water.[1] It is quite unlikely that a substance going from solution to the solid form should dissociate. By analogy we should rather expect the reverse process.

The data for mercuric chloride, which are not given above because a small amount of moisture was found in the sample after the determinations were made, are interesting on this point. Mercuric chloride gives a normal molecular weight corresponding to the formula $HgCl_2$ by the boiling-point method in ether and yet the data show that the relationship $K = \frac{C - C_1}{\sqrt{C}}$ holds very closely.

We have no better explanation to offer at present than to say that perhaps solution compounds similar to the hydrates which we have in aqueous solutions are formed in some cases. These compounds are not stable, however, at the boiling point of the solvent any more than hydrates are at 100° and are decomposed when the solid material is precipitated by the addition of the poorer solvent.

Compounds of mercuric chloride and certain nonaqueous solvents have already been isolated by Dunkelski.[2]

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ON THE DIGESTIBILITY OF BREAD. I. SALIVARY DIGESTION IN VITRO.

[PRELIMINARY PAPER.]

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Summary.

The rate of digestion *in vitro* of the starch of bread can be followed readily and accurately by means of the polariscope, the only optically active substance entering the solution under the conditions herein stated being maltose. A rather large array of subsidiary findings surrounds this main observation, such as confirmation of the specificity of the starch of different plants, a clearer identification of some of the ingredients of the starch of the common cereals and the products of their hydrolyses (including two new substances), the variation of the activity of salivary amylase with the dilution and with the temperature, a comparison of amylases of different origin, and a comparison of the salivary digestibility of a number of standard breads. This paper is a report of progress rather than a finality.

[1] Nernst, "Theoretische Chemie," 483 (1903).

[2] *Loc. cit.*

Historical.

The statements of Maquenne and Roux[1] that in the digestion of amylose no considerable amounts of dextrins enter the solution, but that the dextrins ordinarily observed are derived from the amylopectin of starch are greatly at variance with the older ideas of Musculus and Gruber[2] and of Brown and Heron,[3] still current in American works on physiological chemistry,[4] according to which boiled starch is a homogeneous substance[5] which gradually hydrolyzes by the continual splitting off of maltose or isomaltose, the residual nucleus passing successively through the stages of soluble starch, amylodextrin, erythrodextrin, and an indefinite number of achroödextrins. So confusing is the literature of this subject, much of which is paraphrased in the works cited, that at the present time none of these ingredients has any standing as a pure chemical substance. On this account the polysaccharides recognized in this work are here defined anew, in close agreement with the general trend of past usage, the word dextrin in particular being applied only to substances markedly diffusible through membranes. The true process of salivary digestion seems to lie about half way between the two processes previously referred to.

Experimental.

Polysaccharides.—By working with the starch of bread, as well as with raw and boiled starch, the author was able to recognize more clearly a considerable number of polysaccharides which seem to be chemical individuals, although exhaustive study of each of them is yet to be made. It seems necessary and worth while to record these preliminary observations both for their own sakes and because they were used in indicating *pro tanto* by negation that only maltose was present in the solutions hereafter described.

Cellulose and Amylocellulose.—It should be emphasized that in bread pulp (the white interior of the loaf) the starch granules are not disrupted,[6] and that very little of any soluble carbohydrate is present outside the granules, even though the starch is apparently as digestible as boiled starch (page 1256); whereas in boiled starch the granules are disrupted and, on long boiling (several hours) or by superheating, go completely

[1] Maquenne and Roux, *Ann. chim. phys.*, [8] **9**, 179; Meyer and Jacobson, "Lehrbuch der Organischen Chemie," **1913**, 1032; Hammarsten-Hedin (Mandel), "A Text-book of Physiological Chemistry," **1914**, 229.

[2] *Z. physiol. Chem.*, **2**, 177 (1878).

[3] *Liebig's Ann.*, **199**, 65 (1880).

[4] Hawk, "Practical Physiological Chemistry," **1913**, 61; Mathews, "Physiological Chemistry," **1915**, 328.

[5] Reichert, "Differentiation and Specificity of Starches in Relation to Genera, Species," etc., *Pub. Carnegie Inst. Wash.*, **1913**, p. 160.

[6] Jago, "The Technology of Bread-Making," **1911**.

into solution. Hence the digestion of the cell walls is the more readily observable with starch from bread, making clear the differentiation of the substance composing the walls from the substances found within the granules. This substance we will call amylocellulose, because of its slow digestibility, the cell walls of the large granules of starch from bread becoming disrupted under ordinary conditions of salivary digestion *in vitro* only after more than 24 hours. After 48 hours the process is ended, except for a few chain-like cell fragments and the cell walls of a few very large hexagonal-shaped cells. This definition is at variance with that of Maquenne, who apparently confuses amylocellulose, amylose (the main constituent of cooked starch) and "reverted amylose" (the insoluble and indigestible substance which settles out of starch solutions on standing). Amylocellulose gives no color with iodine water, as may readily be determined by testing after the cell contents have been removed by digestion (*infra*). It probably hydrolyzes through the stage of maltose and constitutes about 10% of the starch granules (dried at 120°), as indicated by the increase in the polarization of the digestion mixture after the contents of the cells have been digested (that is, after four hours, Tables I and IV). The chain-like fragments and the walls of the hexagonal cells are probably cellulose. The interior of these latter cells stains yellow with iodine, indicating the presence of *protein*.[1] All of these features were later recognized in slightly boiled corn and rice starch (page 1257), indicating great similarity between freshly boiled starch and that of bread, and also that the indigestible cells were not derived from the yeast of the bread.

Amylopectin and Rose-amylose.—If raw cereal starch be treated with iodine water in minimal amount a red color is developed. This color disappears at approximately 46° and reappears at the same temperature provided sufficient iodine was originally added to turn the raw starch purple. The blue tint of the purple disappears during the heating owing to loss of iodine from the solution. The substance giving this red color with iodine is rose-amylose, first recognized by Day.[2] It is the substance responsible for most of the confusion existing with regard to the chemistry of starch, since it is usually mistaken for erythrodextrin,[3] and has also been mistaken for "*residual dextrin*," as it is much less readily digested than the dextrins (*infra*). It disappears from ordinary digestion mixtures only after four hours. It is further sharply differentiated from erythrodextrin (*q. v.*) by the difference of 13° in the transition temperatures of these two iodides, by the much greater sta-

[1] Confirmed by digestion with pepsin.

[2] Day, "Digestibility of Starch of Different Sorts as Affected by Cooking," Univ. of Chicago Press, **1908**, 37, 41; Reichert, *Loc. cit.*, p. 195.

[3] Hawk, *Loc. cit.*, pp. 10, 65; Mathews, *Loc. cit.*, p. 899.

bility of its iodide toward alkalies and toward maltose. The iodide of rose-amylose disappears when the calculated alkalinity (using NaOH, Na_2CO_3, or NH_4OH) reaches 0.1 N, whereas the iodide of erythrodextrin disappears when the alkalinity reaches 0.005 N. The difference in the stability of these two iodides to maltose probably accounts for the order of appearance of these iodides and the common blue iodides of starch on the gradual addition of iodine-water to mixtures of these substances. The iodide of rose-amylose appears first, then the ordinary blue iodides, then that of erythrodextrin. There is no rose-amylose in potato starch.

Rose-amylose is probably a product of the hydrolysis of amylopectin, the substance which gives a freshly-boiled cereal starch solution its excessive viscosity when cool. Addition of saliva to such a viscous solution or paste, which previously gave a blue color with minimal amounts of iodine-water, completely destroys the excessive viscosity within half a minute, making the mixture rich in rose-amylose. Arsenic acid of 0.05% or greater concentration (as As_2O_5) completely stops this change of amylopectin into rose-amylose; whereas the same acid at 0.005% lessens the time required for the disappearance of rose-amylose by more than one-half. Santesson[1] found this identical phenomenon in the activity of the catalytic enzyme of frog muscle. Dilute solutions of acetic, butyric and lactic acids facilitate this change but slightly, agreeing with the results recorded later on that the slight variation in the acidity of different samples of bread does not appreciably affect their rate of digestion. No concentration of these acids was found which completely prevents the change of amylopectin to rose-amylose. Hence 0.07% arsenic acid was used to stop all digestion, no other change seeming to take place as readily as this, or to take place at all in solutions containing this concentration of arsenic acid. The fate of rose-amylose is being investigated. It probably finally reaches maltose for the most part. The observation of Tanret[2] that the amylopectin contains all the phosphorus found in starch should be noted in this connection. A rough estimate of the amount of amylopectin in wheat starch can be made from the polarizations hereafter recorded showing about 10% of amylocellulose, 70% of amylose, and (by difference) 20% of amylopectin. This is in fair agreement with the estimate of Maquenne and Roux, who place the "amylose" (which we have seen includes amylocellulose) at 80–85%, the amylopectin at 15–20%.

Achroödextrin.—If bread or boiled starch digestion mixtures be filtered before the addition of iodine, the rose-amylose may readily be detected in the residue, but the filtrate ordinarily gives no color with iodine. Hence the theory of Maquenne and Roux, already cited, that no dex-

[1] *Skand. Arch. Physiol.*, **32**, 405; *Chem. Abs.*, **9**, 2097.

[2] *Bull. soc. chim.*, **17**, 83 (1915).

trins are present. If, however, alcohol be added to the filtrate during the first few minutes under usual conditions, a white precipitate of achroodextrin forms when the alcohol reaches 72 to 80% by volume. Under the conditions of the experiments hereinafter described, a 1% bread suspension gave 0.04% of this dextrin at the end of five minutes, 0.01% at the end of ten minutes, and only a trace at the end of twenty minutes. These values are corrected for a slight precipitate due to the saliva under these conditions. After standing overnight, the dextrin was filtered out in a Gooch crucible, dried at 100°, weighed and redissolved in water. This solution indicated a specific rotation of 170 for this dextrin, a value which is recorded merely to show its properties as a dextrin, the amount worked with being too small to give an accurate result. Further addition of absolute alcohol to the filtrate from this dextrin gave no precipitate or opalescence. Commercial dextrin "precipitated by alcohol" gave 26% of this dextrin.

Maltose was rather sharply identified as follows: A 1.00% (final concentration) suspension of disintegrated bread (Table II), which gave a reading of about 0.1% on the polariscope, which read percentages of *d*-glucose directly, gave readings of about 1.0% within a few minutes after mixture with saliva in moderate amount. In the absence of maltase and proteolytic enzymes the readings slowly increased to about 1.30 (Table I), this slow increase registering for the most part the slow digestion of the rose-amylose and the amylocellulose. The initial rapid rise in the polarization seems to be due to the digestion of the main content of the starch granules (which we may call *amylose*), the optical activity after the digestion of the achroödextrin (first fifteen minutes) being due wholly to maltose. The absence of dextrins is indicated not only by the negative tests with iodine and absolute alcohol, but by the unbroken asymptotic curve (Fig. 1, for Bread No. 1, Table I). The absence of *d*-glucose in appreciable amounts is indicated by negative tests for glucosazone and with a modification of Barfoed's reagent (twice as much copper acetate being used as that ordinarily specified,[1] and the time of boiling being one-half minute), and also by comparison with results obtained when maltase was present (page 1258). A further

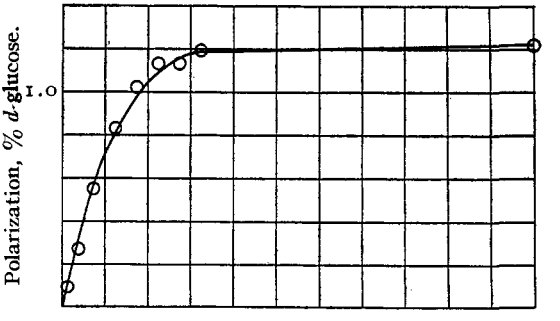

Fig. 1.

[1] Hawk, *Loc. cit.*, p. 443.

pseudo-quantitative proof that only maltose was present in the solutions referred to is the fact that on partial hydrolysis with acids they show the same change of optical activity as a solution of pure maltose having the same polarization. Thus when such solutions are heated half an hour in boiling water with 3% of concentrated hydrochloric acid (by volume), each loses one-third its optical activity. The difficulty of completely hydrolyzing maltose with acids, owing to the reversibility of the reaction, is well known.[1] Finally, the reading 1.30, read as *d*-glucose, corresponds to 0.47% starch, using the equations

$$\% \text{ Maltose} = \text{rotation as } d\text{-glucose} \times \frac{52.5}{138.3},$$

and

$$\% \text{ Starch} = \% \text{ maltose} \times \frac{324}{342},$$

in close agreement with the percentage of starch usually present in bread (50%[2]), and that indicated by the usual method of testing. (This bread contained 40.0% water and 8.0% gluten, besides salt and other minor ingredients.) Maltose shows little multirotation, and isomaltose from starch, if present, has the same specific rotation as maltose. In so far as the optical activity is due to maltose, multiplication of the readings recorded in the tables by 0.36 gives the percentage of the original material represented by the starch digested at the time the reading was made.

On this basis wheat No. 2, Table II (average of three samples), at the end of fifteen minutes (the time the achroödextrin disappears from the filtrate) showed a digestion of 72% of the estimated amount (0.50%) of starch present. Although maltase from the disintegrated bread was present in these experiments, as indicated by the subsequent decrease in the polarizations, yet the rate of such decrease is such that it would be negligible for the first fifteen minutes.

The complete, or nearly complete, change of starch into maltose has been claimed by Maquenne (*loc. cit.*) and by Fernbach and Wolff[3] and others,[4] as determined by copper reduction, polariscopic and other methods.

Erythrodextrin was clearly recognized as follows: Bread was digested in the presence of toluene with saliva diluted with 99 volumes of water. Under these conditions at the end of 15 minutes 60% of the total amount of starch present was found in the filtrate as erythrodextrin, as determined colorimetrically after the addition of arsenic acid and iodine-water. The amount of erythrodextrin in the digestion mixture gradually decreased, dis-

[1] Daish, *J. Chem. Soc.* (*Trans.*), **105**, 2053.

[2] Jago, *Loc. cit.*, p. 540.

[3] *Compt. rend.*, **145**, 80.

[4] Syniewski, *Liebig's Ann.*, **324**, 214.

appearing at the end of two hours. The standard erythrodextrin solution for colorimetric comparison was prepared from a white commercial "Dextrin, Precipitated by Alcohol," which contained only a trace of insoluble matter and of amylodextrin (*infra*), by precipitating the erythrodextrin with 65% alcohol (final concentration). The erythrodextrin began to precipitate when the concentration of the alcohol reached 50%; but achroödextrin did not begin to precipitate in the filtrate from the erythrodextrin until the concentration of the alcohol reached 72%. These sharp limits of precipitation rather indicate that each of these dextrins is a pure substance. The transition temperature of this iodide is 33°. A 0.5% solution of erythrodextrin is completely digested to the achromic point by 1 : 7 saliva in two minutes at the ordinary temperature. During the spontaneous fermentation of starch solutions containing no preservative but iodine, the starch passes through a stage in which the only color given with iodine water is the iodide of erythrodextrin. (For other properties, see rose-amylose.)

Amylodextrin was clearly recognized and estimated in the filtrate of the slow digestion experiment just described. It gives a pure blue color with iodine-water which forms before the iodide of erythrodextrin does in solutions containing both these substances. The transition temperature of the iodide is about 50°. Estimated in the filtrate by colorimetric comparison[1] with wheat starch boiled one hour, only 0.007% was present at the end of 15 minutes, only a trace at the end of 45 minutes, and none at the end of ninety minutes. Amylodextrin is, therefore, more readily digestible than erythrodextrin, which it probably hydrolyzes into. The three dextrins already mentioned not only diffuse through the cell walls of the starch granules of bread, but also through goldbeaters' skin.

Red-amylose and a new substance which we will call *blue-amylose* require brief mention. Ordinary (unfiltered) digestion mixtures of bread or of boiled starch in which the amylose and the rose-amylose have been completely digested, as determined by the methods already given (that is to say, after five hours), and in which no erythrodextrin is present, color a deep red on the addition of iodine-water in comparatively large amounts. The transition temperature of this iodide is 73°; but if the solution is heated somewhat above 80°, the red color no longer reappears on cooling the solution through 73°, but a blue coloration begins to appear as the solution is cooled through 83°. The substance giving the red color under these conditions agrees with Day's[2] description of red-amylose. It is probably a product of the hydrolysis of amylocellulose, and probably hydrolyzes into blue-amylose. Blue-amylose is best obtained by boiling

[1] Dermstedt, *Ber.*, **28**, ref. 1025, among others, employed this principle.

[2] Day, *Loc. cit.*, 37, 41; Reichert, *Loc. cit.*, p. 195.

the residue of a digestion mixture after it no longer colors pink with small amounts of iodine-water (that is, after four hours).

A new dextrin giving with iodine-water a blue iodide (whence we may call it *cyanodextrin*) which forms before the iodide of rose-amylose in mixtures containing both these substances and the transition temperature of whose iodide is sharply 29.5° (uncor.), has also been recognized; but as it does not appear in the sequel of this paper, it will not be further discussed in this place.

It must be added, in order to avoid the erroneous deduction often met with in the literature[1] to the effect that potassium iodide changes the blue iodide of starch to red, that potassium iodide in solutions one-fourth saturated really destroys the blue iodide of starch, but does not affect the iodide of either rose-amylose or erythrodextrin, even when added to saturation. This principle was confirmed by the fact that the amount of erythrodextrin present in a mixture of dextrins and starch could be estimated colorimetrically in the presence of potassium iodide and alcohol (toward which reagent the red iodides are the more stable), even when the amount of starch present was varied within wide limits. Furthermore, solutions of blue iodides containing no erythrodextrin, rose-amylose, or red-amylose do not turn purple or red on the addition of potassium iodide, but pass through green in disappearing.

Conditions of the Digestion.—All the materials used in these experiments were preserved with toluene (except takadiastase, which quickly molds in solution), and the digestions took place in the presence of toluene. The enzymes used were saliva, commercial ptyalin, Horlick's "diastoid," and takadiastase. The three latter were used in final concentration of 1%; the saliva in final concentration of 1 in 8, except where otherwise stated. The standard breads were prepared from the standard flours listed below, and were baked by standard commercial methods by J. C. Summers, of the School of Milling and Baking Technology, conducted by the *Operative Miller* of Chicago:

No. 1. Kansas hard winter wheat flour.
No. 2. Illinois soft winter wheat flour.
No. 3. "Oriental," a spring wheat flour.
No. 4. A standard commerical mixture.

The digestion mixtures usually contained 1% of the original material as their final concentrations, and all the results here recorded have been calculated to this basis. The polariscopic readings are all recorded as read on a Schmidt and Haensch half-shadow saccharimeter reading percentages of *d*-glucose in a 200 mm. tube. The interior of the loaves was used for digestion, either in the form of approximately 1-inch cubes or broken down completely by grinding in a porcelain mortar, except where

[1] Bayer and Field, *J. Chem. Soc.* (*Trans.*), 101, 1403 (1912).

otherwise stated. This grinding does not disrupt the starch granules. In the experiments with cubes, which are not disintegrated by saliva, the results have been corrected for the amount of liquid removed for the separate readings. The digestions were carried out by mixing the materials at 25° and placing them in a water bath contained in an asbestos-lined air oven maintained at 37°. This procedure was adopted to simulate the conditions of normal digestion *in vivo*, the chilling effect of ingested food being observable for several hours. For the separate readings 25 cc. of the mixture were pipetted out into a beaker containing 2 cc. of a 1% solution of arsenic acid. This mixture was then filtered and the polarization read on the filtrate. When the starch granules begin to disintegrate (that is, after 4 or 5 hours), the fragments tend to run through the filter. Except where otherwise stated the polarizations have been corrected for the optical activity due to the sugars in the bread suspension by polarizing the filtrate from a suspension of inch cubes at the end of half an hour. Under these conditions most of the sugars but no dextrins enter the solution; and but little dextrin enters the solution even when the bread is completely broken down. The polarizations are also corrected for the optical activity of the diastoid and the takadiastase. The saliva and ptyalin were optically inactive. Takadiastase attacks both starch and gluten, and also slowly changes the maltose formed from the starch into *d*-glucose. The apparent acidity of the different materials used for digestion was obtained by titrating after half an hour the filtrate from broken down bread suspensions with 0.02 *N* potassium hydroxide and phenolphthalein, the recorded results having been corrected for the apparent acidity of the distilled water used in making the suspensions.

The following table gives the polarization of digestion mixtures made with the breads just described, the bread being in the condition known as "fresh" at the time of the digestion, except where otherwise stated. "Fresh" bread is that baked late in the day for sale the next day. Where the same bread was tested after different periods of fermentation, the digestion experiments were carried out simultaneously, in the same water bath. Three grams of the bread, in cubic form, were placed in the enzyme solution, the final volume being 300 cc. The bread floats throughout the experiment.

These results show, for hard and soft wheat flours, that the time of fermentation of the dough makes no appreciable difference in the digestibility when varied over a range of 400%; nor does such variation appreciably affect the acidity. They show, further, that the starch of the soft wheat digests nearly three times as fast as that of the hard wheat, the spring wheat and the commercial mixture acting approximately like the soft wheat. The digestion coefficients given in the table were obtained

TABLE I.

Polarizations Read as *d*-Glucose.

Wheat No.	1	1	1	1	2	2	2	3	4	Spoiled yeast. 1	2	3
Time of fermentation. Time of digestion (hrs.).	0.5 norm.	norm.	2 norm.	Duplicate norm.	0.5 norm.	norm.	2 norm.	norm.	norm.	norm.	norm.	norm.
0.25	0.09	0.14	0.17									
0.50	(0.18)[1]	(0.23)[1]	(0.26)[1]	0.24	0.67	0.64	0.68	0.65	0.67	0.19	0.81	0.38
0.75	0.27	0.33	0.37									
1.50	0.55	0.64	0.62	0.72	1.12	1.04	1.05	0.90	1.17	0.63	1.24	0.88
2.50	0.83	0.90	0.88	1.03	1.12	1.15	1.13	1.01	1.19	0.89	1.23	0.97
3.50	1.02	1.07	1.08									
4.50	1.13	1.19	1.12		1.12	1.16	1.14					
5.50	1.13	1.17	1.14									
6.50	1.19	1.15	1.23									
22	1.21	1.21	1.34									
23					1.11	1.13	1.00					
45				1.09	.			0.96	0.94			
71					0.77	1.01	0.99					
316										0.89	1.00	0.70
Normal acidity.....	0.00019		0.00026		0.00010		0.00012		0.00012		0.00007	
		0.00015		0.0007		0.00010		0.00012		0.00004		0.00012
Original polarization...	0.08	0.03	0.00	0.02	0.05	0.02	0.04	0.08	0.10	0.09	0.04	0.10
Digest. coeffic.....	15%	19%	22%	20%	56%	53%	57%	54%	56%	16%	67%	32%

[1] Interpolated.

by dividing the polariscopic reading at the end of one-half hour by 1.20 as an approximate average maximum.

The effects produced by the use of yeast which has begun to ferment owing to bacterial activity are marked in the cases of the bread from soft wheat and that from the "oriental" brand, the former being greatly accelerated, the latter retarded. Each of the breads made with spoiled yeast had a bitter taste. The ingestion of a small amount of one of these breads almost immediately caused a burning sensation in the stomach which lasted for ten hours, at the end of which time it was relieved by the ingestion of soda. Hence their digestion *in vivo* might differ still more than is here indicated for their digestion *in vitro*.

That the difference in the digestibility of the breads from hard and soft wheat flours is due wholly to differences in the amount of gluten these breads contain (8% and 6%, respectively), is shown by the following results, obtained by grinding in a mortar some of the pulp of the same loaves of bread used in obtaining the foregoing results. The duplicate determinations were made on the same suspension after standing three days in a glass-stoppered bottle, preserved by toluene.

TABLE II.

				Duplicates.					
Wheat No.	1	1	1	1	1	1	2	2	2
Time of fermentation.	½		2	½		2	½		2
Time of digestion(hrs.).	norm.	norm.	norm.	norm.	norm.	norm.	norm.	norm.	norm.
0.25	..	..	..	..	..	..	0.91	1.12	0.97
0.50	1.08	1.01	1.17	1.09	1.02	1.13	..	..	..
0.75	..	..	..	..	..	..	1.01	1.16	1.18
1.50	1.05	1.08	1.04	1.09	1.13	1.14	1.05	1.08	1.04
2.50	..	..	..	1.08	1.05?	1.09	0.84	1.10	1.10
3.50	1.00	1.00	1.06	1.05	1.14	1.10	0.83	1.05	1.02
4.50	..	..	..	1.00	1.05	1.08	..	..	..
22	0.78	0.89	0.81	..	..	..	0.82	0.99	1.14?
23	..	..	..	0.89	0.97	0.94	..	..	..
71	0.79	0.89	0.79	..	..	..	..	..	..

The starch under these conditions in reality digests about as fast as boiled starch, as judged by the iodine colorations and the polariscopic readings (*infra*).

Other factors, such as slight variations in the acidity of the mixtures, in the temperature of mixing the digestion materials, and in the temperature at which the solutions were read on the poloriscope exerted no appreciable effect.

The following results were obtained with three commerical breads, broken down under water just enough to cause the pulp to sink—a degree of disintegration approximately equal to that produced by normal mastication. The readings are uncorrected for the original polariza-

tions, since these were obtained on the pulp just described, instead of on the undisintegrated pulp, and hence the solutions contained appreciable amounts of dextrins.

TABLE III.

Bread No. Time of digestion. Hours.	5.	6.	7.
0.5	1.12	1.10	1.04
1.5	1.11	1.26	1.28
3.0	1.35	1.22	1.34
23	1.29	1.18	1.24
Normal acidity	0.00018	0.00015	0.00012
Original polarization	0.28	0.20	0.13

These results show that the disintegration of the gluten was sufficient to permit the digestion of the starch to the same extent as that in the breads ground up to flotation. That this agrees closely with the rate of digestion of freshly boiled starch is indicated by the results contained in the following table. The rice starch used in these experiments was boiled five minutes in approximately 2% solution. The concentration of dry (120°) starch in the digestion mixtures was 0.863%. This table also contains data on the digestibility of a home-baked bread (made with hard-wheat flour), and of another commercial bread. The interior of the loaf of these two breads was rasped on a horse-radish grater into fragments about 3 mm. in diameter. The results given in two of the columns were obtained on the same pulps air-dried. The results are not corrected for the original polarizations of the solutions. The starch at its final dilution polarized 0.64; but this seems to be mostly due to dextrins (as the amyloses filter out), which immediately digest, so that this correction should not be applied to the observed readings.

The initial maximum in the polariscopic readings obtained with the starch is due to the presence of a small amount of dextrins in the solution, even with 1 : 7 saliva, so rapid is the hydrolysis of the amyloses present. This may conveniently be shown with iodine after filtering off the rose-amylose, provided a preservative has been added to prevent the digestion of the erythrodextrin during filtration.

If the polarizations given for the starch be divided by 1.726 they become directly comparable with all the other results recorded in this paper, on the assumption that the breads contained 50% of starch, since all are calculated to 1% of the original material for the final concentration in the digestion mixture. This makes the values for the starch, even at the start, when the solution contained dextrins, only slightly higher than the corresponding values obtained with disintegrated bread pulp (Table II).

The colorations of the digesting starch with iodine-water show clearly the progress of the digestion of different ingredients in the starch. The original blue color was partly due to amylodextrin; the red color given at

TABLE IV.

Time of digestion. Hrs.	Color of starch with iodine-water in small amt.	Color with iodine due to	1% diastoid. Freshly boiled rice starch.	1% commercial ptyalin. Home-baked bread No. 8. Moist.	Home-baked bread No. 8. Air-dried.	Commercial bread No. 9. Moist.	Commercial bread No. 9. Air-dried.
0.00	Blue	Starch	0.64				
0.75				0.62		0.68	
1.00	Red ⟶ violet	Rose-amylose	2.62		0.25		0.28
1.75				0.96		0.86	
2.00	Pink ⟶ light purple	Rose-amylose	2.42		0.67		0.60
2.75				1.06		1.04	
3.00	Light purple	Rose-amylose	2.46		0.99		0.88
3.75				1.14		1.10	
4.00	Colorless		2.42		1.28		1.18
4.75				1.22		1.12	
	Large amount of Iodine-water		2.48[1]		1.44		1.34
5.00	Purple	Red-amylose					
6.00	Purple		2.48				
7.00	Purple	Red-amylose	2.50				
24	Trace of purple	Red-amylose	2.70				
25	Pink	Red-amylose	2.60				
26	Pink	Red-amylose	2.64				

the end of one hour with iodine in small amount was due to rose-amylose. the dextrins and amylose having been nearly all changed into maltose; by the end of four hours the amylopectin and the rose-amylose were all digested; by the end of five hours the amylocellulose had digested sufficiently to disintegrate the walls of the starch granules (already disrupted by boiling), so that their fragments came through the filter paper, the mixture now containing red-amylose, requiring a large amount of iodine for the production of its iodide; by the end of twenty-four hours the amylocellulose was nearly all digested. The absence of any maltase (*infra*) is to be noted.

The low digestibility of the air-dried bread is probably attributable to the setting of the gluten during the drying, thus rendering the starch more difficult for the enzymes to get at, rather than to any change in the starch, as surmised by Neumann.[2,3] The home-baked bread was

[1] Filtrate turbid; refilter.

[2] *Zeit. ges. Getreidew.*, **6**, 119 (1914); *Chem. Abs.*, **9**, 2554.

[3] Confirmed by direct experiment after complete disintegration of the gluten by grinding.

much more palatable than the commercial breads, but the polarizations show no reason therefor.

That the source and amount of the enzymes makes but little difference for moderate variation in amount in the rate of digestion of starch is indicated by the following results, obtained with the same bread pulp and the same starch solution, and the enzymes indicated in the table. Fernbach and Wolff[1] found that doubling the concentration of malt extract had no effect on the rate of digestion.

TABLE V.

Time of digestion. Hrs.	1% rice starch.		1% home-baked bread.		
	1% diastoid.	1% taka-diastase.	1% com'l ptyalin.	1% diastoid.	1% taka-diastase.
0.00		(2.4)[2]			
0.50		1.94	(0.47)[2]	0.43	0.40
0.75			0.62		
1.00	2.62	1.70		0.72	0.48
1.75			0.96		
2.00	2.42	1.65		0.92	0.47
2.75			1.06		
3.00	2.46			1.17	0.48
3.75			1.14		
4.00	2.42	1.51			0.46
4.75			1.22		
5.00	2.48	1.43			0.45
6.00	2.48				
7.00	2.50	1.35			0.44
24	2.70	1.18			
25	2.60				
26	2.64	1.16			0.44
29					0.45
122		0.92			0.22

The continuous fall in the readings obtained by the use of takadiastase is noteworthy. The initial value for the starch digestion was obtained by extrapolation, although the maximum must lie somewhere between time 0 and time 0.5 hour. The maximum obtained by extrapolation must be near the true maximum the solution would have shown, however, and agrees approximately with the values obtained by the use of diastoid.

The reason for the steady fall in the polarization obtained with takadiastase and starch is that the takadiastase contains maltase (as is well known[3]), so that the maltose slowly hydrolyzes into *d*-glucose. The final value obtained (asymptotically) after 122 hours equals 38.3% of the intitial (extrapolated) value, in close agreement, considering all the circumstances, with the theoretical value 39.8%, based on the assump-

[1] *Loc. cit.*

[2] Extrapolated.

[3] Daish, *Loc. cit.*, 2054.

tion that the initial optical activity was due to maltose and the final value was due to *d*-glucose, obtained by use of the formula

$$\text{Rotation of } d\text{-glucose} = \text{rotation of maltose} \times \frac{360}{342} \times \frac{52.5}{138.3},$$

where 52.5 is the specific rotatory power of *d*-glucose, 138.3 the specific rotatory power of maltose, and 360 the molecular weight of two molecules of *d*-glucose.

The one-half hour reading obtained by the action of takadiastase on bread also agrees fairly well with those obtained with other enzymes, although the actual amylase present in any of the preparations used was but a small indefinite per cent. of the total weight of the enzyme material. These results, therefore, together with the great activity shown by very dilute saliva, seem to indicate that the rate of digestion does not vary appreciably for moderate variations in the amount of enzyme present. Two influences in this case (takadiastase on bread) tend to diminish the polariscopic readings: first, the slow hydrolysis of the maltose to *d*-glucose; second, the digestion of the gluten, with the elimination of levorotatory substances. The value after 122 hours is only 19% of the average maximum polarization (1.20) of 1% bread preparations.

Takadiastase does not completely digest the amylocellulose even on long standing (122 hours), if it digests it at all; that is to say, if the residue is boiled at any time after the digestion mixture no longer gives any color with iodine-water (end of the digestion of amylopectin and roseamylose), it will, on cooling, give a deep blue with iodine-water.

Conclusions.

1. The specificity and complexity of starches is confirmed.

2. A number of polysaccharides are clearly recognized and differentiated, and the existence of two new ones indicated.

3. The three principal ingredients of cereal starches are amylocellulose (the cell walls), amylopectin, and amylose.

4. The amylose, contrary to the contention of Maquenne and Roux, passes through the stages of amylodextrin and erythrodextrin during salivary digestion, but these dextrins digest so rapidly that their presence in the digestion mixture can conveniently be demonstrated only when the enzyme concentration is very low (*e. g.*, 1 : 99). Furthermore, an appreciable amount of achroödextrin enters the solution with ordinary concentrations of saliva.

5. All of the dextrins under ordinary conditions disappear from the solution within 15 minutes, so that thereafter the further progress of the digestion can be followed by the polariscope, the only optically active substance then present being maltose if maltase has not been added from some outside source. As the amylose is all digested by this time, the

further digestion represents action on amylocellulose and amylopectin and their products of hydrolysis.

6. By slow digestion almost the entire amount of amylose present was obtained in solution as erythrodextrin at the end of 15 minutes. Hence under ordinary conditions the digestion of amylose must be almost instantaneous.

7. Rose-amylose, derived from amylopectin, digests completely in four hours. This has usually been regarded as the end of starch digestion, the rose-amylose being confused with erythrodextrin.

8. The amylocellulose (cell walls) digests only after more than 24 hours.

9. The only differences observable in the rate of digestion of bread made from hard or soft wheat, and fermented more or less than usual, were due to the relative amounts of gluten present. When the gluten was broken down the rate of digestion was sensibly the same.

10. The cause of the greater palatability of home-baked bread was not discovered.

11. Various pronounced effects due to fermentation by spoiled yeast were noted.

12. The activity of amylases is not sensitive to small changes of temperature or of acidity produced by the organic acids found in bread; nor does their activity seem to be proportional to their concentrations.

13. It would seem that under physiological conditions most of the amylose must be changed to dextrins in the mouth, and that these dextrins as well as most of the amylopectin and its products of hydrolysis must be digested in the stomach; whereas the digestion of the amylocellulose must take place for the most part in the intestine.

14. Stale (air-dried) bread digests very slowly unless its gluten be completely broken down.

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NEW BOOK.

Annuaire pour l'An 1916. Public par le Bureau des Longitudes. Price: 1.50 francs (30 cents). Paris: Gauthier-Villars & Cie.

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J. W. RICHARDS.