706. *The Enzymic Synthesis and Degradation of Starch. Part XI. Isophosphorylase.*

By J. M. Bailey and W. J. Whelan.

Isophosphorylase, isolated from the potato, and claimed by Bernfeld and Meutémédian to be responsible for the reversible formation of α-1 : 6-glycosidic linkages in amylopectin, has been prepared by the prescribed technique, and the key experiments of the above authors repeated. Although this repetition revealed an apparent agreement in some of the experimental data, we are forced to the conclusion that these experimental results have been misinterpreted by Bernfeld and Meutémédian and that an enzyme with the properties ascribed to "isophosphorylase" does not exist in the potato.

The work of Peat and his co-workers (*J.*, 1945, 877, 882; 1949, 1448, 1705, 1712; 1950, 84, 93) and of Bernfeld and Meutémédian (*Nature*, 1948, **162**, 297; *Helv. Chim. Acta*, 1948, **31**, 1724, 1735) has given rise to an unusual situation, namely, a demonstration of the existence in the potato of two very different enzymes, Q-enzyme and isophosphorylase, both of which appear to

catalyse the synthesis of the branch linkages in amylopectin. Isophosphorylase, as its name implies, functions by a mechanism of phosphate-transfer, whereas Q-enzyme operates independently of phosphate. In the synthesis of amylopectin from glucose-1 phosphate, each of these enzymes functions in association with the chain-forming enzyme, phosphorylase.

That Q-enzyme and isophosphorylase are not in fact the same enzyme was conclusively proved by Barker, Bourne, Wilkinson, and Peat (*J.*, 1950, 93) by the demonstration that Q-enzyme converted amylose into amylopectin without the mediation of free or ester phosphate. It thus became necessary to examine, in our laboratory, the isophosphorylases isolated from potato by an exact repetition of the extraction process described by Bernfeld and Meutémédian. The results of this examination are now recorded.

No difficulty was experienced in isolating the freeze-dried enzyme preparation by using the exact procedure prescribed by the Swiss authors. Two preparations were made, labelled A and B in Table I, and a study of their properties has forced us to the conclusion that an enzyme with the properties ascribed to isophosphorylase does not exist in the potato; the arguments put forward by Bernfeld and Meutémédian (referred to subsequently as B. and M.) are based on misinterpretations of three main experiments. These will be considered separately, and the results of our repetition of the experiments recorded in each case.

TABLE I.

Action of isophosphorylase on β-dextrin.

Isophosphorylase preparation.	Temp.	pH.	Time of incubation (hrs.).	Isophosphorylase added.*	Degradation of β-dextrin by β-amylase.† With added PO_4'''.	Degradation of β-dextrin by β-amylase.† Absence of PO_4'''.
B. & M.	20°	6·6	2	0 c.c.	0	0
			2	0·3 c.c.	4·5	0·7
			2	0·5 c.c.	7·5	1·2
Present authors (preparation A)	21·7	6·7	2	80 mg.	1·7	1·3
			20	80 mg.	4·4	4·0
Present authors (preparation B)	21·7	6·7	2	80 mg.	0	0
			5	80 mg.	0	0·3

* The weight of enzyme used by B. and M. is not stated.
† As % apparent conversion into maltose.

In the first experiment, limit β-dextrin was incubated simultaneously with β-amylase and isophosphorylase in the presence and in the absence of mineral phosphate. On the Swiss authors' hypothesis, isophosphorylase, in the presence of phosphate, severs the branch links in the β-dextrin and thus removes (as glucose-1 phosphate) the glucose " stubs " which, connected to the molecule by 1 : 6-links, obstruct the action of β-amylase. It should be noted that this hypothesis assumes amylopectin, from which β-dextrin is derived, to possess an irregular structure with multiple branchings. Thus the addition of phosphate to the system, isophosphorylase–β-amylase–β-dextrin, should enable β-amylolysis of the polysaccharide to take place. In the absence of mineral phosphate, no hydrolysis of β-dextrin should occur. In Table I are compared the results quoted by B. and M. and those derived from our repetition of the experiment.

The two sets of data do not agree and we feel ours to be the more reliable because scrutiny of the experimental section of B. and M.'s paper reveals that exceedingly small amounts of maltose were being measured; *e.g.*, percentage conversions into maltose of 0·7, 1·2, 4·5, and 7·5 (Table I) correspond to estimated weights of maltose of 0·030, 0·051, 0·190, and 0·316 mg., respectively. The reducing power was measured by using alkaline 3 : 5-dinitrosalicylate (Meyer, Noelting, and Bernfeld, *Helv. Chim. Acta*, 1948, **31**, 103). In the description of this method it is implied that amounts of maltose of less than 0·2 mg. cannot be detected. This is confirmed by our observations and by Chanda, Hirst, Jones, and Percival (*J.*, 1950, 1289). Consequently, unless the combined reducing powers of the digest components, *i.e.*, β-dextrin, β-amylase, and isophosphorylase, exceeded that of 0·2 mg. maltose, three of the four quoted values of reducing power fell below the limit of sensitivity of the reagent. The β-amylase was stated to be free from reducing substances but lack of similar information about the other components of the digests does not enable the accuracy of measurements of reducing power to be computed. We have used the Shaffer–Hartmann copper reagent which also fails to detect amounts of maltose less than 0·2 mg., but the precaution has been taken of adding to the reagent–digest mixture 0·25 mg. of maltose before commencing the determination.

B. and M.'s second experiment is concerned with the transformation of amylose into

amylopectin. Amylose was incubated with phosphorylase and isophosphorylase for 144 hours in the presence and in the absence of mineral phosphate. In the former case the iodine stain decreased in intensity and the percentage of *apparent* conversion into maltose of the polysaccharide by β-amylase fell from 100 to 65; in the latter case no changes occurred. The changes occurring in the presence of phosphate were interpreted as meaning that " amylose had been transformed into amylopectin " by a mechanism involving the removal, as glucose-1 phosphate, of the non-reducing terminal glucose member of an amylose chain (phosphorylase action), and the subsequent utilisation of the glucose-1 phosphate by isophosphorylase for the attachment of a glucose residue, by 1 : 6-linkage, as a lateral branch; phosphate ion being liberated simultaneously. This glucose unit formed the initial member of a branch chain which was then lengthened by the apposition of glucose units (from glucose-1 phosphate) by phosphorylase action. Multiplication of these exchange reactions should ultimately yield amylopectin (see diagram in *Helv. Chim. Acta*, 1948, **31**, 1730).

In formulating the above scheme it appears to have been overlooked that there is strong evidence to support the view that a single glucose unit attached laterally to an amylose molecule is a chain of insufficient length to act as a " primer " for phosphorylase action, a minimum of three glucose units being necessary. For example, β-dextrin, which contains such single-unit side chains, does not function as a primer (cf. Hestrin, *J. Biol. Chem.*, 1949, **179**, 943). Phosphorylase could not therefore build up a chain of glucose units on such a side chain In interpreting their experimental evidence, B. and M. appear also to neglect the fact that, under the given experimental conditions, phosphorylase will establish the normal equilibrium between free and esterified phosphate and, in the presence of mineral phosphate, an appreciable percentage of the amylose will be transformed into glucose-1 phosphate. This reaction, independently of any branching effected by an isophosphorylase, will result in a fall in the intensity of the iodine stain and in the degree of apparent conversion by β-amylase. From the amount of phosphate used, the given pH of the digest and the free-P : ester-P ratio measured by Hanes (*Proc. Roy. Soc.*, 1940, *B*, **129**, 174) it can be calculated that the amount of glucose-1 phosphate which must have been formed by phosphorylase action corresponds to the conversion of 28% of the amylose. Thus the normal action of phosphorylase on amylose would account for part of the observed diminution in iodine-staining power and nearly the whole of the diminution in the degree of β-amylolysis. Even if no branch linkages had been synthesised the apparent β-amylolysis limit would still have been 72% (initial value was 100%). As the experimentally determined value was 65%, the true change in the β-limit was from 100 to 90%. It must be concluded therefore that very few, if any, branch linkages have been synthesised.

In our repetition of this experiment, we have found that, when allowance is made for the formation of glucose-1 phosphate, the degree of β-amylolysis of the residual polysaccharide is identical with that of the original amylose; the value in each case was 92%. As an additional check, amylose was incubated with phosphorylase and phosphate but no isophosphorylase was added. The conversion into maltose of the residual polysaccharide by β-amylase at the end of the reaction was again 92%. In each experiment when no allowance was made for ester phosphate formation the apparent conversion into maltose was 68%.

The changes which take place in the nature and intensity of the iodine stain of amylose when treated with various combinations of phosphorylase and isophosphorylase in phosphate and acetate buffers have been examined and the results are given in Table II.

When the colours of the iodine-stained solutions were compared, it was seen that all digests except that containing P + IsoP + PO_4 remained blue-staining; the colour of the last became purple after 92 hours. The reason for this difference is not difficult to find. Both the phosphorylase and the isophosphorylase contained traces of α-amylase (cf. P + Ac, IsoP + Ac, Table II). When phosphorylase and phosphate were present a substantial proportion (26%) of the amylose was removed as glucose-1 phosphate. The α-amylase action would proceed independently of the presence of phosphate, and in the two digests P + IsoP + Ac and P + IsoP + PO_4 the red colour would become evident sooner in the latter than in the former digest, not because of isophosphorylase action, but because of the smaller amount of blue-staining amylose present. There is no evidence from changes in A.V. (680 mμ.), wave-length of peak absorption, or degree of β-amylolysis that isophosphorylase causes the formation of a red-staining ramified polysaccharide from amylose. Our *results* agree with B. and M.'s findings but our *interpretation* of them is different.

The third experiment of the Swiss authors is contained in the second of their two main communications (*Helv. Chim. Acta*, 1948, **31**, 1735). Phorphorylase, isophosphorylase, and glucose-1 phosphate were incubated in the presence of increasing quantities of phloridzin, and

the course of reaction was followed by observation of the iodine stains of the synthetic polysaccharides. It was found that as the phloridzin concentration was increased the iodine stain changed progressively from blue through purple and red to brown. Thus, in the absence of phloridzin the stain was blue, with $1{\cdot}2 \times 10^{-3}$M-phloridzin it was red-purple, and with $1{\cdot}6 \times 10^{-3}$M-phloridzin the stain was brown. No " primer " was added to the digests, the amount present as impurity being considered sufficient. The purpose of the phloridzin was to inhibit the phosphorylase without disturbing the amount of " primer." Parnas (" Handbuch der Enzymologie," Nord-Wiedenhagen, Leipzig, 1940, p. 202) is quoted as the source of the statement that phloridzin can cause total inhibition of phosphorylase. It has been overlooked that this statement refers to animal phosphorylase, not potato phosphorylase as used by B. and M. The highest concentrations of phloridzin cause only *ca.* 20% inhibition of potato phosphorylase (Green and Stumpf, *J. Biol. Chem.*, 1942, **142**, 355; Barker, Bourne, Wilkinson, and Peat, *J.*, 1950, 84). By the use of increasing concentrations of phloridzin the Swiss authors assumed that a progressive increase in the isophosphorylase : phosphorylase ratio could be achieved and thereby cause increases in the degree of ramification of the polysaccharide synthesised, this change being detected by the iodine stain. The experimental observations appeared to substantiate the hypothesis.

TABLE II.

Age of digest (hrs.).	Composition of digest: P + IsoP + Ac.	P + IsoP + PO_4.	P + Ac.	P + PO_4.	IsoP + Ac.	IsoP + PO_4.
	(a) Changes in A.V. (680 mμ).					
0·25	1·43	1·43	1·40	1·30	1·42	1·42
17	1·40	1·22	1·47	1·26	1·44	1·42
40	1·40	1·00	1·42	1·22	1·44	1·42
64	1·272	0·800	1·40	1·044	1·308	1·372
92	1·200	0·692	1·336	0·948	1·264	1·264
136	1·104	0·588	1·342	0·828	1·236	1·212
244 *	0·700	0·396	1·136	0·612	0·932	0·876
	(b) Changes in wave-length of peak absorption (mμ.).					
0·25	645	645	645	645	645	645
40	642·5	634	645	647	645	644
64	630	625	639	641	633	636
92	625	615	637	635	631	627
136	617	605	636	627	626	624
244 *	591	579·5	615	607	620	610

Key: P = Phosphorylase; IsoP = isophosphorylase; PO_4 = phosphate buffer; Ac = acetate buffer. pH of all digests = 6·7; Temp. = 21·7°.

* Between 136 and 244 hours some retrogradation occurred in all digests.

We have repeated this experiment and the results are given in Table III. " Primer " (achroic dextrin) was added to some digests because the amount naturally present was small. Moreover, two methods of iodine staining were used. In the first place, we employed B. and M.'s method (*loc. cit.*) in which enzyme reaction is arrested by adding sodium hydroxide solution followed by acetic acid and iodine. The final pH of such a solution (measured by us) is between 5 and 6. The second method, which we prefer, is that of Hassid and McCready (*J. Amer. Chem. Soc.*, 1943, **65**, 1154), in which iodine is added to the digest to inactivate the enzyme, and the pH is subsequently adjusted to <3 by the addition of acid.

It will be seen from Table III that a red iodine stain was observed in the isophosphorylase, phosphorylase, and phloridzin digest. On this basis, B. and M. assume that amylose had been converted into a red-staining polysaccharide. The table shows, however, that the red stain is observed only if action is arrested by the addition of alkali. When acid was used (second method of staining), the iodine stains varied from green to blue and appeared to have no red component. The following observations are pertinent and appear to us to prove that the red colour is an artefact due to the interaction of isophosphorylase and phloridzin. First, digests containing isophosphorylase and phloridzin become brown within 3 hours; all other digests remain colourless. Secondly, from all the digests which gave a colour with iodine a blue-staining precipitate settled after a few hours. The supernatant solutions were yellow except those containing both isophosphorylase and phloridzin, which were stained after alkali treatment; in these the supernatant liquids were pink. Thirdly, when isophosphorylase and phloridzin alone were heated together at the incubation temperature until the brown colour appeared (3 hours), and

the solution was added to iodine solutions buffered at pH values between 2 and 7, it was seen that the mixture was pink when the pH was greater than 5. At pH values lower than 5, no pink colour developed. Presumably, the brown product of interaction of isophosphorylase and phloridzin has indicator properties. Such a product did not form with phosphorylase and phloridzin. Thus the claims that this experiment demonstrated the synthesis of a branched polysaccharide are founded upon a misconception. When the digests are stained in acid solution there is no evidence of the formation of a red-staining polysaccharide.

TABLE III.

Iodine stains of synthetic polysaccharides.

	Addition of NaOH, AcOH, I_2, after digestion for :		Addition of H_2SO_4, I_2, after digestion for :	
Composition of digest.	3 hrs.	19 hrs.	3 hrs.	19 hrs.
P	Yellow	Blue-green	Yellow	Blue-green
P + IsoP	,,	,,	,,	,,
P + Phl	,,	Pale blue	,,	Pale blue-green
P + IsoP + Phl	Pale pink	Red-pink	,,	,,
S + P	Blue-green	Dark blue	Blue-green	Blue-green
S + P + IsoP	Pale blue-green	,,	Pale blue-green	,,
S + P + Phl	Pale grey-green	Blue	Pale green	,,
S + P + IsoP + Phl	Pale red-purple	Purple	,,	,,

Key: S = " Primer "; P = phosphorylase; IsoP = isophosphorylase; Phl = phloridzin ($1{\cdot}5 \times 10^{-3}$M.).

All digests contained glucose-1 phosphate in acetate buffer, pH 7·0. Temp. = 21·7°.

As a final proof of the non-existence of isophosphorylase we performed an experiment suggested by the postulated mechanism of action of this enzyme. If amylose is incubated with glucose-1 phosphate and isophosphorylase, the enzyme should attach single glucose units, by 1 : 6-links, to the amylose chains. These laterally attached units would obstruct the progress of the β-amylase along the chain. Amylose was incubated therefore with glucose-1 phosphate in the presence and also in the absence of isophosphorylase for 4·5 hours at 21°. At the end of this digestion period, the limits of conversion by soya-bean β-amylase of the resulting polysaccharides were almost the same, *viz.*, 91% (with isophosphorylase) and 87% (without isophosphorylase).

The conclusions to be drawn from this repetition of B. and M.'s work may be summarised as follows. Isophosphorylase has been prepared from potatoes by following exactly their directions (*loc. cit.*). Three key experiments have been repeated with this preparation, and in two of these our data correspond with those recorded by the Swiss workers. Because of this agreement it cannot be argued that we have been using an " inactive " specimen of the enzyme. We have shown that these experimental results were incorrectly interpreted by the above authors and do not provide evidence for the existence of an " isophosphorylase." All that the preparation appears to contain is a trace of α-amylase and, perhaps, a trace of phosphorylase. We were not able to reproduce the results of the third experiment (action of isophosphorylase on β-dextrin), but for reasons stated above we consider that the experiment as originally performed could not provide accurate and reliable data. Finally, an experiment devised by ourselves confirmed our view that amylopectin is not synthesised by an " isophosphorylytic " mechanism.

EXPERIMENTAL.

Analytical Methods.—(a) *Determination of phosphorus.* Allen's colorimetric method (*Biochem. J.*, 1940, **34**, 858) was used. Ester-phosphorus was determined by measurement of phosphorus before and after acid hydrolysis (Hanes, *Proc. Roy. Soc.*, 1940, *B*, **129**, 174).

(b) *Determination of reducing sugar.* Shaffer and Hartmann's copper reagent (*J. Biol. Chem.*, 1921, **45**, 377) was used, maltose solution (1 c.c., containing 0·25 mg. of maltose) being added to the digest–copper reagent mixture before it was heated. It was shown, as follows, that phosphate ion, in the concentrations being used, did not interfere with this reagent as it did with Somogyi's reagent (*J. Biol. Chem.*, 1945, **160**, 61). The reducing power of maltose (1 mg.) was determined in the presence of 0·2M-phosphate buffer (pH 6·7), 5 c.c. of reagent being used in a total volume of 10 c.c.

Phosphate buffer (c.c.).	Reduction equivalent (c.c. of 0·005N-$Na_2S_2O_3$) : Somogyi.	Shaffer–Hartmann.
0	4·00	3·39
1·0	4·16	3·50
2·0	2·60	3·50
3·5	0·13	3·42

(c) *Measurement of the iodine stains of polysaccharides.* The expressions " blue value " (B.V.) and " absorption value " (A.V.) are used as defined by Bourne, Haworth, Macey, and Peat (*J.*, 1948, 924) and the methods of determination described by these authors were used with two modifications. The measurements were made with the Unicam Quartz Spectrophotometer and the cell thickness was 1 cm. Accordingly, the values quoted in Table II(*a*) are four-fold multiples so as to be comparable with earlier measurements which were made with 4-cm. cells in a Spekker instrument.

Sources of Enzymes.—(a) *β-Amylase.* Two samples of β-amylase were used, as noted below. They were : (i) crystalline sweet potato β-amylase, kindly presented by Dr. A. K. Balls, and (ii) stock soya-bean β-amylase prepared as by Bourne, Macey, and Peat (*J.*, 1945, 882).

(b) *Phosphorylase.* Potato phosphorylase (P2 fraction) was prepared from King Edward potatoes by the method of Barker, Bourne, Wilkinson, and Peat (*J.*, 1950, 84). It was reprecipitated twice with ammonium sulphate at a concentration of 35 g./100 c.c. and freeze-dried in citrate buffer, pH 6·0, as described by the above authors.

(c) *Isophosphorylase.* Two samples (A and B) of isophosphorylase were prepared from Kerr's Pink potatoes by an exact repetition of B. and M.'s procedure (*Helv. Chim. Acta*, 1948, **31**, 1724). The yields of freeze-dried powder were, respectively, 3·6 g. and 3·2 g. per kg. of potatoes, and the phosphorus contents 0·09 and 0·05%.

Sources of Polysaccharides.—(a) *Amylose.* Potato starch was fractionated by using the aluminium hydroxide method of Bourne, Donnison, Peat, and Whelan (*J.*, 1949, 1) except that, after removal of the amylopectin, the amylose, instead of being precipitated from solution with alcohol as described, was first brought down as the thymol-complex by the method of Bourne, Donnison, Haworth, and Peat (*J.*, 1948, 1687).

(b) *β-Dextrin* (*syn.*, *dextrin-A*). This was prepared from potato amylopectin as in Part X (preceding paper).

Estimation of Reducing Power by Use of 3 : 5-*Dinitrosalicylic Acid.*—Amounts of maltose of 0·5, 1·0, and 2·0 mg. were treated with the above reagent by the method of Meyer, Noelting, and Bernfeld (*loc. cit.*). The optical densities ($D_{1\ cm.}^{500\ m\mu}$) of the solutions as measured with the Unicam spectrophotometer were, respectively, 0·166, 0·416, and 0·995. When these are plotted against maltose concentration the straight line joining the points intercepts the maltose axis at 0·21 mg.

Action of Isophosphorylase on β-Dextrin.—The following solutions were used : (*a*) β-dextrin (98·8 mg. in 100 c.c.), (*b*) β-amylase (2 drops of the suspension of crystalline enzyme in 70 c.c., having the equivalent of 40,000 units of activity, measured as in Part X), (*c*) phosphate buffer (0·2M.; pH 6·7), (*d*) acetate buffer (0·2M.; pH 6·7), (*e*) isophosphorylase (400 mg. in 5 c.c.). The following digests were prepared and incubated at 21·7° under toluene.

Digest no.	Solution (c.c.). (*a*).	(*b*).	(*c*).	(*d*).	(*e*).	Water.	Digest no.	Solution (c.c.). (*a*).	(*b*).	(*c*).	(*d*).	(*e*).	Water.
1	8	1	2	—	1	—	5	—	1	—	2	1	8
2	8	1	—	2	1	—	6	8	1	—	2	—	1
3	8	1	2	—	—	1	7	8	—	—	—	—	4
4	—	1	2	—	1	8							

The experiment was performed in duplicate, the isophosphorylase preparations A and B being used. At intervals (quoted in Table I) the copper-reducing powers of 4-c.c. portions were determined. From these results the extent of action of isophosphorylase was estimated by a method identical with that used by B. and M.

Action of Phosphorylase and Isophosphorylase on Amylose.—The following solutions were used : (*a*) amylose (B.V., 1·43; 250 mg. in 50 c.c.), (*b*) phosphorylase (99·5 mg. in 5·5 c.c.), (*c*) isophosphorylase (preparation B; 327 mg. in 5·5 c.c.), (*d*) phosphate buffer (0·2M.; pH 6·7), (*e*) acetate buffer (0·2M.; pH 6·7). Digests were prepared as follows, diluted to 25 c.c., and incubated at 21·7° under toluene.

Digest no.	Solution (c.c.). (*a*).	(*b*).	(*c*).	(*d*).	(*e*).	Digest no.	Solution (c.c.). (*a*).	(*b*).	(*c*).	(*d*).	(*e*).
1	5	1	1	—	5	5	5	—	1	1·5	3·5
2	5	1	1	1·5	3·5	6	5	—	1	—	—
3	5	1	—	—	5	7	—	1	—	1·5	3·5
4	5	1	—	1·5	3·5	8	—	—	1	1·5	3·5

At intervals (given in Table II) portions (1 c.c.) of digests 1—6 were removed and stained with iodine for the determination of A.V. (520—700 mμ.). After 240 hours the digests were heated at 100° for 10 minutes in closed flasks to destroy the enzymes. Some precipitation of amylose had occurred in all digests during this prolonged period. The precipitate, together with flocculated protein, was removed on the centrifuge. The polysaccharide remaining in digests 2 and 4 was then subjected to β-amylolysis, and the amounts of maltose liberated by soya-bean β-amylase (1300 units) when incubated with the digests (2-c.c. portions in a digest of 6 c.c.) at pH 4·8 and 35° were determined. Because of the retrogradation it was necessary to determine the amount of polysaccharide remaining in solution in digests 2 and 4 by acid hydrolysis. Portions of these digests (4 c.c.) were heated therefore with 5N-sulphuric acid acid (1·72 c.c.) for 2 hours at 100°, neutralised, and diluted to 10 c.c., and the liberated glucose was estimated. It was necessary to correct these values for (*a*) the reducing power of the non-carbohydrate constituents, (*b*) the glucose liberated from the glucose-1 phosphate also present in the digests, and (*c*)

the maltose liberated from the amylose by any amylase impurities in the phosphorylase and isophosphorylase preparations The maltose, although contributing to the apparent polysaccharide content of the digest, would not be attacked by β-amylase, and failure to allow for its presence would give a value for the degree of β-amylolysis which was too low. Corrections for these three factors were applied as follows: (*a*) the reducing powers of the enzyme preparations were determined in digests 7 and 8; (*b*) the ester phosphate was estimated as described above, and the polysaccharide equivalent calculated; and (*c*) the reducing powers of digests 2 and 4 were determined and a correction was applied on the assumption that the reducing power, after subtraction of the reducing power of the enzymes, was due to maltose. This correction for maltose was small and influenced the results by 4 and 2% respectively. The amounts of retrograded polysaccharide were, respectively, 32·5 and 12·5%, and in each digest 26·1% of the amylose had been converted into glucose-1 phosphate.

Polysaccharide Synthesis by Phosphorylase and Isophosphorylase in the Presence of Phloridzin.—The following solutions were used: (*a*) Glucose-1 phosphate, 379·5 mg. of dipotassium salt in 0·2M-acetate buffer (pH 7·0; 50 c.c.), (*b*) phosphorylase (221 mg. in 10 c.c. of water), (*c*) isophosphorylase (preparation B; 82·5 mg. in 4 c.c.), (*d*) "primer" (achroic dextrin, from acid hydrolysis of amylose; 100 mg. in 100 c.c.), (*e*) phloridzin (77·7 mg. in 50 c.c. = $3·29 \times 10^{-3}$M.). These solutions were mixed as shown in Table III, the following amounts (c.c.) being used: (*a*) 2, (*b*) 0·7, (*c*) 0·3, (*d*) 0·05, and (*e*) 2·6. All digests were diluted to 5·65 c.c. with water and incubated at 21·7°. The digests were stained after 3 and 19 hours by two methods: first, by the addition to portions (1·4 c.c.) of sodium hydroxide, acetic acid, and iodine as described by B. and M. (*Helv. Chim. Acta*, 1948, **31**, 1735) and, secondly, by the addition to digest portions (0·5 c.c.) of 2N-sulphuric acid (0·1 c.c.), water (2·2 c.c.), and iodine (0·1 c.c.; 0·2% in 20% potassium iodide solution).

Action of Isophosphorylase on Amylose and Glucose-1 Phosphate.—The following solutions were used: (*a*) amylose (B.V., 1·43; 218·0 mg. in 50 c.c.), (*b*) isophosphorylase (preparation B; 167·0 mg. in 2·5 c.c.), (*c*) glucose-1 phosphate (63·4 mg. in 2 c.c.), (*d*) acetate buffer (0·2M.; pH 7·0), (*e*) stock soya-bean β-amylase (5200 units in 2 c.c.). The following digests were prepared, diluted to 4·5 c.c., and incubated at 21·7° for 4·5 hours under toluene.

Digest no.	Solution (c.c.). (*a*).	(*b*).	(*c*).	(*d*).	Digest no.	Solution (c.c.). (*a*).	(*b*).	(*c*).	(*d*).	Digest no.	Solution (c.c.). (*a*).	(*b*).	(*c*).	(*d*).
1	2	1	0·5	1	2	2	0	0·5	1	3	0	1	0·5	1

The digests were next heated at 100° for 15 minutes in closed flasks to destroy the enzyme, then cooled, and the pH adjusted to 4·8 by addition of 0·1N-acetic acid. β-Amylase (0·5 c.c.) was added, and the solutions were diluted to 5 c.c. and incubated at 35°. Measurements of reducing power were made after 3·25 and 17·25 hours and the degrees of conversion of the polysaccharides into maltose were calculated.

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