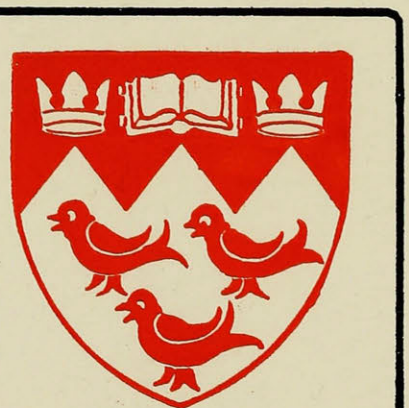
# ACTION OF HISTAMINE ON THE SECRETORY & MOTOR PHENOMENA IN THE DIGESTIVE TRACT



THESIS

# THE ACTION OF HISTAMINE ON THE SECRETORY AND MOTOR PHENOMENA IN THE DIGESTIVE TRACT

By

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# INTRODUCTION

Of the different substances which can be obtained from extracts of various animal organs, histamine deserves special consideration. It has the greatest physiological activity of any of the known amines, producing marked toxic symptoms when given to an animal, and yet it is derived readily from the harmless amino acid histidine, present in nearly all proteins. There are few pharmacological substances which, when injected in small doses, produce such a general reaction as this amine. It has a pronounced effect on the circulatory and respiratory systems. All the digestive glands are stimulated following its administration. It acts on almost all the smooth muscles in the body and chiefly on those responsible for the motility of the digestive tract. In addition the adrenals are stimulated, the output of adrenalin being thereby increased, as is possibly that of other hormones. Histamine is thought to be partly responsible for the phenomenon of shock, for the formation of wheals and some other skin reactions, and may be a contributing factor in the production of symptoms of intestinal intoxication.

The presence of histamine in the intestinal contents has been established, its formation being brought about by certain decarboxylase bacteria which remove $CO_2$ from the amino acid histidine. Histamine may also be extracted from practically all tissues, and recently evidence has been brought forward to show that it may actually be present in the living cell. However, almost

nothing is known about the form in which this very active substance may exist in normal tissues, and the question as to the part, if any, that it plays in normal body processes is not yet answered.

In the following investigation an attempt has been made to explain the nature of the action of histamine on the secretory processes of some of the digestive glands and on the motility of the intestinal tract. This study, consisting chiefly in an experimental analysis of the problem, must be regarded as preliminary work which will aid future investigators to understand the role played by histamine in body processes and the importance of its presence in the intestinal tract. The experimental part of this work is preceded by a review of the literature concerning the action of this substance on the digestive tract. Since no such review has yet been published, it was considered that a complete discussion of this question would be desirable.

# PART I.

## A REVIEW OF THE LITERATURE CONCERNING THE ACTION OF HISTAMINE ON THE ALIMENTARY TRACT

### Historical and General Discussion

Histamine was first prepared synthetically by Windaus and Vogt (1907). Three years later its physiological activity was discovered by Barger and Dale (1910) in the course of an investigation of ergot and its extracts. About the same time Kutscher (1910) independently described the isolation of a base from ergot with properties similar to that discovered by Barger and Dale. Ackermann (1910) showed that histamine could be obtained by subjecting histidine to the action of putrefactive bacteria. A detailed study of the physiological action of histamine was performed by Dale and Laidlaw (1910) on both carnivora and rodents. They noted that intravenous injection of the base was followed by severe symptoms characterized by a marked fall in blood pressure, disturbances of respiration, and if the dose were large enough by collapse and death. Histamine was also found to have a marked action on the digestive system, producing salivation, vomiting and defaecation, in addition to the above-mentioned effects. Since the discovery of the properties of histamine by Dale and his co-workers a great deal of interest has been shown both in its physiological action and in the question as to whether it is present in the tissues and plays a part in pathological or normal body processes.

Considerable evidence has been brought forward to show that histamine is a constituent of extracts of body tissues. Popielski

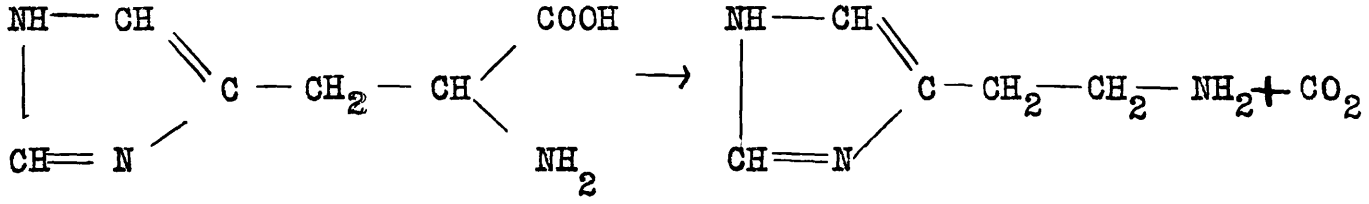
(1909) first demonstrated that such extracts when injected into an animal had a marked depressor action on the blood pressure in addition to other toxic symptoms. Popielski supposed that this specific action of tissue extracts was due to the presence of a hypothetical substance, vaso-dilatin. The resemblance of vaso-dilatin to histamine was first pointed out by Barger and Dale (1910), who found that the depressor substance extracted from the intestinal mucous membrane of the ox was identical in its physiological action to histamine and indistinguishable from vaso-dilatin save that it did not affect the coagulability of the blood. Since then a number of investigators have confirmed the presence of a depressor substance in tissue extracts. Abel and Kubota (1919) were the first to make the claim that this substance was actually histamine or some substance very closely related to it. Their evidence for this assumption was not completely convincing and left some doubt as to the identity of the two. However, more recently Best, Dale, Dudley and Thorpe (1927) have demonstrated that histamine itself is one of the chief depressor constituents of tissue extracts, and furthermore they have brought forth evidence to show that this substance may also be present in the living cells of the body.

There is much support for the assumption that the liberation of histamine from the tissues may play a part in certain pathologica conditions. For example, the work of Dale and Laidlaw (1910), Cannon (1923) and Bayliss (1923) has demonstrated that there is a close resemblance between surgical shock and the shock produced by

large doses of histamine. Substances which closely resemble histamine have been proved to be liberated into the circulation following tissue injury or trauma which result in the production of symptoms similar to those seen in shock. The work of Sir Thomas Lewis and his co-workers has also shown that it is probable that in minor injuries or irritations of the skin the liberation of a histamine-like substance is responsible for the subsequent capillary dilatation and skin reactions observed in such cases.

## FORMATION OF HISTAMINE IN THE INTESTINAL TRACT

The precursor of histamine, or β-iminazolylethylamine, as it was formerly called, is the amino acid histidine, which is present in most proteins, and which is formed normally in the process of digestion in the katabolism of the protein molecule. Under certain conditions, that is, in the presence of decarboxylating bacteria, histidine may be transformed into histamine. The reaction is a simple one involving only a removal of $CO_2$ from the amino acid.



Histidine Histamine

A bacillus capable of producing this transformation was first isolated from the intestinal tract of both man and the guinea-pig by Mellanby and Twort (1912). At about the same time Berthelot

and Bertrand (1912) independently discovered a similar organism. Hanke and Koessler (1922) investigated twenty-nine strains of B. coli and found that six of them were capable of producing histamine from histidine, provided certain salts and a source of carbohydrate and nitrogen were present. It was also shown by Gerard (1922) that the formation of histamine in isolated closed loops of the intestine was dependent on the presence of bacteria, as no histamine was found in the contents of a similar loop which had been previously sterilized. However, as histamine was demonstrated in the sterile mucosa of the closed loop, it must not be concluded from the above experiment that it can only be formed through the agency of bacteria.

Histamine has been shown to be present in the intestinal contents. Hanke and Koessler (1924) demonstrated that this substance in relatively large amounts was found in the faeces and caecal contents of normal persons. Dog's faeces and also dog's liver contained histamine, though none of the base was demonstrated in the one human liver examined. Meakins and Harington (1921) also showed the presence of a substance in the contents of the human ileum and caecum which was pharmacologically identical with histamine. They were, however, unable to demonstrate such a substance in the faeces and concluded that it was oxidized in its passage through the large intestine.

Hanke and Koessler believe that histamine-producing organisms are constant inhabitants of the small intestine, and that histamine is normally present in the intestinal contents. On the other hand, other investigators consider that it is produced chiefly in

disturbances of the alimentary tract, and that its absorption is responsible for a variety of disorders. Mutch (1913) determined the presence of a histamine-forming organism in the ileum and caecum of certain patients showing signs of chronic poisoning from constipation. Mellanby (1915) considered that in the diarrhoea and vomiting of children there might be either an increased formation or an increased absorption of this substance. Eustis (1912) has suggested that certain cases of asthma may be due to the absorption of toxic amines from the intestinal tract. He showed that, while normal human beings as well as animals have the power of detoxicating histamine, this power is lacking in the asthmatic individual. As it had been shown (Cannon, Dragstedt and Dragstedt, 1920) that in the closed gut the proteolytic bacteria multiply and outgrow all other strains, it was considered that increased formation of toxic amines in closed segments might be responsible for the symptoms of intestinal obstruction. Gerard and Meakins and Harington have investigated the problem as to whether the absorption of histamine, the most active of the amines, might be the effective agent in producing these symptoms. The former investigators demonstrated the presence of the amine in isolated closed loops of intestine and is inclined to the view that the absorption of histamine and other amines may play a part in obstruction toxaemia. On the other hand, Meakins and Harington consider the balance of the evidence against this view.

## THE QUESTION OF THE ABSORPTION OF HISTAMINE FROM THE INTESTINAL TRACT

Since the conditions necessary for the formation of histamine, i.e. the presence of histidine and the decarboxylating bacteria, are fulfilled in the intestine, and since histamine is a very toxic substance, the question of its absorption by the organism is an important one.

Koessler and Hanke (1924) found that, if a small amount of histamine was placed in the mouth of a guinea-pig, the animal died shortly afterwards, showing that relatively large quantities must be absorbed by the mucous membrane. However, the evidence is not so clear cut as regards the absorption of histamine from other parts of the alimentary tract. Divergent opinions concerning this question are expressed in the literature. Mellanby (1916) working on cats reported that a histamine solution was not absorbed from a loop of gut from which the circulation was cut off, whereas, if the circulation was not interfered with, the histamine disappeared. He found that the absorbing power of the gut increased from the duodenum to the caecum, and that the presence of food, fluid or acid hindered the absorption, while alkali improved it. Further evidence that histamine may be absorbed from the intestine is afforded by the results of Meakins and Harington (1923), who also worked on anaesthetized cats. They showed that, following the injection of histamine in an intestinal loop, a marked fall in blood pressure occurred. On the other hand, Wangensteen and Loucks (1928), similarly using the fall in blood pressure as an

index of absorption were unable to confirm the results of Meakins and Harington on dogs. They could obtain no fall in blood pressure even though doses of histamine as large as 50-100 mg. were introduced into the intestine.

The remainder of the experimental evidence, in support of the findings of Wangensteen and Loucks, does not favour the absorption of histamine as such from the intestine in quantities sufficient to produce a fall in blood pressure or any other toxic symptoms. Thus Ivy, McIlvain and Javois (1923) gave .75 gm. to dogs and 225 mg. to a man without any marked symptoms. Oehme (1913) administered .5 gm. to rabbits by mouth and observed no ill effects. Koessler and Hanke (1924) showed that guinea-pigs and dogs could be fed very large doses of histamine without symptoms of intoxication, provided it was introduced directly into the stomach. Upon attempting to recover this histamine quantitatively several hours later they found that a small fraction was present in the mucous membrane and liver, part remained in the gut unchanged, while a large amount had vanished and could not be accounted for. This raises the question as to the fate of the histamine which disappears from the intestine and whether or not the liver plays a part in its detoxication or destruction.

## Part played by the Liver in the Detoxication of Histamine

It was first shown by Ewins and Laidlaw (1910) that the liver destroyed certain amines. This work was extended by Guggenheim and Loeffler (1916) who concluded that most proteinogenous amines are detoxicated in the animal organism in their passage through

the liver. However, the katabolism of histamine in this way could not be proved. Other investigators (Meakins and Harington, and Dale and Laidlaw) have also perfused histamine solutions through the liver, finding that only very small amounts of this substance were destroyed. The suggestion of Meakins and Harington was that the part played by the liver in protecting the organism from absorbed histamine is more mechanical than chemical, due to the cushioning effect of its capillary network which prevents a sudden large amount of this substance from entering the circulation. However this type of protective action has been shown to be true for any capillary network and cannot be considered to be specific for the liver (Koessler and Hanke).

Additional evidence for the conclusion that the liver does not detoxicate large quantities of histamine is furnished by experiments with Eck fistula dogs. Ivy (1924) found that these animals were as tolerant as normal dogs to doses of .5-.75 gm. of histamine given by stomach tube. Furthermore it has been shown that the injection of histamine into the portal circulation produces the usual effect on blood pressure (Popielski, 1920; Lim and Ammon, 1923), though Lim and Ammon found that the secretory action on the stomach was diminished in the passage through the liver.

Since it has been found that large quantities of histamine disappear from the lumen of the intestine without producing intoxication symptoms, and since there is very little evidence that the liver destroys large amounts of the amine, Hanke and Koessler have brought forward the explanation that the mucous membrane of the gut has the property of rendering inert most of the absorbed

histamine before it enters the portal circulation. In support of this explanation Best has very recently shown that the intestinal mucous membrane, as well as the kidneys, possesses an enzyme which has the power of detoxicating histamine. However, it must be pointed out that the possibility of the absorption of very minute non-toxic amounts of histamine from the intestine is not precluded, since it has been proved that a sufficient amount of this substance may enter the circulation from the intestine to activate a gastric secretion. This question will be considered further on.

It may be concluded that histamine is present in the intestinal contents and that the conditions necessary for its formation are fulfilled in the intestinal tract. However, there is meagre evidence that toxic amounts of this substance are absorbed under normal conditions. As the liver has very little power of detoxicating histamine, it is probable that this process is brought about by enzymes present in the intestinal mucous membrane.

## HISTAMINE AND GASTRIC SECRETION

It was first shown by Popielski (1920) that histamine was a very powerful stimulant of gastric secretion. Because of the great similarity in its action to the "gastrin", extracted by Edkins from the pyloric mucous membrane, the two were thought by some investigators to be the same substance. A study of the question of the identity of histamine and gastrin was performed by Koch, Luckhardt and Keeton (1920). They found that although

there was a striking resemblance in the behaviour of the two substances, certain chemical differences were manifest, e.g. gastrin was more stable than histamine and was not precipitated by certain acids. It was concluded that the resemblance was not sufficient to warrant the assumption that the two were identical substances. Despite this fact, an increasing interest has been shown in the remarkable action of histamine on gastric secretion and in the importance of its presence in the intestinal tract as well as other parts of the body.

As has been previously pointed out, histamine can be obtained from both the normal mucous membrane of the alimentary tract and the intestinal contents. In view of this and the fact that very minute amounts of the amine favour the secretion of gastric juice, it is possible that this substance may play a part in the physiological processes of digestion. Since the intestine serves as a pathway for the stimulation of the glands of the stomach, the presence of histamine there may be one of the important factors in producing the intestinal phase of gastric secretion as well as the continuous gastric secretion.

It is interesting to note here that it has also been suggested that the formation of histamine or closely similar substances may occur under certain pathological conditions and influence the course of gastric secretion. Kalk (1929) has demonstrated in patients with supersensitive skins which show a good dermatographism, that intensive stimulation of the skin causes a gastric secretion similar to that evoked by the subcutaneous injection of

1 mg. of histamine. In certain skin diseases in dogs, similar to eczema, Archawsky (1929) has also shown that the products arising from the decomposition of protein due to the destruction of tissue and skin stimulate the gastric glands to long continued activity.

## Mode of Introduction of Histamine

### A. Subcutaneous and Intramuscular Injection

As previously pointed out it is a well established fact that histamine is a stimulant of gastric secretion. It was found in man and the following animals, dog, cat, rabbit, guinea-pig, pigeon and frog, that the subcutaneous injection of a dose of .5 - 2 mg. produced a flow of gastric juice. The effect is more pronounced in carnivora than in rodents and cold-blooded animals. Keeton, Koch and Luckhardt (1920) showed that, while cats and dogs gave a good gastric response within the hour following histamine administration, in rabbits the secretion did not occur until the second hour. Rothlin and Gundlach (1921) found that the minimum amount of histamine which would react on gastric secretion when given subcutaneously to a Pavlov's pouch dog was .033 mg. per kilo. Frogs respond only in 45% of cases to histamine stimulation, though if previously accustomed to higher temperatures the number that will react positively is considerably increased (Popielski, 1929).

Intramuscular injection of histamine has a very similar effect to intravenous administration save that the absorption takes place slightly more rapidly and the general reaction, e.g. flushing of the skin, mucous membrane and conjunctiva is a trifle more marked.

There is also evidence to show that histamine may be absorbed

from the surface of the skin. Koskowski (1922) found that in pigeons, if an area of skin were plucked of its feathers and slightly scratched, the local application of a histamine solution had the same action on the gastric secretion as subcutaneous injection.

B. Intravenous Injection

Although it was generally found that histamine was a very effective stimulant to gastric secretion when administered subcutaneously or intramuscularly, several investigators have reported that the intravenous injection of this substance had no effect on gastric secretion, in spite of the fact that it was usually accompanied by marked general symptoms. Popielski (1920) injected histamine several times in doses as large as .8 mg. into the great saphenous vein of an unanaesthetized dog and obtained no secretion of gastric juice, though the animal showed marked signs of discomfort after each injection of the drug. Similar results on Pavlov's pouch dogs are reported by Rothlin and Gundlach, using doses of .4 - .05 mg. (1921). Koskowski (1922) in an investigation on pigeons also found that intravenous injection of histamine was without effect on the gastric secretion, though it acted very strongly when given subcutaneously. In attempting to explain the difference in the action of histamine after the two methods of administration, Koskowski showed that it was not due to destruction of this substance by the blood, since rabbit's blood treated with histamine and injected subcutaneously into pigeons was followed by a copious secretion of juice.

The above-mentioned investigators did not take into consideration that the size of the dose and the rate of injection of histamine into the circulation were important factors in the production of a gastric secretion. In their experiments the amine was injected rapidly in comparatively large amounts. Since then it has been shown that the failure to obtain a positive effect upon intravenous injection was due to the method of administration. Lim (1922) first observed a gastric secretion after intravenous injection of a minute amount of histamine (.001 mg.). He did not attribute his success to the small size of the dose employed, but rather to the fact that the abdominal vagi were severed and the animals under anaesthesia. However, Gutowski (1924) has definitely proved that, if a slow intravenous injection is employed (i.e. 5 c.c. containing 1 mg. of histamine in 50 minutes), a rich secretion of gastric juice is obtained comparable to that received after a subcutaneous injection of 1 mg. of histamine. Moreover it was shown that the amount of secretion was conversely proportional to the rapidity with which the histamine entered the circulation, thus if the injection rate were increased, the gastric secretion diminished. At about the same time Ivy and Javois (1924) independently showed, using a somewhat similar technique (i.e. .0027 mg. of histamine per kilo per minute into the circulation of a dog), that histamine was an effective stimulus to gastric secretion. It is interesting to note that, after an intravenous injection has been effective in producing a secretion, subsequent subcutaneous injections have very little effect.

C. Application to Different Parts of the Digestive Tract

Although very large amounts of histamine may be introduced into various parts of the digestive tract without causing any of the shock-like symptoms produced by intravenous injection, still sufficient of the substance may be absorbed through the intestinal mucosa to activate a gastric secretion. As has been mentioned in the previous section, only very minute doses of histamine injected into the blood will cause a secretion, and thus it may be effective as a stimulant to the gastric glands in amounts which have no effect on the blood pressure.

From the Stomach. - Relatively very large amounts of histamine must be introduced into the stomach to produce a gastric secretion. Ivy, McIlvain and Javois (1923) gave 225 mg. by stomach tube to a man and 100 mg. to a dog and obtained a flow of gastric juice. Local application of a histamine solution to the gastric mucosa is also effective in producing a secretion if given in sufficiently high concentration. The minimal amount which will activate a secretion from a Heidenhain's or Pavlov's pouch by this method is 50 mg. (Lim, Ivy and McCarthy, 1925). Larger amounts than this locally applied provoked a good secretion both from the pouch and the main stomach.

From the Intestine. - Smaller amounts of histamine are more active in producing a gastric secretion from the intestine than from the stomach, although several investigators have obtained negative results from this method of administration. Popielski (1920) injected 3.2 mg. into the duodenum of the dog with no stimulation of the gastric secretion, and Rothlin and Gundlach (1921)

reported that intestinal application of 10 mg. of the amine did not activate the gastric glands. However, Koskowski (1922) found that 30 - 40 mg. injected into the intestine of a pigeon gave a flow of gastric juice as great as that produced by subcutaneous injection of 1 mg. Ivy and McIlvain (1923) without fail found that continuous application of a 1/1000 histamine solution for 20 minutes to the duodenal or jejunal mucous membrane resulted in a good secretion of gastric juice which lasted from one to two hours. Similar results have been obtained by Nechoreschew (1929) who applied a solution containing 2 mg. to an isolated intestinal loop.

It is interesting to note that intestinal application of histamine is more effective than meat extract, Witte's peptone or spinach extract in producing a gastric secretion (Ivy and McIlvain).

It may be concluded that histamine is a powerful stimulant of gastric secretion when given subcutaneously or intramuscularly. It is also effective when introduced directly into the circulation, provided precautions are taken that the dose entering the blood is very minute, the amount of secretion being inversely proportional, within limits, to the amount of histamine in the blood. Absorption of histamine may also take place from the duodenum, jejunum, and to a lesser extent the stomach, in an amount sufficient to stimulate gastric secretion without producing any toxic effects.

## Curve of Gastric Secretion obtained after Histamine

A comparison of the volume and acidity curves obtained after food with those obtained after histamine shows that the two run a more or less parallel course, although the normal maximum values reached with histamine are usually higher than those obtained after the test meal (Brancati, 1928; Rachon and Walawski, 1928).

The majority of investigators have found both in man and in dogs with a Pavlov's pouch or gastric fistula, that the latent period of secretion varies from 5 to 15 minutes following the subcutaneous injection of histamine in doses of from $\frac{1}{2}$ to 2 mg., though Carnot, Koskowski and Libert (1922) have reported a longer latent period of 35 - 55 minutes for man. Apparently the latent period is approximately the same as that found by the majority of workers (i.e. 5 - 15 minutes), when histamine is administered in another way than subcutaneously. For example, Ivy and McIlvain (1923) report that after the application of histamine to an isolated intestinal loop of a dog a gastric secretion started in 10 - 30 minutes. Lim (1922) found in dogs and cats that following a slow intravenous injection, the secretion began in three minutes. Gutowski (1926) stated that the latent period of secretion was the same following subcutaneous or intravenous injection, although the subcutaneous injection produced a longer continued secretion.

The duration of the secretion produced by histamine varies with the dose of the drug administered. However, with the usual subcutaneous injection of 1 - 2 mg. it lasts for approximately 1 - 2 hours. With larger doses the effect is longer. Popielski

(1920) found that subcutaneous injection of 3.2 mg. in a dog with a gastric fistula evoked a secretion which continued six hours, whereas a smaller dose administered to the same animal on another occasion only caused a secretion for 1 hour and 30 minutes.

The maximum rate of gastric secretion after histamine injection is usually established in the first half hour. This secretion then begins to decrease and at the end of 1 hour to 1 hour, 30 minutes is practically over. There are, however, considerable variations in these values. Grompertz and Vorhaus (1925) investigated the response of a number of individuals and they found that in the majority the maximum rate of gastric secretion was reached in 30 - 60 minutes. In others, a smaller number, it did not take place for 60 - 90 minutes, while in a few it occurred in 15 - 30 minutes.

Although the character of the curves after histamine are approximately the same in different individuals, Bloomfield and Polland (1929) found a great difference in the total volume of juice collected. According to Mogena (1927) this amount varies from 180 - 200 c.c., most of which is secreted in the first 15 minutes after the histamine has begun to act.

## The Acidity of the Histamine Gastric Juice

Carnot, Koskowski and Libert (1922), among the first investigators to study the composition of the histamine gastric juice, found that the total and free HCl as well as the peptic activity were increased. Since then many investigators

(Katzelenbogen and Choisy, 1928 ; Rachon and Walawski, 1928; Brancati, 1928; Moretti, 1927; Grompertz and Vorhaus, 1926) have compared the gastric juice obtained with histamine to that obtained after stimulation with food, the general agreement being that the degree of acidity is greater after histamine than after an ordinary test meal. On the other hand, in contrast to this opinion Grimbert and Fleury (1929) find that the histamine juice is distinctly lower in free acidity than juice secreted by a dog during sham feeding or the appetite juice of man. However, as the authors themselves point out, the factor of swallowed saliva was not excluded in their experiments, and thus the reliability of the results may be questioned. Webster (1929) has also seen that, in a dog with an oesophagotomy and gastric fistula, sham feeding gave a juice higher in acid than histamine.

The increase in hydrochloric acid following the injection of histamine becomes evident in approximately 15 minutes to 30 minutes, and then rises rapidly to a maximum (Matheson and Ammon, 1923). There are differing opinions concerning the time relationship between the rate of secretion and the secretion of acid. According to Matheson and Ammon there is a definite sequence in the histamine gastric secretion, the increasing acidity reaching a maximum some minutes before the establishment of the maximum rate of secretion. Similar results are reported by Mogena and Fernandez (1928) and Katzelenbogen and Choisy. On the other hand, Carnot, Koskowski and Libert, and Rachon and Walawski observed that the acidity reached a maximum later than the volume, while Bloomfield and Polland found that the two curves reach a maximum

at approximately the same time.

The values given for the free and total acidity of the histamine gastric juice vary considerably. Bloomfield and Polland investigated a large number of individuals and they found that the normal range for total acidity was from 90 - 125 c.c. (measured by titration against n/10 NaOH). The free acidity values were slightly lower. Wide differences in this range are observed in pathological cases.

## The Chlorides of the Histamine Gastric Juice

Following the injection of histamine there is an increase in the total chloride (i.e. mg. per 100 c.c.) over that observed in the fasting juice (Polland, Roberts, Bloomfield, 1928, and Berglund, Wahlquist and Sherwood, 1927). This increase begins promptly after histamine and reaches its maximum in about twenty minutes. From then on the concentration remains high for approximately 30 minutes, after which it falls slightly. The maximum chloride concentration reached after histamine is usually over 525 mg. per 100 c.c. as compared with 200 - 400 mg. in the fasting juice (Bloomfield, Roberts and Polland).

The curve of the total chlorides coincides with the curve of the volume of secretion only in the first part of its course. It reaches a maximum in about the same time but remains high and does not diminish with the volume curve. However, the relation between the chloride curve and that of the free acidity is more marked. Berglund, Wahlquist and Sherwood found a close

correspondence between the total chlorides and free acidity. Although the total chloride curve exceeded that of the free acidity by a fairly constant amount, the two were parallel throughout their course. The amount of difference in the two curves is considered to be only due to the neutralizing of some of the juice by mucus and base secreted by the gastric glands. Thus the total chloride may be considered as an index of the acid secreting power of the stomach.

Attempts made to correlate the total chlorides of the gastric juice with the chlorine of the blood failed, although a definite relation between the highest chloride figure for gastric juice and the alkali reserve was established (Berglund, Wahlquist and Sherwood). In connection with this fact Fonseca and Carvalho (1927) have observed that after the injection of histamine and subsequent secretion of acid gastric juice the alkali reserve markedly increases. The explanation that this increase is due to the secretion of hydrochloric acid is supported by the finding that it was not observed in cases of achylia which did not respond to histamine.

## The Peptic Activity of the Histamine Gastric Juice

Carnot, Koskowski and Libert (1922), employing the Metts method, first demonstrated that the proteolytic power of human gastric juice, secreted after the injection of histamine, was increased. Since then the fact has been confirmed in humans by a number of investigators. A comparison of the peptic activity after histamine and after a test meal was made by Rachon and

Walawski on an individual with a gastric fistula. They found that the first portions of the histamine juice were richer in enzymes than those secreted after food. In contrast to these results Molinari-Tosatti (1929) noted that the juice secreted after a meal in dogs with Pavlov's pouch was richer in enzymes than that secreted by histamine. Other investigators have also failed to observe an increase in the proteolytic power of dog's gastric juice after histamine, though the acid and volume increased (Rothlin and Gundlach, 1921; Vineberg[*], 1929).

Some writers claim that the peptic activity reaches a maximum at the same time or slightly previously to the maximum acidity (Lim, Matheson and Schlapp; Matheson and Ammon; Teschendorf, Rachon and Walawski). Grompertz and Vorhaus found in their study of abnormal cases that the peptic activity in many instances reached its maximum slightly after the acidity. Carnot, Koskowski and Libert have obtained different results from any of the above-mentioned writers, namely, that the increase in enzymes is greatly retarded and only takes place after the acid and volume have decreased.

## The Mechanism of the Action of Histamine on Gastric Secretion

The mode of action of histamine on the secretory mechanism of the stomach is not quite clear. Since this substance is somewhat similar in its chemical structure and physiological action to pilocarpine, Rothlin and Gundlach (1921) considered that it was similar to a parasympatheticomimetic drug, i.e. it

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[*] Personal communication.

stimulated the endings of the vagus nerve. There is, however, considerable evidence against this view, as it has been shown that the action of histamine as a gastric stimulant is independent of the secretory nerves.

The gastric response to a meal may be markedly inhibited by local application of atropine to the gastric mucosa or its injection into the circulation or intramuscularly (Dodds and Bennett, 1921). However, this drug does not have a similar action on the gastric secretion produced by histamine. Popielski (1920) found that the response to histamine was not affected by either intrathoral section of the vagi or by atropine. His results were confirmed by Koskowski (1922), Delhougne (1926), Katzenelbogen and Choisy (1927), and Lim (1922). The last named investigator found that neither local application of atropine to the stomach nor its intravenous injection in doses as large as .65 mg. had any effect on the histamine secretion. However, Keeton, Luckhardt and Koch (1920) observed that larger doses of atropine caused a certain diminution in the amount of the histamine gastric juice. Lim, Ivy and McCarthy (1925) also noted a similar diminution, though if large enough amounts of histamine were given it was almost negligible.

Experiments on animals with denervated gastric pouch and stomach afford further evidence that the action of histamine is independent of the secretory nerves. Ivy and Javois (1925) noted a marked stimulation of the gastric glands of a Heidenhain's pouch with this substance. Suda (1924) completely denervated the

stomach (Bickel's pouch) and found that histamine would still produce a secretion. Furthermore Ivy and Farrell (1925) described the secretion of gastric juice from a part of the stomach which had been denervated and transplanted into an active mammary gland.

The idea was first put forward by Popielski and later agreed upon by most investigators that histamine affected the secreting organs directly by acting on the cells of the gastric glands. It has been recently suggested (Lee, 1929; Molinari-Tosatti, 1929) that the vaso-dilator action of histamine is probably a highly important factor in its secretory effect. In support of this idea Mladoveanu (1928) showed that inhalation of amyl nitrate or subcutaneous injection of sodium nitrate, both of which produce a marked vaso-dilatation, has a stimulating action on gastric secretion similar to that produced by histamine. However, there is no evidence that vaso-dilatation in itself can provoke a secretion; indeed a number of facts are against this assumption. For example, Barcroft (1914) by using yohimbin caused a marked vaso-dilatation in the salivary glands and was not able to observe any secretion. Similarly doses of histamine ($\frac{1}{4}$ mg.) administered to anaesthetized animals produced the same vascular conditions in the submaxillary gland, and yet the secretion was absent or very scanty unless previous stimulation of the nerve had taken place.

It may be concluded that the action of histamine on the gastric glands is independent of the secretory nerves. It is probable that this substance is stimulating the secreting cells directly, its secretory action being enhanced by its vaso-dilator properties but not due to them.

## The Clinical Use of Histamine as a Test of Gastric Function

Recently histamine has been used extensively as a test of gastric function. Most clinicians who have employed it for this purpose agree that it yields more accurate data about the gastric function than the various food test meals. The advantages of the histamine test meal may be briefly stated. It gives an almost pure gastric juice for cytological and chemical analysis in which the values are not distorted by the admixture of food particles. Moreover the stimulus can be accurately standardized, so that the test is susceptible of identical repetition. These conditions cannot be fulfilled with the usual test meal, as the composition of the food, rate of eating, swallowed saliva, all are subject to variation. On the other hand, it has been shown that repeated examinations of the same patient, using the histamine test, have coincided very well. Histamine is also a better stimulus to the cells of the gastric mucosa than the other commonly used tests. Thus frequently a food or even an alcohol meal will fail to cause an acid secretion, whereas histamine will give a good result.

The histamine test is very simple to carry out. In the method chiefly in use the analysis is performed by withdrawing a sample of juice every fifteen minutes after the histamine injection for a period of one to two hours. Usually before the test the patient is starved overnight or for a period of eight hours and then the Refuss stomach tube is introduced. The fasting contents of the stomach are removed, after which the histamine is injected

subcutaneously and the fractional analysis begun. Certain modifications of the fractional method described above have been described by Bloomfield and Polland (1929) and by Lim, Matheson and Schlapp (1923). The chief object of these modifications is to prevent any of the gastric juice going over into the duodenum and thus causing a duodenal secretion. The regurgitation of duodenal contents and subsequent neutralization of gastric juice is also hindered. In both of the modifications a continuous aspiration of the stomach is begun immediately after histamine administration and continued throughout the course of the experiment. Lim, Matheson and Schlapp also introduced a second tube into the duodenum and removed the contents continuously. By this method a juice of very high acidity is obtained, and the total volume of gastric juice secreted may be measured.

The usual dose of histamine employed in the test is 1 mg. of the drug injected subcutaneously in 1 c.c. of solution, although some investigators employ .5 mg. and others 1 mg. for every 10 kilos of body weight. Grompertz and Cohen (1929) have objected to the larger doses for they found that .25 mg. was sufficient to produce a gastric secretion without having any noticeable ill effect. Lee (1929) has endorsed the smaller dose for he found that .0017 mg. per pound of body weight yielded a good result.

Though there is no harmful effect following the above mentioned doses of histamine, most patients after receiving these amounts show a flushing of the face and neck. With the larger doses, 1 mg. upwards, there may be a slight headache or dizziness

lasting for half an hour, a fall in blood pressure from 6 - 8 mg. Hg. and an increase of 5 - 10 beats in the pulse (Grompertz and Vorhaus, 1926; Grompertz and Cohen; Gallart, Vilardell and Babot, 1927; Andresen, 1926; Matheson and Ammon, 1923). There is also a wheal formed at the point of injection of the drug, which is surrounded by an area of erythema and characterized by a burning and stinging sensation. These unpleasant symptoms are obvious with doses of 1 mg. and more, but are greatly reduced with smaller doses. Mogena and Fernandez (1928) have found that $\frac{1}{4}$ - $\frac{1}{2}$ mg. of adrenalin injected at the site of injection of the histamine allays the wheal formation and vaso-dilatation which otherwise take place.

The histamine test has been used in a large number of pathological as well as normal cases and has proved to be of great value in diagnosing certain conditions.

## The Diagnostic Value of the Histamine Test

It has been found that many cases, which had been diagnosed as achlorhydria upon failure to react to one of the usual test meals, gave a good secretion of acid gastric juice after the injection of histamine. Thus Grompertz and Vorhaus report that of 17 cases which were unresponsive to the Ewald test meal, 10 cases or 59% reacted with a secretion of acid to histamine. Katsch and Kalk (1926) saw that 50% of the cases which did not secrete acid with caffein or alcohol reacted positively with histamine. In a comparison of the percentage of achylias obtained with a cracker and $H_2O$ meal and those obtained by

histamine, Andresen found that with histamine the achylia was 20% less common. Similar findings have been obtained by Mogena, Lunding (1929), Bockus and Bank (1927), Stuber and Nathanson (1926), Dobson (1925) and Torchiani (1927). Henning (1928) has cautioned against taking the results of a single histamine test as affording sufficient information concerning the hydrochloric secreting power of the stomach. He reports a single case in which three entirely different results with histamine were obtained in a patient with achlorhydria. On the other hand, Bloomfield and Polland, and Cade and Milhaud (1928) found that the histamine test gave strikingly constant results in repeated examinations of the same individual.

Though there is no specific reaction to histamine in any given pathological condition, it has been found that there is a failure to react to this test in any cases where actual destruction of the secreting cells has taken place. Thus it is generally agreed that in pernicious anaemia no hydrochloric acid is secreted in response to histamine. A number of investigators have also reported a negative reaction to histamine in cases of chronic gastritis (Grompertz and Vorhaus; Mogena; Vandorfy, 1928; Stuber and Nathanson; Andresen). In chronic wasting diseases such as pulmonary tuberculosis as well as in a number of other disturbances failure to respond to histamine is sometimes noted.

Following operation on the digestive tract the response to histamine appears to be diminished. Ciocca (1929) investigated 18 cases from the fifteenth to twenty-fifth day after operation and found that the quantity of acid was markedly decreased

especially in resection cases. Lee has suggested the advisability of determining the pre- and post-histamine reactions in cases of gastric operation as an aid to forming a prognosis.

A large number of cases of carcinoma of the digestive tract have been reported in which there was failure to secrete acid in the histamine test (Mogena and Fernandez; Vandorfy; Derer and Separovic, 1928; Berri, 1926; Grompertz and Vorhaus; Bloomfield and Polland). However, this response is not necessarily characteristic of all cancer cases. Andresen found that in one out of four cases of gastric carcinoma there was a normal and occasionally an increased acid curve with histamine. Mogena also noted similar cases.

In contrast to the above-mentioned conditions, in which the reaction to histamine is often absent or diminished, in duodenal ulcer cases there is frequently hypersecretion following histamine (Andresen, Ciocca, Mogena, Katzenelbogen and Choisy, Bloomfield and Polland). Usually the heightened response is more marked in the case of duodenal than in the case of gastric ulcer. However, Delhougne (1926), Stuber and Nathanson, and Bockus and Bank have found ulcer cases in which histamine did not give an increased reaction over that obtained with test meals.

Despite the fact that there is not a constant response to histamine in cases of carcinoma and ulcer, several writers have stressed the value of the test as an aid in differentiating between these two pathological conditions. Bloomfield and Polland cite five cases in which the histamine test was of value

in making a diagnosis between carcinoma and ulcer. In these cases high acid and high secretory volumes were shown to be compatible with the presence of ulcer, whereas distinctly low volumes and low acid were usual in cases of carcinoma. Cases in which a similar differentiation was made are quoted by Berri and Andresen.

The use of the histamine test has been criticized by Winkelstein and Marcus (1929), who claim that the neutral red test gives as much information without any of the unpleasant effects of histamine. However, Lee reports cases in which there was no response to neutral red and yet histamine stimulated a secretion of acid. Furthermore, if the smaller doses of histamine are employed, the patient is not inconvenienced by any unpleasant symptoms. Bockus and Bank, on the other hand, believe that the food test meal yields more valuable information than the histamine test because food is a more normal stimulus to the gastric glands. However, the numerous cases which do not respond to the ordinary methods of testing gastric function and yet react to histamine show that the latter test affords much information which cannot be obtained by employing only the fractional test meal.

## THE ACTION OF HISTAMINE ON PANCREATIC SECRETION

Extracts of many animal tissues when injected into the blood have a secretagogue action on the pancreas. Similar substances are found in plants, e.g. in spinach (Bickel, 1917) and in cabbage, yeast, oats, etc. (Uhlmann, 1918). Popielski considered that the stimulating principle in all these substances was vaso-dilation, which was responsible for their physiological action. As vaso-dilation was later identified with histamine, the latter substance, according to one prevailing opinion, was considered to be the active substance in secretin preparations. However, evidence to show that histamine and secretin were not identical substances was brought forward by Parsons (1925). First it was found that in certain very active secretin preparations there was not sufficient histamine to be of physiological importance. Also histamine was more stable in relation to heat and was precipitated by certain reagents, whereas secretin was not. Furthermore a number of investigators have prepared a secretin which was highly active in stimulating the pancreas but was without effect on the blood pressure. It is now generally agreed that histamine is not the active principle in secretin, though the two substances are in many ways closely allied.

Dale and Laidlaw (1910) first demonstrated that intravenous injection of histamine produced a pancreatic secretion. Since then the fact has been confirmed by several investigators (Popielski, 1920; Gutowski, 1926; Molinari-Tosatti, 1929).

According to Popielski the secretion begins very soon after the injection of the amine (50 - 60 seconds) and lasts for fourteen minutes. In converse to its action on gastric secretion, histamine does not activate the pancreas when injected subcutaneously. Apparently this difference is due to the amount of the substance required to produce stimulation. Thus Gutowski found that, while a small quantity of histamine in the blood was favourable to gastric secretion, it had a very slight effect on pancreatic secretion. By increasing the concentration of histamine the gastric secretion was diminished while the flow of pancreatic juice was markedly increased. The amount of histamine required to stimulate the pancreas in animals is a dose of the order of $\frac{1}{4}$ - 1 mg. administered intravenously.

According to Molinari-Tosatti histamine gives a pancreatic juice with high tryptic activity similar to that of secretin, although the relative quantity of juice secreted with histamine is much less.

It is not clear whether histamine in stimulating pancreatic secretion is acting on a nervous mechanism or directly on the cells themselves. Although Popielski found that atropine did not inhibit the action of histamine, Dale and Laidlaw and Molinari-Tosatti are of the opposite opinion. The latter investigators used doses of this drug as large as 30 mg. and found that the action of histamine was abolished, whereas that of secretin was not. In consideration of this fact they suggest that the secretory action of histamine on the pancreas is twofold, due chiefly to stimulation of the nerve fibres but also to the

increased blood flow produced in the gland. Further data concerning the effect of histamine on the pancreatic gland are presented in the experimental part of this thesis.

## THE ACTION OF HISTAMINE ON INTESTINAL SECRETION

The problem of the action of histamine on the secretion of the small and large intestine has been studied by Koskowski (1926). It was found that in dogs with a Thiry Vella fistula the subcutaneous injection of histamine gave a secretion of juice from the isolated loop beginning after a latent period of one minute and continuing for several minutes. This juice was richer in invertase and amylase than that obtained by mechanical stimulation or by pilocarpine. The secretion from the large intestine was studied by means of dogs with an isolated colon. In these animals the subcutaneous injection of histamine was followed by a very scanty secretion of a few drops of alkaline mucus. The latent period was three minutes and the secretion continued for fifteen minutes.

The mechanism of the action of histamine on the intestinal glands appears to be different from that of its action on the gastric glands. In view of the fact that the time taken by the intestinal glands to respond to this substance is much less than that taken by the gastric glands, and since atropine abolishes its action, it seems probable that in the case of intestinal secretion histamine is stimulating a nervous mechanism.

## THE ACTION OF HISTAMINE ON THE SECRETION OF BILE

Popielski (1920) first observed, following the intravenous injection of .8 mg of histamine in a dog, that there was an increased flow of bile from the duodenal fistula. Alpern (1923) later confirmed this fact, using subcutaneous injection of doses of .05 mg to .2 mg per kg on dogs with a fistula of the gall bladder. However, in the latter case the factor of acid gastric juice entering into the duodenum was not controlled, and it may be that the flow of bile was a secondary effect and not immediately due to the injection of histamine. That this may indeed be the case is indicated by the results of Lim, Matheson and Schlapp. They were unable to observe any increase in the secretion of bile or pancreatic juice in man after the subcutaneous injection of histamine, provided the passage of gastric juice into the intestine was prevented.

## THE ACTION OF HISTAMINE ON MOVEMENTS OF THE ALIMENTARY TRACT

It has long been known that histamine induces powerful contractions of smooth muscle in different parts of the body. Thus Quagliariello (1914) showed that it stimulated isolated strips of bronchi, arteries, uteri, and large and small intestines. An exception to the above list was found in the oesophagus of the turtle which was inhibited by the drug (Carlson and Luckhardt, 1921).

When histamine is administered to an intact unanaesthetized animal it also produces a contraction of the musculature of the alimentary tract, as is evinced by vomiting and diarrhoea.

## Action of Histamine on Isolated Strips of Intestine

The action of histamine on isolated strips of gut has been studied by the following investigators in the cat, dog, guinea-pig, rabbit (Dale and Laidlaw, 1910; Quagliariello, 1914; Vanysek , 1914; Olivecrona, 1921; Guggenheim, 1924; Kendall and Varney, 1927; Bishop and Kendall, 1928; Kendall and Bishop, 1928). The figures quoted in the table below will show that this substance is active in extremely small quantities in stimulating the intestinal muscle.

| Name of Investigator | Animal from which intestine was taken | Concentration of Histamine which is effective when applied to intestine |
|---|---|---|
| Guggenheim | Guinea-pig | 1 part in 500,000,000 (minimum concentration) |
| Quagliariello | " | 1 part in 17,500,000 |
| Lim and Chen | Cat | 1 " " 400,000 |
| Dale and Laidlaw | " | 1 " " 500,000 |
| Vanysek | " | 1 " " 500,000 |

It is generally agreed that histamine causes a contraction of the isolated intestine in all the animals examined. This effect is very marked in the case of the guinea-pig, smaller con-

:centrations than in any of the other animals examined producing an effect here. In cats histamine is also a very powerful stimulant of the intestine, while in rabbits it has a comparatively weak effect (Olivecrona). The action of histamine on the isolated gut is to produce an increase in tonus and in the tonus contractions. This effect is transient, the time for it to wear off varying with the dose of the drug. Very large amounts of histamine tend to produce paralysis of the gut and thus abolish further contractions.

The Action of Histamine from the Mucosa on Isolated Loops of Gut

Histamine also stimulated contractions when applied to the mucosal aspect of isolated portions of gut. Loops of intestine have been used for these experiments, into which the histamine solution might be introduced without coming into contact with the serosa (Kendall and Varney, and Olivecrona). Within 30 - 60 seconds after the application of histamine to the mucosa, slow progressive contractions of the loop commence. Doses of histamine of the order of $\frac{1}{4}$ mg will produce contraction from the mucosa, whereas very large amounts (100 mg) have an inhibiting effect. The evidence is in favour of the slow absorption of histamine from the lumen of the gut in quantities sufficient to produce contractions.

It is interesting to note that an acid solution of histamine when applied to the mucosa has relatively little effect on the movements of the gut. Neutralization of the solution enhances this effect, whereas the addition of alkali markedly increases the stimulating action of the drug. Formaldehyde, on the other hand, abolishes the rhythmicity of the gut, which may be restored by

histamine (Kendall and Varney). It may be mentioned that histamine will not cause a contraction of isolated strips of intestine if formaldehyde has been previously added to the solution in which they are suspended.

## Action of Histamine on Movements of the Gut in the whole Animal and in Man

The movements of the gut, after intravenous injection of histamine, in acute experiments on whole animals have been studied by Haramaki (1922) and Nechoreschew (1929). The former observed a markedly increased peristalsis in the small intestine of a rabbit with an intestinal window, following the intravenous injection of histamine. Similar results are reported by Nechoreschew in the case of decerebrate cats with suprarenals tied off. He found that in these animals histamine produced an increase of the gastric motility. However, these findings could not be confirmed in the decerebrate dog. Since adrenalin is known to be antagonistic to histamine, the failure in this case was probably due to the fact that the blood supply to the adrenals had not been cut off.

There are conflicting opinions concerning the action of histamine on movements of the gut in the unanaesthetized intact animal. On one hand Nechoreschew has seen that in dogs with chronic gastric and duodenal fistula subcutaneous injection of this drug or its introduction into the stomach or duodenum influenced the periodic gastric motility. This effect was made manifest

either by a summation of the hunger contractions, or, if histamine was given between the periods of activity by a new set of contractions. Ivy and Vloedmann (1923), on the other hand, were unable to demonstrate that subcutaneous injection of histamine in dogs with Thiry Vella and gastric fistulae produced any change in the movements of the stomach or intestine. The subcutaneous dose of histamine used was in each case the same, i.e. $\frac{1}{4}$ to $\frac{1}{2}$ mg, so that this factor does not account for the divergence of results obtained.

The findings in man are in support of those obtained by Nechoreschew on dogs, namely, that subcutaneous injection of histamine in doses of $\frac{1}{4}$ to $\frac{1}{2}$ mg influences the gastric motility. Thus Tattoni (1928) and Fonseca and Carvalho (1927) found that histamine produced an increased peristalsis and hastened the emptying of the stomach. In these experiments the movements of the gut were observed radioscopically after a baryta meal.

It may be concluded that histamine is a powerful stimulating agent when applied in moderate doses to isolated strips of gut. Furthermore it is effective in producing contractions of isolated loops of intestine when absorbed through the mucosa. In the whole animal intravenous injection of this drug also causes an increase in the movements of the stomach and intestine. There is a divergence of results concerning the effect of subcutaneous injection of histamine, several writers having observed that it had a positive effect on gastric motility, others reporting a negative action. Further experiments on the action of histamine on intestinal motility are reported and discussed in the experimental part of this paper.

## PART II. (EXPERIMENTAL)

### HISTAMINE AND THE SALIVARY GLANDS

The study of the action of histamine on the salivary glands was undertaken for several reasons. In the first place, very little work had been done on this question compared with the investigations performed on the gastric glands and other parts of the alimentary tract. In the literature there were only a few general statements to the effect that histamine activates a salivary secretion (Dale and Laidlaw, 1910; Ackermann and Kutscher, 1910; Fröhlich and Pick, 1912). This action was observed in a whole animal with the nerves of the submaxillary gland intact, as well as after section of the chorda tympani. Since atropine paralyzed the action of histamine, the current view was that this substance acted in the same way as pilocarpine on the nervous mechanism of the gland. No attempt had been made to analyze this question further.

Another reason for choosing the salivary glands for a detailed study of the action of histamine was because of the experimental advantages involved. The well known innervation of these glands, especially the submaxillary, the possibility of controlling the blood flow through this organ as well as of enclosing it in a plethysmograph, afforded the means for a thorough investigation. However, these advantages did not supersede all the difficulties which might be encountered in such an investigation. Although the salivary glands appear to be comparatively simple organs,

in reality they have quite a complicated structure. For example, five groups of histological elements may be differentiated in the submaxillary gland of a cat or dog: (1) the mucous cells, (2) the serous cells of the demilune, (3) the cuboid cells of the intercalary ducts, (4) the rodded epithelium of the intralobular ducts, and (5) the myoepithelial cells. Besides this the microchemical reaction of different groups of cytological elements forming the submaxillary gland are different. Thus Bensley (1928) separates the cytological elements of the salivary glands into homeochrom and tropochrom cells according to the metachromatic staining reaction after formalin fixation.

A. Methods

Cats and dogs were used for the experiments. In most cases they were anaesthetized with a chloroform ether mixture followed by intravenous injection of chloralose, 0.1 g per kg body weight. In a later series of experiments dial "Ciba" was used as an anaesthetic, since it was learned that chloralose had the property of increasing the output of adrenalin (Swale Vincent), which substance is antagonistic to histamine. The dose of dial was .7 g per kg body weight injected intraperitoneally. Some experiments were also performed on decerebrate cats to avoid any anaesthesia during the actual experiment. The chorda and sympathetic nerves were severed, a cannula was inserted in the duct of the submaxillary gland and connected to an electric drop recorder (Gibbs, 1927). The drops registered by this recorder were very small, 45-60 being required to make 1 cc. In some experiments the blood flow was also

measured by Maevsky's method or by Gesell's bloodless method. The method elaborated by Maevsky is as follows. A careful dissection of the gland was made until the vein draining it was discovered; all others leading into the external jugular vein were tied. The blood vessel was dissected down the neck for several inches, ligated in two places and severed between the threads. This prevents loss of blood from the lower part of the vein, and also enables the end near the gland to be pulled out from the surrounding structures. A small cut was then made in this portion, making the introduction of a cannula unnecessary and preventing blood clotting to a certain degree. The falling drops were counted and marked by an electric signal marker.

The doses of histamine phosphate, from 0.25 to 0.5 mg for cats and from 1 to 2 mg for dogs, were injected into the femoral vein, followed by 2 cc. of saline to wash the cannula.

B. General Action of Histamine on the Submaxillary Gland

In the earlier experiments performed on chloralosed cats and dogs, histamine in the above mentioned doses was found to give different results from those obtained by the previous investigators. In these experiments the secretion obtained with histamine was very scanty and sometimes even absent. An investigation was therefore carried out to determine whether narcotics were responsible for inhibiting the histamine action. During the study certain facts were brought to light which showed that there was an antagonism between the action of histamine and adrenalin on the salivary glands. This problem is discussed later in Section G.

In the first stage of this work a large number of experiments was carried out on cats and dogs anaesthetized with chloralose, in which the gland was denervated before the experiment. In over 50 per cent of the experiments on cats histamine, whether injected intravenously, subcutaneously or intramuscularly, had no effect in causing a flow of saliva. In the remainder of the experiments histamine provoked a slight secretion varying from one to three drops. Similar results were observed with dogs, half of the experiments giving a negative effect, while the others produced a secretion ranging from 1 to 12 drops.

Other experiments were performed on cats with only ether and chloroform anaesthesia, to determine if chloralose was responsible for the variable effect obtained above with histamine. It was found that these two narcotics gave the same results as chloralose, and did not increase the effect of histamine on the gland.

To remove the influence of anaesthetics entirely, a few experiments were carried out on decerebrate cats. The results in all cases were positive, although a rather meagre secretion was obtained. Yet the effect of histamine seemed to be more pronounced in the absence of anaesthetics.

Thus histamine in doses of .25 to .5 mg for cats, and from 1 to 2 mg for dogs, under chloralose anaesthesia, causes only in 50 per cent of cases a slight spontaneous secretion. The effect is the same under ether or chloroform anaesthesia but it is increased in the absence of anaesthetics.

As the previous experiments were done with a denervated gland,

the next step was to find out whether or not the integrity of the chorda tympani and sympathetic altered the action of histamine. In attempting to determine this point two types of experiment were performed: one, on an intact gland under chloralose, the nerves being severed later during the experiment without altering the effect of histamine; another, in which cannulae were inserted in the ducts of both glands, one of which was denervated and the other intact. From the results of both classes it is evident that the action of histamine is independent of the integrity of the extrinsic nerves.

Upon repeated injections of histamine the secretory effect of the drug greatly diminishes. Apparently the gland soon becomes refractory, and does not act as at the beginning of the experiment. Therefore in order to activate the same volume of secretion, increasingly larger doses of histamine must be given.

As is well known, on intravenous injection histamine has a powerful depressor action on the blood pressure. This effect did not vary markedly in the different types of experiments, histamine usually causing a marked fall which was sometimes preceded by a slight transitory rise. However, as repeated injections of the drug are given, its effect on the blood pressure gradually decreases. This diminution of the vascular reaction to histamine was in many experiments parallel with the decrease of the secretory effect.

The intravenous injection of histamine also produces a long-continued acceleration of the blood flow through the gland. In the dog this may be followed later by a diminution in the rate of flow. The increase of the blood flow takes place in spite of a

marked fall in blood pressure, and it is usually not greatly affected by successive doses of histamine. The conditions of the blood flow will be discussed later on.

Since it did not appear from these preliminary experiments as if histamine were acting either as a parasympathicomimetic or yet as a sympathicomimetic drug on the submaxillary gland, a special analysis of its mode of action was necessary. The results of this investigation are presented in the following pages.

Methods of Analysing the Action of Histamine. - The action of histamine was investigated from two aspects; as an agent acting on the secretory elements of the gland, and as a drug which may affect the contractile elements of the organ.

In the gastric glands, histamine probably acts on the secretory cells because its action is not abolished by atropine. It may have a similar effect on the salivary cells. Furthermore, we know histamine causes smooth muscle to contract, and there seems to be evidence for the existence of contractile elements in the submaxillary gland. Therefore, histamine may have a mechanical effect pressing out saliva in addition to a secretory action. From these considerations it was of interest to investigate the action of histamine both on the neuro-glandular structure and on the contractile elements of the salivary gland.

Different types of experiments were devised for this purpose. In one series the effect of histamine was tried before the stimulation of the secretory nerves. In another histamine was injected shortly after the gland had been activated by pilocarpine

or by stimulation of the chorda tympani. To obtain still further information about the histological elements on which histamine was acting, different drugs such as ergotamine, atropine, pituitrin and adrenalin were used. Furthermore records of the blood flow through the gland during the histamine stimulation, as well as plethysmographs of the gland have been taken.

Does histamine act on the neuro-glandular elements of the submaxillary gland and influence the subsequent effect of nerve stimulation? - The following method was employed to study the action of histamine, on the response of the gland to excitation of the secretory nerves. The chorda was stimulated for a certain period at intervals of 12 to 14 minutes, to avoid the phenomenon of augmented secretion, so that the normal rate of secretion could be determined. After 12 minutes histamine was injected, and as soon as the blood pressure had approximated its original level, the chorda was again stimulated. The following figures are typical examples of the results obtained throughout this group of experiments.

Table I

| Cat. Chloralose. | Secretion per minute drops | Blood pressure mm. Hg |
|---|---|---|
| 1 hour, 46 minutes: Chorda, coil 13, stimulated for 15 seconds | 20 | 168 |
| 1 hour, 58 minutes: Chorda, coil 13, stimulated for 15 seconds | 19 | 158 |
| 2 hours, 9 minutes: Histamine, $\frac{1}{4}$ mg | 1 | 74-130 |
| 2 hours, 12 minutes: Chorda, coil 13, stimulated for 15 seconds | 18 | 130 |
| 2 hours, 24 minutes: Chorda, coil 13, stimulated for 15 seconds | 18 | 114 |

It is evident from these figures that the chorda secretion is not increased after histamine. As similar experiments with the sympathetic also gave negative results, it may be concluded that histamine does not increase the excitability of the gland, so that a subsequent stimulation of either of the secretory nerves will produce an augmented effect.

Influence of histamine on pilocarpine secretion. - As such a slight reaction was observed from histamine on the resting gland, it was decided to investigate its effect during activity of the organ. Pilocarpine was first given in a dose sufficient to provoke a good secretion during which histamine was injected. Many experiments were done on cats and dogs of which a typical one is quoted below.

Table II.

| Cat. Chloralose. | Secretion per minute | Blood Flow | Blood Pressure |
|---|---|---|---|
| 10 hours, 30 minutes: | | | |
| 1 minute before histamine | 0 | 20 | 138 |
| 1 minute after $\frac{1}{4}$ mg histamine | 0 | 48 | 84-102 |
| 2 minutes after $\frac{1}{4}$ mg histamine | 0 | 38 | 102 |
| 10 hours, 43 minutes: | | | |
| 1 minute before pilocarpine | 0 | 26 | 62 |
| 1 minute after $\frac{1}{4}$ mg pilocarpine | 13 | 26 | 44-64 |
| 2 minutes after $\frac{1}{4}$ mg histamine | 19 | 48 | 64 |
| 10 hours, 46 minutes: | | | |
| 1 minute before histamine | 24 | 48 | 68 |
| 1 minute after $\frac{1}{4}$ mg histamine | 3 | 20 | 50-62 |
| 2 minutes after $\frac{1}{4}$ mg histamine | 16 | 38 | 62 |

A marked inhibition in the secretion is the usual effect of histamine after pilocarpine in the cat. This inhibition is not

dependent on the blood pressure, as the fall after pilocarpine may be as great as that after histamine. It is interesting to note that in a few instances with cats, histamine produced an acceleration of the pilocarpine secretion, but this was observed when the effect of pilocarpine was wearing off. Different results were obtained in the dog, histamine usually giving an acceleration and an inhibition only after large doses of pilocarpine.

An analysis of this peculiar phenomenon showed that the inhibition in secretion was due to a parallel inhibition of the blood flow through the gland, which is exemplified in the above experiment. As histamine by itself usually produces an acceleration of the blood flow, this diminution is thought to be a reversal of function brought about by pilocarpine and will be fully discussed later on. Since pilocarpine apparently interferes with the action of histamine on the blood flow and hence on the secretion, no conclusions regarding the usual effect of histamine can be drawn from these experiments. However, it may be noted that even in the later stages of the histamine action, when the blood flow through the gland returned to normal, there was no summation of the secretory effect of either drug.

The above experiments with the introduction of histamine before the stimulation of the chorda tympani, as well as those in which it was injected after pilocarpine, show that histamine does not raise the excitability of the endings of the secretory nerves or of the neuro-cellular junctions.

Augmented Histamine Secretion. - Quite different results from the above were obtained when histamine was injected after stimulation of the parasympathetic nerve. In an early paper Dale and Laidlaw described an experiment in which profuse secretion of saliva was observed when histamine was injected following stimulation of the chorda tympani. No experiments with the resting salivary gland were performed by these investigators. It should be pointed out that the salivation seen in unanaesthetized animals following histamine administration cannot be taken as evidence of the direct secretagogue action of this drug, since the phenomenon is accompanied by central effects, e.g. nausea and vomiting.

On consideration of the experiments of Dale and Laidlaw it seemed that the secretion was an augmented effect due to the previous stimulation of the secretory nerve. To investigate this part of the problem a large number of experiments, of which a typical one is cited below, were carried out.

Table III

| Cat. Chloralose. | Secretion per minute | Blood Pressure |
|---|---|---|
| Histamine, $\frac{1}{4}$ mg | 0 | 120<br>50-120 |
| 2 hours, 14 minutes<br>Chorda tympani, coil 11, stimulated 30 sec. | 18 | 124 |
| 2 hours, 15 minutes:<br>Histamine, $\frac{1}{4}$ mg<br>1 minute after beginning chorda stimulation | 9 | 124<br>50-120 |
| 2 hours, 40 minutes<br>Chorda tympani, coil 11, stimulated 60 sec. | 33 | 125 |
| 2 hours, 42 minutes:<br>Histamine $\frac{1}{4}$ mg | 9 | 125<br>48-125 |

In this experiment a good secretion from histamine was obtained after chorda stimulation, although histamine alone had no effect. This augmented secretion appears to be proportional to the chorda secretion up to a certain point, beyond which it is independent, e.g. in the experiment presented in Table 3 the augmented secretion in two cases was 9 drops after a chorda secretion of 18 drops and another of 33 drops.

In some of the experiments on cats an augmented secretion was not obtained after chorda stimulation. This may be due either to the presence of chloralose, as it was observed that the augmented effect was more pronounced in the decerebrate preparations, or to poisoning by histamine of the mechanism in the gland responsible for the augmented effect, because the dose of histamine was not sufficiently large. Much better results were obtained with dogs, as the augmented secretion never failed to appear, although as in cats it may vanish after large amounts of histamine are injected, due probably to the paralyzing effect of this drug on the gland.

This augmented secretion with histamine after nerve stimulation seems to be peculiar to the chorda tympani, because a number of similar experiments were carried out with the sympathetic nerve and only negative results were obtained, except in one case after atropine, where histamine following stimulation of the sympathetic produced a very slight flow of saliva.

On consideration of these facts it is noted that histamine injected before stimulation of the chorda does not influence the secretory effect produced by the stimulation. On the other hand, a previous stimulation of this nerve greatly increases the effect

of histamine on salivation.

C. The Vascular Reaction of the Submaxillary Gland to Histamine under Different Conditions

Before discussing the mechanism of the action of histamine on the salivary gland, it is necessary to consider the blood flow through this organ under different experimental conditions. (The methods used in measuring the blood flow are discussed above in section A.)

An injection of histamine in both cats and dogs causes an increase in the blood flow through the salivary gland, as may be seen from experiments A and B (Tables IV and V).

In the cat, after the injection of histamine, there is invariably a long continued acceleration in the blood flow, but in the dog this may later be followed by a diminution in the rate of flow.

The increase of blood flow in drops per minute which takes place after histamine in spite of a marked fall in the blood pressure, varies in different experiments as may be seen from Table VI.

Repeated injections of histamine did not markedly change the reaction of the blood vessels except in one experiment on a dog, when a temporary inhibition of the blood flow was caused after several doses of histamine.

Atropine in a dose sufficiently great to paralyze the secretory action of the chorda tympani does not affect the change in blood flow through the gland caused by histamine. For example

see experiment C (Table VII).

The action of histamine after atropine is independent of the blood pressure, because the fall after each injection was practically the same, being 60 per cent of the previous level.

Table IV

| Experiment A. Cat | Blood Flow in drops per minute |
|---|---|
| Before injection of histamine | 5 |
| After injection of 0.25 mg histamine | 7 |
| Second minute | 12 |
| Third minute | 10 |
| Fourth minute | 8 |

Table V

| Experiment B. Dog | Blood Flow in drops per minute |
|---|---|
| Before injection of histamine | 29 |
| After injection of 1 mg histamine | 69 |
| Second minute | 69 |
| Third minute | 43 |
| Fourth minute | 39 |

Table VI

| | Dog | | | Cat | | |
|---|---|---|---|---|---|---|
| | Experiments | | | Experiments | | |
| | a | b | c | d | e | f |
| Before histamine | 6 | 20 | 21 | 43 | 10 | 19 |
| After histamine | 15 | 48 | 35 | 56 | 79 | 62 |

Table VII

| Experiment C. Dog, 28 kg | Blood Flow in drops per minute |
|---|---|
| Before histamine | 43 |
| After histamine 1 mg | 56 |
| Atropine 12 mg, chorda paralyzed | |
| Before histamine | 77 |
| After histamine 1 mg | 116 |

Slightly different conditions were observed when the chorda tympani was stimulated before the injection of histamine (Babkin and MacKay, 1930). In these experiments stimulation of the chorda gave as usual a marked increase in the blood flow through the gland. Injection of $\frac{1}{4}$ mg of histamine 1 minute, 30 seconds after the beginning of the stimulation of the secretory nerve, when the chorda secretion had stopped completely, gave an additional increase in the blood flow through the gland. This increase was, however, insignificant and of short duration. It lasted about 10 seconds and was quickly replaced by an inhibition of the blood flow. This inhibition coincided with the maximal fall of the blood pressure. When, however, the latter returned to its normal level, the inhibition was replaced by a moderate acceleration. This effect of histamine was the same whether or not the capsule covering the gland was intact.

The Influence of Pilocarpine. - The vascular reaction of the pilocarpinized submaxillary gland is quite different from that of the unactivated gland. Instead of causing an acceleration of the blood flow, under these conditions histamine produces the reverse

effect, namely an inhibition of the blood flow.

There are several indications in the literature that under certain conditions the action of the chorda tympani or of pilocarpine on the blood vessels of the gland may be reversed. However, different opinions are expressed concerning the explanation of this effect. Frŏhlich and Loewi (1906) suppose that the chorda contains two kinds of fibres, vaso-dilators and vaso-constrictors, of which the former greatly preponderate in number. The action of the vaso-constrictors could be demonstrated only on an animal under the influence of amyl nitrite or sodium nitrite, both of which give a nearly maximal dilatation of the blood vessels. Under these conditions, stimulation of the chorda or injection of pilocarpine produces an inhibition in the blood flow through excitation of the vaso-constrictor fibres. Atropine in doses of 1 mg paralyzes this action. Bayliss (1908) attempted to repeat these experiments of Frŏhlich and Loewi, but he could not confirm their results. Maevsky (1922), however, showed that the chorda tympani might have a vaso-constrictor influence in certain stages of pilocarpine poisoning, and that atropine in doses sufficient to paralyze the secretory action of pilocarpine restored the vaso-dilator action of the chorda. The explanation offered by Maevsky is that pilocarpine reverses the action of the chorda, rather than that this nerve contains two distinct kinds of fibres.

The subsequent experiments on a cat and a dog show that the usual effect after histamine, i.e. an increase in the blood flow, is reversed following previous injection of pilocarpine.

Table VIII

| Experiment D. Cat | Saliva per minute | Blood Flow per minute | Blood pressure |
|---|---|---|---|
| Time: 3 hours, 40 minutes | | | |
| 1 minute before histamine | 0 | 10 | 162 |
| 1 minute after 0.25 mg histamine | 0 | 79 | 111-114 |
| 3 hours, 49 minutes | | | |
| 1 minute before pilocarpine | 0 | 24 | 126 |
| 1 minute after 0.25 mg pilocarpine | 32 | 32 | 82-150 |
| 3 hours, 50 minutes | | | |
| 1 minute before histamine | 39 | 39 | 152 |
| 1 minute after 0.25 mg histamine | 22 | 16 | 98-119 |

It is evident from experiment D (Table VIII) that the action of histamine after pilocarpine is quite different from the usual effect produced by histamine alone. This experiment is merely quoted as an example from a large number of similar cases.

In all experiments where blood pressure is noted, the first figure alone represents the blood pressure in millimeters of Hg just before the intravenous injection of different substances. The first figure when two figures are together represents the lowest fall of blood pressure immediately after the injection. The second is the average of the blood pressure at the end of the first and second minutes after the injection. The figures of the salivary secretion and the blood flow refer to the minute before and the minute after an injection of the drug.

From the figures in experiment D, it is seen that when histamine is injected, after the gland is poisoned with pilocarpine, it gives an inhibition of the blood flow accompanied by an inhibition of the secretion. This may be termed a reversal effect

of histamine following pilocarpine.

In a dog, on the other hand, the usual effect of histamine after pilocarpine is different from that found in the cat (Table IX).

Table IX

| Experiment E. Dog, 26 kg | Secretion per minute | Blood Flow per minute | Blood pressure |
|---|---|---|---|
| Time: 2 hours, 12 minutes | | | |
| 1 minute before histamine | 0 | 29 | 248 |
| 1 minute after 1 mg histamine | 0 | 69 | 162-226 |
| Time: 2 hours, 40 minutes | | | |
| 1 minute before pilocarpine | 0 | 52 | 230 |
| 1 minute after 1 mg pilocarpine | 3 | 75 | 144-226 |
| Time: 2 hours, 41 min., 30 sec. | | | |
| 1 minute before histamine | 6 | 56 | 226 |
| 1 minute after 1 mg histamine | 26 | 76 | 148-209 |
| 3.5 mg pilocarpine injected at intervals | | | |
| Time: 3 hours, 45 minutes | | | |
| 1 minute before histamine | 6 | 38 | 234 |
| 1 minute after 1 mg histamine | 5 | 24 | 158-220 |

As one may observe from these figures taken from experiment E, it is possible to obtain the reversal action of pilocarpine in the dog, but this is much more difficult than in the cat, requiring large doses of pilocarpine which almost paralyze the secretory action of the chorda. Appropriate amounts of histamine are also necessary in order to obtain a diminution of the blood flow.

Explanation of the Reverse Action of Histamine after Pilocarpine. - In an attempt to understand the mechanism underlying the observed results it is necessary to consider certain factors, which may play a part in the phenomenon of the reversal action

following pilocarpine.

Subsequent to the injection of both histamine and pilocarpine, there is a marked fall in blood pressure. If this fall were greater after histamine following pilocarpine than after pilocarpine alone, the result might be an inhibition in the blood flow. The reversal effect, however, in many experiments, can take place independently of the fall in blood pressure. Experiment F (Table X) is quoted as an example.

Table X

| Experiment F. Cat, 2.4 kg | Secretion per minute | Blood Flow per minute | Blood pressure |
|---|---|---|---|
| 1 minute before pilocarpine | 0 | 10 | 100 |
| 1 minute after 0.25 mg pilocarpine | 8 | 43 | 65 returned to 115 |
| 1 minute before histamine | 8 | 58 | 120 |
| 1 minute after histamine | 4 | 13 | 80 returned to 100 |

In this experiment and in similar ones on dogs, e.g. see experiment E, the inhibition observed is not due merely to the decrease in blood pressure, because there is a more marked fall after pilocarpine alone, than after histamine following pilocarpine.

The explanation may be advanced that the reversal action is caused by certain mechanical conditions of circulation existing in the gland, caused by the relative state of tonus of different parts of the blood vessels and the level of the blood pressure.

Dale and Richards (1918) and Dale and Burn (1926) have shown that histamine in the cat dilates the capillaries and constricts the arterioles, at the same time producing a fall in the blood

pressure. It has a similar action in the dog except that instead of constricting the arterioles it dilates them. The difference between the two types of animals is that histamine exerts its dilator effect at different levels of the arterial tree, beginning its action between the arterioles and capillaries in the cat, and above the arterioles in the dog.

Pilocarpine also produces an increased blood flow through the gland analogous to that produced by stimulation of the chorda. If pilocarpine caused a maximum dilatation of the glandular blood vessels, then the following injection of histamine would have no further dilatory effect on the capillaries, but at the same time would cause a constriction of the arterioles. This constriction together with the fall in the general blood pressure after the injection of histamine, might be responsible for the inhibition in the blood flow.

This supposition appears rather unlikely as histamine may have its inhibitory effect, when the previous dilatation caused by pilocarpine is far from maximal. In experiment D (Table VIII) previously quoted, histamine caused an increase in flow from 10 to 79 drops per minute, while the next injection of pilocarpine only gave an increase from 24 to 32 drops per minute. These results give the impression that it is not merely the changed mechanical condition of circulation in the submaxillary gland that is responsible for the reversal effect.

An objection could be raised to the previous statement, "that the dilatation caused by pilocarpine is far from maximal",

when one considers the secretion of saliva as well as the blood flow. Thus after histamine there was an increase in blood flow from 10 to 79 drops with no secretion, whereas after pilocarpine there was an increase from 24 to 32 drops in blood flow and at the same time a secretion of 32 drops. If one considers that the saliva came from the blood the total blood flow in this case might be regarded as 64 drops. Thus the supposition could be advanced that the diminished blood flow through the pilocarpinized submaxillary gland after histamine was merely due to a large part of the fluid passing from the blood into the saliva, thereby diminishing the output from the glandular vein. However, there is no justification for such an assumption, since the secretion in the pilocarpinized gland was diminished, along with the blood flow, after the injection of histamine.

Effect of Atropine. - Since the inhibition of blood flow and secretion, after histamine following pilocarpine, depends to a certain extent on the dose of the latter drug, a possible explanation of this phenomenon is that it is a true reversal effect due to poisoning by pilocarpine. Reasoning from this supposition, atropine was injected in a dose sufficient to abolish the secretory action of pilocarpine, to find out whether the vasodilator properties of histamine would be restored. As an example, the figures in Table XI were taken from one of the experiments.

In this experiment the blood pressure was low, but in the other similar experiments, the same effect was obtained with a higher level of the blood pressure. The results from this group

of experiments are as follows: when atropine is injected in a dose sufficient to paralyze the effect of its antagonist pilocarpine, histamine loses its action of diminishing the blood flow through the gland and frequently regains its vaso-dilator properties, as seen in experiment G (Table XI). Subsequent large doses of pilocarpine again restore to histamine its property to diminish the blood flow through the gland. This abolition and restoration of the reversal effect after histamine following pilocarpine may sometimes be repeated in the same animal. But in other experiments after pilocarpine, atropine and histamine have been injected, the chorda loses not only its secretory action but also its influence on the blood vessels.

Table XI

| Experiment G. Cat | Secretion per minute | Blood Flow per minute | Blood pressure |
|---|---|---|---|
| Time: 10 hours, 30 minutes | | | |
| 1 minute before histamine | 0 | 20 | 138 |
| 1 minute after 0.25 mg histamine | 0 | 48 | 84-98 |
| 2 minutes " " " " | 0 | 38 | 98 |
| 3 minutes " " " " | 0 | 32 | 98 |
| 4 minutes " " " " | 0 | 24 | 90 |
| Time: 10 hours, 43 minutes | | | |
| 1 minute before pilocarpine | 2 | 26 | 62 |
| 1 minute after 0.25 mg pilocarpine | 13 | 26 | 44-59 |
| 2 minutes " " " " | 19 | 48 | 59 |
| Time: 10 hours, 46 minutes | | | |
| 1 minute before histamine | 24 | 48 | 68 |
| 1 minute after 0.25 mg histamine | 3 | 20 | 50 returned to 60 |
| 2 minutes " " " " | 16 | 38 | 59 |
| Two doses atropine (0.25 mg each) were injected | | | |
| Time: 10 hours, 54 minutes | | | |
| 1 minute before histamine | 0 | 24 | 60 |
| 1 minute after 0.25 mg histamine | 0 | 33 | 50 returned to 68 |
| 2 minutes " " " " | 0 | 30 | |

(See Figures 1, 2, and 3 for an example of the effect of histamine alone, histamine after pilocarpine and histamine after atropine on the blood flow and salivary secretion.)

No special investigations were carried out to determine the true mechanism of the reversal effect of pilocarpine. Therefore only a tentative explanation may be offered here. It seems, however, as previously stated, that the mechanical conditions of circulation in the gland cannot play a part in this reversal effect.

One possible explanation of the phenomena may be that which was given by Fröhlich and L oewi, that is, that the chorda tympani contains both vaso-constrictor and vaso-dilator fibres. In analogy with these findings, it may be supposed in my experiments that pilocarpine paralyzes the vaso-dilator fibres of the chorda, so that histamine can act only on the vaso-constrictor fibres. Fröhlich and Loewi think that the diminution in the blood flow after the stimulation of the chorda or the injection of pilocarpine in the case where nitrite was given before, is proof of the existence of vaso-constrictor fibres in the chorda. Their explanation depends on the supposition that the vaso-dilator fibres must be in a state of nearly maximal excitation due to the nitrite, before the inhibition caused by the constrictor fibres can be produced. But in my experiments on cats, both small and large doses of pilocarpine produced the reversal effect. In the cases where small doses are given, there cannot be maximal excitation of the vaso-dilator fibres. This is also supported to a certain degree by the fact that the blood flow through the gland after pilocarpine is not always maximal.

The second possible explanation is that the phenomenon is a

true reversal of function brought about by pilocarpine. We do not know what is the mechanism of this reversal function, when the same stimulus will produce an opposite reaction, but the data in this paper do not exclude this possibility.

There are a number of similar instances of reversed action cited in the literature, of which two examples have a bearing on this paper. Dale (1906) found that stimulation of the sympathetic nerve after ergotoxine gave vaso-dilatation instead of vaso-constriction. He is inclined to think that there are two kinds of fibres in the sympathetic, and that the vaso-constrictors are paralyzed by this drug. Dale and Laidlaw (1911) have also found that after cytisine the stimulation of the chorda tympani produces an inhibition in the salivary secretion.

D. Analysis of the Action of Histamine on the Submaxillary Gland

Babkin and McLarren (1927), who investigated the augmented secretion from the sympathetic after chorda stimulation, advance the view that it has two phases. These are: a mechanical phase, due to the action of motor fibres in the sympathetic nerve supplying the contractile elements of the gland, and a secretory phase, which is due to an increased secretory response of the gland after stimulatio of the parasympathetic nerve. There is reason to believe that there may be two such phases of the augmented secretory effect, which histamine produces after previous stimulation of the chorda tympani.

In the first place, the action of histamine on the motor mechanism is to be considered. Experiments were done on cats and

dogs in which saliva was blown back into the gland and then histamine was injected into the blood. The resultant effect was a forcing out of the fluid, probably caused by the action of histamine on the contractile elements of the gland. (See Table XII.)

Table XII

| Dog, 7 k | Secretion per minute drops | Divisions | Blood pressure |
|---|---|---|---|
| 1 minute before histamine | 0 | Not recorded | 146 |
| 1 minute after $\frac{1}{4}$ mg histamine | 10 | Not recorded | 44-176 |
| 2 minutes after $\frac{1}{4}$ mg histamine | 2 | Not recorded | 176 |
| 3 minutes after $\frac{1}{4}$ mg histamine | 1 | Not recorded | 176 |
| Saliva blown in | | 70 divisions | |
| 1 minute before histamine | - | - | 120 |
| 1 minute after $\frac{1}{4}$ mg histamine | 21 | 45 | 40-80 |
| 2 minutes after $\frac{1}{4}$ mg histamine | 5 | 4 | 80 |
| 3 minutes after $\frac{1}{4}$ mg histamine | 2 | 4 | 98 |

In this experiment a glass tube was inserted between the cannula and the drop recorder, so that the movement of saliva could be measured on the scale as well as by the drop recorder. The divisions noted above refer to the movement of saliva on the scale per minute, while the drops are those marked by the recorder during the same time. If the amount of spontaneous secretion with histamine is subtracted from the amount of saliva obtained with with histamine, after fluid is blown into the gland, it will be seen that there is an excess of 15 drops.

Another form of experiment furnishes evidence that histamine stimulates a certain pressor mechanism in the gland. In a dog the

salivary cannula was connected with graduated tubing and a mercury manometer. By raising the pressure in the manometer, the saliva could be forced back into the gland. When the backward movements of the fluid stopped, histamine was injected intravenously and a pressing out of the content of the gland was noted. The greatest amount of saliva was pressed out in the first 15 seconds after histamine (+), then gradually diminished, after which the saliva passed back into the gland (—). Actual figures of this experiment are as follows (Table XIII):

Table XIII

| Dog | Divisions |
|---|---|
| Massage | 5 |
| Pressed back into gland | 78 |
| Histamine 1 mg | Saliva in divisions every 15 seconds: +9, +6, +1, 0, -1, -1, -3, -2, -2, -2, -2, -1, -1, -1. |

The pushing out of saliva was obtained in the cat but not to such a marked extent as in the dog experiments similar to the above, the reason apparently being that the motor mechanism is more easily paralyzed by histamine in the cat.

True augmented secretion with histamine. - The data show that the whole phenomenon of the augmented secretion with histamine cannot be explained by mechanical effects only. On account of this

the second phase, namely, a true augmented secretion, must also be considered. Such an effect was demonstrated in cats and dogs, where the histamine secretion after the chorda was greater than the actual chorda secretion itself (see Table XIV).

Table XIV

| Cat, 4.1 k. Chloralose. | Secretion per minute | Blood pressure |
|---|---|---|
| 11 hours, 26 minutes: | | |
| 1 minute before histamine | 0 | 120 |
| 1 minute after $\frac{1}{4}$ mg histamine | 1 | 88-105 |
| 11 hours, 30 minutes: | | |
| Chorda, coil 14 for 30 seconds | 27 | |
| 11 hours, 31 minutes: | | |
| 1 minute before histamine | 0 | 134 |
| 1 minute after $\frac{1}{4}$ mg histamine | 22 | 99-112 |
| 2 minutes after $\frac{1}{4}$ mg histamine | 6 | 112 |
| 3 minutes after $\frac{1}{4}$ mg histamine | 2 | 114 |
| 4 minutes after $\frac{1}{4}$ mg histamine | 1 | |

Further evidence for the secretory action of histamine was gained from the following experiment on a dog. The last injection of histamine gave 7 divisions of saliva, but by subsequent massage of the gland 19 divisions were obtained. After 13 minutes, when the possible effect of the previous injection of histamine had worn off, 53 divisions were blown back into the gland; an injection of histamine then gave 29 divisions and a subsequent massage 20. Now the gland was massaged till no more saliva could be pushed out. Histamine was then injected and gave only 1 division. During massage following the histamine, 13 divisions were pressed out. The figures are tabulated in Table XV.

Table XV

| Dog | Secretion Divisions per minute |
|---|---|
| 3 hours, 1 minute: | |
| Histamine | 7 |
| Massage | 19 |
| Saliva blown in | 53 |
| 3 hours, 14 minutes: | |
| Histamine, 1 mg | 29 |
| Massage | 20 |
| Massage | 1 |
| 3 hours, 26 minutes: | |
| Histamine | 1 |
| Massage | 13 |

The probable explanation of this experiment is that the amount of saliva secreted under the third injection of histamine filled only the ampulla and ducts of the gland, but was not enough to appear in the main duct.

Gradual diminution of the augmented histamine secretion. - The augmented effect with histamine following chorda stimulation may last for 10 to 12 minutes, although its effect gradually diminishes during that time, as occurred in the following experiment, which could be repeated several times on the same animal (Table XVI).

The length of time the augmented secretion with histamine lasts corresponds to the time of the raised excitability of the gland following chorda stimulation.

A possible explanation of the "die away" effect with successive doses of histamine following chorda stimulation is that the stimulation produces a condition of raised excitability which

gradually diminishes and wears off in 10 or 12 minutes. During this period of raised excitability histamine is able to cause a true secretion, but as the excitability diminishes, the action of histamine also becomes less. As the figures show, the effect must be more than a pressing out, because the augmented effect is at first greater than that due to the chorda stimulation.

Table XVI

| Cat | Secretion per minute | Blood pressure |
|---|---|---|
| Before histamine | 0 | 32 |
| 1 minute after $\frac{1}{4}$ mg histamine | 0 | |
| 12 hours, 34 minutes: | | |
| Chorda, coil 14, stimulated for 30 seconds | 6 | |
| 12 hours, 35 minutes: | | |
| Before histamine | 0 | 32 |
| 1 minute after 1 mg histamine | 9 | 46-40 |
| 2 minutes after 1 mg histamine | 3 | 40 |
| 3 minutes after 1 mg histamine | 1 | 40 |
| 12 hours, 39 minutes: | | |
| 1 minute before histamine | 0 | 42 |
| 1 minute after 1 mg histamine | 7 | 54-50 |
| 2 minutes after 1 mg histamine | 1 | 50 |
| 12 hours, 40 minutes: | | |
| 1 minute before histamine | 0 | 42 |
| 1 minute after 1 mg histamine | 4 | 54-50 |
| 12 hours, 44 minutes: | | |
| 1 minute before histamine | 0 | Clot |
| 1 minute after 1 mg histamine | 2 | |

From the results reported above one may see that histamine has a twofold action on the submaxillary gland; it stimulates the contractile elements and activates the neuro-glandular apparatus of

the organ. A previous stimulation of the chorda tympani greatly facilitates the secretory action of histamine, so that the results of Dale and Laidlaw, who obtained a good secretion from histamine injected intravenously following chorda stimulation, are probably due to this phenomenon.

Action of Atropine. - There are some differences of opinion concerning the action of atropine on the histamine salivary secretion. Dale and Laidlaw (1910) reported that this drug completely abolished the effect of histamine. However, in my investigation different results have been obtained; in the majority of experiments atropine only diminished the action of histamine, although in a few cases it abolished it. The evidence obtained from these experiments does not warrant the conclusion that the two drugs are completely antagonistic to each other, although in a few cases atropine completely abolished the action of histamine. Thus in Table XVIII an example may be seen of the action of histamine after paralysis of the chorda tympani. It should be pointed out that in these experiments the chorda was stimulated just before the injection of atropine, so that previous to the administration of histamine the gland was filled with saliva. In another type of experiment (MacKay, 1927, 1929) after paralysis of the secretory nerve by atropine saliva was blown back into the gland and histamine administered. Especially good results were obtained with the latter drug in this type of experiment. The following explanation of the fact that the histamine action is diminished but not abolished by

atropine may be offered.

As stated above histamine is considered to have a twofold action on the submaxillary gland activated by previous stimulation of the chorda: (1) the pressing out effect, (2) the true secretion, which is increased by previous stimulation of the parasympathetic nerve. From the experimental evidence it appears that atropine affects slightly or not at all, the pressor mechanism of the salivary gland which is activated by histamine. On the other hand, this drug abolishes the favorable conditions, created by previous nerve stimulation, for the action of histamine. In this way the true augmented secretion is abolished and the net effect of histamine diminished. Besides this it appears that atropine produces a general poisoning of the gland cells. This is evinced by the fact that in addition to paralyzing the chorda this drug not infrequently diminishes the action of the sympathetic as well. As it has been supposed that one phase of the histamine action is directly on the secreting cells, the diminution of this action after atropine may be explained by the fact that these cells are poisoned by the drug. These findings, namely, that atropine diminishes but does not abolish the histamine action on the salivary gland, have been supported by Dr. Stavrakis in our laboratory (work not yet published). By using much larger doses of histamine, 2 to 5 mg, he could see quite definitely that atropine did not abolish the "pressor" effect of histamine on the salivary gland, whereas the secretory action of this substance was greatly diminished.

E. The Relation of Histamine to the Sympathetic Innervation of the Submaxillary Gland.

The question next arose as to whether histamine would act on the submaxillary gland after the sympathetic nerve was paralyzed by ergotamine. A series of experiments investigating this problem have been performed and are discussed below.

The augmented secretion, obtained when the sympathetic nerve is stimulated after the chorda, has been chown to be due partly to the action of motor fibres and to a lesser extent to the secretory fibres. Since there is a similarity between the twofold action of the sympathetic and that of histamine, it was considered possible that both might be acting on the same mechanism in the gland. Therefore it was important to study the histamine salivary secretion after the sympathetic nerve had been paralyzed with ergotamine.

Ergotamine methasulphonate, Sandoz preparation, was used for these experiments. The salt was dissolved in distilled water just before using and was injected intravenously. Repeated injections of small doses ($\frac{1}{8}$ to $\frac{1}{4}$ mg in a cat, $\frac{1}{2}$ mg in a dog) did not prove as effective as a single injection of a bigger dose (4 to 5 mg per kg body weight).

The sensitivity of different parts of the sympathetic nervous system to ergotamine was found to vary considerably. By stimulating the peripheral end of the cervical sympathetic, following the injection of this drug, the first noticeable effect was the disappearance of the sympathetic salivary secretion; later the vaso-constrictor action was abolished and finally the dilatation of the pupil. These facts are in accordance with the observations of Anrep (1922), who

could see that ergotamine first paralyzed the motor fibres for the submaxillary gland and later the vaso-constrictor fibres. When the ergotamine effect was fully developed, it was found that adrenalin (1 cc. 1/10,000) injected intravenously did not have its usual pressor or secretory effect and in some instances even produced a slight fall of blood pressure.

In all cases the animals were anaesthetized with chloralose (0.1 g per kilo weight); a cannula was then inserted into the submaxillary duct and connected to a Gibbs' drop recorder measuring 46 drops in 1 cc. The chorda lingual and cervical sympathetic nerves were cut and blood pressure recorded from the carotid artery. In each experiment a fresh solution of histamine (acid phosphate) was used. It should be noted here that the response of the salivary glands to histamine was not always uniform in different animals. A special investigation of some of the factors underlying this individual variability will be reported later.on.

The results obtained with ergotamine definitely show that paralysis of the sympathetic nervous system does not abolish the augmented histamine secretion obtained after stimulation of the chorda. In some experiments ergotamine had no effect on the quantity of saliva secreted after histamine but in others a diminution in secretion was observed. This diminution might be partly attributed to a decreased excitability of the gland in general during the course of the experiment.

The following experiment is quoted as an example (see Table XVII). In all the tables the length of the time of stimulation of the nerve and the distance between the primary and secondary induction coils in centimetres are given. (See also Figure 4.)

Table XVII

November 14, 1928. Cat, 2.65 kg. Chloralose.

| Time | Procedure | Saliva | Remarks |
|---|---|---|---|
| | | drops | |
| 11 hrs. 3' | Sympathetic for 30", coil 11 | 3 | |
| 11 hrs. 9' | Chorda for 30", coil 12 | 28 | |
| 11 hrs. 10' | Sympathetic for 30", coil 11 | 14 | |
| 11 hrs. 22' | Chorda for 30", coil 12 | 27 | |
| 11 hrs. 23' | Histamine $\frac{1}{4}$mg | 8 | Fall of blood pressure, returned in 4' to normal. |
| 11 hrs. 37' | Ergotamine 10 mg | 0 | Slight rise and marked fall in blood pressure for 10', never returned to normal. |
| 11 hrs. 45' | Chorda for 30", coil 12 | 12 | |
| 11 hrs. 46' | Sympathetic for 30", coil 10 | 0 | No dilatation of pupil. No rise in blood pressure. |
| 11 hrs. 49' | Chorda for 30", coil 12 | 13 | |
| 11 hrs. 50' | Histamine $\frac{1}{4}$ mg | 4 | Small fall in blood pressure. |
| 12 hrs. 5' | Suprarenals excised | 0 | |
| 12 hrs. 14' | Sympathetic for 30", coil 10 | 0 | No dilatation of pupil. No rise in blood pressure. |
| 12 hrs. 16' | Chorda for 30", coil 10.5 | 11 | |
| 12 hrs. 17' | Adrenalin 1 cc. 1/10,000 | 0 | No rise in blood pressure. |
| 12 hrs. 18' | Histamine $\frac{1}{2}$ mg | 4 | Slight fall in blood pressure. |

One may see from the above table that after complete paralysis of the sympathetic and adrenalin actions, histamine still produced its typical effect. A dose of atropine sufficient to paralyze the secretory action of the chorda tympani on an ergotamized cat diminished but did not quite abolish the histamine action. An example is shown in the experiment below (see Table XVIII).

Table XVIII

November 5, 1928. Cat, 2.5 kg. Chloralose.

| Time | Procedure | Saliva | Remarks |
|---|---|---|---|
| | | Drops | |
| | Ergotamine 6 mg previously injected. | | |
| 1 hr. 30' | Chorda for 30", coil 12 | 17 | |
| 1 hr. 31' | Sympathetic for 30", coil 9 | 0 | No dilatation of the pupil. |
| 1 hr. 32' | Histamine $\frac{1}{4}$ mg | 3 | |
| 1 hr. 41' | Ergotamine 2 mg | 0 | Fall in blood pressure. |
| 1 hr. 47' | Sympathetic for 30", coil 9 | 0 | Slight dilatation of pupil. |
| 2 hrs. 0' | Chorda for 30", coil 11 | 18 | |
| 2 hrs. 1' | Atropine $\frac{1}{4}$ mg | 0 | |
| 2 hrs. 2' | Chorda for 30", coil 11 | 0 | |
| 2 hrs. 3' | Histamine $\frac{1}{4}$ mg | 1 | |
| 2 hrs. 6' | Chorda for 30", coil 11 | 0 | |
| 2 hrs. 7' | Sympathetic for 30", coil 9 | 0 | Very slow and slight dilatation of pupil. |
| 2 hrs. 11' | Saliva blown back into the | duct | |
| 2 hrs. 12' | Histamine $\frac{3}{8}$ mg | 2 | |

Thus the pressor effect of histamine on the salivary gland is maintained practically unchanged after paralysis of the sympathetic nervous system. On the other hand, as previously mentioned, paralysis of the parasympathetic diminishes, though it does not abolish the action of this drug.

As some of the contractile elements of the gland are under the control of the sympathetic, it is assumed that histamine after ergotamine must be acting on these elements at a point peripheral to the endings of the nerve. Since the action of adrenaline is also abolished by ergotamine (see Table XVII), according to the current view of the relations between a nerve and a cell, histamine must act at a point peripheral to the neuro-cellular junction, i.e. on the cells themselves.

F. The Type of Contractile Elements in the Submaxillary Gland activated by Histamine

Since histamine has been shown to have a double effect on the submaxillary gland of both dogs and cats, producing a true secretion as well as causing a pressing out of saliva, the next step was to investigate the nature of the contractile mechanism of the gland concerned in the latter process.

Pituitrin. - It was first important to determine whether this contractile mechanism was of a muscular nature. A comparison was therefore made between the action of histamine and pituitrin, the latter of which produces a contraction of all kinds of smooth muscle. The effect of pituitrin on salivary secretion was very small and

could not be compared with the action of histamine. Neither by itself nor after stimulation of the chorda tympani did pituitrin give any noticeable effect, hardly one drop of secretion being pressed through the drop recorder. An example of the striking difference between the action of histamine and pituitrin can be seen in the following experiment (see Table XIX). The same results were obtained in analogous experiments on dogs.

Table XIX

December 10, 1928. Cat. Decerebrate.

| Time | Procedure | Saliva | Remarks |
|---|---|---|---|
| | | drops | |
| 11 hrs. 52' | Chorda for 30", coil 16 | 29 | |
| 11 hrs. 53' | Histamine $\frac{1}{4}$ mg | 14 | Marked fall in blood pressure. |
| 12 hrs. 7' | Chorda for 30", coil 16 | 28 | |
| 12 hrs. 8' | Pituitrin 1 cc. ($2\frac{1}{2}$ units Burroughs Wellcome pituitary extract) | 1 | Rise in blood pressure |

In another form of experiment the chorda was rhythmically stimulated and an even flow of saliva produced. Histamine and pituitrin were injected during this secretion. Again the result was quite different for these two substances, as may be seen from the experiment below (Table XX).

Table XX

December 6, 1928. Cat, 2.5 kg. Chloralose.

| Time | Procedure | Rate of Salivary Secretion every 30" in drops | Remarks |
|---|---|---|---|
| 12 hrs. 31' | Rhythmic stimulation of chorda, coil 21 | 30" before injection of pituitrin 4 | |
| 12 hrs.31'30" | Pituitrin ½ cc. (1¼ units Burroughs Wellcome pituitary extract) | 30" after injection of pituitrin 2 | Rise in blood pressure |
| | | 1' after injection of pituitrin 0 | |
| | | 1'30" after injection of pituitrin 2 | |
| 1 hr. 14' | Rhythmic stimulation of chorda, coil 18.5 | 30" before injection of histamine 5 | |
| 1 hr.14'30" | Histamine ¼ mg | 30" after injection of histamine 4 | Fall in blood pressure |
| | | 1' after injection of histamine 5 | |
| | | 1'30" after injection of histamine 4 | |

There is quite a marked inhibition of the salivary flow following the injection of pituitrin and a very insignificant one after histamine. This last is probably due to the fall of blood pressure and diminished supply of blood to the gland. Only in the case of a very great fall in the blood pressure after a larger dose of

histamine ($\frac{1}{2}$ mg) was there a short inhibition of the secretion induced by a rhythmic stimulation of the chorda.

The probable explanation of the inhibitory effect of pituitrin on the salivary flow, induced by a rhythmic stimulation of the chorda tympani, is that it is due to a constriction of the glandular blood vessels. Special experiments on dogs were performed to clear up this problem. These animals, anaesthetized with chloralose, were prepared as usual and in addition to the secretion the blood flow through the gland was recorded. For this purpose Gesell's (1924) blood volume flow recorder was used.

An example may be cited from one of several analogous experiments. The rate of the blood flow was equal to six strokes of Gesell's apparatus in thirty seconds. Stimulation of the chorda for fifteen seconds gave a profuse flow of saliva and increased the blood flow from six to forty-three strokes in thirty seconds. Injection of $\frac{1}{2}$ cc. pituitrin gave one drop of saliva and altogether stopped the circulation through the gland for nine and a half minutes. A subsequent stimulation of the chorda only increased the rate of blood flow from one stroke in thirty seconds to sixteen strokes, and the secretory effect was much less than that observed before the injection of pituitrin. A second stimulation of this nerve still did not give the full amount of secretion and vaso-dilatation. It was only after the third stimulation of the chorda that the secretory and vaso-dilator effects were practically equal to those obtained before the injection of pituitrin. Thus though pituitrin acts very powerfully on the blood vessels it failed to press out saliva from

the gland.

Kogan, Ponirovski and Raiski (1925) found that the intravenous injection of pituitrin caused an inhibition of the secretion activated by pilocarpine in a dog with a permanent fistula of the mixed salivary gland. This diminution of the salivary flow was very prolonged, lasting for twenty-five minutes after 1 cc. pituitrin. The conclusion reached by these workers was that these two substances, pilocarpine and pituitrin, in so far as their effect on the salivary secretion was concerned, were antagonistic to each other. But the experiment quoted above, in which the blood flow through the gland was measured, shows that in discussing the antagonism between pilocarpine and pituitrin the circulation in the gland must be an important factor. In the experiments of Kogan, Ponirovski and Raiski the inhibition of the pilocarpine secretion produced by pituitrin could be due, not to the secretory antagonism between the two substances, but to a long continued shortage of blood supply to the gland.

Adrenalin. - Since adrenalin has the same effect on the salivary glands as sympathetic stimulation, a few experiments were performed with this substance and its action was compared with that of pituitrin.

A dose of adrenalin may be found which by itself is not large enough to activate the secretion of saliva but which will, after stimulation of the chorda, give an augmented effect. However, in considering this augmented effect it must not be thought of as a pure "pressing out," the possibility of a true augmented secretion

being denied. Some experiments indicated that repeated injections of a small dose of adrenalin gave an augmented secretion without previous stimulation of the chorda. While subminimal doses of adrenalin may produce a secretion in an activated gland, it was found that pituitrin, under the same conditions, had practically no effect. The rise of blood pressure after administration of the two substances was approximately equal. (See Table XXI.)

Table XXI

January 20, 1928. Dog. Chloralose.

| Time | Procedure | Saliva | Blood Flow | Blood Pressure |
|---|---|---|---|---|
| | | drops | | mm. Hg |
| 12 hrs. 21' | Adrenalin 1 cc. 1/10,000 | 0 | Inhibition | Rise from 130 to 200 |
| 12 hrs. 27' | Sympathetic for 15", coil 12 | 14 | Marked inhibition | No change |
| 1 hr. 27' | Chorda for 15", coil 15 | 113* | Marked acceleration | No change |
| 1 hr. 32' | Adrenalin 1 cc. 1/10,000 | 4 | Slight inhibition | Rise from 118 to 160 |
| 1 hr.34'30" | Sympathetic for 15", coil 12 | 14 | Marked inhibition | Rise from 116 to 124 |
| 1 hr. 56' | Pituitrin 1 cc. | 1 | Very marked inhibition | Fall from 112 to 5, then rise to 188 |

* Long continued secretion.

Thus when adrenalin alone did not produce any visible effect it gave a secretion if the gland had previously been activated. Pituitrin, on the other hand, acted neither after stimulation of the

secretory nerves, nor in a non-activated gland. For an example of pituitrin action after stimulation of the sympathetic see Table XXI, and after stimulation of the chorda see Table XIX.

Since it has been proved that the sympathetic nerve, like histamine, activates both secretory elements and a contractile mechanism in the submaxillary gland, it seems legitimate to suppose that the two are affecting the same histological structure, but at different points. Thus the nerve acts on the neuro-cellular junction, whereas histamine stimulates the cells themselves. If this supposition is correct, it explains why the histamine effect is not abolished by ergotamine.

What kind of contractile elements are stimulated by the sympathetic nerve and by histamine? - Neither the physiological investigations reported above, nor a special histological examination by Dr. D. J. Bowie, undertaken in our laboratory, revealed the presence of contractile elements of a muscular nature in the submaxillary gland. The assumption has been made, therefore, that the motor mechanism in the gland activated by the sympathetic nerve and by histamine is not composed of muscle tissue. In view of this fact it was considered necessary to perform a special investigation of the problem (Babkin and MacKay, 1930).

Three suppositions concerning the nature and action of the pressor mechanism have been made and tested experimentally:-

(1) The large blood vessels in the submaxillary gland run along the salivary ducts and surround them closely (Flint, 1902). It was thought that the changes in diameter of these vessels brought about

during stimulation of the sympathetic nerve, or after histamine administration, might cause saliva to be pressed out from the ducts already filled by previous chorda stimulation. This assumption, however, seems to be quite improbable according to the experiments of Sinelnikoff (1921). He found that the pressor effect on the salivary gland could be observed after stimulation of a previously cut cervical sympathetic nerve in which the vaso-constrictor fibres had already degenerated. Similarly in the previously discussed experiments with pituitrin (MacKay, 1929), this drug which produced a long continued spasm of the blood vessels did not press out any saliva from the ducts.

(2) The pressing out of saliva from the previously activated gland might be due to histamine suddenly producing a filling of the glandular blood vessels and thus exerting a pressure on the already filled ducts. Special experiments to settle this point were performed and have already been described in section C above. In these experiments when histamine was injected after stimulation of the chorda, the increase of the blood flow was so insignificant and of such short duration that it could not be responsible for the pressing out of saliva. An example of such an experiment is shown in Figure 5.

(3) Finally the pressing out of saliva might be due to some special motor mechanism in the gland, which is activated by the sympathetic nerve or by histamine. An attempt to settle this question was made by means of experiments with a plethysmograph in which the submaxillary gland was enclosed. Control experiments

with stimulation of the chorda and sympathetic gave the same results as those of Bunch (1900), namely, a marked shrinking of the gland. Intravenous injection of histamine after chorda stimulation gave first a very short and insignificant increase in the volume, which was followed by a marked and long continued diminution. The initial increase in volume is attributed to the initial vaso-dilatation with histamine which is discussed above (see Figure 6). On the other hand, if histamine was injected without previous activation of the gland, it did not produce a marked secretion or a diminution of the volume of the organ (see Figure 7). That the shrinking of the activated gland after histamine is not due to the fall in blood pressure, is proved by an experiment with bleeding of the animal in which the pressure fell from 88 to 47 without affecting the volume of the gland.

To what structure is the shrinking of the gland after histamine due? - There are two mechanisms which may be responsible for the decrease in volume of the submaxillary gland:-

(1) Since stimulation of the chorda tympani produces a shrinking of the gland, and since, according to Bunch,"the effect of the secretory nerves is simply and solely on the secretory cells", histamine may have a similar effect. This supposition is probable because histamine produces a secretion in a gland whose nerves have previously been paralyzed by atropine and by ergotamine, whereas adrenalin and pilocarpine under these conditions have no effect. According to the present views the myoneural junctions are paralyzed by atropine and ergotamine. Therefore since histamine is acting

to a certain degree after these drugs, it may be concluded that it affects the secretory elements somewhere peripheral to the neuro-cellular junction. Very little is known about the actual changes occurring in the cell during the true secretory process activated by the chorda tympani or through histamine. However, further investigation must be performed in future in connection with this problem.

(2) Histamine, besides its true secretory effect, has been shown to have the property of pressing out the contents of the gland, either after stimulation of the chorda or when the glandular ducts are filled from outside. As has been previously pointed out, since there is no evidence for the presence of muscle tissue in the gland, this tissue cannot be responsible for the motor phenomena. However, there are other cytological structures in the salivary glands which were supposed to possess contractility. The most interesting of these elements are the so-called "myo-epithelial cells", "basket cells" or "Korbzellen". These cells have been described by Zimmermann (1927) and by Metzner (1907). They lie between the basal membrane of the gland and the base of the secretory cells and surround them with long processes. The myoepithelial cells are not of mesodermal origin but develop from epithelium. Long ago the property of contractility was ascribed to them by the histologists.

The questions as to whether these cells actually do contract and whether they are controlled by the sympathetic nerve and can be influenced by histamine cannot be answered here but must be left for future investigations.

Another supposedly contractile structure within the cell body itself was described by Zimmermann (1925, 1927). This structure is situated in that part of the secretory cell which is filled with zymogen granules. It is a special nucleus thought to be contractile and has been called the "diplosoma". This "kinocentre" must not be confused with the cell nucleus which is the "chemocentre". The "diplosoma" has been demonstrated in various secreting cells including the serous and mucous cells of the salivary gland. However, if these structures actually do possess the power of contracting, there is as yet no evidence justifying the conclusion that they are under the control of the sympathetic or can be affected by histamine.

In summing up there seems to be no doubt that certain motor phenomena may be observed in the submaxillary glands of both dogs and cats, following stimulation of the secretory nerves and after the administration of histamine. This contractility cannot be attributed to the presence of smooth muscles, as no such tissue could be found either in the ducts or in the gland itself. Other cytological structures have been suggested tentatively as being capable of producing contraction of the gland. However, an investigation of the nature of this contractile mechanism must be left to future investigators.

G. Histamine and Adrenalin in Relation to the Salivary Secretion.

The study of the influence of histamine on salivary secretion revealed the fact that even after stimulation of the chorda tympani its effect was not always constant. In some experiments small doses of histamine were practically ineffective, while in others

this substance gave a good secretion of saliva, even when it was injected without previous stimulation of the chorda tympani.

In addition to the variability in the response of the salivary glands to histamine, it was observed in some experiments that there was a difference in the reaction of the blood pressure following injection of this drug. The usual effect produced by histamine on the blood pressure in both dogs and cats was a sharp fall, followed by gradual return to the normal. The time taken for this recovery was different in different experiments or at varying stages in one and the same experiment.

However, in many instances a quite different reaction on the blood pressure was noticed. After an initial fall the curve began to rise, and in about a minute to a minute and a half reached a far greater height than before the injection of histamine. Eight corresponding experiments on cats, which demonstrate these blood pressure changes, are presented in Table XXII. In all these experiments, save one, histamine gave no secretion or a very scanty one. The exception is the experiment of February 19 when histamine after chorda stimulation gave five drops. A typical curve showing the rise of the blood pressure after injection of histamine is seen in Figure 8.

In these curves a rather long latent period before the rise of the blood pressure and a subsequent slow recovery and return to normal suggested that the phenomenon was of a secondary character. Some substance could enter the circulation, affect the blood vessels and produce an inhibition of the secretion. Since there are

indications that histamine causes increased output of adrenalin and the effect is in part a direct one on the adrenals (Kellaway and Cowell, 1923), it was decided to determine the influence of the removal of adrenals on histamine salivary secretion.

Table XXII

| Date | Blood Pressure | | Time taken after histamine for rise in blood pressure to occur | |
|---|---|---|---|---|
| | before histamine | after histamine | | |
| | mm. Hg | mm. Hg | minutes | seconds |
| January 31 | 116 | 74-178 | 1 | 45 |
| February 10 | 82 | 54-136 | 1 | 45 |
| February 21 | 104 | 72-124 | 1 | 30 |
| February 24 | 116 | 90-130 | 1 | 25 |
| October 24 | 72 | 40-128 | 1 | 00 |
| November 1 | 28 | 16- 72 | 1 | 10 |
| February 19 | 92 | 72-116 | 1 | 10 |
| February 11 | 80 | 64-150 | | 45 |

Three figures are given for blood pressure; the first is the normal pressure before the injection of histamine, the second is the maximum fall and the third the maximum rise after the intravenous injection of 0.25 mg of histamine.

In all the experiments the cats were anaesthetized with chloralose, except the experiments of February 19 and February 11, when they were decerebrate.

In several experiments on both cats and dogs when histamine at first was practically ineffective, the adrenals were removed and the subsequent action of this substance noted. A uniform effect was observed in all cases. (a) The histamine salivary secretion after chorda stimulation was quite markedly increased, and (b) there was

never a rise of the blood pressure after injection of histamine. These relations can be seen in Figures 9 and 10, quoted as an example.

Before the extirpation of adrenals stimulation of chorda tympani for thirty seconds produced 15 drops of saliva. (Cat, both chorda tympani and cervical sympathetic cut, Gibbs drop recorder, one drop equal to 1/46 cc.) Injection of 0.25 mg of histamine thirty seconds later produced an insignificant fall of the blood pressure and then a marked rise. No salivary secretion was noted (Figure 9). After the extirpation of both adrenals the stimulation of chorda tympani for thirty seconds produced 12 drops of saliva. One-fourth milligram of histamine now gave 7 drops of saliva. No rise of the blood pressure was observed (Figure 10). Since removal of the adrenals increased the secretory action of histamine, the next step was to inject adrenalin to see if the histamine effect would be diminished again. A slow continuous injection of adrenalin 1/20,000 was begun. An attempt was made to adjust the rate of injection so that the blood pressure would be raised a certain degree but kept at as constant a level as possible. During the injection of adrenalin the same stimulation of chorda tympani for thirty seconds produced 22 drops of saliva, and a subsequent dose of 0.25 mg of histamine only one drop (Figure 11).

The following experiment on a dog is very instructive, since it shows not only a change in the histamine salivary secretion after the removal of the adrenals, but also a change in the character of chorda secretion which again returned to the normal conditions under the influence of adrenalin (Table XXIII).

The dog was anaesthetized with Dial and a cannula inserted in the submaxillary duct. The chorda tympani and the cervical sympathetic nerves were cut and prepared for stimulation. In this experiment histamine in a dose of 0.25 mg acted at first very slightly, only one drop being formed when it was injected alone, while after stimulation of the chorda it had no effect on secretion. A larger dose of 0.5 mg gave an augmented effect of 8 drops. The adrenals were then excised and the same procedure with histamine repeated. A dose of 0.25 mg, which previously had no action after chorda, now gave 7 drops of saliva, while a dose of 0.5 mg produced 36 drops as compared with a previous secretion of 8 drops. Although the histamine augmented secretion was increased five-or sixfold, it was seen that when this substance was injected without previous stimulation of the parasympathetic nerve, it produced the same slight secretory effect (1 drop) as in the beginning of the experiment. This fact, that previous stimulation of the chorda greatly increases the histamine action, has been previously discussed. A slow intravenous injection of adrenalin (1/30,000) was then begun. During the injection of adrenalin the chorda was stimulated and was followed by histamine. This substance now again exhibited only the slight secretory action which it possessed before excision of the adrenals. After the injection of adrenalin was stopped histamine again recovered its secretory effect. The first injection, following the chorda stimulation, produced only a slight secretion (3 drops), but after two subsequent stimulations of the chorda the same dose of histamine gave six and seven drops respectively.

Table XXIII

June 17. Dog, 17 kg. Dial 10 cc.

| Time | Procedure | Saliva | Latent period of Secretion | Duration of the Secretion | Blood Pressure | |
|---|---|---|---|---|---|---|
| | | | | | Initial | After certain procedure |
| | | drops | sec. | min. sec. | mm.Hg | mm.Hg |
| 11.31 | Histamine 0.25 mg | 1 | | | 180 | 70-170 |
| 11.42 | Chorda 15", coil 20 | 59 | 2 | 57 | 170 | |
| 11.43 | Histamine 0.25 mg. | 0 | | | 170 | 72-170 |
| 12.00 | Chorda 15", coil 20 | 70 | 3 | 1 12 | 170 | |
| 12.01 | Histamine 0.5 mg. | 8 | 21 | 1 00 | 170 | 60-140 |
| 12.11 | Adrenals excised | | | | 140 | 100 |
| 12.33 | Chorda 30", coil 20 | 49 | 15 | 3 08 | 100 | |
| 12.36 | Histamine 0.25 mg. | 7 | 44 | 2 00 | 100 | 54-106 |
| 12.50 | Histamine 0.25 mg. | 1 | | | 110 | 62-100 |
| 1.35 | Chorda 30", coil 20 | 68 | 14 | 3 06 | 128 | |
| 1.38 | Histamine 0.5 mg. | 36 | 24 | 1 33 | 128 | 58-120 |
| 2.15 | Chorda 15", coil 20 | 21 | 10 | 1 08 | 120 | |
| 2.16 | Histamine 0.25 mg. | 22 | 27 | 1 42 | 120 | 74-110 |
| 2.29 | Histamine 0.25 mg. | 1 | | | 110 | 74-110 |
| 2.37 | Adrenalin (1/30,000) continuous injection begun | 0 | | | 100 | 152 |
| 2.40 | Chorda 15", coil 20 | 46 | 3 | 50 | | |
| 2.41 | Histamine 0.25 mg. | 1 | | | 160 | 94-240 |
| 2.42 | Adrenalin injection stopped (12 cc. 1/30,000 injected since 2.37) | | | | | |
| 2.49 | Chorda 15", coil 20 | 45 | 3 | 1 06 | 100 | |
| 2.50 | Histamine 0.25 mg. | 3 | 48 | 32 | 104 | 60-100 |
| 3.18 | Chorda 15", coil 20 | 56 | 3 | 1 18 | 106 | |
| 3.19 | Histamine 0.25 mg. | 6 | 37 | 27 | 108 | 68-80 |
| 3.30 | Chorda 15", coil 20 | 63 | 4 | 1 18 | 90 | |
| 3.31 | Histamine 0.25 mg. | 7 | 36 | 1 14 | 90 | Clot |

As has been mentioned above, in this experiment the character of the chorda secretion changed after removal of the adrenals and the injection of adrenalin. Because of these changes the latent period of the secretion from the beginning of stimulation until the first drop of saliva appeared, and the total duration of the secretion have been recorded in Table XXIII. In the beginning of the experiment the latent period was short, only a few seconds, and the duration of the secretion after stimulation was not prolonged after a minute and a few seconds. Removal of the adrenals was followed by certain well marked changes, the latent period being prolonged for ten to fifteen seconds and the after effect also lengthened for several minutes. In spite of this the actual volume of secretion was not increased and in several instances was lessened.

It is interesting to note that the stimulation of the sympathetic nerve in both dogs and cats diminished the subsequent effect of histamine. The impression is created that histamine has a relation to some of the structural elements of the submaxillary gland innervated by the sympathetic nerve. In Table XXIV there is a corresponding example. As usual the effect of histamine diminished with the repeated injections, which explains the small secretion at 12.35 p.m.

Table XXIV

Cat. Dial.

| Time | Procedure | Saliva |
|---|---|---|
| | | drops |
| 11.20 | Chorda 30", coil 20 | 17 |
| 11.21 | Histamine 0.25 mg | 5 |
| 11.54 | Chorda 30", coil 20 | 19 |
| 11.55 | Histamine 0.25 mg | 4 |
| 12.08 | Sympathetic 30", coil 15 | 7 |
| 12.10 | Chorda 30", coil 18 | 22 |
| 12.11 | Sympathetic 30", coil 15 | 7 |
| 12.12 | Histamine 0.25 mg | 1 |
| 12.35 | Chorda 30", coil 18 | 16 |
| 12.36 | Histamine 0.25 mg | 2 |

From the experiments reported above it may be concluded that adrenalin is antagonistic to histamine in so far as the latter substance is acting on the submaxillary secretion. It was observed that the histamine augmented action may be increased considerably by removal of the adrenals and abolished again by injection of adrenalin. From these facts one may partly explain the inhibitory influence of small doses of adrenalin as well as the weak secretory action of histamine not infrequently observed in our experiments. Indeed in many instances the intravenous injection of histamine produced a rise of the blood pressure similar to that given by adrenalin. The great number of unsuccessful experiments with

histamine were observed on those animals which were anaesthetized with chloralose. This fact is in accordance with the data of Swale Vincent and Thompson (1928) who believed that chloralose stimulated the production and discharge of adrenalin from adrenal glands.

No attempt has been made to analyze the inhibitory character of the action of adrenalin on the histamine salivary secretion, as this problem would require a special investigation because of the complicated relations existing in the salivary glands. However, the facts noted here are being reported because they are of interest in connection with the view expressed by Dale (1920), Cramer (1926)and others that there are certain antagonistic relations existing between histamine and the adrenal glands.

# PART III. (EXPERIMENTAL)

## THE ACTION OF HISTAMINE ON THE PANCREATIC GLAND

As has been mentioned in Part I of this thesis, several investigators have found that histamine, when injected intravenously in dogs, activates a pancreatic secretion (Dale and Laidlaw, 1910; Popielski, 1920; Molinari-Tosatti, 1929). In the present investigation this result was confirmed in dogs, and an attempt was made to analyze further the secretory action of histamine. The effect of this substance on the pancreas of the rabbit was also studied for the first time.

### Methods

Dogs and rabbits have been used for these investigations. In the experiments on dogs various anaesthetics were tried, namely, dial, sodium luminal and a mixture of chloralose and urethane. The last-named drug combination was found to be most satisfactory for these experiments, since dial given intraperitoneally produced an irritation of the viscera causing an exudate of fluid into the peritoneal cavity, and sodium luminal provoked a great peripheral dilatation of the blood vessels. The dose of chloralose and urethane was .1 g chloralose per kilo per half the body weight, and 1 g of urethane per kilo per half the body weight. Urethane alone in a dose of 1 g per kilo body weight was used for the rabbits.

Previous to the injection of the above anaesthetic the dogs were given ether, and a cannula was inserted into the femoral vein

for administering the chloralose urethane mixture. Tracheotomy was performed and a cannula inserted into the carotid artery for recording blood pressure. The abdomen was then opened by a mid-line incision and the common bile duct and pylorus tied to prevent complication of the experiments by the passage of bile and acid gastric contents into the duodenum. A cannula was inserted in the pancreatic duct and was connected with a Gibbs' drop recorder. In some cases the animals were starved for twenty-four hours before the experiment, while in others they received food the previous day.

After intravenous injection of urethane into the ear vein the rabbits were prepared in the same way as the dogs, namely, by introducing tracheal, arterial and pancreatic cannulae and by tying off the common bile duct and pylorus.

## General Action of Histamine on Pancreatic Secretion

### A. Dogs.

In accordance with the results of previous investigators, it was found that intravenous injection of histamine produces a flow of pancreatic juice. A dose of $\frac{1}{4}$ mg of the drug is sufficient to activate a fair secretion in an average sized dog. With increasingly larger doses the secretory effect is increased, though the two are not necessarily in direct proportion to each other; thus with twice the amount of histamine the secretion was increased but not doubled. In contrast to gastric secretion it was found that the pancreatic secretion begins very rapidly after the injection of histamine, the latent period being in most experiments only from 40-70 seconds. The effect of the drug is transitory, the secretion being usually

complete in several minutes, although its duration depends to some extent on the size of the dose. Unlike the salivary glands, in the pancreas the effect of repeated injections of histamine is not diminished. Indeed if the doses are given within a short time of each other there may be a summation effect. A typical example of the action of histamine on pancreatic secretion may be seen in Figure 12.

B. Rabbit.

As no previous investigator had studied the action of histamine on pancreatic secretion in the rabbit, it was considered worth while to investigate the problem. This question was especially interesting since certain peculiarities had been observed in the reaction of the circulatory system of the rabbit to histamine.

It was found that histamine when injected into a rabbit had a very different effect on pancreatic secretion than when administered to one of the carnivora. In the rabbit, as is characteristic of this animal, a continuous spontaneous secretion of pancreatic juice was observed throughout the experiment. When histamine was injected, instead of increasing the rate of the spontaneous secretion, it produced a marked inhibition in the flow of juice. An example of the inhibition in secretion may be seen in Figure 13. This inhibition did not become evident for several minutes after the histamine administration and it reached a maximum some time later. The reaction of the blood pressure was found to vary in different rabbits, which fact corresponds to the observations of Dale. There was sometimes, as in the experiment quoted above, a marked rise which took place immediately after histamine and lasted for two or

three minutes. The inhibition in pancreatic secretion was not observed until the blood pressure had returned to its normal level. It should be pointed out that the rabbit is very sensitive to histamine and usually dies after several injections. The drug has a very marked action on the respiration, producing long continued spasms in the breathing.

No attempt was made to find out why the rabbit pancreas responded so differently to histamine from that of the dog. However, it is possible that this substance produces a contraction of the secretory ducts and thereby inhibits the spontaneous flow of juice. A further investigation of this problem is necessary.

Analysis of the Action of Histamine on the Pancreas of the Dog

Since it had been found that the action of histamine on the stimulated salivary gland was very different from its effect on the resting gland, it was decided to investigate whether previous activation of the pancreas would facilitate its effect on pancreatic secretion.

Is there an augmented pancreatic secretion with histamine analogous to that in the salivary glands? - In these experiments the action of histamine was first observed, the gland was then activated by injection of hydrochloric acid into the duodenum. When the secretion from acid was practically stopped, histamine was administered again. An example of such an experiment is seen in Table XXV.

Table XXV

April 26, 1929. Dog, 21 lb. Dial.

| Time | Procedure | Pancreatic Secretion |
|---|---|---|
| 3 hours, 6 minutes | ½ mg histamine | 7 drops |
| 3 hours, 15 minutes | 40 cc. HCl injected into duodenum | Profuse long continued secretion |
| 4 hours, 58 minutes | ½ mg histamine | 7 drops |

As is seen in this table the effect of histamine is the same when it is injected alone as after previous stimulation of the gland with acid. Thus it may be assumed that, unlike the salivary glands, previous activation of the pancreas does not usually increase the response to histamine, although such an effect was observed in one experiment. The different result obtained in this experiment may be explained as follows. Since histamine activates the movements of the gut, it could cause the acid to be pushed along the intestine and thus stimulate a new area of mucous membrane. It is known that acid alone inhibits intestinal motility and its effect on the same part of the mucous membrane gradually wears off, probably by failing to produce secretin (Rasenkow, 1929).

It may be concluded that histamine does not produce an augmented effect in the activated pancreas analogous to that found in the salivary glands. However, it must be remembered that in the salivary glands histamine is stimulating a glandular structure activated by a parasympathetic nerve. In the case of the secretion of pancreatic juice provoked by the introduction of acid into the

duodenum, we are dealing with a pure hormonal action absolutely independent of the nervous system. Therefore this difference in the effect of histamine on the two glands may be due to the difference in the mechanism activating them.

The action of histamine on the pancreas stimulated by pilocarpine is also different from its action on the salivary glands similarly treated. Instead of producing an inhibition in the pilocarpinized pancreas histamine causes a summation effect by markedly increasing the rate of secretion.

As the investigation on salivary glands showed (see Part II), the inhibition of pilocarpine secretion by histamine is of vascular origin. It may be that in the pilocarpinized pancreas the blood vessels react differently to histamine than in the salivary glands. A further examination of this problem was not performed.

Action of Atropine. - As pointed out in the discussion of the literature in Part I, there is a difference in opinion concerning the action of atropine on the pancreatic secretion produced by histamine. Thus Dale and Laidlaw and Molinari-Tosatti report that atropine abolishes the pancreatic secretion, whereas Popielski finds that it does not have this effect. In view of the contradictory evidence offered in the literature, it was thought advisable to clear up this point.

Several experiments have been performed in which the action of histamine was observed before and after varying doses of atropine. The results of these three experiments may be seen in Table XXVI. An example of the action of histamine after atropine is given in Figure 14.

Table XXVI

April 26, 1929. Dog, 11.5 kg. Dial.

| Time | Procedure | Secretion in drops | Remarks |
|---|---|---|---|
| 3 hrs. 58' | Histamine $\frac{1}{4}$ mg | 7 | |
| 4 hrs. 13' | Atropine 7 mg | 0 | |
| 4 hrs. 18' | Histamine $\frac{1}{4}$ mg | 3 | |
| 4 hrs. 40' | HCl in duodenum, 65 cc. | Profuse secretion | |
| 5 hrs. 30' | Histamine $\frac{1}{2}$ mg | 9 | |
| 5 hrs. 37' | Histamine 1 mg | 17 | |
| 5 hrs. 54' | Stimulated vagus, coil 13 | 0 | No fall in blood pressure |

March 27, 1929. Dog. Dial.

| | | | |
|---|---|---|---|
| 11 hrs. 53' | HCl in duodenum, 20 cc. | Profuse secretion | |
| 12 hrs. 29' | Histamine $\frac{1}{4}$ mg (just at end of acid secretion) | 13 | |
| 1 hr. 10' | Histamine $\frac{1}{4}$ mg | 13 | |
| 2 hrs. 52' | Histamine $\frac{1}{4}$ mg | 12 | |
| 3 hrs. 2' | Histamine $\frac{1}{4}$ mg | 13 | |
| 3 hrs. 16' | Histamine $\frac{1}{4}$ mg | 17 | |
| 4 hrs. 15' | Stimulated peripheral end of vagus | | Fall in blood pressure |
| 4 hrs. 16' | Atropine 5 mg. Stimulated peripheral end of vagus | | No fall in blood pressure |
| 4 hrs. 19' | Histamine $\frac{1}{4}$ mg | 13 | |
| 4 hrs. 32' | Atropine 5 mg | | Fall in blood pressure |
| 4 hrs. 33' | Stimulated peripheral end of vagus | | No effect on blood pressure |
| 4 hrs. 35' | Histamine $\frac{1}{4}$ mg | 11 | |

Table XXVI (cont.)

January 10, 1930. Dog, 19.7 kg. Chloralose and urethane.

| Time | Procedure | Secretion in drops | Remarks |
|---|---|---|---|
| 4 hrs. 40' | Hcl in duodenum, 50 cc. | Profuse, long continued secretion | |
| 5 hrs. 12' | Histamine $\frac{1}{2}$ mg | 17 | |
| 5 hrs. 27' | Atropine 5 mg. Stimulated peripheral end of vagus | | Very slight fall in blood pressure |
| 5 hrs. 30' | Atropine 2 mg. Stimulated peripheral end of vagus | | Very slight fall in blood pressure |
| 5 hrs. 32' | Atropine 2 mg. Stimulated peripheral end of vagus | | No fall in blood pressure |
| 5 hrs. 34' | Histamine $\frac{1}{2}$ mg | 13 | |
| 5 hrs. 35' | Histamine $\frac{1}{4}$ mg | 4 | |
| 5 hrs. 40' | Histamine 1 mg | 19 | |

In all cases the action of histamine persisted after the administration of atropine. The latter drug was given in the three experiments in doses of 7, 9 and 10 mg respectively. This amount was sufficient to abolish completely the action of the vagus nerve, so that stimulation of its peripheral end did not produce a fall in blood pressure or a pancreatic secretion. In two of the experiments the histamine secretion was slightly diminished after the dose of atropine, but in another it remained unchanged.

The difference in the results of Molinari-Tosatti may be due to the large amounts of atropine used by this investigator. He reports that the histamine secretion is abolished only after 30 mg of the drug, although this dose is much greater than that required to paralyze the vagus nerve endings in the heart and pancreas. It is possible that such large doses of atropine as 30 mg in addition to paralyzing the nerve endings are poisoning the secretory cells themselves. However, this suggestion cannot be applied to the experiment of Dale and Laidlaw, who found that, after 5 mg of atropine, histamine had lost its secretory effect on the pancreas.

It may be concluded that histamine activates a pancreatic secretion. Its effect is not augmented after previous secretion with acid. On the other hand, a summation of the effect of histamine and pilocarpine could be observed. Atropine in doses sufficient to paralyze the endings of the vagus nerve does not abolish the histamine action.

## The Enzyme Content of the Pancreatic Juice secreted after Histamine

It was reported by Molinari-Tosatti that the pancreatic juice secreted after histamine had the same content of enzymes as that secreted after secretin. In the present investigation an attempt has been made to find out if histamine does give a juice characteristic in its enzyme content. Two stimuli were chosen: (1) pilocarpine, (2) hydrochloric acid introduced into the duodenum. Pilocarpine has been used in preference to stimulating the vagus

nerve, since this last procedure gives a very scanty amount of secretion sometimes not sufficient for enzyme determinations. The injection of acid was preferred to the intravenous injection of secretin because it is the most natural stimulus. Besides this Babkin and Savitch (1908) showed that the secretion juice is richer in enzymes than that secreted by HCl. This difference is probably due to the presence of impurities which are found in all secretin preparations. Therefore secretin pancreatic juice cannot be looked on as truly representative of the humoral secretion.

In the following experiments we had therefore two kinds of pancreatic juice. The one activated by pilocarpine was rich in enzymes and organic material, whereas that evoked by acid was poor in enzymes and organic material.

Method used for Determination of Enzymes. - Mellanby's method of milk coagulation has been used for the enzyme determinations. In this method the pancreatic juice is collected, a certain quantity activated by enterokinase and left in a water bath for one hour at 38° C. This tube is placed in the water bath and examined every fifteen seconds for signs of coagulation. The first appearance of fine suspended particles in the milk is taken as an end point. As this method had originally been devised for determinations of enzymes in cat's pancreatic juice, several changes were introduced to make it satisfactory for dog's pancreatic juice. Since the dog's juice was very rich in enzymes, it was found necessary to dilute it ten times before activating with enterokinase. One cc.

of this mixture, containing .1 cc. juice, .9 cc. $H_2O$ and .01 cc. enterokinase, was added to 5 cc. of the milk and $CaCl_2$ mixture. This was made by taking 5% of fresh skim milk and 5% of n/20 $CaCl_2$. It was found that very satisfactory readings could be obtained by using the above mentioned dilutions, whenever possible duplicate determinations being carried out.

The Enzyme Content of the Pancreatic Juice. - It is known that the content of enzymes in the pancreatic juice depends on two variables:-

(1) The amount of juice secreted is an important factor, since it has been shown, using the same stimulus, that the concentration of enzymes is in inverse proportion to the volume of juice. Thus smaller doses of a substance may activate a juice richer in enzymes than larger doses of the same substance, because the volume of secretion will be different in each case.

(2) The nature of the stimulus is also important in determining whether the juice has a high or low enzyme content. For example, pilocarpine independently of the volume of secretion gives a juice rich in enzymes. The action of this drug is on the endings of the vagus nerve. On the other hand, hydrochloric acid, which is acting after paralysis of the parasympathetic nerve by atropine, gives a juice much lower in enzymes than pilocarpine. Hydrochloric acid stimulates the pancreas by acting directly on the secretory cells. It was considered of interest to note whether histamine had a type of secretion characteristic of either of these stimuli.

From the experimental evidence it appears that the juice secreted after histamine alone resembles roughly that secreted after hydrochloric acid. However, as the volume of the histamine juice is usually lower than that of the acid, the enzymes are more concentrated and give a slightly higher value.

On the other hand, when histamine is injected after a previous stimulus, the character of its secretion changes considerably and resembles the juice produced by the first stimulus. Thus histamine after acid gives a juice resembling the acid secretion, whereas after pilocarpine it gives a secretion like that of the latter drug. An interesting fact observed in these experiments is that after pilocarpine histamine gives a very viscid juice richer in enzymes than the pilocarpine juice itself. An example of a typical enzyme experiment is given in Table XXVII.

It will be seen from this table that the spontaneous secretion is relatively low in enzymes. The first injection of pilocarpine called forth a secretion which was rich in enzymes. This drug was followed by three injections of histamine, the first giving a juice higher in enzymes than the pilocarpine, possibly indicating a pushing out of pilocarpine juice from the gland. The content of enzymes progressively fell in the two subsequent samples of histamine juice. The second injection of pilocarpine gave a juice high in enzymes but not so high as the first. This observation fits in with the well known fact that the pancreatic gland during its activity gradually loses its stored organic material and enzymes. A second time the injection of histamine after pilocarpine gave a juice richer in enzymes than the pilocarpine itself. The histamine was followed

by three injections of acid. An example of the dilution factor can be seen here, as the acid which gave the largest volume of juice had the lowest enzyme content. Histamine after acid evoked a more scanty secretion than after pilocarpine and despite this fact was much poorer in enzymes.

Table XXVII

January 30. Dog, 12 kg. Chloralose & urethane.

| Time | Procedure | Amount of Secretion | Duration of Secretion | Coagulation time of milk | Blood Volume |
|---|---|---|---|---|---|
| | | cc. | minutes | seconds | mm. Hg |
| 11 h. 45' | Spontaneous secretion | .1 | | 190 | 190 |
| 12 h. 4' | Pilocarpine 2.5 mg | 1.00 | 40 | 45 | 130 |
| 1 h. 6' | Histamine ¼mg | ( .2 | 12 ) These | | |
| 1 h. 30' | " " | ( .1 | 10 ) mixed | 20 | 150 |
| 2 h. 35' | " " | .6 | 10 | 32 | 150 |
| 3 h. 40' | " " | .7 | 7 | 105 | 135 |
| 4 h. 10' | Pilocarpine 1¼ mg | .75 | 25 | 75 | 135 |
| 4 h. 37' | Histamine ¼mg | .6 | 10 | 45 | 130 |
| 5 h. 20' | " " | .4 | 9 | 45 | 130 |
| 5 h. 40' | HCl 20 cc. | .4 | 12 | 65 | 140 |
| 6 h. 00' | " " | .5 | 15 | 110 | 140 |
| 6 h. 35' | " " | .9 | 20 | 200 | 150 (clotting) |
| 7 h. 10' | Histamine ¼mg | .1 | 10 | 105 | 130 |
| 8 h. 20' | " " | Very small amount | | | |
| 8 h. 30' | " ½mg | .25 | 13 | 90 | 110 |

From this and other analogous experiments it is concluded that histamine does not cause the secretion of a characteristic type of juice.

The following explanations may be offered for the fact that histamine alone or after acid gives a juice with moderate enzymes, whereas after pilocarpine it activates a secretion very rich in enzymes. It is evident that the histamine secretion is not great; therefore a single injection of the drug in the doses used in this experiment did not produce a secretion sufficient to wash out that secreted by the previous stimulus. Thus the sample of juice secreted after histamine is a mixture of the histamine juice and that of the previous stimulation. It is probable that the pure histamine juice has an average content of enzymes.

## Some Data concerning the Restoration of Pancreatic Secretion

It was first observed by Bayliss and Starling (1902) that, when repeated injections of hydrochloric acid were made into the duodenum, the response of the pancreatic gland diminished. Since then a number of investigators, including Rasenkow (1929), have confirmed this fact. The latter investigator also found that, when secretin was prepared from the intestinal mucous membrane of a dog that had ceased to react to acid, and injected into a second dog, it stimulated a flow of juice. This experiment showed that the failure to respond to acid was not due to a depletion of the secretin stored in the mucous membrane. Upon further investigation Rasenkow found that the injection of certain cleavage products of proteins when introduced into the intestine would restore the

action on the pancreas of acid from the duodenum. Thus peptones and albumoses but not amino acids when injected into the duodenum brought back the response to acid, although they would not produce a secretion by themselves. This work was continued further by Kosektojanz (1929), who proved that acid digests of meat and milk in gastric juice had a similar action to peptones and albumoses in restoring the action of acid. On the other hand, acid digest of bread did not have this effect.

Since most Witte's peptone contains histamine and since it is probable that this substance is present in digests of proteins, the question arose as to whether histamine itself played a part in restoring the action of acid in Rasenkow's experiments. As histamine is present normally in the intestines and as it is known to have a secretory action on the pancreas, this question was not without physiological interest.

A number of experiments have therefore been performed in an attempt to find out whether histamine has the property of peptone to restore the stimulating effect of hydrochloric acid on pancreatic secretion. The dogs used for this investigation were prepared in the same way as those used for other types of pancreatic experiments, i.e., with cannulae introduced into the duodenum and pancreatic duct. Chloralose and urethane proved the most satisfactory anaesthetic.

Several difficulties were encountered in this work. In the first place, in many animals it was impossible to fatigue the pancreatic gland with acid, each injection producing a profuse

secretion which did not show any signs of diminishing with repeated injections. This condition was observed most frequently in dogs that had received food on the day before the experiment or where there were any food particles in the intestine. On the other hand, if the animals were starved and the intestines empty, often repeated injection of acid failed to produce a secretion. In the latter cases sometimes even acid and peptone would not stimulate the gland. However, in a few instances, it was possible to find the right condition, namely, a pancreatic gland that first secreted with acid yet failed to respond after repeated injections of this substance.

Five such cases have been observed. In three of these histamine introduced into the duodenum had a positive effect, that is, it restored the action of hydrochloric acid on the pancreas. In the other two experiments histamine failed to act. In one of the latter negative experiments peptone also had no effect, while in the other it was not tried.

An example of one of the positive experiments is given in Table XXVIII. It may be seen in this table that seven injections of acid into the duodenum first gave a very small pancreatic secretion and then failed to act. 25 mg of histamine were then introduced into the duodenum and left for 25 minutes without producing a secretion. Acid was again administered repeatedly at approximately 25-minute intervals. Although the first injection of acid after histamine had no effect, the subsequent injections began to act and gradually gave a very good secretion, reaching a maximum in about two hours.

Table XXVIII

January 8, 1930. Dog, 7.2 kg. Urethane.
(Dog starved 24 hours before the experiment)

| Time | Procedure | Pancreatic Secretion in drops |
|---|---|---|
| 11 h. 25' | 30 cc. 2% HCl in duodenum | 3 |
| 11 h. 45' | " " " | 3 |
| 12 h. 15' | " " " | Small secretion |
| 12 h. 35' | " " " | 9 |
| 1 h. 5' | " " " | 1 |
| 1 h. 30' | " " " | 0 |
| 2 h. 35' | " " " | 0 |
| 3 h. 0' | 25 mg histamine in duodenum | 0 |
| 3 h. 25' | 30 cc. 2% HCl in duodenum | 0 |
| 3 h. 50' | " " " | 1 |
| 4 h. 15' | " " " | 3 |
| 4 h. 40' | " " " | 21 |
| 5 h. 5' | " " " | 76 |
| 5 h. 40' | " " " | 49 |
| 6 h. 15' | " " " | 76 |
| 6 h. 53' | " " " | 23 |
| 8 h. 15' | " " " | 8 |
| 8 h. 45' | " " " | 4 |
| 9 h. 10' | " " " | 13 |
| 9 h. 45' | " " " | 10 |
| 10 h. 5' | " " " | 4 |

In other experiments the data of Rasenkow have been confirmed with Witte's peptone. It should be noted that both in the experiments with histamine and Witte's peptone the maximum effect of acid was not usually reached until the third or fourth injection of this substance after histamine or peptone had been introduced into the intestine.

Thus in three instances histamine has had the same effect as the products of protein cleavage in restoring the action of acid on the pancreatic gland. However, the evidence is far from conclusive that this is an absolute fact and not an occasional occurrence. It is also too much to assume that histamine is responsible for the restoration obtained with Witte's peptone or other protein decomposition products. Further data on this very interesting question are necessary, but if it can be proved that histamine in the intestine actually does facilitate the action of the pancreas, it will be evidence that it may play a physiological part in digestive processes.

# PART IV. (EXPERIMENTAL)

## THE ACTION OF HISTAMINE ON THE MOTILITY OF DIFFERENT PARTS OF THE INTESTINAL TRACT

The possibility of the formation of histamine in the intestine makes the study of its action on the motility of different parts of the alimentary canal a very important one. Most of the previous workers who studied this problem investigated the effect of histamine only on isolated segments of gut. The work of these investigators is completely discussed in the review of the literature in Part I of the thesis. Although this method of using isolated pieces of gut is of a certain importance in the analysis of the action of different substances on the intestinal muscle, it gives practically no information concerning the action of a drug in the whole animal. It was therefore considered desirable to reinvestigate the problem of the action of histamine on the motility of the gut, using another method than that of the isolated segments. In this work the experimental problem was chiefly concerned with the effect of intravenous injections of the drug on the movements of different parts of the small intestine. This study must be looked on as preliminary to a more detailed investigation of the action of histamine on the intestine when it is introduced subcutaneously and injected into the lumen of the gut itself.

### Methods

Dogs and cats were used for this investigation. These animals received only milk the morning before the experiment. In all cases they were first anaesthetized with ether and a mixture of chloralose

and urethane ( .1 g of chloralose per kg of half the body weight and 1 g of urethane per kg of half the body weight), injected intravenously through a cannula previously introduced into the femoral vein. Tracheotomy was then performed and in some cases a blood pressure cannula inserted into the carotid artery. A mid-line abdominal incision was made and in most experiments ligatures placed around the splanchnics and vagi. Three segments of gut were then isolated as follows. A piece of duodenum about two inches long was measured off below the entrance of the pancreatic and common bile ducts. A longitudinal slit was made through the muscular layer at one end of the segment, and the mucous and submucous layers were separated with a blunt instrument from the muscular layer and tightly ligated. A cannula was then inserted into the end of the duodenal segment through a small incision in the mucous membrane. This cannula was tied in place by a second ligature around the mucous membrane at the point where the latter was separated from the muscular layer. In this way one end of the duodenal segment was isolated and a cannula inserted into its lumen without ligaturing the muscular layers or in any way interfering with the continuity of Auerbach's plexus or the blood supply. The other end of the duodenal segment was treated in exactly the manner described above, except that a T tube was introduced instead of a straight cannula. Loops of jejunum and ileum were also prepared like the duodenum with a cannula in each end. In this way three segments of gut were isolated without the muscle layers being injured and without any ligatures being placed around the intestine. After the segments had been isolated, each in turn was

washed through with warm Ringer solution. The cannulae were then clamped off and the whole animal put in a bath of Ringer solution at 38° C. In this way the intestines were kept immersed constantly in a warm isotonic solution.

The movements of the isolated segment were recorded by the so-called "filling method" described by Babkin (1916). The T cannula in the end of each segment was attached to a Marey's capsule and the other opening closed by a clamp. Warm Ringer solution was then introduced in turn into each of the three segments through the straight cannulae in their proximal ends. During the filling of the loops the tubes leading to the Marey's capsule were clamped off and the other part of the T tube opened. It was usually necessary to adjust the tension in the loops in order to obtain good contractions of the gut. Precautions were also taken to try to keep as near the same tension as possible in the three segments at any one time. The intestine was kept fully immersed in the bath by attaching Spencer Wells forceps to the mesentery, care being taken that the three isolated segments were at the same distance from the surface of the solution. In addition to recording the movements on a smoked paper, it was necessary to watch the intestines with the eye in order to distinguish contractions of the longitudinal layer from those of the circular layer. However, as will be seen from the figures given below, quite characteristic and typical tracings are obtained for some forms of intestinal movements such as peristalsis and rhythmic segmentation.

The doses of histamine used varied from $\frac{1}{4}$ to 2 mg, the drug being in most experiments administered intravenously.

## The Effect of Intravenous Injection of Histamine on the Denervated Gut

In most cases the nerves were intact at the beginning of the experiment and the action of histamine on the innervated gut was observed. Following this the splanchnics and vagi were severed by quickly pulling the ligatures previously placed around them.

In this way the intestine could be denervated without disturbing the position of the animal in the bath. As the greater part of each experiment was performed on the denervated gut, its reaction will be discussed first.

### A. Cats

It was observed in all the experiments that after section of the nerves the motility of the intestine appreciably increased, as did also its response to histamine. A typical picture of the reaction of the duodenum, jejunum and ileum to intravenous injection of $\frac{1}{4}$ mg of this drug may be seen in Figure 15. As may be seen from this figure, different parts of the intestine react differently, the response being most marked in the ileum, less marked in the jejunum and least in the duodenum. In addition to there being a difference in the strength of the reaction, it will be seen that there is also a characteristic type of movement in each of the three parts of the gut. This characteristic response to histamine was invariably observed in the experiments on cats.

The effect of histamine on the different parts of the intestine may be divided into three phases. First, it will be noted that there is a very strong initial contraction in all three loops. This con-

traction takes place very rapidly after injection of histamine and is very pronounced. Direct observation with the eye showed that it was due to an intense shortening of the longitudinal layer, this phenomenon being sometimes so marked that the intestine became quite blanched. It did not appear that any contraction of the circular layer was involved in this initial stage. This contraction forms a steep curve on the tracing which quickly relaxes and is followed by the second phase. In this phase there is a period of inhibition in which the intestine is relaxed from the first initial contraction. A marked vaso-dilatation is seen in this stage, the intestines being quite flushed. The period of inhibition is usually most pronounced and of longest duration in the duodenum. It is also well marked but of shorter duration in the ileum. Sometimes in the jejunum complete relaxation does not take place and the period of inhibition is not observed. This reaction is not shown in Figure 15 but was seen in a number of experiments. In the third phase the duodenum gradually returns to normal, sometimes with heightened tonus and increased contractions which are often of the type of rhythmic segmentation. In the jejunum the contractions in the third phase are chiefly due to shortening of the longitudinal layer, although occasionally rhythmic segmentation may be observed. The third phase in the ileum is quite characteristic and differs from that seen in the other two parts of gut. This phase usually begins after the intestine is relaxed and takes the form of several peristaltic waves which run over the ileum. During this period of activity there is also an increase in the tonus of the lower portions of the gut. Such peristaltic contractions were

not observed in the duodenum or jejunum following histamine.

It is interesting to note that local application of a histamine solution, containing $\frac{1}{4}$ mg in 1 cc., to the outside of the three segments of gut evoked the typical response observed with intravenous injection of the drug. Thus the duodenum reacted the least with an initial shortening of the longitudinal layer, followed by a few contractions which were chiefly due to further shortening and relaxation of this layer. In the jejunum the type of response was the same as in the duodenum but more marked. The ileum reacted most strongly to local application of histamine, giving first a shortening of the longitudinal layer which was followed by active peristalsis. An example of the reaction of the three segments of the intestine to local application of histamine is seen in Figure 16.

Several experiments were performed with only two loops of gut, the upper part of the jejunum and the lower part of the ileum. In these experiments the blood pressure was recorded on the tracing simultaneously with the movements, as it was thought that the second phase of relaxation following the initial phase of contraction might be due to the fall in blood pressure. However, as this relaxation was not observed until after the blood pressure was returning to the normal level, it does not appear as if the condition of the general circulation was responsible for it. This does not mean that circulatory changes occurring in the gut itself do not play any part in producing the phase of relaxation. The third phase, i.e. increased contraction, was observed only after the blood pressure had again reached its normal level. For an example of this type of experiment see Figure 17.

B. Dogs

Somewhat similar results could be seen in dogs with denervated intestine. In these animals, as in cats, the activity was greater in the lower parts of the intestine than in the duodenum. All three segments responded with a three-phase of curve, i.e. contraction, relaxation and series of contractions, although the period of relaxation was much longer than that observed in cats. However, in dogs the specificity of reaction in the ileum was not so marked as in cats, since peristaltic contractions were sometimes observed in the jejunum and even in the duodenum following histamine.

## The Effect of Intravenous Injection of Histamine on the Innervated Gut

The effect of intravenous injection of histamine on the intestine with the nerves intact is the same as its effect on the denervated gut but much weaker.. (See Figure 18 and compare with Figure 17 which is taken from the same experiment after the splanchnic and vagi nerves had been severed.)

It has been noted in both the intact and denervated intestine that after repeated injections of histamine the second and third phases of its action tend to diminish and finally disappear. This is especially true of the peristalsis observed in the ileum.

## The Action of Atropine on the Movements of the Gut stimulated by Intravenous Injection of Histamine

It has been found that intravenous injection of atropine in doses of 1 mg greatly diminishes but does not abolish the action of $\frac{1}{4}$ mg of histamine. (See Figure 19 and compare with Figure 15 from the same experiment before the injection of atropine.) The effect of atropine is most marked on the duodenum, as it practically abolishes the action of histamine on this portion of the gut. In the jejunum the effect of the former drug is not quite so marked as in the duodenum, while in the ileum it is still less pronounced. With larger doses of atropine (4 to 5 mg), which were sufficient to abolish completely the action of acetyl choline, histamine in doses of 1 to 2 mg still gave a motor reaction. This reaction was weak and incomplete in the duodenum and jejunum but more marked in the ileum. In the latter portion of the gut a peristaltic wave was even observed with the above-mentioned dose of histamine after atropine. Thus it may be concluded that after paralysis of the parasympathetic nerve endings the ileum is most reactive to histamine.

It is interesting to note that similar results were obtained when histamine was applied locally to the intestine after atropine, i.e. its effect was most marked on the ileum, much less so on the jejunum and practically abolished in the duodenum.

## The Action of Histamine, introduced in another way than intravenously, on the Movements of the Gut

Only a few experiments have been performed in which histamine was administered in another way than intravenously. Thus the results given in this section must be looked on only as a preliminary report of investigations which will be continued later.

It was reported by Ivy and Vloedmann (1923) that histamine given subcutaneously had no effect on the movements of the stomach of dogs with gastric fistula. In accordance with their results we have found that subcutaneous injection of 1 mg of the drug in cats has no effect on the motility of any of the three segments of isolated gut. Larger doses of histamine than 1 mg have not been tried.

No special investigation was carried out of the effect of histamine on the movements of the gut when introduced into the lumen of the gut itself. However, an interesting fact was observed in one experiment after histamine had been injected into the loop of ileum in the course of the experiment. In this particular experiment on a cat a certain peculiarity was noted at the beginning, namely, that the jejunum reacted better to intravenous injection of histamine than the ileum, although after acetyl choline the reaction in the two portions was practically the same. 6 mg of histamine were then introduced into the ileum and produced a spasm of the muscle. After this had passed off a subsequent injection of histamine intravenously activated a greater response in the ileum than in the duodenum and what is more interesting produced a small peristaltic

contraction in this portion of the gut.

Another interesting observation of the unusual action of histamine was made in an experiment on a dog. This animal had refused food and had been suffering from diarrhoea before the experiment. Upon intravenous injection of histamine it was noted that its usual action on the motility of the gut was reversed, that is, it produced the greatest activity in the duodenum, less in the jejunum and least in the ileum. In this case it was observed that there was a profuse secretion of yellowish fluid into all parts of the gut, so that all the intestines were distended. Several hundred cubic centimetres of this fluid were drawn out from the gut by means of a hypodermic syringe. The isolated segments were then washed through several times with Ringer solution and refilled. After this procedure the intravenous injection of histamine was followed by its usual effect, the contractions being most marked in the ileum and less in the upper portions of the gut.

It may be concluded that histamine introduced intravenously provokes a typical motor reaction in different parts of the small intestine. This reaction is specific regarding its strength, course and character. Local application of the drug to the different parts of the intestines also evokes a characteristic response similar to that produced by intravenous administration. Paralysis of the parasympathetic nerve endings greatly diminishes but does not abolish the action of histamine on the motility of the gut.

## SUMMARY

### PART II

1. Histamine (in doses of $\frac{1}{4}$ to 1 mg) causes in 50 per cent of cases a slight spontaneous secretion of saliva in cats and dogs under chloralose. The effect is the same under ether and chloroform anaesthesia, but is increased in the absence of anaesthetics.

2. Histamine, when injected before stimulation of the secretory nerves or after pilocarpine, does not raise the excitability of the endings of the secretory nerves.

3. A previous stimulation of the parasympathetic secretory nerve greatly increases the secretory effect of histamine on the salivary glands. This effect has been called the "augmented histamine secretion."

4. Intravenous injection of histamine either in the cat or the dog produces a great increase in the blood flow through the submaxillary gland with the chorda and sympathetic nerves cut. If the chorda is previously stimulated, subsequent injection of histamine still produced an increase in blood flow but much less than in the previous case.

5. Previous injection of pilocarpine before the administration of histamine causes the latter to diminish the blood flow through the gland instead of producing its usual increase. Larger doses of pilocarpine are required to produce this reversal effect in the dog than in the cat.

6. This diminution of the blood flow produced when histamine is administered after pilocarpine cannot be explained either by a greater passing of fluid into the salivary ducts, since the flow of saliva was simultaneously diminished, or by mechanical conditions of circulation in the gland.

7. Since atropine restores the vaso-dilator action of histamine previously abolished by pilocarpine, a possible explanation of the phenomenon is that it is a true reversal effect due to poisoning by pilocarpine.

8. An investigation of the augmented secretion produced when histamine was injected after stimulation of the chorda showed that this substance has probably a twofold effect on the gland. First, a secretory effect due to its action on the secretory cells. This is greatly increased by previous nerve stimulation. Second, a mechanical effect whereby saliva is pressed out from the gland.

9. Though atropine occasionally abolishes the "augmented histamine secretion", in most cases it only diminishes its action. As the pressor action of histamine has been shown to be present after atropine, it is thought that the latter drug chiefly diminishes the histamine effect by abolishing its secretory action on the cells themselves.

10. The "augmented histamine secretion" is not abolished by paralysis of the sympathetic nerve by ergotamine.

11. The "pressor" action of histamine was demonstrated in a gland in which both the secretory nerves had been paralyzed by atropine and ergotamine. Since this "pressor" mechanism is thought

to be under the control of the sympathetic, if histamine activates it it must be at a point peripheral to the neuro-cellular junction. The evidence that ergotamine paralyses this junction is that it abolishes the action of adrenalin.

12. A comparison of the action of histamine and pituitrin after stimulation of the chorda tympani showed that these two substances were not acting in the same way on the submaxillary gland. This is considered as evidence for the view that the pressor mechanism or contractile elements in the gland activated by histamine are not of a muscular nature. Thus pituitrin did not give a secretion after stimulation of either of the secretory nerves despite the fact that it has a marked motor action on the blood vessels of the gland.

13. In contrast to the action of pituitrin subminimal doses of adrenalin which alone did not give a secretion may give a slight "augmented effect" in the activated gland.

14. Experiments with a plethysmograph revealed a shrinking in the volume of the submaxillary gland after stimulation of the secretory nerves and after histamine. As no smooth muscles could be demonstrated in this organ, other cytological structures, which might be responsible for these changes in volume as well as for the motor phenomena after histamine, have been discussed.

15. It was found that in certain experiments on cats in which the secretory action of histamine was poor this substance gave a slight fall succeeded by a marked rise in blood pressure instead of its usual effect.

16. Removal of the adrenal glands in these experiments abolished this rise of the blood pressure and considerably increased the secretory action of histamine after stimulation of the chorda. Similar results were observed in experiments on dogs.

17. Continuous intravenous injection of a dilute solution of adrenalin in these adrenalectomized animals again greatly diminished the augmented histamine secretion. The suggestion has been made that the failure to obtain a secretion with histamine in certain experiments may be due to its action on the adrenal glands and the subsequent secretion of adrenalin.

## PART III

1. Histamine injected intravenously in dogs stimulates a pancreatic secretion.

2. Unlike the salivary glands previous activation of the pancreas, by injection of acid into the duodenum, does not increase the histamine effect.

3. When histamine is injected intravenously during a pancreatic secretion evoked by pilocarpine, it produces a summation effect.

4. The intravenous injection of histamine in the rabbit produces an inhibition of the spontaneous pancreatic secretion characteristic of that animal.

5. Atropine in doses sufficient to paralyze the endings of the vagus nerve has very little effect on the pancreatic secretion produced by histamine.

6. The pancreatic juice secreted after histamine alone has an enzyme content somewhat similar to that secreted after acid. However, the character of the histamine juice secreted after a previous stimulus varies with the stimulus. Thus the histamine juice secreted after pilocarpine is similar to a pilocarpine juice having a high enzyme content. On the other hand, the juice secreted after acid has a composition as regards enzymes similar to the acid juice.

## PART IV

1. Histamine when injected intravenously in the whole animal activates a quite characteristic response in different parts of the intestine. Its effect is most marked in the ileum and decreases towards the duodenum.

2. Local application of a histamine solution to the outside of the gut also evokes the typical reactions in different portions of the intestine which are seen after intravenous injection of the drug.

3. After paralysis of the parasympathetic nervous system by atropine the action of histamine on the intestine is greatly diminished but not abolished. This inhibitory effect of atropine on the response to histamine is most marked in the duodenum and least in the ileum.

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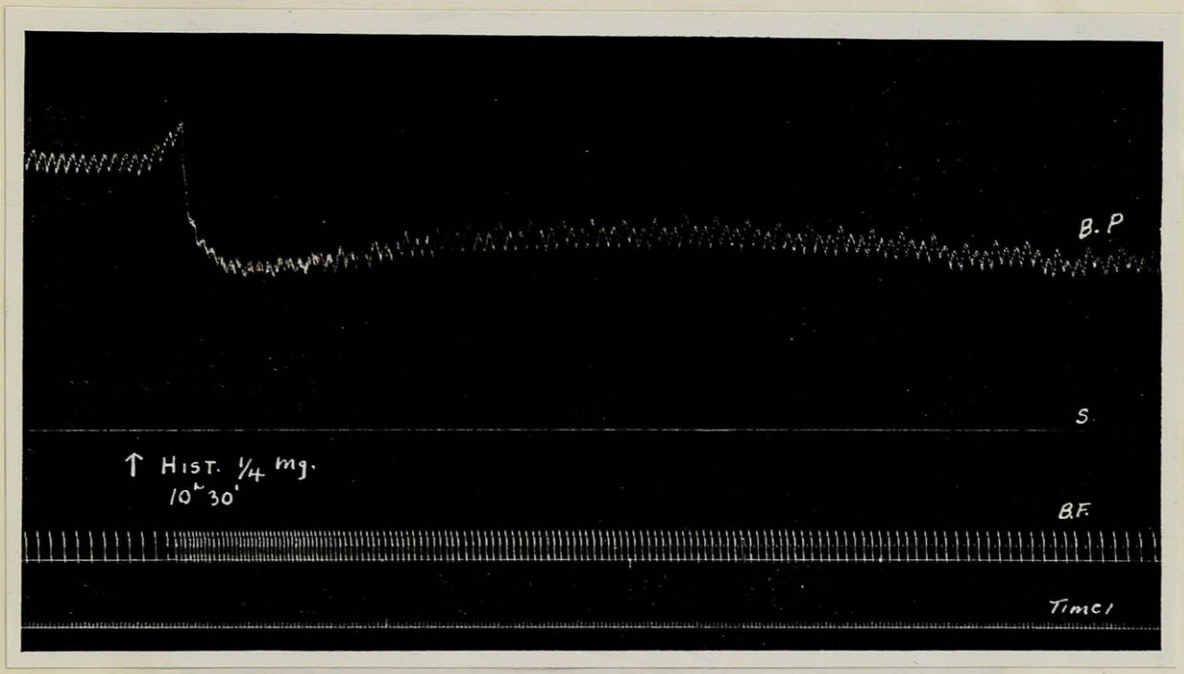


Figure 1. - Cat, chloralose, showing the effect of intravenous injection of $\frac{1}{4}$ mg histamine on the blood pressure and on the blood flow through the gland. In this figure as well as in Figures 2 and 3 the top line = blood pressure; second line = secretion; third line = blood flow; last line = time marker 1".

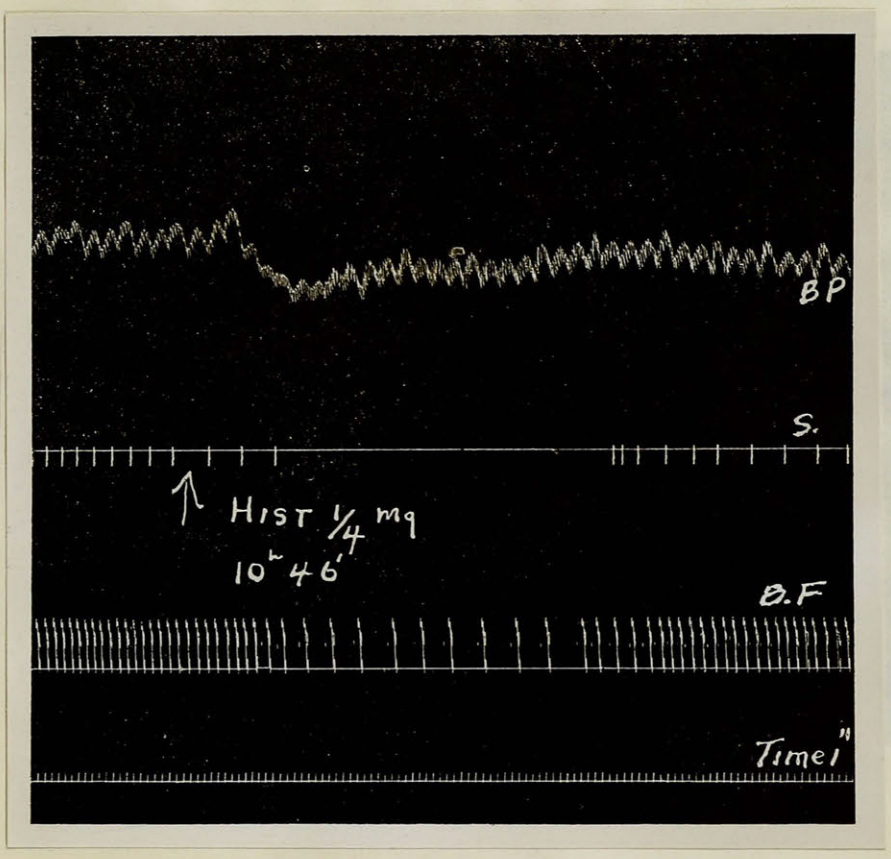


Figure 2. - Same experiment as Figure 1, showing the reversal effect of histamine after pilocarpine on the secretion and blood flow.

Top line = blood pressure; second line = secretion; third line = blood flow; last line = time marker 1".

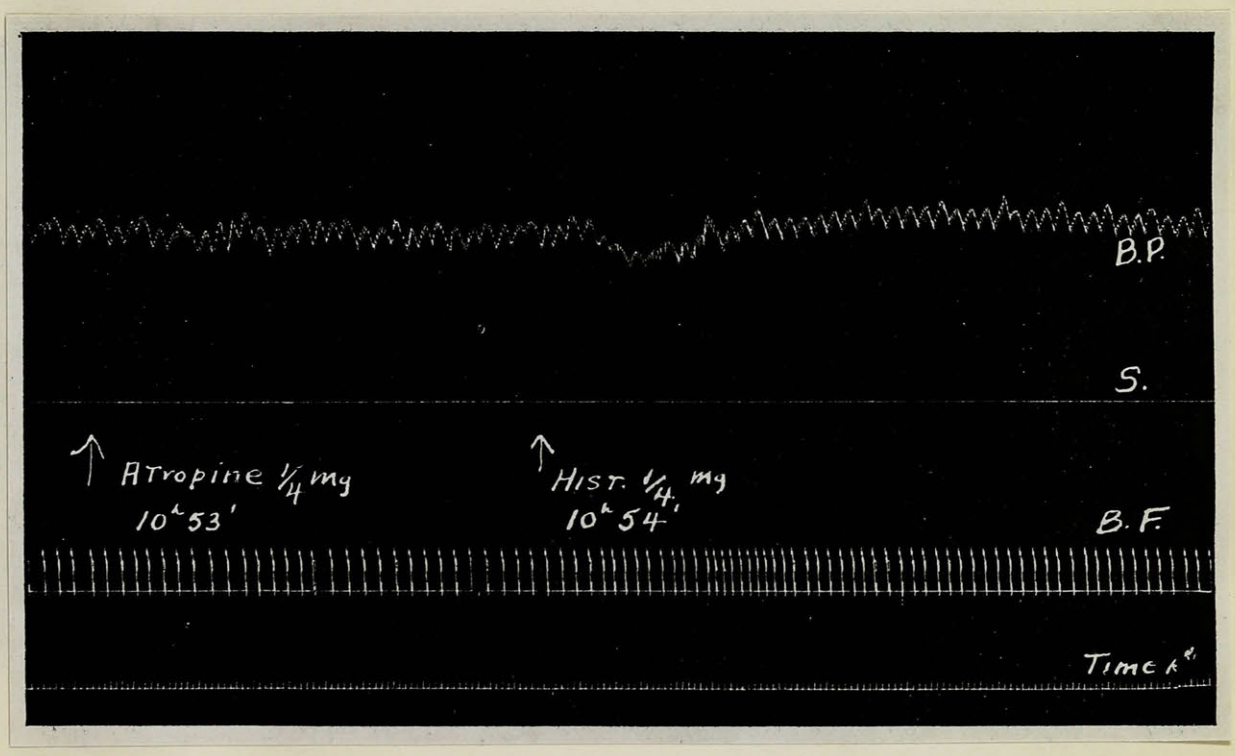


Figure 3. - Same experiment as Figure 1. In this figure the vaso-dilator effect of histamine is restored after atropine, although the acceleration in blood flow is less than in Figure 1.

Top line = blood pressure; second line = secretion; third line = blood flow; last line = time marker 1".

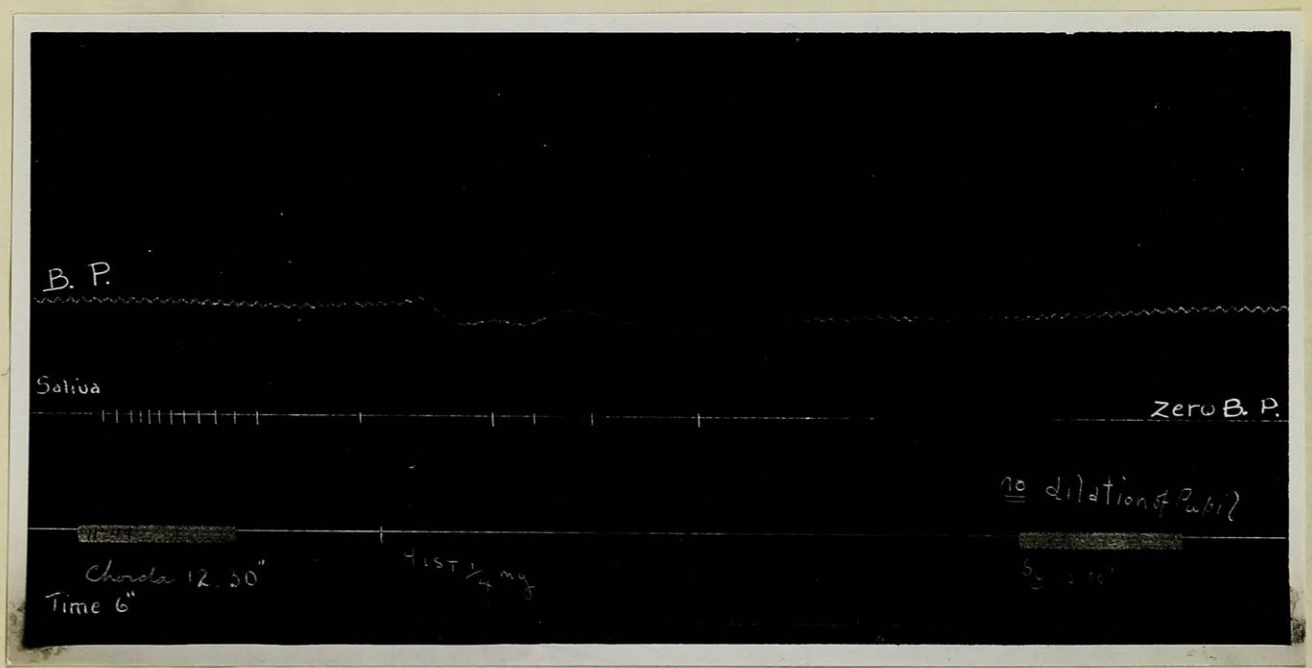


Figure 4. - Cat, chloralose, showing the action of histamine after ergotamine, which had been previously injected. Stimulation of the central end of the cervical sympathetic nerve did not produce any effect on the pupil or on blood pressure, thus showing that the nerve was paralyzed.

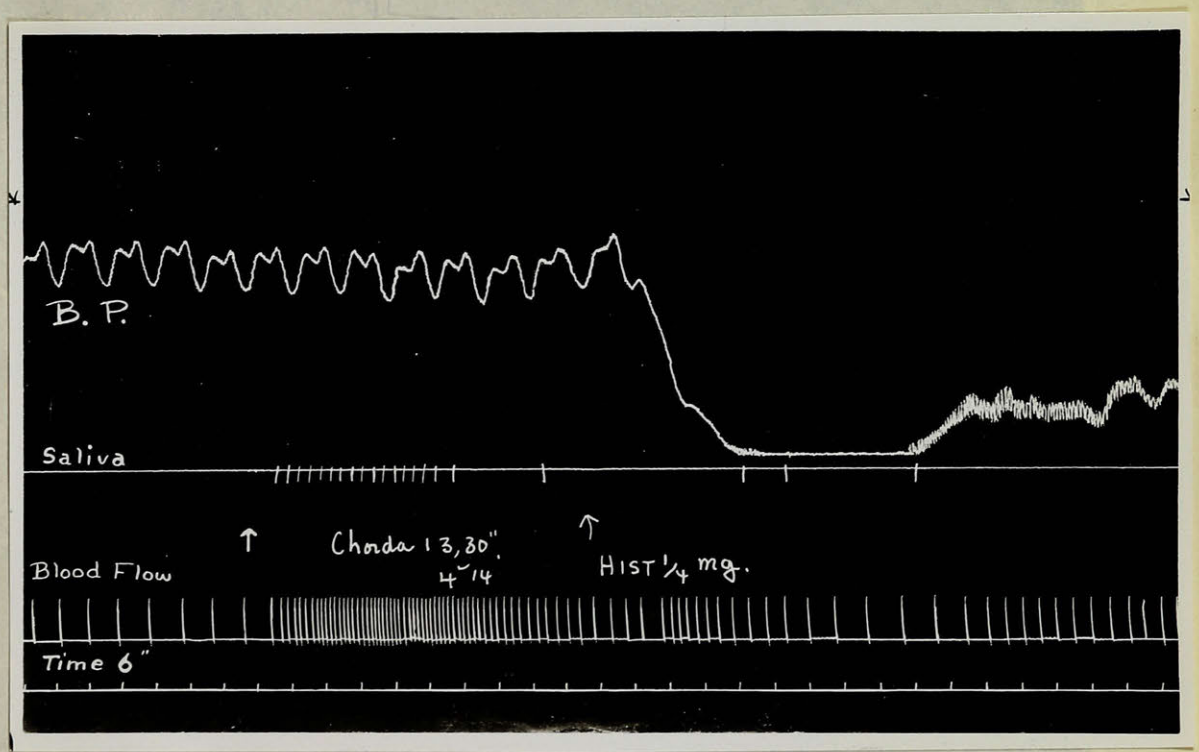


Figure 5. - Cat, dial, showing the effect of intravenous injection of histamine on secretion and blood flow when administered after previous stimulation of the chorda. Maeoski's method of measuring the blood flow through the gland was employed. The capsule of the gland was also cut open.

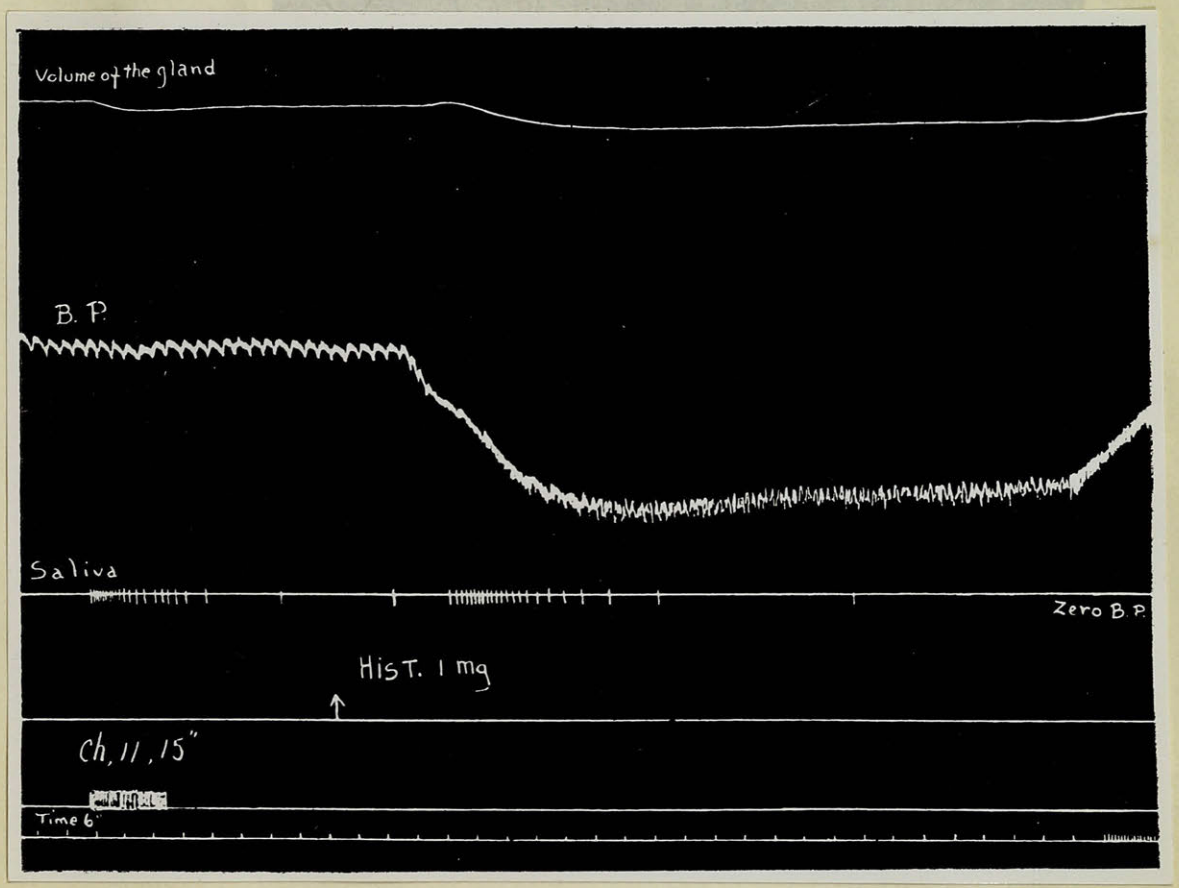


Figure 6. - Dog, dial. Plethysmograph of the right submaxillary gland showing the augmented effect of histamine after previous stimulation of the chorda.

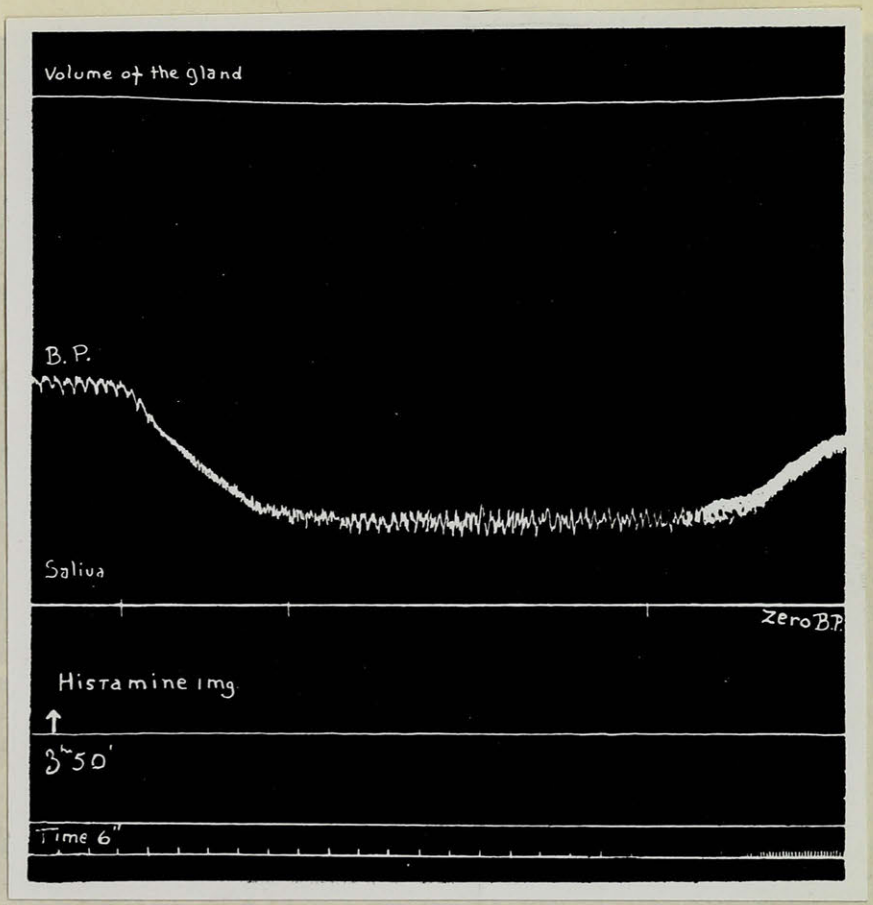


Figure 7. - Same dog as in Figure 6. Plethysmograph of the sub-maxillary gland showing the effect of histamine without previous stimulation of the chorda. Before the injection of histamine there was a slight spontaneous secretion of saliva.

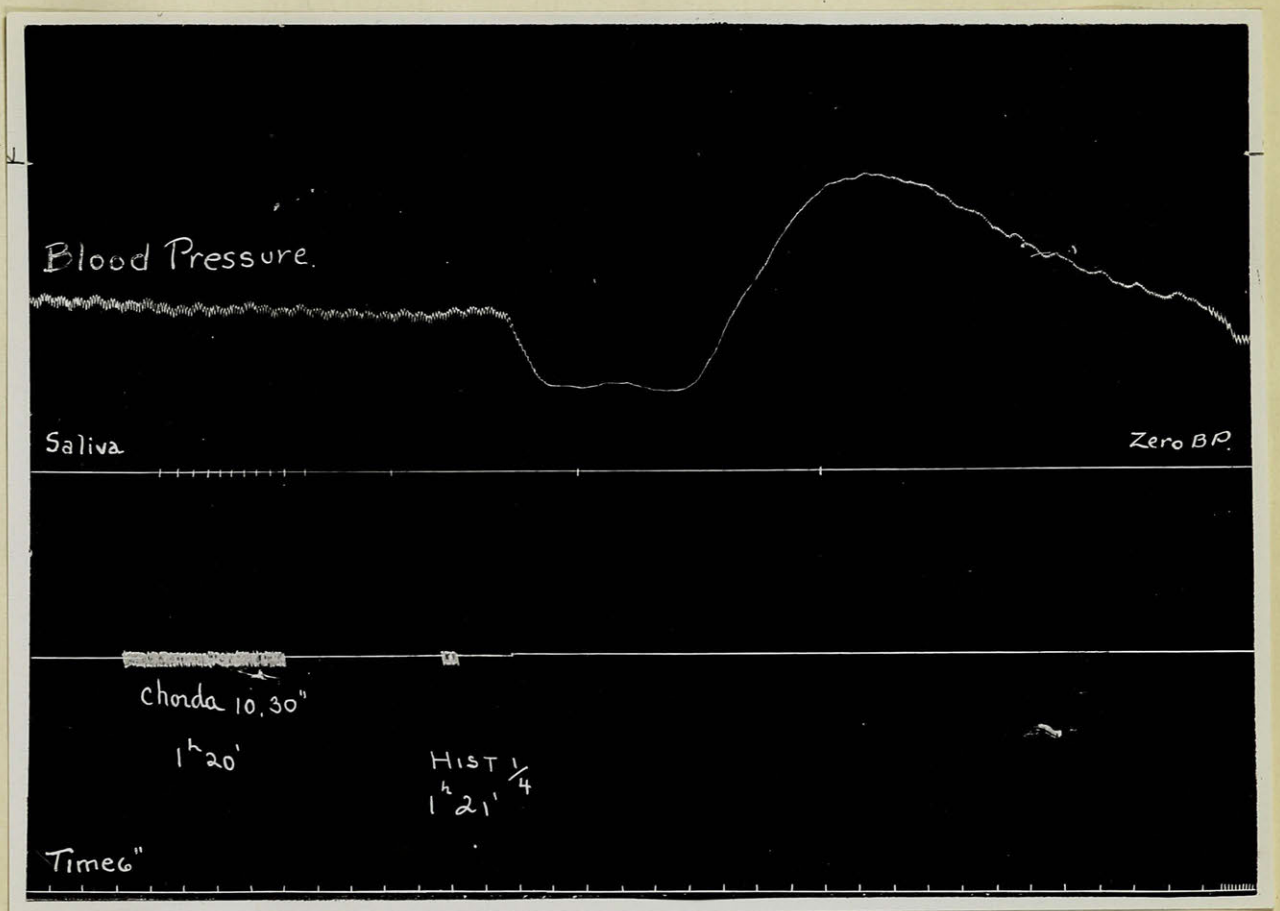


Figure 8. - Cat, chloralose, showing a rise in blood pressure after the injection of histamine. Note the poor augmented secretion.

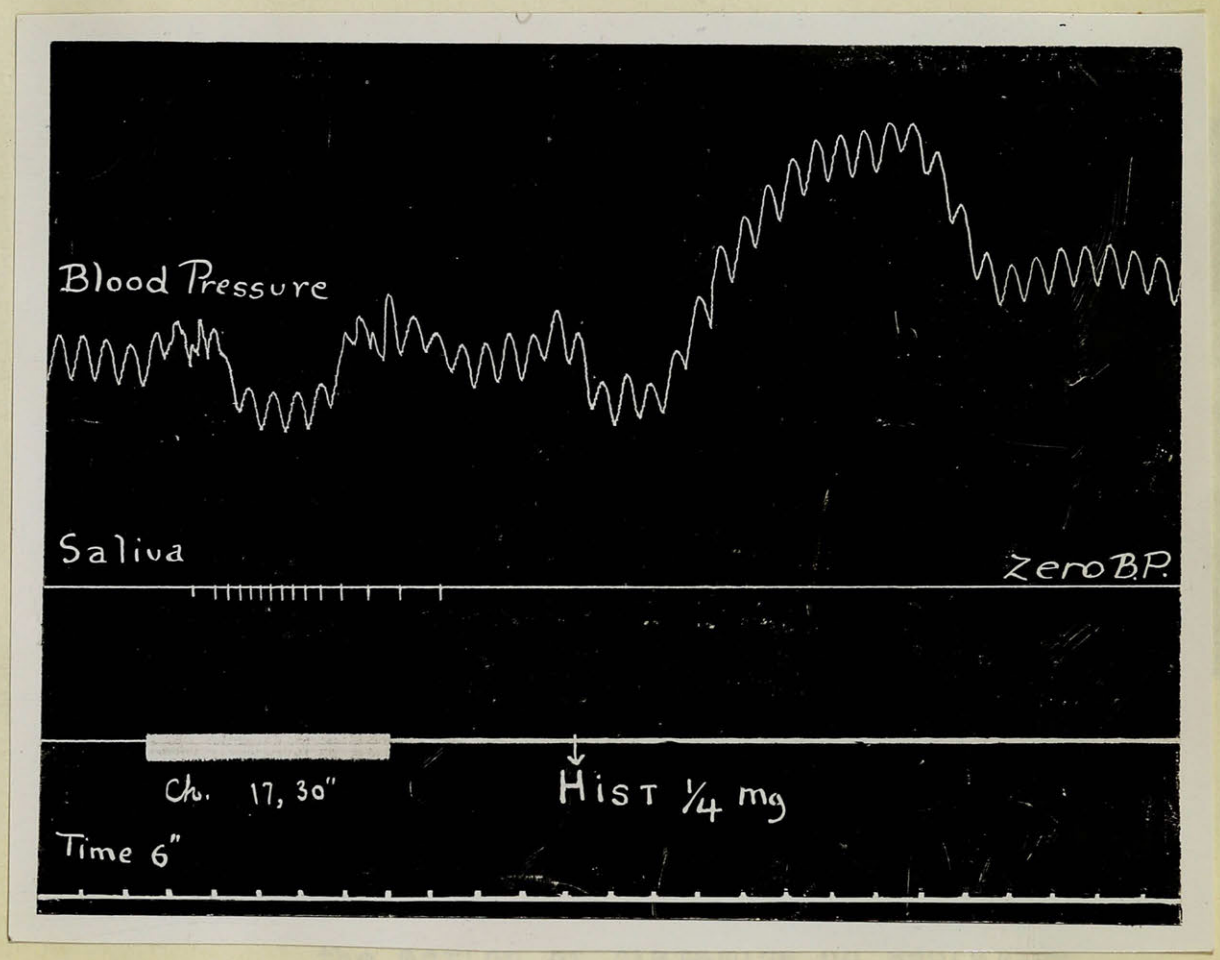


Figure 9. - Cat, decerebrate. This figure shows the effect of histamine before removal of adrenals. Note the rise in blood pressure and the absence of secretion.

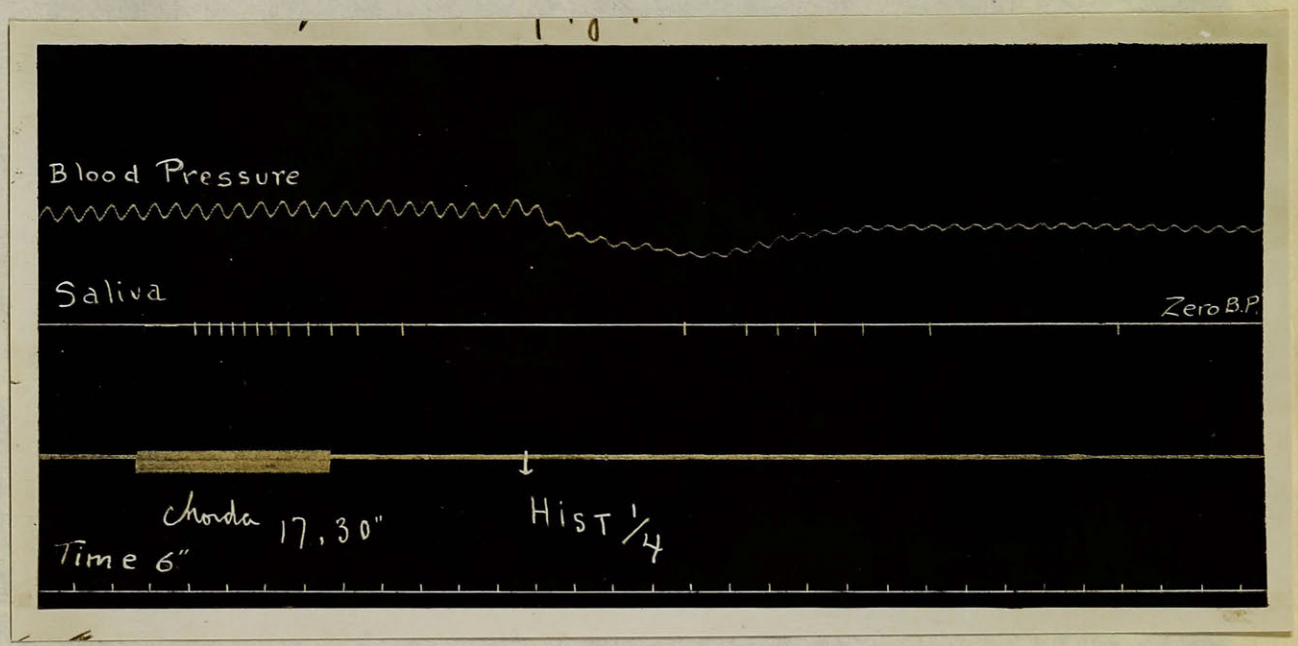


Figure 10. - Same cat as in Figure 9, showing the action of histamine on secretion and blood pressure following removal of the adrenals.

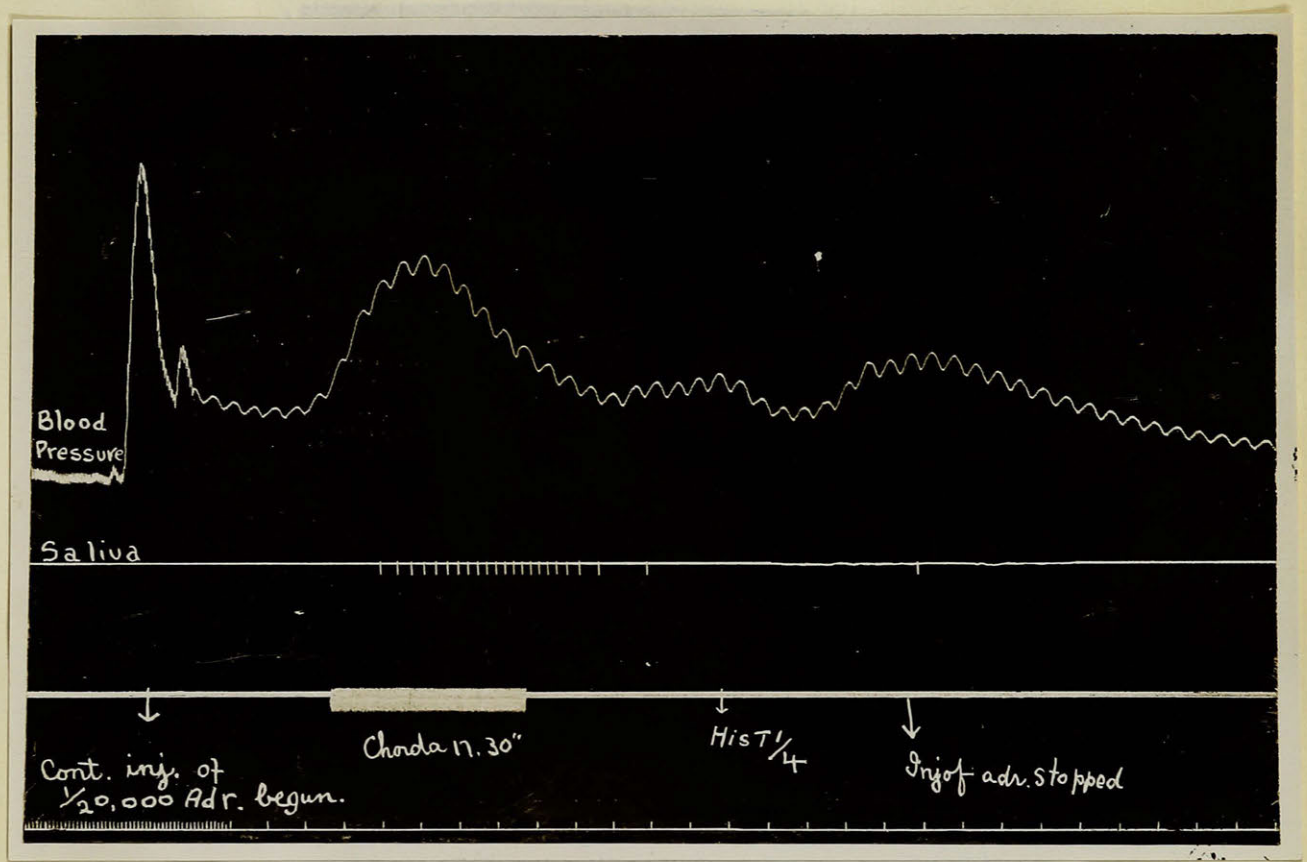


Figure 11. - Same cat as in Figures 9 and 10, showing the abolition of the secretory effect of histamine by adrenalin.

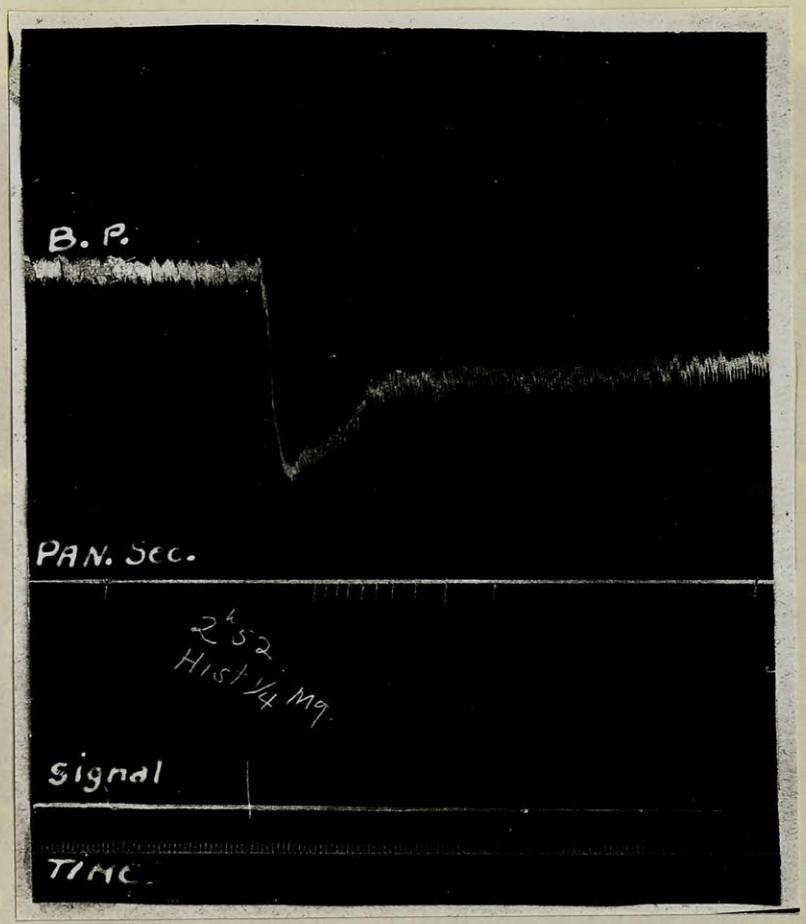


Figure 12. - Dog, chloralose and urethane, showing the typical action of histamine on pancreatic secretion.

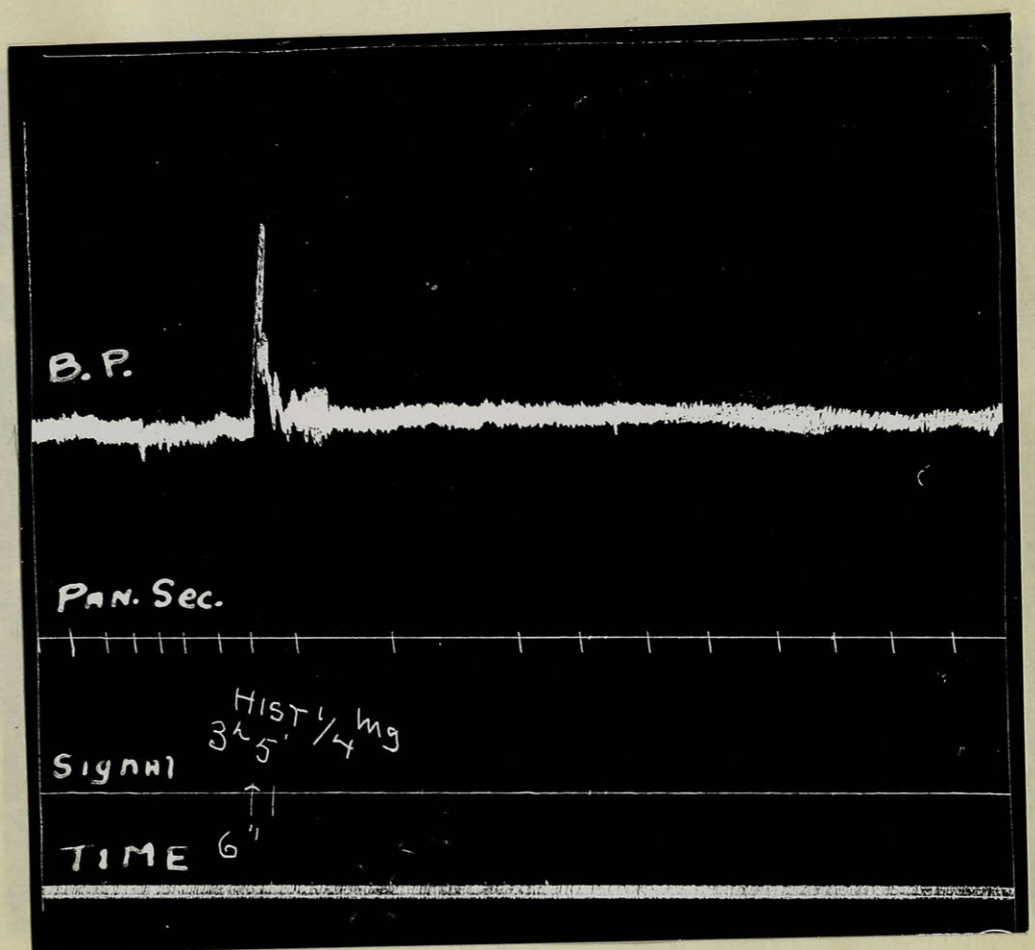


Figure 13. - Rabbit, urethane. Shows a different effect of histamine on pancreatic secretion from that observed in the dog in Fig. 12. Note the rise in blood pressure and the inhibition in the pancreatic secretion.

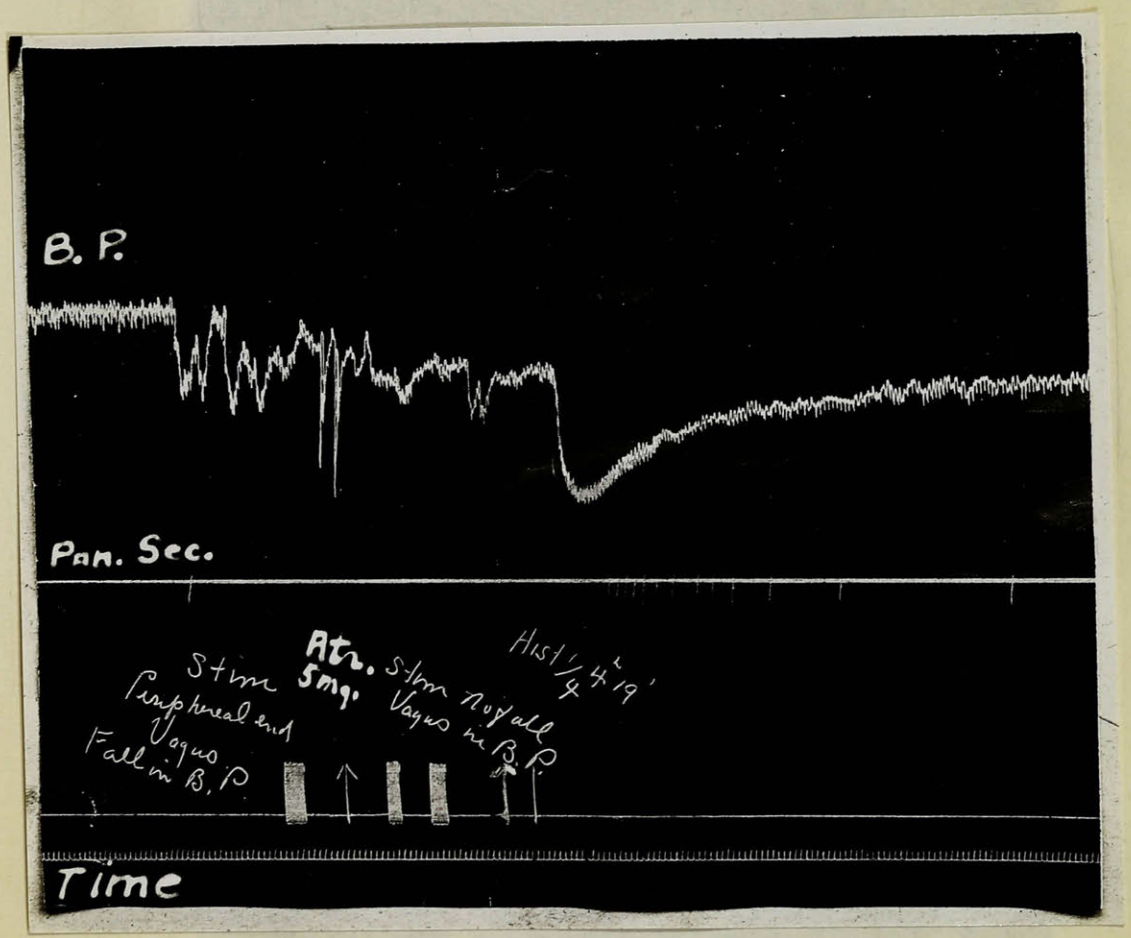


Figure 14. - Dog, chloralose and urethane, same experiment as Fig. 12. This figure shows that the action of histamine on pancreatic secretion is not abolished by previous injection of atropine.

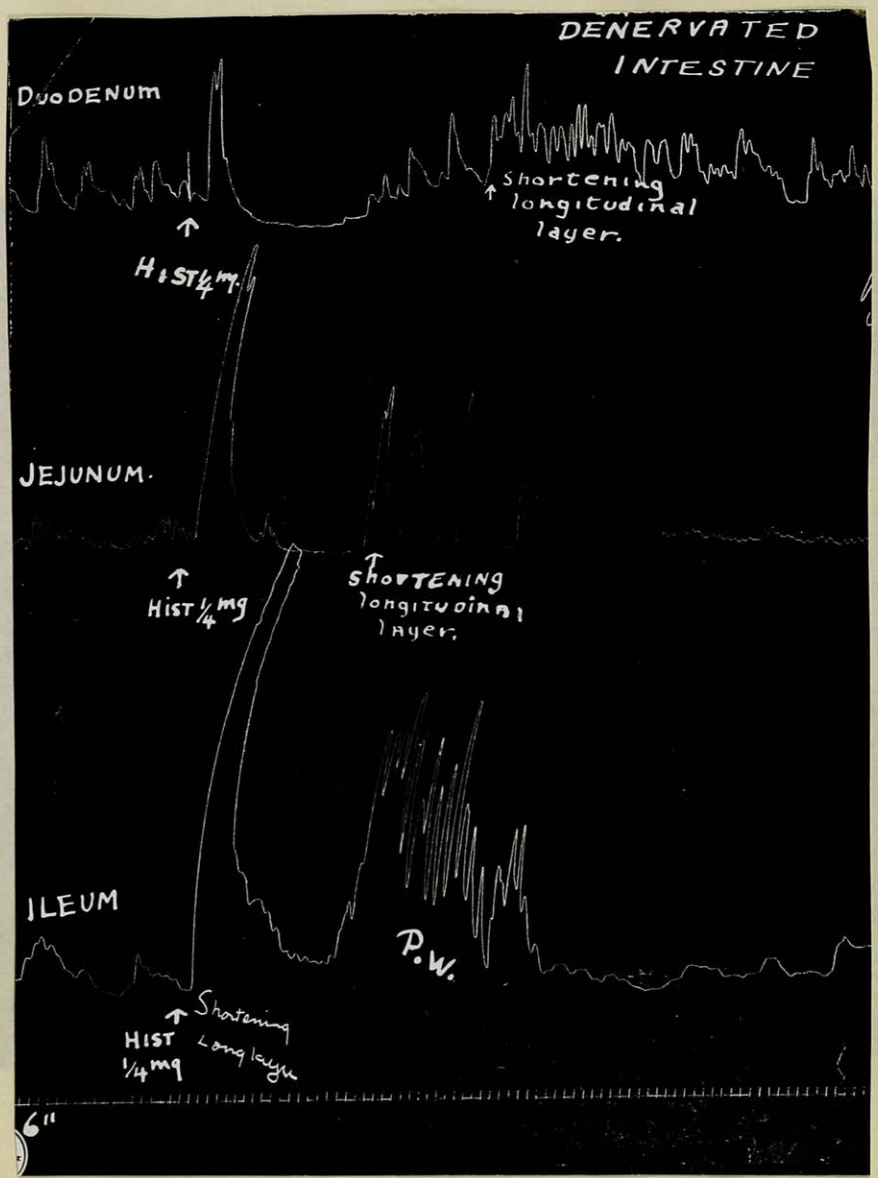


Figure 15. - Cat, chloralose and urethane, showing the different reaction of different parts of the denervated intestine to histamine injected intravenously.

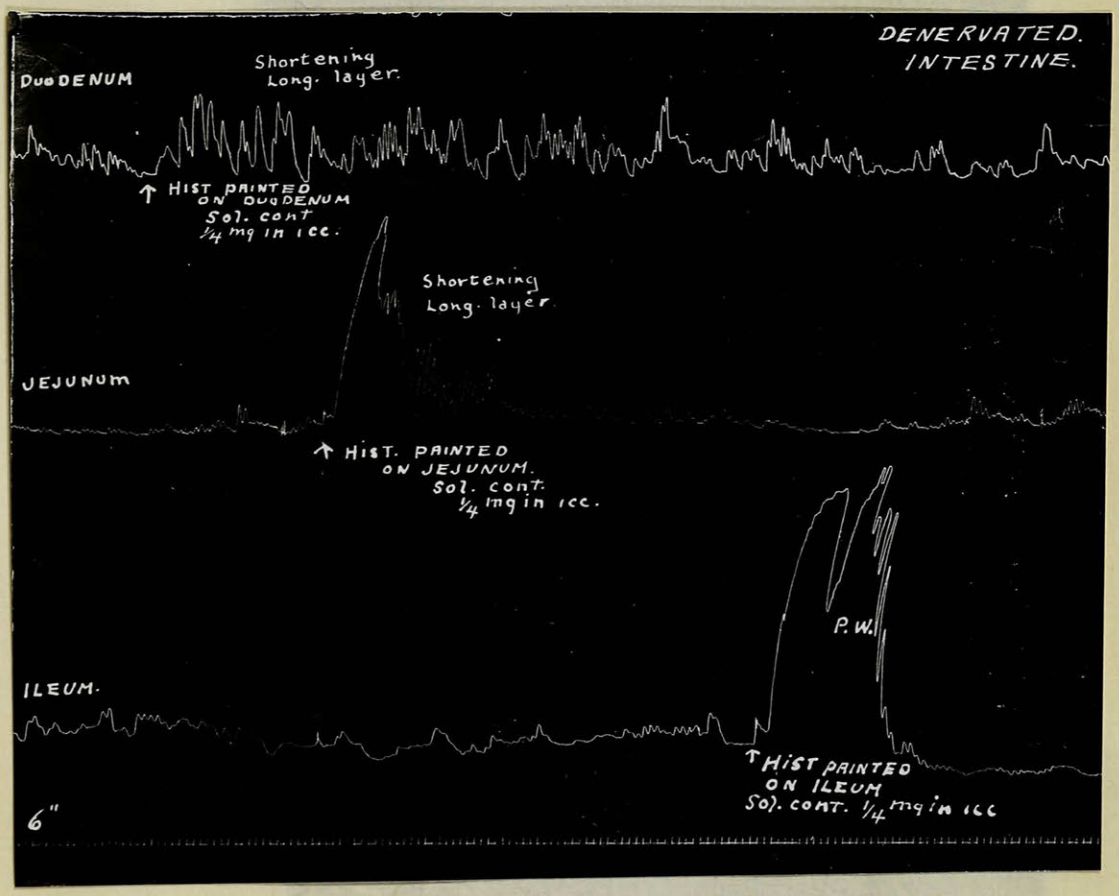


Figure 16. - Cat, same experiment as Fig. 15. This figure shows the typical reaction of different parts of the gut following local application of histamine to the outside of the intestine.

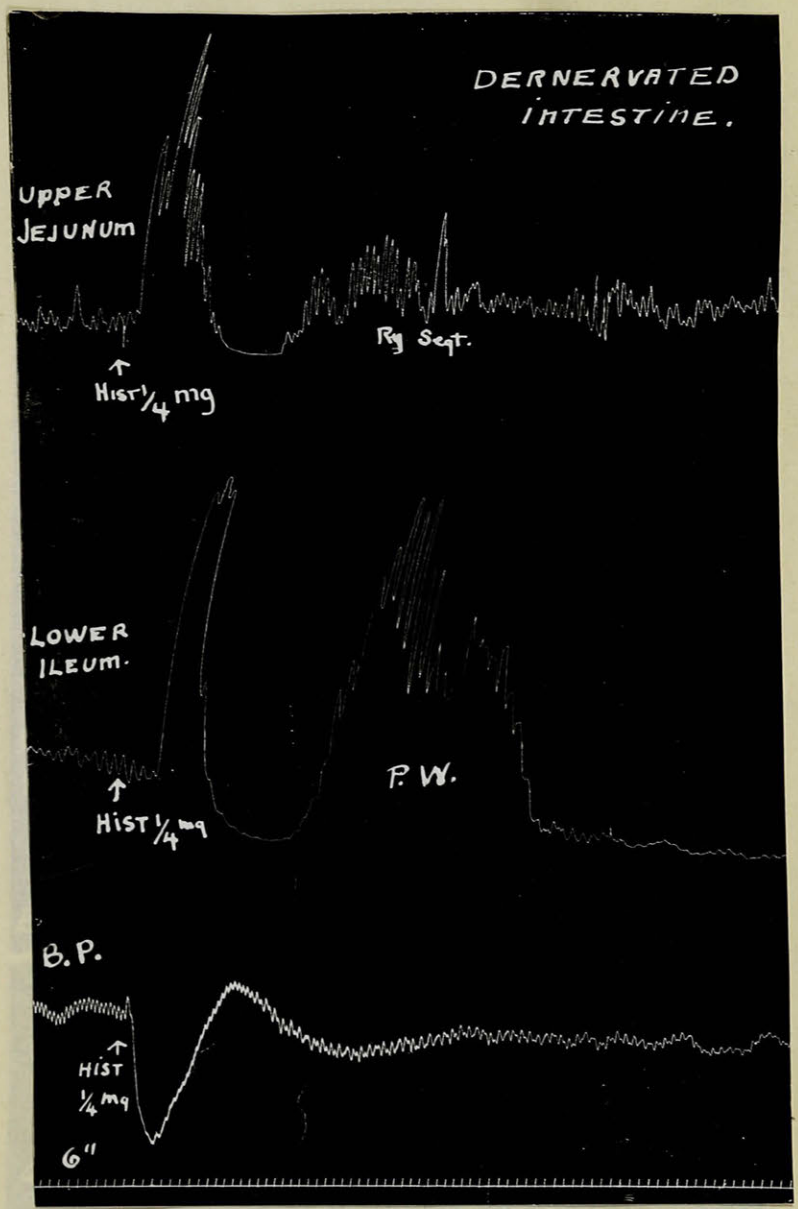


Figure 17. - Cat, chloralose and urethane. Shows the fall in the blood pressure and the typical response of the different portions of the intestine caused by histamine. This figure is taken after the splanchnics and vagi had been severed.

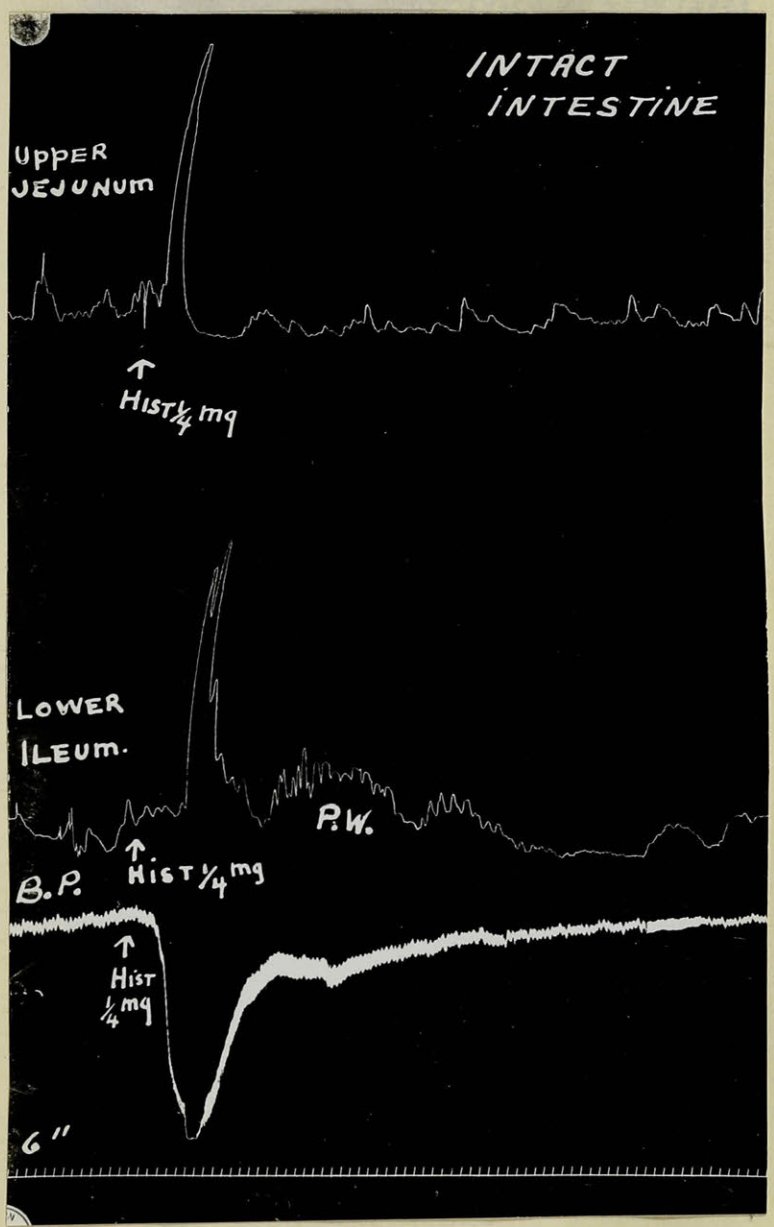


Figure 18. - Same experiment as Fig. 17, before the splanchnics and vagi had been severed. Note the diminished movements.

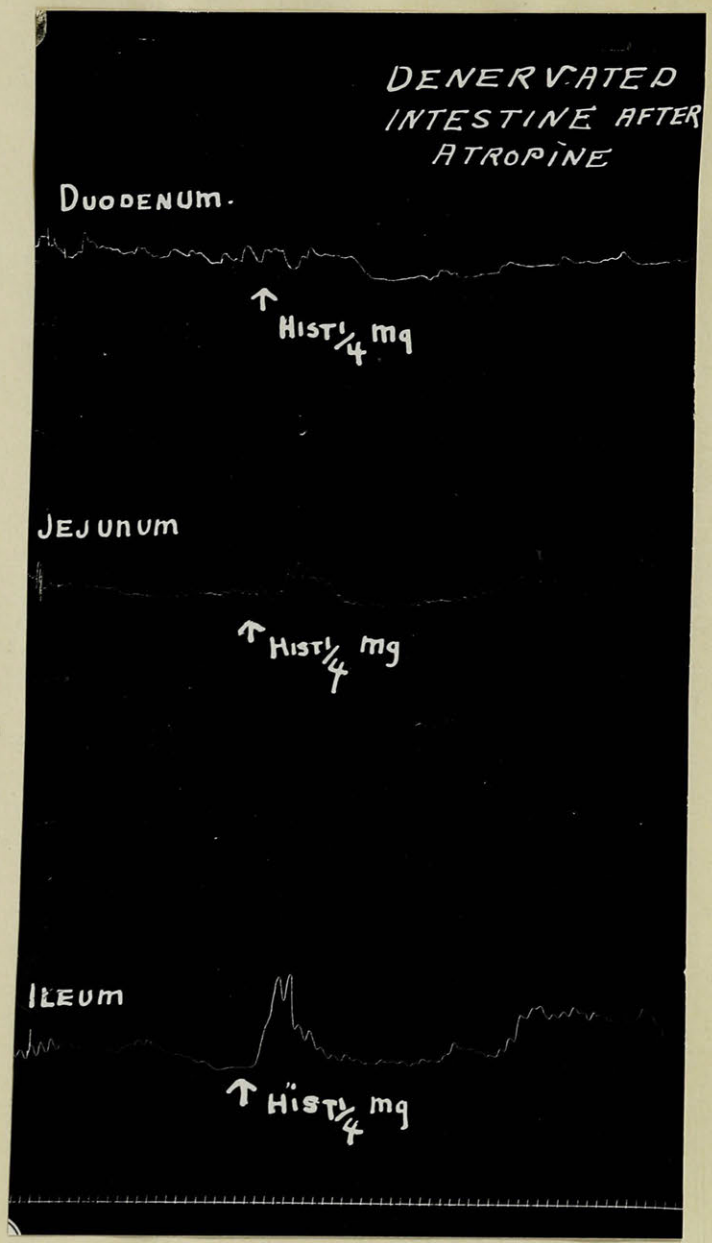


Figure 19. - Cat, chloralose and urethane. Same experiment as Figures 15 and 16, showing the decreased action of histamine after administration of 3 mg of atropine.