Futterman, S., and Kinoshita, J. H. (1959), *J. Biol. Chem. 234*, 3174.
Horecker, B. L., and Kornberg, A. (1948), *J. Biol. Chem. 175*, 385.
Ishilawa, A. (1935), *Z. klin. Path. u. Hämatol. (Japan) 4*, 305.
Jones, A. R., Dowling, E. J., and Skraba, W. J. (1953), *Anal. Chem. 25*, 394.
Kaplan, N. O., Colowick, S. P., and Neufeld, E. F. (1953), *J. Biol. Chem. 205*, 1.
Karnovsky, M. L., Foster, J. M., Gidez, L. I., Hagerman, D. D., Robinson, C. V., Solomon, A. K., and Villee, C. A. (1955), *Anal. Chem. 27*, 852.
Karnovsky, M. L., and Wallach, D. F. H. (1961), *J. Biol. Chem. 236*, 1895.
Kemp, A., and van Heijningen, A. J. M. K. (1954), *Biochem. J. 56*, 646.
King, E. J. (1932), *Biochem. J. 26*, 292.
Kinoshita, J. H. (1957), *J. Biol. Chem. 228*, 247.
Kinoshita, J. H., Masurat, T., and Helfant, M. (1955), *Science 122*, 72.
Kornberg, A. (1955), *in* Methods in Enzymology, vol. 1, Colowick, S. P., and Kaplan, N. O., ed., New York, Academic Press, Inc., p. 441.
McLean, P. (1960), *Biochim. et Biophys. Acta 37*, 296.
Navazio, F., Ernster, B. B., and Ernster, L. (1957), *Biochim. et Biophys. Acta 26*, 416.
Nelson, N. (1944), *J. Biol. Chem. 153*, 375.
Noble, E. P., Stjernholm, R. L., and Ljungdahl, L. (1961), *Biochim. et Biophys. Acta 49*, 593.
Potter, V. R., and Niemeyer, H. (1959), *in* Ciba Foundation Symposium on the Regulation of Cell Metabolism, Wolstenholme, G. E. W., and O'Connor, C. M., ed., Boston, Little, Brown and Co., p. 230.
Robinson, C. V. (1950), *Science 112*, 198.
Rous, P. (1925), *J. Exp. Med. 41*, 399.
Sbarra, A. J., and Karnovsky, M. L. (1959), *J. Biol. Chem. 234*, 1355.
Sbarra, A. J., and Karnovsky, M. L. (1960), *J. Biol. Chem. 235*, 2224.
Stähelin, H., Suter, E., and Karnovsky, M. L. (1956a), *J. Exp. Med. 104*, 121.
Stähelin, H., Karnovsky, M. L., and Suter, E. (1956b), *J. Exp. Med. 104*, 137.
Umbreit, W. W., Burris, R. H., and Stauffer, J. F. (1957), Manometric Techniques, ed. 3, Minneapolis, Burgess Publishing Co., p. 172.
van Vals, G. H., Bosch, L., and Emmelot, P. (1956), *Brit. J. Cancer 10*, 792.
Wenner, C. E., Hackney, J. H., and Moliterno, F. (1958), *Cancer Res. 18*, 1105.

Myoinositol Polyphosphate Intermediates in the Dephosphorylation of Phytic Acid by Phytase*

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Phytic acid, on partial dephosphorylation with phytase, yields a series of optically active polyphosphate esters which heretofore had not been characterized. These degradation products have now been identified as myoinositol 1- and 2-monophosphate; myoinositol 1,2-diphosphate; myoinositol 1,2,3- and L-1,2,6-triphosphate; myoinositol 1,2,5,6-tetraphosphate plus another uncharacterized isomer. The structure of the myoinositol pentaphosphate has not been established. Some of the apparent specificities of phytase indicated by this series of characterized isomers are discussed.

Posternak and Posternak (1929) reported that optically active myoinositol polyphosphate esters were produced by the partial dephosphorylation of phytic acid by the enzyme "phytase." The enzyme preparation used in these experiments was an unfractionated aqueous extract of wheat bran. The products of the reaction were isolated as barium salts by fractional precipitation, and their characterization as myoinositol di-, tri-, and tetraphosphates was based on elemental analysis.

Subsequently, Courtois and co-workers carried out an extensive investigation on this reaction, the results of which have been summarized in a review article (Courtois, 1951). By fractionation of extracts from a variety of plant sources, they obtained in all cases two phosphatase components. One of these, designated as the phosphomonoesterase fraction, showed no phosphatase activity on phytic acid but was active on glycerol 2-phosphate. The second component, called the phytophosphatase fraction, was active in dephosphorylating phytic acid and myoinositol polyphosphate esters, but was also equal to or better than the phosphomonoesterase in dephosphorylating glycerol 2-phosphate. No enzyme fraction was obtained that possessed greater phosphatase activity on phytic acid than on simple phosphate esters. In all, the studies failed to demonstrate an enzyme specific for the dephosphorylation of phytic acid.

The nature of the myoinositol polyphosphate products of the reaction was also investigated by Courtois (1951). The studies involved a comparison of the rates of oxidation of myoinositol hexa-, penta-, tetra-, and triphosphate to the rates of oxidation of reference compounds. The oxidants employed were bromine in bicarbonate solution, potassium bichromate in nitric acid, and sodium periodate in sulfuric acid. The myoinositol polyphosphate esters either were not oxidized or, in the case of tri- and tetraphosphates, were oxidized at a rate much slower than the reference compounds. This behavior was interpreted as indicating the absence of vicinal hydroxyl groups in these esters. Therefore, Courtois proposed that the enzymatic dephosphorylation of phytic acid proceeded as follows.

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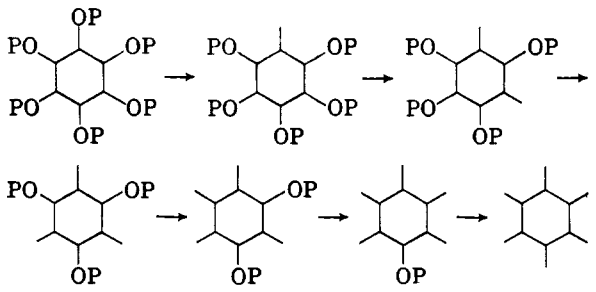


Each intermediate was defined to the extent that it arose from the preceding one by the removal of a phosphate group located *meta* to a free hydroxyl group. However, no absolute structure was proposed for any of the compounds in the sequence. In fact, while the triphosphate fraction was known to be optically active (Posternak and Posternak, 1929), the structure proposed for this isomer has a plane of symmetry. It was suggested (Courtois and Joseph, 1948) that the presence of a small amount of an asymmetric triphosphate could account for this discrepancy.

Desjobert and Petek (1956) have demonstrated that the myoinositol polyphosphate intermediates from the phytase reaction are chromatographically homogeneous. Penta-, tetra-, tri-, and diphosphates, isolated by the method of Posternak and Posternak (1929), gave discrete phosphate-containing spots on paper chromatograms. A simultaneous investigation by Arnold (1956) revealed that the myoinositol polyphosphate fractions were also electrophoretically homogeneous.

It has been established that the major monophosphate product from the reaction is myoinositol 2-phosphate (Iselin, 1949; Fleury *et al.*, 1954; Pizer and Ballou, 1959). The work described in this paper was undertaken to establish the structures of the myoinositol polyphosphates produced in the dephosphorylation of phytic acid by phytase. The following compounds were identified: myoinositol 1- and 2-monophosphate; myoinositol 1,2-diphosphate; myoinositol 1,2,3- and L-1,2,6-triphosphate; myoinositol 1,2,5,6-tetraphosphate plus another uncharacterized isomer. The structure of the myoinositol pentaphosphate component was not established. A preliminary report of this work has appeared (Tomlinson and Ballou, 1961a).

Experimental

Analytical Methods.—Phosphorus determinations were performed by the method of Fiske and SubbaRow (1925) and myoinositol assays according to Atkin, Williams, Schultz, and Frey as described by Snell (1950). A Rudolph Photoelectric polarimeter was used to measure optical rotations on aqueous solutions of the myoinositol polyphosphate cyclohexylamine salts. Infrared spectra of specimens at 1% concentration in potassium bromide pellets were determined with a Baird-Atomic spectrophotometer, Model 4-55.

Chromatography and Electrophoresis.—The solvent systems and conditions for chromatography and electrophoresis of the myoinositol phosphate esters and the sugar polyols have been described in an earlier publication (Tomlinson and Ballou, 1961b).

Materials.—Sodium phytate was prepared from purified calcium phytate (A. E. Staley Mfg. Co., Decatur, Ill.), and phytase was obtained from wheat bran by the methods described by Peers (1953).

Enzymatic Dephosphorylation.—One ml of 0.05 M sodium phytate was mixed with 5 ml of 0.2 M lithium acetate buffer, pH 5.2, containing 10 mg of magnesium sulfate. After the addition of 1 ml of enzyme solution, the total volume was made up to 20 ml by dilution with 13 ml of water. Each mixture was incubated at 55°, and the reaction was followed by the determination of orthophosphate in 0.1-ml aliquots. A control reaction, run without added enzyme, liberated no inorganic phosphate, precluding chemical hydrolysis. Each reaction was stopped by neutralizing the solution to pH 7 with 1 N lithium hydroxide and then boiling it for 5 minutes. The mixture was evaporated to dryness and the residue was extracted with 25 ml of absolute ethanol. The insoluble material (lithium salts of phosphate esters) was separated by centrifugation and dissolved in 4 ml of water. This solution was treated with Dowex 50 (H) to remove the cations. After filtration to remove the resin, the filtrate was made alkaline with 1 N ammonium hydroxide, then concentrated under vacuum to a volume of 0.5 ml. This solution was used for chromatographic and electrophoretic examination (Fig. 1).

Lithium acetate buffer was omitted in the large-scale dephosphorylations. Twenty ml of enzyme solution was added to 380 ml of 5.5×10^{-3} M lithium phytate solution, pH 5.2, containing 20 mg of magnesium chloride. The mixture was incubated at 55° until 50% of the total phosphorus had been liberated as inorganic phosphate. After the solution was adjusted to pH 7 with 1 N lithium hydroxide, it was boiled for 5 minutes and then evaporated under vacuum to a dry residue. The salts were dissolved in 100 ml of water and centrifuged to remove the protein precipitate. This clear solution was used for ion-exchange chromatography.

Ion-Exchange Chromatography.—The myoinositol polyphosphate esters were resolved by ion-exchange column chromatography on Dowex 1 (Cl) resin, with a lithium chloride solution used as the eluent (Table I and Fig. 2) (Hurlburt *et al.*, 1954; Grado and Ballou, 1961).

Identification of Isomers.—The polyphosphate isomers were characterized by their responses to specific tests. The experimental technique and discussion of these tests have been published in detail previously (Tomlinson and Ballou, 1961b).

(a) Partial Dephosphorylation with Alkali.—Each polyphosphate was partially dephosphorylated with concentrated NH_4OH by a sealed tube technique. Subsequently, the myoinositol monophosphates present in the partial hydrolysate were identified by chromatographic comparison with authentic synthetic standards.

(b) Acid-Catalyzed Phosphate Migration.—Acid-induced phosphate migration occurs more readily across *cis*- than *trans*-oriented vicinal

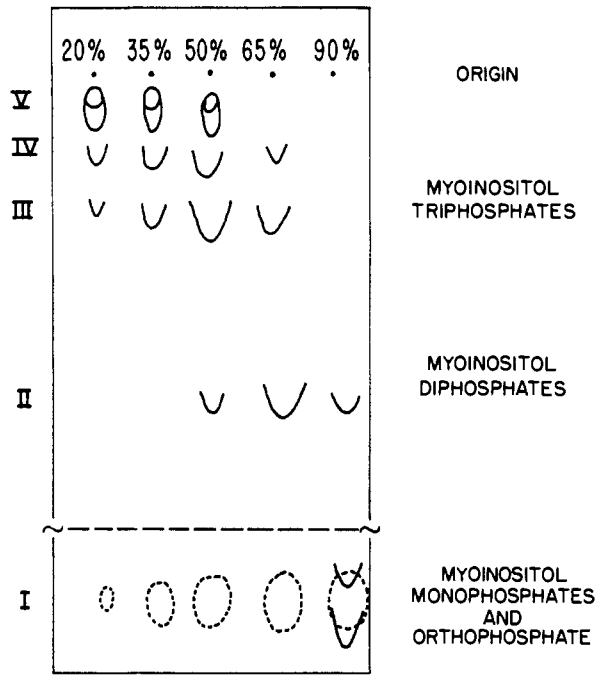


FIG. 1.—Composite chromatogram, run in solvent A, showing the phosphate components present at different stages during the progressive dephosphorylation of phytic acid by phytase. The numbers across the top of the chromatogram represent the percentage of total ester phosphorus liberated as orthophosphate in each experiment. The Roman numerals relate the chromatographic components to the fractions obtained by ion-exchange separation (Fig. 2). To the right of the chromatogram the components are tentatively identified as mono-, di-, and triphosphates, based on the R_F values of synthetic and natural isomeric myoinositol polyphosphates obtained in other work (Grado and Ballou, 1961; Tomlinson and Ballou, 1961b). Inorganic phosphate is indicated by a dotted line.

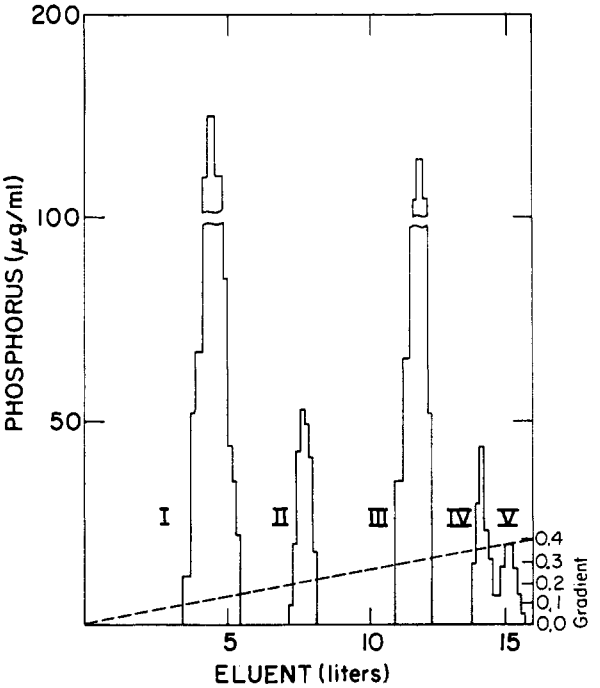


FIG. 2.—The elution pattern obtained on ion-exchange separation on a Dowex 1 (Cl) column of the phosphate products from a large scale enzymatic dephosphorylation of phytic acid stopped after liberation of 50% of the total ester phosphorus.

hydroxyl groups (Pizer and Ballou, 1959). The presence of a readily migratable phosphate group, *i.e.*, one located adjacent to a *cis*-oriented hydroxyl, was determined by acid treatment of the polyphosphate under controlled conditions of pH, temperature, and time. The results from the test were assessed by chromatography.

(c) PERIODATE OXIDATION, REDUCTION, AND DEPHOSPHORYLATION (GENERAL).—On oxidation of a myoinositol polyphosphate there is obtained a phosphorylated dialdehyde, which can be converted to a polyol by reduction and dephosphorylation. The identification of this latter product enables one to restrict the location of the phosphate groups on the original ester to a limited number of positions.

(d) PERIODATE OXIDATION OF THE TETRAPHOSPHATE COMPONENT (MODIFIED CONDITIONS).—It was necessary to use more vigorous conditions than those employed for di- and triphosphate esters to effect oxidation of the tetraphosphate component. Oxidations proceeded satisfactorily at pH 1 and 25° or pH 5 and 100° and when followed by reduction and dephosphorylation gave a known hexitol.

(e) PERIODATE OXIDATION OF THE TETRAPHOSPHATE COMPONENT (ALKALINE PH).[1]—To destroy the oxidizable ester and demonstrate the presence of an unoxidizable ester in the tetraphosphate component, periodate oxidation was carried out as follows. Ten μmole of myoinositol tetraphosphate fraction IV was heated for one hour at 100° in 5 ml of 0.1 M sodium periodate adjusted to pH 8.4. The pH of the solution remained at 8.4 throughout the heating period. The liberation of orthophosphate was measured colorimetrically at 10-minute intervals on 0.2-ml aliquots of the solution, and ceased at a value of about 40% of the total phosphorus after 20 minutes. At the end of this

TABLE I

ANALYSIS OF THE PHOSPHATE-CONTAINING FRACTIONS OBTAINED BY ION-EXCHANGE SEPARATION OF A PARTIAL DIGEST OF PHYTIC ACID WITH PHYTASE

Fraction	Phosphorus/ Myoinositol Ratio	R_{EP}[a] A	R_{EP}[a] B	Electrophoretic Mobility, $R_{picrate}$[b]
I		0.65	0.70	1.4
II	2	0.27	0.55	1.7
III	3	0.17	0.42	2.0
IV	4	0.09	0.35	2.4
V	5.4	0.05	0.23	2.6

[a] Movement relative to that of glycerol 1-phosphate; Solvent A—isopropanol, concd. ammonia, water (7:1:2) (Markham and Smith, 1952); Solvent B—1-propanol, concd. ammonia, water (5:4:1) (Desjobert and Petek, 1956). [b] Movement relative to that of picric acid.

[1] The expected oxidation product from myoinositol 1,2,5,6-tetraphosphate is 2,3,4,5-tetraphospho-6-aldo-D-glucose. Conditions of alkali and heat would cause this intermediate to undergo β-elimination of the phosphate groups on positions 3 and 4, thus allowing further oxidation and complete degradation of the compound.

heating period the solution was cooled, and 80 mg of sodium borohydride was added. The mixture was allowed to stand for 8 hours at room temperature; then it was diluted with water and made acidic by the addition of Dowex 50 (H). When the evolution of hydrogen had ceased, the solution was filtered and concentrated under vacuum. The concentrate, 2 ml, was placed in a small sealed tube and heated at 110° for 48 hours. The contents of the tube were evaporated to dryness under vacuum several times with 1% methanolic hydrogen chloride to remove the boric acid. The residue was dissolved in water, and the solution was deionized by treatment with a mixed bed resin and concentrated for chromatographic and electrophoretic examination. Only myoinositol was detected.

Preparation of Polyol Derivatives.—Ribitol and arabitol were obtained in milligram quantities from a large-scale oxidation of fraction III. They were isolated by chromatographic separation on Whatman 3 MM paper using ethyl acetate, pyridine, saturated aqueous boric acid (65:25:20) as the solvent system (Grado and Ballou, 1961). The compounds were eluted from the paper with water. The eluates were concentrated to dryness under vacuum; then the residue was distilled several times with methanol to remove the boric acid. Dibenzylidene ribitol, prepared from the ribitol specimen by the procedure of Fischer (1893), had a m.p. of 163–165° and an infrared spectrum identical with that of an authentic sample which melted at 163–165°. The melting point of a mixture of the two was not depressed. The arabitol component was acetylated in an acetic anhydride–pyridine mixture. A crystalline product was obtained by adding water to a cold methanol solution of the acetylated compound. The m.p. (73–75°) and infrared spectrum of this derivative were identical with those of an available specimen of authentic L-arabitol penta-acetate, m.p. 76° (Pigman and Goepp, 1948), and differed from those of DL-arabitol penta-acetate, m.p. 95°. The specific rotation of our compound was +37° (in chloroform). The value recorded for D-arabitol penta-acetate is +37.2° (in chloroform) (Hockett and Hudson, 1935).

Chromic Acid Oxidation of Fraction V.—Twenty-seven μmole of mixed myoinositol pentaphosphate and phytic acid was added to 20 μmole of chromic anhydride in 2 ml of acetic acid. In four attempts to carry out an oxidation without cleaving the myoinositol ring, the following conditions were employed: 3 hours at 23°, 3 minutes at 100°, 12 hours at 40°, and 18 hours at 40°. After the oxidation, the solution was neutralized with 1 N sodium hydroxide and 80 to 100 mg of sodium borohydride was added. Five hours later the excess borohydride was destroyed by adding either 1 N acetic acid or Dowex 50 (H) to the solution. The products were dephosphorylated by acid hydrolysis in a sealed tube at 110° for 48 hours or by adjusting the pH of the solution to 5.2 and using phytase. The dephosphorylated solution was evaporated to dryness under vacuum several times with 1% methanolic hydrogen chloride to remove the boric acid. The residue was then dissolved in water, treated with a mixed-bed resin, and concentrated under vacuum for chromatographic (Ballou and Anderson, 1953; Angyal and McHugh, 1957) or electrophoretic (Angyal *et al.*, 1957; Frahm and Mills, 1959) examination of the products.

Results and Discussion

The polyphosphate esters, present at successive stages of dephosphorylation of phytic acid by phytase, were found to appear and disappear in a manner that was correlated with the production of inorganic phosphate, thus confirming the stepwise nature of the dephosphorylation process described by Desjobert and Petek (1956). Seven phosphate-containing components were detected chromatographically. Comparison with reference compounds showed that three of these were orthophosphate, myoinositol 2-phosphate, and myoinositol 1-phosphate. This is the first report that myoinositol 1-phosphate is present at the monophosphate stage in the phytase reaction. On the basis of their chromatographic behavior compared to that of synthetic and natural myoinositol polyphosphates observed in other work (Grado and Ballou, 1961; Tomlinson and Ballou, 1961b), it appeared that two of the four remaining components were a di- and triphosphate (see Fig. 1).

A large-scale dephosphorylation reaction, incubated until 50% of the total ester phosphorus had been liberated as orthophosphate, was fractionated by ion-exchange chromatography and yielded the elution pattern shown in Figure 2. Five components were isolated, each of which was correlated with one of the phosphate-containing spots on the chromatogram shown in Figure 1. The phosphorus-to-myoinositol ratios, chromatographic values, and electrophoretic mobilities of these components are recorded in Table I. Fraction I was orthophosphate. No myoinositol monophosphate was detected, since at 50% dephosphorylation the amount of monophosphate ester is negligible. The phosphorus-to-myoinositol ratios indicated that fractions II, III, and IV were di-, tri-, and tetraphosphate esters. Fraction V was assumed to be composed of a mixture of myoinositol pentaphosphate and phytic acid on the basis of its phosphorus-to-myoinositol ratio. The molecular rotations of fractions II, III, and IV are given in Table II. Although not identical with those found by Posternak (1929), they are of the same sign and magnitude. It is clear that these fractions contain asymmetric

Table II

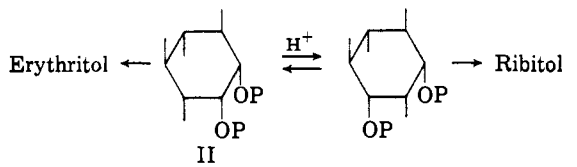
Rotations of the Myoinositol Polyphosphate Fractions Obtained by Ion-Exchange Separation of a Partial Digest of Phytic Acid

Fraction	[M][a] (this work)	[M][b] (by Posternak [1929])
II	−30.5	−45.1
III	−150.2	−118.5
IV	−90.2	−51.2

[a] (c 1.5, aqueous solution of cyclohexylamine salts.)
[b] Calculated from the specific rotations recorded by Posternak (1929), using the expression [M] = $[\alpha]^{25}_{589}$ (molecular weight/100).

myoinositol phosphates which have arisen through a stereospecific dephosphorylation of the phytic acid.

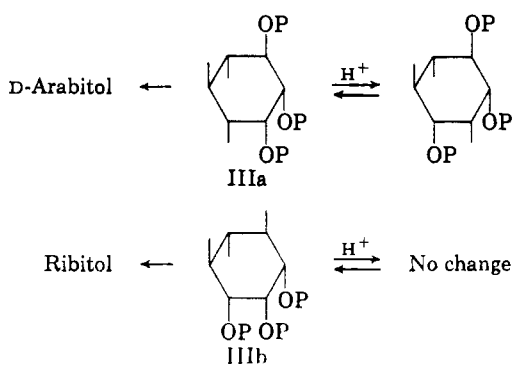
Fraction II was oxidized with sodium periodate and the dialdehyde phosphate product was reduced and dephosphorylated to yield erythritol. Erythritol would be obtained from a myoinositol derivative blocked in the 1- and 2-positions. After mild treatment of fraction II with acid to cause selective phosphate migration (Pizer and Ballou, 1959), chromatographic examination of the products showed the presence of a second phosphate-containing component. The acid-treated mixture yielded ribitol and erythritol by the periodate oxidation technique. The formation of ribitol indicates substitution of the myoinositol in the 1- and 3-positions. These results are explained by the following reaction sequence.



II

Partial dephosphorylation of II with alkali produced myoinositol 1- and 2-phosphate. Thus, II must be predominantly one of the enantiomeric myoinositol 1,2-diphosphates.

Fraction III, which was chromatographically homogeneous, yielded arabitol and ribitol from the periodate oxidation sequence, which proved that it is a mixture of myoinositol 1,2,6- and 1,2,3-triphosphate (IIIa and IIIb). By mild acid treatment of fraction III, a mixture of isomers was produced which could be separated chromatographically, indicating that phosphate migration had occurred. However, the polyols obtained from



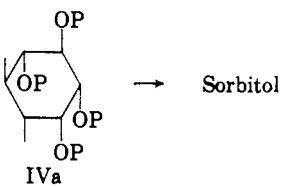
IIIa

IIIb

periodate oxidation of the acid-migration mixture were the same as those from the original. Myoinositol 1,2,3-triphosphate (IIIb) is not expected to be affected by mild treatment with acid, but the 1,2,6-triphosphate (IIIa) isomer should migrate under these conditions to yield myoinositol 1,3,6-triphosphate. The latter should produce altritol in the oxidation sequence with sodium periodate. The failure to detect altritol suggests that this isomer is resistant to oxidation. Partial dephosphorylation of fraction III with base yielded myoinositol 1-, 2-, and 4-phosphates.

The two polyols obtained by periodate oxidation of fraction III were converted to dibenzylidene-ribitol and arabitol penta-acetate. Both derivatives were identical with authentic samples. The specific rotation of the arabitol derivative established that it had the D-configuration. Therefore, the myoinositol 1,2,6-triphosphate has the L-configuration.

Sodium periodate did not oxidize fraction IV when the standard conditions were employed. However, sorbitol was obtained from oxidations carried out at an elevated temperature or low pH. Only myoinositol 1,2,5,6-tetraphosphate (IVa) would yield this hexitol. This tetraphosphate intermediate could lead to myoinositol 1,2,6-triphosphate, but not myoinositol 1,2,3-triphosphate, by the enzymatic hydrolysis of a single phosphate



IVa

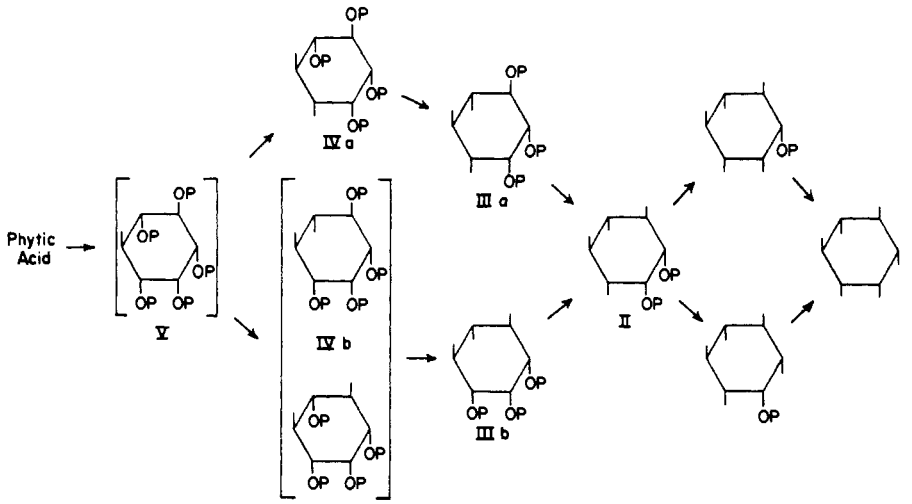
group. When the periodate oxidation of fraction IV was carried out at an elevated temperature and alkaline pH, the liberation of orthophosphate reached a maximum and stopped at about 40% of the total ester phosphorus. The polyol product obtained from the reduction and dephosphorylation of this mixture was myoinositol, which suggests the presence of a second tetraphosphate (IVb) in fraction IV that remained unoxidized. This unoxidized tetraphosphate may be the precursor of myoinositol 1,2,3-triphosphate. If so, it must be either myoinositol 1,2,3,5- or 1,2,3,4-tetraphosphate.

Fraction V probably contained a pentaphosphate ester of myoinositol (Table I), but various attempts at its characterization were unsuccessful.

The scheme of dephosphorylation of phytic acid depicts the compounds studied in this work arranged in order of decreasing number of phosphate groups and in a sequence reflecting obvious structural relationships. The figures enclosed in brackets are structures proposed for incompletely characterized components.

Certain features of this scheme suggest the way in which the hydrolysis of phytic acid by phytase occurs. Following the pentaphosphate stage, it would appear that there are two pathways of dephosphorylation. Although the pentaphosphate was not characterized, L-myoinositol 1,2,3,5,-6-pentaphosphate (V) is the only one which could be a common precursor of all of the lower esters found in the mixture. Thus, if the initial attack on phytic acid is selective for one phosphate group, then it must be that one on the L-4-position. All of the characterized esters in the series have their phosphate groups situated adjacent to one another. This is understandable if, after the initial dephosphorylation, each subsequent phosphate group removed is a less hindered one next to a free hydroxyl group.

The 1,2,5,6-tetraphosphate (IVa) is probably the precursor of IIIa, and since IIIa has the L-



Scheme of Dephosphorylation of Phytic Acid

configuration it is likely that this tetraphosphate has the same configuration. The amount of sorbitol obtained in the periodate oxidation was not adequate to confirm this supposition.

Similarly, the 1,2-diphosphate derived from IIIa must have the L-configuration. However, the 1,2-diphosphate from IIIb could be of either configuration or a racemate, depending on the specificity shown by the phytase at this stage. The fact that II is optically active does not prove it is a pure enantiomer, but only that it predominates in one, probably the L-form.

Although some of the intermediates have been shown to be asymmetric, all yield as the major monophosphate product a symmetrical compound. This must be due to the relative inaccessibility of the single axially oriented phosphate group on position 2. This same effect is probably involved in the selective formation of the 1,2,5,6-tetraphosphate (IVa) and the 1,2,6-triphosphate (IIIa).

References

Angyal, S. J., McHugh, D. J., and Gilham, P. T. (1957), *J. Chem. Soc.* 1423.

Angyal, S. J., and McHugh, D. J. (1957), *J. Chem. Soc.* 1432.

Arnold, P. W. (1956), *Biochim. et Biophys. Acta 19*, 552.

Ballou, C. E., and Anderson, A. B. (1953), *J. Am. Chem. Soc. 75*, 648.

Courtois, J., and Joseph, G. (1948), *Bull. soc. chim. biol. 30*, 610.

Courtois, J. (1951), *Bull. soc. chim. biol. 33*, 1075.

Desjobert, A., and Petek, F. (1956), *Bull. soc. chim. biol. 38*, 871.

Fischer, E. (1893), *Ber. 26*, 633.

Fiske, C. N., and SubbaRow, Y. (1925), *J. Biol. Chem. 66*, 375.

Fleury, P., Desjobert, A., and Lecocq, J. (1954), *Bull. soc. chim. biol. 36*, 1301.

Frahm, J. L., and Mills, J. A. (1959), *Australian J. Chem. 12*, 65.

Grado, C., and Ballou, C. E. (1961), *J. Biol. Chem. 236*, 54.

Hockett, R. C., and Hudson, C. S. (1935), *J. Am. Chem. Soc. 57*, 1753.

Hurlburt, R. B., Schmitz, H., Brunn, A. F., and Potter, V. R. (1954), *J. Biol. Chem. 209*, 23.

Iselin, B. M. (1949), *J. Am. Chem. Soc. 71*, 3822.

Markham, R., and Smith, J. D. (1952), *Biochem. J. 52*, 552.

Peers, F. G. (1953), *Biochem. J. 53*, 102.

Pigman, W. W., and Goepp, R. M., Jr. (1948), Chemistry of the Carbohydrates, New York, Academic Press, Inc., p. 235.

Pizer, F. L., and Ballou, C. E. (1959), *J. Am. Chem. Soc. 81*, 915.

Posternak, S., and Posternak, T. (1929), *Helv. Chim. Acta 12*, 1165.

Snell, E. E. (1950), *in* Vitamin Methods, Gyorgy, P., ed., vol. I, New York, Academic Press Inc., p. 445.

Tomlinson, R. V., and Ballou, C. E. (1961a), Abstracts of Papers, 139th Meeting American Chemical Society, p. 12D.

Tomlinson, R. V., and Ballou, C. E. (1961b), *J. Biol. Chem. 236*, 1902.