11. As shown by the percentage of total solids exclusive of potassium chloride destroyed, and by the percentage of total sulfur converted into hydrogen sulfide, a larger percentage of sulfur-containing material than of total peptone was broken down, the ratio being about 3 : 1. The fact that the bodies of the bacteria were included in the total solids exclusive of potassium chloride, would not materially influence the ratio.

12. A larger percentage of easily oxidized sulfur than of total sulfur was converted into hydrogen sulfide by the bacteria, the ratio being about 4 : 3. It is evident that if a synthetic medium is to be prepared for the detection of the bacteria producing hydrogen sulfide, work upon which is herewith promised, that the sulfur should be introduced in an easily oxidized form.

13. A larger percentage of loosely bound sulfur than of total sulfur was converted into hydrogen sulfide by the bacteria, the ratio being about 3 : 2.

14. A very slightly greater percentage of loosely bound sulfur than of easily oxidized sulfur was convered into hydrogen sulfide by the bacteria, the ratio being about 10 : 9. There was about twice as much easily oxidized sulfur as there was loosely bound sulfur, both before and after the bacteria had acted upon the medium.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF COLUMBIA UNIVERSITY, NO. 245.]

STUDIES ON AMYLASES. VIII. THE INFLUENCE OF CERTAIN ACIDS AND SALTS UPON THE ACTIVITY OF MALT AMYLASE.

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The reaction most favorable to the activity of malt amylase has been given as acid, neutral and alkaline, by different investigators, and there are also striking discrepancies in the statements regarding influence of salts. To some extent, though not entirely, the confusion has arisen through lack of (or failure to use) adequate or uniform criteria of neutrality.

Baswitz[1] observed that carbon dioxide increased the diastatic activity of malt extract. Later Mohr[2] confirmed the observation and found that very small amounts of lactic acid could also be used with beneficial effects.

Kjeldahl[3] found that very small additions of sulfuric acid (making the reaction of the medium equivalent to 0.0005 *N*) increased the saccharo-

[1] *Ber.*, 11, 1443 (1878); 12, 1827 (1879).

[2] *Ibid.*, 35, 1024 (1903).

[3] Dingler's *Polytech. Jour.*, 235, 379, 452 (1880).

ogenic[1] activity of malt extract, while slightly larger amounts were deleterious.

Detmers,[2] studying the amyloclastic[1] action of malt extract, found slightly greater action on passing a current of carbon dioxide through the solution, or on adding small amounts of citric, phosphoric or hydrochloric acid.

Chittenden and Cummins,[3] studying the action of malt extract upon a paste made from carefully washed potato starch, found the optimum activity in a neutral solution containing a small amount of peptone.

Duggan[4] also reported neutrality as the optimum condition for the action of malt amylase, and in a second paper[5] recommended the observation of optimum diastatic activity as a method of determining absolute neutrality—a suggestion which was later elaborated in the light of the ionization theory by Wood.[6]

Lintner[7] likewise specified neutrality as the optimum reaction, while Reychler[8] recommended the addition of potassium acid phosphate and Effront[9] reported activation of the enzyme by hydrochloric, hydrofluoric, sulfuric, and phosphoric acids as well as by acid phosphate. Effront further pointed out that the effect of adding free acid is largely dependent upon the purity of the starch and water employed. He was probably the first to realize the great importance of purity of materials in studying enzyme action. Petit[10] also found slight acidity favorable and reported hydrochloric and lactic acids to show optimum activation at those concentrations which best favor the coagulation that occurs on heating a water solution of the enzyme.

Ford,[11] on the other hand, insists that malt amylase exerts its optimum activity in strictly neutral solution. He attributes the favorable effects, sometimes resulting from the addition of acids or acid salts, to neutralization of alkaline impurities in the starch or the malt extract used.

Schneidewind, Meyer and Münter[12] find acetic and citric acids favorable at a concentration of 0.001% but injurious at 0.01%. They also report

[1] These terms are used in preference to "saccharifying" and "liquefying" as explained in a previous paper.

[2] *Z. physiol. Chem.*, **7**, 1 (1882).

[3] *Studies Yale Laboratory Physiol. Chem.*, 1884–5, p. 36.

[4] *Am. Chem. J.*, **7**, 306 (1885–6).

[5] *Ibid.*, 8, 211 (1886).

[6] *Ibid.*, **15**, 663 (1893).

[7] *J. prakt. Chem.*, [2] **36**, 481 (1888).

[8] *Bull. soc. chim.*, [3] 1, 286 (1889).

[9] *Compt. rend.*, **115**, 1324 (1892); *Bull. soc. chim.*, [3] **9**, 151 (1893).

[10] *Compt. rend.*, **138**, 1231 (1904).

[11] *J. Soc. Chem. Ind.*, **23**, 414. 477 (1904); see also Ford and Guthrie, *J. Chem. Soc.*, **89**, 76 (1906).

[12] *Landwirt Jahrbücher*, **35**, 911 (1906).

favorable effects from very small additions of acid phosphate and of alum.

Maquenne and Roux[1] report that the maximum initial activity is obtained in solutions neutral to methyl orange, but that a solution alkaline to this indicator shows the optimum total digestion of starch into maltose and dextrin.

Fernbach and Wolff[2] find optimum amyloclastic and saccharogenic activities in solutions neutral to methyl orange. Addition of secondary sodium phosphate to such solutions depressed the activity; primary sodium phosphate showed either no effect or a slight activation, the latter attributed by these authors to a failure to secure complete neutrality in the control solution.

Windisch and Derz[3] found free sulfuric acid always harmful even in minute amounts, while primary potassium phosphate increased the activity of the amylase.

Heyl[4] finds that primary potassium phosphate exerts both an activating and a conserving influence upon the enzyme, while neutral phosphate mixture depressed the activity of a freshly prepared malt extract, but increased that of an old extract. He concludes, however, that the activating effect of the phosphate is influenced by the proteins present in the malt extracts with which he worked.

Van Laer,[5] working at 25°, found that acid phosphate and small additions of phosphoric, acetic, and tartaric acids increased the saccharogenic activity of malt extract without apparent effect upon the amyloclastic action, while in parallel experiments with a commercial amylase preparation the addition of acid seemed always to retard the diastatic action, possibly due to a difference of reaction between the commercial preparation and the malt extract.

In view of the above discrepancies and the fact that experiments to test the influence of salts have usually been subject to uncertainty regarding the reaction of the medium, it seems unnecessary to review the earlier results obtained with salts at this time. Even the extended study by Hawkins[6] which demonstrated an activation of amyloclastic power by sodium and potassium chlorides is difficult to interpret in other respects because of the lack of neutrality in the digestion mixtures.

Experimental.

In view of the importance of an adequate knowledge of the influence of acids and salts on the activity of the enzyme and especially the definite determinations of the optimum hydrogen ion concentration, the following

[1] *Compt. rend.*, 142, 1059 (1906).
[2] *Ibid.*, 142, 1216; 145, 261 (1906–7).
[3] *Woch. Brau.*, 30, 533; *Chem. Abs.*, 8, 268.
[4] *J. prakt. Chem.*, [2] 86, 433 (1912).
[5] *Orig. Com. 8th Intern. Congr. Appl. Chem.*, 14, 203 (1912).
[6] *Botan. Gazz.*, 55, 265 (1913).

series of experiments covering several acids and salts have been carried on in this laboratory during the past two years. The general plan has been to study one acid at a time, making a systematic series of quantitative determinations of diastatic power with increasing amounts of added acid until the optimum acidity is plainly exceeded and a distinct deleterious effect of the acid is observed, repeating the experiments in the region of the optimum with smaller increments of acid if necessary, in order to establish the optimum point with sufficient precision. The solution showing the optimum acidity was then exactly duplicated (except that no enzyme was added) and its hydrogen ion concentration determined by the electrometric method.

Since previous workers have employed almost exclusively malt extracts which necessarily contained relatively large and variable amounts of salts and proteins, their results, even if concordant, would not have been conclusive. In the present experiments purified enzyme preparations were used which, while varying considerably in activity and in no case absolutely pure, were much purer and more active than any material available to previous investigators of the influence of acids and salts.

Materials.

Amylase.—The amylase preparations used were made in this laboratory by Miss M. D. Schlesinger as described elsewhere.[1] Before performing an experiment, an accurately weighed portion of the dry preparation would be dissolved in pure water and kept in an icebox at 7° to 8° until used.

Substrate.—The substrate used was soluble starch prepared from potato starch by the Lintner[2] method of treatment with dilute hydrochloric acid. Four samples of this starch were used, one as purchased and three after further purification. Their properties are as follows:

	Moisture. %.	Reduction. Mg. Cu_2O per 2 g. dry starch.	Acidity. Cc. of 0.01 *N* acid per 2 g. dry starch.
Starch 1	6.25	32.4	0.85
Starch 2*a*	11.28	27.3	1.00
Starch 2*b*	3.00	32.0	1.00
Starch 6	9.50	34.5	1.76

The "reduction" is the amount of cuprous oxide reduced by the starch when 100 cc. of a 2% solution are mixed with 50 cc. of Fehling solution and heated in a bath of boiling water for 15 min.

The acidity of the starch is determined by titration of a solution with 0.01 *N* sodium hydroxide, using rosolic acid as indicator.

Starch 1 was a commercial product. Starches 2*a* and 2*b* were prepared by repeatedly washing Starch 1 with distilled water. Starch 6 was also purified from a commercial preparation.

[1] THIS JOURNAL, 35, 1617–22 (1913).

[2] *J. prakt. Chem.*, [2] 34, 378 (1886).

Water.—Ordinary distilled water was twice redistilled: first from alkaline permanganate solution into a reservoir whence it siphoned into a second flask from which it was distilled again. Phosphoric acid was added to this portion in order to hold any ammonia that might have come from the first distillation. Block tin condensers were used and the final distillate was caught in "Nonsol" bottles, contact with the air being avoided as far as possible.

Activating Agents.—These were carefully purified before trying their effect on the action of the amylase. The salts of the best obtainable grade were recrystallized three times, twice from distilled water and finally from triple-distilled water. The inorganic acids were of the purest "analyzed" reagent quality and were used directly as obtained from the manufacturers, it being deemed impracticable to purify them further. The organic acids were redistilled.

Apparatus.

Thermostat.—The digestions of starch by the amylase were carried out in a bath of ten gallons' capacity. The water in this thermostat was heated to, and kept at 40° by means of three 32 candle power carbon filament lamps, one of which burned continuously while the other two were in series with a relay and a large bulb of mercury containing a nickle contact wire in its narrow neck which served as the temperature control. This temperature regulator was very sensitive, keeping the bath within 0.02° above or below 40°. The water in the bath was stirred constantly and vigorously to ensure an even temperature throughout.

Glassware.—All glassware used to contain the starch solutions and digesting mixtures was kept filled with water when not in use, to avoid weathering and consequent introduction of alkali in the enzyme solutions, the danger of which has already been pointed out by Ford.[1] Before using the glassware each piece was carefully rinsed several times with tap water, then with distilled water and finally with triple-distilled water. The flasks or cylinders were then inverted to drain for a few minutes before using.

Hydrogen-ion Apparatus.—For the measurement of hydrogen ion the gas chain method was used in essentially the same manner as described by L. Michaelis.[2] A standardized Weston cell was used as the standard source of potential and a tenth normal calomel electrode was used in the hydrogen cell. A galvanometer was used instead of the electrometer described by Michaelis. For the hydrogen electrode, No. 14 gauge platinized platinum wire fused in glass tubing filled with a mercury connection was adjusted in the glass cell containing the solution, the hydrogen

[1] *J. Soc. Chem. Ind.*, 23, 414 (1904).

[2] Abderhalden's *Handbuch der Biochemischen Arbeitsmethoden*, Vol. V, Part 1, pp. 500–524.

ion concentration of which was to be tested. This cell differed from Michaelis' in that a stream of hydrogen (washed by saturated mercuric chloride, strong potassium permanganate solution, strong alkaline pyrogallol, and passed through a tube of cotton to remove spray) was passed through it. Michaelis uses still hydrogen.

After finding the potential given by an unknown solution the hydrogen-ion concentration was found by referring to the chart plotted by S. P. L. Sörensen.[1] If this chart is not available the concentration can be calculated by means of the formula

$$\log H = -\frac{E - 0.3377}{0.0001983\ T}$$

where H = concentration of hydrogen ion which we are seeking.

E = difference of potential in volts between the 0.1 *N* calomel electrode and the hydrogen electrode used.

T = absolute temperature.

The figure 0.3377 is the potential difference of the 0.1 *N* calomel electrode over the normal hydrogen electrode as determined by Sörensen at 18°.

Method of Determining Saccharogenic Power.

To determine the saccharogenic power an amount of soluble starch sufficient to make 600 cc. of 2% solution was weighed out and transferred to a beaker. Enough sodium hydroxide was added to neutralize the acidity of the starch and the mixture stirred to a paste with about 25 cc. of pure water. This paste was then poured into a 1 l. Erlenmeyer flask containing 300 cc. of boiling pure water, and the contents were boiled for two or three minutes to ensure complete solution of the starch. The flask was then partially cooled and the contents were adjusted to a volume of 420 cc. Seventy cc. were then poured into each of six 100 cc. graduated cylinders. The reagents whose effect on the saccharogenic power were to be determined were then added to the cylinders and the volume of solution in each cylinder was adjusted to the 100 cc. mark with pure water and the cylinders were then placed in the thermostat.

While the cylinders were resting in the water bath, the portions of enzyme solution were carefully measured into six 200 cc. Erlenmeyer flasks by means of a 1 cc. pipet which is accurately standardized and graduated to 0.01 cc. These small Erlenmeyer flasks were then placed in a brass rack and the contents of the cylinders were poured into their respective flasks at intervals of 10 sec. and the rack of flasks with their digestion mixtures placed at once in the water bath. At the expiration of 30 min., 50 cc. portions of mixed Fehling solution were poured quickly into each flask at 10 sec. intervals, corresponding with the addition of

[1] *Comptes-Rendus des Travaux du Laboratoire de Carlsberg*, Vol. 8, 1re Livraison, 1909; also *Biochem. Z.*, **21**, 131; **22**, 352 (1909).

the starch solution to each flask, so that each mixture had digested for exactly 30 min.

The rack was then placed in boiling water for 15 min., after which the contents of the flasks were filtered through Gooch crucibles with suction. The precipitated cuprous oxide was carefully washed with hot water, followed by alcohol and finally ether. The crucibles were dried in an oven and weighed. After the weight of cuprous oxide, due to the reducing power of starch, is deducted from the total, the saccharogenic power is calculated from the scale of Sherman, Kendall and Clark.[1] Blank experiments showed that the acids and salts studied were all without effect upon Fehling solution.

Data of Typical Experiments.

In order to present the results of the experiments as concisely and clearly as possible, no attempt will be made to record every quantitative

TABLE I.—EFFECT OF ACIDS ON SACCHAROGENIC ACTIVITY.

Acid added.[2]	Normal concent. of acid.	Reduction in mg. of Cu_2O.	Power.
1.—Acetic acid. (0.2 mg. Malt Amylase 46B; 100 cc. 2% Starch *2a* in each case.)			
None	Neutral	166.8	266
1.0 cc. 0.02 *N*	0.0002	284.8	472
2.0	0.0004	283.5	470
3.0	0.0006	299.8	503
5.0	0.001	294.4	490
10.0	0.002	273.3	452
20.0	0.004	237.8	388
30.0	0.006	201.4	322
2.—Propionic acid. (0.2 mg. Malt Amylase 46B; 100 cc. 2% Starch *2a* in each case.)			
None	Neutral	171.3	272
0.1 cc. 0.1 *N*	0.0001	210.3	339
0.2	0.0002	268.6	443
0.3	0.0003	279.3	463
0.4	0.0004	276.3	461
0.5	0.0005	282.9	468
1.0	0.001	297.2	495
2.0	0.002	262.0	431
3.0	0.003	250.8	411
5.0	0.005	218.0	336
10.0	0.01	138.9	220
20.0	0.02	52.1	80
30.0	0.03	42.1	64
3.—Phosphoric acid. (0.2 mg. Malt Amylase 46B; 100 cc. 2% Starch *2a* in each case.)			
None	Neutral	181.7	291
0.1 cc. 0.1 *N*	0.0001	205.7	332
0.2	0.0002	214.7	348
0.5	0.0005	286.1	474
1.0	0.001	228.2	371
2.0	0.002	67.0	103
4.—Phosphoric acid, second series. (0.07 mg. Malt Amylase 118; 100 cc. 2% Starch 6 in each case.)			
None	Neutral	65.2	271
1.0 cc. 0.01 *N*	0.0001	65.1	271
3.0	0.0003	114.7	514
4.0	0.0004	168.0	763
5.0	0.0005	196.1	899
6.0	0.0006	170.9	777
5.—Phosphoric acid, third series. (0.07 mg. Malt Amylase 111A; 100 cc. 2% Starch 6 in each case.)			
None	Neutral	57.0	250
4.6 cc. 0.01 *N*	0.00046	140.4	633
4.8	0.00048	138.6	627
5.0	0.0005	147.7	669
5.2	0.00052	148.1	669
5.4	0.00054	145.2	655

[1] THIS JOURNAL, 32, 1082 (1910).

[2] As explained above, under "Method of Determining Saccharogenic Power," the added acid is contained within the final volume of 100 cc., the volume and the concentration of starch of the completed digestion mixture being the same in all cases.

TABLE I.—EFFECT OF ACIDS ON SACCHAROGENIC ACTIVITY—(*Continued*).

Acid added.[1]	Normal concent. of acid.	Reduction in mg. of Cu_2O.	Power.
6.—Hydrochloric acid. (0.2 mg. Malt Amylase 43B; 100 cc. 2% Starch *2a* in each case.)			
None	Neutral	134.6	213
0.5 cc. 0.01 *N*	0.00005	148.0	234
1.0	0.0001	171.8	274
1.5	0.00015	214.1	346
2.0	0.0002	224.8	366
2.5	0.00025	208.7	337
3.0	0.0003	176.9	282
5.0	0.0005	74.5	117
7.0	0.0007	36.1	55
10.0	0.001	18.3	27
15.0	0.0015	10.5	15
7.—Nitric acid. (0.2 mg. Malt Amylase 46B; 100 cc. 2% Starch *2a* in each case.)			
None	Neutral	162.4	258
0.5 cc. 0.01 *N*	0.00005	201.9	325
1.0	0.0001	255.1	418
1.5	0.00015	290.2	482
2.0	0.0002	292.1	485
2.5	0.00025	275.3	454
3.0	0.0003	259.8	427
5.0	0.0005	100.8	156
10.0	0.001	21.6	33
15.0	0.0015	12.8	18
8.—Nitric acid, second series. (0.07 mg. Malt Amylase 118; 100 cc. 2% Starch 6 in each case.)			
None	Neutral	61.2	267
0.5 cc. 0.01 *N*	0.00005	70.4	309
1.0	0.0001	112.7	507
1.5	0.00015	176.9	807
2.0	0.0002	183.8	841
2.5	0.00025	163.1	740
9.—Sulfuric acid. (0.2 mg. Malt Amylase 43B; 100 cc. 2% Starch *2a* in each case.)			
None	Neutral	126.8	200
0.5 cc. 0.01 *N*	0.00005	137.9	218
1.0	0.0001	172.6	271
2.0	0.0002	212.0	342
2.5	0.00025	198.4	318
10.—Sulfuric acid, second series. (0.2 mg. Malt Amylase 46B; 100 cc. 2% Starch *2a* in each case.)			
None	Neutral	158.0	250
2.0 cc. 0.01 *N*	0.0002	276.7	458
3.0	0.0003	243.0	397
5.0	0.0005	122.1	192
10.0	0.001	14.1	4
15.0	0.0015	5.1	1

determination, nor to give the data in the order in which they were determined. The object is rather to give the data of the principal experiments in sufficient detail to show the nature of the evidence and in such order as to simplify the discussion.

The data showing influence of acids upon saccharogenic activity are tabulated first.

The optimum solution in the case of acetic, is that one containing 3.0 cc. of 0.02 *N* acetic acid per 100 cc. of 2% starch solution, which in terms of total concentration of acetic acid is 0.0006 *N*. Measurement of hydrogen ion concentration in such a solution of acetic acid and soluble starch gave the Sörensen exponent $p_H^+ = 4.2$.

The optimum concentration of propionic acid is 0.001 *N*. The determination of hydrogen ion in a propionic acid starch solution of this concentration showed $p_H^+ = 4.4$.

[1] As explained above, under "Method of Determining Saccharogenic Power," the added acid is contained within the final volume of 100 cc., the volume and the concentration of starch of the completed digestion mixture being the same in all cases.

In the experiments with acetic and propionic acids recorded in *1* and *2* of Table I the enzyme 46B and the Starch *2a* were used throughout. Of the experiments with phosphoric acid which follow, the first series (*3*) was made with the same enzyme and starch while in the second series (*4*) both the enzyme and the starch were different, and in the third series (*5*) still a different enzyme preparation was used, but the starch was the same as in the second series. The purer enzyme preparation gave a lower initial and a higher optimum power but the optimum concentration of acid was the same in the three cases.

In the case of phosphoric acid it will be noted that the optimum activity is found in a solution containing this acid in the concentration of 0.0005 *N*. Measurement of hydrogen ion in this solution showed $p_H^+ = 4.6$.

Inasmuch as the activity was hardly appreciably diminished at a phosphoric acid concentration of 0.00054 *N*, the hydrogen-ion concentration was also measured at that point and found to be $p_H^+ = 4.5$.

Passing now to the experiments with strong acids, it will be seen from the table that hydrochloric acid was tested with the same starch as was used with acetic, propionic and the first series with phosphoric and with a very similar enzyme preparation. The first series with nitric employs the same enzyme and starch as the acetic, propionic and (first) phosphoric; the second series with nitric corresponds exactly in starch and enzyme used, with the second series of experiments on phosphoric. The first series with sulfuric corresponds to the experiments with hydrochloric and the second series with sulfuric to the first with nitric. It is therefore believed that the results obtained with the six different acids are fairly comparable with each other and at the same time adequately safe-guarded against any constant error such as might have been possible had all work been done with one sample of enzyme or of starch.

In Series *6*, Table I, the optimum concentration of hydrochloric acid was 0.0002 *N*. The soluble starch solution to which hydrochloric acid had been added to this concentration showed $p_H^+ = 4.2$.

With amounts of acid above the optimum the depression of the power is naturally much greater than for corresponding excesses of the weaker acids.

For nitric acid the optimum point is again found in the 0.0002 *N* concentration of acid, and the activity rapidly falls off in the solutions having a greater concentration of acid. Measurement of hydrogen ion in the optimum solution showed $p_H^+ = 4.4$.

As in the case of hydrochloric acid, the optimum activity with sulfuric acid is obtained in solution having a concentration equal to 0.0002 *N*. Measurement of hydrogen ion in this optimum solution showed $p_H^+ = 4.3$ to 4.4.

Closely connected with the problem of the influence of the acids is that of their neutral salts. Sodium and potassium chlorides, nitrates, and

sulfates have therefore been studied, as well as the primary phosphates which, being both salts and acids, will be considered later. In addition to what has been said above regarding the findings of earlier workers it should be stated that Osborne, in 1895, pointed out[1] that the addition of a small amount of sodium chloride assisted his purified malt amylase preparation to exert its true diastatic power.

In our experiments it has been observed that so long as commercial soluble starch, even of high grade (Starch 1) was used as substrate, the small additions of the salts above mentioned have very little effect, but when the more highly purified soluble starch was used the effect was considerable as will be seen from the tables which follow.

Limitations of solubility and a tendency to salting out of the starch when treated with the Fehling solution precluded experiments at higher concentrations than are shown in the tables.

TABLE II.—EFFECT OF SALTS ON SACCHAROGENIC ACTIVITY.

Amount of salt added.[2]	Molar concent. of salt.	Reduction in mg. of Cu_2O.	Power.
11.—Sodium chloride, first series. (0.07 mg. Malt Amylase 111A; 100 cc. neutral 2% Starch 6 in each case.)			
None	0.00	48.7	214
0.4 cc. 4 *M*	0.16	121.6	547
0.5	0.2	129.3	580
0.6	0.24	126.1	565
0.7	0.28	136.4	614
0.8	0.32	139.6	631
12.—Sodium chloride, second series. (0.07 mg. Malt Amylase 118; 100 cc. neutral 2% Starch 6 in each case.)			
None	0.00	63.7	281
1.0 cc. 4 *M*	0.04	104.2	464
2.0	0.08	119.9	537
4.0	0.16	130.0	584
6.0	0.24	147.8	669
10.0	0.40	144.2	650
13.—Potassium chloride. (0.07 mg. Malt Amylase 118; 100 cc. neutral 2% Starch 6 in each case.)			
None	0.00	64.6	286
1.0 cc. 4 *M*	0.04	92.7	414
2.0	0.08	117.8	528
4.0	0.16	140.5	636
6.0	0.24	139.5	632
10.0	0.40	159.7	726
14.—Potassium chloride, second series. (0.07 mg. Malt Amylase 111A; 100 cc. neutral 2% Starch 6 in each case.)			
None	0.00	48.7	214
4.0 cc. 4 *M*	0.16	122.6	551
6.0	0.24	134.7	608
10.0	0.4	138.3	622
15.0	0.6	131.8	594
20.0	0.8	117.0	523
15.—Sodium nitrate. (0.07 mg. Malt Amylase 118; 100 cc. neutral 2% Starch 6 in each case.)			
None	0.00	62.2	272
1.0 cc. 4 *M*	0.04	74.1	322
2.5	0.10	77.5	341
5.0	0.20	90.7	408
10.0	0.40	107.7	482
20.0	0.80	109.5	491
16.—Potassium nitrate. (Same enzyme and starch as above.)			
None	0.00	62.2	272
2.0 cc. 2.5 *M*	0.05	68.0	300
5.0	0.105	79.3	350
10.0	0.25	98.0	437
20.0	0.5	126.9	570
30.0	0.75	110.8	496

[1] THIS JOURNAL, 17, 587 (1895).

[2] The indicated amounts of reagent solutions are included within the final volume of 100 cc. which, as explained above, was the same in every case.

TABLE II.—EFFECT OF SALTS ON SACCHAROGENIC ACTIVITY—(*Continued*).

Amount of salt added.[1]	Molar concent. of salt.	Reduction in mg. of Cu_2O.	Power.
17.—Sodium sulfate. (0.07 mg. Malt Amylase; 100 cc. neutral 2% Starch 6 in each case.)			
First series. (Prep 118.)			
None	0.00	65.4	287
1.0 cc. 0.33 *M*	0.0033	70.4	309
5.0	0.0166	84.2	373
10.0	0.0333	99.5	446
20.0	0.0666	118.5	533
30.0	0.1000	139.7	631
Second series. (Prep. 111A.)			
None	0.00	55.8	245
30.0	0.1	125.2	561
40.0	0.134	141.6	641
50.0	0.167	140.9	636
18.—Potassium sulfate. (0.07 mg. Malt Amylase; 100 cc. neutral 2% Starch 6 in each case).			
First series. (Prep. 118.)			
None	0.00	65.5	290
1.0 cc. 0.67 *M*	0.0067	80.3	354
5.0	0.0334	109.1	501
10.0	0.0668	135.3	608
20.0	0.134	156.8	712
30.0	0.2	166.0	754
Second series. (Prep. 111A.)			
None	0.00	55.8	245
30.0	0.2	153.1	693
40.0	0.268	163.6	745
19.—Primary sodium phosphate. (0.07 mg. Malt Amylase; 100 cc. 2% Starch in each case.)			
First series. (Prep. 111A; Starch 6.)			
None	0.00	51.7	243
2.5 cc. 0.2 *M*	0.005	111.5	500
5.0	0.01	125.2	561
10.0	0.02	145.4	655
20.0	0.04	152.0	688
30.0	0.06	159.5	726
Second series. (Prep. 118; Starch 2*b*.)			
None	0.00	6.9	29
10.0 cc. 0.2 *M*	0.02	163.2	740
20.0	0.04	188.7	865
30.0	0.06	202.6	934
20.—Primary potassium phosphate. First series. (0.2 mg. Malt Amylase 43B; 100 cc. 2% Starch 2*a* in each case.)			
None	0.00	106.9	167
25.0 cc. 0.2 *M*	0.05	202.1	325
30.0	0.06	200.5	323
Second series. (0.07 mg. Malt 111A; 100 cc. 2% Starch 6 in each case.)			
None	0.00	57.3	249
5.0 cc. 0.2 *M*	0.01	130.4	584
10.0	0.02	143.7	650
30.0	0.06	152.8	693
Third series. (0.07 mg. Malt 118; 100 cc. 2% Starch 6 in each case.)			
None	0.00	65.3	285
30.0 cc. 0.2 *M*	0.06	185.8	860

In the case of the primary sodium phosphate it will be seen that the optimum activities observed were at 0.06 *M*.

Since, as already explained, the experimental method did not permit of carrying the comparison to concentrations higher than 0.06 *M*, the possibility of activation still higher than shown at that point is not excluded, but the form of curve obtained on plotting the results makes it probable that approximately the optimum activation is reached at 0.06 *M*.

Measurements of hydrogen-ion concentration in 0.06 *M* sodium and potassium acid phosphates gave Sörensen exponents of 4.6 and 4.2, respectively.

[1] The indicated amounts of reagent solutions are included within the final volume of 100 cc. which, as explained above, was the same in every case.

The Influence of Acids and Salts upon the Amyloclastic Action.

Determination of Amyloclastic Power.—For determination of amyloclastic power of the amylase a procedure based upon the method of Wohlgemuth[1] was used. This procedure is as follows: Forty-two test tubes very carefully cleaned and dried are placed in a special wire frame or basket and the whole set in a bath of ice water. Then 2.5 cc. of 2% starch solution are carefully measured into each of the tubes by means of a buret which has a very long delivery tip reaching to the bottom of the test tube, thus avoiding the lodging of any of the solution on the sides of the tube.

To the starch solution in the tubes the solution of activating agent is added in the same manner, then enough pure water is added to each tube to make the final volume exactly 5 cc. and the concentration of starch 1%. After each addition of activating agent and water the tube is shaken to mix thoroughly. Very carefully measured portions of enzyme solution are now introduced into each tube and the tube agitated to ensure a perfect mixture. No reaction takes place because the solutions are kept cold by the ice water in which the basket of tubes rests.

The basket of tubes is transferred from the ice water to the constant temperature 40° bath and at the expiration of 30 min. is taken out and placed in the ice water to stop the action. Thus the tubes are subjected to an average temperature of 40° for 30 min. After a few minutes 0.1 cc. of 0.1 *N* iodine in potassium iodide solution is added to each tube. It is extremely important that the same amount of iodine solution be added

TABLE III.—EFFECT OF ACETIC ACID ON AMYLOCLASTIC ACTION.
(Malt Amylase preparation 1'18; Starch 6.)

Concentration of acid. *Power.*	Weights of enzyme in mg. 0.028.	0.035.	0.042.	0.049.	0.056	0.063.
.....	Red-violet	*Red	Red	Orange-red	Orange	Orange
143,000	(heavy)					(faint)
0.00008 *N*	Violet-red	*Red	Orange-red	Red-orange	Orange	Yellow-
143,000						orange
						(faint)
0.00016 *N*	Violet-red	*Red	Orange-red	Red-orange	Orange	Yellow-
143,000		(light)			(faint)	orange
						(faint)
0.00024 *N*	Violet-red	*Red	Orange-red	Orange	Orange	Yellow-
143,000						orange
0.00032 *N*	Violet-red	*Red	Orange-red	Red-orange	Orange	Orange
143,000						(faint)
0.00040 *N*	Violet-red	*Red	Red (light)	Red-orange	Orange	Orange
143,000						(faint)
0.00080 *N*	Violet	Violet-red	*Red	Red (light)	Orange-red	Red-orange
119,000						

* The asterisk designates the end point tube.

[1] *Biochem. Z.*, 9, 1 (1908).

to each tube since variation in the iodine concentration will give serious differences in color value. In this laboratory a dropping bottle is used that delivers drops of 0.1 cc. volume. About 20 cc. of distilled water are then poured into each tube, filling it within about 2 cm. of the top, and the contents thoroughly mixed. Each given set of tubes (containing the same amount of added reagent) is then observed for the end point, *i. e.*, the tube of lowest enzyme concentration which shows none of the familiar blue or violet color due to starch, and the weight of 1% starch solution, 5000 mg., is divided by the weight in milligrams of enzyme present to give the numerical value of the amyloclastic power of the enzyme.

TABLE IV.—EFFECT OF PROPIONIC ACID ON AMYLOCLASTIC ACTION.
(Malt Amylase preparation 118; Starch 6.)

Contentration of acid. *Power.*	Weights of enzyme in mg. 0.028.	0.035.	0.042.	0.049.	0.056.	0.063.
.....	Red-violet	*Red	Orange-red	Red-orange	Red-orange	Orange
143,000						
0.00008 *N*	Violet-red	*Red	Orange-red	Red-orange	Orange	Orange
143,000						
0.00016 *N*	Violet-red	*Red	Orange-red	Red-orange	Orange	Orange
143,000						
0.00024 *N*	Violet-red	*Red	Orange-red	Red-orange	Orange	Orange
143,000						
0.00032 *N*	Violet-red	*Red	Orange-red	Red-orange	Red-orange	Orange
143,000						
0.00040 *N*	Red-violet	*Red	Orange-red	Orange-red	Orange	Orange
143,000						
0.00080 *N*	Violet	Violet-red	*Red	Red	Orange-red	Orange-red
119,000						

TABLE V.—EFFECT OF PHOSPHORIC ACID ON AMYLOCLASTIC ACTION.
(Malt Amylase preparation 111A; Starch 6.)

Concentration of acid. *Power.*	Weights of enzyme in mg. 0.020.	0.024.	0.028.	0.032.	0.036.	0.040.
.....	Violet	Violet-red	Violet-red (light)	*Red (light)	Orange-red	Red-orange
157,000						
0.00008 *N*	Red-violet	Violet-red	*Red	Orange-red	Red-orange	Orange
179,000						
0.00016 *N*	Violet-red (heavy)	*Red	Orange-red	Red-orange	Orange	Yellow-orange
208,000						
0.00024 *N*	Violet-red	*Red	Orange-red	Red-orange	Orange	Yellow-orange
208,000						
0.00032 *N*	Red-violet	Violet-red	*Red (light)	Red-orange	Orange	Orange
179,000						
0.0004 *N*	Red-violet (heavy)	Violet-red (heavy)	*Red	Orange-red	Orange-red	Red-orange
179,000						
0.0006 *N*	Blue	Blue-violet	Red-violet	Violet-red	Violet-red	*Red
125,000						

* The asterisk designates the end point tube.

For example, six portions of enzyme solution containing 0.2 mg. per cc. were measured into six tubes as follows: 0.3 cc., 0.4 cc., 0.5 cc., 0.6 cc., 0.7 cc., and 0.8 cc. After carrying out the procedure just described the following colors were obtained: 1, Blue; 2, Blue; 3, Red-Violet; 4, Violet-Red; 5, Red; 6, Orange-Red. The Wohlgemuth end point was reached in tube No. 5. This tube contained 0.7 cc. or 0.14 mg. of enzyme, therefore the power for 30 min. digestion at 40° ($D_{30'}^{40°}$) is equal to 5000/0.14 = 35,714 or in round numbers 35,700.

TABLE VI.—EFFECT OF HYDROCHLORIC ACID ON AMYLOCLASTIC ACTION.
(Malt Amylase preparation 111A; Starch 6.)

Concentration of acid. *Power.*	Weights of enzyme in mg. 0.020.	0.024.	0.028.	0.032.	0.036.	0.040.
.....	Violet	Red-violet	†Violet-red (pale)	†Orange-red	Red-orange	Red-orange
167,000						
0.00004 *N*	Violet	Violet-red	*Red	Orange-red	Orange	Orange
179,000						
0.00008 *N*	Violet-red	Violet-red (pale)	*Red (light)	Red-orange	Orange	Yellow-orange
179,000						
0.00012 *N*	Red-violet	Violet-red	†Violet-red (pale)	†Red-orange	Orange	Orange
167,000						
0.00014 *N*	Violet	Violet-red	Violet-red	*Red	Orange-red	Red-orange
157,000						
0.00016 *N*	Blue	Red-violet	Violet-red	*Red	Red (pale)	Red (pale)
157,000						
0.00020 *N*	Blue	Blue	Violet	Red-violet	Red-violet	Violet-red (pale)
113,000						

TABLE VII.—EFFECT OF NITRIC ACID ON AMYLOCLASTIC ACTION.
(Malt Amylase preparation 111A; Starch 6.)

Concentration of acid. *Power.*	Weights of enzyme in mg. 0.024.	0.028.	0.032.	0.036.	0.040.	0.044.
...	Red-violet (heavy)	Violet-red	*Red	Orange-red	Red-orange	Orange
157,000						
0.00004 *N*	Violet-red (heavy)	*Red	Red (light)	Orange-red	Red-orange	Orange
179,000						
0.00008 *N*	Violet-red	*Red	Red-orange	Red-orange	Orange	Yellow-orange
179,000						
0.00012 *N*	Violet-red (heavy)	*Red	Red	Red (light)	Orange-red	Red-orange
179,000						
0.00014 *N*	Blue-violet	Violet-red	*Red	Red (light)	Orange-red	Orange-red
157,000						
0.00016 *N*	Blue	Red-violet	Violet-red	Violet-red (faint)	*Red (light)	Orange-red
125,000						
0.0002 *N*	Blue	Blue	Violet	Red-violet (heavy)	Violet-red (heavy)	Violet-red
less than						
113,000						

* The asterisk designates the end point tube.

† In these cases the quality of colors was such that the end point was judged to be half-way between the two tubes.

In testing the effect of any reagent to be examined, six or seven tubes are ordinarily tested with the same amount of reagent but with varying amounts of enzyme solution. Seven or six such sets of tests, each set containing a different amount of reagent, are made for every reagent to be examined. Hence each set of tubes contains the same concentration of reagent whose effect is to be determined, but differing concentrations of enzyme while the several sets of tubes differ from each other in that they contain varying concentrations of activating agent.

TABLE VIII.—EFFECT OF SULFURIC ACID ON AMYLOCLASTIC ACTION.
(Malt Amylase preparation 111A; Starch 6.)

Concentration of acid. *Power.*	Weights of enzyme in mg. 0.024.	0.028.	0.032.	0.036.	0.040.	0.044.
..... *157,000*	Violet	Violet-red	*Red	Orange-red	Orange-red	Red-orange (faint)
0.00004 *N* *157,000*	Red-violet	Violet-red	*Red	Orange-red	Orange-red	Red-orange (faint)
0.00008 *N* *179,000*	Violet-red	*Red	Red	Orange-red	Red-orange	Red-orange (faint)
0.00012 *N* *167,000*	Red-violet	†Violet-red	†Orange-Red	Orange-red	Red-orange	Red-orange (faint)
0.00014 *N* *157,000*	Violet	Violet-red (heavy)	*Red	Red (light)	Red (light)	Orange-red
0.00016 *N* *139,000*	Violet (heavy)	Violet-red (heavy)	Violet-red (heavy)	*Red (dark)	Red	Red
0.0002 *N* *less than 113,000*	Blue	Blue	Violet (heavy)	Violet-red (heavy)	Violet-red (heavy)	Violet-red

TABLE IX.—EFFECT OF SODIUM CHLORIDE ON AMYLOCLASTIC ACTION.
(Malt Amylase preparation 118; Starch 6.)

Concentration of salt. *Power.*	Weights of enzyme in mg. 0.021.	0.028.	0.035.	0.042.	0.049.	0.056.
..... *143,000*	Blue	Violet-red	*Red	Orange-red	Red-orange	Orange
0.08 *M* *143,000*	Blue	Violet-red	*Red	Orange-red	Red-orange	Orange
0.16 *M* *158,700*	Violet	†Violet-red	†Red	Orange-red	Orange	Orange
0.32 *M* *143,000*	.....	Violet-red	*Red	Red	Red-orange	Orange
0.48 *M* *119,000*	.....	.....	Violet-red	*Red	Orange-red	Orange-red
0.64 *M* *119.000*	.....	.....	Violet-red	*Red	Red	Orange-red
0.8 *M* *102,000*	.....	.....	.....	Violet-red	*Red	Orange-red

* End point was found with 0.5 mg. enzyme.

† In these cases the quality of colors was such that the end point was judged to be half-way between the two tubes.

In the tables which follow the "*power*," *i. e.*, the numerical result calculated as above from the amount of enzyme preparation required to carry the digestion to the Wohlgemuth end point, is given in the first column with the concentration of reagent, while the columns following show the colors[1] obtained in each test of the set. One reason for recording all of these colors is to facilitate comparison with somewhat analogous

TABLE X.—EFFECT OF POTASSIUM CHLORIDE ON AMYLOCLASTIC ACTION.

(Malt Amylase preparation 43B; Starch 6.)

Concentration of salt.	Weights of enzyme in mg.					
Power.	0.6.	0.8.	0.9.	1.0	1.2.	1.4.
.....	Violet-red	Violet-red	Violet-red	*Red	Orange-red	.....
54,300		(light)	(light)	(light)		
0.008 *M*	Red-violet	*Red	Red (light)	Red-orange	.....	.....
62,500						
0.08 *M*	Violet-red	*Red	.....	.....	Orange	.....
62,500					(light)	
0.16 *M*	Violet-red	†Violet-red	†Red-	Red-orange	Red-orange	.....
59,500		(light)	orange			
0.32 *M*	Blue	Violet	*Red	Orange	Orange	.....
54,300						
0.4 *M*	Blue	Blue-violet	.....	†Violet-red	†Red-	Yellow-
49,000					orange	Orange
0.8 *M*	Blue	Blue	.....	Blue	Blue	Violet-red
35,200[2]						

TABLE XI.—EFFECT OF SODIUM NITRATE ON AMYLOCLASTIC ACTION.

(Malt Amylase preparation 118; Starch 6.)

Concentration of salt.	Weights of enzyme in mg.					
Power.	0.014.	0.021.	0.028.	0.035.	0.042.	0.049.
.....	Blue	Red-violet	Violet-red	*Red	Orange	Orange
143,000						
0.08 *M*	Blue	Red-violet	*Red	Red (light)	Red-orange	Orange
179,000						
0.16 *M*	Blue	Red-violet	*Red	Red (light)	Orange	Yellow-
179,000						orange
0.32 *M*	Blue	Violet	Violet-red	*Red	Red	Orange
143,000						
0.48 *M*	Blue	Violet	Violet-red	*Red	Red	Orange
143,000			(dark)	(dark)		
0.64 *M*	Blue	Violet	Violet-red	*Red	Red	Red
143,000		(dark)	(dark)	(dark)		
0.8 *M*	Blue	Blue-violet	Red-violet	Violet-red	*Red	Red
119,000					(dark)	

† In these cases the quality of colors was such that the end point was judged to be half-way between the two tubes.

[1] The terminology used in describing the colors is that of the Milton Bradley Standard Color Chart as given by Mullikin in his "Identification of Pure Organic Compounds."

[2] End point was found with 1.5 mg. enzyme.

studies of other amylases in which the results are similarly expressed, such as those of Long and Johnson.[1]

Another reason is that to judge of the amyloclastic activity of a purified preparation of malt amylase on the basis of the Wohlgemuth end point exclusively is likely to be misleading because of the relative persistence of material giving a slight blue or violet iodine reaction when starch is digested by preparations of this character. Thus in the case of the example given above, while the Wohlgemuth end point was found in tube 5 containing 0.7 cc. of enzyme solution or 0.14 mg. of the enzyme preparation, yet in tube 3 having 0.5 cc. or 0.10 mg. there was no

TABLE XII.—EFFECT OF POTASSIUM NITRATE ON AMYLOCLASTIC ACTION.
(Malt Amylase preparation 118; Starch 6.)

Concentration of salt.	Weights of enzyme in mg.					
Power.	0.014.	0.021.	0.028.	0.035.	0.042.	0.049.
.....	Blue	Blue-violet	Violet-red	*Red	Orange-red	Red-orange
143,000						
0.05 *M*	Blue	Blue-violet	Violet-red	*Red	Red-orange	Orange
143,000						
0.1 *M*	Blue	Violet	Violet-red	*Red	Red-orange	Orange
143,000+				(light)		
0.2 *M*	Blue	Violet	*Red	Orange-red	Red-orange	Orange
179,000						
0.4 *M*	Blue	Violet	Violet-red	*Red	Red-orange	Orange
143,000+			(light)	(light)		
0.5 *M*	Blue	Violet	Violet-red	*Red	Orange-red	Red-orange
143,000						
0.75 *M*	Blue	Blue-violet	Violet	Violet-red	*Red	Orange-red
119,000						

TABLE XIII.—EFFECT OF SODIUM SULFATE ON AMYLOCLASTIC ACTION.
(Malt Amylase preparation 111A; Starch 6.)

Concentration of salt.	Weights of enzyme in mg.					
Power.	0.0175.	0.0210.	0.0245.	0.0280.	0.0315.	0.0350.
.....	Blue-violet	Violet	Red-violet	Violet-red	*Red	Orange-red
160,000						
0.0133 *M*	Blue-violet	Violet	Red-violet	Violet-red	*Red	Red-orange
160,000						
0.0267 *M*	Violet	Red-violet	Violet-red	*Red	Red	Red-orange
180,000						
0.04 *M*	Violet	Red-violet	Violet-red	*Red	Orange-red	Red-orange
180,000						
0.053 *M*	Violet	Red-violet	Violet-red	*Red	Red-orange	Red-orange
180,000+			(pale)	(light)		
0.067 *M*	Violet	Red-violet	Violet-red	*Red	Orange-red	Red-orange
180,000						
0.133 *M*	Blue-violet	Red-violet	Violet-red	*Red	Orange-red	Red-orange
180,000						

* The asterisk designates the end point tube.

[1] THIS JOURNAL, 35, 895 (1913).

typical starch-iodine blue. If this were taken as the end point the power of the enzyme would be 50,000 or about 40% higher. Examination of the color readings in Tables XVI will show that in many, if not in most, cases the amount of enzyme required to carry the digestion to the "red" end point is one-third to one-half greater than that which carries it to the disappearance of the typical "blue." In view of the fact that this point has been dealt with in a recent paper from this laboratory,[1] it need not be further discussed here, nor in connection with the tables which are given:

There is no apparent activation of the amyloclastic action by the addition of acetic acid, but beyond the concentration at which the optimum saccharogenic action was obtained there is depression of the amyloclastic as of the saccharogenic power.

The results in Table IV are closely analogous to those just noted in the case of acetic acid.

with phosphoric acid there is a distinct activation. It will be noted that the optimum concentration of phosphoric acid for amyloclastic action as judged by the Wohlgemuth end point is much lower than the optimum for saccharogenic activity. Attention may also be called to the persistence of colors intermediate between blue and red which has already been referred to at the top of the page.

For HCl, as in the case of phosphoric acid, the optimum is much lower than for saccharogenic action and the concentration which gives optimum saccharogenic action has a distinct inhibitory influence upon the amyloclastic action as measured by this method. The unsatisfactory nature of the end point is again apparent.

What has been said of the influence of hydrochloric acid upon amyloclastic action will be seen to apply also to nitric and sulfuric acids.

TABLE XIV.—EFFECT OF POTASSIUM SULFATE ON AMYLOCLASTIC ACTION.
(Malt Amylase preparation 111A; Starch 6.)

Concentration of salt.	Weights of enzyme in mg.					
Power.	0.0175.	0.0210.	0.0245.	0.0280.	0.0315.	0.0350.
.....	Violet-blue	Blue-violet	Red-violet	Violet-red	*Red	Orange-red
160,000						
0.0267 *M*	Violet-blue	Violet	Violet-red	*Red	Orange-red	Red-orange
180,000						
0.0534 *M*	Violet	Red-violet	Violet-red	*Red	Orange-red	Red-orange
180,000	(dark)					
0.08 *M*	Blue-violet	Red-violet	*Red	Red (light)	Orange-red	Red-orange
204,000						
0.107 *M*	Violet	Red-violet	*Red	Red (light)	Orange-red	Red-orange
204,000	(dark)					
0.1335 *M*	Blue-violet	Red-violet	Violet-red	*Red	Orange-red	Red-orange
180,000				(light)		

* The asterisk designates the end point tube.

[1] THIS JOURNAL, 35, 1784 (1913).

With the chlorides, nitrates, and sulfates of sodium and potassium, as with the corresponding acids, the optimum concentrations are much lower for the (apparent) amyloclastic than for the saccharogenic action.

TABLE XV.—EFFECT OF PRIMARY SODIUM PHOSPHATE ON AMYLOCLASTIC ACTION.
(Malt Amylase preparation 118; Starch 6.)

Concentration of salt. *Power.*	Weights of enzyme in mg. 0.028.	0.035.	0.042.	0.049.	0.056.	0.063.
.....	Violet	*Red	Orange-red	Red-orange	Orange	Yellow-orange
143,000						
0.008 *M*	Violet-red	*Red	Orange-red	Red-orange	Orange	Yellow-orange
143,000+						
0.016 *M*	†Violet-red (light)	†Red (light)	Orange-red (light)	Orange	Yellow-orange	Orange-yellow
159,000						
0.024 *M*	†Violet-red	†Red (light)	.....	Yellow-orange	Yellow-orange (faint)	Orange yellow
159,000						
0.032 *M*	†Violet-red	†Red (light)	Orange-red (faint)	Orange (faint)	Yellow-orange	Orange-yellow
159,000						
0.04 *M*	Violet-red	*Red	Orange-red	Orange	Yellow-orange	Orange-yellow
143,000+						
0.08 *M*	Violet-red	*Red	Orange-red	Red-orange	Orange	Yellow-orange
143,000						

TABLE XVI.—EFFECT OF PRIMARY POTASSIUM PHOSPHATE ON AMYLOCLASTIC ACTION.
(Malt Amylase preparation 43B; Starch 6.)

Concentration of salt. *Power.*	Weights of enzyme in mg. 0.4.	0.6.	0.8.	0.9.	1.0.	1.2.
.....	Blue	Blue	Violet-red	*Red	Orange-red	Red-orange
55,600						
0.008 *M*	Blue	Violet-red	*Red (light)	.....	Orange	Yellow-orange
62,500						
0.02 *M*	Blue	Red-violet	*Red (light)	.....	Orange-red	Orange (light)
62,500						
0.04 *M*	Blue	Blue	Red-violet	Violet-red	Violet-red	*Red
41,700						
	0.6.	0.8.	1.0.	1.2.	1.4.	1.6.
0.06 *M*	Blue	Red-violet	Violet-red	Violet-red	*Red	Orange-red
35,700						
	0.8.	1.0.	1.2.	1.4.	1.6.	1.8.
0.08 *M*	Violet	Violet	Red-violet	Violet-red	Violet-red	*Red
29,400						
	1.0.	1.2.	1.4.	1.6.	2.0.	2.4.
0.1 *M*	Blue-violet	Red-violet	Violet-red	Violet-red	Violet-red (pale)	*Red (pale)
22,200						

* The asterisk designates the end point tube.

† In these cases the quality of colors was such that the end point was judged to be half-way between the two tubes.

In the case of the acid phosphates, as of the free acids and the neutral salts, optimum amyloclastic activities, as measured by the Wohlgemuth

method, are reached at concentrations much lower than those which give optimum saccharogenic action, and the latter concentrations distinctly depress the amyloclastic action.

Summary.

The effects of acetic, propionic, phosphoric, hydrochloric, nitric and sulfuric acids, and the chlorides, nitrates, sulfates and primary phosphates of sodium and potassium have been studied with special reference to the determination of concentrations favoring optimum activity of malt amylase.

Previous studies made in this laboratory, showing that the measurement of amyloclastic action by determining the conditions under which the starch-iodine end point occurs, gives with purified malt amylase results which are of little value compared with those obtained by the measurement of the saccharogenic action, have been confirmed. Full data of the amyloclastic action have been given in the foregoing text. The conclusions which follow refer, except as otherwise explained, to the saccharogenic action.

Special attention has been given to the purification of the amylase preparations, the starch, the activating agents, and the water used. While we have not, as in the case of pancreatic amylase, observed any case of complete inactivation of the enzyme through deficiency of electrolyte alone, it is plain that the activities shown by purified materials in the absence of added electrolytes are to be regarded as abnormally low.

All of the electrolytes above mentioned increased the activity of malt amylase. The activities observed at the optimum concentrations of these different activating electrolytes varied from less than double to more than thirty-fold the corresponding activities in the absence of the electrolyte; depending chiefly upon the purity of the enzyme preparation and the starch employed. In comparing the activating influence of different electrolytes more weight should be attached to the maximum saccharogenic power demonstrated in the case of each electrolyte by the same enzyme preparation than to the increase of power above that shown when no electrolyte was added.

In those cases in which the use of the same enzyme permits comparison of results obtained in different series of experiments, it appears that the acids had a greater activating influence than the neutral salts, while the acid phosphates of sodium and potassium gave as high an activation as did any of the free acids. The main object held in view in the arrangement of the experiments was, however, not to compare the acids and salts with each other but to establish the optimum concentration of each of the activating agents considered separately, and particularly the optimum of each acid in terms of hydrogen-ion concentration.

The weak acids (acetic, propionic, phosphoric), the strong acids (hydro-

chloric, nitric, sulfuric), and the acid phosphates of sodium and potassium, all showed optimum activation in those concentrations which have essentially the same actual acidity. This optimum hydrogen-ion concentration, as determined by the electrometric method and expressed by Sörensen's exponent, was found in each case between the limits p_H^+ 4.2 to 4.6.

Additions of free acid in concentrations greater than the optimum have a marked depressing influence upon the activity of the enzyme which is naturally more striking in the case of the stronger acids. Acetic and propionic acids in quantities ten times the optimum decrease the activity about one-half; hydrochloric, nitric and sulfuric acids reduce the activity more than one-half when present in concentrations two and one-half times the optimum, while in presence of five to eight times the optimum of these strong acids the enzyme action was almost entirely destroyed. In determining the diastatic power of malt preparations, acid phosphate may conveniently be used to ensure activation with little danger of excessive acidity.

Whether the activating agent be an acid or a salt, the amyloclastic action, as measured by the Wohlgemuth method, reaches an optimum at a concentration of the activating agent much below that which gives optimum saccharogenic action. Those concentrations which give the optimum saccharogenic activity are so far above the optimum for amyloclastic action (Wohlgemuth method) as to show a distinct inhibitory influence.

We desire to express our indebtedness to the Carnegie Institution of Washington for use of malt amylase preparations which had been purified in connection with investigations conducted by aid of its grants and described in other papers from this laboratory.

For the data given in Tables X and XVI we are indebted to our former associate, Dr. C. F. Hinck.

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STUDIES ON AMYLASES. IX. FURTHER EXPERIMENTS UPON THE PURIFICATION OF MALT AMYLASE.

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In continuing our experiments upon the purification of malt amylase[1] the operations of precipitation, solution, and dialysis which are involved in the purification process have been studied in some detail, with a view to determining quantitatively the loss of diastatic power at each step and,

[1] THIS JOURNAL, 35, 1617–23 (1913).