XXXI. THE INFLUENCE OF DIFFERENT SUBSTANCES ON THE DIASTATIC ACTIVITY OF SALIVA.

By HILDA WALKER.

From the Department of Physiology, University of Birmingham.

(Received February 20th, 1925.)

THIS research was undertaken to determine the action of sweet substances on the diastatic activity of saliva, both as regards the secretion of ptyalin, and its activity when produced.

Recent work, which has a bearing on the subject, is as follows.

Iljin [1922] has found that salts accelerate the production of diastatic enzymes, probably by hastening the transition from the inactive zymogen to the active zymase. Rockwood [1918] showed that the ammonium salts of inorganic acids increased the activity of ptyalin, as did also those of the stronger organic acids. Groll [1918] showed that anions exert a greater influence than cations. The halogen ions increase and those of the sulphates and nitrates inhibit the activity of ptyalin. Ambard [1923] has found that starch adsorbs a definite amount of salivary amylase. Neilson and Lewis [1908] determined the effect of diet on the amylolytic power of saliva. They found that after feeding for some days on a carbohydrate diet the amylolytic power is increased, whilst it is decreased on a protein diet. Sanctis-Monaldi [1920] found that the secretion of parotid saliva was increased by various stimuli, such as bread, meat and acid, but its amylolytic power remained unchanged.

Method. Various substances were put into the mouth and rolled between the tongue and palate, and the activity of the saliva so secreted was compared with that of the saliva previously secreted in the empty mouth. The saliva was as a rule collected between 10 and 11 a.m., breakfast having been taken about 8 or 8.30. In each experiment the mouth was rinsed out three times with tap water and three drinks of water were taken; the saliva then collected was called *N*. After its collection the same procedure was gone through; the substance to be tested was put into the mouth and the saliva so produced called *S*. In each case the saliva was collected into a dry dish. 1 cc. was transferred to a 100 cc. flask, which was then filled to the mark with distilled water. The liquid was then filtered. Throughout the experiment the flasks, dishes and test-tubes were boiled before use.

The estimation of the diastatic activity was carried out by a modification of Wohlgemuth's method.

In a series of twelve test-tubes the diluted filtered saliva was added to a 0.9 % solution of sodium chloride according to the following table, each test-tube thus containing 1 cc. of liquid.

Test-tube	cc. diluted saliva	cc. 0.9 % NaCl
1	0.35	0.65
2	0.33	0.67
3	0.31	0.69
4	0.29	0.71
5	0.27	0.73
6	0.25	0.75
7	0.23	0.77
8	0.21	0.79
9	0.19	0.81
10	0.17	0.83
11	0.15	0.85
12	0.13	0.87

To each tube was then added 2 cc. of 0.1 % solution of starch. Soluble starch was used; the solution was free from chloride and neutral to litmus.

The set of tubes was then put into an incubator for 30 minutes. The temperature of incubation was as a rule 38°, but in various experiments there were slight variations above and below this.

After incubation the tubes were cooled by transference for 3 minutes to water at the temperature of the laboratory. To each tube was then added one drop of a *N*/50 solution of iodine.

Instead of taking the achromic point as a measure of diastatic activity it was found more convenient to make a series of standard colours ranging from blue through purple, red-purple, red-brown to yellow, and by comparison of the experimental colours with those of a standard, to plot a graph for the diastatic activity. The preparation of the standard colours was difficult, as it was not easy to match the exact tint, and when this was done, often after a few days it was found that a precipitate had appeared, or that the colour had faded. Mixtures of the following solutions were found to be most satisfactory: methylene blue, Uffelmann's reagent, magenta, carbol fuchsin and Bismarck brown. The exact strength and proportion of each solution cannot be given, as in each case it was a matter of repeated trial, adding first a little of one and then a little of another until a match was obtained to the colours produced by iodine on partially digested starch at different stages.

The colours were as follows:

- A* = Blue ≡ no digestion.
- B* = Blue with a tinge of purple.
- C* = Purple.
- D* = Red-purple.
- E* = Magenta.
- F* = Red.
- G* = Pale red-brown.
- H* = Pale red-brown, but more brown and less red than *G*.
- I* = Pale brown with a tinge of red.
- J* = Paler brown with a tinge of red.
- K* = Yellow-brown.
- L* = Yellow ≡ complete digestion.

Subject H. W.
Graph 1.
Cane Sugar

I. J.
Graph 2.
Cane Sugar

H. W.
Graph 3.
Normal Saliva made up with
10 per cent. Cane Sugar

H. W.
Graph 4.
Maltose

W. M.
Graph 5.
Maltose

H. W.
Graph 6.
Lactose

D. P.
Graph 7.
Lactose

H. W.
Graph 8.
Lactose

H. W.
Graph 9.
Lactose

H. W.
Graph 10.
Glucose

H. W.
Graph 11.
Glucose, Cane Sugar

H. W.
Graph 12.
Fructose

H. W.
Graph 13.
Galactose

H. W.
Graph 14.
Glycerol

H. W.
Graph 15.
Saccharin

E. W. C.
Graph 16.
Saccharin

The numbers on the base lines are those of the series of tubes.

Since the experiments were comparative no determinations of the p_H were made. It is assumed that in each experiment it was the same for the normal and stimulated saliva, as the substances used were as far as possible pure. In any case the dilution was so great that a slight variation in the p_H would make very little difference.

In graph 1 it will be seen that the starch in the tube containing the smallest amount of normal saliva has undergone no digestion, while the corresponding tube of saliva after cane sugar shows considerable digestion. In each series the amount of digestion increases with increasing quantities of saliva, but in the *S* set digestion is complete in tube 7 and the following tubes, and presumably with a larger quantity of starch the curve would continue its upward direction, while in the *N* set, tube 2 with greatest digestion is only equivalent to tube 11 of the *S* set. A similar result is seen in graph 2.

From these graphs it is seen that cane sugar in the mouth has a very definite accelerator action on the saliva produced.

In order to determine whether this increase in activity was due to an increase in the quantity of ptyalin, or to an activating effect of the sugar upon it when produced, some normal saliva was collected in the usual way; of this 1 cc. was made up to 100 cc. with distilled water, and 1 cc. was made up to 100 cc. with 10 % cane sugar solution. The result is shown in graph 3.

From this it is seen that the presence of cane sugar increases the activity of ptyalin.

Maltose (graph 4) was found also to accelerate the action of saliva. In order to determine whether this was due to activation of the ptyalin, 1 cc. of the maltose saliva was made up to 100 cc. with 10 % maltose solution. The effect, as seen in graph 5, was to depress the action, and therefore the stimulating effect of maltose in the mouth must be due to a greater secretion of ptyalin.

The results of lactose in the mouth are shown in graphs 6 and 7; it appears to have an inhibitory action on saliva. In the next experiment 1 cc. of normal saliva was made up to 100 cc. with 10 % lactose solution, and the activity of this solution was compared with that of the normal saliva made up with saline in the usual way. The result is recorded in graph 8, from which it appears that the presence of lactose in solution enhances the activity of ptyalin. The result of a further confirmatory experiment is shown in graph 9.

Glucose in the mouth also has an inhibitory effect (graph 10).

In graph 11 the result of a comparison between normal, cane sugar, and glucose saliva is shown. The inhibitory effect of glucose was also determined by making up 1 cc. of the cane sugar saliva to 100 cc. with 10 % glucose solution. The graph shows the very marked inhibitory action of glucose.

Fructose in the mouth has a depressing effect, and that this is due mainly, if not entirely, to an inhibition of the ptyalin is seen from graph 12, which shows the result of making up 1 cc. of normal saliva with 10 % fructose solution, in which case there is practically no digestion at all.

Galactose seems to have very little effect, though its presence in solution slightly increases the activity of ptyalin (graph 13).

Glycerol (graph 14) in the mouth has a retarding influence. Saccharin, of which the sodium salt was used, has an activating effect (graphs 15 and 16).

SUMMARY.

From these experiments it is apparent that when various substances are taken into the mouth two effects have to be considered:

(1) an increase or decrease in the quantity, or possibly the quality, of the ptyalin secreted,

(2) a stimulating or depressing action on the enzyme produced by the substance being tested.

Of the substances examined, cane sugar and saccharin in the mouth appear to have the most marked effect in producing a very active saliva. In the case of cane sugar this is largely, if not entirely, due to its stimulating action on the ptyalin.

The behaviour of maltose and lactose is interesting; while maltose increases the quantity of ptyalin produced, its presence depresses the activity of the enzyme. Lactose, on the other hand, tends to inhibit the production of ptyalin, but enhances its activity.

Of the monosaccharides glucose and fructose both lower the activity of saliva, and in each case it is largely due to the depressing action of the sugar on ptyalin.

Further investigations on the same lines are proposed.

My best thanks are due to Professor Carlier (at whose suggestion the work was begun) for much helpful advice and criticism.

REFERENCES.

- Ambard (1923). *Bull. Soc. Chim. Biol.* **5**, 693.
Groll (1918). *Arch. Néerl. Physiol.* **2**, 516.
Iljin (1922). *Biochem. Z.* **132**, 511.
Neilson and Lewis (1908). *J. Biol. Chem.* **4**, 501.
Rockwood (1918). *J. Biol. Chem.* **33**, ix.
Sanctis-Monaldi (1920). *Arch. Fisiol.* **18**, 168.