fluences which cause a strong electrolyte to deviate from the Mass-Action law at high concentrations *gradually and steadily* become smaller and smaller and finally disappear at infinite dilution." The new method then "consists simply in plotting values of K_E, the Mass-Action expression, against corresponding values of the concentration, employing different assumed values of Λ_0, and rejecting those values which cause the curve in dilute solutions to exhibit radical changes in direction."

Reference to the literature will show that this identical method, founded upon precisely the same assumption, was employed by the present writer six years ago,[1] in the determination of the velocity of the hydrogen ion.

Further and extended comment upon Washburn's articles must be postponed until the writer is free to return to pure scientific work.

JAMES KENDALL.

[CONTRIBUTION FROM THE HAHNEMANN MEDICAL COLLEGE AND HOSPITAL OF CHICAGO.]

ON THE DIGESTIBILITY OF BREAD.

III. ERYTHRODEXTRIN IN STARCH HYDROLYSIS.

BY J. C. BLAKE.

Received December 22, 1917.

It was shown in the second paper of this series[2] that amylolytic activity may be followed quantitatively with great facility by the digestion of erythrodextrin to the achromic point. Efforts made to obtain pure erythrodextrin, partly for the purpose of standardizing amylolytic agents and partly for clinical use in this connection, led to some new discontinuous hydrolyses of starch and to some new methods of estimating the relative concentrations of some of its decomposition products. It is believed that as a consequence of this work pure erythrodextrin will soon be prepared.

New Methods of Analysis.

The relative concentrations of 4 of the decomposition products present in boiled starch partially hydrolyzed with dil. hydrochloric acid were estimated by reading the colorations given with iodine water of different concentrations against Lovibond color glasses by means of a Duboscq colorimeter. The smallest amount of iodine gives a yellow color, thought to be due to protein.[3] Further addition of iodine gives an orange color, then a red or purple, the red color being due to erythramylum.[4] Further

[1] *J. Chem. Soc.*, 101, 1279 and 1291 (1912).

[2] THIS JOURNAL, 39, 315 (1917).

[3] Blake, THIS JOURNAL, 38, 1247 (1916). It is well known that protein gives a yellow color with iodine, especially well seen with the gluten of bread.

[4] This name, given to this substance by Brücke at the time he named the dextrins (*Ber. Wien. Akad.*, 65, 126 (1872)), from which he clearly distinguished it, has preference over the name rose-amylose proposed by Day ("Digestibility of Starch," etc., University of Chicago Press, 1908, pp. 37, 41). The name cellulose originally given to it by C. Nägeli ("Die Stärkekörner," Zurich, 1858; *Ber. Akad. München*, 1862, p. 281) is, of

addition of iodine gives purples, violets and blues, the blue being due to amylodextrin (*infra*). A subsequent addition of iodine gives the red color of erythrodextrin, a very large excess of iodine finally giving the yellow (and red) color of the iodine itself. The color of iodine water is 2 red plus 5 yellow of the Lovibond scale; that is, the red equals 40% of the yellow.

The quantitative estimation of the relative concentrations of these substances is based on the fact that increasing amounts of iodine give in each case a sharp maximum of the color due to each of these four substances. This is shown in the following tables, taken at random from several hundred made in this connection. It should be said that under all the conditions used for the readings recorded in this paper the protein and the erythramylum remain associated with the cell walls of the starch granules in such fashion that they do not pass through an ordinary filter paper. All of the work here recorded was done with 4% Oswego Corn Starch, except where otherwise stated.

TABLE I.—MAXIMA DUE TO PROTEIN (YELLOW) AND ERYTHRAMYLUM (RED).
10 cc. of hydrolysis mixture, unfiltered.

Saturated iodine water.	Lovibond slides.			Total color.		
Cc.	Blue.	Red.	Yellow.	Blue.	Red.	Yellow
1/2	0	2.1	2.7	0	2.1	2.7
2	1.1	4.5	2.2	7.0	27.0	13.2
4	2.9	5.0	2.2	29.0	59.0	22.0
6	5.0	8.0	2.2	50.0	80.0	22.0
11	5.0	5.0	Trace	95.0	95.0	Trace
12 1/2	6.8	5.0	Trace	148.0	86.0	Trace

The maxima of 22 yellow and of 95 red are sharply defined. None of this color is due to free iodine.[1] In fact, no free iodine is present, as free iodine is present only after the blue maximum has been passed (not reached in this table) and the maximum due to erythrodextrin has been nearly reached. The yellow color due to the protein is obscured by the large amount of blue color in the last two readings of the table.

course, unsuitable. Nägeli later called this substance starch cellulose (Czapek, "Biochemie der Pflanzen," **1905**, p. 318. Griessmayer (*Ann.*, **160**, 46 (1871)), called it a dextrin whose iodide forms before the blue iodide of starch. It is the "soluble starch" of Musculus and Gruber (*Bull. soc. chim.*, [2] **30**, 67); the "amylodextrin" of W. Nägeli (*Beiträge zur Näheren Kenntnis der Stärkegruppe*, **1872**, ref. Arthur Meyer, "Untersuchungen über die Stärkekörner," **1895**, p. 27; Czapek, *Loc. cit.*, p. 320; Brown and Morris, *J. Chem. Soc.*, **47**, 527 (1885)). The latter authors sharply differentiate between soluble starch and this amylodextrin of W. Nägeli, whereas Musculus and Meyer (*Z. physiol. Chem.*, 4, 454 (1880)) conclude that erythrodextrin is a mixture of this "soluble starch" and "dextrin" (achroödextrin)—a pronouncement coloring much of the more recent literature (cf. Meyer and Jacobson, "Lehrbuch der Organischen Chemie," **1913**, pp. 1033–4), and doubtless accounting for the fact that Merck's "highest purity dextrin" is almost free from erythrodextrin.

[1] Free iodine, as here understood, gives a red color when added to erythrodextrin in solution.

TABLE II.—MAXIMA DUE TO AMYLODEXTRIN (BLUE) AND ERYTHRODEXTRIN (RED).

0.200 cc. of hydrolysis filtrate.

Iodine water. Cc.	Total blue.	Total red.
40	59	11
70	60	25
115	30	22
135	25	20

Here the maxima (60 blue and 25 red) are equally sharp, numerous other readings having shown that there are no other maxima between the readings here recorded. The last reading is appended to show that a very large amount of iodine water does not give an increase in the total red color when much blue is present. Under these conditions the maximum red is attained when the yellow tint first reappears in the solution, a reading for the red greater than the true maximum being obtained only when the yellow due to the free iodine is greater than 15% of the total red color.

Typical readings showing this use of the yellow glass are as follows:

TABLE III.—MAXIMA DUE TO AMYLODEXTRIN AND ERYTHRODEXTRIN WITH EXCESS OF IODINE (YELLOW).

0.500 cc. hydrolysis filtrate.

Iodine water. Cc.	Lovibond glasses.			Total color.		
	Blue.	Red.	Yellow.	Blue.	Red.	Yellow.
5	5.0	2.1	0	50	21	0
8	7.0	5.0	0	63	45	0
10	7.0	6.0	0	74	64	0
13	6.0	6.0	0	66	66	0
17	6.0	7.0	0	60	70	0
21	6.0	8.0	0	58	78	0
26	5.0	6.0	0.5	60	72	6.0

The maxima here (74 blue and 78 red) are sufficiently distinct. Since the red of the iodine water (last reading) equals 40% of the yellow (measured on the Lovibond scale), the error in the red due to the iodine would be 2 out of 72 in this experiment, indicating a true reading of 70. This is thought to be within the experimental error, but whether applied or not, gives a reading distinctly less than the previous reading (78).

In case no blue color is present, the maximum red due to the erythrodextrin is not developed until the yellow due to the iodine equals the total red as shown in the following table, again showing that the blue and yellow largely neutralize each other in solutions when both colors are present:

TABLE IV.—MAXIMUM DUE TO ERYTHRODEXTRIN IN THE ABSENCE OF AMYLODEXTRIN.

0.100 cc. hydrolysis filtrate.

Iodine water. Cc.	Lovibond glasses.			Total color.			Total red minus 40% of total yellow.
	Blue.	Red.	Yellow.	Blue.	Red.	Yellow.	
0.51	0	5.0	2.2	0	6.5	2.86	5.5
1.41	0	5.9	5.0	0	9.8	7.5	6.8
2.64	0	5.0	5.0	0	10.0	10.0	6.0
4.47	0	5.0	7.2	0	9.5	14.0	4.0
9.05	0	3.0	5.5	0	13.0	24.0	3.0

Here the error due to the excess of iodine is material, changing the total red maximum of 10 to 6.8 (last column) as the red due to the erythrodextrin. If still more iodine is added (last reading) the error becomes excessive. No uncertainty for comparative results would be introduced, however, by reading the maximum where the red equals the yellow. No solutions free from amylodextrin are included in the hydrolyses here reported.

A very interesting case is the following, No. 14 of the hydrolysis of boiled starch recorded farther on, in which relatively much erythramylum, little amylodextrin, and much erythrodextrin were present. The readings in this case furnish an excellent test of the method employed, since the red color of the erythramylum tends to merge with the red color subsequently furnished by the erythrodextrin.

TABLE V.—ESTIMATION OF ERYTHRAMYLUM IN THE PRESENCE OF MUCH ERYTHRODEXTRIN.

10 cc. hydrolysis precipitate ("artificial starch") re-suspended in water.

Iodine water.	Lovibond glasses.			Total color.		
Cc.	Blue.	Red.	Yellow.	Blue.	Red.	Yellow.
2.2	0	0.9	0.5	0	9.9	5.5
2.8	0	2.1	0.5	0	25.0	6.0
4.6	0	3.0	0.5	0	31.0	5.0
5.9	1.8	5.0	0.5	26.0	72.0	7.0
7.1	2.9	8.0	0	29.0	80.0	0
11.0	5.0	5.9	0	77.0	91.0	0
15.0	5.0	5.0	0	128.0	128.0	0
19.0	6.1	5.0	0	159.0	131.0	0
22.0	6.8	5.9	0	177.0	153.0	0
26.0	6.8	5.9	0	191.0	165.0	0
30.0	5.0	5.0	0	206.0	206.0	0
35.0	5.0	5.0	0	217.0	217.0	0
42.0	6.1	5.9	0	247.0	239.0	0
49.0	6.8	8.0	0	272.0	320.0	0

Here no maximum in the red appears, and yet 131 was at once taken as representing the erythramylum, because the addition of 4 cc. of iodine water to the solution just previously read gave no material increase in the red coloration (128 to 131), and the subsequent large increase in the red was attributed to erythrodextrin. The value 131 falls on the smooth curve for erythramylum of Fig. 2. Moreover the ratio of iodine water to carbohydrate solution (1.9 : 1) is approximately the same (1.1 : 1) as that used for the maximum color due to erythramylum in Table I, and the difference is primarily due to the larger amount of reducing substances in the solution last reported which act on the iodine. Such a mixture is also obtained by the spontaneous fermentation of cereal starch (but not of potato starch) solutions after about a week, a fact which lead Griessmayer[1]

[1] *Loc. cit.*

to confound erythramylum with the dextrins. A mixture similar to that recorded in the last table probably lead Musculus and Meyer[1] to the conclusion already expressed, that erythrodextrin is a mixture of "soluble starch" (erythramylum) and achroödextrin.

It should be stated as the most convenient further method of confirmation of these conclusions, both in this case and in all others, that the order of disappearance of these colors when water is added to the mixture is the opposite of that of their appearance, the red color due to erythrodextrin going first, then the blue, then the red of erythramylum, and finally the yellow due to protein. In cases where a large excess of iodine water has been added to a solution containing erythrodextrin and but little amylodextrin, the final color on the addition of water is yellow instead of blue (due to iodine in excess as determined by the odor, the color and the test for free iodine already given).

Temperature of Crystallization of "Artificial Starch."

It is well known that partially hydrolyzed starch partially crystallizes on cooling,[2] the precipitate thus formed being known variously as "artificial starch," "soluble starch" (wholly different from Lintner's "soluble starch," however), and amylodextrin. The temperature at which this crystallization takes place was determined by immersing a thermometer in the solution held in a large test tube to the point at which the crystals were forming. This formation starts at the bottom owing to the fact that the cold liquid accumulates there.

Temperature of Transformation of the Iodides of Amylodextrin and Erythrodextrin.

This temperature was determined in a manner somewhat similar to that used for the determination of the temperature of crystallization, except that in this case the thermometer was held in the upper third of the hot liquid until the blue due to the iodide of amylodextrin or the red due to that of erythrodextrin entirely obscured the yellow color due to the iodine. The temperature at which the iodide first appears varies somewhat with the concentration of the dextrin and greatly with the concentration of the iodine, as is shown by the following results obtained with the filtrate from No. 1 of the hydrolysis of boiled starch reported farther on. The temperature at which the blue iodide appears increases as the concentration of the iodine increases. Since it is nearly constant above 12 volumes of iodine water, 15 volumes were added in making the determinations recorded farther on. The lower temperature obtained in the last reading (No. 1 Series) is due to the great dilution of the dextrin, as confirmed by separate experiments, thus accounting for the temperature of 50° given

[1] *Loc. cit.*

[2] Musculus, *Ann. chim.*, [3] **2**, 392 (1874) and ref. cit. in footnote 3; also Meyer u. Jacobson, *Loc. cit.*, pp. 1024, 1032.

TABLE VI.—TEMPERATURE OF FORMATION OF IODIDES.

Vol. dextrin sol.	Vol. sat. iodine water.	Temp. of formation.
	Filtrate from No. 1.	
1	6	61.5°
1	12	71.5°
1	18	72.5°
1	24	74.0°
1	48	71.5°
	Filtrate from No. 15.	
1	7	57°
1	15	59°
1	30	56°

in Paper I of this series.[1] The results obtained with the filtrate of No. 15, containing erythrodextrin but only a little amylodextrin, the color of the iodides in this case being nearly pure red, show that 15 volumes of iodine water would give practically the maximum temperature at which the iodide forms in all the solutions of the series tested. The decrease in the temperature of formation of this iodide which occurs with dilution of the dextrin (plainly seen in Fig. 2, Nos. 12 to 15) accounts for the temperature of 33° given in the paper last referred to (p. 1251).

"Roasting" Starch Moistened with Dilute Acids.

In approximate agreement with the commercial method of making dextrin, 14.00 g. of Oswego corn starch were moistened with 8.50 cc. of tenth-normal hydrochloric acid and put in an electric oven previously heated and regulated to 85°. The starch was so protected from the source of heat that no considerable differences in temperature could be detected in the region it occupied. The mixture was weighed at the end of 3 and of 4 hours, showing by difference that it had reached constant weight at the end of 3 hours. At intervals of one hour from 3 hours on portions of 1.0000 g. were removed, placed in 12.50 cc. of water and allowed to stand 2 hours, with frequent stirring. The material was then filtered in a Gooch crucible and washed 8 times, with suction to dryness at each washing. Although three washings usually remove all soluble material by this process, yet the eighth wash water still colored blue with iodine, due to the presence of a substance but slightly soluble in water (amylodextrin, pages 631, 635 (3)[2]. The residue was dried at 100° to constant weight. Part of the filtrate (unmixed with wash water) was evaporated to constant weight at 100°, from which the solid contents of the entire filtrate were determined. The filtrate was also polarized on a glucosimeter and its relative content of amylodextrin and erythrodextrin determined by the methods just described, except that Nessler tubes took the place of the colorimeter in this experiment.

[1] The reappearance at 50° of the red color due to erythrodextrin was plainly visible in this solution, due to the great dilution of the blue color by the iodine water.

[2] O'Sullivan, *J. Chem. Soc.*, **35**, 772 (1879).

TABLE VII.—HYDROLYSIS OF "ROASTED" (85°) ACID-STARCH.

Expt. No.	Time of heating. Hours.	Residue from 1 g. Gram.	Solid contents of filtrate. Gram.	Sum of Cols. 3 and 4.	Polarization on glucosimeter.	Blue for 1 cc. Lovibond scale.	Red for 1 cc. Lovibond scale.
1	3	0.884	0.111	0.995	2.83	17	46
2	4	0.790	0.210	1.000	5.66	17	86
3	5	0.747	0.253[1]	(1.000)	5.76	20	83
4	6	0.651	0.436	1.087	9.60	17	116
5	7	0.591	0.407	0.998	11.48	17	120
6	8	0.712	0.290	1.002	8.40	13	65
7	9	0.663	0.331	0.994	9.92	13	77
8	48	0.514	0.458	0.972	13.40	5	115

These results are plotted in Fig. 1. The fact that the weight of the residue plus the weight of the solid contents of the filtrate equals the weight of the material used for examination (1.000 g.) confirms the weighings and the completeness of the drying, shows that no hydration takes place

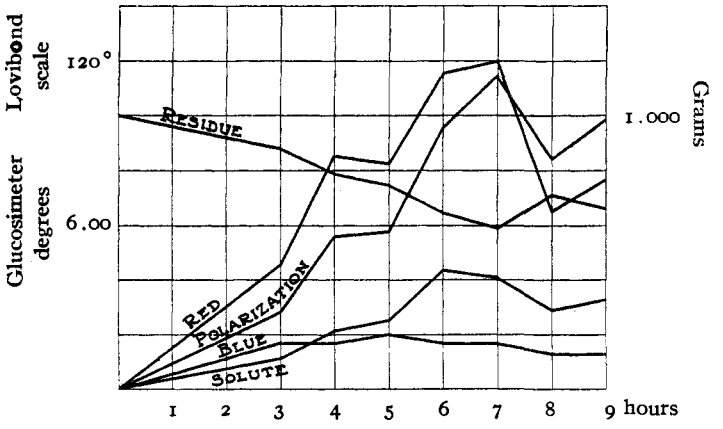

Fig. 1.—Hydrolysis of starch by "Roasting."

during solution of the dextrins (with the possible exception of No. 4) and shows that the extraction was virtually complete (since the wash water was discarded) at the end of 2 hours except for No. 8, in spite of the fact that the wash water still colored blue with iodine.

The general parallelism of the curves for the erythrodextrin (red), for the polarization and for the solid contents of the filtrate offer a confirmation of the general correctness of each of these determinations, especially with regard to their discontinuities. These show clearly the 3 maxima (later shown still more clearly, here shown at the end of 4 hours, 7 hours and 48 hours) due to the decomposition into erythrodextrin of the 3 main polysaccharides of cereal starch (which we have called amylose, amylopectin, and amylocellulose[2]). Further reference to these curves will be

[1] By difference. This material was slightly charred and weighed only 0.202 g.

[2] *Loc. cit.*, Paper I.

made in the next section. Here we need only point out in addition to what has been said the cause of the maximum shown by the curve for the residue at the end of 8 hours and the corresponding minima shown by all the other curves. This maximum is doubtless due to the fact that the disintegration of the amylocellulose has proceeded far enough to enable it to hold the semi-colloidal dextrins from going into solution to some extent (cf. pages 635–6, conclusions 2 and 8). This explanation accounts for the minima of the other curves, a phenomenon sharply to be distinguished from the final minimum obtained by hydrolysis in solution which is due to the hydrolysis of erythrodextrin into achroödextrin (Fig. 2, erythrodextrin curve after 22 minutes). In other words, if this absorption of erythrodextrin by higher polysaccharides had not taken place, no drop in the curve should have occurred at the end of 7 hours, but nearly a horizontal extension like that at the end of 4 hours, showing but slight destruction of the erythrodextrin. From these considerations we derive the conclusion that erythrodextrin is the final product of the hydrolysis of the three main constituents of cereal starch under these conditions (aside from any sugars which may be formed simultaneously), a conclusion confirmed by the slight amount of achroödextrin[1] in the filtrate.

Starch Boiled with Dilute Hydrochloric Acid.

It was found that the addition of water to the starch-acid mixture of the previous experiment before placing it in the oven increased the formation of erythrodextrin. Hence it was thought that boiling starch with acid sufficiently dilute ought to yield erythrodextrin in maximal quantities. Numerous experiments made with this idea in mind led at length to the following results:

Twenty-eight grams of Oswego corn starch, suspended in 60 cc. of water, were added by means of a rotating funnel with bent stem to 620 cc. of water boiling vigorously and rapidly rotating. Under these conditions all of the starch at once forms a homogenous mixture, which soon boils quietly as a glassy paste. At the end of 5 minutes the cells are entirely normal in appearance, and the liquid outside of the cells gives no color with iodine,[2] as may readily be seen under the microscope when iodine water is added in large excess (the starch-iodide coagulates, leaving clear intercellular spaces). At the end of 5 minutes 20 cc. of 1.13 N hydrochloric acid were added through a vertical condenser originally attached to the flask, the boiling was continued and at stated intervals 44.0 cc. (calculated to the room temperature) were blown out of the flask into a large test tube containing 6.00 cc. of 0.52 N sodium carbonate solution (methyl orange indicator). The hydrolysis mixture was, therefore, approximately 0.032 N hydrochloric acid,

[1] Precipitated by 80 to 90% alcohol from the filtrate of 67% alcohol (Paper I, p. 1249).

[2] Cf. C. Nägeli, "Die Stärkekörner," cit., p. 381.

although the acidity varies somewhat during the progress of the hydrolysis. The alkali used was approximately twice that required to neutralize the hydrochloric acid added, and was sufficient to leave all the final mixtures decidedly alkaline to phenolphthalein. This strength of alkali was used after extended experiments had shown that it gave the best precipitation of "artificial starch" in the early stages of the hydrolysis. The alkali stops the hydrolysis, but a preservative must be added to prevent fermentation (1 cc. of 1% arsenic acid[1] was added to each tube after the first precipitation).

The hydrolysis mixture was blown into the alkali in such fashion as to mix the two liquids as much as possible, and the mixture was then thoroughly stirred with a thermometer until the opalescence disappeared from the upper part of the liquid. This mixture was then allowed to cool spontaneously, and the temperature of crystallization noted, as already pointed out.

The precipitate thus formed, together with cell fragments, more or less swollen at different stages, was allowed to settle for 48 hours, when its volume was determined in terms of the total volume of the mixture (making allowance for the irregularity of the bottom of the test tube).

The supernatant liquid was then closely decanted (by continuous suction) and filtered. In this filtrate the relative concentration of amylodextrin and erythrodextrin were determined as already pointed out and the polarization was observed. In this filtrate, also, the transition temperature of the predominent iodide was determined.

The precipitate was suspended in the original volume of water and again allowed to settle. Since most of the precipitate dissolved under these conditions,[2] the material left (large cell fragments) will be called the residue. The volume of the residue was determined after 48 hours' settling; the supernatant liquid (called the wash water) was filtered off and its relative concentration of amylodextrin and erythrodextrin determined. The wash water, plainly, gives an idea of the composition of the crystalline precipitate, the "artificial starch." The residue, while re-suspended in the wash water before the second filtration, was used to determine the protein and the erythramylum. The results are reported in the following table.

These results are plotted in Fig. 2, with the arbitrary changes in units (volumes) noted there to bring them all on the same scale in the clearest relationship.

We have here a fairly well defined picture of the profound changes taking place in the hydrolysis of starch after the first 4 minutes and up to the time of the formation of achroödextrin as the principal polysaccharide. From

[1] Blake, Paper I, p. 1248.

[2] Cf. O'Sullivan, *J. Chem. Soc.*, **35**, 772 (1879).

TABLE VIII.—HYDROLYSIS OF CORN STARCH AT 100° BY 0.032 *N* HCl.

Expt. No.	Interval, Minutes.	Temperature of crystallization of "artificial starch."	Precipitate after 48 hours. Volume %.	Residue after 48 hours. Volume %.	Filtrate. Polarization, 200 mm. tube.	Filtrate. Blue in 0.500 cc. Lovibond scale.	Filtrate. Trans. temp. of blue iodide.	Filtrate. Red in 0.500 cc. Lovibond scale.	Filtrate. Transition temp. of red iodide.	Wash water. Blue in 1.000 cc. Lovibond scale.	Wash water. Red in 1.000 cc. Lovibond scale.	Residue re-suspended in original volume of water. Erythramylum in 10 cc. Lovibond scale.	Residue re-suspended in original volume of water. Protein in 10 cc. Lovibond scale.
1	4	68.0°	19[1]	4.2[2]	Turbid	95	72.0°	47	50°	30	19	87	6
2	5	68.0	18[1]	5.5	Turbid	88	72.0	49	...	36	17	103	7
3	6	68.0	16[1]	6.6	Turbid	105	71.5	54	...	36	24	132	8
4	7	71.0	18[1]	7.7	Turbid	98	71.2	70	...	39	23	120	7
5	8	72.5	15.0	5.6	Turbid	90	71.0	68	...	30	18	131	7
6	11	71.5	13.2	4.9	Turbid	85	68.5	72	...	27	22	95	22
7	12	71.5	12.8	3.6	10.48	73	69.0	74	...	24	19	87	15
8	13	71.5	13.9	4.0	10.47	75	68.5	75	...	22	22	135	9
9	14	71.0	13.5	4.4	10.42	72	67.0	74	...	14	16	110	7
10	15	71.0	14.2	3.8	10.40	74	68.3	78	...	18	21	115	14
11	20	69.0	..	...	Lost	...	...	...	...	Lost	Lost	...	..
12	22	68.5	13.6	2.6	10.30	38	...	104	63	7	22	136	13
13	23	67.0	13.6	4.2	10.23	20	...	83	62	14	31	135	8
14	24	67.0	13.5	4.2	10.26	16	...	75	61	16	28	131	6
15	30	64.0	12.3	3.5	10.26	8	...	67	59	7	25	82	8

[1] Includes slight amount recovered from filtrate on longer standing.

[2] 10% additional forms on longer standing, making 14%. This additional 10% is plainly part of the precipitate, as distinguished from the residue.

the similarity of the hydrolysis of boiled and unboiled starch (not detailed here), it seems that no pronounced chemical changes take place during the 5 minutes' boiling with water. One very noteworthy change takes place, however, during the first 4 minutes' boiling with acid of this strength; the viscous substance (amylopectin?) disappears. The loss of viscosity may be more readily followed by the use of acid of much less concentration. Apparently no precipitate will settle out until this viscous substance has been destroyed. It seems that the turbidity destroyed by the alkali in these experiments, mentioned above, is due to the last traces of this sub-

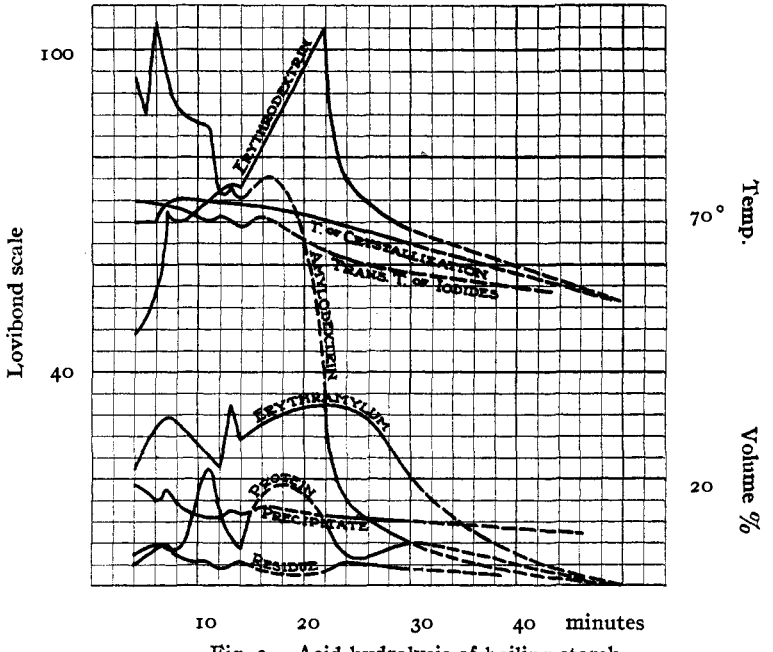

Fig. 2.—Acid hydrolysis of boiling starch.

Erythrodextrin in 0.50 cc. of filtrate.
Amylodextrin in 0.50 cc. of filtrate.
Erythramylum in 2.5 cc. of residue, resuspended in water.
Protein in 10.0 cc. of residue, resuspended in water.

stance. This change is probably concerned with the first maximum and first minimum observed in the blue coloration which iodine produces (amylodextrin curve, 4 and 5 minutes). It should be stated that the extrapolated portions of the curves, represented by dots, are based largely on other concordant experiments and represent the general trend of the curves only. The cell walls are all disrupted in less than one minute after the addition of the acid.

The phenomena really represented in these curves begin with the marked changes which take place between the times 5 minutes and 6 minutes.

Here the protein reaches its first maximum, the blue reaches its second maximum and the erythramylum has nearly reached its first maximum.

The next minute, 6 to 7, is equally eventful: the erythrodextrin, the erythramylum, the precipitate and the residue reach their first maxima (if we except the initial maximum of the precipitate, which apparently antedates the 4-minute interval at which these curves begin) and the temperature of the crystallization rapidly increases. The sudden and pronounced maximum of the precipitate (at 7 minutes) is plainly due to the almost identical maximum in the residue at the same time, and this is due to some change in the material of the cell walls, as the great development of erythramylum also testifies.

The next few minutes show the rather rapid destruction of all these substances by the further action of the acid. A reading at 9 or 10 minutes would have been helpful in plotting this portion of the curve. The times actually read were around the 3 maxima predicted from other experiments and the results show that the predictions were verified.

The next phenomenon of importance is the remarkable liberation of protein that occurs between 10 and 11 minutes. Here the blue shows a slight maximum, as in the previous case, and again, within 2 minutes, we have another simultaneous maximum of the erythramylum and the erythrodextrin. The smallness of the latter seems to be due to the sharpness of the former, and seems to indicate that the erythrodextrin is a decomposition product accompanying the formation of erythramylum, a conclusion greatly strengthened by the simultaneous appearance of maxima of these two substances in great splendor at the end of 22 minutes.

The third maximum in the protein, interpolated as at 18 minutes, is, unfortunately, uncertain, both by reason of the intermission from 15 to 20 minutes and the early loss of the test started at 20 minutes. This loss is the more regrettable because the possible simultaneous maximum in the blue, which occurred on the two former occasions, is also in doubt. A slight maximum in the blue here is indicated in the projected curve and is also found in the curves of other experiments.

The remarkable constancy of the polarization should be pointed out, the final drop in the curve coming only after most of the erythrodextrin has been hydrolyzed and the concentration of maltose has greatly increased. This observation tends to confirm the commonly accepted opinion that the specific rotatory power of all the common dextrins is the same.

The only other phenomenon which need be pointed out is the maximum in the residue at 23 minutes which accompanies the maximum of the erythramylum at this point, as on two previous occasions. This leaves the slight maximum in the residue at 14 minutes as the only irregularity apparently unrelated to other phenomena. It is, therefore, probably erroneous.

Conclusion.

It hence appears that boiled corn starch disintegrates in at least three stages, protein and amylodextrin preceding erythramylum and erythrodextrin in order of formation. These three stages probably correspond with those shown by the "roasting" process, the final practical disappearance of the amylodextrin in both cases occurring at the third and greatest maximum of the erythrodextrin. They also probably correspond with the 3 stages of salivary digestion of starch recorded in the first paper of this series.

Subsidiary Findings.

A number of subsidiary findings deserve notice.

1. "Artificial starch," if crystallized out without previous filtration, is a mixture of amylodextrin, erythrodextrin, erythramylum, protein and cell-fragments.

2. "Artificial starch" is a solid solution of amylodextrin and erythrodextrin in higher polysaccharides. This conclusion follows from the observation that the precipitate dissolved in the wash water (Table VII) has the same composition as the mother liquor from which it was originally crystallized, so far as amylodextrin and erythrodextrin are concerned. The ratio of blue to red in both cases is the same, within the experimental error, except in the last 4 readings, where the blue was so small as to render the relation less reliable. Compare finding No. 8, *infra*.

3. Amylodextrin seems to be a pure crystalline substance, moderately soluble in cold water, coloring blue with iodine, the maximum transition temperature of the iodide being 74°. In a mixture this iodide forms after that of protein, after most of the iodide of erythramylum, and before most of the iodide of erythrodextrin. It is precipitated by alcohol of about 40% by volume.

4. Erythrodextrin seems to be a pure substance, tending to intercrystallize with amylodextrin, and hydrolyzing into achroödextrin. It is precipitated by alcohol of 49 to 67% by volume. The maximum transition temperature of its iodide is 64°. It seems to be the final polysaccharide formed in the "roasting" process of manufacturing dextrin when the acid is sufficiently dilute; that is, it is heat stable.

5. Amylodextrin probably hydrolyzes into erythrodextrin, a decrease in this curve corresponding in all cases to an increase in the erythrodextrin curve (Fig. 2).

6. Erythramylum seems to be a pure substance, formed simultaneously with erythrodextrin. Otherwise its relationships to the dextrins are almost wholly unknown, although it probably hydrolyzes slowly into amylodextrin. It may be separated from amylodextrin in solution by precipitation with alcohol of about 35% and it tends to precipitate out of aqueous solution spontaneously on standing. It is obtained in solution only by the almost

complete disintegration of the walls of the starch granules, as by excessive heat in the roasting process of dextrin manufacture. Its iodide in such a solution is not decolorized by toluene, whereas that of erythrodextrin is readily decolorized by that solvent.

7. In a number of cases long, colorless needles were obtained, probably as a decomposition product of the small amount of cellulose the starch contains. The best crystals were obtained as white needles by adding raw corn starch to boiling 0.011 *N* hydrochloric acid and boiling 150 minutes. At the end of this time the hydrolysis was somewhat more advanced than that represented by the final reading in Table VII, the amylodextrin having just entirely disappeared and the temperature of crystallization having fallen to 45°. At this time, however, these white crystals rather suddenly appeared, of less specific gravity than the (sphero-) crystals previously observed and forming at the constant temperature of 75°. This formation increased steadily in the next 1.5 hours, both crops of crystals being obtained.

8. Lintner's "soluble starch" is nearly pure amylodextrin, formed from the amylocellulose of potato starch and still held in the form of the original cells by a thin layer of insoluble material, which disintegrates in water at about 50°. The transition temperature of its iodide in 1% solution is 69.5°. It crystallizes with difficulty and only at low temperatures (below 50°) even from 10% solution, showing the important role of the higher polysaccharides in the ordinary crystallization of "artificial starch" (cf. Table VII).

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RESEARCHES ON THIOCYANATES AND ISOTHIOCYANATES.

XII. THE POLYKETIDE ISOTHIOCYANATE—ETHYL ISOTHIOCYANPROPIONATE.

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Received January 2, 1918.

The first polyketide isothiocyanate (mustard oil) to be described in the literature, whose constitution has been definitely established, is ethyl isothiocyanacetate (I), which has been synthezised by Johnson and Hemingway.[1] This is obtained in good yield by the action of thiophosgene on ethyl aminoacetate or its hydrochloric acid salt, while its isomer (II) results by interaction of potassium thiocyanate with ethyl chloroacetate in alcohol solution. Both esters boil at practically the same temperature without appreciable decomposition, and it has been our experience that neither isomer exhibits any tendency to undergo molecular rearrangement

[1] THIS JOURNAL, 38, 1550 (1916).