of this precipitate, when recrystallized from a mixture of chloroform and alcohol by the method already described, gave 0.49 g. of recrystallized product. 0.63 g. of the precipitate obtained from the vinegar distillate gave 0.48 g. of recrystallized product when similarly treated. These two recrystallized products as well as the corresponding product obtained from the diacetyl solution were identical in both macroscopic and microscopic appearance. All three preparations melted at the same temperature, separately and when mixed, namely, 243-244° (uncor.), and all three responded with equal readiness to v. Pechmann's test[1] for α-diketones.

From the results recorded above it is evident that the reduction of Fehling's solution by cider vinegar distillates is due largely, if not wholly, to acetylmethylcarbinol. On the assumption that Fehling's solution oxidizes acetylmethylcarbinol to acetic acid it was found possible, from the amount of cuprous oxide produced by a given volume of distillate or of acetylmethylcarbinol solution of varying concentrations, to calculate very closely the amount of precipitate which would be obtained with the phenylhydrazine reagent. This would be possible only if the reactions involved were at least approximately quantitative. The identification of acetic acid as the oxidation product formed in the action of Fehling's solution upon acetylmethylcarbinol has not yet been carried out, but there can be little doubt that acetic acid is the final oxidation product of this reaction. This would be in agreement with the fact that in oxidizing with silver oxide the distillate which he obtained from wine vinegar Farnsteiner found only acetic acid.

Further investigations in connection with the occurrence of acetylmethylcarbinol in vinegars and other products and of the conditions under which it is formed are in progress.

Summary.

The volatile reducing substances in cider vinegar consist largely, if not wholly, of acetylmethylcarbinol.

Acetylmethylcarbinol (methylacetol, dimethylketol, or dimethylglycolose) is a normal constituent of cider vinegar.

WASHINGTON, D. C.

ON THE DIGESTIBILITY OF BREAD.

II. SALIVARY DIGESTION OF ERYTHRODEXTRIN IN VITRO.

BY J. C. BLAKE.

Received December 18, 1916.

One conclusion of the first article of this series, namely,[2] that the activity of the amylases worked with did not seem to vary appreciably with moderate variations in their concentrations, seemed to press for further ex-

[1] *Ber.*, 21, 2752–3 (1888).

[2] THIS JOURNAL, 38, 1259 (1916).

planation. Hawk[1] states that the linear law has been proved for lipase, sucrase, rennin, and trypsin, and states further[2] that "salivary amylase acts more efficiently when the saliva is diluted from 4 to 7 times." Arrhenius[3] refers to the fact, shown by London, that the digestion of small quantities of amylodextrin follows the monomolecular formula.

The following experiments were made with the sample of dextrin referred to in the first article of this series. It had the following composition:

Cellulose	Trace
Amylodextrin	Trace
Erythrodextrin	52.7%
Achroödextrin	22.8
Maltose	8.3
Water	12.2
Undetermined	4.0

The dextrins were determined by precipitation with alcohol from 1% solution, the erythrodextrin by 60%, the achroödextrin from the filtrate of the erythrodextrin by 80% alcohol. Although it is hoped that experiments can soon be made with pure polysaccharides, yet it seems worth while to record the experiments made with this material. Preliminary attempts to prepare large quantities of erythrodextrin by precipitation with alcohol from more concentrated solutions showed that the separation from achroödextrin became incomplete.

Before making the digestions the cellulose and most of the amylodextrin (or starch) were filtered out through an asbestos filter. This filtrate gave pure colors with iodine during the digestion, varying from plum-colored through brick-red to orange and yellow. The faint traces of amylodextrin (which furnished the blue of the plum-color) digested almost instantly, the red colors being due to erythrodextrin.

The saliva was collected from day to day for about ten days without specific stimulation, and was preserved by toluene. All of the dilutions were made from this specimen with the same sample of ordinary distilled water by successive dilutions. The dextrin solutions were prepared in a similar way from a 20% solution (40 g. in 200 cc. of solution) of the air-dried dextrin, and were also preserved by toluene.

The temperature coefficient of the digestion was first determined, in order to ascertain at what temperatures it showed minima. In all of these digestions 5 cc. each of the dextrin solution and the saliva solution were heated for five minutes at the indicated temperatures in a water bath; then they were quickly and thoroughly mixed together and re-immersed in the bath. At various intervals two drops of a saturated aqueous

[1] "Practical Physiological Chemistry," 9 (1916).

[2] *Loc. cit.*, p. 57.

[3] "Quantitative Laws in Biological Chemistry," 95 (1916); cf. also Mathews, "Physiological Chemistry," p. 333 (1916).

solution of iodine were placed on a test tablet, and a drop of the digestion mixture added. The achromic point was taken as that moment at which the test thus made gave no greater color than that of two drops of the iodine-water similarly placed. The results are given in the following table, and are plotted in Fig. I:

TABLE I.

Digestion of 2.5% Dextrin (Final Concentration) by $^1/_{32}$ Saliva (Final Concentration) at Various Temperatures.

Temperature.	Time to achromic point in minutes = t.	$1/t$. Hours.	Temperature.	Time to achromic point in minutes = t.	$1/t$. Hours.
25.0	8	7.5	57.5	$3^1/_2$	17
32.5	6	10	60.0	4	15
40.0	$4^1/_2$	13	61.0	5	12
45.0	4	15	62.0	14	4
47.5	3	20	63.0	60	1
51.0	3	20	65.0	∞ [1]	0
55.0	3	20			

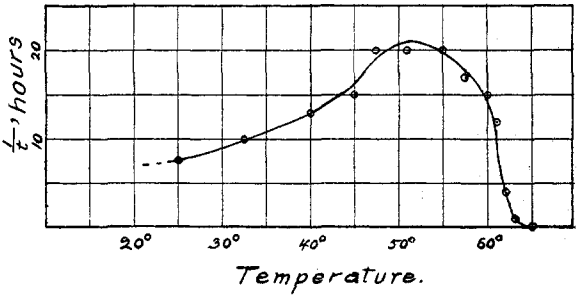

Fig. 1.

The expression $1/t$ represents the speed of the digestion, and shows a fairly sharp maximum at 51° instead of 35° to 45° as usually stated.[2] They also confirm the statement made in the previous paper,[3] that the temperature coefficient is small at the ordinary temperature. Because of this fact, and because of the ease of maintaining the temperature constant, the following digestions were carried out at 25.0° instead of at body temperature.

The form of this curve also supports its theoretical explanation. The initial increase in amylolytic activity as the temperature rises is attributable to the general quickening of chemical reactions which increase of temperature induces, and even agrees quantitatively with this explanation; that is to say, an increase of temperature from 25° to 45° doubles the rate of digestion just as an increase of 20° doubles the rate of chemical reactions in general. If no other factor intervened, the curve should continue asymptotically upward with further rise of temperature, instead of suffering an inflection. This inflection at 46°, the subsequent maximum and final cessation of digestion are due to the fact that at 46° the heat begins to "kill" the enzyme. That destruction of the enzyme at

[1] Incomplete after 45 hours at ordinary temperature.

[2] Hawk, *Loc. cit.*, p. 6; Mathews, *Ibid.*, p. 331 (1916).

[3] Blake, *Ibid.*, p. 1255.

temperatures below 75° is not instantaneous is well known.[1] This slow destruction of the enzyme at temperatures above 46° accounts also for the continuation of the curve to 65°, although its general trend would indicate a sharp ending at 62°. That the enzyme is destroyed by the heating, and not merely inhibited, is indicated by the fact that the experiment made at 65° failed to reach completion in 48 hours even though the mixture had cooled to the ordinary temperature.

TABLE II.

Digestion of 2.5% Dextrin (Final Concentration) at 25.0° with Various Concentrations of Saliva.

Final concentration of saliva = q.	Time to achromic point in minutes = t.	qt.	Final concentration of saliva = q.	Time to achromic point in minutes = t.	qt.
1/2	5/12	0.21	1/64	21	0.33
1/4	1	0.25	1/128	40	0.31
1/8	2	0.25	1/256	125	0.49
1/16	5	0.31	1/512	<24 hrs.	<3
1/32	9	0.28			

The values for qt are constant within the limits of experimental error down to a dilution of $^1/_{128}$ saliva, showing clearly that the rate of digestion is directly proportional to the concentration of the saliva, except when the ratio of substrate to saliva becomes very great (*vide infra*), a well known phenomenon,[2] due to the union of the enzyme with the substrate preliminary to hydrolysis.

In the light of these experiments, taken in connection with those already referred to in the first article of this series, it would seem that the reason why the kinds of amylases tested gave the same apparent rate of digestion, although of different concentration of enzyme, was because the amylase used (*e. g.*, $^1/_8$ saliva) contained sufficient enzyme, to digest completely all the amylose present (<0.5%) within the shortest time recorded (15 minutes), so that the increased polariscopic readings noted thereafter represented the slow digestion of the amylopectin (or rose-amylose) and the amylocellulose, which are present in starch in relatively small amount.

TABLE III.

Digestion of Various Concentrations of Dextrin by Saliva of 1/128 (Final) Concentration at 25.0°.

Final concentration of dextrin, % = q.	Time to achromic point in minutes = t.	t/q.	Final concentration of dextrin, % = q.	Time to achromic point in minutes = t.	t/q.
0.078	1	12.8	1.25	$15^1/_2$	12.4
0.156	$1^3/_4$	11.2	2.50	44	17.6
0.312	$3^1/_2$	11.2	5.00	170	34.0
0.625	7	11.2	10.00	<24 hrs.	<144

[1] Mathews, *Loc. cit.*, p. 331.

[2] Arrhenius, *Ibid.*, pp. 34-5.

The constancy of the value of t/q until the ratio of saliva to dextrin becomes very minute shows that the digestion varies directly as the concentration of the erythrodextrin. The ratio of saliva to dextrin at which the digestion becomes extremely slow is the same in both cases, although the actual concentrations in the one case are four times as great as in the other: $^1/_{128}$ saliva : 10% dextrin : : $^1/_{128}$ saliva : 2.5% dextrin. This stoichiometrical relation again indicates the formation of a chemical compound between the enzyme and the substrate, and should in time lead to molecular-weight determinations.

That the assumed compound is a union of the enzyme with the substrates (the dextrins) and not with the product of their hydrolysis (maltase)[1] is indicated by the experiments recorded in Table IV, which show that maltose exerts a very minor retarding influence on the digestion.

TABLE IV.

Digestion of 1.25% Dextrin (Final Concentration) by 1/128 Saliva (Final Concentration) at 25.0° in the Presence of Various Concentrations of Maltose.

5% dextrin. Cc.	1/32 saliva. Cc.	Water. Cc.	1.95% maltose (dried at 100°). Cc.	Time to achromic point in minutes.	Retardation due to maltose. Minutes.
2	2	4	0	10	..
2	2	3	1	$12^1/_2$	$2^1/_2$
2	2	2	2	14	4
2	2	1	3	$17^1/_2$	$7^1/_2$
2	2	0	4	$19^1/_2$	$9^1/_2$

The retardation produced by maltose is slight compared to the retardations previously noted, and varies directly as the concentration of the maltose (Fig. 2). It should be remembered in this connection that the erythrodextrin digests so much faster than the achroödextrin[2] that but little maltose would be formed before the achromic point is reached. The relatively slow digestion of the achroödextrin can be conveniently followed by the polariscope. Thus in a preliminary experiment of this kind this 5% dextrin solution was mixed with one volume of $^1/_{16}$ saliva (filtered) and read on the polariscope from time to time at the room temperature. The readings decreased asymptotically from 7.30 to 5.90, becoming constant at the end of 170 hours. Assuming only maltose to be present in the final solution,[3]

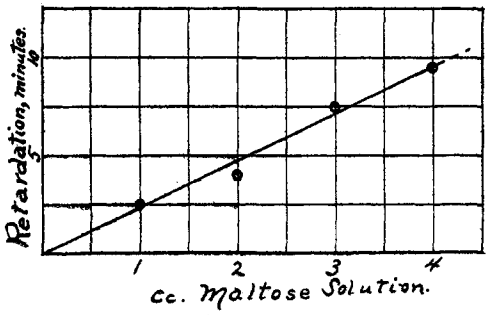

Fig. 2.

[1] Mathews, *Loc. cit.*, p. 333.

[2] Blake, *Ibid.*, p. 1248.

[3] *Ibid.*, p. 1249.

this determination, when corrected for the small amount of maltose originally present in the dextrin, gives a value of 170 for the specific rotation of achroödextrin, which is the value twice obtained by direct determination by the alcoholic method of separation already described.

Conclusions.

1. The digestion of erythrodextrin by ptyalin is a monomolecular reaction.

2. The optimum temperature for this digestion is 51°. The temperature coefficient is relatively small at the ordinary temperature, being about the same as that of chemical reactions in general. The enzyme is destroyed at 65°.

3. The digestion becomes disproportionately slow when the ratio of substrate to enzyme becomes very large. This ratio is constant for different concentrations, and probably represents a compound formed by the union of the enzyme and the substrate preliminary to hydrolysis.

4. The retarding influence of maltose is shown to be relatively small and to vary directly as its concentration.

5. The relatively slow digestion of achroödextrin is again pointed out, and a preliminary redetermination of its specific rotation made.

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[CONTRIBUTION FROM THE PHYSICAL LABORATORY, PRINCETON UNIVERSITY, AND THE CARBOHYDRATE LABORATORY, BUREAU OF CHEMISTRY, UNITED STATES DEPARTMENT OF AGRICULTURE.]

STUDIES ON THE FORMS OF *d*-GLUCOSE AND THEIR MUTAROTATION.

BY C. S. HUDSON AND J. K. DALE.

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CONTENTS.—1. Introduction. 2. The Purification of Glucose. 3. The Preparation of Pure β-Glucose. 4. The Preparation of Pure α-Glucose. 5. The Variation of the Rate of Mutarotation with the Concentration of Sugar. 6. The Influence of Temperature upon the Rate of Mutarotation. 7. The Influence of Temperature on the Catalysis of the Mutarotation by Acids.

1. Introduction.

It is generally recognized that the mutarotation of glucose is due to a balanced reaction which may be expressed in the form

$$\alpha\text{-Glucose} \rightleftarrows \beta\text{-Glucose.}^1$$

α-Glucose is the usual form in which the sugar crystallizes and its initial specific rotation, when the sugar is dissolved in cold water, is near +110°. This value falls gradually to +52° during the course of the mutarotation reaction. β-Glucose is the form which was first isolated in the crystalline

[1] For a summary of the literature on this subject, see THIS JOURNAL, 32, 889 (1910).