GASTRIC SECRETION AND MOTILITY IN CERTAIN VERTEBRATES

T H E S I S

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By

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INTRODUCTION

THE PROBLEM.

With the view of understanding better the gastric functions in man, and because of man's position in the zoological scale, most of the experimental work in the physiology of the stomach has been carried out on mammals. However, the results have only emphasized the complicated nature of the secretory and motor functions of the stomach and have raised questions which still remain to be answered.

Gastric secretion is continuous and spontaneous in some mammals but intermittent in others. It is not known why the gastric glands of some species should require a nervous or humoral stimulus to induce them to secrete while those of other species require none. In the mammal the vagus nerve is the secretory nerve for acid and pepsin while the sympathetic (splanchnic) is the secretory nerve for mucus. Does this mean that the parietal and peptic cells receive only para-sympathetic fibres and the mucous cells only sympathetic fibres? In mammals histamine causes the secretion of acid only and not of pepsin. Why histamine should act selectively on the parietal cells is not known. This substance has become of increasing importance to the gastro-enterologist as a means of testing gastric functions and information about its mode of action would be of great value.

It is usually stated that the vagus is motor to the mammalian stomach while the splanchnic is inhibitory. This assumption has been the basis of treatment for a number of various "gastric disorders". While it is true that vagus

stimulation and pilocarpine usually do produce contractions of the stomach, this is not always the case. Sometimes the vagus is inhibitory and the splanchnic excitatory. The reason for this variation in the effect of the autonomic nerves is not known.

I thought it possible that a study of the secretory and motor functions of the stomach of the lower vertebrates might reveal important information about the phylogenetic history of the mammalian stomach. I undertook this investigation of gastric secretion and motility in the less organized vertebrates in the belief that it would lead to an approach of a better understanding of the complicated secretory and motor processes of the stomach of the more highly organized mammals.

The following vertebrate animals were employed:- Elasmobranch fishes (skate), Amphibia (Necturus or mud-puppy, and frog), and birds (pigeon and chicken). Early in the research many differences in gastric physiology between these lower vertebrates and the mammals were found. With the view of determining whether the gastric processes of the lower forms were recapitulated in the ontogeny of the mammal, I used the stomach of the mammalian foetus and new-born (cat and dog). Finally, since the lower vertebrates (except birds) were cold-blooded animals, I decided to investigate a mammal that has a low body temperature. For this purpose I chose the groundhog during hibernation.

EVOLUTION OF DIGESTIVE SYSTEM.

1. Extracellular Digestion.

The first animals to evolve from the primitive holozoic plant-animal cells were unicellular and, like the present-day Protozoa, probably possessed the properties of intracellular digestion only. The evolution of animals from this unicellular organism followed two main lines, one in which digestion remained intracellular and the other in which digestion became extracellular. With the exception of the Porifera or Sponges, all the Metazoa possess a true gut. In these Metozoa both intracellular and extracellular digestion occur together in those invertebrates where the gut is primitive in structure (such as the coelenterates) or where the animal feeds on liquid or finely divided food (such as the rotifers, arachnida, and mollusca). In the higher invertebrates and in all the chordates the mode of digestion is entirely extracellular.

With the appearance of extracellular digestion, structural changes of the gut have occurred to deal with the ingestion, storage, conduction, maceration, digestion and absorption of food and the formation and elimination of faeces. These changes in structure have involved the appearance of special glandular mechanisms for the production of digestive enzymes and of muscular and nervous mechanisms for the maceration and propulsian of food.

Digestion takes place through the agency of proteases, carbohydrases, and lipases secreted by different glandular elements. In the invertebrates these various glands are situated in different parts of the gut, but almost all of the enzymes are ultimately conducted to the same part of the gut and mixed together. Digestion in invertebrates is thus performed at the meeting place of all the enzymes and the food is there attacked by them simultaneously. On the other hand, in the vertebrates there is a localization of digestion as well as a localization of secretion of enzymes. Digestion takes place in different regions of the digestive tract in a series of steps. This arrangement permits different phases of digestion to be carried out in different portions of the gut, each at its favorable pH (Vonk, '37).

2. Optimum pH of Enzymes.

Each of the digestive enzymes has its own pH optimum. For pepsin, the optimum pH (in the vicinity of 2) is maintained by the secretion of acid by the gastric glands. Such a high acidity could be maintained efficiently only through the localization of digestion, as in the vertebrates where the secretion of acid occurs in the stomach. The acid secreted by the vertebrate stomach is hydrochloric, though lactic and phosphoric have been identified also.

In the invertebrates the reaction of the digestive tube is mainly neutral or alkaline but in a number of species an acid reaction is encountered. In the oyster, scallop, and clam the

pH of the entire gut is around 5.7 (Yonge, '26). Very rarely is a pH below 5 encountered. No invertebrates secreting hydrochloric acid are known for certain, the principle acid of the invertebrates being phosphoric acid. Where cells capable of secreting acid are absent, the pH may be influenced in other ways. Acid may be formed in the gut by the action of micro-organisms on the food or through the production of CO_2 during metabolism (Yonge, '37).

3. Invertebrate and Vertebrate Digestive Tracts.

Certain differences exist between the alimentary tracts of invertebrates and vertebrates. The more general ones may be summarized briefly as follows:-

In vertebrates the term "stomach" refers to an anatomical entity possessing glands capable of secreting acid and pepsin and in which peptic digestion takes place. In the invertebrates the term "stomach" refers only to that part of the digestive tract where most of the enzymes meet to perform the major part of digestion; it is not homologous in all animals but rather analagous.

In all vertebrates digestion is entirely extracellular; in the invertebrates both intracellular and extracellular digestion may exist (Kruger, '34; Yonge, '37).

In the vertebrates digestion occurs in a series of steps, different enzymes acting in different portions of the digestive tract. In the invertebrates all the enzymes act simultaneously in one portion of the tract (Kruger, '34).

Pepsin is secreted only by the vertebrate stomach (Vonk, '37).

Hydrochloric acid is secreted only by the vertebrate stomach and the reaction of the invertebrate stomach is never as acid as the vertebrate stomach (Yonge, '37).

In the vertebrates absorption of the products of digestion occurs in the intestine and, excepting for alcohol and certain drugs (which cannot be regarded as products of digestion), not in the stomach. In invertebrates, both secretion and absorption may occur in the same region (Jordan, '27).

A nervous mechanism regulating secretion or motility, or both, is probably present in all vertebrates. It is only in the more developed invertebrates that such a mechanism exists.

THE VERTEBRATE STOMACH.

1. Gastric Glands Secreting Acid and Pepsin.

It is only in mammals that pepsin and acid are secreted by two morphologically different elements of the gastric mucosa. In the mammal the acid is secreted by the parietal cell and the pepsin by the peptic or chief cell. In fishes, amphibia, reptiles, and birds, the gastric gland consists of mucous cells of the neck and a single type of cells of the body of the gland. The latter, designated as the "cells of the chief glands," or "Hauptdruzenzellen", secrete both the acid and the pepsin. (Langley, 1881; Harvey and Bensley, 1912; Collip, 1920; Plenk, 1932). If, then, in the non-mammalian gastric glands both the acid and the pepsin are secreted by the same cell, the question arises, "to which cell of the mammalian gastric gland is this cell homologous, the peptic cell or the parietal cell?"

Granting that the peptic cell (or else the parietal cell) of the mammalian gastric glands is the homologue of the acid- and pepsin - secreting cell of the other vertebrates, to what cell is the parietal (or the peptic, as the case may be) homologous? The scheme suggested by Lim ('22) is very interesting but does not really answer the question. According to Lim, the gastric glands are first formed from non-mucoid cells. Later these cells became mucoid in character throughout the whole stomach. The next

type of cell to differentiate is the parietal cell, and later still the peptic cell. (See, however, Plenk, '32).

Most of the attempts to settle this question have been made on amphibia. Unfortunately, however, in amphibia the picture is greatly obscured by the presence in some species, notably the frog, of cells in the oesophageal glands which are capable of secreting pepsin. Partsch (1877), Swiecicki (1876) and Kranenburg ('01) regarded the single type of cell of the body of the frog's gastric glands as the homologue of the parietal cell of the mammalian gastric glands; while they believed that it secreted acid they did not believe that it could secrete pepsin as well. They relegated the function of secreting pepsin to the oesophageal glands, and Partsch and Swiecicki declared their belief that the peptic cells of the frog's oesophagus were homologous with the chief cells of the mammalian gastric glands. Lim ('22) found what he believed to be peptic cells confined exclusively to the oesophageal glands and concluded that the stomach contained only mucoid and parietal cells. Kranenburg ('01) and more recently Hirano ('26-'27) identified the neck cells of the frog's gastric glands with the chief cells of the mammalian gastric glands and the cells of the body of the gastric glands of the former with the parietal cells of the latter.

2. Secretion of Acid and Pepsin.

The proteolytic enzyme present in the vertebrate stomach is universally agreed to be pepsin, which (according to Vonk '37) is found only in the gastric and Brunner's glands of vertebrates.

But not all investigators believe that the pepsins in all vertebrates are identical. Scheunert ('09) found fish pepsin to be more active than mammalian pepsin at low temperatures. Riddle ('09) concluded from his experiments on fish, amphibia and reptiles (two species of each) that there was a progressive loss in peptic activity from fish to reptiles. The pepsin of fish (Racokzy, '13; Vonk '37) and of the frog (Pjatnitzky, '31) is less resistant to high temperatures than that of mammals.

However, Kenyon ('25) found that there is no loss in peptic activity in ascending the vertebrate scale; the rate of peptic digestion is remarkably uniform for fishes, amphibia, reptiles, and mammals (birds not investigated). Furthermore, Vonk ('29) found that the pH optima of the pepsins of the dog-fish, pike, frog, and pig are almost identical and situated at about pH2.

In addition to the gastric and Brunner's glands, in some species pepsin is also secreted by the oesophageal glands. Notable among these is the frog (vida supra), sturgeon (Kingsley, '13) and the members of the carp family (vide Biedermann, ('11). Just why pepsin should be present in the carp's oesophagus when the animal possesses no stomach and does not secrete acid is inexplicable.

An interesting point is also raised by MacKay ('28) in her work on the digestive system of Zoarces anguillaris. She showed that although this fish possesses a true anatomical stomach, the pepsin which is extractable is weak and its pH optimum is at 1.2

whereas the stomach, even when filled with food, is only slightly acid; the reaction lying between pH 6.5 and 8.4. It would seem that although pepsin may be secreted by the Zoarces' stomach, it is doubtful that it is of use in protein digestion.

3. Continuous and Intermittent Gastric Secretion.

Gastric secretion may be either continuous or intermittent. Both types of secretory activity of the gastric glands exist among the invertebrates and vertebrates. In the invertebrates, according to Yonge ('32), a continuous secretion is characteristic of those animals which feed continuously on finely divided particles of food. An intermittent or periodic secretion is present in predatory animals or in those which feed at intervals.

In the vertebrates a continuous gastric secretion has been reported for many species. According to Dobreff ('27) the shark secretes acid throughout life; this secretion is continuous and spontaneous. Braitmaier ('04) found gastric secretion continuous in the fowl. In most of the herbivorous animals (e.g., calf, sheep, goat, horse) the secretion of gastric juice is continuous (Bickel, '05; Grosser, '05; Rakoczy, '10; Savitch and Tichomivor, '11; Popov, '32; Poltyreff and co-workers, '36). A continuous secretion is also present (probably) in the rabbit (Gamble and McIver, '25).

In carnivorous animals (cat, dog) some stimulus, conveyed either through a nerve or through the body fluids, is always necessary in order to activate the glandular apparatus. This has been shown repeatedly in our laboratory for the cat and dog.

Gastric secretion is said to be intermittent in man also (Babkin, '32; Winkelstein, '35) though this has not yet been definitely established. Babkin ('32) reviewed the question of intermittent and continuous gastric secretions and pointed out the various factors which may lead to a continuous activation of the gastric glands.

4. Intrinsic Innervation.

Within the wall of the gut there exists an intrinsic nervous system consisting of Auerbach's and Meissner's plexuses. Whether the constituent nerve fibres form a true syncytial network or are of synaptic nature is still unsettled though the bulk of the evidence points to the latter condition. Debatable too is the question of the primary origin of this intramural nervous mechanism. A mesodermal origin, a vagal origin, and a splanchnic origin have been variously advocated, each theory being supported by prominent embryologists and histologists (van Campenhout, '30). The vagal fibres, on entering the muscle, end up in connection with the intrinsic plexus, on the other hand the sympathetic fibres pass through the plexus and go direct to the muscle.

It is now generally agreed that the rhythmic contractions are myogenic in origin. Pieces of muscular tissue of the gut will contract in the absence of both Auerbach's and Meissner's plexuses (Gaskell, '20; Alvarez, '22). However, for the purpose of bringing about a co-ordinated movement of the gut for the propulsion of food, the intrinsic innervation is necessary (Bayliss and Starling, '01; and many others).

According to Alvarez ('19) the rhythmicity of the primitive gastrointestinal tract was graded downward from pharynx to anus. Such a gradient is still present in the stomach of the higher vertebrates. The contraction in response to direct faradic stimulation of the stomach muscle has the shortest latent period in the cardiac region and the longest in the region of the pyloric antrum. A similar gradient in metabolism also exists in both the stomach muscle and the mucous membrane.

The recent work of Berkson ('33 a,b) indicates that the intrinsic nerve plexus has a physiological rhythm, somewhat analogous to the sino-auricular node. He was able to record regular characteristic variations in electrical potential from both excised and isolated loops of intestinal tissue. He showed that these changes in potential were of nervous and not muscular origin.

5. Some Peculiar Digestive Tracts.

While much may be, and indeed has been, written about the peculiar structural and physiological differences in the stomach of the ruminants and other herbivora, I shall here limit myself to a few examples among the lower vertebrates, chiefly the fishes. These differences in structure are without reference to the systematic position of the species but show the remarkable adaptations of the stomach to the feeding habits of the animal.

A true stomach is not present in all vertebrate animals nor, when present, is it always used exclusively for the purposes

of digestion. Thus Tait ('35) mentions certain fishes which utilize part of the stomach and intestine as a respiratory organ. Of interest are the deep-sea fish, ceretoida, cited by Tate Regan ('25). The adult male of this species is devoid of the entire gastrointestinal tract. Early in life the male attaches himself to the female and anastomoses are established between the body of the female and the lips and tongue of the male. For the remainder of his life the male obtains nourishment solely through the blood stream of his mate and his digestive tract soon degenerates and disappears or becomes vestigeal.

Oppel (1896) lists various species of fishes possessing an intestinal tract but lacking a stomach. Chief among these are the members of the carp family or cyprinidae. The carp has been recently reinvestigated by Kenyon ('25), Vonk ('27) and Beauvalet ('33 a); all agree that glands capable of secreting acid and pepsin are absent. Also devoid of gastric glands are the mummichug or Fundulus heteroclitus (Babkin and Bowie, '28), and the lung-fishes or Dipnoi (Plenk, '32).

AMPHIBIAN STOMACH METAMORPHOSIS.

1. Stomach Musculature.

During the period of metamorphosis certain changes take place in the structure of the amphibian alimentory canal. These changes are linked up with the period of starvation during metamorphosis, the changes from the larval to the adult types of diet, and the changes in the mode of respiration.

At the maximum larval length the period of fasting starts and lasts for 10 days in the frog tadpole and 9 days or less in the Amblystoma larva. During metamorphosis a reduction in the length of the entire digestive tube takes place, amounting to 56% and 46.9% by weight in the frog and Amblystoma respectively (Kuntz, '24).

The stomach becomes relatively thicker and more compact. The muscularis, which in the larva consisted only of circular muscle fibres, may now have longitudinal fibres added to it. The increase in thickness of the stomach wall is not due to mitosis but to a rearrangement and aggregation of the tissue elements when the whole digestive tract undergoes reduction in length.

2. Gastric Glands.

Both the larval and adult Amblystoma are carnivorous. Peptic digestion is possible in the larva, it is decreased during metamorphosis, and is later restored in the adult. Thus Kuntz ('24)

found the following:-

Development	Relative Peptic Power
Before Metamorphosis	1.00
Early "	0.05
Late "	0.11
After "	1.64

Both the frog larva (Kuntz, '24) and the newt larva (Okada, '33) have not the capacity of peptic digestion, perhaps because of their herbivorous diet. The ability to secrete acid is developed only after metamorphosis. In the newt Okada ('33) found the pH of the stomach to be:-

Development	pH
Larva	7.6 tp 7.8
During metamorphosis	7.8
Adult	5.6

Cells secreting acid and pepsin are present in the larval Amblystoma but absent from the larval newt and frog. In the latter animals these cells begin to develop during the period of metamorphosis, but it is not known from what cellular elements they arise. The gastric glands become closer aggregated and separated from the muscularis by the intervention of sub-mucous connective tissue.

3. Ciliated Epithelium.

In some of the lower vertebrates the whole or a part of the gastraintestinal tract is lined by ciliated epithelium. Admitting the cilia to be quite numerous to be effective, the role of cilia in propelling food down the gut should be considered.

In the very early stages of development of all amphibia there is a total absence of ciliated epithelium from the entire alimentory canal (Gage and Gage, 1890). Cilia appear upon the oesophageal epithelium, at least, after the food yolk has disappeared and the larva is ready to gather its own food. Just what part of the alimentory canal becomes ciliated and how long the cilia remain is determined by the dietary habits and the mode of respiration of the animal. In the larval stages of aquatic carnivorous species (e.g. Salamanders) the ciliated epithelium is confined to the oesophagus. In the larval vegetable feeders (frogs and toads) ciliated epithelium is found in the oesophagus, stomach, part of the intestine, and the cloaca. During metamorphosis all the cilia disappear, and after metamorphosis, when the animal has become carnivorous and an air-breather, cilia reappear, but only in the oesophogus. It is a general rule that the buccal cavity is void of cilia in all aquatic forms but contains cilia in those forms in which water is rarely taken into the mouth.

II

REVIEW OF LITERATURE

AMPHIOXUS AND CYCLOSTOMES - DIGESTIVE TRACT.

1. Amphioxus.

In Amphioxus and the Cyclostomes the alimentary canal is of primitive structure and runs straight from mouth to anus. A stomach in the histological sense is absent in Amphioxus. The animal possesses a pharynx, into which open numerous gill-slits, and an intestinum. These are innervated by visceromotor fibres from the dorsal roots (Boeke, '35). Excepting observations under the microscope of peristaltic waves travelling along the intestinum, nothing is known about the secretory or motor functions of the digestive tract.

Behind the branchial arches, and ventral to the chorda dorsalis, there is on each side a very thin triangular muscle composed of cross-striated muscle fibres. The two (right and left) muscles enclose the post-branchial gut. Apparently the functions of these muscles is to elevate and constrict the gut. The muscles contract synchronously and rhythmically and thus give rise to rhythmic contractions of the gut. The triangular muscles and the muscular coat of the intestinum are both innervated by the same plexus and not by somatic nerves (Boeke, '35). According to Boeke, the enteric plexus consists of a syncytial arrangement of ganglion cells.

2. Cyclostomes.

The Cyclostomes fall into two chief orders, the Myxinoidei (type Myxine or Atlantic Hagfish) and the Petromyzantes

(type Petromyzan or Lamprey). In Petromyzon the large intestine possesses a spiral valve. According to Jacobshagen ('31), the large intestine is developed before the anterior part of the digestive tract or "stomach". He interprets this as evidence that the posterior part of the digestive tract is phylogenetically older than the stomach region.

In Myxine autonomic nerve fibres come from only the VII and X cranial nerves, and both or only the latter represent the vagus nerve of the higher vertebrates to supply the entire digestive tract. No outflow of autonomic fibres occurs from the spinal nerves so that only the cranial automonic fibres supply the viscera.

It was shown by J. Muller as long ago as 1837 that the two vagi run backwards and unite in the region of the oesophago-stomachal junction to form the nerve intestinales. This nerve passes dorsally along the mid-line of the stomach-intestine and constitutes the only nerve supply to the organ.

Myxine has little, if any, muscular tissue in the wall of its stomach-intestine; the tract consists almost wholly of mucosa membrane and connective tissue (Dahlgreen, '32; Turnbull, '30; cited by Patterson, '32). Patterson and Fair ('33) found that in this animal peristalsis of the stomach-intestine is very weak and that stimulation of the vagus resulted in its relaxation or dilatation. Since the only nerve supply to the stomach-intestine causes a relaxation and since peristalsis is practically

non-existent, the question arises: how is food passed down the alimentary tract towards the anus? Patterson has shown that the stomach-intestine is held tightly to the body wall by means of connective tissue. The body wall itself is highly muscular and waves of peristalsis travel along it somewhat as in the earthworm. These waves carry along whatever food may be present within the inner tube (i.e., stomach-intestine). The literature reveals nothing about the secretions of the digestive tract.

ELASMOBRANCHS - GASTRIC SECRETION.

1. Secretion of Acid and Pepsin.

The subject of gastric secretion in the Elasmobranch fishes has not received much attention. According to Wienland ('01), the acid secreted by the ray's gastric glands is formic acid. However, von Herwerden ('08) found the acid secreted by the Elasmobranch stomach to be HCl and this finding was later confirmed by von Herwerden and Ringer ('11).

A greater acidity was found by von Herwerden ('08) in the stomachs of Elasmobranch fishes than Teleost fishes. Vonk ('27) found the pH of the stomach contents of the dogfish to average 2.8 and of various Teleosts to average 5.2.

Bodansky and Rose ('22) found the pepsin extractable from the gastric mucosae of three species of Elasmobranchs to have a pH optimum of 3. This approximates the values given by Vonk ('27) who found the optimum pH of dogfish (Acanthias) pepsin to be 2.0 and similar to mammalian pepsin. Beauvalet ('33) believed that pepsin is present only in the upper part of the stomach and trypsin in the lower part.

According to Van Slyke and White ('11), who investigated digestion in the stomach and intestine of the dogfish, 2 or 3 days are required for complete digestion.

2. Influence of Autonomic Nervous System.

Wienland ('01) observed in rays that there were in the stomach alternate periods of acidity and alkalinity. He associated this phenomenon with certain peculiar sphincter-like structures occuring in the veins of the stomach (Sappey, 1880; Meyer, 1880; vide Oppel, 1896). Wienland concluded that gastric secretion was under the control of these sphincters; closed sphincters resulted in an alkaline secretion and open sphincters in an acid secretion. The influence of vasomotor nerves on gastric secretion he did not investigate.

A continuous and spontaneous gastric secretion of high acidity occurs in the shark (Dobreff, '27). Because he was unable to influence this continuous secretion by the use of sympatho- and parasympatho-mimetic drugs, Dobreff denied the existence of a secretory innervation of the stomach and claimed that the mechanism of gastric secretion is purely humoral.

Ungar ('35) perfused the stomach of Elasmobranchs with pure and modified sea-water and obtained a gastric secretion. He found this secretion to be increased by acetylcholine and that atropine abolished the acetylcholine effect.

3. Effect of Histamine.

MacKay ('31) found that histamine had no effect on the circulatory system of the skate. Ungar ('35) found that the

secretion obtained by perfusing the Elasmobranch stomach with sea-water was increased in quantity when histamine was included in the perfusing fluid.

ELASMOBRANCHS - GASTRIC MOTILITY.

1. Movement of Stomach.

Athas ('20) described rhythmic and tonic contractions of the isolated stomach suspended in a bath of physiological medium. By keeping the solution well oxygnated the tissue retained its contractile activities for as long as 7 days.

Alvarez ('27) studied the peristaltic movements of the stomach in the skate and dogfish. In the skate he observed deep peristaltic waves, occasional antiperistaltic waves, and small rhythmic contractions in the main portion of the stomach. The peristaltic waves did not always spread over the rest of the stomach while in the pyloric portion chiefly small contraction waves occurred. In the dogfish Alvarez found peristalsis to be similar to that seen in the mammalian stomach except that there was less complete correlation between the movements of the fundus and the pars pylorica.

According to Jacobshagen ('31) the spiral canal is developed before the stomach and the midgut before either. The possible significance of this in connection with the nature of peristalsis is not clear.

2. Influence of Autonomic Nervous System.

The stomach of the Elasmobranchs possesses a well developed sympathetic and parasympathetic innervation. A number

Table 1.

Influence of Parasympathetic Nervous System on Motility of Elasmobrand Stomach.

Author	Experiment	Region of Stomach Affected	Result
Stannius, 1849	Vagus stimulation	Whole stomach	Contractions
Bottazi, 1902	"	"	Contractions
Muller and Liljestrand, 1918	"	"	Moderate contractions; inhibition in 5 out of 16 cases
Young, 1933	"	Cardiac portion	Contractions
Dreyer, 1928	Pilocarpine on muscle strips	Longitudinal coat of ascending limb	No effect
Nicholls, 1933	"	All segments	Contractions
Nicholls, 1933	Acetylcholine on muscle strips	"	Contractions

Table 2.

Influence of Sympathetic Nervous System, on Motility of Elasmobranch Stomach.

Author	Experiment	Region of Stomach Affected	Result
Bottazi, 1902	Splanchnic stimulation	Whole stomach	Contractions
Muller and Liljestrand, 1918	"	"	Contractions
Young, 1933	"	Pylorus & pyloric portion	Contractions
Nicholls, 1935	Splanchnic stimulation of isolated strips	Various regions	Contractions
Lutz, 1931	Adrenaline injections in animal	Whole stomach	Contractions
Dreyer, 1928	Adrenaline on muscle strips	All segments	Contractions
Nicholls, 1933	"	"	Contractions

of investigators have studied the influence of the autonomic nervous system on the gastric motility. The results are summarized in Tables 1 and 2.

The remarkable feature of the action of the sympathetic and parasympathetic nervous systems on the gastric motility of the Elasmobranch is that both are of the excitatory type. This was shown by a number of experiments in which the vagus was stimulated (Stannius, 1849; Battazi, '02; Muller and Liljestrand, '18; Babkin and MacKay-Sawyer, '32; Young, '33) and by other experiments in which the splanchnic was stimulated or adrenaline injected (Battazi, '02; Muller and Liljestrand, '18; Lutz, '31; Young, '33; Nicholls, '35). In support of this are the experiments with adrenaline, acetylcholine, and pilocarpine, all of which were found to increase tonus and cause contractions of isolated strips of stomach (Dreyer, '28; Nicholls, '33, '34 and '35).

Apparently the antral region in the skate is more sensitive to adrenaline than are the muscles of the other region. This region is inhibited by adrenaline in concentrations of 1/1,000,000 and greater, but more dilute solutions, (e.g. 1/2,000,000) cause stimulation (Nicholls, '33). If the excitability of this region is lowered, as it presumably is through storage for 3 or 4 days in physiological media at 0°C., adrenaline in all effective doses is excitatory.

Since both sympathetic and parasympathetic nerves extite gastric movements in the Elasmobranch stomach, the question arises, "are either of them also inhibitory?" According to Muller and Liljestrand ('18) the vagus contains both motor and inhibitory fibres for the stomach. They found that in some experiments stimulation of the vagus superimposed on stimulation of the

anterior splanchnic nerves inhibited the movements activated by the latter.

NECTURUS - STOMACH.

1. Structure.

The histological structure of the stomach of Necturus has been studied by Kingsbury (1894). The muscularis externa is well developed and consists of both circular and longitudinal coats which increase in thickness as the pylorus is approached. At the cardiac end the two layers are closely connected by bundles of fibres which may represent the oblique muscle layer of the mammalian stomach. The muscularis mucosa in the cephalic portion of the stomach is composed of a single coat of longitudinal fibres; to this there is added in the pyloric portion a second inner coat of circular fibres.

The branches of the vagi originate from a glossopharyngeal-vagus complex and supply the gills, lungs, stomach, and intestine (Norris and Buckley, '11). This close linking together of the nerves of respiratory and digestive organs makes possible reciprocal reflexes. Mechanical stimulation of the gills leads to reflex inhibition of stomach contractions, possibly by means of axon reflexes (Patterson, '28).

2. Gastric Motility.

The only work on gastric physiology of Necturus has been that of Patterson ('23, '28) who studied the influence of the vagus nerve on gastric motility. He found that electrical stimulation or mechanical traction of the vagus, when the stomach was exhibiting motor activity, resulted in immediate inhibition of

the gastric movements. Bilateral vagotomy led to a marked augmentation of the normal contractions. His conclusion was that the vagus exerts predominantly on inhibitory influence on the motor activity of the stomach. He believed it probable that the splanchnic nerves supply motor excitatory fibres to the stomach.

FROG - GASTRIC SECRETION.

1. Oesophageal Secretion.

It is well known that the oesophagus of the frog possesses glands which are capable of secreting pepsin. The literature is well reviewed by Biedermann ('11) and Plenk ('32) but information on the nervous or chemical mechanisms regulating the secretion of pepsin is wanting.

2. Seasonal Variations in Secretion.

Riddle ('09) found that the digestive power of the gastric juice was greatly reduced in the frog during the winter months (November to March). Peptic secretion was 1/3 greater in July than in March. Delrue ('33) found that secretion from the isolated gastric mucosa was greatly reduced in frogs during November as compared with frogs during summer.

3. Influence of Autonomic Nervous System.

Contejean (1893; see also Gaultier, '07) believed that in the frog the vagus was the secretory nerve to the stomach. He

found that reduction in the circulation brought about a dimunition of the secretion of acid only but not of pepsin and concluded that the two secretions were independent.

On the other hand Smirnov ('18, '21, '23) concluded that the vagus played no part in gastric secretion. Using frogs with gastric fistulae he found that an acid pepsin-rich juice could be obtained when inert substances (pieces of cork or rubber) were placed in the stomach. Cutting of both vagi in no way interferred with the production of this juice. He advanced two possible explanations:- either Auerbach's plexus alone is responsible for gastric secretion, or else it is a result of a reflex through short sympathetic paths, the reflex passing through the sympathetic ganglia lying outside the stomach.

Boenheim ('18) studied the chloride concentration of the fluid secreted by the isolated perfused frog's stomach. He found that pilocarpine increased and atropine usually decreased the chloride concentration. Delrue ('30 a,c) found that pilocarpine alone had no effect on the secretion from the isolated frog's gastric mucosa but that if perfused together with an extract of stomach tissue pilocarpine produced a secretion.

4. Effect of Histamine.

Keeton, Koch and Luckhardt ('20) found that histamine, when injected into the lymph sac of the bullfrog, stimulated an acid gastric secretion. Popielski ('29) obtained a gastric secretory response to histamine only in 45% of the frogs kept at

room temperature but in 75% of the frogs previously kept at 37° C. for 3 to 4 weeks. From this he concluded that histamine was ineffective in cold-blooded animals.

Hogartz ('32) found that histamine increased the chloride content of the frog's gastric mucosa, a fourfold rise being recorded after 5 hours. Delrue ('30 a,c) obtained an acid secretion from the frog's isolated gastric mucosa when histamine was added to the external (sub-mucosa) side of the perfusing medium.

FROG - GASTRIC MOTILITY.

1. Seasonal Variations in Motility.

Patterson ('16) found that gastric hunger contractions in the bull-frog cease at 13° C. Cessation was not in tonus but in atony of the stomach. Patterson observed that the gastric mechanism was capable of movement at all seasons of the year and even in hibernation, if the temperature were suitable. Normally, during winter hibernation the gastric hunger contractions would presumably be paralyzed.

Babkin ('24) found that the capacity of the stomach of Rara temporaria and R. esculenta varied with the season. Tosioka ('26) studied gastric movements in the toad (Bufo Japonica) by means of the X-ray. Material required much more time to pass through the digestive tract in winter than in summer. Furthermore, the movements of the empty stomach of the hibernating toad did not resemble the hunger contractions of the summer animal.

TABLE 3.

Motility of Frog's Stomach.

Author	Effect of Vagotomy
Volkmann, 1841	Initiated lively peristaltic movements.
Ravitch, 1861	Stomach paralyzed.
Goltz, 1872	Increased tonus and increased motility.
Contejean, 1893	Increased motility.
Steinach, 1898	Increased tonus and increased motility.
Patterson, 1920	Decreased tonus.
Carlson and Luckhardt, 1921	Increased motility.
Itagaki, 1930	Decreased tonus and increased motility.

Author	Effect of Vagus Stimulation
Goltz, 1872	Increased motility.
Contejean, 1893	Weak current contracted; strong current inhibited.
Dixon, 1902	Decreased tonus and increased contractions.
Hopf, 1911	Increased motility.
Patterson, 1920	Initiated peristalsis.
Itagaki, 1930	Increased tonus; contractions not influenced.
Yuh, 1931	Increased tonus.
Aikawa, 1931	Either inhibition or augmentation of tonus and contractions.

TABLE 4.

Motility of Frog's Stomach.

Influence of Stimulating the Sympathetic Nervous System.

Author	Experiment	Result
Waters, 1885	Spinal nerves 4,5,6	Initiated peristaltic movements.
Steinach and Wiener, 1895	Posterior roots of spinal nerves 2 to 6; ventral roots ineffective.	Initiated peristaltic movements.
Contejean,1893	Splanchnic nerve.	Strong tetanic contraction.
Dixon, 1902	Mixed spinal nerves 3,4,5.	Increased tonus and increased motility.
Langley and Orbeli, 1910	Ventral roots of spinal nerves 2, 3,4,5.	Contraction of whole gut.
Itagaki, 1930	Dorsal roots of spinal nerves 3, 4,5.	Increased tonus and stronger movements.
Aikawa, 1931	Spinal nerves 4, 5,6.	Either increased or decreased tonus and rhythmic contractions
Yuh, 1931	Spinal nerves 3, 4,5.	Increased tonus and increased contractions
Asimoto,1936	Dorsal roots of spinal nerves 3,4, 5; ventral roots ineffective	Initiated strong peristaltic movements

TABLE 5.

Gastric Motility in Frog.

Author	Effect of Adrenaline
Boruttan, 1899	Rapid loss in tonus.
Meyer, 1906	Small doses increased and large inhibited contractions.
Kautzsch, 1907	Loss in tonus.
Aikawa, 1931	Contractions inhibited and tonus lowered.
Yuh, 1931	Loss in tonus, increase in strength of contractions.
Sakura, 1932	Small doses increased and large inhibited contractions.
Epstein, 1932	Loss in tonus.
Epstein, 1931	(Toad:- loss in tonus).
Sapeika, 1934	(Clawed toad:- loss in tonus).

Author	Effect of Parasympatho-mimetic Drugs
Feuhner, 1920	Pilocarpine & physostigmine - feeble contractions.
Aikawa, 1931	Pilocarpine and acetylcholine: tonus and contractions increased, effects abolished by atropine.
Yuh, 1931	Pilocarpine:- tonus and contractions increased, effect abolished by atropine.
Rabbeno, 1932	Pilocarpine:- tonus lost and contractions arrested.
Epstein, 1932	Pilocarpine:- Increased contractions. Physostigmine: no effect.
Epstein, 1931	(Toad - same as frog, Epstein 1932).
Sapeika, 1934	(Clawed toad - acetylcholine increased tonus, effect abolished by atropine. Pilocarpine ineffective).

Bevagna ('35) found that a greater faradic current was required to put into a tonic state of contraction the stomach of the toad and frog during hibernation than during the summer.

2. Influence of Autonomic Nerve Stimulation.

The effects of nerve stimulation and the use of autonomic drugs on the intact stomach and stomach strips of the frog and toad are listed in tables 3, 4, and 5. The majority of the investigators found that cutting of the vagi increased gastric tonus and initiated or else increased gastric contractions. Stimulation of the vagi usually augmented the gastric contractions and increased the tonus although inhibition of both contractions and tonus has been recorded also.

All the investigators listed in table 4 agree that sympathetic stimulation excites contractions of the frogs stomach, and most of them also recorded a heightened tonus. It is to be noted that while the efferent sympathetic fibres of the splanchnic nerve leave the cord in the 3rd, 4th, and 5th spinal nerves and possibly in the 2nd and 6th as well, there is no unanimity on whether they course via the dorsal or the ventral spinal roots.

The results of autonomic nerve stimulation and nerve section lead to the conclusion that the vagus of the frog contains both excitatory and inhibitory fibres to the stomach while the sympathetic contains chiefly, if not solely, excitatory fibres.

3. Effect of Autonomic Drugs.

At first glance, no harmony appears to exist in the results of experiments involving the use of various sympatho- and

parasympatho-mimetic substances on excised strips of stomach. These are listed in table 5. However, such variable results are what we should expect. If the parasympathetic nerve supply to the stomach contains both excitatory and inhibitory fibres, then the effect of parasympatho-mimetic substances would depend on the relative concentration employed, a point which was not always taken into consideration in these experiments. With regards to the effect of adrenaline, Sakura ('32) and Meyer ('06) noted contractions with small doses and relaxation with large doses. Either the sympathetic contains both excitatory and inhibitory fibres, or else small doses of adrenaline are "physiological" while large doses empoison the tissue.

STOMACH OF REPTILES.

1. Gastric Motility.

Our knowledge of the gastric physiology of the reptile is very fragmentary indeed. A thorough perusal of the literature has revealed exactly nothing on gastric secretion or digestion except that it takes almost a month for the turtle to excrete an experimental meal.

Patterson ('16) found that rhythmic hunger contractions in the snapping turtle would occur for periods of 5 to 6 hours with intervening periods of quiescence lasting one or two hours. The gastric hunger mechanism was thus intermediate between the continuous contractions of the frog and the intermittent contractions of the dog.

2. Innervation of Stomach.

Bercovitz and Rogers ('21) found that faradisation of the vagus in the turtle caused contractions of the stomach but the effects weakened with repeated stimulation. Single shocks were ineffective and slowly interrupted shocks caused relaxation. Veach ('25) stimulated the peripheral end of the vagus (turtle) with a low frequency current and observed a marked motor effect on the stomach. Both the tonus and the magnitude of contractions were increased. On the other hand, high frequency stimulation resulted in an initial contraction which was followed by relaxation to a tonus level below the normal. The rhythmic activity ceased despite continuous excitation of the vagus.

Thorell ('27) found that adrenaline inhibited all parts of the turtle's stomach except the cardiac portion. Kaltreider ('30) studied the effects of drugs on the isolated pylorus of the terrapin. Pilocarpine produced slow and powerful contractions; frequently also the tonus was increased. Atropine antagonized the pilocarpine. Adrenaline usually relaxed, but sometimes stimulated, the sphincter. Kaltreider concluded that the pyloric sphincter received both sympathetic excitatory and inhibitory fibres and that the excitatory predominated.

BIRDS - GASTRIC SECRETION.

1. Anatomy.

In birds the oesophagus is a thin-walled organ capable of great distension. In the granivorous birds a portion of the oesophagus may be dilated to form a sac-like affair for the storage of grain. This organ, called the crop, is a true part of the oesophagus and does not secrete any enzyme, but contains only mucous glands. In the fowl, the crop is a thin muscular ovoid dilation of the oesophagus, in the pigeon the crop consists of two lateral pouches.

The oesophagus posterior to the crop leads into the stomach. The stomach of birds, unlike that of mammals, is not uniform in shape but ranges from a single simple organ in some birds, to a complex double organ in others. The simple form is found in the pelican, heron, petrel, and other carnivorous birds, where it is a single sac provided with a thick zone of glands. In certain of the granivorous birds (chicken, pigeon) the stomach is divided into an anterior secretory portion, known as the proventriculus or secretory stomach, and a posterior muscular portion, the ventriculus or gizzard. The proventriculus is thin-walled and glandular, its volume is inconsiderable, and it has a narrow cavity. On the other hand, the gizzard is a thick-walled organ with powerful muscles and is lined by a thick layer of horny substances. Stones in the gizzard of grain-eating birds take the place of teeth and fulfil the mechanical function of trituration. The proventriculus is only the site of secretion

and acts as a passage-way; the mechanical and chemical processes of digestion occur in the gizzard (Mangold, '29;but see Groebels, '30).

The muscular gizzard may well be looked upon as the homologue of the pyloric portion of the mammalian stomach whereas the proventriculus may be regarded as the homologue of the fundic portion. Apparently the degree of musculature of the gizzard is dependent on the nature of the food. In birds of prey the gizzard is replaced by a thin fleshymembrane of uniform thickness (Chauvreau, 1891). John Hunter (cited by Garrod, 1872) by giving a duck animal food rather than grain, was able to cause a great diminution in the musculature of the gizzard. More recently (Maw, '36) chickens which were fed on hard coarse material developed gizzards to a much greater size than did chickens fed the same material in the ground form.

Changes in the structure of the gizzard may occur naturally. Thus Edmonstave (cited by Semper, 1880) found that in the Shetland Islands certain fowl (Tarus tridactylus) change their type of gizzard twice yearly. In summer the birds feed on grain and have the typical granivorous gizzard, while in winter they feed on fish and develop the carnivorous type of stomach. In certain birds that feed on hard fruits (Corpophoga latrans) the musculature of the gizzard is not well developed nor are any stones ingested to aid trituration (Garrod, 1876). Instead, the surface epithelial lining is raised into horny cones, the opposing surfaces of which grind together in the manner of teeth.

2. Glands of Crop and Proventriculus.

The crop does not secrete any digestive ferments; these, when found present in the crop, can be explained as being there because of regurgitation. In some species of pigeon the crop epithelium of both parents undergoes active proliferation during the first few weeks after the hatching of the eggs. The epithelium is shed and forms a milky fluid, the so-called "crop milk" which is used for nourishing the young pigeons during their first few days of life. This was described by John Hunter (1792) and Hasse (1865) and more recently by Dabrowski (32 a,b) and Patel ('36). The "crop milk" is rich in fat and protein but is said to contain very little carbohydrate (Dabrowski, '32 a,b).

The glands of the proventriculus of granivorous birds are described as compound tubular glands, resembling the mammalian gastric gland, but having only one variety of cell elaborating both pepsin and HCl (Teichmann, 1899; Bowie '37; and others); the cells of these glands are laden with peptic zymogen granules (Paira-Mall, 1900). According to Plimmer and Rosedale ('22) the only ferment present here is pepsin; Shaw ('13) however has detected rennin as well. The gizzard definitely does not secrete any pepsin (Paira-Mall, 1900; Dulzette, '27; Fritz, Burrows and Titus, '36) though in the pigeon Dulzette has found amylase and saccarase present.

3. Early Researches.

Reaumur (1756) introduced lead tubes containing dry sponges into the alimentary tract of certain birds of prey. The

tubes were recovered when the birds vomited them up and the juice was then squeezed out. The juice was salty and bitter to the taste and acid to litmus, and had a softening and slightly solvent action on meat. Spallanzani (1785) collected in much the same manner as Reaumur the digestive juices from the vulture, owl, eagle and other birds. He found the juices to dissolve cheese, meat and bread. Carminati (1785) always found in carnivorous birds a strong acid which he believed was formed from the meat ingested and not secreted by the bird's stomach. Tiedemann and Gmelin (1831) also used Reaumur's method for collecting gastric juice from birds. They described the acid as HCl, but in some instances they obtained evidence of the presence of acetic acid and even of nitric acid. These experiments are more of historical interest than informative.

4. Recent Investigations.

The first to establish a gastric fistula in the secretory stomach of birds was Zitovitch ('15). In chickens and ducks he found a weakly acid juice, insignificant in digestive power. Teasing the bird increased the acidity up to 0.48% HCl and also the digestive power of the juice. The juice digested coagulated proteins only moderately well. Rusinov ('17) experimented on geese which had both a fistula of the proventriculus and oesophagotomy. The digestive power of sham-fed juice was weak; the juice did not digest coagulated protein but did fibrin. The average acidity was recorded as 0.16% HCl. Karpov ('19) had two geese with oesophagotomy and gastric fistula. The

sham-fed juice was unable to digest coagulated protein (Mett's method) and was reckoned to be ten to twelve times weaker in peptic activity than dog's gastric juice. Fibrin, glutein, elastin and gelatin were best digested, in the order named. The acidity was lower than dog's gastric juice and sometimes reached 0.27% HCl. The conclusion was that the lower acidity of the goose's gastric juice was in confirmity with a vegetable diet in which the proteins require a lower optimum acidity. Groebbels ('30) working on the goose found that while digestion of carbohydrates begins in the gizzard, digestion of proteins begins in the proventriculus. The greatest flow of gastric juice occurred on feeding potatos, less on bread and oats, and least on turnip.

Popoff and Kudriavtsef ('30) investigated the stomach of chickens with gastric fistulas. The juice obtained was always a mixture of proventricular juice and intestinal juice. They attribute this to the fact that in birds a reflex on the pyloric sphincter is absent, and add that sham-feeding experiments are useless since a pure juice cannot be obtained anyhow. The gastric juice after a meal of barley, oats or boiled potato was able to digest coagulated eggwhite and showed some free acid. After feeding raw meat, the chicken's gastric juice did not contain free acid nor did it have any effect on coagulated eggwhite. However, when HCl was added to the juice secreted on meat, it acquired the properties to digest coagulated egg-white

and the highest total acidity of any juice was obtained after the feeding of meat.

Of extreme interest is the work of Meyer ('29) who studied the comparative digestive powers of the gastric secretions of different animals on various proteins. He found that protein remains undigested longer in the stomach of the chicken than it does in the stomach of carnivorous and insectivorous birds and the dog. His results are therefore in conformity with those of the above workers, namely that the secretion from the granivorous stomach is not as active peptically as the secretion from the carnivorous stomach.

According to Braitmaier ('04) cited by Carlson, '23) the secretion of the fasting stomach in birds is continuous. According to Groebels ('30) protein digestion begins in the proventriculus. Lenkeit ('31) however, claims that the digestive juices of the glandular stomach do not possess any digestive powers until they reach the gizzard and become there admixed with the food.

5. Influence of the Autonomic Nervous System.

From the experiments of Zitovitch ('15) who found that teasing the bird (chicken and duck) increased the output of acid and pepsin, it would seem that in these birds the secretory nerve to the proventriculus is the vagus. On the other hand, the experiment of Rusinov ('17) and Karpov ('19) on geese with oesophogotomy would show that the role of the vagus nerve is a weak one. They found that the digestive power of sham-fed juice was negligible. However, I question the value of results based on experiments with sham-feeding in birds.

As Popoff and Kudriavsef ('30) have pointed out, the gastric juice obtained from birds with permanent gastric fistulas is never pure but is always contaminated by intestinal juice. The alkaline intestinal juice, together with bile, would reduce a good deal the peptic activity of the gastric juice, especially so if the pepsin were originally present in low concentration.

Axenfeld(1890) stimulated the vagi in pigeons and found that a secretion of acid juice was induced. Collip(22) by means of a lumbar puncture needle was able to aspirate the contents of the proventriculus of the chicken; he found that both sham-feeding and pilocarpine produced an acid secretion.

6. Effect of Histamine.

The secretory effect of histamine on birds has been studied only by Koskowski ('22) and Danysz-Michel and Koskowski ('22). In an attempt to prove that vitamin B and histamine were identical substances, they injected histamine into pigeons with temporary gastric fistulas. They found that 0.1 mg histamine introduced subcutaneously resulted in a secretion of acid juice exactly 8 minutes later. The secretion was at the rate of 6 to 7 cc. per hour. The peptic power of the juice apparently was not studied.

BIRDS - GASTRIC MOTILITY.

1. Movements of Crop.

The crop, although not part of the stomach proper, fulfills one of the functions of that organ, namely storage of food. It has been observed to undergo strong spastic contractions when empty and in that respect also resembles the true stomach. It is for these reasons that I shall consider here briefly the motility of the crop. The movements of the crop may be seen plainly in the bird when the feathers are removed. The movements may be recorded on a kymograph by means of the classical balloon-tambour method; according to Patterson ('33) the formation of a crop fistula interferes only to a slight degree with the normal movements.

Rogers ('16 a,b) by means of the balloon method, studied the movements of the crop in the pigeon. He found the empty crop to contract in a series of wave-groups, 8-16 wave-groups at intervals of 10-60 minutes. Hunger was very closely associated with a marked hypermotility of the crop; these hunger contractions were inhibited by food, water and external influences (fear, surprise). Rogers found also a close association between hunger and restlessness in the bird even when it was decerebrated. As the crop became empty the bird became restless; the restlessness,

like the hunger contractions, was inhibited by food and water. Probably the inhibitory effects of external influences account for the claims of Doyon (1894) and Rossi (1905) that the empty crop is quiescent.

Ashcraft (1930) studied the crop of fowls about three weeks after decerebration. He verified the observations of Rogers on the pigeon that the bird is restless when the crop is empty and contracting. Waves of peristalsis pass across the crop in about seven seconds.

Henry, Macdonald and Magee (1933) also noted how greatly the crops contracts under the influence of visual and auditory stimuli. It is well known that a hungry bird, made restless by hunger contractions of its crop, will peck at substances within its field of vision. It was found by Beck (1930) that in fowls which fasted 24 hours, the food intake (voluntary feeding) was dependent on vision and not on taste or smell.

According to Patterson (1933) the hunger contractions of the crop are not continuous, as they are in the frog, but intermitten and similar in character to those of the mammal. From this point of view Patterson rightly regards the crop as similar to the cardiac portion of the mammalian stomach, although dissimilar anatomically and in regards to other functions.

Macowan and Magee (1931) by means of the X-ray observed boluses to be sent from the fowl's crop every one to five minutes and to reach the proventriculus in about thirty seconds. Steinmetzer (1924) fed hens pellets of flour containing barium sulphate and by

means of the X-ray observed them to stay in the crop for two hours. The rate at which the fowls crop empties was found by Ihnen ('28b) to depend on the degree of fullness of the stomach, the consistency of the food, and on the water content of the ingesta. The correlation between the work of the crop and the gizzard is also emphasized by Mangold ('29) and Lenkeit ('31). The food leaves the crop only when the gizzard is ready for its reception.

2. Innervation of Crop.

Rogers (1916a) found pilocarpine to increase both the visceral tone and the rate of contractions of the pigeon crop whilst atropine reduced them. Atropine also abolished the restlessness in the hungry bird. Ihnen ('28a,b) studied the innervation of the crop in the fowl and dove. The recurrent branch of each vagus moves the corresponding side of the crop. Unilateral vagotomy did not influence the activity of the crop but bilateral vagotomy produced crop paralysis. Stimulation of the recurrent branch of the vagus produced contractions of the crop only, the oesophagus was little involved. The upper oesophagus is innervated by the Nn. oesophagei which control the crop sphincter and peristalsis of the oesophagus. Section of either vagus in chickens prevented the crop from emptying in the normal time but complete recovery was soon established. Bilateral vagotomy produced permanent crop paralysis and eventually death from starvation. Complete extirparion of the crop in the

fowl was not fatal as long as the central and distal ends of the oesophagus were anastomozed but there was difficulty in assimilating food.

Hanzlik and co-workers (Hanzlik and Shoemaker, '26; Hanzlik, Butt and Stockton, '27) found that splanchnic stimulation produced contractions of the crop and that the spontaneous crop movements were not abolished when the sympathetic is degenerated.

3. Movements of Proventriculus.

Ashcraft ('30) found that regular contractions at the rate of about one per minute occurred in the proventriculus of the fasting fowl. Unlike the hunger contractions of the crop, those of the proventriculus were not associated with restlessness on the part of the bird. Henry, Macdonald and Magee ('33) by means of the X-ray recorded the passage of a bolus of food through the proventriculus of the fasting hen to take only about fourteen seconds.

4. Innervation of Proventriculus.

Axenfeld (1890) found that stimulation of the vagus in pigeons produced movements of the proventriculus. In 1925 Doyan published results of work which he did in 1894 on pigeons, chickens and ducks (Doyan, '25). He found that the effect of vagus stimulation depends on whether the stomach is at rest or is moving. If the stomach were quiescent, then vagus stimulation initiated motility; if the stomach already showed peristalsis, vagus stimulation produced inhibition. According to Doyan, the movements of the proventriculus and gizzard usually alternated. On the

whole, the influence of splanchnic nerve stimulation was that of inhibition when the stomach was motile, and motor when the stomach was at rest. Pilocarpine produced strong contractions which, when followed by either vagus or splanchnic stimulation resulted in relaxation.

Nolf ('27) described the neurones to the proventriculus of the chicken to be of two orders: 1) those which control rhythm, and, 2) inhibitory or stimulatory neurones. Both are paralyzed by nicotine. Probably these proventricular neurones are partly vagus and partly sympathetic in origin. The proventriculus receives direct preganglionic fibres from the vagus and post-ganglionic fibres from the sympathetic.

5. Movements of Gizzard.

When young fowl are fed soft mushy food for any length of time, the muscular development of the gizzard is greatly inhibited. This was apparently first observed by John Hunter. Of kindred interest is the research of Brandes (1896). Meat was fed to pigeons for seven months. The meat was dissolved in the proventriculus and did not stay long in the gizzard but was rapidly passed into the intestine. Apparently only bulky foods remained for any length of time in the gizzard. Mangold ('29) found that the length of time that food remains in the gizzard depends on the quantity and consistency of the food.

Steinmetzer ('24) by means of the X-ray followed the course of barium sulphate pills in the digestive tract, and found

them to remain in the gizzard for two hours. According to Henry, Macdonald and Magee, ('33) the gizzard contracts every 2.5 minutes and discharges columns of food about once every second.

During periods of fasting, the gizzard of the fowl contracts vigorously (Ashcraft, '30). The influence of mechanical stimulation of the gizzard by the pebbles present within on the rate and strength of contractions has been studied by Jaeckel ('24), Kath ('25, cited by Mangold, '29) and Mangold ('27).

Total extirpation of the gizzard in the chicken is not fatal (Fritz, '35); only the ability to digest coarse foods is decreased (Fritz, Burrows, and Titus, '36).

6. Innervation of Gizzard.

Doyon (1894; '25) employed the balloon method of recording the contractions of the gizzard in the pigeon, chicken and duck. He found that stimulation of the vagus produced marked contractions of the gizzard if it were at rest, but inhibited contractions, if they were already present. Splanchnic nerve stimulation after double vagotomy produced isolated contractions of the gizzard.

Mangold ('29) found that stimulation of the peripheral end of the cut vagus induced increased movements of the gizzard. Bilateral vagotomy resulted in slower and more irregular contractions. Henry, Macdonald and Magee ('33) studied the action

of drugs on the gizzard by means of tambour recordings. They found atropine to inhibit the contractions, while pilocarpine increased either the rate or the amplitude. Adrenaline slowed the rate but increased the amplitude of the contractions. They concluded, therefore, that the vagus was mainly excitatory, whilst the splanchnic was mainly inhibitory.

Our knowledge of the innervation of the gizzard comes chiefly from the work of Nolf ('25a,b,c, '27, '30). Nolf found that the mechanical activity of the fowl's gizzard was reduced after double vagotomy. Stimulation of the vagus with a weak current was exclusively inhibitory in effect, whilst a strong current was predominantly motor in effect. The same could be said for splanchnic nerve stimulation. Sympathetic fibres to the gizzard leave by way of spinal nerves D3 to D6 inclusive. According to Nolf, each of these four nerves contain motor and inhibitory fibres, as does also the vagus. Therefore there is no true antagonism between the vagus and the sympathetic. Each nerve may act as its own inhibitor. The inhibitory effect of the vagus opposes efficiently the motor effects of both vagus and sympathetic; this is true also for the inhibitory effect of the sympathetic. In accord with this lack of differentiation between the functions of the vagus and sympathetic nerves, Nolf found that atropine abolished the motor effects of the vagus as well as of the sympathetic. The isolated gizzard is under control of an intrinsic innervation; the extrinsic innervation affects amplitude and rate of contractions, but not the muscular tonus. The gizzard is in nervous connection with the proventriculus, by means of synaptic nerves since nicotine paralyzes the co-ordination between the two organs.

STOMACH OF MAMMALIAN FOETUS.

1. Gastric Secretion.

Little is known about the physiology of the stomach of the mammalian foetus. The foetuses so far investigated have been those of the rabbit, cat, dog and man. The results are not strictly comparable for, as Bancroft ('36) has pointed out, "not only have different mammals different periods of gestation but they are born in very unequal degrees of development."

The development of the gastric glands has been studied chiefly in man. According to Cho ('31) simple glands appear at the site of the future cardiac glands in the 90 mm foetus and these assume their characteristic shape at 190 mm. In the human embryo of 5½ months (Zimmermann, '25) and 6 months (Taguchi, '22), undifferentiated cells, mucoid cells, and parietal cells have been found. Peptic cells appeared only in much older foetuses. In the cat, no petic cells are present at birth; these appear only after the first week (Lim, '22).

Sutherland ('21) found in the dog and cat that an acid secretion in response to gastrin could be obtained before term. Keene and Hewer ('29) first found HCl in the stomach of the human foetus at 19 weeks and this agrees with Cardin ('33) who concluded that acid secretion is begun in the human foetus only after the fourth month.

Keene and Hewer ('29) found proteolytic enzymes in the human foetus only after 16 weeks. However, in the dog's stomach Gmelin ('02) could obtain a secretion of pepsin only towards the end of the first month of post-natal life. Holter and Anderson ('34) also found it impossible to obtain in the newborn dog a pepsin-active secretion by any means, including test-meals and pilocarpine. The first enzymatically active secretion was obtained only after the sixth week. In the cat, Lim ('22) could obtain a secretion of pepsin only after the third week.

The above data supports the theory of Lim ('22) who believes that during development the peptic cells are the last to become differentiated. It would seem that in the human, both peptic and parietal cells are present at birth whereas in the dog and cat peptic cells do not appear until some time after birth.

2. Gastric Motility.

According to Tani ('27) the stomach of the rabbit foetus shows spontaneous movements when the foetus reaches 7.5 gm. body weight. The stomach increases in sensitivity to drugs during growth but the stomach of the newborn rabbit is less sensitive than that of a pre-term foetus. Adrenaline accelerates the spontaneous movements.

Koshtoyantz and Mitropolitanskaya ('34) found no automaticity of the human foetal gastrointestinal tract at 5½ weeks. The first spontaneous movements appeared after the 6th or 7th week. McLachlin ('36) observed rhythmic contractions in circular rings of the gastrointestinal tract of an 8 weeks' foetus in which the longitudinal muscle layer had not yet appeared.

Tani ('27) found that adrenaline had a restraining action on the gastric motility of the human foetus and Inada ('28) found peristalsis to be excited by parasympatho-mimetic and depressed by sympatho-mimetic drugs. According to McLachlin ('36), whose conclusions were similar to the above, the human foetal gastrointestinal tract is sensitive to acetylcholine at an earlier age than to adrenaline. Carlson and Ginsburg ('15) studied the newborn human infant and puppies born before term. They concluded that "in the normal mammal the gastric hunger mechanism is completed, physiologically, and is probably active some time before birth."

STOMACH OF HIBERNATING MAMMALS.

So far as a search of the literature reveals (for excellent reviews on hibernation, vide Gorer, '30; Johnson, '31; Ferdmann and Feinschmidt, '32) nothing is known about the digestive physiology of the hibernating mammal. In the marmot, Biering and Kollmann ('28) found the acinous cells of the pancreas partially atrophied but the islet cells increased in size during hibernation. Probably most of the food ingested has passed through the body before the animal goes into hibernation (Johnson, '31). Short periods of wakening, observed to occur during hibernation of the thirteen-lined ground squirrel, have been attributed to the effect of stimuli produced by faeces within the rectum (Johnson, '31).

III

METHODS

GASTRIC SECRETION.

1. Acute Experiments.

Many of the acute experiments on animals for the study of gastric secretion were performed under suitable anaesthesia. Each anaesthetic was chosen after careful control experiments had shown that it had neither stimulatory or inhibitory influences on the secretory processes. The following anaesthetics were employed:-

Skate - Dial "Ciba"; 0.2 to 0.3 cc or less per kilogram body weight; intra-abdominal injection.

Pigeon - Nembutal (Abbott's sterile solution No.844); 20 to 25 mg. per kilogram body weight; intra-abdominal injection.

Chicken - Same as pigeon.

Groundhog - Mixture of chloralose (1.5%) and urethane (15%); 2.5 cc per kilogram body weight; intravenous injection.

In many instances no anaesthetic was used; instead the brain, and frequently also the spinal cord, were destroyed. This method of immobilization was used in the skate and the frog. The spinal cord of the skate was destroyed by inserting a heavy wire probe through a cut in the ligament between the skull and the first vertebra and passing it down the spinal cord as far as the region

of the vent. The site of the insertion was then sutured. Animals with the cord so destroyed were kept alive for ten to sixteen days without feeding, during which time their secretion was studied. In the frog, either the brain or the spinal cord alone or the whole central nervous system was destroyed by pithing.

Inasmuch as the destruction of various portions of the brain and cord was done more for the sake of ruling out the influence of the central stations of the autonomic nervous system than for the purpose of immobilizing the animal, the extent of the lesions was always verified by post mortem examination. Further details of the different experimental procedures involved will be considered in the appropriate places.

2. Chronic Experiments.

In certain experiments on the skate the unanaesthetized animal was employed intact. The animal was taken from the water and a long narrow glass tube with rounded end was passed through the mouth into the stomach. By lifting the posterior part of the animal's body above the level of the tube and keeping the tube at a downward slant, any fluid which might be present in the stomach could be drained out. As a means of testing the reaction of the gastric mucosa (irrespective of that of the gastric juice), a long wire, to one end of which were attached blue and red litmus and congo red papers, was passed through the glass tube into the stomach.

All experiments on the gastric secretion of Necturus

were made on the intact unanaesthetized animal. The animal was lifted out of the water by means of a moist cloth and placed on a moist board. Care was taken not to touch the gills or injure the skin. The mouth was forced open by means of blunt forceps and a narrow glass tube with rubber tip was passed into the stomach. All the Necturi were approximately of the same size and previous dissection of a number of dead animals was made to determine the length of the stomach. An equivalent length from the rubber tip was marked off on the glass tube, and the tube was never inserted into the stomach beyond this marking. This precaution prevented any possible injury to the gastric mucosa which might have resulted from passing the tube too far into the animal.

A number of experiments were performed on chickens with permanent fistula of the proventriculus. The chicken was operated on whilst under ether or else Nembutal anaesthesia. The proventriculus was exposed and one end of a silver cannula of appropriate size inserted; the site of the insertion was then sutured with interrupted stitches. The other end of the cannula was brought through the muscles and skin by a stab wound. The muscles and skin were sutured separately with interrupted stitches. The portion of the cannula with the proventriculus did not occlude the lumen and complete continuity of the gastra-intestinal tract existed. Birds with such permanent fistulas lived for many months.

Permanent gastric fistulas were also established in groundhogs by the well-known method of Pavlov. However, the natural ferocity of the groundhog when awake made investigation of its gastric secretion very hazardous indeed. Consequently

the experiments on gastric secretion of the waking groundhog are too few and inconclusive to be included in this dissertation.

3. Drugs Employed.

In the study of gastric secretion, various sympatho and parasympatho-mimetic substances and chemical stimulants were used. These were:- acetylcholine hydrochloride (Merck) and acetylcholine hydrobromide (Eastman-Kodak); adrenaline hydrochloride (Parke-Davis); atropine sulphate (Merck); ergotamine metansulphonate (Sandoz); pilocarpine hydrochloride (Merck); and histamine dichloride (Hoffmann-LaRoche). The histamine was administered subcutaneously; all the other substances were given intravenously.

In addition, alcohol, glucose, and sodium chloride were used in experiments on the pigeon. These were either placed into the duodenum or injected intravenously.

4. Electrical Stimulation of Nerves.

Electrical stimulation of nerves was performed by means of an induction coil. The Harvard inductorium was employed for the skate and frog and the Porter inductorium for the chicken and groundhog. The strength of the induced current was controlled by varying the distance between the primary and secondary coils and recorded in terms of the number of centimeters distance between the coils.

The vagi or splanchnic nerves were cut and their ends carried on strings. For stimulation purposes the peripheral or

central ends were placed on electrodes and good contact between the nerve and the electrodes was assured by slight tension on the string attached to the nerve. The nerve was kept constantly moistened by physiological saline solution. Fatigue of the vagi was prevented by stimulating them alternately, each vagus being stimulated for only ten-minute periods. In addition, the point of contact between the nerve and the electrodes was altered from time to time. With the above precaution, the vagi could be stimulated for as long as 10 hours without becoming unduly fatigued. A similar procedure was followed in stimulating the splanchnic nerves of the skate and the frog.

For stimulating the medulla oblongata in the frog pin electrodes were used.

5. Analysis of Gastric Juice,

The peptic power of gastric juice was determined mainly by its digestive action on coagulated egg-white. For this, the Nirrenstein and Schiff's modification of Mett's method (Hawk and Bergeim, '27) was used. Because of the low peptic activity of the pigeon's and hibernating groundhog's gastric secretions, the Mett tubes were prepared at 75° C rather than at the more customary 85° C.

Extracts of the mucous membranes of the oesophagus and stomach were prepared by the method of Hammarsten ('19). The oesophageal secretion of the frog always consisted of clumps of mucus. This secretion could not be treated like the fluid gastric secretion when determining its peptic activity. Accord-

ingly, extracts of the oesophageal secretions were also prepared by Hammarsten's method.

The pH of gastric juice was determined by means of a capillator colorimeter (British Drug Houses). The free and total acid were determined in the usual manner, using Topper's reagent and phenolphthaline as indicators.

Chlorides were deterimined by the method of Wilson and Ball ('28) in the gastric juice and blood. However, at the time I worked on the skate the only method available for chloride determinations was that of Mohr and consequently the values obtained for blood plasma chlorides are only approximate. Mohr's method was also used for the determination of the chloride concentration in seawater.

Freezing point determinations of the blood and gastric juice of the skate and of sea-water were made by means of a Beckmann thermometer.

The methods used in the chemical analyses of the pigeon's and chicken's gastric juices are given in the section describing the results.

GASTRIC MOTILITY.

1. Balloon and Tambour Method.

Through small incisions in the stomach of the skate two balloons were introduced, one into the fundic and the other into the pyloric portion, of the stomach; these were connected to Marey's

tambours and the contractions recorded on smoked paper. The stomach was constantly kept moist with a solution of the following composition (Nicholls, '34):-. Na Cl, 16.3; urea,21.6;K Cl 0.9;$CaCl_2$,1.1;$NaHCO_3$,0.38;NaH_2PO_4,0.06;glucose,1.0gm;H_2O, 1 liter.

In the frog the stomach was disconnected from the oesophagus and two cannulas were inserted, one into the cardiac and the other into the pyloric end of the stomach. The stomach was filled with distilled water and the anterior (cardiac) cannula connected with a Marey tambour. The pyloric cannula was used to regulate the amount of fluid within the stomach. The surface of the stomach was moistened with frog Ringer. Contractions were recorded on smoked paper.

The recorded movements were controlled by direct observations of the stomach.

2. Registration of Contractions of Strips of Muscle.

To study the motility of the Necturus stomach, the animal was decapitated, the stomach removed and cleaned of mesentry, and placed on a clean glass plate covered with Ringer solution of the composition noted below. The stomach was then divided by means of a sharp scalpel into three sections - a short piece just posterior to the oesophagus, a somewhat longer piece from the main body, and a short piece lying just anterior to, but not including, the pylorus. The portions of tissue intervening between these sections as well as the oesophagus were discarded.

Circular and longitudinal strips were cut from these sections of the Necturus stomach. The circular strips were approximately 0.5 cm. in width while the longitudinal strips were about 0.5 cm. wide and 2 cm. long. The strips were attached to an aluminum recording lever and suspended in a bath of Ringer solution of the following composition:- NaCl, 7.0 gm; K Cl, 0.3 gm; $NaHCO_3$, 0.2 gm; and $CaCl_2$, 0.25 gm. per litre of distilled water.

Gastric motility in the foetal and newborn cat and newborn dog was similarly studied by means of strips of the stomach muscle. The foetal cats were obtained by anaesthetizing the mother with ether and performing a laparatomy. Rapidity was essential when removing the foetuses, since handling of the uteri initiated gasping reflexes in the foetus which resulted in their swallowing amniotic fluid.

The foetal cat as well as the newborn cat and dog were decapitated, and the stomachs removed and cleared of mesentry. A strip of stomach tissue or (where the stomach was very small) the whole stomach was attached to a lever and suspended in a bath of mammalian Ringer solution kept throughout the experiment at 37°C.

In the case of the Necturus, either a 100 cc or a 500 cc bath was used; in the case of the Mammalian foetus and newborn, a bath of 500 cc capacity was used. Air was bubbled through the solution at a constant rate. The force of the air-bubbles hitting the tissue was insufficient to disturb the otherwise sensitive recording lever.

3. Drugs Employed.

In the skate atropine sulphate (Merck) and adrenaline hydrochloride (Parke-Davis) were injected intravenously. Where isolated muscle strips were used, the above, and also the following, substances were added to the bath:- acetylcholine hydrochloride (Merck) and acetylcholine hydrobromide (Eastman-Kodak); barium chloride (Baker); chloretone (Parke-Davis); ephedrine hydrochloride (Parke-Davis); ergotamine metansulphonate (Sandoz); and pilocarpine hydrochloride (Merck).

4. Electrical Stimulation of Nerves.

When the balloon method of registering the movements of the intact stomach was used (skate and frog), the vagi, splanchnic, and sciatic nerves were stimulated by an induced current. The method was similar to that already outlined above.

5. Storage of Tissue.

Although most of the experiments with strips of stomach tissue were performed very soon after the stomach was excised from the animal, in several instances the strips, or else the whole stomachs, were first stored in a refrigerator before using. The tissue was kept in appropriate medium at a temperature of 4^{o} to 6^{o} C. When the mammalian tissue was taken from cold storage, it was warmed very gradually over a period of two hours to 37^{o} C. Rapid warming was found to be injurious although rapid cooling was not.

IV

EXPERIMENTAL RESULTS

ELASMOBRANCHS -
GASTRIC SECRETION.

1. Continuous Secretion.

The reaction of the gastric mucous membrane of a healthy fasting skate (unfed for 14 to 20 days) is always acid. Secretion is continuous although very little fluid can be aspirated from the stomach. It is with great difficulty that even 0.5 cc. can be aspirated from such an animal. This secretion does not depend on the integrity of the parasympathetic nervous system since it was not abolished by section of the vagi or injection of large doses of atropine. This point was proved in several experiments of which the following are representative examples.

Exp. 34 - R. diaphanes, ♀ 1.475 kg. Aug.17: 4.00 p.m., 0.5 cc. Dial given intra-abdominally. Stomach empty, reaction of mucosa very acid. 4.25 p.m., abdomen opened; oesophagus and pylorus tied with strong ligatures. Abdominal muscles and skin sutured separately. 4.35 p.m., operation ended. August 18: 4.25 p.m., animal in good condition. Spinal cord cut below medulla, and abdomen immediately opened. Stomach contained about 2.0 cc. of acid mucoid fluid, pH 3.8. Reaction in fundus more acid than in pyloric canal.

In two other experiments (exp. 26, Aug.13, and exp.27, Aug.14) a long glass tube was tied by strong ligatures into the lower end of the oesophagus, so that one end protruded a little from the mouth. The free end of the tube was corked so that sea-water could not enter the stomach. The abdominal wall was closed by stitches. The animals survived for 26 hours and 35 hours respectively.

In both cases death was presumably due to compression of one of the abdominal venous sinuses owing to change in the position of the stomach end of the tube during the swimming movements of the animals. Nevertheless, in both cases the reaction of the stomach was definitely acid during the experiment and also immediately after death. The spontaneous secretions aspirated several times during the experiment were scanty. For example, in one of the animals the total secretion during 24 hours did not exceed 2.00 cc. of mucoid fluid, and in the other only 0.4 cc. (pH 3.3) was obtained 18 hours after tying of the vagi.

In another experiment (exp.24, Aug.11) the spinal cord was cut below the medulla and the whole brain then destroyed. Respiration ceased but the spinal reflexes still persisted. Two hours after the operation the spinal reflexes were found to be weaker but the reaction in the stomach was still strongly acid. Although the heart was still beating slowly 3 hours and 15 minutes after the operation, the spinal reflexes had gradually disappeared. The stomach contained about 12 cc. of sea-water and the reaction of the stomach had changed to neutral. Therefore the sea-water must have entered the stomach just before death, and probably as the result of paralysis of the muscles of the pharynx or of the cardia.

Five experiments were performed in which 2 to 5 mg. atropine sulphate were injected subcutaneously and intramuscularly. In all but one of the experiments the reaction in the stomach remained acid but no increase in secretion occurred in any of them. The following is quoted as an example.

Exp.39 - R. diaphanes, medium size. Aug.21:2.20 p.m, stomach empty and acid. 4 mg. atropine sulphate injected subcutaneously and intramuscularly. Stomach empty for the next 5 hours, but reaction of the mucosa strongly acid. 7.35 p.m, 0.5 cc. thick mucus aspirated, with pH of 2.8 to 3.2. Aug.22:9.30 a.m, stomach empty and acid.

The exceptional case mentioned, in which the reaction did not remain acid, was as follows.

Exp.20 - R.erinacea, ♀, about 1 kg. Aug.8: 5 mg. atropine sulphate were injected at one time - 3 mg. subcutaneously and, through error, 2 mg. intra-abdominally. For 4 hours the stomach was empty and its reaction acid. Then during the next 24 hours aspiration resulted each time in from 7 to 10 cc. of a neutral or slightly alkaline fluid, presumably sea-water. During the 24 hours following, the fluid became slightly acid again. The volume of fluid which could be aspirated gradually fell, and after 6 or 7 days the volume had again become small and the reaction acid. In this experiment the atropine probably disturbed the motor innervation of the pharynx or cardia, so that sea-water passed into the stomach.

It is important to note that in severe operations or shortly before death the acid secretion ceases and the reaction of the mucosa becomes neutral.

Exciting the animal does not in any way affect the spontaneous gastric secretion, nor does it make any difference whether it is kept for days in the shallow laboratory aquarium, exposed to the full light of day, or in tanks in a dark cool cellar.

2. Effect of Mechanical Stimulation.

Distension of the stomach in a spinal skate for several hours with an inflated balloon or filling it with bits of rubber or pieces of herring did not provoke a gastric secretion. Therefore in the skate neither the local intramural nerve plexuses nor the autonomic nervous system mediate any secretory reflexes arising from the gastric mucosa itself.

In this respect the skate's stomach is apparently similar to the mammalian stomach where mechanical stimulation of the gastric mucosa is without effect on the secretion. On the other hand, as I have shown, mechanical stimulation of the frog's stomach results in a secretion of acid gastric juice(vide infra).

3. Influence of the Parasympathetic Nervous System.

Several experiments were performed on spinal skates, in which a rubber balloon was introduced into the upper part of the oesophagus or else into the pharynx. The balloon was alternately distended and deflated ~~repeatedly~~ with air in order to produce a swallowing reflex and so to stimulate a gastric secretion through the vagi. To preserve the vagi intact, a longitudinal incision was made through the muscular layer of the oesophagus a little above the cardia. The submucous and mucous membranes were separated from the muscular layer with a blunt instrument. A glass tube, to which a rubber balloon was attached, was passed through the incision and into the upper part of the oesophagus, where it was tied to the submucous and mucous layers so as to leave the musculature free and the vagi intact. The oesophagus was thus

separated completely from the stomach. A glass cannula was introduced into the pylorus and the pylorus was then tied at the duodenal junction. The animal was left lying supine on a board and respiration was applied in the usual manner by passing sea-water through one of the spiracles.

No definite results were obtained by stimulating the swallowing movements. For three or four hours the balloon was repeatedly distended and deflated. Each time the balloon was distended the animal performed swallowing movements, and the water which filled its buccal cavity was often expelled through the mouth. No secretion of gastric juice resulted and the reaction of the mucous membrane remained slightly acid or neutral.

No attempt was made to stimulate the visceral branches of the vagus because the operation required for this is difficult and might be too drastic for the animal; the results moreover would be doubtful owing to the possible inhibitory effect of the operation on gastric secretion.

The results of the experiments described above, when considered in conjunction with the negative results obtained by Dobreff ('27), make it seem very doubtful that the vagus nerve participates in gastric secretion. However, it is still possible that the vagus does convey some secretory impulses to the gastric mucosa in this species; if so, then special methods would be required for their study.

4. Influence of the Sympathetic Nervous System.

In several acute experiments (using Dial "Ciba" as an anaesthetic) the anterior splanchnic nerves were stimulated. The

abdomen had been previously opened, the oesophagus and pylorus tied, and a cannula fixed into the fundus. Although the nerves were stimulated intermittently for periods of 3-1/2 to 4 hours and typical motor responses were always produced, no definite secretory effects were obtained. It is true that in some experiments the reaction of the gastric mucosa, which from acid had become neutral during the operation, again turned acid during the course of the experiment. However, this might have been due to the recovery of the animal from the operative shock, and not to the secretory effect of splanchnic stimulation. Since the inhibition of secretion could be possibly the result of the operative procedure, we carried out some experiments with adrenaline.

In acute experiments as well as in normal animals subcutaneous injection of adrenaline in various amounts (including massive doses) did not provoke a gastric secretion. Moreover, in many cases there was even an inhibition of the spontaneous secretion or of the secretion which had previously been produced by the presence of food in the stomach. The following examples illustrate this statement.

Exp.47, Aug.31 - R.erinacea, ♀ medium size. 8 cc. of adrenaline (1 : 5,000) were injected over a period of 8 hours. At the end of this period a little fluid and food, both having a neutral reaction, could be aspirated from the stomach. The reaction of the mucosa was also neutral.

Exps. 57 to 59, Sept. 5 - Three R. erinacea, medium size. These experiments were of more than 21 hours' duration. 2 cc. of adrenaline (1 : 10,000) were injected every 1-1/2 hours, so that

each animal received altogether 22 cc. of adrenaline. At the end of the experiments the findings were:

Exp. 57 - stomach contained food; reaction acid.

Exp. 58 - stomach empty; reaction neutral.

Exp. 59 - stomach empty; reaction neutral.

Therefore the conclusion may be drawn that the sympathetic nervous system does not convey secretory impulses to the gastric glands in the skate as it does in amphibia such as the frog. The inhibitory influence of splanchnic stimulation and of adrenaline on gastric secretion in the skate is probably due to vasoconstriction.

5. Destruction of the Spinal Cord.

As reported above, elimination of the vagus influence on the gastric mucosa did not produce any marked effect on the secretory function of the gastric glands.. Quite different resuts, however, were obtained when the sympathetic nervous system was eliminated by destruction of the spinal cord from the cervical region down to the tail. Destruction of the spinal cord results in a "paralytic" secretion of acid gastric juice. This comparatively voluminous acid secretion appeared in from about 24 hours to 3 or 4 days after the cord was destroyed, the time varying in the 10 experiments performed. The secretion continued to the end of the experiment, the longest duration of any of the experiments being 16 days, during which the animal did not of course receive any food. The amount of fluid which could be aspirated from the stomach varied widely in different animals, ranging from 1 cc. to 15 cc.

TABLE 6. "Paralytic" secretion of gastric juice in the skate after destruction of the spinal cord.

Exp.18. - R. erinacea, Aug. 7, stomach empty reaction of gastric mucosa very acid. Spinal cord destroyed at noon. Killed Aug. 16.				Exp.51. - R. erinacea, Aug. 31, stomach empty, reaction of gastric mucosa very acid. Spinal cord destroyed at noon. Killed Sept. 15.			
Date	Vol. cc.	pH	Δ° C.	Date	Vol. cc.	pH	Cl gm. %
Aug. 8	no fluid	acid		Sept. 1	5.0	6.2	1.780
" 9	13.0	3.0		" 2	1.2	3.2	1.801
" 10	7.0	3.2		" 4	1.0	3.0	1.854
" 11	2.0	3.0		" 5	2.0	2.5	1.818
" 12	1.8	2.8		" 6	3.0	2.5	1.813
" 13	9.2	2.8		" 7	0.5	2.5	.....
" 14	9.5	2.8	1.662	" 8	2.0	2.0	1.760
" 15	10.0	3.0	1.667	" 9	1.0	2.0	1.960 (?)
	5.0	3.4	1.325	" 11	0.5	2.0	.....
				" 12	4.5	2.1	1.670
				" 14	5.0	2.0	1.760
Aug. 16				Sept.15			
Juice	5.0	4.9	1.245	Juice	5.0	2.0	1.760
Blood			1.515	Blood)			
				plasma)			0.929
Sea-)				Sea-)			
water)			1.686	Water)			1.804

N.B. - Average Δ of the local sea-water during summer = 1.72° C.

per 24 hours, though the exact amount of juice secreted could not always be determined, since the integrity of the gastro-intentinal tract was preserved and part of the secretion probably passed into the intestine. Although sea-water may possibly have entered the stomach just after the operation, it is very improbable that it did so in the later stages of the experiment. It is possible that, immediately after the cord was destroyed, the oesophagus lost its tone or the tone of the sphincters was diminished so that water passed into the stomach. Sea-water, which has an average pH of 8.2, would be quite sufficient to neutralize the acid secretion. If poured on the acid mucosa of the stomach and kept there for one or two minutes, it turns the mucosa neutral or alkaline.

Two examples are quoted here of long-continued "paralytic" gastric secretion in skates with spinal cord destroyed, exps. 18 and 51 (table 6). The first animal was sacrificed 9 days, the second 16 days after the destruction of the cord.

The freezing point depression of the acid gastric juice in the first skate (exp. 18) gradually diminished until it reached an extremely low value, Δ = 1.245 °C. It was lower than the freezing point of the blood (Δ = 1.515°C.) and of the sea-water (average Δ = 1.72° C.) It is a remarkable fact that the Δ of the blood in this animal was much lower than the freezing point of the sea-water.

These findings on the blood of the skate are in accordance with the results previously obtained by Chaisson ('33), in which destruction of the spinal cord resulted in a lowering of the osmotic pressure of the blood.

In the second animal (exp.51) the chloride concentration of the acid gastric secretion varied from day to day, reaching on several occasions a concentration higher than that of the local sea-water (St.Andrews, N.B). On Sept.15, when the animal was killed, the Cl concentration of the juice was equal to 1.76 mg. per cent, whilst that of the sea-water was 1.804 mg. per cent. The blood plasma of this skate contained somewhat more Cl (0.929 gm. per cent) that is found in the normal animal (about 0.905 gm. per cent).

Injection of adrenaline stopped the acid "paralytic" secretion. In several experiments the stomach contained a neutral or slightly alkaline fluid; this was presumably sea-water because its Cl concentration and Δ were approximately those of sea-water. Experiment 33 may be cited as representative of several such experiments.

Exp.33 - R. diaphanes, 1.25 kg. Had been kept in the laboratory for 19 days without food. Aug.18: Stomach acid, no fluid. Spinal cord destroyed. Aug.22: Stomach empty and acid. 10 a.m., 2 cc. adrenaline (1 :10,000) injected subcutaneously and intramuscularly. 11.05 a.m, 0.5 cc. fluid aspirated from stomach, pH 7.2. 12.05 p.m, 23.0 cc. fluid, pH 7.6. 2.05 p.m., 4.5 cc. fluid, pH 7.6. The Cl of these fluids was 1.79 gm. per cent. (Another indication that the fluid was sea-water is that it could be aspirated from the stomach as soon as the animal was replaced in the aquarium tank but not when it was kept on a board.). Aug.23: 8.15 a.m, 3.0 cc. acid fluid aspirated, pH 3.1, Cl 1.98 gm. per cent. The Cl concentration of the sea-water at this time was 1.80 mg.

per cent. Aug.24: 8.15 a.m, 3.6 cc. fluid, pH 3.7.

The evidence in favour of the theory that an actual secretion of gastric juice results from destruction of the spinal cord is as follows.

(1) The reaction of the gastric contents was acid, attaining sometimes a pH as high as 2.0.

(2) The osmotic pressure of the gastric contents was lower than that of the sea-water.

(3) The Cl concentration of the gastric contents did not always correspond to that of the sea-water but was sometimes lower and at other times higher.

(4) The secretion possessed a moderate peptic power, varying from 40 to 150 Mett's units.

(5) During the secretion of the "paralytic" juice both the secretion and the blood itself became gradually depleted of some substance, as may be judged from the gradual rising of the freezing points.

Control experiments, in which the spinal cord was not destroyed but was cut below the medulla, showed that this procedure does not produce a "paralytic" secretion. Only a few cc. of gastric juice could be aspirated occasionally. Thus:

Exp.31 - R. diaphanes, 1.50 kg. Aug.16: Stomach empty and reaction acid. Spinal cord cut below medulla. Stomach empty but strongly acid, until Aug.19. Aug.19: 3 p.m., 3.1 cc of fluid, pH 3.8, were aspirated. During the following two days the stomach remained empty but its mucous membrane was strongly acid

in reaction. Aug.21: Animal killed. Blood Δ = 1.725°C.; sea-water Δ = 1.737° C.

Therefore the essential condition for the production of a "paralytic" gastric secretion seems to be the disconnection of the peripheral parts of the sympathetic nervous system from its central stations in the spinal cord. The greater part of the cord must be completely destroyed. When on some occasions a probe of insufficient thickness was used for the purpose of destroying the cord, presumably some points of the cord remained intact, since the animal, when touched, moved its pectoral fins and tail slightly. In such cases an acid gastric secretion did not appear, but instead the stomach was filled with a large amount of neutral fluid, presumably sea-water, just as in the first few hours after complete destruction of the spinal cord or after injection of adrenaline.

6. Effect of Histamine.

This substance, known to be a very powerful stimulant of the gastric glands in warm-blooded animals, when introduced subcutaneously into the skate even in massive doses, neither augmented nor inhibited the spontaneous gastric secretion in normal animals. Thus in one experiment (exp. 45, Aug.26) 2 mg. histamine monochloride were administered subcutaneously every two hours for a period of ten hours (total dose 10 mg.) without any effect.

In another series of experiments (exps. 53 to 56, Aug.19) four skates were injected simultaneously with 2 mg. of

histamine and 2 cc. of adrenaline (1 : 5,000). In three of the animals the initial acid reaction of the stomach became neutral in 12 hours.

In connection with the above it is interesting to note that MacKay ('31) found that histamine has no effect on the circulatory system of the skate.

7. Influence of Secretion on Osmotic Pressure of Blood.

The osmotic pressure of skate's blood is somewhat higher than that of the sea-water in which the animal lives. Thus Chaisson ('33) and Chaisson and I ('35) found the Δ of skate's blood to be 1.80°C. and of sea-water 1.72°C. About half of the blood Δ is due to its high urea content (Smith, '29).

As noted above, destruction of the skate's spinal cord resulted in a "paralytic" secretion. In view of the fact that Babkin and Komarov ('31) found that gastric juice of the skate contained about 1% urea, I was inclined to believe that the lowered Δ's of gastric juice and blood during secretion (table 6) were due to loss of urea.

Since then Dr. MacIntosh of our laboratory has re-investigated the problem ('36). He found that gastric juice urea fell progressively during "paralytic" secretion but that blood urea remained constant. He concluded that the fall of the blood Δ was due to the depletion of some substance other than urea.

EXPLANATION OF LETTERING IN FIGS. 1, 2, 3, 4.

A.I.A.	anterior intestinal artery.
A.G.A.	anterior gastric artery.
A.G.Sp.	anterior gastro-splenic artery.
C.A. or C.A.A.	coeliac axis.
C.B.	chromaffin body.
C.B.D.	common bile duct.
D.A.	dorsal intestinal artery.
H.A.	hepatic artery.
I,I.A.	intra-intestinal artery.
L.I.	large intestine.
L.Sp.A.	left anterior splanchnic nerve.
L.V.	left vagus nerve.
P.	pylorus.
Pl.	plexus.
P.G.Sp.	posterior gastro-splenic artery.
P.I.A.	posterior intestinal artery.
P.S.A.	pancreatico-splenic artery.
R.Sp.A.	right splanchnic nerve.
R.V.	right vagus.
S.	sinus.
Sp.A.	anterior splanchnic nerve.
Sp.M.	middle splanchnic nerve.
S.G.	sympathetic ganglion ("gastric ganglion").
$S.G._1$	first small sympathetic ganglion.
$S.G._2$	second small sympathetic ganglion.
$S.G._3$	third small sympathetic ganglion.
S.M.A.	superior mesenteric artery.
V.G.A.	ventral gastric artery.

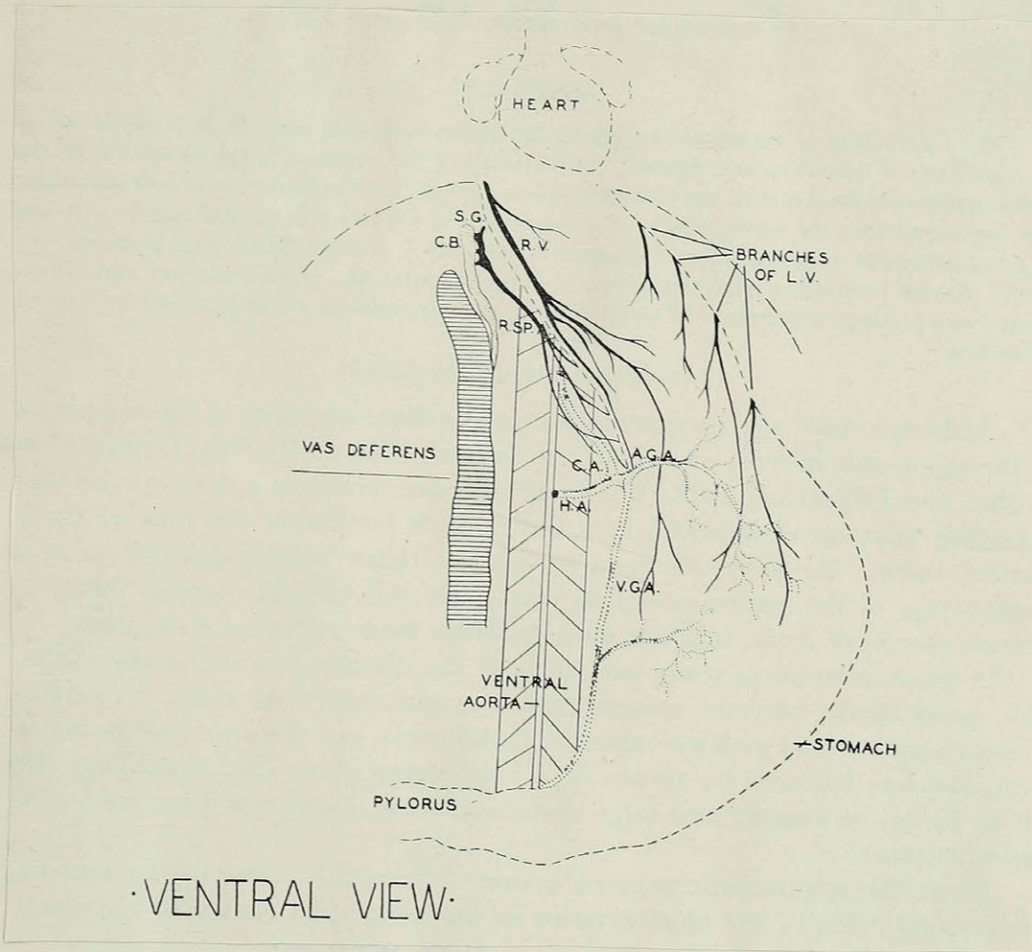


FIGURE 1. Semi-diagrammatic representation of distribution of the visceral branch of the vagus in the skate.

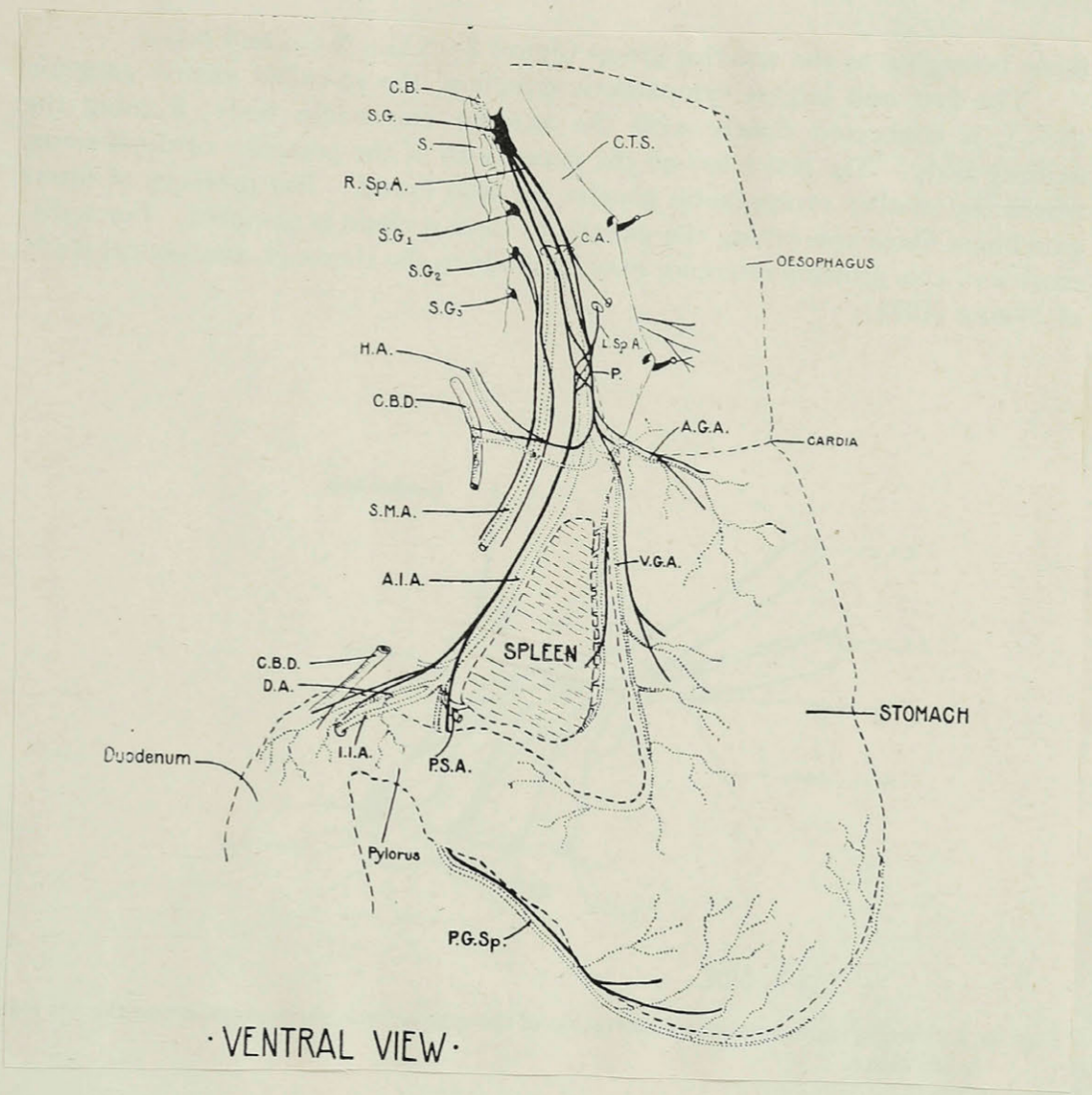


FIGURE 2. Semi-diagrammatic representation of the sympathetic nervous system on the right side in the skate.

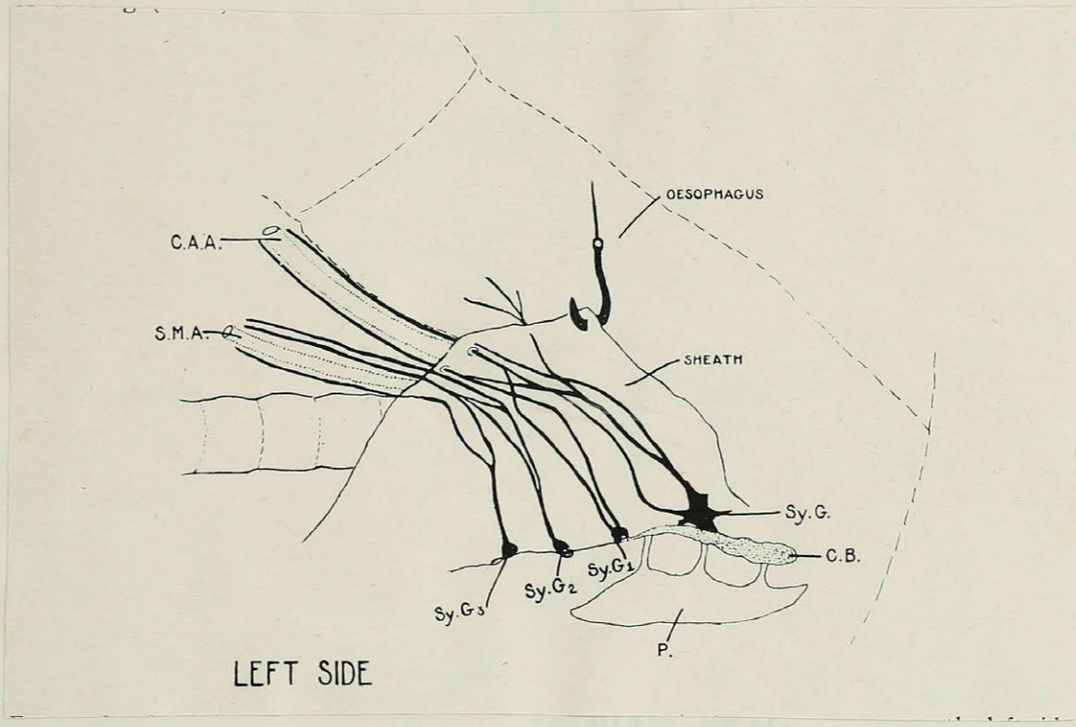


FIGURE 3. Semi-diagrammatic representation of the sympathetic nervous system on the left side in the skate.

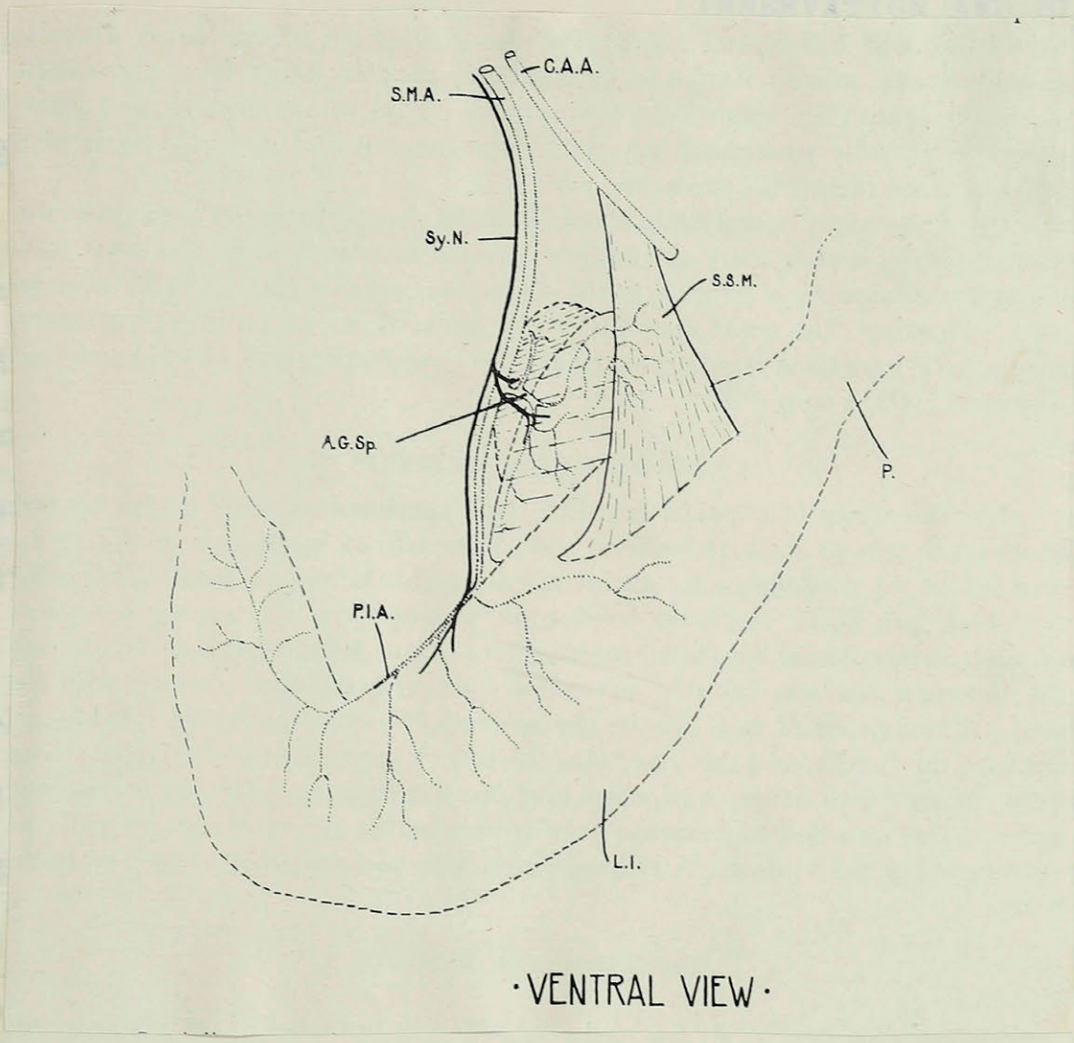


FIGURE 4. Semi-diagrammatic representation of the sympathetic nervous supply to the intestine in the skate.

ELASMOBRANCH STOMACH - INNERVATION AND BLOOD SUPPLY.

1. Blood Vessels.

The following anatomical relations were found to exist in Raja stabuliforis (from 20 to 25 kgm weight) and large specimens of R. diaphanes. The blood vessels were injected with pyronine and Janus green and the course of the nervous branches was followed by means of a magnifying glass. Daniel's ('28) terminology for the designation of the arteries has been used.

The coeliac axis at the level of the cardia gives off the gastro-hepatic branch and the anterior intestinal branch (A.I.A.). The gastro-hepatic artery is divided into the hepatic artery (H.A.), sending branches to the liver and gall-bladder, the anterior gastric artery (A.G.A.) and the ventral gastric artery (V.G.A.) (figures 1 and 2).

The anterior intestinal artery runs along the common bile duct to the duodenum. It gives off the following main branches: posterior gastro-splenic artery (P.G.Sp.), intra-intestinal artery (I.I.A.), dorsal intestinal artery (D.A.) and pancreatico-splenic artery (P.S.A.) (figure 3).

The superior mesenteric artery (figures 2 and 4) supplies the dorsal part of the pyloric division of the stomach, the dorsal lobe of the pancreas and the spleen through the anterior gastro-splenic artery (A.G.Sp - figure 4). Another branch of the superior

mesenteric artery is the posterior intestinal artery (P.I.A. - figure 4). It runs along the dorsal wall of the intestine, almost up to the most posterior part, and gives off segmental annular branches.

2. Visceral Branch of the Vagus.

Both intestinal branches of the vagus (figure 1) enter the abdominal cavity as two large nerve trunks. They lie under the tough membrane covering the oesophagus and both give off numerous branches to the oesophagus. Several branches also descend to the body of the stomach and about the middle of this organ disappear within the muscular tissue, so that they cannot be followed further with the naked eye. Muller and Liljestrand ('18) were able to trace the right vagus nerve to the anterior part of the cardiac division, where it divides into two branches. One of these follows the course of the gastric artery and one of the main branches of the anterior splanchnic nerves to the incisura angularis, where it breaks up into a number of small branches supplying the pyloric canal. The other branch of the vagus reaches the middle splanchnic nerves and supplies the intestine.

3. Sympathetic Innervation.

On each side of the vertebral column may easily be seen a large and three or four comparatively smaller sympathetic ganglia, which send nerve branches to the gastro-intestinal tract chiefly along the arteries described above. Other sympathetic ganglia are

found lying caudad, but these are much smaller than those belonging to the anterior group (figure 2 - S.G.$_1$, S.G.$_2$, and S.G.$_3$).

The first and largest sympathetic ganglion, the so-called gastric ganglion (S.G.), is connected closely with the anterior chromaffin body, forming the axillary body. The latter lies on the dorsal wall of the posterior cardinal sinus, where the smaller sympathetic ganglia are also found. The presence of inter-ganglionic fibres connecting the ganglia to form a chain is disputed. For a discussion of this problem reference may be made to the thorough anatomical study of Young ('33).

Fine rami communicantes may easily be seen running from the spinal nerves to the gastric ganglion.

The large sympathetic ganglion on the right side (figure 2) usually gives off three nerve branches, which are called by Muller and Liljestrand ('18) anterior splanchnic nerves. These nerves pass caudalwards and join the coeliac axis. They run along the artery for a certain distance and are enclosed in the same sheath which surrounds the artery. In this region the nerves supply a few branches to the oesophagus and are connected by some fine fibres with the vagus nerve. At the level of the distal end of the oesophagus they join with the branches of the left anterior splanchnic nerves (figures 2 and 3) to form a plexus (figure 2 - Pl) around the artery. From here on both sympathetic systems arising from the gastric sympathetic ganglia must be considered conjointly.

The left gastric sympathetic ganglion (figure 3) also usually gives off three nerve branches which join the coeliac axis

and add to the formation of the plexus around it. On their course to the coeliac axis they also give off a few branches to the oesophagus.

At the level of the cardia the nerves surrounding the coeliac axis form three main divisions which follow the course of the three arteries, viz, the hepatic (H.A.), the gastric (A.G.A. and V.G.A.) and the anterior intestinal (A.I.A.). Accordingly the sympathetic nerves supply the liver and the bile passages, the cardiac part of the stomach and a greater part of the lesser curvature, and also the spleen. The sympathetic nerve, following the anterior intestinal artery, divides at the pyloro-duodenal junction into two branches, one going to the duodenum, the other following the posterior gastro-splenic artery and supplying the pylorus and the greater curvature of the pyloric part of the stomach (figure 2). The two or three small sympathetic ganglia (figure 2 - $S.G._1$, $S.G._2$ and $S.G._3$) on the right side give off nerve branches to the superior mesenteric artery, the course and divisions of which they follow (figures 2 and 4). Muller and Liljestrand ('18) call these sympathetic fibres the "Nn. splanchnici medii". Before joining the superior mesenteric artery the nerve fibres from the corresponding ganglia on the left side form a kind of plexus on the oesophagus (figure 3). The sympathetic nerves following the course of the superior mesenteric artery supply the intestine, spleen and perhaps the pancreas.

Muller and Liljestrand ('18) describe a third set of fine nerve fibres in elasmobranchs, arising from the sympathetic

ganglia which form the caudal end of the chain (Nn.splanchnici posteriores). They run partly free, and partly along the inferior mesenteric artery, to the caecum and rectum. It is very difficult to see them with the naked eye.

One of the many remarkable features of the sympathetic nervous system of elasmobranchs is that there are no large sympathetic ganglia on the way from the lateral sympathetic ganglia to the abdominal organs, such as exist in mammals. However, the great majority of the nerve fibres which leave the lateral sympathetic ganglia in elasmobranchs are non-medullated, post-ganglionic fibres. (Bottazzi, '02; Young '33).

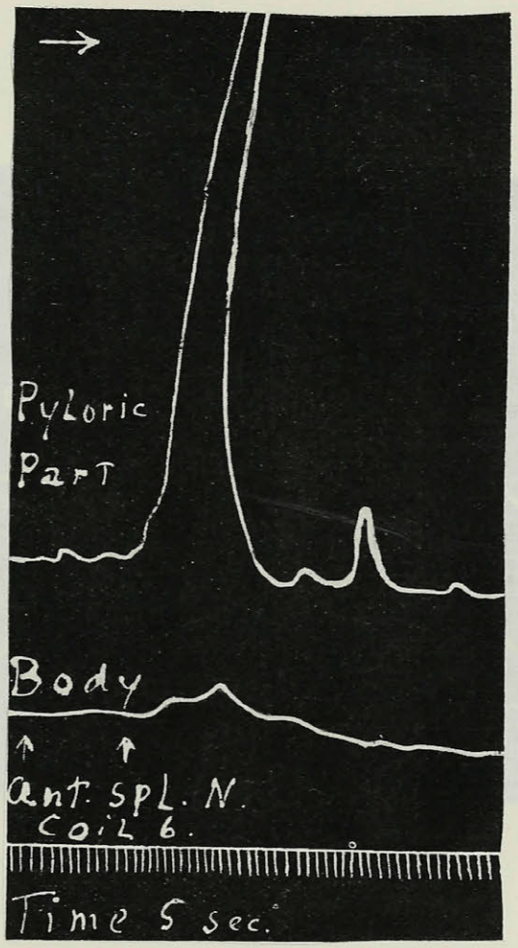


FIGURE 5. Exp. Aug. 15. R. stabuliforis, 27 kilos. One balloon introduced into the pyloric canal (upper curve), another into the body of the stomach (lower curve). Stimulation of anterior splanchnic nerves for 60 sec.

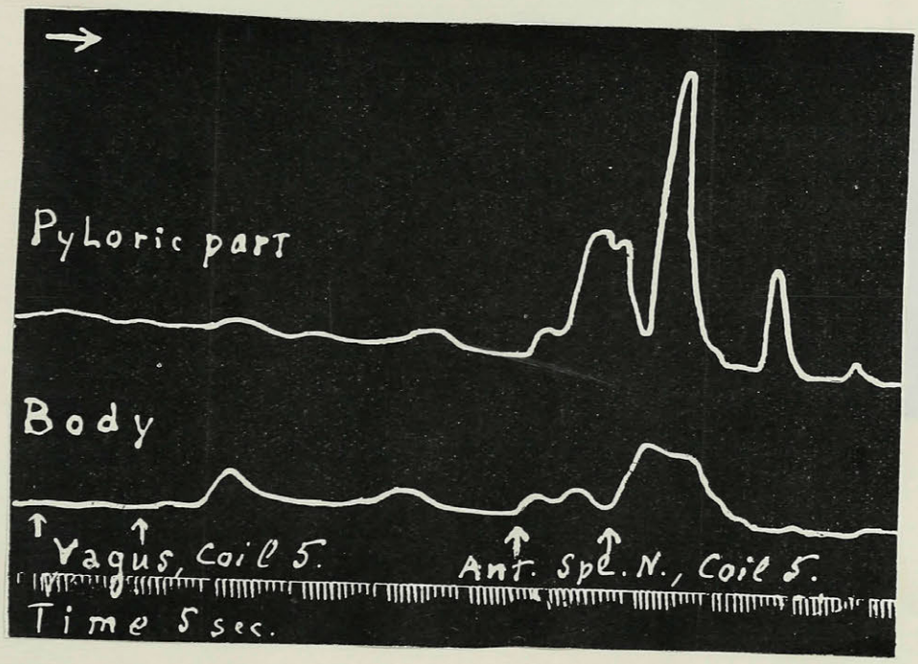


FIGURE 6. Exp. Aug. 15 (See figure 5). Summation of effect of stimulation of the vagus and anterior splanchnic nerves.

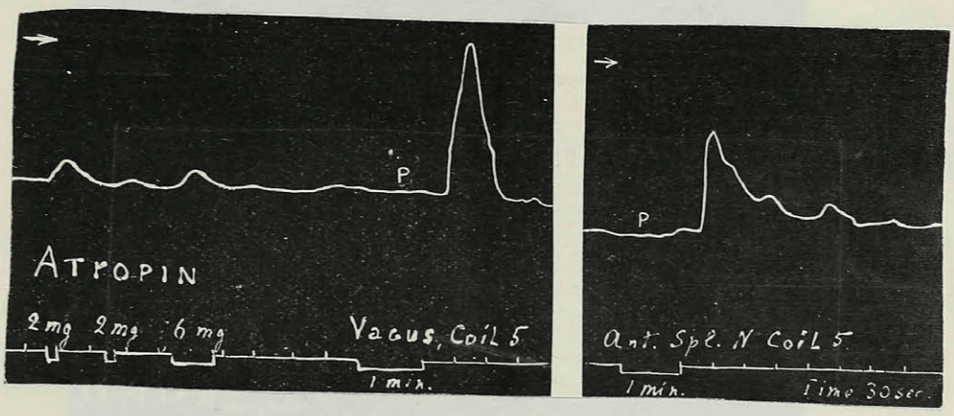


FIGURE 7. Exp. Aug. 23. R. diaphanes, 5-1/2 kilos. Balloon inserted into the body of the stomach. Influence of atropine on the effect of vagus and anterior splanchnic nerve stimulation. Stimulation of the latter nerve applied almost immediately after stimulation of vagus. P., contraction of the pylorus and pyloric canal which was not recorded.

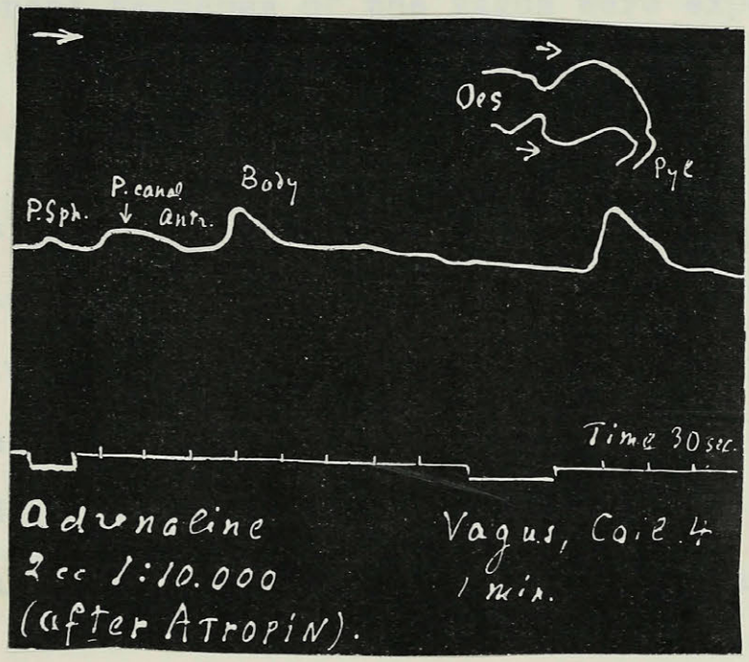


FIGURE 8. Exp. Aug. 23. Continuation of the experiment represented in figure 7. Previous injection of atropine did not hinder the stimulatory effect of adrenaline. Stimulation of vagus initiated a wave of contraction at the cardia, which did not travel very far.

ELASMOBRANCHS-
GASTRIC MOTILITY.

1. Influence of Vagus Stimulation.

Different branches of the vagus were exposed at the oesophagus and stimulated. The upper branches of the vagus bear relations to the oesophagus only; stimulation with a faradic current produces almost immediate contraction of the oesophagus. (According to Meneely '33, the oesophagus of the dogfish, Squalus acanthias, consists of striated muscle). The other branches of the vagus have relation to both the oesophagus and the stomach.

Stimulation of the vagus showed two remarkable features:-

1. immediate contraction of the oesophagus, and contraction of the stomach which began 25 to 45 seconds after the cessation of stimulation;
2. initiation of contractions in different parts of the cardia and fundus on stimulation of different branches of the vagus, but a spread of contraction over whole organ if the current applied to a vagal branch was strong enough. The motor effects of vagus stimulation are shown in Figs. 6 , 7 , and 8 .

A typical course of events when one of the vagal branches (in this case that on the right side of the oesophagus) was stimulated for 30 seconds with a moderately strong

induction current (Harvard inductorium, coil 8 cm.) was as follows:

Experiment, 2, Aug.9. Raja stabuliforis. - Almost immediate contraction of the lower end of the oesophagus. Five to ten seconds after the beginning of stimulation, contraction of the pyloric sphincter occurred, lasting 5 to 10 seconds. Next there was a period with very few, if any, contractions, covering the rest of the stimulation period and the period immediately following cessation of stimulation. But 20 to 25 seconds after the end of the stimulation forward and backward movements of the lesser curvature and slight contractions of the pyloric canal began. A pulsating ring appeared in the cardiac part, and at times the body of the stomach would perform rotating movements.

2. Influence of Anterior Splanchnic Nerves.

The anterior splanchnic nerves, when stimulated, produced a much stronger and more prolonged effect than did the vagus. Almost immediately after stimulation a contraction of the lower end of the oesophagus would frequently appear. During the first 5 to 10 seconds the pyloric sphincter contracted. During the remainder of the stimulation period (30 to 60 seconds) there were no movements whatsoever. The period of 25 to 30 seconds immediately following cessation of stimulation was also one of quiescence, after which a very strong contraction began at the pyloric canal and spread craniad. This contraction passed

through the antrum pylori* and constricts the whole stomach; as the organ became narrower, it moved bodily caudad. The pyloric canal was constricted in toto, had a pencil-like appearance and was whitish in colour. Once or twice a contraction of the duodenum was noted, but this phenomenon was not so constant as the contraction of the stomach which could always be observed during and after stimulation of the nerves until they lost their excitability. About 1½ minutes after the end of stimulation the whole stomach had constricted and then relaxed, these movements being repeated possibly several times (figure 5).

3. Influence of Nn. Splanchnici Medii.

Stimulation for 30 seconds (coil 5 cm.) gave the following results. During the stimulation only a slight contraction of the pylorus was noted. One to two minutes after the cessation of stimulation a strong ring of constriction appeared at the lower end of the large intestine and spread about one-third of the way up.

After several stimulations of the vagus and the anterior splanchnic nerve, in some experiments spontaneous contractions occurred in the form of travelling rings. These started at the cardiac end of the stomach and descended to about the middle of the fundic part.

* Professor Babkin and I propose tentatively to designate as "antrum pylori" the part lying between the corpus or body of the stomach and the pyloric canal in the skate. It is necessary to give a separate name (although perhaps a temporary one) to this region of the stomach on account of its special reaction to sympathomimetic drugs, described by Nicholls ('33).

Muller and Liljestrand ('18) observed that vagus stimulation inhibited movements which had been provoked by splanchnic stimulation. In most of these experiments it was found that stimulation of both nerves, either simulataneously or in rapid succession, produced no diminution in effect. In some cases, however, it was observed that preliminary stimulation of the vagus actually increased the effect and shortened the latent period of splanchnic stimulation (see figure 6).

4. Effect of Atropine

Atropine in large doses (6 to 10 mg.) did not prevent the positive effects of either vagus or splanchnic stimulation or of intravenous injection of adrenaline (figures 7 and 8). A small dose of adrenaline (2 cc. of 1:10,000 solution injected into the lateral vein of a skate of 5 kg.) produced quite distinct movements of the stomach in exactly the same order as stimulation of the anterior splanchnic nerves usually did (see figure 8). Atropine by itself stimulated movements of the stomach (figure 7) and in fact even increased the effect of vagus stimulation. Nicholls ('34) demonstrated that the effect of vagus stimulation on a strip of gastric muscle of skate is not affected by atropine, whereas the positive effect of acetylcholine is abolished by atropine.

NECTURUS
GASTRIC SECRETION.

1. Continuous Secretion.

In a healthy Necturus gastric secretion is continuous and spontaneous. Although the animals were kept in tanks of running water and not fed for periods as long as four months, small amounts (about 0.3 cc.) of acid fluid could always be aspirated from the stomach. This secretion is noteworthy in that it was almost entirely without peptic activity as tested by Mett's method. Except in sick animals (skin infected by a fungus growth), the volume of juice that could be aspirated was always small and always acid (pH range 1.0 to 3.5, average about 2.0).

The stomachs of a number of animals were aspirated on several occasions and the concentration of acid in the collected samples of the gastric juice was determined. As will be seen from the following table, very little free acid was found, most of the acid being "bound".

HCl m.eq./l.	Sample Number					
	A	B	C	D	E	Average
Total	50.4	40.0	20.0	32.0	29.6	34.4
Free	4.0	0.0	0.0	2.4	7.2	2.7

On several occasions very little fluid could be aspirated from the stomach. However, the gastric mucosa of these animals was definitely acid to some congo red paper which was passed into the stomach.

TABLE 7.

Influence of Acetylcholine on Gastric Secretion in Necturus.

Expt. No.	Concentration in mg.	Time Interval. Hours	Gastric Juice	
			cc.	pH or reaction
40	0.1	3	0.4	1.6
14	"	12	0.3	2.4
13	"	25	0.2	Congo Red +
15	"	25	0.2	Congo Red +
39	0.2	3	0.3	1.4
16	"	13	0.3	2.0
29	0.5	5	0.4	1.8
12	"	7	0.4	1.2
41	"	18	0.5	1.4
45	1.0	3	1.4	6.2
42	"	18	0.3	1.2
25	2.0	5	0.3	Congo Red +
26	"	5	0.3	Congo Red +
27	"	5	0.3	Congo Red +
28	"	5	0.6	Congo Red +
44	"	12	0.4	1.6
43	"	18	0.3	Congo Red +

Influence of Pilocarpine on Gastric Secretion in Necturus.

22	0.2	3	0.4	3.5
23	"	3	0.4	1.8
24	"	5	0.6	2.0
46	"	18	0.3	Congo Red +
47	"	18	0.3	Congo Red +

Table 8 .

Effect of Adrenaline on Gastric Secretion in Necturus.

Expt. No.	Coneentration	Time Interval. Hours	Gastric Juice	
			cc.	pH or reaction
10	1/5,000	10	0.3	Blue Litmus -
35	"	18	0.3	6.4
30	1/10,000	5	0.3	Blue Litmus -
31	"	5	0.2	Blue Litmus -
32	"	6	0.5	6.8
33	"	6	0.3	Congo Red +
34	"	6	0.2	6.8
1	"	19	0.4	6.0
2	"	19	0.6	6.8
3	"	19	0.3	Blue Litmus +, Congo Red -
6	"	21	0.4	Blue Litmus +, Congo Red -
7	"	48	1.1	3.6
5	1/20,000	6	1.0	2.0
36	"	15	0.6	6.5
10	1/25,000	2	0.2	Blue Litmus +, Congo Red -
11	"	5	0.5	6.0
21	"	5	0.4	6.2
20	"	6	0.5	6.5
8	"	9	0.8	6.8
37	"	20	0.6	6.5
18	1/50,000	2	0.1	Congo Red +
20	"	3	0.8	7.0
19	"	6	0.5	6.8
38	"	18	0.4	Blue Litmus +, Congo Red -

2. Influence of Acetylcholine and Pilocarpine.

Normally, about 0.3 cc. of juice of pH 1.0 to 3.5 could be aspirated from the Necturus' stomach. As will be noted from table 7 , administration of acetylcholine (0.1 to 2.0 mg.)and pilocarpine (0.2 mg) were without effect over periods of 3 to 25 hours on gastric secretion. Except in experiment No.45, neither the volume of the juice that could be aspirated nor the acidity were changed.

Only 3 experiments were performed with atropine, each Necturus receiving 0.5 mg., 1.0 mg., and 2.0 mg. respectively. Even after 8 hours, the juice aspirated from the stomach was similar to that of the normal animal.

The only conclusions that can be drawn from these experiments is that parasympatho-mimetic substances are without influence on the gastric secretion of Necturus. In this respect the Necturus would appear to be similar to the skate, where it was found that the vagus nerve probably does not participate in gastric secretion.

3. Influence of Adrenaline.

The results of administration of adrenaline in different concentrations (1/5,000 to 1/50,000) after various time intervals (2 to 48 hours) are tabulated in table 8 . It will be observed that except in 4 experiments (Nos.5,7,8,33), adrenaline had an inhibitory influence on gastric secretion. However, I do not know yet whether this inhibition is the effect of direct action of the adrenaline on the gastric glands or the result of vasoconstriction.

NECTURUS

GASTRIC MOTILITY.

1. Normal Contractions.

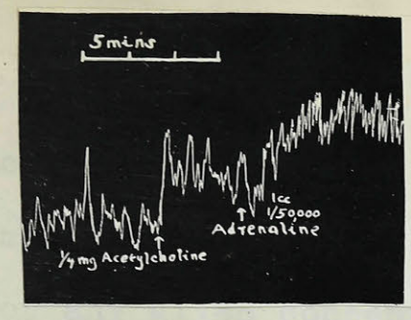
Compared with the smooth muscle of warm-blooded animals, the rate of contraction of Necturus stomach tissues is much slower. The rhythmicity of these spontaneous contractions is, however, constant. Even when the movements are accelerated, as, for instance, when acetylcholine is added, the rate is still less than in the normal dog's stomach.

The chief difference between circular and longitudinal strips of Necturus stomach lay in their rates of contraction. For example, circular strips from the fundic region contracted on the average 8.5 times per ten minutes whilst longitudinal strips from the same region contracted 4.5 times per ten minutes (average). As a rule, the amplitude was greater in strips taken from the fundus (either circular or longitudinal) than in strips from the cardiac third or pyloric third of the stomach.

In strong contrast with mammalian stomach strips, in the Necturus the rate of supply of air-bubbles to the bath is without noticeable influence on either the tonus or rhythmicity. In the case of the mammalian foetus' stomach (vide infra) shutting off of the air supply brings on almost complete cessation of rhythmic contractions as well as a fall of the tonus.

2. Influence of Acetylcholine.

Circular Strips.--Strips taken from the circular coat of all three regions were stimulated by all concentrations of



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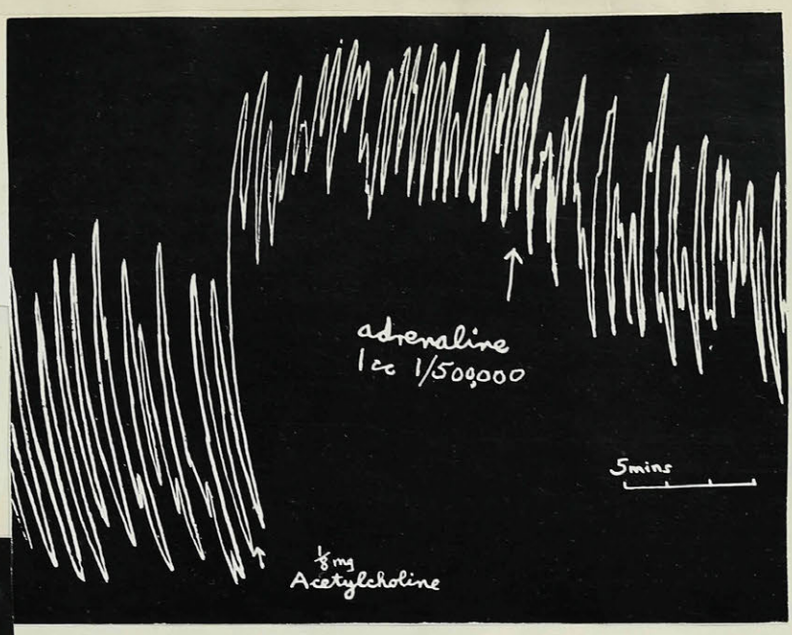

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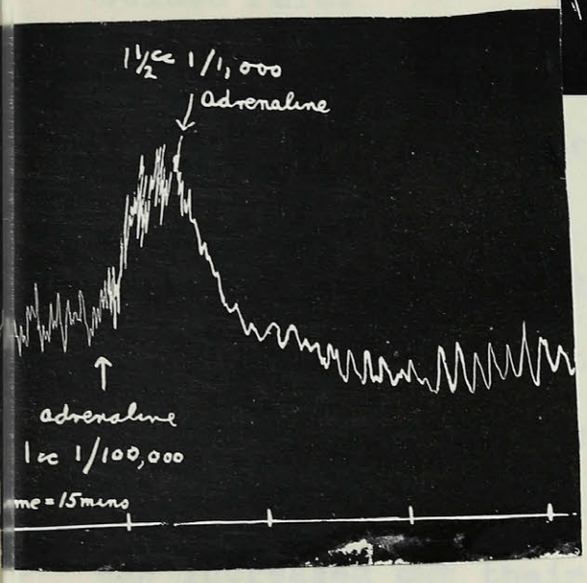

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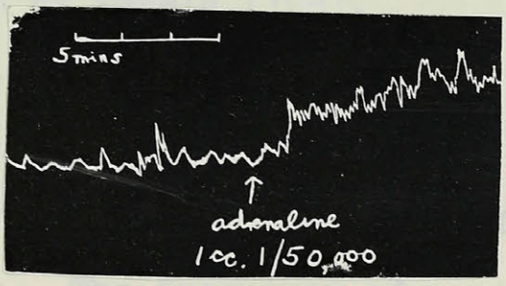

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Fig. 9 - Fundus, circular. Showing synergistic action of adrenaline after acetylcholine. Bath 500 cc.

Fig.10 - Fundus, longitudinal. Bath 500 cc.

Fig.11 - Fundus, circular. Showing mutuo-antagonistic effects of adrenaline in different concentrations. Bath 500 cc.

Fig.12 - Pyloric, circular. Bath 100 cc.

acetylcholine. The rise in tonus was usually very marked and rapid, and the rhythmicity was frequently increased (Fig. 9). For any given concentration of acetylcholine the effect was more marked on strips from the middle (fundic) third of the stomach than on strips from other regions, though this was not an absolute rule.

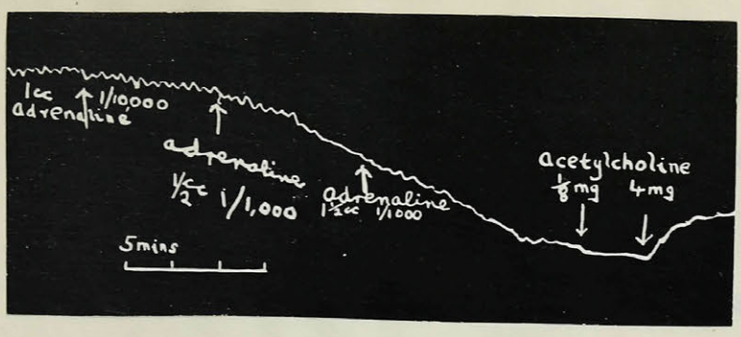
The effect of acetylcholine in increasing both the tonus and the rate of contractions has already been shown in respect of the mammalian (cat) stomach by Esveld ('28).

Longitudinal Strips.--On longitudinal strips taken from all three regions of the stomach, acetylcholine had a definite stimulatory effect, the posterior part being least affected. The tonus rise was abrupt and high. The frequency of contractions was increased but their amplitude was diminished (Fig. 10).

3. Influence of Adrenaline.

Cardia, Circular Strips.--The effects of adrenaline on the circular strips taken from the upper portion of the stomach, just below the oesophagus, are similar to those on circular strips taken from the middle or fundic portion and can best be considered when dealing with that region.

Fundus, Circular Strips.--Adrenaline had two dissimilar and, in certain respects, mutuo-antagonistic effects on the circular fibres of the fundus, depending on the concentration of adrenaline. In concentrations of 1/500,000 to 1/1,000,000,000 adrenaline had a stimulatory effect. The tonus rose markedly and the frequency of contractions was increased from 8.5 to 13 per ten minutes (averages).



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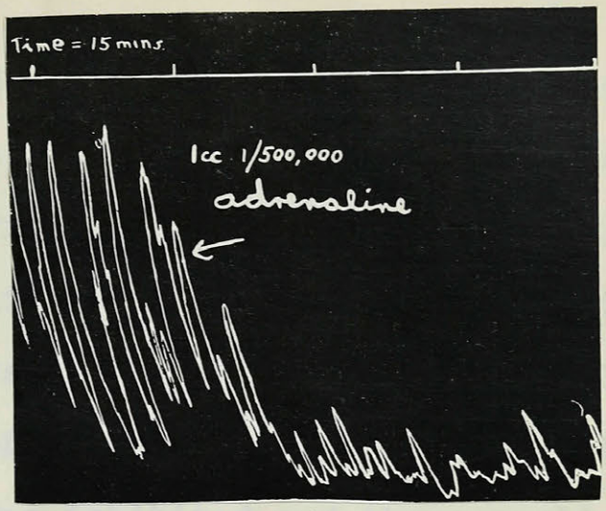

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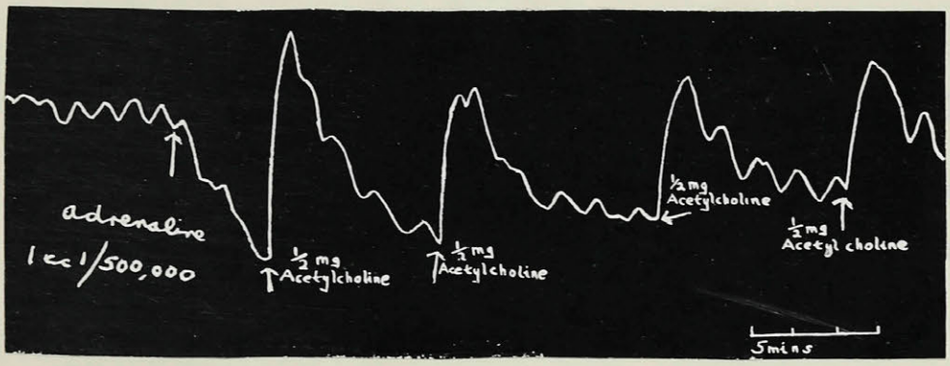

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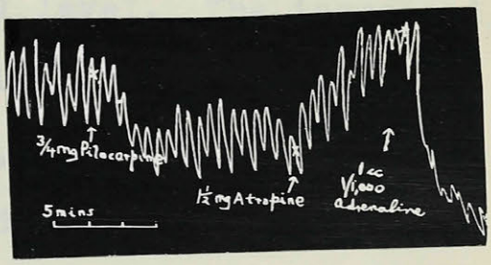

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Fig.13 - Cardia, longitudinal. Showing comparative insensitivity of cardiac region to adrenaline. Bath 100 cc.

Fig.14 - Fundus, longitudinal. Bath 500 cc.

Fig.15 - Pyloric, longitudinal. Single coat of longitudinal fibres. Showing pyloric region to be most sensitive to adrenaline. Bath 500 cc.

Fig.16 - Fundus, longitudinal. Bath 500 cc.

In concentrations greater than 1/500,000 the effect of adrenaline was distinctly inhibitory, both as to tonus and frequency of contractions (the latter decreasing from 8.5 to 4 per ten minutes).

As already noted, adrenaline added in small concentrations caused a rise in tonus and augments the rate of contraction. If now there was a further addition of adrenaline in large concentration, the previous rise in tonus was inhibited and there would even occur a fall in tonus below the normal level. The increased rhythmicity was also again decreased (Fig.11). The reverse, a rise of tonus due to a large dose, did not, of course, occur. If adrenaline in high concentration was added slowly to the bath, there occurred at first a rise in tonus, but this was soon followed by a return to, and often to a drop below, the original level.

Pyloric, Circular Strips.--Strips of circular muscle taken from the hinder third, or pyloric portion, of the stomach were very sensitive to the presence of adrenaline. The effect of a very small amount of adrenaline (such as 1/1,000,000,000) was marked; an almost immediate rise in tonus and an increase in both the height and frequency of the contractions were noted (Fig.12). Larger concentrations of adrenaline affected this region as they did the fundic portion; there resulted an inhibition of tonus together with a great decrease (sometimes total cessation) of amplitude and frequency of the contractions.

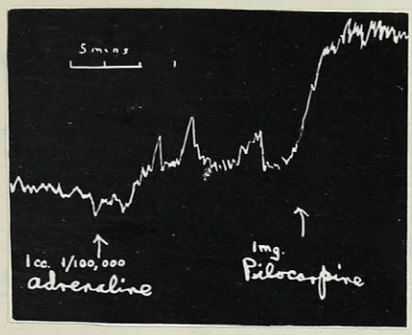
Cardia, Longitudinal Strips.--The reaction to adrenaline of longitudinal strips of (stomach) muscle from this region was

on the whole similar to that of strips from the main portion of the stomach, except that the sensitivity was much less. Small doses of adrenaline were ineffective whilst adrenaline in large concœntrations produced a fall in tonus and abolished all rhythmic movements (Fig.13).

Fundus, Longitudinal Strips.--The reaction of longitudinal strips taken from the middle region or fundus to all concentrations of adrenaline was that of inhibition. Concentrations of 1/1,000,000 to 1/1,000,000,000 always resulted in a fall of tonus and a decrease of the height and frequency of the rhythmic movements. In adrenaline solutions limited to the above concentrations, the weaker solutions, whilst bringing about a fall in tonus, did not always produce complete cessation of the rhythmic contractions: for this the larger concentrations were necessary (Fig. 14).

The effect of adrenaline in repeated small doses was usually like that of a single large dose--lowering of tonus and inhibitions of contractions. However, it may be said that in concentrations of 1/100,000 or less the degree of fall was proportional to the concentration.

Pyloric, Longitudinal Strips.--Of the three segments into which the stomach was divided in these experiments, the pyloric or hinder third was the most sensitive to adrenaline. I have already noted above how sensitive circular strips from this region were to adrenaline. Longitudinal strips were even more sensitive. The fall in tonus produced by adrenaline in small concentrations was so profound as to make a return to the normal



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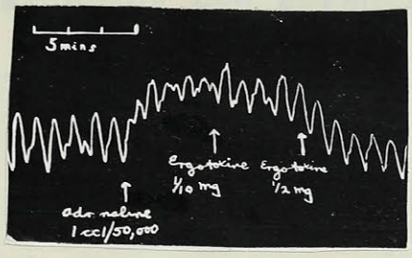

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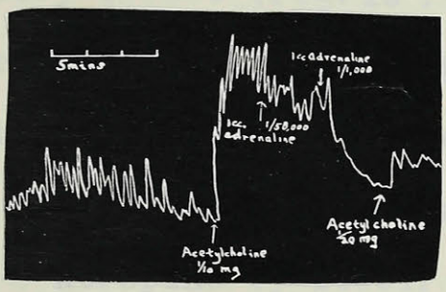

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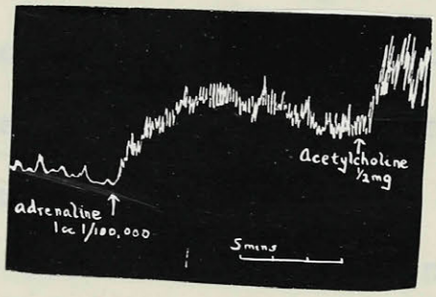

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Fig.17 - Fundus, circular. Showing the synergistic action of pilocarpine after adrenaline.

Fig.18 - Fundus, circular.

Fig.19 - Fundus, longitudinal.

Fig.20 - Pyloric, circular. Showing the synergistic action of acetylcholine after adrenaline.

Volume of bath in Figs. 17, 18, 19, and 20, all 500 cc.

level difficult even with acetylcholine (Fig. 15). However, this will be discussed below when considering the effects of adrenaline and acetylcholine in combination.

4. Influence of Pilocarpine, Atropine, Ergotamine.

These drugs were used occasionally. Pilocarpine when added to strips of circular muscle, produced a rise in tonus but, when added to strips of longitudinal muscle, apparently had an inhibitory effect (Fig. 16). On circular strips of muscle pilocarpine antagonized large doses of adrenaline but (similarly to acetylcholine, as will be discussed below) produced an additive effect with small doses of adrenaline (Fig. 17).

Atropine by itself was without influence on either tonus or rhythmicity of both kinds of strips. However, it antagonized and abolished the stimulatory action of acetylcholine on both circular and longitudinal muscle strips and it also antagonized the inhibitory influence of pilocarpine on longitudinal strips (Fig. 16).

Ergotamine, like atropine, had little, if any influence by itself but antagonized the stimulatory effects of small doses of adrenaline on the circular strips (Fig. 18). What the result would be of administering ergotamine after large doses of adrenaline is not known. It would be of great interest to determine whether the effect of ergotamine depended on the concentration of adrenaline.

5. Influence of Adrenaline and Acetylcholine in Combination.

Probably the most interesting observations in these

experiments were made when adrenaline was given shortly after acetylcholine, or vice versa. As I have already described, acetylcholine produced a rise in tonus and an increase in rhythmicity in both the longitudinal and circular muscle coats. On the other hand whilst adrenaline produced inhibition only on longitudinal muscle, its effect on circular muscle varied with the concentration, producing inhibition in high concentrations and excitation in low concentrations.

Longitudinal Strips.--When adrenaline was added to the bath containing pulsating strips of longitudinal muscle and then acetylcholine was added, the result was that of pure antagonism, i.e. the acetylcholine raised again the tonus which had been lowered by the adrenaline. The same antagonism was seen when the adrenaline was preceded by the acetylcholine--the adrenaline brought down the tonal level which had been raised previously by acetylcholine (Figs. 10 and 19).

As already noted, adrenaline had its profoundest effects on strips taken from the pyloric portion. After a small dose of adrenaline, the fall in tonus was so great and persistent that it required repeated doses of acetylcholine to antagonizethis effect and bring the tonus back to normal again. This is brought out clearly in Fig. 15 , where a strip of longitudinal coat, separated from the circular, was used.

Circular Strips.--The effect on circular muscle of these two drugs was also very regular but not so simple as in the case ofthe longitudinal muscle. This complexity was due to the fact that the effect of adrenaline alone depended on its concentration,

and the synergistic or antagonistic effects of acetylcholine and adrenaline depended also the concentration of adrenaline.

A high concentration of adrenaline, which resulted in a fall of tonus, had its effect abolished by acetylcholine. A low concentration of adrenaline, which resulted in a rise in tonus, would have its effect reinforced by a subsequent administration of acetylcholine to produce a summation of effects (Fig. 20). The stimulatory effect of acetylcholine would be abolished by a subsequent dose of adrenaline in high concentration but would be enhanced by adrenaline in low concentration (Fig.20). These results are made clear from the graphs and are summarized in table 9 . A synergistic effect was also obtained with pilocarpine and low concentrations of adrenaline (Fig.17), but antagonism existed between pilocarpine and adrenaline in high concentrations.

TABLE 9

Effect on circular strips of muscle of two drugs, or of the same drug in different concentrations, when given in consecutive doses.

	Second Drug		
First Drug	Acetylcholine	Adrenaline low concentration	Adrenaline high concentration
Acetylcholine		Summation	Antagonism
Adrenaline low concentration	Summation		Antagonism
Adrenaline high concentration	Antagonism	No effect	

6. Influence of Ephedrine and Chloretone.

Several experiments were made with ephedrine to determine if its supposed sympathomimetic effect could be brought out on the Necturus stomach. It was found that whilst ephedrine by itself had almost no effect, it did, however, appear to "antagonize" the acetylcholine effect on longitudinal strips just as adrenaline would. However, massive doses of ephedrine (15 to 25 mg) were needed and its effect could not be always washed out (as could the adrenaline effect).

Chloretone was used to check the results obtained with adrenaline since chloretone is the preservative present in adrenaline stock solutions and its possible pharmocological action could not be neglected. It was found to have no effect whatsoever.

FROG
GASTRIC SECRETION.

1. Control Experiments.

During the late spring and summer months, frogs secrete spontaneously an acid gastric juice of moderate peptic power. Only about 45% of a large number of frogs tested in the early part of September showed stomachs which were empty and did not secrete any juice. On the other hand, empty non-secreting stomachs were shown by 80% of the frogs tested in mid-January. Similar seasonal variations in the gastric capacity of the intact frog (Babkin, '24), motility of stomach strips (Epstein, '32) and secretion from the isolated gastric mucosa (Delrue, '33) have been recorded.

In control experiments where the stomach was ligated at the oesophagus and pylorus but no stimulants were administered, a small secretion of mucous nature was obtained. The secretion, which was rarely acid and had a moderate peptic power (average, 729 Mett units) was probably due to the mechanical stimulation of the stomach by the ligatures or to a hypermotility of the stomach pressing out pepsin which was held in the folds of the gastric mucosa. The oesophageal secretion in control experiments consisted of thick clumps of mucus of relatively low peptic power (average, 576 Mett units).

2. Influence of Mechanical Stimulation.

The animals were immobilized by destroying the brain and medulla. As will be shown later, this procedure did not

Table 10.

Animal No.	Beads Introduced Into	Reaction	
		Pyloric part	Cardiac part
124-B	Pyloric part	Acid	Neutral
134-B	Pyloric part	Acid	Neutral
124-A	Cardiac part	Acid	Faintly acid
124-C	Cardiac part	Acid	Neutral
134-A	Cardiac part	Acid	Neutral

affect the secretory response of the stomach to mechanical stimulation. The oesophagus was separated from the stomach. One-third of the way up from the pylorus the stomach was divided to form a pyloric portion and a cardiac portion. Just previous to this, beads had been placed either in the pyloric portion or in the cardiac portion. The animals were kept covered with cotton moistened with Ringer. The contents of the two portions were tested after various time intervals. A typical series showing the reactions at the end of 3 hours is given in table 10 . It is apparent that mechanical stimulation of either the pyloric part or the cardia of the stomach produces an acid secretion from the pyloric part only.

Mechanical stimulation of the gastric mucosa induced a reflex secretion not only of acid but of pepsin as well. By placing glass beads in the stomach, the frog could be made to secrete continuously. This secretion (pH 2.2 to 4.3; peptic power about 1000 Mett units) was produced in about 45 minutes and ceased 12 to 72 hours after the removal of the beads from the stomach. In several experiments beads were placed in the stomachs of unoperated frogs. The beads apparently were too large to be passed into the intestine; they were retained by the stomach for 2 months during the whole of which time the stomach secreted acid juice continuously.

Inert substances when present in the stomach also induced a pepsin-rich secretion from the oesophageal glands but only when the spinal cord was intact. The effect on gastric secretion of stimulating the oesophageal mucosa alone by inert

substances was uncertain. In only three of nine experiments was an acid secretion obtained from the stomach. However, the area of oesophageal mucosa was relatively small and it is possible that in most of these experiments the oesophagus was not stimulated at all.

3. Influence of the Parasympathetic Nervous System.

Faradic stimulation of the vagus or of the medulla oblongata for various lengths of time did not produce an acid gastric secretion, though occasional weak movements of the empty stomach were set up. At times a large volume of viscid mucus, definitely alkaline, was produced.

In a number of experiments the whole spinal cord was destroyed or removed and only the medulla and brain left intact. Beads were then placed in the stomach. The only result was the production of a thick alkaline mucus; even after 24 hours there was no indication of an acid juice. This absence of an acid secretion could not have been due to a state of shock following the destruction of the cord, since in other experiments destruction of the brain and medulla only did not interfere with the production of an acid secretion.

Although destruction of the central nervous system does not stop circulation in the viscera (Langley and Orbeli, '10), yet to rule out the possible effects of the fall in blood pressure brought about when the cord was destroyed a series of experiments with yohimbine hydrochloride was performed. Two-tenths milligram of yohimbine was injected into either the dorsal or the abdominal

Table 11.

Drug	Number of Animals Used	Condition of Animal	Reaction
Pilocarpine	7	Normal	All neutral or slightly alkaline
	4	Brain and medulla destroyed	All neutral or slightly alkaline
	5	Cord destroyed	All alkaline
Acetylcholine	7	Normal	All alkaline
	2	Brain and medulla destroyed	All neutral or alkaline
	4	Cord destroyed	All alkaline
Atropine	6	Normal	All acid
	6	Brain and medulla destroyed	5 acid, 1 neutral
	8	Cord destroyed	7 neutral or slightly alkaline, 1 acid

lymph sacs of normal frogs, of frogs with beads in the stomach, and of frogs with beads in the stomach and the spinal cord destroyed. Yohimbine itself, as judged by its action on the normal animal, was without apparent effect on gastric secretion, although the abdominal and visceral blood vessels were markedly distended with blood. Whereas in animals with the cord intact mechanical stimulation produced an acid secretion, in animals with the cord destroyed mechanical stimulation did not. These experiments indicate that the vagus plays little, if any, part in the production of an acid gastric secretion in the frog, as contrasted strongly with the action of the vagus in the dog and cat.

This conclusion is further borne out by the following experiments with pilocarpine (1 to 20 mg.), acetylcholine (0.01 to 2 mg.) and atropine (1 to 5 mg.). These drugs were injected into the abdominal lymph sacs of normal frogs, or frogs with brain and medulla destroyed or with cord destroyed. The results are summarized in table 11 .

It is evident that in the frog pilocarpine and acetylcholine do not stimulate an acid gastric secretion as they do in the dog. On the other hand, atropine does stimulate an acid secretion but only in frogs with cord intact. It would appear that in the frog atropine may not only paralyze the parasympathetic nerve endings but may stimulate some part of the sympathetic nerve as well. Haberlandt ('24) found that atropine may stimulate the sympathetic nerve endings in the frog's heart.

Table 12 .

Animal No.	Parts of C.N.S. Destroyed (+)										Reaction
	Brain	Medulla	Spinal cord giving off nerves								
			1	2	3	4	5	6	7	8	9	
111-A	+	+	+	+	+							Neutral
81-B	+	+	+	+	+	+						Neutral
11	+	+	+	+				+	+	+	+	Neutral
81-A	+	+								+	+	Acid
21	+	+						+	+	+	+	Acid
111-B	+	+					+	+	+	+	+	Acid
2912-B			+	+								Faintly Acid
272			+	+	+	+	+					Neutral
23-B			+	+	+	+	+	+				Neutral
2912-A									+	+	+	Acid

Table 13.

Gastric secretion in response to pilocarpine, adrenaline and histamine. Brain destroyed; stomach ligatured at oesophageal junction and at pylorus. Peptic power in Mett units.

Experiment	Number of Animals	Oesophagus			Stomach			Gastric Secretion was Acid
		High	Low	Average	High	Low	Average	
Control	36	998	207	576	973	369	729	Rarely
Pilocarpine	23	615	384	512	718	615	669	Occasionally
Adrenaline	52	1440	466	1092	1600	676	1196	Always
Histamine	51	2112	948	1186	615	256	452	Always

In our experiments it would seem that atropine did not stimulate the sympathetic nerve endings in the gastric mucosa but rather the sympathetic cells within the cord or in the large sympathetic ganglia.

The gastric secretion obtained when pilocarpine or acetylcholine was administered was very thick and viscous, and with a digestive power (average, 669 Mett units; table 13) somewhat lower than that in control experiments. Usually the reaction was neutral or slightly alkaline and only rarely acid (pH range, 7.3 to 5.0, average 7.0). Possibly the parasympathetic nervous system regulates the secretion of gastric mucus in the frog.

The effect of pilocarpine and acetylcholine on the oesophägeal glands was negative with respect to the secretion of pepsin (table 13).

4. Influence of the Sympathetic Nervous System.

When only the spinal cord of the frog is left intact and beads are placed in the stomach, an acid secretion (pH 2.2 to 4.3) is produced in about 45 minutes. To determine what portion of the cord must remain intact in order that mechanical stimulation of the stomach may produce an acid secretion, in a series of frogs various portions of the cord were removed or destroyed by careful pithing, and beads were then placed in the stomach. Control operations, involving similar parts of the cord but not including insertion of beads, were also performed. The extent of the operation was in each case verified at post-mortem.

From table 12 it is evident that the segments of the cord which give off spinal nerves 2 to 5 must remain intact for mechanical stimulation of the gastric mucosa to produce an acid secretion. (Attention iscalled to the fact that comparative anatomists designate the hypoglossal nerve of the frog as the second spinal, the first being hypothetical and non-existent. We take the hypoglossal as the first spinal nerve, as did also Langley and Orbeli, '10).

Spinal nerves 2 to 5 contribute sympathetic fibers to form the splanchnic nerve. In another series of experiments the splanchnic nerves were cut or the sympathetic chains were removed. In such cases introduction of glass beads did not produce an acid secretion.

In frogs without beads in the stomach and only the cord destroyed no acid secretion was observed even 24 hours after the operation. On the other hand, if only the brain and medulla had been destroyed, an acid secretion would often appear but only about 20 hours after the operation.

Stimulation of the peripheral end of the splanchnic nerve in frogs with the cord either intact or destroyed, produced an acid secretion; stimulation of the central end did not. Stimulation of the sympathetic chains also produced an acid juice but only when they were in direct connection with the splanchnic nerve. All these experiments point to the important part which the sympathetic nervous system plays in regulation of the gastric secretion.

The results of experiments with adrenaline give further support to the conclusion that the sympathetic carries secretory fibers to the frog's gastric glands. An acid secretion was obtained after a latent period which was proportional in duration to the concentration of adrenaline. Frogs with the whole or parts of the central nervous system destroyed reacted to adrenaline, in any given concentration lower than 1 : 1000, much more slowly than did normal animals. Concentrations of 1 : 1000 were too high and delayed secretion for about 11 to 15 hours in all cases.

Adrenaline also stimulated the secretion of pepsin (table 13). The secretory effect of adrenaline was, within certain limits, in inverse proportion to the concentrations employed. Adrenaline in concentration of 1/1000 to 1/5000 did not stimulate a secretion of pepsin until about 12 hours after the injection.

The appearance of an acid, pepsin-rich, secretion only after about 12 hours after the administration of a large dose of adrenaline (1/1000 to 1/5000) may perhaps be explained by the fact that by this time the concentration of adrenaline in the blood had been sufficiently reduced to an excitatory level. In several instances an acid and pepsin-rich secretion induced by a previous administration of a small dose of adrenaline was abolished by a large dose of adrenaline. Whether in these instances inhibition of secretion was due to a strong vaso-constriction produced by the large dose of adrenaline, or to a true inhibitory reaction of the drug on the nervous system or the secretory cells themselves, we cannot at present tell.

Large doses of adrenaline (1/1000 to 1/5000) gave rise within 1 or 2 hours to a neutral or slightly alkaline secretion of thick viscous mucus (pH about 7.1) of moderate peptic power (700 Mett units). This secretion was similar in physical appearance to the control secretion but was much more profuse. After about 12 hours or so, this secretion became acid (pH 3.0 to 4.0) and its peptic power rose rapidly. Fifteen hours after the administration of a large dose of adrenaline, a frog with its stomach ligated at both the oesophageal and pyloric ends would contain within its stomach only acid fluid (pH 2.2 to 3.3) of high peptic power (up to 1600 Mett units). Whether the masses of thick mucus secreted earlier had become dissolved or were digested we do not at present know.

The high pepsin content of the juice stimulated by adrenaline cannot be explained as being due to the hypermotility of the stomach induced by the adrenaline pressing out pepsin which is held in the folds of the gastric glands. Kymographic recordings show that the amount of adrenaline which can produce a secretion is apparently too small to produce any very marked changes in the gastric movements. Further, the secretion continues for a much longer time than do the gastric contractions.

In addition to its effect on the stomach, adrenaline also caused a secretion of pepsin from the oesophagus (average, 1092 Mett units; table 13). However, I am not certain that the time of onset of a peptic secretion from the oesophagus is dependent on the concentration of adrenaline used.

5. Reflex Stimulation of Secretion.

It was observed that when animals which had been tested and in which the stomach was found neutral, were made to jump around, or were handled roughly for about 15 minutes, they gave a moderate amount of acid juice an hour or so later. The average peptic power of such juice was about 100 Mett units. This secretion was evidently not the result of mechanical stimulation of the stomach which had been previously aspirated, since the secretion of control animals similarly aspirated but not handled roughly remained neutral for days. Three possible explanations presented themselves: 1) The excitement of the frog might produce some kind of secretion analogous to the first phase of secretion in warm-blooded animals. 2) The secretion might be due to mechanical stimulation of the abdomen, and hence of the stomach, during jumping. 3) Reflex stimulation of some organs of the body other than the stomach - probably the limbs - might result in a secretion.

The first tentative explanation was easily disposed of. Animals with the cerebral hemispheres and even the whole brain destroyed still secreted when handled roughly. It was also found that mechanical stimulation of the abdomen, such as rubbing or tapping for about an hour or more, did not affect secretion.

On the other hand, although frogs with only the brain destroyed did secrete when handled roughly, they did not do so when the cord was destroyed. Further, since frogs with the splanchnic nerve cut and the cord intact did not secrete when handled roughly,

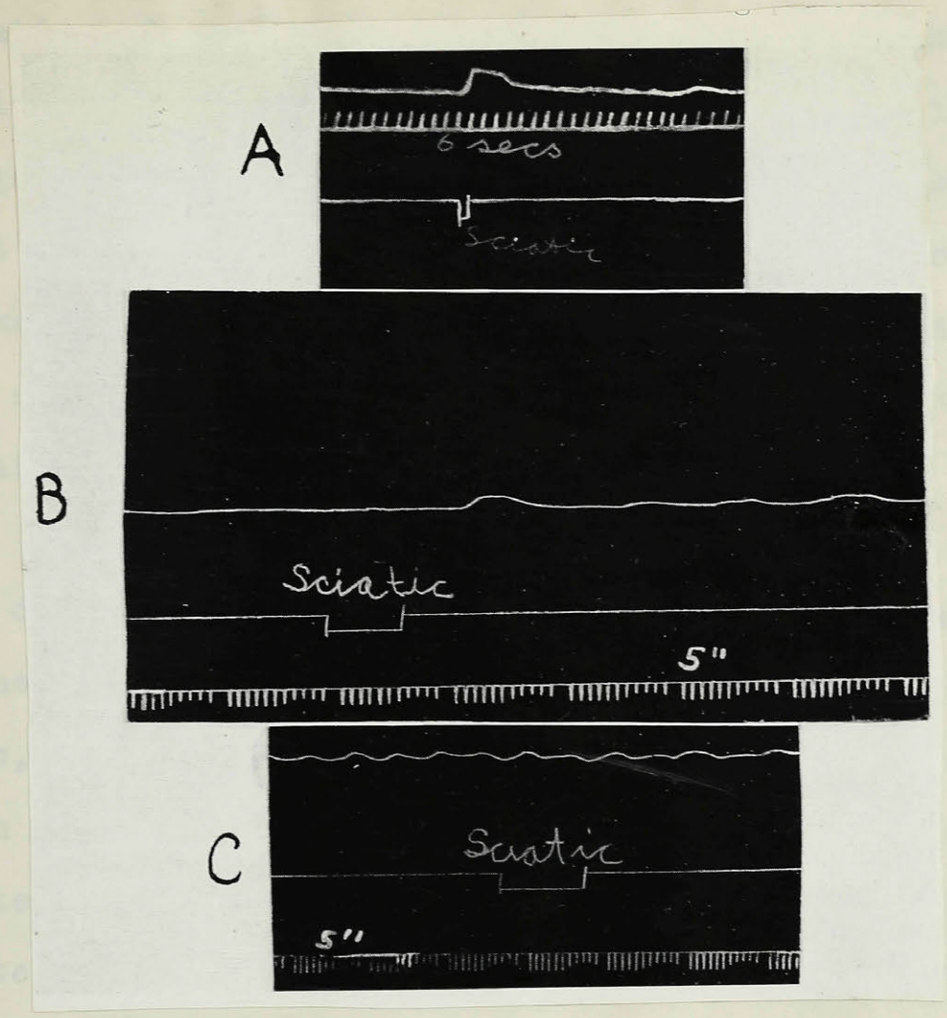


Figure 21.

A - Experiment No. 153. Brain and medulla destroyed, spinal cord intact. Almost immediate response to stimulation of the sciatic nerve.

B - Experiment No. 116. Brain and medulla destroyed, spinal cord intact. Delayed response on sciatic nerve stimulation, suggesting the interplay of adrenaline.

C - Same animal as in "B", after destruction of the spinal cord. Sciatic nerve stimulation without effect.

it was evident that the secretion was the result of a reflex stimulation of some other organ and not of the mechanical manipulation of the stomach itself. Passive exercise of the hind limbs gave a secretion when the brain and medulla were destroyed but not when the cord was destroyed. Special experiments were performed to test the hypothesis that the secretion was due to a reflex stimulation arising from the movements of the hind limbs, stimulation of the skin, etc.

The brain and medulla were destroyed and the frog was pinned down on its back. The abdomen was opened and the stomach disconnected from the oesophagus. One cannula was tied into the cardiac, and another into the pyloric end of the stomach. The stomach was filled with distilled water and the anterior cannula connected to a Marey tambour. The pyloric cannula was used to regulate the amount of fluid within the stomach. The sciatic nerve of one of the limbs was exposed and divided.

Faradic stimulation of the peripheral end of the sciatic nerve did not result in any contraction or relaxation of the stomach. When the central end of the sciatic or the lumbar plexus was stimulated, a strong contraction of the stomach was usually obtained if the stomach had previously been quiescent; if the stomach had been contracting automatically the result was often a relaxation. If the cord was destroyed the automatic contractions assumed a more regular character, but stimulation of the central end of the sciatic nerve was without any effect (fig.21).

To test the possibility of a reflex secretion, the above procedure was repeated but no fluid was placed in the stomach nor

were cannulae tied into it. Secretion was obtained in the stomach when the central end of the sciatic nerve or of the lumbar plexus was stimulated but only if the cord was intact. No secretion resulted from stimulation of the peripheral end of the sciatic, nor from stimulation of the central end of the sciatic, if the cord was destroyed or the splanchnic nerves cut.

Since the suprarenals were not removed in these experiments, the motor and secretory effects of sciatic stimulation might have been partly due to adrenine discharge. At times the stomach contractions took place immediately after stimulation of the sciatic nerve; at other times several minutes elapsed between stimulation and response, and this would suggest the action of adrenine.

6. Effect of Histamine.

Keeton, Koch and Luckhardt ('20) apparently were the first to study the secretory effect of histamine on the frog. They found that histamine when injected into the lymph sac stimulated an acid gastric secretion in the bullfrog but not the grass frog. Hogartz ('32) found that histamine raised the chloride content of the frog's gastric mucosa. Delrue ('33) obtained an acid secretion from the frog's isolated gastric mucosa when histamine was added to the sub-mucosa side of the perfusing medium.

Popielski ('29), believing histamine to be ineffective in cold-blooded animals, tried its effect on frogs (Rana temporaria) whose body temperature had been gradually raised over a period of 3 to 4 weeks to 37° C. Whereas at room temperature only 45% of the

Table 14.

Gastric Secretion in Response to Histamine.

Date	Experiment No.	HCl-Milli Equivalent per Liter		Pepsin Units	Cl-Mg. Per Cent
		Free	Total		
4- 2-36	217-239	65.5	95.2	576	..
11- 2-36	307-327	68.8	84.8	576	..
14- 2-36	328-341	..	..	615	397.0
20- 2-36	364-378	71.2	92.0	416	424.5
11- 3-36	449-463	72.0	86.0	576	..
20-10-36	742-748	40.0	48.0	256	..
3-11-36	772-775	60.0	68.0	..	..

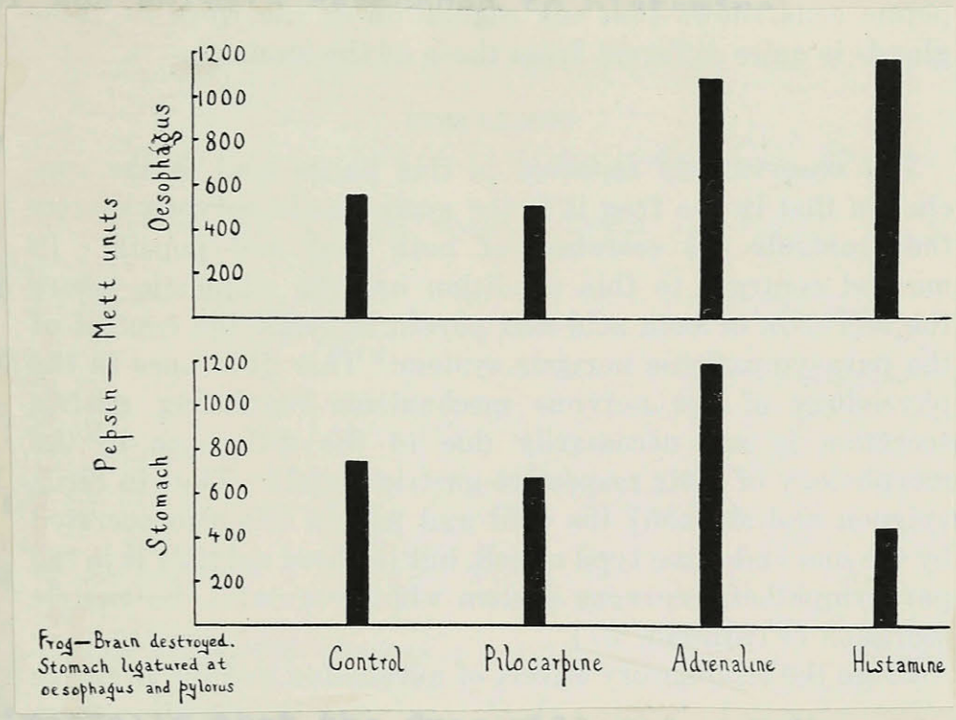


Figure 22.

Concentration of pepsin (in Mett units) in secretions from oesophagus and stomach stimulated by various substances.

frogs which had been given histamine (2 mg. per animal) responded with an increased gastric secretion, at 37° C. about 75% of the animals responded to histamine.

However, it should be pointed out that frogs used by Popielski were secreting spontaneously and that histamine increased only the volume of secretion. Mere increase in body temperature is capable of increasing the volume of secretion. Further, a temperature of 37° C. is physiologically unsuitable for the frog. This is shown by Popielski's own record that 20% of the animals kept at that temperature died. Patterson ('16) found gastric hunger contractions of the frog to cease at 38° C. while Babkin ('24) found them to cease at 35° C. Muller ('22) had shown experimentally that the frog will not survive a temperature of 40° C.

In our experiments, the frogs were kept at room temperature (about 22° C.) Histamine always induced a gastric secretion of large volume which was high in acidity (average 79 mill equiv.) but poor in pepsin (average 452 Mett Units; tables 13 and 14). Indeed the peptic power of the gastric juice was even lower than that of the control secretion (average 729 Mett units). Possibly histamine not only does not stimulate but actually inhibits pepsin secretion as is believed by Alley ('35).

Most surprising and inexplicable was the observation that histamine stimulated the oesophageal glands to secrete pepsin in high concentrations (average 1186 Mett units; table 14 and fig. 22). In view of the fact that histamine is known not to be a stimulant of the peptic cells of the mammalian stomach or of the pepsin-secreting elements of the stomach of other vertebrates, this secretory

effect on the frog's oesophageal peptic cells shows that the regulation of the work of these glands is quite different from those of the stomach.

FROG - GASTRIC MOTILITY.

1. Effect of Stimulation of Splanchnic Nerves.

Inasmuch as numerous previous workers had already investigated the effect of splanchnic nerve stimulation on the motility of the frog's stomach (see Table 4), only a few experiments were performed on the bull-frog, Rana catesbiana. Stimulation of a splanchnic nerve with a moderate current (coil 10 cm.) resulted in a series of waves passing along the stomach and in an increase in tonus. In one animal the brain was destroyed and one splanchnic nerve cut. On stimulating the central end of the cut splanchnic, lively peristalsis was exhibited by the stomach. This effect was abolished when the other splanchnic nerve was cut.

2. Effect of Stimulation of Sciatic Nerve.

Stimulation of the central end of the sciatic nerve resulted in contractions of the stomach only as long as both the spinal cord and one of the splanchnic nerves were intact. The reflex effects of sciatic nerve stimulation have already been considered above. See also Fig. 21.

BIRDS
GASTRIC SECRETION.

1. Control Experiments.

In only 17 out of 98 (or 17%) of the pigeons used in these experiments was a secretion obtained without the previous administration of some stimulus. In 4 of these 17 instances the secretion was viscid, alkaline, and of small volume. In nearly every case the gizzard of these birds contained food in various stages of digestion and the duodenum was filled with digesting food and bile. On the other hand the gizzard of pigeons which did not secrete without any definite stimulus was empty (except for pebbles) and the mucosa of the proventriculus was alkaline or neutral but not acid. From our experiments we cannot conclude that gastric secretion in the pigeon is spontaneous and continuous and, although fewer experiments were done on the chicken, the same applies for the chicken. According to Braitmaier('04) gastric secretion in the bird is continuous but our findings do not confirm this. In the pigeon and chicken gastric secretion is therefore intermittent as it is also in the dog, the winter frog, and possibly also in the turtle and man. A spontaneous and continuous secretion is found in the skate, Necturus, and summer frog (vide supra), and hibernating ground-hog (vide infra).

The variations in the composition of the gastric juice of the digestive phase as found in 13 of the 17 experiments mentioned above were as follows:-

Rate of secretion.....0.2 to 4.2 cc/hr.

Free acidity.....40 to 136 milli equiv.

Total acidity.....60 to 148 milli equiv.

Pepsin.....0 to 36 Mett units.

The anaesthetic used (Nembutal) did not stimulate nor otherwise affect the course of gastric secretion.

2. Effect of Histamine.

It was found that if more than 0.2 mg. histamine were administered to a pigeon, more than (or) 0.6 to a chicken, the gastric juice obtained was contaminated by blood. A single dose of 0.5 mg histamine produced a blood-tainted secretion which lasted for almost two hours; a further hour's interval of rest was required before the juice obtained from a subsequent injection of 0.1 mg histamine was clear of blood. The ulcerative effects of large doses of histamine have also been observed in mammals by several investigators..

Histamine (0.1 to 0.2 mg. per pigeon and 0.1 to 0.4 mg per chicken) stimulated the secretion of a large volume of colorless acid juice. The initial secretion commenced after a latent period of from 7 to 15 minutes and the effect of a single dose lasted about 1 hour. To study the course of gastric secretion under histamine the injections were made every 45 minutes. The secretion was not abolished by atropine. The range of the acidities of the juice was as follows:-

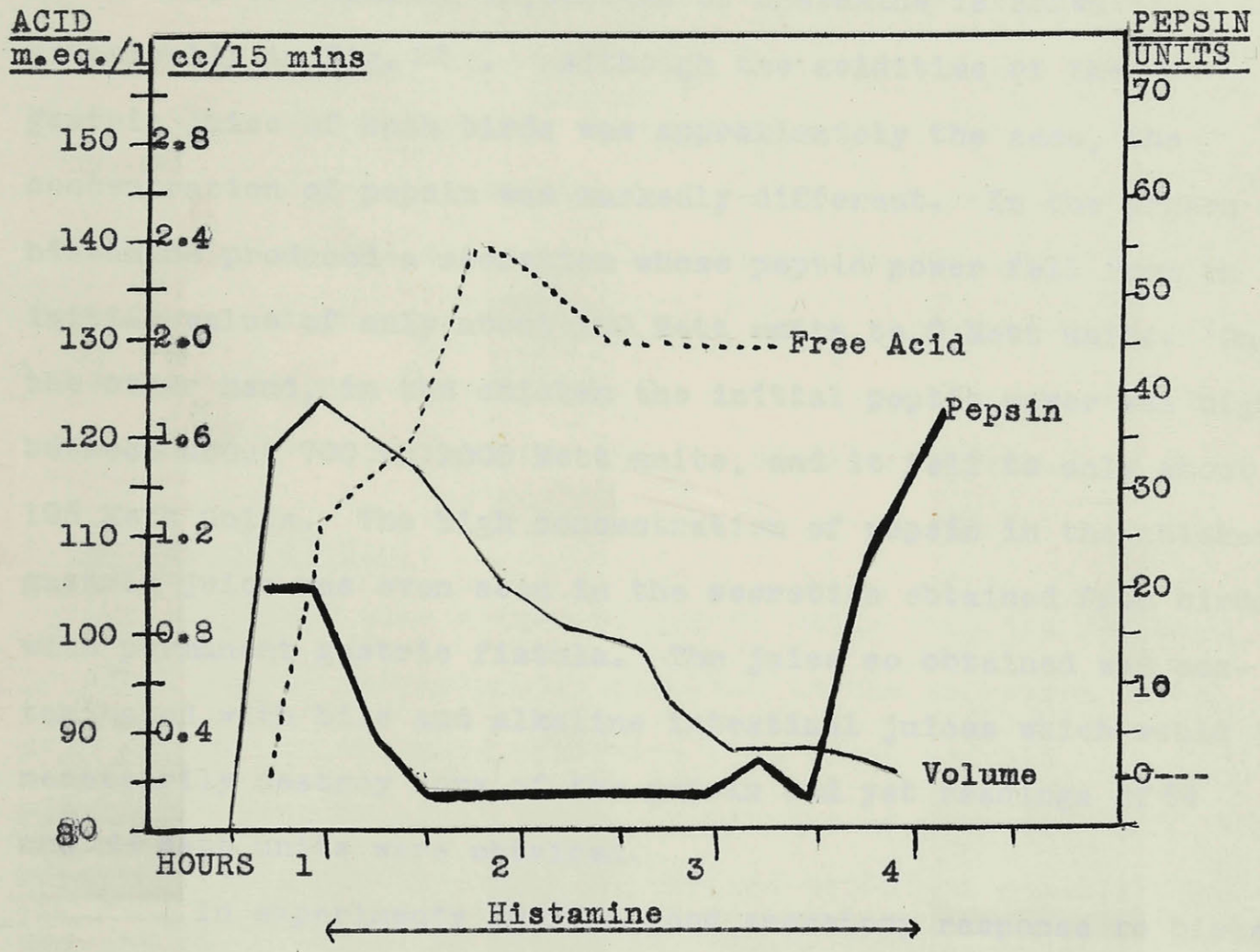


FIGURE 23

Effect of Histamine on the Gastric Secretion of the Pigeon

	Pigeon	Chicken
Free acid, milli equiv.....	70 to 160	80 to 150
Total acid, milli equiv.....	120 to 195	120 to 180

The effect of repeated injections of histamine is shown graphically in Fig. 23 . Although the acidities of the gastric juice of both birds was approximately the same, the concentration of pepsin was markedly different. In the pigeon histamine produced a secretion whose peptic power fell from an initial value of only about 100 Mett units to 0 Mett units. On the other hand, in the chicken the initial peptic power was high, between about 700 to 1000 Mett units, and it fell to only about 125 Mett units. The high concentration of pepsin in the chicken's gastric juice was even seen in the secretion obtained from birds with permanent gastric fistula. The juice so obtained was contaminated with bile and alkaline intestinal juices which would necessarily destroy some of the pepsin and yet readings of 64 and 144 Mett units were obtained.

In experiments where a good secretory response to histamine was obtained, there occurred a great diminution in the volume of the secretion after $2\frac{1}{2}$ to 3 hours. Corresponding to this fall in volume there was a marked increase in the peptic power of the juice, (Fig.23). As will be seen below, this diminution in the volume of secretion was due to the great loss of body fluids which went to form the gastric juice. For the sake of brevity we may term the decresed secretion as "exhaustion" secretion and the corresponding increase in pepsin concentration as "exhaustion" rise in pepsin.

The above experiments lead to the conclusion that in the pigeon and chicken histamine stimulates the secretion of only acid and not of pepsin. Although both the acid and the pepsin are secreted by the same cell, histamine stimulates selectively only the acid-producing mechanism of the cell. Furthermore, since atropine does not abolish the secretion, histamine does not act on the cell in the same manner as does acetylcholine. The bird's proventriculus is thus similar to the stomach of the dog (Babkin, '30; Bowie and Vineberg, '35), hibernating ground-hog, and the frog in that in these animals histamine does not stimulate the secretion of pepsin.

3. Effect of Alcohol.

Alcohol when either placed directly into the duodenum or administered intravenously resulted in the secretion of an acid juice. It was more effective when given by vein, a secretion being obtained within 15 minutes as compared with 35 minutes when given by duodenum. Either method of administration was not as effective as histamine. Alcohol behaved like histamine in that it did not stimulate the secretion of pepsin. The composition of gastric juice stimulated by alcohol was as follows in the pigeon.

	Per duodenum (Expt.No. A31)	Intravenous (Expt.No.B31)
cc alcohol given	30	5
% alcohol used	5	5
Latent period, mins.	35	15
cc secreted in 15 mins.	0.8	1.3
Total acid, milli equiv.	130	162
Pepsin, Mett units.	0	0

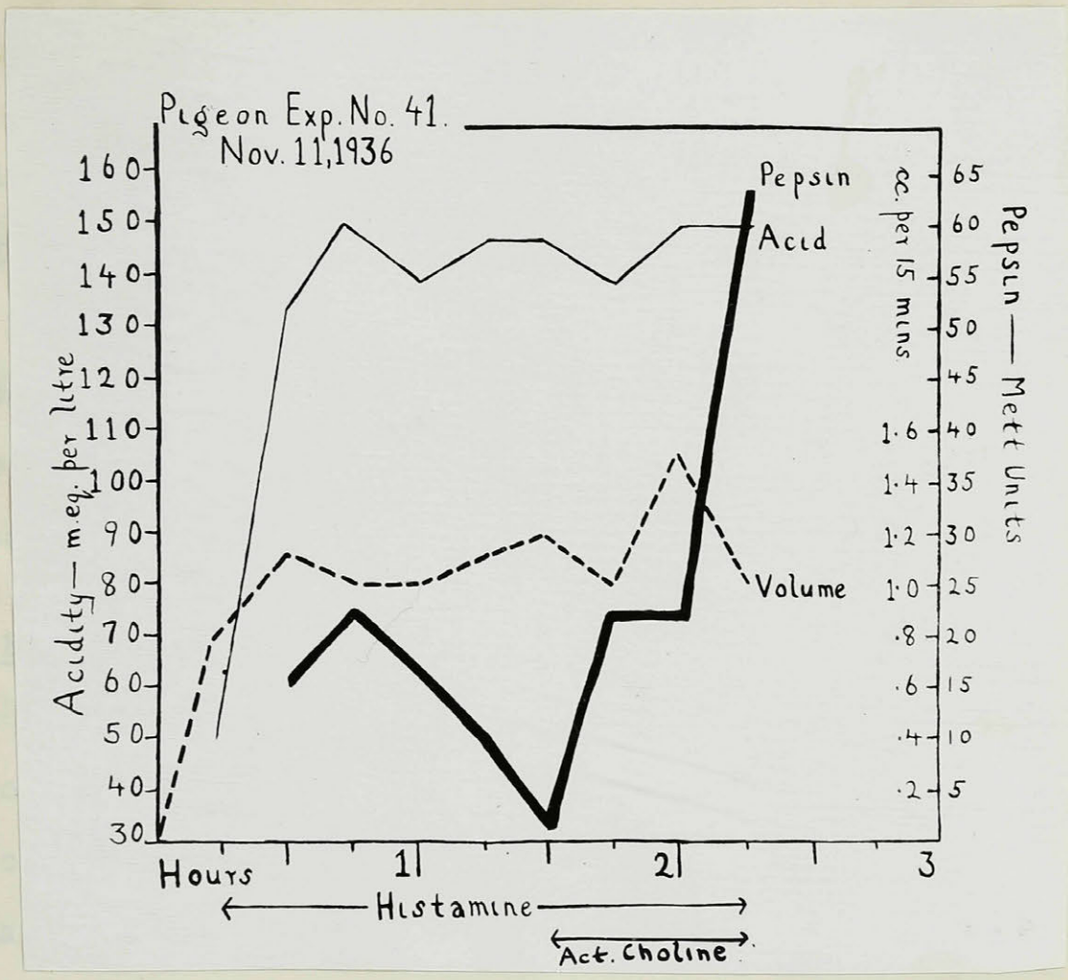


FIGURE 24.

Pigeon - the effect on gastric secretion of histamine followed by acetylcholine.

4. Effect of Adrenaline.

Adrenaline (1 cc per pigeon of 1/10,000) did not stimulate the secretion of gastric juice. When given to a pigeon already receiving histamine, there was a dimunition in the rate of secretion without any marked effect on the concentration of the pepsin. The inhibition was probably due to a general vaso-constriction rather than to any direct specific effect of the adrenaline on the secretory cells.

5. Effect of Acetylcholine.

Acetylcholine when given by itself to either pigeon or chicken induced the secretion of only small amounts of juice which was very rich in pepsin and of high acidity. In order to obtain larger amounts of gastric juice, acetylcholine was administered after a secretion had been previously induced by histamine. Histamine alone gave a secretion extremely poor in pepsin but the subsequent injection of acetylcholine (0.1mg per kgm body weight) increased greatly the concentration of pepsin (Fig. 24). The acetylcholine was given 1 to 1½ hours after the first dose of histamine and while the volume of secretion was still high. The resulting increase in the concentration of pepsin must have been due to the specific effect of acetylcholine on the pepsin-secreting functions of the cell and could not be regarded as similar to the "exhaustion" rise in pepsin concentration seen towards the end of an experiment with histamine alone (vide supra).

To further study the effects of acetylcholine on gastric secretion 10cc of 0.9% saline was given intravenously to a pigeon

which had received histamine. Shortly after the volume of circulating fluid was thus increased by the saline, acetylcholine was administered. The result was a gastric juice with an increased concentration of pepsin.

These experiments show that the secretion of pepsin is under parasympathetic control. Since the secretion produced by the acetylcholine is of only small volume, it would appear that this substance is more specific for the secretion of organic material than for the water of the secretion.

6. Effect of Pilocarpine.

Pilocarpine (0.5 mg per kgm) produced in the pigeon a secretion of moderate volume and of moderate peptic power. When given alone it resulted in a secretion somewhat larger in volume than that obtained from acetylcholine alone. When superimposed on histamine the rise in concentration of pepsin was not as great as when acetylcholine was similarly used. This is brought out in the following table:-

	Expt. No.	cc per 15 mins	Pepsin units
Pilocarpine	80	0.9	16
Acetylcholine	53	0.4	64
Pilocarpine and Histamine	84	1.5	23
Acetylcholine and Histamine	58	1.6	52

The gastric secretion induced by pilocarpine was abolished by atropine (1 mg per pigeon). The secretion produced by pilocarpine

and histamine was neither abolished nor diminished by atropine. These experiments confirm the conclusions already drawn that gastric secretion in the pigeon is under the control of the parasympathetic nervous system and not of the sympathetic as well. Since pilocarpine produced a larger secretion of juice than did acetylcholine and its enzmyatic content was poorer, it would seem that pilocarpine is not as specific a ~~secret~~ secretogogue for pepsin as acetylcholine.

7. Influence of Blood Volume.

When histamine was administered to a pigeon or chicken, the volume of the secretion rose as did also the acidity while the concentration of pepsin fell rapidly (Figure 23). After some time the volume of the secretion began to diminish until it reached a low level in about 2½ to 3 hours. When the volume had diminished quite markedly there occurred a sudden rise in the concentration of pepsin. The acidity usually remained high till the end.

A pigeon receiving repeated doses of histamine would secrete 8 to 12 cc. of juice within 2½ to 3 hours. During this period there was no compensation for the comparatively large volumes of fluid that the bird lost in the form of gastric juice. This loss in fluid often resulted in a loss in body weight of about 5 or 6%. Although no data exist on the blood volume of birds, a loss of 1/20 th of the body weight in the form of gastric juice must concentrate the blood greatly. A definite concentration of the blood was noted whenever an artery was severed in a bird that had already secreted 10cc and more of gastric juice. The blood was very thick and the flow extremely sluggish. This contrasted sharply with the fluid blood that spurted from the cut artery of a normal bird.

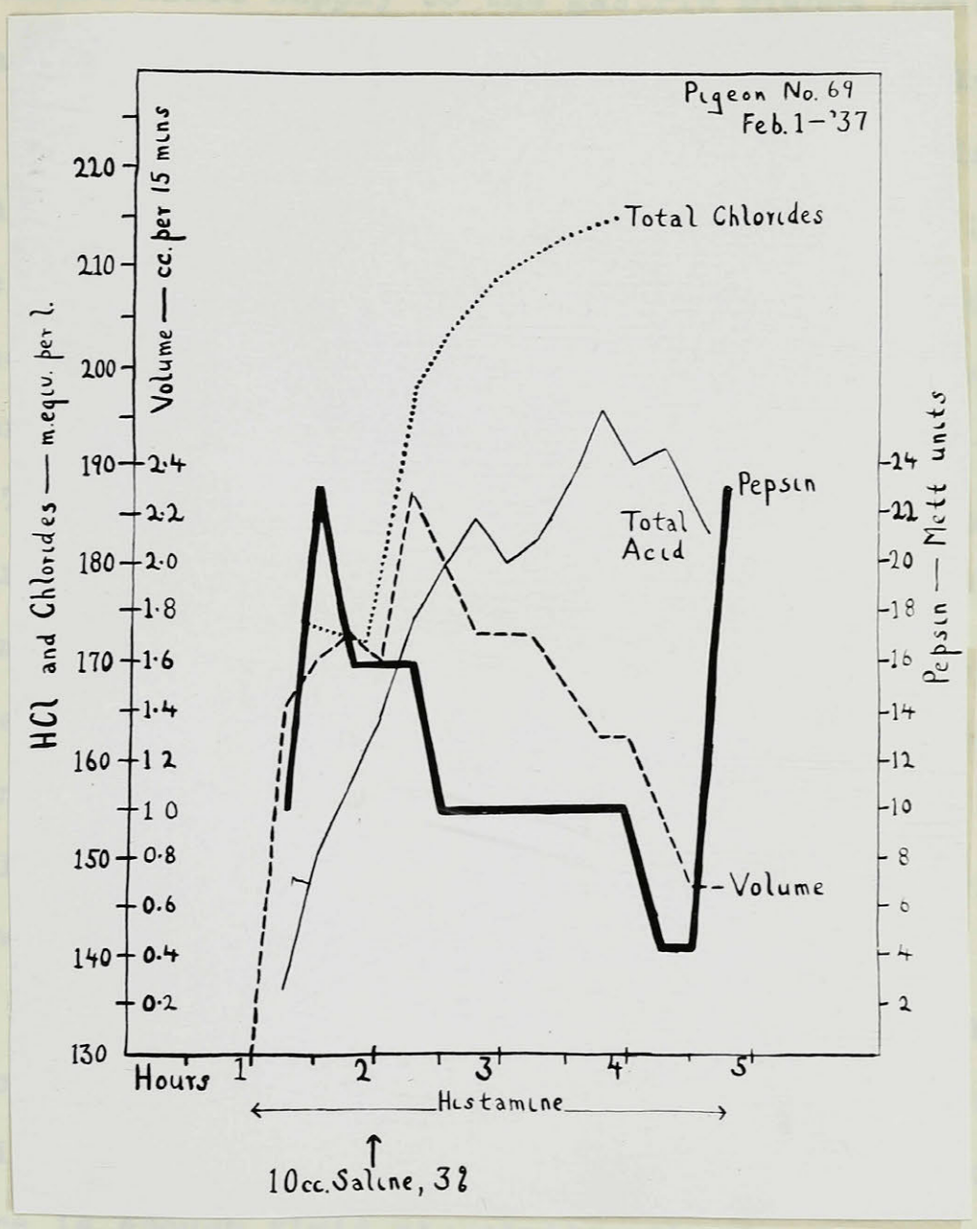


FIGURE 25.

Influence of intravenous saline on the gastric secretion induced by histamine. Pigeon.

The diminished blood supply to the gastric glands could therefore be an explanation of the diminished secretion after 2½ or 3 hours in spite of the repeated administration of histamine. The exhaustion of the secretory cells or their empoisoning by histamine were two other possibilities to be considered. To study the role of the volume of body fluid in gastric secretion, solutions of sodium chloride of various concentrations were administered intravenously. To differentiate between the effect of altering the osmotic pressure of the blood and the specific effect of sodium chloride, isosmotic solutions of glucose were also injected. These injections were made at the rate of 2cc or less per minute since too rapid an introduction of fluid killed the bird.

The results obtained in experiments where sodium chloride was injected intravenously clearly show that the secreting cells are not empoisoned by histamine nor do they become exhausted. The diminution in secretion that occurs after 2½ or 3 hours in histamine experiments (Fig. 23) is apparently due to the loss of body fluids. As long as there is enough fluid circulating through the body there is a secretion of gastric juice in response to histamine. This was shown in many experiments of which No. 69, presented graphically in figure 25, is representative, after the injection of 10cc of 3% saline, the volume of the gastric juice secreted rose and then fell gradually. The total volume of the secretion over the period of the experiment (5 hours) was 19.8cc. Assuming that all of the 10cc of injected fluid was eventually secreted as gastric juice (without taking into account loss through diuresis, intestinal secretion, etc.) the pigeon secreted an additional 9.8 cc. of juice, which is

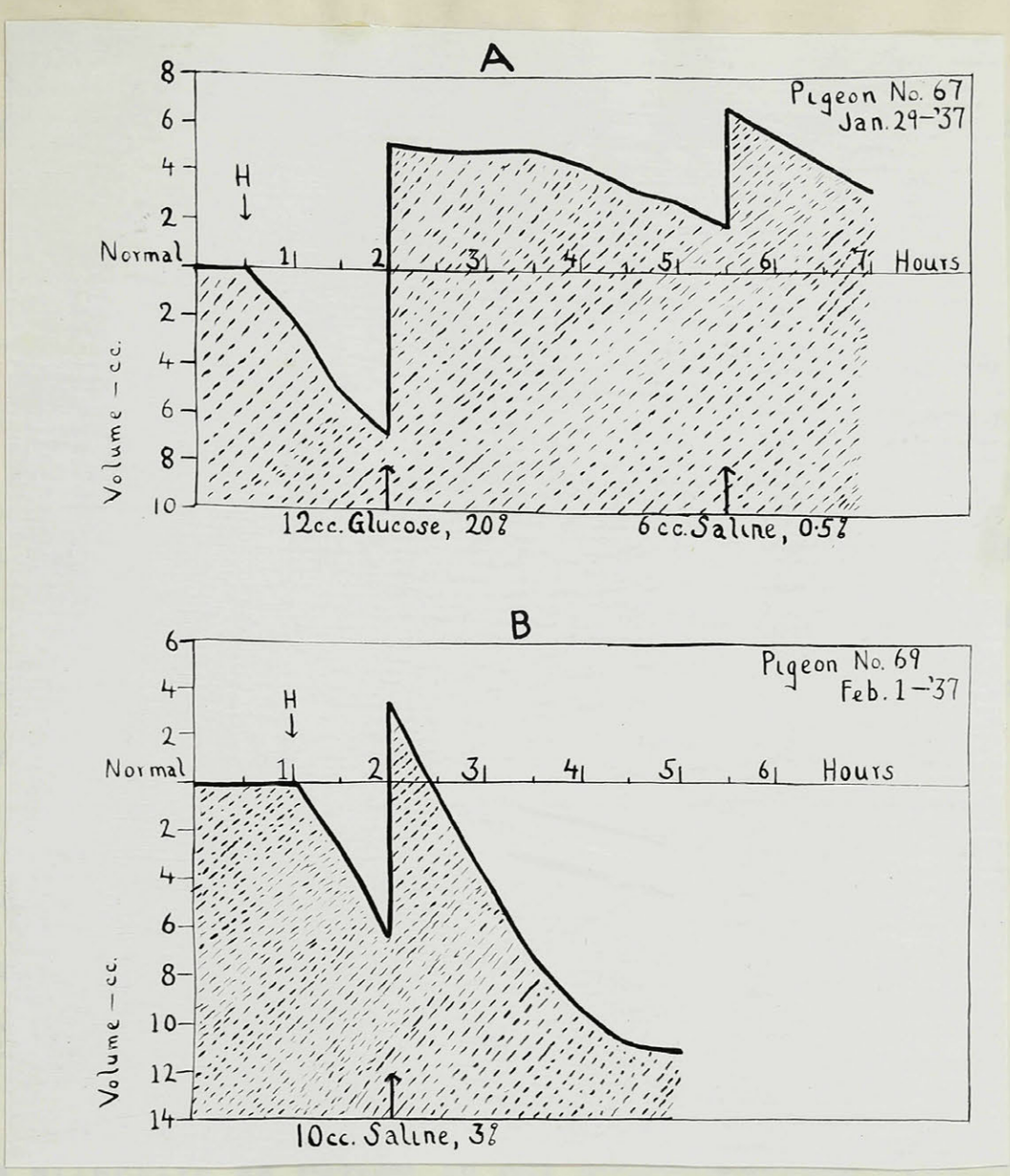


FIGURE 26

Pigeon. Volume of fluid within body shown below heavy line. Histamine injections every 45 minutes commenced at arrow.

A. 20% glucose inhibits loss of body fluids; pigeon is not secreting.

B. 3% sodium chloride does not inhibit loss of body fluids.

Figure 26-B is based on experiment shown in figure 25. No allowance made for diuresis, intestinal secretions, etc.

about the average that a pigeon can secrete under histamine alone. At the end of 4½ hours the pigeon had secreted 9.1cc. above the amount of saline injected. The volume of body fluid is again diminished and an "exhaustion" rise in the concentration of pepsin occurs.

In several experiments saline was given intravenously every one-half hour in amounts equivalent to the volume of juice secreted (by histamine stimulation) during the preceding half-hour. The result was a continuous secretion of acid juice, poor in pepsin, at a volume that was constant for as long as 6 hours. In other words, as long as there was a compensation of the fluid lost in the form of gastric juice, there was a steady secretion and no "exhaustion" rise in pepsin concentration occurred.

8. Effect of Glucose

Glucose injected intravenously in concentrations of 5.6% was as effective as saline in any concentrations (0.4 to 3.3%) in maintaining a secretion induced by histamine and in preventing an "exhaustion" rise in pepsin. (When placed into a loop of intestine, 5.6% glucose was less effective). Glucose in concentrations of 10% delayed somewhat the secretion and stimulated the output of pepsin.

Glucose in the concentration of 20% inhibited very markedly the secretion. Figures 26-A and B are attempts to show graphically the volumes of fluid present in the body during secretion. In figure 26-A, the administration of 12cc of 20% glucose resulted in an arrest of the loss of body fluid (i.e., cessation of secretion). Although histamine injections were repeated every 45 minutes,

Table 15 .

"Histamine" Gastric Juice of Pigeons and Chickens.

Exp.No.	HCl, m.eq./l.		Cl, m.eq./l.	Solids , %.			N, mg. %.			Pepsin Units
	Free	Total		Total	Organic	Ash	Total	N.P.N.	Protein	
Pigeon										
47	139.0	158.0	142.4	.28	.10	.18	14.5	-	-	4
49	151.2	156.8	188.0	.29	.11	.18	14.5	-	-	16
55	153.6	158.4	171.6	.28	.16	.12	14.6	11.1	3.5	10
57	156.4	162.4	195.0	.33	.09	.24	12.9	9.2	3.7	-
74	152.8	160.8	170.4	.38	.20	.18	24.4	18.0	6.4	-
76	150.4	154.4	170.4	.20	.14	.06	9.5	6.3	3.2	7
86	--	--	--	--	--	--	28.6	22.0	6.6	-
95	154.8	160.8	170.4	--	--	--	9.6	7.2	2.4	-
96	--	--	--	--	--	--	14.8	10.3	4.5	5
98	146.8	155.6	168.0	--	--	--	14.5	10.3	4.2	1
99	147.2	153.2	172.0	--	--	--	12.8	8.2	4.6	4
Chicken										
26	146.4	152.8	174.4	.43	.31	.12	30.2	17.6	12.6	174
27	126.0	136.0	162.8	--	--	--	57.1	44.3	14.0	125

secretion resumed only after a long period of inhibition. This is in sharp contrast with figure 26-B which shows that 3% sodium chloride (approximately isosmotic with 20% glucose) had no such effect.

Many more experiments are required to determine the reason for the differences in effect between isosmotic solutions of sodium chloride and glucose in higher concentrations. It is evident, however, that glucose has a specific trophic effect on the pepsin-secreting functions of the secretory cells since its adminstration results in an increased output of pepsin.

9. Composition of Pure Gastric Juice.

Pure gastric juice of pigeons and chickens was obtained in acute experiments by means of histamine. The results of analytical assays are shown in Table 16. Chlorides were determined by the method of Wilson and Ball ('28) and nitrogen by the micro-Kjeldahl method of Pregl ('30). Non-protein nitrogen was determined by the acetone method worked out in our laboratory by S.A.Komarov (unpublished).

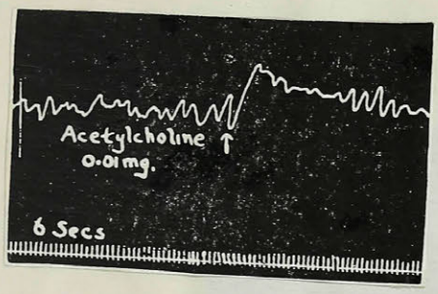
Not enough analyses of chicken's gastric juice have been made to permit comparison between the secretions of pigeons and chickens. However, the higher nitrogen content and peptic power of the chicken's juice are evident.

MAMMALIAN FOETUS
GASTRIC MOTILITY.

1. Normal Contractions.

The stomach of the cat foetus as well as that of the newborn cat and dog is very sensitive to the slight succussion produced by the air bubbling through the bath. Shutting off of the air supply greatly diminishes the rhythmic contractions and also produces some fall of the tonus. Similar observations on strips of stomach from the adult cat and dog were made by Brown and M'Swiney ('26).

The contractions occurring in the foetal cat's isolated whole stomach under the conditions of the experiment take the form of either peristaltic or antiperistaltic waves. The normal peristaltic type was observed to be the more frequent. Thus in the stomach of a foetus 92 mm. body length a wave of constriction originating at or near the gastro-oesophageal junction would reach the pyloric region in 18 to 25 seconds. A second wave might commence when the first was half-way down the stomach, but usually the second wave would originate only when the preceding wave was well within the region of the pyloric antrum. Both the peristaltic and antiperistaltic waves occurred in groups, 3 or 4 constrictions following consecutively with relatively long intervals between the wave-groups. A series of 3 waves of a total duration of about 45 seconds might be followed by a resting phase of about 60 seconds or more before a new series of waves would commence.



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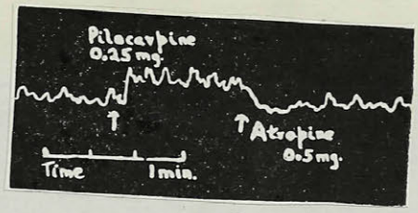

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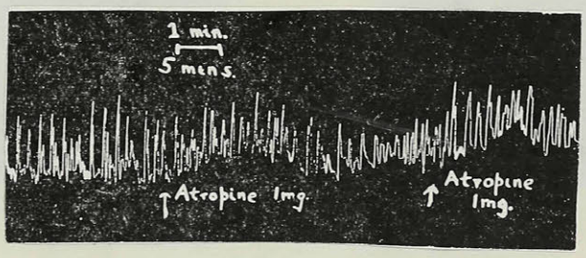

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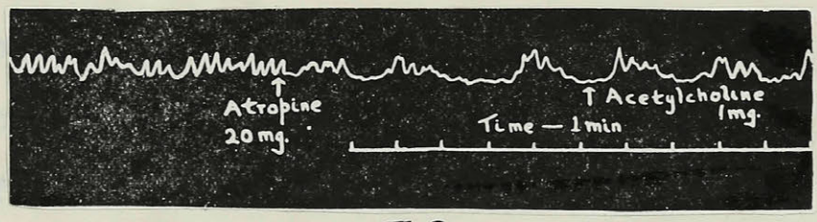
All records are from the stomach of the foetal cat. In each case the capacity of the bath was 500 cc The body lengths (crown-rump) of the foetuses were:-

Fig. 27 - 162 mm. Effect of acetyclcholine (1/50,000,000).

Fig. 28 - 89 mm. Atropine (1/1,000,000) abolishes the effect of pilocarpine (1/2,000,000).

Fig. 29 - 110 mm. Temporary rise of tonus induced by small doses of atropine (1/5,000,000).



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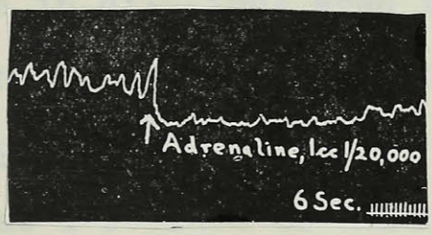

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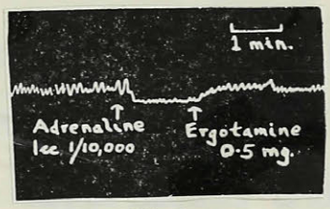

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Fig. 30 - 98 mm. Periodic changes in tonus and rhythmicity induced by large doses of atropine (1/25,000).

Fig. 31 - 162 mm. Effect of adrenaline (1/10,000,000).

Fig. 32 - 116 mm. Ergotamine (1/1,000,000) antagonizes the effect of adrenaline (1/5,000,000).

2. Influence of Acetylcholine.
Pilocarpine and Atropine.

Parasympathomimetic drugs have a definite stimulatory effect, raising the tonus and in some instances increasing the rate of contractions of the stomach of both the foetal and newborn cat and dog. (Fig. 27). Acetylcholine initiated rhythmic movements at a higher level of tonus in those cases in which the foetal stomach did not show spontaneous contractions. The stomach in a cat foetus 98 mm. long was sensitive to acetylcholine in dilutions even in excess of 1/50,000,000, and to pilocarpine in a dilution of 1/2,500,000. The foetal stomach is slightly more sensitive to acetylcholine than is the newborn cat's stomach as shown by my experiments and also more sensitive than is the adult cat's stomach.

Atropine when given after either acetylcholine or pilocarpine antagonized these (Fig. 28) and brought about partial to complete cessation of motility. Very small doses of atropine (1/500,000) when not preceded by previous treatment of the stomach by other drugs raised the tonus for a short period of time (Fig. 29). Larger doses of atropine (1/25,000) arrested the motility of the stomach often at a high tonus level or else induced periodic tonic and rhythmic contractions which were not affected by subsequent addition of acetylcholine (Fig.30). This twofold effect of atropine is in agreement with Magnus' theory ('25) of atropine action on the myenteric plexus.

3. Influence of Adrenaline and Ergotamine.

Adrenaline had predominantly an inhibitory effect on both tonus and contractions (Fig. 31)though in low concentrations its effect on gastric tonus was frequently excitatory especially in the case of the large foetuses and the newborn cat. An initial rise in tonus was sometimes seen in stomachs from the larger foetuses: this would perhaps indicate that the stomachs of the smaller foetuses were the most sensitive to adrenaline.

Ergotamine produced only an occasional rise in tonus. However, this drug had a very marked inhibitory effect on adrenaline. Used after adrenaline, ergotamine restored the tonus level of the muscle which adrenaline had lowered (Fig. 32).

4. Influence of Adrenaline and Acetylcholine in Combination.

When acetylcholine was followed by adrenaline the reaction was sometimes synergistic but most often the result was one of antagonism. The type of response to adrenaline after acetylcholine apparently depended on the concentration of adrenaline. The occasional synergistic effects produced on the foetal stomach by these 2 drugs in combination were strikingly similar to those which I observed on strips of stomach of Necturus. Apparently no such synergism occurs in the adult's cat's stomach.

TABLE 16 .

Animal No.1	Date 1935	Body Temperature	GASTRIC JUICE	
			Volume aspirated	pH
		°C	cc.	
	Jan.22	8	2.0	..
	Jan.24	9	1.1	3.8
	Jan.28	5	0.1	Acid to litmus
	Jan.31	7	Several drops	Acid to litmus
	Feb. 1	9	0.4	3.8

TABLE 17.

Animal No.	Date 1935	Body Temperature	Gastric Juice				
			Volume aspirated	Acidity		Pepsin	Cl_2
				Free	Total		
		°C	cc.	m.eq./l.		Mett's units	mg. per cent
1	Jan.22	8	2.0	18.0	30.0	0	...
3	Feb.21	..	1.4	24.0	53.2	0	...
6	Mar. 8	9	1.5	...	...	0	521

GROUNDHOG-
GASTRIC SECRETION.

1. Control Experiments.

The stomach of the hibernating ground-hog was always found to contain a small volume (about 5 cc.) of clear or slightly milky-white fluid of pH 3.5 to 4.1. No food was found in either the stomach or small intestine, although some traces of food were found in the small intestine of one of the animals. The duodenum frequently contained a slightly alkaline liquid but no apparent traces of bile. Masses of faecal material, more or less solid, filled the colon of most of the animals. In several instances the bladder was found to be filled with urine.

Small quantities of clear, acid fluid could be aspirated daily from the stomach of hibernating animals. Table 16 gives the volume and the pH of the juice found in one of the animals over a period of a number of days.

An analysis of the gastric juice aspirated on several occasions from different animals in deep hibernation revealed the data shown in table 17. The low peptic power of the aspirated fluid is particularly to be noted.

2. Continuous Secretion.

It is of interest to record that, although the body temperature (rectal) was often as low as 4° to 5° C., spontaneous

gastric secretion still occurred. For mammals this is of course a very low temperature. This activity of the ground-hog's stomach at a low temperature is all the more striking when compared with the activity of the frog's stomach. In the frog gastric motility ceases when the body temperature is below 13° C. (Patterson, '16), and digestion is arrested when the body temperature is at or below 6° C. (Turbin, '25).

On several occasions the stomach of semi-torpid animals was aspirated. In these the amount of juice which could be aspirated (about 0.7 cc.) was not much greater than during deep hibernation, and the pH (3.6) was about the same. In these instances the body temperature varied between 22° and 32° C.

In acute experiments carried out on hibernating ground-hogs, it was found that the stomach secreted continuously. The rate of secretion from the entire stomach was about 0.4 cc. per hour (in experiments of 10 or more hours' duration). The juice so obtained was usually unable to digest coagulated protein (Mett's method), had a pH of about 3.5, and was comparatively low in free HCl (23.2 to 48.8 m.eq.per liter) and total HCl (52 to 56 m. eq. per liter).

Extracts made from the gastric mucosa (method of Hammarsten, '19) were very low in peptic power. A histological study of all regions of the stomach of the hibernating ground-hog is being made in the department of histology of McGill University, and those sections which have so far been studied

TABLE 18.

Experiment of March 13,1935. Animal No.7. Deep hibernation. Initial body temperature 8°C.; body temperature during experiment 30° to 32° C.

Combined Samples	Time Interval	Procedure	Gastric Juice				
			Volume	Mucus (sediment) volume	Acidity		Pepsin
					Free	Total	
Number	Minutes		cc.	cc.	m.eq./l		Mett units
1	30	Control	0.2	...	...	...	...
2	30 30 30 30	Control	0.1 0.2 0.2 0.2	0.05	23.2	56.0	48
3	15 15 15	Histamine: 1 mg.	0.1 0.2 0.4	0.00	44.8	80.0	16
4	15 15 15	Histamine: 1 mg.	0.2 0.4 0.6	0.05	92.8	125.6	36
5	15 15 15	Histamine: 1 mg.	0.4 0.6 0.5	Trace	109.6	144.8	16
6	15 15	Control	0.5 1.0	Trace	108.0	134.4	9
7	15	Control	1.3	Trace	102.0	130.0	9
8	15 15	Vagus stim.: coil 10¼ cm.	1.0 1.1	0.05	103.2	130.4	0
9	15 15	Vagus stim.: coil 10 cm.	1.1 1.2	0.05	110.4	128.8	4
10	15 15	Vagus stim.: coil 10 cm.	1.6 0.7	0.05	115.2	137.6	9
11	15 15	Vagus stim.: coil 9¾ cm.	1.1 1.1	0.10	104.0	134.4	0
12	15 15	Vagus stim.: coil 9¾ cm.	0.8 1.2	0.10	116.0	133.6	16
13	15 15	Vagus stim.: coil 9½ cm.	0.8 1.9	0.15	112.8	132.0	36
14	15 15	Vagus stim.: coil 9½ cm.	0.9 1.0	0.10	112.8	128.0	36
15	15	Control	0.5	0.00	117.6	131.2	..
16	15 15	Histamine: 1 mg.	0.6 0.6	0.00	...	...	23
17	15 15	Histamine: 1 mg.	0.6 0.7	Trace	124.0	136.0	16
18	15 15	Histamine: 1 mg.	0.7 0.7	0.00	124.8	140.0	4

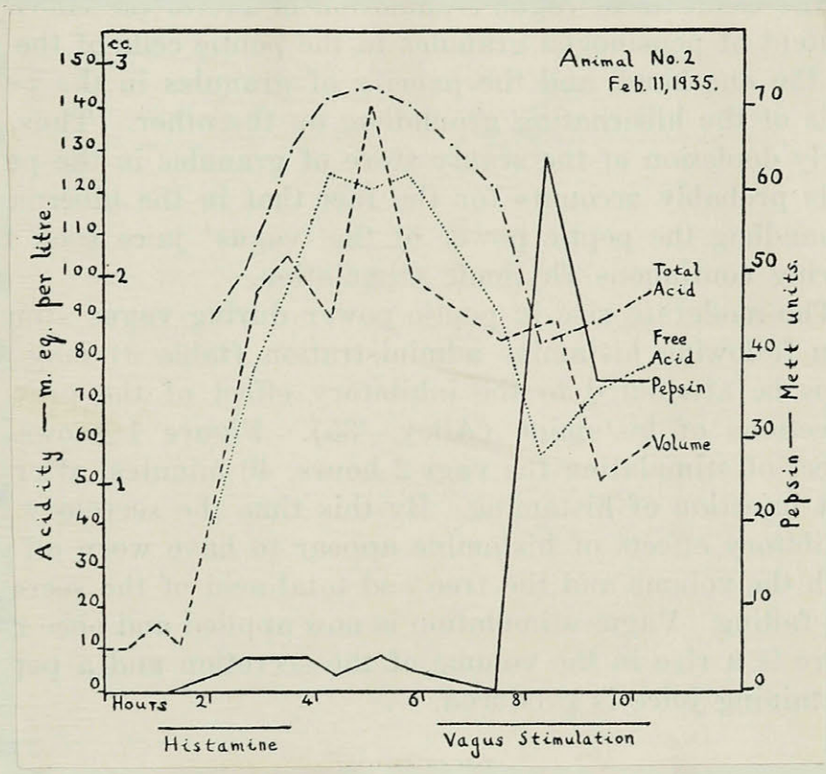

FIGURE 33.

The effect of histamine and vagus stimulation on the gastric secretion of the groundhog during hibernation.

show that the pepsinogen granules are very few in comparison with those found in the normal waking animal (Bowie, '35). Histologically the gastric mucosa of the hibernating groundhog appeared to be similar to that of the dog after long periods of vagus stimulation (Babkin, '34; Bowie and Vineberg, '35). Control sections of the stomach, obtained from the waking animal during the summer, show the peptic cells to be fuller of pepsinogen granules than those of the hibernating animal, though not to the same degree as the peptic cells of the normal canine stomach. According to Bowie ('35) both in the rabbit's stomach and in the stomach of the waking groundhog the peptic cells contain fewer pepsinogen granules than are present in the stomach of the cat or dog.

3. Effect of Histamine.

Repeated subcutaneous injections of 0.5 mg. or 1 mg. of histamine in the hibernating groundhog (given ever 45 minutes) caused a flow of gastric juice. After a short latent period the volume of the secretion rose steadily, reaching its maximum about 4 hours after the beginning of the injections. The concentrations of free and total hydrochloric acid also rose. The pepsin content of the juice was negligible at the beginning of the experiment, and it was in no way increased by the histamine administration.

The first part of table 18 shows the typical results of histamine administration, and figure 33 shows graphically the

effects of a similar procedure. It will be noted that, with repeated injections of histamine, the volume of juice secreted rose gradually, and that there was a rise of both the free and the total acidity parallel to the volume increase. The pepsin either remained low, or, when on rare occasions it was at first comparatively high (table 18), it fell progressively.

Observations on dogs in this laboratory have shown that histamine does not produce an output of pepsin in the gastric juice (Babkin, '30; Vineberg and Babkin, '31); indeed, histamine may even inhibit the discharge of the enzyme from the peptic cells (Alley, '35). Whereas in the dog whatever pepsin may be present in the 'histamine' gastric juice is due to a passive washing-out of the enzyme from the glands, in the hibernating groundhog there is very little, if any, pepsin present in the 'histamine' gastric juice, because the enzyme content of the glands is very small.

4. Effect of Vagus Stimulation.

Figure 33 and table 18 also show the effect of vagus stimulation on the gastric secretion of the groundhog while it is hibernating. During the experiments the body temperature (rectal) of the animals rose gradually to between 28° and 32° C. but never went higher.

Rhythmic stimulation of the vagi in the neck was carried out with a moderately strong induction current (coil 10.5 to 8.5 cm.). A weaker current, although effective, is

known to produce only a scanty flow of slightly alkaline or slightly acid mucus (Vineberg, '31). Unlike the effect of histamine, the effect of stimulating the vagi is exerted on both the acid-producing and pepsin-producing elements of the gastric mucosa. After a short latent period there is an increase in the free and total acidities over those of the continuous secretion, and pepsin begins to appear in the juice. The pepsin, however, reaches only a moderate concentration (64 Mett units, fig. 33), as compared with the peptic power of dog's gastric juice evoked by vagus stimulation (1300 to 1800 Mett units, Vineberg, '31).

In several experiments, instead of vagal stimulation, 1 mg. of pilocarpine nitrate was administered intravenously every hour. The effects on gastric secretion were similar to those of vagus stimulation.

The marked difference in the degree of response of the peptic elements to vagus stimulation is due to the enormous content of pepsinogen granules in the peptic cells of the dog one the one hand, and the paucity of granules in the peptic cells of the hibernating groundhog on the other. Thus, the early depletion of the scanty store of granules in the peptic cells probably accounts for the fact that in the hibernating groundhog the peptic power of the 'vagus' juice soon falls during continuous rhythmic stimulation.

The moderate rise in peptic power during vagus stimulation following histamine administration (table 18) may perhaps

be attributed to the inhibitory effect of the previous injections of histamine (Alley, '35). Figure 33 shows the effect of stimulating the vagi 2 hours, 40 minutes, after the last injection of histamine. By this time the secretory and inhibitory effects of histamine appear to have worn off and both the volume and the free and total acid of the secretion are falling. Vagus stimulation is now applied and once more there is a rise in the volume of the secretion and a pepsin-containing juice is produced.

V

DISCUSSION.

GASTRIC SECRETION

1. SKATE

In the skate, the gastric glands are in a continuous, though comparatively insignificant, state of (secretory) activity. Very strong evidence was obtained in this investigation in support of the view that this spontaneous secretory activity does not depend on the direct influence of either the parasympathetic or the sympathetic nervous system, but that it is greatly influenced by changes in the abdominal circulation. This condition is all the more remarkable considering that the motor innervation of the stomach in elasmobranchs is well developed.

Destruction of the spinal cord leads to a "paralytic" gastric secretion unusually large in volume. It is highly probable that this secretion is of vascular origin and depends on the lowered tonus and possibly on the resultant increase in the permeability of the blood vessels supplying the gastric mucosa. The phenomenon of "paralytic" secretion of gastric juice in the elasmobranchs in reminiscent of the effect which unilateral section of the splanchnic nerve produces on kidney secretion in warm-blooded animals. In both cases changes in the circulation within the organ greatly increase the activity of the secretory epithelium of that organ.

In the elasmobranchs the urea content of the blood is high (Smith '29). When the secretion lasts long enough in a starving "paralytic" skate, it is found that the blood becomes gradually depleted of its electrolytes, as may by judged from the diminution of its osmotic pressure (Chaisson, '33). The blood

chlorides are not diminished nor, according to MacIntosh ('36), is the blood urea. Urea is found in the skate's gastric juice (Babkin and Komarov '31) and probably occurs there through active secretion by the gastric glands. During the course of "paralytic" secretion the osmotic pressure of the gastric juice falls (table 6); according to MacIntosh (36) this is due to a decrease in the amount of urea secreted. The skate's stomach may be likened to the skate's kidney in that a high threshhold for the passage of urea exists in both organs (Smith '33). Further research is required to determine what components of the blood are lost during "paralytic" secretion and are not replaced.

2. NECTURUS

Like the skate, in Necturus the gastric glands are in a continuous state of spontaneous secretory activity. Secretion was not influenced by parasympatho-mimetic substances such as pilocarpine and acetycholine. After the administration of adrenaline, a fluid of little acidity could be aspirated from the stomach. The volumes aspirated were somewhat larger than in control experiments. This might be due to the swallowing of water as the result of paralysis of the oesophageal muscles by the adrenaline, since adrenaline lowers the tonus of the frog's oesophagus, (Carlson and Luckhardt '21, Rabbeno, '32). Whether the inhibitory effect of adrenaline on gastric secretion is Necturus is due to its direct action on the secretory cells or to its vaso-constrictor action on the visceral blood vessels we do not know.

3. FROG

The experiment of the frog show that, in the production of acid and pepsin secretion, the sympathetic nervous system plays a very important part, whereas it seems that the vagus nerve does not play an important part. The results obtained agree with those of Smirnov ('18, '21, '23), in so far as they show that mechanical stimulation causes an acid secretion which persists in spite of the cutting of the vagi; they disagree, however, with his interpretations of the phenomena.

The intimate mechanism of the activation of secretion could not be a purely local affair, since destruction of the cord prevented a secretion. The absence of secretion, when the cord was destroyed, could not be assumed as being due to a very low blood pressure, since yohimbine gave presumably a low blood pressure but did not interfere with mechanical secretion when the cord was intact. Further, cutting of the splanchnic nerve, even when the cord was intact, prevented a secretion. Clearly no innate mechanism of the stomach itself is alone responsible for secretion.

Secretion might be the result of a true reflex or an axon reflex. Tonkich ('25) found that in frogs with the whole central nervous system destroyed stimulation of the central end of the splanchnic nerve resulted in an accelerated heart beat, and stimulation of the central end of the vagus resulted in paling and movements of the stomach. She believes that the impulse from excitation of the splanchnic nerve runs through the sympathetic trunk and reaches the heart by way of the vago-sympathetic nerves, ahd goes in the reverse direction when the central end of the vagus

is stimulated. In other words, the interactions between the heart and gastro-intestinal tract are realized by means of an axon reflex. But secretion cannot be due to an axon reflex since it is absent when the cord is destroyed and the splanchnic nerve and sympathetic chains are left intact. There is also indirect evidence, based on the fact that motor and secretory reflexes do not appear on stimulation of the sciatic and lumbar nerves after destruction of the cord, that the cord plays a very important role in acid secretion. Gastric secretion is probably not due to an axon reflex but to a true reflex involving afferent paths from the stomach along the sympathetic nervous system to the region of the cord giving off spinal nerves 2 to 5, and from there back through secretory efferent paths also in the sympathetic nervous system.

The reflex secretion resulting from sciatic nerve stimulation is also due to a true reflex and not to an axon reflex, since it is abolished when the cord is destroyed. The centers for this reflex would appear to lie in the cord and not necessarily in the brain, since the reflex is still obtained when the brain is destroyed. Langley ('24), working on the cat found an analogous reflex from the sciatic nerve: stimulation of this nerve is decerebrate cats with cord also cut at the first cervical caused anti-peristalsis of the proximal colon and occasional inhibition of the rhythmic movements of the ileum. it also produced an immediate rise in blood pressure, often followed later on by a secondary rise; the secondary rise Langley attributed to the adrenal mechanism. Head and Riddock ('17)

found in human cases of complete lesion of the spinal cord in the lower cervical and upper thoracic regions that sweating was initiated when the plantar surface of the foot was stimulated.

From the secretory effects of sympathetic nerve stimulation and of adrenaline, and the negative effects of vagus stimulation and of pilocarpine and ocetylcholine, it would seem improbable that the secretory cell is innervated by both sympathetic and parasympathetic fibres. Rawlinson ('33) has concluded that the various types of secretory elements of the cat's salivary gland are each innervated by only one kind of autonomic fibre.

Recently D. J. Bowie of the department of histology of McGill University has demonstrated that the cells of the chief glands of the frog's stomach contain granules which can be stained by a method (Bowie'36) specific for only pepsin zymogen granules. These granules are definitely not mitochondria. The granules in these cells of the frog's stomach differ from the pepsin granules of the frog's oesophageal glands in that they are smaller, less numerous and stain somewhat less intensely. The pepsin granules of the frog's oesophageal glands resemble more the pepsin granules of the chief cells of the mammalian gastric glands than do the pepsin granules of the frog's gastric glands.

A striking point in the physiology of the gastric secretion in the frog is the high concentration of pepsin in the gastric juice. The lowest average figure for pepsin was obtained in histamine gastric juice and was equal to 452 Mett units (table 13). The highest average figure was 1196 Mett units and was obtained in adrenaline gastric juice. In the dog and cat histamine stimulated a secretion of gastric juice extremely poor in pepsin. The average figures for pepsin of canine juice of the 'chemical' are about

75 to 100 Mett units and about 200 to 400 Mett units for 'sham fed juice. It is only under the influence of electrical stimulation of the vagi that the peptic power of canine gastric juice reaches a value of 1000 or more Mett units. In this respect, the frog's gastric juice approaches the enzymatic properties of human gastric juice where pepsin values as high as 1936 Mett units ('fasting' juice) and 1603 Mett units ('histamine' juice) have been obtained (Toby, '37).

How are we to explain the presence of pepsin secreting cells in the frog's oesophagus? To this question, we have as yet no answer. Morphologically the frog's oesophageal glands are more akin to the frog's gastric glands than they are to the mammalian oesophageal glands. We may suppose with Biedermann ('11) that the oesophageal glands of the frog represent gastric glands which, like them, still continue to secrete pepsin but, unlike them, have lost the power to secrete acid.

4 BIRDS

Gastric secretion in the pigeon is apparently intermittant and not continuous since in these experiments a continuous secretion was found to exist only in association with the presence of food in the gizzard. Since acetylcholine and pilocarpine but not adrenaline stimulate gastric secretion, then secretion must be under parasympathetic control only. This lends support to the idea that only one kind of autonomic nerve fibre innervates a particular type of secretory cell.

It is important to note that the injection of histamine resulted in the secretion of a juice high in acidity but poor in pepsin. Similar results have been obtained in the dog (Bowie and

Vineberg, '35) and man (Toby '37). Therefore in the mammalian gastric glands histamine stimulates only the parietal cells and in birds only the acid-producing functions of the single type of cell which secretes both acid and pepsin. The modern conception is that histamine is a normal participant in gastric secretion in mammals. Since histamine is ineffective in the skate but is effective in the frog and bird, it is possible that histamine begins to assume a role in the normal gastric secretory processes in all vertebrates higher than the Elasmobranch fishes.

The gastric glands of the bird are similar to the pancreatic of the mammal in one respect. In the bird's stomach, acetylcholine stimulates the secretion of only small amounts of pepsin-rich juice while histamine stimulates the secretion of large volumes of fluid poor in pepsin. In the pancreas, vagus stimulation results in the secretion of a small quantity of juice rish in enzymes while secretin induces the secretion of large volumes of juice poor in enzymes. In both glands only one kind of secretory cell is concerned—the cells of the chief glands in the stomach and the acinous cells in the pancreas.

The experiments show that secretion depends on the volume of body fluid available. When this is depleted the secretion diminishes but it is restored when saline or low concentrations of glucose are administered.

Increasing the total blood chlorides by injecting sodium chloride resulted in an increase in the chlorides of the gastric juice. The rise in chlorides of the gastric juice following the introduction of saline is noticeable in figure 25. A concentration of 215 m.eq.per liter was obtained as compared with the

normal chloride concentration of about 170 m.eq.per liter in histamine juice (table 15). However, no special study of this problem was made on the pigeon as did Gilman and Cowgill ('31) on the dog.

Glucose in concentrations of 10% or higher had a specific trophic effect on the secretion of pepsin. According to Dr. Day who worked in our laboratory on dogs with gastric fistulae, glucose administered intravenously stimulated the output of pepsin only when a concentration of 40% or higher was used. The lower concentration effective for the pigeon may perhaps be explained by the higher blood glucose normally present in birds (about 200 mg.% in the chicken, according to Cassidy, Dworkin, and Finney, '26).

Hebb ('37) found that administration of glucose enhanced the normal secretory effect of vagal stimulation on the pancreas of the rabbit. A relationship between the blood sugar concentration and gastric hunger contractions and tonus has also been found (Bulatao and Carlson, '24; Farrell and Ivy, '26). The various digestive processes of secretion and motility are therefore intimately related to sugar metabolism but the mechanism involved still remains to be elucidated.

5. HIBERNATING GROUNDHOG

In the study of gastric secretion in the goundhog during hibernation, two important points were brought out:- (1) the continuous secretion of a moderately acid gastric juice and (2) the lack of pepsin in such juice.

In connection with the first point, it is to be noted that many herbivorous animals have large digestive glands (e.g.,

the parotid glands, gastric glands, and the pancreas) which secrete spontaneously and continuously. However, the continuous secretion of certain glands of herbivorous animals does not depend on the continuous presence of food in the gastro-intestinal tract. This was shown most convincingly in the experiments of Baxter ('31) on the rabbit's pancreas. Even when the entire gastro-intestinal tract was removed, the pancreatic gland continued to secrete for 5 or more hours. This secretion may be regarded as spontaneous and independent of any special secretory stimuli. Special secretory stimuli (e.g. nervous or chemical) may of course increase the volume of the secretion but its continuousness is due to the special properties of the secretory cells.

In carnivorous animals (e.g., dog, cat and man) gastric secretion is intermittent (Babkin, '32; Winkelstein, '35). In these animals some stimulus, conveyed either through the nerve or through the body fluids, is always necessary in order to activate the glandular apparatus. The continuous gastric secretion in the groundhog during hibernation is a striking example of a spontaneous secretion maintained even after many weeks of fasting. So far no adequate explanation of this fact can be given.

No less interesting is another fact observed during this investigation, namely, the peculiar composition of the gastric juice in the hibernating groundhog. As noted above, the juice although containing acid, was in most cases almost devoid of pepsin. The fact that the peptic cells of the hibernating groundhog contain very few zymogen granules (Bowie, '35) may explain in part the extremely weak digestive power of the juice. However, another

explanation may be advanced. It has been definitely established by Bowie and Vineberg ('35) that in the dog the discharge of pepsinogen granules from the peptic cells is under the control of the vagus. It was shown in these experiments that in groundhogs also the discharge of pepsin is regulated by the vagus. It may be supposed that during hibernation the function of the nervous system is depressed and the vagus cannot produce its usual effect on the gastric glands. It is perhaps significant that in the bat parasympathomimetic drugs take longer to act when the animal is in a hibernating state than when awake (Adler, '26). Thus it would seem that only a comparatively simple process like the formation of acid is maintained in the gastric glands of hibernating mammals.

GASTRIC MOTILITY

1. SKATE

The remarkable feature of the action of the sympathetic and parasympathetic nervous systems on the gastric motility in the skate is that both are of the excitatory type. This was shown by experiments in which both kinds of nerves were stimulated (Bottazzi, '02; Babkin and MacKay-Sawyer; '32; Lutz, '31) as well as by experiments in which sympatho - and parasympatho - mimetic drugs were employed (Dreyer, '28-29; Lutz, '31; Nicholls, '33; Young, '33). In the present investigation, not only was this confirmed, but it was also demonstrated that both nerves act synergetically. That is, neither one in any way hinders the positive effect of the other, and in certain cases one may even increase the positive effect of the other. Moreover, repeated stimulation of the vagus and the interior splanchnic nerves sometimes induced automatic contractions of the stomach which were of peristaltic type. Muller and Liljestrand ('18) noted in some of their experiments that stimulation of the vagus superimposed on stimulation of the anterior splanchnic nerves inhibited the movements activated by the latter. As stated above, no such inhibition was observed in these experiments. The possible explanation of Muller and Liljestrand's observation may be that usually the contractions appear after the cessation of stimulation of either of the nerves, and not during their stimulation. We cannot yet give any explanation of this peculiar relation of autonomic nerves to the tissue innervated by them in elasmobranchs. Muller and Liljestrand found that, if both nerves were stimulated simultaneously (the strength of the current

employed is not stated by them), this period of inhibition could be very much empasized. Undoubtedly more experiments are necessary to justify their claim that the vagus contains both motor and inhibitory fibres for the stomach. Therefore, in elasmobranchs the relations between the two divisions of the autonomic nervous system, with regard to the activity of the muscle of the stomach, differ from those existing in mammals.

Nicholls ('33) showed that the spontaneous contractions of an isolated strip of antral muscle of the skate are inhibited by adrenaline in concentrations of 1:1,000,000 and greater, but that more dilute solutions (e. g. 1:2,000,000) cause stimulation. As mentioned above, we never observed any inhibitory effect of sympathetic (splanchnic nerve) stimulation on the motility of the stomach in situ. However, a careful study of the curves of contraction recorded by means of a balloon introduced into the distal part of the stomach (figure 8) shows that a wave of contraction spreading orally from the pyloric sphincter leaves the antrum unaffected. Nicholls ('34) also showed that the antral muscle of the skate's stomach, whose excitability had presumably been lowered through storage for 3 or 4 days in physiological media at 0° C., was stimulated by all effective does of adrenaline It may be supposed that the muscle of the antral region in the skate is more sensitive to adrenaline than are the muscles of other regions, and larger doses of this drug have an inhibitory action on it. The absence of marked relaxation of the antral region of the stomach in the intact animal during stimulation of the splanchnic nerves could be attibuted on this ground to an insufficient liberation of "sympathin". It would seem that during

the evolutionary history of the vertebrates the inhibitory properties of the sympathetic nervous system towards the muscles of the gastro-intestinal tract developed very gradually. Young ('33) quite independently, also emphasizes the fact that there is very little evidence in elasmobranch fishes of a differtiation of the autonomic nervous system into the two antagonistic subdivisions found in mammals.

It is of great interest also to note that the smooth muscles of the arteries in the skate react to both adrenaline and acetylcholine by contraction (Babkin, Bowie and Nicholls, '33). Thus in animals so ancient from an evolutionary point of view as the elasmobranchs, differention of the muscular reaction towards parasympathetic and sympathetic "chemical transmitters" or "local harmones" has not yet appeared.

Another interesting feature of the effect of autonomic nerve stimulation on the stomach in the skate is that movements begin with contraction of the pyloric sphincter. These movements spread orally along the pyloric canal towards the body of the stomach and gradually involve the whole organ in a state of activity. It is therefore remarkable that the automatic movements of the stomach are always of a peristaltic and not of an antiperistaltic character. It is true that weak stimulation of of separate branches of the vagus produces local contractions of the body of the stomach, but a stronger current as a rule initiates a general motor reaction. On stimulation of the anterior splanchnic nerves with an induction current of moderate strength, a general motor reaction of the whole stomach is the usual result.

These physiological findings may to a certain degree be explained by the histological structure of the "gastric plexus" described by E. Muller ('20). According to him the "gastric plexus" in selachians (Squalus acanthias) is a true nerve net in which the neurofibrils pass directly from one cell to another. He believes that within the plexus one may distinguish both vegal and sympathetic cells. The cells of the plexus are connected with the endings of nerve fibres which Muller believes are vegal in character. From his description one does not get a clear view of the relationship between the sympathetic fibres and the gastric plexus. But if the extrinsic vagus and sympathetic nerve fibres are in some way connected with the nerve cells of the "gastric plexus", then the nerve impulse arriving at any point of this structure will activate movements in the whole organ.

2. NECTURUS

Three main points of interest were brought out in the investigation of gastric motility in Necturus:-

(1) The differences in the effects of small doses and large doses of adrenaline on circular strips of stomach.

(2) The different effect of small doses of adrenaline on the circular strips and of adrenaline in any concentration on the longitudinal strips.

(3) The positive synergistic effect on circular strips of adrenaline in low concentrations with acetylcholine in all concentrations.

Concerning (1) we have seen that in the Elasmobranch fishes splanchnic nerve stimulation and also adrenaline produce a motor effect. According to Nicholls ('33) strips taken only from the antrum reacted with inhibition to larger doses of adrenaline in Raja diaphanes and R. erinacea, but in R. clavata all concentrations of adrenaline were excitatory (Nicholls, '34).

In Necturus adrenaline has inhibitory effect in large concentrations and a excitatory effect in low concentration on the contractions of circular strips which depend on its circular coat. In the frog too small doses of adrenaline excite and large doses inhibit contractions (see table 5) . We see then that in the vertebrate forms lower than Necturus adrenaline is predominantly excitatory in its effect on the muscles of the stomach. In Necturus its inhibitory effect, which is reminiscent of that in mammals, begins to appear. No adequate explanation can be given at present for the dual effect of adrenaline on the circular muscles. Nicholls ('33) suggested that the inhibitory effect of adrenaline on the antral muscles in some species of skates depended on either a greater sensitivity of this region to adrenaline as compared with other regions of the stomach, or the presence of two types of sympathetic fibres in the antral region, excitatory which are stimulated by small doses, and inhibitory which are stimulated by larger doses or adrenaline. Without special experimentation, neither of the two suppositions can be accepted or rejected. Other explanations of an equally hypothetical nature, based on the modern conception of humoral transmission of nerve impulses, may be advanced also.

Regarding (2), longitudinal strips from the fundic and pyloric portions of the stomach reacted to all concentrations by an inhibition of the spontaneous movements. Inhibition by adrenaline of longitudinal strips from the cardiac or fore part occurred only with the larger concentrations; small doses were practically ineffective. On the other hand, circular strips from these three regions reacted by an increased motor activity to adrenaline in lower concentrations (1/500,000 or less). The differences in reaction of the circular strips from the longitudinal strips were undoubtedly determined by the unequal responses of the two coats of the stomach to adrenaline. Possibly the explanation of this phenomenon may be the same as that advanced in relation to the differences in reaction of circular strips to different doses of adrenaline.

From the point of view of the digestive work of the stomach in toto, the different reactions of the circular and longitudinal muscle layers may be regarded rationally. The stomach becomes elongated because of the relaxed longitudinal muscles and is now capable of accomodating a greater amount of food. At the same time the contracting circular coat of muscle propels the food masses towards the pylorus.

Point (3) is that adrenaline in high concentrations and acetylcholine are antagonistic to each other, the one relaxing and the other contracting the musculature of the stomach of Necturus. On the other hand, a marked synergistic effect is seen on circular strips when the adrenaline is in low concentration.

The phenomenon deserves great attention from the viewpoint of the evolution of function. As was pointed out above, the innervation of the stomach as well as the intestine (Nicholls, '33b) of the elasmobranch fishes is entirely motor. The arteries in these fishes also react by contraction to both adrenaline and acetylcholine (Babkin, Bowie and Nicholls, '33). Stimulation of the vagus nerve not only does not inhibit the motor effect of subsequent stimulation of the sympathetic nerves but actually facilitates it.

In the teleost fishes of the genus Epinephelos (red hind grouper, etc.) Bernheim ('34) found that adrenaline added to an intestinal strip which as been made to contract by mechanical stimulation produces a relaxation. But if the adrenaline is added to an intestinal strip partly contracted by acetylcholine or pilocarpine, a further contraction occurs. Under conditions, by means of adrenaline, it is possible to more than double the height of the original contraction.

The synergistic effect of adrenaline and acetylcholine on the muscles of the Necturus stomach belongs to the same group of phenomena.

In the frog, the splanchnic nerves are excitatory in their effect on the stomach, whilst the vagi may also be excitatory, depending on the state of tonus existing at the time of vagus stimulation.

In warm-blooded animals the antagonism between the sympathetic and parasympathetic drugs is very marked. However, here too we find many instances of their synergistic action. The classical example of such synergies is the "augmented salivary secretion"

recently reinvestigated from this point of view by MacIntosh and Rawlinson ('35) and Fleming and MacIntosh ('35). Recently also Samaan ('35) showed that repeated stimulation of the cardioaccelerator nerve in dogs and cats, as well as small doses of adrenaline renders the cardiac muscle more susceptible to vagal impulses. Bernheim and Blockson ('32) found that adrenaline added to guinea-pig ileum previously contracted by pilocarpine or physostigmine causes a further contraction which may or may not be followed by relaxation.

3. MAMMALIAN FOETUS

Whether the stomach of the foetus has already became innervated when it first begins to show signs of spontaneous contractions, we do not know. According to Tani ('27) spontaneous gastric contractions are present early in the developement of the rabbit foetus but the stomach's sensitivity to drugs increases only gradually during growth. This might indicate that the gastric muscles are endowed with the power of contracting even before they became innervated, supporting the contention of Alvarez ('22) and others that rhythmic contractions of the gut are myogenic in nature.

We do not know whether it is the sympathetic or the parasympathetic nervous systme that innervates the stomach first. The evidence in favor of both views has been reviewed by Van Campenhout ('30) who also points out that this question is intimately related to the problem of the origin of the intrinsic innervation of the gut. The cat foetuses employed in my experiments were probably too old to enable one to determine by the responsiveness of the stomach to drugs which of the autonomic nerves reaches the

stomach first. McLachlin ('36) found the human foetal gastro-intestinal tract to respond to acetylcholine at an earlier age than to adrenaline. This may mean that the gastro-intestinal tract receives sympathetic fibres later than it does parasympathetic. According to Gaskell ('20), Kappers ('29) and Dale ('34) the parasympathetic is a more diffuse and widespread mode of innervation than the sympathetic and the sympathetic system is a less primitive structure.

No definite traces of the more primitive type of motor response to adrenaline, such as exists in the skate, were found to be recapitulated in the stomach of the cat foetus. The usual effect of adrenaline was that of inhibition. Adrenaline given after acetylcholine usually antagonized the excitatory effect of the latter but occasionally the two drugs acted synergistically (as they do in the skate and on circular strips of Necturus stomach).

No doubt the similar action of the sympathetic and parasympathetic in the Elasmobranchs is related to the fact that in these fishes the autonomic innervation of the gastro-intestinal tract is more nearly segmental than in mammals (Young, ('33). In the mammals there is a greater extent of overlapping between the two nerve systems innervating the digestive tube with the result that antagonism between them is a paramount feature . We do not know the processes which were involved in the establishment of antagonism between the sympathetic and parasympathetic nervous systems. Bases on the modern conception of neuro-humoral transmission of nervous impulses, we may say that in the lower vertebrates the gastric muscle, which may be considered the effective

organ of the transmitter or local hormone (Loewi, '35), reacts alike to both sympathin (adrenaline) and vagus substance (acetylcholine).

However, it is possible that in the lower vertebrates a substance which is neither adrenaline nor acetylcholine, but intermediate between the two, is the hormone liberated at the endings of both sympathetic and parasympathetic nerves. Stehle, Melville and Oldham ('36) have pointed out the similarity between the side-chain of adrenaline and the choline molecule. They suggest that choline, which occurs widely in the body, is the source of both neuro-hormones. Possibly in the lower vertebrates the synthesis of neither hormone is carried to completion but only to intermediary stages, resulting in substances which are much more similar pharmocologically than adrenaline and acetylcholine. Certainly a good deal more research must be done before anything definite may be stated.

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VI

CONCLUSIONS.

SECRETION

Although little is known about the role of the nervous system in secretion in invertebrates, it is know that a humoral mechanism is present even where digestion is intracellular (as in the Protozoa). This apparently is the earliest means of regulating secretion to be developed and persists in all vertebrates. It is found in the Elasmobranch fishes (since a gastric secretion occurs when food is ingested), amphibia, birds and mammals.

Regulation of secretion by means of a nervous system is of more recent origin in the developemental history of the vertebrates since in the Elasmobranch fishes it plays only a subsidiary role through the sympathetic action on the blood vessels of the viscera. This would also seem to be the case for Necturus although of this we are not certain. In the higher vertebrates, from the frog up, the nervous system assumes an important role.

The results of these experiments add support to the view that the different types of secretory cells are each innervated by only one of the autonomic nervous systems. In the mammals we have a strong evidence that the parietal and peptic cells are each innervated by only parasympathetic fibres and not by sympathetic as well. In the non-mammalian vertebrates acid and pepsin are secreted by the same cell. In the frog, this cell receives only sympathetic fibres while in the bird it receives only parasympathetic fibres. A good deal more research work must be done before we can understand how the transition from a sympathetic to a parasympathetic type of nervous regulation took place.

Histamine, if it is at all effective as a secretagogue, stimulates in all the animals we have studied a secretion of acid only and not of pepsin. In mammals, where it probably participates

in the normal processes of secretion, histamine acts selectively on the parietal cell (Bowie and Vineberg '35) and in other vertebrates it acts selectively on the acid-producing functions of the single cell that secretes both acid and pepsin.

MOTILITY

In the Elasmobranch fishes both the sympathetic and parasympathetic nervous systems are excitatory for gastric motility. Neither nerve is inhibitory but rather, when acting together, a summation of motor effects occurs.

In the frog too, the sympathetic and parasympathetic are motor for the stomach. But on vagus stimulation we may have inhibition as well as excitation so that probably both motor and inhibitory fibres are present in the vagus.

Necturus, although situated lower in the phylogenetic scale than the frog, is in respect to gastric motility more closely related to the mammal than to the frog. The inhibitory effect of adrenaline is evident and acetylcholine is predominately motor. However, its relationship to the frog is not entirely divorced since, as Patterson ('28) has shown, vagus stimulation usually results in inhibition of gastric tonus and contractions. Furthermore, consideration must also be given to the fact that while the Necturus stomach consists of both circular and longitudinal coats, the frog stomach is greatly lacking of a longitudinal coat.

In the mammals the parasympathetic is excitatory and the sympathetic inhibitory to the stomach. However, the effects of these nerves may depend to some extent on the tonus of the stomach and, as McSwiney and co-workers have shown (Brown and McSwiney, '32),

their effects may be reversed under certain conditions.

The similarity between the sympathetic and the parasympathetic effects in the lower vertebrates may perhaps be accounted for by the more nearly segmental innervation of the gastro-intestinal tract by these nervous systems. It is only in the higher vertebrates, were a much greater degree of overlapping of the nerve supplies occurs, that antagonism appears.

It is evident that the muscles of the stomach came under nervous control sooner than the gastric glands. A humoral mechanism for regulating gastric motility is present in the frog (Babkin, '24),birds and mammals and probably also in the fishes and reptiles although about this we have no information.

VII

SUMMARY.

ELASMOBRANCH FISHES.

1. Secretion.

(1) A fasting skate continuously secretes a small volume of gastric juice of high acidity. The secretion ceases shortly before death.

(2) This spontaneous secretion is not influenced either by section of both vagi or by administration of atropine. Stimulation of the anterior splanchnic nerves or administration of adrenaline inhibits the secretion.

(3) Destruction of the spinal cord, and hence elimination of the influence of the central part of the sympathetic nervous system, provokes a "paralytic" gastric secretion. This secretion commences 24 hours to three or four days after the operation, and may last for many days (longest term of observation, 16 days).

(4) The volume of the "paralytic" secretion varies from day to day. It is acid, having an average pH lying between 2.0 and 3.5. The freezing point of this secretion rises gradually towards zero, though the Cl concentration remains more or less constant. Adrenaline stops the paralytic secretion for several hours.

(5) The theory is advanced that the"paralytic" secretion is due to some vascular changes in the abdominal viscera, arising as a result of the disconnection of the peripheral parts of the sympathetic nervous system from its central apparatus.

(6) Administration of histamine has apparently no effect on secretion.

2. Motility.

(1) The course and distribution of the vagus nerve and the anterior splanchnic nerve to the gastro-intestinal tract in Raja diaphanes and Raja stabuliforis are described.

(2) Both these nerves produce movements of the stomach in the skate. Neither of them inhibits these movements.

(3) Weak stimulation of various branches of the visceral vagus stimulates local contractions in different areas of the stomach. Stronger stimulation of the vagus sets up movements of the whole organ, beginning at the pylorus and proceeding craniad.

(4) Any effective stimulation of the anterior splanchnic nerve produces much stronger contractions than does vagus stimulation. The contraction always starts at the pylorus and moves towards the oesophagus.

(5) Stimulation of the vagus does not inhibit, but rather seems to facilitate the action of the sympathetic nerve.

(6) Atropine sulphate stimulates movements in the stomach and does not abolish the effect of vagus and sympathetic stimulation and of adrenaline.

NECTURUS.

1. Secretion.

(1) A healthy Necturus secretes continuously and spontaneously a small volume of gastric juice which is acid but of low peptic activity.

(2) This spontaneous secretion is not influenced by the administration of acetylcholine, pilocarpine or atropine.

(3) Adrenaline inhibits this spontaneous secretion.

2. Motility.

(1) Adrenaline in small concentrations raises the tonus and increases the frequency of contractions of circular strips of Necturus stomach.

(2) In larger concentrations adrenaline inhibits both the tonus and frequency of contractions of circular strips.

(3) Adrenaline in all concentrations inhibits both the tonus and frequency of contractions of longitudinal strips.

(4) Circular and longitudinal strips from the posterior (pyloric) part of the stomach are more sensitive to adrenaline than strips from other regions of the stomach.

(5) Acetylcholine stimulates both circular and longitudinal strips from all regions.

(6) Pilocarpine, like acetylcholine, stimulates circular strips but, unlike acetylcholine, has an apparent inhibitory effect on longitudinal strips.

(7) On circular strips, adrenaline in small concentrations followed by acetylcholine or pilocarpine (or vice versa) result in a synergistic action. Adrenaline in large concentration is antagonistic to acetylcholine or pilocarpine.

(8) On longitudinal strips, adrenaline in all concentrations is antagonistic to acetylcholine.

FROG.

1. Secretion.

(1) During the late spring and summer the fasting frog secretes continuously and spontaneously an acid gastric juice of moderate peptic power. During winter the fasting frog does not secrete gastric juice.

(2) The oesophageal glands of the frog secrete pepsin in high concentration but not acid.

(3) Neither vagus stimulation nor the administration of acetylcholine or pilocarpine stimulate the gastric secretion of either acid or pepsin or the oesophageal secretion of pepsin.

(4) The sympathetic nervous system contains secretory fibres for the production of acid and pepsin by the gastric glands and of pepsin by the oesophageal glands.

(5) Mechanical stimulation of the gastric mucosa by inert substances a secretion of pepsin from the oesophagus and of both acid and pepsin from the stomach as the result of a true reflex mediated by way of the splanchnic nerves and spinal cord.

(6) Reflex secretion by the stomach can be obtained by stimulation of the central end of the sciatic nerve even when the brain and medulla, but not the spinal cord, have been destroyed.

(7) Histamine stimulates the secretion of pepsin by the oesophageal glands and of acid but not of pepsin by the gastric glands.

2. Motility.

(1) Stimulation of the splanchnic nerve causes a rise in tonus and increases the contractions of the stomach.

(2) Stimulation of the central end of the sciatic nerve results in reflex movements of the stomach even when the brain and medulla are destroyed but not when the spinal cord is destroyed or the splanchnic nerves are sectioned.

Secretion

(1) Gastric secretion in the pigeon and chicken is intermittant and occurs only in response to the some stimulus.

(2) Acetylcholine stimulates the secretion of small amounts of juice which is very rich in pepsin and of high acidity. Pilocarpine is sowhat less effective.

(3) Adrenaline does not stimulate the secretion of gastric juice.

(4) Histamine stimulates the secretion of only acid and not of pepsin as well. Histamine secretion is not abolished by atropine.

(5)Alcohol stimulates the secretion of acid but it is not as effective as histamine.

(6) The volume of gastric juice secreted by histamine stimulation depends on the volume of body fluids. Compensation of the loss of body fluids (as gastric juice) by means of 0.4 to 3.3% sodium chloride or 5.6% glucose results in an increase in the volume of juice secreted.

(7) 10% glucose delays and 20% glucose completely inhibits the secretion of gastric juice induced by histamine.

(8) Glucose in concentrations of 10% or higher stimulates the output of pepsin.

(9) Pigeon's gastric juice is much poorer in pepsin than chicken's gastric juice.

(10) The data of detailed analyses of 11 samples of pigeon's and 2 sample of chicken's gastric juices are given.

MAMMALIAN FOETUS

Motility

(1) Spontaneous contractions of the stomach of the cat foetus are already present in foetuses of 58mm. body length.

(2) Both peristaltic and antiperistaltic waves of contractions can be observed in the isolated stomach of the foetal cat.

(3) Acetylcholine and pilocarpine raise the tonus and often increase the rate of contractions. The effect of these drugs is abolished by atropine.

(4) Atropine by itself has a double - inhibitory and excitatory - effect depending on the concentration.

(5) Adrenaline in small doses stimulates and in large doses inhibits the motility.

(6) Ergotamine paralyzes the effect of adrenaline.

(7) The usual effect of adrenaline after acetylcholine is that of antagonism but sometimes the two substances act synergistically.

HIBERNATING GROUNDHOG

Secretion

(1) In the hibernating groundhog there is spontaneous, continuous gastric secretion.

(2) This secretion was observed to occur notwithstanding that the body temperature of the animal was sometimes as low as $4^{\circ}C$.

(3) The spontaneous secretion is only of a moderate acidity and is almost entirely devoid of pepsin.

(4) Vagus stimulation, and likewise pilocarpine, activates the production of both acid and pepsin by the gastric glands.

(5) Histamine produces an increased output of acid, but does not stimulate the production of pepsin by the gastric glands.

VIII

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