XXXII. THE RELATION OF SALIVARY TO GASTRIC DIGESTION.

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INTRODUCTION.

The exact significance of the presence of ptyalin in the saliva has been a matter of dispute.

That an enzyme should be secreted in the mouth to be presently destroyed in the stomach, whilst a more efficient diastase is present in the secretion of the pancreas, is rather curious. The researches of Edkins on gastric secretin, however, show that dextrans may act as stimulants to the formation of this hormone. Since dextrin is one of the products of hydrolysis of starch resulting from the action of ptyalin, we can assign at least one valuable function to this enzyme. It occurred to me that another important function might on theoretical grounds be assigned to this ferment. Hedin [1906, 1912] has shown that charcoal and certain powders have the power of adsorbing enzymes, thereby hindering their activity. It is logical to suppose that colloidal starch solutions might adsorb pepsin and hence inhibit the activity of this enzyme. Saliva by hydrolysing the starch might prevent the adsorption of pepsin by the colloidal carbohydrate and would thus aid gastric digestion. The following experiments were conducted in order to elucidate this question.

METHOD.

Pepsin was allowed to act on fibrin in the presence of either colloidal starch solutions or starchy foods and the rate of digestion compared with that which occurred after previous hydrolysis of the carbohydrate by saliva

or takadiastase. The Grützner method was used to determine the rate of digestion, a solution of carmine serving as a standard colour against which the digestion tubes were compared. The tubes were placed in a shaker (to avoid errors due to diffusion) within a thermostat at 39° C.

In the various experiments pepsin was placed in contact with carbohydrate material for some time in order that adsorption might take place; this interval of time is indicated throughout the paper by the word *contact*.

The following solutions were prepared:

- (1) A 2 per cent. starch solution.
- (2) A 2 per cent. solution of takadiastase.
- (3) 0.25 g. pepsin (Merck) dissolved in 100 cc. of 0.3 per cent. HCl.

The diastase solution was divided into two equal portions, one of which was boiled to destroy the ferment. Saliva was used in many experiments.

Exp. 1. A. 4 cc. unhydrolysed starch + 1 cc. pepsin HCl.

B. 4 cc. hydrolysed starch + 1 cc. pepsin HCl.

Contact 150 min. Equal quantities of fibrin were now added.

Fibrin digestion. *A* required 13 min.; *B* required 55 min.

There is evidently a marked difference in the activity of the pepsin after being in contact with colloidal starch.

In this experiment the contact was 150 min., which will be shown in the next experiment to be unnecessarily long.

Exp. 2. Boiled Starch.

A. 1 cc. pepsin HCl + 4 cc. hydrolysed starch.

B. 1 cc. pepsin HCl + 4 cc. unhydrolysed starch.

Unboiled Starch.

C. 1 cc. pepsin HCl + 4 cc. (starch + diastase).

D. 1 cc. pepsin HCl + 4 cc. (starch + boiled diastase).

Contact 20 min. Equal quantities of fibrin were then added.

Fibrin digestion. *A* required 13 min.; *B* required 60 min.; *C* required 13 min.; *D* required 13 min.

It is evident that:

- (a) Adsorption of pepsin is very marked in 20 min.
- (b) Unboiled, i.e. non-colloidal starch suspensions do not hinder peptic digestion.

Exp. 3. Soluble starch was prepared by treating pure potato starch with 7.5 per cent. HCl in thermostat at 40° until the resultant gave a clear solution

in warm water and a blue reaction with iodine. The acid was removed by washing with water to which alcohol had been added.

Erythroextrin was not specially prepared for this experiment but a dextrin supplied by Merck, giving a rich red colour with iodine, was used. Saliva was substituted for takadiastase in all further experiments—except where otherwise indicated.

The carbohydrate solutions were all 2 per cent.

- A.* Unboiled starch suspension.
- B.* Unhydrolysed starch solution.
- C.* Hydrolysed starch solution.
- D.* Soluble starch.
- E.* Erythroextrin.

3 cc. of each solution were taken and 1 cc. boiled filtered saliva added to each of tubes *A*, *B*, *D*, and *E*. 1 cc. unboiled saliva added to *C*. 1 cc. pepsin HCl added to *A*, *B*, *C*, *D*, *E*. Contact 30 min. Equal quantities of fibrin were then added.

Fibrin digestion. Time in min.

	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>
I.	10	49	10	32	10
II.	10	50	10	35	14

It is evident from these results that soluble starch causes a certain amount of adsorption, but that the power is not retained by the erythroextrin molecule. Unboiled starch, as in the previous experiment, did not exhibit any adsorptive effects.

Exp. 4. Further evidence as to the adsorption of pepsin by starch solutions is given by the following figures:

A. 1 cc. 0.25 per cent. pepsin solution + 3 cc. hydrolysed starch (2 per cent.).

B. 1 cc. 0.25 per cent. pepsin solution + 3 cc. unhydrolysed starch (2 per cent.).

Added equal quantities of fibrin to each. Contact 21 min. Removed fibrin and quickly washed with water. Added 5 cc. 0.3 per cent. HCl to *A* and to *B*.

Fibrin digestion. *A* required 15 min.; *B* required 46 min.

Another experiment showed that colloidal starch solution had little adsorptive effect upon HCl.

Exp. 5. A further test was performed to see if the presence of maltose might act as a catalyst to the action of pepsin HCl. Tubes were arranged as follows:

- A.* 4 cc. water (distilled).
- B.* 4 cc. 2 per cent. maltose.
- C.* 3 cc. water + 1 cc. saliva.

To each was added 1 cc. pepsin HCl. Contact 30 min. Equal quantities of fibrin were then added.

Fibrin digestion. *A* required 9 min.; *B* required 9 min.; *C* required 9 min.

Maltose has no catalytic effect upon pepsin HCl digestion and therefore cannot account for the difference between the rates of digestion of fibrin in the presence of unhydrolysed and hydrolysed starch.

Experimental work up to this juncture had been confined to pure starch solutions or the derivatives of starch. The experiments were next extended to certain food stuffs.

Exp. 6. Maizena. A preparation of maizena was made by adding the material to boiling water.

Tube *A.* 4 cc. maizena + 1 cc. boiled saliva.

Tube *B.* 4 cc. maizena + 1 cc. unboiled saliva.

Contact with saliva 60 min. *A* blue with iodine. *B* no colour with iodine.

1 cc. pepsin HCl added to each. Contact 20 min. Equal quantities fibrin then added.

Fibrin digestion. *A* required 30 min.; *B* required 12 min.

By the time that *A* had acquired the colour of the standard solution the fibrin in *B* had almost completely disappeared, although there was of course a considerable amount of fibrin still undigested in *A*.

Exp. 7. Rice. Boiled rice ground in a mortar. Equal weighed quantities placed in tubes *A* and *B*.

2 cc. boiled saliva added to *A*.

2 cc. unboiled saliva added to *B*.

Contact with saliva 60 min.

Added to each 1 cc. pepsin HCl. Contact 35 min. Fibrin was then added.

Fibrin digestion. *A* required 37 min.; *B* required 17 min.

Microscopical examination of material from tubes *A* and *B* after one hour's contact with saliva showed the following appearances:

A. Particles of comparatively massive appearance (as compared with those in *B*), staining darkly with iodine.

B. Particles of feathery disintegrated nature, giving a very slight stain or none whatever with iodine.

Exp. 8a. Potato. Potato boiled for three-quarters of an hour. Equal weighed quantities taken.

A. Potato + 2 cc. boiled saliva.

B. Potato + 2 cc. unboiled saliva.

Contact with saliva 45 min.

Added 1 cc. pepsin HCl to each. Contact 23 min.

Added 2 cc. 0.3 per cent. HCl to make total volume = 5 cc. and then added fibrin.

Fibrin digestion. *A* required 45 min.; *B* required 35 min.

Microscopical examination of iodine stained specimens of *A* and *B* showed that colloidal starch was still present within the cells of *A*, whereas it had been dissolved completely out of the cells of *B*.

Exp. 8b. The experiment was repeated using potato which had been boiled for two hours and had a very mashed appearance. Iodine stained specimens showed much cell debris. The cell contents had disappeared in many cases, leaving the skeleton of cellulose unstained.

Equal quantities *A* and *B* were taken as before and treated in exactly the same manner as in the previous experiment except that the contact with pepsin HCl was 20 min.

Fibrin digestion. *A* required 42 min.; *B* required 30 min.

It will be noted that potato exercises a comparatively small inhibitory effect upon peptic digestion.

This is probably accounted for by the fact that much of the colloidal starch still remains enclosed within the potato cells and hence does not exert an adsorptive effect upon pepsin.

Exp. 9. Porridge. Porridge ground in mortar to uniform consistency. Equal weighed quantities taken.

A. Porridge + 2 cc. boiled saliva.

B. Porridge + 2 cc. unboiled saliva.

Contact with saliva 120 min.

Added 1 cc. pepsin HCl. Contact 15 min.

Added 2 cc. 0.3 per cent. HCl to make total volume 5 cc., and then added fibrin.

Fibrin digestion. *A* required 29 min.; *B* required 10 min.

Treatment with iodine and microscopical examination showed that the starch had completely disappeared from *B*, whereas it was abundant in *A*. Disintegrated cells were present in both preparations.

Exp. 10. Bread. Bread was finely ground in a mortar. Equal weighed quantities taken and treated exactly as in Experiment 9, with the exception that salivary digestion occupied 70 min. and the contact with pepsin HCl was 30 min.

Fibrin digestion. *A* required 45 min.; *B* required 28 min.

Exp. 11. Gum Acacia. A 4 per cent. solution of gum acacia in water was made.

A. 3 cc. gum acacia + 1 cc. boiled saliva.

B. 3 cc. gum acacia + 1 cc. unboiled saliva.

Contact with saliva 75 min.

Added 1 cc. pepsin HCl. Contact 30 min. Fibrin was then added.

Fibrin digestion. After 65 min. there was equality in the liberation of colour in *A* and *B*, but both tubes were far below the standard tube in depth of colour.

The experiment was repeated with the following modifications:

A. 3 cc. gum acacia + 1 cc. saliva + 1 cc. pepsin HCl. Contact 35 min. Added fibrin.

B. 1 cc. pepsin at 39° for 35 min. Then added fibrin, followed immediately by 3 cc. gum acacia + 1 cc. saliva.

C. 1 cc. pepsin kept at 39° for 35 min. Then added 3 cc. gum acacia + 1 cc. saliva followed immediately by fibrin.

Fibrin digestion. After 90 min. there was equal liberation of colour in the three tubes, but still far from the standard colour tube. It appears that saliva does not alter the adsorptive power of gum acacia, and further that this adsorption is very complete and takes place almost instantaneously.

Exp. 12. Biscuit. A plain starchy biscuit was powdered in a mortar and equal quantities weighed out.

Microscopical examination of an iodine stained specimen revealed the fact that most of the starch grains were unburst. Equal quantities of taka-diastase (2 per cent. solution) boiled and unboiled respectively were added to tubes *A* and *B*. Contact with diastase 30 min.

No appreciable change—discernible to the eye—had taken place at the

end of this interval, and the enzyme was therefore allowed to act all night—4.25 p.m. to 9.30 a.m.

1 cc. pepsin HCl added to *A* and *B*. Contact 20 min. Fibrin was then added.

Fibrin digestion. *A* required 47 min.; *B* required 32 min.

Owing to the large amount of material undissolved which modified the colour of the liberated carmine, it was difficult to satisfy oneself as to the exact end point.

Starch grains were abundant in *A*, even after prolonged contact with diastase.

It is interesting to note in connection with the foregoing experiments that in the cat, dog, horse, sheep and ox the saliva is non-amylolytic. The last three animals consume large quantities of starchy food stuffs. The starch grains however are intact—unless the food has been artificially prepared by cooking, steaming, etc.—and do not form colloidal solutions and hence do not hinder gastric digestion.

CONCLUSIONS.

1. Peptic digestion is delayed in the presence of colloidal starch solutions through adsorption of enzyme, the time interval being increased four-fold in the presence of 2 per cent. starch solution.

2. There is a stage in the disruption of the starch molecule at which the phenomenon of adsorption of pepsin is lost. This occurs between the amylo-dextrin and erythro-dextrin stage.

3. Unboiled starch does not hinder the action of pepsin, a factor of importance in the dietetics of herbivora.

4. Cooked farinaceous foods—rice, potato, bread, porridge, maizena—all hinder peptic digestion if not first subjected to salivary digestion.

5. Gum acacia causes marked inhibition of peptic activity, and this is not prevented by previous contact with saliva.

6. The saliva of man, in virtue of its ptyalin, plays a considerable part in aiding gastric digestion by hydrolysing colloidal starch, which would otherwise adsorb pepsin.

REFERENCES.

- Hedin (1906), *Biochem. J.* 1, 484.
Hedin (1912), *Zeitsch. physiol. Chem.* 82, 175.