HUMORAL AND LOCAL HORMONAL MECHANISMS

THESIS

HUMORAL AND LOCAL HORMONAL MECHANISMS REGULATING THE ACTIVITY OF THE DIGESTIVE GLANDS

By

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ABSTRACT.

The digestive glands are activated by nervous and humoral stimuli: the latter include both specific digestive hormones and active substances derived from the food and absorbed into the blood; the former are now believed to act through the liberation of 'local hormones'. The significance of these various stimuli is discussed in the introduction.

I have investigated the mechanisms underlying certain phenomena of salivary secretion, viz. the 'augmented secretion' and the response of the 'paralytic' gland to sympathetic stimulation. These have been shown to depend not on a motor action of the sympathetic, but on an increased irritability of the secretory cells; and the theory is advanced that this increased irritability is due to a passage of acetylcholine from the alveolar to the neighbouring demilune cells. The choline-esterase content of the cat's submaxillary gland is unaffected by degenerative section of the chorda tympani.

An experimental study has been made of the humoral transmission of the effects of parasympathetic nerves. Evidence has been secured which supports the view that the effect of the lingual nerve and of the chorda tympani are mediated by the liberation of acetylcholine; similar experiments attempting to demonstrate a humoral transmission of the effect of the vagus yielded negative results.

In a study of the second, or 'chemical' phase of gastric secretion, it has been demonstrated that: (1) the nitrogenous basic extractives

extractives of fish muscle play an important part in the production of the second phase; (2) the activity of the gastric glands is increased by choline, a substance which is present in the diet and which may be of importance for the normal secretory activity of the stomach; (3) histamine is not, as has been supposed, the hypothetical 'gastric hormone'.

It could not be shown that histamine functions as a local hormone in the first or reflex phase of gastric secretion.

It has been shown that alcohol may readily become a conditioned stimulus for gastric secretion and so may evoke the secretion of a gastric juice of adequate digestive power.

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PART I. GENERAL INTRODUCTION.

That the activities of the glands of digestion are largely controlled by the nerves which supply them, was one of the first discoveries of modern physiology. The nervous mechanism of secretion, so clearly seen to be capable of excitation by highly specific stimuli, and of producing finely graded effects, appeared at first to be adequate for mediating all the responses of the digestive glands; and even so acute a worker as Pavlov (1) did not suspect the existence of any other sort of regulation. (The long familiar, and still mysterious stimulation of bile formation by the derivatives of cholic acid, normal constituents of the bile itself, appeared to be analogous rather to the increase in urine formation provoked by administration of the constituents of urine.) In 1902, however, Bayliss and Starling (2) obtained secretin from the mucous membrane of the duodenum, and subsequent workers showed that it was indeed, as its finders had asserted, a normal stimulant for pancreatic secretion, discharged into the blood on chemical excitation of the duodenal mucosa, and passing to and stimulating the acinar cells of the pancreas. This was the first discovered instance of a humoral regulation of the digestive glands: in the succeeding years such instances have been multiplied.

The secretin mechanism was also the first example of the coordination of distant organs by means of a specific substance liberated by one, and carried by the blood to the other. The existence /

existence of many more such chemical messengers - the "hormones" of Bayliss and Starling - has since been demonstrated; and much attention has been paid to the possibility that the activities of various other digestive glands are regulated by hormones. Thus secretin has been found to accelerate the formation of bile by the liver (perhaps indirectly through its action on the pancreas (3)), and to stimulate the secretion of Brünner's glands of the upper duodenum. The hypothetical hormones 'cholecystokinin' (4) and 'villikinin' (5), which are apparently liberated during digestion from the intestinal mucosa, and control the motility of the gall-bladder and the intestinal villi respectively, may also be mentioned. The gastric glands, too, are believed by many to be stimulated by a hormone, 'gastrin', discharged from the mucosa of the pyloric region when food is present there, and carried by the blood to the fundus. The evidence for the existence, as genuine hormones, of 'cholecystokinin', 'villikinin' and 'gastrin', is still inconclusive. For example, the activity of the gastric glands following the taking of a meal can certainly not be accounted for by nervous influences alone: some form of humoral excitation must be involved; but it has not been shown that the humoral stimulant is actually a specific hormone, rather than some substance or substances derived from the food. A similar objection may be raised to the acceptance of 'cholecystokinin' and 'villikinin'.

There is thus a second kind of humoral regulation to be considered, a regulation by secretagogue bodies which are absorbed as such from the food, as contrasted with regulation by specific digestive /

digestive hormones. For the participation of such a mechanism there is considerable suggestive evidence, and certain writers (e.g. Rasenkov (6) and Krimberg (7)) believe that this is the only way in which the taking of food can produce humoral stimulation. The active substances are probably not the ordinary products of hydrolysis of protein, carbohydrate and fat, since the fatty acids and sugars have no secretagogue action when injected intravenously, and the amino acids appear to act only in large doses, if at all; more probably they are highly active bodies which are present in small quantity in the free state or in labile combination. Especial attention has been directed to the nitrogenous basic extractives of food of animal origin, which include various physiologically active substances (8, 9). Nevertheless it has not been proved with certainty that any of these substances is present in the ordinary diet in effective quantities, or can during the digestion of a meal reach a concentration in the blood sufficient to activate the digestive glands.

A third type of humoral regulation is to be mentioned. This depends on the effect of non-specific substances which are related to general tissue metabolism, as distinguished from the special hormones and secretagogues whose presence in the circulation is a consequence of the taking of food. Among the phenomena of this class may be cited, to choose a few examples: the inhibitory effect on gastric secretion of lowered CO_2 tension of the plasma (Brown and Vineberg (10)); the stimulating effect of hypoglycaemia on the gastric secretion (Okada (11)); the stimulating effect of hypoglycaemia /

:glycaemia on the discharge of enzymes by the pancreas (LaBarre and Destrée (12), Hebb (13)); the increase in the excitability of the gastric glands following prolonged administration of a high protein diet (14); and perhaps the achlorhydria of pernicious anaemia. In many of these cases the mechanism involved is not purely humoral but, to use Okada's term, humoneural, the activity of the nervous centres for secretion being modified by changes in the composition of the milieu interne; in other cases it is the secretory elements themselves whose excitability is changed.

In all these three types of regulation, the cells of the digestive glands react to changes in the chemical composition of their environment. It was formerly customary to contrast sharply the 'chemical' type of control with nervous control, in which the stimulus for cellular activity was some subtle change of electrical forces at colloid interfaces. Modern researches have shown that the difference is not so fundamental as it seemed, since in either case the response depends on the liberation of definite chemical substances which can excite the cell: in nervous regulation, the chemical stimulants are liberated close to, or within the cells on which they act; in humoral regulation, the chemical stimulants are liberated in distant tissues, and are carried by the blood to their site of action. In view of this essential similarity, the chemical transmitters of nerve action have been referred to as 'local hormones'. The name is not a very happy choice, but none better is available, and I shall use it in this sense in the following pages.

The /

The difference between the local hormones and the 'true' hormones becomes less sharp, when it is considered that the former are not necessarily incapable of acting on structures distant from those in which they are set free. Thus sympathin, the adrenaline-like substance which mediates the effect of most post-ganglionic sympathetic ('adrenergic') fibres, normally passes into the circulation and may exert on distant organs effects similar to those produced by their sympathetic nerve supply. The action of acetylcholine, which appears to be the chemical transmitter of the effects of a variety of nerve fibres (post-ganglionic parasympathetic, some post-ganglionic sympathetic, pre-ganglionic sympathetic and parasympathetic, and somatic motor fibres: 'cholinergic' fibres), is normally restricted more narrowly to the region where it is set free. The reason for this is that the blood, and the tissues generally, contain a specific enzyme which rapidly inactivates acetylcholine by hydrolyzing its ester linkage. This enzyme can, however, itself be inactivated by administering eserine to the animal, and when this is done acetylcholine is destroyed only slowly in the body; stimulation of a parasympathetic nerve may then produce parasympathetic effects on other organs, as well as on the ones directly innervated. Such humoral transmission of the effects of nerves has been, in fact, the method by which much of the evidence for the existence of local hormones was obtained. In the other method which has been most often used, the local hormone is detected by assaying on biological reagents the venous blood or perfusate from a stimulated organ.

Both /

Both methods thus involve the passage of the local hormone from the cells in which it acts into the blood vessels, and therefore depend on the possibility of humoral transmission of the nervous effect.

Besides this transportation by the circulation, it is theoretically possible that the local hormone may pass by simple diffusion to cells near its site of liberation and exert its characteristic effect on these. Evidence that this may occur has been brought by Dale and Gaddum (15) from their experiments on the contracture of denervated voluntary muscle following stimulation of its vasodilator nerves, and by Parker (16) from his work on the nervous control of the chromophore cells of fishes.

There are other local hormones than those which transmit the action of specific types of nerve fibre. Histamine, as the mediator of the vascular response of skin to local injury, must be placed in this class; recent work has shown that histamine is liberated also in skeletal muscle as the local hormone of 'reactive hyperaemia' (17) and of the vasodilatation which accompanies activity (18). Adenosine has also been asserted to cooperate in the production of hyperaemia in muscle (19). The evidence for a variety of other hypothetical local hormones, such as the 'heart hormone' promoting the rhythmic activity of cardiac muscle (20, 21), is extremely unconvincing.

Direct evidence for the participation of local hormones is rather meagre. For the submaxillary gland it has been shown (22, 23), and abundantly confirmed, that stimulation of the chorda tympani /

tympani causes an acetylcholine-like substance to pass into the circulation; this substance can be recovered in a quantity sufficient to account for a large part of the gland's secretory activity (24). It has not yet, however, been shown that a similar mechanism operates in any of the other salivary glands, or in the pancreatic gland. Dale and Feldberg (25) observed that vagal stimulation increased the rate at which acetylcholine was liberated by the stomach, but did not determine whether the acetylcholine was set free by the glands, musculature or blood vessels. That sympathetic stimulation causes the discharge of sympathin has been demonstrated for the salivary glands (26), but not for the other glands of digestion, in which indeed (except for the mucous cells of the gastric surface epithelium (27)) no direct secretory action of the sympathetic has been established. The action of drugs and the analogy with other parasympathetic nerves do, of course, strongly suggest a cholinergic innervation for the salivary glands, the mucous cells of the oesophagus, the parietal, peptic and mucoid cells of the stomach, and the acinar cells of the pancreas. The glands of the intestinal mucosa, which have no close relation to the vagus but respond to mechanical and local chemical stimuli, may possibly be controlled by a local hormone which is not acetylcholine. Finally it may be conceived that histamine, since it occurs in the gastric mucosa in high concentration, and is a powerful stimulant for the parietal cells, may be involved in the chemical transmission of the secretory effect of the vagus.

This /

This brief survey of the humoral and local hormonal mechanisms regulating the digestive glands will serve as an introduction to the experimental part of this work. To give, within the limits of a thesis, a comprehensive review of even the more important contributions in these fields, is obviously impossible.* The problems with which I have been myself concerned, although they belong in this general category are not all closely related. For this reason it seems unwise to try to present, in a single introductory statement, a summary of all the relevant literature. I shall therefore preface the various sections with an account of the previous experimental work dealing with the respective problems.

The experimental work falls into five main parts, which I shall now enumerate.

(1) Studies in salivary secretion. As a consequence of the new light shed on the normal mechanisms of glandular activity by the development of theories of chemical transmission, a re-investigation of certain phenomena of glandular secretion appeared to be desirable. Two such phenomena exhibited by the salivary glands, the so-called 'augmented secretion' and the peculiar behaviour of the submaxillary gland deprived of its parasympathetic innervation, have been investigated. In continuation of the latter work /

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* The literature on humoral regulation of the digestive glands has been reviewed by Babkin (28, 29) and by Ivy (30). Gaddum (31) has recently published a comprehensive summary of our present knowledge of local hormone regulation.

work, a study has been made of the relation of the choline-esterase content of the submaxillary gland to the presence of an intact parasympathetic nerve supply.

(2) Studies on the humoral transmission of the effects of parasympathetic nerves. Much of the evidence for the chemical transmission of nervous action has been obtained from experiments in which the stimulation of a nerve could be shown to affect organs other than those which it directly supplied. I have tried by the use of such methods to establish more firmly the hypothesis of chemical transmission for certain organs.

(3) Studies on the second phase of gastric secretion. A series of problems relating to the mechanism of the second or humoral phase of gastric secretion have been investigated. (a) A study has been made, with the aid of both chemical and physiological methods, of the extent to which the secretagogue activity of fish muscle can be accounted for by its organic basic constituents. (b) The effect of choline on gastric secretion was studied: since this substance is widely distributed in foodstuffs and possesses physiological activity, it was of interest to know how far, if at all, it might be involved in the initiation of the second phase. (c) It has been suggested that 'gastrin', the hypothetical hormone of the second phase, is identical with histamine. I have tested this theory by determining the histamine concentration of the blood before and after the taking of a meal.

(4) The effect of vagal stimulation on the histamine content of /

of the gastric venous blood. If, as has been suggested above, the secretory action of the vagus on the parietal cells is mediated by histamine, proof of this might be supplied by showing that the liberated histamine set free by vagal stimulation would appear in the venous blood of the stomach. This possibility has been examined in acute experiments on dogs.

(5) Alcohol as a conditioned stimulus for the gastric glands. This is a study of the effects of dilute alcohol on the gastric secretion, and of the possible modification of these effects through the influence of the central nervous system. Since it is therefore a study of the integration of nervous and humoral stimuli, it has seemed proper to include it in this thesis.

PART II /

PART II. STUDIES IN SALIVARY SECRETION.

I. THE EFFECT OF ATROPINE ON THE AUGMENTED SALIVARY SECRETION IN THE CAT.

Introduction.

As I have pointed out in the general introduction, the modern conception of the chemical transmission of the nervous impulse makes desirable a re-interpretation of many well-known facts of glandular activity. This statement appears especially applicable to certain phenomena which reveal an interaction of different nerves on the same secreting organ. Two of the most striking examples of this class have to do with the salivary glands. The first of these is the so-called 'augmented secretion'; the second is the peculiar effect of degenerative section of the parasympathetic supply. The latter phenomenon will be dealt with in detail in the following section. The present section describes an attempt to gain a clearer understanding of the intimate mechanism of the augmented secretion.

The name 'augmented secretion' is due to Langley (32), who observed in 1889 that the volume of saliva secreted by the submaxillary gland of the dog in response to sympathetic stimulation is greatly increased by previous excitation of the chorda tympani. He ascribed the effect to an increased irritability of the secretory cells persisting after stimulation of the chorda. This interpretation has been criticized by Mathews (33) and especially /

especially by Anrep (34), who consider the phenomenon due merely to an expression of saliva from the intra-glandular ducts by some contractile mechanism under sympathetic control. Mathews would even deny any true secretory innervation by the sympathetic. The experiments of Goldenberg (35), in Babkin's Odessa laboratory, suggest that the Mathews-Anrep explanation is inadequate. This worker found that the secretory response to stimulation of either nerve is increased by any previous excitation of the gland: there are thus four types of augmented secretion, viz. sympathetic after chorda, chorda after chorda, chorda after sympathetic, and sympathetic after sympathetic. Further support of the original view of Langley was furnished by Babkin and McLarren (36) and Babkin and MacKay (37), who have concluded that both of the mechanisms suggested are probably involved, namely, expression of saliva from the ducts owing to the activity of some contractile structure (presumably the myo-epithelial cells), and increased irritability of the glandular tissue: in so far as the latter factor is effective, there is a 'true augmented secretion.'

Added interest is lent to this question by the growing tendency to regard each of the various glandular organs as composed of several types of structural units differing in their mode of activation (cf. Babkin (35)). Thus Rawlinson (39), whose histological material was obtained from very carefully controlled physiological experiments, finds that in the cat's submaxillary gland, stimulation of the chorda tympani produces changes in the mucous cells, while stimulation of the sympathetic produces changes in /

in the demilune cells; the action of each nerve being confined to the one type of cell. Following this conception, one might explain the 'true augmented secretion' as due to the passage, from one to the other set of cells, of some product of secretory activity, or, more probably, of a local hormone. Before adopting such a view, however, one must have conclusive proof of the existence of a true augmented secretion, as distinguished from a purely mechanical expulsion of saliva: the hitherto available evidence, although strong, cannot be considered final.

In this connection my attention was drawn to some observations of Langley (32) on the effect of atropine in various doses on the augmented secretion in the dog. After large doses of atropine, stimulation of the chorda evoked no secretion, and failed to augment the secretory power of the sympathetic. Small doses greatly diminished the secretory activity of the chorda, but had comparatively little effect on its augmentor activity. While Langley cited these results in support of his own theory for the origin of the augmented secretion, it is clear that they might also be explained, on the basis of the Mathews-Anrep view, by a motor action of the sympathetic. I therefore decided to re-investigate the effect of atropine on the augmented secretion, in order to see whether, with suitable doses of the drug, stimulation of the chorda might still increase the sympathetic secretion, while evoking no flow of saliva itself. The experiments now to be described show that this may in fact occur.

Experimental.

Cats under dial anaesthesia were used in all the experiments. The chorda tympani and cervical sympathetic were cut and taken on ligatures for stimulation, the strength of the current and the position of the stimulating electrodes being constant throughout each experiment: undue cooling and drying of the exposed nerves was carefully avoided. As large a cannula as possible was inserted into the submaxillary duct, and connected to a graduated glass tube of narrow bore lying horizontally at the level of the gland; by this means a salivary flow of 0.005 c.c. could easily be detected. In several experiments the secretion was registered by means of Gibbs' drop-recorders and the blood-flow through the gland simultaneously recorded by Maevsky's technique (40).

(An alternative method was devised for recording the blood-flow through the submaxillary gland in the heparinized animal: it worked very well in the two experiments in which it was used, but was discarded on account of the high cost of heparin. The jugular vein was dissected out, and all its tributaries ligated with the exception of the one (or two) draining the submaxillary and sub-lingual glands, so that blood from these structures alone entered the jugular. A cannula was then inserted into one of the large branches of the jugular (in this case v. posterior facialis), the mouth of the cannula pointing toward but not quite reaching the jugular itself: the cannula was connected by means of a rubber tube, filled with 0.9 per cent NaCl, to a Gibbs' drop-recorder. When the blood-flow through the gland was to be recorded, the cannula /

cannula was pushed forward until it entered the jugular, which was clamped centrally: the blood flowing through the gland then passed out by the cannula and operated the drop-recorder. When the record was finished, the jugular clamp was released, the cannula was withdrawn to its original position, disconnected, and rinsed by the injection of a little saline; the valves at the mouth of the posterior facial prevented any reflux of blood through it, although the operation was repeated several times. The method appears to be an excellent one when the submaxillary blood-flow of the cat is to be measured at intervals during a long experiment. The loss of blood is minimized as compared to Maevsky's method, and no complicated apparatus, requiring special calibration, is required, as with the methods of Rein and Gesell.)

Atropine sulphate was injected in doses usually of $\frac{1}{8}$ mg., until strong and prolonged faradization of the chorda caused no movement of the fluid in the graduated tube. If an additional small dose was then given, the complete paralysis of the chorda secretion lasted from 1 to 3 hours, and recovery, when it did occur, was very slow. The dose required varied from 0.07 to 0.15 mg. per Kg. In a certain proportion of the animals the chorda secretion was not entirely abolished by such doses of atropine, although it was very greatly reduced: further atropinization then paralyzed both the secretory and the augmentor action of the chorda. The majority of the animals, however, gave results of the type described below.

Throughout each experiment the sympathetic was stimulated at constant /

constant intervals (4 to 8 minutes in different experiments) for periods of 1 minute. When the secretory response became constant, or, as more often happened, showed a steady decline, the chorda was stimulated, either for the 60 seconds just preceding the sympathetic stimulation, or for 30 seconds just preceding the sympathetic stimulation, or for 30 seconds, with an interval of 30 seconds' rest before the sympathetic stimulation. (The latter procedure eliminates a possible augmentor effect of the chorda vasodilatation, which passes off quickly when stimulation is stopped.)

Figure 1 illustrates a typical experiment. The sympathetic was stimulated at 6-minute intervals for periods of 1 minute. The vertical columns represent the volume of saliva secreted with each stimulation. The chorda, which produced no secretion throughout, was stimulated for the 60 seconds just preceding every third sympathetic stimulation. The augmenting influence of the chorda can be clearly seen; in some instances it appears to extend to the second subsequent sympathetic stimulation, a fact often noted.

Exactly similar results were obtained by substituting injection of adrenaline for stimulation of the sympathetic. In the experiment summarized in Fig. 2, 1 c.c. of a 1:10,000 solution of adrenaline chloride was injected at intervals of 8 minutes. The chorda stimulations, none of which caused any visible secretion, were applied for 3 minutes, and were followed by 1 minute's rest before the next injection of adrenaline.

Results /

(To follow page 16.

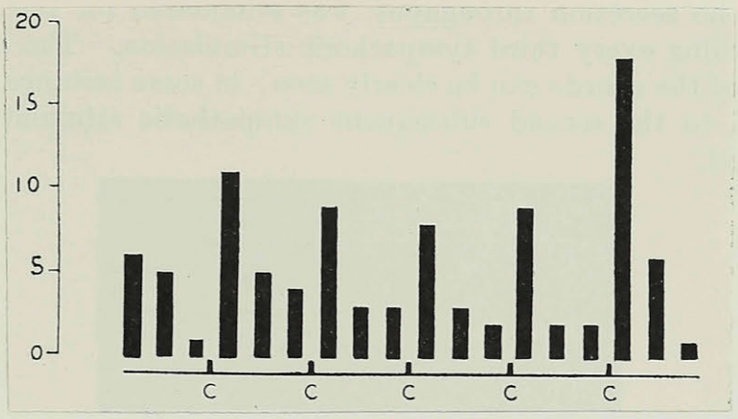

FIGURE 1. - The sympathetic nerve was stimulated for periods of 1 minute at 6-minute intervals. The vertical columns represent the submaxillary secretion in divisions of the graduated tube (1 division = 4.5 c.mm.). The points marked C on the lower line indicated that the chorda, which alone produced no secretion, was stimulated for the 60 seconds just preceding the sympathetic stimulation.

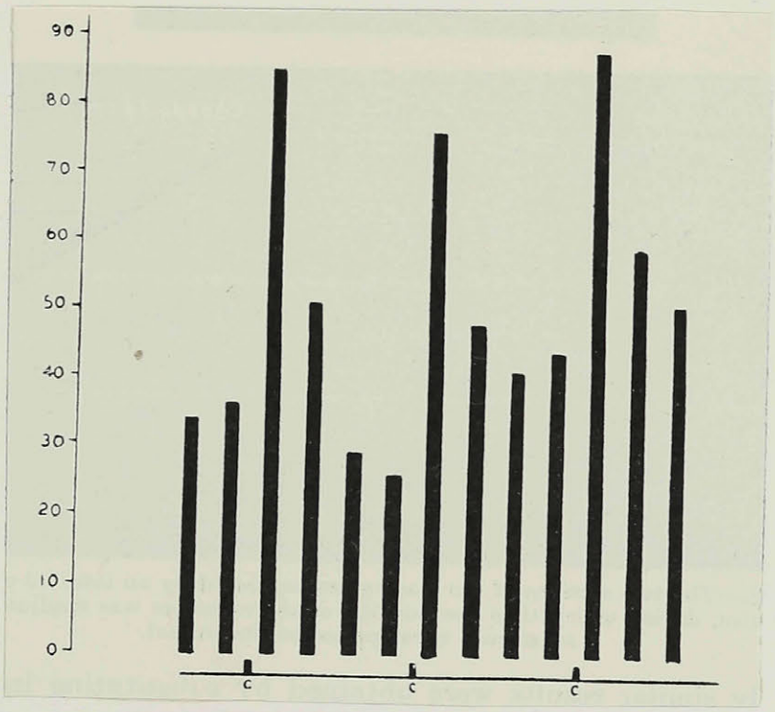

FIGURE 2. - 1 c.c. of 1:10,000 adrenaline solution was injected at 8-minute intervals. The vertical columns represent the salivary secretion in divisions of the graduated tube (1 division = 4.5 c.mm.). The points marked C on the lower line indicate that the chorda, which alone produced no secretion, was stimulated for 3 minutes, with 1 minute's rest before the next injection of adrenaline.

Results analogous to these were obtained in 5 experiments with sympathetic stimulation, and in 8 experiments with adrenaline. In each of these the chorda augmentation was observed several times before the chorda itself recovered its secretory power.

The latent period of the adrenaline (or of the sympathetic) secretion was definitely shortened by previous stimulation of the atropinized chorda (cf. Fig. 3).

When the chorda was stimulated just before the end of a secretion produced by the sympathetic or by adrenaline, the salivary flow was markedly accelerated, although previously (and subsequently) the chorda alone was quite ineffective in producing any flow of saliva (Fig. 3). This temporary recovery of the secretory power of the chorda continues not longer than a minute or two after the adrenaline secretion has stopped; not more than a drop or two of saliva can be expressed in this way by chorda stimulation, the gland ceasing to secrete although the excitation is continued. A similar effect is seen when the chorda is stimulated during the first stages of recovery from atropine: there is a scanty secretion, lasting less than half a minute; after a minute's rest, a second stimulation produces a still smaller secretion; while subsequent stimulations are ineffective.

Simultaneous records of blood-flow and secretion showed: (1) that stimulation of the atropinized chorda does not affect the vascular action on the gland of a subsequent injection of adrenaline; and (2) that the secretion provoked by adrenaline is at its height during the preliminary phase of vasoconstriction, and slackens /

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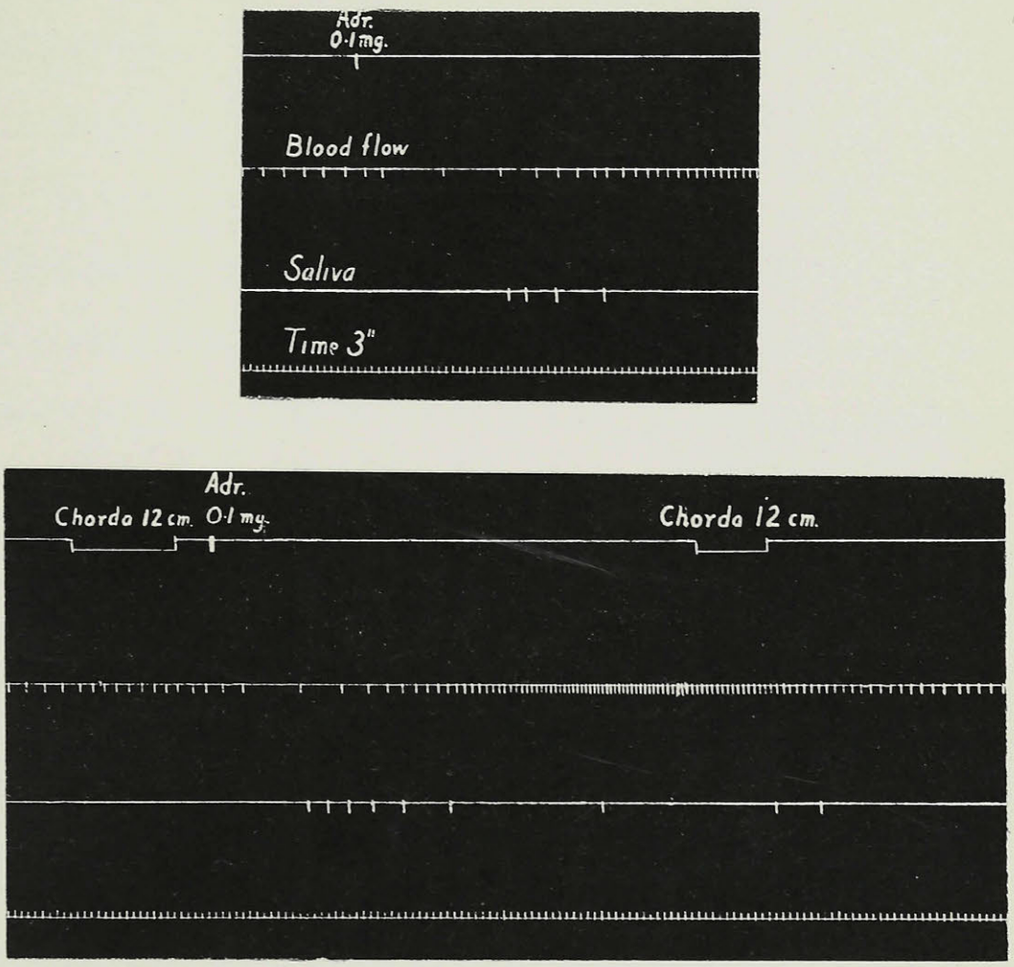

FIGURE 3. - For explanation see text. The two sections of the tracing were made at an interval of about 10 minutes, during which time the position of the recorders was readjusted but no stimuli were applied to the animal.

slackens during the phase of dilatation (see Fig. 3). In the experiment from which the tracing was taken, the vascular action of the chorda was not great, but the augmentor action was definitely shown.

Discussion.

On the basis of Mathews' hypothesis for the origin of the augmented secretion, it might be objected that in these experiments the secretory power of the chorda may not be quite abolished, but that enough saliva is secreted under its influence to distend the alveoli and intra-glandular ducts without causing any movement of the fluid in the graduated tube; stimulation of the sympathetic, by activating some contractile mechanism, would then cause the discharge of this saliva. This objection is negatived by the following considerations:-

(1) The paralysis of the chorda was frequently tested by prolonged stimulation with strong currents. No movement whatever of the fluid in the gauge could be observed, although a flow of less than 0.005 c.c. could easily be detected. It is probable that the secretion of any considerable volume of saliva would raise the pressure within the ducts sufficiently to cause at least some slight motion of the fluid column, as the frictional resistance of the cannula and gauge was small (1 to 2 cm. H_2O). It will be remembered that stimulation of the chorda in the absence of atropine will cause discharge of saliva against a pressure of at least 2000 times this magnitude (Hill and Flack (41)).

(2) /

(2) The augmentation was usually evident in at least the second sympathetic (or adrenaline) secretion following stimulation of the atropinized chorda.

(3) Not only did the chorda augment the sympathetic secretion, but the secretory power of the chorda itself was temporarily restored by stimulation of the sympathetic.

Furthermore, the augmented secretion is not dependent on vascular changes within the gland, since (1) it is abolished by doses of atropine insufficient to affect greatly the vasodilator action of the chorda; (2) the chorda vasodilatation passes off quickly after stimulation stops, while the augmenting action of the chorda persists for at least 10 minutes; (3) the vascular action of adrenaline is not much modified by previous stimulation of the atropinized chorda; and (4) the sympathetic (or adrenaline) secretion does not appear to be closely dependent on the richness of the blood supply to the gland, since the most rapid flow of saliva occurs during the phase of vasoconstriction.

It must therefore be concluded that in the cat at least, while the sympathetic does activate some motor phenomenon, a heightened irritability of the secretory cells is an essential factor in the production of the augmented secretion. Langley in his original paper suggested that of the cells of the salivary glands some might be innervated by the cranial nerve and some by the sympathetic, and "that when either nerve is stimulated there is an irradiation of impulses of less intensity to cells in the neighbourhood of those directly affected, that on stimulation of the /

the chorda tympani the cells connected with it are left for a time in a state of weak excitation, so that the irradiation of impulses reaching the gland by the sympathetic is much greater than normal." He adds, however: "This, it is true, involves the difficulty of the way in which an irradiation of the impulses can take place; an irradiation either by nervous structures or by gland cells - and especially perhaps the former - is not very easy as the facts stand to clearly comprehend." In the light of the modern view of the chemical transmission of autonomic nerve impulses, it is tempting to suggest that the "irradiation of impulses" of Langley's hypothesis be translated as diffusion of a chemical transmitter, acetylcholine or sympathin, from one to the other set of cells. Such a view is readily harmonized with the finding of Rawlinson that the alveolar and demilune cells are innervated respectively by the parasympathetic alone and by the sympathetic alone. Apparently the cellular changes revealed by histological examination are those characterizing the active secretory processes, and not those characterizing the preliminary 'sensitization' which accompanies the activity of neighbouring cells. It may be recalled here that atropine, as was shown by Gibbs and Szeloczey (24) and by Henderson and Roepke (42) does not interfere with the liberation of acetylcholine in the submaxillary gland under the influence of chorda stimulation. The augmented sympathetic-after-chorda secretion thus presents an interesting example of synergism between two chemical transmitters.

The glandular material from these experiments has been studied histologically /

histologically by Dr. H. E. Rawlinson. Since he was able to use glands which had been subjected to very little or no effective chorda stimulation (it will be remembered that there was no recourse to ether in securing narcosis), this important source of ambiguity was eliminated. Rawlinson concludes (43) that "the augmented secretion in the atropinized gland is due to an increased activity of cells under sympathetic control (demilunes), and the contribution from those more directly under the control of the chorda (alveolar cells) is negligible." Since atropine appears to act by preventing the liberated acetylcholine from exerting its regular effect on the receptor substance (cf. Gaddum (44)), so that the sequence of metabolic changes leading up to secretion is interrupted at this point, I suggest that the hypothetical substance passing from the alveolar to the demilune cells is probably acetylcholine itself, rather than some other substance set free at a later stage of the secretory process.

That the augmented secretion is due to a passage of acetylcholine from the alveolar to the demilune cells, is thus at the least an attractive hypothesis. The increased sensitivity of the latter cells would presumably be due to the persistence, not of the acetylcholine itself (which would be destroyed by tissue esterases), but of some change produced by it in the demilune cells.

Summary.

1. After small doses of atropine, the secretory response of the cat's submaxillary gland to sympathetic stimulation and to adrenaline /

adrenaline is increased by previous stimulation of the chorda tympani, although the latter nerve is itself unable to cause any secretion.

2. Stimulation of the chorda tympani must therefore cause a true increase in the irritability of the secretory cells to sympathetic stimulation.

3. It is suggested that the mechanism of the augmented secretion may depend on the diffusion of acetylcholine from the alveolar cells, innervated by the chorda, to the demilune cells, innervated by the sympathetic.

II. THE EFFECT OF SYMPATHETIC STIMULATION AND OF AUTONOMIC DRUGS ON THE PARALYTIC SUBMAXILLARY GLAND OF THE CAT.

Introduction.

It was found by Maevsky (45) that after degenerative section of the chorda tympani, the cat's submaxillary gland shows a greatly increased response to sympathetic stimulation and to adrenaline. Maevsky explained on this basis the accelerating effect of dyspnoea on the paralytic secretion, a fact previously noted by Langley (46). In the preceding section of this thesis I have shown that the augmented salivary secretion is caused, at least in part, by the synergistic action of the parasympathetic and sympathetic nerves supplying the secretory cells of the gland. The phenomenon noted by Maevsky appeared to be a related one, and it seemed desirable to discover /

discover whether in this case too the heightened response to sympathetic stimulation was due to a true increase in the irritability of the secretory cells themselves. The demonstration of such an interaction of sympathetic and parasympathetic effects would, as I have pointed out previously, be of considerable significance for the adaptation of our views on glandular activity to the modern theory of the humoral transmission of the nerve impulse. I have therefore re-investigated this phenomenon in somewhat greater detail.

Experimental.

Cats were used in all the experiments. The chorda-lingual nerve had been cut aseptically on one side from 10 to 40 days before the operation. In the acute experiments the animals were anaesthetized with a chloralose-urethane (1:10) mixture injected intravenously after ether induction, or by intraperitoneal administration of dial or nembutal: the latter procedure eliminated the possibility that the normal gland might be exhausted through reflex ether salivation. Both submaxillary ducts were cannulated and the secretion measured by means of Gibbs' drop-recorders or by permitting the saliva to flow into graduated glass tubes of narrow bore. Both cervical sympathetic nerves were cut and ligated peripherally for stimulation; the chorda-lingual nerve on the unoperated side was also cut. In one or two experiments the salivary and the blood flow from both glands were measured simultaneously by Maevsky's technique (47). The femoral artery rather than the carotid was used in recording the blood pressure /

pressure.

In 14 experiments it was uniformly found, in confirmation of Maevsky, that the sensitivity of the gland to sympathetic stimulation was greatly increased by previous section of the chorda tympani. The threshold current producing secretion was lower in the paralytic than in the normal gland. With currents of the same strength and duration the secretion of the paralytic gland was from 50 to 800 per cent greater than that of the normal gland; furthermore, the latent period was shorter and the duration of the secretion was much prolonged. (See Fig. 4 and Table I.)

Analogous results were obtained by intravenous injection of adrenaline (Fig. 5). (See also Table I.) The secretory response of the paralytic gland appeared to be uninfluenced by variation of the interval between the preliminary denervation and the acute experiment within the limits noted (10 to 40 days).

These results are not due to a general increase in the irritability of the denervated gland, for the parasympathomimetic drugs pilocarpine (in 7 out of 8 experiments) and acetylcholine (in 3 experiments) acted more strongly on the normal than on the paralytic gland. This may be related to the loss in weight which follows section of the chorda. In contrast to these drugs, choline produced, in 3 out of 4 experiments, a greater secretion from the paralytic than from the normal gland. (See Table I.)

The increased sensitivity of the paralytic gland to adrenaline might be ascribed to a changed vascular response of the gland to this substance following degeneration of the chorda tympani. To test /

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TABLE I.

	Denervated Gland	Normal Gland
Experiment 1.		
Stimulation of r. and l. sympathetic nerves, inductorium:		
Threshold distance for secretion	15 cm.	9 cm.
Experiment 2.		
Stimulation of r. and l. sympathetic nerves, 30 sec., coil 12.5 cm.:		
Volume of secretion	0.39 c.c.	0.08 c.c.
Latent period	4 sec.	22 sec.
Duration of secretion	4 min.	15 sec.
Adrenaline chloride, 0.1 mg.:		
Volume of secretion	0.52 c.c.	0.15 c.c.
Latent period	9 sec.	28 sec.
Duration of secretion	6-7 min.	40 sec.
Adrenaline chloride, 0.1 mg., after cocaine hydrochloride, 8 mg.:		
Volume of secretion	0.78 c.c.	0.22 c.c.
Increase in volume, per cent	50	47
Experiment 3.		
Acetylcholine chloride, 0.5 mg.:		
Volume of secretion	0.07 c.c.	0.15 c.c.
Choline chloride, 10 mg.:		
Volume of secretion	0.12 c.c.	0.08 c.c.
Experiment 5.		
Pilocarpine nitrate, 1 mg.:		
Volume of secretion per min., beginning 2 min. after injection	0.15 c.c.	0.53 c.c.

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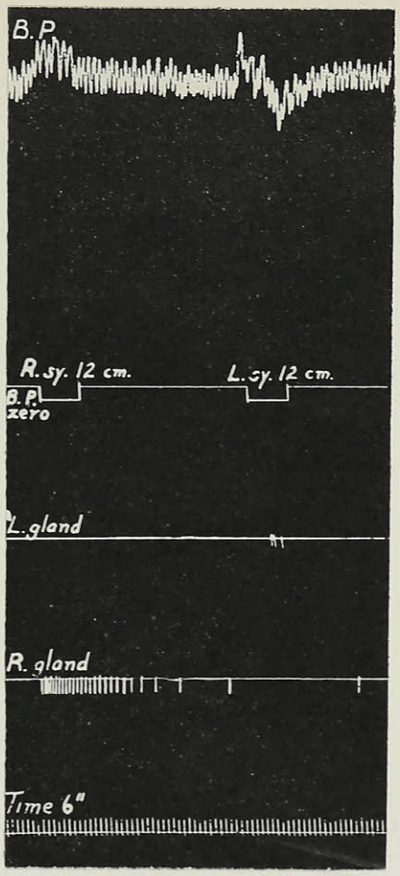

FIGURE 4. - Right chorda tympani cut 4 weeks previously. From top to bottom: blood pressure; signal line marking stimulation of right and left cervical sympathetic nerves; secretion of left submaxillary gland; secretion of right submaxillary gland; time in 6-second intervals. The paralytic gland responds more strongly than the normal to sympathetic stimulation.

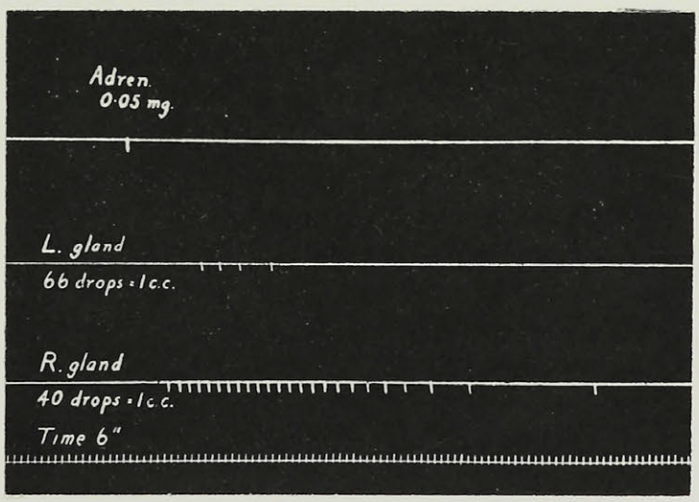

FIGURE 5. - Right chorda tympani cut 3 weeks previously. Injection of 0.05 mg. of adrenaline evoked a much greater secretion from the paralytic than from the normal gland.

test this possibility, simultaneous records were made of the blood flow and salivary flow from both glands after injection of adrenaline. The results of such an experiment are shown graphically in Figure 6, rate of flow being plotted against time. In both glands adrenaline produced a brief slowing, followed by a marked and prolonged acceleration, of the blood flow; in the paralytic gland, however, the vasoconstriction was seen to be slight when allowance was made for the volume of fluid lost to the saliva, and the vasodilatation was considerably greater than in the normal gland. The difference in the vascular effect was nevertheless insufficient to account for the difference in the secretory effect, particularly as in both glands the discharge of saliva was most rapid in the initial phase when the blood flow was little increased, and slackened during the subsequent phase of vasodilatation. A tracing from a similar experiment, in which the preliminary vasoconstriction was unusually small, is reproduced in Figure 7.

The secretory action of the sympathetic on both normal and paralytic glands was abolished by ergotoxine (2.0 to 5.0 mg. per Kg.). Atropine in moderate doses (0.5 to 2.0 mg. per Kg.) had little effect on the response of either gland to sympathetic stimulation or to adrenaline. The effects of these drugs on the response of the paralytic gland are illustrated by the following protocol.

Cat /

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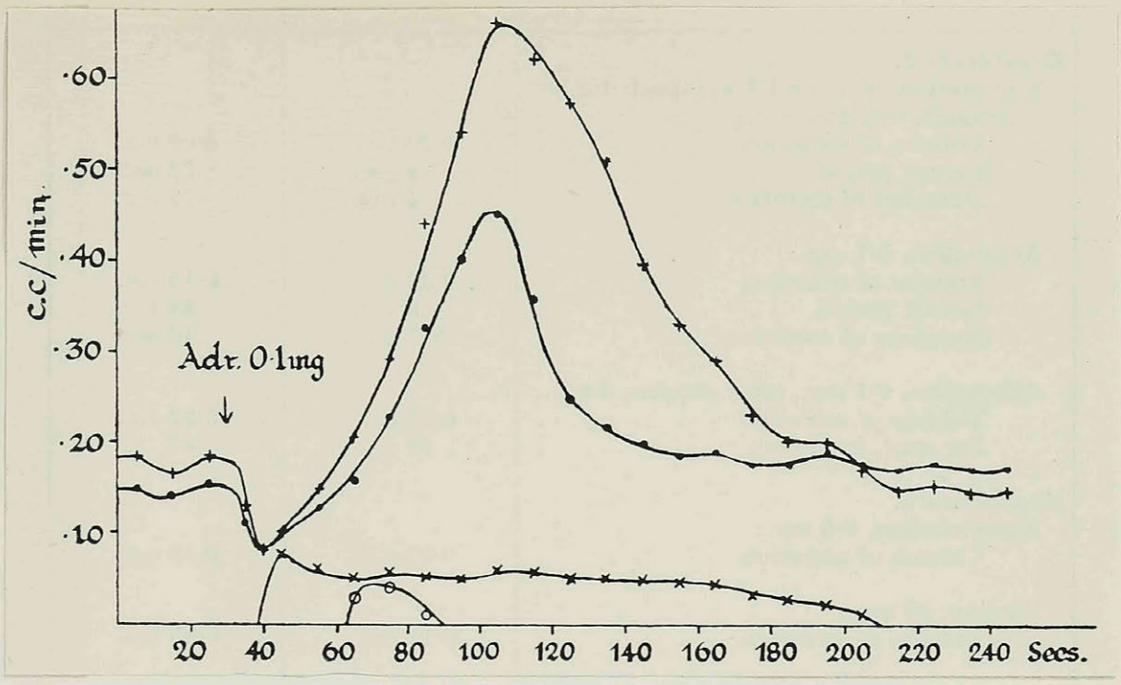

FIGURE 6. - Right chorda tympani cut 5 weeks previously. + = venous outflow, right submaxillary; • = venous outflow, left submaxillary; x = secretion, right submaxillary; o = secretion, left submaxillary. Ordinates mark rate of flow in c.c. per minute; abscissae, time in seconds. Arrow marks point of injection of 0.1 mg. of adrenaline. Discussion in text.

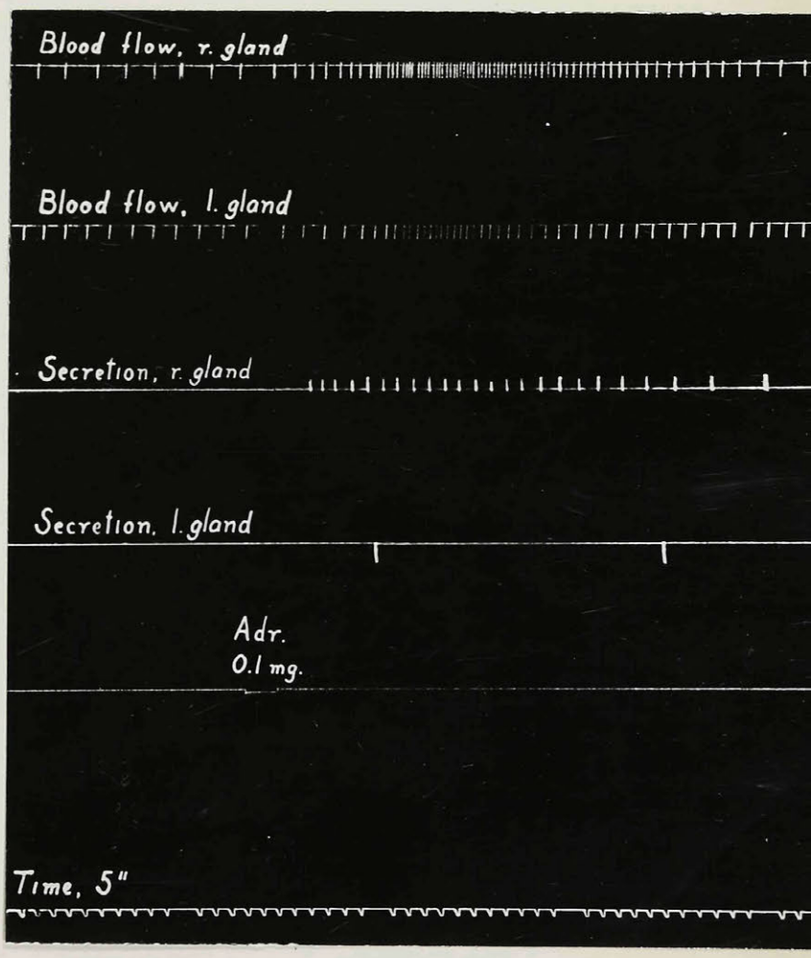

FIGURE 7. - Right chorda tympani cut about 3 weeks previously. Injection of 0.1 mg. of adrenaline evoked a profuse secretion from the paralytic gland, but a very small secretion from the normal gland. The blood flow through either gland was much accelerated, the increase being greater in the paralytic than in the normal gland; but the difference in vasomotor effects was insufficient to account for the difference in the secretory effects.

Cat, female, 1.8 Kg. Chloralose-urethane anaesthesia. Right chorda tympani cut 26 days previously. Right cervical sympathetic cut and peripheral end placed on electrodes for stimulation. Secretion of right submaxillary gland measured. Femoral vein cannulated for injection of drugs.

Time	Procedure	Secretion c.c.
12:45	Stimulation of right sympathetic, 30 seconds, coil distance 9 cm.	0.35
1:00	Repeat.	0.37
1:20	Adrenaline chloride, 0.05 mg.	0.50
1:25	Atropine sulphate, 0.5 mg.	-
1:40	Stimulation right sympathetic as above.	0.28
2:00	Adrenaline chloride, 0.05 mg.	0.55
2:20	Stimulation of right sympathetic as above.	0.30
2:50	Repeat.	0.28
2:55	Atropine sulphate, 0.5 mg.	-
3:05	Stimulation of right sympathetic as above.	0.25
3:20	Adrenaline chloride, 0.05 mg.	0.50
3:25	Ergotoxine ethanesulphonate, 10 mg. slowly.	-
3:40	Stimulation of right sympathetic as above. (No effect on pupil.)	0.00
3:50	Adrenaline chloride, 0.05 mg. (Blood pressure lowered.)	0.00

It was noted that in the paralytic gland the power of atropine to abolish the secretion evoked by pilocarpine was much reduced (Fig. 8). In some cases atropine even accelerated the flow of secretion from the paralytic gland, while quickly stopping the more copious secretion produced by the normal gland: larger doses halted /

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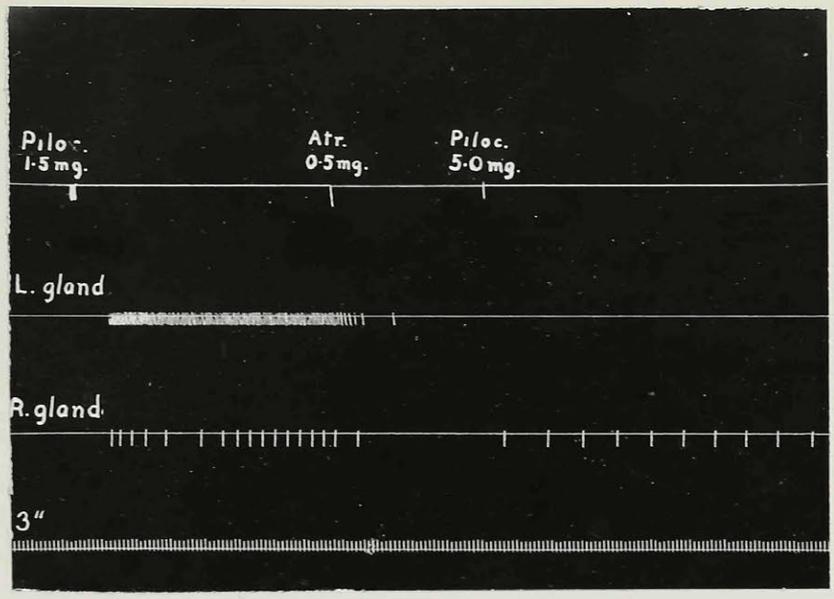

FIGURE 8. - Right chorda tympani cut 24 days previously. Adrenals intact. Injection of 1.5 mg. of pilocarpine caused both submaxillary glands to secrete, the flow from the normal gland being more copious than that from the paralytic gland. The injection of 0.5 mg. of atropine quickly stopped the secretion from either gland. A larger dose of pilocarpine (5.0 mg.) then evoked a secretion from the paralytic, but not from the normal gland.

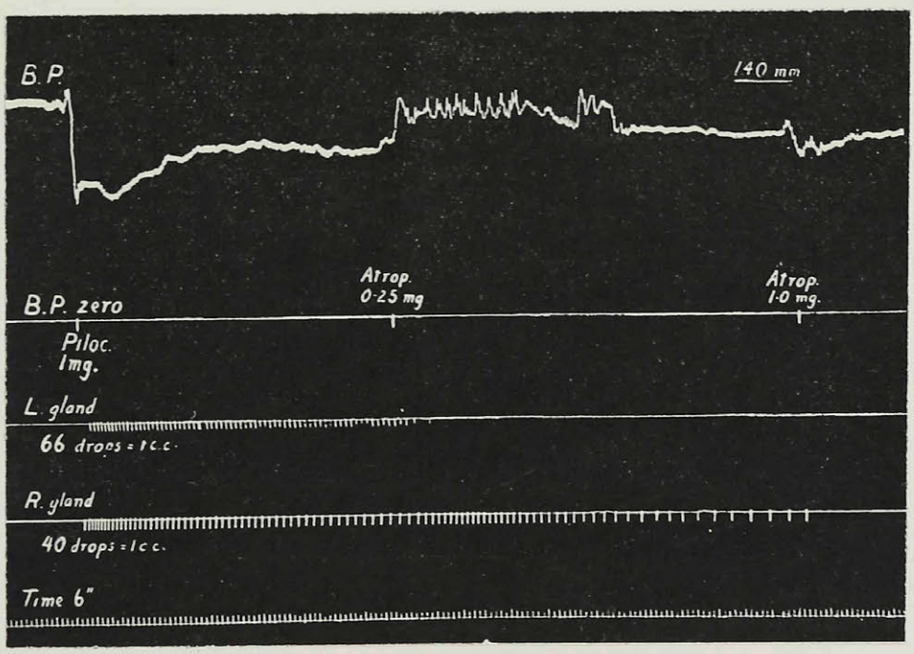

FIGURE 9. - Right chorda tympani cut 19 days previously. Adrenals intact. A very small dose of atropine stops the pilocarpine secretion from the normal gland, but accelerates the secretion from the paralytic gland.

halted the salivary flow from the paralytic gland as well (Fig. 9). This peculiar effect was not seen in animals whose suprarenals had been removed (Fig. 10), and thus presumably depends on a liberation of adrenaline under the influence of pilocarpine, either reflexly through the blood-pressure fall, or, more probably, through a direct action of pilocarpine on the suprarenal medulla (cf. Dale and Laidlaw (47)): the secretory effect of pilocarpine on the paralytic gland is thus in part really an effect of adrenaline. Feldberg, Minz and Tsudzimura (48, 49) have shown that the cholinergic action of the splanchnics in causing discharge of adrenaline from the suprarenals is not entirely 'nicotine-like' but partly 'muscarine-like', so that such an action of pilocarpine is not surprising, and may explain many anomalous responses to this supposedly purely parasympathomimetic drug.

In two experiments it was found that after a small dose of eserine (0.15 mg. per Kg. of eserine sulphate), stimulation of the sympathetic nerve on the paralytic side was followed, after a latent period of 25 to 30 seconds, by a secretion from the opposite gland (Fig. 11). Whether this was due to humoral transmission of the secretory effect by liberated acetylcholine (Secker (50)) or sympathin (26), or merely to potentiation by a small blood-pressure rise of a latent secretagogue action of eserine (Feldberg and Guimarais (51)), was not determined. Several attempts to repeat this observation were unsuccessful.

Discussion /

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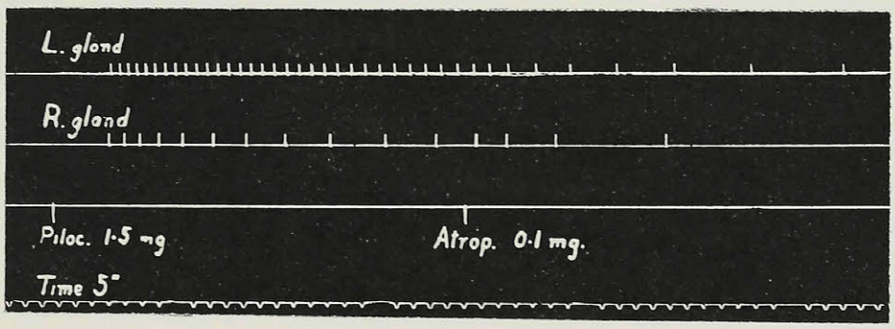

FIGURE 10. - Right chorda tympani cut 11 days previously. Adrenals removed. Atropine stops the pilocarpine secretion equally in the normal and paralytic glands.

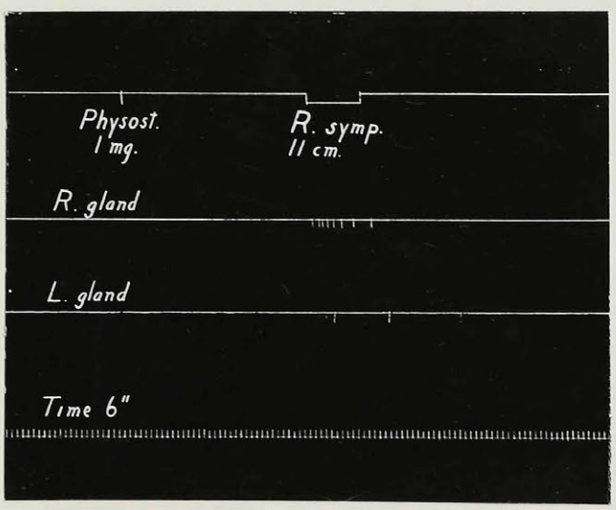

FIGURE 11. - After the injection of eserine, stimulation of the cervical sympathetic trunk on the side of the paralytic gland was followed by a secretion from the opposite gland as well.

Discussion.

It is not yet clear why section of the chorda tympani should increase the secretory response of the submaxillary gland to sympathetic stimulation. It seems certain, however, that the effect cannot be explained on the basis of Mathews' (33) view of a purely motor action of the sympathetic (page 10). Thus the secretion may last for 6 to 8 minutes, and the stimulation may be repeated directly afterward with the same result. Furthermore, the quantity of saliva secreted after a single injection of 0.1 mg. of adrenaline chloride may reach 0.95 c.c., or well over half the volume of the paralytic gland. Mere compression of the alveoli or ducts could scarcely expel so great a volume of fluid.

It must therefore be concluded that in the paralytic gland there is an increase in the irritability of the secretory cells themselves for sympathetic stimulation. The simplest explanation of the facts is to suppose, with Langley (46), that the post-ganglionic neurones of the chorda are in a state of slight continuous activity, the result perhaps of stimulation by the degenerating pre-ganglionic fibres. The well-known 'paralytic secretion' may be considered another indication of this activity of the post-ganglionic neurones. If this explanation be correct, the increased response of the paralytic gland to sympathetic stimulation is analogous to the augmented sympathetic-after-chorda secretion, and, like it, presents an interesting example of synergism between sympathetic and parasympathetic nerves. The tentative explanation of the latter phenomenon, viz. sensitization of the demilune cells by /

by acetylcholine diffusing into them from the alveolar cells, is perhaps applicable here too.

It must nevertheless be pointed out that the heightened irritability produced by degenerative section of the chorda, unlike that which follows normal chorda stimulation, is not abolished by moderately large doses of atropine (e.g. 0.5 to 1.0 mg. per Kg. of atropine sulphate). No ready explanation of this fact in terms of the above-mentioned hypothesis is available.

The remarkable sensitivity of the paralytic gland to adrenaline in the circulation is worthy of special notice. Thus it responds not only to very small doses of the injected hormone, but also to adrenaline discharged by the suprarenal medulla either through physiological stimulation (asphyxia) or through the action of drugs (pilocarpine). It might very well serve in experimental work (with acute section of the cervical sympathetic) as an indicator for adrenaline or sympathin in the circulation.

Summary.

1. The submaxillary gland of the cat, after degenerative section of the chorda tympani, shows a greatly increased response to sympathetic stimulation and to adrenaline.

2. This increased response is due to a true heightened irritability of the secretory cells for sympathetic stimulation.

3. Pilocarpine and acetylcholine act more strongly on the normal than on the 'paralytic' gland.

4. The paralytic gland is a very sensitive indicator for adrenaline in the circulation.

5. /

5. It is suggested that the increased sensitivity of the paralytic gland to sympathetic stimulation is analogous to the augmented sympathetic-after-chorda secretion.

III. THE CHOLINE-ESTERASE CONTENT OF THE NORMAL AND OF THE PARALYTIC SUBMAXILLARY GLAND IN THE CAT.

Introduction.

In the preceding section I have suggested that the heightened irritability of the paralytic gland may be due to the continuous passage to the demilune cells of acetylcholine released by the alveolar cells. Degenerative section of the chorda tympani, however, does not raise but lowers the acetylcholine equivalent of the submaxillary gland (Chang and Gaddum (52)). The latter is, however, a measure of the total acetylcholine, combined and free, in the gland; and it could still be possible that in the paralytic gland there is a higher concentration of the free ester. It might thus be thought that denervation impairs the ability of the gland to inactivate acetylcholine which has been liberated within it: in other words, that the denervated gland contains a reduced supply of choline-esterase, the specific enzyme which hydrolyzes esters of choline (53, 54, 55). This enzyme occurs in different tissues, as well as in the blood, in widely varying concentration (Plattner and Hintner (56)). There has hitherto been no evidence to indicate whether or not the amount present in any organ is affected by section of the nerve supply to the organ. I have therefore /

therefore made several rough determinations of the rate at which acetylcholine is destroyed by simple extracts of paralytic, as compared with normal, submaxillary glands.

Experimental.

Methods for this determination have been published by Plattner and Hintner (56), Rona and Ammon (57), Ammon (58), and Stedman, Stedman and Easson (55): of these the first uses the biological test on the frog's heart to measure the amount of acetylcholine remaining after treatment of a known quantity of the ester with organ extracts; the others use respectively electrometric, titrimetric and manometric measurement of the rate of hydrolysis. An attempt was made to employ the method of Plattner and Hintner for the determination of the submaxillary gland esterase. The attempt was unsuccessful, owing to the unsuitability for the assay of the hearts of the frogs locally available (very small specimens of R. pipiens): the concentration of acetylcholine necessary to produce a minimal inotropic effect on these was only slightly less than that producing complete standstill of the heart. I therefore adopted the method of assay on the eserinized frog's rectus, as recommended by Chang and Gaddum (52). This muscle is a less sensitive reagent for acetylcholine than the eserinized leech muscle, but is equally specific, and more uniform in its responses (52, 59). Over the frog's heart it has the great advantage of being practically unaffected by a variety of other substances likely to be present in tissue extracts. It is in fact

so/

so insensitive to all constituents of gland extracts other than acetylcholine, that it was found possible to dispense with the trichloracetic acid precipitation used by Plattner and Hintner, and add the crude acetylcholine extract directly to the bath in which the test-muscle was suspended. Control experiments showed that the presence of gland materials did not affect the reaction of the rectus to acetylcholine simultaneously or subsequently applied to it.

The details of the method used are as follows.

All branches of the carotid artery and jugular vein on both sides were ligated, with the exception of those passing to the submaxillary glands. The glands were perfused with large quantities of Ringer's solution at body temperature until quite free from blood (blood is especially rich in choline-esterase), then dissected out of their capsules and excised. Excess fluid was rapidly removed with blotting paper, and the glands were weighed and ground with washed quartz sand in 3 volumes of M/3 phosphate buffer (pH 7.4). (The denervated gland weighed on the average 20 per cent less than the normal, confirming Langley (46)). At least 30 minutes' vigorous grinding was required for each gland; otherwise low figures for the esterase content were obtained. The residue from the grinding, after twice washing and centrifuging, yielded very little esterase activity on repeated grinding: the extraction, if not quantitative, therefore removed the great bulk of the enzyme.

The extracts were covered and allowed to stand 3 to 4 hours.

Six /

Six volumes of phosphate buffer were then added to make the final dilution of the tissue tenfold, and the mixture again centrifuged and decanted. For the assay, 1 c.c. of the extract was added to 1 c.c. of a 1:10,000 solution of acetylcholine chloride, and the mixture allowed to stand at room temperature for a definite time (30 to 300 seconds). 1 c.c. of the mixture was then added to the bath (volume 100 c.c.) containing the eserinized rectus, and the contraction produced compared with that caused by a known quantity of acetylcholine. The action of the esterase was quickly stopped by the eserine in the bath. At least three determinations were made with each extract. (See Figure 12, for excerpts from a tracing made during the biological assay of two such extracts.)

From the values obtained, a curve was plotted, from which was read the time required for 50 per cent destruction of the added acetylcholine. The following results were obtained, the values for the glands from the same animal being grouped in pairs.

Gland	Time required for 50% destruction of added acetylcholine minutes
Normal (right)	$1\frac{3}{4}$
" (left)	$1\frac{3}{4}$
Normal (right)	$1\frac{1}{4}$
Normal (left)	$2\frac{1}{2}$
Paralytic (right)	2
Normal (left)	$1\frac{3}{4}$
Paralytic (right)	$2\frac{1}{4}$
Normal (Plattner and Hintner)	5

The /

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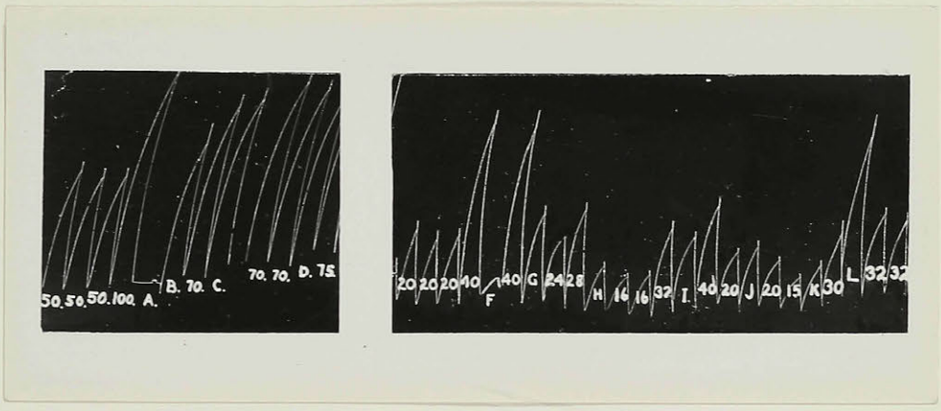

FIGURE 12. - Partial record of the biological estimation of the choline-esterase content of the normal and paralytic gland from a cat. The tracing shows the contraction of the eserinized rectus abdominis of a frog produced by the indicated doses (in y) of acetylcholine chloride. Letters indicate the responses produced by certain doses of acetylcholine which had been treated with gland extracts for various lengths of time, as follows:

A: 100 y, treated for 300 seconds with paralytic gland extract.
B: 100 y, " " 75 " " " " "
C: 100 y, " " 82 " " " " "
D: 100 y, " " 60 " " " " "
E: 100 y, " " 64 " " normal " "
F: 40 y, " " 180 " " paralytic " "
G: 40 y, " " 75 " " " " "
H: 40 y, " " 150 " " " " "
I: 40 y, " " 50 " " " " "
J: 40 y, " " 120 " " normal " "
K: 40 y, " " 200 " " " " "
L: 100 y, " " 60 " " " " "

The acetylcholine inactivating power of the cat's submaxillary gland is thus somewhat higher than that determined for the dog's submaxillary gland by Plattner and Hintner, the dilutions of the tissue being the same in both cases.

Since the catalytic destruction of acetylcholine is a monomolecular reaction, the assay can be checked by ascertaining the rate of inactivation at different times after the addition of the esterase. This, of course, is proportional to the concentration of the unhydrolyzed ester, i.e.

$$k = \frac{1}{t} \log\left(\frac{a}{a-x}\right)$$

where k is constant and a and $a-x$ are the concentrations of acetylcholine after 0 and t seconds respectively. Table II illustrates the results when this calculation is made.

TABLE II.

Gland	t	a-x(a=100)	log(a-x)	$\log\left(\frac{a}{a-x}\right)$	$k=\frac{1}{t}\log\left(\frac{a}{a-x}\right)$
Right (normal)	75	60.0	1.778	0.222	0.00300
	90	55.0	1.740	0.260	0.00290
	240	18.0	1.255	0.745	0.00310
Right (paralytic)	50	75.0	1.875	0.125	0.00250
	60	70.0	1.845	0.155	0.00258
	75	67.5	1.829	0.171	0.00228
	150	42.5	1.628	0.372	0.00248
Left (normal, same cat)	60	75.0	1.875	0.125	0.00208
	120	57.5	1.760	0.240	0.00200
	200	47.5(?)	1.677	0.323	0.00162
	240	30.0	1.477	0.523	0.00218

Solutions /

Solutions of acetylcholine subjected to prolonged treatment with gland extracts lost all perceptible activity (Fig. 12, A).

Discussion.

Considering the multiple sources of error, the values for k from the same gland agree tolerably well. Statistical analysis of a much greater series of determinations would be necessary to determine whether denervation of the gland has any definite influence on its esterase content. Nevertheless it is clear that this is of the same order of magnitude in the normal and denervated gland, and that no difference exists which can adequately explain the tremendously increased sensitivity of the paralytic gland to sympathetic stimulation. It is, however, still possible that degenerative section of the chorda tympani, as I have suggested in the previous section, stimulates the production of free acetylcholine within the gland.

The production of enzymes by other glandular tissues continues after section of the nerves which normally control the discharge of these enzymes. A familiar example is provided by the Heidenhain pouch of the stomach, which has no vagal innervation but continues indefinitely to elaborate pepsin. Nothing is known of the conditions under which choline-esterase is formed in the body. Its high concentration in the blood would suggest, indeed, that the intervention of the nervous system is unnecessary.

Summary /

Summary.

1. A simple method is described for the approximate estimation of the choline-esterase activity of the submaxillary gland.

2. The choline-esterase content of the submaxillary gland of the cat is not significantly affected by degenerative section of the chorda tympani.

PART III /

PART III. STUDIES ON THE HUMORAL TRANSMISSION OF THE EFFECTS OF PARASYMPATHETIC NERVES.

Introduction.

In the general introduction I have referred briefly to the development of the modern theory of the chemical transmission of nerve impulses. I must now treat in somewhat more detail of the experimental methods used, and of the extent to which experiments on the individual organs have supported the theory. During the sixteen years which have elapsed since Loewi (60) described his original experiments, the literature of this subject has grown to unwieldy proportions. The several hundreds of papers which have appeared are of very unequal merit. A large number of them represent merely the application of slightly different techniques for the verifying of already well-established facts. Many more are based on the use of methods of extraction and assay which modern knowledge has shown to be not completely reliable. Finally, a not inconsiderable number are demonstrably worthless, and can only be regarded as attempts to find experimental support for an already foregone conclusion. It is interesting to note that, in the early days of work in this field, the negative results out-numbered the positive, and many experienced workers denied even Loewi's fundamental findings; as the main facts became well established, however, very few found any difficulty in confirming them, no matter how ill adapted their experimental technique to the problem in hand. It is perhaps unfortunate that of the major developments /

developments of modern physiology the problems of chemical transmission alone are accessible to investigators who use only the simple tools of the founders of the science. It has consequently attracted many who see a possibility of getting spectacular results with a minimum of labour, and who remain unconscious of the difficulties of analysis and interpretation inherent in almost all researches in this field. The applicability of these remarks will be justified in the review of the literature.

My experiments on the humoral transmission of parasympathetic effects have been carried out chiefly on the heart, the tongue, and the salivary glands. I shall therefore review only that part of the literature which deals with the evidence for the chemical transmission of the parasympathetic action in these organs. Attention must first be given to the methods used in the experiments on which this evidence is based.

Loewi (60) in his pioneer work studied the liberation of a parasympathomimetically active substance into the Ringer's solution perfusing the excised frog's heart subjected to vagus stimulation. A similar technique has been adopted by all subsequent workers on the frog's heart, and by many who investigated the heart and other organs of the mammal with the same end in view. While positive results obtained in this manner are highly suggestive, they are open to criticism unless supported by other evidence. An organ perfused with physiological salt solution cannot be regarded as completely normal. There always remains the possibility, however unlikely, that stimulation of its nerves may evoke changes not produced /

:duced in the organ normally supplied with blood (cf. the emphasis of this point by Lapicque (61) and Ivy (30)); for example, a simple lowering of cell permeability (which may be below normal following perfusion with a colloid-free solution) may permit escape of the pre-formed acetylcholine of the tissue. The objection applies especially to mammalian tissues, which suffer more than those of the frog from the anoxia accompanying saline perfusion.

The alternative method is therefore to demonstrate the release of a parasympathomimetically active substance from an organ whose circulation is intact. The presence of this substance in the circulation may be indicated by its effect on another organ of the same animal, or of a second animal in cross-circulation experiments; or the venous blood from the organ may be assayed on one of the standard biological indicators. Blood has, however, owing to its high content of choline-esterase, the power of inactivating acetylcholine very rapidly. Thus horse's blood destroys over 50 per cent of added acetylcholine in 10 seconds (54). Since the quantities of acetylcholine set free are invariably very small, the possibility that its concentration in the blood will be sufficient to excite another organ is practically negligible, unless special precautions are taken to inhibit its destruction. This fortunately can readily be done, by giving the animal a small dose of eserine, which annuls the activity of choline-esterase (Engelhart and Loewi (53)). The liberated acetylcholine thus escapes destruction in the circulation, as well as in the organ where it is set free, and

in /

in the indicator organ. No confidence whatever can be placed in experiments which claim to demonstrate the transference of the parasympathomimetic action from one organ to another in the presence of blood and in the absence of eserine. It also must be pointed out that the use of the frog's heart for the assay of venous blood from a stimulated organ is a procedure very liable to error. The frog's heart is readily inhibited by various substances formed in blood subjected to handling in vitro, especially adenylic acid and K ions (from laked corpuscles). The eserinized dorsal body wall of the leech is unaffected by these substances, and may be considered a practically specific test-organ for acetylcholine. Its use for this purpose was introduced by Minz (62) in 1932, and at the time when my work began its use for the detection of acetylcholine discharged by the heart had not been described, although Feldberg (63) had recently used it to detect the chemical transmitter of the lingual nerve in the venous blood of the tongue. While the evidence for a cholinergic mechanism supplied by the leech muscle test properly performed is probably unequivocal, it is still desirable to have confirmatory evidence, secured by the aid of a very different method.

With this preliminary critique of the methods used for detecting the humoral transmission of parasympathetic effects, I shall review briefly the work relating to the possibility of such transmission in the heart, the salivary glands, and the tongue.

1. /

1. The heart: humoral transmission of the vagus effect.

(a) The frog heart. Loewi (60) stimulated the vago-sympathetic trunk attached to a frog's heart suspended on a Straub cannula, and found that when the vagal action predominated (i.e., when the heart was inhibited) the perfusion fluid acquired the capability of inhibiting a second heart to which it was applied. Loewi's original report could not be confirmed by a number of workers, notably Asher (64, 65, 66), Bohnenkamp (67), Atzler and Müller (68), and Nakayama (68). Further work by Loewi and his collaborators Navratil and Engelhart (70, 71, 72), however, proved conclusively the chemical transmission of the vagal effect in the perfused frog's heart. Confirmatory evidence was supplied by Brinkman and van Dam (73), Ten Cate (74), Kahn (75) and Bain (76). The chemical and pharmacological properties of the Vagusstoff were meanwhile exhaustively studied by Loewi and his collaborators (60, 70, 72) and by Plattner (77), and it was shown that these were indistinguishable from those of a choline ester.

(b) The mammalian heart. The early work on chemical transmission in the mammalian heart was, in contrast, very inconclusive. The first attempted demonstration was made by Duschl and Windholz (78), who used the so-called method of parabiosis: two rats were joined so that their blood vessels anastomosed; after they had recovered, vagal stimulation of one was followed by bradycardia in the other. No eserine was used, and the experiments were poorly controlled. In Duschl's later experiments (79) blood was withdrawn by cardiac puncture from cats and rabbits, whose hearts had been stopped by vagal stimulation, and injected into other animals, in

which /

which it slowed the heart and lowered the blood pressure. Neither the donor nor the recipient was eserinized. It is scarcely credible that the minute trace of 'Vagusstoff' which might escape destruction by the blood could have any perceptible action after its dilution in the recipient's circulation: if this were the case, the recovery of the heart from vagal stimulation would always be slow, owing to persistence of the chemical transmitter in the circulation, whereas it is in fact almost instantaneous. The conditions were, however, favourable for the formation in the injected blood of adenosine, which acts somewhat similarly on the heart and vessels (Zipf (80, 81), Barsoum and Gaddum (82)). The positive results reported by Popper and Russo (83), Brinkman and van der Velde (84), Zunz and Govaerts (85), Rylant and Demoor (86, 87), and Viale (88), all of whom used closely similar methods, are open to the same criticism. Rasenkov and Ptschelina (89) tested the systemic blood obtained during vagal stimulation on the blood pressure and pancreatic secretion of a second animal: their professedly positive results have been adversely criticized by Babkin et al. (23).

Hansen and Rech (90) recorded simultaneously the maternal and foetal electrocardiographs in the pregnant guinea-pig, and found stimulation of the maternal vagus to cause slowing of the foetal heart. It may be doubted, however, if this effect was due to acetylcholine set free by the vagal stimulation, since (a) no eserine was used, and (b) the observed latent period of 10 seconds appears a very short time for acetylcholine to be liberated, pass to the left side of the heart, through the uterine vessels, the /

the placenta and the foetal circulation, and exert its action on the embryonic heart.

Investigators who perfused the mammalian heart with saline solutions did, however, obtain fairly clear-cut results. Thus the perfusate of the isolated heart of the rabbit was found to acquire, during vagal stimulation, the power of stimulating the isolated intestine of a rabbit (Jendrassik (91)) or of inhibiting the heart of a second rabbit (Rylant (87)). Such results are highly suggestive; yet they cannot, as I have pointed out, be regarded as conclusive evidence for a cholinergic mechanism in the normally insanguinated heart.

The first investigators in this field who took into account the lability of acetylcholine in blood and used eserine to preserve it, were Plattner (92) and Tournade, Chabrol and Malméjac (93). Plattner withdrew blood directly from the coronary sinus of the eserinized animal under vagal stimulation, and tested it on the frog's heart, with completely negative results, although he was able to detect the discharge of acetylcholine when the heart was perfused with Locke's solution rather than blood. Both Plattner and Tournade criticize sharply the inadequate methods of their predecessors. Freeman, Phillips and Cannon (94) shunted the portal blood into the inferior vena cava so that the entire area supplied by the vagus could contribute the chemical transmitter to the general circulation. As indicators for the vagal substance they used the denervated iris, danervated heart (stimulating the vagi below the heart), and denervated submaxillary gland. Their results /

results were uniformly negative. Finally it may be noted that Henderson and Roepke (95) occasionally found vagal stimulation in the eserinized cat to provoke salivary secretion from a denervated gland: while assuming that the effect was due to acetylcholine carried to the submaxillary gland in the blood, they did not analyze the phenomenon more fully; in particular they did not exclude the possibility of reflex adrenaline liberation.

Unequivocal proof of the liberation of acetylcholine (or a very similar substance) by the mammalian heart with intact blood supply, was furnished only in 1933 by Feldberg and Krayer (96). They eserinized their experimental animals (cats and dogs) very heavily, using atropine to prevent the blood-pressure fall, and found that coronary sinus blood obtained during vagal stimulation contracted the eserinized leech, whereas 'resting' blood was inactive (the action of acetylcholine on the leech is not antagonized by atropine); they obtained confirmatory results with the cat's blood-pressure assay. Their report was not accessible to me until after the beginning of my own work on the humoral transmission of the vagal effect.

As for the other organs innervated by the vagus, viz., bronchi, oesophagus, musculature and glands of stomach and intestine, and pancreas, satisfactory evidence for chemical transmission has been slow in coming. Thornton (97) and Saalfeld (98) independently demonstrated in the effluent obtained during vagal stimulation from the guinea-pig lung perfused with eserinized Ringer, a substance acting like acetylcholine on the eserinized leech and on the blood-pressure /

:pressure of the cat. Dale and Feldberg (25) made the corresponding experiment for the dog's stomach, this being either artificially perfused or receiving its normal blood supply, and found that the continuous discharge of an acetylcholine-like body into the portal vein was accelerated on stimulation of the vagus.

Proof for the humoral transmission of the action of the vagus on the intestine and on the pancreas is still completely lacking. The evidence from analogy with other organs receiving a parasympathetic innervation, and from the action of drugs, is, of course, extremely suggestive.

2. The salivary glands: humoral transmission of the chorda tympani effect.

Babkin and Gibbs observed about 1926 that faradization of the chorda tympani in the eserinized animal provoked a sharp fall of blood-pressure, which occurred 20 to 25 seconds after the beginning of the stimulation, and could not be accounted for by the local vasodilatation. The phenomenon could not be satisfactorily explained until after the protective action of eserine on acetylcholine had been demonstrated (53). After further experiments by Gibbs and Wolff, the work was published in 1932 (22) and was the first satisfactory demonstration of a cholinergic innervation of the salivary glands. Positive results were independently announced by Beznak (99), who used a cross-circulation method: he did not employ eserine, and his data are not very convincing. Supplementary evidence was shortly brought, however, by Gibbs and Szeloczey (24) with the aid of an ingenious saline perfusion method which /

which permitted the intermittent restoration of the normal circulation, so that the condition of the gland remained more or less normal. They found that the effluent obtained during chorda stimulation acted like acetylcholine on the cat's blood-pressure and on the rabbit's intestine.

A later series of experiments by Babkin, Alley and Stavraky (23) showed that stimulation of one chorda tympani in the eserinized cat could lead, after a definite latent period, to secretion by the denervated submaxillary gland of the opposite side. The secretion was not due to the concomitant blood-pressure fall, to a reflex arising from the carotid sinus, or to reflex discharge of adrenaline. Clamping the vein of the gland abolished the effect, which was therefore due to the humoral transmission of a para-sympathomimetic substance set free from the active gland. In the absence of eserine, of course, neither the depressor action nor the crossed secretory action occurs. In view of the specificity of the inhibition of choline-esterase by eserine (58), the active substance must be regarded as a choline ester.

Further investigation, with positive results, was carried out by Henderson and Roepke (95), who used saline perfusion methods combined with the frog's heart assay. The depressor effect of chorda stimulation in the intact eserinized animal was confirmed by Feldberg (99), who brought forward additional evidence for the interpretation proposed by Babkin, Gibbs and Wolff.

3. /

3. The tongue: humoral transmission of the lingualis (chorda) effect.

After the hypoglossal nerve, which carries the voluntary motor fibres to the tongue, has been cut and allowed to degenerate, stimulation of the lingual evokes a peculiar slow contracture of the voluntary muscle of the tongue (Vulpian and Phillipeaux (100)). This effect of the lingual nerve can be mimicked by injecting nicotine (101) or acetylcholine (102) into the lingual artery; in the latter case, the effect is due to the 'nicotine-like' action of acetylcholine (103 - see also Dale, 104). The fibres in the lingual responsible for the effect are derived from the chorda tympani, and Heidenhain (101) suggested long ago that they were the vasodilator fibres. Bremer and Rylant (105) and Dale (106) suggested that the Vulpian-Phillipeaux contracture might be caused by a substance normally transmitting the vasodilator action of the lingual, but capable also of acting on the tongue musculature when this was sensitized by previous denervation. Dale and Gaddum (15) observed that the contracture produced by stimulation of the lingual nerve was intensified by eserine, and pointed out that this fact alone was strong evidence for attributing the phenomenon to cholinergic fibres; they also showed that the failure of atropine to prevent the contracture produced by lingual stimulation, while abolishing that produced by injected acetylcholine, was not necessarily opposed to this interpretation. Bain (107) perfused the dog's tongue with Ringer, and showed that the effluent received during stimulation of the lingual contracted the intestine of the rabbit /

rabbit. Finally Feldberg (63, 108) made the decisive experiment of assaying the venous blood of the tongue on the eserinized leech and on the blood-pressure of the cat: an acetylcholine-like substance was present only when the animal was eserinized and the lingual nerve was being stimulated.

It will be apparent from this review of the literature that in the fall of 1933, when I began my experiments on humoral transmission, the evidence for the chemical transmission of the action of the vagus in the mammalian heart was not yet entirely conclusive. The liberation of an acetylcholine-like substance had, indeed, been observed in the heart perfused with saline solution and subjected to vagal stimulation. Similar experiments on the heart with intact blood supply, however, had uniformly failed to yield analogous results. (The work of Feldberg and Krayer (96), in which positive evidence was obtained by testing coronary sinus blood directly on the muscle of the leech, was not yet available to me.) In view of the ease with which Babkin, Alley and Stavraky (23) had been able to demonstrate the transmission of the chorda tympani effect to the opposite submaxillary gland, it appeared worth while to try to use this organ as an indicator for acetylcholine liberated under the influence of the vagus. Such an attempt had already been made, unsuccessfully, by Freeman, Phillips and Cannon(94). These workers, however, tried to sensitize the gland to the humorally transmitted vagus-substance by previous denervation, a procedure which, on the contrary, lowers its sensitivity to acetylcholine (see page 21); furthermore /

furthermore, they did not attempt to sensitize the gland by previous stimulation, as Babkin et al. did with improved results. I therefore attempted to adapt the method of Babkin, Alley and Stavraky to the detection of the hypothetical 'vagus substance' in the circulating blood, intending, in preliminary experiments at least, to make no distinction between the heart and the other organs supplied by the vagus. Since the distribution of the vagus is so much greater than that of the chorda tympani, it seemed reasonable to hope for positive findings; even if most or all of the acetylcholine discharged into the portal circulation were destroyed by the liver (101), still the bronchi, oesophagus, auricle and special tissues of the heart might serve effectively as sources of the local hormone. Experiments of this type, with various technical modifications introduced when the expected results failed to materialize, comprise the bulk of the work to be described in this section of the thesis. I have also, in a number of experiments, used a similar method to demonstrate the release of a parasympathomimetic substance during stimulation of the lingual nerve. Finally, I have endeavoured to improve the method of Hansen and Rech (90) (passage of vagus substance from mother to foetus) for investigations of this kind, and to apply it to detecting in the circulation the chemical transmitter of the effects of the vagus and the chorda tympani.

Experimental.

1. An attempt to demonstrate the vagal substance in the circulating blood, using the submaxillary gland as indicator.

Cats were used in all the experiments. They were anaesthetized with a chloralose-urethane (1:10) mixture, injected intravenously after ether induction. Tracheotomy was performed, but artificial respiration was rarely resorted to unless the chest was to be opened. The lingual nerve was severed on one side proximal to the origin of the chorda tympani, and the peripheral end taken on a ligature and fixed on the stimulating electrodes, care being taken to keep the nerve uniformly warm and moist. A cannula was inserted into the submaxillary duct on the same side, and connected to a Gibbs' drop-recorder for registration of the salivary flow. Both cervical sympathetic nerves were cut, well craniad to eliminate any chance of current spread from the stimulated vagi. Both vagi were cut in the neck, and the peripheral ends taken on ligatures for stimulation. In some experiments the thorax was later opened for stimulation of the vagi below the heart. Blood-pressure was recorded with the mercury manometer from the carotid of the side opposite the stimulated gland, or from a femoral artery.

For stimulation of the chorda, the induction coil was placed in series with a metronome by which the current could be interrupted at brief regular intervals; in this way a very uniform flow of saliva can be obtained over a long period without damage to the nerve, and the gland is very sensitive to slight changes in the composition of the blood. In nearly all of the experiments, vagus stimulation /

stimulation was carried out when the gland was secreting at a uniform rate, either through chorda stimulation or after injection of a small dose of pilocarpine. In a few experiments, the chorda was first stimulated, evoking a rapid secretion which slowly subsided after the excitation ceased, and vagal stimulation was applied during the after-effect.

In the anaesthetized animal before administration of eserine, stimulation of one or both vagi never accelerated the salivary flow; usually there was some inhibition of the secretion produced by the interrupted chorda stimulation (Fig. 13). After the injection of eserine (0.15 to 0.30 mg. per Kg.), the secretion was greatly accelerated, and a weaker stimulating current was applied to the chorda so that the blood-pressure fall would not be too great. (Continuous strong chorda stimulation in the eserinized cat brings the arterial pressure down rather rapidly to a very low level, e.g. 30 to 50 mm. Hg, apparently through failure of the organism to destroy acetylcholine set free by the salivary glands: prolonged stimulation of the chorda is in fact less well withstood than prolonged stimulation of the vagus. Weaker stimulation, producing a salivary flow of about 1 drop in 5 or 10 seconds, does not seriously affect the blood-pressure.) Faradization of the vagus (15 to 60 seconds) now had a variable effect. There was generally, accompanying the fall in blood-pressure, a slight initial slowing of the secretion. This was, in about one-half of the experiments, succeeded by a more or less definite acceleration of the secretion beyond the normal rate, this acceleration being sometimes, but not always /

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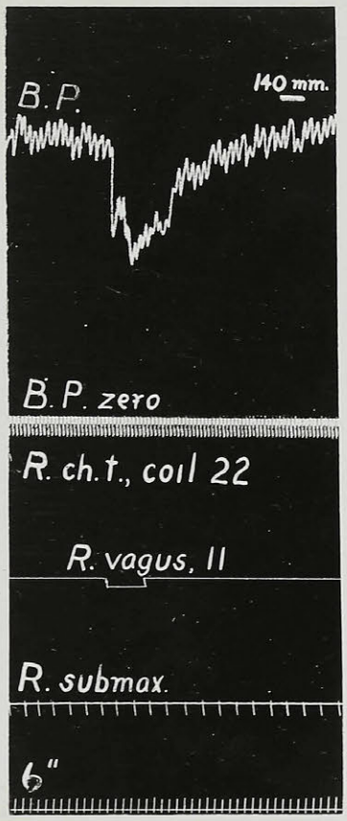
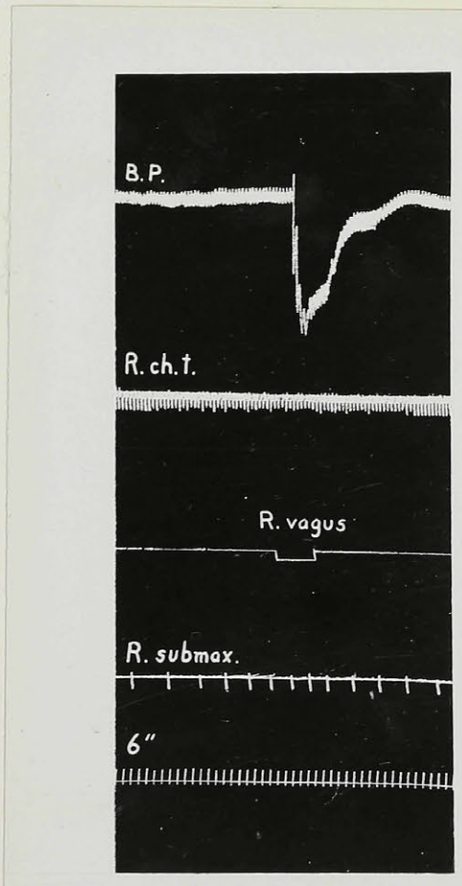


FIGURE 13. - Reading from top to bottom: blood pressure; zero line of blood pressure and signal line showing rhythmic stimulation of right chorda tympani; signal line showing stimulation of peripheral end of right vagus; secretion of right submaxillary gland in drops; and time record. Stimulation of the peripheral end of the vagus in the absence of eserine fails to affect the rate of secretion of saliva.

FIGURE 14. - Explanation of record as in Fig. 13. In the eserinized cat, stimulation of the peripheral end of the vagus may accelerate the flow of saliva from the submaxillary gland secreting under the influence of rhythmic stimulation of the chorda tympani.



always, accompanied by a partial recovery of the blood-pressure. A typical tracing is reproduced in Figure 14. The effect could be obtained repeatedly in the same animal. The latent period was about 20 seconds, i.e. about the same as for transference of the chorda effect to the opposite side, and it seemed at first that a humoral transmission of the vagal effect was actually being observed.

Control experiments soon showed, however, that excision of the suprarenals almost completely abolished the effect. Figure 15 shows the effect on the salivary secretion of stimulating the peripheral end of the vagus in the same eserinized animal, (a) before and (b) after removal of the suprarenals. The acceleration of the secretion in the former case was therefore undoubtedly due to stimulation of the gland by adrenaline, discharged from the suprarenals through a pressor reflex evoked by the fall of blood-pressure. Reflex liberation of adrenaline in response to a lowering of blood-pressure is a familiar phenomenon (110, 111); the chorda stimulation, of course, facilitates the secretory action of adrenaline on the submaxillary.

In all the subsequent experiments (8 in number) the suprarenals were removed at the start. In only two experiments was a very doubtful acceleration of the salivary flow seen to follow vagus stimulation; in the remainder the only result was pure inhibition of the flow. Various modifications of the experimental technique failed, in the absence of the suprarenals, to give any but negative results. Thus it was endeavoured to reduce the circulation by tying the aorta and inferior vena cava in the chest, sometimes with ligation /

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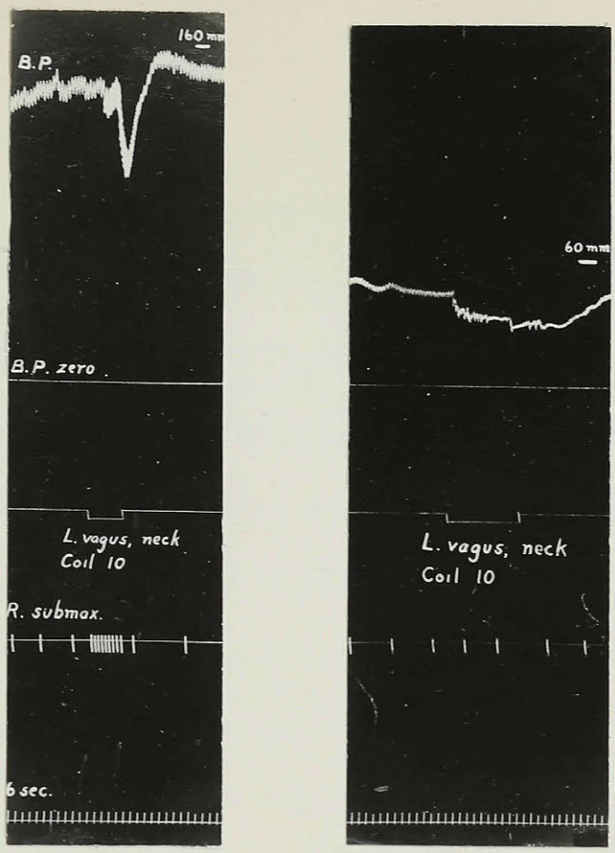

FIGURE 15. - Eserinized cat, right submaxillary gland secreting under the influence of rhythmic chorda stimulation. Before adrenalectomy (a), stimulation of the peripheral end of the contralateral vagus greatly accelerated the salivary secretion: note also the rise of blood pressure on cessation of vagal stimulation. After adrenalectomy (b), stimulation of the vagus failed to accelerate the salivary flow. The blood pressure fell during the abdominal operation, but otherwise the animal remained in apparently good condition for a long time afterward.

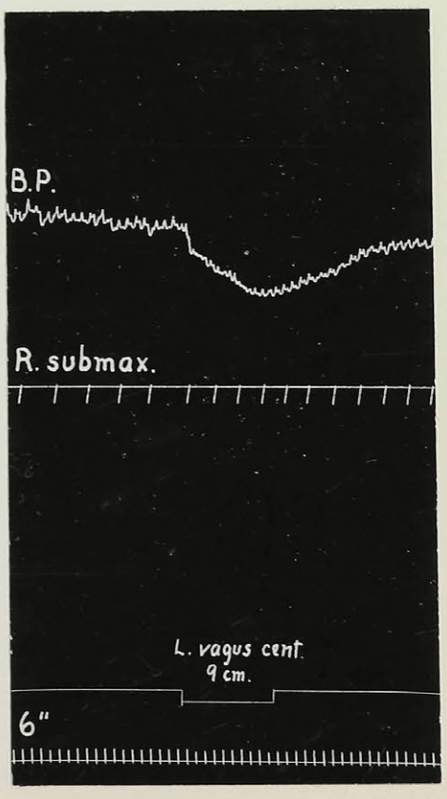

FIGURE 16. - Right submaxillary gland, denervated, secreting under the influence of pilocarpine and eserine. Adrenals removed. Stimulation of the central end of the opposite vagus accelerated the flow of saliva.

ligation of the subclavian vessels also, so that the vagus substance would not be diluted so greatly by the systemic blood. Again, massive doses of eserine (1 mg. per Kg.) were used, the blood-pressure being maintained at its normal level with the help of saline injections. Pilocarpine was used instead of chorda stimulation to sensitize the gland, in order to avoid a possible continuous rise in the concentration of acetylcholine in the blood, which might make the effect of acetylcholine liberated by the vagus less conspicuous. For a similar reason the chorda in other experiments was not excited continuously, but only prior to stimulation of the vagus: a positive effect of the latter would then be manifest as an acceleration of the after-secretion following stimulation of the chorda. With none of these alternative procedures did stimulation of the vagus ever produce anything more than an insignificant acceleration of the salivary flow, even with the suprarenals intact; and with the suprarenals removed all the results were uniformly negative.

Stimulation of the <u>central</u> end of the vagus did, however, in two experiments increase the flow of saliva from a denervated submaxillary gland secreting under the influence of pilocarpine and eserine. The effect was obtainable from either vagus (both were cut) and there was no dilatation of the pupil, ruling out a possible spread of current to the sympathetic. The effect persisted after (a) tying off of the suprarenals, and (b) ligation of the thoracic aorta and inferior vena cava. The acceleration came on after a latent period of 25 to 30 seconds, the latent period for the reflex blood-pressure/

blood-pressure fall being only 4 to 5 seconds. (See Figure 16.) The experiments are suggestive of a humoral transmission of the effect of cholinergic vasodilator fibres, which, however, are not numerous in the cat(111); no further analysis of the effect was made.

Stimulation of the central end of the sciatic nerve in one or two experiments accelerated the salivary flow in the eserinized animal. The effect was abolished by adrenalectomy, and so duplicates the effect observed by Ostrogorsky (112) and shown by Florovsky (113) to be due to reflex liberation of adrenaline.

2. Experiments on the humoral transmission of the effect of the lingual (chorda) nerve.

Cats were used in these experiments; the preliminary preparations were as described in the preceding section. Blood-pressure was recorded from the femoral artery. Both submaxillary ducts were cannulated, and the right one connected to a drop-recorder. The right lingual nerve was cut about 1 cm. above, and again just below its point of separation from the chorda, so that stimulation of the intervening portion would affect only the salivary glands. The fibres in the lingual nerve, the humoral transmission of whose effect was studied in these experiments, are derived from the chorda tympani. To make the description clearer, however, I shall here use the 'chorda' to mean only the small nerve leaving the lingual to run to the salivary glands, and I shall use 'lingual' to mean both the combined chorda-lingual trunk and its continuation (composed of both true chorda and true lingualis fibres) into the tongue. The left chorda was cut at its junction with the lingual, and the latter nerve /

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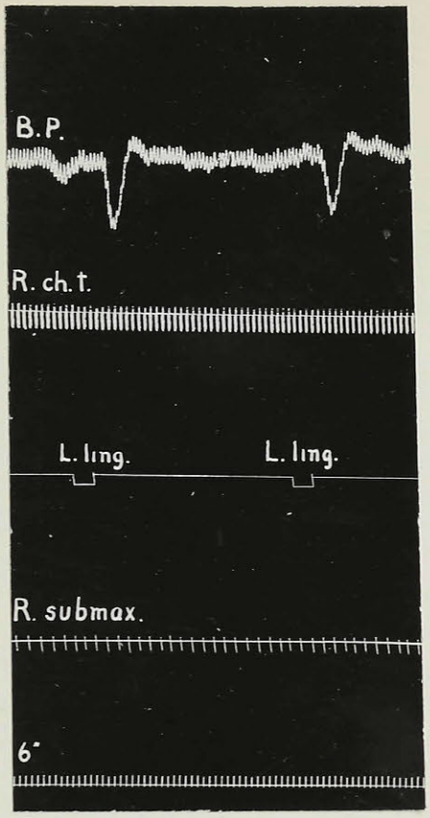


FIGURE 17.

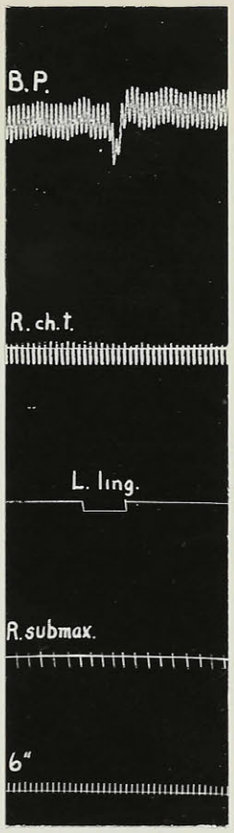
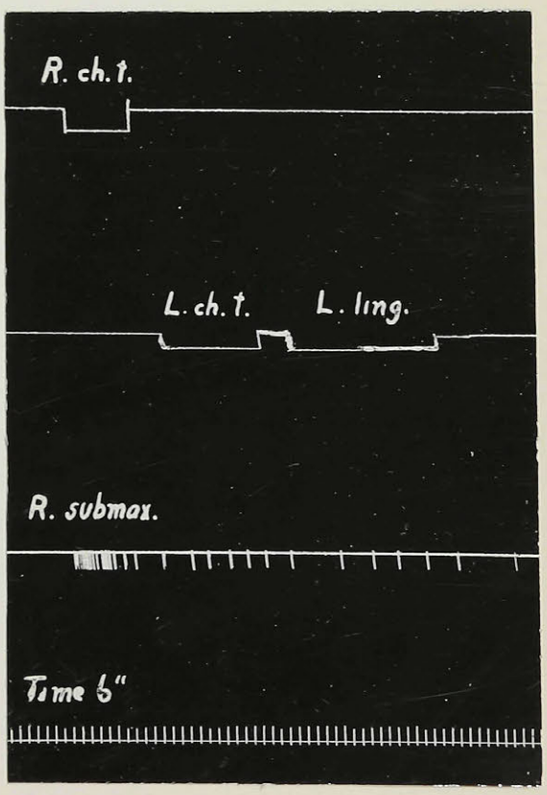


FIGURE 18.

FIGURE 17. - Eserinized cat, right submaxillary gland secreting under the influence of rhythmic chorda stimulation. Stimulation of the left lingual nerve (of which all branches to the salivary glands had been cut) produced after a latent period of about 20 seconds a sharp fall of blood pressure.

FIGURE 18. - Eserinized cat, right submaxillary gland secreting under the influence of rhythmic chorda stimulation. Stimulation of the peripheral end of the left lingual nerve slightly lowered the blood pressure and produced a small but definite acceleration of the salivary flow.

FIGURE 19. - Eserinized cat, secretion of right submaxillary gland recorded. During the secretory after-effect of stimulation of the right chorda tympani, stimulation of the peripheral end of the left chorda produced, after 20-25 seconds' latent period, an acceleration of the salivary flow from the right gland. As the secretion was again slackening, stimulation of the peripheral end of the left lingual nerve (in which only fibres to the tongue remained) produced a second acceleration of the flow, the latent period being about 25 seconds.



stimulation again lowered the blood-pressure and markedly accelerated the secretion from the right gland. This acceleration was normally followed by a steady decline in the rate of secretion. If, however, the left lingual were now stimulated, there was a definite quickening of the salivary flow from the right gland, appearing after a latent period of about 20 seconds and persisting after the stimulation was stopped (Figure 19). This effect could be produced after removal of the suprarenals. The acceleration produced by lingual stimulation was never as great as that produced by chorda stimulation, and correspondingly could not be demonstrated in a proportionately larger number of experiments. In all cases in which the chorda effect was marked, nevertheless, the lingual effect could also be produced. (Out of 7 experiments, a positive effect was seen in 4, and could be obtained several times in each of these.)

3. <u>The foetal heart as an indicator for the humoral transmission parasympathetic effects</u>.

These experiments were performed on pregnant cats near term. Since the number of such animals available was limited, only three complete experiments can be reported. The cats were anaesthetized with chloralose-urethane, and prepared for the recording of salivary secretion and blood-pressure. The chorda tympani and vagus were cut, and their peripheral ends made ready for stimulation The abdomen was opened and a large brass ring stitched into the incision to facilitate observation of its contents. A small opening was made in one horn of the uterus on the side opposite to the placental attachment of an embryo, which was then exposed by cutting /

cutting of the foetal membranes. It was left in position with its blood supply intact, and could be observed for at least 30 to 60 minutes without the supervention of breathing movements or asphyxia. Undue cooling or drying was avoided by the free use of warm Ringer to bathe the uterus. The foetal heart rate was counted by direct inspection and recorded on the kymograph by a hand signal. The maternal heart rate was read from the blood-pressure record. The results were plotted graphically.

No control experiments without eserine were made. In the two experiments in which the vagus was stimulated, it was without effect on the foetal heart rate. Stimulation of the chorda did, however, in two out of three experiments, slow the foetal heart. The effect came on abruptly after a latent period of 15 to 30 seconds and lasted for at least a minute after the cessation of stimulation, the recovery being generally incomplete. So long as the maternal chorda remained unstimulated, however, the heart of the embryo always maintained a very constant rate, so that the effect must be ascribed to the chorda stimulation and not to accidental circulatory changes. The same effect was obtained at least three times in each of the positive experiments; since it was never seen with the vagal stimulation, it is presumably not due to asphyxia of the embryo or to other possible results of the fall of the maternal blood pressure. Figure 20 summarizes graphically a typical experiment.

Discussion /

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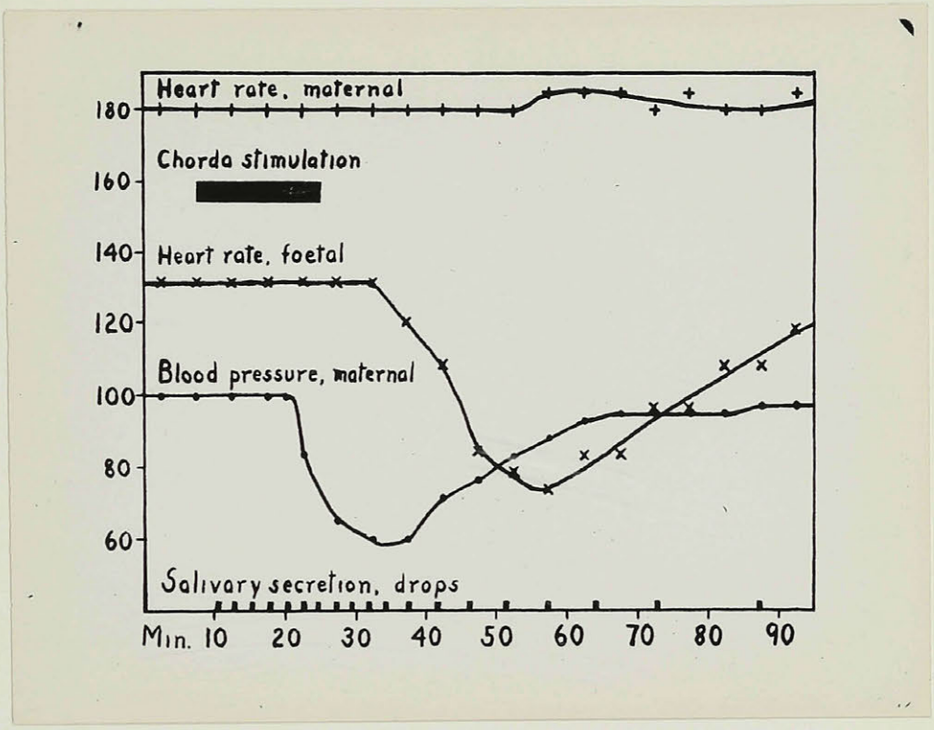

FIGURE 20. - Diagram constructed from the kymographic record of an acute experiment on an eserinized pregnant cat. The ordinates give the maternal and foetal heart rates in beats per minute and the maternal blood pressure in millimetres of mercury. Stimulation of the chorda tympani produced (a) a secretion of saliva which continued for some time after the circulation stopped, (b) a fall in the maternal blood pressure, occurring after a latent period of about 15 seconds, (c) a slowing of the foetal pulse, occurring after a latent period of about 28 seconds.

Discussion.

The experiments on humoral transmission of the vagus effect illustrate a danger of which workers in this field may easily be unaware. It is generally accepted that a sensitization by eserine of a reaction within the organism is *prima facie* evidence for a cholinergic mechanism governing that reaction. Now I have found here that vagal stimulation accelerates the secretion of the denervated salivary gland, only if the animal has been previously treated with eserine. The substance in the blood which mediates this effect is, however, not acetylcholine but adrenaline. The explanation of the eserine sensitization in this case doubtless depends on the fact (Feldberg, Minz and Tsudzimura (49)) that the splanchnic innervation of the suprarenal medulla is cholinergic, so that eserinization accentuates the adrenaline discharge reflexly produced by the depressor action of the vagus. The submaxillary gland secreting under chorda stimulation is, of course, very sensitive to adrenaline (cf. Part I, Section I). Satisfactory evidence for the transmission of the parasympathetic effect within the animal from one organ to another can therefore be obtained only when the intervention of the suprarenals is excluded.

It is clear that vagal stimulation in the eserinized animal is very much less effective than stimulation of the chorda tympani for the liberation of the chemical transmitter into the circulation. This fact does not at all speak against the validity of the theory of chemical transmission as applied to the mammalian vagus. The soundness /

soundness of the theory was already strongly indicated by the experiments with saline perfusion, and by the fact that eserine intensifies and prolongs the vagal inhibition of the heart (114, 115 - frequently verified in the present investigation); and more recently the positive findings of Feldberg and Krayer have settled the matter beyond doubt. The distribution of the vagus, even excluding the abdominal organs, includes, besides the auricles and special tissues of the heart, the bronchioles and oesophagus, and so is presumably at least as great as that of the chorda tympani. It is therefore interesting to speculate on the reasons why so much acetylcholine enters the blood stream when the chorda tympani is stimulated, and so little when the vagus is stimulated. The following possibilities may be suggested (special reference is made to the heart, but most of what is said applies also to the bronchioles):

(1) The cardiac mechanism (choline-esterase) for inactivating acetylcholine continues to work effectively in the presence of eserine: improbable, since the vagal inhibitory effect is so much enhanced by this drug;

(2) The quantity of acetylcholine set free by effective vagal stimulation within the heart tissues is much less than that set free by effective chorda stimulation within the submaxillary gland: this is improbable, since the threshold dose for injected acetylcholine is about the same for the two organs; unless

(3) Acetylcholine is liberated in the heart in especially close relation to the receptive substance, so that concentration at the point /

point of action is high, while the total amount liberated is low. This also is improbable, since atropine readily annuls the cardiac action of the vagus, but only with difficulty the vasodilator action of the chorda tympani (cf. Dale's (116) discussion of the effect of atropine on the pseudo-motor tongue contraction).

(4) The liberated acetylcholine can readily escape from the tissues of the submaxillary gland, but not from the cardiac tissues. This appears to be the best hypothesis available. Thus considerable quantities of acetylcholine appear to escape into saline perfusates of the mammalian heart subjected to vagal action, but not into the venous blood of the heart which retains its blood supply, even when due precautions are taken to inactivate the blood esterase (Plattner (93)). Furthermore, Dale and Gaddum (15) have provided strong evidence that acetylcholine, set free in relation to the blood vessels of the tongue, can pass to and act upon the denervated musculature of that organ; and my own work on the augmented secretion, taken in connection with the histological findings (Rawlinson (43)), supports the idea that acetylcholine formed in the alveolar cells of the submaxillary gland passes to and acts upon the demilune cells. The cells of the submaxillary gland and the tongue are thus 'leaky' for acetylcholine, so that when it has been set free within these organs it passes easily into the blood. The heart apparently is not leaky for acetylcholine, which can get in, but not out, so long as the permeability of the cardiac tissue remains normal. This hypothesis fits well with the respective vascular effects of the vagus and the chorda-lingual: vasodilatation is frequently associated with /

with increased permeability; the vagus has a vasoconstrictor action on the coronary vessels, while the chorda and lingual are powerful vasodilators.

The leech muscle test for acetylcholine in the blood, as used by Feldberg and Krayer (96) is clearly much more sensitive, if less elegant and spectacular, than the test on the submaxillary gland. Feldberg and Krayer found that the cat's heart, when subjected to vagal stimulation, discharged about 0.2y per minute of acetylcholine into the coronary sinus blood. This quantity would not appreciably affect the submaxillary gland (sensitivity 1-2 y). Furthermore, the use of the leech assay enables the experimental animal to be treated with relatively hugh doses of eserine, which greatly increase the stability of acetylcholine in the blood and tissues. Thus Feldberg and Krayer employed 1 mg. per Kg., as contrasted with 0.15 to 0.30 mg. per Kg. in my experiments; the depressor action of these doses was avoided by using atropine, which does not interfere with the leech assay but renders the submaxillary gland test impossible. Finally, for the leech test a 1:1 dilution of the venous blood suffices, whereas the coronary sinus blood becomes diluted perhaps 20 times by admixture with the systemic blood.

The leech muscle assay must now be considered the method of choice when the liberation of acetylcholine from a stimulated organ is to be tested. Other methods may lend confirmatory evidence if the quantity of ester set free is sufficiently great.

Little further need be said about the experiments on the transmission of the effect of the lingual nerve. They supply con-

:firmatory evidence of the liberation, during stimulation of this nerve, of a substance possessing parasympathomimetic properties (lowering of blood pressure, stimulation of salivary secretion). Since the effect is seen only in the eserinized animal, and does not depend on the presence of the suprarenals, the substance can be assumed to be a choline ester. This conclusion is the same as that drawn on other grounds by Dale and Gaddum (15) and Feldberg (108). The experiments, like those of the above-named workers, were conducted on the normally insanguinated organ, and are thus not open to the objections which may be raised to saline perfusion methods as applied to such problems.

It may be noted in passing that both the depressor and the 'crossed' secretory effects obtained by stimulation of the lingual nerve, are smaller than those obtained by stimulation of the chorda tympani, which indeed appears to be in a class of its own so far as the production of these phenomena is concerned.

In regard to the experiments on the transmission of the vagus and chorda effects to the foetus, I shall only point out that my failure to obtain inhibition of the embryonic heart on vagal stimulation in the eserinized cat, seems to contradict the conclusions drawn by Hansen and Rech from their experiments on the uneserinized guinea-pig. In the latter animal the vagus has a powerful bronchoconstrictor action, and they have not proved that the foetal bradycardia which they observed was not caused by asphyxia or by lowering of the maternal blood pressure. The latent period for the action on the foetal heart in their experiments (10 seconds) seems /

seems also, as I have said before, too short to fit a theory of humoral transmission.

The transmission of the chorda effect in my own experiments appears to be a genuine case of humoral transportation, since it occurred when the fall of the maternal blood pressure was less than it was when the vagus was stimulated, although the latter procedure did not affect the foetal heart. Nevertheless not all the other possible explanations have been eliminated, e.g. redistribution of the maternal blood through the depressor action of the chorda, action of the chorda substance on the uterine musculature, etc. The difficulty of interpreting the results forces one to conclude that the method is not very useful for researches of this kind.

It may be appropriate at this point to ask what, if any, is the physiological significance of this transmission of the parasympathetic effect from one organ to another. The answer, clearly, is "None". So effective is the inactivating mechanism of the blood and tissues that it is impossible for acetylcholine liberated by nerve action to pass through the systemic and pulmonary circulations and reach another organ in sufficient concentration to have any effect on it, unless it is protected from destruction by the presence of eserine. Proof of the humoral transmission of the parasympathetic effect from one organ to another has never been furnished by any experiments performed on the uneserinized animal with intact circulation.

Under normal conditions, therefore, the effect of acetylcholine produced by parasympathetic stimulation is confined to the

organ /

organ in which it is liberated: it is purely and simply a 'local hormone'. This does not exclude the possibility that it may diffuse to, and act upon, cells in the same organ other than those in which it was set free. That this may happen under physiological conditions is evidenced by Dale and Gaddum's work on the pseudo-motor contraction, and by Rawlinson's and my own on the augmented and paralytic salivary secretion. It may be noted here that the chemical transmitter of sympathetic post-ganglionic effects is much more stable than acetylcholine, and does normally act on distant structures as well as at its site of liberation. This arrangement may be considered teleologically economical: the sympathetic nervous system normally acts as a unit, the parasympathetic nervous system does not.

Summary.

1. An unsuccessful attempt was made to prove a cholinergic mechanism for the cardiac vagus, by demonstrating a humoral transmission of the parasympathetic effect from the heart to the salivary gland of the eserinized animal. Apparently positive preliminary results were accounted for by reflex discharge of adrenaline as a result of the vagal stimulation. The reasons for the failure of the attempt are discussed.

2. Stimulation of the lingual nerve may produce in the eserinized animal a fall of blood pressure and a secretion from the denervated contralateral submaxillary gland. This confirms the conclusion of Dale and Gaddum and of Feldberg, that the vasodilator fibres /

fibres of the lingual nerve are cholinergic.

3. Slowing of the foetal heart was observed following stimulation of the maternal chorda tympani, but not of the naternal vagus, when eserine had been administered. The findings are discussed from the standpoint of humoral transmission of the parasympathetic effect.

4. The possible physiological significance of humoral transmission of nerve effects is considered.

PART IV /

PART IV. STUDIES ON THE SECOND PHASE OF GASTRIC SECRETION.

I. INTRODUCTORY: THE PRESENT STATUS OF THEORIES OF THE SECOND PHASE.

I have now to describe several groups of experiments which were undertaken as a contribution to our knowledge of the second phase of gastric secretion. The problem of the second phase is a very complicated one, and in spite of much research on the subject very little is yet known of the mechanisms involved. Detailed reviews of the literature have been published by Babkin (28, 29, 117) and by Ivy (30), and since the appearance of these no important advances have been made. For convenience of treatment, however, I shall present here a brief outline of the present status of our knowledge of the second phase, with a discussion of the various theories which have been advanced to explain the experimentally ascertained facts.

It has long been a familiar fact that the secretory response of the stomach to the taking of a meal may be divided into two phases, which are controlled by very different mechanisms. The first phase is reflex in origin, being aroused by the sight, smell, taste, swallowing or thought of food; it depends on the integrity of the vagus nerves, and the gastric juice secreted during it is characterized by a high concentration of pepsin. The second phase, which normally overlaps the first, is a response to the presence of food in the digestive tract, and can occur when the meal is intro-

duced /

:duced directly into the stomach of an experimental animal without its knowledge; the second phase does not depend on the integrity of the extrinsic nerve supply, though its course may be modified through the activity of this. The juice secreted during the second phase is comparatively poor in pepsin. For the purposes of this outline, I shall consider the second phase to include the secretory activity evoked by the presence of food either in the stomach or in the intestine.

The secretory effect of food introduced into the alimentary tract, when the first phase has been eliminated, must be due either to the mechanical action of the food, or to its chemical properties.

'Mechanical' and 'chemical' stimulation. Mechanical stimulation of the resting gastric mucosa, as performed in the most diverse ways by Pavlov and his pupils (see Babkin (28)), uniformly failed to excite any gastric secretion. When the gastric glands are already active, however, mechanical stimulation may somewhat increase the secretion (Krshyshkovsky (118)); and on this basis may doubtless be explained the experiments of Lim, Ivy and McCarthy (1919), who deny Pavlov's results, since these workers used dogs whose 'pouches' gave a continuous spontaneous secretion. A reflex activation of the secretion by distension of the stomach may doubtless occur, and properly calls for consideration with the first phase; thus in Savich's(120) experiments distension of the pylorus caused vomiting, and this is generally associated with a gastric secretion of nervous origin. Violent mechanical /

mechanical stimulation might be expected to excite the gastric glands through the liberation of histamine in the gastric mucosa, where it occurs in high concentration. It is clear, however, that mechanical factors can play at most a very subsidiary role in the normal stimulation of the gastric glands. Thus food extracts when introduced into the stomach evoke a copious secretion, while the same volume of water evokes practically none. The second phase is, therefore, as all modern writers agree, almost exclusively a 'chemical' phase.

The site of action of the chemical stimulants. Chemical stimulation must depend on the contact of the food with the mucous membrane of the digestive tract. It is thus important to know within which regions of the digestive tract the presence of food can excite gastric secretion. This question can be answered with considerable accuracy, as follows:-

(1) The introduction of food, or of products of food digestion, into the fundus of the stomach never brings about any secretion of gastric juice. The only known chemical stimulants which can act on being introduced into the isolated fundus, are alcohol (121) and histamine (119), the latter only in massive doses.

(2) In the pylorus, on the other hand, a variety of chemical stimulants can excite gastric secretion. This has been demonstrated with especial clarity by Sokolov (122) and by Lönnquist (123), who used dogs equipped with Pavlov pouches and permanent fistulas of the stomach and duodenum; the stomach and intestine were completely separated at the pyloric sphincter. Introduction of food

or /

or chemical stimulants into the isolated stomach evoked a secretion of juice from the pouch. Still clearer evidence was advanced by Zeliony and Savich (124) and by Volborth (125) from experiments on dogs having the pyloric part separated from the rest of the stomach; in these animals the introduction of various substances into the isolated pylorus excited the glands of the fundus. Edkins and Tweedy (126) obtained similar results in acute experiments. Smidt (127) found that resection of the pyloric part of the stomach inhibited the production of the second phase in a Pavlov pouch. The role of the pylorus in the production of the second phase is therefore well established. The only negative results, those of Ivy and Whitlow (128) have been criticized on technical grounds by Babkin (29).

(3) Chemical stimulants, when introduced into the small intestine and prevented from refluxing into the stomach, can also stimulate gastric secretion. In general, the effective substances are the same as those acting from the pylorus. They act, however, much less strongly in the intestine.

(4) In the large intestine, the only effective chemical stimulant is alcohol.

The nature of the chemical stimulants. The substances which have been shown to stimulate gastric secretion through their chemical action may be enumerated. Meat and meat extracts head the list, followed by milk, soaps, fatty acids, vegetable extracts, and soda (in the stomach only); water and dilute salt solutions act weakly. Various workers have found that products of protein digestion /

digestion stimulate secretion, but it has not been shown that these products were free from secretagogues originally present as extractives in the food. Most of the amino-acids are inactive (129), and the ascription of activity to others may be due to methodical errors (Babkin (29)); thus I have found (unpublished observation) that glycine has only a questionable secretagogue action even in very large doses. A large number of amines, some of which may be present in meat extracts, have been found by Ivy and Javois (9) to excite gastric secretion; here, too, unfortunately errors of technique render the positive findings open to question (Babkin (29)). It is of especial interest to note that the action of histamine from the intestine is very slight.

Finally it should be noted that certain substances inhibit gastric secretion on being introduced into the digestive tract. Of these fat is by far the most important: all fats when introduced into the digestive tract lower the volume, acidity, and especially the digestive power of the juice secreted under varying modes of stimulation, and prolong the duration of the secretion. The inhibition affects both the first (reflex) and the second (chemical) phase, but the former more than the latter. The products of fat digestion, however, the soaps and fatty acids, appear to stimulate gastric secretion.

The mechanism of chemical stimulation. A variety of theories has been proposed in order to explain the way in which the contact of chemical stimulants with the pyloric and intestinal mucosa can stimulate the glands of the fundus. The possible mechanisms may be listed as follows:-

(1) /

(1) The second phase may be reflexly produced, the reflex arc being either 'long' (through the central nervous system) or 'short' (in the enteric plexus from pyloric or intestinal to fundic mucosa). Such a mechanism must be of minor importance, since the second phase occurs normally when food is introduced into the denervated isolated pylorus (Savich (130)), or when the effect of feeding is tested on a denervated fundic pouch Rheinboldt (131)); secretion in response to a meal occurs even in small fundic pouches transplanted to the mammary gland.

(2) The second phase may be due to the stimulation of secretory centres by changes in the composition of the blood following the absorption of products of digestion, the efferent path being the vagus nerves (Okada (11)). It has not been claimed that this mechanism - the so-called 'humoneural' regulation - is the only one involved in the production of the second phase. According to Okada, hypoglycaemia stimulates, and hyperglycaemia inhibits gastric secretion, while amino-acids in the blood stimulate. The data at hand are too few to enable one to assess properly the importance of this mechanism. The impression one receives, however, is that this is not very great. Thus large doses of insulin or of amino-acids are necessary to produce small secretory effects; again, the secretory response of the Pavlov pouch to 'gastrin' preparations is not affected by insulin (132).

(3) The second phase may be due to a specific gastric hormone 'gastrin', set free from the pyloric mucosa by the action of chemical stimulants, and carried by the blood to the fundic glands, on which it acts. Although this theory has had many warm supporters, the /

the action of 'gastrin' is still uncertain. The essential fact on which the 'gastrin' hypothesis is based is that extracts, especially acid extracts of the pyloric mucosa stimulate gastric secretion when injected parenterally (Edkins (133)). Similarly prepared extracts of most other tissues (with the exception, however, of the fundic mucosa (134)) are less active, while the other digestive glands respond less strongly than the gastric glands to such preparations. Popielski (135) has observed, however, that all active extracts possess a non-specific vasodilator action (presence of 'vasodilatin'); he accepted the suggestion of Dale and Laidlaw (136) that 'vasodilatin' was identical with histamine. Histamine is, of course, an extremely powerful stimulant of the gastric secretion, and most of the later work in this field has been directed toward ascertaining whether histamine and 'gastrin' are in fact the same. I shall have occasion to discuss this work in some detail later on (page 114), and shall now state only what appear to be the main conclusions, which are:- (1) Histamine is present in the gastric mucosa in pharmacologically significant concentration, and doubtless is responsible for much, if not all, of the activity of 'gastrin'; (2) the chemical properties of 'gastrin' are not quite identical with those of histamine, but it is possible that the reactions of histamine may be modified by the presence of impurities in the stomach extracts (cf. Gaddum (31) - page 12); (3) certain experimenters (Koskowski and Kubikowski (137)) claim to have been able to show an increase in the histamine content of the blood following digestion /

digestion of a meal: their work has been adversely criticized by Babkin (117); (4) there are certain apparent differences in the composition of the gastric juice produced by histamine and by chemical stimulation respectively: I shall discuss this point later. Recent workers have been strongly inclined to conclude the identity of histamine with the active principle of the extracts of gastric mucosa. Ivy et al. (138) say that "either histamine is the gastric hormone, or there is no gastric hormone, or the gastric hormone has never been extracted from the pyloric mucosa."

(4) The final possibility is that the second phase is due to the presence in the food, or in its digests, of active secretagogues which are absorbed as such, and, passing in the blood to the fundus, stimulate its glands directly. On this view, to which I shall refer as the 'absorption theory', the hypothesis of a hormone formed in the pylorus is unnecessary. To prove this theory, it must be shown that such constituents (a) act either on oral or parenteral administration and (b) are capable of being absorbed from the gut and of reaching in the blood a concentration sufficient to excite the gastric glands. Judged by these standards, the evidence for the 'absorption theory' is quite unsatisfactory. (1) Various meat extracts, for example, stimulate gastric secretion both on introduction into the stomach and on subcutaneous administration, but it has not been shown that the same substances are responsible in both cases. Indeed, a great part of the activity observed on subcutaneous injection is doubtless due to the presence of histamine, which does not act from the /

the gut. (2) Again, when present in the pylorus, fatty acids and soaps stimulate, while neutral fat inhibits secretion, yet the former substances are received into the blood in the form of neutral fat: this fact appears to be explicable only on the basis of a hormone theory. (3) Atropine paralyzes the secretagogue action of Liebig's extract when the latter is introduced into stomach, but not when it is injected intravenously: this objection may be avoided by assuming that atropine inhibits the absorption of secretagogues, or that the secretory action following intravenous injection of the extract is due to a substance (e.g. histamine) which does not act from the gut. (4) If mere absorption of secretagogues is all that is necessary for the production of the second phase, one would expect this to be performed more efficiently by the intestine than by the pylorus, yet chemical stimulants act more strongly from the stomach than from the intestine. To this objection it might be replied that the pylorus is perhaps especially adapted for this particular task of absorbing secretagogues, or else that the secretagogues are better absorbed from an acid medium. (5) Kim and Ivy (139) found that the same solution of liver extract could be applied repeatedly to a pouch of the entire stomach without diminution of its secretagogue activity this is apparently strong evidence for the 'gastrin' theory; but the secretory effects obtained were small, ahd it is possible that the pouch did not absorb well; furthermore, the acidity of the solution was increased by each application, and possibly this promoted absorption of the secretagogues. The authors sometimes observed the /

the opposite effect, viz. diminution of the secretory effect on repeated application.

It will be obvious from this brief discussion that neither the 'gastrin' theory nor the 'absorption' theory rests on a very sound experimental basis. The final decision between the two must no doubt await the identification of the secretagogue bodies present in the circulating blood during the second phase.

Are the peptic cells stimulated during the second phase? Leaving for the present our discussion of these rival theories, we may consider the characteristics of the secretion produced during the second phase. The most striking feature of the juice of the second, as compared with the first phase, is its lower digestive power. Since the acidity is nearly the same in either case when the rate of secretion is the same, it must be concluded that in the second phase the peptic cells are relatively less active than the parietal cells. Nevertheless when the juice of the second phase is compared to that produced under the influence of histamine, it is clearly seen to have a higher content of pepsin, even when the rate of secretion is the same with both types of stimulation. This, as Babkin (117) has emphasized, is a strong argument against the belief that histamine alone is the humoral mediator of the second phase. It suggests that during the second phase the peptic cells, as well as the parietal cells, become active, perhaps through stimulation of the cells of the intra-gastric plexuses (Babkin (117)). This suggestion must, however, be received with caution, since histamine has been shown to have an inhibitory effect on the discharge /

:charge of pepsin; the very low digestive power of histamine juice might thus be explained as due to a retention of the spontaneous output of the peptic cells; while during the second phase this spontaneous output persists, being neither inhibited by histamine, nor accelerated through a humoral stimulation of the peptic cells or the nerves controlling them. I shall refer later to the question of whether the peptic cells participate actively in the second phase.

Having given this introductory outline of the present state of our knowledge of the second phase, I shall now present my experimental work on this subject. This may be divided into three sections, as follows:-

(1) I have investigated, with the aid of both chemical and physiological methods, the constituents of fish muscle which exhibit secretagogue activity on introduction into the digestive tract. Although it seems clear that only systematic work of this sort can make clear the nature of the secretagogue substances of foodstuffs, the literature contains only a few isolated instances of attempts to correlate the chemical and physiological properties of these bodies.

(2) In view of the physiological activity of choline, and its wide distribution in foodstuffs, I have made a detailed study of its action on gastric secretion, in the hope of determining its possible significance for the production of the second phase.

(3) Since there is strong evidence for the identity of histamine with the secretagogue body extracted from the pyloric mucosa /

mucosa, and since it has been suggested that histamine is actually the gastric hormone, I investigated the histamine content of the blood before and after the taking of food. If histamine is really the hormone of the second phase, its concentration in the blood must necessarily be increased during the digestion of a meal.

The account of each of these experimental studies will be preceded by a short review of the related literature and followed by a brief discussion.

II. THE NITROGENOUS BASES OF HADDOCK MUSCLE AND THEIR EFFECT ON GASTRIC SECRETION.

I referred in the General Introduction to the importance of the nitrogenous basic constituents of protein food as stimulants for the digestive glands. The experimental work on this subject has been scanty and unsystematic. The literature contains various isolated allusions to the gastric secretory action of various basic substances which may occur in the diet. Little effort has been made, however, to learn to what extent the secretagogue activity of these substances can explain the second phase of gastric secretion. Thus it is not sufficient to show that a substance known to be present in food can stimulate the gastric glands: it must be ascertained how far the secretory response of the stomach to a meal containing this substance can be accounted for by the quantity present in the meal. Furthermore, it must be shown that the substance is active when introduced into the digestive tract: various substances which act when injected parenterally are, on introduction into /

into the gut, either not absorbed, or inactivated by the intestinal wall or by the liver. Due attention must also be paid to the possibility: (a) that active substances may be formed as artefacts during chemical procedures for the isolation of secretagogues; (b) that food, especially animal food, may contain active substances, liberated by post-mortem autolysis or by the preliminary phases of digestion, which are not detected in chemical studies in which all such uncontrolled decomposition is avoided; (c) that a substance, though possessing little direct activity of its own, may yet intensify the secretagogue action of other substances; and (d) that the activity of a substance may depend on the state of nutrition of the experimental animal.

The first systematic attempt at analysis of the secretagogue activity of the nitrogenous basic fraction was made by Krimberg and Komarov (8). The phosphotungstic-acid-precipitable fraction of beef muscle extract was further fractionated by the silver-baryta method into the so-called purine, histidine-arginine, and lysine fractions. The secretagogue activity of each of these fractions and of the total nitrogenous-basic fraction was tested on dogs equipped with Pavlov pouches, the extracts being administered intravenously. By this method it was found that the secretagogue activity of the total nitrogenous-basic fraction was practically all recovered in the histidine-arginine fraction, the lysine and purine fractions being almost completely inactive. Krimberg and Komarov were unable to determine how much of the total activity of the beef muscle was accounted for by the nitrogenous bases, since the crude protein-free extract /

extract was too toxic to give intravenously. The chief criticism to be made of this work is the mode of giving the extracts; it is always possible that substances acting intravenously may fail to act from the gut, and vice versa.

The work of Campbell (unpublished) is especially interesting. He prepared extracts of the muscle of various fishes, and tested them on a dog equipped with a Pavlov pouch, introducing the solutions directly into the stomach through a gastric fistula. Practically the entire activity of the crude protein-free extract of cod-muscle was found to be due to its content of nitrogenous bases. In the nitrogenous-basic fraction 47 per cent of the activity was accounted for by the histidine-arginine fraction and 19 per cent by the lysine fraction, which contained 8 and 38 per cent respectively of the non-protein nitrogen of the muscle. The juice produced by the lysine fraction was very rich in pepsin; as the secretion, however, was small, this was perhaps due not to a true stimulation of the peptic cells, but merely to a washing out from the glands of accumulated pepsin. Campbell unfortunately did not have the opportunity to complete this work; he was able to make only one series of experiments on one dog.

Kim and Ivy (139) examined the effect of liver extract and meat (Liebig's) extract, administered to dogs by different routes. They found that removal of the vasodepressor substances by treatment of the crude extract with Lloyd's reagent did not appreciably decrease the secretagogue value of the extracts when this was tested by application to an entire-stomach pouch; on introduction into the intestine, however, the crude extract was more effective than the purified /

purified. The greater part of the secretagogue activity of fresh liver was recovered in the 70%-alcohol-soluble, 95%-alcohol insoluble fraction; further, the active substances were not precipitated by phosphotungstic acid. The latter finding is in contrast to Campbell's results on fish muscle; possibly, however, the secretagogue constituents of the two tissues differ.

I have been able to find in the literature no reports, other than that by Kim and Ivy, of any work in which the chemical distribution of secretagogues in food has been correlated with their secretory action on enteral administration. It seems clear that our better understanding of the second phase must await a systematic study of this sort. I have therefore repeated in part the work of Campbell, since his data were so few in number. The first part of this work was chemical, involving the preparation of the total nitrogenous-basic fraction of haddock muscle, and the further fractionation of this by the silver-baryta method. The second part of the work was physiological, and involved the testing of the secretagogue power of the various extracts on a dog equipped with a Pavlov pouch and gastric fistula.

The chemical distribution of the non-protein nitrogen of fish muscle is of interest per se, as well as in its relation to gastric secretion. The literature on this subject, which consists largely of isolated observations as to the presence or absence of various individual substances, is summarized by Kapeller-Adler and Krael (140). Systematic fractionation of the nitrogenous-basic fraction, as has been so often carried out for mammalian muscle, especially

by /

by Kossel and his students (141), has been performed for fish muscle only by Komarov (142, 143) and Campbell (144). As compared with mammalian muscle, the muscle of haddock (142) as well as of cod, salmon, herring and skate (144) is characterized by a somewhat higher content of basic nitrogen, which is due chiefly to a high content of nitrogen in the lysine fraction; the percentage of the non-protein nitrogen accounted for by the histidine-arginine fraction and by the "humine bodies" was relatively low in fish muscle. The fractionation technique used in my experiments was practically identical with that adopted by Komarov and by Campbell, and my results are quite similar to theirs.

I shall describe first the details of the fractionation of the fish-muscle extract, giving the values obtained for partition of the non-protein nitrogen; and secondly, the physiological assay of the various fractions for their gastric secretory activity. The physiological investigations consist of two parts. The effect of the extracts in evoking a flow of gastric juice (production of fluid and acid) was studied by introducing the different solutions directly into the stomach of a dog equipped with a gastric fistula and a Pavlov pouch. Such a method is, however, not generally suitable for determining a possible action of the chemical stimulants on the peptic cells, since the resting gastric glands always contain considerable pepsin, which is washed out when secretion begins; if the volume of the secretion is small, the presence of this previously secreted pepsin obscures any moderate effect of the chemical stimulants on the peptic cells. I have therefore injected the fractions /

fractions intravenously during the course of a histamine secretion in the anaesthetized animal. The prolonged action of histamine produces a juice whose digestive power becomes progressively lower and lower until it approaches zero (145, 146); injection of a parasympathomimetic substance like pilocarpine (147) or choline (see page 104) now greatly increases the peptic activity. If the fractions were to contain appreciable quantities of choline, or of substances acting similarly on the peptic cells, this would presumably be evidenced by an increase in the peptic power of the 'histamine' juice. It might be thought more desirable to introduce the extracts into the digestive tract during the action of the histamine: this was prevented by obvious technical difficulties, e.g. the difficulty of ensuring the absorption of extracts introduced into the stomach of the anaesthetized and and histaminized animal.

Experimental.

1. The partition of the non-protein nitrogen of haddock's muscle.

The fractionation was carried out according to Komarov's (142) modification of the method of Kossel and Kutscher (141), with certain minor changes. The crude haddock-muscle extract, representing 4740 gm. of tissue, was prepared as described by Komarov (142) from the fresh tissue taken immediately after death, by extraction with alcohol and hot distilled water, and freed from protein by heating at the isoelectric point. It gave no precipitate on treatment with trichloroacetic acid and was regarded as practically protein-free.

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The extract was made up to about 3 litres with distilled water and concentrated *in vacuo* to about 1 litre. (All evaporations during this work, unless otherwise mentioned, were performed in this way, *i.e.* under a pressure of 10 to 20 mm. Hg at a water-bath temperature not exceeding 45° C. Toluene was added as preservative between the various stages of fractionation.) The concentrated extract was then heated to 85° for 15 minutes and a small precipitate of protein-like material filtered off by suction. The filtrate was cooled and made up to 2000 c.c. 500 c.c. of this was measured out and reserved for analyses and physiological experiments; the remainder was used for further fractionation.

Phosphotungstic acid precipitation. The extract was concentrated to about 800 c.c. and transferred to a 3-litre beaker. It was then precipitated with 50% phosphotungstic acid solution, 50% H_2SO_4 being coincidently added with vigorous stirring to make the concentration of H_2SO_4 in the solution approximately 5 per cent. When precipitation was nearly complete, the solution was tested for free nitrogenous bases by filtering 2 to 3 c.c. and adding 2% phosphotungstic acid, any precipitate being conserved. After about 800 gm. of phosphotungstic acid and 300 c.c. of sulphuric acid had been added, the filtrate showed slight turbidity on treatment with an equal volume of 50% (but not 2%) phosphotungstic acid. The solution was permitted to stand overnight to complete the precipitation. It was then filtered and washed once with 5% sulphuric acid. The filtrate was again treated with 100 c.c. of 50% phosphotungstic acid and 10 c.c. of 50% sulphuric acid, and after standing /

standing a second small precipitate was filtered off. The filtrate now gave no further precipitate on treatment with 50% phosphotungstic. The precipitates were combined and washed thoroughly with 5% sulphuric acid. The filtrate (containing mono-amino acids, urea, non-nitrogenous extractives, etc.) was discarded. The precipitate was decomposed by grinding in the mortar with solid barium hydroxide, any great excess of the latter being avoided; there was nevertheless probably some loss of volatile-base nitrogen during this procedure. The solution of liberated free nitrogenous bases was quickly filtered off and acidified with sulphuric acid; the precipitate was washed with distilled water and immediately returned to the mortar, ground with distilled water, and filtered. This process was repeated several times, hot water being used for the final extraction of the precipitate. The last washings were practically colourless. The combined filtrates, totalling about 4 litres in volume, were nearly freed from sulphate by treatment with $Ba(OH)_2$ and $BaCO_3$; the $BaSO_4$ formed was filtered off and washed with hot distilled water. The faintly alkaline, nearly SO_4-free solution was concentrated to about 1.5 litres under CO_2. It was then made up to 2000 c.c., of which 500 c.c. was taken for analyses and experiments, and the remainder (representing $\frac{9}{16}$ of the original extract) was used for further fractionation.

Purine fraction. The main bulk of the fraction was neutralized with 10% HNO_3, concentrated to about 100 c.c., and made slightly acid to Congo by further addition of HNO_3. 20% $AgNO_3$ was added to Kossel's end-point. After standing overnight in the ice-box, the precipitate /

precipitate of silver compounds of purine bases (first silver precipitate) was filtered off and well washed with distilled water. It was decomposed by treating with a stream of H_2S for several hours, with frequent shaking. After the precipitate of AgS had been filtered off, the light yellow solution, containing the purine bases in the form of nitrates, was concentrated to about 300 c.c. It was filtered and made up to 500 c.c., of which 100 c.c. was taken for analyses; the remainder was evaporated to dryness on the steam-bath and kept in the desiccator.

Histidine fraction. The filtrate and washings from the first silver precipitate were combined and exactly neutralized with warm saturated $Ba(OH)_2$ and the precipitate (second silver precipitate) filtered off and washed with cold distilled water. The precipitate was decomposed by treatment with H_2S in the same way as the purine fraction. After the solution of bases had been filtered off, the precipitate was treated once more with H_2S. The combined filtrates were concentrated to 150 c.c. and filtered from a slight residue of inorganic material. The filtrate was made up to 500 c.c., a 100-c.c. sample taken for analyses, and the remainder dried on the steam-bath and placed in the desiccator. (The names 'histidine', 'arginine' and 'lysine' are applied to the fractions because these amino-acids can be quantitatively separated from protein hydrolysates by the respective procedures.)

Arginine fraction. The filtrate from the second silver precipitate was precipitated with warm saturated $Ba(OH)_2$, a slight excess only being added. Ice-cold distilled water was used for washing. The /

The filtrate, containing the bases of the lysine fraction, was received directly into 5% sulphuric acid. The whole procedure, including filtering and washing, was performed in less than 40 minutes under ice-cooling. The washed precipitate was immediately suspended in distilled water and made slightly acid with H_2SO_4. It was then decomposed with H_2S in the same way as the second silver precipitate, and filtered, the residue being repeatedly suspended in warm distilled water and again decomposed until the filtrate was colourless. The combined filtrates were concentrated, freed from traces of sulphate by careful treatment with dilute $Ba(OH)_2$, and further concentrated to about 200 c.c. The extract was then filtered and made up to 500 c.c., of which 100 c.c. was taken for analyses and the remainder evaporated to dryness.

Lysine fraction. The filtrate from the final silver precipitate was freed of Ag by treatment with H_2S. Ag_2S was filtered off, H_2S removed by aeration, and excess of H_2SO_4 removed by neutralization with $Ba(OH)_2$. The extract was then concentrated to a small volume, precipitated with phosphotungstic acid in the presence of H_2SO_4, exactly as was the crude extract, and the precipitate decomposed as before with $Ba(OH)_2$. The filtrate was collected into 5% H_2SO_4 to remove excess baryta, and $BaSO_4$ filtered off. The filtrate was concentrated to about 200 c.c., made distinctly alkaline with $Ba(OH)_2$, and treated with a vigorous stream of CO_2. After the $BaCO_3$ had been filtered off, remaining traces of baryta were removed by cautious treatment with H_2SO_4. The still alkaline Ba- and SO_4-free filtrate, containing bases in the form of carbonates /

(To follow page 86.

TABLE III.

The results are expressed as mg. per 100 gm. of the wet tissue and as percentages of the total non-protein nitrogen. The data of MacIntosh and of Komarov are for haddock, those of Campbell for cod, a closely allied species.

Nitrogen in the form of:	Haddock				Cod		Beef	
	MacIntosh		Komarov		Campbell		Komarov	
	mg. %	N.P.N. %	mg. %	N.P.N. %	mg. %	N.P.N. %	mg. %	N.P.N. %
Non-protein	466.4	100.0	380.0	100.0	419.2	100.0	402.0	100.0
Nitrogenous Bases	279.7	60.0	239.4	63.0	253.6	60.5	215.4	53.6
Purine Fraction*	10.1	2.2	15.4	4.0	24.5	3.8	3.5	0.9
Histidine Fraction	1.8	0.4						
Arginine Fraction	21.7	4.7						
Histidine-arginine Fraction	23.5	5.0	51.5	13.6	32.7	7.8	89.8	22.3
Lysine Fraction	198.3	42.5	145.0	38.2	157.9	37.7	65.6	16.3
'Humine Bodies' in p.t.a. precipitate	47.8	10.2	27.5	7.3	38.5	9.2	56.5	14.1

* Purine bases were present as nitrates in the fish-muscle fractions, as free bases in the beef-muscle fraction; the N values for the former are therefore relatively high.

morning or pre-feeding secretion, when care was taken to avoid psychical stimulation, was less than 1 c.c.

The experimental procedure was the same for all the experiments. The dog was placed on the stand at 9 a.m., and a perforated rubber catheter placed in the pouch in the usual way for continuous collection of the secretion. A few drops of gastric juice, probably secreted during the night, was usually obtained, but if the flow did not cease within 30 minutes the experiment was not continued. The extract to be used was measured out in an amount corresponding to 200 gm. of the fresh tissue, dissolved in water, and made up to 100 c.c. It was warmed to 37° C. and injected directly into the stomach of the dog by means of a funnel connected with rubber tubing to the gastric fistula; care was taken to avoid the introduction of air into the stomach. The solution was run in slowly and was followed by 25 c.c. of water to rinse out the apparatus, which was then disconnected and the fistula stoppered. The volume of the pouch secretion was measured at 30-minute intervals. The duration of the secretion was 2 to 2½ hours following administration of the active fractions, and about 1 hour following administration of saline or of the inactive fractions, though in both cases there was a considerable flow (0.5 c.c. or more) of thick mucus subsequent to this. The greatest volume of secretion occurred during the 2nd and 3rd half-hour periods with the lysine fraction, during the 1st and 2nd periods with the other preparations. All possible care was taken to avoid psychical disturbances which might affect the secretion. Usually the dog slept through most of the experimental period.

The /

The volumes given in the table indicate the quantity of clear juice secreted, any mucus having been immediately removed with a glass rod. When a quantity of juice was obtained, it was analyzed for free HCl and total acid by titration with Töpfer's reagent and phenolphthalein respectively, and pepsin by Nirenstein and Schiff's (148) modification of Mett's method.

Two experiments were performed with each of the fractions whose preparation has been described, with the exception of the fraction of the nitrogenous bases, where an insufficient quantity of the extract had been retained for this purpose. In this case a 'synthetic' fraction of the nitrogenous bases was prepared by combining the purine, histidine, arginine, and lysine fractions in quantities equivalent to 200 gm. of muscle each, and making up to 100 c.c. with water. The results of duplicate experiments always agreed well.

Control experiments were carried out with 100 c.c. volumes of (a) water, (b) 0.9% NaCl, and (c) 5% NaCl, instead of the haddock extracts. These cover the osmotic pressure range of the extracts. It is clear that the effect of osmotic pressure variations is unimportant (Table IV).

The results of the experiments are summarized in Table IV. The second column of figures gives the results of a preliminary incomplete series of experiments: between these and the experiments summarized in the first column of figures, the dog suffered a prolapse of the pouch, following which the composition of the pouch juice /

juice was altered. The third column of figures gives the results obtained by Campbell on administration of cod-muscle extracts, in quantities corresponding to 200 gm. of muscle, to a Pavlov-pouch dog.

The values for acidity and digestive power have been omitted, as there are wide variations between the results of duplicate experiments, even although all determinations were made immediately at the end of the experiment. The cause of these discrepancies was no doubt contact of the juice before leaving the pouch with relatively large quantities of mucus, which would be associated with previously formed alkali and pepsin.

TABLE IV.

Fraction	Volume of Secretion (c.c.)		
	1st Series	2nd Series	Campbell
Protein-free filtrate	3.05, 3.35	5.75, 5.40	6.59
Nitrogenous Bases fr., original	2.40	4.90	6.55
Nitrogenous Bases fr., 'synthetic'	4.05		
Purine fraction	1.00, 0.95		
Histidine fraction	0.90, 0.90	1.75	
Arginine fraction	2.55, 2.30	3.15	
Histidine-arginine fr.			3.25
Lysine fraction	2.90, 3.15		1.53
Distilled water	1.00		1.85
0.9% NaCl	0.90		0.31
5.0% NaCl	0.60		

(b) /

(b) Intravenous injection into anaesthetized animals secreting under the influence of histamine. These experiments, devised with the intention of ascertaining the effect of the haddock-muscle extractives on the secretion of pepsin, were performed on dogs anaesthetized with chloralose-urethane. The trachea, external jugular vein (for injections), and sometimes the carotid artery (for blood-pressure records) were cannulated. Both vagi were cut in the neck to avoid possible reflex activation of the peptic cells. The stomach was ligated at the pyloric sphincter, and a doubly flanged metal cannula stitched into the ventral wall near the greater curvature. The abdominal incision was closed, and the stomach washed out with large quantities of warm water. After it had drained completely, histamine (2 to 4 mg.) was injected subcutaneously at hourly intervals, and the secretion collected as it flowed from the cannula during 30-minute periods. When a copious secretion had been established for 2 hours or more (so that the digestive power of the juice could b e expected to have become very low), one of the haddock-muscle fractions was given intravenously, the injection being made very slowly, and collection of gastric juice continued. Free and total acidity, chlorides (149) and pepsin were determined.

The results are best illustrated by the quotation of two experimental protocols. Two such experiments were performed with the lysine fraction, one with the total nitrogenous-basic fraction. The crude protein-free filtrate was too toxic to inject. The histidine-arginine fraction was not used. In each of the experiments /

iments the digestive power of the gastric juice secreted under the influence of histamine fell off rapidly almost to zero. Injection of the lysine fraction (which would contain any choline present in the free form in the tissue) failed completely to raise the peptic power; and injection of the total-bases fraction had only a very faint effect of this kind. As the protocols show, the rate of secretion tended to rise with repeated subcutaneous injections of histamine, but varied considerably even though care was taken to keep the animal's condition as constant as possible; the effect of the basic fractions on the volume of the secretion, although apparently the rate of flow was increased, was not shown to be specific. The acidity and chloride content of the juice, which reach very high levels with the continued action of histamine, were likewise not appreciably affected.

Experiment June 13, 1935.

Dog, male, 5.6 Kg., Chloralose-urethane anaesthesia. Gastric fistula inserted, pylorus ligated, vagi cut. Stomach washed out three times with 500-c.c. portions of warm tap-water. Cannulas in external jugular vein for injections and in carotid artery for recording blood-pressure. Operation begun 11 a.m.

Time		Vol. c.c.	Free acid m.eq./l.	Total acid	Cl	Pepsin Mett U.	B.P. mm.Hg
12:00-12:30	Stomach draining.	0.4					
12:30	Histamine (2 mg.) subcutaneously.						
12:30- 1:00		1.8	48	54	118	41	
1:00- 1:30		3.8	71	77	140	19	
1:30	Histamine (2 mg.) subcutaneously.						
1:30- 2:00		5.2	73	82	144	10	
2:00- 2:30		3.2	70	75	152	5	
2:30	Histamine (2 mg.) subcutaneously.						
2:30- 3:00		5.4	89	92	156	5	
3:00- 3:30		8.5	119	123	157	3	
3:30	Histamine (2 mg.) subcutaneously.						
3:30- 4:00		12.9	132	135	157	1	
4:00- 4:30		18.7	148	152	164	0	60
4:30	Histamine (2 mg.) subcutaneously.						
4:30-4:40	Lysine fraction (20 c.c. = 200 gm. haddock muscle).						

4:30 /

(Experiment June 13, 1935 - cont.)

Time	Vol. c.c.	Free acid m.eq./l.	Total acid m.eq./l.	Cl	Pepsin Mett U.	B.P. mm.Hg.
4:30-5:00	24.7	156	161	169	0	120
5:00-5:30	21.6	162	166	173	0	105
5:30 Histamine (2 mg.) subcutaneously.						
5:30-6:00	23.5	158	164	169	0	
6:00-6:30	16.1	157	161	166	0	110

Experiment May 8, 1935.

Dog, male, 12 Kg. Chloralose-urethane anaesthesia. Gastric fistula inserted, pylorus tied, vagi cut. Stomach washed out three times with 1000-c.c. portions of warm tap-water. Still a little bile-stained mucus remaining in stomach. Operation begun 12:00 noon.

Time	Vol. c.c.	Free acid m.eq./l.	Total acid m.eq./l.	Cl	Pepsin Mett U.
1:15-2:00 Stomach draining.					
2:00-2:30	0.9				
2:30-3:00	0.2				
3:00 Histamine (3 mg.) subcutaneously.					
3:00-3:30	10.0	105	121	167	41
3:30-4:00	12.9	118	134	169	11
4:00 Histamine (3 mg.) subcutaneously.					
4:00-4:30	13.3	124	141	170	6
4:30-5:00	15.6	133	148	169	6
5:00 Histamine (3 mg.) subcutaneously.					
5:00-5:30	15.2	144	155	173	4
5:30-5:40 100 c.c. 1.35% NaCl (isotonic with N-bases fr.) intrav.					
5:30-6:00	22.0	140	152	171	3
6:00 Histamine (3 mg.) subcutaneously.					
6:00-6:30	14.6	137	148	167	3
6:30-7:00	18.0	131	145	172	2
7:00 Histamine (3 mg.) subcutaneously.					
7:00-7:20 100 c.c. N-bases fr. (= 200 gm. haddock) intravenously.					
7:00-7:30	22.6	132	146	171	2
7:30-8:00	20.7	129	144	176	1
8:00 Histamine (3 mg.) subcutaneously.					
8:00-8:30	14.3	116	140	169	1
8:30-9:00	18.2	94	132	166	4
9:00 Histamine (3 mg.) subcutaneously.					
9:00-9:30	16.8	88	132	-	6
9:30 10 c.c. protein-free filtrate (≡ 20 gm. haddock) intrav.					
9:35 Dog died.					

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(To follow page 93.

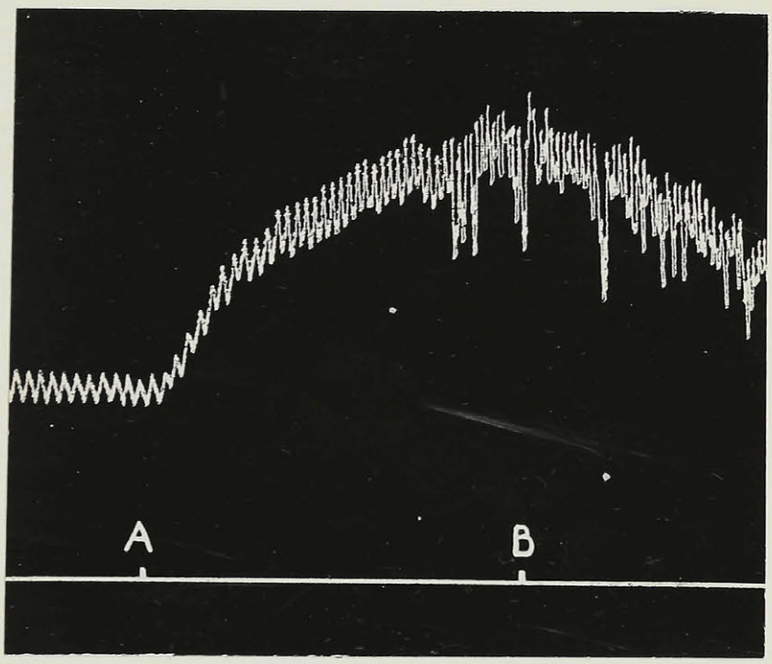

FIGURE 21. - Blood-pressure tracing from a dog (5.6 Kg.) anaesthetized with chloralose-urethane, and receiving 2 mg. of histamine dihydrochloride subcutaneously every hour. From A to B, injection of 20 c.c. of a solution of the lysine fraction of the nitrogenous bases of haddock muscle (corresponding to 200 grams of muscle). The blood pressure rose sharply and remained above the initial level for at least 2 hours. The irregularities at the top of the curve are due to gasping and to slight clonic convulsions; these effects soon passed off.

The effect on the blood pressure of injection of the lysine fraction is shown in Figure 21. The blood pressure rose during the injection from 60 to 120 mm. Hg, with slight clonic convulsions lasting a minute or so. Thereafter it fell off slowly, but remained higher than before the injection. The total nitrogenous-bases fraction caused large variations in the blood pressure, followed by a prolonged fall. The effect of the histidine and arginine fractions on the blood pressure was not determined.

Discussion.

(a) The distribution of the non-protein nitrogen of haddock muscle. Considering the long and complicated chemical procedure involved, and the natural variations among individuals of the same or closely related species, the values for the partition of non-protein nitrogen obtained in this work are in good agreement with those of Komarov and of Campbell. It will be noted that the total non-protein nitrogen of haddock muscle, and the proportion of this accounted for by the nitrogenous bases, is roughly the same as in mammalian muscle; the creatine content has also been shown to be about the same as that of mammalian muscle (150), and doubtless accounts for most of the non-basic non-protein nitrogen (creatine is, however, partially precipitated by phosphotungstic acid).

The high figures obtained for the total non-protein nitrogen in my work are largely due to the presence of a high proportion of non-basic nitrogen, and may possibly be due to slight autolysis before the preliminary extraction. The somewhat lower values for purine nitrogen are thought to be due to incomplete decomposition of the

first silver precipitate.

The results corroborate the finding of Komarov and of Campbell that fish muscle, in comparison with meat, is characterized by a high proportion of nitrogen in the lysine fraction and a low proportion in the histidine-arginine fraction. (See Table IV.) As to the nature of the compounds responsible for this difference, there is no evidence available. Known compounds, indeed, account for only a small part of the total basic nitrogen. Thus the volatile base (NH_3, trimethylamine, etc.) nitrogen is less than 5 per cent of the total basic nitrogen, and imidazole bodies (histamine, carnosine) are practically absent; the amino nitrogen is about 10 per cent of the whole (Campbell, 144). The absence of parasympathomimetic activity in the lysine fraction (see below) would indicate that the high values for the lysine fraction are not due to choline or its derivatives. In view of the physiological activity of the arginine and lysine fractions, further study of their composition would be very desirable.

It may be mentioned that the histidine and arginine fractions were separately isolated from fish muscle for the first time. The former is seen to contain only a very small part of the total basic nitrogen.

The nature of the pressor substance of the lysine fraction is unknown.

(b) <u>The secretagogue action of the haddock-muscle extractives</u>. In agreement with the results of Campbell, the fraction of the nitrogenous bases is found to retain the bulk of the secretagogue activity /

activity of the crude protein-free extract of the fish muscle. Further, the potency is confined to the arginine and lysine fractions, the purine and histidine fractions being no more active than the water and saline controls. My results differ from those of Campbell in that the lysine fraction possessed greater secretagogue activity than the arginine fraction. It must also be pointed out that the 'synthetic' nitrogenous bases fraction, made up by combining the four basic fractions, each in a quantity corresponding to 200 gm. of muscle, was found to be more active than the original nitrogenous bases fraction. The lysine fraction alone, in fact, was more active than the original total-bases fraction. The explanation, no doubt, is that there was some production of secretagogue bodies during the procedure of fraction, most probably during the third silver precipitation with warm saturated $Ba(OH)_2$, although this operation was performed very rapidly. Thus methylguanidine might be formed by the hydrolysis of creatine. In the experiments of Campbell (see Table IV) the sum of the secretory responses to the histidine-arginine and lysine fractions was less than the response to the total basic fraction. The question of how far the secretory activity of the crude extract may be traced to these fractions, must therefore be left open. The probability of artefact formation during the preparation of the original nitrogenous bases fraction appears very much less, since there was never more than a small excess of alkali present. There is thus valid reason to believe that the secretagogues of the crude fish-muscle extract are chiefly nitrogenous bases; further, the active /

active substances are not precipitated by silver on the acid side of neutrality (purine and histidine fractions are inactive). Since the activity is apparently divided between the arginine and lysine fractions, there is probably more than one active substance involved. Unfortunately no determinations were made of the secretagogue activity of the non-basic constituents of the crude extract ("monoamino acid fraction").

The question of whether the peptic cells participate in the second phase of gastric secretion is a difficult one to answer. It is a simple matter to introduce extracts of muscle, etc. into the digestive tract and measure the enzyme content of the juice secreted. But the volume of juice obtained in this way from a stomach pouch is never very great, and the pepsin which it contains is certainly not all secreted under the action of the extract, but is previously present in the glands in association with mucoprotein, and is merely washed out by the fluid from the parietal cells. Thus when histamine is injected, the first portions of juice collected contain considerable pepsin, and only later is the almost enzyme-free juice, characteristic of the action of histamine, obtained.

The method which I have adopted, of administering the extract to be tested after the animal has undergone prolonged treatment with histamine, appears to be much better suited for investigating this point. Since the stomach is secreting a juice almost free from pepsin, any small secretory activity of the peptic cells should be readily observed. The chief objections to this method are (1) that the /

the extracts must be administered parenterally, and (2) that since histamine has a certain inhibitory influence on the discharge of pepsin (Alley (151)), a mild stimulation of the peptic cells will be ineffective. I shall show in the next section that choline greatly increases the peptic power of the 'histamine' secretion, so that the second objection loses in force. As to the first objection, it may be replied that, if the extractive substances of muscle stimulate the peptic cells by being absorbed into the blood, they should act on intravenous injection also; there is no evidence at all for the alternative explanation that they may act by producing a 'gastric hormone', which would in any case have to be very different from the hypothetical hormone stimulating the parietal cells.

It is noteworthy that the lysine fraction corresponding to 200 gm. of haddock muscle contained insufficient choline to stimulate the peptic cells appreciably. The total amount of choline in 200 gm. of cod muscle is about 150 mg. (152), but this is presumably mainly in the form of phosphatide; such a dose of free choline markedly stimulates the discharge of pepsin (see the next section). The amount of free choline in extracts prepared as here described may actually be less than that in the corresponding quantity of fish muscle prepared as food, since in the preliminary extraction little opportunity was given for autolysis, which very rapidly liberates choline from an inactive water-soluble precursor (153, 154).

Further /

Further evidence that there is no greatly increased activity of the peptic cells during the second phase has been obtained in a new series of experiments, which I shall briefly mention here. A dog with a Bickel (completely denervated) pouch was injected with 0.3 mg. of histamine at half-hour intervals, and the pouch juice collected. Both the concentration of pepsin in the juice and the total amount of pepsin discharged became progressively lower in each half-hour period. The animal was then fed, the histamine injections being continued as before, so that the inhibitory effect of histamine on the discharge of pepsin would remain constant. The rate of secretion naturally rose, since the stimulus of feeding was combined with the stimulus of histamine; the concentration of pepsin fell, which might be explained on the basis of dilution by the greater volume of juice; but the output of pepsin also continued to fall, and remained very low as long as the histamine injections were continued. The second phase therefore seems to involve no very important increase in the rate at which pepsin is excreted by the peptic cells. (For evidence that such an increase is, however, at least theoretically possible, see the next section.)

Summary /

Summary.

1. The nitrogenous basic extractives of haddock muscle have been quantitatively fractionated by the silver-baryta method.

2. The secretagogue activity on the gastric glands of a crude protein-free extract of haddock muscle was found to be due in large part to its content of nitrogenous bases. Of these, the arginine and lysine fractions only were active.

3. It could not be demonstrated that these fractions were capable of effectively stimulating the peptic cells.

III. /

III. THE EFFECT OF CHOLINE ON THE GASTRIC SECRETION.

Introduction.

The preceding section of this thesis has dealt with the nitrogenous basic constituents of muscle as stimulants for gastric secretion. Most of these compounds have doubtless no secretagogue activity. The chemical nature of the ones which are effective is quite unknown. Histamine, which is easily the most powerful of all known stimulants of the gastric glands, does not act from the digestive tract, and so is not responsible for the secretagogue effect of muscle extracts. The same may be said for adenosine, which is destroyed by tissue autolysis and by digestion; for creatine, carnosine, carnitine and methylguanidine, whose action is too weak to class them as excitors of the second phase; and indeed for practically all the known bases of muscle. Choline, however, is both widely distributed in foodstuffs and possesses of well-marked physiological activity. It has been shown by Ivy and Javois (9) to excite gastric secretion on introduction into the digestive tract, but no detailed analysis of its action has been made. The desirability of such an analysis is obvious from the point of view of the possible share of choline in the production of the second phase. Besides this, choline has typically parasympathomimetic properties, and so might be expected to stimulate the peptic cells, which are normally controlled by the vagus: the question of the participation of these cells in

the /

the second phase has been raised in the preceding section.

I have therefore studied in some detail the effect of choline on the gastric secretion. I have fortunately been able to perform most of my experiments on unanaesthetized dogs equipped with gastric fistulas and pouches of the stomach, administering choline either alone or in combination with other stimulants of the gastric secretion; I have also made a number of acute experiments to clear up certain points, especially in connection with the effect of choline on the peptic cells.

Experimental.

Most of the experiments were performed on three dogs equipped with pouches of the stomach, as follows:

'P': a female, weighing 14 Kg., about 10 years old, equipped for 6 years with a Pavlov pouch (vagal and sympathetic innervation preserved) and a gastric fistula. (This was the dog used in the experiments with the nitrogenous bases.)

'H': a female, weighing 21 Kg., about 6 years old, equipped for 3 years with a Heidenhain pouch (sympathetic innervation only). This dog possessed only one kidney.

'B': a male, weighing 19 Kg., about 3 years old, equipped for about a year with a Bickel pouch (complete extrinsic denervation) and a gastric fistula.

The animals were all kept in good health over a long period. They received a standardized diet of lean beef-heart, oatmeal porridge, milk, and salt. All experiments were begun in the morning /

morning while the gastric glands were at rest. In the case of the Pavlov-pouch dog especially, due precaution was taken to avoid psychical stimulation of the secretion, and experiments were never begun when the resting secretion exceeded 0.5 c.c. for 30 minutes.

Free and total acidity and pepsin were determined in the pouch juice by the methods already described. The volume of visible mucus in the secretion was also measured.

The acute experiments, which were designed to test more exactly the effect of choline on the peptic cells, and were performed according to a technique exactly similar to that used in the acute experiments with the nitrogenous bases (see page 91).

The experiments fall into several groups, which will be described in order, as follows:-

(1) Experiments on the effect of choline injected intravenously.
  (a) The effect of choline alone.
  (b) The effect of choline combined with alcohol.
  (c) The effect of choline combined with histamine (acute experiments).

(2) Experiments on the effect of choline introduced into the digestive tract.
  (a) The effect of choline alone.
  (b) The effect of choline on the secretion produced by feeding.

(3) Experiments on the effect of choline introduced into the portal circulation (acute experiments).

(4) Experiments on the effect of atropine on the secretory action of choline.

(5) Experiments on the secretory effect of lecithin.

1. <u>The effect of choline injected intravenously</u>.

(a) <u>Choline alone</u>. Choline chloride (5 to 15 mg. per Kg.) injected intravenously during the rest of the gastric glands, evoked /

evoked in all three animals a scanty flow of gastric juice. The juice was of low acidity but rich in pepsin, and contained much mucus. The injection was followed by salivation, lachrymation, and nasal secretion; the pulse was at first slowed and then became rapid and shallow, and there was a marked hyperpnoea. These effects passed off within a few minutes. Gastric secretion began in about five minutes and continued for about an hour and a half. The secretory effect of choline is perhaps best illustrated by comparing it with the effect of the animal's regular meal on the preceding day (see Table V). Similar results were obtained in the 6 experiments in which choline was given intravenously.

TABLE V.

Dog	Stimulation	Vol. c.c.	Mucus c.c.	Free Acid m.eq./l.	Total Acid m.eq./l.	Pepsin Mett u.	Duration hrs.
'H'	Choline chloride (300 mg.) intrav.	3.6	1.5	0	39	460	1¼
	Daily meal	31.0	2.5	106	116	64	6
'B'	Choline chloride (300 mg.) intrav.	2.2	1.5	15	51	550	1¾
	Daily meal	21.0	1.0	118	132	41	6
	Choline chloride (1 gm.) by gastric fistula	5.3	3.6	0	20	350	2

(b) Choline combined with histamine. Since the secretion produced by choline consists largely of mucus, which does not drain readily from the pouch, it was endeavoured to get a clearer picture of the effect on the peptic cells by superimposing the action /

action of choline on that of histamine in unanaesthetized dogs; just as, in the experiments described in the preceding section (page 91 et seq.) I attempted to detect any action of the haddock extracts on the peptic cells, by injecting the extracts intravenously during the course of a secretion evoked by histamine. The anaesthetized dogs were given histamine subcutaneously (0.2 or 0.3 mg. per Kg. of the dihydrochloride at half-hourly intervals), and choline chloride (5 to 20 mg. per Kg.) was injected intravenously after a good secretion had become established. The effect of choline was uniformly (6 experiments) to raise greatly the peptic power of the juice produced by histamine. The rate of secretion, which is very high with such doses of histamine, was generally reduced; the acidity also fell off somewhat. A typical experimental protocol is presented. (Figure 25 also illustrates the effect of choline on the secretion produced by histamine.)

Experiment July 17, 1936.

Male dog, poodle type, 6 Kg. Chloralose-urethane anaesthesia. Pylorus tied, vagi cut, small gastric fistula inserted. Dog placed in prone position. Operation finished 12:15 p.m. Stomach washed out with 1 litre of warm tap-water.

Time		Vol. c.c.	Free Acid m.eq./l.	Total Acid m.eq./l.	Chloride m.eq./l.	Pepsin Mett u.
12:15-12:45		0.7				
12:45- 1:15		0.1				
1:15	Histamine (2 mg.)	subcutaneously.				
1:15- 1:45		15.0	82	88	114	13
1:45- 2:15		24.3	138	144	172	4
2:15	Histamine (2 mg.)	subcutaneously.				
2:15- 2:45		28.1	148	152	175	1
2:45- 3:15		26.4	149	153	171	1
3:15	Histamine (2 mg.)	subcutaneously.				
3:15- 3:45		26.9	148	152	171	1
3:45- 4:15		21.3	142	149	171	1
4:15	Histamine (2 mg.)	subcutaneously.				

4:15 /

(Experiment July 17, 1936 - cont.)

Time		Vol. c.c.	Free Acid m.eq./l.	Total Acid m.eq./l.	Chloride m.eq./l.	Pepsin Mett u.
4:15-4:45		24.2	140	147	171	1
4:45	Choline chloride (100 mg. - 5 c.c.) very slowly intrav.					
4:45-5:15		12.0	138	146	174	108
5:15	Histamine (2 mg.) subcutaneously. Choline chloride (100 mg.) intravenously.					
5:15-5:45		4.4	113	126	169	100
5:45	Choline chloride (100 mg.) intravenously.					
5:45-6:15		3.6	116	127	168	135

(c) Choline combined with alcohol. Analogous experiments were made on unanaesthetized animals. Alcohol introduced into the stomach of a dog with a permanent gastric fistula stimulates the secretion by the pouch of a juice of very low digestive power. If choline is injected during the course of this secretion, the peptic power of the juice rises markedly and the rate of secretion is also somewhat increased. Out of 8 experiments of this sort, the injection of choline produced in 6 experiments a definite increase in the pepsin concentration of the juice produced under the influence of alcohol; in 2 experiments the increase was doubtful or absent. One experimental protocol will suffice.

Experiment /

Experiment June 11, 1936.

Dog 'P'. Placed on stand 9:40 a.m.

Time		Vol. c.c.	Mucus c.c.	Total Acid m.eq./l.	Pepsin Mett u.
9:40- 9:55		0.6	(0.2)	(52	(310
9:55-10:15		0.2	-	(	(
10:15	175 c.c. of 5% ethyl alcohol - 25 c.c. of water introduced into stomach through gastric fistula.				
10:15-10:30		2.6	(0.1)	64	230
10:30-10:45		2.0	-	104	108
10:45-11:00		1.0	-	124	117
11:00	200 mg. of choline chloride intravenously.				
11:00-11:15		1.8	(0.2)	114	256
11:15-11:30		1.5	(0.2)	130	269
11:30-11:45		0.4	-	(	(
11:45-12:00		0.3	(0.3)	(105	(324
12:00-12:15		0.2		(	(
		9.8	0.8		

Without choline, this volume of alcohol normally gave a secretion of 6 to 7 c.c. (For explanation of the comparatively high pepsin content of the 'alcohol' secretion, see page 143).

Choline injected intravenously is thus a rather weak stimulus for the production of water and HCl by the gastric glands, and a strong stimulus for the discharge of pepsin.

2. The effect of choline introduced into the digestive tract.

(a) The effect of choline alone. Of presumably greater significance is the effect on gastric secretion of choline introduced into the digestive tract. This effect was conveniently studied by introducing choline directly into the stomach by the gastric fistula, so that psychical influences were avoided. Given in this way choline, even in large doses (0.5 to 1.0 gm.) caused no visible systemic disturbances, as it did when given intravenously, but it did again evoke a rather scanty gastric secretion, which as before was of very low acidity but rich in pepsin and mucus. The

secretion began only after a latent period of about 1 hour, and lasted for about 2 hours (Table V; see also Fig. 22). The effective dose appeared to be about twice as high as for intravenous injection.

(b) The effect of choline on the secretion produced by feeding. Choline given in conjunction with a meat meal was found to produce a striking change in the secretory response of the stomach to the latter. The procedure was as follows. The dog (in these experiments, 'B') was placed on the stand in the morning, and 5 c.c. of a 2% solution of choline chloride was introduced into the stomach by the gastric fistula. After 3½ hours, when the small secretory effect of the choline had passed off, the animal was fed a meal of 700 gm. of lean beef-heart. In control experiments, an equivalent quantity of NaCl (419 mg.) was substituted for the choline chloride. Figure 22 illustrates the effect, on the secretory response to a meat meal, of previous treatment with (a) NaCl and (b) choline chloride.

It will be noted that after choline, the secretion produced by meat reaches its peak earlier and is much greater in volume. The digestive power is high in the first hour after feeding, but this is probably due to a washing out from the glands of previously secreted pepsin (cf. page 81); it falls rapidly to normal levels. The augmenting effect of choline on the secretion thus persists after its preliminary direct effect wears off.

In two series of experiments, the animal received each day of the week a meal of 700 gm. of lean beef-heart. (The meat was minced and /

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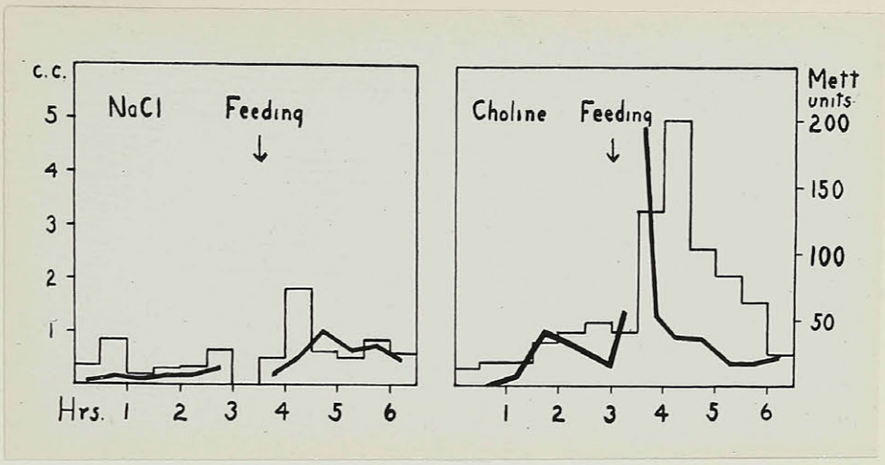

FIGURE 22. - Dog 'B'; Bickel pouch. The diagrams show the volume of gastric juice secreted by the pouch (rectangular lines) and the concentration of pepsin in the juice (heavy lines). The two experiments illustrated were performed (2 days apart) under fully comparable conditions. In the first experiment, the animal received 419 mg. of NaCl by gastric fistula, and $3\frac{1}{2}$ hours later, 700 gm. of meat by mouth. In the second experiment, it received, instead of the NaCl, an equivalent quantity (1 gram) of choline chloride. The secretory response to the meat meal was greatly increased by the previous administration of choline. The graph appears to indicate that feeding was carried out before the response to choline had ceased: however, nothing but faintly acid mucus was secreted during the hour preceding the meal.

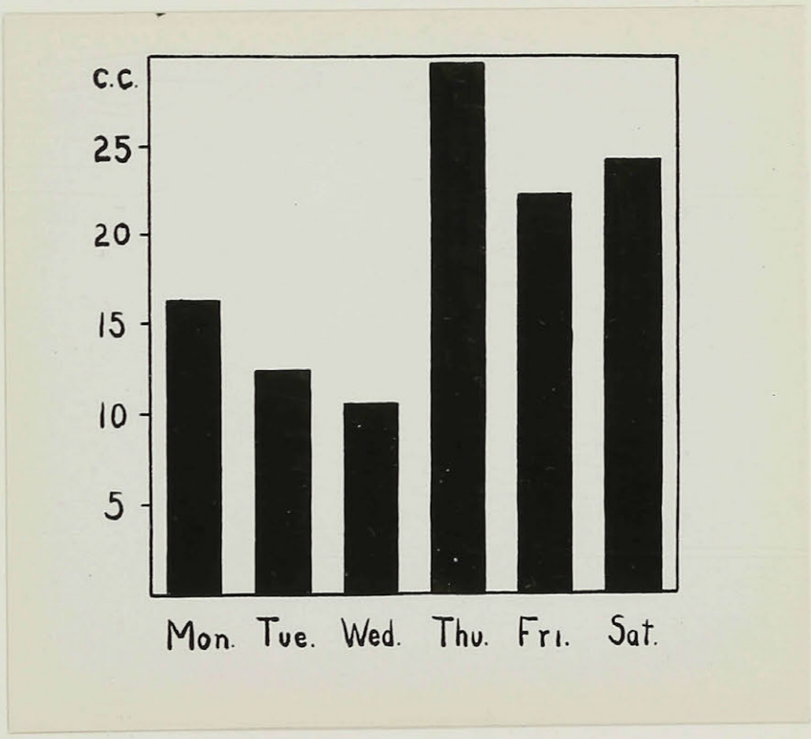

FIGURE 23. - Dog 'B'; Bickel pouch. The black rectangles represent the volume of gastric juice secreted in response to the standard daily meal of lean beef-heart. Before the Thursday meal, the animal received 1 gm. of choline chloride by gastric fistula. Before the Tuesday meal, it received an equivalent quantity of NaCl in the same way. Note the persistence of the choline effect. The volume of juice secreted on Monday, after a day's starvation, is (as frequently happens) above normal.

and thoroughly mixed at the beginning of the week, and kept in the ice-box until used, so that it was of uniform composition throughout. The daily secretory response to this meal under ordinary conditions varied less than 10 per cent. If, however, choline were previously administered by gastric fistula, the meat produced a secretion 60 to 120 per cent greater than the normal. This augmenting effect of choline was still evident on the next day, when the secretion evoked by the standard meal was still definitely higher than the normal. In control experiments in which the choline chloride was replaced by NaCl, the volume of the secretion remained normal. Figures 23 and 24 illustrate the effect of choline administration on the volume of gastric juice secreted in response to a standard meat meal. The series of experiments from which Figure 24 was drawn was made about a month later than that summarized in Figure 23, the animal being kept on a meat diet during the interval: it will be noted that the 'basal' secretory response is higher in the later experiments.

A similar augmenting effect of choline on the secretory response to the next day's meal was noted in many of the earlier experiments, in which choline was given either intravenously or per fistulam: as the composition of the daily diet was not carefully controlled, these results are not quoted.

3. The effect of choline injected into the portal circulation.

It appeared possible that the weaker secretagogue action of choline on introduction into the stomach, as compared with intravenous injection, might be due to its removal from the circulation by the liver. That the liver does exert such an effect is indicated

by /

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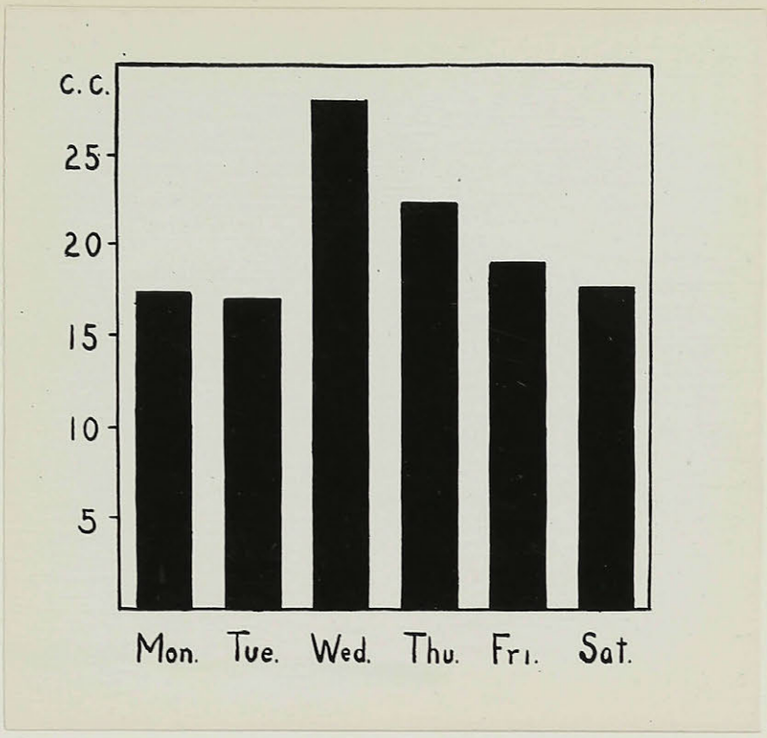

FIGURE 24. - Dog 'B'; Bickel pouch. Secretory response of the pouch to a standard meat meal. Before the Wednesday meal, the animal received 1 gm. of choline chloride by gastric fistula; before the Friday meal, an equivalent quantity of NaCl.

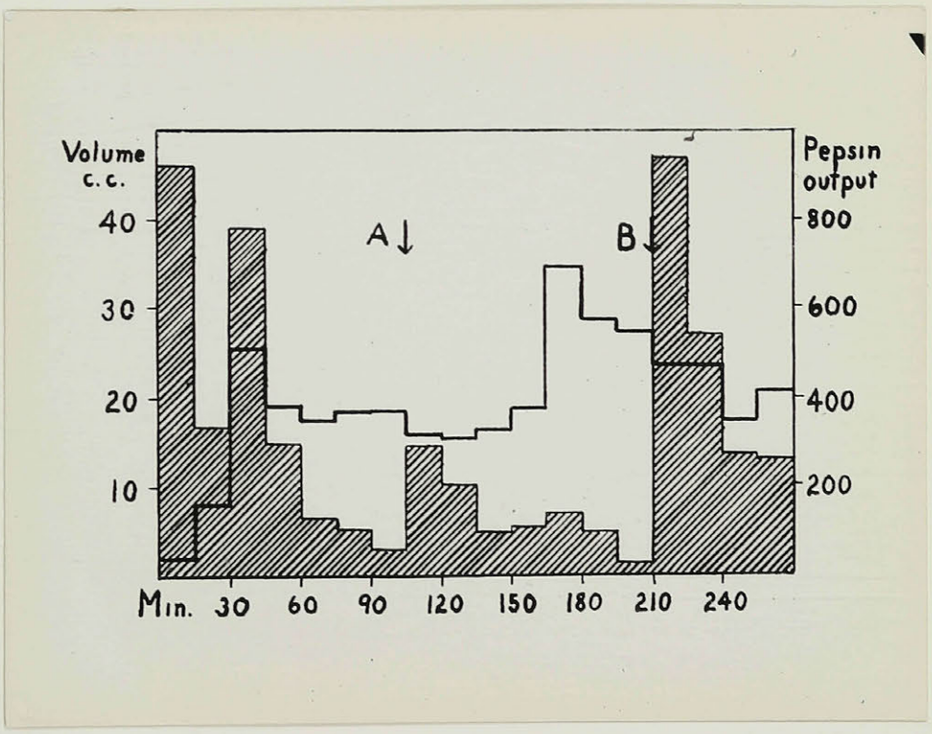

FIGURE 25. - The heavy rectangular line represents the volumes of gastric juice secreted in each 15-minute period by an anaesthetized dog receiving 1.2 mg. of histamine subcutaneously every half-hour. The shaded areas represent the total output of pepsin during each period. Note the steady decline in pepsin secretion after the first 45 minutes. At A, 100 mg. of choline chloride was injected into a branch of the superior mesenteric vein; at B, the same dose was injected into the jugular vein. Note the greater effect on enzyme discharge of B as compared with A. Choline had no striking effect on the volume of the secretion produced by histamine.

by two experiments performed on anaesthetized sogs secreting under the influence of histamine. In these experiments the same dose of choline chloride was injected slowly into (a) a branch of the superior mesenteric vein, (b) the jugular vein, the experimental technique being exactly similar to that described on page 91. The increase in the output of pepsin following injection (a) was less than half of that produced by injection (b) and did not last so long. Reversing the order of the injections did not change the results. Figure 25 represents graphically the results of one such experiment.

4. The effect of atropine on the secretory action of choline.

The direct secretory action on the gastric glands of choline given either intravenously or by gastric fistula, is fully antagonized by atropine (0.2 mg. per Kg.). Whether or not the secondary augmenting action of choline is also antagonized could not be ascertained, since atropine interferes with the digestion of the meat meal.

5. The effect of lecithin on the gastric secretion.

Since the choline of food occurs largely in combination as lecithin, it was of interest to try the action of this substance on the gastric secretion. This work is still in progress and no definite conclusions can yet be drawn. A purified preparation of lecithin, supplied by the Department of Biochemistry, McGill University, has been used, the dose being 6 gm. (theoretically equivalent to 1 gm. of choline). On being introduced into the stomach /

stomach of dog 'B', it produced only a very small secretion of the Bickel pouch, and gave no evidence of stimulating the peptic cells. In the first series of experiments, in which the dog had been placed only recently on a basic diet of meat, previous treatment with lecithin markedly increased the secretory effect of the standard meal; in the second series, before which the dog had been fed only meat for several weeks, lecithin inhibited the secretory response to the meal. Possibly lecithin has potentially both stimulating and inhibiting properties, derived from its choline and fatty acid components respectively; and the state of nutrition of the animal determines which of these will be exhibited.

Discussion.

Since the effect of choline is qualitatively similar whether it is given intravenously or by gastric fistula, the weaker action in the latter case being apparently accounted for by the intervention of the liver, it is highly probable that the secretagogue action of choline on the gastric glands is direct, through absorption from the gut, rather than indirect, through formation of a hormone. In agreement with this idea is the long latent period for its action when given by stomach, suggesting that it acts from the lower part of the intestine. These facts thus appear to support the 'absorption' rather than the 'gastrin' theory: more satisfactory evidence would be supplied by showing the choline content of the plasma to be raised following ingestion of choline.

The method by which choline exerts its augmenting effect on the /

the secretion of the denervated pouch is not yet clear. One possibility is that it may antagonize the inhibitory action of fat on the gastric secretion. Some fat was unavoidably present in the meal of lean beef, and it may be that choline removed the inhibitory effect of this by improving the ability of the liver to metabolize fat, as Best and his co-workers (155, 156) suggest. It is also possible that the 'augmenting' effect may be a direct sensitization of the parietal cells by choline. The response of the parietal cells to a standard stimulus undoubtedly may be modified by the state of nutrition of the animal. In Rasenkov's laboratory, Koshtoyanetz has shown that the prolonged administration of a diet of meat alone increases the response of a denervated pouch to a variety of test meals, and I have seen a similar effect in my own experiments. It is possible that this phenomenon may depend on the choline content of meat. All this is, nowever, highly hypothetical.

The direct effect of choline on the gastric glands is that of a typical parasympathomimetic drug; it is similar to, but much weaker than that of pilocarpine. Of especial interest is the fact that choline stimulated the peptic cells when introduced into the digestive tract. Since it undoubtedly occurs to some extent free, or in very labile combination, in practically all animal foods (157, 158), it is at least theoretically possible for the peptic cells to undergo stimulation during the second phase. Whether this stimulation is quantitatively important is another matter; the experiments described in the preceding section (pages 91 et seq.) suggest that it is not important.

The /

The doses of choline used in these experiments were rather large, and it is questionable how far they can be compared to the quantities of choline supplied in the diet. The total choline content of skeletal muscle was found by Best, Fletcher and Solandt (152) to be 760 and 1000 mg. per Kg. for ox and rat muscle respectively. Most of this is in the form of phosphatide, which, though it is attacked by the lipases of the digestive tract (159, 160) has not been shown to give rise to free choline during digestion. The free choline of living muscle, as of other tissues, is probably low; recent work has shown, however, that choline is readily liberated by post-mortem enzyme action from a water-soluble precursor present in many tissues (153), so that very large quantities of choline have been found in tissues which were not fixed by heating immediately on removal from the body. Thus liver has been found to contain up to 720 mg. per Kg. (161) and skeletal muscle 150 to 200 mg. per Kg. (157, 158), even when promptly worked up; this liberated choline is not destroyed by further autolysis. Commercial meats may therefore be presumed to contain very considerable quantities of free choline. The amounts of choline given by stomach in our experiments are thus likely somewhat higher than occur free in the diet, but since the action of these doses was so striking, it is probable that, at least in foods of animal origin, the choline present is a significant factor in the causation of the second phase of gastric secretion. The direct effect of free choline must be considered unimportant, in view of the high digestive power of 'choline' juice as compared with the juice of the /

the second phase; it is, however, probable that the secondary 'augmenting' effect is of significance for the normal production of the second phase. The final decision on this point awaits the outcome of further work on the effect of lecithin.

Summary.

1. Choline administered to dogs either intravenously or directly into the stomach, stimulates the gastric glands: the production of pepsin and mucus is stimulated more than the production of water and HCl.

2. Choline has also a secondary augmenting effect on the secretory response to a subsequent meal. This augmenting effect persists for at least a day.

3. The mechanism of the secondary augmenting action, and the possibility that choline is concerned in the normal production of the second phase, are discussed.

IV. /

IV. THE HISTAMINE CONTENT OF THE BLOOD AS AFFECTED BY FEEDING.

Introduction.

The most important piece of experimental evidence for the existence of a 'gastric hormone' is the fact that simple extracts of the pyloric mucosa, injected subcutaneously, are strong stimulants for the gastric secretion. Sacks, Ivy, Burgess and Vandolah (138) showed in 1932 that the pyloric mucosa contains large quantities of histamine, which they isolated as the picrate and identified chemically. The physiological activity of the extracts was largely accounted for by the histamine which could be isolated from them, and Sacks and his co-workers advanced further evidence to show that histamine was not only the principal, but the only gastric secretagogue present: for example, the activity of the extracts was entirely destroyed by preparations of histaminase. The presence of high concentrations of histamine in the pyloric mucosa was verified by Gavin, McHenry and Wilson (162), using biological methods for the estimation of histamine; they also concluded that the secretagogue activity of pyloric mucosa extracts was due to histamine. Extracts of fundic mucosa, however, in contrast to Edkins' (133) original statement, were equally potent. Certain apparent differences between the chemical properties of 'gastrin' and histamine (Keeton, Koch and Luckhardt (163)) are doubtless due to impurities present in the crude extracts of mucosa (163, 164).

There is thus good reason to believe that "the active component /

:ponent in extracts of the gastric mucosa, possessing the secretagogue activity ascribed by Edkins to 'gastrin', is really histamine" (Gaddum (31), page 30). Sacks, Ivy, Burgess and Vandolah (138) are strongly inclined to regard histamine as being actually the gastric hormone; they conclude cautiously, however, that "either histamine is the gastric hormone, or there is no gastric hormone, or the gastric hormone has never been extracted from the pyloric mucosa."

If histamine is really the gastric hormone, set free by the pyloric mucosa and borne by the blood to the fundic glands, its effective concentration in the systemic blood must be increased during the digestion of a meal. Koskowski and Kubikowski (137) have asserted that such a rise in the blood histamine content does follow the ingestion of a meat meal: their methods of assay, however, were extremely crude, and if they made any control experiments, they have failed to report them. Lim and Necheles (165) have described the detection, by a method of vividialysis, of a gastric secretory excitant in the portal and systemic blood of fed dogs, but say little about its chemical or pharmacological properties. The changes in the pharmacological activity of the blood, asserted by Rasenkov (166) to accompany digestion, are not such as would be expected to arise from an increase in the blood histamine.

It is clearly very desirable that some accurate information should be obtained concerning the effect of feeding on the histamine concentration of the blood. The direct estimation of histamine in small quantities of blood was impossible until recently, when Barsoum /

Barsoum and Gaddum (82) published a method by which extracts of blood may be freed from active substances other than histamine, and their biological activity then compared with that of known quantities of histamine, the test-organ being the isolated intestine of the guinea-pig. I have therefore used the method of Barsoum and Gaddum for the determination, in dogs, of the effect of feeding on the histamine concentration of the blood.

The method of Barsoum and Gaddum, as described by its authors, is unfortunately quite unsatisfactory unless the amount of histamine present is large. Very small quantities of histamine added to dog's blood are recovered to the extent of 50 per cent or less. A slight modification of the method, however, viz. the use of hot instead of cold absolute alcohol at one stage of the extraction, permits satisfactory recovery (80 to 95 per cent) of added histamine in quantities as small as 0.0002 mg. Since Barsoum and Gaddum give no particulars at all of their technique for the assay of blood extracts on the isolated intestine, and since I have modified their chemical procedure somewhat, I shall describe in detail the method for estimation of the blood histamine which I have found to be most satisfactory.

Experimental.

The preparation of extracts of blood for the estimation of histamine. 10 c.c. of blood, obtained from a limb vein or by cardiac puncture, are run immediately into 15 c.c. of 10% trichloroacetic acid and after vigorous stirring are permitted to stand for 2 hours. The precipitate of proteins is centrifuged down /

down, and the supernatant fluid decanted and filtered. The precipitate is re-extracted by thorough stirring with (10 + 5 + 5) c.c. of warm (65°) trichloroacetic acid solution, each portion being removed by centrifuging and filtered. (It is difficult to extract a bulky precipitate with small quantities of fluid by washing on the filter paper, as proposed by Barsoum and Gaddum; also, the use of warm trichloroacetic acid solution for washing the precipitate lessens the possibility of loss of histamine through absorption.) The combined filtrates are extracted four times with ether (reagent, Merck) in a separatory funnel, the ether layer being re-extracted each time with 1 to 2 c.c. of distilled water, to avoid mechanical loss of histamine. The extract, which is now about neutral to Congo paper, is treated with 10 c.c. of concentrated HCl and heated for 2 hours in a boiling water bath. It is then concentrated to a small volume <u>in vacuo</u>, quantitatively transferred to a 50-c.c. Erlenmeyer flask, and dried completely <u>in vacuo</u>; in the final stages care is taken to avoid charring of the residue by excessive heating (although such charring does not appear to cause any loss of histamine). The yellowish-brown residue is now extracted with 3-c.c. portions of absolute alcohol previously saturated with NaCl. The residue is very thoroughly broken up by means of a glass stirring rod, the flask kept at 70° C. in the water-bath for several minutes, and the maceration of the precipitate repeated; the extract is then cooled, the precipitate allowed to settle, and the supernatant fluid decanted through a 3-cm. filter paper (S. & S. No. 589[1]). This procedure is repeated three times.

It /

It must be emphasized that repeated treatment with hot alcohol, and very thorough breaking up of the residue is indispensable for satisfactory recovery of histamine. Apparently the histamine is absorbed by "humine" bodies produced during the acid digestion (cf. Gerard (167)). The combined alcoholic filtrate is evaporated to dryness in vacuo, the residue taken up in hot distilled water, and the aqueous extract cooled and filtered. The clear straw-coloured filtrate is neutralized with x c.c. of N NaOH (0.28-x) c.c. of N NaCl added to adjust the osmotic pressure, and the volume made up to 2.5 c.c. A small precipitate which may form on neutralization settles rapidly and does not interfere with the determination.

It is hardly necessary to say that strict quantitative technique must be used throughout, and that extreme care must be taken to avoid contamination with histamine from extraneous sources. The spilling of crystals or strong solutions of histamine in the laboratory is especially dangerous, since the total quantities of histamine worked up are so minute.

The biological assay of histamine-containing extracts on the isolated guinea-pig intestine. Essentially the method consists in comparing the size of the contraction of the isolated intestine produced by treatment with a certain volume of the extract, with the contraction produced by treatment with a known quantity of histamine.

The following technique gave uniformly satisfactory results.

A large guinea-pig, not necessarily starved, is killed by a blow on the head. The abdomen is immediately opened, and several inches of the lower ileum freed of mesentery by a rapid stroke of the /

the dissecting forceps, and emptied of food residues by gently pressing out with Ringer-moistened fingers. The loop of intestine is excised, washed briefly in Ringer at room temperature, and placed in fresh Ringer until required for use.

Strips about 2 cm. long in the relaxed condition are used. One end is tied to a hook of platinum wire (Fig. 26), the other to one end of a long ligature for attaching to the writing-lever. The hook is seized with fine forceps and attached to the air-inlet at the bottom of the tissue-bath (Fig. 26).

The tissue-bath was designed especially for this work. It is of Pyrex glass and has a capacity of about 2.5 c.c. It is kept immersed in a Dale-Evans constant-temperature water-bath at 37° C. The inlet is connected with the reservoir for warm Ringer, and is narrowed to reduce the communication with the dead spaces; the outlet is of wider bore so that the Ringer may be quickly changed. A sphygmomanometer bulb conveniently supplies pressure for forcing the Ringer into the bath. Air bubbles through the bath at the rate of about 2 bubbles per second. A small capillary siphon hung over the edge of the tissue-bath keeps the level of the Ringer the same as that of the water in the thermostat, and therefore constant; but does not (a) empty so rapidly as to interfere with the proper admixture of added histamine solutions, or (b) siphon tap-water into the emptied bath. (See Figure 27 for a diagrammatic representation of the set-up.)

A 'Fix-it' lever with Lovatt Evans frontal writing-point is used, the kymograph moving at its slowest speed. The tension of the /

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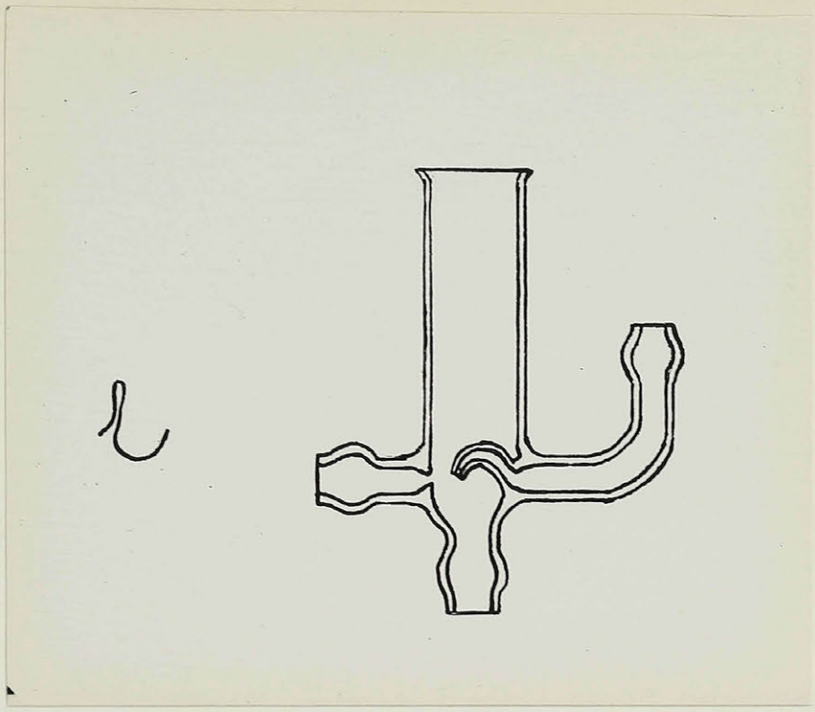

FIGURE 26. - At left, platinum hook used for affixing intestinal strip to air-inlet of tissue-bath; at right, semi-diagrammatic representation of the tissue-bath itself. Both actual size.

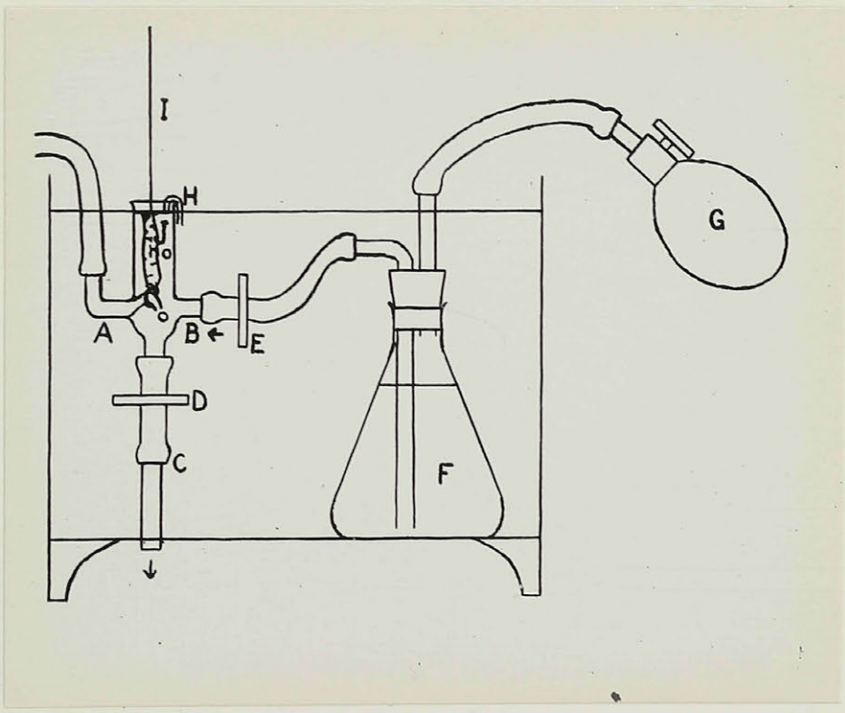

FIGURE 27. - Semi-diagrammatic representation of the apparatus for biological assay of histamine. A, air-inlet tube of tissue-bath; B, inlet for Ringer; C, outlet for Ringer; D, E, clamps; F, reservoir for warm Ringer; G, sphygmomanometer bulb for forcing fresh Ringer into the tissue-bath; H, capillary siphon for maintaining fluid level within tissue-bath; J, strip of guinea-pig intestine. The volume of the water-bath is actually about 6 litres.

the strip is about 0.5 gm., the magnification about 10 times.

The Ringer solution used has the following composition:

NaCl	0.85 per cent
$NaHCO_3$	0.10 " "
KCl	0.042 " "
$CaCl_2$	0.024 " "
$MgCl_2$	0.010 " "
NaH_2PO_4	0.005 " "
Glucose	0.10 " "
Atropine sulphate	1:15,000,000

Glucose and phosphate are not indispensable, but appear to prolong the period of usefulness of the gut; the high K content minimizes possible interference by excess of K in the extracts; Mg reduces the spontaneous activity of the strip. Atropine is indispensable, as the blood extracts frequently contain choline-like compounds in appreciable quantity.

The histamine solutions (Histamine dihydrochloride, Hoffmann-LaRoche) are made up in 0.97% NaCl to be approximately isotonic with the Ringer's solution. Fresh standard solutions are always used.

A constant volume of standard histamine solution (or of blood extract), generally 0.1 c.c., is always added. The additions are made at constant intervals of 3 minutes from a Pyrex dropping pipette having a rubber bulb, and a constricted tip, so that the injected solution mixes rapidly with the fluid in the bath; the pipette is rinsed out immediately with fluid from the bath. Control injections of coloured solutions showed that mixing was complete within 2 to 3 seconds: this must be ensured if uniform results are to be obtained. The histamine solution is allowed to remain in contact with the intestinal strip for 25 seconds, and the fluid is then quickly changed, with no further rinsing until the next addition /

addition. A stop-watch is used to keep the time intervals constant. I have found it well to tap the water-bath with the fingers while the strip is contracting to minimize the effect of friction of the writing-point, although this is extremely small.

The sensitivity of the intestine increases with the first few additions of histamine, so that the histamine content of the extracts, if these are applied at this time, must be determined by interpolation. After about 6 or 8 additions of histamine, the strip gives nearly constant responses to the same dose of histamine, and continues to do so for 2 hours or more, so that at least 40 contractions with the extracts or the histamine standards may be recorded. At least 3 determinations are made with each extract; these should not differ by more than 10 per cent. The guinea-pig's intestine generally gives a definite response to histamine in $1:10^9$ concentration: since the volume of added fluid is about 5 per cent of the bath volume, this means that 0.002 y of histamine can be determined, corresponding to 5 y per litre of blood. If the extracts have an unusually high content of histamine, they are suitably diluted with saline so that the standard volume of 0.1 c.c. produces a sub-maximal effect.

The shape of the contraction curve produced by the extracts is normally the same as that produced by pure solutions of histamine; if it is not the same, as may happen when a strip is damaged or has been used too long, the results are unreliable.

Numerous control experiments have shown that the response of the intestine to histamine is not affected by the presence of other constituents /

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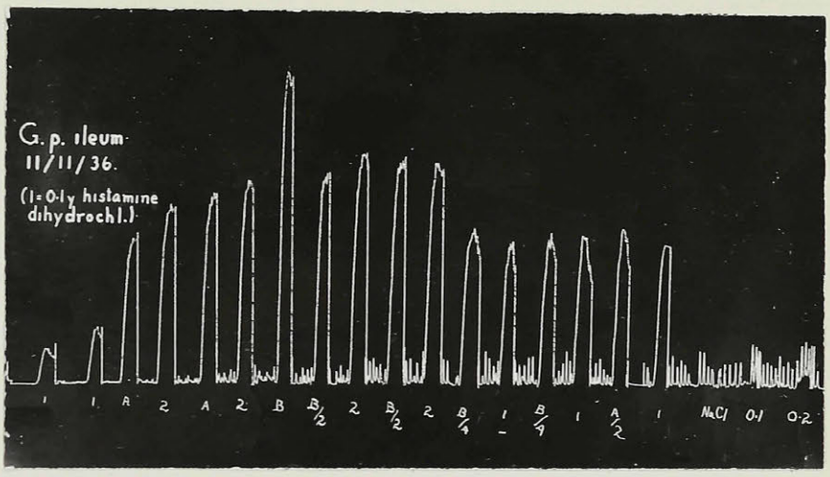

FIGURE 28. - Part of a tracing made during the assay of 2 extracts of blood (see page 122). The 2 blood samples, taken at the same time, had an original volume of 10 c.c., but to one of them (extract B) was added 0.5 y of histamine dihydrochloride. The final volume of each extract was 2.5 c.c. Extract A was found to be equal in potency to a 2.1×10^{-7} solution of histamine dihydrochloride, corresponding to a blood histamine concentration of 32 y per litre. Extract B was found to be equal in potency to a 4×10^{-7} solution of histamine dihydrochloride, corresponding to a blood histamine concentration of 60 y per litre. The 0.5 y of added histamine dihydrochloride was therefore about 94 per cent recovered.

Note, at the beginning of the assay, the increasing sensitivity of the strip; and at the end of the tracing, the determination of the minimal effective dose (concentration in the bath of 1:1,600,000,000).

constituents of the extracts. Histamine added to blood in a quantity approximately equal to that already present (0.3 y in 10 c.c.) was at least 80 per cent recoverable. The extracts appear to maintain their potency indefinitely if kept in the ice-box.

Figure 28 shows the tracing made during the assay of two extracts of blood. The two blood samples, of volume 10 c.c. each, were taken at the same time, but to one of them (B) was added 0.5 y of histamine dihydrochloride. The tracing shows that the added histamine was 90 per cent recoverable. The minimal effective concentration of histamine in this assay was about 6×10^{-10}.

The 'over-dosage' test for histamine. Barsoum and Gaddum (82) found that strips of the fowl's rectal caecum, which have been treated with excess of histamine, contract maximally, but soon relax, and are then quite insensitive to histamine, but continue to respond to interfering substances which may be present in the extracts. If an extract contracts the rectal caecum before such 'over-dosage' with histamine, but fails to do so afterward, strong evidence is furnished that the active principle of the extracts is really histamine. This useful qualitative test is easily adapted to the guinea-pig ileum by using a Ringer's solution containing histamine in $1:10^6$ concentration. When first placed in this solution, the intestine contracts maximally, but with repeated renewal of the histamine-containing Ringer in the bath slowly relaxes, and in about 30 minutes approaches its former length. It then responds nearly as well as originally to K, Ba, OH^- and (when not atropinized) to choline and acetylcholine, but is quite insensitive to ordinary doses of histamine. The extracts used in these experiments invariably failed /

failed to stimulate the atropinized intestine when this had been treated with histamine in excess.

Feeding Experiments. These were performed on large dogs which had been starved for 24 hours. Blood for the determinations was withdrawn from a leg vein, care being taken that the vessels of the limb were not occluded for more than the few seconds required for introduction of the needle. The animals nearly always remained quiet while the needle was being inserted and the blood withdrawn. Anrep and Barsoum (18) have shown that in the absence of asphyxia or activity the venous blood from a muscle has the same histamine content as the arterial blood, so that any increase in the histamine content of the latter would be duplicated in the former: it is therefore permissible to use systemic venous blood for experiments of this kind. In two experiments, arterial blood was obtained directly from the heart by cardiac puncture: exactly the same results were found in these experiments.

The results of all experiments performed are given in Table VI. It will be noted that the histamine equivalent of the 'fed' blood exceeded that of the 'resting' blood in 2 experiments only. The meat meals given were nearly all very large, and provoked a profuse secretion from the denervated pouches of dogs 'B' and 'H', which were used in experiments 6 to 10.

TABLE VI /

TABLE VI.

The histamine content of dog's blood before and after a meal.

Expt. No.	Dog	Meal	Time between feeding and taking of 2nd (or 3rd) blood sample (min.)	Histamine content of blood (γ per litre)	
				Resting	Fed
1	'S'	800 gm. beef-heart	110	23	20
2	'D'	1000 gm. " "	120	180	180
3	'D'	1250 gm. " "	110	140	130
4	'N'	300 gm. " "	120	9	9
5	'N'	1000 gm. " "	190	7	8
6	'B'	2 lb. 'Chum'	80, 220	13	13, 14
7	'H'	2 lb. "	80, 220	8	8, 8
8	'B'	2 lb. "	320	< 6	< 6
9	'H'	2 lb. "	320	8	8
10	'B'	700 gm. beef-heart	80, 250	17	19, 15
11	'X'	700 gm. " "	75, 210	4	< 4, 12
12*	'X'	700 gm. " "	150	11	15
13*	'X'	700 gm. " "	180	33	24

* In experiments 12 and 13, the blood was obtained from the left ventricle by cardiac puncture.

It will be noted that the blood histamine remains more or less constant in the same animal. Dog 'D', which gave very much higher values than any of the others, carried a gastric fistula which had been partly torn out and around which the tissue was badly ulcerated by the action of the gastric juice; this may be the reason for the high concentration of histamine in the blood.

A note on a choline-like substance present in blood extracts.

The extracts of blood frequently, but not always, possessed a 'choline-like' activity, a rough measurement of which could be made by subtracting the histamine equivalent as determined on the atropinized gut from the histamine equivalent as determined on the unatropinized gut. In 3 out of 5 experiments, the quantity of

this/

this was considerably increased during the digestion of a meal; in 1 experiment, it was unchanged; and in 1 it was lowered. Since it has not been shown whether choline in the blood would be all recovered by the extraction procedure used in the work on histamine, I tried in one experiment to estimate the free choline of the blood by acetylating aliquots of the extracts after they had passed the ether extraction stage, and assaying against acetylcholine on the eserinized frog's rectus. The values found for free choline varied in the same direction as those calculated for the 'choline-like' substance detected by the guinea-pig ileum test, thus:

TABLE VII.

Determination	Blood Sample		
	A (before feeding)	B (7.5 min. after feeding)	C (210 min. after feeding)
	concentration per litre		
(1) Histamine, as determined on atropinized g.p. ileum	4 y	-	12 y
(2) 'Histamine', as determined on unatropinized g.p. ileum	24 y	6 y	18 y
(3) 'Choline-like substance', (2) minus (1), measured as histamine	20 y	6 y	6 y
(4) Choline, as determined on eserinized rectus after acetylation of extract, measured as choline	5 mg.	1.5 mg.	1.5 mg.

The values here obtained for the 'free' choline of the blood are of the same order as those found by Hunt (168) and by Heesch (169). Whether choline was the only substance in the extracts whose /

whose action on the guinea-pig intestine was annulled by atropine, cannot be determined from these data; nor can it be established whether or not the level of such compounds in the blood is affected by feeding.

Discussion.

The experiments afford conclusive evidence that the histamine content of the systemic blood is not increased during the digestion of a meal. Histamine therefore cannot function as a hormone for gastric secretion in the sense that secretin functions as a hormone for pancreatic secretion. This does not eliminate the possibility that the liberation of histamine forms a part of the intimate secretion-producing mechanism of the parietal cells; thus it might be supposed that secretagogues in the blood might cause the bound histamine in these cells to be set free, whereupon the secretory mechanism would be set in motion. If histamine does function in this way, its action is purely local, and it does not escape into the blood in appreciable quantity.

The only possible objection to this conclusion depends on the newly discovered fact that the histamine determined by the method of Barsoum and Gaddum appears to be contained largely within the corpuscles (170, 171), and is therefore probably unable to produce its characteristic effects. It might then be supposed that, although the <u>total</u> histamine of the blood remains the same during digestion, the <u>free</u> histamine of the plasma is increased; but the latter may be so small in quantity as compared with the histamine of the corpuscles /

corpuscles, that any change in it fails to be detected. Against this supposition may be quoted one experiment, in which the subcutaneous injection of an effective dose of histamine into an anaesthetized cat increased the histamine content of the blood as estimated in the usual way; in this experiment the 'resting' histamine content was 9 y per litre; 30 minutes after subcutaneous injection of 2 mg. of histamine dihydrochloride, the value was 37 y per litre; a subsequent injection of 5 mg. more raised the value in 10 minutes to 68 y per litre. If the pyloric mucosa liberates histamine in effective quantities, one would expect a similarly measurable increase in the histamine content of the blood. Nevertheless it is not yet absolutely certain that during the second phase the plasma histamine remains below the physiologically active concentration.

Since it has not been proved that the pyloric mucosa contains any secretagogue other than histamine, this work lends support to the theory that the gastric glands are activated during the second phase by secretagogues derived from the food, absorbed by the pyloric or intestinal mucosa, and carried by the blood to the fundus. There is one alternative hypothesis, namely that the gastric hormone has not yet been extracted from the pyloric mucosa. Dr. Komarov* in this laboratory has very recently prepared an extract of gastrid mucosa which possesses considerable secretagogue activity and is presumably free from histamine; the nature and mode of action of its active principle are still obscure. At the present time it can only /

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* Unpublished data.

only be said that the evidence so far presented for the existence of a true gastric hormone is extremely unsatisfactory.

Summary.

1. The histamine content of the systemic blood of dogs is unaffected by feeding.

2. Since histamine is apparently the active principle of 'gastrin' preparations, this fact speaks against the existence of a true hormone for gastric secretion.

PART V /

PART V. THE EFFECT OF VAGAL STIMULATION ON THE HISTAMINE CONTENT OF THE GASTRIC VENOUS BLOOD.

Introduction.

The experiments described in the preceding section prove that histamine is not the gastric hormone, carried by the blood from the pyloric mucosa to the fundic cells. What then is the function of histamine in the gastric mucosa? The fact that histamine is present in extraordinarily high concentration in this tissue, for whose activity it is so powerful a stimulant, suggests that it may play some role in the production of gastric juice. The easiest guess is that it may be liberated within, or near, the parietal cells in response to stimulation of the vagus, which carries the secretory fibres to the gastric glands, and that it sets these cells into activity. Histamine would thus function, not in the second phase as a 'true hormone', but in the first phase as a 'local hormone'.

A number of theoretical considerations make this hypothesis attractive. (1) The normal physiological activity of histamine appears to be the mediation of local response to stimulation: cf. its action in cutaneous vasodilatation in response to injury (Lewis and his co-workers (172)), in the bronchospasm of anaphylactic shock (173), in reactive hyperaemia (17), and in the vasodilatation of active muscle (18). (2) The gastric mucosa is the only tissue in which a high concentration of histamine is associated with absence of histaminase (174), so that nothing would stop the local action of /

of histamine once it was set free. (3) There is evidence in the literature that the gastric juice is richer in histamine than the blood plasma, suggesting that histamine is produced during gastric secretion and does not merely pass into the juice from the blood. (4) Histamine inhibits the secretory response of the stomach to sham-feeding: it is known (see page 122 ; cf. Feldberg and Schiff (175)) that the effect of histamine tends to vary inversely with the concentration already present. (5) The composition (acidity, Cl content) of 'histamine' juice is very nearly the same as that of 'vagus' juice, suggesting that the same stimulus activates the parietal cells; certain differences (higher combined acidity, protein, non-protein nitrogen in 'vagus' juice) may be accounted for by the participation of the peptic and mucoid cells in the response to nerve stimulation.

The hypothesis was therefore worth testing. I have adopted the simplest possible method of doing this, namely stimulating the vagus in acute experiments, and noting whether the gastric venous blood withdrawn during stimulation was richer in histamine than the arterial blood, or than control samples of venous blood from the unstimulated organ. The assumption made was the one so often justified in work on neurohumoral transmission, namely that, if a 'local hormone' is liberated within the cells of an organ, some of it will escape into the blood-stream. I have also determined, by a modification of the method of Barsoum and Gaddum (82), the histamine content of gastric juice produced by (a) vagal stimulation in acute experiments, (b) sham-feeding, and (c) injection of histamine.

It /

It may as well be stated at the outset that I have found no good evidence for such liberation of histamine by the gastric mucosa during effective stimulation of the vagus. In the earlier experiments, evidence was apparently obtained for histamine libera- during vagal stimulation. These results could not be duplicated in later experiments performed with better technique, and I now believe that such apparently positive effects were due to excessive handling of the stomach during the withdrawal of gastric venous blood. In several later experiments there was undoubtedly a good gastric secretion, which was not inhibited by the procedure for obtaining blood, yet the histamine content of the blood was no higher than that of control samples.

While a large number of acute experiments (21 in all) were performed, with a number of variations in the technique, I shall give only an outline of the methods used for obtaining gastric venous blood, and the complete protocol of one typical experiment. The methods for determining the blood histamine have been fully described in the preceding section.

Experimental.

Gastric venous blood was obtained from anaesthetized dogs by one of the two following methods.

(a) The spleen, pancreas, entire large and small intestine, and most of the omentum were removed after ligation of their vessels, care being taken to preserve gastric branches of the pancreatico-duodenal and splenic veins. A cannula was inserted into the stump of /

of the superior mesenteric vein near its union with the gastro-splenic, its tip pointing toward the liver. On removing the clamp from the superior mesenteric vein and clamping the portal vein, the entire venous blood of the stomach could be withdrawn from the cannula.

(b) The vessels of the spleen were tied off as close as possible to that organ. The gastrosplenic vein in its upper part now received only gastric blood (from the greater curvature), which could be withdrawn through a cannula inserted into one of the splenic branches. At the time of withdrawing the blood the latter vein was unclamped and the main gastrosplenic clamped. This method was satisfactory only in large dogs.

In the experiment presented, especial precautions were taken to prevent the liberation of histamine through handling of the stomach. Stimulation of the vagus produced a good flow of gastric juice, but failed to increase the histamine content of the gastric venous blood.

Experiment December 16, 1936.

Male dog, setter type, 16 Kg. Chloralose-urethane anaesthesia, well maintained throughout, only about 5 c.c. extra anaesthetic being required (after blood sample no. 4 was taken). Precautions taken for temperature regulation, etc. Pulse strong, respiration regular, throughout experiment.

Cannulas in femoral vein and artery and in trachea. Vagi taken on ligatures for stimulation. Rubber catheter introduced into stomach *via* oesophagus for collection of gastric juice. Pylorus tied. There was only one main splenic vein: its splenic and omental branches were tied and cannula inserted into the largest splenic branch. Point of clamping splenic vein was only about 2 cm. from point of insertion of cannula, so that when blood was taken /

taken the stomach was not handled or even exposed. Gastroepiploic vein tied near pyloric sphincter. The skin was dissected off the abdominal wall for some distance to make a pouch in which the spleen was laid, great care being taken to prevent occlusion of gastric vessels; cannula was accessible merely by opening skin incision. Operation finished 12:05. Stomach contained 4 c.c. mucous fluid: acidity 24, 40; pepsin 250.

12:40 Blood sample no. 1, 8.5 c.c., from gastric vein.

12:43 Blood sample no. 2, 10.0 c.c., from femoral artery.

12:45 Vagal stimulation begun, right and left vagi alternately for 5-min. periods. Metronome (see page 50) gave 1-second stimulation to 5-seconds rest. Coil 8.5 cm. Both vagi acting well on pulse.

2:00 No secretion. Coil 7 cm.

2:10 Secretion commenced. First portions rich in mucus.

2:35 25.7 c.c. gastric juice obtained.

2:45 Total secretion of gastric juice 40.1 c.c.

2:52 Total secretion 53.4 c.c.

3:00 Blood sample no. 3, 6.5 c.c., from gastric vein. Blood flow through stomach appeared to be reduced.

3:10 Blood sample no. 4, 10 c.c., from femoral artery. Blood pressure much lower than previously.

3:12 Total secretion 80.5 c.c. No interruption during taking of blood samples.

3:20 Total secretion 95.3 c.c.

3:25 Blood sample no. 5, 9 c.c. Blood flow appeared somewhat greater than when taking sample no. 3, but less than when taking sample no. 1.

3:30 Total secretion 109.8 c.c. Stimulation stopped. Secretion stopped within 1-2 min.

3:35 Blood sample no. 6, 9 c.c., from gastric vein. About same rate of flow as in no. 5.

Extracts for the estimation of histamine were prepared in the usual way from all blood samples. Assay on the guinea-pig intestine showed that all samples had practically the same histamine content of less than 6 γ per litre (unusually low) except for /

for sample no. 1, which contained 8 y per litre. Histamine added to the extracts was satisfactorily determined.

The values observed for the blood histamine in anaesthetized dogs were of the same order as those found in unaesthetized dogs, viz. 5 to 25 y per litre, calculated as histamine base. Extensive operative interference did not appreciably affect the histamine content of the systemic blood; however, all samples were taken at least 30 minutes after the end of the operation.

Histamine in canine gastric juice is easily determined by the method of Barsoum and Gaddum. The only modification required is that the mixture of trichloroacetic acid and gastric juice must be heated to 70° C. for 5 minutes, since trichloroacetic acid precipitates the dissolved mucin of gastric juice only with the aid of heat. Gastric juice obtained by injection of histamine gives a negligible precipitate with trichloroacetic acid. Added histamine is recovered practically completely in the final extract.

Table VIII gives the results of the histamine determinations made on gastric juice. The gastric juice, except in the 2 acute experiments, was obtained from the same dog with oesophagotomy and a gastric fistula. A portion was taken for acid and pepsin determinations: the remainder was filtered and worked up immediately. It will be seen that the histamine content of 'vagus' juice is of the same order as that of blood; in the one case where it is much higher, the animal had recently torn out the gastric fistula, and the histamine may have come from the lacerated mucous membrane. The single sample of 'histamine' gastric juice tested was not especially /

especially rich in histamine.

TABLE VIII.

Stimulation	Free Acid m.eq./l.	Total Acid m.eq./l.	Pepsin Mett u.	Histamine y/litre
Sham-feeding	142	152	324	116
" "	139	150	undet.	7
Histamine	148	160	13	10
Sham-feeding	141	152	400	40
" "	128,134	140,146	174,310	7, 9
" "	141	146	183	15
" "	141	149	255	9
Vagus stimulation, acute experiment	120	142	762	5
Vagus stimulation, acute experiment	undet.	undet.	282	22

Discussion.

These experiments fail completely to lend any support to the idea that histamine is the mediator of vagal secretory impulses to the parietal cells. Such negative findings do not, however, disprove the hypothesis. The quantities of histamine which are effective at the site of liberation may be very small, so small that when they diffuse into the circulation they do not perceptibly increase the total histamine content of the gastric venous blood. As the histamine of the blood is, apparently, nearly all bound in the corpuscles, it is possible that the plasma histamine might be increased many times, without any measurable change taking place in the /

the histamine value for the whole blood. More refined methods of experimentation will be necessary to show whether such a rise in the plasma histamine does or does not occur when the gastric glands are activated by vagal stimulation.

My finding that dog's gastric juice has a rather low histamine content may be contrasted with that of Brown and Smith (176), who found that human gastric contents contained up to 500 y per litre of a 'histamine-like substance'. Their method of assay, however, was less satisfactory than that used in this work. It is, of course, quite possible that human gastric juice contains more histamine than canine gastric juice.

It is to be noted that the concentration of histamine in the gastric juice is undoubtedly higher than that in the plasma. This must mean that during the process of secretion there is free histamine within the gland cells; and it may be supposed that this free histamine was itself capable of stimulating the cells. The total histamine present in a given amount of gastric juice is, however, very much less than that which would have to be parenterally administered to evoke the secretion of a similar volume of juice.

The question of whether histamine participates in the nervous control of gastric secretion must therefore be left, for the present, an open one. It is hoped that further work will shed more light on the problem.

Summary./

Summary.

1. Stimulation of the vagus does not perceptibly increase the histamine content of the gastric venous blood, as determined by the method of Barsoum and Gaddum.

2. The possibility is discussed that histamine mediates the secretory action of the vagus on the gastric glands.

PART VI /

PART VI. ALCOHOL AS A CONDITIONED STIMULUS OF GASTRIC SECRETION.

Introduction.

During the work on the effect of choline on gastric secretion, I had occasion to administer alcohol to a dog equipped with a Pavlov pouch and gastric fistula (see page 105), in order to produce a flow of gastric juice of low peptic power. In the first experiments performed, alcohol fulfilled this purpose very well; and the data obtained fully confirmed the finding of the Pavlov school (177; see also Babkin (28), pp. 402-407) that alcohol does not stimulate the secretion of pepsin - that alcohol, in other words, excites the parietal cells but not the peptic cells. Continuation of the experiments showed, however, that the effect of alcohol was not necessarily thus restricted, and that in certain circumstances it could activate the peptic cells as well. This secondary working of alcohol was analyzed, and it was found that the effect was an indirect one, depending on the fact that the animal had grown accustomed to alcohol, and had learned to relish it. While this work does not, strictly speaking, come under the heading of this thesis, I shall nevertheless include it, since it forms an interesting example of the way in which nervous influences may modify the activity of a stimulus which acts ordinarily in purely humoral fashion. The experiments are also of interest from the standpoint of the therapeutic use of alcohol as a stomachic.

Our present knowledge of the effect of dilute alcohol on gastric digestion may be briefly summarized as follows. Alcohol in weak solution (5 to 20 per cent), introduced into any part of the digestive /

digestive tract, evokes quickly a copious flow of gastric juice, which has a high acidity and a very low digestive power. The secretory effect of alcohol does not depend on the integrity of the vagi, since it can be observed in an animal equipped with a Heidenhain pouch. In vitro, alcohol in 1 or 2 per cent concentration has no significant effect on the peptic activity of gastric juice (177): when weak alcoholic drinks are taken in conjunction with a meal, the concentration of alcohol in the stomach is probably not greater than this.

The effect on the gastric secretion of adding alcohol to a meal was systematically studied by Zitovich (177), using dogs equipped with pouches of the stomach. He found that when 5 or 10 per cent alcohol was added to a meal of meat, bread or milk, the volume of gastric juice secreted in response to the meal was greatly increased. Alcohol in the same concentration also counteracted the inhibitory effect of fat on the gastric secretion. The secretagogue action of alcohol was especially striking when the first or reflex phase of the secretion was eliminated by introducing the test-meal directly into the stomach through a gastric fistula. The addition of alcohol then restored the normal course of the secretion, and shortened the emptying time of the stomach by one hour. The peptic power of the juice secreted during the first two hours was, however, lower (average, 41 Mett units) than when the meal was given by mouth, with water instead of dilute alcohol (average, 177 Mett units). Zitovich's work thus shows why, when through loss of appetite the gastric glands fail to give their normal /

normal prompt response to the taking of a meal, the ingestion of a beverage of low alcoholic content will help to restore the proper digestion of the food. Nevertheless, the secretion activated by alcohol was not, in his experiments, a perfect substitute for the normal reflex secretion, since the enzyme content of the juice was comparatively low.

It might thus be concluded from the literature that alcohol is an effective stimulant for the parietal cells but does not excite the peptic cells. My own experiments, which show, as mentioned above, a stimulation by alcohol of the discharge of pepsin, will now be described.

Experimental.

The animal used was a dog equipped with a Pavlov pouch of the stomach and a gastric fistula. The experiments were done in the morning, the usual precautions being taken to prevent psychical stimulation of the secretion. 200 c.c. of 5% ethyl alcohol of good quality, warmed to 37° C., was introduced slowly into the stomach through the fistula while the gastric glands were at rest, and the resultant pouch secretion was collected. In control experiments, two other dogs were used, one with a Heidenhain pouch (vagal innervation destroyed) and one with a Bickel pouch (completely denervated) and a gastric fistula. (These dogs have been referred to as 'P', 'H', and 'B' respectively in preceding pages.) The Bickel-pouch dog could be similarly given alcohol by the gastric fistula; the Heidenhain-pouch dog could be given alcohol by rectum. None of the dogs had ever received alcohol before.

Free /

Free and total acidity and pepsin were determined on the pouch juice as previously described.

The results of the first series of experiments with the Pavlov-pouch dog were uniform. Soon after the dilute alcohol was injected, the animal's breath smelled of alcohol and she began to lick her lips. Within 2 or 3 minutes gastric juice began to flow from the pouch. The secretion was brisk for about an hour, and stopped after about 2 hours, when the main stomach was found to be empty. A typical protocol from the series is presented.

Experiment February 4, 1936.

Dog 'P', female, 14 Kg.

Time		Vol. c.c.	Free Acid m.eq./l.	Total Acid m.eq./l.	Pepsin Mett u.
9:05	Animal placed on stand. Stomach empty.				
9:05- 9:20	Pouch secreted 0.15 c.c. of mucous fluid.				
9:18- 9:20	200 c.c. of 5% ethyl alcohol at 37° C. introduced through the gastric fistula.				
9:20- 9:50		3.90	111	120	60
9:50-10:20		2.90	129	138	73
10:20-10:50		0.95	100	109	97
10:50-11:20		0.30			
		8.05			

The same experiment was repeated many times on this dog between the end of January and the middle of December 1936. The course of the alcohol secretion, and its total volume and acidity, were always about the same. The volume varied between 7.3 and 9.8 c.c., although latterly it was generally near the upper limits. The concentration of pepsin in the juice, however, underwent a striking /

striking change. After the first month or two, during which the experiments were repeated about twice a week, the values for pepsin became gradually higher and higher with each consecutive injection of alcohol. A protocol from this later period will illustrate the change.

Experiment September 8, 1936.

Dog 'P'.

Time		Vol. c.c.	Free Acid m.eq./l.	Total Acid m.eq./l.	Pepsin Mett u.
9:25	Animal placed on stand. Stomach empty.				
9:25- 9:55	Pouch secreted 0.5 c.c. Acid free 0, total 14; pepsin 369.				
9:53- 9:55	200 c.c. of 5% alcohol at 37° C. introduced through the gastric fistula.				
9:55-10:25		4.6	82	95	403
10:25-10:55		1.7	108	128	400
10:55-11:25		0.8	76	100	416
11:25-12:15		0.6	60	72	538
		7.7			

The course of the change in the secretory response is illustrated also in Figure 29. The left-hand graph (a) shows the volume of juice secreted in eight experiments, selected as typical of different periods during the investigation. The right-hand graph (b) shows the concentration of pepsin in the juice secreted in these same experiments.

The average value for pepsin concentration increased from 33 Mett units in January to 462 in September - i.e., there was an increase /

(To follow page 142.

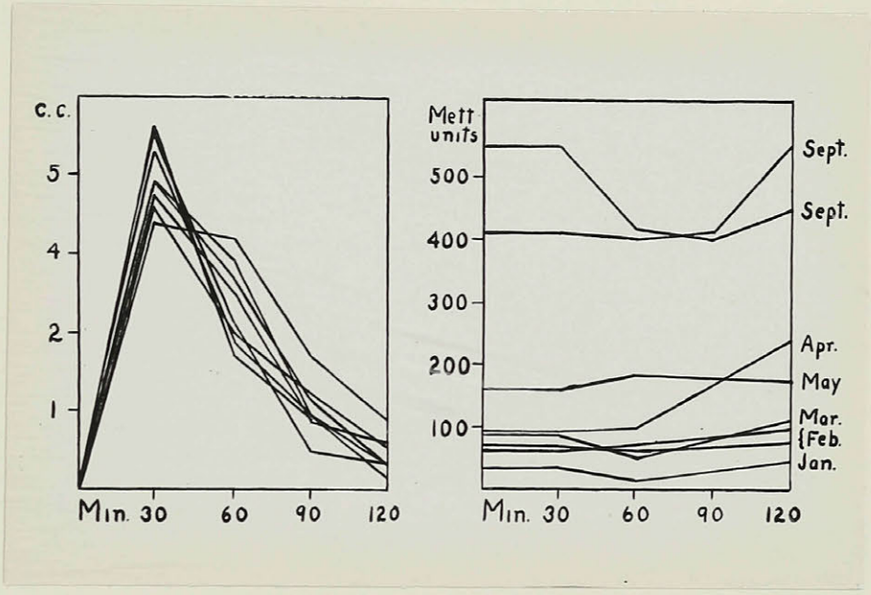

FIGURE 29. - (a) Volume of juice secreted (Pavlov pouch) after the introduction of 200 c.c. of 5% alcohol into the stomach. 8 experiments.

(b) Concentration of pepsin in the gastric juice in the same 8 experiments. Habituation to alcohol raised the digestive power of the juice but had comparatively little effect on the volume secreted.

increase in the digestive power of 1400 per cent. Thus alcohol, which at first did not stimulate the output of pepsin, came in this dog to be a very strong stimulus for the peptic cells.

The other two dogs, whose pouches had no vagal innervation, gave very uniform low values for pepsin (average, less than 50 Mett units) during this whole experimental period.

It was suspected that the explanation of the changed reaction of the Pavlov-pouch dog was that alcohol had become in this animal a positive conditioned stimulus for gastric secretion. There were several reasons for supposing this. During the action of alcohol the dog was clearly slightly intoxicated, and appeared to be in particularly good humour. Although the alcohol was introduced directly into the stomach and the animal was not allowed to smell or taste it, it was soon excreted by the lungs, and the dog could be seen to lick her lips at intervals during the experiment. After the experiments had gone on for some time, she would wag her tail violently, show the greatest pleasure when preparations for the experiment were being made, and follow the movements of the experimenter with much interest. Once or twice a small amount of gastric juice was secreted even before the alcohol was introduced into the stomach. On several occasions the dog was offered 5% alcohol to drink, and took considerable quantities freely although water was refused. The drinking was followed by a fair secretion of gastric juice of high peptic power. Thus in one of these experiments (October 12, 1936), when the dog took 175 c.c. of 5% alcohol, the total volume of secretion for 2 hours was 7.1 c.c., and the average peptic /

peptic power 271 Mett units.

It therefore seemed reasonable to conclude that the dog had learned to like alcohol, or perhaps rather the effects of alcohol, and that alcohol had thus become a positive conditioned stimulus for the secretion of pepsin. The actual stimulus would probably be the taste of the alcohol as it was excreted in the breath.

This conclusion is strongly supported by the following experiment, in which the alcohol was given by rectum, instead of through the gastric fistula as usual. The animal had never before received a rectal injection, and objected violently. The digestive power of the juice secreted in response to the alcohol was low, and remained low after a second portion of alcohol had been given through the gastric fistula in the usual way, although the volume of the secretion was nearly normal. In previous and subsequent experiments, in which the alcohol was introduced directly into the stomach under more pleasant circumstances, the digestive power of the juice secreted was very high. The protocol of this experiment is given complete.

Experiment October 14, 1936 /

Experiment October 14, 1936.

Dog 'P'.

Time		Vol. c.c.	Total Acid m.eq./l.	Pepsin Mett u.
10:00	Dog placed on stand.			
10:00-10:30	Pouch secretion 0.5 c.c.			
10:30	Small water enema given to wash out bowel. Dog very excited, struggled, tried to bite.			
10:30-10:50	No secretion.			
10:48-10:50	200 c.c. of 5% alcohol by rectum. Same reaction shown by animal.			
10:50-11:20		2.2	90	122
11:20-11:50		1.7	120	52
11:50-12:20		0.7	70	41
		4.6		
12:23-12:28	Attempted to give 2nd portion of alcohol per rectum. Animal much agitated. Attempt unsuccessful.			
12:28-12:30	200 c.c. of 5% alcohol by gastric fistula. Dog did not object.			
12:30- 1:00		3.5	119	59
1:00- 1:30		2.5	144	31
1:30- 2:00		0.9	undet.	58
		6.9		

It is clear that in this experiment the discharge of pepsin was inhibited by the defence reaction of the animal. It is a familiar fact that such emotional reactions frequently inhibit alimentary conditioned reflexes for a considerable period. Since the secretion of pepsin is controlled by the vagus, we can say that the pepsin output was small, owing to inhibition of the vagal secretory /

secretory centres, although there was still a flow of juice owing to the peripheral action of the alcohol.

Several experiments were performed in which it was attempted, by administration of atropine, to eliminate the vagal participation in the secretory effect of alcohol. The results of these experiments were, however, not very clear-cut. Large doses (0.3 mg. per Kg. of atropine sulphate) entirely abolished the secretion provoked by alcohol, chiefly by interfering with its absorption, since the stomach contained nearly the original volume of solution after 1½ hours. This is in agreement with the finding of Orbeli (178). Smaller doses of atropine diminished both the volume of the juice and the total output of pepsin, although the evacuation time of the stomach appeared to be not greatly lengthened.

Discussion.

Alcohol differs from all other chemical stimulants of the gastric secretion in that it acts well from any part of the digestive tract. The composition of the juice secreted in response to alcohol (unless alcohol has become a conditioned alimentary stimulus) is very similar to that of the secretion provoked by histamine, i.e. the acidity is high and the enzyme concentration low. Alcohol therefore, like histamine, acts chiefly on the parietal cells. Since alcohol also resembles histamine in its action on the skin capillaries, it is not impossible that some of its effects may be due to liberation of histamine, but for this there is no direct evidence. The primary secretagogue action of alcohol, like that

of /

of histamine, is a peripheral one, since it does not require the integrity of the extrinsic innervation of the stomach. Whether the antagonistic effect of atropine is due merely to interference with the absorption of alcohol, or whether the intrinsic gastric nerves are involved in the action of alcohol, has not been determined.

The change in the composition of the juice secreted under the influence of alcohol, after this substance had become a conditioned stimulus for gastric secretion, is a good example of the way in which nervous influences may modify the influence of humoral excitants. Such an integration of the action of nervous and humoral stimuli undoubtedly plays a part in the normal regulation of gastric secretion. It is of interest to note that in this case the nervous stimulus acts primarily on the peptic cells, but hardly at all on the parietal cells: another instance of the way in which the several types of cells composing each glandular organ are activated by different mechanisms (see page 121).

From the practical point of view, it may be observed that positive alimentary conditioned reflexes can no doubt be formed with dilute alcoholic beverages generally. Even in the absence of such reflexes, alcohol through its peripheral action may improve a faulty digestion by increasing the flow of juice. If positive conditioned reflexes have been developed, the beneficial effect of alcohol is augmented, since the juice secreted has a greater digestive power. It might be suggested that, if dilute alcohol is to be prescribed as an aid to gastric digestion, the beverage preferred by the patient would probably possess the greatest virtue as a stomachic: proper consideration /

consideration must of course be given to the effect on digestion of constituents of the beverage other than alcohol.

Summary.

1. In the unhabituated subject, dilute alcohol on absorption stimulates the gastric glands directly, evoking a copious flow of gastric juice of high acidity and low digestive power. On continued administration alcohol may become a conditioned stimulus for gastric secretion, and in these circumstances causes the secretion of a juice of high digestive power.

2. The significance of this is discussed from the standpoint of the use of alcoholic drinks as stomachics.

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