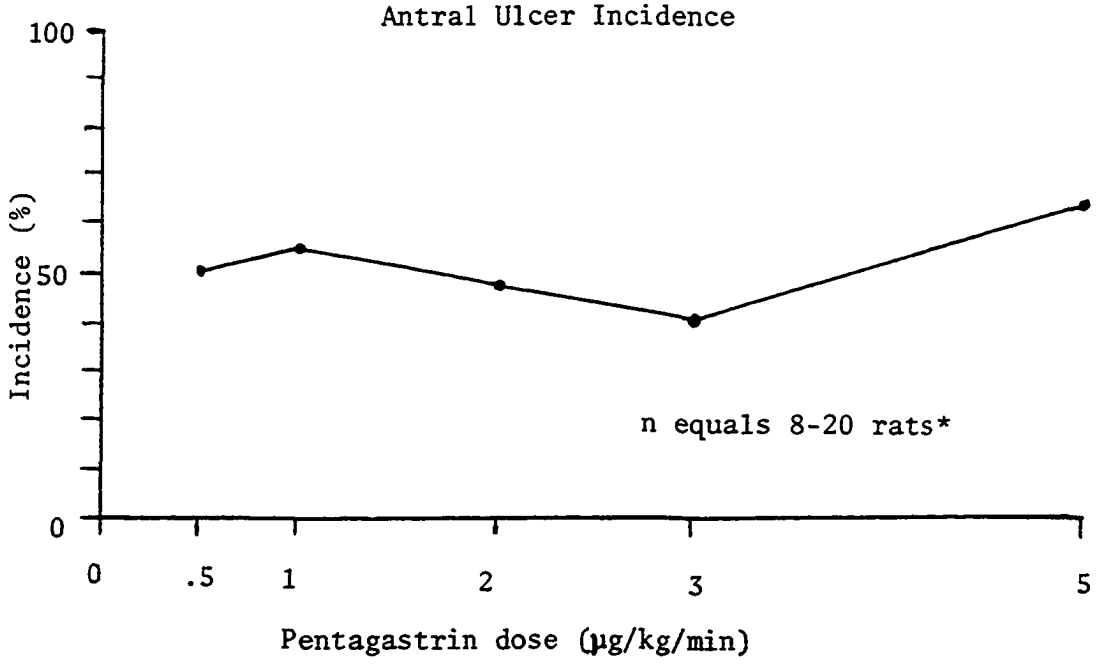


Figure 19
Antral Ulcer Incidence



* number of rats which received doses indicated

at a dose of 5.0 µg/kg/min. No duodenal ulcers developed in response to a 72 hour infusion with pentagastrin doses of 1.0 and 2.0 µg/kg/min. Because no perforated duodenal ulcers or deaths were produced in response to pentagastrin infusion, these data have not been graphed. Because no rat had more than one duodenal ulcer, the data for the average number of duodenal ulcers per rat have not been graphed. The average severity units per rat was .03 at a pentagastrin dose of 2.0 µg/kg/min and .31 at a dose of 5.0 µg/kg/min. These data have not been graphed. The average severity units per ulcer was .50 at a pentagastrin dose of 2.0 µg/kg/min and .83 at a dose of 5.0 µg/kg/min. These data have not been graphed.

Rats which received pentagastrin alone were not examined for the presence of forestomach ulcers.

Corpus ulcers were only observed in one rat which received a pentagastrin dose of 1.0 µg/kg/min for an incidence of 10%. These data have not been graphed.

Antral ulcers (Figure 19) were produced in response to pentagastrin at all doses tested. The incidence of antral ulcers ranged from 40% at a dose of 3.0 µg/kg/min to 63% at a dose of 5.0 µg/kg/min. 74% of all antral ulcers produced by pentagastrin were located in the pylorus.

Among rats which received pentagastrin alone, yellow material was observed in the duodenum and/or jejunum of 100% of rats with duodenal ulcers and 52.6% of rats without duodenal ulcers.

Histamine and pentagastrin

Histamine and pentagastrin doses administered in combination ranged from .2 to 10 µg/kg/min for pentagastrin and ranged from .5 to 1.0 mg/kg/min for histamine.

Histamine and pentagastrin administered in combination produced duodenal ulcers (Figure 20) at all doses tested. The incidence of ulceration reached a maximum of 100% at a dose of 1.0 mg/kg/min histamine and 10 µg/kg/min pentagastrin. The incidence of perforated duodenal ulcers (Figure 21) reached a maximum of 30% at a dose of 1.0 mg/kg/min histamine and 10 µg/kg/min pentagastrin. At each of the seven other dose combinations which produced duodenal ulcer perforations the incidence of perforation was 11% or less, representing a maximum of one rat per group. The incidence of mortality (Figure 22) reached a maximum of 33% at a dose of .75 mg/kg/min histamine and .5 µg/kg/min pentagastrin. Up to 100 or more ulcers of .1 mm diameter or smaller were observed in the corpus of 80% of the rats which died. The average number of duodenal ulcers per rat (Figure 23) ranged from a low of .25 to a high of 1.32. The average severity units per rat (Figure 24) ranged from a low of .25 to a high of 2.0. The average severity units per ulcer (Figure 25) ranged from a low of .83 to a high of 1.67. Both the average severity units per rat and the average severity units per ulcer reached a maximum at a dose of 1.0 mg/kg/min histamine and 10 µg/kg/min pentagastrin.

Because no forestomach ulcers were produced in response to combined doses of histamine and pentagastrin, the data have not been graphed.

Figure 20
Duodenal Ulcer Incidence

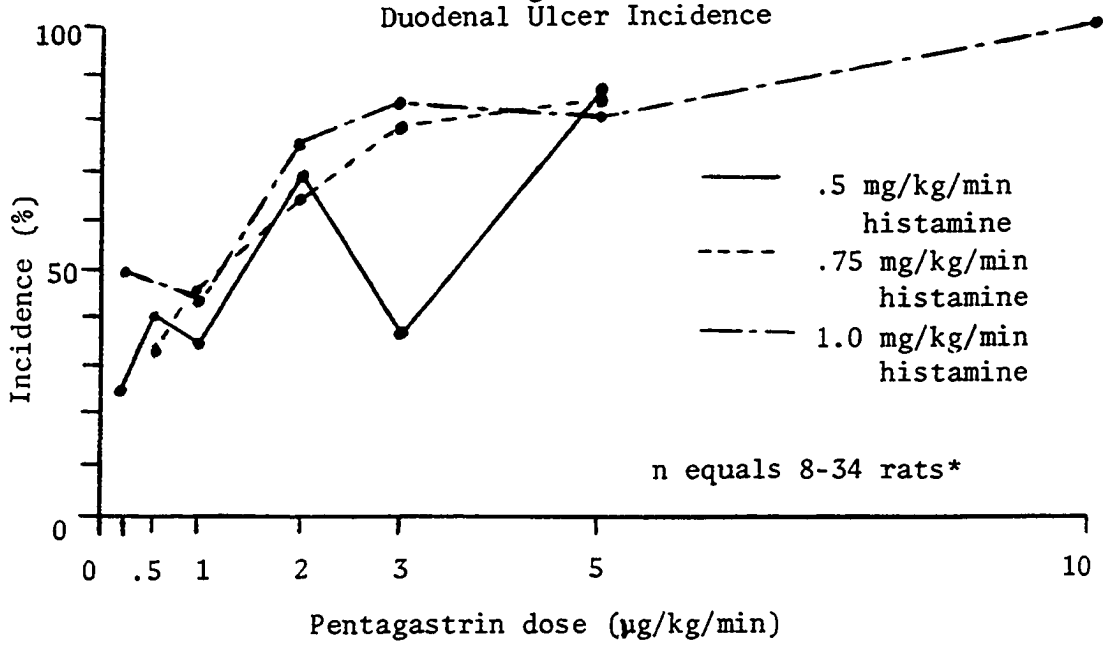
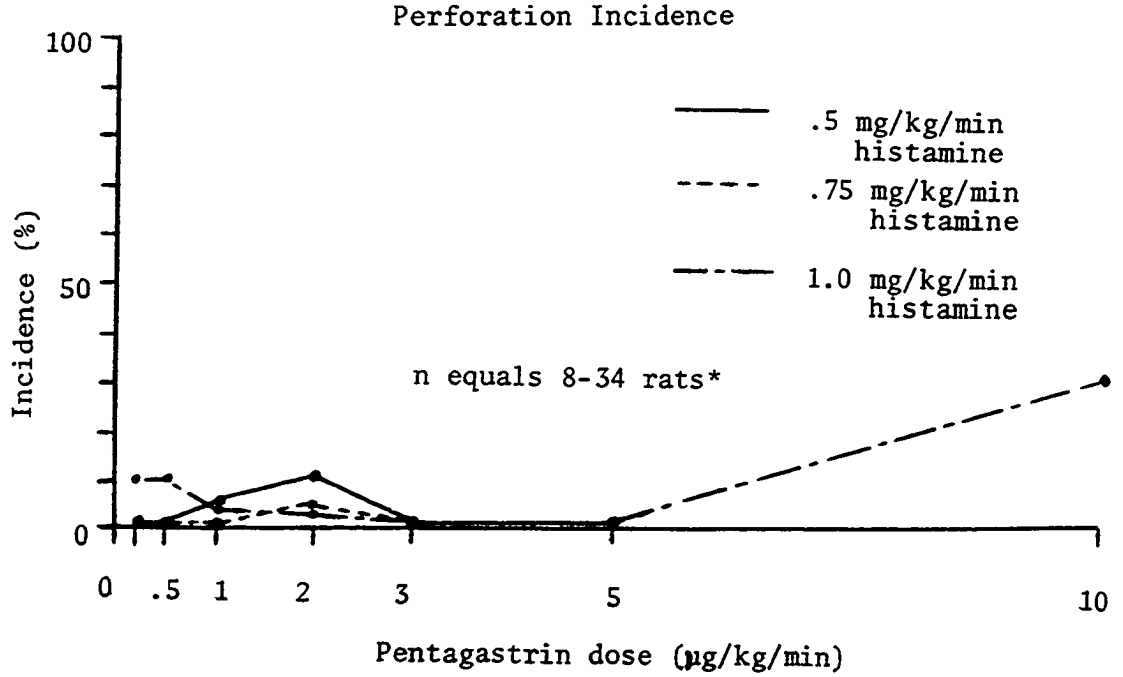


Figure 21
Perforation Incidence



* number of rats which received doses indicated

Figure 22
Mortality Incidence

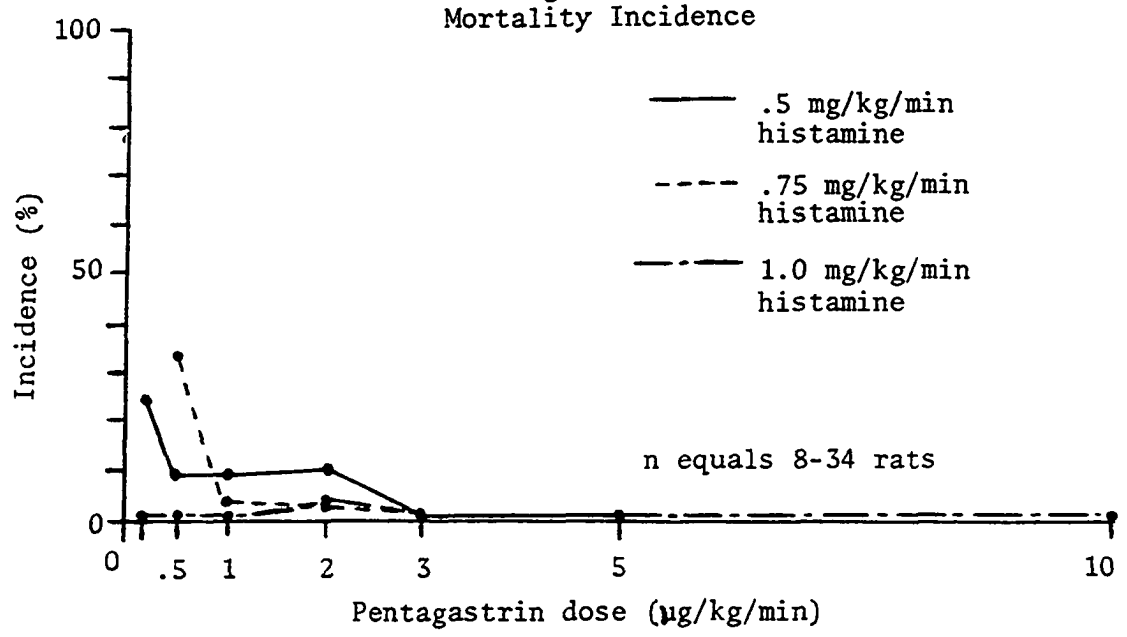


Figure 23
Duodenal Ulcers Per Rat

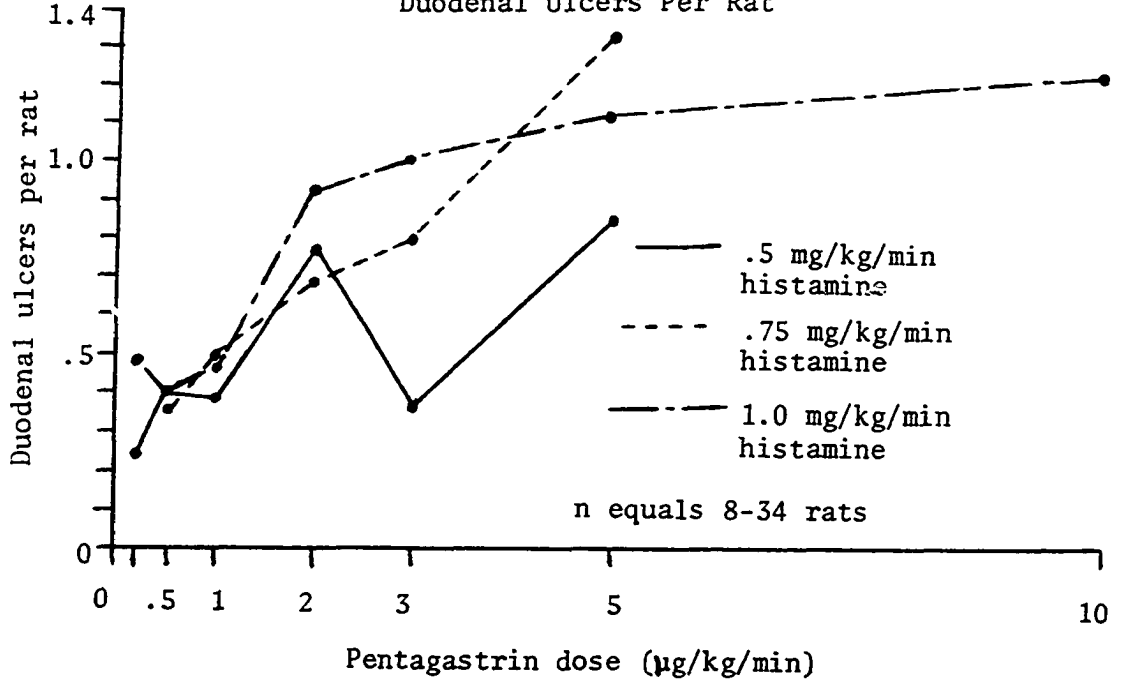


Figure 24
Severity Units Per Rat

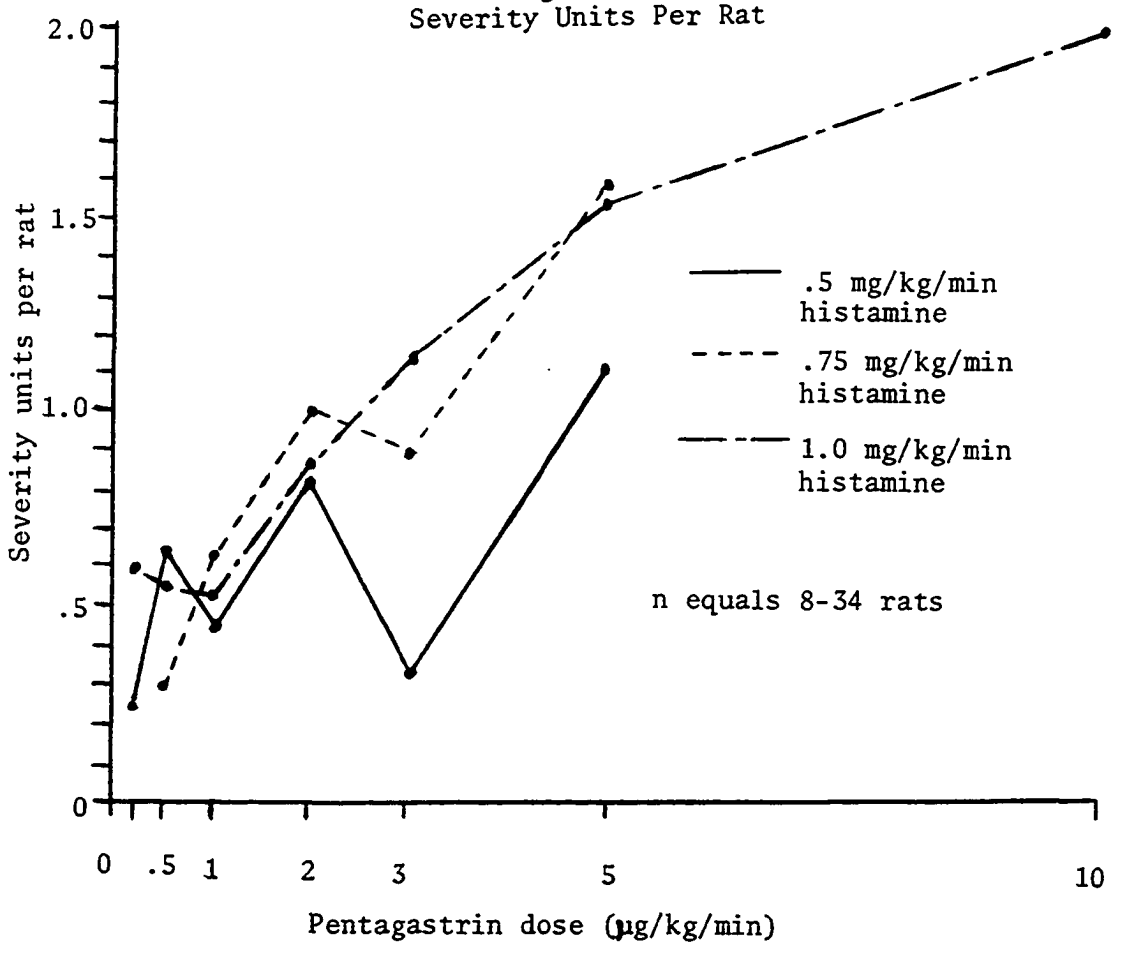


Figure 25
Severity Units Per Ulcer

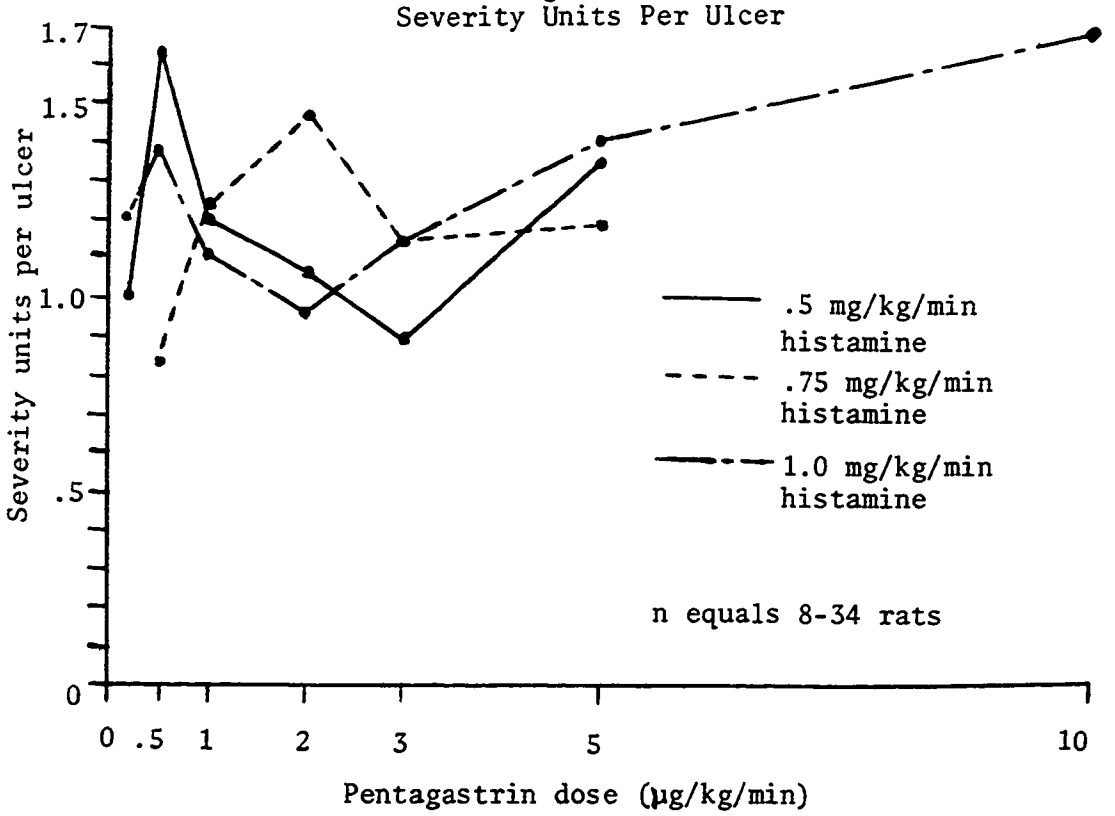


Figure 26
Corpus Ulcer Incidence

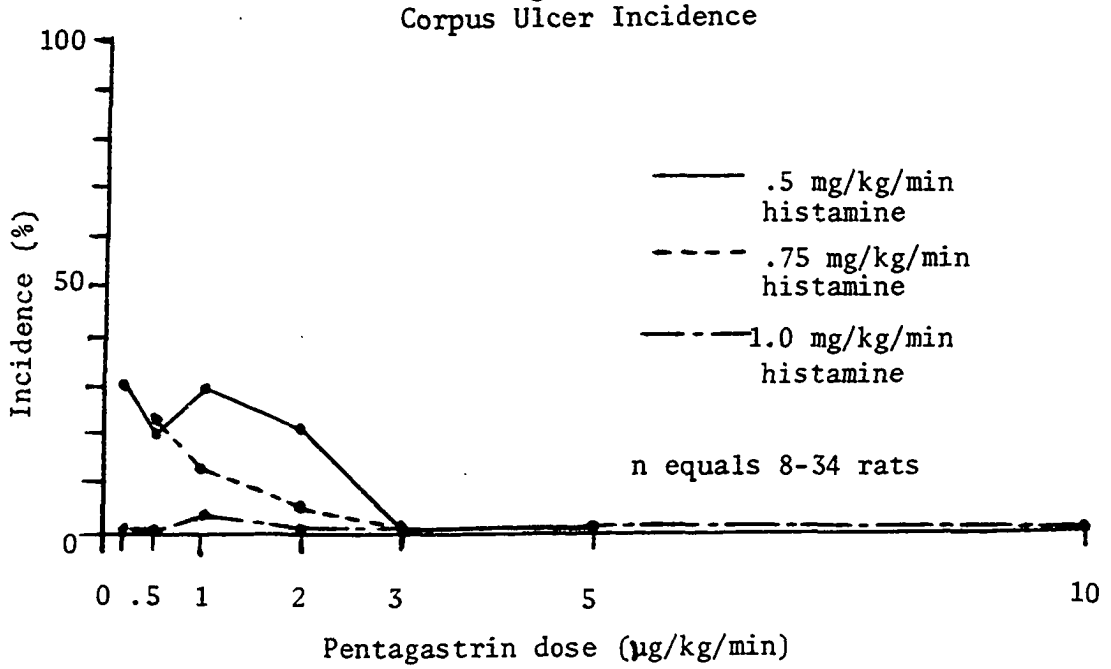
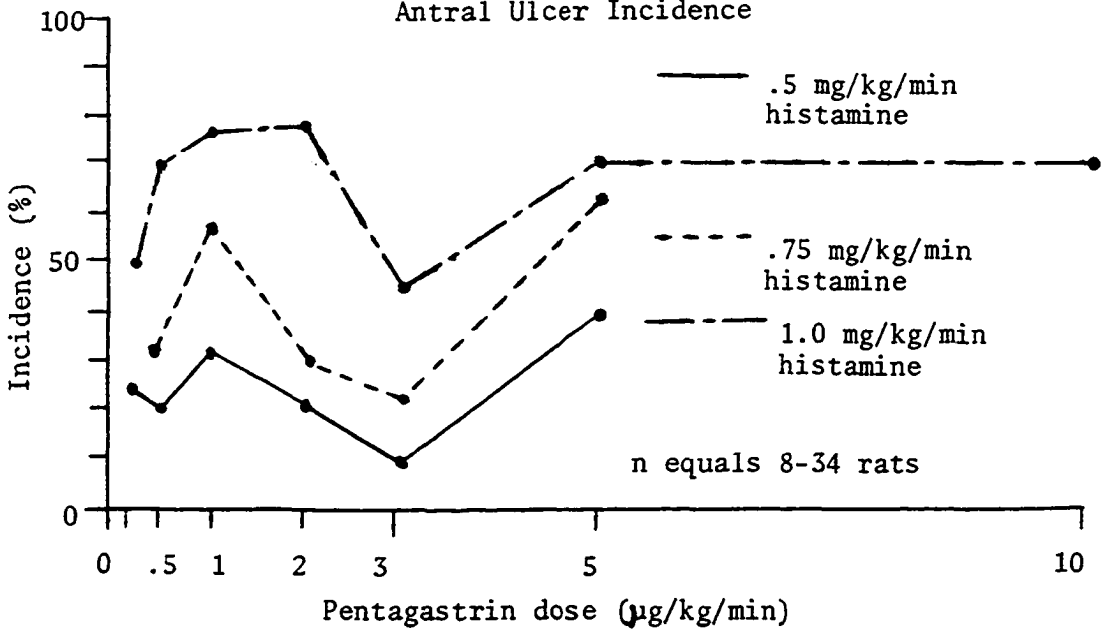


Figure 27
Antral Ulcer Incidence



Corpus ulcers (Figure 26) were produced in response to combined doses of histamine and pentagastrin at pentagastrin doses of 2.0 µg/kg/min and lower. A maximum corpus ulcer incidence of 30% was produced at a dose of .5 mg/kg/min histamine and .2 µg/kg/min pentagastrin. Death was observed in 57.1% of the rats which developed numerous (up to 100 or more) corpus ulcers.

Antral ulcers (Figure 27) were produced at all combined doses of histamine and pentagastrin. A maximum antral ulcer incidence of 78% was reached at a dose of 1.0 mg/kg/min histamine and 2.0 µg/kg/min pentagastrin.

Among rats receiving combined doses of histamine and pentagastrin, yellow material was observed in the duodenum and/or jejunum of 88.3% of rats with duodenal ulcers and 58.5% of rats without duodenal ulcers.

Histamine and carbachol

Histamine and carbachol doses administered in combination ranged from .05 to 1.0 mg/kg/min for histamine and ranged from .1 to 1.0 µg/kg/min for carbachol. Histamine and carbachol administered in combination produced duodenal ulcers (Figure 28) at all doses tested. A duodenal ulcer incidence of 100% was produced in response to four dose combinations. Perforated duodenal ulcers (Figure 29) were produced in response to a dose of 1.0 mg/kg/min histamine and .2 µg/kg/min carbachol and in response to all combined doses containing .3 µg/kg/min or more carbachol. A maximum incidence of duodenal ulcer perforation of 100% was produced in response to two dose combinations. A maximum incidence of mortality (Figure 30) of 83% was produced in response to a dose of 1.0 mg/kg/min histamine and 1.0 µg/kg/min carbachol. For each of the

Figure 28
Duodenal Ulcer Incidence

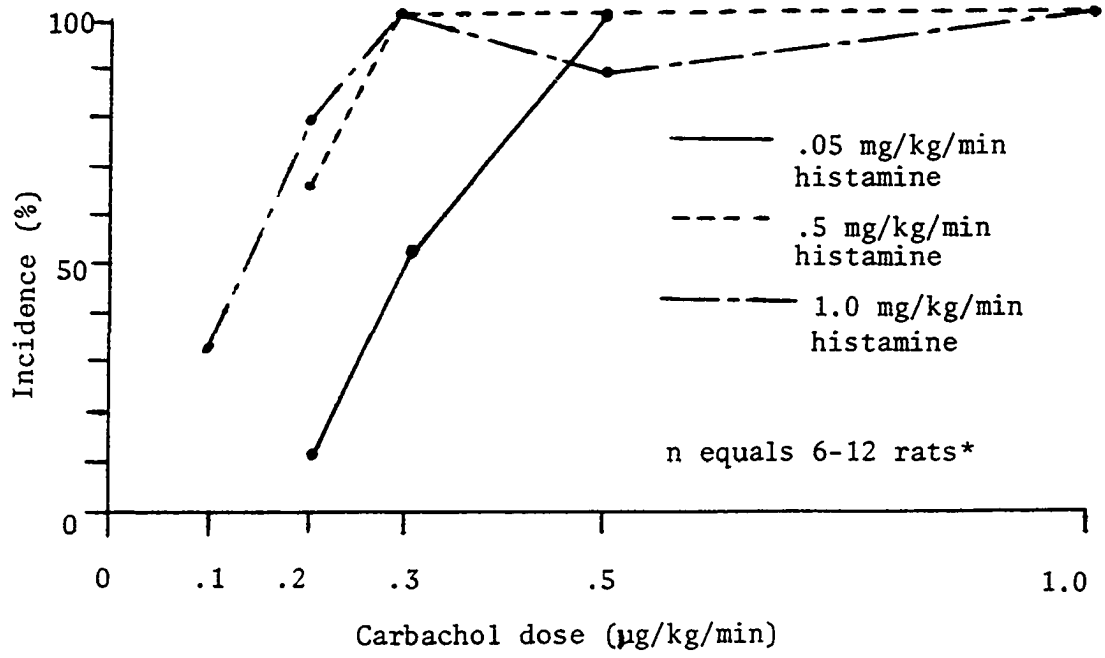
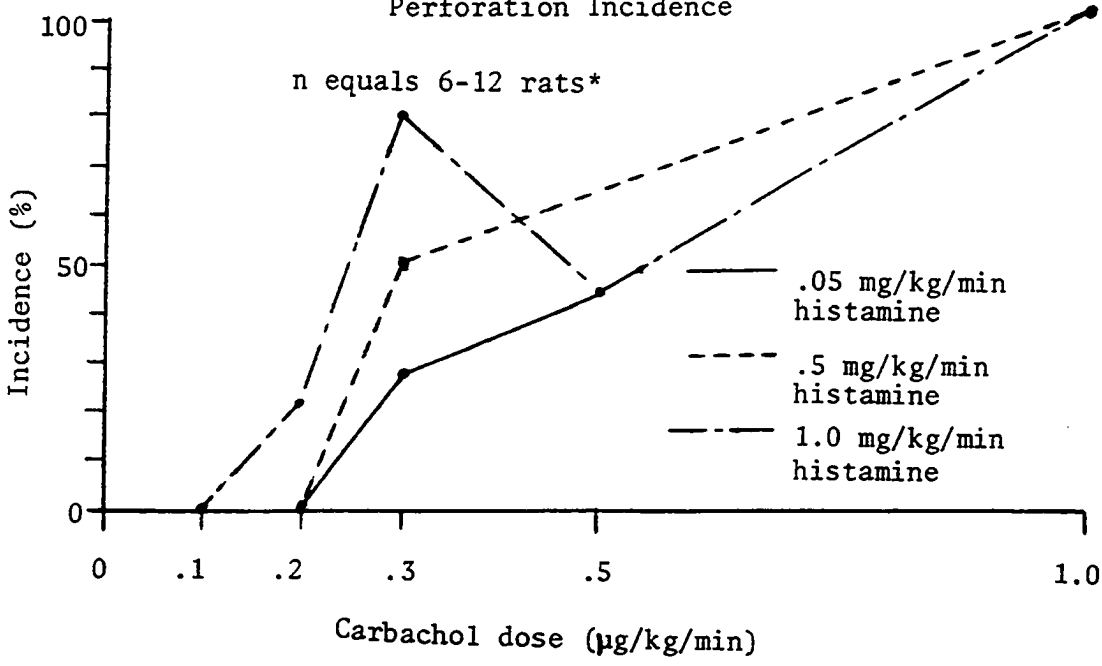


Figure 29
Perforation Incidence



* number of rats which received doses indicated

Figure 30
Mortality Incidence

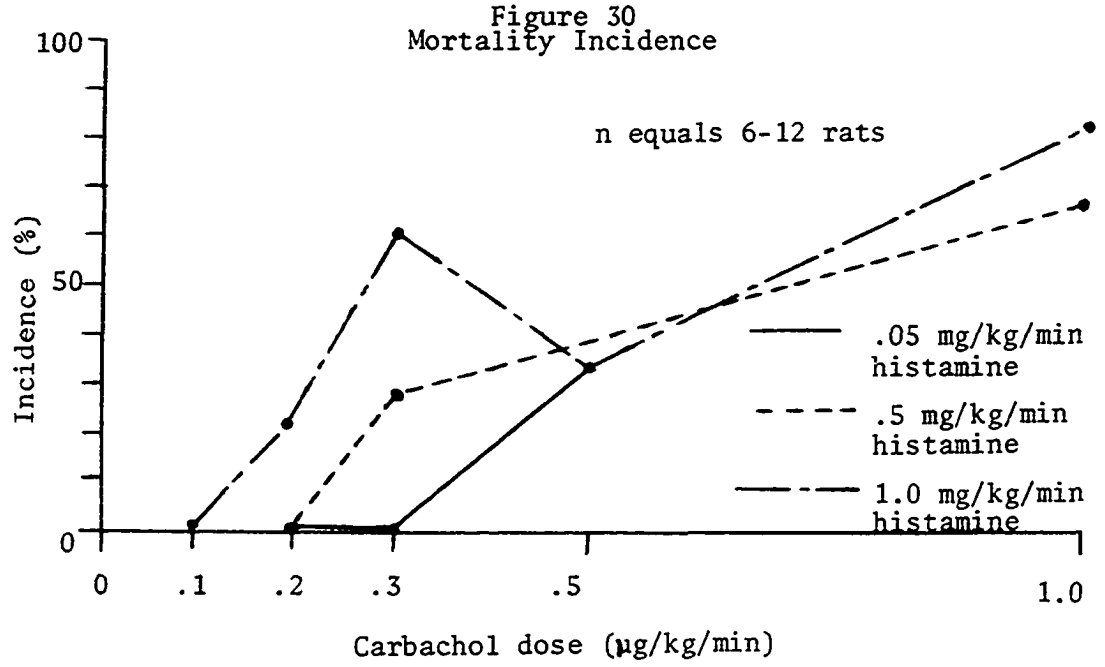


Figure 31
Duodenal Ulcers Per Rat

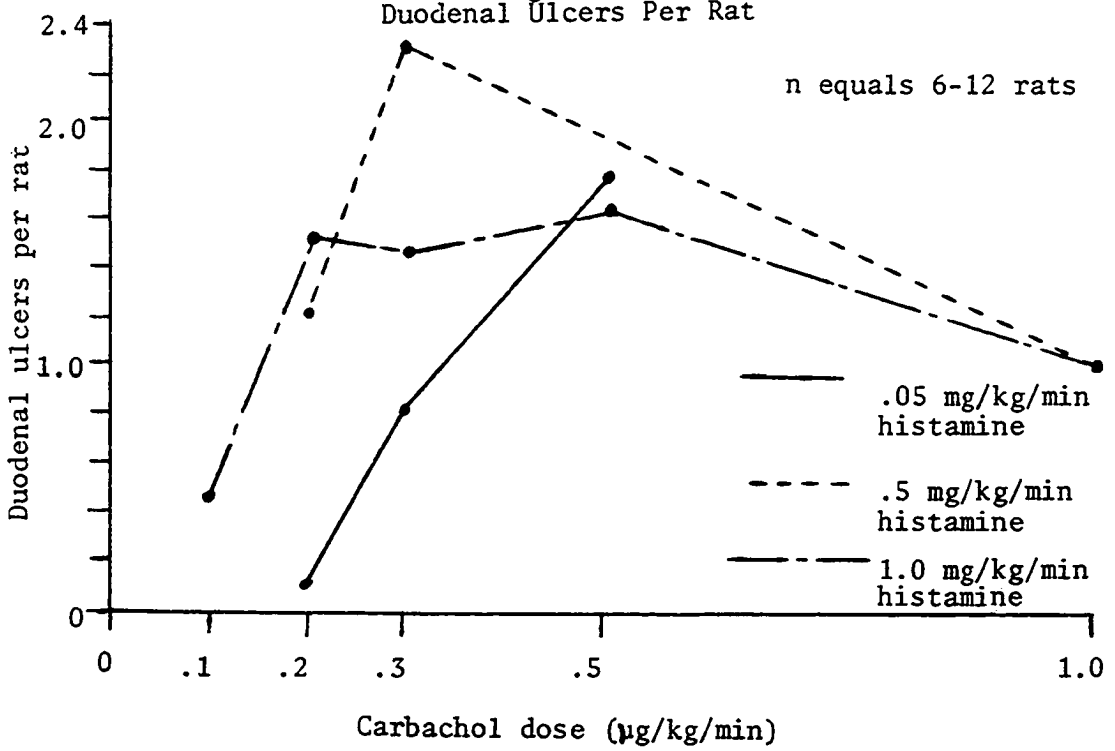


Figure 32
Severity Units Per Rat

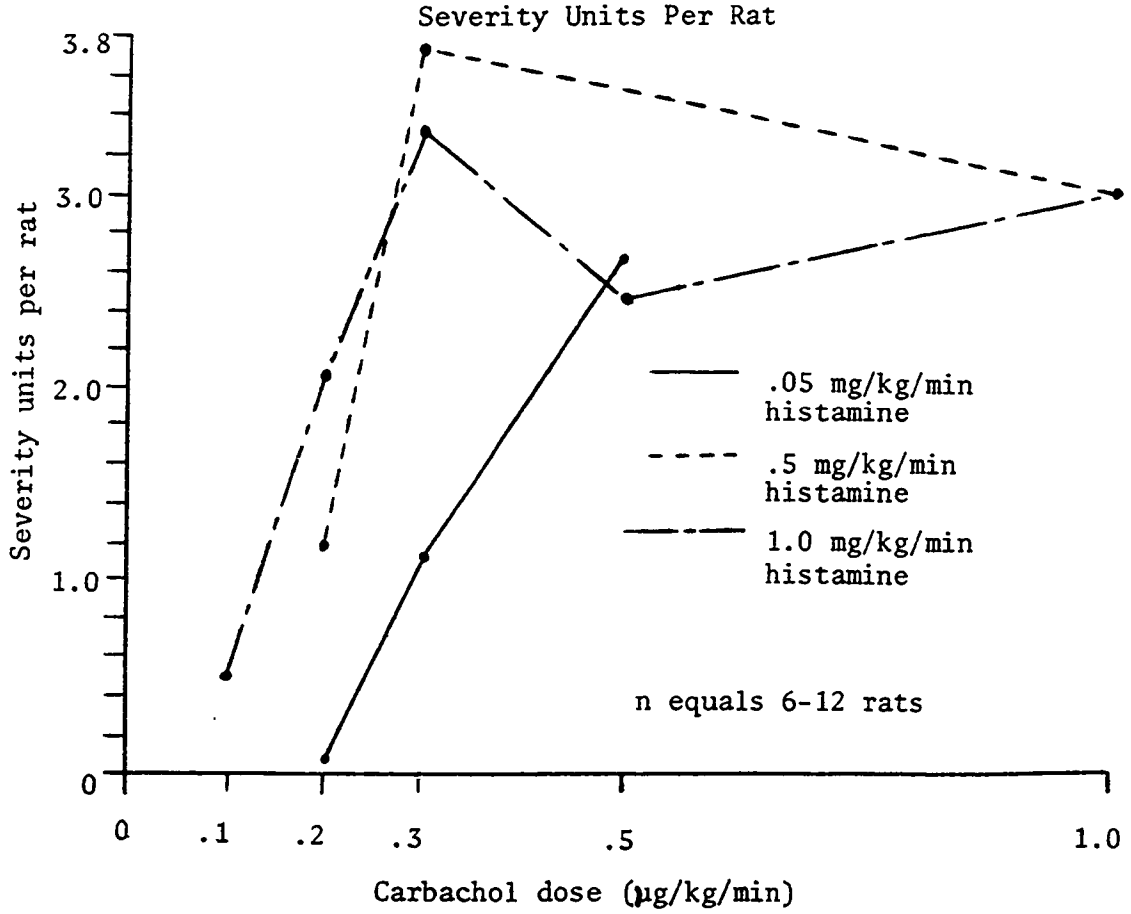


Figure 33
Severity Units Per Ulcer

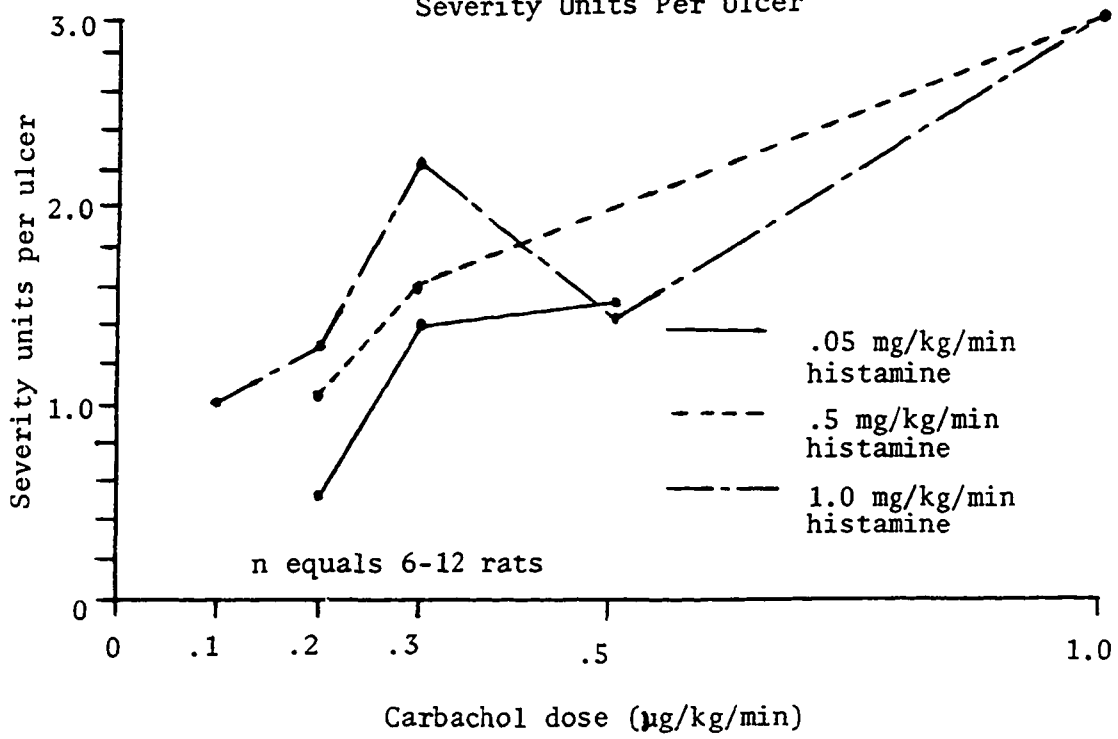


Figure 34
Corpus Ulcer Incidence

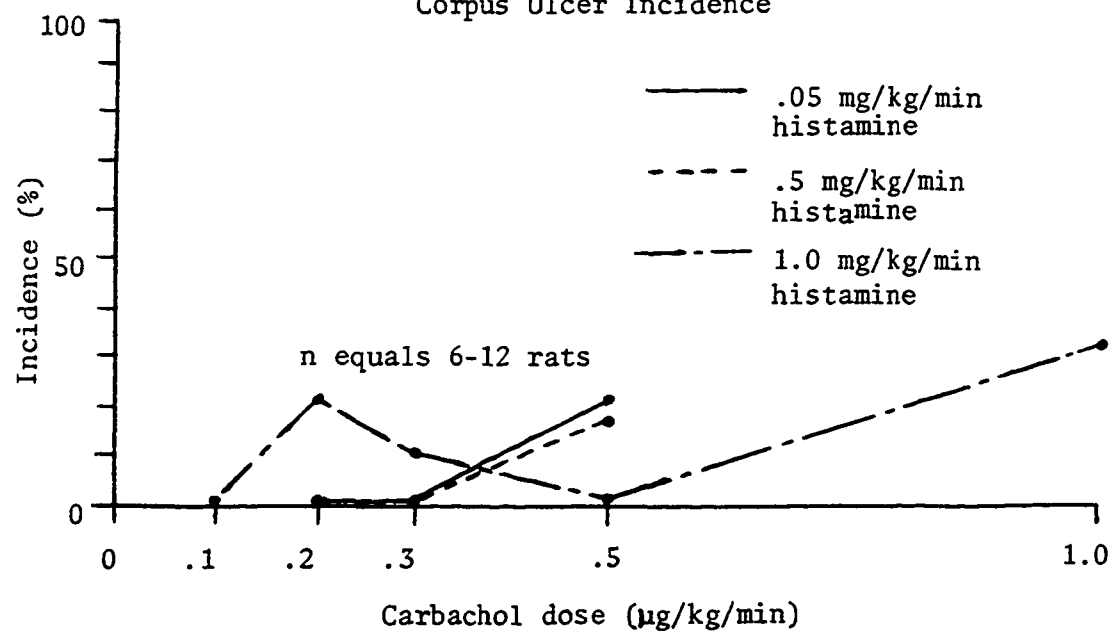
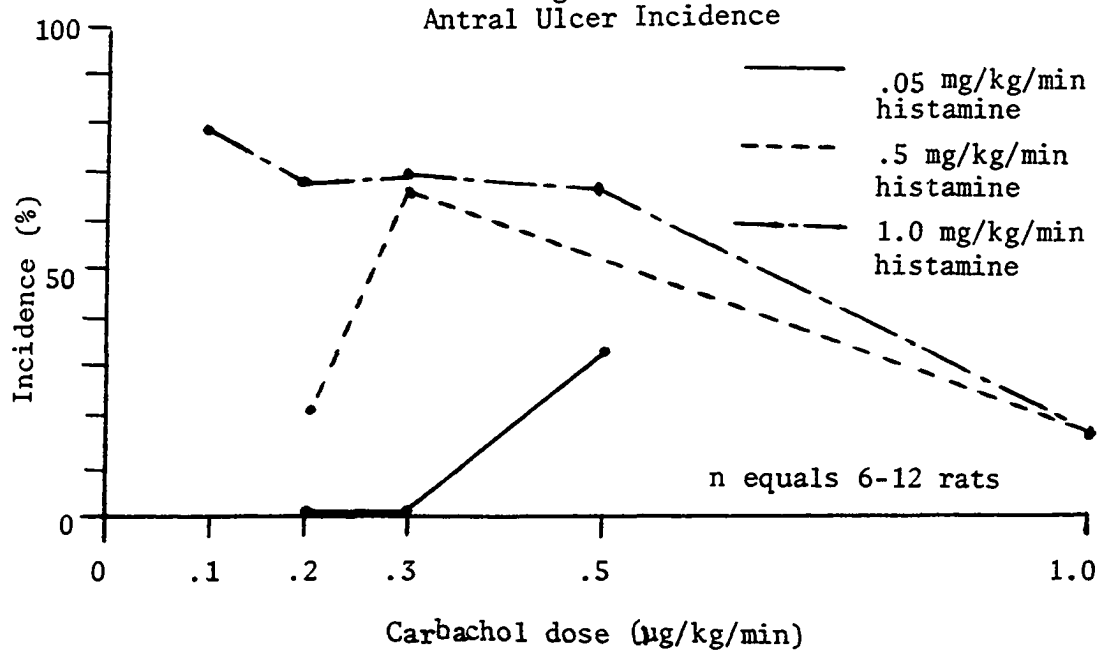


Figure 35
Antral Ulcer Incidence



three histamine doses administered in combination with carbachol the maximum average number of duodenal ulcers per rat (Figure 31) exceeded 1.6. However, at the highest dose of carbachol (1.0 µg/kg/min) administered in combination with histamine the average number of duodenal ulcers per rat was 1.0. At this dose each rat had one duodenal ulcer. The maximum average number of duodenal ulcers per rat was 2.33 at a dose of .5 mg/kg/min histamine and .3 µg/kg/min carbachol. The average severity units per rat (Figure 32) reached a maximum of 2.75 at a combined dose of .5 mg/kg/min histamine and .3 µg/kg/min carbachol. At the highest carbachol dose (1.0 µg/kg/min) administered in combination with histamine, the average severity units per rat was 3.0. The average severity units per ulcer (Figure 33) reached a maximum of 3.0 (each ulcer perforated) in response to a carbachol dose of 1.0 µg/kg/min administered in combination with histamine.

Forestomach ulcers were produced only at a dose of .05 mg/kg/min histamine and .2 µg/kg/min carbachol (33% incidence and were not graphed.)

The maximum corpus ulcer incidence (Figure 34) of 33% was produced in response to a dose of 1.0 mg/kg/min histamine and 1.0 µg/kg/min carbachol.

Antral ulcers (Figure 35) were produced in response to a dose of .05 mg/kg/min histamine and .5 µg/kg/min carbachol and in response to histamine doses of .5 and 1.0 mg/kg/min administered in combination with carbachol. Antral ulcer incidence reached a maximum of 78% in response to a dose of 1.0 mg/kg/min histamine and .1 µg/kg/min carbachol.

Among rats receiving combined doses of histamine and carbachol, yellow material was observed in the duodenum and/or jejunum of 17.7% of rats with duodenal ulcers and 6.2% of rats without duodenal ulcers.

Discussion

Histamine alone did not produce the desired duodenal ulcer incidence of 100%. The reduction of duodenal ulcer incidence at the highest histamine dose is probably related to the high incidence of mortality produced at that dose. The mortality of rats receiving the highest histamine dose did not appear to be due to any ulcer perforation or excessive hemorrhage. Therefore, the reduction of histamine-induced ulcer incidence may be due to death of the rats resulting from a pharmacological effect of histamine not associated with ulcer production. The incidences of forestomach, corpus and antral ulcers produced by histamine alone did not appear to be affected by the incidence of duodenal ulcers. Histamine alone produced a maximum incidence of: 100% for corpus and antral ulcers, 95% for mortality, 73% for duodenal ulcers, 14% for duodenal ulcer perforation and 10% for forestomach ulcers.

Carbachol alone did not produce the desired duodenal ulcer incidence of 100%. Duodenal ulcer incidence, the average number of duodenal ulcers per rat, the average severity units per rat, and the average severity units per ulcer all show a similar increase, peak, and decrease in value in response to increased carbachol doses. The reduction of duodenal ulcer incidence with carbachol is probably due to two effects of high doses on the action of cholinergic agents: increased mucus secretion (Robert, Nezamis and Phillips, 1965), and decreased acid secretion (Pevsner and Grossman, 1955). Cholinergic agents also increase gastrointestinal motility (Grollman, 1962) and the resultant relatively rapid flow of gastric juice through the duodenum may account for decreased ulcer incidence. With carbachol alone, the decrease in

duodenal ulcer incidence at high doses makes the relationship between stomach and duodenal ulcer incidences unclear. Forestomach ulcer incidence decreased in the presence of duodenal ulcers while antral ulcer incidence was apparently unaffected and corpus ulcer incidence followed a pattern similar to that of duodenal ulcer incidence except that the peak and decline of corpus ulcer incidence occurred at higher doses of carbachol than the peak and decline of duodenal ulcer incidence. Carbachol alone produces a maximum incidence of: 89% for forestomach ulcers, 70% for duodenal ulcers, 39% for corpus and antral ulcers, 20% for duodenal ulcer perforation and 10% for mortality.

Pentagastrin alone did not produce the desired duodenal ulcer incidence of 100%. Pentagastrin alone was not tested at high doses and it is therefore unknown whether or not pentagastrin alone is capable of producing a high incidence of duodenal ulceration. The incidences of corpus and antral ulcers did not appear to be affected by the incidence of duodenal ulcers. Pentagastrin alone produced a maximum incidence of: 63% for antral ulcers, 38% for duodenal ulcers and 10% for corpus ulcers. (No duodenal perforations or mortality were produced and the forestomach was not observed for ulcers.) Pentagastrin produced a greater incidence of yellow material in the duodenum and/or jejunum than any other experimental agent or combination of agents.

Histamine and pentagastrin administered in combination produced the desired duodenal ulcer incidence of 100%. For every combined dose of histamine and pentagastrin, the incidences of duodenal ulceration and duodenal perforation were greater than the additive effects of the agents used singly at the same doses which were administered in combination. Such effect indicates synergism. Increases in duodenal ulcer

incidence, average number of duodenal ulcers per rat and average severity units per rat in the presence of relatively steady average severity units per ulcer indicate that increased combined doses of histamine and pentagastrin produced greater numbers of duodenal ulcers rather than increased severity of existing ulcers. The incidence of antral ulcers did not appear to be affected by the incidence of duodenal ulcers. The incidence of corpus ulcers tended to decrease in the presence of an increased incidence of duodenal ulcers. Histamine and pentagastrin administered in combination produced a maximum incidence of: 100% for duodenal ulcers, 78% for antral ulcers, 33% for mortality, 30% for duodenal ulcer perforation and 30% for corpus ulcers. (No forestomach ulcers were produced.) The reasons are unknown for decreased incidences of corpus ulcers and mortality in response to increased combined doses of histamine and pentagastrin. Further, the extent (if any) of interrelationship between corpus ulcer incidence and mortality incidence with histamine and pentagastrin is unknown.

Histamine and carbachol administered in combination produced the desired duodenal ulcer incidence of 100%. For every combined dose of histamine and carbachol the incidences of duodenal ulceration, duodenal ulcer perforation, and mortality were greater than the additive effects of the agents used singly at the same doses which were administered in combination. Such effect indicates synergism. The reduction in the average number of duodenal ulcers per rat at higher combined doses can be explained by the formation of one duodenal ulcer which rapidly perforated, often killing the animal early during the experiment. At lower doses several duodenal ulcers formed in some animals, one of which may have eventually perforated, thereby resulting in a high average

number of duodenal ulcers per rat, and a high result for the average severity units per rat. The increase in average severity units per ulcer, and the decrease in the average number of ulcers per duodenum demonstrate this tendency toward formation of a small number of very severe ulcers in every animal at higher combined doses. The relationship between the incidence of perforation and the incidence of mortality suggests that death resulted from perforation and peritonitis (as shown by the presence of fluid in the abdomen) at least for combinations of histamine and carbachol. With histamine and carbachol in combination the incidence of antral ulcers decreased in the presence of high incidences of duodenal ulcers, and more specifically, in the presence of high incidences of duodenal perforations. Histamine and carbachol administered in combination produced a maximum incidence of: 100% for duodenal ulcers and duodenal ulcer perforation, 83% for mortality, 78% for antral ulcers, and 33% for forestomach and corpus ulcers. The reasons for the decrease in antral ulcer incidence in response to increased combined doses of histamine and carbachol are unknown.

By far the most efficient production of duodenal ulcers was achieved with combined doses of histamine and carbachol. The doses used in combination of both histamine and carbachol were low when compared to the doses of either compound used alone to produce ulcers. These very low doses were often sufficient to produce not only ulcers in every animal examined, but in some cases perforated ulcers in every animal and a high incidence of deaths.

The combination of histamine and pentagastrin was also efficient, as compared to the effects of these agents administered singly. However, the incidences of ulceration, perforation and death were consid-

erably lower and slower to develop than was true with histamine and carbachol.

None of the agents administered singly produced an incidence of duodenal ulceration greater than seventy-five percent. Pentagastrin alone was not tested at high doses, carbachol alone showed a reduction of duodenal incidence after a maximum incidence of seventy percent, and histamine alone showed a decrease in incidence coupled with high mortality after a maximum incidence of seventy-three percent.

Among the agents administered alone, carbachol showed the greatest potency in terms of dose. On a weight basis, carbachol was somewhat more active than pentagastrin, and both were far more active than histamine. In combinations, carbachol again was more active than pentagastrin in producing an increase in duodenal ulcers (at any given histamine dose), while on a weight basis, histamine was again least active. On a molar basis however, pentagastrin was more active than carbachol and both were far more active than histamine, whether administered singly or in combination.

Stomach ulcers (forestomach, corpus and antral ulcers), although of subordinate interest, are also significant, particularly in the relation of their incidence to duodenal ulcer incidence. The reasons for the relation between stomach and duodenal ulcer incidences are unknown. Decreases in stomach ulcer incidences in the presence of high incidences of severe duodenal ulcers may be due to some tissue reaction to the formation of a severe ulcer or the effects of high doses.

In all cases of duodenal ulceration there seemed to be a specific sequence to the appearance of the associated changes. First to appear even before ulceration, was duodenal dilatation, and this persisted

through most of the following stages. With relatively mild duodenal ulcers a transverse red line was evident in the outer wall of the duodenum. With more severe ulcers, this red line disappeared or was concealed by the appearance of the ulcer through the wall. Perforated ulcers sometimes showed without requiring the removal of the duodenum, but more frequently their existence was revealed by the presence of fluid in the abdomen and erosion of adjacent organs. The final change associated with duodenal ulcers was death. Except in the case of histamine alone, death was always apparently due to a perforated duodenal (or rarely antral) ulcer.

CHAPTER III

EFFECT OF PENTAGASTRIN ON GASTRIC SECRETION

Introduction: Pentagastrin as the Pharmacological Agent

The purpose of this portion of the investigation was to study the mechanism of action of pentagastrin in the production of duodenal ulcers. Prior to ulcer production experiments with the other pharmacological agents, experiments with pentagastrin indicated that it was a potentially active agent in the production of duodenal ulcers. Therefore, at that time, pentagastrin was chosen as the pharmacological agent for gastric secretion study. The mechanism of action of duodenal ulcer production with pentagastrin was probably through a modification of duodenal content and/or direct modification of the duodenal tissue (Gobbel and Adkins, 1967). Direct tissue modification was not studied.

Gastric secretion was the component of the duodenal content which was studied in this investigation. Gastric secretion was studied because acid and pepsin are produced in the stomach, (Glass, 1968) and because gastrin did not stimulate Brunner's glands (Cooke and Grossman, 1965), but does increase secretions from the duodenal mucosa (and exocrine pancreas) as measured by bicarbonate output (Roberts, Schulke and Winship, 1968). Therefore, the mechanism of action of pentagastrin in altering duodenal content may have been through modification of gastric secretion.

The effect of pentagastrin on several components of gastric secretion has been previously reported. Pentagastrin has been observed

to stimulate secretion of acid, pepsin, and total gastric juice volume (Gregory and Tracy, 1964). With gastrin, the pepsin response has been noted to be relatively slow to develop. Over a four hour period no pepsin response was observed (Schoenfeld, Siplet and Komarov, 1966), but by six hours a weak pepsin response was present (Lee and Thompson, 1968). Further, pentagastrin has also been observed to produce increased gastric mucosal blood flow in dogs (Swan and jacobson, 1967). No studies of the effect of pentagastrin on mucus components of gastric secretion have been reported. Since histamine, carbachol and pentagastrin were all stimulators of gastric secretion, the information gained regarding pentagastrin might relate to the mechanism(s) of action of the other experimental agents.

Methods

Infusion technique

The procedure for infusion used to determine the effect of pentagastrin on gastric secretion consisted of a four hour subcutaneous administration of either saline or a given dose of pentagastrin at a flow rate of 1.08 mg/hr to chronic fistula rats. The fistula rats had stainless steel cannulas implanted in the forestomach using a modified procedure of Komarov, Bralow and Boyd (Komarov, Bralow and Boyd, 1963).

The same group of sixteen fistula rats was used throughout the investigation, and therefore each rat served as its own control. Pentagastrin administration was alternated with saline administration throughout the experimental series, thereby assuring continued control information.

The rats were fasted twenty-four hours prior to administration of saline or pentagastrin, and at least three days elapsed between experiments to allow for recovery from the effects of fasting, treatment, and fluid and electrolyte loss. Just prior to infusion the rats were weighed and the average fasted weight was the basis for calculations of pentagastrin dose.

The infusion and collection period lasted four hours, during which gastric juice was collected continually via the cannulas. The basic experimental series consisted of first, establishing a maximum four hour secretion level, and second, decreasing the doses of pentagastrin until its effect on volume was negligible as compared with control values.

In another series of experiments the effect of prolonged infusion was studied. These three experiments involved the same fistula rats as the basic experimental series, but involved an infusion period of twenty-four hours with a rate of infusion of .54 ml/hr, followed by the four hour infusion and collection period. This series of experiments involved the use of saline and a pentagastrin dose of 2 µg/kg/min. The three experiments used to determine the effect of prolonged infusion were: 24 hours of saline infusion followed by four hours of saline infusion (during collection), 24 hours of saline infusion followed by four hours of pentagastrin infusion, and 24 hours of pentagastrin infusion followed by four hours of pentagastrin infusion.

Variables studied

The gastric juice samples for each rat were processed separately, and volumes were recorded both hourly and for the entire four hour infusion period. Samples from the total four hour production for each

rat were used in all chemical determinations. Determinations were made of both concentration and total output for acid; pepsin; and three mucus components; hexosamine, sialic acid, and fucose. At least one-half milliliter of gastric juice was used for every chemical determination.

Acidity was measured by titration of gastric juice with .01 N sodium hydroxide to pH 7, using a Fisher pH meter with a glass electrode. Pepsin was determined by a modified method of Anson (Anson, 1938). Hexosamine was determined by a modified method of Boas (Boas, 1953). Sialic acid was determined by a modified method of Warren (Warren, 1959). Fucose was determined by a modified method of Dische and Shettles (Dische and Shettles, 1948).

Results

Introduction

Saline was administered to the fistula rats in nine successive experiments. During this time, the average weight of the fed rats increased 13%, from 257 to 290 grams. Because no effect of weight increase on four hour gastric juice volume was evident with saline infusion, the effect of weight increase on other components of gastric secretion was disregarded. Therefore, results from all nine saline administrations have been pooled, and the average value for each component is indicated on its graph.

Hourly volume results are given in Table 1 of the appendix and in graphs. Four-hour collection results from the basic experimental series are given in Table 2 of the appendix and in graphs. The results

for four-hour collection following twenty-four hours of infusion are given in Table 3 of the appendix only.

Hourly volumes

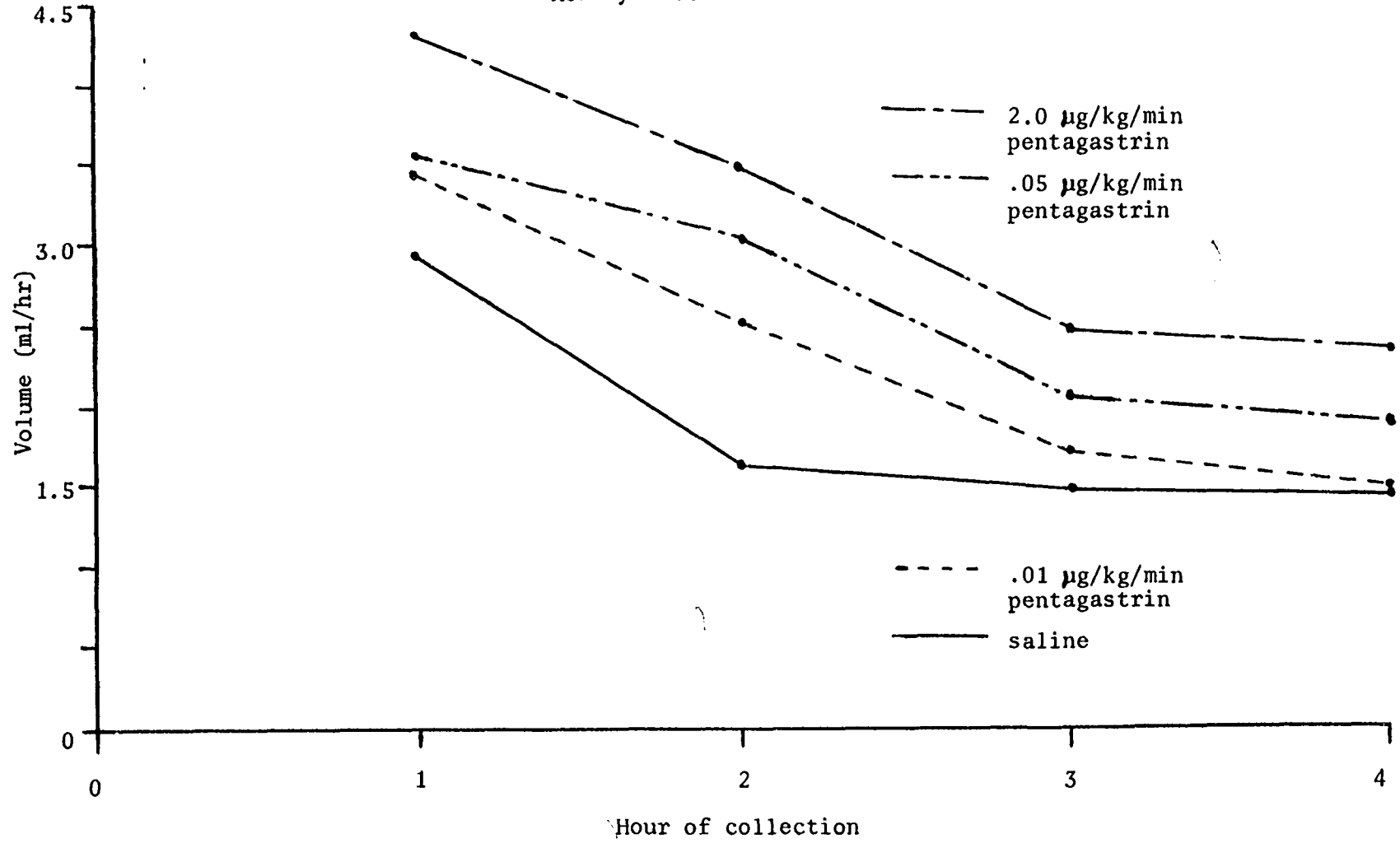
The greatest hourly output of gastric juice in response to saline (Figure 36) came during the first hour of collection. The gastric juice volumes leveled off during the second, third and fourth hours of collection.

The three pentagastrin doses shown in figure 36 produced progressively greater total volumes of gastric juice. Furthermore, at each hour of collection, increased pentagastrin doses produced increased hourly volumes. For every pentagastrin dose, the hourly volume of gastric juice was greater than the average control value for that hour. For every pentagastrin dose, the leveling off of hourly volume did not occur until at least the third hour. For every pentagastrin dose, the greatest hourly volume was produced during the first hour of collection.

Four-hour collection

The average volume of gastric juice secreted during the four-hour saline infusions was 7.5 ml (Figure 37). Acid concentration averaged 94.6 mEq/l (Figure 38) while acid output averaged .714 mEq/4 hours (Figure 39). Pepsin concentration averaged 31.8 mEq tyrosine/ml/min (Figure 40) while pepsin output averaged 237 mEq tyrosine per 4 hours (Figure 41). Hexosamine concentration averaged .11 mg/ml (Figure 42) while hexosamine output averaged .80 mg/4 hours (Figure 43). Sialic acid concentration averaged .22 μM (Figure 44) while sialic acid output averaged 1.7 μmoles/4 hours (Figure 45). Fucose concentration

Figure 36
Hourly Gastric Juice Volumes



averaged .075 µg/ml (Figure 46) while fucose output averaged .53 µg/4 hours (Figure 47).

Increased pentagastrin doses produced increased average volumes of gastric juice (Figure 37) collected during four-hour infusions. The average volume of gastric juice leveled off at pentagastrin doses of .15 µg/kg/min and higher. For every pentagastrin dose, the average gastric juice volume was greater than the average control value.

Increased average acid concentrations (Figure 38) were produced in response to increased pentagastrin doses. The acid concentration at a dose of .005 µg/kg/min was less than the average control value, but the acid concentrations at higher doses were greater than the average control value. The average acid output (Figure 39) increased in response to increased pentagastrin doses. For every pentagastrin dose, the average acid output was greater than the average control value.

The average pepsin concentration (Figure 40) generally decreased in response to increased pentagastrin doses. All values for pepsin concentration obtained during pentagastrin infusion were less than the average control value. The average pepsin output (Figure 41) showed inconsistent variation in response to increased pentagastrin doses. The average pepsin output varied both above and below the average control value.

The average hexosamine concentration (Figure 42) generally decreased in response to increased pentagastrin doses. Hexosamine concentration values for all pentagastrin doses except .005 µg/kg/min were less than the average control value. The average hexosamine output (Figure 43) showed inconsistent variation in response to increased

Figure 37
Four Hour Volume

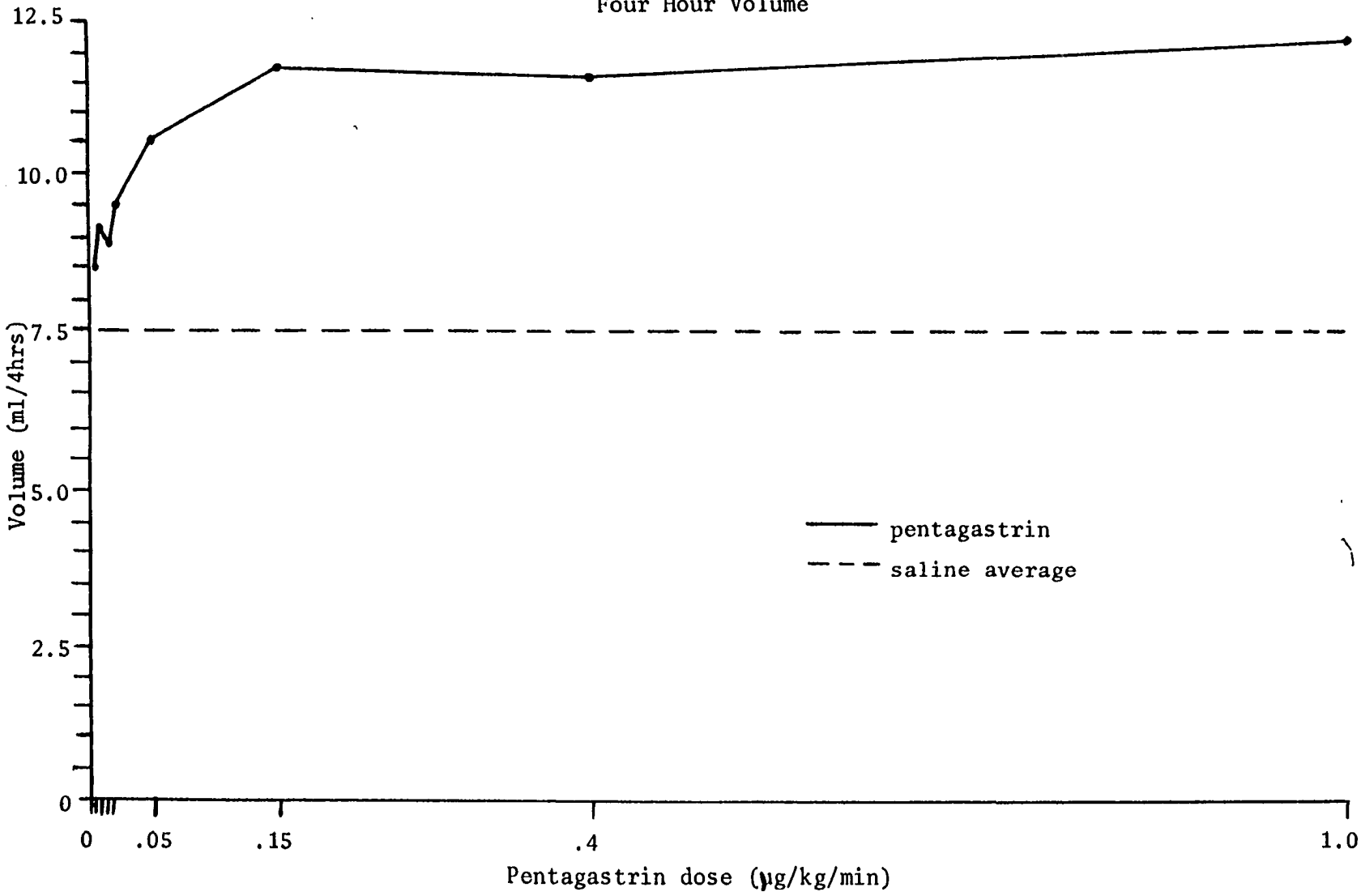


Figure 38
Acid Concentration

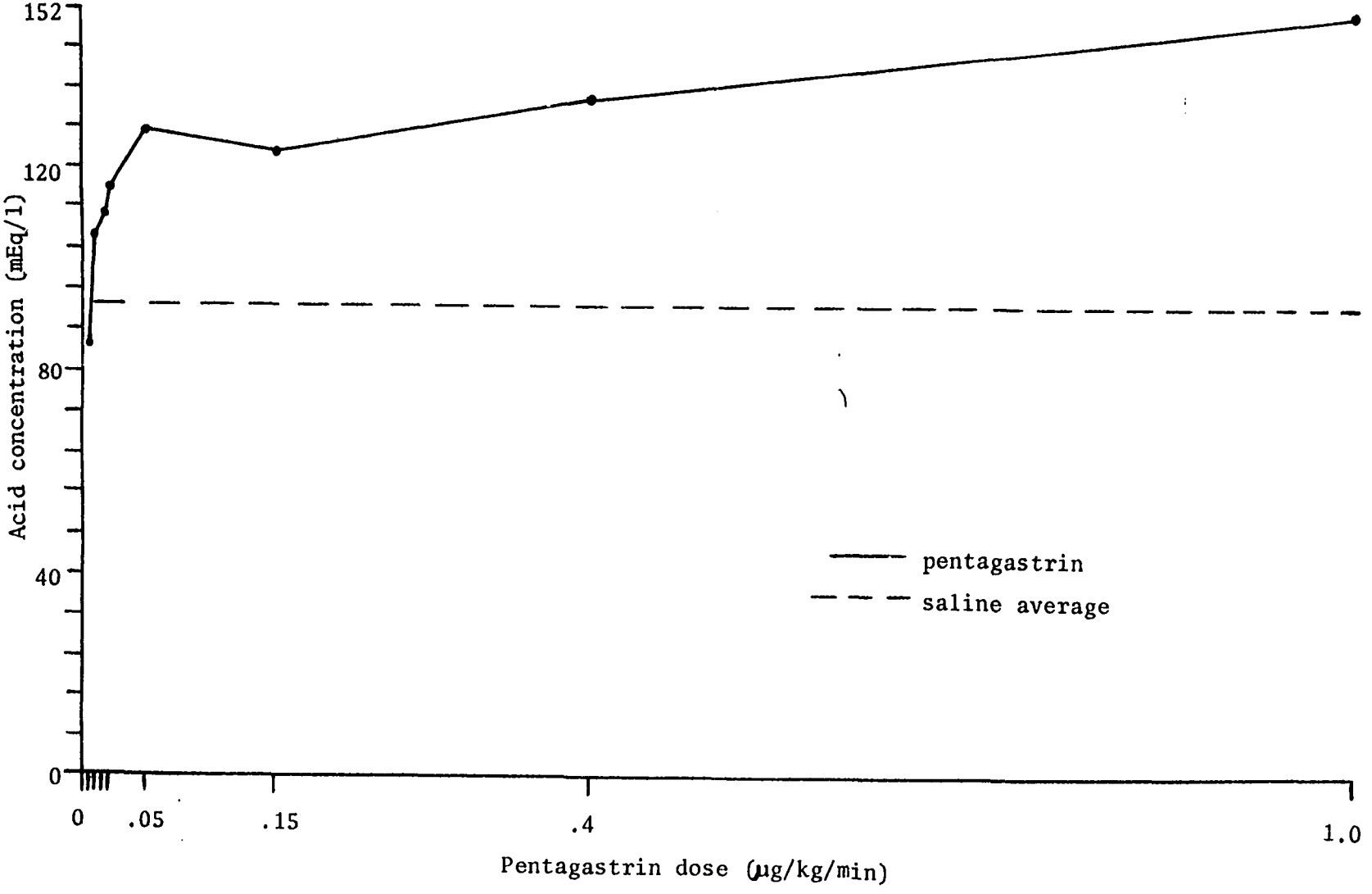


Figure 39
Acid Output

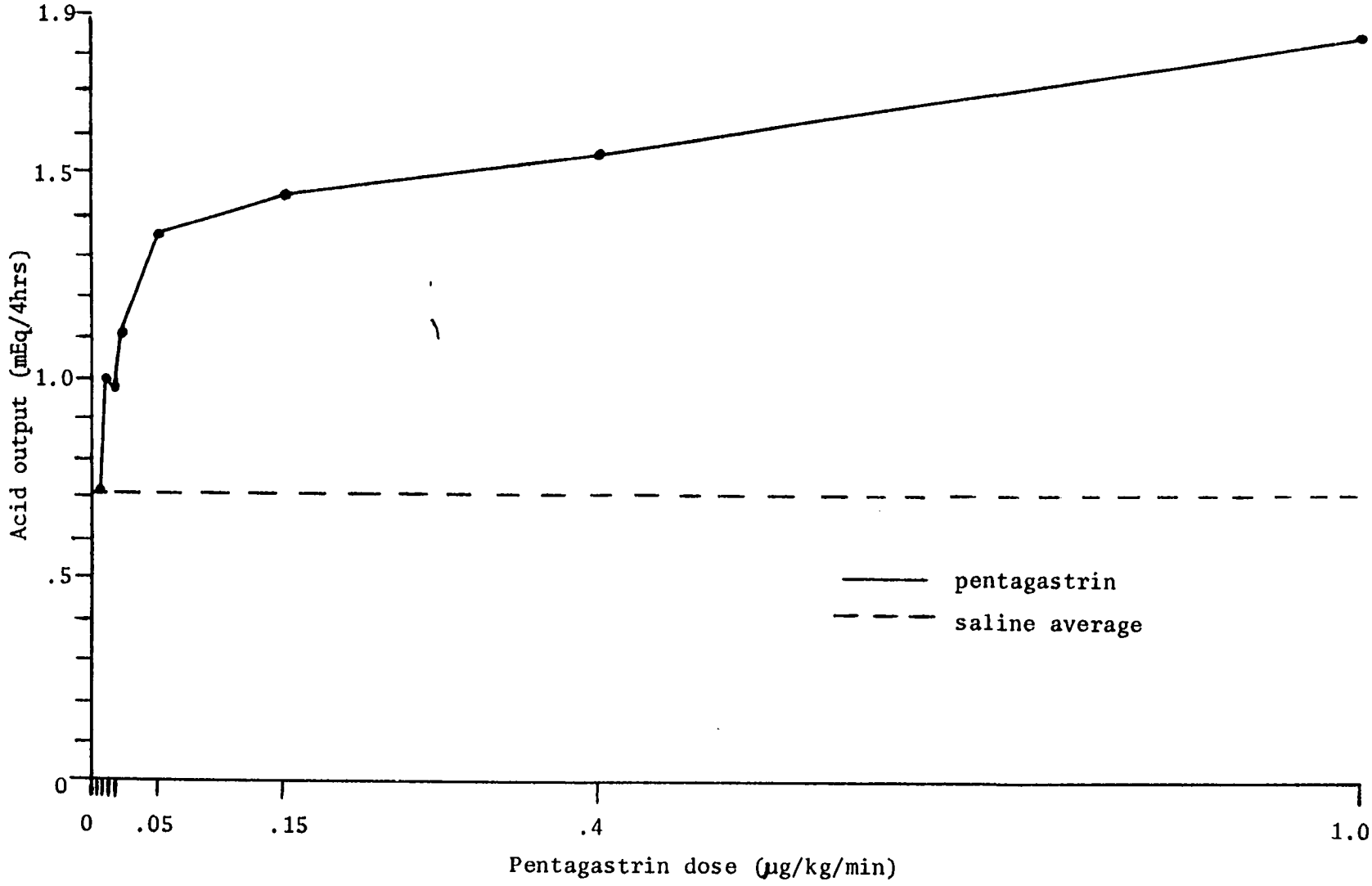
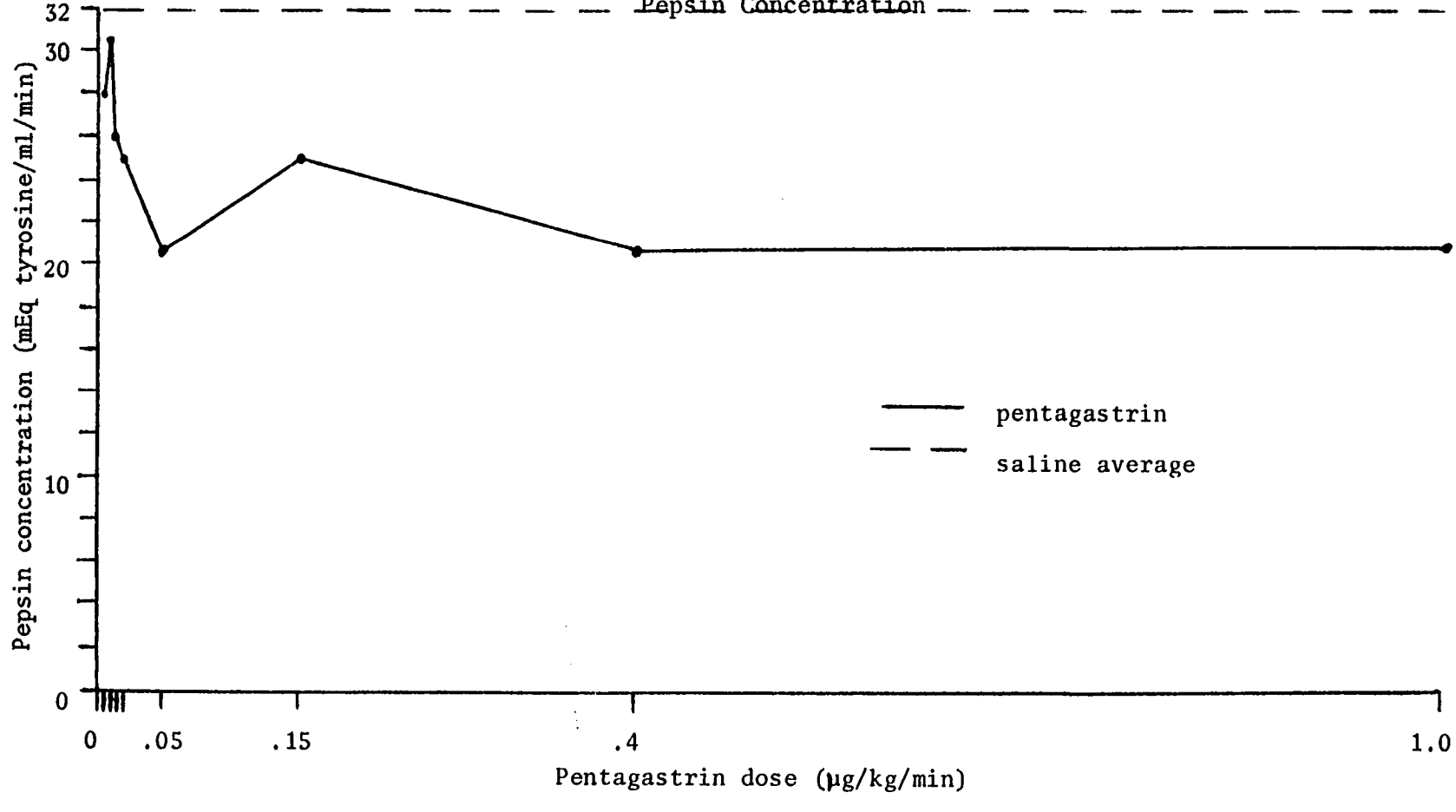


Figure 40
Pepsin Concentration



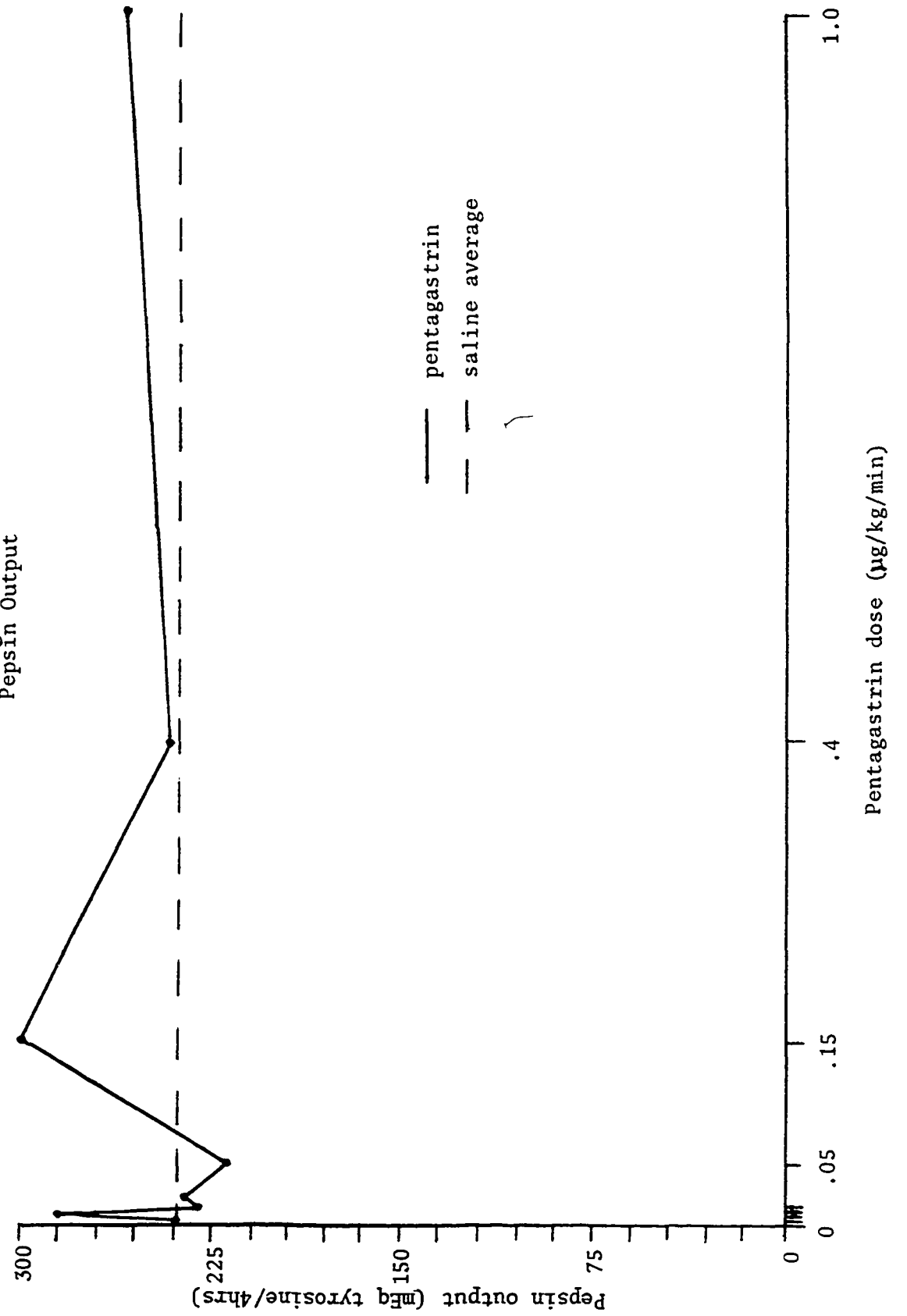


Figure 41
Pepsin Output

Figure 42
Hexosamine Concentration

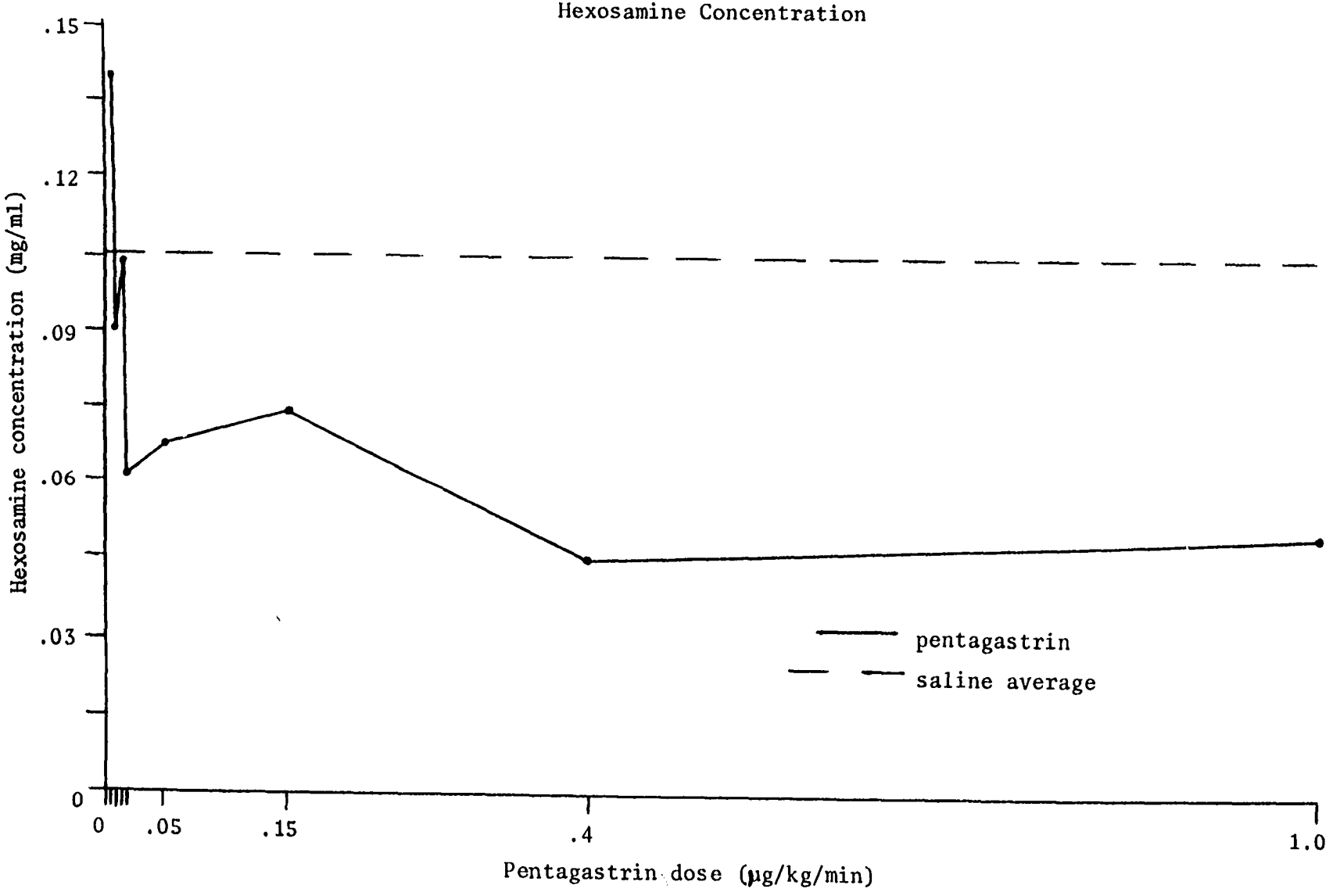
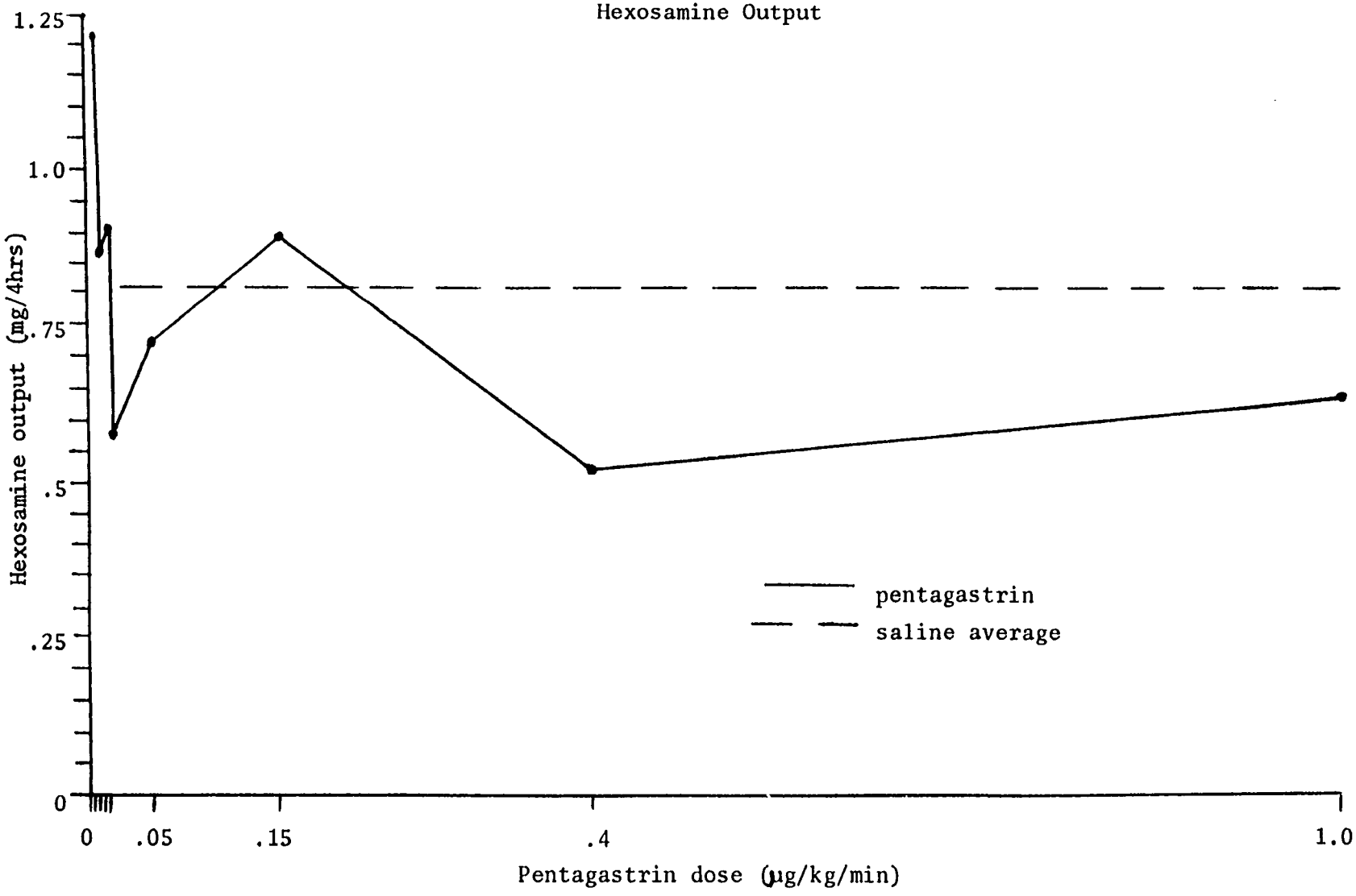


Figure 43
Hexosamine Output



pentagastrin doses. The average hexosamine output varied both above and below the average control value.

Increased pentagastrin doses produced decreased sialic acid concentrations (Figure 44). Sialic acid concentrations for all pentagastrin doses except .005 µg/kg/min were lower than the average control value. The average sialic acid output (Figure 45) showed inconsistent variation in response to increased pentagastrin doses. The average sialic acid output varied both above and below the average control value.

The average fucose concentration (Figure 46) showed inconsistent variation in response to increased pentagastrin doses. Except for a dose of .4 µg/kg/min, all pentagastrin doses produced fucose concentrations less than the average control value. The average fucose output (Figure 47) showed inconsistent variation in response to increased pentagastrin doses. All pentagastrin doses except .4 µg/kg/min produced fucose output less than the average control value.

Four-hour collection after 24-hour infusion

Twenty-four hours of pentagastrin infusion followed by another four hours of pentagastrin infusion (during collection) produced the greatest acid concentration and fucose output, and the least pepsin output, hexosamine output and sialic acid output. The greater gastric juice volume and acid output were produced by 24 hours of saline infusion followed by four hours of pentagastrin infusion. The least volume and acid output, and the greatest pepsin output, hexosamine output, and sialic acid output were produced by 24 hours of saline infusion followed by another four hours of saline infusion.

Figure 44
Sialic Acid Concentration

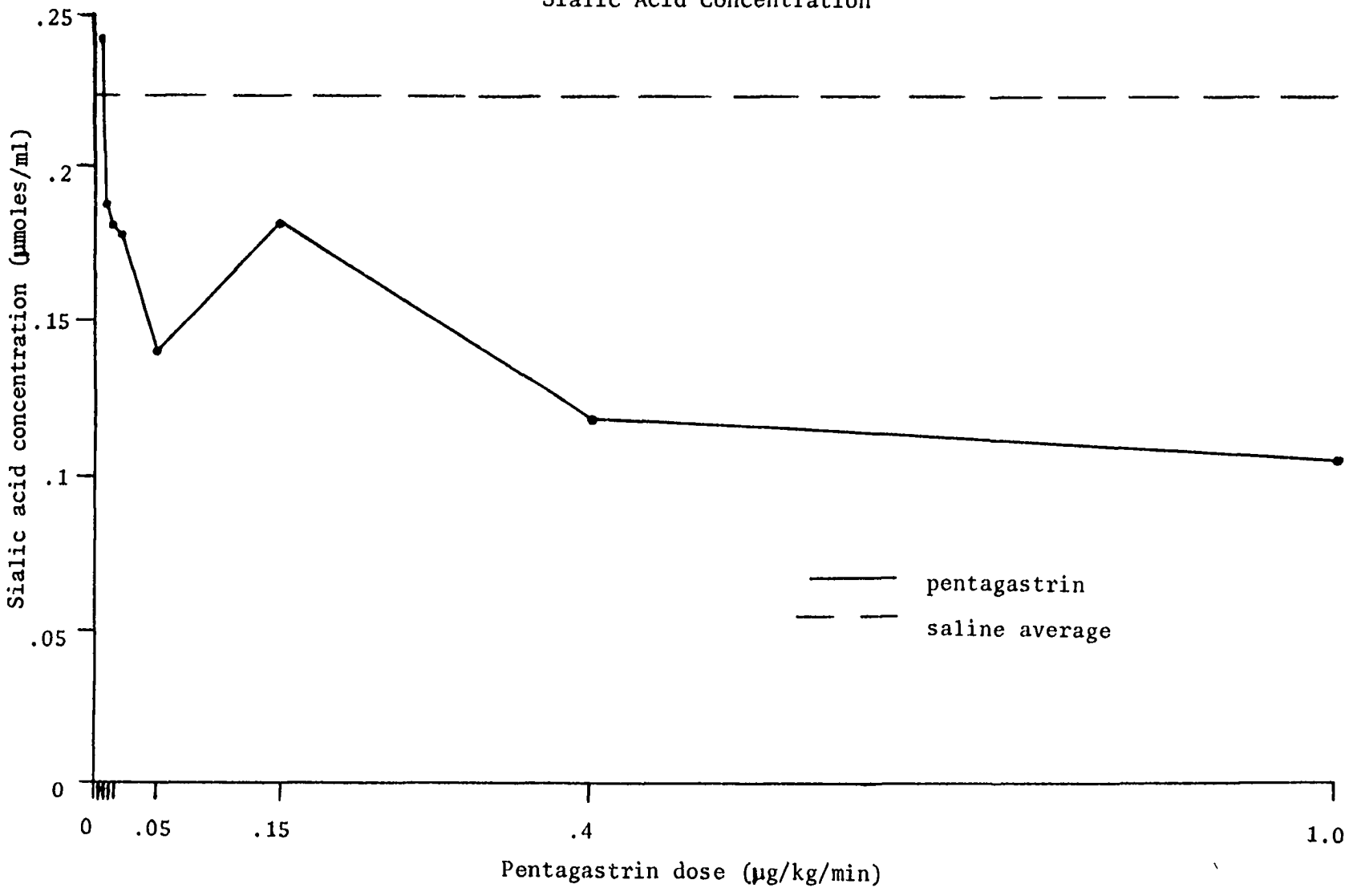


Figure 45
Sialic Acid Output

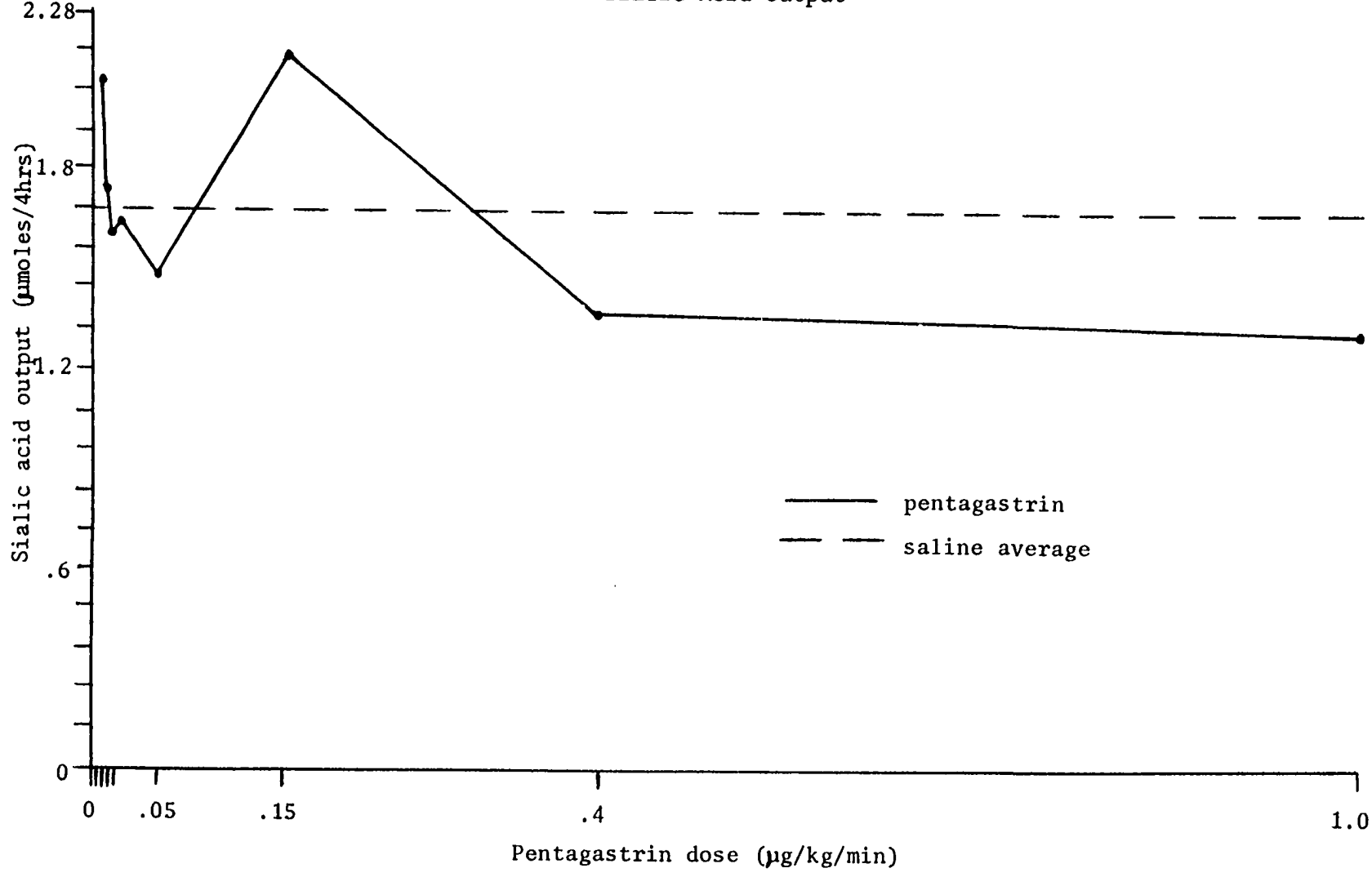


Figure 46
Fucose Concentration

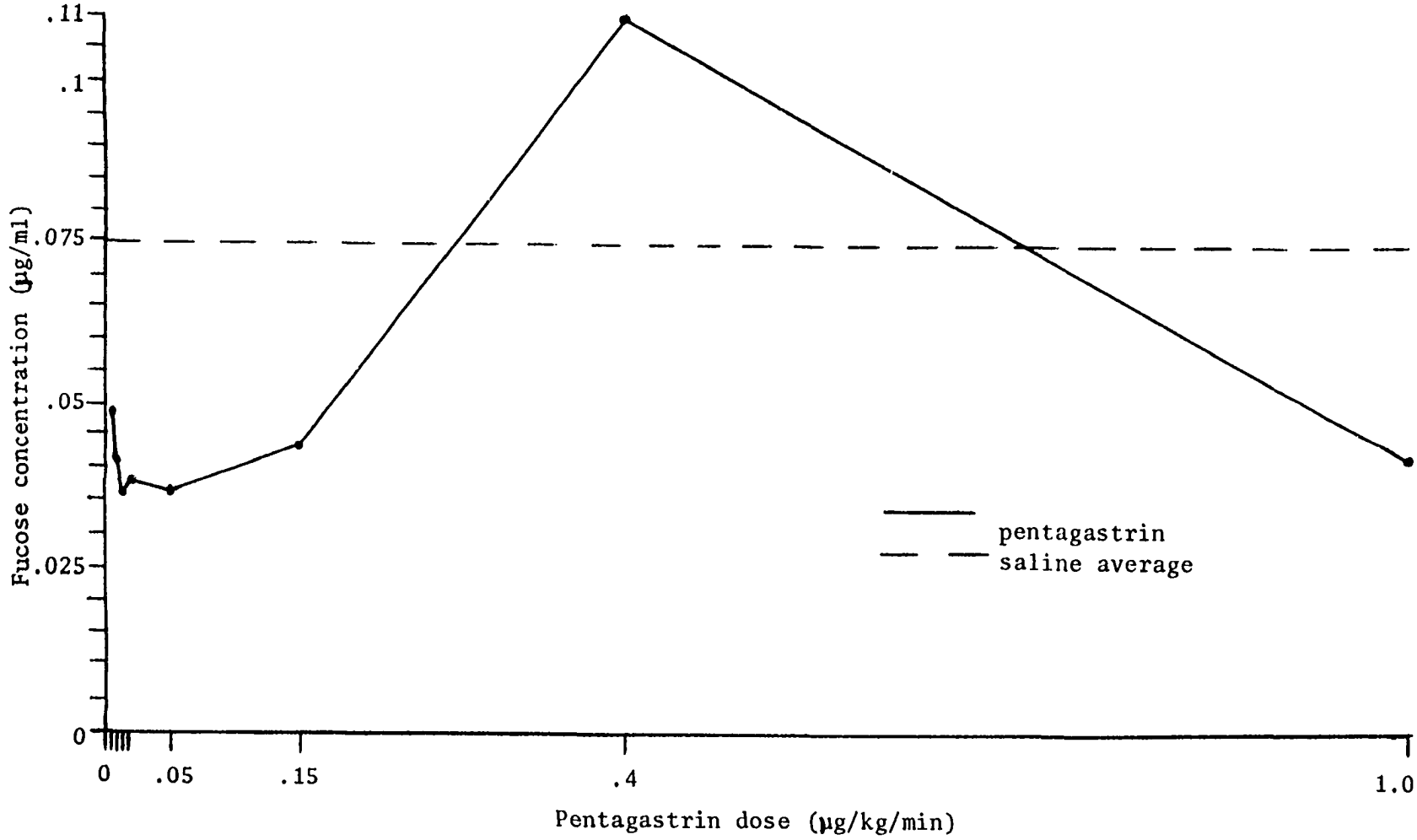
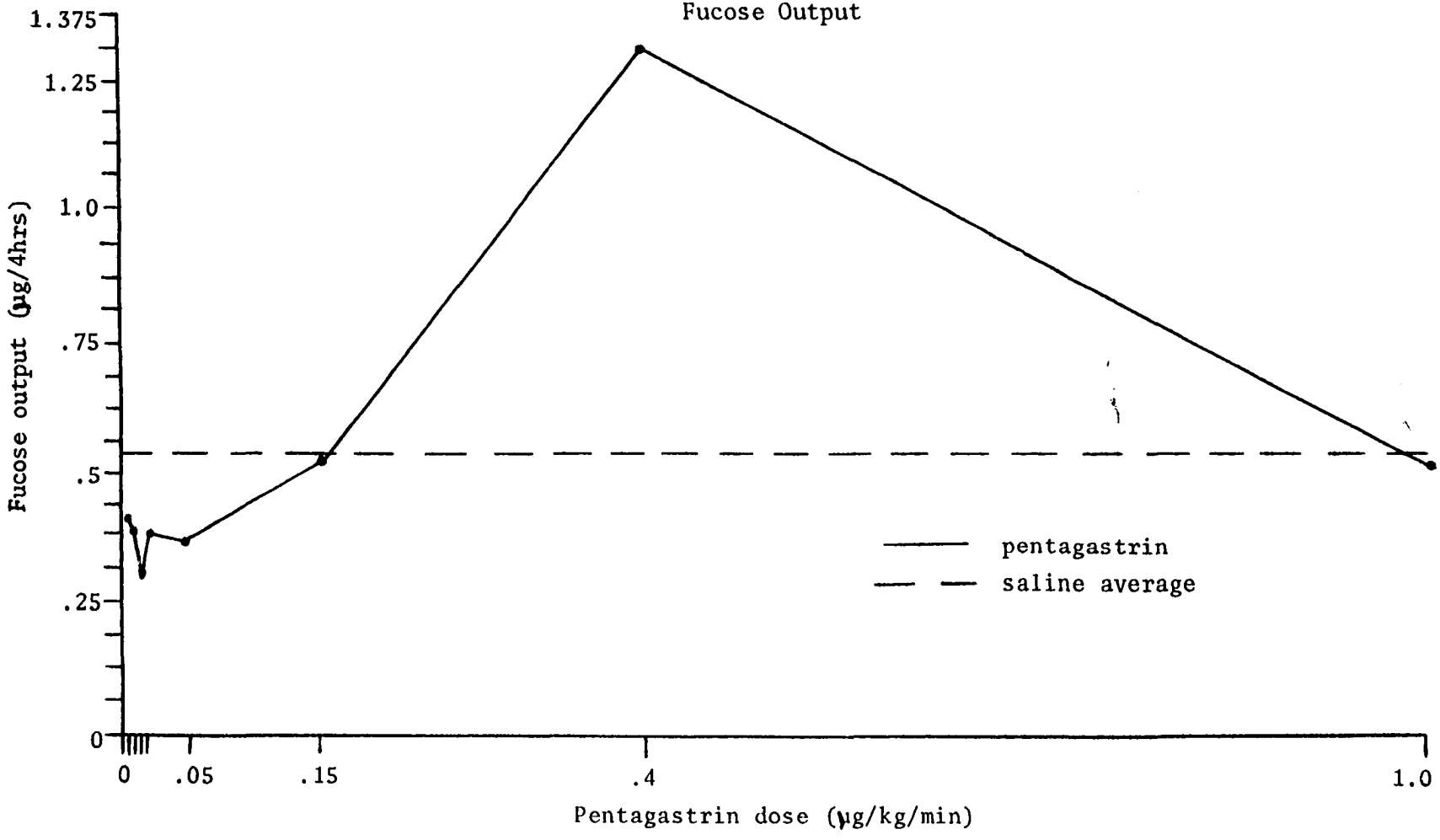


Figure 47
Fucose Output



Discussion

Hourly volumes of gastric juice were greater with pentagastrin than with saline and the volume of gastric juice secreted during any one hour was in direct relation to the dose of pentagastrin. The greatest volume of gastric juice was always produced during the first hour of collection, and generally, during each succeeding hour less gastric juice was secreted than during the preceeding hour. With pentagastrin, a leveling off of hourly volume occurred later than with saline.

Four hour collections with pentagastrin produced expected dose related increases in gastric juice volume, acid concentration and acid output. A decrease in pepsin concentration, and a pepsin output relatively close to control values were also present as expected. Hexosamine and sialic acid decreased in concentration, but remained near saline values in output. Fucose concentration and output were generally lower than the average control values (although fucose output was closer to its average control value).

Results from experiments involving 24 hours of infusion followed by the usual four hours of infusion (during collection) suggest that the prolonged infusion process itself produced decreases in gastric juice volume and fucose output (as shown by comparison of saline controls). Prolonged pentagastrin infusion produced less gastric juice volume, acid output and sialic acid output than the usual four hour pentagastrin infusion. However, the acid output produced after prolonged pentagastrin infusion was still greater than that produced by saline controls, while pepsin output, hexosamine output and sialic acid output were less than were produced by saline controls. Prolonged pentagastrin infusion pro-

duced a gastric juice volume which was greater than the volume produced by prolonged saline infusion but which was relatively close to the volume produced by the usual four hour saline infusion.

Results of all of the gastric secretion experiments indicate that the effect of pentagastrin on the output of gastric secretion components involved increases in volume and acid output, and slowly appearing decreases in pepsin output, hexosamine output and sialic acid output. The output level of fucose was affected little if at all. The only gastric secretion component to show an increase in concentration was acid while the concentrations of pepsin, hexosamine and sialic acid tended to decrease in response to pentagastrin. Therefore, ulcers produced in response to administration of pentagastrin may have been due to the prolonged production of large amounts of very acid gastric juice possibly coupled with decreased production of mucus.

The role of increased gastric mucosal blood flow in pentagastrin-induced ulcer formation in rats is unclear. Quite possibly, increased mucosal blood flow is involved in the production of increased gastric juice secretion, but is not directly involved in ulcer formation. The role of pentagastrin in promoting tissue vulnerability is also unclear. The presence of a mucosal barrier to ulceration has been observed in dogs (Hollander, 1954). The extent to which this exists in the stomach or duodenum of the rat is unknown, but it may be that with pentagastrin the mucosal barrier is weakened in the duodenum, thereby making it more vulnerable to attack by gastric secretions.

Ulcers produced in response to infusion of the other secretogogues may also have been due to secretion of large amounts of very acid gastric juice coupled with decreased mucus secretion. The observed differences

in ulcer production by the secretogogues and combinations of secretogogues probably reflect specific potencies of these agents in their promotion of tissue vulnerability, increased acidic gastric secretion and decreased mucus secretion.

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APPENDIX

Abbreviations and Terms Used in Tables

Table Number	Abbreviation or Term	Meaning	Units of Expression
1, 2 and 3	Standard Error	Standard Error of the Mean	Absolute Number
2 and 3	Volume	Volume	ml/4 hours
2 and 3	Acid C	Acid Concentration	mEq/l
2 and 3	Acid O	Acid Output	mEq/4 hours
2 and 3	Pepsin C	Pepsin Concentration	mEq tyrosine/ml/min
2 and 3	Pepsin O	Pepsin Output	mEq tyrosine/4 hours
2 and 3	Hexosamine C	Hexosamine Concentration	mg/ml
2 and 3	Hexosamine O	Hexosamine Output	mg/4 hours
2 and 3	Sialic Acid C	Sialic Acid Concentration	µm/ml
2 and 3	Sialic Acid O	Sialic Acid Output	µm/4 hours
2 and 3	Fucose C	Fucose Concentration	µg/ml
2 and 3	Fucose O	Fucose Output	µg/4 hours

Pentagastrin Dose (µg/kg/min)	Hourly Secretion (ml/hr) 1st Hour	2nd Hour	3rd Hour	4th Hour
Saline	2.93	1.66	.149	1.47
Standard Error	.088	.063	.045	.041
.005	3.03	2.18	1.81	1.49
Standard Error	.254	.182	.149	.101
.01	3.46	2.53	1.72	1.51
Standard Error	.335	.230	.209	.188
.015	3.39	2.00	2.15	1.35
Standard Error	.285	.250	.112	.076
.02	3.38	2.21	2.20	1.71
Standard Error	.203	.158	.123	.101
.05	3.53	3.03	2.11	.189
Standard Error	.318	.211	.202	.136
.15	5.11	2.22	2.68	1.73
Standard Error	.313	.167	.205	.117
.4	3.98	3.14	----	----
Standard Error	.259	.264	----	----
1.0	3.81	3.67	2.75	2.05
Standard Error	.207	.174	.158	.114
2.0	4.30	3.47	2.46	2.33
Standard Error	.297	.196	.074	.118

Table 1.
Average Gastric Juice Volumes

Gastric Juice Component	Pentagastrin Dose	Saline	.005	.01	.015	.02	.05	.15	.4	1.0	2.0
	Volume	7.53	8.51	9.23	8.89	9.50	10.56	11.74	11.60	12.30	12.53
	Standard Error	.17	.61	.65	.49	1.26	.73	.69	.81	.56	.52
	Acid C	93.6	85.0	107.4	109.8	116.1	127.9	124.2	133.4	152.1	145.4
	Standard Error	1.4	4.9	3.2	1.5	2.3	2.5	3.2	2.9	2.7	2.4
	Acid O	.714	.719	.992	.975	1.11	1.35	1.45	1.54	1.86	1.81
	Standard Error	.021	.065	.080	.055	.084	.097	.083	.109	.080	.064
	Pepsin C	31.8	28.0	30.6	26.1	25.0	20.7	25.1	20.6	21.0	15.3
	Standard Error	.3	1.0	.6	1.3	1.4	.6	1.0	.7	1.0	.5
	Pepsin O	237	239	286	232	237	219	297	242	260	194
	Standard Error	5	19	23	17	18	16	23	20	18	13
	Hexosamine C	.106	.141	.092	.104	.065	.068	.074	.045	.050	----
	Standard Error	.002	.007	.005	.006	.006	.004	.005	.004	.004	----
	Hexosamine O	.083	1.219	.865	.908	.597	.722	.882	.520	.633	----
	Standard Error	.022	.122	.090	.060	.056	.068	.085	.064	.066	----
	Sialic Acid C	.222	.241	.188	.182	.179	.139	.182	.117	.106	.119
	Standard Error	.004	.012	.010	.010	.010	.008	.014	.006	.006	.007
	Sialic Acid O	1.67	2.08	1.74	1.60	1.65	1.48	2.15	1.37	1.32	1.50
	Standard Error	.04	.19	.14	.11	.09	.14	.22	.12	.10	.11
	Fucose C	.0750	.0491	.0409	.0359	.0382	.0358	.0430	.1090	.0419	.0359
	Standard Error	.0040	.0064	.0118	.0063	.0056	.0100	.0059	.0196	.0056	.0053
	Fucose O	.534	.405	.382	.301	.381	.362	.533	1.307	.518	.444
	Standard Error	.027	.062	.114	.049	.059	.095	.086	.226	.076	.062

Table 2.
Four Hour Collection

Treatment	24 Hours Saline followed by 4 Hours Saline	24 Hours Saline followed by 4 Hours Pentagastrin	24 Hours Pentagastrin followed by 4 Hours Pentagastrin
Volume	6.71	11.50	7.83
Standard Error	.50	.66	.59
Acid C	105.9	133.9	142.7
Standard Error	4.4	2.7	2.2
Acid O	.698	1.54	1.11
Standard Error	.049	.09	.08
Pepsin C	39.1	17.7	22.4
Standard Error	1.7	.5	.7
Pepsin O	269	205	178
Standard Error	27	15	17
Hexosamine C	.113	.052	.064
Standard Error	.009	.004	.007
Hexosamine O	.780	.594	.521
Standard Error	.098	.053	.071
Sialic Acid C	.247	.121	.120
Standard Error	.020	.010	.008
Sialic Acid O	1.75	1.40	.930
Standard Error	.19	.16	.080
Fucose C	.056	.031	.057
Standard Error	.004	.004	.006
Fucose O	.377	.344	.469
Standard Error	.047	.048	.077

Table 3.
Effect of Prolonged Pentagastrin Infusion on Gastric Juice