

THE CHEMICAL MECHANISM OF GASTRIC SECRETION¹. BY J. S. EDKINS. (One Figure in Text.)

(From the Physiological Laboratory of S. Bartholomew's Hospital.)

Preface. The earliest phase in the process of gastric digestion has been shown by Pawlow and his pupils to be chiefly dependent upon a psychic condition resulting in nervous impulses passing by the vagi to the gastric glands and bringing about their activity. This form of secretion is comparatively transitory and the later stages are regarded as due to certain exciting substances, present in the food or formed in the early stages of the digestive process, acting on the peripheral end-apparatus of the sensory nerves of the stomach and thereby evoking a reflex secretion of the juice. Direct stimulation of the glandular mechanism by means of the exciting substances becoming absorbed is thought by Pawlow to be very improbable though difficult to actually disprove.

Since the work of Bayliss and Starling, which showed that the pancreas could be caused to secrete without the intervention of any nervous mechanism but simply by the direct exciting effect of something which could be introduced into the general circulation in the process of absorption from the alimentary canal, it has of course been possible that the so-called chemical secretion of the stomach might be explained in a similar manner. Certain substances absorbed in the stomach might in the process of absorption extract from the gastric mucous membrane certain chemical principles which when introduced into the blood stream would finally act as specific excitants of the flow of gastric juice.

The problem involved a consideration of (1) what substances, when absorbed, would be most potent in introducing these chemical excitants into the circulation, and (2) what part of the alimentary tract would be the most probable path for the absorbed substances to take.

In connection with the first part of the problem it suggested itself

¹ Some of the more important facts embodied in this paper were published in a preliminary communication to the Royal Society in May 1905.

that just as gastric digestion was preparatory to pancreatic secretion, particularly in respect of the production of the acid necessary for the development of Bayliss and Starling's *secretin*, so the changes brought about in salivary digestion or early gastric digestion might lead to the formation of substances which had particular influence in the development of some *gastric secretin*.

In connection with the second part of the problem it was assumed that generally no marked exodus of gastric digestive products into the duodenum would occur before the chemical secretion became established. The absorption leading to any introduction of a gastric secretin must therefore take place mainly in the stomach itself. It has been shown by v. Mering and myself that no absorption of water occurs ordinarily in the stomach. v. Mering has further proved, however, that glucose and peptone amongst other digestive products are absorbed. The path of this absorption is at first sight somewhat difficult to locate. But though it may be difficult to understand how a process of absorption can occur in any part of the stomach, still the difficulty is by far the greatest in respect of the *fundus glands*. It seemed therefore desirable to test in the first place the simple glands of the stomach, such as are found in the pyloric region or in the true cardiac region of such animals as possess that region at all developed¹, to see if they would yield an extract which on injecting intravenously would lead to any alteration in gastric secretion. The method adopted and the results of the different experiments as regards gastric secretion is as follows.

Method. When investigating the absorption of water in the alimentary canal, I was able to show that normal saline solution introduced into the stomach would remain for a prolonged period (1—2 hours) unabsorbed, and without change in reaction. At no time did I find any evidence in the normal animal of a secretion of the smallest perceptible quantity of acid. Adopting in the present experiments therefore a similar procedure to that which was used then, I first ligatured the cardiac orifice of the stomach, and introduced a cannula, through the duodenum, into the pyloric end, tying this in at the level of the pyloro-duodenal junction. The ligature at the pyloric end was passed beneath the peritoneum so as not to interfere with any large blood-vessels. That at the cardiac end was tied sufficiently tightly

¹ I wish to point out that I have adopted the stricter nomenclature of reserving the term *cardiac region* for that part of the stomach nearer the cardiac orifice which possesses simple glands and describing as *fundus region* that part which possesses compound glands, containing both central and parietal cells.

to physiologically sever the vagus fibres. The cannula was connected with a reservoir of normal saline solution in such a way that the amount passing into the stomach could be measured, and either a known quantity was first passed into the stomach or the stomach was dilated to its full capacity by the saline solution being supplied at a certain pressure. In the following experiments the animals were almost invariably used in the fasting condition and the effects are much more evident in this state. The extracts were made either from the stomach of the cat or the pig and were injected into the animal under experiment through the jugular vein.

The animals were anæsthetised at first with chloroform only, and during the progress of the experiment the anæsthesia was maintained by a mixture of chloroform and ether (2 : 3). As much of the abdominal contents as projected through the incision were covered with flannels wrung out of hot water.

At the end of the different stages of the experiment the normal saline solution was removed and tested for hydrochloric acid and in many cases pepsin.

THE EFFECT OF INJECTING EXTRACTS OF DIFFERENT REGIONS OF THE STOMACH UPON GASTRIC SECRETION.

Watery extracts of mucous membrane. The result of injecting an extract made by exposing the scrapings of the mucous membrane to the action of simple tap-water are not invariably the same. Frequently no evidence of secretion is obtained, sometimes a small amount of secretion results, but at no time does any important degree of gastric secretion appear.

Extracts of different regions of the stomach made in dextrin (5 p.c.). The extracts in these experiments were made by stripping off the pyloric mucous membrane, chopping it finely and then pounding it up in a mortar with sand. The mass resulting was shaken up with 50 c.c. of 5 p.c. dextrin solution (Schuchardt's pure dextrin) and after standing for some time was filtered.

An illustrative experiment is recorded.

Cat, fasting for about 12 hours. Preparation as given under 'Method.' Washed out stomach by pyloric cannula with warm normal saline, a small amount of gastric contents removed. Stomach then connected with N.S.S. reservoir at pressure of 5 cm. of the liquid. On removing clamp 166 c.c. passed into cavity.

- 4.10. Injected 6 c.c. of 5 p.c. *dextrin* into jugular.
- 4.45. Removed gastric contents, recovering about the same quantity as passed from reservoir = *A*.
- 4.55. Reconnected with reservoir, passing in about 134 c.c.
Then injected 6 c.c. of *extract of pyloric mucous membrane in 5 p.c. dextrin* into jugular.
- 5.35. Removed liquid from stomach, again the same in quantity practically as introduced = *B*.
A was neutral. *B* was acid to litmus, gave Günzberg's reaction, and estimated against centinormal sodium hydrate, it was found that 20 c.c. = 3.5 c.c. $\frac{\text{NaOH}}{100}$.
- On testing for peptic activity, there was found to be no marked difference between the two.

The experiment showed that extracts of the pyloric mucous membrane made in the cold with dextrin will cause a slight secretion, whilst the dextrin by itself will cause none. Several experiments were made under practically the same condition with dextrin extracts and usually a very small amount of acid was shown in *B*.

Extracts of the fundus mucous membrane were also tried but no secretion was shown. The N.S.S. from the stomach, which being made with tap-water was very slightly alkaline, was equally alkaline at the end of a series of injections of the fundus mucous membrane.

But the effect of the extract being made in dextrin was not markedly different from that obtained when made with water alone.

Extracts of different regions of the stomach in glucose 5 p.c. The extracts were at first made as described under the dextrin experiments, i.e. in the cold. But a much larger secretion of gastric juice resulted than with dextrin extracts. The percentage of acid in the recovered fluid varies a great deal even under the same conditions but was uniformly much higher than in the dextrin extracts. The glucose seemed however only to influence the activity of the extract when the extract is made at a low temperature. A cold water extract of the pyloric mucous membrane will sometimes manifest exciting power though on taking an average of several experiments such extracts are uniformly less potent than such as are made in glucose. I have never observed a marked reaction to the injected extract if made in cold water alone, whilst if made in glucose the percentage of acid in the N.S.S. recovered from the stomach may reach practically .1 p.c.

An illustrative experiment is recorded.

Cat, large, fasting for 24 hours. Washed out stomach with N.S.S., which showed a slight bile stain. Anæsthesia A.C.E. only. Connected stomach with reservoir and passed in slowly 86 c.c. N.S.S.

- 2.30. 1st injection of 3 c.c. of *simple glucose 5 p.c.* into jugular vein.
Six injections of the same amount made at intervals of 10 mins.
- 3.25. Disconnected and removed liquid from stomach = *A*. Reconnected and refilled stomach to 83 c.c.
- 3.30. 1st injection of *extract of pyloric mucous membrane in 5 p.c. dextrin* into jugular vein. Repeated injections at 3.40, 3.50, 4.0, and 4.10.
- 4.15. Disconnected and removed liquid from stomach = *B*. (92 c.c.)
A was neutral to litmus (neutral). *B* was strongly acid to litmus, and gave a marked Günzberg's reaction. When titrated against centinormal sodium hydrate it was found that 10 c.c. *B* = 23 c.c. $\frac{\text{NaOH}}{100}$, i.e. that *B* = '084 p.c. HCl.
- As regards the peptic activity of *A* and *B*, tubes containing carmine fibrin showed no change with *A* after 3 days in the cold, *B* was completely digested in the cold after 18 hours.

Experiments were also made with extracts of the fundus mucous membrane. No secretion was observable. A marked depression frequently leading to the death of the animal always occurred after injection of fundus extracts. Some depression occurs after pyloric extracts, but this is less marked. Records of the blood pressure in the different cases are shown later.

Boiled extract of pyloric mucous membrane. Boiling the pounded mucous membrane with glucose or simply with water leads to a far more active extract being formed. In these cases there seems to be no advantage in using glucose or dextrin as a vehicle, though they appear to have influence when the extract is made in the cold. Moreover the mucous membrane of a corresponding part of the stomach of another animal of different species seems to be practically as efficacious as that of an animal of the same variety as the subject of the experiment.

Illustrative experiment.

Extract was made by boiling the pyloric mucous membrane of the pig in glucose 5 p.c. This was concentrated *in vacuo* over sulphuric acid for 24 hours.

Cat, female, in fasting condition, stomach empty, washings slightly alkaline.

- 2.55. Connected N.S.S. reservoir and passed in 20 c.c.
- 2.56. Injected into jugular vein 2 c.c. pyloric extract.
- 3.6. " " " 2.5 c.c. "
- 3.16. " " " 3 c.c. "
- 3.26. " " " 3 c.c. "
- 3.36. Disconnected from reservoir and removed liquid from stomach. Liquid was strongly acid, and gave a very intense Günzberg. 10 c.c. was neutralised by 26.3 c.c. $\frac{\text{NaOH}}{100}$, and was therefore '096 p.c. HCl.

When using the gastric mucous membrane of the pig, the necessity of boiling (or adopting some more vigorous method of extraction or

conversion than simple exposure of the pounded mucous membrane to water at a temperature not greater than 40° C.) seems to be essential at any rate for the secretion of acid.

Experiments were made in which an extract of the pig's pyloric mucous membrane at 40° C. for one hour was tested as an excitant. There was never any acid secreted as the result. Though there was evidence of pepsin, the amount progressively diminished in the series of observations on any animal, independently of the nature of the extracting solution, and it was difficult therefore to regard this as anything else than passive extraction of pepsin from the mucous membrane of the observed animal.

Two explanations of these negative results presented themselves. Either the active substance was not extractable by simple tap-water at a low temperature, or else a precursor of the substance passed into solution which was not converted into the active substance under the conditions permitted. To this point I recur later.

Boiled extracts of different regions of pig's stomach. This afforded an opportunity of comparing the reaction at one time of extracts of different regions, and especially of noting whether the true cardiac region which is well developed in the pig's stomach acted similarly to the pyloric and differently to the fundus region. The results showed that cardiac extracts were much the same as the pyloric as regards potency, the fundus region being as in other experiments inert.

The extracts were made in boiling 5 p.c. glucose.

Cat, fasting, washings of stomach neutral.

- 2.45. Connected with N.S.S. reservoir and introduced 20 c.c.
 2.46. Injected 2.5 c.c. extract of *true cardiac region*.
 2.56. " 3 c.c. " " "
 3.6. " 3.5 c.c. " " "
 3.16. Removed liquid from stomach = A.
 Stomach washed till washings faintly alkaline. Re-established connection with reservoir and passed in 20 c.c. N.S.S.
 3.20. Injected 3 c.c. extract of *pyloric region*.
 3.30. " " " "
 3.40. " " " "
 3.50. Removed liquid from stomach = B.
 Again washed out stomach till alkaline. Re-established connection with reservoir and passed in 20 c.c. N.S.S.
 3.57. Injected 3 c.c. extract of *fundus region*, and at 4.7 and 4.17.
 4.27. Removed liquid from stomach = C.
 Re-established connection with reservoir and passed in 20 c.c. N.S.S.
 4.28. Injected 3 c.c. extract of *pyloric region* again, and at 4.38 and 4.48.
 4.58. Removed liquid from stomach = D.

Comparison of the liquids :

	Litmus	Günzberg's test	Percentage of acid
A.	Strongly acid	Marked	·0146 p.c. HCl
B.	Strongly acid	Marked	·0146 p.c. HCl
C.	Neutral	None	—
D.	Acid	Distinct but small	·0057 p.c. HCl

It must be pointed out that some exhaustion in the process of secretion is frequently seen. Hence the injection of fundus extract producing no result might be explained as the result of exhaustion. But the subsequent evoking of further secretion by pyloric extract forbids this. In this experiment the reaction was very small all through, but it clearly exhibited the different results which always obtain with the different extracts.

Peptone and peptone extracts of gastric mucous membrane. Injecting 'peptone' alone, either Witte's peptone or a meat extract such as Darby's fluid-meat, which contains extractives as well results, unlike glucose, in some secretion. But a 'peptone' extract of mucous membrane has more power as an excitant than the peptone alone. It seems however that a boiled extract in peptone has not necessarily any superior power to one made in glucose.

A comparison of the effects of different forms of peptone was shown by taking in order 1. Witte's peptone alone. 2. A neutral Witte's peptone extract of cardiac mucous membrane of pig. 3. An acid Witte's peptone extract of cardiac mucous membrane afterwards neutralised. 4. The liquid resulting from digesting the cardiac mucous membrane. 5. A glucose extract of mixed pyloric and cardiac mucous membrane. As regards efficiency as excitants of gastric secretion the glucose extract was first (but this included some pyloric mucous membrane), next came, approximately equal, the neutral and acid Witte's peptone extracts, and last and again approximately equal, the digestion extract and Witte's peptone alone.

It is to be noted that in a protracted experiment, using one form of excitant only, that taking periods of about half-an-hour, the secretion gradually tends to lessen in percentage of HCl. This is especially the case, if in the commencing periods the secretion is at all excessive.

The effectiveness of different regions of the stomach when extracted in peptone was also examined. In one experiment in which the extracts were made in Darby's fluid-meat with ·2 p.c. hydrochloric acid, (boiled, neutralised, and filtered), it was found that the fluid-meat alone produced a secretion with the acid = ·013 p.c. HCl, the cardiac extract

caused secretion with acid = $\cdot 027$ HCl, the fundus extract caused no secretion, the subsequent injection of pyloric extract caused about $\cdot 018$ p.c. HCl. These extracts were used successively on the same animal with the above results.

Glycerine Extracts. If the mucous membrane be pounded up and left in glycerine for some days, the glycerine will be found to have extracted to some degree the exciting substance. Glycerine extracts of the pyloric mucous membrane and the fundus mucous membrane of the pig's stomach were prepared.

In this case, as would be expected, the pyloric extract reacted markedly, the fundus extract showed a minimal reaction, there being evidence of a trace of mineral acid, though it could not be detected by Günzberg's reaction. It is possible that the separation of the fundus mucous membrane had not been carried out quite accurately, as this was the only case in which a vestige of reaction was shown by an extract of the fundus mucous membrane.

Acid Extracts. Extracts were made both of the pyloric and fundus mucous membrane in $\cdot 4$ p.c. HCl (using similar quantities of mucous membrane), allowing to stand with occasional shaking for two hours, then neutralising and filtering. In these cases no reaction was shown with the fundus mucous membrane.

Fasting cat, washings from stomach clean.

- 2.30. Connected stomach with reservoir and passed in 20 c.c. N.S.S.
Injections of 2.5 c.c. *fundus extract* at intervals of 5 mins.
- 3.3. Removed liquid from stomach = *A*. *A* found to be slightly alkaline.
- 3.6. Reconnected with reservoir and passed into stomach 20 c.c. N.S.S.
3 injections of 2.5 c.c. *pyloric extract* at intervals of 5 mins.
- 3.36. Removed liquid from stomach = *B*. *B* gives marked Günzberg's reaction, and percentage of acid = $\cdot 033$ HCl.

Relation of acid and pepsin in secretion. In most cases the peptic activity of the liquid removed from the stomach was tested in addition to the percentage of acid. Generally speaking the development of acid and pepsin run on similar lines. But this is not invariable, perhaps owing to individual idiosyncrasy, and in some cases the only reaction to the injection of extracts is shown by variations in the quantity of ferment exhibited by the different samples of liquid removed.

Duration of the secretion. It is difficult with the method adopted to state how soon after the first injection the secretion is first evident. In

an animal that reacts well after a period of ten minutes the liquid in the stomach may become perceptibly acid. In an animal which has been subjected to injections at intervals of five minutes, it is found that after the expiration of ten minutes from the last injection, secretion may still be continuing, but after a further period of ten minutes has elapsed, *i.e.* twenty minutes altogether from the last injection, no acid reaction in 20 c.c. of normal saline solution placed in the stomach and remaining there for ten minutes is shown.

Influence of atropin. Experiments were made to test whether the secretion brought about by extracts of pyloric mucous membrane was affected by the animal being deeply under the influence of atropin. The atropin in no way diminished the reaction of the animal, if anything, the progressive exhaustion of reaction to the injection seemed to be diminished. It seems probable therefore that the effects resulting from the injections are not to be explained by any excitation of a local nervous mechanism, but to the direct action of the active substance upon the protoplasm of the secreting cells.

Extracts made from cat's pyloric mucous membrane in .4 p.c. HCl for 1 hour, then neutralised and filtered. The neutralisation involved rather considerable dilution.

Fasting cat, washings from stomach clean and neutral.

11.30. Connected stomach with reservoir and passed in 20 c.c. N.S.S.

11.35. 1st injection of 2.5 c.c. *pyloric extract* into jugular vein. Repeated at 11.40 and 11.45.

11.50. Injected 2.5 c.c. of 1 p.c. solution of atropin sulphate dissolved in N.S.S., subcutaneously, half into each leg = .025 grs. atropin sulphate.

12.5. Removed liquid from stomach = *A*. *A* is acid to litmus and gives marked Günzberg's reaction. Percentage of acid = .036 HCl.

Washed out stomach, reconnected and passed in 20 c.c. N.S.S.

12.9. 1st injection of 2.5 c.c. same pyloric extract. Repeated at 12.14 and 12.19.

12.39. Removed liquid from stomach = *B*. *B* is acid to litmus, gives marked Günzberg's reaction. Percentage of acid = .043 HCl.

The activation of extracts of pyloric mucous membrane. It was pointed out earlier in this paper, that a simple watery extract of the pig's pyloric mucous membrane, made at a temperature not exceeding 40° C., was inactive, but an extract made with boiling water possessed marked power as an excitant. In the case of the cat's pyloric mucous membrane the exciting substance seems to be less stable and not infrequently a cold water extract exhibits a small amount of activity. This suggests that boiling leads to a more perfect extraction or a conversion of a precursor of the exciting substance, itself inactive, into the completely developed active substance. Extracts made with

hydrochloric acid similarly are markedly active. Experiments were made to see if the negative effect of an inert extract was due to the fact that the extract contained such precursor in the inert state, but capable of being activated by treatment with acid. The results showed consistently that an inert extract could be made active by such treatment with acid.

The pyloric mucous membrane of a cat was scraped, pounded with sand and left to stand with occasional shaking for 4 hours in 25 c.c. tap-water. Then filtered, and two parts each of 10 c.c. were taken.

H = 10 c.c. watery extract + HCl to bring mixture to .35 p.c., placed in incubator at 35° C. for 20 mins. then neutralised with NaOH.

P = 10 c.c. watery extract + a mixture of the quantities of HCl and NaOH added above.

The salts were the same therefore in both and there was similar dilution.

Fasting cat, washings of stomach clean, 20 c.c. of N.S.S. introduced into stomach.

4.1. 1st injection of *simple watery extract*, repeat at 4.6 and 4.11.

4.32. Removed liquid from stomach = *A*. *A* is neutral.

4.34. Reconnected with reservoir and passed in 20 c.c. N.S.S.

1st injection of *extract treated with HCl*. Repeat at 4.39 and 4.44.

5.4. Removed liquid from stomach = *B*. *B* is acid and gives marked Günzberg's reaction.

Acid = .032 p.c. HCl.

The effect of glucose in activating an extract was also tested. It was found that the activity of a poorly exciting watery extract could be increased, a more vigorously exciting extract was not improved. The results in the direction of activation were however much less conspicuous with glucose than HCl.

Finally the effect of HCl upon a boiled extract was tested. In this case no increase of power was exhibited, showing that the boiling did not simply plentifully extract the mother substance, but also completely converted it into the active excitant.

Extract made by boiling in tap-water the pounded pyloric mucous membrane of a cat's stomach. Then filtered.

H = 10 c.c. extract + 3 c.c. HCl 1 p.c. for 1 hour, then neutralised with NaOH.

P = 10 c.c. extract + mixture of 3 c.c. HCl 1 p.c. and NaOH to neutralise.

Extracts were therefore similarly diluted and contained same amount of salts.

Cat, fasting, stomach washings clean. Introduced 25 c.c. N.S.S. into stomach.

12.0. 1st injection of *simple boiled extract*. (*P*) Repeat 12.5 and 12.10.

12.30. Removed liquid from stomach = *A*. *A* strongly acid, intense Günzberg reaction.

Acid = .164 p.c. HCl.

Stomach washed out and reconnected with reservoir and passed in 25 c.c. N.S.S.

12.35. 1st injection of boiled extract treated with HCl. (*H*) Repeat 12.40 and 12.45.

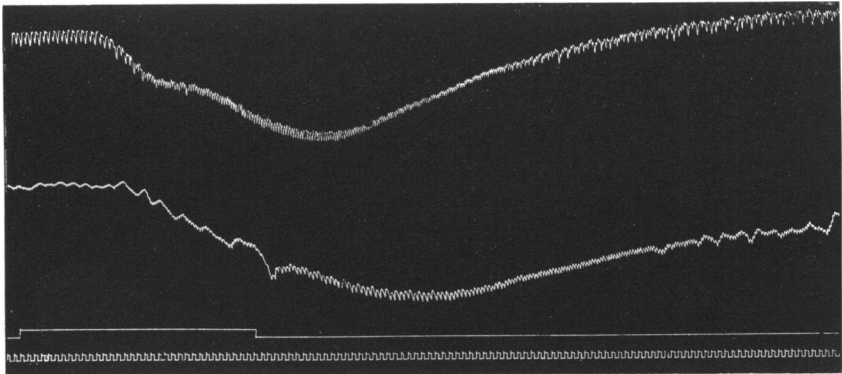
1.5. Removed liquid from stomach = *B*. *B* very acid, very intense Günzberg's reaction.

Acid = .125 p.c. HCl.

In this case in the second stage the percentage of acid was rather

less than in the first. With the considerable reaction that the animal showed, this was probably due to some exhaustion and it seems improbable that there was much difference between the activities of the two extracts.

The effect of the extracts on the blood-pressure. As was found by Bayliss and Starling with pancreatic secretion, extracts made in a manner similar to those in the preceding experiments show a marked influence upon blood-pressure, by means of some depressant substance being simultaneously extracted. The only point of interest in my experiments in this connection is that extracts made from the fundus mucous membrane show this effect more markedly than do extracts from the pyloric. This was not infrequently the cause of premature death of the animal when fundus extract was injected. It seemed also that extracts made in normal saline solution contained much greater quantities of the depressant than when made in simple tap-water. This led me early to give up using normal saline as the vehicle of extraction. The two tracings appended show the difference between pyloric and fundus extracts in this respect.



Upper tracing, blood-pressure in carotid after injection of pyloric extract. Lower tracing, a record taken later in same animal after injection of fundus extract.

SUMMARY OF RESULTS.

I. Extracts made of the pyloric mucous membrane in boiling water or HCl .4 p. c. contain an active substance which on injection into the blood vessels of an animal leads to a secretion of gastric juice.

2. Extracts made in cold water, peptone, glucose or glycerine also contain variable amounts of this substance.

3. Extracts of the fundus mucous membrane, however made, do not contain this substance.

4. The inactive condition of some extracts is due to the substance being present in an undeveloped state, boiling the substance or treating with acid will lead to the complete development. This is however only the case with extracts made from the pyloric or true cardiac mucous membrane, but not the fundus mucous membrane.

5. Atropin does not diminish the reaction of an animal to this excitant.

6. The substance is not a ferment as boiling an extract leads to an increase rather than a diminution of its properties.

The costs of this research have been defrayed by a grant made by the Royal Society.