177. *The Amylolytic Degradation of Starch. A Revision of the Hypothesis of Sensitisation.*

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An extension of the work of Haworth, Kitchen, and Peat (1943) on the amylolysis of starch has provided data which necessitate a revision of the tentative hypothesis then advanced to account for the " sensitisation " of the limit dextrin-A by the action of salivary amylase. Dextrin-A is resistant to the hydrolytic action of β-amylase until it is " sensitised " by contact

with salivary α-amylase. To explain this effect the suggestion was made that the function of the α-amylase in sensitisation was the scission and resynthesis of the 1 : 6-glucosidic linkages constituting the cross (or polymeric) links in the branched structure of dextrin-A (and of amylopectin from which it is derived).

The experimental results described in this communication cannot be reconciled with the suggested mode of action of α-amylase but are explicable in terms of the conception of α-amylolysis as being the hydrolytic scission of the normal 1 : 4-glucosidic bonds of "inner" chains (an inner chain is a chain of 1 : 4-linked α-glucose members situated between two branch points). Contrary to our earlier view it would seem that the cross linkages of branched structures like amylopectin are resistant to hydrolysis by α-amylase.

Sufficient information is not yet available to enable a decision to be reached on the molecular constitution of ramified structures such as amylopectin. The properties of amylopectin can be equally well interpreted in terms of the branched or laminated formulation of Haworth and Hirst which was the simplest expression capable of illustrating the facts then available, or by an extension of this idea in which more than one cross linking is introduced. The extreme case is illustrated by the formulation by K. H. Meyer of an irregular model with multiple branching of chains.

Some comments are made on Myrbäck's hypothesis concerning the biogenesis of starch.

In 1943, Haworth, Kitchen, and Peat (*J.*, 1943, 619) recorded some observations on the degradation of starch by amylolytic action to account for which a hypothesis was provisionally advanced concerning the mechanism of the action of α-amylase on starch. The facts were briefly as follows: (i) the hydrolysis of starch by β-amylase proceeded until 60% of the starch had been converted into maltose; (ii) the remaining 40% consisted of a limit dextrin (dextrin-A) which was resistant to the further action of β-amylase; (iii) when dextrin-A was submitted to the action of salivary amylase, the dextrin was "sensitised" in the sense that it was once more susceptible to the hydrolytic action of β-amylase, by the agency of which it was converted into maltose (40%) and a second limit dextrin (dextrin-B, 60%); (iv) the sensitisation of dextrin-A appeared to be effected by a very brief contact with salivary amylase (the α-amylase was in each experiment destroyed by boiling before the β-amylase was added) and to be accomplished without the liberation of reducing groups.

It appeared possible at that time to accommodate these facts on the view that the points of junction of the repeating unit chains of the branched component of starch (amylopectin) constituted obstructions to the end-wise attack of β-amylase, the action of which on amylopectin ceased when the branch points were reached. The sensitising action of salivary amylase was then conceived as a removal of the obstructions by scission of the glucosidic linkages (believed to be 1 : 6-linkages) constituting the branch points. To account for the fact that the sensitised dextrin-A did not behave towards β-amylase as if it consisted entirely of unbranched chains of the amylose type, it was necessary to assume either that the salivary amylase attacked only a selected number of the 1 : 6-cross linkages or that the scission of 1 : 6-linkages by this enzyme was a reversible reaction and that the polymeric or cross linkage between two unit-chains was reconstituted in a different position. Of the alternative hypotheses, the latter was preferred for reasons given in the paper cited.

The experimental work described in the 1943 paper was actually carried out much earlier and was recorded in thesis form in 1937. Great advances have been made during the past decade in the technique of starch analysis and in the investigation of amylase reactions, and the purpose of this communication is to describe the results of the application of these more refined methods to the study of the problem outlined above. The observations of Haworth, Kitchen, and Peat have been for the most part confirmed and extended, but in one essential particular we have now found that the chosen methods of sugar analysis used earlier were not sufficiently precise for the purpose and led to the erroneous conclusion that the sensitisation of dextrin-A occurred without the concomitant liberation of reducing groups.

The chief refinements introduced in the present work consisted in (*a*) the use of a β-amylase preparation from soya bean which was shown to be free from contamination by α-amylase and maltase; (*b*) the preparation of dextrin-A under rigidly controlled conditions by the action of the soya bean β-amylase on an amylopectin separated from potato starch by the thymol method (Haworth, Peat, and Sagrott, *Nature*, 1946, **157**, 19); (*c*) the use of copper reagents (Shaffer–Hartmann and Somogyi) adapted to a semi-micro-scale, to estimate the extent of hydrolysis effected at any stage in a digestion; (*d*) the quantitative determination of the iodine-staining power of a digest (the Absorption Value, A.V.) and the diminution of this value [expressed as a percentage of the Blue Value (B.V.) of the original dextrin-A], as hydrolysis proceeded; and (*e*) a strict quantitative control of enzyme concentration and of the reaction time.

The β-amylase was used throughout these experiments in such concentration that, while it effected a rapid hydrolysis of amylose, its action on the dextrin-A was negligibly small. The

effectiveness of the action of salivary amylase was varied by changing either the concentration or the time of action of this enzyme.

The observations made on the sensitisation of dextrin-A are summarised in the Table, and some of these results are given graphical expression in the Figure.

The Successive Actions of α- and β-Amylase on Dextrin-A.

Digest.	Dilution of stock solution of salivary α-amylase (×).	Duration of action of α-amylase (mins.).	Iodine colour (% diminution of A.V. after α-amylase action) (6800 A.).	% Apparent conversion into maltose effected by : α-Amylase alone.	α-Amylase followed by β-amylase (total).	β-Amylase (alone) after sensitisation (difference).
1	10,000	*ca.* 0·5	0	0	0	0
2	1000	,,	0	0	0	0
3	100	,,	0	0	0	0
4	100	,,	1·1	0	0	0
5	50	,,	12·1	—	10·45	—
6	50	,,	12·9	—	11·8	—
7	20	,,	54·2	—	21·4	—
8	20	,,	48·7	—	18·6	—
9	10	,,	88·5	11·75	28·2	16·45
10	10	,,	84·6	15·1	32·4	17·3
11	500	5	0	0	0	0
12	200	5	2·7	—	10·4	—
13	500	15	2·1	—	—	0
14	100	5	23·0	—	13·5	—
15	200	15	17·6	—	12·5	—
16	50	5	67·0	8·7	21·9	13·2
17	100	10	51·1	5·3	16·35	11·05
18	100	15	52·2	—	19·1	—
19	200	30	47·8	6·1	19·2	13·1
20	100	20	75·6	12·5	26·8	14·3
21	50	15	90·5	16·7	30·2	13·5
22	20	15	Achroic	37·5	44·1	6·6
23	10	15	,,	41·0	46·6	5·6
24	5	15	,,	47·5	51·9	4·4
25	1	15	,,	52·8	58·3	5·5
26	1	30	,,	57·7	62·2	4·5
27	1	60	,,	—	67·3	—
28	1	60	,,	63·3	70·5	7·2
29	1	120	,,	71·4	74·3	2·9
30	200	5	23·5	2·1	14·7	12·6
31	200	10	34·0	2·9	15·6	12·7
32	200	15	54·5	5·7	18·9	13·2
33	50	0·5	49·5	4·45	—	—

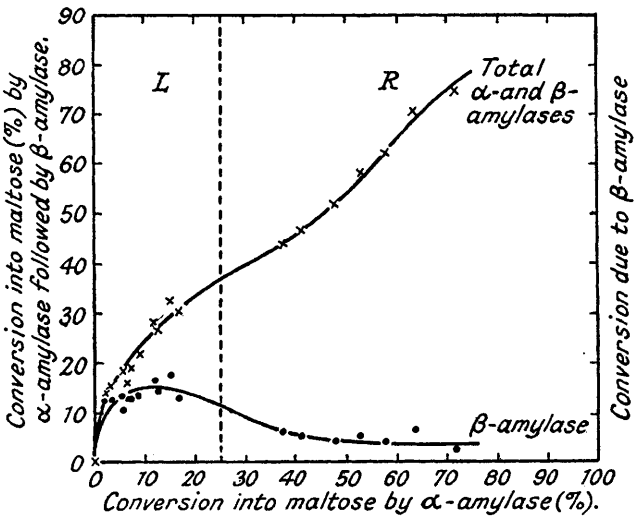
It is at once apparent that an essential step in the sensitisation of dextrin-A by the action of α-amylase is the hydrolytic fission of some of the glucosidic linkages. When the concentration and time of action of the salivary amylase are so ordered that no diminution of A.V. or increase in reducing power of the digest is observed, then no sensitisation of the dextrin-A occurs. Otherwise expressed, some degree of hydrolysis of dextrin-A by the sensitising enzyme is an essential prerequisite for its further hydrolysis by β-amylase.

If sensitisation connotes hydrolysis and total resynthesis of cross-linkages, no liberation of reducing groups would occur during the process, and this is contrary to the experimental facts. If it be supposed that the action of α-amylase is purely hydrolytic and is confined to the scission of 1 : 6-linkages, it can be calculated, on the basis of a unit-chain length of 12 glucose members (Haworth, Hirst, Kitchen, and Peat, *J.*, 1937, 791), that dextrin-A would yield a mixture of unbranched dextrins the reducing power of which would be approximately one-sixth of that of maltose, *i.e.*, the scission (without resynthesis) of all the 1 : 6-links in dextrin-A would be accompanied by an increase in reducing power corresponding to an apparent conversion into maltose of about 17%. It is now shown that this supposition also is not in keeping with the known facts. Thus, the scission of all the cross-linkages in dextrin-A would remove all the postulated obstructions to β-amylase hydrolysis, and the subsequent action of this enzyme on the sensitised dextrin would be expected to lead to complete, or nearly complete, conversion into maltose. The results given graphically in the Figure (region L) show quite clearly that this is not so. The action of β-amylase on an α-amylase-treated digest, having a reducing value

corresponding to 17% apparent conversion into maltose, ceases when the total conversion figure reaches 30—32%. In other words, β-amylase is able to effect a further conversion of 13—15% only, instead of the 80% expected if the above postulate were true.

Finally, if sensitisation consists in the hydrolysis of a proportion only of the 1 : 6-linkages, then as the percentage apparent conversion into maltose by α-amylase increases, the subsequent percentage conversion by β-amylase should increase also, as the obstructing branch points are progressively removed. Again, this is contrary to the facts. The graph shows that, as the percentage conversion by α-amylase was increased from 2 to 18%, the subsequent action of β-amylase further degraded the sensitised dextrin-A by an amount which was approximately the same (13% conversion into maltose) for each specimen.

When the results of the prolonged exposure of dextrin-A to the sensitising action of α-amylase are examined (Region R in the Figure), the persistence of 1 : 6-linkages is even more strikingly indicated. In this series of experiments, the greater part of the degradation of dextrin-A was effected by the agency of the α-amylase and obviously involved the scission of 1 : 4-links. The activity of the enzyme was sufficiently high to yield products giving no colour with iodine (100% diminution of B.V.). The subsequent action of β-amylase on these achroic dextrins led



Sensitisation of Dextrin-A.

to further hydrolysis, but a limiting factor was still present, and 100% conversion into maltose was never observed, even when three-quarters of the dextrin-A had been initially hydrolysed by α-amylase.

It would seem that these diverse observations can be correlated only on the view that α-amylase, equally with β-amylase, is unable to resolve the polymeric linkages, and that the hydrolytic activity of both amylases is confined to the chain-forming 1 : 4-glucosidic linkages

The tentative proposal advanced to explain the observations of Haworth, Kitchen, and Peat is thus shown to be inadequate by the further investigation of the phenomenon of sensitisation described in this communication. The branch points (*i.e.*, the polymeric 1 : 6-linkages) which obstruct the action of β-amylase are not hydrolysed in the initial sensitising period of α-amylolysis but persist in the dextrins formed during that period (these dextrins are described as α-dextrins by Myrbäck). No occasion arises, therefore, to postulate a re-synthesis of 1 : 6-linkages as an integral part of the sensitising action.

The sensitisation of dextrin-A is, in fact, explicable in terms of the liberation (by α-amylase scission of 1 : 4-linkages) of new " outer " chains with non-reducing terminal groups. These " outer " chains are susceptible to endwise attack by β-amylase, the action of which proceeds, with the separation of maltose units, until a branch-point is again reached.

Our hypothesis of sensitisation has been criticised by K. Myrbäck (Svedberg Anniversary Volume, 1944, pp. 474—483) as not being in line with the general conception of the course of α-amylolysis. In the light of the results of the further studies described above, we acknowledge the soundness of this criticism and recognise that sensitisation is explicable as being the result of

the scission by α-amylase of some of the 1 : 4-linkages constituting " inner " chains (an inner chain being a chain of 1 : 4-linked α-glucose members situated between two branch points).

While agreeing with Myrbäck's views on the mode of action of α-amylase on starch and its components, we are not yet convinced that the properties of amylopectin (and of the dextrin-A derived from it) are better explained by the irregular, highly ramified structure proposed by K. H. Meyer and Bernfeld (*Helv. Chim. Acta*, 1940, **23**, 880) than by a simpler laminated formulation of which that originally proposed by Haworth, Hirst, and Isherwood (*J.*, **1937**, 577) is an example. Myrbäck prefers the Meyer formulation of amylopectin and bases his preference on certain assumptions with regard to the mode of synthesis of starch in the plant. This author states (*loc. cit.*) that " in a certain plant under certain conditions, the probability of attachment of a new glucose unit in the position 6 (which means ramification of the chain) is in constant ratio with the probability of attachment in the position 4 (which means lengthening of the chain). Assuming that γ is the ratio between these probabilities, it is possible to calculate the probable constitution of the molecules formed when synthesis has proceeded for some time ".

We have some reason to believe, however, that the synthesis of amylopectin in the plant does not consist entirely in the building up of chains by the apposition of single glucose units. Studies of the biogenesis of starch in the potato indicate that two enzymes are operative in the synthesis of the branched chain component. The one, P-enzyme or phosphorylase, builds up from glucose-1 phosphate short-chain dextrins (*pseudo*amylose) consisting, on an average, of 20 glucose members joined by 1 : 4-linkages; the other enzyme, Q-enzyme, effects the mutual union by 1 : 6-linkages of these *pseudo*amylose chains and thus gives rise to the branched structure of amylopectin (Bourne and Peat, *J.*, 1945, 877; Bourne, Macey, and Peat, *J.*, 1945, 882). Q-enzyme is incapable of using glucose-1 phosphate as a substrate.

Cori and Cori (*J. Biol. Chem.*, 1943, **151**, 57) have shown that a " cross-linking " enzyme similar to Q-enzyme is an essential factor in the synthesis of glycogen in animal tissues. This factor, like Q-enzyme, functions only in conjunction with a phosphorylase (a P-enzyme). It exerts no action, *per se*, on glucose-1 phosphate.

Furthermore, the presence of a polysaccharide catalyst is necessary as a " starter " even in the synthesis of unbranched chains from glucose-1 phosphate by the agency of P-enzyme. The catalyst appears to constitute a pattern or nucleus on which the chains being synthesised grow by the addition of glucose units, one at a time.

It is not proposed, at present, to amplify or to criticise Myrbäck's hypothesis of the course of α-amylolysis. The main purpose of this communication is to state our revised view that the sensitisation of dextrin-A by α-amylase cannot be divorced from the normal hydrolytic function of α-amylase, this normal function being the scission of 1 : 4-glucosidic linkages. The cross linkages, constituting branch points, appear to be resistant to the hydrolytic activity not only of β-amylase but also of the α-amylase of saliva, and these 1 : 6-glucosidic linkages persist in the dextrins produced by the action on amylopectin of either enzyme.

Experimental.

α-Amylase from Saliva.—A stock solution of salivary α-amylase was prepared by diluting early-morning saliva with an equal volume of water and removing the precipitate of mucin by the centrifuge. Maltase was shown to be absent by incubation with maltose, no increase in reducing power being detected by the Shaffer–Hartmann method. When used in digests containing dextrin-A as substrate, this stock enzyme solution was suitably diluted with water, the greatest dilution being 10^4-fold.

β-Amylase from Soya Bean.—Soya bean was chosen as the source of β-amylase because it had been claimed that the enzyme product, in contrast to that from wheat and barley, was free from contamination by α-amylase. The method of isolation has already been reported (Bourne, Macey, and Peat, *loc. cit.*). The earlier observation that there was no maltase impurity in the product was confirmed.

Estimation of Reducing Sugar.—For the estimation of reducing sugars produced by amylolysis two copper reagents were employed, namely those of Shaffer and Hartmann (*J. Biol. Chem.*, 1921, **45**, 377) and Somogyi (*J. Biol. Chem.*, 1937, **117**, 771). In each case the amount of maltose produced in a digest was estimated from a linear calibration graph (maltose *v.* equivalent volume of thiosulphate solution), which had been obtained by using standard solutions of pure maltose.

The Shaffer–Hartmann reagent, which was stable for periods of at least 4 weeks, was satisfactory for the estimation of 0·4—3·0 mg. of maltose in 5 c.c. of solution, and, of course, more concentrated sugar solutions could be suitably diluted. However, it failed completely to detect smaller amounts of the sugar, *i.e.*, the calibration graph intercepted the axis at about 0·4 mg. owing to re-oxidation of the cuprous oxide by atmospheric oxygen (Somogyi, *loc. cit.*). When it became necessary to measure momentary actions of amylolytic enzymes the method was no longer adequate, without modification, to estimate the traces of maltose thus produced. In such a case a known amount of maltose was incorporated in the digest, and the increase in sugar was then a measure of the extent of amylolysis.

On grounds of convenience this modified procedure was subsequently replaced by the method of Somogyi the use of which enabled maltose concentrations of 0·02—0·90 mg./5 c.c. to be measured. The

copper reagent, which had been specially designed for this purpose, contained sodium sulphate (20%) to reduce the solubility of atmospheric oxygen, and consequently the calibration graph passed through the origin. When the reagent was stored for more than a few days it was apparent that some change had occurred, necessitating a second calibration. Therefore all determinations by the Somogyi method were made as soon as possible after the standardisation.

Measurement of Absorption Value (A.V.).—The expression A.V. is used to describe the reading on the logarithmic scale of a Spekker Photoelectric Absorptiometer when the absorption of light by a solution of a polysaccharide–iodine complex, contained in 4 cm. cells, was measured. The light filters employed were of the Ilford gelatin type (Nos. 601—608, transmitting light of 4300—6800 A.). The wave-length of the light used is stated whenever an absorption value is quoted, *e.g.*, A.V., 0·21 (6800 A.). No stipulation is made with regard to the absolute or relative concentrations of polysaccharide and iodine; indeed in some cases, for example during the course of an amylolytic digest, these are not known.

Measurement of Blue Value (B.V.).—The blue value (B.V.) of a polysaccharide has a special significance; it is the A.V. observed when a polysaccharide is stained with iodine under the standard conditions prescribed by Hassid and McCready (*J. Amer. Chem. Soc.*, **1943**, **65**, 1154) and when Ilford gelatin light filters, transmitting light of 6800 A., are employed. Under these conditions the coloured solution contains the polysaccharide (1 mg./100 c.c.), iodine (2 mg./100 c.c.) and potassium iodide (20 mg./100 c.c.). Thus the blue value, in contrast to an absorption value, is characteristic for a given polysaccharide. Since the concentration of the actual coloured material, *i.e.*, the polysaccharide–iodine complex, cannot be readily ascertained, there is no point in evaluating a hypothetical extinction coefficient which, in any case, would be proportional to the blue value.

Although Hassid and McCready (*loc. cit.*) stated that polysaccharide–iodine colours developed to their full intensity almost immediately, we have found that for some polysaccharides, especially for those staining red, this was not quite true. As a precautionary measure, therefore, the coloured solutions were allowed to stand for several hours before the blue value was ascertained. The statement by the same authors that the colours were stable for several days was verified.

Preparation of Dextrin-A from Amylopectin.—A sample of amylopectin, which had been isolated from potato starch by fractional precipitation with thymol (Haworth, Peat, and Sagrott, *loc. cit.*) and had B.V., 0·216, was used as the substrate for the production of dextrin-A by the agency of soya bean β-amylase.

A suspension in cold water of the amylopectin (dry weight, 50·8 g.), which initially had been moistened with a small volume of alcohol, was slowly stirred into boiling water, giving an approximately 3% paste (1500 c.c.), which was rendered less viscous by the addition of sodium chloride (0·5 g.). After being boiled for 20 minutes longer, the solution was cooled to 35·5°, stirring being continued throughout the process. Meanwhile the enzyme solution was prepared by shaking soya bean β-amylase (0·40 g.) with distilled water (350 c.c.) for approximately 1 hour and removing the undissolved residue by the centrifuge. The enzyme solution and the paste were thoroughly mixed, accurately diluted to 2 l., covered with a layer of toluene, and incubated at 35·5°.

The progress of the amylolysis was followed by removing, at intervals, aliquot portions (1 c.c.) of the digest, which were diluted with sufficient water to permit accurate analysis for maltose by the Shaffer–Hartmann method. The weights of maltose in the digest were nil, 28·37 g., and 29·45 g. after zero time, 27 hours, and 42 hours respectively. To ensure completion of the reaction a further volume of β-amylase solution (0·10 g. in 50 c.c. water; prepared as above) was introduced at this stage. After total incubation times of 68 and 88 hours, 29·65 g. and 29·80 g. of maltose were present. The mean (29·63 g.) of the last three estimations was assumed to be the limiting amount of maltose, and, since some amylopectin (0·44 g.) which had not been gelatinised was recovered from the walls of the reaction vessel by hardening with alcohol, this value represented a 55·7% conversion into sugar. The relative constancy in the amount of maltose produced in 42—88 hours indicated the absence of all but a trace of α-amylase in the enzyme.

After being boiled for 10 minutes to inactivate the enzyme and filtered to remove coagulated protein, the filtrate was mixed with an equal volume of alcohol, which was sufficient to precipitate practically all the dextrin-A, leaving most of the maltose in solution. The product was hardened by grinding it with alcohol, washed with ether, and dried in a vacuum over phosphoric oxide. Yield, 22·8 g.(45·3%); ash, 0·55%; reducing power ≡ 1·6% maltose.

The remaining maltose and inorganic impurities were removed by dissolving the dextrin in warm water (1300 c.c.) and dialysing in cellophane bags against frequent changes of distilled water for 2 days. The residue was precipitated with alcohol (2 volumes), hardened, washed, and dried as above. Yield, 18·9 g. (37·5%; based on amylopectin); B.V., 0·200; reducing power <0·05% maltose. No change in either B.V. or reducing power was induced by boiling the dextrin in aqueous solution (50 mg. in 30 c.c.) for 3 minutes.

Further Treatment of Dextrin-A with β-Amylase.—Three digests having the following compositions were incubated under toluene at 35·5°.

Digest (A): dextrin-A (40 mg.), β-amylase solution (10 c.c.; 0·1% prepared as above), water (40 c.c.).
Digest (B): β-amylase solution (10 c.c.; 0·1%), water (40 c.c.).
Digest (C): dextrin-A (40 mg.), water (50 c.c.).

At intervals a portion (5 c.c.) of each digest was removed and the reducing power was estimated directly by the Somogyi method. The amounts of "apparent" maltose in the digests were (A), 1·02 mg. (13 and 17 hours); (B), 0·40 mg.(13 and 17 hours); (C), 0·00 mg. (13 and 17 hours). Therefore 0·62 mg. of maltose was produced by β-amylolysis in digest (A), representing 1·18% conversion into sugar.

Typical Digests Involving the Treatment of Dextrin-A with Saliva and Subsequently with Soya Bean β-Amylase.—(a) *Prolonged action of α-amylase (region R of graph).* The reducing power of the stock solution of mucin-free saliva, estimated by the Shaffer–Hartmann method, corresponded to 2·82 mg. of maltose/10 c.c. A portion of this enzyme was incorporated in digest (29), which contained: dextrin-A (50 mg.), mucin-free saliva (10 c.c.), and water (20 c.c.).

The digest was incubated at 15° for 2 hours and then boiled for 3 minutes, a process which had been shown to effect the complete destruction of the enzyme. The boiled solution was accurately diluted to 50 c.c., giving a standard solution of which 1 c.c. corresponded to 1 mg. of dextrin-A. Two aliquot portions (5 c.c. each ≡ 5 mg. dextrin-A) were withdrawn at this stage; the reducing power of the first portion, determined by the Shaffer–Hartmann method, indicated 4·05 mg. of "apparent" maltose. Thus the entire standard solution contained the equivalent of 40·5 mg. of maltose, 2·8 mg. being due to the saliva itself. The maltose (37·7 mg.) produced by α-amylolysis represented a 71·4% conversion of the dextrin-A.

The second portion (5 c.c.) of the standard solution was introduced into a graduated flask (500 c.c.) containing water (200 c.c.) and slightly acidified with hydrochloric acid (3 drops; 5N). After the addition of iodine–potassium iodide solution (5 c.c.; 0·2% iodine in 2% potassium iodide) it was diluted to 500 c.c. and left for several hours. The A.V. (6800 A.) revealed that the polysaccharide no longer stained with iodine, the fall in A.V. (6800 A.) during α-amylolysis being 100%.

To the remainder of the standard solution (40 c.c. ≡ 40 mg. dextrin-A) was added β-amylase solution (10 c.c.; 0·1%, prepared as above). This digest was incubated at 35·5° under toluene, the reducing power in aliquot portions being estimated periodically by the Shaffer–Hartmann method. After 2, 21, and 27 hours, the "apparent" maltose was 32·4, 33·8, and 34·1 mg., the mean of the last two readings being accepted as the limiting concentration. Since 2·2 mg. and 0·4 mg. were contributed by the saliva and β-amylase themselves, 31·4 mg. had been produced by the consecutive actions of these two enzymes from dextrin-A (40 mg.), representing a 74·3% conversion.

(b) *Less drastic treatment with saliva. Addition of maltose (region L of graph).* Part of the stock solution of mucin-free saliva was diluted 200-fold. Its reducing power was thereby diminished to a value (0·014 mg./10 c.c.) which made a negligible contribution to the reducing power of the following digest (19): dextrin-A (50 mg.), maltose (15·9 mg.), diluted mucin-free saliva (200-fold; 10 c.c.), and water (20 c.c.). When the incubation had proceeded for 30 minutes at 15°, the α-amylase was destroyed by boiling and the experiment was continued as in (a).

The Shaffer–Hartmann method revealed that the standard boiled solution remaining after α-amylolysis contained 19·1 mg. of maltose. Of this, 15·9 mg. had been introduced deliberately and thus 3·2 mg. resulted from enzyme action, the conversion being 6·06%. The A.V. (6800 A.) was 52·2% of that given by the dextrin-A under similar conditions.

During the subsequent action of β-amylase the limiting amount of maltose (21·2 mg.) was reached after 16 hours. Since 12·7 mg. had been introduced deliberately (3·2 mg. had been removed for the 2 estimations) and 0·4 mg. was contributed by the β-amylase itself, 8·1 mg. of maltose had been produced from dextrin-A (40 mg.); a conversion of 19·2% had been effected by the two enzymes.

(c) *Less drastic treatment with saliva. No maltose added (region L of graph).* Again mucin-free saliva which had been diluted 200-fold was employed and its contribution to the reducing power of the digest could be neglected. The digest (30), which was incubated at 15° for 5 minutes, contained: dextrin-A (50 mg.), diluted mucin-free saliva (200-fold; 10 c.c.), and water (20 c.c.).

The solution was boiled for 3 minutes and accurately diluted to 50 c.c. A portion (5 c.c.) of this standard solution, when stained with iodine as in (a), showed a 23·5% reduction in A.V. (6800 A.). A second portion (5 c.c.), analysed directly by the Somogyi method, contained 0·109 mg. of maltose, making a total of 1·09 mg. in the entire standard solution. This represented a 2·06% conversion into maltose by α-amylase.

The remainder (40 c.c. ≡ 40 mg. dextrin-A) was incubated with β-amylase (10 c.c.; 0·1% solution, prepared as above) under toluene at 35·5°. Aliquot portions (5 c.c.), analysed by the Somogyi method, contained 0·66 mg. of maltose when the limit was reached. Thus 6·6 mg. were present in the digest, 0·4 mg. being contributed by the β-amylase itself and 6·2 mg. resulting from the two enzyme actions, representing a 14·7% conversion of the dextrin-A.

THE UNIVERSITY, BIRMINGHAM. [*Received, June 21st,* 1947.]