366. *The Enzymic Synthesis and Degradation of Starch. Part IV. The Purification and Storage of the Q-Enzyme of the Potato.*

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An improved method is described for the isolation and purification of the Q-enzyme of potatoes. The Q-enzyme prepared by this method effects almost a 90% diminution of the absorption value of amylose with the concomitant liberation of only 2% (as maltose) of reducing end-groups. The preparation is free from α- and β-amylase, maltase, and fatty acids.

The amylose-degrading action of Q-enzyme functions at optima of pH 7·0 and temperature, 21° ± 1°.

Although Q-enzyme is unstable in aqueous solution, it can be stored for long periods without appreciable loss in activity when it is freeze-dried.

In 1940, Hanes (*Proc. Roy. Soc.*, 1940, *B*, **129**, 174) isolated from potato juice a phosphorylase (P-enzyme), which catalysed the synthesis, from dipotassium glucose-1 phosphate, of a polysaccharide resembling the amylose component of potato starch in its degree of hydrolysis with β-amylase and in its ability to give a blue stain with iodine. An end-group assay, carried out

two years later by Haworth, Heath, and Peat (*J.*, 1942, 55), revealed that this polysaccharide consisted of unbranched chains, averaging 80—90 units in length, of 1 : 4-linked α-glucose members, and thus confirmed that it was indeed an amylose. Since the major component of potato starch is not amylose, but amylopectin, a red-staining polyglucose having a branched structure in which chains of approximately 20 glucose members are united by cross-linkages, it was evident that a second enzyme figured prominently in the synthesis of starch *in vivo*. This supplementary enzyme was isolated from potatoes by Haworth, Peat, and Bourne (*Nature*, 1944, **154**, 236) and was named, provisionally, Q-enzyme. Unlike P-enzyme, it does not use glucose-1 phosphate as a substrate (Bourne and Peat, *J.*, 1945, 877). The synthesis of amylopectin from the Cori ester probably involves catalysis by P-enzyme of the end-wise apposition of glucose residues until the linear chains reach such a length that they serve as a substrate for Q-enzyme, which establishes cross-links between the chains to give a branched structure. In this respect Q-enzyme is similar to an enzyme found in liver and heart (Cori, Swanson, and Cori, *Federation Proc.*, 1945, **4**, 234). Q-Enzyme possesses a dual function since it converts amylose itself into amylopectin by a hydrolytic, as opposed to a phosphorolytic, process (Bourne and Peat, *J.*, 1945, 877; Bourne, Macey, and Peat, *J.*, 1945, 882).

Q-Enzyme has hitherto been isolated by treating potato juice with kaolin under the conditions described by Waldschmidt-Leitz and Mayer (*Z. physiol. Chem.*, 1935, **236**, 168) for the adsorption of amylases, and thereafter precipitating the Q-enzyme from the supernatant liquid with neutral ammonium sulphate (16 g./100 c.c.), leaving the bulk of the P-enzyme in solution. The enzyme was further purified by re-fractionation with ammonium sulphate (Bourne and Peat, *J.*, 1945, 877). Although this method yields a Q-enzyme reasonably free from α- and β-amylases when applied to freshly-harvested potatoes, it gives enzyme preparations progressively richer in amylases from potatoes which have been stored. In addition, the efficiency of the adsorption process varies with different batches of kaolin.

We have now developed an alternative method of isolation by which Q-enzyme preparations substantially free from the other enzymes of this group are obtained as free-flowing powders. A preliminary account of this method was given by Peat, Bourne, and Barker in 1948 (*Nature*, **161**, 127). The method gives reproducible results with potatoes during their " resting period," but not, as yet, with potatoes in which sprouting has commenced. Since however it is now possible to store Q-enzyme for prolonged periods, we have adopted the policy of preparing a stock of the enzyme from young potatoes for use at less favourable seasons.

The new method, developed from one applied to yeast for a different purpose by Minagawa (*J. Agric. Chem. Soc. Japan*, 1932, **8**, 1068), involves (i) precipitation from potato juice of Q-enzyme and other proteins by the addition of lead acetate at pH 7·25, (ii) elution of the lead–protein complex with sodium hydrogen carbonate solution, through which a stream of carbon dioxide is passed, and (iii) precipitation of the enzyme (fraction Q1) from the supernatant liquid with neutral ammonium sulphate (19 g./100 c.c.). A small amount of enzyme (fraction Q2) remains in solution, and is precipitated, together with P-enzyme, when the concentration of ammonium sulphate is increased to 35 g./100 c.c. Fraction Q1 can be further purified by reprecipitation with neutral ammonium sulphate (19 g./100 c.c.), giving fraction Q3. Each stage of the method has been carefully investigated to ensure that it is carried out under optimum conditions.

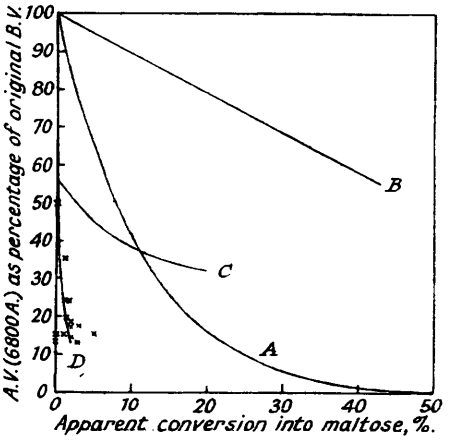
Fraction Q1 shows only a slight copper-reducing value and is free from maltase inasmuch as no increase in reducing power follows when it is incubated with maltose in acetate buffer at pH 7. Fraction Q3, which is also free from maltase, is non-reducing. None of the many samples of fractions Q1 and Q3 prepared by us has been contaminated with the fatty acids which are normally present in potato juice. Fatty acids form insoluble complexes with amylose (Schoch and Williams, *J. Amer. Chem. Soc.*, 1944, **66**, 1232), and their presence in an enzyme preparation precludes its use in quantitative studies on starches or on starch fractions containing amylose. These acids were probably removed as lead salts during the isolation of the enzymes.

Bourne, Macey, and Peat (*J.*, 1945, 882) distinguished Q-enzyme from the normal amylases by plotting " absorption value (A.V.) (6800 A.) " against " apparent percentage conversion into maltose " during the incubation of the enzymes with amylose. Each enzyme gave a characteristic curve, which was independent of enzyme concentration. A typical graph for Q-enzyme (fraction Q1), prepared by the new method, is shown in Fig. 1; this graph (*D*) is plotted from results obtained with about ten different samples of fraction Q1. It demonstrates unequivocally that this Q-enzyme preparation is different from either α- or β-amylase (curves *A* and *B*), and is moreover appreciably purer than the Q-enzyme previously isolated by the kaolin method

(curve *C*). Fraction Q1 effects an 87% reduction in A.V. (6800 A.) at a stage when the "apparent percentage conversion into maltose" is only 2%. If the Q1 preparation is contaminated by either α- or β-amylase, the concentrations of these impurities must be extremely small, too small, in fact, to be detected by the usual methods of estimation. The efficiency of the removal of lead ion during the treatments with sodium hydrogen carbonate and ammonium

FIG. 1.

The hydrolysis of amylose by Q-enzyme and amylases.



A.—α*β-Amylase from saliva.*
B.—*β-Amylase from soya bean.*
C.—*Q-Enzyme from young potatoes (kaolin method).*
D.—*Enzyme fraction Q1 (lead acetate method).*
Curves A, B, and C were given by Bourne, Macey, and Peat (J., 1945, 882).

sulphate is demonstrated by the fact that the introduction of only 6×10^{-4}M-lead acetate decreases the activity of a Q1 preparation by about 80%.

The optimum conditions of temperature and pH for the amylolytic activity of Q-enzyme have been ascertained as follows. Aliquot portions of a solution of fraction Q1 were incubated with amylose in the presence of veronal buffers, which differed in their pH values only by virtue of

FIG. 2.

The effect of pH on the hydrolysis of amylose (B.V., 1·04) by Q-enzyme at 20·5°.

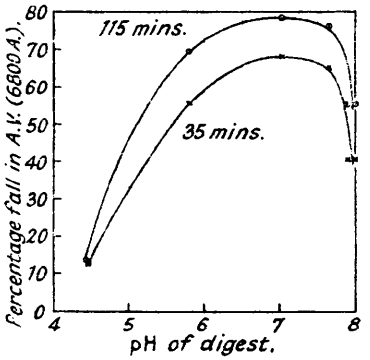
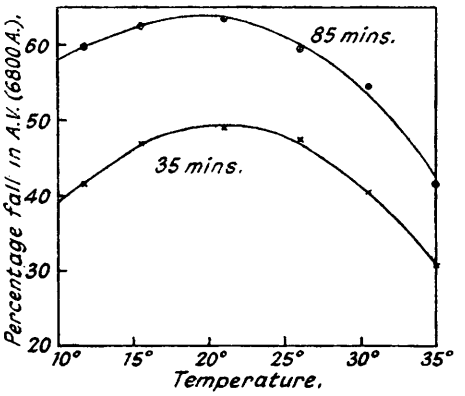


FIG. 3.

The effect of temperature on the hydrolysis of amylose (B.V., 1·04) by Q-enzyme at pH 7·04.



the amount of sodium hydroxide added (Fig. 2). The optimum pH for the enzyme is 7·0; the activity declines sharply above pH 7·5 and below pH 6·5. The optimum pH for this preparation of Q-enzyme is higher than that for either β-amylase (*ca.* 5·0) or P-enzyme (*ca.* 6·0), but is approximately the same as that for α-amylase (*ca.* 7·0). In a second series of digests, fraction Q1 was incubated with amylose (pH 7·04) at temperatures ranging from 11° to 35°

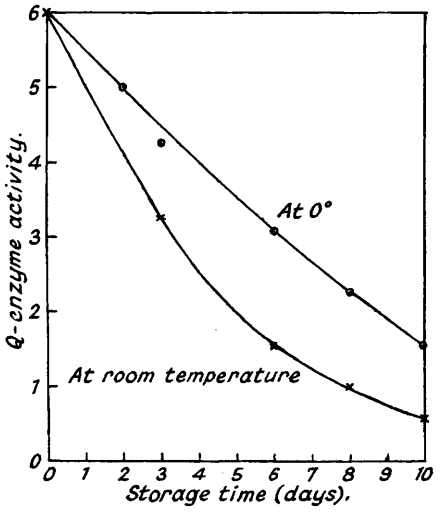
(Fig. 3). The optimum temperature (21° ± 1°) was unusually low in comparison with the optima of other enzymes concerned in starch metabolism. The rate of hydrolysis of amylose by Q-enzyme falls rapidly below 17° and above 26°. At 35°, the usual temperature for incubations of this kind, the enzyme exhibits less than 50% of its maximum activity.

One of the major difficulties encountered in the earlier work on Q-enzyme was the impossibility of keeping a stock of the enzyme. For each experiment, however small, it was necessary to prepare fresh enzyme and to check its purity immediately before use. Furthermore, the work was confined to a short period of the year, following the harvest, when the amylase content of potatoes was small. For this reason, we investigated the conditions of storage of Q-enzyme specimens prepared by the new method, and report that fraction Q1 may be stored for long periods with only a slight loss in activity.

There are obvious difficulties in the way of estimating Q-enzyme activity in terms of absolute units, and therefore to place these storage experiments on a quantitative basis, the Q-enzyme specimen being tested was stored under a variety of conditions and any change of activity which occurred was estimated by comparison with the effect of dilution with water of a solution of that particular sample of enzyme. As criterion of activity was taken the diminution of the A.V. (6800 A.) of amylose (B.V. 1·04; see Experimental) produced in unit time by the enzyme acting under optimum conditions of temperature and pH. The effect of storage of a solution of a Q1 fraction at room temperature and at 0° is shown in Fig. 4, from which it is seen that at room temperature only 50% of the activity remained after 3·4 days, whereas at 0° 6·2 days were required for the same loss of activity. A second experiment showed that the half life of the enzyme was about 15 and 4 minutes at 45·5° and 55·5°, respectively.

FIG. 4.

The stability of enzyme fraction Q1 in aqueous solution at room temperature and 0°.



It was clearly not feasible to store a Q1-fraction in the dissolved state, and attention was directed to the stability of the dry enzyme. The use of alcohol as a precipitant and drying agent was precluded by the fact that Q-enzyme is extensively inactivated by alcoholic precipitation, even at 0° (Bourne, Macey, and Peat, *J.*, 1945, 882). It was decided, therefore, to freeze-dry Q-enzyme at various stages during its isolation from potato juice by the lead acetate method. Potato juice, itself, was dried in the frozen state on several occasions with little, or no, loss of Q-enzyme activity, since it was possible to isolate from the products samples of the Q1 fraction as active as those normally obtained from juice which had not been freeze-dried. (The Q-enzyme content of potato juice cannot be measured directly because of the presence of amylose-precipitating fatty acids.) As a routine method for the storage of Q-enzyme, freeze-drying of potato juice has two disadvantages, first, that the removal of large volumes of water is time-consuming, and secondly, that the dry product is extremely hygroscopic. Each of these difficulties was eliminated when the lead–protein complex was freeze-dried, but, in this case, there was a loss of nearly two-thirds of the activity during the drying process.

The best method of storage was as the powder obtained when a frozen solution of a Q1 preparation, as distinct from the lead complex, was dried. On average, 70% of the enzyme remained active after drying. Samples prepared in this manner have been stored for long periods with little further loss in activity; one specimen, for example, retained 84% of its initial activity after 50 days at 0°.

There is an interesting difference in the behaviour of fractions Q1 and Q3 during freeze-drying. When a frozen solution of a Q3 fraction was dried, the activity decreased by more than 90%, whereas the loss incurred by fraction Q1 was less than 30%. It is probable that the inactivation was caused by an increase in acidity, resulting from the removal of ammonia from ammonium sulphate present in the enzyme solutions. In the case of a Q1 preparation, non-enzymic proteins and traces of sodium hydrogen carbonate serve as buffers and prevent a marked fall in pH, whereas the purer Q3 fraction is not adequately buffered in this way. Thus, when a small volume of boiled potato juice was added to the Q3 fraction before freeze-drying, the loss of activity was not

more than 20%. It may well be that there is a similar explanation of the destruction of P-enzyme during freeze-drying (*cf.* Weibull and Tiselius, *Arkiv Kemi, Min., Geol.*, 1945, **19**, *A*, No. 19). We intend to study the freeze-drying of both purified P-enzyme and Q-enzyme in the presence of strong buffers in order to elucidate these points.

The freeze-drying of Q1 fractions effects, unexpectedly, a further purification. Whereas a Q1 preparation normally contains about 10% of the P-enzyme initially present in the juice, the freeze-dried product contains only about 4%. This P-enzyme content is further reduced to below 1% by reprecipitation with neutral ammonium sulphate at 19 g./100 c.c. There is thus strong support for the belief, expressed by Bourne and Peat (*J.*, 1945, 877), that Q-enzyme, when free from P-Enzyme, has no action on glucose-1 phosphate.

As a result of these studies, we have prepared a stock of very active Q-enzyme (at the Q1 stage of purification) for further investigations concerning the synthesis of amylopectin and the conversion of amylose into amylopectin. Some of the results of these investigations are reported in the communication (Part V) which follows.

Experimental.

Analytical Methods.—(a) *Measurement of the iodine stains of polysaccharides.* The two expressions, Absorption Value (A.V.) and Blue Value (B.V.), employed in this paper to describe the intensities of the iodine stains of polysaccharides, were defined by Bourne, Haworth, Macey, and Peat (*J.*, 1948, 924). Whereas the B.V. is determined under standard conditions of concentration and at λ 6800 A. and is therefore characteristic of the polysaccharide in question, the A.V. is simply a convenient term used for the comparison of intensities made under any given set of conditions.

(b) *Determination of reducing sugar.* The copper reagent of Shaffer and Hartmann (*J. Biol. Chem.*, 1921, **45**, 377) was used for the determination of reducing sugars. For the determination of small quantities of sugar ($< 0{\cdot}2$ mg. of glucose and $< 0{\cdot}4$ mg. of maltose), a known weight of the appropriate sugar was introduced and the amount originally present was calculated by difference.

(c) *Determination of inorganic- and ester-phosphorus.* A colorimetric method (Allen, *Biochem. J.*, 1940, **34**, 858) was used for the determination of inorganic phosphorus (free P). The total phosphorus (free P + ester-P) was determined, after acid hydrolysis, by the same method and the ester-P was calculated by difference.

(d) *Determination of phosphorylase.* Phosphorylase activity was measured by the method of Green and Stumpf (*J. Biol. Chem.*, 1942, **142**, 355) and is expressed in the units defined by them.

Preparation of Starch Fractions.—The amylose and amylopectin used in this work were derived from potato starch by selective precipitation of the former with thymol, according to the standard procedure of Bourne, Donnison, Haworth, and Peat (*J.*, 1948, 1687).

Standard Method for the Isolation of Q-Enzyme.—All operations were performed at room-temperature.

(a) *Extraction of potato juice.* King Edward potatoes (1 kg.) were peeled, thinly sliced, and soaked for 20 minutes in water (1 l.), containing 0·5% of sodium hydrosulphite [dithionite]. The slices were drained, washed, minced, and pressed. The light-yellow juice was clarified by centrifuging and immediately subjected to the following fractionation procedure.

(b) *Preparation of lead acetate solution.* A solution (approx. 1 l.) of lead acetate (190 g.) was adjusted with N-sodium hydroxide to pH 7·25, and set aside for a few days. After removal of the precipitated material, the solution was diluted with water to 3 l.

(c) *Treatment with lead acetate and fractionation with ammonium sulphate.* To each 100 c.c. of juice, 0·02N-sodium hydroxide (40 c.c.) was added, with stirring; the pH of the solution was then 7·2. After the addition of water (30 c.c.), lead acetate solution (30 c.c.) was introduced and the yellow lead–protein precipitate was removed by the centrifuge, the supernatant liquid (pH 6·85) being discarded. The precipitate was stirred with 0·2N-sodium hydrogen carbonate (100 c.c.) for 5 minutes, and a stream of carbon dioxide was then passed through the suspension for 2½ minutes. The insoluble residue was removed by the centrifuge and discarded. To the supernatant liquid (*ca.* 110 c.c.) ammonium sulphate solution (pH 7; 50 g./100 c.c.) was added to give a final ammonium sulphate concentration of 19 g./100 c.c. The precipitate (fraction Q1) was collected and redissolved in water (20 c.c.). In some cases it was re-fractionated by a similar precipitation with ammonium sulphate, the product (fraction Q3) being redissolved in water (20 c.c.).

Standard Digest for the Hydrolysis of Amylose by Q-Enzyme.—Amylose (dry weight, *ca.* 220 mg.; B.V., 1·04) was moistened with ethanol (1 c.c.), and 0·5N-sodium hydroxide (12 c.c.) added. Dissolution of the amylose was completed by heating in a boiling water-bath for 3 minutes. The solution was cooled and diluted with water to 100 c.c. A portion containing 186·6 mg. of amylose was exactly neutralised to phenolphthalein with 0·5N-sulphuric acid and diluted to 100 c.c. This solution, which contained 14 mg. of amylose in 7·5 c.c., was incorporated in a digest, consisting of amylose solution (7·5 c.c.), maltose solution (1·5 c.c., equiv. to 3·0 mg. of maltose), M-acetate buffer (2·5 c.c.; pH 7·04), and enzyme solution (2·5 c.c.), making a total volume of 14 c.c. After a suitable period of incubation at 20·5°, an aliquot portion (4 c.c.) was submitted to the Shaffer–Hartmann determination of reducing sugar. Another portion (1 c.c.) was stained with iodine (2 mg.)–potassium iodide (20 mg.) in a final volume of 100 c.c., the A.V. being measured in the usual manner. A control digest, containing water (7·5 c.c.) instead of the amylose solution, was simultaneously incubated at 20·5°, and the reducing power was measured periodically. The difference between the reducing values of the two digests was a measure of the reducing groups liberated by the action of the enzyme on amylose and is expressed in terms of "apparent maltose".

Development of the Standard Method for the Isolation of Q-Enzyme.—(a) *Optimum conditions for precipitation of the lead complex.* Portions (100 c.c.) of the same sample of potato juice were adjusted to

varying pH values with 0·02N-sodium hydroxide and diluted with water so that, on addition of lead acetate solution (30 c.c., see above) of the same pH, the final volume was 200 c.c. The precipitated lead–protein complex was collected by the centrifuge, the Q-enzyme being subsequently eluted as described above and precipitated once with ammonium sulphate, as in the earlier method, except that the concentration of ammonium sulphate was 16 g./100 c.c. The enzyme was redissolved in water (20 c.c.) and tested for Q-enzyme activity by incubation with amylose.

To the supernatant liquid remaining after the removal of the lead–protein complex, ammonium sulphate solution (pH 7; 50 g./100 c.c.) was added to give a salt concentration of 16 g./100 c.c. The precipitate was stirred with water (20 c.c.) and centrifuged, and the clear solution tested for Q-enzyme activity.

pH of juice and lead acetate.		Enzyme fraction during lead acetate treatment.	Test for Q-enzyme (19 hrs.).	
Before mixing.	After mixing and centrifuging.		Fall (%) in A.V. (6800 A.).	Apparent conversion into maltose (%).
6·15	5·41	Precipitate	82·6	0
		Solution	5·8	1·4
6·76	6·09	Precipitate	86·6	2·4
		Solution	3·9	0
7·54	7·09	Precipitate	87·6	0
		Solution	20·2	1·0
8·49	8·48	Precipitate	76·0	1·0
		Solution	50·0	0

A graph of the above results revealed that the maximum amount of Q-enzyme was precipitated from potato juice by lead acetate when the two solutions had pH 7·25 before mixing.

(b) *Optimum conditions for elution of the lead complex.* Portions (100 c.c.) of the same sample of potato juice were treated with lead acetate by the standard method, and the precipitated lead–protein complexes eluted by stirring for 5 minutes at room temperature with various solutions (100 c.c.). In each case, after centrifuging, ammonium sulphate solution (pH 7·0; 50 g./100 c.c.) was added to the supernatant liquid to give a final concentration of ammonium sulphate of 18·3 g./100 c.c. Each precipitate was collected by centrifuging, stirred with water (20 c.c.), freed from insoluble material, and tested for Q-enzyme activity by incubation with amylose.

Solution used for elution (100 c.c.).	Fall (%) in A.V. (6800 A.) of amylose (30 mins.).
(1) $NaHCO_3$–CO_2 as in standard method	67·8
(2) Retreatment after (1)	26·0
(3) Water	1·0
(4) $NaHCO_3$–CO_2 after elution with water as in (3)	55·8
(5) $NaHCO_3$ as in standard method (no CO_2)	63·3
(6) 0·01N-NaOH	1·9

(c) *Optimum conditions for the precipitation of the Q1-enzyme fraction.* Portions (100 c.c.) of the same sample of potato juice were treated with lead acetate and then with sodium hydrogen carbonate–carbon dioxide, as in the standard method. To the eluate, varying proportions of ammonium sulphate solution (pH 7; 50 g./100 c.c.) were added, such that the final concentrations of ammonium sulphate were those recorded in the following table. Each enzyme precipitate was separated, redissolved in water (20 c.c.), and tested for Q-enzyme activity by incubation with amylose.

Concentration of ammonium sulphate (g./100 c.c.).	Fall (%) in A.V. (6800 A.) of amylose (30 mins.).	Units (Green & Stumpf) of P-enzyme from 100 c.c. of juice.
12·5	11·5	—
14·7	60·6	—
18·3	71·3	—
19·0	72·3	17·1
23·5	66·5	—
30·3	61·5	—
32·3	60·4	—
35·0	—	150

When the above results were plotted, it was clear that the most suitable concentration of ammonium sulphate for the precipitation of the Q1 fraction was 19 g./100 c.c. At this concentration only about 11% of the P-enzyme in the juice was precipitated with Q1.

Inhibition of Q-Enzyme by Lead Ions.—A sample of fraction Q1, prepared from potato juice by the standard method, was dissolved in water (20 c.c.). Portions of this solution were incubated with amylose, as in the standard estimation of Q-enzyme, with two variations: (*a*) the amylose solution was neutralised with acetic acid instead of sulphuric acid, and (*b*) lead acetate was incorporated in the digests in the concentrations shown in the following table.

Molarity of lead acetate in digest.	Fall (%) in A.V. (6800 A.) of amylose (30 mins.).
None	71·9
$1·49 \times 10^{-6}$	70·7
$2·98 \times 10^{-5}$	69·6
$1·98 \times 10^{-4}$	63·1
$5·95 \times 10^{-4}$	53·8

Optimum pH for the Action of the Q1-Enzyme Fraction on Amylose.—A sample of fraction Q1, prepared from potato juice (300 c.c.) by the standard method, was dissolved in water (60 c.c.). Portions (2·5 c.c.) of the solution were incubated with amylose under the conditions prescribed above, except that the acetate buffer was replaced by veronal buffers of different pH values; the latter buffers differed only in the amount of sodium hydroxide added. Since the veronal buffers were dilute, they did not retain their initial pH values when incorporated in the digests and it is, therefore, the pH values of the digests which are recorded in Fig. 2. The freedom, from amylases, of the Q1 fraction used was demonstrated by the fact that, at pH 7·04, which is near the optimum for α-amylase, the "apparent conversion into maltose" was only 0·5% when the fall in A.V. (6800 A.) was 78·5%.

Optimum Temperature for the Action of the Q1-Enzyme Fraction on Amylose.—A sample of fraction Q1, prepared from potato juice (300 c.c.) by the standard method and dissolved in water (60 c.c.), was incubated with amylose, as in the standard digest, except that (*a*) the acetate buffer was replaced by veronal buffer, such that the digests had pH 7·04, and (*b*) the temperature of incubation ranged from 11° to 35°. The results are recorded in Fig. 3. The Q1 specimen employed was entirely, or almost entirely, free from amylases, since in 300 minutes at 21° there was a fall of 75·2% in A.V. (6800 A.), whilst the "apparent conversion into maltose" was only 0·9%.

Stability of the Q1-Enzyme Fraction in Solution.—Samples of fraction Q1 prepared by the standard method from 100 c.c. of potato juice were dissolved in water (20 c.c.) and stored at different temperatures, namely, at 0°, room-temperature (*ca.* 17°), 35·5°, 45·5° and 55·5°. At intervals, a portion (2·5 c.c.) of each solution was incubated with amylose, under the conditions of the standard digest, and the Q-enzyme activity calculated by reference to curves showing the effect of dilution on the activity of the original Q1 solution. The rates of decay of the enzyme in solution at room temperature and at 0° are shown in Fig. 4. Each of the enzyme solutions employed was shown to be essentially free from α- and β-amylases by its ability to effect a fall of 75% in the A.V. (6800 A.) of the amylose, with less than 2% "apparent conversion into maltose."

Freeze-drying of Q-Enzyme Samples.—(a) *Potato juice.* When potato juice was frozen in an alcohol–carbon dioxide mixture and dried at 0·01 mm., very little, if any, loss of Q-enzyme activity occurred. This method of obtaining solid enzyme was unsuitable for routine purposes, because of the large volume of water to be removed in the drying process and the extremely hygroscopic nature of the product.

(b) *The lead–protein complex.* In four experiments, fraction Q1 was prepared from potato juice (100 c.c.) by the standard method, with the exception that the lead–protein complex was freeze-dried, as above, before the elution stage. Each Q1 specimen was dissolved in water (20 c.c.), and its activity determined by reference to the dilution curve (see above). The enzyme samples thus obtained had about 40% of the activity of an average sample of fraction Q1 prepared by the standard method without freeze-drying.

(c) *Fraction Q1.* Fraction Q1, isolated by the standard method from potato juice (100 c.c.), was dissolved in water (20 c.c.) and the solution divided into two equal portions, one of which served as a control. The other was freeze-dried, as above, and the product redissolved in water (10 c.c.). The Q-enzyme contents of the freeze-dried and control enzymes were compared by the method already given. The results obtained with two different samples of Q1 are shown below and are typical of 30 such experiments. Each of these enzyme preparations effected a fall of at least 80% in the A.V. (6800 A.) of amylose, with an "apparent conversion into maltose" of only 1%.

Q-Enzyme.	Treatment.	Fall (%) in A.V. (6800 A.) of amylose (30 mins.).	P-Units from 100 c.c. of juice.
First sample	Control	67·8	14·5
	Freeze-dried	64·7	5·9
Second sample	Control	72·6	—
	Freeze-dried	69·8	—

(d) *Fraction Q3.* The Q3 fraction isolated from potato juice (150 c.c.) was redissolved in water (30 c.c.), and the solution divided into three equal parts, one of which served as a control. The second was freeze-dried, as above, and the third freeze-dried in the presence of potato juice (5 c.c.), which had previously been boiled for 10 minutes and centrifuged. The two freeze-dried samples were redissolved in water (10 c.c.). The Q-enzyme activities of the three solutions were measured, as above. The Q3 fraction used in these experiments effected a fall of 80% in the A.V. (6800 A.) of amylose with no detectable increase in reducing power.

Treatment of enzyme solution.	Fall (%) in A.V. (6800 A.) of amylose (30 mins.).	P-Units from 100 c.c. of juice.
Control	70·4	2·6
Freeze-dried	2·8	—
Freeze-dried with boiled juice added	69·0	—

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