673. *The Action of β-Amylase on Amylopectin and on Glycogen.*

By T. G. Halsall, E. L. Hirst, L. Hough, and J. K. N. Jones.

The action on waxy maize starch, waxy sorghum starch, and rabbit-liver glycogen of crude β-amylase prepared from wheat flour has been compared with that of crystalline β-amylase, and the limit dextrins so obtained have been examined. The starch limit dextrins produced by the crystalline enzyme have much higher molecular weights than those given by the crude enzyme, but the ratio of terminal to non-terminal glucose residues present in the dextrins is the same (approx. 1 : 10). About 50% of the glucose residues of the original polysaccharide are eliminated by the action of the enzyme, leaving, both for starches and for glycogen, a limit dextrin containing twice the proportion of end-groups originally present.

Recent advances in knowledge of the chemical structure of starch and glycogen have served to promote a greater understanding of the action of the amylases on these polyglucoses (cf. Myrbäck and Sillen, *Nature*, 1949, **163**, 410). The experimental evidence indicates that β-amylase attacks amylose from the non-aldehydic end of the chain (Hanes, *Canadian J. Res.*, 1935, **136**, 185; Myrbäck and Ahlborg, *Biochem. Z.*, 1941, **307**, 132, 140) and that the degradation

process involves complete hydrolysis to maltose, probably as a result of the liberation of successive terminal maltose residues. On the other hand the amylopectin component, which has a ramified structure and a very high molecular weight (10^6 or greater), is only partly converted by β-amylase into maltose and gives rise to a dextrin, designated limit dextrin or α-amylodextrin, which is resistant to β-amylase (cf. Kerr, " Chemistry and Industry of Starch," New York, 1944, p. 8; Meyer, Bernfeld, Rathgeb, and Gürtler, *Helv. Chim. Acta*, 1948, **31**, 1536). It is considered that β-amylase attacks from the non-reducing ends of the chains, removing the terminal maltose residues until the branching points are reached, whereupon hydrolysis ceases and side chains are left consisting of one, two, or perhaps three glucose residues. This limit dextrin is of interest from a structural viewpoint since it is believed to contain the branching points of the parent polysaccharide. Many workers (Haworth, Hirst, Kitchen, and Peat, *J.*, 1937, 791; Haworth, Hirst, and Waine, *J.*, 1935, 1299; Meyer, Bernfeld, and Press, *Helv. Chim. Acta*, 1940, **23**, 1465) have examined its properties, in particular by the methylation method, in order to determine the proportion of terminal glucose residues present in the dextrin. In most of this work the source of the limit dextrin has been whole starch and in these circumstances the structural conclusions are valid only if the amylose component has been converted completely into maltose. Unless special precautions are taken there is some uncertainty about this in view of the tendency for amylose to undergo retrogradation into the insoluble form, which is not attacked by β-amylase (Meyer, Bernfeld, Gürtler, and Noelting, *Helv. Chim. Acta*, 1948, **31**, 108). It is necessary also to be sure of the purity of the enzyme, since it would appear from the present experiments that traces of α-amylase can appreciably affect the molecular weight of the resulting dextrin. These considerations probably account for some of the differences which have been reported to exist between dextrins prepared from different starches and from different samples of the same type of starch. Another possibility which has not been sufficiently stressed in discussions on the structure of these substances is that the study of starches of different botanical origins has not yet progressed sufficiently to permit a decision as to whether or not the various amylopectin components are structurally identical.

Ungerminated cereal grains form the most useful source of β-amylase and, in some cases, have been reported to be entirely devoid of α-amylase (Mills and Bailey, *Cer. Chem.*, 1938, **15**, 351). On the other hand, Hopkins, Murray, and Lockwood (*Biochem. J.*, 1946, **40**, 507) are of the opinion that β-amylase prepared from ungerminated cereals is always contaminated with a little α-amylase. We have carried out many extractions of β-amylase from ungerminated wheat grains, but all these were observed to contain slight traces of α-amylase which could not be removed. The soya bean has been reported to be a source of β-amylase free from α-amylase (Newton, Hixon, and Taylor, *Cer. Chem.*, 1943, **20**, 23). Nevertheless, in the course of many experiments we invariably found appreciable α-amylase activity in the aqueous extract of soya flour, thus confirming Teller's observations (*J. Biol. Chem.*, 1936, **114**, 425).

The limit of maltose formation from native and soluble starches obtained by various workers varies from 59 to 67%. Solutions of solubilised starch have been most widely used as substrates for amylolytic degradation, but this procedure introduces complications since the starch may have undergone structural changes during the process of solubilisation. We have observed, however, that intensively dried starch gives clear solutions closely resembling those of soluble starch and forms a suitable substrate for amylolytic degradation. The starch granules are apparently disrupted during the desiccation process, thus facilitating both dispersion in water and reaction with β-amylase. The preparation of the substrate is important and in order to ensure homogeneity we have forced the starch paste through a homogeniser before the addition of the enzyme.

A sample of dried potato starch was treated at pH 4·8 with an amorphous β-amylase (prepared from ungerminated wheat flour) which had been treated previously with 20% aqueous alcohol for 18 hours, in order to inactivate as far as possible the α-amylase and maltase. Hydrolysis proceeded rapidly until the reducing power reached a constant value corresponding to 60—62% conversion into maltose. The crude limit dextrin (A) obtained by precipitation with alcohol was fractionated by redissolving it in water and adding increasing quantities of alcohol. A main fraction was precipitated at a concentration of 40—45% of alcohol, and smaller fractions possessing different properties were precipitated at higher and at lower alcohol concentrations. Investigation of the main fraction by the periodate oxidation method and the methylation method of end-group assay gave respectively 10—11 and 12—13 glucose residues per non-reducing end group. These figures closely resemble those obtained by Haworth, Hirst, Kitchen, and Peat (*loc. cit.*), who found by the methylation method 11—12

glucose residues per non-reducing end group for a number of specimens of α-amylodextrin. A similar figure (10) has been reported by Swanson (*J. Biol. Chem.*, 1948, **172**, 825) as the result of colorimetric studies of the iodine complex of the limit dextrin. Since the results of end-group assay by the methylation method are in close agreement with those obtained by the potassium periodate oxidation method, it follows that no appreciable proportion of the glucopyranose residues in the limit dextrin (A) can be linked to other residues solely through the positions $C_{(1)}$ and $C_{(6)}$.

In order to avoid complications from the amylose component of whole starch, the action of wheat β-amylase at pH 4·8 on waxy maize starch, which contains a negligible proportion of amylose (Bourne and Peat, this vol., p. 5) has been investigated. The properties of the resulting limit dextrin (B) have been compared with those of another limit dextrin (C), prepared by using crystalline β-amylase isolated from sweet potatoes (Balls, Thomson, and Walden, *J. Biol. Chem.*, 1946, **163**, 571). The crystalline β-amylase was supplied to us through the kindness of Dr. A. K. Balls.

The limit dextrin (B), isolated after 54% conversion of the waxy maize starch into maltose by an amorphous wheat β-amylase preparation containing a trace of α-amylase, was shown, by the potassium periodate method, to give one mole of formic acid per 10 glucose residues. Since a yield of 12% of tetramethyl methylgucoside was obtained on hydrolysis of the methylated derivative, indicating one non-reducing terminal glucopyranose residue per 9 glucose residues, it follows that the formic acid produced by periodate oxidation must have come from the non-reducing terminal residues only. Glucopyranose residues linked solely through $C_{(1)}$ and $C_{(6)}$ are therefore not present in the dextrin. The waxy maize starch itself possesses one non-reducing terminal residue per 22 glucose residues.

The limit dextrin (C) prepared from the same sample of waxy maize starch, by the action of crystalline β-amylase, had a very high molecular weight, since it gave a methylated derivative the viscosity of which in *m*-cresol indicated a molecular weight of at least 4×10^5, it being assumed that the viscosity–*M* relationship is the same as for amylopectin. The dextrin itself dissolved in water only with difficulty, giving an extremely viscous solution, which gave a deep reddish-purple colour with iodine, whereas a solution of limit dextrin (B) gave a red colour. Fractionation experiments indicated that dextrin (C) was essentially homogeneous. The limit dextrin (B) (prepared by use of the non-crystalline enzyme) was much more soluble in water than limit dextrin (C), required a larger concentration of alcohol for its precipitation from aqueous solution, and gave solutions of low viscosity. These observations indicate that it was of lower molecular weight. Dextrin (C) was shown by potassium periodate oxidation to possess one terminal non-reducing glucose residue per 11 glucose units, this result being strikingly similar to that obtained for the limit dextrin (B). The products obtained after 53% conversion into maltose during the hydrolysis of waxy maize starch with crystalline β-amylase were examined by the method of paper partition chromatography (Partridge, *Nature*, 1946, **156**, 270), maltose alone being detected. These results suggest that the action of a trace of α-amylase in the β-amylase is to lower the molecular weight of the resulting dextrin, without affecting the proportion of end groups.

The properties of another limit dextrin (D), prepared by the action of crystalline β-amylase on waxy sorghum starch, another natural amylopectin, were very similar to those of the limit dextrin (C) obtained from waxy maize starch. This dextrin (D) dissolved with difficulty in water, giving very viscous solutions, and was homogeneous according to the evidence of fractionation experiments. Oxidation with a solution of potassium periodate indicated that one in 12 of the glucose residues was so combined that it gave formic acid. This figure is slightly higher than that for the waxy maize starch limit dextrin and it is of interest that the original waxy sorghum starch had 25 glucose residues per non-reducing end group, as compared with 22 for waxy maize starch.

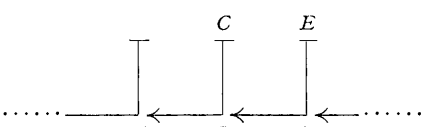
The action of crystalline β-amylase on waxy sorghum starch resulted in 52% conversion into maltose. This figure may be compared with those recorded by Meyer, Bernfeld, Rathgeb, and Gürtler (*Helv. Chim. Acta*, 1948, **31**, 1540), who prepared corn amylopectin by electro-dialysis of defatted autoclaved corn starch, dissolved it in alkali, and degraded the solution at pH 5·3 with an amorphous β-amylase prepared from wheat. The limit of hydrolysis corresponded to 62% conversion into maltose, which is a higher value than had been recorded previously.

The limit dextrin prepared from rabbit-liver glycogen by the action of non-crystalline wheat β-amylase differed markedly from the waxy starch dextrins. It gave a limpid aqueous solution which was scarcely coloured on addition of iodine. With potassium periodate this dextrin

(designated E in the Experimental section) gave one mole of formic acid per 9 glucopyranose residues. Its fully methylated derivative gave on methanolysis 2 : 3 : 4 : 6-tetramethyl methylglucoside in 12% yield, corresponding to 10 glucose residues per non-reducing end group, whereas the original glycogen contained 18 glucose residues per non-reducing end group (Haworth, Hirst, and Isherwood, *J.*, 1937, 577; Halsall, Hirst, and Jones, *J.*, 1947, 1399). Another sample of this rabbit-liver glycogen was treated with crystalline β-amylase, and the limit dextrin (F) was isolated after 53% conversion of the polysaccharide into maltose. This dextrin had properties similar to those of the glycogen dextrin (E). On oxidation with potassium periodate it gave one mole of formic acid per 10 glucose residues. It is especially noteworthy that Meyer and Fuld (*Helv. Chim. Acta*, 1941, **24**, 375) have reported that a sample of glycogen possessing one end group per 11 glucose residues was degraded by β-amylase to the extent of 47% maltose and that the limit dextrin which resisted further attack by β-amylase had one terminal group per 5·5 glucose residues.

These results provide further evidence for the view that β-amylase acts by successive removal of maltose molecules from the branched chains present in amylopectin and in glycogen but is incapable of attacking the glucosidic linkages at the points of branching. In each branch it leaves at least one glucose residue which is present in the limit dextrin as an end group and, in the instances studied, some 50% of the glucose residues were eliminated, leaving limit dextrins containing twice the proportion of end groups present in the original polysaccharide. The observations could be explained either on the basis of a statistical average of branches of various lengths randomly attached to each other or by an ordered arrangement of chains of fixed length attached to each other near their mid-points, but the present evidence affords no means of making a decision. Even a trace of α-amylase activity profoundly affects the molecular size of the limit dextrins, and the need for caution in the interpretation of enzymic experiments is emphasised.

A new factor has emerged in the observation that the amylopectin limit dextrins obtained by the action of crystalline β-amylase have very high specific viscosities which differentiate these dextrins sharply from glycogen and glycogen limit dextrins, and thus invalidate the argument used by Meyer, Bernfeld, Boissonas, Gürtler, and Noelting (*J. Physical Coll. Chem.*, 1949, **53**, 328) in favour of one particular (irregularly branched) form of the accepted ramified structure for amylopectin and glycogen. On the other hand amylopectin and glycogen display close chemical resemblances in that β-amylase removes similar proportions of maltose from both, and in both cases the limit dextrins contain double the proportion of end groups possessed by the parent substance. The structural differences which give rise to this behaviour still await elucidation. The viscosities of these solutions render it probable that glycogen molecules are spherical in shape in contrast to amylopectin molecules to which the Staudinger viscosity relationships are applicable, suggesting a linear type of structure. These properties, together with the work of Bell (*J.*, 1948, 992) with its implication that $C_{(1)}$–$C_{(3)}$ links may be present in glycogen, suggest that this polysaccharide may possess a multibranched structure of the type suggested by Meyer for amylopectin, whereas a more open, less branched structure may be present in amylopectin. The simplest form of ramified structure is the one depicted by Haworth, Hirst, and Isherwood, (*J.*, 1937, 578), but it has never been claimed by the authors that this particular modification provides the only possible interpretation of the experimental evidence. Using it as a model, however, we see that the structure can be written as a linear macromolecule with short side chains, as indicated in the figure, in which *ABC*, *BDE*, etc., represent sets of 20 glucose residues united linearly through $C_{(1)}$ and $C_{(4)}$. At *A*, *B*, *D*, etc., the free reducing group of one set is attached glucosidically to $C_{(6)}$ of one of the middle glucose residues of another set, giving a linear molecule . . . . *ABD* . . . . with side chains *BC*, *DE*. . . . . Such a macromolecule would be expected to follow the Staudinger viscosity laws but, owing to the side chains, with a constant, K_m, different from that for cellulose, and this has indeed been found to be the case for methylated starch derivatives (Hirst and Young, *J.*, 1939, 1471). The disaggregation of methylated amylopectin to material of lower molecular weight but containing the same proportion of end groups (Bawn, Hirst, and Young, *Trans. Faraday Soc.*, 1940, **36**, 880) must also be considered in connection with the structure of amylopectin, and on the present evidence this phenomenon is difficult to reconcile with the idea of an irregularly multiple-branched structure for this polysaccharide.



Experimental.

The crystalline β-amylase used in these experiments was kindly provided by Dr. A. K. Balls of the Enzyme Research Laboratory of the United States Department of Agriculture. The crystals (40 mg.) were received suspended in *ca.* 0·8 saturated ammonium sulphate solution (pH 3·8—3·9) and had been isolated from sweet potatoes. They were dissolved in water (100 c.c.), and the solution was kept at 0° covered with a layer of toluene to maintain aseptic conditions. No α-amylase was present (Wijsman's test). The absence of maltase was shown as follows. A solution of maltose (0·198%; 40 c.c.) was mixed with acetate buffer (pH 4·8; 5 c.c.) and enzyme solution (0·25 c.c., diluted to 5 c.c. with water). The reducing power was then determined by Somogyi's method (*J. Biol. Chem.*, 1945, **160**, 61), and again after the solution, covered with an aseptic layer of toluene, had been in the incubator at 37° for 44 hours. At the same times the reducing powers of a solution of water (40 c.c.), acetate buffer (pH 4·8; 5 c.c.), and enzyme solution (0·25 c.c. diluted to 5 c.c.) and of a solution of water (45 c.c.) and acetate buffer (pH 4·8; 5 c.c.) were determined, the solutions being kept under the same conditions as the maltose solution. The amount of copper reduced was determined by the iodometric method and the free iodine was titrated with 0·005N-sodium thiosulphate solution:

Time of incubation, hrs.	0·005N-$Na_2S_2O_3$ used (c.c. per 5 c.c. of solution). Maltose solution.	Blank solution.	Enzyme solution.
0·0	1·61	4·63	4·64
44	1·63	4·64	4·61

These results show that maltase activity is absent, that the enzyme itself has no reducing powder, and that it did not develop reducing power on storage.

Waxy Maize Starch Limit Dextrin.—Waxy maize starch (9·99 g. dry weight) was made into a cream with water (130 c.c.) and was poured into water kept just below 100°. The dispersed starch was kept at this temperature for 30 minutes, after which the solution was cooled and acetate buffer (pH 4·8; 50 c.c.) and enzyme solution (20 c.c.) were added. The solution was covered with a layer of toluene and was kept at 37° in the incubator for 70 hours. The reducing power of a 5-c.c. sample of solution, determined by the hypoiodite method of Jeanloz (*Helv. Chim. Acta*, 1946, **29**, 57), then corresponded to the conversion of 53% of the starch into maltose. The limit dextrin was precipitated from solution by addition of methyl alcohol (1·5 l.) and was separated on the centrifuge. It was hardened under methyl alcohol, powdered, filtered off, washed with methyl alcohol and ether, and dried (yield, 4·6 g.). The solution left after the separation of the dextrin was evaporated to a syrup, which was examined by the paper chromatographic technique. Maltose was the only sugar found. The syrup deposited crystalline maltose monohydrate, $[\alpha]_D$ +129°, final value in water (*c*, 0·5).

The limit dextrin (4·6 g.) was dissolved in hot water (125 c.c.), giving a viscous solution, and cooled; alcohol was then added slowly with stirring. When 50 c.c. of alcohol had been added (making the alcohol concentration 28%) precipitation began. The dextrin solution was then set aside overnight, after which the supernatant liquid was decanted. The solid was hardened under methyl alcohol, powdered, filtered off, washed with methyl alcohol and ether, and dried (fraction I, 4·4 g.). More alcohol (150 c.c.) was added to the mother-liquor (alcohol concentration then 62%) whereupon a very slight precipitate was deposited (*ca.* 50 mg.).

Fraction (I) of the dextrin had the following properties: $[\alpha]_D^{15}$ +161° in N-sodium hydroxide (*c*, 0·43). Its aqueous solution was neutral and gave a deep reddish-purple colour with iodine. The reducing power was negligible; ash content, nil. The limit dextrin (Fraction I) (230·6 mg.) was dissolved in water (110 c.c.) containing potassium chloride (5 g.), and sodium metaperiodate solution (0·3M.; 10 c.c.) was added. The oxidation and the determinations of the resulting formic acid were carried out according to the procedure already described (*J.*, 1947, 1399). After 150 hours 5·4 mg. of formic acid had been formed, corresponding to one mole of formic acid per 11 glucose residues.

Methylation of Waxy Maize Starch Limit Dextrin.—The limit dextrin (fraction I) (2·4 g.) was dissolved with stirring in cold aqueous sodium hydroxide (3N.; 50 c.c.). The viscous solution was stirred rapidly and 40% aqueous sodium hydroxide (100 c.c.) and methyl sulphate (50 c.c.) were added gradually at room temperature. A vigorous reaction occurred. Stirring was continued overnight, acetone was then added, and the partly methylated dextrin preferentially dissolved in the acetone layer, which was separated. Two more methylations under similar conditions were carried out and the methylated dextrin was isolated by pouring its solution in acetone into boiling water, with stirring. The precipitated dextrin was purified by dissolution in acetone, and evaporation of the filtered solution to dryness under reduced pressure (yield, 1·7 g.); $[\alpha]_D$ +208° in chloroform (*c*, 0·18); $\eta_{sp.}^{20}$ 1·30° in *m*-cresol (*c*, 0·4). If the viscosity–*M* relationship observed for methylated starch (*J.*, 1939, 1475) holds in this case, the molecular weight would be *ca.* 400,000 (Found: OMe, 42·3%).

Waxy Sorghum Starch Limit Dextrin.—Waxy sorghum starch (10·0 g. dry weight) was converted into the limit dextrin by the method described above. The solution was incubated at 37° and the reducing power was determined by the hypoiodite method after 2½ hours and after 44 hours. The conversion into maltose at these times was 50·5 and 51%, respectively. The dextrin was precipitated from solution by addition of methyl alcohol (1·5 l.) and was separated on the centrifuge. It was hardened under methyl alcohol and then powdered, filtered off, washed with methyl alcohol and ether, and dried (yield, 4·75 g.). The residual solution was evaporated to a syrup (5·3 g.), from which maltose monohydrate was obtained, $[\alpha]_D$ +129° (equilibrium value in water). Examination of the syrup by the paper chromatographic technique showed that maltose alone was present.

The dextrin was dissolved in water (125 c.c.), giving a viscous solution. Ethyl alcohol (50 c.c.) was added, and the solution became less viscous but no precipitation occurred. A further portion of alcohol (25 c.c.) was added (alcohol concentration 37%) and the dextrin separated as a jelly. After the addition

of a further quantity of alcohol (25 c.c.) the dextrin was isolated by decanting the supernatant liquid (X). The dextrin was hardened under methyl alcohol, powdered, filtered off, washed with methyl alcohol and ether, and dried (fraction A, 4·5 g.); $[\alpha]_D^{20}$ +154° in N-sodium hydroxide (*c*, 0·45); reducing power, nil; ash, nil. The aqueous solution of the limit dextrin was neutral and gave a deep reddish-purple colour with iodine. A sample of this material (230·6 mg.) was dissolved in water (110 c.c.) containing potassium chloride (5 g.), and sodium metaperiodate solution was added (0·3M.; 10 c.c.). The oxidation was then carried out as in the case of waxy maize starch dextrin (Found, after 150 hours: formic acid, 5·3 mg., corresponding to the formation of one mole of acid per 12 glucose residues). Addition of further alcohol to the supernatant liquor X (above) caused the precipitation of less than 0·1 g. of a sticky solid. Evaporation of the liquor left only a trace of syrup.

Dextrin from Rabbit-liver Glycogen.—Rabbit-liver glycogen (9·160 g.) was dissolved in water (215 c.c.), and acetate buffer (pH 4·8; 25 c.c.) and enzyme solution (10 c.c.) were added. The solution was covered with a layer of toluene and kept at 37° in the incubator for 45 hours. The reducing power of a 5-c.c. portion was determined by the hypoiodite method and corresponded to the conversion of 53% of the glycogen into maltose. The dextrin was then precipitated by addition of ethyl alcohol (1·5 l.). The supernatant liquid was decanted, and the dextrin was hardened, powdered, filtered off, washed with methyl alcohol and ether, and dried as described previously (yield, 4·15 g.). The supernatant liquid was evaporated to a syrup from which maltose monohydrate ($[\alpha]_D$ +129°, equilibrium value in water) was obtained (yield, 5·1 g.). Examination of the syrup by the method of paper chromatography showed that maltose was the only sugar present.

The glycogen limit dextrin was dissolved in water (100 c.c.), giving a limpid, slightly opalescent solution. Ethyl alcohol (50 c.c.) was added; the opalescence increased slightly but no precipitation occurred. A further portion of ethyl alcohol (25 c.c.) (alcohol concentration, 43%) was added and towards the end of the addition a gummy material was precipitated. The solution was decanted and methyl alcohol was added to the precipitate to harden it. The product was powdered, washed, and dried as usual (fraction I, 4·1 g.). Ethyl alcohol (100 c.c.) was added to the decanted liquor, and on storage a small amount of material (<0·05 g.) separated and was removed. On addition of a further portion of alcohol (100 c.c.) no precipitate was obtained. On evaporation of this liquor only a trace of gummy material was obtained.

Fraction I of the limit dextrin from glycogen had the following properties: $[\alpha]_D^{20}$ +161° in N-sodium hydroxide (*c*, 0·55); its aqueous solution was neutral and gave no colour with iodine; reducing power, nil; ash, nil. This limit dextrin (219·6 mg.) was dissolved in water (110 c.c.) containing potassium chloride (5 g.), and sodium metaperiodate solution (0·30M.; 10 c.c.) was added. The oxidation and the determination of the resulting formic acid were carried out by the method already described (Found after 150 hours: formic acid, 5·9 mg., corresponding to the formation of one mole of acid per 10 glucose residues).

Preparation of β-Amylase from Soya Flour.—Soya flour (75 g.) was shaken for 18 hours with 20% aqueous alcohol (350 c.c.). The insoluble material was removed on the centrifuge, and the alcohol concentration of the extract increased to 65%. The white flocculent precipitate was separated on the centrifuge, washed with alcohol and ether, and dried under reduced pressure over phosphoric oxide. The light brown powder (*ca.* 1 g.) was stored at 0°. Both α- and β-amylase were detected by Wijsman's method. After several hours' incubation with maltose at pH 4·8, no increase in reducing power was found and hence maltase was absent.

Attempts to remove the α-amylase by fractional precipitation with alcohol were unsuccessful.

Preparation of β-Amylase from Wheat Flour.—Wheat flour (100 g.), prepared by milling ungerminated wheat grains, was shaken with 20% aqueous alcohol (350 c.c.) for 1 hour, the solid was removed on the centrifuge, and the solution shaken for a further 18 hours. The alcohol concentration of the extract was raised to 50%, the precipitate was removed by centrifuging, and the alcohol concentration of the chilled extract increased to 80%. The resulting precipitate was isolated by centrifuging, washed with alcohol and ether, and dried under reduced pressure over phosphoric oxide for 24 hours; yield, *ca.* 1 g. The white β-amylase powder was kept at 0°.

α-Amylase activity was tested for by Wijsman's method. A small area of colourless gelatin was found and hence a trace of α-amylase was present. Maltase activity was tested for by the method described above. After several hours' incubation no increase in reducing power was found. Hence maltase activity was absent.

Action of Wheat β-Amylase on Potato Starch.—Dry potato starch (50 g.) was stirred with cold water (125 c.c.), and the thin cream so produced poured with stirring into boiling water (500 c.c.). The resultant starch solution was put through a homogeniser in order to ensure that all the starch was dispersed. The pH of the solution was adjusted to 4·8, and a 1% solution of wheat β-amylase powder (125 c.c.) added. A little toluene was added, and the whole incubated at 37°. The hydrolysis was followed by determining the reducing power of the reaction mixture by the alkaline hypoiodite reagent. After 2 hours there was no further change in the reducing power, which corresponded to 60—62% conversion into maltose. Alcohol (2 l.) was added to the solution, and the white sticky precipitate hardened by heating under reflux with methyl alcohol. The yield of dextrin was 38% of the original starch.

The dextrin (18 g.) was dissolved in hot water (400 c.c.), and alcohol (200 c.c.) added in small quantities with stirring. The white sticky precipitate was isolated on the centrifuge, heated under reflux withm ethyl alcohol for one hour, and dried *in vacuo* over phosphoric oxide at 60°; yield of fraction 1, 3 g.

The alcohol content of the clear supernatant liquid was increased to 43%, and the colloidal precipitate (fraction 2) was isolated on the centrifuge. The sticky precipitate was hardened by heating under reflux with methyl alcohol for one hour and then dried *in vacuo* over phosphoric oxide at 100°; yield, 8 g.

This procedure was repeated three more times, the alcohol concentration being increased to 50, 60, and 85%, and fractions 3, 4, and 5, respectively, were isolated.

Fraction.	% Alcohol used for precipitation.	Yield from crude dextrin, %.	Blue value.*	Iodine colour.	Reducing value (% maltose).
1	0— 33	16	0·18	Purple	0·7
2	33— 43	55	0·125	,,	1·3
3	43— 50	16	0·12	Mauve	2·1
4	50— 60	3	—	Red	—
5	60— 84	3	0·05	,,	5·8
6	84—100	2	0·05	Red-brown	—

Fraction.	$[\alpha]_D^{16}$ (*c*, 1·0) in water.	$[\alpha]_D^{16}$ (*c*, 1·0) in 5% NaOH.	No. of glucose residues per terminal group.†
1	—	137°	11—12 (A)
2	227°	186	10 (A) 12 (B)
3	187	163	9 (A) —
4	—	—	12 (A) —
5	176	129	6 (A) —
6	—	—	4 (A) —

* For method, see Hassid and McCready, *J. Amer. Chem. Soc.*, 1943, **65**, 1157.

† (A) Determined by potassium periodate oxidation. (B) Determined by the methylation method (cf. Brown and Jones, *J.*, 1947, 1344).

Fraction 2 (see above) was methylated with sodium hydroxide and methyl sulphate (nine times). The methylated dextrin had the following properties: $[\alpha]_D^{21}$ +190° in chloroform (*c*, 2·0) (Found: OMe, 43·3%), and its aqueous solution gave no colour with iodine; it (0·817 g.) was dissolved in 1% methanolic hydrogen chloride (30 c.c.), and the solution boiled under reflux for 6 hours, neutralised with alcoholic potash, precipitated potassium chloride filtered off through a cotton-wool plug, and the cotton-wool washed with methanol, the filtrate being evaporated to a syrup on a steam-bath, and the last traces of solvent being removed under a vacuum (yield of methyl glucosides, 0·935 g.). The tetramethyl methylglucoside (72·1 mg.; η_D^{16} 1·4446; OMe, 59·3%) in the syrup was isolated by the extraction method of Brown and Jones (*loc. cit.*). This corresponds to 1 terminal glucose residue per 12 glucose residues.

Action of Wheat β-Amylase on Waxy Maize Starch.—(i) Waxy maize starch (0·1 g.) was brought into solution by heating it at 80° in water (50 c.c.) for 15 minutes. After the solution had cooled to 37°, acetate buffer (pH 4·8; 10 c.c.) and an aqueous solution of wheat β-amylase (1%; 25 c.c.) were added, the volume being made up to 100 c.c. by addition of water. A layer of toluene was added, and the mixture was incubated at 37°; at intervals 5-c.c. portions were removed for estimation of reducing power by the Somogyi micro-method: conversion (%) into maltose 52 (1 hour); 52 (2 hours); 54 (3 hours); 54 (4 hours).

(ii) Waxy maize starch (25 g.) was stirred with cold water (60 c.c.) and the cream so produced was poured with stirring into boiling water (250 c.c.). The solution was then put through a homogeniser to ensure complete dispersion of the starch. The pH of the solution was then adjusted by addition of a buffer solution to 4·8 and an aqueous solution of wheat β-amylase powder (0·5%; 65 c.c.) was added. A little toluene was added, and the mixture incubated at 37°. After 19 hours the reducing power had become constant and a small flocculent precipitate had separated. This was removed on the centrifuge. It gave a blue colour with iodine and did not contain nitrogen. Alcohol (1 l.) was added to the clear solution and the precipitated limit dextrin was separated, hardened under methyl alcohol, powdered, filtered off, and dried (yield 9 g.). This material was dissolved in water (200 c.c.), giving a limpid solution. On addition of alcohol (145 c.c.) a yellow syrup separated and the supernatant liquid was decanted. On addition of methyl alcohol to the syrup it hardened, giving a white solid which was powdered and dried [fraction (*a*); yield, 4 g.]. The alcohol concentration of the decanted solution was increased to 70% and the syrup which was precipitated was allowed to settle. The turbid supernatant liquid was decanted and the syrup was hardened under methyl alcohol powdered, and dried [fraction (*b*); yield, 4 g.]. The turbid liquor (above) clarified on storage overnight and a syrup settled out. This was separated, hardened under methyl alcohol, powdered, and dried [fraction (*c*); yield, 0·3 g.]. The properties of these fractions are tabulated below.

	Reducing value (as maltose), %.	$[\alpha]_D$ in N-NaOH.	No. of glucose residues per terminal group.*	Blue value.	Colour with iodine.
Fraction (*a*)	0·4	+149° (*c*, 0·7)	10 (A)	0·06	Red
Fraction (*b*)	0·9	+160 (*c*, 1·0)	9 (A) (B)	0·04	,,
Fraction (*c*)	2·1	+143 (*c*, 0·6)	—	0·02	,,

* For (A) and (B), see preceding table.

Fraction (*b*) was methylated with sodium hydroxide and methyl sulphate (nine times). The methylated dextrin had $[\alpha]_D^{18}$ +196° in chloroform (*c*, 2·8) (Found: OMe, 42·2%). The methylated dextrin (0·8 g.) was dissolved in 1% methanolic hydrogen chloride, and the solution boiled under reflux for 6 hours, and worked up as above. The tetramethyl methylglucoside (116 mg.; η_D^{16} 1·4440) in the syrup was isolated by Brown and Jones's extraction method (Found: OMe, 60·4%). This corresponds to 1 terminal group per 9 glucose residues.

Action of Wheat β-Amylase on Rabbit-liver Glycogen.—(i) Rabbit-liver glycogen (0·1 g.) was converted into dextrin in the way previously described: conversion (%) into maltose, 45·5 (0·3 hour); 52 (1 hour); 52 (2 hours); 53 (4 hours).

(ii) Rabbit-liver glycogen (15 g.) was made into a slurry with water (50 c.c.). This was poured with stirring into boiling water (300 c.c.) and cooled; the pH of the solution was adjusted to 4·8.

An aqueous solution of wheat β-amylase powder (0·5%; 50 c.c.) was then added, together with a little toluene. After 19 hours' incubation at 37° the reducing power had become constant. Alcohol (650 c.c.) was then added and the precipitated dextrin was separated, hardened under methanol, powdered, and dried (yield, 6 g.). It was fractionated by successive addition of alcohol to its aqueous solution (see above).

Fraction.	Composition of aqueous alcohol used in precipitation, %.	Reducing value (as maltose), %.	$[\alpha]_D^{16}$ in N-NaOH.	No. of glucose residues per terminal group.*
1 (3 g.)	0—40	0·3	+155° (*c*, 0·86)	8·5 (A) 10 (B)
2 (2·5 g.)	40—60	0·5	+169 (*c*, 0·9)	9 (A) 10 (B)

* (A) and (B) have the same significance as in preceding tables.

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THE UNIVERSITY, BRISTOL.
THE UNIVERSITY, EDINBURGH.
THE UNIVERSITY, MANCHESTER.

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