21. *The Enzymic Synthesis and Degradation of Starch. Part VII. The Mechanism of Q-Enzyme Action.*

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The results reported in the earlier communications in this series are reviewed and correlated with the observations recorded in this paper. Evidence is given which proves, beyond reasonable doubt, that Q-enzyme functions by a non-phosphorolytic mechanism. In this and in other properties it appears to be distinct from the *iso*phosphorylase isolated (also from potatoes) by Bernfeld and Meutémédian (*loc. cit.*).

It is concluded that Q-enzyme is a transglucosidase, but not of the phosphorylase type, and that two amylopectin-synthesising systems represented by Q-enzyme and *iso*phosphorylase may exist together in the potato.

Our work on the enzymic synthesis and degradation of starch has now reached a stage where it is desirable to review the results reported in a series of earlier communications (Part I, Bourne and Peat, *J.*, 1945, 877; Part II, Bourne, Macey, and Peat, *J.*, 1945, 882; Part III, Bourne, Sitch and Peat, *J.*, 1949, 1448; Parts IV and V, Barker, Bourne, and Peat, *J.*, 1949, 1705, 1712; Part VI, Barker, Bourne, Wilkinson, and Peat, preceding paper) and to correlate them with the observations recorded in this paper.

In 1944 Haworth, Peat, and Bourne (*Nature*, **154**, 236) reported the isolation from potatoes of an enzyme (Q-enzyme) which catalysed, in association with potato phosphorylase (P-enzyme), the synthesis of amylopectin from glucose-1 phosphate. We were unaware at that time of the " branching factor " prepared from heart, liver, and yeast by Cori *et al.* (*J. Biol. Chem.*, 1943, **151**, 57) which played a similar rôle in the synthesis of glycogen. Shortly afterwards we advanced a hypothesis to account for the properties of Q-enzyme, from which it was inferred that natural amylose could be converted into amylopectin by the agency of this enzyme (Parts I and II). This hypothesis was subsequently substantiated and a preliminary account was given by Peat, Bourne, and Barker (*Nature*, 1948, **161**, 127) of the conversion of amylose into amylopectin. In no case during the isolation and purification of Q-enzyme or during the investigation of its functions was mineral phosphate introduced and for this reason we have always held the view that Q-enzyme operated by a non-phosphorolytic mechanism.

Recently, Bernfeld and Meutémédian (*Helv. Chim. Acta*, 1948, **31**, 1724, 1735) described the isolation from potatoes of an enzyme (named by them, *iso*phosphorylase) which bore some resemblance to Q-enzyme inasmuch as it played a part in the formation of amylopectin from both amylose and glucose-1 phosphate. There was nevertheless a fundamental difference between the two. Whereas we postulated that phosphate-transfer was not involved in the formation of cross linkages by Q-enzyme, the Swiss authors claimed that such transfers were an integral part of the cross-linking mechanism.

Since *iso*phosphorylase induces no qualitative change in the characteristic blue-staining property of amylose unless mineral phosphate and phosphorylase are present, Bernfeld and Meutémédian suggest that the new enzyme functions by catalysing the transfer of phosphate. They believe that P-enzyme first acts on amylose and mineral phosphate to give glucose-1 phosphate, which is then utilised by *iso*phosphorylase to establish 1 : 6-linkages, with the simultaneous liberation of mineral phosphate. The glucose-1 phosphate subsequently liberated by P-enzyme from the end of the original chain is used either (i) by *iso*phosphorylase to establish another branch, or (ii) by P-enzyme to lengthen the branch already established by *iso*-phosphorylase.

The scheme for the conversion of amylose into amylopectin proposed by two of us in 1945 (Part I) was based on the observation that Q-enzyme functioned in the absence of both mineral phosphate and P-enzyme. The small impurities of mineral phosphate and P-enzyme known to be present in the samples of Q-enzyme then available were not regarded as being sufficient to play any part in the conversion. The publication of Bernfeld's views prompted us to examine Q-enzyme activity in greater detail, because, theoretically, a trace of mineral phosphate would be sufficient to facilitate the phosphorolytic mechanism proposed by him.

Conversion of Amylose into Amylopectin.—By treatment with Q-enzyme, linear amylose chains are transformed into the branched amylopectin structure. The evidence upon which this statement is based has been detailed in two earlier papers (Part V and *Nature*, 1948, **161**, 127). Briefly, Q-enzyme causes a rapid diminution of the absorption value, A.V. (6800 A.), of an amylose solution to about 13% of the original value. This red-staining solution contains only 2% of reducing sugar, expressed as maltose, and is hydrolysed at a higher initial rate by

β-amylase than is the original amylose solution, a fact in keeping with the postulate that an increase in the number of chain-ends occurs during Q-enzyme action. When the red-staining polysaccharide produced from amylose in this manner is isolated, it is indistinguishable, in its blue value, in the light-absorption curve of its iodine-stained solution, and in its limiting conversion with β-amylase, from the purest samples of natural potato amylopectin. In this conversion, (amylose ⟶ amylopectin) equilibrium lies completely, or nearly so, on the side of the branched structure.

According to Bernfeld, *iso*phosphorylase, in the presence of P-enzyme and mineral phosphate (1·35 atoms of phosphorus per $C_6H_{10}O_5$ unit), also lowers the limit conversion with β-amylase of an amylose solution (to 65%). In this reaction, the blue-staining amylose is converted into a purple-staining polysaccharide, the latter being favoured at equilibrium. Although the polysaccharide produced in this way has not yet been isolated, it probably possesses a branched structure of the amylopectin type (Bernfeld and Meutémédian, *loc. cit.*).

It was shown in Part I that as the purification of a Q-preparation proceeded, *i.e.*, as the P-enzyme content diminished, its power to effect the synthesis of polysaccharide from glucose-1 phosphate also decreased. This has been repeatedly confirmed and in the present work quantitative experiments show that the addition of glucose-1 phosphate affects neither the rate nor the end-point of conversion of amylose into amylopectin by the purest specimens of Q-enzyme (Table III). Furthermore, the degree of branching of the product of the action of Q-enzyme on amylose is the same whether the Cori ester is present or not (Table VI).

The lack of effect of mineral phosphate on the conversion of amylose into amylopectin by Q-enzyme is shown in Table II. Two parallel digests of amylose and Q-enzyme were made in acetate buffer of pH 7 at 20°. One digest contained 0·04 atom of free-phosphorus per $C_6H_{10}O_5$ unit and the other, to which mineral phosphate was added, contained 35 times as much. Nevertheless, the rate of reaction as measured by the fall of A.V. (6800 A.) was the same in the two digests. Furthermore, the free-phosphate contents did not diminish during the reaction, which was virtually complete in 150 minutes. The same lack of effect on Q-enzyme activity was shown in a second experiment in which the mineral phosphate content was increased from 0·04 to 0·18 atom of phosphorus per $C_6H_{10}O_5$ unit.

It should be pointed out that, before any specific effect of mineral phosphate on the conversion of amylose by Q-enzyme could be ascertained, it was essential to know whether salts in general influenced the reaction. Accordingly, amylose was incubated with Q-enzyme at pH 7·0 in a series of digests which differed only with respect to concentration of citrate buffer. The rate of conversion, measured by the fall in A.V. (6800 A.), rose appreciably as the buffer concentration was increased to $4·5 \times 10^{-3}$M. and thereafter remained constant (Table I). This marked activation of Q-enzyme by salts has also been observed by Gilbert and Swallow (in the press). It was obvious that valid conclusions concerning the effect of mineral phosphate on Q-enzyme activity could be drawn only from those experiments in which other salts were present in sufficient concentration fully to activate the enzyme.

Adopting the same method as that used in studying the effects of glucose-1 phosphate and mineral phosphate, it was demonstrated that the addition of P-enzyme does not influence the conversion of amylose by Q-enzyme, if phosphate is absent and if the enzyme is activated by the presence of other ions (see Tables IV and V).

It must therefore be concluded that the presence of a relatively large amount of mineral phosphate, of P-enzyme, or of glucose-1 phosphate does not influence the conversion of amylose into amylopectin by Q-enzyme, with respect either to rate or to type of reaction. If now Q-enzyme operates as an *iso*phosphorylase the addition of any of these three agents would be expected markedly to affect the rate of conversion as measured by the change in A.V. (6800 A.). The fact that this does not happen furnishes ample support for our view that Q-enzyme does not operate by a mechanism of phosphate transfer in the synthesis of cross-linkages.

Hehre (*J. Biol. Chem.*, 1949, **177**, 267) has reported that an enzyme present in extracts of *Neisseria* converts crystalline corn amylose into a glycogen-like polysaccharide without the release of reducing sugars and without the addition of mineral phosphate. This enzyme would appear to have the character more of Q-enzyme than of *iso*phosphorylase.

Attention was next directed to the question of reversal of synthesis, *i.e.*, to the scission of cross-linkages and the part, if any, played by phosphate in the process. For this purpose the action of Q-enzyme on amylopectin and on the limit dextin-A was studied.

It was important in these experiments to be able to assess the degree of branching in the products of Q-enzyme activity. Such comparisons of extent of ramification were made by measuring the limit of β-amylolysis of each specimen. The smaller the proportion of branches

in the substrate, the higher will be the β-amylase conversion limit. It must be borne in mind, however, that no β-amylase preparation at present available is entirely free from α-amylase and for this reason β-amylolysis must be carried out at an acid pH (usually, 4·7) at which the α-amylase is inoperative (see Peat, Whelan, and Pirt, *Nature*, **1949**, **164**, 499).

When thymol-amylopectin (blue value, B.V., 0·215) was incubated for 3 hours at pH 7·0 with fraction Q3 there was less than 1% increase in the content of reducing sugar, expressed as maltose. The polysaccharide product gave rise, by β-amylolysis at pH 4·7, to a limit dextrin and 47% of maltose, compared with 53% of maltose in the case of the parent thymol-amylopectin (Table VII). Little or no scission of the cross-links could have occurred. Indeed Q-enzyme treatment had brought about a slight increase in the degree of branching, presumably by the conversion into amylopectin of the small amylose impurity known to be present in thymol-amylopectin (Bourne, Donnison, Haworth, and Peat, *J.*, 1948, 1687). Identical results were obtained from a similar experiment in which mineral phosphate (1·35 atoms of phosphorus per $C_6H_{10}O_5$ unit) was introduced during the treatment of amylopectin with Q-enzyme (Table VII). It is clear that, if the synthesis of cross-links by Q-enzyme is a reversible process, then at equilibrium there is an overwhelming preponderance of the branched structure and that this equilibrium is not influenced by the presence of phosphate.

The ability of highly purified Q-enzyme (fraction Q5) to hydrolyse the cross-links of dextrin-A has been investigated (Table VIII). Although there was only a slight development of reducing power, corresponding to 1% conversion into maltose, when dextrin-A was treated for 3 hours with enzyme fraction Q5, the dextrin did become somewhat more susceptible to β-amylolysis. Whereas the original dextrin gave 2% of maltose with β-amylase, the product of Q-enzyme action gave 11·5% of maltose. It was shown once again that the presence of mineral phosphate (1·35 atoms of phosphorus per $C_6H_{10}O_5$ unit) did not materially alter the result. In this respect the action appears to be different from that of *iso*phosphorylase on the dextrin. While it is clear that slight hydrolysis, as opposed to phosphorolysis, of the dextrin had occurred during the Q-enzyme treatment, it is by no means certain that there had been a specific scission of the cross-links by Q-enzyme, for it is well-known that a very minute amount of α-amylase impurity in the enzyme sample could, by scission of 1 : 4-linkages in the " inner " polysaccharide chains, have caused the observed increase in the degree of β-amylolysis of the dextrin (Bourne, Haworth, Macey, and Peat, *J.*, 1948, 924). We therefore note that the action of Q-enzyme on dextrin-A is not facilitated by the presence of mineral phosphate, but, until further information is available, we hestitate to attribute the action on dextrin-A to the scission of 1 : 6-linkages by Q-enzyme.

As is to be expected, whole starch behaves, in most respects, like a mixture of amylose and amylopectin under the action of Q-enzyme which simply transforms the amylose component of potato starch into amylopectin and leaves the amylopectin component essentially unchanged, so that the whole of the starch is converted into the ramified structure (Parts II and V). Q-Enzyme rapidly diminishes the viscosity of a starch paste (Parts I and VI), whereas *iso*-phosphorylase does not (*Helv. Chim. Acta*, 1948, **31**, 1724). It appears improbable that the liquefying property of Q-enzyme can be attributed to either α- or β-amylase, because (*a*) reducing sugars do not appear, (*b*) the solution never becomes achroic, and (*c*) the polysaccharide product is indistinguishable from natural amylopectin. The effect of Q-enzyme in lowering starch viscosity is not influenced by the addition of mineral phosphate (Part VI, Fig. 2).

Purity of the Q-Enzyme Preparations.—It was fully appreciated, early in this work, that the conclusions concerning the mechanism of starch synthesis and degradation depend largely on the degree of enzymic purity. In particular it was important that Q-enzyme preparations should be as free as possible from amylases. The most persistent impurity is α-amylase and tests for its presence are complicated by the doubt, which still exists, whether Q-enzyme *per se* liberates reducing groups during the conversion of amylose into amylopectin or whether this small " apparent conversion into maltose " (*ca.* 2%) is caused by a trace of α-amylase. It is certain, however, that if α-amylase is still present in the purest Q-enzyme samples, it is there in such minute quantities as not to affect the validity of the conclusions we have drawn.

In Parts IV and V of this series and also in the present paper, evidence has been presented to show that Q-enzyme, even at the Q1 stage, contains no more than a trace of either α- or β-amylase, since it effects an 87% reduction in A.V. (6800 A.) of amylose with the concomitant liberation of only 2% of reducing sugar (expressed as maltose). At this point, the solution is stained red by iodine, and does not subsequently become achroic. Fraction Q1 is free from maltase (Part IV), while fraction Q3 contains no phosphatase (no action on sodium glycero-phosphate, Part VI). Fraction Q3 is normally contaminated with a little P-enzyme (Part VI),

but, if during the course of its isolation it is freeze-dried at the Q1 stage in the absence of a buffer, the P-enzyme impurity is preferentially destroyed and in this way samples of fraction Q3 were obtained which were devoid of P-enzyme activity when tested by the method of Green and Stumpf (*J. Biol. Chem.*, 1942, **142**, 355).

Synthesis of Amylopectin from Glucose-1 Phosphate.—Bourne and Peat (1945) advanced the theory that P-enzyme first builds up linear polysaccharide chains by the end-wise apposition, in α-1 : 4-linkage, of glucose units derived from glucose-1 phosphate, and that these amylose-type molecules then serve as a substrate for Q-enzyme, which converts them into amylopectin in the same way as it transforms natural amylose into amylopectin. There could be no doubt that the product of the joint action of P- and of Q-enzyme on glucose-1 phosphate was amylopectin, for this synthetic polysaccharide was isolated and could not be distinguished, in its blue value, in its limit conversion with β-amylase, and in its average chain-length, as determined by end-group assay, from natural amylopectin (Part I).

Bernfeld and Meutémédian also regard the first stage in amylopectin synthesis as the formation, by P-enzyme, of linear chains. The second stage is pictured as the establishment of branch points by the addition to these chains, by *iso*phosphorylase, of single glucose units (derived from glucose-1 phosphate) in 1 : 6-linkage. It is suggested that the branches thus introduced are lengthened by subsequent P-enzyme action and in this way give rise to the " outer " chains of amylopectin. There is some doubt, however, whether the final stage in this scheme of synthesis would in fact occur, for it is known that at least three, and proably more, 1 : 4-linked glucose units must be present in an " outer " chain before the structure will serve as a template for P-enzyme (Weibull and Tiselius, *Arkiv Kemi, Min., Geol.*, 1945, **19**, *A*, No. 19). This question of the relation between structure and activating power has been discussed more fully in Part III of this series.

We have always maintained that Q-enzyme *per se* cannot utilise glucose-1 phosphate as a substrate, because increased purification of the enzyme invariably results in a decreased ability to liberate mineral phosphate from the Cori ester. Indeed this has been the method by which we have measured the progressively decreasing impurity of P-enzyme in Q-enzyme samples. Samples of Q-enzyme have now been prepared in which P-enzyme cannot be detected by the method of Green and Stumpf (*loc. cit.*), which involves incubating the enzyme sample with glucose-1 phosphate in the presence of starch and measuring the amount of mineral phosphate liberated. It follows that Q-enzyme cannot utilise glucose-1 phosphate even in the presence of the template chains furnished by starch. On the other hand, Bernfeld and Meutémédian (*loc. cit.*) demonstrated that *iso*phosphorylase does liberate mineral phosphate from the Cori ester. In this respect Q-enzyme again appears to differ from *iso*phosphorylase, but its behaviour is similar to that of Cori's branching factor from liver (*J. Biol. Chem.*, 1943, **151**, 57).

Conclusions.—The evidence presented furnishes proof that Q-enzyme does not operate by a mechanism involving phosphate, and cannot, therefore, be an *iso*phosphorylase. The conclusion appears to be inevitable that two types of enzyme are responsible for the synthesis of cross-links in branched polysaccharides, one type (*iso*phosphorylase) functioning by means of phosphate transfers and the other (Q-enzyme, Cori's " branching factor ") by a trans-glucosidase action which is independent of phosphate. It is not altogether surprising that there should be two modes of synthesis for it is well-known that starch is degraded by amylolytic as well as by phosphorolytic enzymes.

Q-Enzyme would thus be regarded as belonging, in common with other enzymes concerned in polysaccharide synthesis, to the class of trans-glycosidases, of which the phosphorylases constitute only one type (see Doudoroff, Barker, and Hassid, *J. Biol. Chem.*, 1947, **168**, 725). As discussed by us in Part V of this series, the scission of a 1 : 4-link in the amylose chain probably occurs simultaneously with the formation of a 1 : 6-link and thus appreciable quantities of short unbranched chains (" pseudo-amylose ") are not detected.

Experimental.

Analytical Methods.—The analytical methods employed in this work were discussed in Part IV.

Fractionation of Starch.—The amylose and amylopectin samples were isolated from potato starch by fractional precipitation of the former with thymol (Bourne, Donnison, Haworth, and Peat, *J.*, 1948, 1687).

Isolation of P- and Q-Enzymes from Potatoes.—Details of the method employed for the isolation of these enzymes were given in the preceding paper.

Ionic Activation of the Action of Q-Enzyme on Amylose.—Enzyme fraction Q3 was freeze-dried in the presence of citrate buffer (pH 7·0) according to the standard method described in the preceding paper. When required, the dry powder was dissolved in water (80 c.c. for each 100 c.c. of juice used in the isolation of the enzyme). This Q3 solution, which was $3{\cdot}13 \times 10^{-2}$M. with respect to citrate buffer,

was incorporated in digests containing amylose (14 mg.; B.V. 1·04), maltose (3 mg.), Q3 solution (2·0 c.c.), 0·2M-citrate buffer (pH 7·0; varying volumes), and water to make the total volume 14 c.c. in each case. The digests were incubated at 20·5° and, at intervals, aliquot portions (1 c.c. ≡ 1 mg. of amylose) were stained with iodine (2 mg.)–potassium iodide (20 mg.) in a total volume of 100 c.c. The values of A.V. (6800 A.) thus obtained were related to the concentrations of buffer employed, as is shown in Table I. In addition to these different concentrations of citrate buffer, there were other salts present in constant strength in each digest, namely sodium sulphate ($1·12 \times 10^{-2}$M.) (arising from dissolution of the amylose in aqueous sodium hydroxide and neutralisation of the solution with sulphuric acid) and ammonium sulphate (a small residue left from precipitation of the enzyme). The Q3 fraction used in these digests had been shown, in a separate experiment, to effect a fall of 88% in the A.V. (6800 A.) of the amylose with an " apparent conversion into maltose " of only 3·0% and thus to be essentially free from the normal amylases.

TABLE I.

Ionic activation of the action of Q-enzyme on amylose.

Molarity of citrate buffer (pH 7·0) in digest ($\times 10^2$).	Fall (%) in A.V. (6800 A.) of amylose. 20 mins.	45 mins.	Molarity of citrate buffer (pH 7·0) in digest ($\times 10^2$).	Fall (%) in A.V. (6800 A.) of amylose. 20 mins.	45 mins.
0·45	40·4	61·9	3·33	51·8	69·5
1·16	47·0	65·6	4·76	53·3	69·8
1·88	48·8	66·2	6·18	54·2	69·8

When the results (Table I) were plotted it was evident that the minimum concentration of citrate buffer necessary for full activation of the enzyme was $4·5 \times 10^{-2}$M.

Effect of Mineral Phosphate on the Rate of Conversion of Amylose by Q-Enzyme.—Fraction Q1 was dissolved in a little water and freeze-dried, a process which diminished considerably the small amount of P-enzyme impurity (see Part IV). When required, the dry powder was dissolved in water (17 c.c. for each 100 c.c. of juice used in the isolation of the enzyme). An aliquot portion (5 c.c.) of this enzyme solution was incubated at 20·5° with amylose (20 mg.; B.V. 1·30; dissolved in sodium hydroxide and neutralised with sulphuric acid, as described in Part IV), maltose (8 mg.), and M-acetate buffer (pH 7·0; 5 c.c.) in a digest having a total volume of 50 c.c. At intervals, portions (2·5 c.c. ≡ 1 mg. of amylose) were stained with iodine (2 mg.)–potassium iodide (20 mg.) in a final volume of 100 c.c. The values for % fall of A.V. (6800 A.) are shown, together with the increase in copper reducing value (expressed as maltose), in Table II. The free-phosphorus content of the digest remained constant at 0·170 mg., corresponding to 0·044 atom of phosphorus per $C_6H_{10}O_5$ unit in the amylose. This impurity of mineral phosphate had been introduced mainly with the enzyme solution.

A second digest (total volume, 50 c.c.), which was incubated simultaneously with that already described, contained, in addition to the above components, $3·462 \times 10^{-3}$M-phosphate buffer (pH 7·0; 5 c.c.). This digest contained 0·18 atom of free phosphorus per $C_6H_{10}O_5$ unit. The values of A.V. (6800 A.) and " apparent conversion into maltose " were very similar to those recorded in the first digest (Table II).

In a second experiment with a fresh enzyme solution 1·35 atoms of free phosphorus per $C_6H_{10}O_5$ unit were added. The rate of conversion of the amylose was not increased even by this high concentration of mineral phosphate (Table II).

TABLE II.

Effect of mineral phosphate on the conversion of amylose by Q-enzyme.

Experiment.	Molarity of acetate buffer in digest.	Atoms of free phosphorus per $C_6H_{10}O_5$ unit in the amylose.	Fall (%) in A.V. (6800 A.) of amylose. 25 mins.	150 mins.	Apparent conversion into maltose (%) in 150 mins.
First	0·1	0·04	73·7	84·2	1·0
,, ...	0·1	0·18	74·0	84·3	1·2
Second	0·1	0·04	72·9	86·0	0·0
,, ...	0·1	1·39	73·0	86·4	0·0

Effect of Glucose-1 Phosphate on the Rate of Conversion of Amylose by Q-Enzyme.—Fraction Q3 was prepared from a sample of fraction Q1 which had been freeze-dried as described above. It was dissolved in water (20 c.c. for each 100 c.c. of potato juice used in the isolation of the enzyme) and gave a solution in which phosphorylase could not be detected by the method of Green and Stumpf (*J. Biol. Chem.*, 1942, **142**, 355). This Q3 solution (4·5 c.c.) was incorporated in two digests (volume 50 c.c.) similar to those used in the previous experiment. The control digest was found to contain 0·032 mg. of free phosphorus, *i.e.*, 0·0083 atom of phosphorus per $C_6H_{10}O_5$ unit. The second digest contained the same components as the control, together with 0·1M-dipotassium glucose-1 phosphate solution (1·0 c.c.), *i.e.*, 0·81 mole of the Cori ester per $C_6H_{10}O_5$ unit. The digests were incubated at 20·5° and aliquot portions were stained with iodine in the manner described above. Glucose-1 phosphate did not influence the rate of fall of A.V. (6800 A.), as is shown in Table III.

Effect of Phosphorylase on the Rate of Conversion of Amylose by Q-Enzyme.—The enzyme fractions P2 and Q3 had been freeze-dried in citrate buffer by the standard methods described in the preceding paper. They were dissolved as required in water (20 c.c. for each 100 c.c. of potato juice used in the isolation of the enzymes), giving solutions which contained, respectively, 14·05 and 0·78 units (Green

and Stumpf) of phosphorylase per c.c. These enzyme solutions were incorporated in two digests, prepared in the usual way and having the compositions shown in Table IV. The digests were incubated at 20·5° and, at intervals, aliquot portions (1 c.c. ≡ 1 mg. of amylose) were stained with iodine as usual. As will be seen from Table V, the presence of P-enzyme did not influence the rate of fall of A.V. (6800 A.).

TABLE III.

Effect of glucose-1 phosphate on the rate of conversion of amylose (B.V. 1·30) by Q-enzyme.

Molarity of acetate buffer in digest.	Moles of Cori ester per $C_6H_{10}O_5$ unit.	Fall (%) in A.V. (6800 A.) of amylose. 90 mins.	105 mins.	180 mins.
0·1	Nil	71·7	73·1	78·1
0·1	0·81	71·5	72·8	77·1

TABLE IV.

Effect of P-enzyme on the rate of conversion of amylose by Q-enzyme. Composition of the digests.

	Volumes of component solutions, c.c.					
Digest.	Fraction P2.	Fraction Q3.	Amylose (B.V. 0·98) (14 mg./6·5 c.c.).	0·2M-Citrate buffer (pH 7·0).	Maltose (3 mg./1·5 c.c.).	Water.
A	Nil	1·0	6·5	1·5	1·5	3·5
B	1·0	1·0	6·5	0·5	1·5	3·5

TABLE V.

Effect of P-enzyme on the rate of conversion of amylose by Q-enzyme.

Digest.	Molarity of citrate in digest.	Units of P-enzyme (Green & Stumpf) in digest.	Fall (%) in A.V. (6800 A.) of amylose. 30 mins.	67 mins.	144 mins.	Apparent conversion into maltose (%).
A	$3·04 \times 10^{-2}$	0·78	68·4	76·3	83·1	2·4
B	$3·04 \times 10^{-2}$	14·83	66·3	75·7	82·9	1·8

Effect of Glucose-1 Phosphate on the Degree of Branching of the Polysaccharide Produced from Amylose by Q-Enzyme.—Fraction Q3 was prepared from a sample of fraction Q1 which had been dissolved in water and freeze-dried. The Q3 sample was dissolved in water (20 c.c. for each 100 c.c. of potato juice used in the isolation of the enzyme) and gave a solution in which phosphorylase could not be detected by the method of Green and Stumpf (*loc. cit.*). This enzyme solution (4·5 c.c.) was introduced into two of three digests, which were prepared in the usual way; all three contained amylose (20 mg.; B.V. 1·30), maltose (8 mg.), and M-acetate buffer (pH 7·0; 5 c.c., being sufficient to ensure complete ionic activation of the Q3) in a total volume of 24 c.c. A second series of three digests (24 c.c.) was prepared, each containing, in addition to the components already listed, 0·1M-dipotassium glucose-1 phosphate (1·0 c.c.), *i.e.*, 0·81 mole of the Cori ester per $C_6H_{10}O_5$ unit in the amylose. These six digests were incubated at 20·5° for different times, and N-acetic acid (5 c.c.) was then added to each; this lowered the pH to 4·7 and thus almost completely inactivated the Q-enzyme. To each digest a 0·2% solution

TABLE VI.

Effect of glucose-1 phosphate on the degree of branching of the polysaccharide produced by the action of Q-enzyme on amylose.

Fraction Q3 (free from P-enzyme) + amylose (B.V. 1·30) at pH 7·0. Time of incubation before the addition of β-amylase (hours).	Mole of Cori ester per $C_6H_{10}O_5$ unit.	Apparent conversion into maltose (%) at end of incubation.	β-Amylolysis of product at pH 4·7. Limit conversion into maltose (%).
0	Nil	—	89
0	0·81	—	89
1·5	Nil	—	44
1·5	0·81	—	47
3·0	Nil	1·3	40
3·0	0·81	0·0	45

(10 c.c.) of soya-bean β-amylase was then added, the volume adjusted to 50 c.c., and incubation continued until no further increase in reducing power occurred. The liberation of maltose was followed by means of the Shaffer–Hartmann reagent, and to the figures thus obtained small corrections were applied for the reducing powers of the orginal components of the digests. Table VI shows that the presence of glucose-1 phosphate does not influence, within the experimental error, the structure of the polysaccharide produced by Q-enzyme acting on amylose.

Action of Q-Enzyme on the Cross-linkages of Amylopectin.—The Q3 fraction employed was the same as that used in the last experiment. Portions (4·5 c.c.) of an aqueous solution (20 c.c. for each 100 c.c. of juice used in the isolation of the enzyme) of the enzyme were introduced into three of four identical digests, which were prepared in the usual manner; all four contained amylopectin (20 mg.; B.V. 0·215), maltose (8 mg.) and M-acetate buffer (pH 7·0; 5 c.c., being sufficient fully to activate the enzyme) in a total volume of 30 c.c. A series of four similar digests (30 c.c.) was prepared, each containing, in addition to the above componets, $3·338 \times 10^{-2}$M-phosphate buffer (pH 7·1; 5 c.c.), *i.e.*, 1·35 atoms of free phosphorus per $C_6H_{10}O_5$ unit in the amylopectin. The eight digests were incubated at 20·5° for different periods, adjusted to pH 4·7 with acetic acid, mixed with a 0·2% solution (10 c.c.) of soya-bean β-amylase diluted to 50 c.c., and further incubated until maximum conversion into maltose had occurred. The maltose liberated was estimated by the Shaffer–Hartmann method, allowance being made for the reducing powers of the digest components.

TABLE VII.

Action of Q-enzyme on the cross-links of amylopectin.

Fraction Q3 (free from P-enzyme) + amylopectin (B.V. 0·215) at pH 7·0.			
Time of incubation before the addition of β-amylase (hours).	Moles of free phosphorus per $C_6H_{10}O_5$ unit in the amylopectin.	Apparent conversion into maltose (%) at end of incubation.	β-Amylolysis of product at pH 4·7. Limit conversion into maltose (%).
0	Nil	—	53
0	1·35	—	53
0·5	Nil	0·0	51
0·5	1·35	0·0	47
1·5	Nil	0·0	48
1·5	1·35	0·0	47
3·0	Nil	1·3	47
3·0	1·35	0·0	47

Table VII shows (i) that the Q-enzyme slightly increased the degree of branching of the "thymol"-amylopectin by an amount consistent with the conversion of the small amylose impurity in the polysaccharide into amylopectin and (ii) that the presence of mineral phosphate did not influence the conversion.

Action of Q-Enzyme on the Cross-linkages of Dextrin-A.—Fraction Q5 (see preceding paper), isolated from 100 c.c. of potato juice, was dissolved in 0·2M-citrate buffer (pH 6·5; 13 c.c.) and freeze-dried. When required, the powder was redissolved in water (100 c.c.), giving a solution which contained only 0·014 unit (Green and Stumpf) of phosphorylase per c.c.

The previous experiment was repeated using this enzyme solution with dextrin-A, prepared by β-amylolysis of thymol-amylopectin, as substrate. The results are shown in Table VIII.

TABLE VIII.

Action of Q-enzyme on the cross-links of dextrin-A.

Fraction Q5 + dextrin-A at pH 7·0.			
Time of incubation before the addition of β-amylase (hours).	Moles of free phosphorus per $C_6H_{10}O_5$ unit in dextrin-A.	Apparent conversion into maltose (%) at end of incubation.	Increase in conversion into maltose (%) during β-amylolysis at pH 4·7 for $3\frac{1}{2}$ hours.
0	0	0·0	2·0
0	1·35	0·0	3·5
0·5	0	0·0	6·5
0·5	1·35	0·0	5·5
1·5	0	0·0	10·5
1·5	1·35	0·0	9·5
3·0	0	1·0	11·5
3·0	1·35	2·0	9·5

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