



8-1973

The Influence of Nicotine on the Effect of Secretin on Gastric Secretion in the Rat and Dog

Donald H. Currie

Follow this and additional works at: https://scholarworks.wmich.edu/masters_theses



Part of the [Anatomy Commons](#), and the [Veterinary Anatomy Commons](#)

Recommended Citation

Currie, Donald H., "The Influence of Nicotine on the Effect of Secretin on Gastric Secretion in the Rat and Dog" (1973). *Master's Theses*. 2640.

https://scholarworks.wmich.edu/masters_theses/2640

This Masters Thesis-Open Access is brought to you for free and open access by the Graduate College at ScholarWorks at WMU. It has been accepted for inclusion in Master's Theses by an authorized administrator of ScholarWorks at WMU. For more information, please contact wmu-scholarworks@wmich.edu.



THE INFLUENCE OF NICOTINE ON THE EFFECT
OF SECRETIN ON GASTRIC SECRETION
IN THE RAT AND DOG

by

Donald H. Currie

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment
of the
Degree of Master of Arts

Western Michigan University
Kalamazoo, Michigan
August 1973

-

ACKNOWLEDGEMENTS

I would like to thank Dr. André Robert for his cooperation in allowing me to conduct my research in his laboratory. His advice, guidance and assistance molded for me, an invaluable experience in the field of biological research. My sincere thanks also go to my committee chairman, Dr. Jean M. Lawrence, without whom this opportunity to conduct research with Dr. Robert at the Upjohn Company would not have been possible. Through their constructive criticism and suggestions, both André Robert and Jean Lawrence have been extremely helpful in comprising this manuscript.

My thanks also go to my other committee members, Dr. Leonard Beuving and Dr. Jack Wood, for their suggestions concerned with the preparation of this thesis. The technical advice of Mr. James Nezam-
is and the technical assistance of Mr. Joseph Badalamenti and Ms. Cleo Lancaster was also greatly appreciated.

Donald H. Currie

MASTERS THESIS

M-4856

CURRIE, Donald H.

THE INFLUENCE OF NICOTINE ON THE EFFECT
OF SECRETIN ON GASTRIC SECRETION IN THE
RAT AND DOG.

Western Michigan University, M.A., 1973
Physiology

University Microfilms, A XEROX Company , Ann Arbor, Michigan

THIS DISSERTATION HAS BEEN MICROFILMED EXACTLY AS RECEIVED.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

TABLE OF CONTENTS

CHAPTER		PAGE
I	INTRODUCTION	1
	General Background and Purpose	1
	Literature Review	3
II	MATERIALS AND METHODS	13
	Pharmacological Agents	14
	Preparation of Rats	15
	Preparation of the Dog	16
	Infusion of Rats	16
	Infusion of the Dog	18
	Experimental Procedure in Rats	19
	Experimental Procedure in the Dog	20
	Gastric Juice Collection and Analysis	21
III	RESULTS	27
	Studies in the Rat	27
	Studies in the Dog	29
IV	DISCUSSION	63
	Studies in the Rat	63
	Studies in the Dog	67
	Possible Role of Nicotine in the Pathophysiology of Peptic Ulcer in Smokers	70
V	SUMMARY	74
VI	BIBLIOGRAPHY	76

INTRODUCTION

General Background and Purpose

Epidemiological studies have indicated an increased incidence of duodenal ulcer in cigarette smokers (Barnett, 1927; Smoking and Health, 1964; Monson, 1970). This association between smoking and ulcer is generally not considered to be a cause and effect relationship due primarily to the lack of laboratory evidence for a mechanism connecting any component of cigarette smoke (e.g. tar, nicotine) with duodenal ulcer formation.

Nicotine has been shown to increase the ulcerogenicity of certain gastric secretagogues (pentagastrin + carbachol) for the duodenum in the rat. Robert et al. (1971) have recently demonstrated that when these secretagogues were infused in small doses over a twenty-four hour period, duodenal ulcers were produced in one-third of the animals. When nicotine sulfate was added to the infusion, the ulcer incidence, severity and number per duodenum were increased. This effect of nicotine was dose dependent. Using cats, Konturek et al. (1971) observed a similar potentiating effect of nicotine on duodenal ulcers produced by the continuous infusion of pentagastrin. Toon et al. (1951) have also shown that the chronic inhalation of cigarette smoke in dogs abets the ulcerogenic effect of histamine in beeswax, an effect they believed to be caused by nicotine. Chronic inhalation of denicotinized cigarettes caused no increase in duodenal ulcers. This response was also dose related since an increase in the number of cigarettes smoked increased the incidence of peptic ulcers.

Konturek et al. (1972) have shown that the intravenous infusion of nicotine in dogs, in doses corresponding to amounts absorbed during smoking, decreased secretin-stimulated pancreatic secretion of fluid and bicarbonate. Since gastric acid in the lumen of the duodenum is normally neutralized by pancreatic juice and to a lesser extent by bile, it was suggested that nicotine might exert this pro-ulcer effect by preventing such neutralization.

Secretin also acts to prevent an abnormal acid milieu in the duodenum by inhibiting gastric secretion of acid (Greenlee et al., 1957). The possibility also exists that nicotine could interfere with the inhibitory effect of secretin on the stomach. This has not been determined. If the situation exists whereby nicotine also blocks the effect of secretin on the stomach, the result would be a greater acid milieu in the lumen of the duodenum and this condition could also promote ulcer formation. The effect of nicotine on pancreatic and biliary secretion has been studied in the dog (Konturek et al. 1971). They have shown that nicotine decreases both secretin-stimulated pancreatic secretion and spontaneous biliary secretion in the dog. Furthermore, Konturek et al. (1971) have examined the effect of nicotine on gastric secretion in cats and dogs and have shown that nicotine has no effect on gastric secretion stimulated with pentagastrin. The effect of nicotine on basal and pentagastrin-stimulated gastric secretion has been previously studied by Thompson using pylorus-ligated rats (Thompson and Bruckner, 1969) and rats prepared with chronic gastric fistulas (Thompson, 1970; Thompson, et al. 1970). It was not the purpose of our investigation to examine completely, the effects of nicotine on gastric secretion. However, it was the purpose

of this investigation to learn more about the pro-ulcer effect of nicotine by studying the influence of nicotine on the effect of exogenous secretin on gastric secretion. This was done in rats equipped with chronic gastric fistulas and in a dog prepared with a denervated fundic pouch (Heidenhain) and a gastric fistula. In order to establish a gastric secretory response, pentagastrin was infused subcutaneously in the rats and intravenously in the dog. The effect of an infusion of secretin on pentagastrin-stimulated gastric secretion was then established. Subsequently, nicotine sulfate was infused in combination with secretin to study the influence of nicotine on the effect of secretin on gastric secretion.

Literature Review

As early as 1894 Dolinski and his associates found that when hydrochloric and other acids were allowed to enter the intestine, pancreatic juice began to flow. However, it was not until 1902 when Bayliss and Starling observed an increase in pancreatic secretion when acid was applied to a denervated loop of the jejunum that a hormonal theory for external pancreatic secretion was proposed. These observations of Bayliss and Starling led to the discovery of secretin. Secretin was then defined as a hormone which was released by the upper intestinal mucosa when the latter was exposed to certain chemical substances and which stimulated the secretion of pancreatic juice. From this discovery the theory of a humoral mechanism evolved and was used to designate a process which changes the function of an organ by the carrying to that organ of some blood-borne substance. The actual

proof for such a mechanism did not come about until 1926 when Ivy and Farrell showed that a transplanted portion of the pancreas, completely separated from its original blood and nerve supply, was stimulated to secrete after the ingestion of a meal. They concluded that this was positive proof for the existence of a humoral mechanism for external pancreatic secretion. Much research has been performed in the ensuing seventy-one years following the discovery of secretin in an effort to discover what it is and where and how it functions. Detailed reviews of many of these earlier investigations have been written by Still (1931), Babkin (1950) and Grossman (1950; 1958).

Early attempts to isolate secretin were unsuccessful because appropriate procedures for the purification of polypeptides were not available. Finally in 1966, with the advance of new methods, Mutt and Jorpes were able to describe the molecular structure of the hormone. Secretin was shown to be a polypeptide of twenty-seven amino acid residues comprising eleven different amino acids. It is also known that secretin is strongly basic because of its particular amino acid composition. This alkalinity may in part be the reason that the hormone is released when acid enters the duodenum (Hubel, 1972). Secretin was also synthesized in 1966 by Bodansky and co-workers and its potency was found to be equal to that of natural secretin.

More recent investigations have confirmed that the presence of acid in the duodenum acts as the stimulus for the release of secretin from the duodenal mucosa. Thomas and Crider (1940) have shown that pancreatic bicarbonate secretion begins only when the duodenal contents are more acid than pH 5. The importance of this acid stimulus has been

well established by Hallenbeck (1951) and Grossman (1967) who have observed a two-fold increase in the pancreatic secretory rate upon diversion of the alkaline pancreatic juice from the duodenum. The exact mechanism by which acid acts as a stimulus for the release of secretin is unknown. However, it has been suggested that acid acts in conjugation with other hormonal and neural stimuli which may also play a part in the release of secretin (Hubel, 1972).

Upon release from the duodenal mucosa secretin has been shown to affect a variety of organs. Secretin has been reported to increase the secretory rate of the Brunner's glands of the duodenum in the dog (Cooke and Grossman, 1966; Stening and Grossman, 1969). Cohen (1971) has shown that secretin also exhibits an effect on the human esophagus. Either an intravenous injection or infusion of secretin inhibited a gastrin-induced rise in pressure at the lower esophageal sphincter. Secretin has been shown to affect the liver by causing a moderate stimulation of bile production in a number of animal species but causes only a slight increase in bile secretion in rats. A review of this work on bile secretion has been presented by Hubel (1972). Secretin augments the strength of gall bladder contraction elicited by cholecystokinin (Stening and Grossman, 1969) and affects the kidney by increasing renal excretion of water, sodium and potassium (Barbezat et al., 1971). Secretin has also been shown to increase cardiac output in cats and dogs (Ross, 1970) and to stimulate lipolysis in a number of animal species (Rudman, 1969).

Much of the current research concerns the effect of secretin on the pancreas (endocrine and exocrine) and the stomach. Secretin has

been shown to affect the exocrine pancreas by increasing salt and water secretion. Increased secretion is usually within thirty seconds post-injection and the duration of the response is increased with the dose of secretin used, up to a maximum of 2.5 U/kg (Hickson, 1970). Hickson has also shown that the sensitivity of the pancreas to secretin depends on the species in which the hormone was tested. Using secretin extracts he observed that the threshold dose in dogs was much higher than in pigs, although doses necessary to achieve maximal rates of secretion were similar. Gregory (1962) reported that maximal bicarbonate output in dogs was obtained with 1-2 U/kg of secretin whereas in cats 0.8 U/kg caused maximal bicarbonate production when injected intravenously. A reduction in volume and bicarbonate output was observed in dogs and cats with supramaximal doses of secretin (Henrickson, 1968; Konturek, 1969) and was believed to be caused by side effects such as restlessness and retching which were caused by the secretin preparations. Human studies by Konturek (1970) indicated maximal pancreatic secretion of bicarbonate with only 0.9 U/kg by single intravenous injection or by constant infusion.

Large doses of secretin have also been shown to affect the endocrine pancreas by liberating insulin in fasting animals. Unger et al. (1967) have shown that in dogs, after a single intravenous injection of secretin, in doses which cause maximal pancreatic secretion, the concentration of insulin in the portal venous blood increased and then fell to normal within ten minutes. Similar increases in insulin concentrations of the peripheral blood have been reported in healthy and diabetic humans following intravenous administration of secretin

(Chisholm et al., 1969). The infusion of low doses of secretin have been shown to potentiate the effect of glucose on increasing pancreatic insulin secretion (Dupre, 1969).

The discovery of the inhibition of gastric acid secretion by secretin was first made by Greenlee and his associates (1957). They suggested that secretin was involved in the duodenal inhibition of gastric secretion. This early study revealed that intravenous injections of secretin in dogs inhibited the response of a denervated fundic pouch (Heidenhain) to food and to antral pouch stimulation (i.e. endogenous gastrin) but not to histamine. Jordan and Peterson (1962) also noticed a marked inhibition by secretin of the Heidenhain pouch response to feeding. Single intravenous injections of secretin have also been shown to inhibit Heidenhain pouch secretion stimulated by the continuous infusion of a gastrin extract (Gillespie and Grossman, 1964). Wormsley and Grossman (1964) have observed that a single intravenous administration of secretin inhibited gastrin-stimulated acid secretion from the Heidenhain pouch (denervated) as well as from the gastric fistula (i.e. the remaining portion of the main stomach which is innervated). Since these studies it has been well established that secretin inhibits gastric acid secretion when the stimulus is gastrin or pentagastrin (Nakajima et al., 1969; Johnson and Grossman, 1968; Johnson and Grossman, 1971). Histamine-induced gastric acid secretion in vagally denervated pouches in dogs is either unaffected by secretin (Johnson and Grossman, 1968) or is inhibited only when there is a submaximal gastric secretory response (Nakajima et al., 1969). Gastric secretion stimulated by gastrin or by pentagastrin has also been shown

to be inhibited by either intravenous injections or infusions of secretin in the rat (Tompson and Johnson, 1969; Johannson et al., 1972), cat (Stening et al., 1969) and to a lesser extent in man (Brooks and Grossman, 1970).

Pepsin secretion rises following secretin administration in a number of animal species. Secretin (75 U/kg), either injected or infused, increased the concentration of pepsin from both Heidenhain pouches (denervated) and Pavlov pouches (innervated) in rats stimulated with gastrin (Johannson et al., 1972). However, total pepsin output was observed to rise only in the response of the Heidenhain pouch. Magee and Nakajima (1968) observed an eight-fold increase in pepsin output from the Heidenhain pouch of dogs when secretin (5 U/min) was infused intravenously. This increase in pepsin secretion was present in dogs stimulated with either histamine or gastrin pentapeptide. Stening et al. (1969) have confirmed this pepsinogenic effect of secretin in dogs and have also observed a similar stimulatory effect on pepsin secretion in cats. In these experiments, either an intravenous injection of secretin or the closure of the gastric fistula (causing release of endogenous secretin) increased the pepsin output from the Heidenhain pouch of cats. Injections of 1-4 U/kg of secretin in humans causes pepsin secretion to rise to levels almost three times that of the basal rate (Brooks et al., 1969). Berstad and Peterson (1970) have shown that in man, the combination of secretin and pentagastrin caused an increase in pepsin output higher than can be obtained as a maximal response to either agent alone.

Nicotine has recently been shown to potentiate duodenal ulcers in the rat produced by either the subcutaneous infusion of certain secretagogues (pentagastrin + carbachol) or by the perfusion of 0.12 N hydrochloric acid through the esophagus (Robert, 1972; Robert et al., 1971). The intravenous infusion of nicotine (100 ug/kg/hr) also increased the ulcer severity and incidence in cats infused continuously for thirty-six hours with pentagastrin. This dose of nicotine potentiated the ulcerogenic effect of pentagastrin but was a dose which by itself had no effect on gastric acid secretion (Konturek et al., 1971). Chronic inhalation of cigarette smoke increases the ulcerogenicity for the duodenum of histamine in beeswax in dogs (Toon et al., 1951). This effect was attributed to nicotine since chronic inhalation of denicotinized cigarettes produced no increase in duodenal ulcers. Studies on the effect of tobacco smoking on the etiology of duodenal ulcers in man are unclear. However, cigarette smokers are known to have a higher incidence of duodenal ulcer than non-smokers (Smoking and Health, 1964). Tobacco smoking has also been implicated as a contributory factor in the reduced healing of peptic ulcers in man (Doll et al., 1958; Doll, 1964).

Few studies have been conducted which examine the effect of nicotine on gastric secretion. Those which have been published are contradictory. In gastric fistula rats stimulated with gastrin pentapeptide, the infusion of nicotine (100 ug/kg/hr) has been shown to have no effect on gastric acid secretion (Thompson, 1970). On the other hand, Thompson and Bruckner (1969) have also observed that

nicotine depressed gastric acid and pepsin secretion in pylorus-ligated rats. They have shown that the subcutaneous infusion of nicotine hydrogen tartrate (60-80 ug/kg/hr) in pylorus-ligated rats, caused maximal depression of basal gastric secretion. Thompson et al. (1970) have also shown that subcutaneous injections of various doses of nicotine (50-200 ug/kg) depressed acid and pepsin secretion from pylorus-ligated rats stimulated with gastrin pentapeptide. In gastric fistula rats stimulated with histamine, the subcutaneous infusion of nicotine (100 ug/kg/hr) also decreased acid and pepsin output (Thompson, 1970). Chronic administration of nicotine has been shown to stimulate gastric secretion of acid and pepsin. Chronic subcutaneous injections of nicotine (500 ug/kg/day for 15 days) resulted in an increase in gastric juice volume, acid output and pepsin output in pylorus-ligated rats (Thompson et al., 1970). Chronic oral ingestion of nicotine for fifteen days has also been reported to increase the output of acid and pepsin from the rat stomach after ligation of the pylorus (Thompson et al., 1970). Thompson (1972) has also studied the effects of chronically administered nicotine (2 mg base/kg/day) on gastric secretion in pylorus-ligated rats with hypothalamic lesions. Chronic injections of nicotine increased gastric juice volume, acid output and pepsin output in control rats. The destruction of the anterior hypothalamus abolished this effect of nicotine. Thompson concluded that this stimulatory effect of nicotine on gastric secretion in the rat was mediated through the hypothalamic area.

Konturek et al. (1971) have studied the effect of nicotine

infusion (100 ug/kg/hr) on pancreatic, biliary and gastric secretion in the dog. When nicotine was infused intravenously in dogs equipped with a biliary cannula, the volume and bicarbonate output of unstimulated hepatic bile was decreased by 50 percent. A similar dose dependent inhibition of secretin-stimulated secretion of fluid and bicarbonate from the pancreas was also observed. Secretin-stimulated pancreatic secretion was depressed 62 percent at a dose of 100 ug/kg/hr of nicotine. This same dose of nicotine had no effect on gastric secretion. The continuous infusion of nicotine at doses of 100-200 ug/kg/hr has been shown to have no effect on gastric secretion from fistula cats stimulated with pentagastrin (Konturek et al., 1971). Higher doses of nicotine (400 ug/kg/hr) did decrease gastric secretion but was accompanied by such side effects as retching and restlessness. These same investigators (1972) have studied the effect of nicotine on the pancreatic action of exogenous secretin as well as the action and release of endogenous secretin. Intravenous infusion of nicotine (100 ug/kg/hr) inhibited pancreatic secretion stimulated by exogenous secretin (0.57-3 U/kg/hr) and by endogenously released secretin elicited by the intraduodenal infusion of hydrochloric acid. Nicotine depressed by 50 percent, pancreatic secretion stimulated by 1.5 U/kg/hr (ED₅₀) of secretin. The intraduodenal application of nicotine decreased pancreatic secretion stimulated by endogenously released secretin but not to the intravenous infusion of the hormone. From these studies in the dog, Konturek et al. have proposed a possible mechanism for the pro-ulcer effect of nicotine observed by himself (1971), Robert (1972; Robert et al., 1971) and Toon (1951).

It was suggested that nicotine favors ulcer formation by blocking the stimulatory effect of secretin on pancreatic secretion of fluid and bicarbonate. This alkaline pancreatic secretion is critical to neutralization of gastric acid flowing over the duodenum.

MATERIALS AND METHODS

In this investigation experiments were performed in chronic fistula rats and in a dog prepared with a denervated fundic pouch (Heidenhain) and a gastric fistula. The rat fistula preparation allowed all of the juice from the stomach to be collected externally. In the dog, gastric juice was collected from the Heidenhain pouch. In each experiment it was necessary that the gastric fistula remain open. This prevented gastric juice from the main stomach from passing over the duodenum and thus releasing unknown amounts of endogenous intestinal inhibitors, such as secretin, of gastric secretion.

Pentagastrin, infused subcutaneously in rats and intravenously in the dog, was used to stimulate gastric secretion. During the studies in both species, the gastric secretory response to pentagastrin was first established. The following conditions were then examined. First, it was necessary to establish the effects of an infusion of secretin upon pentagastrin-stimulated gastric secretion. It was also necessary to establish that the doses of nicotine employed during these studies had no effect when infused against a background of pentagastrin-stimulated gastric secretion. This was essential since any inhibitory effect of nicotine infused alone might mask the effect of secretin. Finally, secretin was infused in combination with nicotine to determine if nicotine would alter the effect of secretin on gastric secretion. In the experiments in both species, nicotine was infused one hour prior to the administration of secretin. This allowed a pre-treatment with nicotine and insured adequate absorption. Specific

details concerning the preparation of the animals and the design of the experiments are given below.

Pharmacological Agents

Pentagastrin (an analogue of the C-terminal five amino acids of gastrin, N-5-butyloxycarbonyl beta-ala-try-met-phe-NH₂, Fox Chemical Co.) was used as a stimulant of gastric secretion in both the rat and the dog studies. Pentagastrin was infused subcutaneously in rats at a dose of 0.5 ug/kg/min and was delivered at a rate of 1.08 ml/hr. In the dog, 0.5 ug/kg/min of pentagastrin was infused intravenously at a rate of 2.5 ml/hr. The original compound was a dry chemical which was put into solution with a physiological saline solution. Stock solutions were prepared and kept frozen until the day of experimentation when the appropriate dilutions were made. Physiological saline (0.9%) was used as the control agent against which the effects of pentagastrin could be compared.

Nicotine sulfate (40% commercial, Sigma Chemical Corp.) was first prepared as an aqueous stock solution. Following the calculation of the required dose (ug/kg) the necessary dilutions were made with normal saline. Nicotine sulfate was infused subcutaneously in the rats at a rate of 2.16 ml/hr. A dose of 100 ug/kg/hr was used throughout the rat experiments. A dose of 150 ug/kg/hr was infused intravenously in the dog experiments (2.5 ml/hr).

Purified natural secretin (GDI Laboratory, Karolinska Institutet, Stockholm, Sweden) was prepared in normal saline and was also infused subcutaneously in rats at a rate of 2.16 ml/hr. Doses of 30-60 U/kg/hr

were employed in the rat studies. In the dog studies, partially purified natural secretin (Boots Pure Drug Co., Nottingham, England) was also prepared in normal saline and was infused at a constant rate of 2.5 ml/hr. A dose of 1.5 U/kg/hr was employed in these experiments.

In this investigation different secretin preparations (i.e. Boots and GIH) were used in the rat and dog studies. Tumpson and Johnson (1969) have shown that in fistula rats, a single intravenous injection of GIH secretin at a dose of 75 U/kg maximally inhibits pentagastrin-stimulated gastric secretion. However, in the dog, Johnson and Grossman (1968) have observed near maximal (90 percent) inhibition of gastrin-stimulated gastric secretion, following an intravenous administration of only $\frac{1}{4}$ U/kg/hr of GIH secretin. Therefore, it is apparent from the results obtained by these investigators that the rat is more resistant to the inhibitory effect of secretin on gastric acid secretion. The GIH secretin, being more pure, is nine times more potent than the partially purified Boots secretin preparation (i.e. 1 U of GIH secretin is equivalent to 9 U of Boots secretin; Grossman, 1969). Since large quantities of Boots secretin would be necessary to inhibit gastric secretion in rats, we chose in our rat studies to use the more potent GIH secretin.

Preparation of Rats

Female Upjohn rats (derived from the Sprague-Dawley strain) with a body weight of 200-210 grams were selected for the preparation of a chronic gastric fistula one month prior to experimental use (the method was that of Komarov and Boyd, 1962). Following an overnight

fast, the animals were anesthetized with a single intraperitoneal injection of 0.6 ml of a 2 percent sodium brevital solution (sodium methohexital, Lilly and Co.). A stainless steel cannula was implanted in the forestomach which is the non-glandular portion of the rat stomach (Robert, 1971).

During the one month post-operative period, all animals were placed in stainless steel feeding cages fitted with a narrow mesh screen in order to minimize possible damage to the cannula. During this time, all animals had free access to Upjohn laboratory chow and tap water.

Preparation of the Dog

One male mongrel dog was surgically prepared with a denervated fundic pouch (Heidenhain) and a gastric fistula. Gastric secretory studies were conducted four months after surgery. The dog, weighing twenty-six kilograms, was fed one can of Alpo daily and had free access to Purina laboratory chow (Purina-Ralston Co.). The animal was also given 1.5 litre of 0.9% sodium chloride two times daily to replace the daily fluid and electrolyte loss through the fundic pouch cannula.

Infusion of Rats

The chronic fistula animals were used one month following implantation at which time their body weight averaged 250 grams. All animals were fasted for twenty-four hours prior to the start of each experiment and at least one week was allowed between successive

experiments to allow complete recovery from the effects of fasting and fluid loss. Two hours before the start of each experiment, all animals were given a single subcutaneous 10 ml injection of saline (Robert, 1972). Robert has shown that this saline injection restores body fluids which have been lost through self-imposed dehydration which occurs during the fasting of rats. This saline administration restores body fluids and results in increased gastric secretion.

The average fasted body weight was used as the basis for calculating the doses of the agents that were administered. The prepared solutions were then put in separate 12 ml syringes which were connected by means of 21 gauge clear Tygon tubing (Transflex, Minnesota Manufacturing Co.) to continuous-flow variable speed infusion machines designed by the Upjohn Company. After the solutions began to flow from the tubings, the needle (21 gauge) attached to the other end of the tubing, was introduced subcutaneously in the dorsal region and was secured to the skin with a surgical wound clip. Two needles were placed subcutaneously as far apart as possible. The needle placed in the anterior dorsal region was used for the continuous infusion of either saline or pentagastrin at a rate of 1.08 ml/hr. The other needle was placed posteriorally and was attached to a three way juncture to allow the infusion of a combination of saline, nicotine or secretin through a single needle. This procedure allowed a separate infusion of nicotine and secretin. These substances were administered at a rate of 2.16 ml/hr. Following the placement of the needles, the plugs of the cannulas were removed to allow the drainage

and collection of gastric juice into 12 ml graduated centrifuge tubes.

During the infusion, the animals were placed in individual stainless steel cylindrical cages with flat bottoms and perforations to allow adequate ventilation. These cages permitted limited movement but prevented the animals from bending and chewing the tubing. Food and water were withheld throughout the entire infusion period. For each experiment the infusion and collection lasted five hours during which time the gastric juice was collected via the cannulas.

Infusion of the Dog

The dog was fasted for eighteen hours before each experimental run. The fasted body weight was used as the basis for calculating the appropriate dosage. Studies were conducted two times per week with at least two days between successive experiments.

During each experiment the animal was harnessed in a wooden frame which restricted their movements and allowed complete drainage of gastric juice. For each experiment, pentagastrin, secretin and nicotine sulfate were placed in separate 20 ml syringes. The syringes were connected by means of 21 gauge Tygon tubing to the infusion machine. At the start of each experiment a catheter (Bard Inc.) was inserted into a femoral vein. The catheter was connected to Tygon tubing equipped with a three way juncture to allow the separate infusion of combinations of pentagastrin, secretin and nicotine sulfate. Following the start of the infusion the dog remained on the stand for approximately six hours during which twenty-three successive fifteen

minute collections of gastric juice were made from the Heidenhain pouch. The gastric fistula also remained opened to allow drainage of the gastric juice from the main stomach.

Experimental Procedure in Rats

Initially, preliminary studies were conducted to establish the effect of pentagastrin (0.5 ug/kg/min) on gastric secretion and the effect of various doses of secretin (50-60 U/kg/hr) on pentagastrin-stimulated gastric secretion.

During the experiments in which the effects of nicotine sulfate (100 ug/kg/hr) and secretin (40 U/kg/hr) on gastric secretion were studied, the groups consisted of pentagastrin + saline (Group I), pentagastrin + nicotine (Group II), pentagastrin + secretin (Group III) and pentagastrin + secretin + nicotine (Group IV). Pentagastrin (0.5 ug/kg/min) was infused at the start of each experiment to all experimental animals and was continued throughout the entire five hour period at a rate of 1.08 ml/hr. Maximal gastric secretory response to this dose of pentagastrin was obtained after the first hour of infusion. At the end of this hour, the infusion of nicotine sulfate was started in Groups II and IV and was continued for the remaining four hours. At the end of the second hour of infusion, secretin was infused along with pentagastrin in Group III and with pentagastrin + nicotine sulfate in Group IV for the remaining three hours. Saline was infused in Group I and at various hours in the other groups to insure the infusion of a constant total fluid volume in all animals. Figure 1 illustrates these groups and the intervals of infusion

in each for these experiments.

Results are presented as mean values from 15-19 rats. Significance of the difference in gastric juice volume, acid output, pepsin concentration and pepsin output in response to secretin or nicotine + secretin between control (Group I) and experimentals (Groups II-IV) was determined by the Dunnett Multiple Comparison Procedure.

Experimental Procedure in the Dog

At the start of each experiment, pentagastrin (0.3 ug/kg/min) was infused (2.5 ml/hr) through a catheter inserted into a femoral vein. After the gastric secretory response elicited by pentagastrin remained stable for one hour, secretin (1.5 U/kg/hr) was added to the intravenous infusion for one hour. Following the removal of secretin and the reestablishment of a secretory plateau for one hour, nicotine sulfate was added to the infusion at a dose of 150 ug/kg/hr. After one hour of nicotine infusion, secretin was again added to the infusion along with nicotine. This second administration of secretin lasted one hour and fifteen minutes (i.e. five fifteen-minute collections). Pentagastrin, nicotine sulfate and secretin were all infused by means of separate syringes. Figure 2 illustrates the general experimental procedure for these experiments with nicotine and secretin.

To insure that the first secretin infusion (one hour) had no effect on the results obtained when nicotine and secretin were infused together, four additional control studies were performed during which secretin alone was added to the intravenous infusion of pentagastrin

at two intervals. The first infusion of secretin lasted one hour (i.e. four fifteen-minute collections) and corresponded to the same time intervals in the experimental studies. The second administration of secretin remained for one hour and fifteen minutes and gastric juice was also collected at corresponding time intervals. In this investigation these experiments were called secretin controls (Fig. 2). In these secretin control studies saline was added to the infusion in place of nicotine.

Three pentagastrin control experiments were also performed in which pentagastrin was infused throughout the entire six hour infusion period (Fig. 2). In these control tests saline was infused in place of secretin and nicotine sulfate.

Results from these studies are presented as mean values from at least three replicate experiments of each condition. Significance of the difference in gastric juice volume, acid concentration, acid output, pepsin concentration and pepsin output in response to secretin and nicotine + secretin was determined by the student t test.

Gastric Juice Collection and Analysis

Five hourly collections of gastric juice were made during each rat experiment and twenty-three successive fifteen minute collections were made during each dog experiment. Volume, acid and pepsin determinations were made for each collection. Gastric juice volume collected from the rats was read to the nearest 0.1 ml whereas that of the dog was made to the nearest 0.5 ml. The acidity was determined by titrating to pH 7 (glass electrode) with an Autoburette Titrator

(Copenhagen Radiometer, London Co.) with 0.1 N NaOH. Acid concentration is expressed as mEq/L and acid output as either mEq/hr (rat) or mEq/15 min (dog). Pepsin concentration was determined by the hemoglobin method (Autoanalyzer, Technicon Corp.) and is expressed as uEq tyrosine/ml. Pepsin output (which is the product of volume times pepsin concentration) is expressed as uEq tyrosine/hr for the rat studies and as uEq/15 min for the studies in the dog.

Figure 1. Periods of infusion of pentagastrin (0.5 ug/kg/min), nicotine sulfate (100 ug/kg/hr) and secretin (40 U/kg/hr) in Groups I-IV (13-19 rats). Collections of gastric juice were made at each hourly interval.

Group I = pentagastrin + saline; Group II = pentagastrin + nicotine; Group III = pentagastrin + secretin; Group IV = pentagastrin + nicotine + secretin.

--

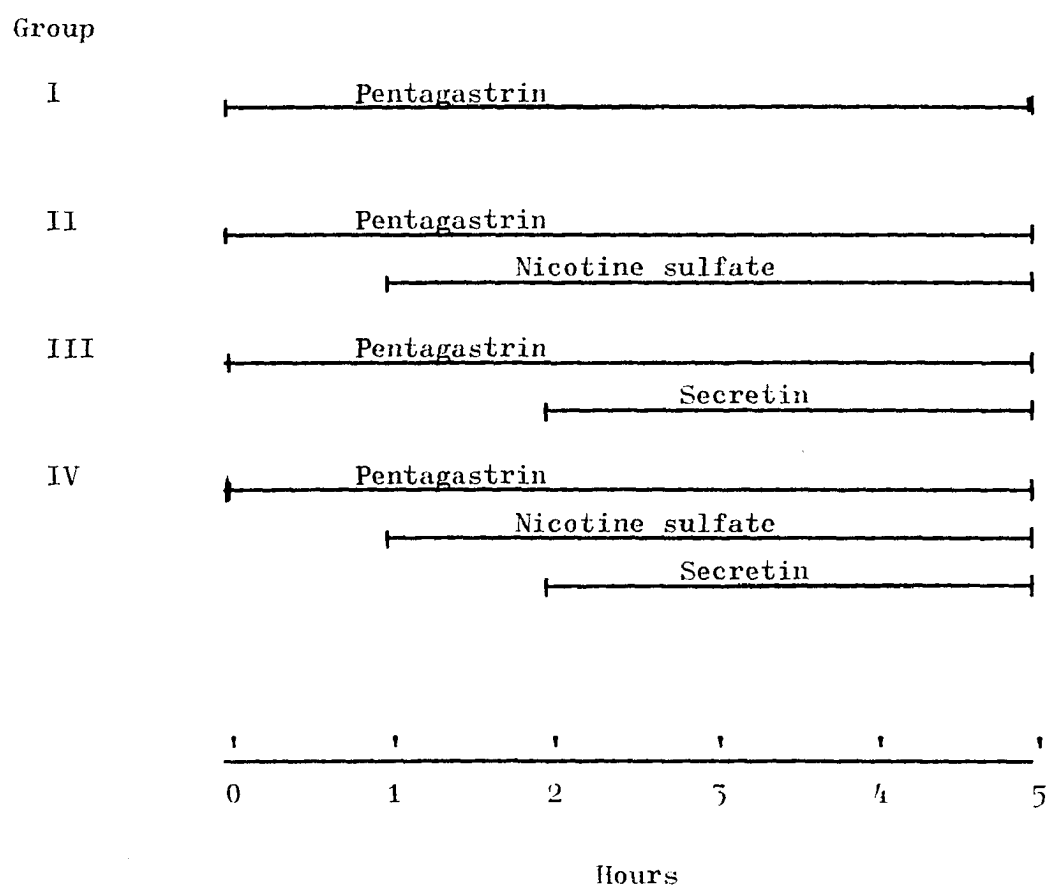


Figure 2. Periods of infusion of pentagastrin (0.5 ug/kg/min), nicotine sulfate (150 ug/kg/hr) and secretin (1.5 U/kg/hr) in the dog experiments. Collections of gastric juice were made at fifteen minute intervals.

Experiment:

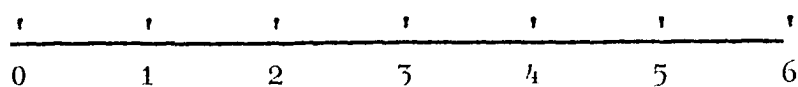
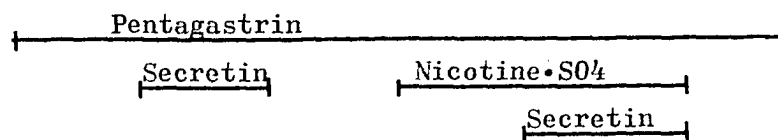
Pentagastrin
control



Secretin
control



Secretin +
Nicotine
experiments



Hours

RESULTS

Studies in the Rat

The increase in gastric secretion in thirteen chronic fistula rats in response to pentagastrin administration (0.5 ug/kg/min) is shown in Figure 3. These results confirm the secretagogue effect of pentagastrin. Table 1 shows that volume, acid output and pepsin output were significantly increased ($P < .01$) from the non-stimulated controls receiving only saline. It was necessary to combine the first two and the last three hourly collections in order to obtain enough gastric juice to make acid and pepsin determinations for the saline controls. Similar results concerning the effect of pentagastrin on gastric secretion in fistula rats have been reported by other investigators (Adashek and Grossman, 1962; Barrett, 1966; Lee and Thompson, 1968 and 1969).

The effect of various doses of secretin (30, 40 and 60 U/kg/hr) on pentagastrin-stimulated gastric secretion was also studied. Figure 4 shows a dose dependent inhibition of the volume of gastric juice collected at hourly intervals. Maximal inhibition was attained two hours after the start of secretin infusion. The results in Table 2 also indicate a dose related inhibition of both volume and acid output. All of the doses of secretin tested produced a significant inhibition ($P < .01$) of the volume and acid output. A dose of 40 U/kg/hr was chosen for use in the subsequent studies with nicotine. This dose of secretin decreased volume and acid output by approximately 60

percent (Table 2). Pepsin concentration was significantly increased ($P < .01$) at each dose of secretin. Pepsin output was significantly higher ($P < .05$) than control values at a dose of 50 U/kg/hr as compared to the other doses (40 and 60 U/kg/hr) which remained close to control values.

Table 5 shows the effect of an infusion of nicotine sulfate (100 ug/kg/hr- Group II), secretin (40 U/kg/hr- Group III) and nicotine + secretin (Group IV) on volume, acid output, pepsin concentration and pepsin output. At each hourly interval following the infusion of nicotine sulfate (Group II), no significant difference from the pentagastrin controls was observed in any of the parameters studied. Maximal inhibition of pentagastrin-stimulated gastric secretion was observed two hours after the addition of secretin to the infusion (Group III). Volume and acid output were significantly reduced ($P < .01$) from the controls (Fig. 5 and 6) and were inhibited approximately 65 percent and 59 percent respectively during the final two hours of secretin infusion. Pepsin concentration was significantly higher ($P < .01$) during each hour of secretin infusion (Fig. 7) whereas total pepsin output remained unchanged (Fig. 8). The combination of nicotine + secretin (Group IV) produced no significant change from Group III (i.e. the infusion of secretin alone). Again, maximal inhibition of pentagastrin-stimulated acid secretion was observed two hours after the addition of secretin to the infusion (Fig. 5 and 6). Volume was inhibited 61 percent and acid output 47 percent and both were significantly lower ($P < .01$) than that of the control group. Pepsin concentration was significantly higher ($P < .01$) during the last two hours of secretin infusion (Fig. 7). Pepsin output was significantly

increased ($P < .05$) following the second hour of the nicotine + secretin infusion but returned to control values during the last hour of infusion (Fig. 8).

Studies in the Dog

As described previously, the secretin + nicotine experiments consisted of two administrations of secretin to a dog stimulated with pentagastrin. Secretin was first infused alone for one hour and was later infused with nicotine. The first intravenous infusion of secretin (1.5 U/kg/hr) significantly decreased ($P < .01$) both the volume and acid output of pentagastrin-stimulated gastric secretion (Fig. 9 and 10). Maximal inhibition of acid secretion at this dose was observed thirty minutes after the start of secretin infusion and lasted throughout the remaining period of infusion. Upon removal of secretin from the infusion, gastric secretion gradually rose and reached normal values within thirty minutes. The subsequent infusion of nicotine sulfate (150 ug/kg/hr) produced no effect on gastric acid secretion. Figures 9 and 10 indicate that the second addition of secretin (in the presence of nicotine) failed to produce a significant decrease in either gastric juice volume or acid output, although both of these parameters decreased slightly. During the periods of maximal inhibition, secretin alone caused a significant decrease ($P < .01$) in acid concentration, whereas secretin + nicotine did not (Table 4). Figure 12 shows that during the periods of maximal inhibition (i.e. thirty minutes after the start of secretin) the volume of gastric juice was decreased by 53 percent from the pentagastrin controls during the

Filmed as received
without page(s) 30.

UNIVERSITY MICROFILMS.

first administration of secretin and was decreased by only 28 percent when secretin was added to the infusion of nicotine. Acid output was decreased by 56 percent during the infusion of secretin and 27 percent during the secretin + nicotine infusion (Fig. 13).

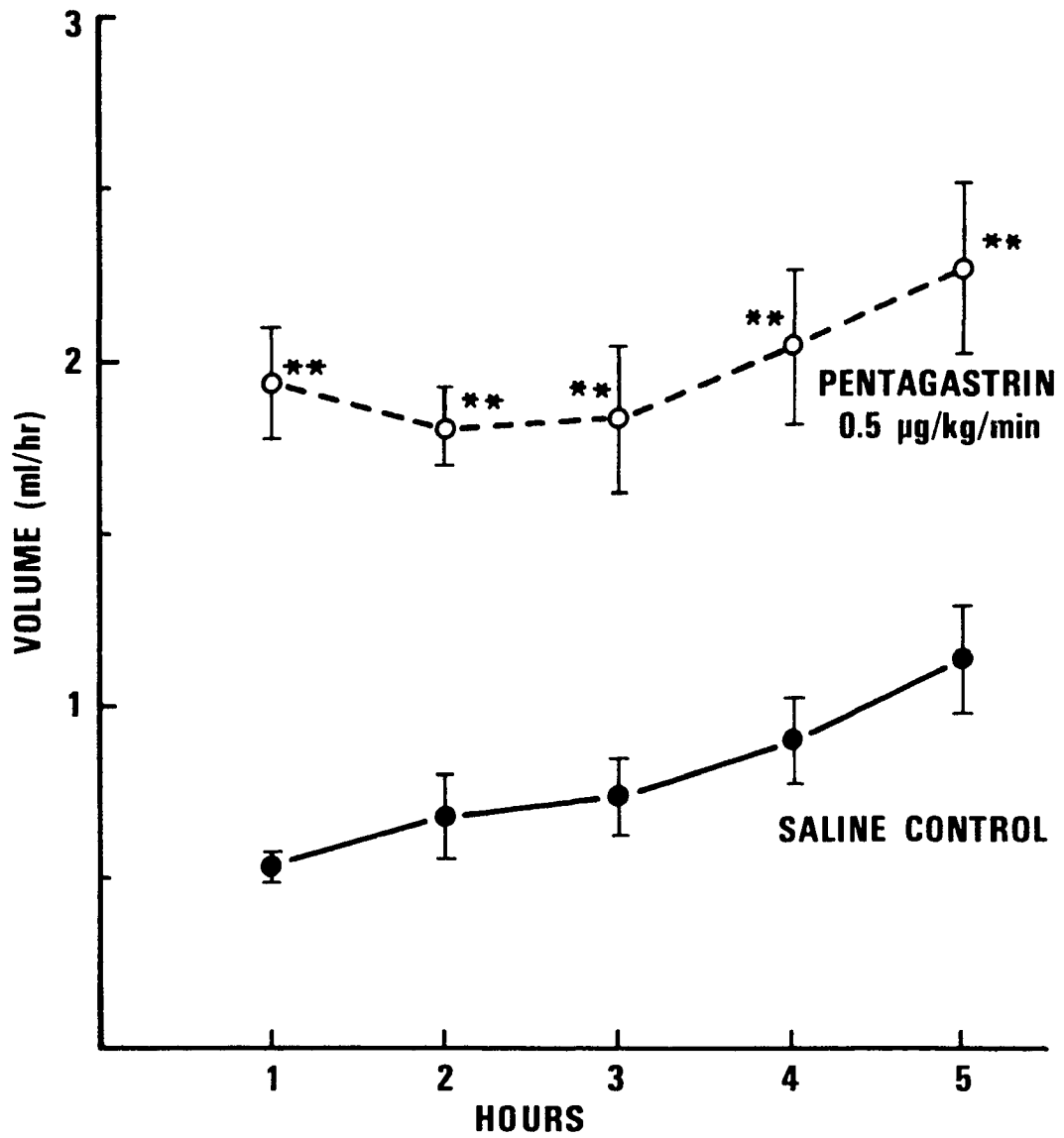
The effect of secretin and secretin + nicotine on pepsin concentration and pepsin output is shown in Figures 14 and 15. The results on pepsin concentration were confusing. Pepsin concentration did not change during the infusion of secretin but when secretin was added to the infusion with nicotine, pepsin concentration appeared to gradually increase (Fig. 14). The first infusion of secretin alone produced a significant decrease ($P < .05$) in pepsin output during these experimental runs. However, the second addition of secretin to the infusion with nicotine did not produce a significant decrease (Fig. 15). Figure 16 shows a 55 percent decrease in pepsin output was produced by secretin whereas only a 7 percent decrease was produced by the nicotine + secretin infusion.

In the secretin control experiments, secretin was added to the infusion of pentagastrin at two intervals. Secretin infusion produced a significant decrease ($P < .05$ and $P < .01$) of volume and acid output during both the first and second administrations (Fig. 9 and 10). Acid concentration was also significantly decreased (Table 4). During the periods of maximal inhibition, secretin produced a 52 percent decrease in volume during the first interval of infusion and a 49 percent decrease during the second administration (Fig. 12). Figure 13 shows that acid output was decreased 53 percent and 51 percent during the first and second administrations respectively.

Figures 14 and 15 show the results of these secretin control experiments on pepsin secretion. The first infusion of secretin showed no effect on pepsin concentration. It is difficult to make any correlation concerning the second period of secretin infusion since a sharp decrease in pepsin concentration resulted thirty minutes after secretin was added to the infusion (Fig. 14). This decrease was then followed by a gradual increase in pepsin concentration. Pepsin output was decreased 50 percent and 39 percent respectively (Fig. 16).

Figure 3. Effect of the infusion of pentagastrin (0.5 ug/kg/min) on the volume of gastric juice collected at hourly intervals. Each point represent the mean of 13 rats in the pentagastrin group and 12 rats in the saline control group (nonstimulated). Significance of the difference from the saline controls is expressed as ** when $P < .01$. Horizontal bars above and below each hour indicate limits of the standard error of the mean for each point.

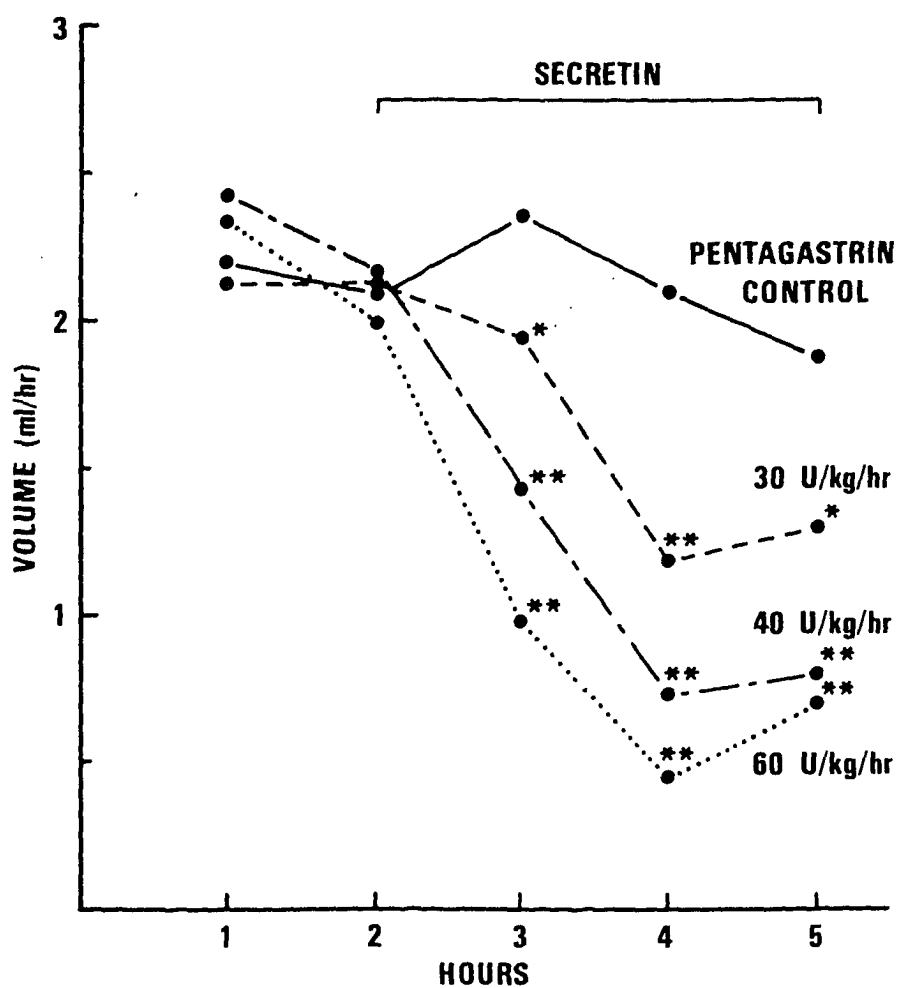
Table 1. Comparison of volume (ml/2 or 3 hr), acid output (mEq/2 or 3 hr) and pepsin output (uEq tyrosine/2 or 3 hr) of nonstimulated fistula rats receiving saline with those infused with pentagastrin (0.5 ug/kg/min). Collections of gastric juice from the first two and last three hours of infusion were combined. Significance of the difference is expressed as ** when $P < .01$.



	SALINE (CONTROL)		PENTAGASTRIN	
	1+2 HR	3+4+5 HR	1+2 HR	3+4+5 HR
VOLUME	1.17	2.84	3.75**	6.16**
ACID OUTPUT	.21	.40	.53**	.90**
PEPSIN OUTPUT	4.31	8.37	7.47**	13.36**

Figure 4. Effect of the infusion of various doses of secretin (30, 40 and 60 U/kg/hr) on the volume of gastric juice collected at hourly intervals in fistula rats stimulated with pentagastrin. Each point represents the mean values for gastric juice volume from 10-15 animals in each group. Significance of the difference from the pentagastrin controls is expressed as * when $P < .05$ and as ** when $P < .01$.

Table 2. Effect of the infusion of various doses of secretin (30, 40 and 60 U/kg/hr) on volume, acid output, pepsin concentration and pepsin output during the last two hours of infusion (i.e. 4 + 5 hours combined). The percent change from the pentagastrin control group is also indicated. Significant differences from the pentagastrin controls are expressed as * when $P < .05$ and as ** when $P < .01$.



PENTAGASTRIN (CONTROL)		SECRETIN - U/KG/HR		
		30	40	60
		%	%	%
		CHANGE	CHANGE	CHANGE
VOLUME (ml/2 HR)	4.01	2.51** -37	1.52** -62	1.18** -71
ACID OUTPUT (mEq/2 HR)	.58	.35** -41	.23** -61	.16** -72
PEPSIN CONC. (uEq Tyr/ml)	1.92	4.72** +146	5.18** +170	4.69** +144
PEPSIN OUTPUT (uEq Tyr/2 HR)	7.34	11.59* +58	8.77 +19	6.25 -15

Table 3. Summary of the effect of an infusion of nicotine sulfate (100 ug/kg/hr), secretin (40 U/kg/hr) and nicotine + secretin on pentagastrin-stimulated gastric secretion in 13-19 fistula rats. Volume, acid output, pepsin concentration and pepsin output from each hourly interval are shown. Significant differences are expressed as * ($P < .05$) and ** ($P < .01$) when treated groups (II-IV) were compared against the pentagastrin controls (I).

Group I = pentagastrin + saline; Group II = pentagastrin + nicotine; Group III = pentagastrin + secretin; Group IV = pentagastrin + nicotine + secretin.

Note: Volume, acid output, pepsin concentration and pepsin output of Group IV showed no significant difference when compared to Group III.

		Group	<u>I</u>	<u>II</u>	<u>III</u>	<u>IV</u>
No. of Animals			19	15	17	15
Hour						
1	Volume (ml/hr)		2.17	2.54	2.42	2.45
	Acid output (mEq/hr)		.28	.32	.32	.31
	Pepsin concentration (uEq tyrosine/ml)		2.58	2.39	2.42	2.05
	Pepsin output (uEq tyrosine/hr)		5.50	5.57	5.85	4.81
2	Volume		1.96	2.30	2.41	2.00
	Acid output		.28	.31	.36	.27
	Pepsin concentration		2.04	1.92	2.21	1.82
	Pepsin output		4.02	4.40	5.51	5.54
3	Volume		1.75	1.88	1.48	1.14 *
	Acid output		.25	.29	.22	.17
	Pepsin concentration		2.09	2.06	3.04 **	2.64
	Pepsin output		3.75	3.87	5.29	3.90
4	Volume		1.69	1.63	.74 **	.85 **
	Acid output		.24	.26	.12 **	.14 **
	Pepsin concentration		2.19	2.11	4.84 **	4.59 **
	Pepsin output		3.65	3.97	4.57	5.32 *
5	Volume		1.63	1.61	.85 **	.90 **
	Acid output		.25	.27	.15 **	.15 **
	Pepsin concentration		2.23	2.19	4.78 **	4.11 **
	Pepsin output		4.08	3.94	4.61	5.40

— .

Figure 5. Effect of nicotine sulfate (100 ug/kg/hr- Group II), secretin (40 U/kg/hr- Group III) and nicotine + secretin (Group IV) on the volume of gastric secretion from rats stimulated with pentagastrin (0.5 ug/kg/min). Significant differences from the control (Group I) are expressed as * when $P < .05$ and ** when $P < .01$. Horizontal bars above and below each hour indicate the limits of the standard error of the mean for each point.

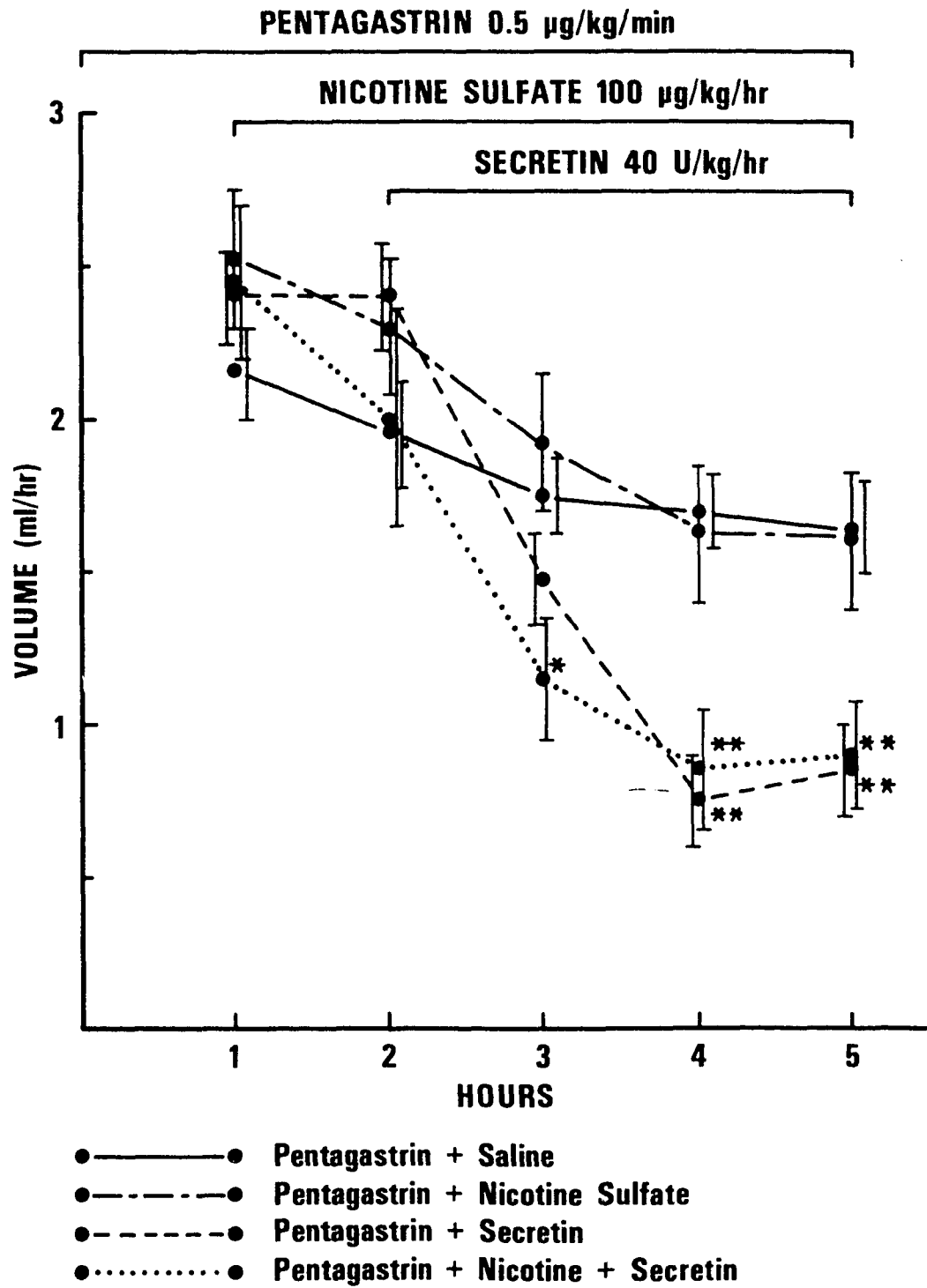


Figure 6. Effect of nicotine sulfate (100 ug/kg/hr- Group II), secretin (40 U/kg/hr- Group III) and nicotine + secretin (Group IV) on acid output from fistula rats stimulated with pentagastrin (0.5 ug/kg/min). Significant differences from the control (Group I) are expressed as * when $P < .05$ and ** when $P < .01$. Horizontal bars above and below each hour indicate the limits of the standard error of the mean for each point.

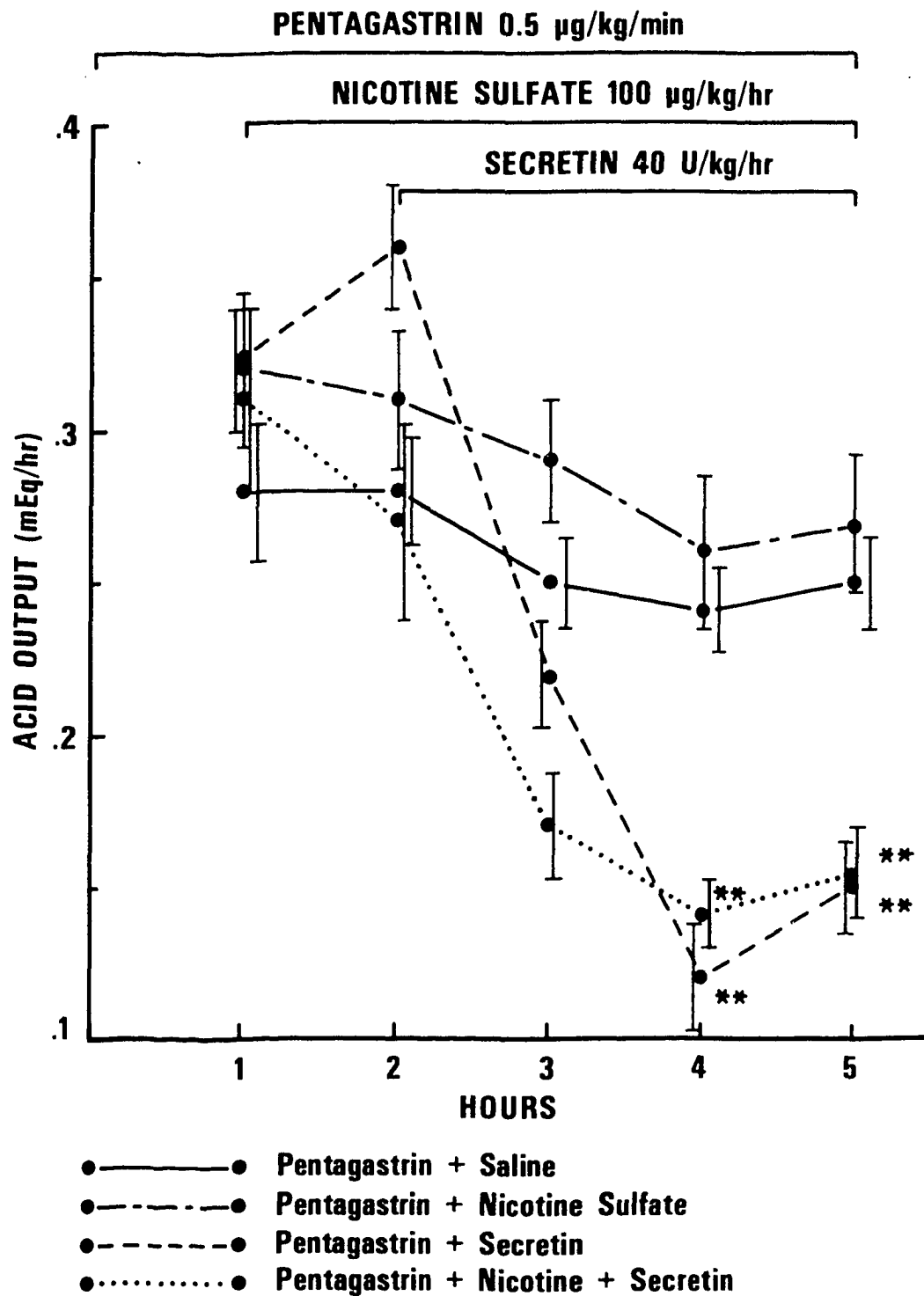


Figure 7. Effect of nicotine sulfate (100 ug/kg/hr- Group II), secretin (40 U/kg/hr- Group III) and nicotine + secretin (Group IV) on pepsin concentration from fistula rats stimulated with pentagastrin (0.5 ug/kg/min). Significant differences from the control (Group I) are expressed as * when $P < .05$ and ** when $P < .01$. Horizontal bars above and below each hour indicate the limits of the standard error of the mean for each point.

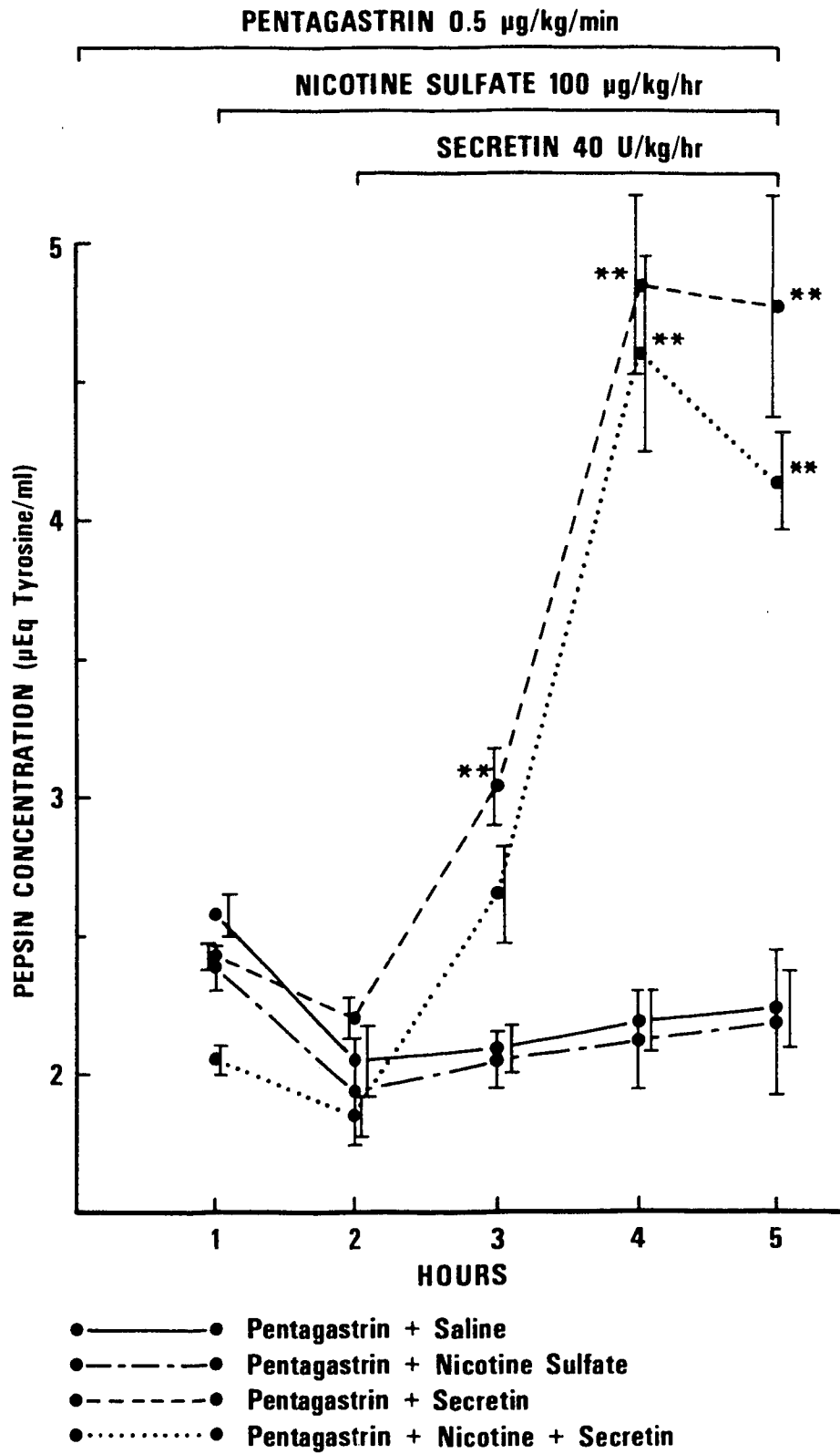


Figure 8. Effect of nicotine sulfate (100 ug/kg/hr- Group II), secretin (40 U/kg/hr- Group III) and nicotine + secretin (Group IV) on pepsin output. Significant differences from the control (Group I) are expressed as * when $P < .05$ and ** when $P < .01$. Horizontal bars above and below each hour indicate the limits of the standard error of the mean for each point.

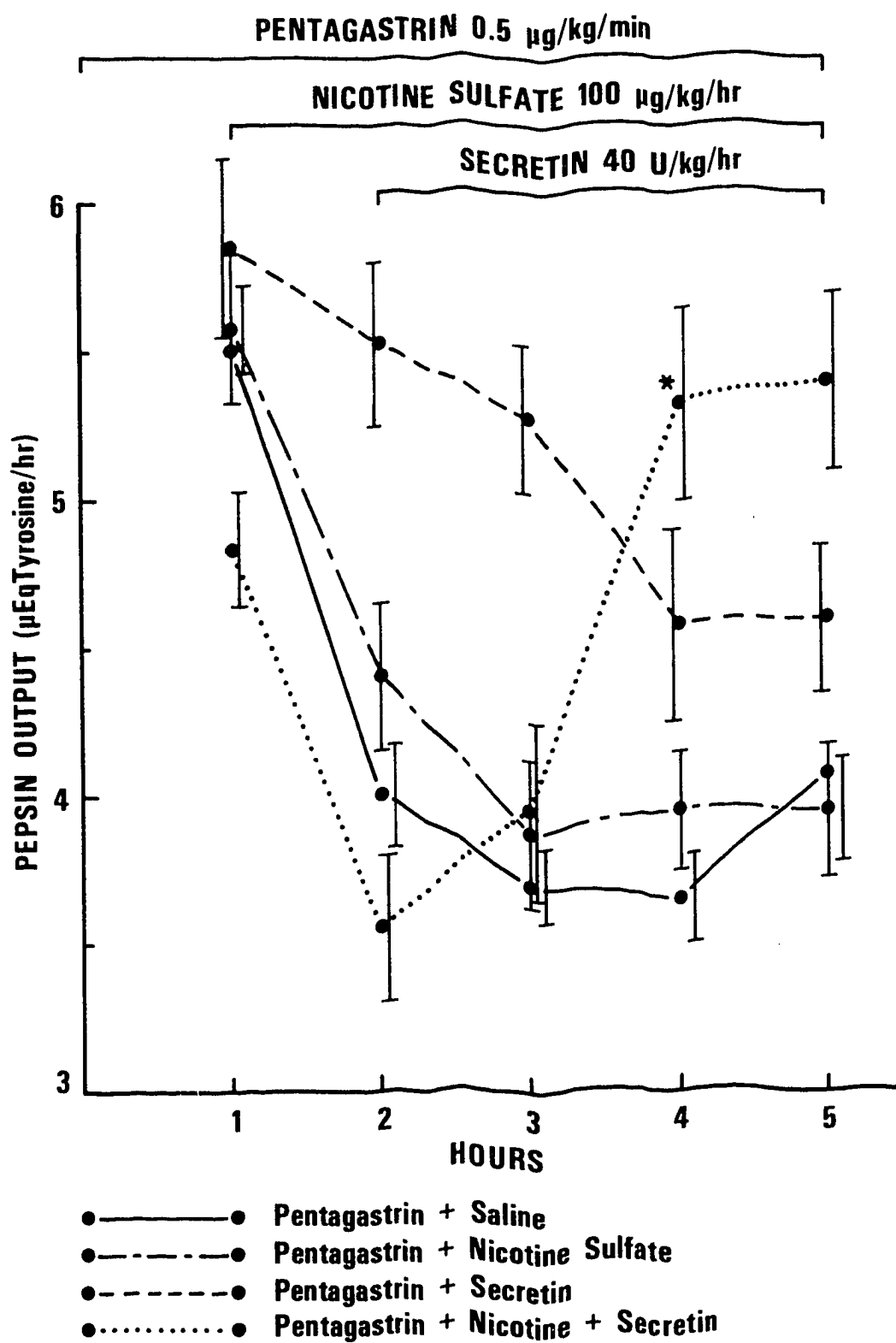


Figure 9. Results obtained from the secretin control and secretin + nicotine experiments on the volume of gastric juice collected from the Heidenhain pouch of a dog stimulated with pentagastrin. Collections of gastric juice were made at 15-minute intervals. Each point represents the mean of three pentagastrin controls, four secretin controls and six secretin + nicotine experiments. Significant differences from the pentagastrin controls are expressed as * when $P < .05$ and ** when $P < .01$.

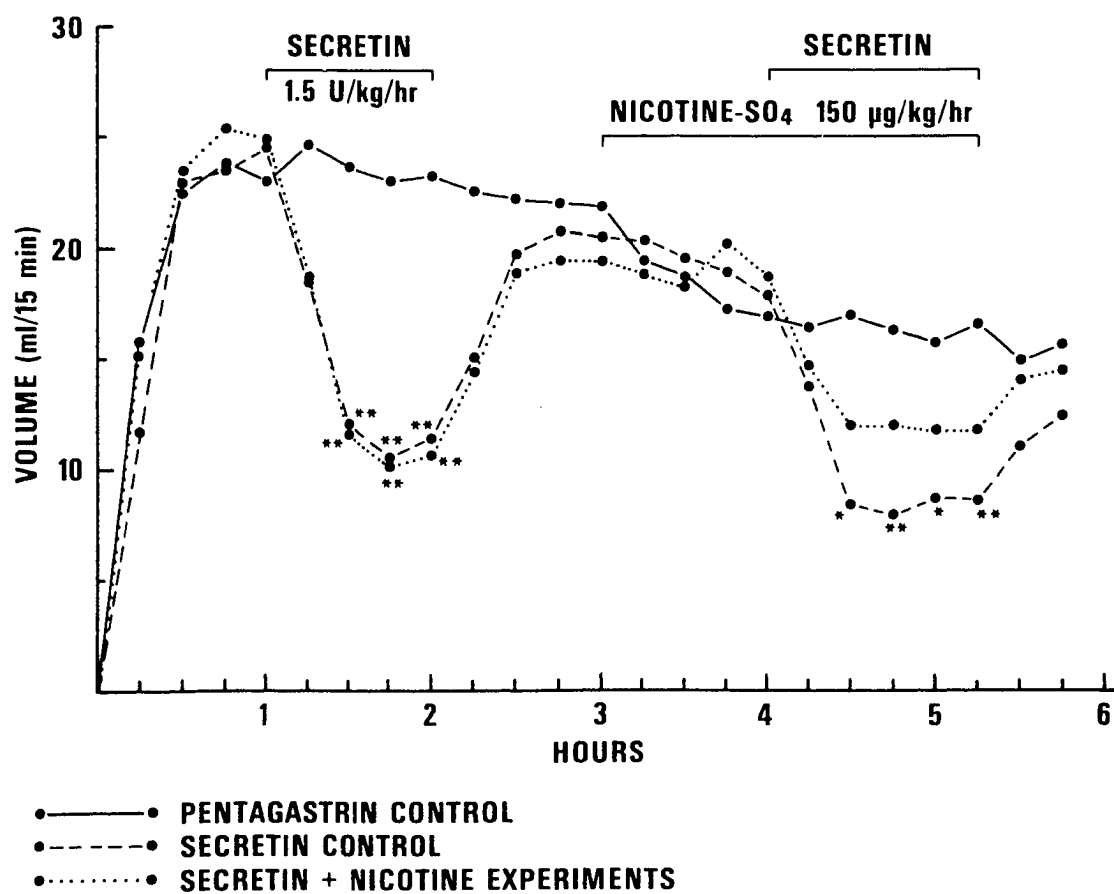


Figure 10. Results obtained from the secretin control and secretin + nicotine experiments on acid output of gastric juice collected from the Heidenhain pouch of a dog stimulated with pentagastrin. Collections were made at 15-minute intervals. Each point represents the mean of three pentagastrin controls, four secretin controls and six secretin + nicotine experiments. Significant differences from the pentagastrin controls are expressed as * when $P < .05$ and ** when $P < .01$.

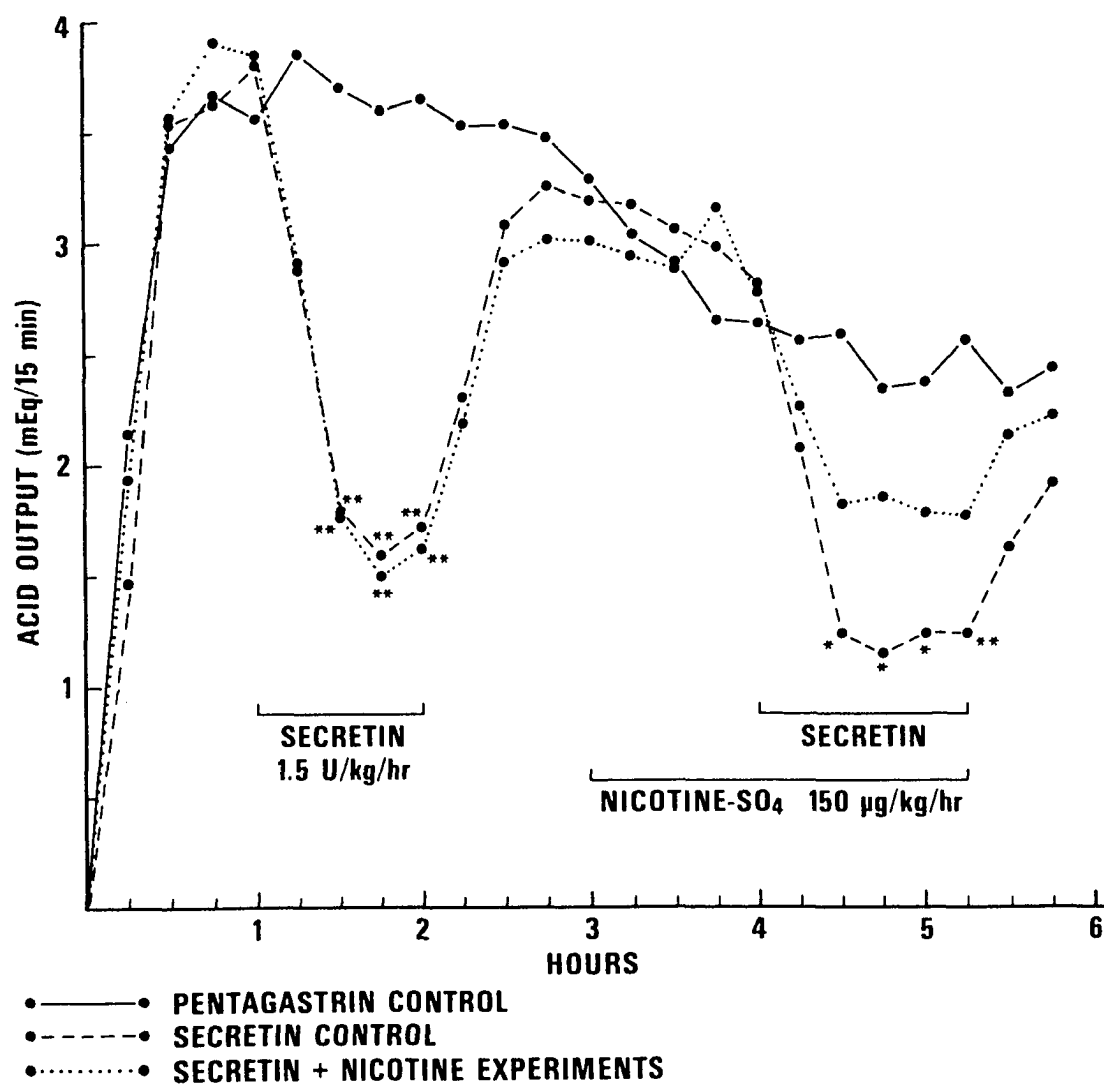


Table 4. Percent decrease in acid concentration from the pentagastrin controls during the periods of maximal inhibition from the secretin control and secretin + nicotine experiments in the dog. Each value is the mean of the last three 15-minute collections during the first administration of secretin and of the last four 15-minute collections during the second administration. Significant differences from the pentagastrin controls are expressed as * when $P < .05$ and as ** when $P < .01$.

EXPERIMENT	1ST ADMINISTRATION - SECRETIN		2ND ADMINISTRATION - SECRETIN OR NICOTINE + SECRETIN	
	ACID CONC. (mEq/LITER)	% DECREASE	ACID CONC.	% DECREASE
PENTAGASTRIN CONTROL -	157 ± 1	---	154 ± 2	---
SECRETIN CONTROL -	149 ± 2*	5	142 ± 3*	8
SECRETIN + NICOTINE - EXPERIMENTS	148 ± 1**	6	149 ± 3	3

Figure 12. Percent change from the pentagastrin controls in the volume of gastric juice collected during the periods of maximal inhibition from the secretin control and secretin + nicotine experiments. Each bar represents the mean of the last three 15-minute collections during the first administration of secretin and from the last four 15-minute collections during the second administration of secretin alone (secretin control) or secretin in the presence of nicotine.

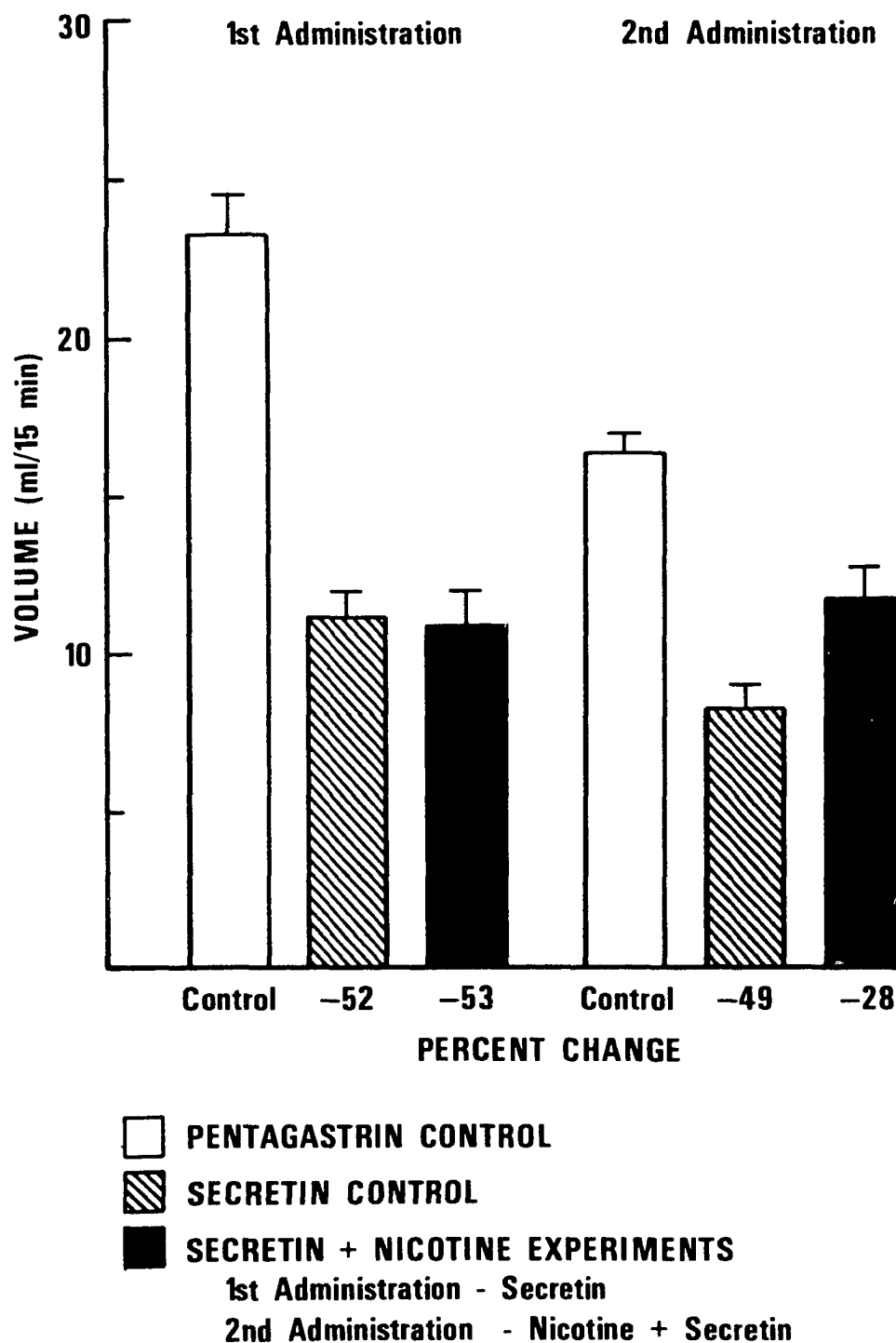


Figure 13. Percent change from the pentagastrin controls in acid output during the periods of maximal inhibition from the secretin control and secretin + nicotine experiments. Each bar represents the mean of the last three 15-minute collections during the first administration of secretin alone (secretin control) or secretin in the presence of nicotine.

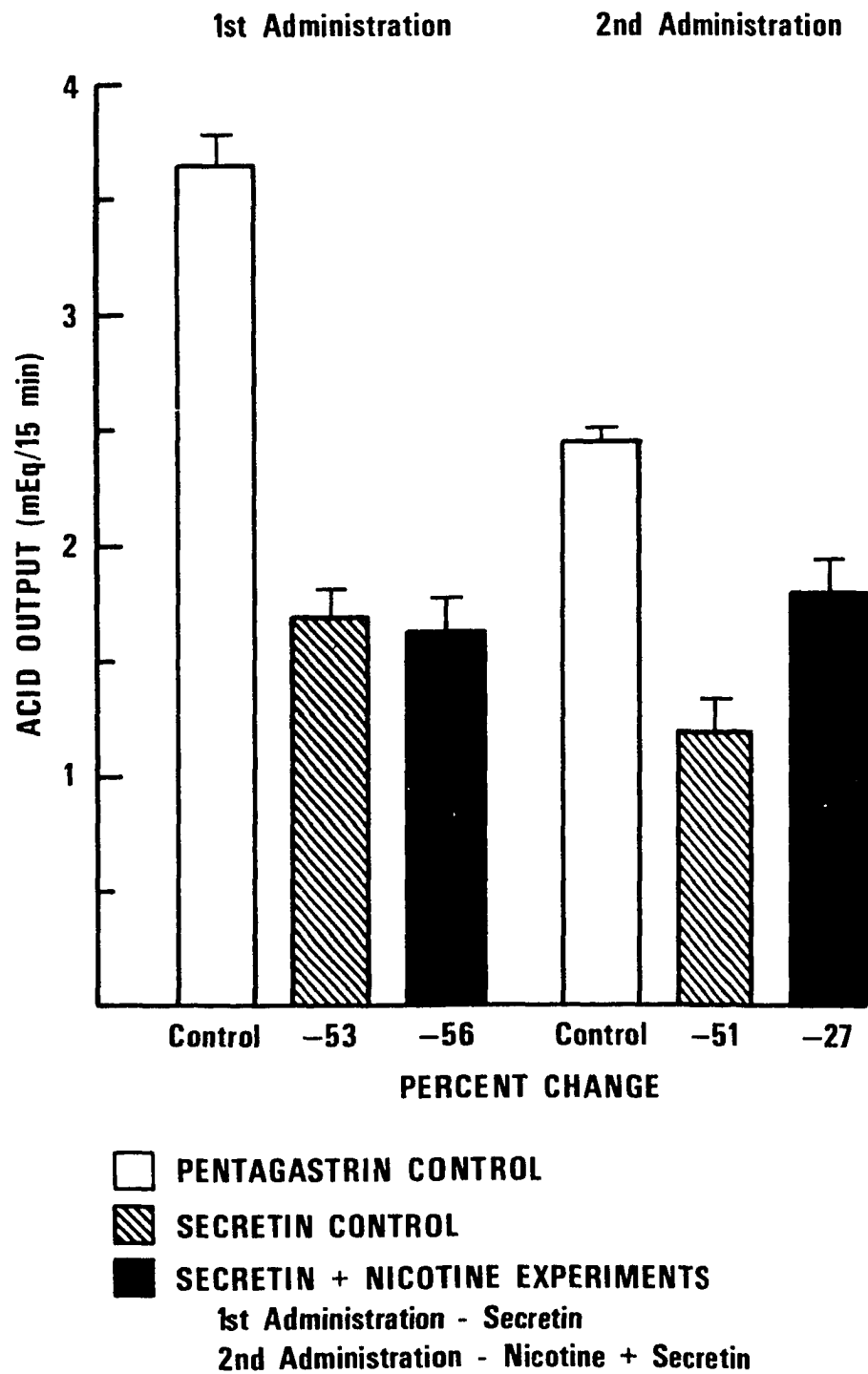


Figure 14. Results obtained from the secretin control and secretin + nicotine experiments on pepsin concentration of gastric juice collected from the Heidenhain pouch of a dog stimulated with pentagastrin. Collections were made at 15-minute intervals. Each point represents the mean of three pentagastrin controls, four secretin controls and six secretin + nicotine experiments. Significant differences from the pentagastrin controls are expressed as * when $P < .05$ and ** when $P < .01$.

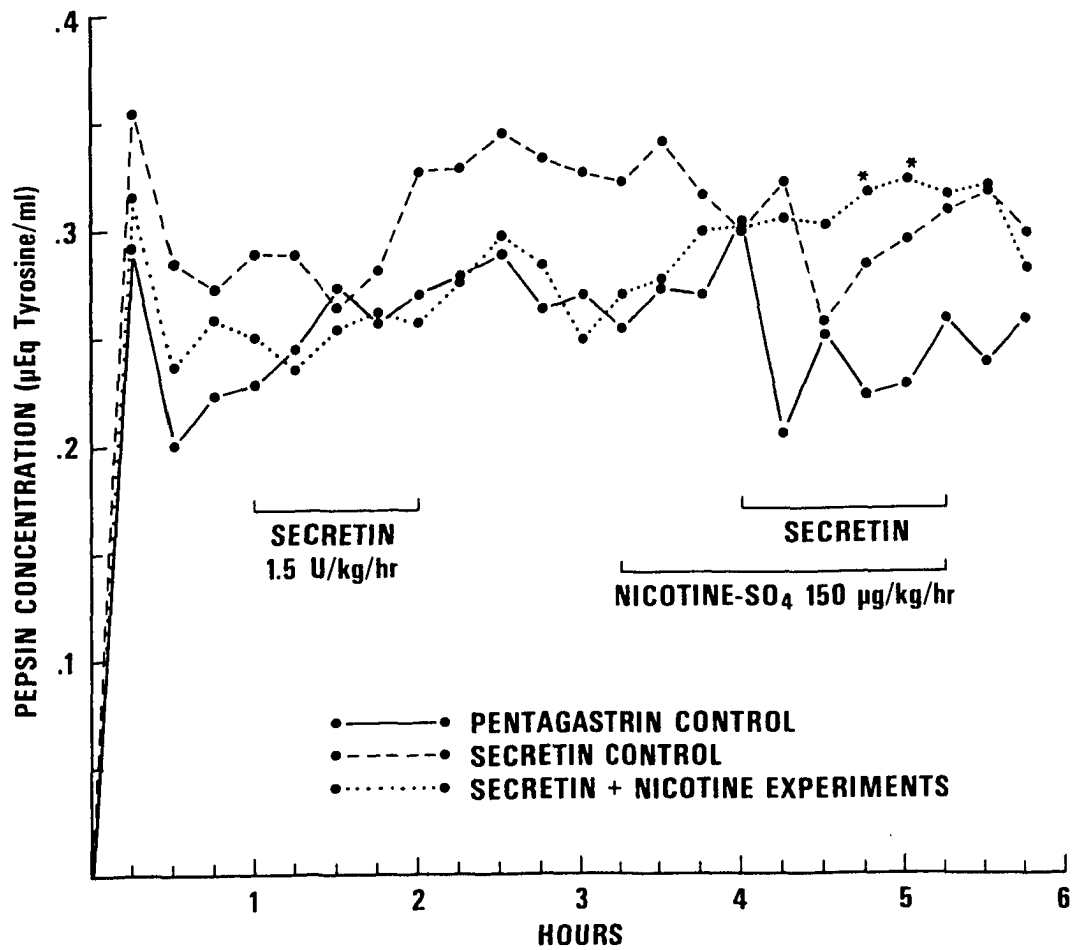


Figure 15. Results obtained from the secretin control and secretin + nicotine experiments on the pepsin output of gastric juice collected from the Heidenhain pouch of a dog stimulated with pentagastrin. Collections were made at 15-minute intervals. Each point represents the mean of three pentagastrin controls, four secretin controls and six secretin + nicotine experiments. Significant differences from the pentagastrin controls are expressed as * when $P < .05$ and ** when $P < .01$.

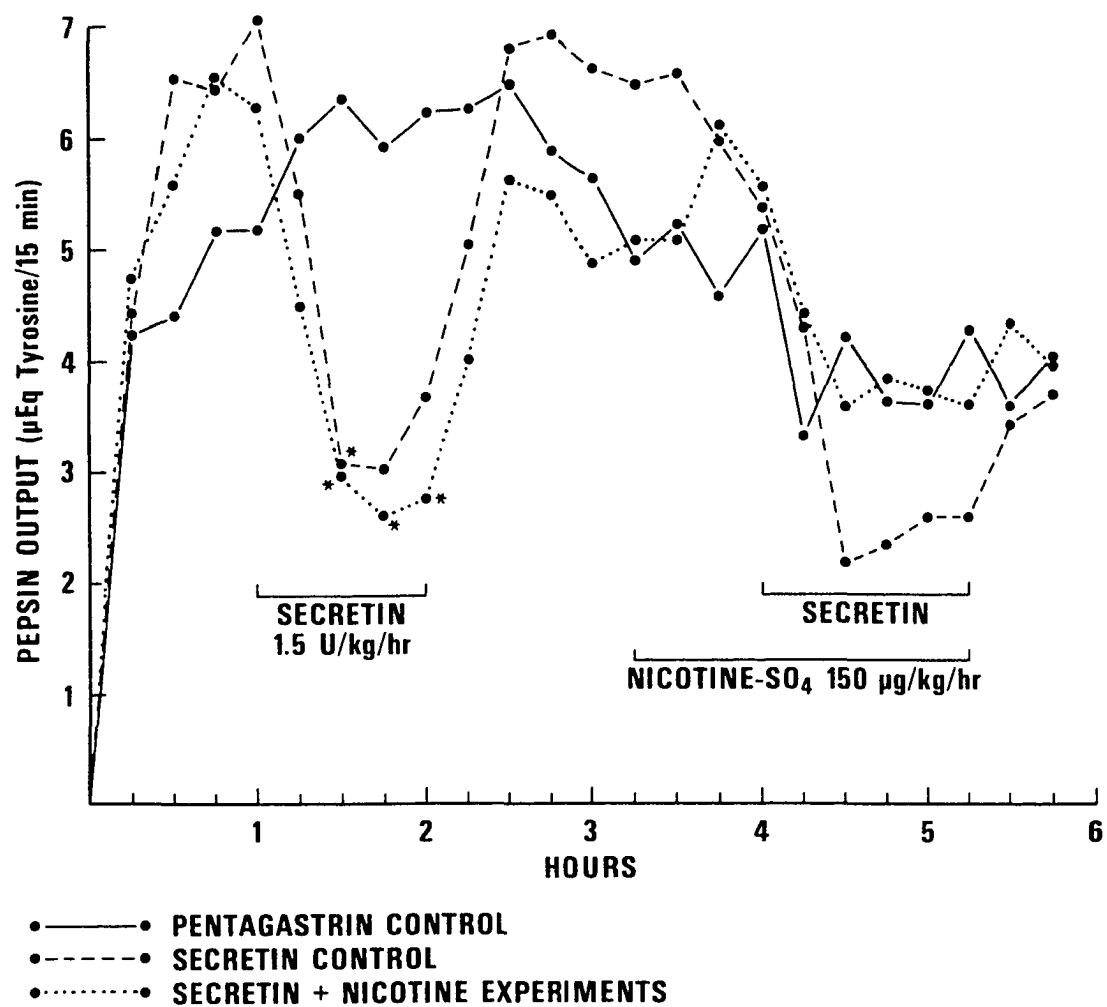
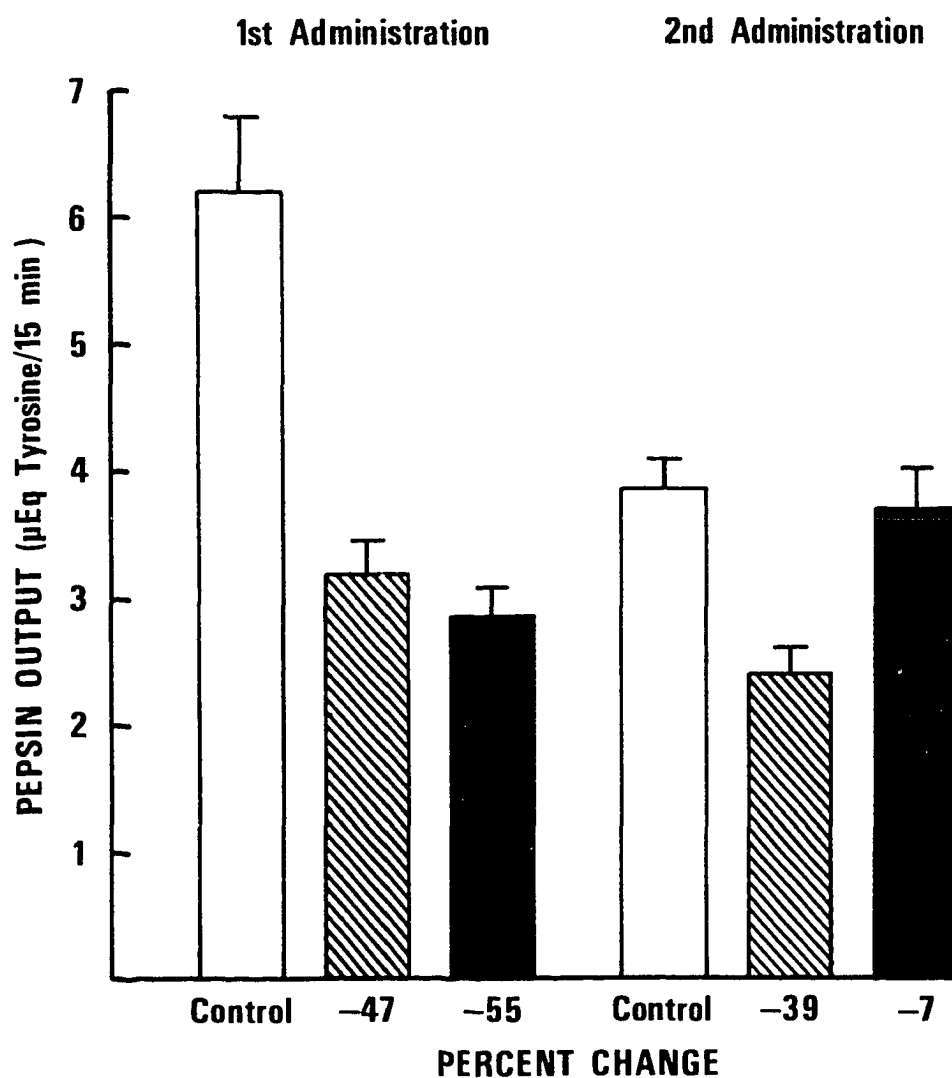


Figure 16. Percent change from the pentagastrin controls in pepsin output during the periods of maximal inhibition from the secretin control and secretin + nicotine experiments. Each bar represents the mean of the last three 15-minute collections during the first administration of secretin and of the last four 15-minute collections during the second administration of secretin alone (secretin control) or secretin in the presence of nicotine.



- ☐ PENTAGASTRIN CONTROL
 - ☒ SECRETIN CONTROL
 - ☒ SECRETIN + NICOTINE EXPERIMENTS
- 1st Administration - Secretin
2nd Administration - Nicotine + Secretin

DISCUSSION

Studies in the Rat

The results of this investigation confirmed that exogenous secretin inhibits pentagastrin-stimulated gastric acid secretion in chronic fistula rats. The response obtained was dose related. A dose of 40 U/kg/hr of secretin was used throughout the remaining studies with nicotine since it was a submaximal dose which produced a significant effect on gastric secretion. The subcutaneous infusion of secretin at this dose inhibited pentagastrin-stimulated gastric juice volume by 62 percent and acid output by 61 percent. Our results are similar to those obtained by Tumpson and Johnson (1969) who also observed that a subcutaneous injection of secretin (75 U/kg) inhibited the gastric acid response of fistula rats stimulated with maximal and supramaximal doses of pentagastrin. A similar inhibition of gastric secretion stimulated with pentagastrin was obtained by Chey (1970). In these studies, a synthetic secretin was used at a dose of 2 U/kg/hr which produced a 50 percent decrease in the maximal acid response elicited by pentagastrin. They also showed that the intraduodenal infusion of hydrochloric acid (i.e. release of endogenous secretin) caused a similar degree of inhibition. This inhibitory effect of secretin was also obtained by Chey on fistula rats prepared with innervated pouches (Pavlov). The intravenous infusion of 37.5 U/kg/hr of secretin decreased the acid response of the innervated pouch stimulated with gastrin by approximately 45 percent. This observation

agrees closely with the results obtained in our study with the chronic fistula rat which also has an innervated stomach. At a slightly higher dose of secretin (40 U/kg/hr), we obtained a 61 percent decrease in acid secretion.

Secretin has also been shown to stimulate pepsin secretion in the rat by increasing both the concentration and output of pepsin (Johannson et al., 1972). Our results showed that doses of 30-60 U/kg/hr increased markedly, the concentration of pepsin in fistula rats stimulated with pentagastrin (Table 2). However, pepsin output was significantly higher than in the pentagastrin controls only at a dose of 30 U/kg/hr. At doses of 40 and 60 U/kg/hr, pepsin output was lower than at a dose of 30 U/kg/hr and was not significantly changed from the pentagastrin controls. Since pepsin output is the product of pepsin concentration times volume, the lower values for pepsin output at the higher doses of secretin (40 and 60 U/kg/hr) was due primarily to the greater decrease in the volume of gastric juice. Johannson et al. (1972) have shown that the continuous infusion of secretin (37.5 U/kg/hr) at a dose which decreased gastric acid secretion by 40-45 percent, caused a significant increase in total pepsin secretion from the response of the innervated pouch stimulated with gastrin. In our investigation, the infusion of 40 U/kg/hr caused a nonsignificant effect, increasing pepsin output by 19 percent (Table 2). However, this dose inhibited gastric juice volume by 60 percent which is a greater inhibition than that obtained by Johannson et al. This greater decrease in gastric juice volume which we observed, resulted in a less marked increase in pepsin output.

Our results showed that in the rat, nicotine did not alter the effect of exogenous secretin on gastric acid secretion (volume and acid output). The subcutaneous infusion of secretin at a submaximal dose of 40 U/kg/hr significantly decreased both the volume and acid output of gastric juice. The combination of nicotine sulfate with secretin did not influence this inhibition produced by secretin. Pepsin concentration was significantly higher following the infusion of secretin. Again, when nicotine sulfate was infused with secretin, nicotine failed to influence the rise in pepsin concentration produced by secretin. Pepsin output was not significantly changed from the pentagastrin controls following the infusion of either secretin alone (Group III) or nicotine + secretin (Group IV).

The dose of nicotine employed in these studies (100 ug/kg/hr) was used for the following reasons: a) First, in the dog, this dose had been shown to have no effect on gastric secretion stimulated with pentagastrin (Konturek et al., 1970). b) Second, in our studies, nicotine sulfate infused subcutaneously in fistula rats at a dose of 100 ug/kg/hr did not affect gastric secretion stimulated with pentagastrin (Table 3). Higher doses of nicotine sulfate (300-600 ug/kg/hr) were also tested but were observed to cause restlessness in the animals and also to inhibit gastric secretion. A similar inhibitory effect of high doses of nicotine on gastric secretion has also been shown in the cat (Konturek et al., 1971). In this study by Konturek, retching and restlessness were also observed. In our studies, any effect of nicotine itself on gastric secretion would be undesirable since this would interfere with the gastric effects of secretin.

c) Third, although nicotine at 100 ug/kg/hr showed no effect on gastric secretion, it is a dose which has been shown to increase the incidence and severity of duodenal ulcers in rats produced by the continuous infusion of pentagastrin (Robert, 1973). Therefore, this was a dose which, in the rat, was absorbed in amounts sufficient enough to produce an observable physiological response. d) Fourth, Thompson (1970) has also shown that the subcutaneous infusion of nicotine (100 ug/kg/hr) had no effect on pentagastrin-stimulated gastric secretion in fistula rats. He did observe that nicotine increased pepsin output in rats stimulated with both maximal and submaximal doses of pentagastrin. The results of our investigation did not confirm this stimulatory effect of nicotine on pepsin secretion.

The opposite results obtained in the present investigation whereby nicotine partially blocks the effect of exogenous secretin on gastric secretion in the dog but not in the rat, may be due to a true difference between these two animal species. The possibility also exists that with the chronic fistula preparation in the rat, not all of the acid flowing from the main stomach was collected externally, allowing some acid to flow over the duodenum. Acid flowing over the duodenum would release endogenous secretin and possibly other intestinal inhibitors of gastric secretion such as cholecystokinin (Wormsley and Grossman, 1964). The exogenous administration of cholecystokinin extracts (CCK) has been shown to inhibit pentagastrin-stimulated gastric acid secretion in rats (Tompson and Johnson, 1969) prepared with gastric fistulas. On the other hand, the intravenous infusion of a synthetic cholecystokinin produced no effect when given to

chronic fistula rats stimulated with either pentagastrin or histamine (Chey et al., 1970). Exogenous cholecystokinin has also been shown to inhibit gastric secretion stimulated with gastrin or pentagastrin in dogs (Gillespie and Grossman, 1964; Stening et al., 1969) and in humans (Chey et al., 1970). Endogenous cholecystokinin has been shown to potentiate the effect of exogenous secretin on the dog pancreas (Meyer et al., 1971). The possibility exists that such a potentiation exists between CCK and secretin on the rat stomach. This has not yet been determined. If such a condition existed during our investigation with the rat, unknown amounts of endogenous CCK and secretin might have been released by acid flowing over the duodenum, the result being an even stronger inhibition of gastric secretion which could not be blocked by nicotine. The observation by Grossman et al. (1970) that nicotine blocks secretin-stimulated pancreatic secretion but does not block pancreatic secretion stimulated by CCK -- lends support to this explanation.

Studies in the Dog

Since Greenlee et al. (1957) reported that extracts containing secretin inhibited gastric secretion of acid in dogs stimulated with food and antral irrigation, their results have been confirmed and extended by many other investigators. Both exogenous and endogenous secretin have been shown to inhibit gastric acid secretion in other species including the rat (Tumpson and Johnson, 1969; Chey et al., 1970; Johannson et al., 1972), cat (Stening et al., 1969) and man (Konturek, 1970; Chey et al., 1970). Our investigation has also

confirmed the gastric secretory inhibition produced by secretin. Continuous infusion of secretin at a dose of 1.5 U/kg/hr produced a significant decrease in gastric juice volume and acid output in a dog stimulated with pentagastrin. This was the response of the denervated fundic pouch (Heidenhain).

Secretin has also been shown to stimulate pepsin secretion in experiments using rats (Johannson et al., 1972), cats (Stening et al., 1969), dogs (Magee and Nakajima, 1968; Stening et al., 1969) and humans (Berstad, 1970). In our investigation in the dog, it was observed that pepsin output decreased following the administration of secretin. The contradictory nature of these results was difficult to explain but was apparently a result of the large decrease in the volume of gastric juice. Stening et al., (1969) observed that when secretin produced a strong inhibition of the volume of gastric secretion using dogs stimulated with gastrin, it was not possible to obtain reliable values for pepsin output. He did observe that secretin increased pepsin concentration. Magee and Nakajima (1969) reported an increase in pepsin output even with a dose of secretin that caused a significant decrease in acid output.

The results of our studies also confirmed that the continuous infusion of nicotine alone (150 ug/kg/hr) produced no effect on the response of the Heidenhain pouch stimulated with pentagastrin. Konturek et al., (1971) have previously shown that an intravenous infusion of nicotine (100 ug/kg/hr) in dogs, had no effect on one-half maximal gastric acid secretion evoked by either histamine or pentagastrin. Nicotine infusion also failed to alter basal gastric acid

output. In this study by Konturek et al., nicotine depressed secretin-stimulated pancreatic and basal hepatic secretion of bicarbonate.

Our findings indicated that intravenously infused nicotine blocked the inhibitory effect of secretin on gastric secretion stimulated with pentagastrin. In the secretin control experiments, the administration of secretin (1.5 U/kg/hr) produced a significant decrease in gastric juice volume and acid output. This dose of secretin was approximately the ED_{50} which was the dose that inhibited gastric secretion by 50 percent. When nicotine was infused along with secretin, a nonsignificant decrease in gastric acid secretion resulted (decreased by only 27 percent). Our results indicated that nicotine blocked the inhibitory effect of secretin (ED_{50}) on gastric secretion by one-half.

It was important that the experiments in this investigation were conducted in a dog with an open gastric fistula. This allowed gastric juice from the main stomach to flow externally so that acid could not enter the duodenum and release unknown amounts of endogenous secretin (Johnson and Grossman, 1968).

A number of other conditions concerning the effect of nicotine on gastric secretion influenced by secretin remain to be examined. The results of this investigation were those obtained from one dog. Experiments of this nature should be performed in a number of additional animals to account for individual variations within a species. Also, the effect of nicotine on gastric secretion inhibited by endogenous secretin has not been investigated. Konturek et al. (1972) have shown that nicotine blocked pancreatic secretion stimulated with

both endogenous and exogenous secretin. In this same study, Konturek et al. have concluded that nicotine was a competitive inhibitor of secretin since the percentage of inhibition by nicotine decreased as the dose of secretin increased. They have shown that at a dose of 1.5 U/kg/hr (ED_{50}) of secretin, nicotine blocked pancreatic secretion stimulated with secretin by 49 percent. Our findings show that nicotine blocked the inhibitory effect of 1.5 U/kg/hr of secretin (ED_{50}) on gastric secretion by 47 percent.

Finally, the results from our investigation showed the gastric response from a denervated portion of the stomach (Heidenhain pouch). The importance of vagal innervation in the regulatory effect of acid in the duodenum on gastric secretion has been shown (Code and Watkinson, 1955). They observed that irrigation of the duodenum with exogenous acid inhibited histamine-stimulated gastric secretion from innervated pouches (Pavlov) but not from denervated pouches (Heidenhain). The validity of this investigation has been questioned by Wormsley and Grossman (1964) since endogenous acid from the main stomach had been allowed to drain into the duodenum during the course of these experiments. In contrast to this earlier study of Code and Watkinson, Wormsley and Grossman have found that vagal innervation was not necessary for the inhibitory action of endogenous duodenal acidification on gastric secretion stimulated with either histamine or gastrin.

Possible Role of Nicotine in the Pathophysiology of Peptic Ulcer in Smokers

The pro-ulcer effect of nicotine on the duodenum has been shown to exist in rats (Robert et al., 1971; Robert, 1972), in cats (Konturek et al., 1971) and in dogs (Toon et al., 1951). Nicotine has also been suggested to be a causative agent involved in the etiology of duodenal ulcers in man (Barnett, 1927; Monson, 1970; Smoking and Health, 1964). Recent work by Konturek et al. (1972) in dogs, has suggested that nicotine exerts this pro-ulcer effect by decreasing the stimulatory effect of secretin on volume and bicarbonate content of pancreatic juice. They have shown that the intravenous infusion of nicotine decreased pancreatic secretion of fluid and bicarbonate into the duodenum when the pancreas had been stimulated by exogenous or endogenous secretin released by the intraduodenal infusion of acid. Duodenal ulcer formation could be due partly to nicotine-induced interference with the neutralizing of gastric acid within the duodenum. A hyperacidic milieu within the lumen of the duodenum would damage the mucosa.

Although Robert (1972) has shown that nicotine potentiates the effect of certain secretagogues on the rat duodenum, it appears from our results in the rat, that this effect cannot be explained as a result of the interference by nicotine on the inhibitory effect of secretin on gastric secretion. However, it is plausible that nicotine favors ulcer formation in the rat by preventing the secretion of pancreatic juice as shown by Konturek et al. (1972) in the dog. A similar study on pancreatic secretion has not been performed in rats. Furthermore, a complete explanation cannot be obtained until the effect of nicotine is studied on gastric secretion inhibited by

endogenous secretin.

The results of this present investigation in the dog, lend support to the mechanism proposed by Konturek for the ulcer effect of nicotine, since nicotine also appears to block the effect of secretin on the stomach. In the dog, nicotine appears to create a hyperacidic condition within the duodenum in two ways. First, nicotine inhibits secretin-stimulated pancreatic secretion, an alkaline secretion which would normally neutralize gastric acid within the duodenum (Konturek et al., 1972). Second, from the results of the present study, it also appears that nicotine blocks the inhibitory effect of secretin on gastric secretion which results in even greater amounts of acid flowing over the duodenum. Although this explanation is plausible, it must be remembered that secretin is not the only intestinal inhibitor of gastric secretion. Cholecystokinin has also been shown to inhibit pentagastrin-stimulated gastric secretion in rats (Tumpson and Johnson, 1969), dogs (Gillespie and Grossman, 1964; Stening et al., 1969) and in humans (Chey et al., 1970) and may be released to inhibit gastric secretion along with secretin. Furthermore, Konturek et al. (1972) have shown that nicotine did not block pancreatic secretion evoked by cholecystokinin whereas it did block pancreatic secretion stimulated with secretin. The condition may also exist where nicotine does not block gastric secretion inhibited by cholecystokinin. However, Johnson and Grossman (1968) provided evidence that secretin is probably the only enterogastrone (i.e. an intestinal inhibitor of gastric secretion) released by acid in the duodenum.

The results obtained from our investigation and from those obtained by Konturek et al. (1972) suggest a possible role of nicotine in the pathophysiology of duodenal ulcer in smokers. If in man, nicotine influences the stimulatory effect of secretin on the pancreas as well as its inhibitory effect on the stomach as it does in the dog, nicotine may also create a hyperacidic milieu within the duodenum. Excess acid in the duodenum is a condition which is known to be ulcerogenic.

SUMMARY

Nicotine, a component of cigarette smoke, has been shown in laboratory animals, to potentiate the ulcerogenic effect of certain secretagogues for the duodenum. Also, smokers have been shown to have a higher incidence of peptic (duodenal) ulcer than people who do not smoke. Although evidence has been provided for this pro-ulcer effect of nicotine, few studies have been performed to explain its mechanism.

Secretin, a hormone released from the duodenum, acts to prevent a hyperacidic condition within the duodenum (i.e. a condition which is ulcerogenic) by stimulating the secretion of alkaline pancreatic juice and by inhibiting gastric secretion of acid.

Recent evidence suggests that in dogs, nicotine may act to create a hyperacidic milieu in the duodenum by blocking the stimulatory effect of secretin on pancreatic secretion.

In studying the effect of nicotine on the gastric-inhibitory effect of secretin, the inhibition by secretin of pentagastrin-stimulated gastric secretion was first confirmed. The dose of secretin infused subcutaneously in gastric fistula rats decreased gastric secretion by 59 percent whereas the dose infused intravenously in a dog decreased gastric secretion by 56 percent. When nicotine was infused with secretin, gastric secretion of acid was depressed approximately 50 percent in the fistula rat. In the dog, the combination of secretin with nicotine decreased gastric acid secretion by only 27 percent. Nicotine infused alone did not influence gastric secretion in either

species.

Two conclusions can be made from these findings. First, in the rat, nicotine does not influence the inhibition of gastric acid secretion produced by secretin. Secondly, in the dog, nicotine partially blocks the inhibitory effect of secretin on gastric acid secretion.

If the response of the human stomach to nicotine is similar to that observed in the dog, nicotine may act to create a hyperacidic condition within the duodenum of smokers in two ways. First, nicotine may also block the stimulatory action of secretin on the production of alkaline pancreatic juice. In addition, nicotine may also block the inhibitory effect of secretin on gastric secretion of acid. However, the results obtained in the rat studies are contrary to this interpretation and emphasize that it is conjectural at this time. Whether nicotine would alter the stimulatory effect of secretin on the rat pancreas remains to be examined.

BIBLIOGRAPHY

- Adashek, K. and M.I. Grossman. 1962. Response of rats with gastric fistulas to injection of gastrin. *Proc. Soc. Exp. Med.* 112:629-631.
- Babkin, B.P. 1950. *Secretory Mechanism of the Digestive Glands.* Second edition. New York, Paul B. Hoeber, Inc. Pp. xxi + 1027.
- Barbezat, G.O., J.I. Isenberg and M.I. Grossman. 1971. Secretin is a diuretic in dog. *Clin. Res.* 19:150.
- Barnett, C.W. 1927. Tobacco smoking as a factor in the production of peptic ulcer and gastric neurosis. *Boston Med. Surg.* 197:457-459.
- Barrett, A.M. 1966. Specific stimulation of gastric acid secretion by a pentapeptide derivative of gastrin. *J. Pharm. Pharmac.* 18:655-659.
- Bayliss, W.M. and E.H. Starling. 1902. The mechanism of pancreatic secretion. *J. Physiol. (London)* 28:325-353.
- Berstad, A. and H. Peterson. 1970. Dose-response relationship of the effect of secretin on acid and pepsin secretion in man. *Scand. J. Gastroenterol.* 5:647-654.
- Brooks, A.M. and M.I. Grossman. 1970. Effect of secretin and cholecystokinin on pentagastrin-stimulated gastric secretion in man. *Gastroenterol.* 59:114-119.
- Brooks, A.M., J. Isenberg and M.I. Grossman. 1969. The effect of secretin, glucagon and duodenal acidification on pepsin secretion in man. *Gastroenterol.* 57:159-162.
- Chey, W.Y., S. Hitanant, J. Hendricks and S.H. Lorber. 1970. Effect of secretin and cholecystokinin on gastric emptying and gastric secretion in man. *Gastroenterol.* 58:820-827.
- Chey, W.Y., B. Sivasomboon, J. Hendricks and S.H. Lorber. 1970. Effects of secretin and cholecystokinin on gastric secretion in rats. *Gastroenterol.* 58:1037.
- Chisholm, D.J., J.D. Young and L. Lazarus. 1969. The gastrointestinal stimulus to insulin release. I. Secretin. *J. Clin. Invest.* 48:1455-1460.

- Code, C.F. and G. Watkinson. 1955. Importance of vagal innervation in the regulatory effect of acid in the duodenum on gastric secretion of acid. *J. Physiol.* 130:233.
- Cohen, S. and W. Lipshutz. 1971. Hormonal regulation of human lower esophageal competence: Interaction of gastrin and secretin. *J. Clin. Invest.* 50:449-454.
- Cooke, A.R. and M.I. Grossman. 1966. Studies on the secretion and motility of Brunner's glands pouches. *Gastroenterol.* 51:506-514.
- Dolinski, I.L. 1894. Influence of acids on secretion of the pancreatic gland. Thesis, St. Petersburg.
- Doll, R. 1964. Medical treatment of gastric ulcer. *Scottish Med. J.* 9:183-196.
- Doll, R., F.A. Jones and F. Pygott. 1958. Effect of smoking on the production and maintenance of gastric and duodenal ulcers. *Lancet* 1:657-662.
- Dupre, J. 1969. Effects of secretin, pancreozymin, or gastrin on the response of the endocrine pancreas to administration of glucose or arginine in man. *J. Clin. Invest.* 48:745-757.
- Gillespie, I.E. and M.I. Grossman. 1964. The inhibitory effect of secretin and cholecystokinin on Heidenhain pouch response to gastrin extract and histamine. *Gut* 5:342-345.
- Greenlee, H.B., E.H. Longhi, J.D. Guerrero, et al. 1957. Inhibitory effect of pancreatic secretin on gastric secretion. *Am. J. Physiol.* 190:396-401.
- Gregory, R.A. 1962. *Secretory Mechanisms of the Gastro-intestinal Tract.* London, Edward Arnold Ltd. Pp. ix + 159.
- Grossman, M.I. 1950. Gastrointestinal hormones. *Physiol. Rev.* 30:33-90.
- Grossman, M.I. 1958. The physiology of secretin. *Vitamins Hormones* (N.Y.) 16:179-205.
- Grossman, M.I. 1969. Stability of secretin. *Gastroenterol.* 57:767.
- Grossman, M.I., J.H. Meyer and L.W. Way. 1970. Pancreatic bicarbonate response to various acids in duodenum of the dog. *Am. J. Physiol.* 219:946-970.

- Hallenbeck, G.H. and D. Annis. 1951. Effect of excluding pancreatic juice from the duodenum on secretory response of the pancreas to a meal. *Proc. Soc. Exp. Biol. Med.* 77:383-385.
- Henriksen, F.W. 1968. The maximal pancreatic secretion in dogs. *Scand. J. Gastroent.* 3:140-144.
- Hickson, J.D. 1970. The secretory and vascular response to nervous and hormonal stimulation in the pancreas of the pig. *J. Physiol. (London)* 206:299-322.
- Hubel, K.A. 1972. Secretin: a long progress note. *Gastroenterol.* 62:318-336.
- Ivy, A.C. and J.I. Farrell. 1926. Contributions to the physiology of the pancreas: II. The proof of a humoral mechanism for external pancreatic secretion. *Am. J. Physiol.* 78:325-338.
- Johansson, I., L. Lundell and S.E. Svensson. 1972. Inhibitory and stimulatory effects of secretin on gastric secretion in conscious rats. *Br. J. Pharm.* 46:94-100.
- Johnson, L.R. and M.I. Grossman. 1968. The enterogastrone released by acid in the duodenum. *Am. J. Physiol.* 215:885-888.
- Johnson, L.R. and M.I. Grossman. 1971. Intestinal hormones as inhibitors of gastric secretion. *Gastroenterol.* 60:120-144.
- Jordan, P.H. and N.D. Peterson. 1962. Effects of secretin upon gastric secretion. *Ann. of Surg.* 156:914-923.
- Komarov, S.A., S.P. Bralow and E. Boyd. 1962. A permanent rat gastric fistula. *Proc. Soc. Exp. Biol. Med.* 112:451-453.
- Konturek, S.J. 1969. Comparison of pancreatic responses to natural and synthetic secretins in conscious cats. *Am. J. Dig. Dis.* 14:557-565.
- Konturek, S.J. 1970. Pancreatic dose-response curves to intravenous secretin in man. *Gastroenterol.* 58:828-832.
- Konturek, S.J., J. Dale, Jacobson and L.R. Johnson. 1972. Mechanisms of nicotine-induced inhibition of pancreatic secretion of bicarbonate in the dog. *Gastroenterol.* 62:425-429.
- Konturek, S.J., T. Radecki, P. Thor, et al. 1971. Effects of nicotine on gastric secretion and ulcer formation in cats. *Proc. Soc. Exp. Biol. Med.* 138:674-677.
- Konturek, S.J., T.E. Solomon, G. McCreight, et al. 1971. Effects of nicotine on gastrointestinal secretions. *Gastroenterol.* 60:1098-1105.

- Lee, Y.H. and J.H. Thompson. 1968. The effect of hog gastrin on gastric secretion in chronic gastric fistula rats. *Experientia* 15:563-565.
- Lee, Y.H. and J.H. Thompson. 1969. Response of rats with chronic gastric fistulas to subcutaneous injections of histamine, ICI-50123, and insulin. *Am. J. Dig. Dis.* 14:14-29.
- Meyer, J.H., L.J. Spingola and M.I. Grossman. 1971. Endogenous cholecystokinin potentiates exogenous secretin on pancreas of dog. *Am. J. Physiol.* 221:742-747.
- Monson, R.R. 1970. Cigarette smoking and body form in peptic ulcer. *Gastroenterol.* 58:337-344.
- Mutt, V. and J.E. Jorpes. 1966. Secretin: Isolation and determination of structure. Proceedings of the Fourth International Symposium on the Chemistry of Natural Products. Stockholm, Sweden.
- Robert, A. 1971. Proposed terminology for the anatomy of the rat stomach. *Gastroenterol.* 60:344-345.
- Robert, A. 1972. Potentiation, by nicotine, of duodenal ulcers in the rat. *Proc. Soc. Exp. Biol. Med.* 139:319-322.
- Robert, A. 1973. Personal communication.
- Robert, A. and J.E. Nezamis. 1972. Stimulation of gastric secretion by a subcutaneous injection of saline. Technical Report, Upjohn Company, Kalamazoo, Michigan. Pp. 3.
- Robert, A., D.F. Stowe and J.E. Nezamis. 1971. Possible relationship between smoking and peptic ulcer. *Nature* 233:497-498.
- Ross, G. 1970. Cardiovascular effects of secretin. *Am. J. Physiol.* 218:1166-1170.
- Rudman, R. 1969. Lipolytic activity of synthetic porcine secretin. *Endocrinology* 85:214-217.
- Smoking and Health. 1964. Report of the Advisory Committee to the Surgeon General of the Public Health Service. U.S. Department of Health, Education, and Welfare, Publ. 1103. Pp. 337.
- Stening, G.F. and M.I. Grossman. 1969. Hormonal control of Brunner's glands. *Gastroenterol.* 56:1047-1052.
- Stening, G.F. and M.I. Grossman. 1969. Potentiation of cholecystokinetic action of cholecystokinin (CCK) by secretin. *Clin. Res.* 17:528.

- Stening, G.F., L.R. Johnson and M.I. Grossman. 1969. Effect of secretin on acid and pepsin secretion in cat and dog. *Gastroenterol.* 56:468-475.
- Stening, G.F., L.R. Johnson and M.I. Grossman. 1969. Effect of cholecystokinin and caerulein on gastrin- and histamine-evoked gastric secretion. *Gastroenterol.* 57:44-50.
- Still, E.U. 1931. Secretin. *Physiol. Rev.* 11:328-357.
- Thomas, J.E. and J.O. Crider. 1940. A quantitative study of acid in the intestine as a stimulus for the pancreas. *Am. J. Physiol.* 131:349-356.
- Thompson, J.H. 1970. Effects of nicotine and tobacco smoke on gastric secretion in rats with gastric fistulas. *Am. J. Dig. Dis.* 15:209-215.
- Thompson, J.H. and W. Bruckner. 1969. Rat gastric secretion following acute exposure to nicotine. *European J. Pharm.* 9:261-265.
- Thompson, J.H., C.A. Spezia and M. Angulo. 1970. Rat gastric secretion and mucosal serotonin following chronic oral nicotine ingestion. *Res. Comm. Chem. Path. Pharm.* 1:721-732.
- Thompson, J.H., C.A. Spezia and M. Angulo. 1970. Chronic effects of nicotine on rat gastric secretion. *Experientia* 26:615-617.
- Thompson, J.H., C.A. Spezia and M. Angulo. 1970. The acute effects of tobacco smoke and nicotine on gastric secretion of acid and pepsin in pylorus-ligated rats. *Res. Comm. Chem. Path. Pharm.* 1:230-246.
- Thompson, J.H. and R. George. 1972. Chronic effects of nicotine on gastric secretion in rats with hypothalamic lesions. *Am. J. Dig. Dis.* 17:513-518.
- Unger, R.H., H. Kettlerer and J. Dupre. 1967. The effects of secretin, pancreozymin and gastrin on insulin and glucagon secretion in anesthetized dogs. *J. Clin. Invest.* 46:630-645.
- Wormsley, K.G. and M.I. Grossman. 1964. Inhibition of gastric acid secretion by secretin and by endogenous acid in the duodenum. *Gastroenterol.* 47:72-81.