824. *The Mode of Action of the Q-Enzyme of* Polytomella coeca.

By S. A. Barker, A. Bebbington, and E. J. Bourne.

The rate of conversion of potato amylose into amylopectin by the Q-enzyme of *Polytomella coeca* is increased markedly when maltosaccharides of short average chain-length are introduced. Other saccharides, devoid of α-1 : 4-glucosidic linkages, do not have this effect. The maltosaccharides probably serve as receptors of dextrin chains transferred by the enzyme, a view which derives support from the fact that, in the presence of ^{14}C-maltose, the protozoal enzyme converts amylose into a polysaccharide which displays ^{14}C-activity.

Although the ability of oligo- and poly-saccharides to function as primers in the phosphorylase-catalysed synthesis of amylose (Hanes, *Proc. Roy. Soc.*, 1940, *B*, **128**, 421; **129**, 174; Cori and Cori, *J. Biol. Chem.*, 1939, **131**, 397), and also in syntheses of other polysaccharides (for a recent review see Barker and Bourne, *Quart. Reviews*, 1953, **7**, 56), is now well-established, no evidence has been obtained hitherto of the need for primers in the conversion of amylose into amylopectin by means of Q-enzyme. We wish to report more fully (see brief note, Barker, Bebbington, and Bourne, *Nature*, 1951, **168**, 834) on such a phenomenon, encountered during an investigation of *Polytomella coeca* Q-enzyme (Bebbington, Bourne, Stacey, and Wilkinson, *J.*, 1952, 240; Bebbington, Bourne, and Wilkinson, *J.*, 1952, 246).

An attempt was made to relate the activities of various *Polytomella coeca* Q-enzyme concentrations (ratios 1 : 2 : 3 : 4) by a method analogous to that of Gilbert and Swallow (*J.*, 1949, 2849), but using potato amylose as the substrate instead of starch. It was apparent that, although some degree of proportionality existed between the concentration of the enzyme and the time taken for a given fall in A.V. (6800 Å) (Table 1, p. 4054), the results did not conform to the kinetic equation for a reaction of the first order (as they did when potato starch was treated with potato Q-enzyme), marked deviations from linearity being revealed when log $(x - x_\infty)$ was plotted against t (where x and x_∞ are, respectively, the light absorption values at time t and after complete reaction). The rate of fall in the value of A.V. (6800 Å), which was very slow initially, increased markedly as the reaction progressed. The slow phase was not caused by the presence of an inhibitor in the enzyme solution, for when a digest was inactivated by heat after being allowed to proceed beyond this stage of the reaction, and a fresh portion of enzyme solution added, this phase was not re-encountered (Fig. 1). The rate of fall of A.V. (6800 Å) was greater with an impure amylose (B.V. 0·95) than with amylose (B.V. 1·25), and it seemed likely that this difference could be attributed to the larger proportion of amylopectin impurity in the former, especially since both amylose samples had been prepared from potato starch by the same method, involving precipitation with thymol (Bourne, Donnison, Haworth, and Peat, *J.*, 1948, 1687). As confirmation of this, it was found that the kinetics of the reaction involving the amylose sample (B.V. 0·95) could be simulated by the addition of potato amylopectin to the amylose of higher B.V.

That the observed effects were not due to the activation of traces of amylases, which might have been present as impurity in the enzyme preparations, was shown by the fact that, in the presence of the activator polysaccharide, there was no detectable increase in the normal small reducing power developed during the course of the Q-enzyme reaction. Furthermore, pretreatment of the activator (amylopectin) with a Q-enzyme preparation did not increase the activating ability, showing that the true activator was probably amylopectin, and not a product resulting from its conversion by the enzyme solution (Table 4, p. 4055). This activation was still exhibited when the Q-enzyme had been purified 7-fold [as determined for a soluble-starch substrate by Gilbert and Swallow's method (*loc. cit.*)]. The purified enzyme showed the same relative rates of reaction with potato amylose (B.V. 1·27) and commercial soluble starch as it did beforehand (Table 11, p. 4057).

The activating powers of a number of polysaccharides were compared by using a standard digest containing potato amylose (B.V. 1·27), obtained by a combination of the methods involving the use of aluminium hydroxide and thymol (Bourne, Donnison, Peat, and Whelan, *J.*, 1949, 1; Hobson, Pirt, Whelan, and Peat, *J.*, 1951, 801). Liver glycogen and the native amylopectin of *Polytomella coeca* had approximately the same influence as potato amylopectin on the reaction rate, whilst the starch-type polysaccharides SII and SIII [prepared from potato amylose by means of the protozoal Q-enzyme (Bebbington, Bourne, and Wilkinson, *loc. cit.*)], and the limit dextrin produced by the action of β-amylase on potato amylopectin, were somewhat less effective (Table 2, p. 4054); inulin, xylan, and dextran (*Betacoccus arabinosaceous*) showed negligible activity (Table 3, p. 4054). Of other carbohydrates examined, D-glucose, D-galactose, and D-fructose had no significant influence on the reaction, whilst among the disaccharides (sucrose, maltose, lactose, and cellobiose) maltose alone greatly increased the rate of fall of A.V. (6800 Å) in the early stages. These results suggest that the activator molecule for Q-enzyme must contain glucose units mutually linked by α-1 : 4-glucosidic bonds.

Potato amylopectin (B.V. 0·15) was hydrolysed with N-sulphuric acid, and its ability to activate the protozoal Q-enzyme was examined at various stages of the hydrolysis.

FIG. 1. *The action of Q-enzyme on a partly converted amylose.*

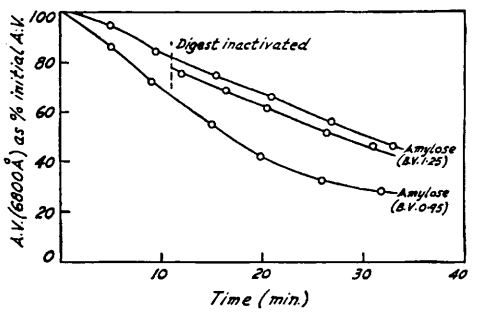
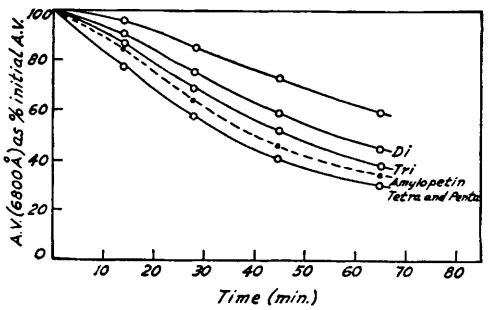


FIG. 2. *The activation of Q-enzyme by linear dextrins.*



The degradation products formed during the initial fragmentation of the amylopectin had about the same activating power as the parent polysaccharide (Table 5, p. 4055). Indeed, there had been very little decrease in activating power by the time that the apparent conversion into glucose, as determined by cuprimetric titration, had attained a value as high as 20% (equivalent to an average molecular size of 5 glucose units); thereafter, the activating power diminished more rapidly as the dextrins were converted finally into glucose.

The activating power of the *Neisseria perflava* polysaccharide, which is known to belong to the amylopectin–glycogen class (Barker, Bourne, and Stacey, *J.*, 1950, 2884), was surprisingly small, due perhaps to entanglement of the chains; mild acidic hydrolysis resulted in the development of an activating power comparable with that of potato amylopectin. The activating power reached a maximum when the reducing value corresponded to a conversion into glucose of *ca.* 10%, and then decreased slowly as the hydrolysis was continued (Table 6, p. 4055). These results, together with those for potato amylopectin, suggest that certain dextrins formed during the hydrolyses were more efficient activators than the polysaccharides themselves, on a weight for weight basis.

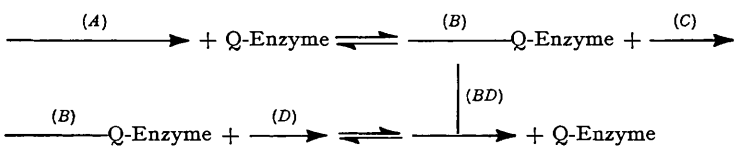
Confirmation of this was obtained when the extent to which the activation is dependent on chain-length was examined by incubation of equal weights of maltose, maltotriose, maltotetraose, and maltopentaose with potato amylose and Q-enzyme. The results (Fig. 2) illustrate that there is an increase in activating power in passing from maltose through maltotriose to maltotetraose; maltopentaose has the approximately same activating power per unit weight as maltotetraose, and both are more effective than an equal weight of potato amylopectin. However, the α- and β-Schardinger dextrins failed

to function as activators; indeed, in each case a slight inhibition of the reaction was observed, which was not apparent after the β-Schardinger dextrin had been submitted to very mild acidic hydrolysis (Table 7, p. 4056).

The dependence of the degree of activation achieved on the concentration of the activator was examined in digests containing Q-enzyme, potato amylose, and different amounts of potato amylopectin. Although small quantities of amylopectin had quite a marked influence on the rate of the amylose conversion (see Table 8, p. 4056), the effect could be increased by further additions of the branched polysaccharide; even when the concentration of the amylopectin was half that of the amylose substrate, the limit of activation, although approached, had not been reached. Conversely, as the amount of amylopectin impurity in a given amylose sample was diminished by further fractionations, so the susceptibility of the sample to attack by the enzyme progressively decreased (Table 9, p. 4056). As was to be expected, mild treatment of an amylose sample with acid increased the rate at which it was subsequently attacked by Q-enzyme (Table 10, p. 4056).

To summarise, it is clear that the rate of conversion of a potato amylose of high blue value by *Polytomella coeca* Q-enzyme is markedly increased by the introduction of maltosaccharides of short average chain-length (2—20 units), but not by any of the other carbohydrates tested. An activator molecule may be as small as maltose or as large as amylopectin, and so reducing groups cannot be responsible for these phenomena. The failure of the Schardinger dextrins to function as activators may be due either to their inability to assume a conformation acceptable to the enzyme, or to the possibility that the enzyme may require the presence of non-reducing end-groups in the activator, as is known to be the case with several other enzymes responsible for syntheses of polysaccharides. In this connection, it will be recalled that an indication has been obtained earlier that, in the initial stages of the action of potato Q-enzyme on amylose, the branches may be introduced near the non-reducing ends of the main chains (Barker, Bourne, Peat, and Wilkinson, *J.*, 1950, 3022).

These observations find a ready explanation if, as has been suggested previously (Hestrin, *Brewers' Digest*, 1948, **23**, 1; Barker, Bourne, and Peat, *J.*, 1949, 1712; Barker, Bourne, Wilkinson, and Peat, *J.*, 1950, 93; Hobson, Whelan, and Peat, *J.*, 1951, 596), Q-enzyme is a transglucosidase. It is probable that each step in the amylose ⟶ amylopectin conversion can be represented by the following scheme, in which the lines signify chains of α-1 : 4-glucopyranose units, and the arrow-heads represent reducing groups:



There is, as yet, no direct evidence to show the minimum chain length which *Polytomella coeca* Q-enzyme requires in the amylose-type substrate (*A*), but the figure is probably not far removed from the 42 glucose units which applies in the case of the potato Q-enzyme (Bailey, Peat, and Whelan, *Biochem. J.*, 1952, **51**, xxxiv; Nussenbaum and Hassid, *J. Biol. Chem.*, 1952, **196**, 785). The maltosaccharide molecule (*D*), which serves as the receptor for the transferred chain (*B*), could be another intact amylose molecule, a residual dextrin (*C*) formed at an earlier stage in the process, or a branched product of the type (*BD*). If the second step controls the rate, the conversion of pure amylose would then be expected to be slow in the initial stages, because of the small number of chains available to serve as receptors (*D*), but the rate would increase progressively as the number of receptor chains was increased by the production of dextrins (*C*) and branched molecules (*BD*). It is likely that the function of the maltosaccharide activators described above is to increase greatly, in the early stages of the amylose conversion, the number of receptor chains of type (*D*).

If this hypothesis be true, then some of the primer molecules should be incorporated into the structure of the branched product of the enzyme action, and this has been shown

to be the case. When potato amylose was treated with the Q-enzyme of *Polytomella coeca* in the presence of ^{14}C-maltose, the polysaccharide product displayed radioactivity after being separated from the excess of ^{14}C-maltose on a paper chromatogram. It remains to be seen whether Q-enzyme samples derived from other sources (*e.g.*, the potato and the broad bean) are influenced by maltosaccharide primers in the same way.

Experimental

Isolation of Q-Enzyme.—The crude extract (100 c.c.), isolated from a culture (4 l.) of *Polytomella coeca* by the standard method of Bebbington, Bourne, Stacey, and Wilkinson (*loc. cit.*), was treated with lead acetate solution (pH 7·25; 18 c.c.), prepared as described by Barker, Bourne, and Peat (*J.*, 1949, 1705), and the resulting lead–protein complex was discarded. The material precipitated by a further portion (7 c.c.) of the lead acetate solution was extracted with 0·2M-sodium hydrogen carbonate (50 c.c.) for 10 min. in the presence of carbon dioxide. The Q-enzyme, precipitated by addition of ammonium sulphate solution (50 g./100 c.c.; pH 6·8; 50 c.c.) to the supernatant liquid, was collected in the centrifuge, after being kept at 0° for 1 hr., and was dissolved in 0·02M-citrate buffer (pH 6·8; 12 c.c.). Several different Q-enzyme solutions were used in the work described.

Deviations from a Reaction of the First Order.—Varying quantities (x c.c.) of the enzyme solution were incorporated in four digests (each 10 c.c.) containing amylose solution (5 c.c.; 10 mg.) and 0·02M-citrate buffer [pH 6·8; $(5-x)$ c.c.]. The rate of reaction at 25° was followed by staining aliquot portions (1 c.c.) with iodine (2 mg.) and potassium iodide (20 mg.) in a total volume of 100 c.c., and measuring A.V. (6800 Å) in the manner described by Bourne, Haworth, Macey, and Peat (*J.*, 1948, 924); the results are given in Table 1. The amylose solution had been prepared by dissolving potato amylose (100 mg.; B.V. 1·25) in 0·5N-sodium hydroxide (4 c.c.), neutralising the solution with sulphuric acid, and diluting it to 50 c.c.

Table 1. *Rates of reaction with various enzyme concentrations.*

Enzyme concn. (x c.c.)	A.V. (6800 Å) after : 5 min.	9 min.	16 min.	22 min.
1	1·11	0·87	0·55	0·40
2	0·84	0·45	0·27	0·21
3	0·59	0·32	0·19	0·16
4	0·43	0·26	0·17	0·14

Examination of the Initial Phase.—Two samples of potato amylose having B.V. 1·25 and B.V. 0·95, respectively, were incorporated in separate digests (10 c.c.) containing amylose solution (see above; 10 mg.; 5 c.c.), 0·02M-citrate buffer (pH 6·8; 4·5 c.c.), and Q-enzyme (0·5 c.c.). In addition, a duplicate of the digest containing amylose (B.V. 1·25) was incubated at 25° for 11 min., deactivated at 100° for 15 min., cooled to 25°, and mixed with a further portion (0·5 c.c.) of the enzyme solution. The rates of reaction, at 25°, are shown in Fig. 1.

Standard Digest for the Measurement of Activation.—The digest (10 c.c.) contained potato amylose solution (see above; B.V. 1·27; 9 mg.; 5 c.c.), activator [5 mg. in 0·02M-sodium sulphate (2 c.c.)], 0·02M-citrate buffer (pH 6·8; 2 c.c.), and Q-enzyme (1 c.c.). The rate of reaction, at 25°, was followed as described above, and the degree of activation was obtained by comparison with a blank digest in which the activator solution was replaced by 0·02M-sodium

Table 2. *Activation by polysaccharides of the starch type.*

Activator added	% Initial A.V. (6800 Å) after : 12 min.	22·5 min.	34 min.	44·5 min.
None	98·5	90·9	85·6	77·1
Polysaccharide SIII	98·0	86·4	76·8	70·2
β-Limit dextrin	97·4	81·9	69·2	58·8
Potato amylopectin	90·5	72·5	58·2	47·1
	11 min.	21 min.	31 min.	41 min.
None	96·7	87·0	78·4	70·3
Polysaccharide SII	95·2	83·7	72·2	63·9
Glycogen	84·0	62·3	47·0	36·0
Potato amylopectin	80·1	59·1	43·0	32·9
Polytomella coeca amylopectin	82·4	61·0	43·8	32·1

sulphate (2 c.c.). The results obtained with various carbohydrates as possible activators are shown in Tables 2 and 3.

TABLE 3. *Activation by other carbohydrates.*

Activator added	% Initial A.V. after 55 min.	Activator added	% Initial A.V. after 55 min.
None	76·6	Cellobiose	76·8
D-Glucose	72·8	Lactose	74·7
D-Galactose	71·0	Inulin	72·5
D-Fructose	72·2	Xylan	71·2
Sucrose	74·3	Dextran	75·2
Maltose	59·0	Potato amylopectin	37·1

Activation by Q-Enzyme-treated Potato Amylopectin (B.V. 0·15).—Amylopectin (25 mg.; 5 c.c.) was incubated with *Q*-enzyme solution (2 c.c.) and 0·02M-citrate buffer (pH 6·8; 3 c.c.) at 25° for 45 min., during which there was a fall of 36·4% in A.V. (6800 Å). A similar digest, in which the enzyme solution had been replaced by 0·02M-citrate buffer (pH 6·8; 2 c.c.), was incubated simultaneously. Both digests were heated at 100° for 15 min., and cooled; aliquot portions (2 c.c.) were incorporated in two standard activator digests (see Table 4).

TABLE 4. *Effect of Q-enzyme on the activating power of potato amylopectin.*

	A.V. (6800 Å) as % initial A.V.:			
Substrate	5 min.	10 min.	25 min.	40 min.
Amylose + amylopectin	96·3	93·7	78·7	66·9
Amylose + treated amylopectin	98·3	95·2	82·6	71·3

Activation by the Hydrolysis Products of Potato Amylopectin (B.V. 0·15).—The polysaccharide (413 mg.) was hydrolysed with N-sulphuric acid (100 c.c.) at 75° initially, and thereafter the temperature was slowly raised to 100°. Aliquot portions (5 c.c.) were neutralised with sodium hydroxide, then diluted to 10 c.c., and the reducing powers estimated by cuprimetric titration by the method of Shaffer and Hartmann (*J. Biol. Chem.*, 1921, **45**, 377). Other portions (2 c.c.) of the neutralised hydrolysate were incorporated, with potato amylose (B.V. 1·27), in a series of standard activator digests. In Table 5, " activating power " is the difference between the fall (%) in A.V. (6800 Å) of the digests containing the activators and the fall (%) in A.V. (6800 Å) of a digest from which the activator had been omitted. The incubation time was 80 min.

TABLE 5. *Effect of hydrolysis on the activating power of amylopectin.*

Activating power of hydrolysis product	35·5	35·6	35·3	32·8	32·1	32·4	28·8	11·0
Apparent conversion into glucose (%)	0·0	0·7	2·2	4·0	7·6	18·5	39·8	84·4

Activation by the Hydrolysis Products of the Neisseria perflava *Polysaccharide.*—The polysaccharide (107 mg.) was hydrolysed with N-sulphuric acid (25 c.c.), as described above for amylopectin. Aliquot portions (2 c.c.) were neutralised with sodium hydroxide, then diluted to 5 c.c., and the reducing and activating powers of each solution determined as above (see Table 6).

TABLE 6. *Effect of hydrolysis on the activating power of* Neisseria perflava *polysaccharide.*

Activating power of hydrolysis product	8·3	27·1	34·8	36·6	40·0	38·4	33·4	20·6
Apparent conversion into glucose (%)	0·0	0·5	1·5	2·8	7·2	16·7	42·9	80·3

Activation by Oligosaccharides of the Maltose Series.—Potato amylose (1·21 g.) was hydrolysed with N-sulphuric acid for 2 hr. at 80° and neutralised with barium carbonate; the components of the filtered solution were separated on a charcoal column according to the methods of Whistler and Durso (*J. Amer. Chem. Soc.*, 1950, **72**, 677) and Bailey, Whelan, and Peat (*J.*, 1950, 3692). Elution with increasing concentrations of aqueous ethanol gave maltose (73·6 mg.), maltotriose (85·9 mg.), maltotetraose (84·7 mg.), and maltopentaose (66·4 mg.). The activating powers of the dextrins (2·5 mg.), which were characterised by filter-paper chromatography, were determined by incorporation in standard activator digests; the results are given in Fig. 2.

Activating Powers of the Schardinger Dextrins.—Schardinger β-dextrin (50 mg.) was hydrolysed with 0·001N-hydrochloric acid (5 c.c.) at 100° for 7 hr. (French, Levine, and Pazur, *J. Amer. Chem. Soc.*, 1949, **71**, 356). The solution was neutralised with sodium hydroxide and diluted

to 10 c.c.; an aliquot portion (2 c.c.) was incorporated in a standard activator digest, and the activating power of the hydrolysate was compared with that of the original β-dextrin (10 mg.), and also with that of Schardinger α-dextrin (10 mg.) (see Table 7).

TABLE 7. *Activating powers of the Schardinger dextrins.*

Activator added to amylose (B.V. 1·27):	None	β-Dextrin	β-Dextrin hydrolysate	α-Dextrin
Fall (%) in A.V. (6800 Å) after 49 min. ...	22·8	14·6	23·0	14·5

Relation between Activating Power and Concentration of the Activator.—Different quantities (x c.c.) of potato amylopectin solution [2·5 mg./c.c. in 0·01M-citrate buffer (pH 6·8)] and 0·01M-citrate buffer [pH 6·8; $(2 - x)$ c.c.] were incorporated as activators in standard digests, and the rates of reaction determined in the usual manner (Table 8).

TABLE 8. *Activating power and concentration of the activator.*

Amylopectin (mg.) added as activator	% of Initial A.V. (6800 Å) after: 9 min.	17 min.	25 min.	36 min.
None	97·1	91·6	84·8	77·7
1·25	93·0	82·2	70·8	58·3
2·50	86·8	72·4	60·4	45·7
3·75	85·5	69·2	57·0	42·0
5·00	84·5	66·9	53·6	40·2

Rates of Reaction with Different Amyloses.—Four samples of potato amylose were prepared. Amylose (B.V. 1·14) was obtained by thymol refractionation of amylose (B.V. 0·95), amylose (B.V. 1·27) by an aluminium hydroxide–thymol fractionation (Bourne, Donnison, Peat, and Whelan, *loc. cit.*; Hobson, Pirt, Whelan, and Peat, *loc. cit.*) of potato starch, amylose (B.V. 1·38) by amyl alcohol refractionation of amylose (B.V. 1·27), and amylose (B.V. 1·39) by amyl alcohol refractionation of amylose (B.V. 1·38). Each amylose was examined as a substrate for Q-enzyme by incorporation in a standard digest. No activator was added, and the concentrations of the polysaccharides were reduced to 8 mg./10 c.c. of digest. The differing susceptibilities to Q-enzyme attack are illustrated in Table 9.

TABLE 9. *Rates of reaction with different amyloses.*

B.V. of amylose substrate	% of Initial A.V. (6800 Å) after: 10 min.	25 min.	45 min.	70 min.	95 min.
1·39	97·1	88·5	75·2	59·8	48·0
1·38	96·8	87·5	73·0	57·1	45·3
1·27	96·5	85·5	68·9	52·0	40·4
1·14	94·0	77·0	57·0	38·0	29·0

An Acid-degraded Amylose as a Substrate for Q-Enzyme.—Potato amylose (B.V. 1·27; 180 mg.) was dissolved in 0·05N-sodium hydroxide (4 c.c.), and diluted to 40 c.c., before 2N-sulphuric acid (10 c.c.) was added. Two aliquots (10 c.c.) were removed, one before and one after the solution had been heated at 70° for 20 min., during which there was a fall of 7·4% in A.V. (6800 Å). The samples were neutralised with sodium hydroxide, and diluted to 20 c.c. Portions (5 c.c.) of the neutral solutions were incorporated as substrates for Q-enzyme in standard digests, in the absence of added activator (Table 10).

TABLE 10. *Acid-degraded amylose as a substrate for Q-enzyme.*

Duration of enzyme action (min.)	10	20	38	65
Fall (%) in A.V. (6800 Å) during enzyme action:				
untreated amylose	0·0	1·4	7·6	19·9
acid-degraded amylose	2·4	9·1	18·2	32·7

Comparison of the Activation of Q-Enzyme Samples of Different Specific Activities.—An enzyme sample (12 c.c.), obtained as above, was purified further by dilution with 0·02M-citrate buffer (pH 6·8; 38 c.c.), addition of lead acetate solution (12·5 c.c.), and elution of the precipitated complex with 0·2M-sodium hydrogen carbonate (25 c.c.), as before. Q-Enzyme was precipitated with ammonium sulphate solution (50 g./100 c.c.; pH 6·8; 25 c.c.), collected in the centrifuge, and redissolved in 0·02M-citrate buffer (pH 6·8; 12 c.c.); it had an absolute activity of 7·7 units compared with 1·1 units shown by the initial enzyme sample. [These activities were determined with soluble starch as substrate, in a somewhat similar fashion to that described by Gilbert and Swallow (*loc. cit.*).] The volumes of the two enzyme solutions

were adjusted with 0·02M-citrate buffer (pH 6·8) so that when aliquot portions (1 c.c.) were incorporated in two digests containing soluble starch the rates of reaction were almost identical. The rates of reaction were then compared when the enzymes were incorporated in standard digests containing amylose (B.V. 1·27), but not added activator (see Table 11).

TABLE 11. *Comparison of two Q-enzyme samples.*

Substrate	Enzyme activity	A.V. (6800 Å) as % of initial A.V.: 14 min.	28 min.	42 min.	63 min.
Soluble starch	1·1	71·4	52·1	43·6	37·7
Soluble starch	7·7	74·5	55·5	45·0	40·2
Amylose	1·1	93·6	81·0	67·2	53·8
Amylose	7·7	94·6	83·1	72·1	58·6

Isolation of ^{14}*C-Labelled* Polytomella coeca *Starch.*—A medium (500 c.c.) containing peptone (0·5 g.), sodium acetate (0·6 g.), $^{14}CH_3 \cdot CO_2Na$ (200 mg.; 12·46 μc), $CH_3 \cdot {}^{14}CO_2Na$ (200 mg.; 15·24 μc), and indicator solution (0·5 c.c. of 0·5% bromocresol-purple and 0·5% of bromothymol-blue) was inoculated with a growing *Polytomella coeca* culture (peptone/ethanol; 2 c.c.), and incubated at 25° for 7 days, during which the pH was kept at 6·0—7·0 by the addition of acetic acid. A continuous stream of sterile air was passed over the surface of the culture in order to remove radioactive carbon dioxide, which was absorbed in saturated barium hydroxide solution. The radioactive starch (290 mg.), isolated from the cell debris with chloral hydrate (33%), showed 2100 counts/min. on a 1 cm.^{2} tray (cf. Bevington, Bourne, and Wilkinson, *Chem. and Ind.*, 1950, 691).

Isolation of ^{14}*C-Labelled Maltose.*—A solution of the radioactive starch in N-sodium hydroxide (15 c.c.) was diluted to 145 c.c., and N-sulphuric acid (15 c.c.) was added. The neutral solution was incorporated in a digest containing 0·02M-acetate buffer (pH 6·8; 120 c.c.) and salivary α-amylase solution (30 c.c.), prepared by the method of Meyer, Fischer, Staub, and Bernfeld (*Helv. Chim. Acta*, 1948, **31**, 2158). After 18 hr. the enzyme was inactivated, and the digest was freeze-dried until the volume had been reduced to 40 c.c. The solution was passed down a charcoal column, and the radioactive maltose (132 mg.) was recovered by elution with water and 5% aqueous ethanol; it was identified by filter-paper chromatography.

Investigation of the Activation of Q-Enzyme using Radioactive Maltose.—A portion (1 c.c.) of a 0·02M-citrate buffer extract of freeze-dried *Polytomella coeca* (60 mg. in 6 c.c.) was incubated with potato amylose (B.V. 1·49; 15 mg. in 6 c.c.) and ^{14}C-maltose (10 mg.). After completion of the enzyme reaction (20 hr.), non-radioactive maltose (10 mg.) was added, the enzyme destroyed by boiling after a further 0·5 hr., and the solution freeze-dried to a white powder (A). A second portion (1 c.c.) of the enzyme extract was incubated with amylose (B.V. 1·49; 15 mg. in 6 c.c.) alone, and after 20·5 hr. the enzyme was destroyed by boiling. A mixture of radioactive maltose (10 mg.) and non-radioactive maltose (10 mg.) was then added, and the white powder (B) obtained by freeze-drying. As a further control, a third portion (1 c.c.) of the enzyme extract was incubated with radioactive maltose (10 mg.) dissolved in water (6 c.c.). After 20·5 hr. the enzyme was destroyed by boiling, potato amylose (B.V. 1·49; 15 mg.) and non-radioactive maltose (10 mg.) were added, and the white powder (C) was obtained by freeze-drying. Equal portions (16 mg.) of (A), (B), and (C) were submitted to paper-chromatographic separation for 60 hr., the upper phase of a mixture of butanol (40%), ethanol (10%), water (49%), and ammonia (1%) being used as the solvent. A measure of the incorporation of radioactive maltose into the product of Q-enzyme action was obtained by radioactive counts along strips (1 cm. wide) cut from the base line of the chromatograms. The average count/min. along the strip from (A) was 26·2 compared with 12·1, 12·7, and 12·2 obtained with (B), (C), and the normal background, respectively. For all counts the standard error was less than 5%.

The authors are indebted to Professor M. Stacey, F.R.S., for his interest, to Dr. J. C. Bevington for providing facilities for the assay of radioactive carbon, and to Professor D. French for samples of the Schardinger dextrins. They also express their gratitude to the British Rayon Research Association and Courtaulds' Scientific and Educational Trust Fund for financial assistance to two of them (S. A. B. and A. B.).

CHEMISTRY DEPARTMENT, THE UNIVERSITY,
EDGBASTON, BIRMINGHAM, 15.

[*Received, July 24th*, 1953.]