XCVII. HEXOSEPHOSPHATES PRODUCED BY HIGHER PLANTS.[1]

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The fact that phosphates stimulate the respiratory sugar metabolism in higher plants suggests that the formation of phosphoric esters may occur as part of this process. Stoklasa *et al.* [1904–1913] obtained cell-free zymase-like preparations from peas, beet-roots, potatoes *etc.*, which actively fermented sugars. Bodnár [1916] confirmed these results under strictly aseptic conditions, since some investigators had attributed Stoklasa's findings to bacterial contamination. Evidence of phosphorylation was first given by Bodnár [1925] who demonstrated that inorganic phosphate disappeared when added to ground peas in presence of toluene. His results were confirmed by Zalesky and Pissarjewski [1927] who did not however regard this process as a necessary step in the respiration and attributed the stimulation to alkalinity of the phosphate rather than to the formation of phosphoric esters. More recently, Rao [1935] has prepared active cell-free aqueous extracts by plasmolysing fermenting peas with light petroleum and states that in such extracts the decomposition of sugar and the phosphorylation are concomitant processes.

While Bodnár's findings indicated the possible parallelism between carbohydrate metabolism in the higher plants and in yeast or muscle [Harden, 1932; Bodnár and Tankó, 1929], little evidence was available regarding the nature of the compounds formed during the phosphorylation. It is known that when preparations of germinating peas, beans, barley *etc.* are allowed to act upon the hexosediphosphoric acid of Harden and Young products are formed which are characteristic of sugar breakdown in yeast or muscle [Neuberg and Gottschalk, 1924; 1925; Neuberg and Kobel, 1929; 1930; 1934, 1; Baba, 1935]. Moreover, with phosphoglyceric acid as a substrate, pyruvic acid is formed [Neuberg and Kobel, 1934, 2].

Barrenscheen and Albers [1928] found that the acid-soluble phosphorus increased during assimilation in irradiated *Elodea canadensis* and during germination of rye. A phosphoric ester was isolated by Barrenscheen and Pany [1930] from the phosphorylated products formed when *Elodea* was kept in a dilute solution of sugar and inorganic phosphate for an irradiation period of 4 hours. In its composition (Ba, P) the ester resembled a hexosemonophosphate, but it differed from the Robison ester [Robison, 1922] in possessing a much lower reducing power (iodimetric, 3·2 %; H.J. 3·5 %). These investigators also isolated the barium salt of a phosphorylated octaamylose from the germ plants of wheat. This substance gave a high dextrorotation, $[\alpha]_D^{24°}$ +44·9°, and after hydrolysis by takadiastase of its magnesium salt, yielded a hexosemonophosphoric ester having H.J. 22·6 % and $[\alpha]_D^{20°}$ −4·3°. On hydrolysis by bone phosphatase the monophosphate yielded fructose, which was identified by means of its methylphenylosazone. The authors also found that the synthetic phosphorylated

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starch, prepared by the method of Kerb, yielded on partial hydrolysis by taka-diastase a compound probably identical with the phospho-octaamylose obtained from plants. These experiments, carried out under conditions approaching the physiological, strongly support the view that hexosephosphates are formed by higher plants. The crude monophosphate isolated from *Elodea* by Barrenscheen and his collaborators resembles none of the esters characteristic of yeast and muscle, and these investigators considered that the phospho-octaamylose of wheat occurred preformed in the starch molecule. Whilst plants may have their own individual peculiarities in combining phosphate with sugar, the results of Neuberg and his co-workers, referred to above, seem to suggest that the phosphoric esters known to take a leading part in the sugar-breakdown by yeast and muscle may also form a part of the phosphorylated compounds produced by higher plants.

The aim of the present research has been to approach the problem of phosphorylation in higher plants from the preparative side and to isolate and identify as far as possible the compounds produced by pea preparations under the conditions devised by Bodnár.

Experimental.

Ground peas were prepared according to Bodnár [1925]. Removal of the proteins was effected by trichloroacetic acid and the phosphorus was estimated by Briggs's method, as modified by Martland and Robison [1926]. Marrow peas (No. 110, supplied by G. Kontsek, Ltd.) yielded active (phosphorylating) preparations simply by grinding the seeds, the skins being removed by sifting through grenadine (metal sieves were avoided). Preparations of Victoria and sugar peas did not phosphorylate unless the peas were first allowed to swell in water; the skins being removed and the seeds dried at 30–32° before being ground and sifted.

The behaviour of the marrow pea-flour indicated that bacterial contamination during the swelling process was not responsible for the phosphorylation. Moreover, if the swelling was allowed to proceed in the presence of chloroform or toluene instead of water alone, equally active preparations were obtained. The pea-flour retained its activity for several months. Using the marrow pea-flour, the addition of inorganic phosphate equivalent to 10 mg. P per g. of flour was found to provide the most suitable experimental conditions; increasing the phosphate to 15 mg. P per g. did not appreciably increase the amount of esterification, whilst the larger proportion of unesterified phosphate interfered with the subsequent isolation of phosphoric esters.

The optimum p_H *for esterification.*

0·3 g. of the pea preparation was added to 1·5 ml. portions of the buffer solution (veronal-acetate buffer [Michaelis, 1931]), each containing 0·9 mg. P as inorganic phosphate. The tubes (10 ml.), to which a drop of toluene was added, were placed in a desiccator containing water and toluene and kept at 32°; several tubes were prepared at each p_H. The inorganic phosphate was estimated at 0, 2 and 5 hours after the addition of 3 ml. of H_2O and 2·5 ml. of 10 % trichloroacetic acid. The p_H was determined on a separate series of tubes of similar composition, using the quinhydrone electrode. As is shown in Table I, the optimum p_H for the disappearance of inorganic phosphate lies slightly on the acid side of neutrality. The observed optimum may be an apparent one, for 1 mg. of organic phosphorus was present in the pea preparation used, and some of this may have been hydrolysed at the more alkaline p_H values.

Table I. *Effect of* p_H *on the disappearance of inorganic phosphate from pea preparations.*

Veronal-acetate buffer. The inorganic P values are calculated for 0·3 g. pea used in each experiment. Estimation of p_H was carried out by means of the quinhydrone electrode at 22°.

Time	p_H					
2 min.	6·16	6·63	6·96	7·63	8·02	8·34
2 hours	6·14	6·54	6·77	7·43	7·87	8·24
5 hours	6·12	6·49	6·69	7·30	7·78	7·99
	mg. inorganic P/0·3 g. preparation					
0 hours	1·16	1·17	1·17	1·17	1·16	1·17
2 hours	1·06	1·00	0·99	1·10	1·19	1·21
5 hours	0·98	0·91	0·86	1·07	1·23	1·28

Rate of phosphorylation.

20 g. of pea-flour in a 200 ml. flask were stirred to a paste with 40 ml. H_2O or phosphate solution (204 mg. P; p_H 7·2) and 2 ml. of toluene. The pea-flour was stirred at intervals and the experiment was stopped at the time indicated by the addition of 70 ml. of H_2O and 70 ml. of 10 % trichloroacetic acid. The protein precipitates were washed with 50 ml. 3 % trichloroacetic acid.

The filtrates were adjusted with NaOH to p_H 8·4 and the precipitates were filtered off, washed and dried (Fraction 1).

Basic lead acetate solution was added in slight excess to the filtrates. The washed basic lead precipitates were suspended in water and decomposed with H_2SO_4; the $PbSO_4$ was centrifuged down and washed repeatedly with acidified H_2O. The centrifugates and washings were adjusted to p_H 8·4 with baryta (without the removal of the $BaSO_4$) and the phosphoric esters precipitated by the addition of 2·5 volumes of 96 % alcohol. The dried precipitate (A), after the total and inorganic P had been estimated, was repeatedly extracted with 15 times its weight of H_2O, added in portions. The solution was again precipitated with alcohol; the dried precipitate was extracted with H_2O as before, and 33 % alcohol, sufficient to give a 10 % solution, was added. The insoluble portion was centrifuged down; the P remaining in the centrifugate represented Fraction 2, *i.e.* the neutral barium salt soluble in 10 % alcohol.

Fraction 3, the barium salt insoluble in 10 % alcohol, is represented by the P content of precipitate A, less that of Fraction 2. The reducing sugars in the filtrate from the basic lead precipitation were determined by the Hagedorn-Jensen method and were calculated as glucose.

The results, summarised in Table II, show that a considerable accumulation of esterified phosphate occurs when the pea-flour preparations are incubated for periods of from 35 to 70 hours in the presence of added inorganic phosphate. The amount of reducing sugar fell in each experiment where phosphorylation took place, suggesting that phosphoric esters of the sugars were formed. The phosphorylation is greatly in excess of that taking place in the autofermentations. The phosphoric esters produced are found mainly in Fraction 3 and to a much less extent in Fraction 2. Fraction 1, which remained at a constant level during the autofermentations, decreased slightly in the experiments in which inorganic phosphate was added; this fraction had a phosphorus content of 16–18 % and gave a negative Molisch reaction. The lead salt was insoluble in acetic acid and the fraction gave the Fischler and Kürten [1932] reaction for phytin.

Table II. *Distribution of organic P in fractions obtained from phosphorylation experiments with pea-flour.*

20 g. of pea preparation were used in each experiment and all figures are calculated for the total trichloroacetic filtrate.

Fractions: 1, insoluble sodium salt.
2, soluble barium salt.
3, sparingly soluble barium salt.

In Exps. 3, 5, 7 the figures in brackets show the increase in esterified P resulting from the addition of inorganic phosphate.

In the autofermentations the p_H dropped from 6·8 to 6·4; in those with added inorganic phosphate from 7·0 to 6·6.

The reducing power was estimated in the filtrates from the basic lead precipitates.

				mg. P in								Reduction H.J. mg. as glucose
				Trichloroacetic filtrate		Fraction 1	Filtrate of fraction 1		Fraction 2	Fraction 3		
Exp.	mg. inorg. P added	°C	Duration hours	Org.	Inorg.	Org.	Org.	Inorg.	Org.	Org.	Inorg.	
1	—	—	0	65	17	48	18	16	4	5	12	190
2	—	32	8	79	8	51	30	7	11	8	7	373
3	204	32	8	130	160	40	90	160	14 (3)	50 (42)	155	172
4	—	32	35	81	11	47	33	12	14	11	10	416
5	204	32	35	273	18	40	229	22	32 (18)	159 (148)	26	205
6	—	21	70	86	4	48	38	4	16	14	3	374
7	204	21	70	274	13	42	232	13	28 (12)	163 (149)	17	148

The effects of fluoride and monochloroacetic acid.

The formation of hexosephosphates was strongly inhibited by 0·002M NaF, and a similar result was obtained with monochloroacetic acid. The hydrolysis in N HCl, at 100° of the trichloroacetic filtrate from an experiment with 0·0002M NaF (which did not inhibit phosphorylation), did not reveal any evidence of the accumulation of more difficultly hydrolysable esters in comparison with the controls.

Isolation and identification of the phosphoric esters formed.

As Table II shows, Fraction 3, the sparingly soluble barium salt, constituted by far the greater part of the phosphoric esters formed during the experimental period. For isolation and identification of the esters, the phosphorylated products obtained from several experiments (carried out under the same conditions as Exp. 7) were combined and fractionated according to the general procedure already outlined above. The basic lead salt was decomposed with H_2SO_4 at 0° to avoid possible hydrolysis, and the neutral barium salt was prepared.

Fraction 3 was further purified by formation of the soluble acid barium salt by the addition of HCl to p_H 3, the insoluble $BaSO_4$ being separated by centrifuging. It was precipitated from the centrifugate at p_H 4 by the addition of 4 volumes of 96 % alcohol. The filtrate from this precipitation was adjusted to p_H 8·4, and the precipitate obtained was combined with Fraction 2.

Fraction 2 was further purified by four reprecipitations from its aqueous solution by alcohol. The portion insoluble in 10 % alcohol was purified by precipitation of the acid salt with alcohol and then combined with Fraction 3.

From 100 g. of pea-flour, to which 1 g. of P as inorganic phosphate had been added, about 6 g. of the crude acid barium salt of Fraction 3 and 1 g. of the crude neutral barium salt of Fraction 2 were obtained.

The diphosphate fraction (*Fraction 3*). This fraction was still contaminated with inorganic phosphate, which was removed by means of magnesia mixture, and the acid barium salt again precipitated (yield, 5 g.). There was no separation of phosphoglycerates when a small amount of the ester was kept at 0° in solution in 20 % alcohol. The ester was further purified by two reprecipitations of the acid barium salt (yield, 3·5 g.). Part of it was converted into the neutral barium salt by dissolving in water, adjusting to p_H 8·4 with baryta and precipitating with an equal volume of alcohol. The analysis of the substance is shown in Table III, No. 1. (All figures are calculated on the dry weight; 90 minutes' drying at 110°

Table III. *Analyses of neutral Ba salts of the phosphoric esters isolated from the phosphorylation experiments.**

No.	Source of Ba salt	Ba %	P %	Reducing power as glucose, % H.J.†	Reducing power as glucose, % Iodine	Fructose (Selivanoff) %	$[\alpha]_D^{20°}$
1	Fraction 3, purified	—	10·0	12	1·4	8	+ 3·0° (c=28·6 %)
1 *a*	Monophosphate obtained from No. 1 by acid hydrolysis	—	7·8	34	2·0	25	+ 2·3
2	Fraction 2, crude	33·8	5·9	21	24·0	—	+ 4·6
3	Fraction 2, purified through brucine salt	34·6	7·7	29·5	28	8	+ 7·3
3 *a*	Mother-liquor of brucine salt	—	2·7	10	14	2	—
4	No. 3 after removal of aldoses by bromine oxidation	—	7·8	22	0·5	17	− 4·3
5	Fraction 2, originally present (0 hour exp.)	—	1·9	9	22	2	−17·2
6	Fraction 2, from autofermentation (21°, 70 hours)	—	2·9	9	20	4	− 6·9
	* Analyses of hexosephosphates for comparison:						$[\alpha]_{5461}^{20°}$
	Hexosediphosphate [1]	—	10·0	12	1·5	9	+ 2·0
	Fructose-6-phosphate [1]	—	7·73	35	1·6	22	+ 2·3
	Robison ester [2]	—	7·85	30	25·0	6	+14·4
	Glucose-6-phosphate [3]	—	7·86	36	45·7	(0·5)	+20·6
	Mannose-6-phosphate [3]	—	7·87	36	28·5	(0·3)	+ 3·6
	Fructose-1-phosphate [4]	—	7·85	26	0·9	24	−39·0
	Galactose-6-phosphate [5]	—	7·86	28	41·0	(0·7)	+30·0

† Determined with addition of 0·5 ml. of 0·5 N NaOH.

[1] Macleod and Robison [1933]. [2] Robison [1922]. [3] Jephcott and Robison [1934]. [4] Tankó and Robison [1935]. [5] Grant [1935].

in the Pregl micro-dryer.) The analysis of the neutral barium salt agrees closely with that of fructosediphosphate. The rotation was measured in acid solution and calculated for the neutral salt.

Phenylosazone. The phenylhydrazine salt of the phenylosazone was prepared and reprecipitated from alcohol-chloroform; the phospho-osazone obtained, dried over H_2SO_4 *in vacuo* had M.P. 152·5–153°. (Found: P, 5·52 %; $C_{24}H_{31}O_7N_6P$ requires P, 5·68 %.)

Rate of hydrolysis. The rate of hydrolysis of the acid salt in N HCl at 100° is shown in Table IV, where it is compared with that found by Macleod and Robison [1933] for 1:6-diphosphofructofuranose; the figures are in excellent agreement.

The monophosphate prepared by the fractional hydrolysis of the acid salt (6 min. in N HCl at 100°) was analysed and found to consist of the Neuberg

Table IV. *Hydrolysis in* N *HCl at 100° of hexosediphosphate isolated from pea products.*

	Hydrolysis %		$k \times 10^3$	
Time (min.)	A Diphosphate from pea products (0·455 mg. P per ml.)	B 1:6-Diphosphofructofuranose* (0·454 mg. P per ml.)	A	B
5	23·7	23·3	22·3	23·0
10	36·5	36·8	15·9	16·8
25	53·5	54·4	9·0	9·4
60	69·5	68·8	5·2	4·7
120	84·1	83·5	4·7	4·6
180	91·4	90·2	4·4	3·7
300	96·8	96·6	3·6	3·8

* Macleod and Robison [1933].

ester, fructose-6-phosphate (Table III, 1*a*). The results show that the hexosediphosphate isolated from Fraction 3 and formed during the phosphorylation taking place with the pea preparations used is identical with the Harden-Young ester formed by yeast preparations, *i.e.* 1:6-diphosphofructofuranose.

The hexosemonophosphate fraction (*Fraction* 2). The analysis of the crude Fraction 2 suggested that it consisted partly of hexosemonophosphates. The reducing power was in good agreement with the P, but the ratio Ba/P was 1·3 and the substance contained about 2 % N, only partly precipitated by phosphotungstic acid. By fractional precipitation with alcohol a fraction was obtained between 40–70 % alcohol, which had the following analysis: P, 3·9 %; Ba/P 1·8; H.J. 12 %; I_2, 38 %. Thus the crude fraction contained a compound of low P and relatively high Ba content (suggesting a carboxylic group) and possessing a high iodine value.

For further purification, Fraction 2 was converted into the brucine salt [Robison and King, 1931] and 90 % of the original phosphorus was found in fractions having P, 2·9–2·5 % (brucine hexosemonophosphate requires 2·95 %). The fractions of lower P content, after recrystallisation from 90 % alcohol, gave P 2·9 %. The fractions were combined and recrystallised from water. During the separation of the first crop crystals were formed resembling those of brucine fructose-1-phosphate [Tankó and Robison, 1935]. The combined crops (P, 2·85–2·94 %) were reconverted into the barium salt and reprecipitated three times in the usual manner. From 1 g. of the crude barium salt of Fraction 2 0·4 g. of the purified monophosphate was obtained. The residue left in the mother-liquors was converted into the barium salt and precipitated several times from its aqueous solution with 96 % alcohol. The analyses (Table III, Nos. 3, 3*a*) show that a hexosemonophosphate was obtained in fairly pure state and separated from the material in the mother-liquors, which had a low P and a high iodine value. The monophosphate obtained (N-free; Ba/P = 1) differed from the Robison ester chiefly in its lower dextrorotation, in spite of its somewhat higher iodine value.

Removal of aldosemonophosphates by oxidation with bromine. A portion of the pure monophosphate (0·3 g.) was oxidised with bromine to remove the aldose components as described by Tankó and Robison [1935]. The analysis of the product (0·05 g.), after the separation of the phosphohexonates, is given in Table III, No. 4. The rate of hydrolysis of this non-aldose portion in *N* HCl at

Table V. *Hydrolyses of hexosemonophosphates obtained from pea products.*

Time min.	Before bromine oxidation (0·297 mg. P per ml.): Hydrolysis % Found	Hydrolysis % Calculated*	$k \times 10^3$	After bromine oxidation (0·294 mg. P per ml.): Hydrolysis %	$k \times 10^3$
5	—	—	—	24·0	23·8
35	20·2	20·0	2·8	49·6	5·7
60	—	—	—	58·0	3·1
180	34·8	33·8	0·60	73·6	3·4
630	48·4	49·5	0·23	82·8	0·41
2160	75·5	—	0·21	—	—
5160	93·7	—	0·19	—	—

* Assuming the composition to be: 54 % of glucose-6-phosphate, $k = 0·23 \times 10^3$; 8 % of mannose-6-phosphate, $k = 0·29 \times 10^{-3}$; 38 % of the compound obtained after bromine oxidation whose hydrolysability is shown in the last two columns.

100° is compared with that for the original monophosphate in Table V. The observed properties of this non-aldose component are difficult to explain on the assumption that it consists only of the two known fructosemonophosphates (fructose-6- and fructose-1-phosphate). The observed laevorotation is too small for the high initial rate of hydrolysis, assuming this to be due to fructose-1-phosphate; the low Selivanoff value suggests the presence of a non-fructose derivative, as does the rapid fall in the rate of hydrolysis after 3 hours. Fructose-6-phosphate ($k = 4·2 \times 10^{-3}$ [Robison, 1932]) under the above conditions would be hydrolysed to the extent of 28·7 % in 35 min., 82·5 % in 180 min.; the non-aldose component of the present monophosphate is hydrolysed 49·6 % in 35 min., 73·6 % in 180 min. The excellent solubility of the non-aldose portion, the correct phosphorus and low iodine values rule out the possibility that unchanged aldose-monophosphate or phosphohexonates can be responsible for these discrepancies. Fructose-6-phosphate is a most likely constituent of the product, accompanied by a more readily hydrolysable compound (possibly fructose-1-phosphate) and one less readily hydrolysable. More attention will have to be directed to the compounds originally present in the pea-flour used, or formed during the auto-fermentation process (see Table III, Nos. 5, 6, for analyses), which possess low P and Selivanoff values, high iodine values, are laevorotatory and are hydrolysed by acids at a rate comparable with that of glucose-6-phosphate.

Phosphohexokinase in pea preparations.

It has been shown that the Robison ester of yeast fermentation consists of a mixture of the 6-phosphates of glucose, fructose and mannose [Robison, 1932] and that a phosphohexokinase occurs in yeast and in muscle, bone and other tissues capable of causing this equilibrium mixture to be formed from any one

Table VI. *Demonstration of phosphohexokinase in pea preparation.*

Substrate: fructose-6-phosphate.

mg. pea preparation per mg. ester P	Time (min.) ...	2	5	15
44·6	Aldose %	33	58	62
22·3	Aldose %	21	38	60

The original I_2 value of the fructose-6-phosphate was 2·5 %.
Concentration of ester (Na-salt) 0·02 molar: $p_H = 7·2$.
The figures for aldose are based on the I_2 values, less the amount due to the pea preparation, and are given as percentages of the total calculated hexose in the substrate.

of the hexosemonophosphates [Lohmann, 1933; Tankó and Robison, 1935; Patwardhan and Robison, unpublished results]. The proportion of 62 % aldose and 38 % non-aldose esters in the monophosphate at present under investigation suggested that such an enzyme might also be present in the plant preparation used.

50 or 100 mg. of the pea-flour were added to 1·4 ml. portions of a solution of the sodium salt of fructose-6-phosphate (1·6 mg. ester-P per ml.). The mixtures were kept at room temperature and the action was stopped by the addition of trichloroacetic acid. The iodimetric reducing power was estimated on the neutralised trichloroacetic filtrate. The iodine value of the pea-flour used was low in comparison with the changes taking place and did not alter significantly during the period of the experiment. The ketose ester (which had only 2·5 % iodine value) was converted to the extent of 62 % into aldose ester. The change was complete in 15 min. and no hydrolysis of the ester took place in that time; nor was fructose-diphosphate hydrolysed when added in place of the monophosphate. There was no appreciable increase in iodine value when fructose and inorganic phosphate were used.

Discussion.

These results indicate that under the optimum conditions (p_H, temperature and concentration of inorganic phosphate) for the rate of phosphorylation of dried pea preparations, a hexosediphosphate and hexosemonophosphates accumulate. These esters have been isolated and their properties compared with those of the phosphoric esters known to play a part in the carbohydrate metabolism of yeast and muscle.

The diphosphate which constituted the major portion of the phosphorylated products was the Harden-Young ester, 1:6-diphosphofructofuranose. No evidence was obtained of the formation of phosphoglycerates. The monophosphate fraction was a mixture of aldose and ketose esters resembling the Robison ester, but containing other components, possibly fructose-1-phosphate and a difficultly hydrolysable non-aldose ester which is being further investigated. The phosphorylation in the pea preparation was inhibited by NaF and by monochloroacetic acid. The preparations contained a phosphohexokinase.

The evidence obtained supports the view that phosphorylation takes place in higher plants, with the production of the same phosphoric esters as are formed in yeast or muscle. In addition esters are present which differ in constitution from the known phosphoric esters and which may be related to the reserve materials of the pea preparations, since these were the source of the hexose constituents of the phosphoric esters produced. The pea preparations used in the experiments hydrolysed hexosediphosphate at very low rates. Preparations obtained in a similar way from fresh green peas hydrolysed the diphosphate very rapidly, and this may explain why such preparations did not produce any accumulation of phosphoric esters. Preparations from germinating marrow peas behaved similarly to those from fresh green peas. Further work will be carried out to define the nature of the monophosphates formed when the hexosediphosphate is hydrolysed by these preparations.

Summary.

1. The optimum conditions of the experiments devised by Bodnár [1925] were investigated in order to increase the amount of organic phosphorus compounds formed from added inorganic phosphate by pea preparations in the presence of toluene, without any extra carbohydrate.

2. The method of separation of the phosphorylated products is described. These compounds are phosphorylated derivatives of the sugars.

3. The greatest part, over 90 % of the esterified phosphorus, has been identified as the Harden-Young ester, 1:6-diphosphofructofuranose.

4. The monophosphate complex has been separated from the other fractions and purified by means of the brucine salt. The pure monophosphate resembled the Robison ester but had a somewhat lower dextrorotation and there was some evidence that this was due to fructose-1-phosphate. Another difficultly hydrolysable non-aldose ester was present and is being further investigated.

5. The presence of phosphohexokinase in the pea preparations has been demonstrated; this enzyme converts fructose-6-phosphate into a mixture of 62 % aldose- and 38 % non-aldose-esters.

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