345. *Thymol and* cyclo*Hexanol as Fractionating Agents for Starch.*

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The separation of starch into its components, amylose and amylopectin, is now carried out by a standardised procedure herein described. The method involves the use of thymol as an amylose-precipitating agent, the amylopectin remaining in solution.

*cyclo*Hexanol is recommended as a fractionating agent in place of thymol when the remaining starch component, namely amylopectin, is desired in a purer condition. *cyclo*Hexanol–amylose contains a higher proportion of amylopectin than does the thymol–amylose.

It is shown that the *cyclo*hexanol–amylopectin fractions of very low blue value, reported by Haworth, Peat, and Sagrott (*Nature*, 1946, **157**, 19), are not normal constituents of starch but are hydrolytic degradation products of amylopectin. When *cyclo*hexanol–amylopectin is submitted for a short period to the action of saliva or of mineral acid, a precipitate forms in the presence of excess of *cyclo*hexanol which has the character of amylose. The amylose–*cyclo*hexanol complex is not itself susceptible to hydrolysis by either the enzyme or the acid used.

The branched and unbranched components of starch have been separated in a number of ways which have been summarised recently by T. Schoch (" Advances in Carbohydrate Chemistry ", Vol. 1, 1945). Haworth, Peat, and Sagrott (*Nature*, 1946, **157**, 19) briefly described the effect of a number of hydroxylic conpounds which functioned as precipitants of the amylose component. Among these precipitants thymol and *cyclo*hexanol appeared to be particularly suitable as agents for the fractionation of starch. It is the purpose of this communication to give a fuller account of the experience gained during the past three years in the use of these methods by a group of workers in these laboratories who have been engaged in the study of various aspects of the chemistry and physiology of starch.

The Thymol Method.—The standard procedure now used by us is described in detail in the experimental section. It is essential that the starch be dispersed as completely as possible in boiling water to which a salt (0·1% NaCl) is usually added, and that any undispersed material be removed before the addition of thymol. The concentration of starch should not exceed 3% in the presence of ions or 1% in their absence. The precipitation of the amylose–thymol complex begins a few hours after the addition of thymol (to saturation) and is usually complete in 48 hours. It is advisable, however, to allow 60 hours to elapse before the precipitate is separated from the amylopectin-containing mother liquor. The amylose–thymol complex is obtained in a more readily separable form if it is precipitated at a slightly elevated temperature (30°). The amylose precipitate is best freed from residual amylopectin solution by being repeatedly washed with water saturated with thymol.

The optimum conditions prescribed above were determined by a systematic variation of the following factors : the initial concentration of the starch ; the viscosity and the pH of the starch

dispersion; the temperature at which the precipitation of the amylose–thymol complex is allowed to proceed; and the time elapsing between the addition of the thymol and the removal of the precipitate. The most important factor appears to be the physical state of the initial dispersion of the starch in water, and this cannot be always rigidly controlled. A further complication is due to the colloidal instability of the unbranched amylose component which is often precipitated spontaneously as an unmanageable gel, enclosing within its substance a relatively large volume of an aqueous solution of the amylopectin component. The larger the scale on which the separation is carried out the greater is the tendency of the partly purified amylose to form such a gel. This tendency appears to be largely conditioned by the degree of dispersion of the original starch paste which in turn depends upon the concentration of the starch and the duration and efficiency of the stirring of the boiling paste.

Some representative results of the separation of the components of potato starch by the use of thymol are shown in Table I. The blue value (B.V.) of the iodine complex and the extent of

TABLE I.

Specimen results by the standard thymol method.

Separation No.	Variations from standard method.	Amylose fraction: yield (%).	B.V.	Amylopectin fraction, B.V.
I	None	23·0	1·15	0·220
II	,,	18·4	1·17	—
III	,,	18·9	1·21	0·230
IV	,,	20·5	1·16	0·211
V	Centrifuging (25,000 r.p.m.) omitted	—	1·12	—
VI	,, ,, ,,	20·0	1·11	—
VII	Precipitation at room temperature (5—20°)	21·0	1·05	0·250
VIII	,, ,, ,, ,,	15·3	1·06	0·168
IX	,, ,, ,, ,,	25·2	1·01	—
X	Washing with thymol-water omitted	18·6	1·03	—
XI	,, ,, ,,	27·7	1·01	—
XII	,, ,, ,,	20·1	0·95	—
XIII	,, ,, ,,	14·2	0·83	—
XIV	Sodium chloride omitted	No separation		

hydrolysis effected by β-amylase are taken as criteria of the degree of separation of the amylose from the amylopectin component. Average specimens of " thymol "–amylose and –amylopectin from potato starch show blue values of 1·0—1·2 and 0·21—0·23 and are hydrolysed by β-amylase to the extent of 96—99% and 47—62% respectively.

cyclo*Hexanol as an Amylose Precipitant.*—The fractionation of potato starch by precipitation of the amylose–*cyclo*hexanol complex yields fractions which are slightly different in composition from those usually obtained by the thymol method. Without at this stage discussing the question of the chemical homogeneity of the components of starch, it is obvious that none of the methods employed yields a " pure " amylose or a " pure " amylopectin, but rather that a given reagent (*e.g.*, thymol) separates starch into two fractions each of which may be regarded provisionally as a mixture of an amylose which, by definition, consists entirely of long glucose chains without cross linkages, and an amylopectin in which no long unbranched chains are present. To distinguish the fractions actually obtained in a given separation from these hypothetical entities, we have adopted the system of incorporating the name of the fractionating agent in the description of the fractions (*e.g.*, thymol–amylose, *cyclo*hexanol–amylopectin, etc.) whenever clarity demands such a distinction.

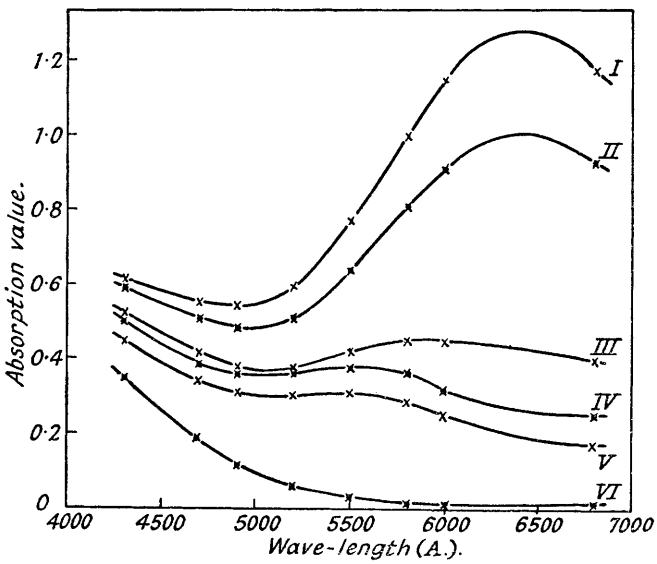
The blue values of average specimens of thymol–amylose and *cyclo*hexanol–amylose (each from potato starch) are 1·1 and 0·9 respectively, the corresponding amylopectins showing B.V., 0·22 and 0·18. For a compariosn of two methods of fractionation it is legitimate to assume that the higher the B.V. of a starch-component the higher the amylose- and the lower the amylopectin-content. On this basis, it is evident that *cyclo*hexanol yields a purer amylopectin than does thymol, whereas thymol gives an amylose containing less of the branched-chain constituent than occurs in *cyclo*hexanol–amylose.

If the absorption values (A.V.) of a standard solution of polysaccharide and iodine are measured using a range of Ilford filters it is possible to plot A.V. against wave-lengths of peak transmission of the filters. It will be convenient to describe such a graph as an absorption curve. The components of starch can be distinguished by the forms of their absorption curves. This is illustrated in the figure, which also shows that the absorption by thymol–amylose is greater at all wave-lengths than that by *cyclo*hexanol–amylose.

Haworth, Peat, and Sagrott (*loc. cit.*) occasionally obtained specimens of *cyclo*hexanol–amylopectin with very low blue values (*ca.* 0·02). Such specimens could never be produced at will, and subsequent work demonstrated that they were not normal constituents of starch but were formed, possibly because of contamination by traces of saliva, by hydrolysis of the true *cyclo*hexanol–amylopectin (B.V., 0·18).

In the course of experiments designed to test the action of salivary amylase in high dilution on amylopectin in the presence of *cyclo*hexanol there was invariably observed the separation of a small precipitate which was stained blue by iodine and showed other properties which classified it as of the amylose and not of the amylopectin type. Control experiments established that this was a true enzyme action and that partial hydrolysis of the amylopectin was a prerequisite for the precipitation of the intermediate amylose fraction. It is interesting to note that the insoluble *cyclo*hexanol–" amylose " complex appears not to be itself susceptible to hydrolysis by the amylase. It was later shown that any starch-hydrolysing agent could effect the separation of the intermediate amylose fraction from amylopectin in the presence of *cyclo*hexanol. Thus, when an amylopectin dispersion was heated for 3 minutes in a boiling water-bath with N-sulphuric

Specimen light-absorption curves of polysaccharide iodine complexes.



I, *Thymol–amylose.* II, cyclo*Hexanol–amylose.* III, *Potato starch.*
IV, *Thymol–amylopectin.* V, cyclo*Hexanol–amylopectin.* VI, I_2–KI *alone.*

acid in the presence of *cyclo*hexanol, a flocculent precipitate was thrown down. This product, which was obtained in 1·5% yield, showed a higher B.V. (0·355) than that of the original amylopectin (B.V., 0·210).

Pacsu and Hiller (*Text. Research J.*, 1946, **16**, 243) have also observed the liberation of an " amylose " component when an amylopectin (*e.g.*, waxy maize) is treated either with 0·1N-sulphuric acid or with α-amylase. It is difficult, however, to reconcile the evidence available with the novel view of these authors that the observation just recorded represents the conversion of a branched amylopectin structure into an amylose with fewer branches.

Experimental.

Absorption Value (A.V.) and Blue Value (B.V.).—The expression " absorption value " is used to describe the reading on the logarithmic scale of a Spekker Photoelectric Absorptiometer when the absorption of light by a solution of a polysaccharide–iodine complex, contained in 4 cm. cells, is measured. The light filters employed were of the Ilford gelatin type (Nos. 601—608, transmitting light of 4300—6800 A.).

The term " blue value " has a special significance; it is the A.V. observed when the polysaccharide–iodine solution is prepared under standard conditions and when Ilford gelatin light filters transmitting light of 6800 A. are employed. Details for the preparation of a standard solution of this type were given by Bourne, Haworth, Macey, and Peat (this vol., p. 924).

Hydrolysis of Starch and its Components.—(a) *By aqueous acid.* The polysaccharide, after being dried

in a vacuum at 60° for about 6 hours, was weighed into a boiling tube and heated under reflux in a boiling water-bath with 7% sulphuric acid for 12 hours. The hydrolysate was then cooled, neutralised with sodium hydroxide, and diluted to 100 c.c. The amount of glucose present in the neutral solution was determined by means of the Shaffer–Hartmann copper reagent (see Bourne, Haworth, Macey, and Peat, *loc. cit.*).

As amylose is only sparingly soluble in water it is advisable to dissolve it initially in 2% sodium hydroxide (10 c.c.) and then add sulphuric acid to give a final volume of 25 c.c. with an acid concentration of 7%

(b) *By β-amylase.* A general method was adopted for the measurement of the extent of hydrolysis of a 1:4-α-glucosidic polysaccharide effected by the β-amylase of soya bean (Bourne, Macey, and Peat, *J.*, 1945, 882). A digest consisting of the dry polysaccharide (about 30 mg.) in water (34 c.c.), acetate buffer (pH 4·8; 6 c.c.), and β-amylase solution (10 c.c.; 0·1%) was covered with a layer of toluene and incubated at 35·5°. At frequent intervals the amount of maltose formed was determined by removing aliquot portions for analysis by the Shaffer–Hartmann method.

When the polysaccharide was not readily soluble in warm water, it was dissolved by warming with 2% sodium hydroxide solution (10 c.c.), the solution being accurately neutralised by the addition of 5N-sulphuric acid and the volume being adjusted to 34 c.c. with water.

A control digest, containing all the ingredients except the polysaccharide, was used in each determination. Only a small reducing power, if any, was developed in these controls. Where necessary a small correction was applied on this account.

Standard Procedure for the Fractionation of Potato Starch by the Thymol Method.—Potato starch (120 g., dry weight) suspended in cold water (500 c.c.) was slowly added, with continuous stirring, to boiling water (3500 c.c.). The viscosity of the paste was lowered by adding sodium chloride (4 g.) and continuing to stir the boiling solution for 20 minutes. During this process the maximum efficiency of stirring should be achieved. The paste was cooled to 70° and then passed through a Sharples supercentrifuge (25,000 r.p.m.) at a rate of 4 l. per hour. Any small undissolved residue which was thereby removed was discarded.

The temperature of the paste was adjusted to 30°, and powdered thymol (8 g.) was added, with stirring. The mixture was kept at 30° for 60 hours, during which time the insoluble amylose–thymol complex settled out. This was removed in a centrifuge (2000 r.p.m.), washed by being stirred with water saturated with thymol (1 l.), and again separated. The washing process was repeated with 2 fresh portions of water saturated with thymol. The precipitate was dehydrated and freed from thymol by being triturated repeatedly with alcohol and finally with ether. It was dried in a vacuum over phosphoric oxide. The treatment with alcohol and then with ether removed all but a trace of thymol from the complex.

The amylopectin fraction was precipitated by the addition of alcohol (4500 c.c.) to the concentrated mother liquor (1500 c.c.) after the removal of the amylose complex. It was washed with alcohol and ether and dried over phosphoric oxide.

The method as described is suitable for quantities of starch not greater than 150 g. On a bigger scale, *e.g.*, on 1 kg., complete dispersion of the starch in water requires a longer period (up to 4 hours) of stirring at the boiling point.

TABLE II.

β-Amylolysis of potato starch and its fractions.

Thymol separation No. (See Table I).	B.V. of polysaccharide.	Conversion into maltose (%).	Thymol separation No. (See Table I).	B.V. of polysaccharide.	Conversion into maltose (%).
Amylopectin :			Amylose :		
VIII	0·168	49·2	X	1·03	81·4
I	0·220	62·0	VIII	1·06	92·2
VII	0·250	47·0	V	1·12	99·0
Starch :	0·400	60·2	I	1·15	98·2
			III	1·21	96·8

Effect of Various Factors on the Degree of Separation of Amylose and Amylopectin Achieved by the Thymol Method.—(a) *Hydrogen-ion concentration.* An approximately 2% dispersion of potato starch was prepared and divided into portions (100 c.c.). To each portion were added, with stirring, a buffer solution (100 c.c.), thus reducing the starch concentration to 1%, and powdered thymol (2 g.). A range of buffer solutions was prepared from the Universal Buffer containing phosphoric, acetic, and boric acids (see Britton's " Hydrogen Ions "). After each mixture had been kept at 30° for 48 hours the amylose and amylopectin fractions were isolated as in the standard method.

TABLE III.

Effect of pH *on the fractionation of starch.*

pH of buffer.	Amylose fraction. Yield (%).	Amylose fraction. B.V.	Amylopectin fraction, B.V.	pH of buffer.	Amylose fraction. Yield (%).	Amylose fraction. B.V.	Amylopectin fraction, B.V.
5·0	27	0·96	0·21	8·0	24	1·03	0·19
6·0	24	0·93	0·21	9·0	27	0·95	0·19
7·0	26	0·96	0·19	10·0	27	0·89	0·19
				11·0	27	0·90	0·20

Since no attempt was made to remove inorganic matter from the products the above figures are only approximate. They are, however, sufficiently accurate to indicate that any variations attributable to pH changes must be very slight.

(b) *Concentration of the starch paste.* Aqueous pastes (100 c.c.) were prepared containing 0·25, 0·5, 1·0, 2·0, and 5% of potato starch. Into each was stirred powdered thymol (2 g.), and the mixtures were kept at 30° for 48 hours before being centrifuged (2000 r.p.m.). The amylose–thymol complex was readily separated in the first three cases, but sedimentation was slow and incomplete from the 2% and 5% pastes. Thus it appeared that starch pastes of 1% or less would be the most suitable for quantitative separation. The difficulties encountered with the more concentrated pastes were attributed to their higher viscosities (no salt was added in these experiments), which prevented the sedimentation of the fine particles of the thymol–amylose complex.

(c) *Initial viscosity of the starch paste.* Four pastes (200 c.c.) (3%) of potato starch were prepared in the usual manner and, after being cooled, were " thinned " by the addition of sodium chloride (0·1%). Traces of insoluble matter were removed by centrifuging. Although these pastes had been prepared under similar conditions they differed appreciably in their relative viscosities as measured by an Ostwald viscometer. Into each paste was stirred powdered thymol (2 g.), and the mixture was kept for 48 hours at 30°. The precipitated amylose–thymol complex was collected in a centrifuge (2000 r.p.m.), and washed 4 times with saturated thymol-water (150 c.c. each), and then with alcohol (twice) and ether (twice).

The amylopectin was recovered from the supernatant liquid by precipitation with alcohol, and washed with alcohol (twice) and ether (twice). Both fractions were dried in a vacuum over phosphoric oxide. The degree of separation achieved with pastes of different viscosity is shown in Table IV.

TABLE IV.

Initial η_r of paste (water = 1).	Blue values.	
	Amylose fraction.	Amylopectin fraction.
83·5	No separation	
25·9	0·84	0·24
15·3	1·03	0·22
12·4	1·10	0·21

In another experiment, a 3% paste of potato starch was prepared and, when cold, sodium chloride (0·1%) was added. The resultant paste, which had $\eta_r = 50·2$, was divided into 4 parts, which were respectively (i) vigorously stirred at room-temperature for 3 hours, (ii) boiled for 20 minutes, (iii) diluted with an equal volume of water to give a 1·5% paste, and (iv) diluted with water to give a 1% paste. The 4 solutions were then subjected to fractionation with thymol by the method already outlined, with the results shown in Table V.

TABLE V.

No.	Initial η_r of paste (water = 1).	Blue values.	
		Amylose fraction.	Amylopectin fraction.
(i)	45·8	No separation	
(ii)	13·3	0·89	0·24
(iii)	5·7	0·94	0·17
(iv)	2·7	1·02	0·16

In a third experiment, a potato starch paste (6%) was divided into 3 portions which were treated with alkali, respectively, as follows :

(i) diluted with sodium hydroxide solution to give a paste which contained 3% of starch and 2½% of sodium hydroxide ;

(ii) made 2½% with respect to sodium hydroxide, stirred at room temperature for 1 hour, neutralised with hydrochloric acid, and diluted with water to give a 3% starch paste ;

(iii) as under (ii), except that the temperature was 50° during the stirring process.

The 3 solutions were then subjected to fractionation with thymol with the results shown in Table VI.

TABLE VI.

No.	Initial η_r of paste (water = 1).	Blue values.	
		Amylose fraction.	Amylopectin fraction.
(i)	19·9	1·00	0·21
(ii)	11·2	1·04	0·24
(iii)	8·5	1·14	0·27

The more drastic the treatment with alkali the more difficult it became to remove the finely-divided amylose complex from the amylopectin solution. Hence the B.V. of the latter increased. However, when the complex was removed in the Sharples supercentrifuge in case (iii) the B.V. of the amylopectin was as low as 0·16.

(d) *Temperature and duration of complex formation.* These factors were not specifically controlled, but the results of several workers indicated that the optimum conditions were those reported in the standard method.

(e) *Treatment of the components with thymol-water.* (i) *Amylose.* A sample of a moist amylose–thymol complex isolated from potato starch was divided into 3 parts. The first part was washed with saturated

thymol-water (3 volumes), the second was washed twice in this way, while the third was not treated. All were washed with alcohol and then ether before being dried in the usual manner. The B.V. of the unwashed material was 1·04, which was increased by the first washing to 1·11 and by the second washing to 1·14.

(ii) *Amylopectin.* A sample of potato amylopectin (3 g.; B.V., 0·240) was shaken at room temperature for 18 hours with saturated thymol-water (100 c.c.). The insoluble residue was removed by centrifuging and discarded. The soluble polysaccharide, which was precipitated by the addition of alcohol, washed with alcohol and then ether, and dried, had B.V., 0·235. A second sample (3 g.) of the same amylopectin which was shaken with saturated thymol-water for 42 hours gave a product having B.V., 0·222. A third sample (3 g.) of the amylopectin was heated at 100° for 2 hours with saturated thymol-water; the product had B.V., 0·221. When the heating at 100° was continued for 12 hours the product had B.V., 0·219.

The Fractionation of Potato Starch by the cyclo*Hexanol Method.*—The general procedure used in these experiments was similar to that recorded for the standard thymol method. The amounts of *cyclo*hexanol used instead of thymol are recorded in Table VII, where a summary of a number of fractionations is given. The amylose fractions were, of course, washed with saturated *cyclo*hexanol–water instead of thymol-water.

TABLE VII.

Specimen results by the cyclo*hexanol method.*

Separation No.	Starch paste (%).	*cyclo*Hexanol c.c./100 c.c. of paste.	Amylose fraction. Yield (%).	Amylose fraction. B.V.	Amylopectin fraction, B.V.
I	3	0·2	37·6	0·72	0·225
II	3	5·0	43·1	0·85	0·220
III	3	5·0	—	—	0·156
IV	1	0·5	30·8	0·93	0·096
V	1	0·5	24·0	0·93	—
VI	1	1·0	23·9	0·93	—
VII	1	2·0	27·2	0·91	—
VIII	1	3·0	27·9	0·94	—
IX	1	4·0	27·0	0·89	0·218
X	1	5·0	23·0	0·94	—
XI	1	6·0	23·8	0·95	—
XII	1	6·0	25·1	0·91	0·094
XIII	1	6·0	—	—	0·193
XIV	1	6·0	—	—	0·182
XV	1	6·0	—	—	0·152

Action of Saliva on Potato Amylopectin in Presence of cyclo*Hexanol.*—A 1% paste of potato starch, to which a trace of sodium chloride had been added, was allowed to stand for 3 days at 30° in the presence of sufficient *cyclo*hexanol to maintain a saturated solution throughout this period. The insoluble *cyclo*hexanol–amylose complex was removed by passing the suspension thrice through a Sharples supercentrifuge (25,000 r.p.m.). Portions (10 c.c.) of the amylopectin solution, still saturated with *cyclo*hexanol, were incubated at 25° with mucin-free saliva (1 c.c.) under different conditions. The precipitated fraction, if any, was separated (2000 r.p.m.), washed twice with alcohol, then with ether, and dried. Its staining power with iodine was tested qualitatively.

A blue-staining precipitate was isolated in several experiments in which untreated mucin-free saliva was used, but there was no precipitate when the saliva had been pre-heated for 10 minutes at 100° or when mercuric chloride (1 part in 1000), which inhibits α-amylase action, was incorporated in the digest.

Effect of Salts on the α-Amylolysis of Potato Amylopectin in Presence of cyclo*Hexanol.*—A paste (0·75%) of potato amylopectin (B.V., 0·210) was covered with a layer of *cyclo*hexanol and kept for 3 days at 30°. All ions were removed from this solution and from mucin-free saliva by passage through ion-exchange resins. Two digests were prepared containing the following constituents:

Digest (A): ion-free amylopectin paste (30 c.c.), ion-free saliva (1 c.c.), and distilled water (8 c.c.).

Digest (B): ion-free amylopectin paste (30 c.c.), ion-free saliva (1 c.c.), distilled water (2 c.c.), and acetate buffer (pH 4·8) (6 c.c.).

The buffer (pH 4·8) was chosen because the natural pH of digest (A) was 4·8. Each digest was incubated at 25°, and at intervals portions (3 c.c.) were withdrawn, immersed in a boiling water-bath for 5 minutes to destroy α-amylolytic activity, and centrifuged. A portion (1 c.c.) of the supernatant layer was introduced into a 500 c.c. flask containing water (200 c.c.), 5N-hydrochloric acid (3 drops), and iodine solution (5 c.c.; 0·2% iodine in 2% potassium iodide solution). After dilution to 500 c.c. the A.V. (6800 A.) was measured. The results are recorded in Table VIII.

TABLE VIII.

Time of incubation (mins.).	Absorption values (6800 A.). (A).	(B).
0	0·31	0·31
60	0·31	0·05
120	0·31	0·03

It was observed that a blue-staining precipitate separated from digest (B), but not from digest (A), in which no enzyme action occurred.

Fractionation of Potato Amylopectin with cyclo*Hexanol and Saliva.*—Potato amylopectin (2·8 g.), dispersed in water (400 c.c.), was covered with a layer of *cyclo*hexanol and kept at 30° for 3 days. It was heated to 40° and mucin-free saliva (5 c.c.) was added. The solution was immediately heated, and in 35 seconds reached 100°, and was kept there for 10 minutes. The precipitated polysaccharide was separated (2000 r.p.m.), washed, twice with alcohol and twice with ether, and dried in a vacuum over phosphoric oxide. Yield, 56 mg.; B.V., 0·66; 94·5% hydrolysed by β-amylase (mean).

It thus appeared to be a degraded amylose type.

Fractionation of Potato Amylopectin with cyclo*Hexanol and Acid.*—Potato amylopectin (1·50 g., B.V., 0·210) was dispersed in water (200 c.c.), covered with a layer of *cyclo*hexanol, and kept at 30° for 3 days. Sulphuric acid (5N; 50 c.c.) was added, making a N-acid solution, and, still covered with *cyclo*hexanol, it was immersed in a bath of vigorously boiling water. After the solution had been above 95° for 3 minutes, it was cooled and centrifuged (2000 r.p.m.). The precipitate was washed thrice with alcohol and then with ether before being dried in a vacuum over phosphoric oxide. Yield, 23 mg.; B.V., 0·355.

The Light-absorption Curve of a Polysaccharide–Iodine Complex.—The dry polysaccharide was dissolved and stained with iodine under the standard conditions prescribed by Hassid and McCready (*J. Amer. Chem. Soc.*, 1943, **65**, 1154), giving a coloured solution which contained the polysaccharide (1 mg./100 c.c.), iodine (2 mg./100 c.c.) and potassium iodide (20 mg./100 c.c.). The absorption values of this solution were determined over the whole range of Ilford gelatin filters (Nos. 601—608). By plotting A. V. against the wave-length of peak filter transmission (4300—6800 A.), a characterstic "absorption curve" for the polysaccharide was obtained. This curve of course incorporated the B.V. at 6800 A.

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