to stand in the air a few minutes, a pink color will be given by phenolphthalein, after the loss of hydrogen sulfide.

Details of Determinations.—The procedure was followed exactly as given. The acids were all pure samples of tested melting point.

Stearic acid was found not to set free any hydrogen sulfide from the solution:

Crotonic acid. Calc. COOH = 52.33%.
Found, 50.93, 51.20, 51.20. Av. 51.11%. Av. × 100/calc. = 97.67.
Anisic acid. Calc. COOH = 29.60%.
Found, 28.99, 28.78, 28.90. Av. = 28.89%. Av. × 100/calc. = 97.60.
Benzoic acid. Calc. COOH = 36.89%.
Found, 35.95, 36.01, 36.05. Av. = 36.00%. Av. × 100/ calc. = 97.59.
Salicylic acid. Calc. COOH = 32.61%.
Found, 31.85, 32.07, 31.83. Av. = 31.92. Av. × 100/calc. = 97.88.
Tartaric acid. Calc. COOH = 60.00%.
Found, 60.67, 60.28, 60.53, 60.65. Av. = 60.47. Av. × 100/calc. = 100.79.
Maleic acid. Calc. COOH = 75.85%.
Found, 76.14, 76.56, 76.33. Av. = 76.34. Av. × 100/calc. = 100.6.

Student's Series.—These were carried out by a student absolutely unfamiliar with the method. The first four determinations are given, and the first is excluded from the average, as a practice determination:

Anisic acid. Calc. COOH = 29.60%.
Found, 27.54, 28.26, 29.01, 28.62. Av. = 28.62. Av. × 100/calc. = 97.74.

Old Apparatus.

For comparison may be taken two sets of results obtained with the old apparatus:

1. By a student unacquainted with either apparatus:
Salicylic acid. Calc. COOH = 32.61%.
Found, 21.74, 20.66, 22.90. Av. = 21.77. Av. × 100/calc. = 66.76.
2. *Cantharic acid.*[1] Calc. COOH = 34.18%.
Found, 30.26, 32.3, 28.56. Av. = 30.37. Av. × 100/calc. = 88.85.

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STUDIES ON WATER DRINKING. XIV. THE DIGESTIVE EFFICIENCY OF SALIVA AS INCREASED BY DILUTION WITH WATER.

BY OLAF BERGEIM AND P. B. HAWK.

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Many investigations have been made on the influence of variations in the concentration of enzymes, of their substrates, and of electrolytes, on the rate of the reaction. Very little work has, however, been done directly on the effect of simultaneous decreases of all three of these, that is, of dilution. For example in the case of the amylases, as was first

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[1] Hans Meyer and Krczmar, *Monatsh.*, 19, 715 (1898).

shown by Kjeldahl,[1] the rate of hydrolysis of starch is directly proportional to the amount of enzyme as long as the substrate is present in considerable excess, or until the digestion has proceeded from 30 to 40% of the way to completion. After this point is reached the rate becomes gradually slower, assuming the form of a logarithmic curve, but no expression for the rate of the reaction has been yet proposed which holds true in all cases, probably on account of the variety of factors which influence this rate. As to the effect of varying the concentration of the substrate, the rate of hydrolysis seems to be directly proportional to the amount of substrate as long as the enzyme concentration is not too small compared with that of the substrate, after which the change is practically the same irrespective of the amount of substrate. For electrolytes which may favor the reaction it has been shown that the optimum concentration depends largely upon the concentration of the enzyme and the substrate, but these remaining constant, the rate of hydrolysis will decrease if the concentration of the electrolyte is shifted in either direction from the optimum, there being, of course, no law for the rate of this change which will hold for all electrolytes.

In the case of dilution, properly so called, *i. e.*, where only the amount of water is varied, the matter is more complicated and becomes still more so if the diluent itself contains ingredients which affect the rate of the reaction. It seems obvious that what the effect of the dilution will be must depend upon the initial concentration of the enzyme, substrate, and electrolytes. For example, if the enzyme and substrate are present in high concentrations, dilution will increase the rate of the hydrolysis by decreasing the concentration of the products of the reaction.

Bradley[2] in his work upon lipase found that when water was present to the extent of 50% of the mixture of lipase and ester, the amount of reversion was negligible, and the reaction proceeded to complete hydrolysis of the ester. In the case of starch the reaction ordinarily proceeds to 80 to 85% maltose, apparently reaching an equilibrium. The reaction can be brought to completion by removing the maltose formed by fermentation or by dialysis. This is not necessarily equivalent to dilution, as in the latter case the products of the reaction are not removed but merely diluted and we must consider at the same time the effect of the simultaneous dilution of enzyme and substrate and electrolytes. If a salt such as sodium chloride or an acid such as hydrochloric is present in a quantity such as to inhibit the reaction, dilution will tend to decrease this inhibition to an optimum point, after which further dilution will tend to decrease the rate of hydrolysis. This is particularly true because, as

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[1] *Meddel. f. Carlsberg Lab.*, 1879; *Abst. Maly Jahr.*, 9, 381.

[2] *J. Biol. Chem.*, 8, 251 (1910).

Paschutin[1] has shown, if neutralized saliva is diluted with water in varying ing proportions, a content of acid, which in undiluted saliva would cause a diminution of diastatic action, in the more dilute solutions actually aids the diastatic process. Similar deductions have been made by Chittenden and Griswold,[2] Vernon,[3] Schierbeck,[4] and Cole.[5] On the other hand, Kübel[6] has shown that the more concentrated the starch solution the less inhibition will be caused by a given amount of sodium chloride and the more of this salt will be required for optimum conditions.

From these considerations it will be apparent that whether dilution increases or decreases the action in a given case will depend upon which of the component factors predominates. On account of the complexity of the problem, prediction from theoretical considerations is not very safe and the only logical way to arrive at a decision is by systematic experimentation.

The object of the present study was to carry out some such experiments upon salivary amylase.

In Fischer's "Physiology of Alimentation," page 104, occurs the following statement: "It has been shown by the work of Cannon that the saliva does not act best in the concentration in which it is poured out upon the food, but when diluted with about three times its bulk of water." When taken into consideration in connection with the water drinking studies in progress in our laboratory this supposed finding of Cannon's was of particular interest. We therefore wrote to Fischer requesting the reference to the original article. Much to our disappointment Fischer informed us that the statement as to the digestive action of diluted saliva had resulted from a misreading of certain of Cannon's conclusions. Inasmuch as experiments[7] previously reported by us had shown a more satisfactory digestion and utilization of carbohydrates when large volumes of water were taken at meal time, it seemed entirely possible to us that a part of this increased carbohydrate digestive and absorptive efficiency might be due to the fact that the concentration of the saliva had been lowered through the medium of the high water ingestion. That such increased efficiency of the salivary function was not essential to the better utilization of ingested carbohydrates was fully appreciated inasmuch as certain of our experiments[8] had shown that copious water drinking brought about a stimulation of the pancreatic function. This augmented flow

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[1] *Arch. Anat. und Physiol.*, 305 (1871).

[2] *Amer. Chem. J.*, 3, 305 (1881).

[3] *J. Physiol.*, 27, 174 (1902).

[4] *Skand. Arch. Physiol.*, 3, 344 (1892).

[5] *J. Physiol.*, 30, 202 (1903).

[6] *Arch. Physiol.*, 76, 276 (1899).

[7] Mattill and Hawk, THIS JOURNAL, 33, 2019 (1911).

[8] Hawk, *Archives of Internal Medicine*, 8, 382 (1911).

of pancreatic juice with its contained pancreatic amylase might well assume all responsibilities regarding the more satisfactory utilization of the carbohydrates of the food. It was nevertheless thought desirable to learn the facts regarding the digestive efficiency of diluted saliva and the experiments reported herewith were subsequently carried out.

In these experiments the action of several different diluting fluids was tested. Distilled water was used to determin the effect of a simultaneous decrease of the concentrations of all of the constituents of the mixture. A solution of 0.3% sodium chloride and 0.02% disodium hydrogen phosphate was employed to determin the effect of decreasing the concentration of enzyme and substrate while maintaining an optimum electrolyte concentration. Tap water was also used for comparison and two softened waters, prepared by mixing one part of lime water and five parts of tap water, were tested in some cases. One of these, designated as "3–11" had remained loosely stoppered for several months, while the one designated as "9–11" was prepared shortly before beginning these experiments. Kahlbaum's soluble starch was used as the substrate. Starch pastes were always made up on the day of the experiment, as the freshly prepared pastes are much more readily digested than those which have undergone the progressive changes that Maquenne and Roux[1] have termed retrogradation and coagulation. The saliva employed was obtained by chewing pure paraffin to stimulate the flow. Fresh, filtered saliva was used in all cases.

The Effect of Dilution on the Time of Digestion of Starch to the Achromic Point.—Into each of a series of Erlenmeyer flasks, of Jena glass and carefully cleaned, varying in size from 50 to 1000 cc., were introduced, by means of a pipet, 10 cc. of a 1% starch paste, made from Kahlbaum's soluble starch. Varying amounts of various waters were then added and the contents were well mixed. Then to each one was added 1 cc. of a 10% solution of saliva in that particular water, this being added at thirty-second intervals. The contents were thoroughly mixed and allowed to stand at room temperature, being covered with watch glasses to prevent evaporation. At intervals a drop of the mixture was removed by means of a pipet and tested on a test tablet with a very dilute iodine solution. The end point was taken where the mixture first ceased to alter the pale yellow color of the iodine solution. All readings were corrected for the difference of time in adding the enzyme solution.

It will be noted from Table I and from the curves (I, II, III and IV) plotted using these data (see Fig. 1):

1. Dilution with distilled water gradually increased the time of digestion for saliva until in a 1 : 5000 dilution very little action took place.

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[1] *Compt. rend.*, **140**, 1303 (1905); *Ann. chim. phys.*, [9] **369**, 179 (1906).

This is the effect that we should expect from the *change in the electrolyte concentration*, which gradually decreases.

TABLE I.—THE EFFECT OF DILUTION ON THE TIME OF DIGESTION OF STARCH TO THE ACHROMIC POINT 10 CC. 1% STARCH PASTE; 0.1 CC. SALIVA.

	Time to the achromic point.			
Total volume of mixture. Cc.	Boiled distilled water. T 27°.	0.3% NaCl; 0.02% Na_2HPO_4. T 27°.	Softened water (3–11). T 20.0°.	Recently softened water. T 17–20°.
11..........	14.0 min.	....	2 h. 38 min.	2 hours
29..........	19.5 min.	9.0 min.	3 h. 52 min.	6 hours
30..........	28.0 min.	10.0 min.	3 h. 52 min.	10 hours
40..........	50.5 min.	11.0 min.	....	10 hours
50..........	1 h. 6.0 min.	11.0 min.	3 h. 36 min.	12 hours
70..........	1 h. 27.5 min.	11.0 min.	4 h. 50 min.	15 hours
100..........	2 h. 7.0 min.	11.0 min.	4 h. 50 min.	19 hours
150..........	6 h. 30.0 min.	11.0 min.	4 h. 50 min.	21 hours
200..........	9 h. 0.0 min.	11.0 min.	6 h. 4 min.	25 hours
300..........	14 h. 0.0 min.	16.0 min.	8 h. 0 min.	30 hours
500..........	40 h. 0.0 min.	17.0 min.	9 h. 0 min.	30 hours

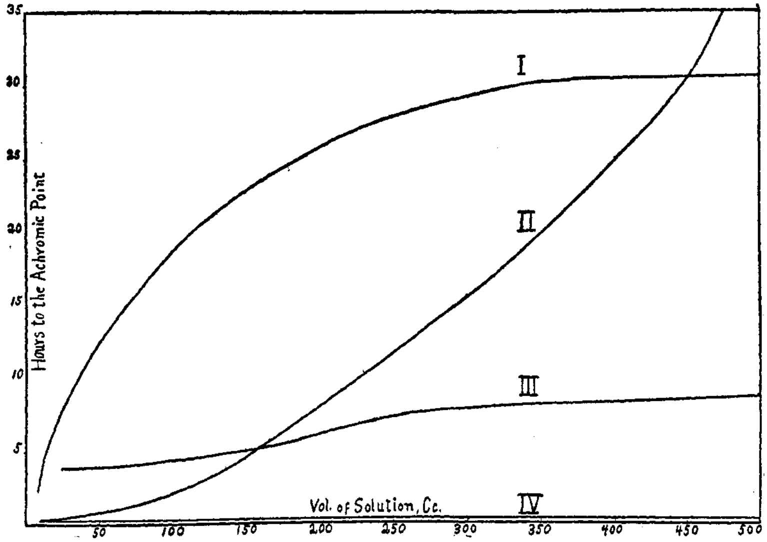

Fig. 1.—Effect of dilution on the time of digestion of starch to the achromic point by salivary amylase. I. Diluent, softened water (9–11); temp. 17–20° C.; 10 cc. 1% starch; 0.1 cc. saliva. II. Diluent, distilled water; temp. 27° C.; 10 cc. 1% starch; 0.1 cc. saliva. III. Diluent, softened water (3–11); temp. 20–24° C.; 10 cc. 1% starch; 0.1 cc. saliva. IV. Diluent, 0.3% NaCl, 0.02% Na_2HPO_4; temp. 27° C.; 10 cc. 1% starch; 0.1 cc. saliva.

2. Dilution with a sodium chloride-disodium hydrogen phosphate solution of approximately the optimum concentration for salivary digestion, by maintaining the electrolyte concentration had very slight effect on the time of hydrolysis of the starch up to a dilution of 1 : 5000.

3. Tap water, softened by the addition of one-fifth of its volume of lime water and allowed to stand for several months, evidently *contained substances inhibiting the action of* the salivary amylase so that the hydrolysis was considerably delayed. It does, however, contain the *electrolytes necessary for the enzyme action*, this fact tending to counteract the inhibition, so that in dilute solutions (1 : 1500 and over) the action was more rapid than with distilled water, which lacks the electrolyte but contains no inhibiting substance.

The principal inhibitory agent in the softened water was found to be *magnesium hydroxide*. The inhibitory relationships of softened water will be fully discussed in a later article.

4. The freshly prepared softened water evidently contains *a considerably larger amount of the inhibiting substance*, so that even in dilutions of 1 : 300 it required ten hours to reach the achromic point compared with half an hour for distilled water and ten minutes with the chloride-phosphate solution.

The Effects of Dilution with Different Waters on the Amount of Reducing Sugar Produced by a Given Amount of Salivary Amylase in a Given Time. —The study of the relative speeds of hydrolysis of starch by enzymes in varying concentrations of starch and enzyme introduce many difficulties in the way of securing strictly comparative data from experiments made at different times. Noticeable variations may be brought about by small changes in the amylolytic activity of the saliva, by very slight variations of temperature during hydrolysis, or in the determination of the reducing sugar. Also bacterial action is never entirely eliminated. For these reasons it is desirable to run at one time a series complete enough to cover the entire significant portion of the curve of the rate of hydrolysis. The following method was used to eliminate these sources of error as far as possible:

Thoroughly cleaned Jena, Erlenmeyer flasks of 200 cc. capacity and of the same shape, so as to expose equal surfaces of the contents to the air in the copper reduction method, were used throughout. Two grams of Kahlbaum's soluble starch in the form of pastes of concentrations from 2 to 20% in the particular water which was to be tested, were introduced. All starch pastes were made up immediately before use. In order to maintain the relative amounts of starch and enzyme, without increasing the total volume of the mixture above 100 cc., which would interfere with the reducing sugar determination, the amounts of starch and saliva were gradually decreased, keeping the total volume constant. The flasks were then placed in a constant temperature bath until they had acquired the temperature of the bath, and the saliva solution, previously warmed to the same temperature, was added from calibrated pipets at 30-second intervals. In no case was the actual volume of the saliva solution added less than 1 cc., so that appreciable errors in measurements were avoided. After remaining in the bath of 30 minutes the action was immediately stopped by the addition of Fehling's solution, allowance being, of course, made for the difference in time of starting the determinations. They were then transferred to a vigorously boiling water bath, the surface of the water in the bath coming to the level of the liquid within the flasks, and kept there for 15 minutes. The reduced cuprous oxide was immediately filtered on Gooch crucibles containing a quarter-inch layer of asbestos specially treated with nitric acid, caustic potash and Fehling's solution. The cuprous oxide was thoroughly washed with hot water, then with alcohol and ether, and weighed after drying to constant weight in an air bath at 110°. The above method for determining the amount of reducing sugar was adapted for the special purpose from that given by Sherman, Kendall, and Clark for the determination of diastatic activity.[1]

In some cases the modification of the copper iodide titration method as recently proposed by Kendall was used.[2] We have found this method to give good results, if the precautions suggested by Kendall are observed. Somewhat less time is required than for the gravimetric determination. In very dilute solutions some difficulty is met with in reading the exact end point, as the starch-iodine color reappears on standing a short time.

Peters[3] has recently published a careful study of the sources of error of the copper iodide method which contains many valuable suggestions.

All columns are corrected for controls and to the basis of 1 gram of starch.

From Table II and Curve V corresponding to it (see Fig. 2) we see how pronounced an inhibition was exerted on the action of salivary amylase by softened water, even after it had stood in contact with the air

TABLE II.—EFFECT OF DILUTION.

Diluent: Soft water (3–11), 9½ months old.

Time: 30 minutes. Temperature: 40°.

No.	No. cc. saliva.	Amount of starch paste.	Water to make vol.:	Mg. of maltose.	Dilution 1:
1	0.63	10 cc. of 20%	11 cc.	693.2	16
2	0.63	25 cc. of 8%	26 cc.	520.0	40
3	0.63	25 cc. of 8%	35 cc.	354.5	56
4	0.63	50 cc. of 4%	51 cc.	222.3	80
5	0.63	100 cc. of 2%	101 cc.	78.5	160
6	0.31	50 cc. of 2%	100 cc.	39.0	320
7	0.06	10 cc. of 2%	100 cc.	24.0	1600
8	0.03	5 cc. of 2%	100 cc.	12.2	3200
9	0.00	25 cc. of 8%	100 cc.	41.8	..
10	0.00	100 cc. of 2%	100 cc.	24.6	..
11	0.00	5 cc. of 2%	100 cc.	0.5	..

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[1] THIS JOURNAL, **32**, 1087 (1910).

[2] *Ibid.*, **33**, 1947 (1911); **34**, 317 (1912).

[3] *Ibid.*, **34**, 422 (1912).

Table III.—Effect of Dilution.

Diluent: Tap water. Time: 25 minutes. Temperature: 24°.

No.	No. cc. saliva.	Amount of starch paste.	Water to make vol.:	Mg. of maltose.	Dilution 1:
1	0.5	10 cc. of 10%	11 cc.	359.3	20
2	0.5	10 cc. of 10%	20 cc.	355.0	40
3	0.5	10 cc. of 10%	60 cc.	268.4	120
4	0.5	10 cc. of 10%	100 cc.	262.4	200
5	0.25	5 cc. of 10%	100 cc.	192.6	400
6	0.20	4 cc. of 10%	100 cc.	154.5	500
7	0.10	2 cc. of 10%	100 cc.	145.0	1000
8	0.05	1 cc. of 10%	100 cc.	131.4	2000
9	0.00	10 cc. of 10%	100 cc.	...	...

Table IV.—Effect of Dilution.

Diluent: 0.3% NaCl, 0.02% Na_2HPO_4. Time: 30 minutes. Temperature: 40°.

No.	No. cc. saliva.	Amount of starch paste.	Water to make vol.:	Mg. of maltose.	Dilution 1:
1	0.04	10 cc. of 20%	11 cc.	251.7	275
2	0.04	10 cc. of 20%	16 cc.	264.3	400
3	0.04	25 cc. of 8%	26 cc.	293.4	650
4	0.04	25 cc. of 8%	36 cc.	301.4	900
5	0.04	50 cc. of 4%	51 cc.	312.1	1275
6	0.04	100 cc. of 2%	101 cc.	335.9	2525

Table V.—Effect of Dilution.

Diluent: 0.3 % NaCl, 0.02 % Na_2HPO_4. Time: 30 minutes. Temperature: 40°.

No.	No. cc. saliva.	Amount of starch paste.	Water to make vol.:	Mg. of maltose.	Dilution 1:
1	0.1	10 cc. of 20%	11 cc.	227.1	110
2	0.1	10 cc. of 20%	16 cc.	364.4	160
3	0.1	25 cc. of 8%	36 cc.	387.7	360
4	0.1	50 cc. of 4%	51 cc.	387.7	510
5	0.05	50 cc. of 2%	100 cc.	399.1	2000

Table VI.—Effect of Dilution.

Diluent: 0.3% NaCl.

No.	No. cc. saliva.	Amount of starch paste.	Water to make vol.:	Mg. of maltose.	Dilution 1:
1	0.1	10 cc. of 10%	11 cc.	198.8	110
2	0.1	10 cc. of 10%	21 cc.	195.2	210
3	0.1	10 cc. of 10%	36 cc.	210.8	360
4	0.1	10 cc. of 10%	61 cc.	204.7	610
5	0.1	10 cc. of 10%	101 cc.	205.3	1010
6	0.02	2 cc. of 10%	100 cc.	199.4	5000
7	0.01	1 cc. of 10%	100 cc.	199.1	10000
8	0.00	10 cc. of 10%	100 cc.	...	...
9	0.00	2 cc. of 10%	100 cc.	...	...
10	0.00	1 cc. of 10%	100 cc.	...	...

for several months with the consequent absorption of carbon dioxide. The activity was ten times as great where only 11 cc. of liquid were used as it was where 100 cc. of this water had been added and 17 times as great as where 200 cc. had been used. From this point on the decrease was not so rapid, as the enzyme was not apparently destroyed, but the amount of starch hydrolysis brought about was very small indeed compared with that which occurs under favorable conditions.

From Table III and the corresponding Curve VI, which deal with the action of tap water (see Fig. 2), we see that this also was a case where the salivary enzyme was forced to act under unfavorable conditions other than that of mere dilution. These conditions were, however, by no means so adverse as in the case of softened water, although the alkalinity to phenolphthalein was greater. This was due partly perhaps to the protecting influence of the large amounts of carbonic acid contained in this water, partly to the lack of the strongly inhibiting hydroxides of the alkaline earths, and also undoubtedly, as was indicated by the very fair rate of hydrolysis in the more dilute solutions, to the favoring action of some of the electrolytes contained in this water which are removed by a lime treatment. It will be noticed that in that part of the curve where the inhibition was practically at its maximum, *i. e.*, in the lower part,

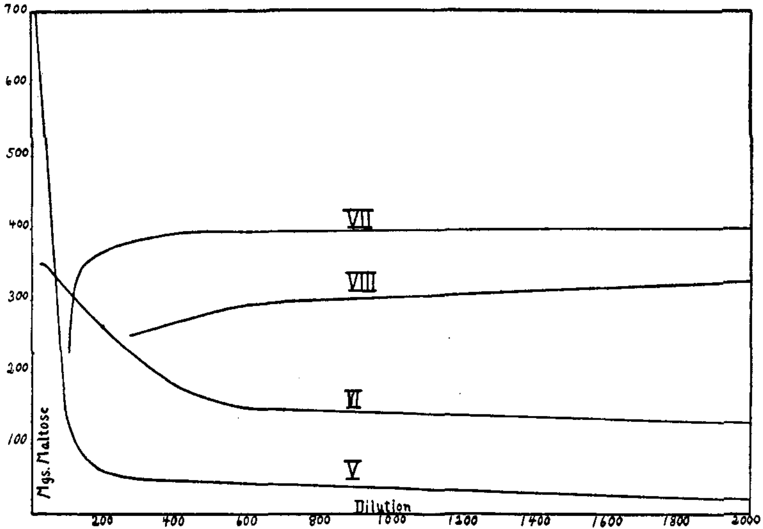

Fig. 2.—Effect of dilution on the rate of digestion of starch by salivary amylase. V, Diluent, softened water (3–11); 2 g. starch; $^5/_8$ cc. saliva; 30 min.; 40° C. VI, Diluent, tap water; 1 g. starch; 0.5 cc. saliva; 25 min.; 24° C. VII. Diluent, 0.3% NaCl, 0.02% Na_2HPO_4; 2 g. starch; 0.04 cc. saliva; 30 min.; 40° C. VIII, Diluent, 0.3% NaCl, 0.02% Na_2HPO_4; 2 g. starch; 0.1 cc. saliva; 30 min.; 40° C.

the activity in the case of tap water was three to five times as great as with the softened water, although for reasons before stated the two curves may be considered comparable only in an approximate way. Neither was there such a pronounced inhibition in the upper part of the curve. Tap water can not, however, be considered a very favorable medium for the action of salivary amylase, as the action was many times slower than in a sodium chloride solution of favorable concentration. The form of the curve of hydrolysis is such as we should expect from the action of substances which give a reaction unfavorable but somewhat less so than in the case of softened water.

In Curves VII, VIII and IX corresponding to Tables IV, V and VI the action of a 0.3% sodium chloride solution was considered, in the first two cases there also being present small amounts of disodium hydrogen phosphate (see Figs. 2 and 3). The most striking fact brought out by these data is that in dilutions of from 600 to 2000 and in the last case even in dilutions as large as 600 to 10,000 the curve for the rate of hydrolysis was practically a straight line. This would tend to show that approximately the optimum concentration of salts was used, and further that when such is the case very large dilutions do not appreciably affect the rate of hydrolysis. On the other hand in the early part of the curve the rate was considerably reduced, due mainly in this case to the high viscosity of the solution, although in the later stages there would doubtless also be an inhibition due to the accumulation of the products of the reaction. In the case where the sodium chloride was used alone we find that while the medium is favorable the maximum hydrolysis does not lie in such great dilutions, which supports work done by various investigators and verified in some of our own, to the effect that small amounts of disodium phosphate will increase the rate of hydrolysis over that for sodium chloride alone.

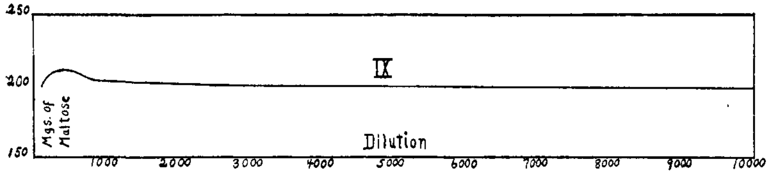

Fig. 3.—Effect of dilution on the rate of digestion of starch by salivary amylase. IX. Diluent, 0.3% NaCl solution; 1 g. starch; 0.1 cc. saliva; 30 min.; 24° C.

Optimum Dilutions and the Effect of Dilution with Different Waters upon the Speed of Hydrolysis of Starch by Saliva in more Concentrated Mixtures.—In the foregoing series of experiments it will be noted that the amounts of saliva are small compared with the amounts of starch and with the total volumes of the solutions.

It will also be noted that in nearly all cases the rate of digestion decreases with the increase in dilution, the only apparent exception being in the cases of dilution with a sodium chloride solution in high starch concentration, where the limiting factor is quite probably the high viscosities of the solutions. In other words little study was made of the changes of velocity of digestion in the neighborhood of the optimum concentration. Under the conditions of the experiments this was indeed impossible, because no accurate work can be done in high concentrations of saliva where the starch is digested to the achromic point almost instantaneously. The study of the digestion in these concentrations was, however, considered to be important as approaching normal conditions of salivary digestion and also to find out how far the inhibiting action of some of these waters extended. The reduction of temperature was employed as the method of slowing the reaction without introducing unknown factors. The following preliminary test was made, the time taken to reach the achromic point being measured.

The digestions were carried out in test tubes, stoppered and placed in an ice bath, starch paste and saliva both being at 0° at the time of mixing. The saliva was added at 30-second intervals and the time readings corrected for this. Some difficulty was found in reading the end point, for which reason the time readings are probably a little high.

TABLE VII.—EFFECT OF DILUTION IN CONCENTRATED MIXTURES. PRELIMINARY TEST.

Diluent: Distilled water. Temperature: 0°. End point: Achromic.

Amount of starch paste.	No. cc. saliva.	Amount of water.	Dilution 1:	Time to the achromic point.
5 cc. of 10%	5	..	2	25 minutes
5 cc. of 10%	5	5 cc.	3	20 minutes
2 cc. of 10%	2	6 cc.	5	16 minutes
1 cc. of 10%	1	8 cc.	10	11 minutes
0.5 cc. of 10%	0.5	9 cc.	20	11.5 minutes
0.1 cc. of 10%	0.1	9.8 cc.	100	16 minutes

This preliminary test brought out the fact that digestion took place quite rapidly even at zero degrees and that the optimum dilution was in the neighborhood of ten.

This test also pointed out the greatest probable sources of error in a study of this kind as being: 1st, the accurate measurement of the time of digestion; 2nd, in having both starch paste and saliva at zero degrees at time of mixing; and 3rd, in the immediate and intimate mixing of the two. To eliminate these sources of error as far as possible, the following method was used:

Thoroughly cleaned, rubber stoppered, hard glass test tubes of 25 cc. capacity were used. They were immersed to within one-fourth inch of the top in an ice mixture. Varying amounts of freshly prepared starch paste were then introduced and an amount of water which, with the saliva to be added later, would make the desired volume. They were well mixed and when the mixture had come to a constant temperature the required amount of saliva, likewise cooled to 0°, was added from cold calibrated pipets at exactly 30-second intervals. The tubes were shaken and immediately put back in the ice mixture. A guide test was run by means of the iodine color reaction and the digestions were stopped before they had proceeded more than about 20% of the way to completion. They were stopped by running in at 30-second intervals, in the same order as before, 5 cc. of Fehling's solution. In some cases the whole process was carried out in a cold room (10°) to still further obviate the danger of a rise in the temperature of the saliva while being transferred.

When the digestions were completed the contents of the tubes were transferred to 200 cc. Erlenmeyer flasks with the aid of 25 cc. of distilled water, 50 cc. of Fehling's solution added, and the flasks placed in a boiling water bath for 15 minutes. The cuprous oxide was then filtered off, dissolved in nitric acid, and titrated as usual by the iodine method.

TABLE VIII.—EFFECT OF DILUTION IN CONCENTRATED MIXTURES.

Diluent: Distilled water. Time: 11.5 minutes. Temperature: 0°.

No.	Amount of starch paste.	No. cc. saliva.	Amount of water.	Mg. of maltose.	Dilution 1:
1	5 cc. of 10%	5	..	171.1	2
2	5 cc. of 10%	5	5 cc.	179.3	3
3	2 cc. of 10%	2	10 cc.	187.5	7
4	1 cc. of 10%	1	8 cc.	179.8	10
5	1 cc. of 10%	1	13 cc.	161.2	15
6	0.5 cc. of 10%	0.5	9 cc.	151.8	20
7	5 cc. of 10%	..	5 cc.	...	..

All columns corrected for control test number 7.

TABLE IX.—EFFECT OF DILUTION IN CONCENTRATED MIXTURES.

Diluent: Filtered tap water. Time of digestion: 10 min. Temp.: 0°.

No.	Amount of starch paste.	No. cc. saliva.	Amount of water.	Mg. of maltose.	Dilution 1:
1	10 cc. of 10%	10	...	378.6	2
2	7 cc. of 10%	7	6 cc.	441.8	3
3	4 cc. of 10%	4	12 cc.	448.6	5
4	3 cc. of 10%	3	14 cc.	458.5	7
5	2 cc. of 10%	2	16 cc.	449.3	10
6	1.5 cc. of 10%	1.5	17 cc.	410.5	13.3
7	1.3 cc. of 10%	1.3	17.4 cc.	321.5	15.4
8	1 cc. of 10%	1	18.0 cc.	305.4	20
9	0.6 cc. of 10%	0.6	18.8 cc.	294.9	35
10	0.4 cc. of 10%	0.4	19.2 cc.	283.0	50
11	0.2 cc. of 10%	0.2	19.6 cc.	287.6	100
12	10 cc. of 10%	..	10 cc.	...	...
13	0.2 cc. of 10%	..	19.8 cc.	...	...
14	0.4 cc. of 10%	..	19.6 cc.	...	...

All columns are corrected for check and calculated to the basis of 1 gram of starch.

TABLE X.—EFFECT OF DILUTION IN CONCENTRATED MIXTURES.

Diluent: 0.3% NaCl solution. Time of digestion: 9 minutes. Temperature: 0°. Carried out in room at 10°.

No.	No. cc. of 10% starch.	No. cc. saliva.	No. cc. of NaCl sol.	No. cc. of $Na_2S_2O_3$.	Mg. of maltose.	Dilution 1:
1*a*	10.0	10.0	..	112.32	455.0	2
1*b*	10.0	10.0	..	111.92		
1*c*	10.0	..	10.0	9.40		
2*a*	5.0	5.0	10.0	64.95	521.0	4
2*b*	5.0	5.0	10.0	65.07		
2*c*	5.0	..	15.0	6.10		
3*a*	3.0	3.0	14.0	36.95	494.7	7
3*c*	3.0	..	17.0	5.50		
4*a*	2.0	2.0	16.0	26.61	491.9	10
4*b*	2.0	2.0	16.0	25.55		
4*c*	2.0	..	16.0	3.87		
5*a*	1.3	1.3	17.4	18.45	493.1	15
5*c*	1.3	..	18.7	3.58		
6*a*	1.0	1.0	18.0	14.42	483.7	20
6*b*	1.0	1.0	18.0	14.42		
6*c*	1.0	..	19.0	3.50		

The duplicates were averaged and corrected for the control test which was run in each case.

From the data on distilled water, Tables VII and VIII and Curve X corresponding to Table VIII, we see that the effect of dilution was gradual, the most rapid hydrolysis taking place in about *seven dilutions* (see Fig. 4). However the activity was only about 5% less in three dilutions and ten dilutions, respectively, and beyond ten dilutions it fell off only very gradually due to the gradual decrease in the electrolyte concentration.

Where tap water was used (Table IX and Curve XI) we again found the optimum to be in the immediate neighborhood of *seven dilutions*, with but slight variation from 5 to 10 dilutions (see Fig. 4). Beyond this, however, the falling off was much more rapid than in the case of distilled water, indicating the presence of inhibiting substances in this water. The greater activity at the optimum over that at two dilutions must probably be credited to decreases in the viscosity of the solution and in the concentration of the products of the reaction.

In the case where a dilute sodium chloride solution was used (Table X and Curve XII) we found, as usual, a greater production of reducing sugar than where other solutions were employed (see Fig. 4). The form of the curve of the rate of the reaction was also slightly different. The optimum conditions were found at *four dilutions instead of seven as in the two previous*

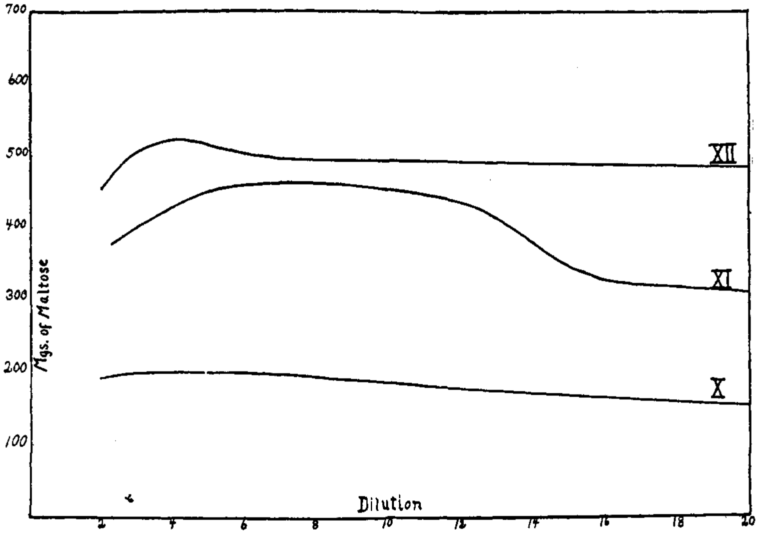

Fig. 4.—Effect of dilution on the rate of digestion of starch by salivary amylase in concentrated mixtures (the volume of 10% starch paste was the same as the volume of saliva used in each instance). X. Diluent, distilled water; temp. 0° C.; time of digestion, 11.5 min. XI. Diluent, tap water; temp. 0° C.; time of digestion, 10.0 min. XII. Diluent, 0.3% NaCl; temp. 0° C.; time of digestion, 10.0 min.

cases, the sodium chloride evidently assisting the action in these solutions of high viscosity. On the other hand we found that the hydrolysis was greater in twenty dilutions than in two or three dilutions and the falling off with the dilution was more gradual, so that all in all the sodium chloride solution was much the most satisfactory medium.

General Discussion.

No attempt has been made in these tests to duplicate physiological conditions which are themselves variable. Neither is it claimed that the results obtained can be applied directly and in all cases to salivary digestion within the organism. The work may, however, give general indications of value and experiments made under controlled conditions must be considered in any attempt at the understanding of the influences which affect the digestive processes as they normally occur.

In this connection it is important to know what really happens to ingested water when it reaches the stomach. Liquid alone, as Grützner[1] and others have shown will remain but a short time in the stomach if the intestin is not already full. Best and Cohnheim[2] have shown that the time water remains in the stomach depends very little on its temperature,

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[1] *Pflüger's Arch.*, 91, 195 (1902); 106, 463 (1906).

[2] *Z. physiol. Chem.*, 69, 116 (1910).

but somewhat upon the quantity ingested, while a sodium chloride solution remains somewhat longer than water. The same appears, in general, to be true, even if the stomach does contain food. Grützner makes the following statement in the summary of his well-known studies along this line: "Mässiges Getränk wahrend der Mahlzeit stört sicherlich die Thätigkeit des gesunden Magens in keiner weise, wie man vielfach angenommen hat." He also cites the work of Leconte[1] who fed two dogs normally, 2 hours later gave one of them water, and 15 minutes later examined the stomach contents of both animals. He found scarcely any difference between the two, the water having largely left the stomach and even the duodenum. It had carried only small particles of food along with it. The water apparently left the stomach along the small curvature without disturbing the food to any extent.

Kaufmann[2] even claims to have demonstrated the presenee of a trough on the smaller curvature, after physostigmine injection along which water might readily leave the stomach. Scheunert[3] disagrees with this finding after numerous experiments on horses and dogs, in which the course of the water through the stomach was followed by means of colored stains contained in the liquid. This author found no evidence of a trough, although a large part of the water did leave along the smaller curvature. To a greater or less extent the water poured over the entire contents of the stomach. He believes that the food may absorb water until it reaches the most desirable consistency, after which no more will ordinarily remain. However, as Grützner has suggested, if the stomach is filled with liquid or semi-liquid food, water may intermix to some extent although probably only when the stomach empties, as he observed a lasting layer formation even with the semi-liquid foods.

In our experiments it was shown that where a 10% starch paste was used as a basis and an equal amount of saliva added, that dilution with from two to five times their combined volume of water (the mixtures then containing from $^1/_2$ to $2^1/_2$% starch) considerably increased the amount of digestion. Further if the electrolyte concentration is kept fairly high (as we should expect upon any ordinary diet), and providing no strongly inhibiting substances are present in the water (which can of course be guarded against), then the rapidity of digestion for the greatest dilutions which could possibly occur in the body would be greater than in this original mixture of 10% starch paste and saliva. We must consider also the fact that the ordinary starchy foods, such as bread and potatoes, have starch concentrations considerably above 10%, as well as the above cited facts with regard to the passage of water through the stomach.

[1] *La Cellule*, 17, 2 fasc., 1900.

[2] *Z. Heilkunde*, 28, 303 (1907).

[3] *Pflüger's Arch.*, 114, 65, 93 (1906); 144, 430, 569 (1912).

It would appear, that as far as salivary digestion is concerned, there is no danger of excessive dilution with any water of fair quality.

On the other hand it seems probable that a dilution as great as that of the optimum for salivary digestion is not reached under ordinary conditions. For this reason the ingestion of large amounts of water would increase the efficiency of salivary amylase through the production of a satisfactory dilution of the digestion mixture.

These results are in accord with work carried out in this laboratory showing a better utilization of carbohydrates during periods of high water ingestion.[1]

Conclusions.

On the basis of experiments made *in vitro* it is apparent that the digestive efficiency of normal human saliva is *increased* when such saliva is *diluted*. The *optimum dilution* is dependent upon the nature of the diluent, being *four volumes* for sodium chloride solution (0.3%) and *seven volumes for water*, either distilled or tap water.

Softened water exerts an *inhibitory* influence, due principally to the presence of *magnesium hydroxide*.

The fact that salivary amylase acts more efficiently when the saliva is diluted is an added argument in favor of *water drinking with meals*.

The influence of dilution, as above set forth, aids in explaining the better digestion of ingested carbohydrates when accompanied by a copious water ingestion.

[Contribution of the Kansas Agricultural Experiment Station.]

ACIDITY IN SILAGE: METHOD OF DETERMINATION.

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As far as ascertained by the writers, acidity in silage is determined by extracting either with water or with alcohol and titrating with a standard alkali solution, using phenolphthalein as indicator. Esten,[2] Hart and Willaman,[3] Dox and Neidig,[4] use water as the solvent. With certain kinds of silage, water gives an extract difficult to filter. Alcohol as a solvent appears not to have been so thoroughly tried by experiment station chemists. To compare water and alcohol as extractive reagents in determining the acidity in silage, was the object of the following experiments.

The work of Dox and Neidig, and of Hart and Willaman has shown that the principal volatil acids in silage are: acetic, propionic, butyric and valeric, acetic constituting 75% or more of the total. Lactic is as-

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[1] Mattill and Hawk, This Journal, 33, 2019 (1911).

[2] Conn. Storrs Expt. Sta., *Bull.* 70.

[3] This Journal, 34, 1619.

[4] Research Bull. No. 7, Iowa Expt. Sta.; This Journal, 35, 93.