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Starch.

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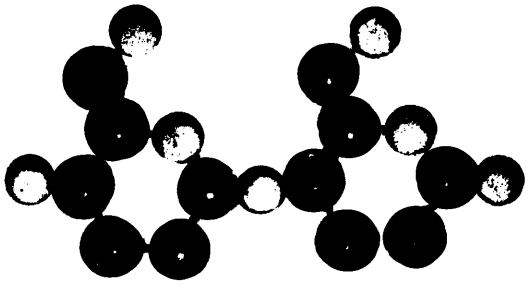
IT is a curious fact that some of the most notable advances in the chemistry of starch have been made during the stress of this and earlier wars: witness the discovery of the iodine coloration by de Claubry (1814); the isolation of glucose from starch in the same year by de Saussure, who also recognised maltose five years later; the detection of sugars in the products of acid hydrolysis in 1811 by the Russian chemist Kirchhoff and his discovery of diastase (β-amylase) about 1815; the separation of amylose and amylopectin in 1915 by Tanret and the purification of the former by adsorption on cellulose. These are all outstanding events in the history of starch. But in the present war we have witnessed the enzymic synthesis in the laboratory of both amylose and amylopectin, which I shall here describe, and the development of facile methods for their recognition and their isolation as two distinctive entities from starch.

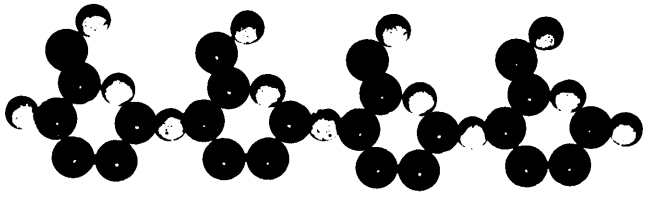
FIG. 1.



In my address today I shall review much of this later development and include in my survey some new researches which have not yet been published. It would perhaps be appropriate on an occasion of this kind to occupy the first few minutes of my address by referring to a few of the fundamental developments which have contributed to our knowledge of the constitution of starch.

We were able in 1927 in my School at Birmingham to establish that the disaccharide maltose, a product of the action of amylases on starch, had the constitution represented, in skeleton form, by Fig. 1, and that starch itself consisted of chains of glucose residues mutually linked, line ahead, by α-1 : 4-glucosidic bonds as shown in Fig. 2. Doubt had been thrown, without real reason, on the view that the maltose

FIG. 2.

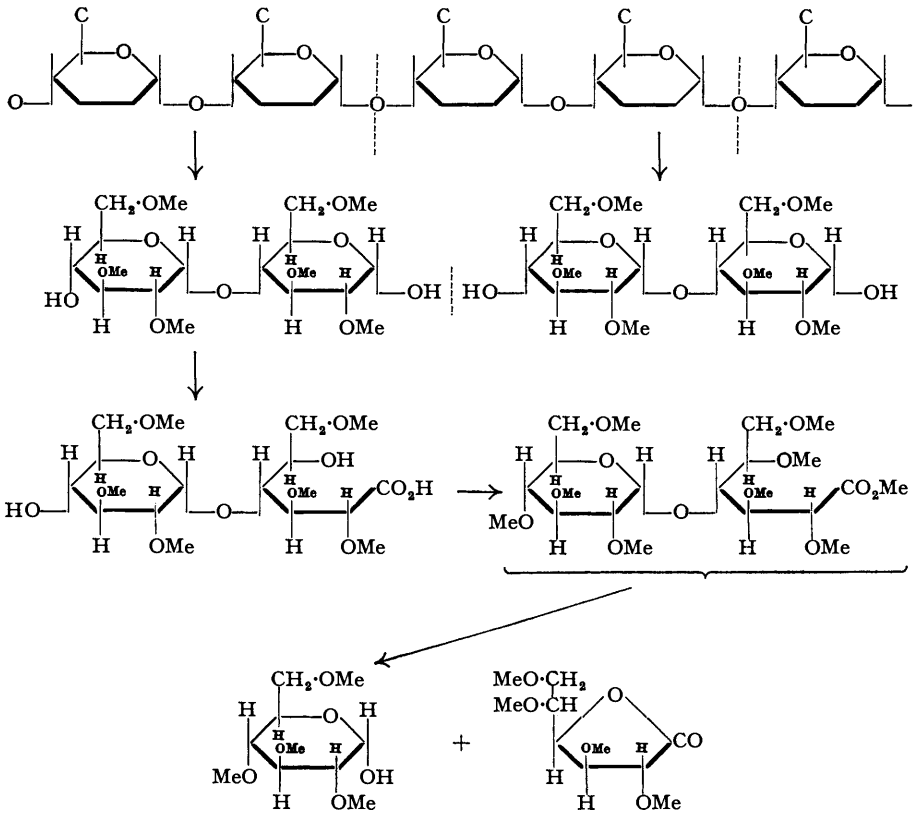


structure was represented in the constitution of starch, the suggestion being that the enzymes present during the breakdown were also capable of uniting two glucose members to give maltose. The implication that maltose units exist preformed in starch was given experimental confirmation in 1930 by Percival and myself (see Fig. 3). We were able to show that methylated starch undergoes scission by acetyl bromide at room temperature to yield products in which the 1 : 4-linkage of maltose survives.

It is of interest to contrast the structures of cellulose and of starch, which differ only in the configuration of the glucosidic bonds. These are α-glucosidic in starch and β-glucosidic in cellulose. I drew attention to this

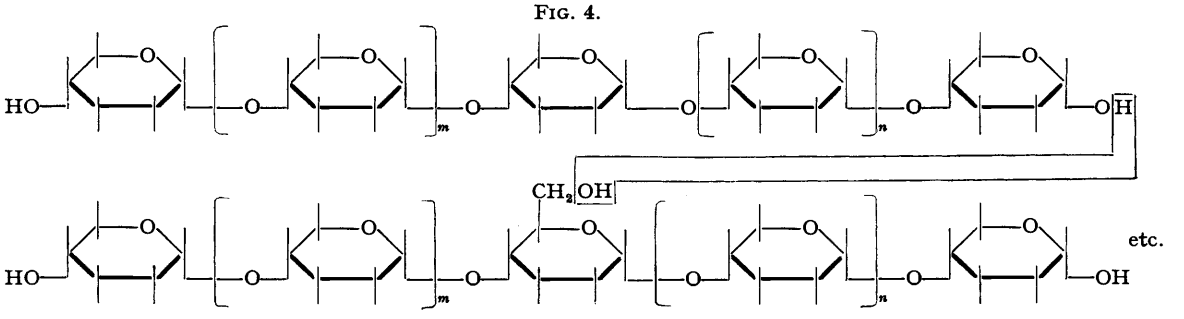
fact in my book in 1929, and indicated that while the β-linkage in cellulose would confer a rectilinear structure on the chains, the starch chains being α-linked would tend to assume a spiral conformation, and this idea has found remarkable confirmation in the views which will be developed later in this address.

FIG. 3.



The method of end-group assay which I developed in the first instance for cellulose and applied to starch in 1932 appeared to show that starch was constituted of short chains of some 24—30 glucose units. As some misapprehension has arisen I wish to repeat here that we have never regarded this short chain as constituting the whole physical molecule of starch; on the contrary, I have always been at some pains to make clear that this is a minimum chain-length and that starches may differ in the sense that this short chain unit may be repeated to a greater or lesser extent in the whole starch complex, some starches having a very large number (say 200) of this repeating unit in their composition and others a smaller number (say 10). My colleague Hirst,

FIG. 4.



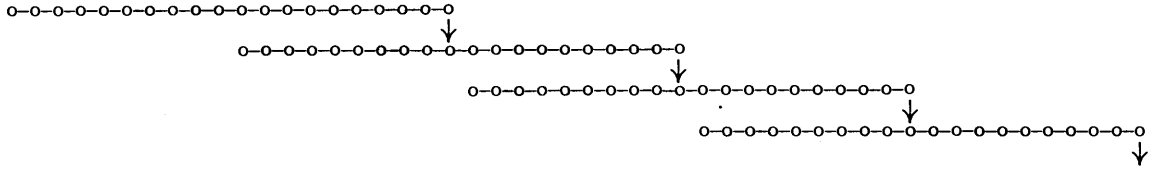
and I, expressed the view in 1937 that this mutual combination of chains may be effected through a primary valency linkage joining the reducing group of one chain and a hydroxyl group (probably that on C_6 of a glucose residue) of a second (Fig. 4).

For convenience, we will refer to this as the "laminated" formulation which is given diagrammatical form in Fig. 5, where the circles express the unit chain of 20 glucose units and the arrow heads show the mode of linking with similar adjoining units. The laminated formula gives expression to the well-known fact that the

molecular weights of starches are many times greater than that of the unit-chain. Direct evidence that the cross-link is indeed a 1 : 6-glucosidic bond and that it probably has the α-configuration was provided subsequently by the work of Hirst, of Freudenberg, and also of Myrbäck who found evidence of an *iso*gentiobiose (Fischer's *iso*maltose) linking in a trisaccharide derived from the enzymic breakdown of starch.

FIG. 5.

Starch.



Up to recent years the balance of evidence appeared to favour the view that native starch, whatever its source, was essentially homogeneous in a chemical, but not a physical, sense. From the days of Maquenne's classical work at the beginning of the century it has been recognised that starch contains at least two components which differ markedly in their physical properties and in respect of the action of amylases upon them; very many methods for effecting the separation of these components, which were named amylose and amylopectin by Maquenne, have been proposed, but all of these methods are not equally efficacious. It is indeed due to the inadequacy of many of the procedures which were described that the recognition of amylose and amylopectin as distinct chemical species has been so long delayed.

It is interesting to find that only three of the older methods proposed do in fact achieve a separation of two chemically distinct entities from starch and that two of these methods originated with Tanret in 1915. One of these, the leaching out of the amylose component with hot water, was greatly improved and developed by M. E. Baldwin in 1930 and applied in 1940 by K. H. Meyer, who obtained in this way a fairly pure amylose, albeit in poor yield, from corn and potato starches. This amylose was shown by Meyer to be constituted not of branched but of long unbranched chains, as we have likewise found to be the case in the synthetic polysaccharide of Hanes.

A better method of separation was provided by Schoch in 1941. This method consists essentially in the heating of a starch paste in an autoclave at 120° with butyl or amyl alcohol whereby the amylose component is precipitated; the amylopectin is separated from the mother liquor by the addition of ethyl alcohol. The weight yield of amylose in several runs was 25% of corn starch. A comparison of the properties of amylose and amylopectin is shown in the table below.

Comparison of Potato Amylose and Amylopectin.

	Amylose.	*Amylopectin.*
Iodine colouration.	Intense blue.	Red-brown.
Blue value (Spekker absorptiometer).	1·35	0·05
β-Amylase action.	Completely converted to maltose.	50% conversion to maltose. Residue of limit-dextrin-A.
Solubility in water.	Sparingly soluble.	Very soluble.
Solubility in ethylenediamine hydrate.	Readily soluble.	Sparingly soluble.
Stability in aqueous solutions.	Undergoes spontaneous precipitation (retrogradation).	Stable indefinitely (no retrogradation).
Intrinsic viscosity in ethylenediamine solution (c in g., %).	1·15 (from arrowroot starch)	0·4 (from arrowroot starch)
End-group assay.	100 or more glucose members.	Repeating unit chains each of about 20 glucose members.
X-Ray diagram.	V-pattern (for alcohol-precipitated amylose).	Amorphous.
	Amylose acetate.	*Amylopectin acetate.*
Form.	Fibrous; forms tough films and fibres.	Amorphous powder; forms brittle films; cannot be spun.
Viscosity (in chloroform): Intrinsic viscosity (c in g., %)	2·45	1·08
$\eta_{sp.}/c$	Increases with concentration.	Independent of concentration.
Molecular size.	Comparatively low.	Very high.

Of these properties, the most easily recognisable are the colours given by iodine and the relative completeness of hydrolysis by β-amylase.

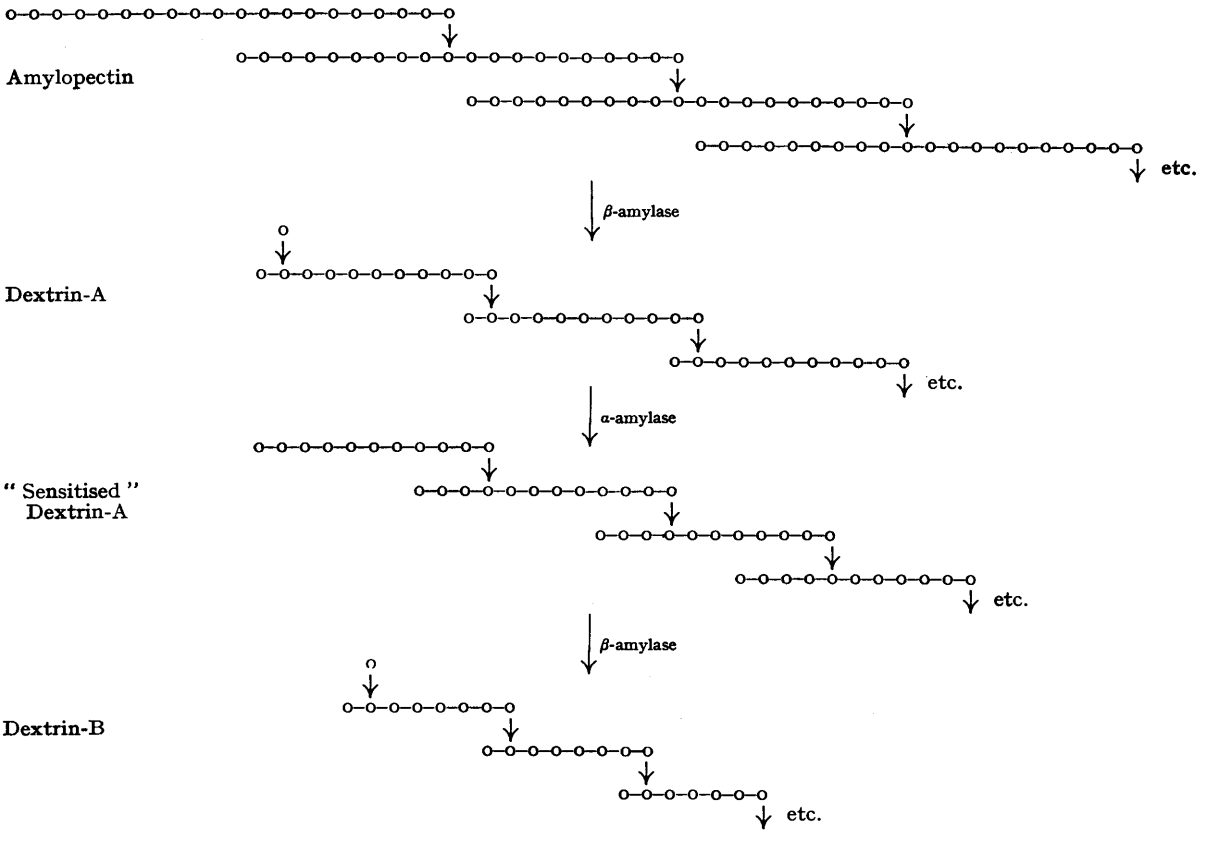
We have been able very recently to develop a new method of separation which yields relatively pure specimens of amylose and particularly of amylopectin from potato starch. This consists, briefly, in saturating a starch paste with thymol at 20—30° while keeping the solution at room temperature for 2—3 days; thymol is very sparingly soluble so that very little is needed. The amylose is thus precipitated and the remaining amylopectin still contains a little amylose which can be removed by saturating an aqueous solution of the amylopectin with *cyclo*hexanol, thus throwing out the residual amylose together with some of the amylopectin.

The amylopectin remaining in solution gives a reddish colour with iodine, whereas amylose gives a pure blue. Other methods of separation are under investigation and with one of these we seem to have achieved the isolation of two very pure components. The intensity of the blue colour given to amylose by iodine is here made the basis for the quantitative analysis of mixtures of amylose and amylopectin. The colour intensity is measured by the method of Hassid and McCready using a Spekker absorptiometer.

The difference in the action of β-amylase on the two components of starch is very marked. Amylose, constituting 20—25% of starch, is converted completely to maltose, whereas the amylopectin is converted to maltose to the extent of 50% only, the residue being a red-staining dextrin (dextrin-A) which resists the further action of β-amylase. We have postulated as the cause of this resistance the presence of the α-linking shown by

FIG. 6.

Degradation of starch by amylases.



the arrow in the formulation of amylopectin in Fig. 6, and the position of this linking may be determined by the extent to which maltose members are released from the left-hand side of the chains shown in this formula for amylopectin. In earlier work (Haworth, Kitchen, and Peat) we have shown that this resistance to β-amylase is overcome in dextrin-A by momentary contact with α-amylase. This may be due to the severance of this α-linking by the α-amylase and the recombination, possibly owing to the presence of a Q-factor in the α-amylase, of the shortened unit chains by the reconstruction of this α-linking in another position. Certainly it is the case that the " sensitised " dextrin-A is now capable of further attack by β-amylase to give maltose and a new dextrin-B as indicated by the lower formulation in Fig. 6.

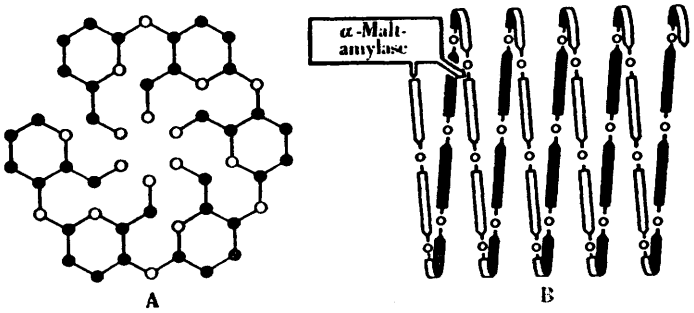
Natural amylose and amylopectin are chemically different in the sense that amylose is composed of largely unbranched chains of 100 or more glucose members whereas amylopectin is given a laminated or branched structure composed of unit chains of about 20 glucose residues.

Amylose has been obtained in crystalline form. This was achieved by Kerr and Severson who used for its separation a combination of the leaching out method of Tanret and the method of Schoch. *X*-Ray examination of this crystalline amylose by Rundle led him to support a helical structure for amylose and for the amylose–iodine complex. The molecular structure suggested is shown in Fig. 7, and is here reproduced from the paper by C. S. Hanes, together with his subscript, in " The New Phytologist " (**1937, 36, 101**).

Each turn of the spiral is made up of six glucose residues and a molecule of iodine is neatly accommodated in each turn, this forming the intensely coloured amylose–iodine complex. This helix theory is, of course, not

new. I had proposed it on geometrical grounds in 1929 and later Hanes adopted the idea to explain the mode of action of α-amylase (see Fig. 7). It is perhaps worthy of remark here that an amylose solution, when free from amylopectin, is colloidally unstable, and that amylose is closely related in properties to cellulose. In particular, amylose acetate, which is fibrous in appearance and indistinguishable by eye from cellulose acetate, forms tough clear films which, when formed under tension, give *X*-ray fibre diagrams of the cellulose type (Whistler). It is possible that in these stretched films the amylose acetate is in an extended, and not in a helical, form.

FIG. 7.

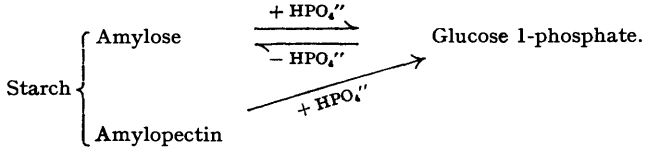


A, *hexagonal arrangement of 6α-linked glucopyranose units (Sachse conformation) modified after Haworth* (1929). B, *hypothetical spiral model of α-linked chain of 30 glucose units, showing possible mode of combination with α-malt-amylase.*

We now come to the important question of the synthesis of starch in the plant. The illuminating researches of C. S. Hanes at the Low Temperature Research Station, Cambridge, ran parallel with the investigations of Cori on the synthesis of glycogen in muscle and it was found that the enzymes concerned in each case used the same substrate, namely glucose 1-phosphate. In 1940, Hanes announced the synthesis *in vitro* of a starch-like polysaccharide from glucose 1-phosphate by the agency of an enzyme, phosphorylase, present in the pea and the potato. In collaboration with Dr. Hanes, we examined this synthetic product and confirmed his impression that it was not identical with natural starch but was rather the amylose component of starch. In other words, phosphorylase effected the synthesis of long, largely unbranched chains of glucose units according to the scheme illustrated in Fig. 8, which we have modified from Hanes's original scheme to be more in keeping with the latest advances.

FIG. 8.

Breakdown of starch and synthesis of amylose by phosphorylase.
(*C. S. Hanes.*)



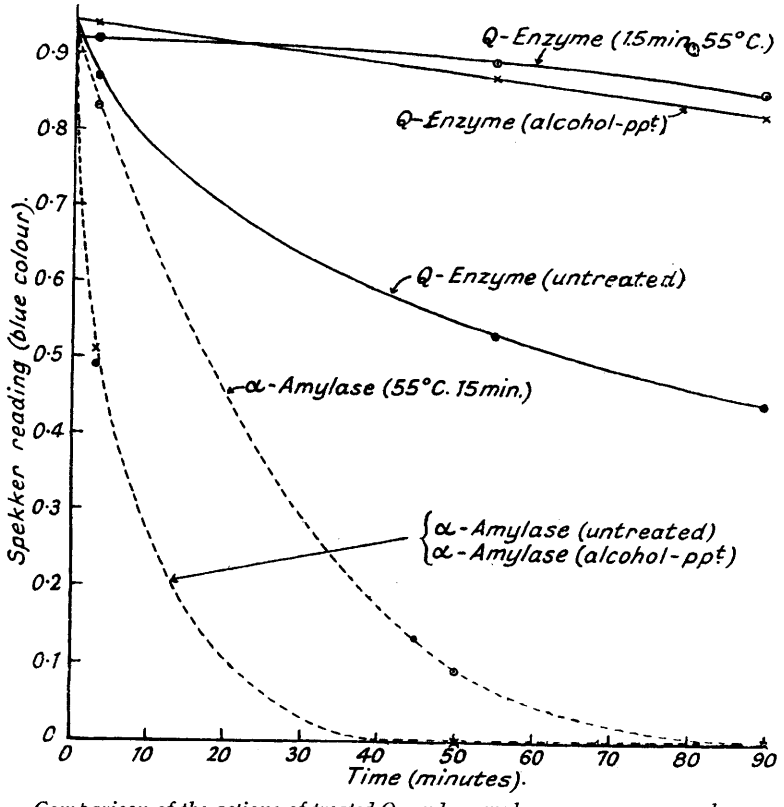
We could not fail to note that only one component of starch, and moreover only that occurring in the smaller proportion, was synthesised by phosphorylase. By what agency was amylopectin, representing about three-fourths of the starch, synthesised in the plant? Clearly phosphorylase represented only a part of the enzyme system concerned with the metabolism of starch, and my colleagues Bourne and Peat therefore made a search for some other factor or factors involved in starch synthesis. They succeeded ultimately in demonstrating the presence of another such agent in potato juice. They established that this new factor was an enzyme, since its activity was destroyed when it was heated to 100°, and I refer to it as the Q-enzyme to distinguish it from the phosphorylase (P-enzyme) with which it is associated. The P-enzyme has now been isolated as a dry powder, apparently stable; the Q-enzyme has been separated from it, but has not yet been obtained in such a state of purity that one can be sure of the complete absence of P-enzyme from the preparation.

With this reservation in mind, I will outline the properties and probable functions of this new factor. The Q-enzyme *per se* appears to have no synthetic function with respect to glucose 1-phosphate. It acts upon starch, however, very definitely as a hydrolytic enzyme, in other words as an amylase. We have satisfied ourselves, however, that it is an amylase of a special type, hitherto unrecognised, and cannot be classified simply as an α- or a β-amylase. The β-amylase of soya bean acting on an amylose separated by thymol produces the immediate liberation of maltose and this reaction proceeds smoothly until the whole of the amylose has been converted into maltose. This observation is in conformity with the view, held generally, that β-amylase makes an endwise attack on the amylose chain and reduces its length by two glucose units at a time. During the course of the reaction the blue colour given by the amylose with iodine becomes less and less intense until ultimately no colour is given. The action of Q-enzyme on amylose is very different. The colour with iodine

slowly changes from blue to a red which thereafter persists, *i.e.*, an achroic point is never reached. The most striking difference, however, lies in the fact that with many preparations of Q-enzyme no reducing power develops during the whole of the time of reaction with amylose; hydrolysis by Q-enzyme evidently does not involve the liberation of a simple sugar. With some preparations of Q-enzyme we have found that sugar production occurs in the later stages of the reaction, but the amount is always very much less than that liberated by an equivalent concentration of α- or β-amylase at the same stage in the reaction. We attribute this occasional production of sugar by Q-enzyme to the presence in the preparations of the latter of an α- or β-amylase. It is known that the potato often contains amylase and we have found this to be especially true of potatoes which are sprouting.

The curves of hydrolysis with Q-enzyme are nearer in type to those of α-amylase than to those of β-amylase. Nevertheless, Bourne and Peat have been able to show that Q-enzyme is not necessarily identical with α-amylase inasmuch as Q-enzyme is inactivated by either heating for a short time at 55° or precipitation from aqueous solution with alcohol. The activity of the α-amylase of saliva is practically unaffected by either of these

FIG. 9.



Comparison of the actions of treated Q- and α-amylase enzymes on amylose.

treatments (Fig. 9). It is to be observed that at this stage no means is available for a comparison of the stability of the enzymes at identical concentrations, since their absolute activities cannot be determined.

I have said that Q-enzyme is not to be regarded as an enzyme capable in itself of synthesising starch from glucose or maltose or glucose 1-phosphate, but, given a suitable substrate, it immediately displays synthetic powers. That substrate is provided through the agency of P-enzyme acting on glucose 1-phosphate, and most probably also by the hydrolytic action of Q-enzyme itself acting upon amylose (and possibly upon amylopectin). We put forward this latter hypothesis, tentatively and with caution, as being the only one which covers the observations that have so far been made. As a working hypothesis, then, we picture the biological synthesis and degradation of whole starch as proceeding according to the scheme shown in Figs. 10 and 11.

This conception of the course of starch metabolism in the potato postulates the formation of an intermediate polysaccharide of unique constitution. This is described in Fig. 10 as " 20 unit-chain (unbranched) " and is to be pictured as composed of single unbranched chains of about 20 glucose units mutually linked by 1 : 4-α-glucosidic bonds. This intermediate product (pseudo-amylose) may be derived (i) from glucose 1-phosphate by the agency of P-enzyme; (ii) from amylose by the hydrolytic action of Q-enzyme; and possibly (iii) from amylopectin in the presence of Q-enzyme. We regard this pseudo-amylose as the only substrate on which Q-enzyme can act synthetically, whereby 1 : 6-cross-linkages are established and amylopectin is formed. The suggestion

is thus made that, when pure Q-enzyme acts on amylose, this long unbranched chain compound is split up into shorter chains (pseudo-amylose) which are then combined laterally with the production of amylopectin. The hypothesis that amylose is converted by Q-enzyme into amylopectin still awaits experimental confirmation.

The conversion of glucose 1-phosphate into amylose by the agency of purified P-enzyme, accomplished in the first place by Hanes, is now to be regarded as taking place through the intermediate formation of pseudo-amylose. In the absence of Q-enzyme, the normal synthetic activity of the P-enzyme does not cease with the production of 20-unit chains but continues until the long, largely unbranched chains of amylose itself are built up. If, however, the P- and Q-enzymes are functioning together in a suitable ratio, the P-enzyme can carry the synthesis only to the stage of pseudo-amylose which thereafter becomes the substrate for Q-enzyme and is converted into amylopectin.

Bourne and Peat have isolated the polysaccharide produced by the completed action of a mixture of P- and Q-enzymes on glucose 1-phosphate; they have shown that it has the characteristics of an amylopectin, having

FIG. 10.

Suggested scheme for synthesis and breakdown of amylopectin and amylose by P- and Q-enzymes of potato.

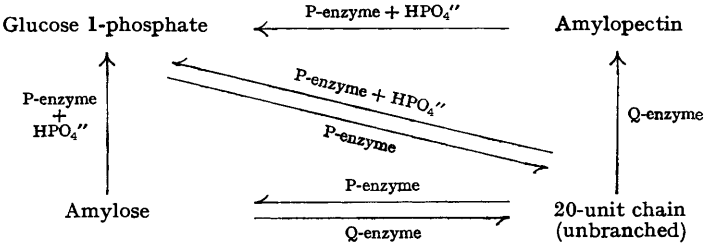
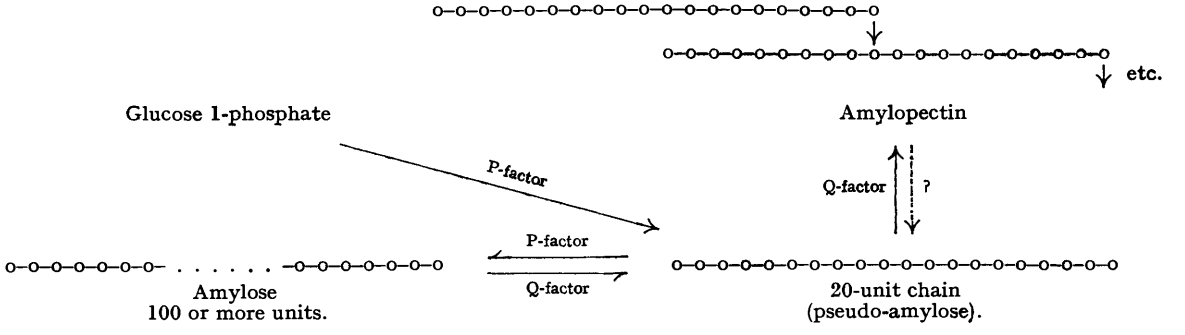


FIG. 11.

Synthesis of amylopectin and amylose.



a branched structure, and by end-group assay have estimated that the repeating chain unit contains an average of 20 glucose members. Incidentally, it is for this reason that we describe pseudo-amylose as being constituted of 20-unit chains.

Fig. 11 also illustrates the katabolic activity of P- and Q-enzymes. Hanes has shown that whole starch is convertible into glucose 1-phosphate by phosphorylase so it must be assumed that the amylose and amylopectin components are both suitable substrates for this enzyme in the presence of phosphate ions. Q-Enzyme, on the other hand, appears to have no phosphorylytic activity. It may be that its hydrolytic activity is confined to amylose as substrate, but it may later appear that amylopectin also forms a suitable substrate for Q-conversion into pseudo-amylose. It would indeed be somewhat remarkable if the synthesis of 1 : 6-links by Q-enzyme were not a reversible reaction.

Much remains to be done. Our hypothesis of the mechanism of starch metabolism is only speculative at present, not because it cannot accommodate all the known facts but because all the knowledge required to substantiate it is not yet available.