INOSITOL PHOSPHATES IN FOODS

BRIAN Q. PHILLIPPY

United States Department of Agriculture
Agricultural Research Service
Southern Regional Research Center
New Orleans, LA 70124
USA

I. INTRODUCTION

Since the middle of the twentieth century, *myo*-inositol hexakisphosphate, which is commonly known as phytate, has been recognized as an antinutrient for its ability to bind to, precipitate and decrease the bioavailability of di- and trivalent cationic minerals. Phytate is present in all seeds, usually at

ADVANCES IN FOOD AND NUTRITION RESEARCH VOL 45
ISBN: 0-12-016445-0

levels between approximately 0.5 and 2% of their dry weight. In diets containing a large proportion of calories derived from grains and/or legumes, an imbalance of phytate and minerals can lead to nutritional deficiencies. Trace minerals such as zinc and iron bind to phytate most tightly and are affected to a greater degree than calcium. A comprehensive review of this subject was published by Reddy *et al.* (1989) and updated by Zhou and Erdman (1995) and Weaver and Kannan (2002).

Until the 1980s, phytate in foods was almost always quantified using methods that were not specific for inositol hexakisphosphate. During that decade, high-performance liquid chromatography (HPLC) procedures for inositol phosphate analysis opened the way for significant advances in the collection of more accurate data. Recognition that inositol phosphates containing different numbers of phosphate groups were present in appreciable amounts in many foods also created a dilemma as to how the older data should be interpreted. This problem was magnified by the discovery that the different inositol phosphates had different effects on the bioavailability of minerals.

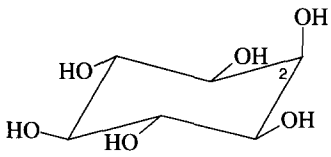
An additional layer of complexity arose at that same time with the ability of some HPLC methods to separate some of the inositol phosphate isomers. Although isomers containing the same number of phosphate groups have not been shown to differ much in their effects on mineral availability, some chemical properties and biological functions have been linked to specific structures. Appreciation of the fundamental importance of inositol phosphates in basic cell physiology coupled with experimental data involving humans and other animals has led to a re-evaluation of the roles of inositol phosphates in food.

In this review the current knowledge about these compounds is compiled and organized in an attempt to provide a context for its interpretation and to create a framework from which scientists can formulate ideas for future research.

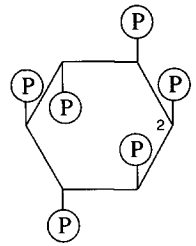
II. CHEMISTRY OF INOSITOL PHOSPHATES

A. NOMENCLATURE

There have been several changes in the rules and conventions for naming inositol phosphates since the first *myo*-inositol monophosphate isomer was identified in soybeans by Ballou and Pizer (1959). This resulted in some confusion in the literature of the following years, when the names assigned to enantiomers became switched and subsequently simplified. The current guidelines were issued in 1989 (NC-IUB, 1989).



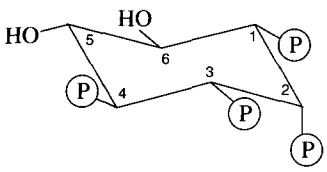
I



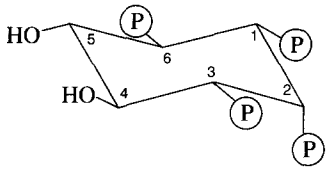
II

Thorough discussions of the naming and numbering of the structures of inositol and inositol phosphates are provided by Posternak (1965) and Cosgrove and Irving (1980). *Myo*-inositol is one of nine isomers of inositol, which is the common name for cyclohexanehexols. It contains five equatorial and one axial hydroxyl groups numbered from one to six, with the axial hydroxyl designated as position number 2 (**I**). The molecule is symmetrical on either side of an axis formed by positions 2 and 5. Thus positions 1 and 3 are equivalent, as are positions 4 and 6. The first numbering convention was to depict the hydroxyls as a fraction, arranged with the most numerous hydroxyls above the inositol ring and listed in the numerator and those below the ring in the denominator. *Cis* hydroxyl groups were assigned the lowest possible numbers. Accordingly, phytate was referred to as *myo*-inositol 1,2,3,5/4,6-hexakisphosphate (**II**). Originally inositol phosphate isomers were numbered according to the conventions of carbohydrate chemistry. Optically active isomers were named based on the configuration of D- and L-glyceraldehyde (Lardy, 1954). Phosphates were given the lowest possible numbers and assigned the prefix D when numbered clockwise with the hydroxyl or phosphate at position 1 projecting upward, and L when numbered counterclockwise. Symmetrical isomers, also known as meso compounds, such as inositol 1,3-bisphosphate, had no D or L.

A major reversal of these rules occurred in 1967, when the International Union of Pure and Applied Chemistry and the International Union of Biochemistry decided to change the nomenclature system for cyclitols (IUPAC-IUB, 1968). Following these rules, the positions were still numbered in the direction that would have the lowest possible number for the first phosphate group, but designated as D if the numbers proceeded in a clockwise direction when the hydroxyl or phosphate at position 1 projected downward, or L if the numbers rotated counterclockwise when the hydroxyl or phosphate at position 1 projected downward. The result of this change was that the D and L assignments of all of the inositol



III



IV

phosphate names in the literature prior to the implementation of these changes became switched. It was also decided at that time to use the Greek prefixes bis, tris, tetrakis, pentakis and hexakis with inositol phosphates to show that the phosphates are singly attached to the inositol carbons. In contrast, pyrophosphate linkages are represented with the Latin prefixes di, tri, tetra, etc., as in nucleotides and inositol polyphosphate pyrophosphates.

An example of these rules is shown below for the enantiomeric pair of D- and L-myo-inositol 1,2,3,4-tetrakisphosphate (**III, IV**). Pairs of enantiomers are referred to as D and/or L when the proportion of isomers is unequal or unknown, and DL when the isomers are in equal proportion, i.e. a racemic mixture.

Another revision followed the discovery in 1983 that D-*myo*-inositol 1,4,5-trisphosphate was a second messenger in signaling events that released calcium from intracellular stores to activate various biochemical reactions. In just a few years a complex pathway of inositol phosphate metabolism was uncovered, and hundreds of scientific articles were published. The abbreviation that became widely used at this time was Ins, preceded by a D or L, as needed, and followed by the phosphate positions enclosed in parentheses, and finally a capital P with a subscript to denote the number of phosphates when more than one. In addition, the prefix *myo* was omitted. Since most of the isomers in these studies had the D configuration, in 1988 the Nomenclature Committee of IUB again decided to modify the rules and name all isomers according to the D orientation and omit the D or L (NC-IUB, 1989). Using the new rules, structures **III** and **IV** would be inositol 1,2,3,4-tetrakisphosphate and inositol 1,2,3,6-tetrakisphosphate, or, in abbreviated form, Ins(1,2,3,4)P_4 and Ins(1,2,3,6)P_4, respectively. Inositol phospholipids, or phosphoinositides, are abbreviated similarly, using PtdIns to represent phosphatidylinositol, e.g. PtdIns(4,5)P_2.

B. ANALYSIS

The specificity and accuracy of the analytical methods for phytate and other *myo*-inositol phosphates has evolved to the point where the means

available to collect information may be greater than the needs of most food and nutrition scientists. The diversity of methods currently in use poses some intricate questions about exactly what data are most desirable and how they should be presented. There is no doubt that the older methods for phytate analysis are not very specific, but some are still used, and, for many food products, provide the only data available in the literature.

Prior to the development of HPLC methods that separate inositol phosphates from one another, most of these compounds usually were measured together to give data intended to represent phytate. These procedures included numerous variations of the ferric chloride precipitation method and the ion exchange method that was approved by the Association of Official Analytical Chemists in 1988. Components of foods such as oxalic acid (McKenzie-Parnell and Guthrie, 1986), gallic acid (Bos *et al.*, 1991), chlorogenic acid (Bos *et al.*, 1991) and polyphosphate compounds that do not contain inositol, such as nucleotides (Phillippy *et al.*, 1988), could also give elevated phytate values. Nevertheless, data obtained from seeds in their native state should be reasonably accurate, since any interfering compounds are likely to be present in small amounts compared to the large quantities of phytate. However, values from foods in which the phytate may have been partially degraded by enzymatic or thermal processes must be viewed with some caution. The low phytate values reported in fruits and vegetables are especially questionable, since those data are the most likely to be significantly inflated due to the presence of nucleotides, oxalate, etc.

HPLC methods specific for phytate first appeared in the early 1980s and were soon expanded to quantify different inositol phosphates present in foods. At about the same time, the burgeoning research in signal transduction revolving around inositol 1,4,5-trisphosphate led to extremely sensitive methods for measuring inositol phosphates in animal cells. The methods developed in these related disciplines have been reviewed (Irvine, 1990; Xu *et al.*, 1992; Skoglund and Sandberg, 2002).

The currently used HPLC methods for the separation of inositol phosphates fall into two basic categories: ion pair and ion exchange. The ion pair procedure developed by Sandberg and Adherinne (1986) has been used the most often by food scientists because it separates inositol tris-, tetrakis-, pentakis- and hexakisphosphates based only on the number of phosphate groups. This simplifies their quantification and provides all the information that is usually wanted. The disadvantages are that isomers are not separated and that other polyphosphates such as nucleotides can interfere (Morris and Hill, 1996). However, nucleotides such as ATP do not appear to be present in sufficient quantities in mature ungerminated

seeds to significantly elevate the data, so this is probably a minor limitation. Some modifications have been suggested to improve the original method (Lehrfeld, 1994). Recently, an inability to use the ion pair method to measure the inositol phosphates in infant cereals was attributed to the combined high mineral and low phytate contents of these foods (Brooks and Lampi, 2001).

The other type of HPLC procedure separates some of the inositol phosphate isomers by ion exchange. The method developed by Phillippy and Bland (1988) separates phytate and some isomers of inositol tris-, tetrakis- and pentakisphosphate. An improved method can now also separate inositol bisphosphates and a few more of the other isomers (Skoglund *et al.*, 1997a, 1998; Carlsson *et al.*, 2001). The ion exchange procedures provide more extensive data than those using ion pairing and are most useful in identifying the specific isomers present in food products or enzymatic reactions. In many cases, however, routine quantification of the numerous individual isomers present in foods may be neither practical nor justified unless the additional information is specifically desired. Whereas the above ion exchange methods employing acidic eluants give better separation of the most highly phosphorylated inositol phosphates, high pH eluants have been found useful in separating those with the lowest numbers of phosphates (Skoglund *et al.*, 1997b, 1998). Ion exchange HPLC can also be used to separate inositol bis- to hexakisphosphates based solely on the number of phosphate groups (Rounds and Nielsen, 1993), but one should beware of the possible interferences from nucleotides such as ADP, which is present in mature soybean seeds in almost a ten-fold excess over ATP (Phillippy *et al.*, 1994). Recently, ion exchange chromatography with high pH eluants and conductivity detection has been used to analyze phytate, other inositol phosphates and inorganic polyphosphates in foods (Sekiguchi *et al.*, 2000; Talamond *et al.*, 2000).

Additional selective methods to analyze inositol phosphates include capillary chromatography and nuclear magnetic resonance (NMR). Several capillary electrophoresis methods have been developed to separate phytic acid and other inositol phosphates, but they do not appear to have been adopted by the scientific community, perhaps because they need further refinement (Skoglund and Sandberg, 2002). NMR methods can be used to simultaneously determine phytic acid and other inositol phosphates in a mixture (Johnson *et al.*, 1995), and ^{31}P NMR has been used to quantify some of the inositol phosphates in complete and digested feeds (Kemme *et al.*, 1999). The newest evaporative light scattering detectors have the potential to significantly lower the HPLC detection limits for the most highly phosphorylated inositol phosphates in food extracts, provided that the background from interfering compounds is not too high.

III. METABOLISM OF INOSITOL PHOSPHATES

A. SYNTHESIS

The pathway for the synthesis of phytate has not yet been defined with complete certainty. One reason for this is that there are numerous branch points between *myo*-inositol and phytate, and a single direct pathway may simply be inadequate to portray this complex web of interconnected reactions. Another reason may be that there is more than one possible route, resulting in redundancy to help ensure the production of sufficient phytate to meet the needs of a particular type of cell. In nature different pathways for phytate synthesis appear to have been favored during the evolution of the diverse species of microorganisms and higher life forms. It is believed that all cells of all organisms probably contain some phytate. Studies of the simplest life forms have provided insights into the possible pathways for phytate synthesis in higher plants and animals.

A complete pathway has been reported for the slime mold *Dictyostelium* (Stephens and Irvine, 1990); the intermediates were identified as Ins(3)P, Ins(3,6)P_2, Ins(3,4,6)P_3, Ins(1,3,4,6)P_4 and Ins(1,3,4,5,6)P_5. Each of these isomers was detected in *Dictyostelium* cells or homogenates incubated with [^{3}H]inositol, and each of them was converted to InsP_6 by *Dictyostelium* homogenates in separate experiments. Ins(1,2,4,5,6)P_5 and Ins(1,2,3,4,6)P_5 were also detected in the cells and homogenates and could be phosphorylated to InsP_6. However, these pentakisphosphates were probably not the main precursor of InsP_6, because they contained lower specific radioactivities than Ins(1,3,4,5,6)P_5, and because they, and not the latter, were observed as dephosphorylation products of InsP_6. *Dictyostelium* contains inositol 3-kinase activity, but Ins(3)P may also be derived in part from glucose 6-phosphate or phosphatidylinositol 3-phosphate (Stephens *et al.*, 1990). Another pathway for InsP_6 synthesis in *Dictyostelium* has been observed, starting with Ins(1,4,5)P_3, which is the inositol phosphate second messenger involved in signaling via calcium release. This pathway was located in cell nuclei, and the intermediates were identified as Ins(1,3,4,5)P_4 and Ins(1,3,4,5,6)P_5 (Van der Kaay *et al.*, 1995). Somewhat similarly, a soluble fraction from the yeast *Schizosaccharomyces pombe* converts Ins(1,4,5)P_3 into Ins(1,3,4,5,6)P_5 and InsP_6 primarily through Ins(1,4,5,6)P_4, but also partially through Ins(1,3,4,5)P_4 (Ongusaha *et al.*, 1998).

In animal cells the first pathway identified for InsP_6 synthesis begins with the cleavage of Ins(1,4,5)P_3 from PtdIns(4,5)P_2, followed by the sequential formation of Ins(1,3,4,5)P_4, Ins(1,3,4)P_3, Ins(1,3,4,6)P_4 and Ins(1,3,4,5,6)P_5 (Shears, 1989). The latter is by far the predominant InsP_5 isomer in animal cells, which also contain the 2-kinase that phosphorylates

it to form $InsP_6$ (Ji *et al.*, 1989; Stephens *et al.*, 1991). Evidence from avian erythrocytes suggests that Ins(1,3,4,5,6)P_5 could also be produced from Ins(1)P via the stepwise formation of Ins(1,6)P_2, Ins(1,4,6)P_3, Ins(1,3,4,6)P_4, Ins(3,4,6)P_3 and Ins(3,4,5,6)P_4 (Stephens and Downes, 1990). In addition, a 3-kinase that phosphorylates Ins(1,4,5,6)P_4 as well as Ins(1,2,4,5,6)P_5 has been detected in rat liver (Craxton *et al.*, 1994). Recently, an inositol polyphosphate multikinase that can convert Ins(4,5)P_2 to Ins(1,3,4,5,6)P_5 via Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 was cloned from a rat cDNA library (Saiardi *et al.*, 2001). The enzymes mentioned above and others form a complex network leading to $InsP_6$ that may provide redundancy to ensure its synthesis and/or regulatory control over the cellular concentrations of its metabolites. Nevertheless, the fundamental route of $InsP_6$ synthesis in animals is currently unresolved (Irvine and Schell, 2001).

The inositol polyphosphate pathway in plants also appears to have alternate routes to make $InsP_6$. As in animals, a likely precursor is Ins(1,3,4,5,6)P_5. Kinases that can phosphorylate Ins(1,3,4)P_3 to Ins(1,3,4,5)P_4 and Ins(1,3,4,5,6)P_5 have been identified in Arabidopsis and soybean seeds (Wilson and Majerus, 1997; Phillippy, 1998a). Some evidence has been obtained for the sequential phosphorylation of Ins(3)P, Ins(3,4)P_2, Ins(3,4,6)P_3, Ins(3,4,5,6)P_4, and Ins(1,3,4,5,6)P_5 in *Spirodela polyrhiza* and *Commelina communis* (Brearly and Hanke, 1996, 2000). Kinases that phosphorylate several of the inositol pentakisphosphates already containing a phosphate at position 2 have also been observed in mung bean and soybean seeds (Stephens *et al.*, 1991; Phillippy *et al.*, 1994), but those isomers may arise from the degradation of $InsP_6$ rather than its synthesis. In addition, an Ins(1,4,5)P_3 6-kinase has been identified in pea roots (Chattaway *et al.*, 1992). Transcripts of the genes for L-Ins(1)P, which is the same as D-Ins(3)P, were observed in the embryo and aleurone layer of developing rice seeds shortly before the appearance of phytate-containing particles called globoids (Yoshida *et al.*, 1999). Furthermore, two maize mutants deficient in $InsP_6$ synthesis produced reduced amounts of $InsP_6$, and one had increased levels of other inositol phosphates, though the affected genes have yet to be identified unequivocally (Raboy *et al.*, 2000). One of two types of barley mutants produced less than one fourth as much $InsP_6$ as the parent line and accumulated 15% of the inositol-bound phosphorus in D and/or L-Ins(1,3,4,5)P_4, hypothetically due to a mutated Ins(1,3,4,5)P_4 6-kinase gene (Hatzack *et al.*, 2000, 2001).

B. DEGRADATION

Animals, plants and microorganisms all make enzymes that break down phytic acid. Phytases are phosphatases with the ability to use $InsP_6$ as a

substrate, whereas phytases and other phosphatases can hydrolyze the various $InsP_5$, $InsP_4$, $InsP_3$, $InsP_2$ and InsP isomers. Monogastric animals including humans lack sufficient phytase in their guts to adequately break down the $InsP_6$ in diets high in whole grains or legumes. Therefore phytases from plants and microorganisms are sometimes utilized to help degrade the inositol phosphates before and/or after foods are eaten. Inositol phosphates in foods can also be degraded by high temperatures and pressures during thermal processes such as frying and canning. The reduction of phytate content during food processing has been thoroughly reviewed by Reddy *et al.* (1989) and Sathe and Venkatachalam (2002).

$InsP_6$ and other inositol phosphates in food may be hydrolyzed by phytases as the food is prepared or while it passes through the gastrointestinal tract. The endogenous phytases of seeds can remove much of the $InsP_6$ in grains and legumes if they are soaked in aqueous solutions for a number of hours prior to cooking (Larsson and Sandberg, 1992; Gustafsson and Sandberg, 1995; Fredlund *et al.*, 1997; Bergman *et al.*, 1999). The absorption of water initiates the germination of seeds and activates any phytases already present. New enzymes are also synthesized from freshly transcribed RNA. Some ungerminated seeds such as rye contain significant amounts of phytase (Greiner *et al.*, 1998), while others such as maize have very little (Laboure *et al.*, 1993). Fermentation can destroy much of the $InsP_6$ in foods due to the action of microbial and plant phytases (Sutardi and Buckle, 1985; Gustafsson and Sandberg, 1995; Türk *et al.*, 1996). After food is eaten, any phytases present in the food can break down phytate in the stomach.

The amount of phytase activity in different types of cells and tissues does not appear to be correlated with their $InsP_6$ content. Ungerminated rye grain contained phytase activity of 3.2 μmol min^{-1} g^{-1} at 35°C, and this activity remained fairly constant during 10 days of germination (Greiner *et al.*, 1998). Although ungerminated spelt and barley have negligible phytase activity, after 2 and 4 days, respectively, of germination 1.1 and 1.35 μmol min^{-1} g^{-1} of activity at 35°C had accumulated (Koneitzny *et al.*, 1994; Greiner *et al.*, 2000b). Maize roots contained 0.50 μmol min^{-1} g^{-1} of phytase activity at 40°C (Hübel and Beck, 1996). In rat small intestine the duodenum, jejunum and ileum contained respectively 6.0, 1.3 and 1.0 μmol min^{-1} g^{-1} of activity at 60°C (Rao and Ramakrishnan, 1985), and in rat intestinal mucosal tissue the phytase activity was 0.36 μmol min^{-1} g^{-1} at 37°C (Yang *et al.*, 1991a). Vegetables contain phytase activities at levels up to 0.15 μmol min^{-1} g^{-1} at 37°C, which is present in green onions (Phillippy, 1998b). The most widely utilized microbial phytases are secreted, although the *Escherichia coli* enzyme is strictly an intracellular protein.

Numerous phytases and other inositol polyphosphate phosphatases have been purified, and some of them have been cloned for experimental and industrial production. The specific activities of some of the purified phytases along with their pH optima are shown in Table I. The phytases with the highest activities *in vitro* are from *E. coli* and *Peniophora lycii*. Though few of the animal phytases have been purified and studied, their potential contributions to the breakdown of phytate in foods may justify a more thorough exploration of ways to increase their impact.

TABLE I
ACTIVITIES OF PURIFIED PHYTASES

Source	Specific activity μmol min^{-1} mg^{-1}	(°C)	pH optimum	Reference
Rice bran	50	(40)	4.4	Hayakawa *et al.* (1989)
Maize seedlings	2.3	(55)	4.8	Laboure *et al.* (1993)
Spelt seedlings	262	(35)	6.0	Konietzny *et al* (1994)
Maize roots	64	(40)	5.0	Hübel and Beck, (1996)
Tomato roots	285	(37)	4.3	Li *et al.* (1997)
Rye seeds	517	(35)	6.0	Greiner *et al.* (1998)
Scallion leaves	500	(37)	5.5	Phillippy (1998b)
Wheat bran	260	(37)	6.0	Nakano *et al.* (1999)
Oat seedlings	307	(35)	5.0	Greiner and Alminger (1999)
Barley seedlings	117	(35)	5.0	Greiner *et al.* (2000b)
Faba bean seedlings	636	(35)	5.0	Greiner *et al.* (2001b)
Rat intestinal mucosa	5.7	(37)	7.5	Yang *et al.* (1991a)
Rat liver	0.01	(37)	7.4	Nogimori *et al.* (1991)
Schwanniomyces castelli	441	(70)	4.4	Segueilha *et al.* (1992)
Aspergillus niger	126	(58)	5.5	Ullah and Gibson (1987)
Thermophilus lanuginosus	110	(37)	6	Berka *et al.* (1998)
Peniophora lycii	987	(37)	4	Lassen *et al.* (1998)
Aspergillus terreus	142	(37)	6.5	Wyss *et al.* (1999)
Aspergillus fumigatus	26	(37)	6.4	Wyss *et al.* (1999)
Emericella nidulans	29	(37)	6.5	Wyss *et al.* (1999)
Myceliophthora thermophila	42	(37)	5.5	Wyss *et al.* (1999)
E. coli M15	811	(37)	4.6	Wyss *et al.* (1999)
E. coli K12	8016	(35)	4.5	Greiner *et al.* (1993)
Klebsiella terrigena	205	(35)	5.0	Greiner *et al.* (1997)
Bacillus sp. DS11	20	(37)	7.0	Kim *et al.* (1998)
Bacillus subtilis	88	(37)	7	Kerovuo *et al.* (1998)

Phytases are often categorized by their specificity in removing the first phosphate group from $InsP_6$ to form $InsP_5$. To some extent, the initial site of attack appears to be related to the pH optimum of the enzyme. At pH 2.0, which is the lower of its two pH optima, the phytase from the fungus *Aspergillus niger* produces mainly Ins(1,2,4,5,6)P_5 (Irving and Cosgrove, 1972). At its higher optimum of pH 5.5 this enzyme still prefers to hydrolyze the 3-phosphate, but forms an increased proportion of Ins(1,2,3,4,5)P_5. The phytase from the fungus *P. lycii* appears to form the same products, with increased hydrolysis of the 4- or 6-phosphate at pH 5.5 compared to pH 3.5, but at both pH values Ins(1,2,3,4,5)P_5 or Ins(1,2,3,5,6)P_5 is the major product (Lassen *et al.*, 1998). NMR was used to identify the products from *P. lycii* phytase but, like HPLC, this technique cannot differentiate between the chemically equivalent 4 and 6 or the 1 and 3 positions. The phytase from the yeast *Saccharomyces cerevisiae* appears to form only Ins(1,2,4,5,6)P_5 at pH 4.5 (Greiner *et al.*, 2001a). *E. coli* phytase has an optimum of pH 4.5 and degrades $InsP_6$ via Ins(1,2,3,4,5)P_5 (Greiner *et al.*, 2000a). The most studied plant phytase, from wheat bran, has maximal activity at pH 5.2 and produces predominantly Ins(1,2,3,5,6)P_5 (Tomlinson and Ballou, 1962). *Paramecium* phytase has a pH 7.0 activity optimum (Freund *et al.*, 1992) and initially forms Ins(1,2,3,4,5)P_5 (Van der Kaay and Van Haastert, 1995). Little is known about the plant alkaline phytases such as the lily pollen enzyme, which has an optimum rate at pH 8 and yields Ins(1,2,3,4,6)P_5 (Barrientos *et al.*, 1994). The relatively nonspecific multiple inositol polyphosphate phosphatase (MIPP) from rat liver forms similar amounts of (Ins(1,2,4,5,6)P_5 and/or Ins(2,3,4,5,6)P_5), (Ins(1,2,3,4,5)P_5 and/or Ins(1,2,3,5,6)P_5), Ins(1,2,3,4,6)P_5 and Ins(1,3,4,5,6)P_5 from $InsP_6$ at pH 7.4 (Nogimori *et al.*, 1991). However, in the presence of 200 μM Al^{3+}, the dominant product from MIPP is Ins(1,2,3,4,6)P_5 (Ali *et al.*, 1995).

Following the removal of the first phosphate from $InsP_6$, some phytases and other phosphatases proceed to remove additional phosphates adjacent to a hydroxyl group. Thus the wheat phytase hydrolyzes Ins(1,2,3,5,6)P_5 to Ins(1,2,5,6)P_4 and Ins(1,2,3,6)P_4, followed by the formation of Ins(1,2,6)P_3, Ins(1,2,3)P_3 and Ins(1,5,6)P_3, and so on, until only Ins(1)P, Ins(2)P and possibly *myo*-inositol remain (Tomlinson and Ballou, 1962; Phillippy, 1989; Nakano *et al.*, 2000). Phytases from other cereal seeds follow a similar sequence, producing Ins(1,2,3,5,6)P_5, Ins (1,2,5,6)P_4, Ins(1,2,6)P_3, Ins(1,2)P_2 and Ins(2)P (Greiner and Alminger, 2001). *A. niger* phytase leaves only Ins(2)P, whereas a combination of the phytase and the pH 2.5 optimum acid phosphatase from *A. niger* removes all six phosphates (Wyss *et al.*, 1999). In contrast to wheat phytase, the

predominant isomer formed by *Dictyostelium* phytase from Ins(1,2,3,6)P_4 is Ins(2,3,6)P_3 (Adelt *et al.*, 2001). The calcium-dependent phytase from *Bacillus subtilis* appears to cleave alternate rather than adjacent phosphates, resulting in Ins(2,4,6)P_3 and Ins(1,3,5)P_3 as the end products (Kerovuo *et al.*, 2000). Inositol phosphates that are intermediates in the synthesis of InsP_6 can be degraded by various phosphatases with characteristic specificies. For example, *Dictyostelium* and the rat liver MIPP can produce Ins(1,4,5)P_3 from Ins(1,3,4,5,6)P_5 using either Ins(1,3,4,5)P_4 or Ins(1,4,5,6)P_4 as an intermediate (Van Dijken *et al.*, 1995). Additional inositol phosphate phosphatases have been reviewed by Shears (1989).

Thermal degradation of phytate is accelerated by low pH and high pressure. Upon hydrolysis of pure InsP_6 in solution by autoclaving for 1 h at 121°C, the reduction in InsP_6 content at pH 4.0, 7.0 and 10.7 was 81, 64 and 43%, respectively (Phillippy *et al.*, 1987). The inositol phosphate breakdown products from InsP_6 autoclaved at pH 4.0, resembled those formed by *A. niger* phytase, with Ins(1,2,4,5,6)P_5 and/or Ins(2,3,4,5,6)P_5 being the predominant InsP_5 isomer, followed by Ins(1,2,3,4,5)P_5 and/or Ins(1,2,3,5,6)P_5 (Phillippy and Bland, 1988). At pH 10.8 the hydrolysis showed much less specificity, which resulted in a more even distribution of isomers.

IV. INOSITOL PHOSPHATES IN SEEDS

A. WHOLE RAW SEEDS

The predominant inositol phosphate in whole grains, legumes and nuts is InsP_6, which may account for approximately 0.4–6% of their dry weight (Reddy, 2002). Compared to the InsP_6 content, the other inositol phosphates in raw and dried seeds are present in relatively minor amounts. For this reason, data obtained using some of the nonspecific methods for phytate analysis that measure all polyphosphate compounds, although not 100% accurate, provide estimates of the InsP_6 in these materials. InsP_6 content is partly determined by the phosphate level of the soil, and InsP_6 accumulates mainly during the final stages of soybean seed development (Raboy and Dickinson, 1987). Levels of substances such as nucleotides that interfere in its analysis would not be expected to be influenced by variations in growing conditions to as great an extent as InsP_6. Mechanical processing of dry seeds such as grinding and milling do not result in significant enzymatic or thermal degradation of InsP_6. However, since InsP_6 is often concentrated in specific areas of seeds (Reddy *et al.*, 1989), separation of different anatomical parts may lower or raise the percentage of InsP_6 in the products as compared to the whole seeds. This is especially true for grains such as rice and wheat, where the InsP_6 is found mostly in

the bran and germ. Processing strategies to extract $InsP_6$ from foods derived from seeds have been extensively reviewed (Reddy *et al.*, 1989; Sathe and Venkatachalam, 2002).

The inositol phosphate contents of some of the most widely utilized seeds used as food are listed in Table II. Literature values have been uniformly converted to g/100 g as shown in parentheses because these units are usually used to portray the nutritional composition of foods and they are easy to compare with percentage data, which has traditionally been used to report phytate content. Values of $InsP_6$, $InsP_5$, $InsP_4$ and $InsP_3$ were converted from μmol/g to g/100 g by multiplying by 0.066, 0.058, 0.050 and 0.042, respectively. As mentioned above, $InsP_6$ levels can fluctuate considerably due to environmental and processing effects, and additional variation can result from genetic differences. Therefore the data in Table II represent random examples that may be more or less typical of each kind of seed as it exists throughout the world. $InsP_6$ comprises more than 75% of the inositol phosphates in most of these seeds, and the $InsP_6$ values fall within or near the ranges of the nonspecific "phytate" values reported elsewhere in the literature. $InsP_6$ and $InsP_5$ account for more than 95% of the total inositol phosphates in most raw grains and legumes. $InsP_3$ is undetectable in most of these seeds and $InsP_4$ is usually less than 5% of the total. Therefore, it may not always be necessary to perform routinely quantification of the $InsP_4$ and $InsP_3$ fractions of raw food materials derived from seeds.

Some raw seeds and related products have not been analyzed for the different groups of inositol phosphates, but data have been obtained using HPLC and NMR methods that specifically measured only $InsP_6$. The $InsP_6$ contents of white rice determined by HPLC and polished rice determined by NMR were 0.23 and 0.42%, respectively (O'Neill *et al.*, 1980; Graf and Dintzis, 1982). These values are comparable to the range of 0.14–0.34% obtained by nonspecific methods (Reddy *et al.*, 1982). Although $InsP_6$ accounted for only 55% of the total inositol phosphates in wild rice (Table II), in a nonquantitative study of farm rice the only inositol phosphate detected other than $InsP_6$ was the monophosphate (Asada *et al.*, 1969). Soybean meal was found to contain 1.44% $InsP_6$ by HPLC (Bos *et al.*, 1991), which is similar to the 1.24% $InsP_6$ in raw soybeans, also determined by HPLC (Talamond *et al.*, 2000). Other values for $InsP_6$ obtained by NMR include 0.84–0.97% for 100% extraction wheat flour, 0.13–0.18% for white wheat flour and 0.75% for Chinese millet (O'Neill *et al.*, 1980). Pearl millet and peanuts analyzed by HPLC contained 0.74 and 0.68% $InsP_6$, respectively (Talamond *et al.*, 2000), and sunflower seeds were determined by HPLC to contain 4.48% $InsP_6$ (Cilliers and van Niekert, 1986).

TABLE II

INOSITOL PHOSPHATES IN RAW SEEDS

Seed	$InsP_3$		$InsP_4$		$InsP_5$		$InsP_6$	
	μmol/g	(g/100 g)	μmol/g	(g/100 g)	μmol/g	(g/100 g)	μmol/g	(g/100 g)
Wheat[abc]	–		0.52	(0.03)	0.55–3.64	(0.03–0.21)	10.27–16.36	(0.68–1.08)
Corn[ac]	–		–		0.47–0.95	(0.03–0.05)	14.18–15.91	(0.94–1.05)
Wild rice[d]	0.67	(0.03)	1.40	(0.07)	3.14	(0.18)	6.36	(0.42)
Oat[abc]	–		0.58	(0.03)	0.25–3.16	(0.01–0.18)	9.80–17.25	(0.65–1.14)
Rye[ab]	–		0.79	(0.04)	0.68–4.24	(0.04–0.25)	9.31–15.30	(0.61–1.01)
Barley[abcef]	0.05–0.36	(0.00–0.01)	0.12–0.84	(0.01–0.04)	0.44–3.16	(0.03–0.18)	2.94–17.87	(0.19–1.18)
Sorghum[ac]	–		–		0.47–1.55	(0.03–0.09)	7.12–13.98	(0.47–0.92)
Triticale[a]	–		0.48	(0.02)	3.12	(0.18)	15.15	(1.00)
Sesame[a]	–		1.30	(0.06)	17.81	(1.03)	81.21	(5.36)
Lupin[g]	–		–		0.00–1.03	(0.00–0.06)	4.76–10.86	(0.31–0.72)
Pinto beans[adh]	–		0.17–2.96	(0.01–0.15)	0.89–8.48	(0.05–0.49)	8.94–14.1	(0.59–0.93)
Great Northern beans[dh]	–		0.19–0.48	(0.01–0.02)	1.04–2.19	(0.06–0.13)	12.7–17.0	(0.84–1.12)
Navy beans[dh]	–		0.14–0.96	(0.01–0.05)	1.24–1.80	(0.07–0.10)	12.4–16.5	(0.82–1.09)
Baby Lima beans[h]	–		0.23	(0.01)	2.13	(0.12)	9.96	(0.66)
Lima beans[d]	–		0.77	(0.04)	1.50	(0.09)	12.78	(0.84)
Roman beans[h]	–		0.02	(0.00)	1.95	(0.11)	10.6	(0.70)
Red kidney beans[h]	–		0.16	(0.01)	1.84	(0.11)	13.5	(0.89)
Red chili beans[h]	–		0.02	(0.00)	2.18	(0.13)	11.9	(0.78)
Red (Guerniquesa) beans[g]	0.21	(0.01)	–		0.33	(0.02)	9.52	(0.63)
Brown beans[i]	–		–		1.01	(0.06)	12.6	(0.83)
Black beans[fhj]	–		0.10–0.16	(0.00–0.01)	1.05–1.87	(0.06–0.11)	5.82–15.91	(0.38–1.05)
Black (Tolosana) beans[g]	0.21	(0.01)	–		0.34	(0.02)	8.55	(0.56)
Faba beans[g]	0.43	(0.02)	0.26–0.94	(0.01–0.05)	1.29–3.66	(0.07–0.21)	5.23–9.24	(0.34–0.61)
Soybeans[f]	–		0.28	(0.01)	1.41	(0.08)	5.79	(0.38)
Chickpeas (garbanzo beans)[fgh]	0.62	(0.03)	0.00–0.22	(0.00–0.01)	0.53–1.76	(0.03–0.10)	3.91–6.00	(0.26–0.40)

TABLE II *(continued)*

INOSITOL PHOSPHATES IN RAW SEEDS

Seed	$InsP_3$		$InsP_4$		$InsP_5$		$InsP_6$	
	μmol/g	(g/100 g)	μmol/g	(g/100 g)	μmol/g	(g/100 g)	μmol/g	(g/100 g)
Blackeye peas (cowpeas)[h]	0.01	(0.00)	0.26	(0.01)	2.52	(0.15)	12.6	(0.83)
Pigeon peas[h]	–		0.04	(0.00)	2.41	(0.14)	7.96	(0.52)
Green split peas[ah]	–		0.17–0.58	(0.01–0.03)	1.36–2.47	(0.08–0.14)	6.06–6.48	(0.40–0.43)
Yellow split peas[h]	–		0.12	(0.01)	1.49	(0.09)	8.82	(0.58)
Lentils[ghk]	0.32–0.38	(0.01–0.02)	0.21–0.50	(0.01–0.02)	0.83–1.39	(0.05–0.08)	3.70–10.77	(0.24–0.71)

[a]Data derived from Lehrfeld (1989).
[b]Data derived from Larsson and Sandberg (1992).
[c]Data derived from Kasim and Edwards (1998).
[d]Data derived from Lehrfeld (1994).
[e]Data derived from Bergman *et al.* (1999).
[f]Data derived from Trugo *et al.* (1999).
[g]Data derived from Burbano *et al.* (1995).
[h]Data derived from Morris and Hill (1996).
[i]Data derived from Gustafsson and Sandberg (1995).
[j]Data derived from Greiner and Konietzny (1998).
[k]Data derived from Kozlowska *et al.* (1996).

B. PROCESSED FOODS

Processing methods that degrade $InsP_6$ result in the formation of inositol phosphates containing fewer than six phosphate groups. As the degradation proceeds, the inositol phosphate concentrations individually peak and then fall at rates determined by the processing conditions and the composition of the food. $InsP_6$ may remain the predominant inositol phosphate even after most of it has been hydrolyzed if the other inositol phosphates accumulate to only low levels due to a slow $InsP_6$ hydrolysis rate. Intermediates will accumulate when their rate of formation is faster than their rate of degradation. One factor that can limit their rate of formation is that the solubilities of their salts with multivalent minerals are inversely proportional to the number of phosphate groups. The solubilities of the inositol phosphates help to determine their aqueous concentrations and the reaction rates of phytases and other phosphatases during enzymatic hydrolysis. In addition, the rate of hydrolysis depends on the amount of enzyme, and higher concentrations of intermediates transiently accumulate when more enzyme is present.

$InsP_6$ in foods may be destroyed enzymatically, nonenzymatically or by a combination of these methods. Canning at high temperatures and pressures quickly inactivates phytases and results in nonenzymatic $InsP_6$ degradation. Similarly, the toasting of breakfast cereals at very high temperatures results in nonenzymatic destruction of $InsP_6$. Fermentation of breads results in the enzymatic breakdown of much of the $InsP_6$ due to yeast and cereal phytases (Harland and Harland, 1980; Larsson and Sandberg, 1991; Türk *et al.*, 1996, 2000). Soaking and hydrothermal processing of legumes and grains prior to cooking also cause much enzymatic loss of $InsP_6$ (Gustaffson and Sandberg, 1995; Fredlund *et al.*, 1997). Quick soaking for 2 h and overnight soaking for 18 h gave similar levels of $InsP_3$, $InsP_4$, $InsP_5$ and $InsP_6$ in various cooked beans (Morris and Hill, 1996).

The inositol phosphates in various processed foods are shown in Table III. The data are on a dry weight basis except for some of the flours and breakfast cereals, which are low moisture foods. Compared to the levels in whole raw seeds (Table II), the level of $InsP_6$ was higher in wheat bran, rice bran and wheat germ but lower in corn bran, wheat flour, corn flour and sorghum flour. $InsP_6$ was the predominant inositol phosphate in breakfast cereals, cooked beans, peas and lentils, and breads, except for two of the sour breads. There was much greater variation in the $InsP_6$ values for the different breakfast cereals (0.6–8.82 μmol/g) and breads (0.17–8.26 μmol/g) compared to the cooked legumes (4.93–10.1 μmol/g). The levels of $InsP_6$ in the cooked legumes were greater than 50% of their levels in the raw seeds.

TABLE III

INOSITOL PHOSPHATES IN PROCESSED FOODS

Food	$InsP_3$		$InsP_4$		$InsP_5$		$InsP_6$	
	μmol/g	(g/100 g)	μmol/g	(g/100 g)	μmol/g	(g/100 g)	μmol/g	(g/100 g)
Wheat bran[abcd]	–		0.8–2.48	(0.04–0.12)	1.0–11.93	(0.06–0.69)	46.9–84.4	(3.09–5.57)
Corn bran[a]	–		–		–		0.8–1.1	(0.05–0.07)
Rice bran[de]	–		1.30	(0.06)	9.82–13.36	(0.57–0.77)	99.2–103.5	(6.55–6.83)
Wheat germ[a]	–		1.58	(0.08)	9.48	(0.55)	30.1	(1.99)
Wheat flour, 85% extraction[f]	–		–		0.07	(0.00)	1.24	(0.08)
Wheat flour, 55% extraction[f]	–		–		0.00–0.02	(0.00)	0.14–0.17	(0.01)
Corn flour, 95% extraction[g]	–		–		1.33	(0.08)	10.1	(0.67)
Corn flour, 65% extraction[g]	–		–		0.88	(0.05)	2.14	(0.14)
Corn flour[e]	1.0	(0.04)	1.9	(0.09)	2.5	(0.14)	1.5	(0.10)
Oat whole meal flour[c]	–		–		0.22	(0.01)	10.8	(0.71)
Rye whole meal flour[c]	–		0.06	(0.00)	0.50	(0.03)	10.3	(0.68)
Whole rye meal (1.8–2.0% ash)[h]	–		–		1.4	(0.08)	15.0	(0.99)
Rye meal (0.5–0.6% ash)[h]	–		–		0.8	(0.05)	7.2	(0.47)
Sorghum flour[g]	–			–	1.03	(0.06)	5.92	(0.39)
Quinoa flour[i]	–		0.1	(0.01)	0.1–0.2	(0.01)	8.6–11.4	(0.57–0.75)
Soy-based infant formula[j]	–		0.18	(0.01)	0.95	(0.05)	3.64	(0.24)
Bran breakfast cereals[k]	0.4	(0.02)	1.7	(0.08)	4.9	(0.28)	12.0	(0.79)
Bran flake cereal[j]	1.41	(0.06)	2.63	(0.13)	6.42	(0.37)	8.82	(0.58)
Wheat breakfast cereals[k]	0.7	(0.03)	1.7	(0.08)	3.1	(0.18)	4.9	(0.32)
Corn breakfast cereals[k]	–		–		0.1	(0.01)	0.6	(0.04)
Corn flakes cereals[h]	–		–		0.52	(0.03)	1.04	(0.07)
Rice breakfast cereals[k]	0.1	(0.00)	0.2	(0.01)	0.6	(0.03)	0.9	(0.06)
Oat breakfast cereals[k]	0.2	(0.01)	1.2	(0.06)	3.3	(0.19)	7.6	(0.50)
Mixed grain breakfast cereals[k]	0.1	(0.00)	0.5	(0.02)	1.6	(0.09)	3.3	(0.22)
Müsli cereals[h]	–		1.5	(0.07)	2.6	(0.15)	8.3	(0.55)
White wheat bread[gj]	–		0.17	(0.01)	0.25–0.34	(0.01–0.02)	0.17–0.93	(0.01–0.06)

TABLE III *(continued)*
INOSITOL PHOSPHATES IN PROCESSED FOODS

Food	$InsP_3$		$InsP_4$		$InsP_5$		$InsP_6$	
	μmol/g	(g/100 g)	μmol/g	(g/100 g)	μmol/g	(g/100 g)	μmol/g	(g/100 g)
French bread[h]	–		–		–		0.75	(0.05)
Wheat and oat bread[h]	3.13	(0.13)	1.99	(0.10)	2.56	(0.15)	8.26	(0.54)
Wheat and rye bread[h]	–		–		–		2.62	(0.17)
Sour rye and wheat bread[h]	3.02	(0.13)	–		0.60	(0.03)	0.60	(0.04)
Sour wheat and potato bread[h]	1.43	(0.06)	1.59	(0.08)	–		0.48	(0.03)
Sour buckwheat bread[h]	–		–		–		0.61	(0.04)
Crispbread[h]	–		1.0	(0.05)	1.8	(0.10)	5.9	(0.39)
Whole wheat pasta[h]	–		–		1.5	(0.09)	13.5	(0.89)
Regular pastas[h]	–		1.1	(0.05)	0.8	(0.05)	6.5	(0.43)
Four cereals pasta[h]	–		–		–		5.4	(0.36)
Wheatmeal porridge flakes, raw[h]	–		–		2.5	(0.14)	13.9	(0.92)
Ricemeal porridge flakes, raw[h]	–		–		0.4	(0.02)	2.7	(0.18)
Oatmeal porridge flakes, raw[h]	–		1.3	(0.06)	4.0	(0.23)	14.1	(0.93)
Ryemeal porridge flakes, raw[h]	–		–		2.3	(0.13)	14.0	(0.92)
Barleymeal porridge flakes, raw[h]	–		–		0.7	(0.04)	4.2	(0.28)
Pinto beans, cooked[l]	0.20	(0.01)	0.91	(0.04)	3.33	(0.19)	8.14	(0.54)
Great Northern beans, cooked[l]	0.23	(0.01)	1.05	(0.05)	3.60	(0.21)	9.24	(0.61)
Navy beans, cooked[l]	0.13	(0.00)	0.68	(0.03)	3.07	(0.18)	8.80	(0.58)
Baby Lima beans, cooked[l]	0.25	(0.01)	1.08	(0.05)	3.07	(0.18)	7.08	(0.47)
Roman beans, cooked[l]	0.08	(0.00)	0.73	(0.04)	3.25	(0.19)	9.17	(0.60)
Red kidney beans, cooked[l]	0.19	(0.01)	1.02	(0.05)	2.81	(0.16)	9.12	(0.60)
Red kidney beans, canned[j]	0.45	(0.02)	0.91	(0.04)	3.19	(0.18)	7.51	(0.50)
Red chili beans, cooked[l]	0.07	(0.00)	0.81	(0.04)	3.37	(0.19)	10.1	(0.67)
Black beans, cooked[l]	0.18	(0.01)	0.98	(0.05)	3.62	(0.21)	9.96	(0.66)
Chickpeas, cooked[l]	0.10	(0.00)	0.56	(0.03)	2.04	(0.13)	5.18	(0.34)
Blackeye peas, cooked[l]	0.22	(0.01)	0.89	(0.04)	3.38	(0.20)	9.67	(0.64)
Pigeon peas, cooked[l]	0.22	(0.01)	0.96	(0.05)	2.77	(0.16)	5.97	(0.39)

TABLE III *(continued)*

INOSITOL PHOSPHATES IN PROCESSED FOODS

Food	$InsP_3$		$InsP_4$		$InsP_5$		$InsP_6$	
	μmol/g	(g/100 g)	μmol/g	(g/100 g)	μmol/g	(g/100 g)	μmol/g	(g/100 g)
Green split peas, cooked[l]	0.07	(0.00)	0.45	(0.02)	1.73	(0.10)	4.93	(0.32)
Yellow split peas, cooked[l]	0.05	(0.00)	0.52	(0.03)	1.53	(0.09)	7.35	(0.48)
Lentils, cooked[l]	0.44	(0.02)	0.97	(0.05)	3.62	(0.21)	7.09	(0.47)
Bean sprouts, canned[j]	0.20	(0.01)	0.22	(0.01)	0.60	(0.03)	1.57	(0.10)
Tofu[j]	–		–		1.21	(0.07)	22.0	(1.45)
Corn dough[g]	–		–		3.31	(0.19)	2.24	(0.15)
Cassava dough[g]	–		–		0.34	(0.02)	0.21	(0.01)
Banku[g]	–		–		2.34	(0.14)	2.12	(0.14)
Ekuegbemi[g]	–		–		1.03	(0.06)	1.52	(0.10)
Fufu[g]	–		–		0.60	(0.03)	0.98	(0.06)
Gari[g]	–		–		0.17	(0.01)	0.59	(0.04)
Fanti kenkey[g]	–		–		2.26	(0.13)	1.15	(0.08)
Ga kenkey[g]	–		–		2.05	(0.12)	1.18	(0.08)
Wheat-based meal, cooked[m]	2.79	(0.12)	2.98	(0.15)	4.53	(0.26)	12.3	(0.81)
Rice-based meal, cooked[m]	1.62	(0.07)	1.98	(0.10)	2.53	(0.15)	5.32	(0.35)
Maize-based meal, cooked[m]	1.86	(0.08)	1.42	(0.07)	2.84	(0.16)	7.73	(0.51)
Pearl millet-based meal, cooked[m]	2.29	(0.10)	2.74	(0.14)	4.40	(0.25)	11.0	(0.72)
Finger millet-based meal, cooked[m]	2.17	(0.09)	1.68	(0.08)	3.50	(0.20)	10.9	(0.72)
Sorghum-based meal, cooked[m]	2.21	(0.09)	2.30	(0.11)	3.66	(0.21)	9.82	(0.65)

[a]Data derived from Lehrfeld (1989).
[b]Data derived from Sandberg and Ahderinne (1986).
[c]Data derived from Sandberg and Svanberg (1991).
[d]Data derived from Kasim and Edwards (1998).
[e]Data derived from Lehrfeld (1994).
[f]Data derived from Brune *et al.* (1992).
[g]Data derived from Ferguson *et al.* (1993).
[h]Data derived from Plaami and Kumpulainen (1995).
[i]Data derived from Valencia *et al.* (1999).
[j]Data derived from Phillippy *et al.* (1988).
[k]Data derived from Morris and Hill (1995).
[l]Data derived from Morris and Hill (1996).
[m]Data derived from Agte *et al.* (1999).

Brune *et al.* (1992) studied the breakdown of $InsP_6$ during breadmaking. Wheat flour of 85% extraction contained 0.829 and 0.42 g/kg of $InsP_6$ and $InsP_5$, respectively. Bread made from this flour had 0.049, 0.008, 0.011 and 0.040 g/kg $InsP_6$, $InsP_5$, $InsP_4$ and $InsP_3$, respectively, based on the dry weight of the flour used. In contrast, wheat flour of 55% extraction contained 0.089–0.106 and 0.005–0.006 g/kg $InsP_6$ and $InsP_5$, respectively. The levels of $InsP_6$, $InsP_5$, $InsP_4$ and $InsP_3$ in bread made from this flour were 0.003–0.013, 0.001–0.005, 0.001–0.002 and 0.005–0.020 g/kg, respectively. The loss of $InsP_6$ during breadmaking was 94% for the 85% extraction flour.

The effect of pH on the hydrolysis of $InsP_6$ in wheat breads containing varying amounts of rye bran, oat flour or oat bran was investigated by Larsson and Sandberg (1991). The acidities of the doughs were raised by the addition of sour dough or lactic acid to pH 3.9–5.5, which resulted in breads with pH 4.2–5.7. The sum of $InsP_6$ and $InsP_5$ was reduced by 79–97% in the breads containing sour dough, whereas the total of $InsP_6$ plus $InsP_5$ decreased 44–97% in breads with lactic acid. Optimum hydrolysis occurred when the pH was between 4.3 and 5.1 in both the dough and the bread. Less $InsP_6$ plus $InsP_5$ was destroyed in breads containing oat bran than in the breads made with rye bran or oat flour owing to lower phytase activities in the oat bran.

In breads made from equal parts of whole and white wheat flours, the addition of lactic acid and *A. niger* phytase completely eliminated $InsP_6$ and $InsP_5$ (Türk and Sandberg, 1992). Results in the same study also showed that unfermented milk inhibited the destruction of $InsP_6$ and $InsP_5$ to a greater extent than could be accounted for by its calcium content. Türk *et al.* (1996) investigated the isomeric structures of the inositol phosphates present in whole wheat bread. Ion chromatography revealed that D- and/or L-Ins(1,2,3,4,5)P_5 was the predominant $InsP_5$, followed by D- and/or L-Ins(1,2,4,5,6)P_5, Ins(1,2,3,4,6)P_5 and Ins(1,3,4,5,6)P_5. Similar amounts of what appeared to be D- and/or L-Ins(1,2,3,4)P_4 and D- and/or L-Ins(1,2,5,6)P_4 were also present, most likely due to the action of the wheat and yeast phytases.

Kozlowska *et al.* (1996) ground raw lentils containing 10.77, 1.07, 0.50 and 0.37 μmol/g $InsP_6$, $InsP_5$, $InsP_4$ and $InsP_3$, respectively, into flour which was then fermented for 4 days. Suspensions of 79 g lentil flour per liter of sterile tap water fermented at 28 or 42°C lost 73 and 78%, respectively, of their $InsP_6$. In contrast, suspensions of 221 g flour per liter fermented similarly had $InsP_6$ decreases of only 63 and 68% at 28 and 42°C, respectively. Interestingly, $InsP_3$ appeared to increase to fairly high levels, 1.51 and 1.91 μmol/g, respectively, in the latter two suspensions after 4 days. However, additional work is needed to determine the

composition of these fractions, since nucleotides such as ATP will elute along with $InsP_3$ in the ion pair HPLC analysis (Morris and Hill, 1996).

Optimized procedures have been developed to degrade the inositol phosphates in pea flour (Fredrikson *et al.*, 2001b). At pH 7.5 and 45°C, 66% of the sum of $InsP_6$, $InsP_5$, $InsP_4$ and $InsP_3$ was eliminated by endogenous phytase activity within 10 h. The predominant $InsP_5$ and $InsP_4$ breakdown products were Ins(1,2,3,4,6)P_5 and D- and/or L-Ins(1,2,3,4)P_4, which is a pattern similar to that of the alkaline phytase of lily pollen (Barrientos *et al.*, 1994). A modified process employing an exogenous microbial phytase for just 1 h was used to prepare a dephytinized pea protein isolate flour intended for test production of infant formulas (Fredrikson *et al.*, 2001a).

To increase the reduction of $InsP_6$ during the soaking of black beans containing 15.91, 1.72, 0.16 and 0 μmol/g of $InsP_6$, $InsP_5$, $InsP_4$ and $InsP_3$, respectively, the effects of pH, temperature and exogenous enzymes were evaluated (Greiner and Konietzny, 1999). When the pH of the soaking buffer was adjusted to pH values from 4.5 to 8.0, the greatest degradation of $InsP_6$ occurred at pH 6.0 during a 15 h incubation at 50°C and resulted in 7.64, 1.40, 3.85 and 4.75 μmol/g of $InsP_6$, $InsP_5$, $InsP_4$ and $InsP_3$, respectively. As mentioned above for lentils, large apparent $InsP_3$ values could conceivably be due to nucleotides such as ATP. Whereas soaking overnight in water at room temperature followed by cooking resulted in only an 8% decrease in the sum of $InsP_6$ and $InsP_5$, soaking at pH 6.0 and 60°C for 15 h led to a 54% reduction after cooking. Addition of *E. coli* or rye phytase to the soaking buffer, 50 mM sodium acetate pH 5.5, during the last 2 h of a 15 h soak, had no effect at 25°C, but reduced the sum of $InsP_6$ and $InsP_5$ by 29 and 34%, respectively, at 50°C. Cooking led to decreases in beans soaked with *E. coli* or rye phytases at both temperatures such that the sums of $InsP_6$ and $InsP_5$ decreased by 46 and 39%, respectively, in beans soaked at 25°C and by 82 and 70%, respectively, in beans soaked at 50°C. Germination of black beans at 25°C in the dark required 14 days to achieve a 47% reduction in the sum of $InsP_6$ and $InsP_5$.

Grains can also be soaked during their initial treatment in procedures such as steeping, malting or hydrothermal processing. Soaking leads to the breakdown of $InsP_6$ by endogenous phytases in wheat, rye, oat, barley and corn (Sandberg and Svanberg, 1991; Larsson and Sandberg, 1992; Hull and Montgomery, 1995; Fredlund *et al.*, 1997). Bergman *et al.* (1999) optimized the hydrothermal processing of barley to degrade $InsP_6$ and increase the level of *myo*-inositol. Variables were the temperature (T_1) of the first 1 h wet and 5 h dry steeps, the temperature (T_2) of the second 1 h wet and 15 h dry steeps, and the concentration and volume of lactic

acid in the wet steeps. $InsP_6$ was lowered by 96% to a final concentration of 0.5 μmol per g dry weight using 48 and 50°C for T_1 and T_2, respectively, and 3.2 volumes of 0.8% lactic acid. *Myo*-inositol was increased from 0.56 to 2.68 μmol per g dry weight with 48°C for T_1, during which the dry steep was prolonged to 21 h, 1.5 volumes of 0.8% lactic acid and without the second steeps. The best combination, a 95% decrease in $InsP_6$ and a *myo*-inositol value of 2.23 μmol per g dry weight, was achieved using 48 and 50°C for T_1 and T_2, respectively, and 1.5 volumes of 0.8% lactic acid. Under the latter conditions the final contents of $InsP_6$, $InsP_5$, $InsP_4$, $InsP_3$, $InsP_2$ and InsP were 0.66, 0.15, 0.79, 1.73, 1.1 and 0.9 μmol per g dry weight, respectively.

Germination is responsible for the $InsP_6$ breakdown in malting processes for legumes and grains. Trugo *et al.* (1999) studied the effect of malting on the inositol phosphates in soybean, black bean, chickpea and barley seeds. After 2 days of malting the sum of $InsP_5$ and $InsP_6$ was reduced by 25% in black beans, which had the greatest losses. Higher levels of $InsP_3$ and $InsP_4$ were generated during malting, but $InsP_6$ was the predominant inositol phosphate present in all of the malted products. Additional reports on seed germination showed that little of the inositol phosphates in lentils was lost after 3 days but more than 70% was hydrolyzed by day 6 (Ayet *et al.*, 1997), and 22–38% of the inositol phosphates in two species of lupin was destroyed in 4 days (de la Cuadra *et al.*, 1994). Slight increases in $InsP_3$ and $InsP_4$ were observed during germination of the lentils and one of the species of lupin.

Valencia *et al.* (1999) compared soaking and fermentation of raw and germinated quinoa flours followed by cooking. Cooking alone lowered the $InsP_6$ content of raw flour from 8.6–11.4 μmol/g to 6.9–9.5 μmol/g. Soaking in a suspension with three parts of water for 12–14 h at room temperature prior to cooking lowered the $InsP_6$ to 2.0–4.3 μmol/g, whereas fermentation with *Lactobacillus plantarum* prior to cooking gave 1.0–2.0 μmol/g $InsP_6$. The most extensive reduction of $InsP_6$ was in germinated flour that was fermented prior to cooking, which gave 0.2–0.3 μmol/g $InsP_6$, 0.0 μmol/g $InsP_5$ and 0.0–0.1 μmol/g each of $InsP_4$ and $InsP_3$. Soaking of maize flour or pounded maize was recently found to reduce the $InsP_5$ and $InsP_6$ content by more than half and was preferable to fermentation because the conditions were easier to control (Hotz and Gibson, 2001).

V. INOSITOL PHOSPHATES IN FRUITS AND VEGETABLES

Extremely little is known about the inositol phosphates in fruits and vegetables. Data obtained using nonspecific methods for phytate analysis

indicate that the amounts of $InsP_6$ in these foods are considerably lower than in seeds. A notable exception is avocado fruit, which contains approximately 0.5% $InsP_6$ on a dry weight basis (Phillippy and Wyatt, 2001). Avocado fruit, like seeds, contains a high level of fat that needs to be protected from oxidation. Therefore the fact that avocado fruit contains large amounts of $InsP_6$ bolsters the hypothesis that *in vivo* $InsP_6$ serves as an antioxidant to prevent iron-catalyzed free radical formation (Graf *et al.*, 1987). Some vegetables that grow underground in the form of bulbs, roots and tubers may have higher amounts of inositol phosphates than green vegetables. Onions, parsnips and carrots have been reported to contain 0.38, 0.24 and 0.04% $InsP_6$, respectively, according to dry weight, whereas turnips, beet roots, celery and cabbage all had 0.02% or less $InsP_6$ (Harland and Morris, 1995). In all of these vegetables $InsP_6$ was the predominant inositol phosphate, and smaller amounts of $InsP_5$, $InsP_4$ and $InsP_3$ were also measured. Potatoes analyzed by NMR were reported to contain 0.09% $InsP_6$ (O'Neill *et al.*, 1980), but HPLC results with several varieties of store-bought potatoes have revealed $InsP_6$ concentrations on average of about 0.3% on a dry weight basis (B. Q. Phillippy, unpublished data). Post-harvest changes in the inositol phosphates of these foods have never been studied, and it is not known whether the above data are typical for these foods or how much variation might be expected.

Plant cells also contain Ins(1,4,5)P_3, which is involved in calcium signaling and growth (Stevenson *et al.*, 2000). Ins(1,4,5)P_3 is produced upon the hydrolysis of PtdIns(4,5)P_2 by phospholipase C following stimulation. Ins(1,4,5)P_3 then binds to receptors in the vacuole to release calcium stores, which evoke a biological response (Munnik *et al.*, 1998). The levels of Ins(1,4,5)P_3 are low compared to those of $InsP_6$ and some of the other inositol phosphates. Red beets contain 8–11 pmol Ins(1,4,5)P_3/g of fresh weight (Beno-Moualem *et al.*, 1995), and the stems of maize plants have 139–276 pmol Ins(1,4,5)P_3/g of fresh weight (Perera *et al.*, 1999). In vegetative plant tissues Ins(1,4,5)P_3 appears to be a minor component of the $InsP_3$ fraction, which includes Ins(1,2,3)P_3, Ins(3,4,6)P_3, D- and/or L-Ins(1,5,6)P_3, D- and/or L-Ins(2,4,5)P_3 and D- and/or L-Ins(1,2,5)P_3 (Brearley and Hanke, 2000). Other inositol phosphates identified in *Spirodela polyrhiza* L. (duckweed) turions, which are similar to tubers, include Ins(3,4,5,6)P_4, Ins(1,3,4,5,6)P_5 and D- and/or L-Ins(1,2,4,5,6)P_5 (Brearley and Hanke, 1996).

VI. INOSITOL PHOSPHATES IN ANIMALS

A. ABSORPTION AND TISSUE CONTENT

The fate of inositol phosphates eaten by animals is particularly complex. Many factors interact as the inositol phosphates make their way through

the digestive tract to determine whether they will be excreted, degraded or absorbed. The composition of the diet may be most important, since food can contribute phytases and other phosphatases, minerals that affect the solubilities of the inositol phosphates, ionic components that can bind to the inositol phosphates, and components that may interact more indirectly. Nondietary factors that may also play a role in the destiny of the inositol phosphates include the genetic disposition of the animal as well as its physiological and nutritional status.

Significant enzymatic degradation of inositol phosphates can occur in the stomach of monogastric animals including humans if the right type and amount of phosphatases are present in the diet (Sandberg and Andersson, 1988; Sandberg *et al.*, 1996; Mullaney *et al.*, 2000). Additional phytases and phosphatases will be active in the intestines and may come from cells in the intestinal mucosa (Yang *et al.*, 1991b; Maenz and Classen, 1998) or from microorganisms (Moore and Veum, 1983; Lopez *et al.*, 1998). Adaptation to $InsP_6$ increased the phytase activity in the duodenum and ileum of rats, whereas wheat bran increased phytase activity only in the ileum (Lopez *et al.*, 2000b). Resistant starch fed to rats adapted to wheat bran doubled the disappearance of $InsP_6$ in the feces, most likely by promoting the growth of digestive microflora that express phytase activity (Lopez *et al.*, 2000a). Inositol liberated from inositol phosphates during digestion is actively absorbed in the small intestine and is transported through the blood for absorption by other tissues (Holub, 1982).

Inositol phosphates may have some limited ability to be absorbed, but the situation is far from clear. Sakamoto *et al.* (1993) found that [^{3}H]$InsP_6$ in drinking water appeared in gastric mucosal cells as a mixture of inositol and inositol mono- through hexakisphosphates, but only inositol and the monophosphate were detected in the blood plasma. Similarly, Vucenik and Shamsuddin (1994) showed that [^{3}H]$InsP_6$ incubated with HT-29 (human colon adenocarcinoma) cells appeared as a mixture of inositol and inositol mono- through hexakisphosphates in the cytosol. In contrast, YAC-1 (mouse lymphoma) and K562 (human erythroleukemia) cells exposed to [^{3}H]$InsP_6$ contained some of the less phosphorylated inositols but no detectable $InsP_6$. These results show that the cells contain phytase that can degrade $InsP_6$, but they do not show which compound(s) were absorbed, since the phytase and other phosphatases could have acted outside of the cells.

Grases *et al.* (2001a) recently demonstrated that the addition of dodecasodium phytate at a level of 1% by weight to diets devoid of $InsP_6$ and other forms of inositol for 12 weeks resulted in dramatic increases by more than ten-fold in the level of $InsP_6$ in the brain, liver, kidney, bone, urine and plasma of rats. Because inositol was not used as a control, it

could not be estimated how much of the tissue $InsP_6$ may have originated from synthesis via inositol liberated from $InsP_6$ by phytases in the gut. However, the results do show that tissue levels of $InsP_6$ respond to dietary manipulation in rats. The addition of $InsP_6$ to the rat diet resulted in a smaller increase in $InsP_5$ compared to $InsP_6$ in the tissues and fluids (Grases *et al.*, 2001b), indicating that $InsP_6$ absorbed intact may have served as a precursor for the $InsP_5$.

In a study with human volunteers, Grases *et al.* (2001c) determined that the plasma levels of $InsP_6$ were normally $260\pm30\,\mu g\,L^{-1}$ but fell to $70\pm10\,\mu g\,L^{-1}$ after 15 days on an diet containing no cereal products, legumes, nuts, potatoes or coffee. Maximum concentration of $InsP_6$ in plasma was reached 4 h after ingestion of 1400 mg dodecasodium phytate, and urinary $InsP_6$ levels were directly related to plasma levels. It was noted that the overall percentage of absorption was low and that maximum urinary excretion, and thus maximum plasma levels, could be obtained by consumption of a diet containing normal amounts of $InsP_6$. Inositol levels in the diets were not measured nor was inositol used as a control to compare the effects of dietary inositol and dietary $InsP_6$ on $InsP_6$ levels in plasma and urine.

Adaptor protein 2 (AP-2) is an $InsP_6$ receptor associated with the plasma membrane (Voglmaier *et al.*, 1992). By binding to AP-2, cytosolic $InsP_6$ prevents the binding of clathrin to AP-2 to form coated pits that take part in endocytosis (Gaidarov *et al.*, 1996). However, the assembled coat structures containing clathrin and AP-2 have a greater affinity for di-octanoylphosphatidylinositol 3,4,5-trisphosphate than for $InsP_6$, suggesting that endogenous phosphoinositides occupy the AP-2 binding sites in the plasma membrane (Gaidarov *et al.*, 1996). It is not known whether $InsP_6$ can be absorbed by endocytosis via these or other receptors.

Good evidence for the intact absorption of inositol phosphates was reported by Ozaki *et al.* (2000). Using polyamines including aminoglycosides such as neomycin, synthetic spherical dendrimeric polyamines with 12 or 32 primary amines and polybasic nuclear histone proteins as carriers, phosphoinositides or inositol phosphates were translocated into a variety of cells. Although the efficiency of cellular uptake was best for inositides with lipophilic moieties, an undetermined portion of 77 μM $Ins(1,4,5)P_3$ preincubated with 50 μM type III-S histone from calf thymus was taken up by NIH3T3 mouse fibroblasts and induced rapid cytosolic calcium mobilization. Thus polybasic compounds can neutralize the charge of inositol phosphates in a manner analogous to the intracellular delivery of oligonucleotides. Foods obtained from plants and animals contain a variety of compounds containing amines that could be tested for their ability to carry inositol phosphates into cells.

The large number of $InsP_2$, $InsP_3$ and $InsP_4$ isomers identified in animal cells has made quantification of their individual concentrations a tedious prospect. Some effort has been made to determine the amounts of those isomers which are most abundant. Ins(1,4,5)P_3 is present in animal cells at resting concentrations of about 0.1–2 μM with several-fold increases observed upon stimulation (Shears, 1989). Unstimulated levels of Ins(1,3,4)P_3 in some cells are 1–4 μM (Shears, 1989), whereas Ins(1,2,3)P_3 levels of 0.6–13.1 μM have been reported in various cell types (Barker *et al.*, 1995).

The predominant inositol phosphates in most types of animal cells appear to be $InsP_6$ and Ins(1,3,4,5,6)P_5. In heart, kidney, spleen, liver and blood but not muscle of Buffalo rats $InsP_6$ and Ins(1,3,4,5,6)P_5 were typically present at levels of at least 5–15 nmol/g wet weight (Szwergold *et al.*, 1987). $InsP_6$ levels alone were determined to be 1.04–2.80, 3.20–4.10, 30.0–42.0 and 1.07–1.21 μg/g in kidney, liver, brain and bone, respectively, of rats, and 0.14–0.31 and 0.43–2.52 μg/ml in plasma and urine, respectively, of humans (March *et al.*, 2001). In various types of human blood cells $InsP_6$ ranged from 10 to 105 μM, whereas Ins(1,3,4,5,6)P_5 concentrations were 3–55 μM (Pittet *et al.*, 1989; Bunce *et al.*, 1993; Guse *et al.*, 1993). Other isomers of $InsP_5$, $InsP_4$ and $InsP_3$ were present in these cells at lower levels, several of them between 1 and 10 μM. $InsP_2$ isomers were somewhat more abundant than $InsP_4$ or $InsP_3$, and the total monophosphates amounted to 27–93 μM (Bunce *et al.*, 1993).

The inositol polyphosphate pyrophosphates consist of *myo*-inositol esters where five or six of the hydroxyl groups are substituted with different combinations of monophosphate and diphosphate groups yielding various configurations of $InsP_6$, $InsP_7$ and $InsP_8$. Radiolabeling experiments in AR4-2J pancreatoma cells indicates that their concentrations are probably low compared to concentrations of many of the other inositol polyphosphates (Shears *et al.*, 1995).

Quick-frozen muscles from frogs and rats contained about 2–3 nmol $InsP_6$/g wet tissue (Table IV). Assuming that the muscles contained approximately 75% H_2O, the $InsP_6$ levels on a dry weight basis were about 10 nmol g^{-1}, which is 1000-fold less than the $InsP_6$ concentration found in raw seeds (Table II). After $InsP_6$, the most abundant inositol phosphate in muscle appears to be D- and/or L-Ins(1,4)P_2.

The inositol phosphate concentrations in animal products other than muscle used as food have been largely ignored owing to their low levels in comparison to the levels found in seeds. In fresh turkey blood the predominant inositol phosphate was Ins(1,3,4,5,6)P_5 at 1.1 mM, followed by 27 μM Ins(1,4,5,6)P_4, 6.6 μM $InsP_6$, 5.3 μM Ins(1,3,4,6)P_4, 1.8 μM Ins(1,5,6)P_3, 1.2 μM Ins(1,3,4,5)P_4, 1.1 μM Ins(2,4,5)P_3, 1.0 μM Ins(1,4,5)P_3 and 0.4 μM Ins(1,3,4)P_3 (Radenberg *et al.*, 1989). In contrast, refrigerated

TABLE IV
MASSES OF SOME INOSITOL PHOSPHATES IN SKELETAL MUSCLES[a]

	Frog (nmol/g wet weight)	Rat (nmol/g wet weight)
$InsP_6$	2.44–2.91	1.85–3.03
$Ins(1,3,4,5,6)P_5$	0.52–0.65	0.48–0.65
D- and/or L-$Ins(1,2,4,5,6)P_5$	0.14–0.24	<0.02–0.15
D- and/or L-$Ins(1,4,5,6)P_4$	0.12–0.17	0.24–0.50
D- and/or L-$Ins(1,2,5,6)P_4$	0.03–0.05	<0.03–0.06
D- and/or L-$Ins(1,3,4,5)P_4$	0.12–0.19	0.17–0.59
D- and/or L-$Ins(1,4,5)P_3$	1.21–1.46	0.69–1.48
D- and/or L-$Ins(1,3,4)P_3$	0.07–0.18	0.13
$Ins(1,3,5)P_3$ and/or $Ins(2,4,6)P_3$	0.05–0.13	<0.03
D- and/or L-$Ins(1,4)P_2$	2.03–2.69	3.29–4.07
$Ins(1,3)P_2$	0.28–0.87	1.33–1.85

[a] Data compiled from Mayr and Thieleczek (1991).

calf brains contained mostly $InsP_6$ followed by $Ins(1,3,4,5,6)P_5$ (Phillippy and Bland, 1988). Interestingly, in rats injected with [^{3}H]inositol, more labeled $InsP_6$ was present in the hippocampus after 24 h than in the other regions of the brain (Vallejo *et al.*, 1987). The intracellular level of $InsP_6$ in the rat hippocampus was estimated to be 13 μM, and similar concentrations were detected in the cerebellum, cortex and striatum (Yang *et al.*, 2001).

B. BIOLOGICAL FUNCTIONS

All of our knowledge about the biological functions of inositol phosphates has come about within the last twenty years (reviewed in Irvine and Schell, 2001). In 1983 Streb and coworkers discovered that $Ins(1,4,5)P_3$, which is formed by the action of phospholipase C on $PtdIns(4,5)P_2$, releases calcium from a nonmitochondrial source in pancreatic acinar cells (Streb *et al.*, 1983). Subsequently other inositol phosphates were identified and their metabolic relationships were elucidated. In addition to calcium mobilization, a variety of other signaling functions have been associated with certain inositol phosphates (Shears, 1998). In particular, $InsP_6$ seems to be involved in numerous cellular processes as a result of its proclivity to bind to cationic minerals and proteins.

Binding of ligands such as hormones, neurotransmitters and growth factors to their receptors in the plasma membrane causes the hydrolysis of $PtdIns(4,5)P_2$ by phospholipase C to yield diacylglycerol and $Ins(1,4,5)P_3$, which translocates through the cytoplasm as a second messenger (Berridge, 1993). $Ins(1,4,5)P_3$ receptors are calcium channels found in the

membranes of cellular organelles including the endoplasmic reticulum (Taylor *et al.*, 1999), sarcoplasmic reticulum (Tasker *et al.*, 2000), nuclear membrane (Humbert *et al.*, 1996) and plasma membrane (Tanimura *et al.*, 2000). Upon binding, Ins(1,4,5)P_3 activates its receptor, which results in the opening of the calcium channel and the release of stored calcium or uptake of extracellular calcium. Many cellular processes including growth, fertilization, secretion, contraction and sensation have been linked to Ins(1,4,5)P_3 signaling (Berridge, 1993). In addition, Ins(1,4,5)P_3 is involved in the regulation of cellular proliferation and apoptosis through this pathway (Patel *et al.*, 1999; Jayaraman and Marks, 2000).

Different inositol phosphates can bind to and activate Ins(1,4,5)P_3 receptors in *Xenopus* oocytes or Chinese hamster ovary cells. Some metabolites of Ins(1,4,5)P_3 such as Ins(1,3,4,5)P_4 and Ins(1,3,4,6)P_4 display activity, although they are less potent than the former (Delisle *et al.*, 1994; Burford *et al.*, 1997). Highly active isomers that are not naturally abundant include Ins(4,5)P_2, Ins(2,4,5)P_3, DL-Ins(1,4,6)P_3 and DL-Ins(1,2,4,5)P_4. Interestingly, some isomers formed by plant phytases, such as Ins(1,2,3)P_3, may have a low calcium-releasing activity. Other isomers that released calcium from *Xenopus* oocytes included DL-Ins(1,2,6)P_3, DL-Ins(1,5,6)P_3, DL-Ins(1,2,3,6)P_4, DL-Ins(1,2,5,6)P_4 and DL-Ins(1,2,3,5,6)P_5, although the relative activities of the enantiomers in these pairs were not determined (Delisle *et al.*, 1994).

In addition to membrane-bound receptors, inositol phosphates also interact with soluble proteins. Ins(1,4)P_2 binds to and activates 6-phosphofructokinase (Mayr, 1989). Ins(1,4)P_2, Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 bind to and inhibit fructose 1,6-bisphosphate aldolase A (Koppitz *et al.*, 1986). Aldolase-bound Ins(1,4,5)P_3 may also act as a pre-existing pool of the second messenger Ins(1,4,5)P_3 that is discharged by fructose 1,6-bisphosphate during glycolysis in muscle (Thieleczek *et al.*, 1989).

Ins(1,2,6)P_3 is known to be produced from Ins(1,2,5,6)P_4 by phytases from plants and microbes (Phillippy, 1989; Türk *et al.*, 2000). D- and/or L-Ins(1,2,6)P_3 has been identified as a minor inositol trisphosphate in some animal cells (McConnell *et al.*, 1992). Also known as the pharmaceutical drug α-trinositol, Ins(1,2,6)P_3 has analgesic and anti-inflammatory properties and is an antagonist of neuropeptide Y (Bell and McDermott, 1998). Although its mode of action is unknown, Ins(1,2,6)P_3 may inhibit signal transduction by binding to proteins such as receptors for Ins(1,3,4,5)P_4 (Bell and McDermott, 1998).

Ins(1,2,3)P_3 contains the functional 1,2,3-trisphosphate grouping that binds iron such that it cannot catalyze the formation of hydroxyl free radicals (Hawkins *et al.*, 1993; Spiers *et al.*, 1995, 1996). While acting as intracellular antioxidants, Ins(1,2,3)P_3 and other inositol phosphates containing this

grouping might safely transport iron between sites within the cell (Barker *et al.*, 1995). Chelation of iron to the 1,2,3-trisphosphate grouping may also reduce the likelihood for lipid peroxidation catalyzed by iron bound to other anionic compounds (Phillippy and Graf, 1997). *In vitro* Ins(1,2,3)P_3 was nearly as effective as InsP_6 at preventing iron-catalyzed hydroxyl radical formation (Spiers *et al.*, 1995, 1996), whereas InsP_6 was significantly better than Ins(1,2,3)P_3 at inhibiting iron-catalyzed lipid peroxidation (Phillippy and Graf, 1997).

Ins(1,3,4,5)P_4 appears to act synergistically with Ins(1,4,5)P_3 in the mobilization of calcium from intracellular stores. However, results from different studies have been variable and its mechanism is unclear (Smith *et al.*, 2000). Low concentrations of Ins(1,3,4,5)P_4 may facilitate calcium influx by inhibiting Ins(1,4,5)P_3 5-phosphatase, whereas higher concentrations may inhibit calcium signaling by binding to Ins(1,4,5)P_3 receptors (Hermosura *et al.*, 2000). Ins(1,3,4,5)P_4 and other inositol phosphates with structural similarities to PtdIns(3,4,5)P_3 also compete with the latter for binding to proteins containing pleckstrin homology (PH) domains. This helps to regulate the recruitment of signaling molecules containing PH domains such as Gapl, protein kinase B (also known as Akt) and phospholipase C to cellular membranes (Kavran *et al.*, 1998).

Ins(3,4,5,6)P_4 inhibits chloride secretion by epithelial cells following prolonged stimulation of Ins(1,4,5)P_3 production (Vajanaphanich *et al.*, 1994). Receptor-mediated inositol phosphate turnover increases the conversion of Ins(1,3,4,5,6)P_5 to Ins(3,4,5,6)P_4, which inactivates chloride channels in the plasma membrane (Xie *et al.*, 1996). In human pancreatoma epithelial cells, Ins(3,4,5,6)P_4 specifically attenuates the longer term activation of calcium-dependent chloride channels by type II calmodulin-dependent protein kinase following the acute phase of calcium mobilization (Ho *et al.*, 2001). Deficits in chloride channel activity regulated by Ins(3,4,5,6)P_4 may be involved in the kidney and lung pathology resulting from diabetes and cystic fibrosis, respectively (Ismailov *et al.*, 1996). However, effective Ins(3,4,5,6)P_4 concentrations may be essential for the chloride ion regulation of metabolic functions such as the salt and fluid secretion of intestinal epithelial cells (Vajanaphanich *et al.*, 1994).

Ins(1,3,4,5,6)P_5 has not been assigned any specific functions other than being a key intermediate in the formation of Ins(3,4,5,6)P_4 and InsP_6, and more recently as a substrate for the tumor suppressor protein PTEN (phosphatase and tensin homolog deleted on chromosome ten), which is a protein phosphatase, a PtdIns(3,4,5)P_3 3-phosphatase and an Ins(1,3,4,5,6)P_5 3-phosphatase (Caffrey *et al.*, 2001). However, its structural similarity to certain InsP_4 isomers and InsP_6 results in some sharing of functionality. For example, both Ins(1,4,5,6)P_4 and Ins(1,3,4,5,6)P_5 interact strongly

with the PH domain of protein kinase B (Razzini *et al.*, 2000). Similarly, both Ins(1,3,4,5,6)P_5 and $InsP_6$ bind L- and P-selectins (Cecconi *et al.*, 1994). In birds and some reptiles Ins(1,3,4,5,6)P_5 binds to hemoglobin and regulates its affinity for oxygen (Gersonde and Ganguly, 1986). $InsP_6$ has the ability to serve the same function, but neither inositol phosphate is naturally present in mammalian erythrocytes in sufficient amounts. Therefore, $InsP_6$ has been incorporated into human red blood cells in order to increase the delivery of oxygen to tissues (Boucher *et al.*, 1996).

Numerous roles for $InsP_6$ in cells have been suggested to date (Shears, 2001), and it is very likely that more remain to be discovered. With its high negative charge density, much of $InsP_6$ is probably bound to cellular membranes through bridges of metal cations such as Mg^{2+} and Fe^{3+} (Poyner *et al.*, 1993). By chelating iron in a manner that prevents it from catalyzing the formation of hydroxyl free radicals, $InsP_6$ may be a critical antioxidant (Graf and Eaton, 1990; Hawkins *et al.*, 1993). Some of the proteins $InsP_6$ strongly binds to include L- and P-selectins (Cecconi *et al.*, 1994), AP-2 (Timerman *et al.*, 1992; Voglemaier *et al.*, 1992), AP-3 (Norris *et al.*, 1995; Ye *et al.*, 1995), coatomer (Fleisher *et al.*, 1994), synaptotagmin (Fukuda *et al.*, 1994; Llinas *et al.*, 1994), myelin proteolipid protein (Yamaguchi *et al.*, 1996) and guanylate cyclase (Suzuki *et al.*, 2001). A protein kinase stimulated by $InsP_6$ phosphorylates pacsin/syndapin I and thereby increases its association with dynamin I at nerve terminals (Hilton *et al.*, 2001). $InsP_6$ also binds to PH domains, although with less affinity than some of the other inositol phosphates (Kavran *et al.*, 1998). In the hippocampus, cerebellum, cortex and striatum regions of rat brain, $InsP_6$ levels were elevated upon activation and lowered by inhibition of neuronal activity (Yang *et al.*, 2001).

Specific functions of $InsP_6$ in insulin-secreting cells are derived through the inhibition of phosphatases and the activation of protein kinase C. By inhibiting serine-threonine phosphatases, $InsP_6$ may enhance phosphorylation of voltage-gated L-type calcium channels resulting in calcium influx over the plasma membrane (Larsson *et al.*, 1997). This in turn leads to an increase in cytoplasmic free calcium and insulin release. Activation of protein kinase C by $InsP_6$ may also lead to insulin secretion by promoting the recruitment and transport of granules to the site of exocytosis or by altering the conformation of proteins responsible for vesicle fusion (Efanov *et al.*, 1997). Enhancement of calcium influx by $InsP_6$ has also been observed in other cells and organelles such as cerebellar neurons and liver mitochondria (Nicoletti *et al.*, 1989; Copani *et al.*, 1991). Recently, $InsP_6$ was observed to increase L-type calcium channel activity in hippocampal neurons by increasing the activity of adenylyl cyclase, which raised cyclic AMP levels, which in turn enhanced the activity of protein

kinase A (PKA) (Yang *et al.*, 2001). Thus L-type calcium channel activity in hippocampal neurons may be enhanced by $InsP_6$ through increased phosphorylation at PKA phosphorylation sites of the channel, in addition to the inhibition of serine/threonine protein phosphatases.

$InsP_6$ is also required for the export of mRNA from the nucleus to the cytoplasm, where it can be translated into protein (York *et al.*, 1999; Feng *et al.*, 2001). It is possible that $InsP_6$ functions by binding proteins associated with the nuclear pore complex or the shuttling heterogeneous nuclear ribonucleoprotein complexes. The three enzymes needed for synthesis of the $InsP_6$ required for mRNA export in *Saccharomyces cerevisiae* were phospholipase C, Ins(1,4,5)P_3 6-kinase/Ins(1,4,5,6)P_4 3-kinase and Ins(1,3,4,5,6)P_5 2-kinase (York *et al.*, 1999; Saiardi *et al.*, 2000a). The intermediate Ins(1,4,5,6)P_4 was also shown in *S. cerevisiae* to be needed to regulate the transcription of six genes involved in the synthesis or breakdown of argenine (Odom *et al.*, 2000).

The requirement of $InsP_6$ for efficient DNA repair of double-stranded breaks is an additional nuclear function that was recently discovered (Hanakahi *et al.*, 2000). $InsP_6$ is part of the nonhomologous end-joining apparatus that consists of the XRCC4/DNA ligase IV complex and DNA-dependent protein kinase (DNA-PK), which is comprised of a catalytic subunit and the DNA end-binding protein Ku. $InsP_6$ binds to DNA-PK, but the mechanism by which $InsP_6$ promotes end-joining is unknown. Maximum activity was obtained with 1 μM $InsP_6$, although Ins(1,3,4,5,6)P_5 and Ins(1,3,4,5)P_4 were also somewhat effective ligands.

Ins(1,3,4,5,6)P_5 and $InsP_6$ can have phosphates enzymatically added onto one or two of their carbon-bound phosphates to form diphosphorylated inositol phosphates such as $InsP_7$ and $InsP_8$. These inositol pyrophosphates are found in animals, plants and microbes and thus far include $[PP]_2$-$InsP_4$, PP-$InsP_5$, $[PP]_2$-$InsP_3$ and PP-$InsP_4$ (Yang *et al.*, 1999; Saiardi *et al.*, 2000b). $InsP_6$, PP-$InsP_5$, and $[PP]_2$-$InsP_4$ are synthesized by Ins(1,3,4,5,6)P_5 2-kinase, $InsP_6$ kinase and PP-$InsP_5$ kinase, respectively, which can also catalyze their reverse reactions to produce ATP (Phillippy *et al.*, 1994; Voglmaier *et al.*, 1996; Huang *et al.*, 1998). Thus $InsP_6$ and the inositol pyrophosphates may function partly as cellular energy stores. $InsP_7$ and/or $InsP_8$ are involved in the homologous recombination mode of repair of double-stranded DNA breaks (Luo *et al.*, 2002). The family of diphosphoinositol polyphosphates may regulate assembly of vesicles used for endocytosis and the trans-Golgi transport of proteins (Saiardi *et al.*, 2000b), and $InsP_7$ may also have a role in vesicle exocytosis (Luo *et al.*, 2001). In addition, the fact that $InsP_6$ kinase stimulates the uptake of inorganic phosphate may mean that $InsP_7$ or $InsP_8$ also help to regulate that process (Schell *et al.*, 1999). An especially intriguing new finding is

that the post-transcriptional induction of an $InsP_6$ kinase is largely responsible for the growth inhibition and apoptosis of human ovarian carcinoma cells following treatment with interferon-β (Morrison *et al.*, 2001).

VII. NUTRITIONAL IMPORTANCE OF INOSITOL PHOSPHATES

A. BIOAVAILABILITY OF MINERALS

The history of the inositol phosphates from a nutritional perspective has followed an uneven path. As described in the review by Reddy *et al.* (1989), the propensity of $InsP_6$ to impair the absorption of minerals such as calcium and iron was recognized early in the twentieth century. By chelating and precipitating multivalent cationic minerals, $InsP_6$ was found to lower the bioavailability of the macrominerals calcium and magnesium as well as trace minerals such as iron and zinc. Since the precipitated mineral complexes contained $InsP_6$, the inositol and phosphate moieties of this compound would also be unavailable for absorption, although this has not been viewed as a significant concern for humans. A change in the paradigm started in the 1980s when some positive nutritional attributes of $InsP_6$ were discovered. Presently, nutritionists are attempting to clarify the roles of inositol phosphates in the diet and to determine the most appropriate levels for consumption.

There is general agreement that diets containing high levels of $InsP_6$ can reduce the bioavailability of polyvalent cationic minerals (Zhou and Erdman, 1995; Rickard and Thompson, 1997; Harland and Narula, 1999). What is not clear is how much $InsP_6$ is too much. As data on this subject have grown more extensive, it has become apparent that a simple rule cannot encompass all situations. Instead, special considerations must be made for different minerals, different groups of people and diets of different composition.

Although phytate can probably chelate all polyvalent cationic minerals, some minerals have received more attention than others in rough correlation to their perceived nutritional importance. Zinc and iron have been of primary concern because they readily form poorly available insoluble complexes with $InsP_6$ and are critical for growth and development (Zhou and Erdman, 1995). Calcium has also received considerable attention, but it is now recognized that $InsP_6$ inhibits calcium absorption less than oxalic acid does (Heaney *et al.*, 1991; Frossard *et al.*, 2000). Interactions of $InsP_6$ with magnesium, copper, selenium, manganese, cobalt, nickel, cadmium, lead, aluminum and mercury have also been studied to varying degrees

and cannot be ignored when considering the nutritional importance of inositol phosphates.

In vivo studies with Japanese quail, rats and humans have shown that moderate amounts of $InsP_6$ and $InsP_5$ decrease the absorption of zinc, whereas $InsP_4$ and $InsP_3$ have no effect (Tao *et al.*, 1986; Lönnerdal *et al.*, 1989; Sandström and Sandberg, 1992). In Caco-2 cells, the inhibition of the uptake and transport of zinc was directly proportional to the number of phosphate groups on $InsP_3$, $InsP_4$, $InsP_5$ and $InsP_6$ (Han *et al.*, 1994). *In vitro* studies showing that $InsP_6$ and $InsP_5$ bind more zinc than $InsP_4$ and $InsP_3$ at an inositol phosphate to zinc molar ratio of 1 : 14 indicated that chelation and precipitation are most likely the mechanisms responsible for inhibiting zinc absorption (Persson *et al.*, 1998). Simpson and Wise (1990) also found that zinc was more soluble in the presence of $InsP_3$ and $InsP_4$ than $InsP_5$ and $InsP_6$ at molar ratios of 1 : 1, but at higher ratios of inositol phosphate to zinc the highest percentage of soluble zinc was observed in the presence of $InsP_6$. However, raising the molar ratio of calcium to zinc above 15 : 1 resulted in lower zinc solubilities in the presence of any of those inositol phosphates. In recent studies in rats and humans, respectively, whole wheat flour and oat bran, which have high levels of $InsP_6$, decreased the fractional absorption of zinc in comparison with low-fiber diets but enhanced the total zinc absorption owing to the high zinc contents of those ingredients (Levrat-Verny *et al.*, 1999; Sandström *et al.*, 2000). In rats fermentation of fibers in the large intestine increased the absorption of various minerals by increasing their solubility via a lower pH and microbial production of phytase (Lopez *et al.*, 1998, 2000a). Thus the high zinc content of whole grains may compensate for the negative effect of $InsP_6$ on zinc absorption in some diets. Additional work is needed to determine whether these findings can be reproduced with other diets such as those containing legumes and the effects of different levels of calcium.

In a study of iron absorption from bread in humans, the sum of $InsP_3$, $InsP_4$, $InsP_5$ and $InsP_6$ was related to the inhibition of absorption (Brune *et al.*, 1992). In experiments performed at molar ratios of inositol phosphate to iron of 0.04 : 1 to 3.6 : 1, the solubility of iron following *in vitro* digestion was decreased by $InsP_6$ and $InsP_5$ but slightly increased by $InsP_4$ and $InsP_3$ (Sandberg *et al.*, 1989). The reason for the apparent different responses in the above studies to $InsP_4$ and $InsP_3$ in humans and *in vitro* was recently discovered. While $InsP_3$ and $InsP_4$ added individually to wheat rolls had no effect on iron absorption in humans, they did increase the negative effect of small amounts of $InsP_5$ and $InsP_6$ when all four were added together (Sandberg *et al.*, 1999). Thus $InsP_3$ and $InsP_4$ potentiated the negative effect of $InsP_5$ and $InsP_6$, presumably by binding some of the soluble iron and

cross-linking it to insoluble complexes formed by iron and $InsP_5$ and $InsP_6$, thereby increasing the portion of iron that was insoluble. In Caco-2 cells 10 : 1 molar ratios of inositol phosphate to iron gave small differences in uptake of iron between $InsP_3$, $InsP_4$, $InsP_5$ and $InsP_6$, but the transport of iron across the cells was dramatically reduced in proportion to the number of phosphates (Han *et al.*, 1994). Additional Caco-2 experiments with a 2 : 1 molar ratio of inositol phosphate to iron showed $InsP_6$ and $InsP_5$ inhibited iron uptake after 1 h, but various isomers of $InsP_4$ and $InsP_3$ only inhibited uptake after 4 h (Skoglund *et al.*, 1999). Similar to zinc, whole wheat flour enhanced the total iron absorption in rats, but unlike with zinc, the per cent iron absorption was also increased relative to white wheat flour (Levrat-Verny *et al.*, 1999). In an earlier rat study, Saha *et al.* (1994) found that the fractional absorption of iron and other minerals from whole wheat flour decreased with increasing phytate content, but was still high, 66% for iron, at the highest level of phytate. It would be helpful to do a similar study in humans. A consequence of the addition of dodecasodium phytate at a level of 1% to the diet of rats was a decrease in the iron concentration in their brains (Grases *et al.*, 2001d). Degradation of $InsP_6$ by phytase has been used to increase the bioavailability of iron from soy-based infant formula and wheat bread (Davidsson *et al.*, 1994; Sandberg *et al.*, 1996).

Several strategies have been used to lower the amount of $InsP_6$ in seeds. Maize, barley, rice and soybeans with mutations in genes involved in $InsP_6$ synthesis have been developed with levels of $InsP_6$ reduced by about half or more (Larson and Raboy, 1999; Hatzack *et al.*, 2000; Larson *et al.*, 2000; Wilcox *et al.*, 2000). When the low-phytic acid maize was used to make tortillas, iron absorption by men was 8.2% compared to 5.5% from tortillas made from wild-type maize (Mendoza *et al.*, 1998). When porridge was prepared from the two types of maize, which had been fortified with additional iron, no effect on the iron absorption in women was observed (Mendoza *et al.*, 2001). Because 92% of the activity of transgenic *Aspergillus fumigatus* phytase expressed in rice was lost after boiling the seeds for 20 min in water, phytase expression targeted to the endosperm to prevent the storage of phytic acid in the edible part of the seeds is currently under investigation (Lucca *et al.*, 2001). Prospective work with an *E. coli* phytase introduced into *Arabidopsis* has produced transgenic seeds with reduced levels of phytate and increased free phosphate (Coello *et al.*, 2001). Low-phytic acid grains and legumes have much anticipated potential for those who must satisfy their nutritional needs with a limited food intake and cannot supplement or fortify their diets with extrinsic minerals. A recent study unexpectedly found that variations in $InsP_5$ plus $InsP_6$ from 19.6 to 29.2 μmol/g in 24 genotypes of beans (*Phaseolus*

vulgaris L.) with 52–157 μmol/g of endogenous iron had no effect on iron bioavailability in rats (Welch *et al.*, 2000). However, the iron bioavailability from the beans was high, 53–76%, possibly due to unknown promoter substances and the rat intestinal phytase.

Two studies have compared the effects of various inositol phosphates on the availability of calcium. The absorption of calcium in fasted rats was 83% from a solution containing $InsP_6$, whereas $InsP_5$, $InsP_4$ and $InsP_3$ resulted in absorption of more than 98% of the dose at an inositol phosphate to calcium molar ratio of 4 : 1 (Lönnerdal *et al.*, 1989). In another rat study using purified inositol phosphate isomers, $InsP_6$ decreased calcium absorption, while Ins(1,2,3,5,6)P_5 and Ins(1,2,5,6)P_4 had no effect (Shen *et al.*, 1998). However, Ins(1,2,3,6)P_4 significantly increased calcium absorption at an inositol phosphate to calcium molar ratio of 1 : 25. It is possible that a portion of the Ins(1,2,3,6)P_4 entered cells and bound to receptors in the plasma membrane, thereby opening calcium channels, or this isomer may have formed a soluble calcium complex that was more readily absorbed. The bioavailability of calcium from grain and legume products such as whole wheat flour and soy flour is generally high (Mason *et al.*, 1993; Saha *et al.*, 1994), and components besides $InsP_6$ in wheat bran and beans may be more inhibitory to its absorption (Weaver *et al.*, 1993, 1996). In recent studies calcium transport across Caco-2 cell monolayers was reduced 16% by 2 mM $InsP_6$ (Kennefick and Cashman, 2000), and the fractional calcium absorption was decreased 16% by a diet containing 7.5 mmol/kg $InsP_6$ in rats (Harrington *et al.*, 2001).

Although the effects of different inositol phosphates on the bioavailability of polycationic minerals other than zinc, iron and calcium have not been investigated, it can be assumed that $InsP_6$ and $InsP_5$ will generally have the greatest negative impact. Interactions with other components of the diet, especially calcium and other minerals, also play an important role in determining availability. *In vivo* studies have continued to investigate concerns for the adverse effects of $InsP_6$ on the absorption of selenium and magnesium (Saha *et al.*, 1994; Pallauf *et al.*, 1998; Rimbach and Pallauf, 1999). In 10 mM metal chloride and 62.5 μM $InsP_6$ solutions at pH 6.0 and 7.0 more than half the Mg^{2+} precipitated within 2 h while all the Ca^{2+} remained in solution, but below pH 5.5 and 6.0, 10 mM Ca^{2+} and Mg^{2+}, respectively, were 100% soluble at all concentrations of $InsP_6$ from 62.5 μM to 20 mM (Nolan *et al.*, 1987). In solutions of 1 mM $InsP_6$ and 1–3 mM copper(II) or zinc(II) ions at pH 5.9, copper(II) ions were more soluble than zinc(II) ions (Champagne and Hinojosa, 1987), which explains why $InsP_6$ can enhance the bioavailability of copper in the rat (Lee *et al.*, 1988). However, soluble zinc to copper molar ratios increased as the total metal ion to $InsP_6$ molar ratios increased from 2 : 1 to 12 : 1 (Champagne and Hinojosa, 1987), which

may help to explain why $InsP_6$ has also been found to inhibit copper absorption in rats (Lopez *et al.*, 1998). In pH 7.0 solutions containing 10 mM $InsP_6$ and 1 mM metal ions, copper(II) remained soluble while zinc phytate slowly precipitated (Champagne and Fisher, 1990). Between pH 5 and pH 6, $InsP_6$, $InsP_5$, $InsP_4$ and $InsP_3$ can bind more copper than zinc, and the amount of metal bound is proportional to the number of phosphate groups (Persson *et al.*, 1998). Additional mineral nutrients including manganese, cobalt and nickel are known to bind to $InsP_6$ (Vohra *et al.*, 1965), and others such as chromium, molybdenum and vanadium are likely to do so as well.

Aluminum and the heavy metals also form complexes with inositol phosphates. Aluminum binds to the second messenger Ins(1,4,5)P_3 more strongly than to ATP, but the biological significance of this is not clear (Mernissi-Arifi *et al.*, 1995). Aluminum and lead formed insoluble complexes with $InsP_6$ at metal to $InsP_6$ molar ratios of 5 : 1 to 3 : 1, whereas mercury was completely soluble at all $InsP_6$ concentrations (Bullock *et al.*, 1995). At pH 5.0 in solutions containing 10 mM $InsP_6$ and 10 mM metal ions the solubilities of aluminum and lead were 69% and 4%, respectively. Mercury competes with calcium in binding to $InsP_6$ (Bullock *et al.*, 1995) and forms complexes with Ins(1,2,6)P_3 (Lapp and Speiss, 1991). Thus one must consider the possibility that inositol phosphates might enhance the absorption of mercury, as has been shown for $InsP_6$ and copper. Cadmium binds to $InsP_6$, $InsP_5$, $InsP_4$ and $InsP_3$ less strongly than copper and zinc do at low pH values in the vicinity of pH 4 (Persson *et al.*, 1998). In a recent study in rats, *Aspergillus niger* phytase addition to a maize-soybean diet to increase zinc absorption did not alter cadmium concentrations in the liver and kidney, but nonsignificant increases in femur lead were observed (Rimbach *et al.*, 1998).

B. PREVENTION OF HEALTH DISORDERS

The backbone of most inositol phosphates in cells is *myo*-inositol. The nutritional importance of *myo*-inositol has long been recognized for its roles in the utilization of fat, as a growth promoter, and its ability to improve nerve conductance in diabetics (Holub, 1982, 1986). These functions may be partly or mostly derived from the use of inositol as a precursor of phosphatidylinositols and inositol phosphates. An extensive review of the metabolism of *myo*-inositol in plants was published recently (Loewus and Murthy, 2000). Inositol phosphates from seeds are a significant food source of *myo*-inositol, as are the phospholipids and free inositol from many plant- and animal-based foods (Berdanier, 1992). The

total *myo*-inositol contents of the majority of fruits, vegetables, grains and nuts analyzed after digestion with 6 N HCl for 40 h at 120°C were between 0.2 and 2.0 mg/g (Clements and Darnell, 1980). *Myo*-inositol has been evaluated for its ability to improve the mental health of patients with various psychiatric disorders (Kofman *et al.*, 1998; Seedat and Stein, 1999; Kofman *et al.*, 2000; Einat and Belmaker, 2001; Nemets *et al.*, 2001). In addition to *myo*-inositol, smaller amounts of *epi*- and *scyllo*-inositol are present in human brains (McLaurin *et al.*, 2000). Phosphatidyl-*scyllo*-inositol appears to be synthesized more rapidly than phosphatidyl-*myo*-inositol in barley seeds (Carstensen *et al.*, 1999), but little is known about the metabolism or function of *scyllo*-inositol in animals. D-*Chiro*-inositol, which may be of benefit to diabetics (Steadman *et al.*, 2000), and *myo*-inositol levels in urine of older men and women, appear to be related to insulin secretion (Campbell *et al.*, 2001).

Myo-inositol and $InsP_6$ have synergistic or additive effects in inhibiting the development of cancer (Shamsuddin, 1999). In mice, dietary *myo*-inositol has been shown to be effective in preventing cancer of the colon (Shamsuddin *et al.*, 1989), lung (Estensen and Wattenberg, 1993; Hecht *et al.*, 1999; Wattenberg *et al.*, 2000; Hecht *et al.*, 2001), forestomach (Estensen and Wattenberg, 1993) and liver (Nishino *et al.*, 1999). The anticancer action of $InsP_6$ is extensively documented as reviewed by Shamsuddin (1995, 1999) and Jenab and Thompson (2002). In rats, mice or humans $InsP_6$ has antitumor effects in cells or tissues of the blood, colon, liver, lung, mammary gland, prostate and skin. Although exogenous $InsP_6$ and wheat bran containing a similar amount of $InsP_6$ had similar effects on biomarkers of colon cancer risk in rats (Jenab and Thompson, 1998, 2000), the former was more effective in reducing the number of mammary tumors (Vucenik *et al.*, 1997).

While most studies on the anticancer effects of $InsP_6$ have yielded positive results, a few contradictory reports have raised concerns regarding cancers of the urinary tract and rhabdomyosarcomas, which are muscle tumors occurring mainly in young people. Rats given drinking water containing 1.25% or 2.5% $InsP_6$ *ad lib* for 2 years passed blood in the urine from hemorrhage of necrotic renal papillae and developed renal papillomas (Hiasa *et al.*, 1992). In rats given a combination of three cancer initiators and fed diets with or without 2% $InsP_6$ for 32 weeks, $InsP_6$ increased the incidence of urinary bladder papillomas (Hirose *et al.*, 1999). However, in rats given a single different initiator and treated similarly with $InsP_6$ or its salts, $InsP_6$ alone had no effect while the dodecasodium salt of $InsP_6$ significantly increased the incidence of urinary bladder hyperplasias and papillomas (Hirose *et al.*, 1999). It was concluded that the effect of $InsP_6$ itself was equivocal, but the dodecasodium salt of $InsP_6$ promoted carcino-

genesis. Alkaline salts are known to promote urinary bladder carcinogenesis by raising the urinary pH (Lina *et al.*, 1994), which causes the formation of cytotoxic calcium phosphate precipitates (Cohen *et al.*, 2000). In serum-free medium, micromolar levels of $InsP_6$ stimulated the growth of two human rhabdomyosarcoma cell lines but inhibited the growth of a third rhabdomyosarcoma and two human colon carcinoma cell lines (Germain and Houghton, 1997). When rhabdomyosarcoma cells susceptible to growth inhibition by $InsP_6$ were xenografted into nude mice, tumor size after 2 and 5 weeks was 25-fold and 49-fold smaller, respectively, in mice treated with $InsP_6$ than in untreated controls (Vucenik *et al.*, 1998). Additional studies to further clarify the potential risks associated with the consumption of $InsP_6$ in regards to urinary tract cancers and rhabdomyosarcomas would be helpful.

There are a variety of mechanisms by which inositol and $InsP_6$ may inhibit the development of cancer. There is evidence that *myo*-inositol suppresses the phosphatidylinositol 3-kinase pathway and thereby protects against the inhibition by carcinogens of cell differentiation (Jyonouchi *et al.*, 1999). The effect of *myo*-inositol within the cell is likely to be mediated through one or more of its phosphorylated metabolites. Signal transduction through Ins(1,4,5)P_3 is also elevated in human carcinomas, and inhibitors of phosphatidylinositol 4-kinase and phosphatidylinositol 4-phosphate 5-kinase induce differentiation and apoptosis of cancer cells (Weber *et al.*, 1999). Razzini *et al.* (2000) propose that Ins(1,4,5,6)P_4 and Ins(1,3,4,5,6)P_5, but not $InsP_6$, inhibit the growth of various types of cancer cells by binding to pleckstrin homology (PH) domains of Akt (protein kinase B), which is activated by the lipid products of phosphatidylinositol 3-kinase. It has been suggested that $InsP_6$ inhibits cell transformation into a cancerous phenotype by directly inhibiting phosphatidylinositol 3-kinase (Huang *et al.*, 1997; Dong *et al.*, 1999) or by inhibiting the phosphorylation of extracellular signal-related protein kinases (Erks), c-Jun NH_2-terminal kinases (JNKs) or an inhibitor of nuclear factor κβ (Iκβ) (Chen *et al.*, 2001). Proteins that become upregulated following $InsP_6$ treatment include *p53* in HT-29 colon carcinoma cells (Saied and Shamsuddin, 1998) and hepatic glutathione *S*-transferase in mice (Singh *et al.*, 1997). Ornithine decarboxylase, which is essential for the promotion of some tumors, is downregulated by $InsP_6$ in mouse keratinocytes (Nickel and Belury, 1999). Furthermore, $InsP_6$ inhibits endocytosis by impairing the binding of erbB1 to AP2 in prostate cancer cells (Zi *et al.*, 2000). Although it seems possible that some $InsP_6$ may enter cells intact, convincing proof for this is lacking. An alternative mechanism through which extracellular $InsP_6$ conceivably could combat cancer is through mineral deprivation; intracellular zinc deficiency leads to cell death by apoptosis (Truong-Tran *et al.*, 2000). Iron chelators such

as O-Trensox and desferrioxamine induce apoptosis in human hepatoblastoma HepG2 and hepatocarcinoma HBG cells, although addition of iron or zinc during treatment restores both proliferation and inhibition of apoptosis (Rakba *et al.*, 2000). Another relevant observation is that $InsP_6$ enhances natural killer cell activity (Baten *et al.*, 1989). $InsP_6$ is also a substrate for the $InsP_6$ kinase that transduces the signal from interferon-β for the growth inhibition and apoptosis of ovarian carcinoma cells (Morrison *et al.*, 2001).

The antioxidant attributes of inositol phosphates may contribute to their anticancer activity as well as the prevention and amelioration of other conditions associated with excessive oxidation or inflammation. $InsP_6$ chelates iron within its 1,2,3-trisphosphate grouping, thus preventing iron-catalyzed hydroxyl free radical formation (Hawkins *et al.*, 1993). Ins(1,2,3)P_3, D/L-Ins(1,2,3,4)P_4, Ins(1,2,3,5)P_4, D/L-Ins(1,2,3,4,5)P_5 and Ins(1,2,3,4,6)P_5 also possess this property (Hawkins *et al.*, 1993; Spiers *et al.*, 1995, 1996; Phillippy and Graf, 1997). $InsP_3$, $InsP_4$ and $InsP_5$ fractions derived from $InsP_6$ by hydrolysis with microbial phytase prevent the iron-catalyzed decomposition of lipid peroxides, which liberates peroxyl and/or alkoxyl radicals, whereas $InsP_2$ has no effect (Miyamoto *et al.*, 2000). Studies have shown that dietary $InsP_6$ reduces lipid peroxide formation in the liver of lactating mice and the colon of pigs with high iron intake (Singh *et al.*, 1997; Porres *et al.*, 1999). In rats subjected to oxidative stress, dietary $InsP_6$ and Ins(1,2,3,6)P_4 decreased the production of lipid peroxides in the small intestine and colon, whereas only $InsP_6$ gave an antioxidative response in the lung (Burgess and Gao, 2002). However, $InsP_6$ had no effect on lipid peroxides, protein oxidation, α-tocopherol or reduced glutathione in the liver of growing rats (Rimbach and Pallauf, 1998). In HL-60 human leukemia cells and calf thymus DNA exposed to H_2O_2, $InsP_6$ reduced the formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine, a biomarker of oxidative DNA damage; and cleavage at the 5′-guanine of GG and GGG sequences in c-Ha-*ras*-1 and *p53* gene DNA fragments in the presence of H_2O_2 and copper was decreased by $InsP_6$ (Midorikawa *et al.*, 2001). *In vitro* $InsP_6$ inhibited the oxidation of 6-hydroxydopamine by iron or manganese, accelerated catalysis by vanadium and had no effect in the presence of copper (Bandy *et al.*, 2001). Formation of the *p*-quinone oxidation product proceeded most rapidly when the reduction potential of various metal-ligand complexes fell between the reduction potentials of 6-hydroxydopamine and molecular oxygen. Copper was the most effective catalyst, and the rate of 6-hydroxydopamine oxidation by copper phytate was increased three-fold in the presence of 100 mM Na_2SO_4.

The effectiveness of $InsP_6$ as an antioxidant food preservative has been demonstrated repeatedly (Empson *et al.*, 1991; Lee and Hendricks, 1995;

Hix *et al.*, 1997; Lee *et al.*, 1998; Cornforth, 2002). Unlike iron, copper appears to bind preferentially to the 5-phosphate of $InsP_6$ (Champagne *et al.*, 1990), but the ability of copper to produce hydroxyl free radicals is unhindered by $InsP_6$ (Madurawe *et al.*, 1997). In contrast to $InsP_6$, the common food additive ethylenediaminetetraacetic acid (EDTA) stimulates iron-catalyzed but inhibits copper-catalyzed hydroxyl radical formation (Madurawe et al., 1997).

$Ins(1,2,6)P_3$, which is the drug α-trinositol and is produced by many plant and microbial phytases, exhibits analgesic and anti-inflammatory properties. It has been speculated that $Ins(1,2,6)P_3$ may function in part by binding to one or more of the proteins in the phosphatidylinositol signaling pathway (Bell and McDermott, 1998), but its effectiveness has only been reported for parenteral systemic administration and topical treatment of burned skin (Tarnow *et al.*, 1998). It might be revealing to investigate the antioxidant and anti-inflammatory potencies of $Ins(1,2,3)P_3$ and $Ins(1,2,6)P_3$ in some model feeding studies.

$InsP_6$ lowers blood glucose, cholesterol and triglycerides (Rickard and Thompson, 1997; Jariwalla, 1999). This would be especially helpful for people susceptible to diabetes or heart disease. The mechanism for these effects is not completely clear but may be related to the inhibition of digestive enzymes by $InsP_6$ and/or a reduction in the plasma ratio of zinc to copper. *Myo*-inositol and $InsP_6$ were equally effective in preventing the elevation in liver lipids following sucrose feeding in rats (Katayama, 1999). It was proposed that both compounds worked by depressing hepatic lipogenesis rather than by inhibiting intestinal enzymes. Nonetheless, the old saying that a meal that keeps a person from getting hungry for a long time 'sticks to the ribs' may refer to foods high in $InsP_6$, which probably slows digestion by adhering to enzymes and other proteins in the lumen of the stomach and on the surface of the gastric mucosa. Indeed, α-amylase and lipase activities were significantly inhibited *in vitro* by 2–4 mM inositol phosphates containing one to six phosphates, and the decrease in digestibility and degree of inositol phosphorylation were highly correlated (Knuckles and Betschart, 1987; Knuckles, 1988).

Various other health benefits from $InsP_6$ consumption have been postulated. Some of those who eat diets high in red meat may accumulate excess iron, which can promote the development of infections, cancer and other degenerative diseases, and foods rich in $InsP_6$ may help prevent the over-accumulation of absorbed iron (Weinberg, 1999). This is a somewhat complex issue, especially in light of the recent observation that foods containing $InsP_6$ may lead to increased iron absorption simply because they increase the amount of iron consumed (Levrat-Verny *et al.*, 1999). In some instances the iron from foods naturally high in $InsP_6$ may be less

available on a per cent basis than the iron in meat and thus less likely to be absorbed in overabundance. There has been an accumulation of evidence that dietary $InsP_6$ helps to prevent the formation of kidney stones (Zhou and Erdman, 1995). It has been suggested that $InsP_6$ excreted in the urine is responsible for preventing stone formation by inhibiting crystallization of calcium salts (Grases and Costa-Bauzá, 1999; Grases *et al.*, 2000). However, 12 h following intragastric administration of [^{3}H]$InsP_6$ to rats the radioactivity in the urine appeared to be associated with Ins and InsP but not with any of the inositol polyphosphates (Sakamoto *et al.*, 1993). Thus any intact absorption and subsequent excretion of $InsP_6$ must be very low. $InsP_6$ has also been linked to upregulation of neutrophil functions (Eggleton, 1999) and the inhibition of platelet aggregation (Vucenik *et al.*, 1999).

VIII. SUMMARY AND CONCLUSIONS

Advances in the analytical methods for inositol phosphates have enabled an increase in our knowledge of their nutritional roles in recent years. HPLC methods provide the separations needed to identify and quantify individual inositol phosphates in foods. The metabolic pathways for the synthesis and degradation of $InsP_6$ are multibranched and dependent upon the particular mix of enzymes and substrates present in the same cellular compartment. $InsP_6$ is the most abundant inositol phosphate in the raw seeds of most grains and legumes and generally is present at concentrations between 0.4 and 1.2% of the dry weight. $InsP_6$ and $InsP_5$ account for more than 95% of the total inositol phosphates in most raw grains and legumes and predominate in processed foods, which sometimes also contain substantial levels of $InsP_4$ and $InsP_3$. Avocado fruit and some vegetables contain appreciable amounts of $InsP_6$, but very little data is available in this area. Inositol phosphates appear to be mostly hydrolyzed to inositol prior to absorption in the guts of animals. Numerous inositol phosphate isomers in animal cells display an increasingly diverse range of biological functions. The fractional absorption of dietary minerals such as zinc and iron is decreased by $InsP_6$ and $InsP_5$, and these effects might be potentiated by $InsP_4$ and $InsP_3$. *Myo*-inositol and $InsP_6$ may help to prevent various health disorders by a number of possible mechanisms. More research is needed before the optimum levels of $InsP_6$ in the diets of people differing in age, sex and health concerns can be estimated.

IX. FUTURE RESEARCH NEEDS

The most immediate research need is to determine the appropriate levels of $InsP_6$ in human diets. Some $InsP_6$ may be desirable for its potential ability to prevent or delay various health disorders, but too much can result in mineral deficiencies. It may be helpful to establish a tolerance zone or range, which lies between the optimal and toxic doses, as has been suggested for essential trace elements such as selenium and chromium (Katz, 1996). This could be accomplished by feeding studies in which the effects of $InsP_6$ on indices of potential health benefits and on the bioavailabilities of minerals are monitored simultaneously. Since the tolerance zone likely depends on factors such as age, sex, health status and diet composition, experiments performed on different population groups would be necessary in order to be able to estimate the appropriate level of $InsP_6$ in someone's diet. It will be important to identify trade-offs between the positive and negative nutritional aspects of $InsP_6$ if overlapping effects are observed and to attempt to resolve any potential conflicts. Then we will have a better idea of how much $InsP_6$ it would be prudent to remove from our foods by breeding or during processing.

More accurate data on the inositol phosphates in foods are needed. Since a number of inositol phosphates are bioactive and may be absorbed intact by cells under certain conditions, more data on their concentrations in foods should be obtained. The inositol phosphates and their natural variation in fruits and vegetables need to be analyzed, since comprehensive and accurate data for these foods is lacking. It might be a good idea to replace the nonspecific Association of Official Analytical Chemists' method for phytate analysis with one of the HPLC methods if there is sufficient interest among researchers.

Biological studies are needed to clear up some lingering questions about the fate of inositol phosphates and their associated minerals within the gut. The possibility of adaptation to $InsP_6$ in the human diet needs to be investigated more thoroughly. Recent reports showing greater overall absorption of zinc and iron from diets containing high-phytate foods should be followed up with more definitive studies. The potential for absorption of inositol phosphates complexed with food components should be evaluated. It is not currently known to what extent any of the bioactive inositol phosphates consumed in foods can be absorbed before they are enzymatically degraded or whether there may be significant population group differences in inositol phosphate absorption.

Potential health benefits of *myo*-inositol and inositol phosphates in the diet have been identified. More research is needed to define their mechanisms of action and whether the effects are mediated predominately

within the lumen of the gut or following absorption, on the surface of cells or intracellularly.

DISCLAIMER

Mention of names of companies or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture over others not mentioned.

ACKNOWLEDGEMENT

The author thanks Dr John M. Bland for the computer-generated chemical structures.

REFERENCES

Adelt, S., Plettenburg, O., Dallmann, G., Ritter, F. P., Shears, S. B., Altenbach, H.-J. and Vogel, G. 2001. Regiospecific phosphohydrolases from *Dictyostelium* as tools for the chemoenzymatic synthesis of the enantiomers D-*myo*-inositol 1,2,4-trisphosphate and D-*myo*-inositol 2,3,6-trisphosphate: non-physiological, potential analogues of biologically active D-*myo*-inositol 1,3,4-trisphosphate. *Bioorg. Med. Chem. Lett.* **11**, 2705–2708.

Agte, V. V., Tarwadi, K. V. and Chiplonkar, S. A. 1999. Phytate degradation during traditional cooking: significance of the phytic acid profile in cereal-based vegetarian meals. *J. Food Compos. Anal.* **12**, 161–167.

Ali, N., Craxton, A., Sumner, M. and Shears, S. B. 1995. Effects of aluminum on the hepatic inositol polyphosphate phosphatase. *Biochem. J.* **305**, 557–561.

Asada, K., Tanaka, K. and Kasai, Z. 1969. Formation of phytic acid in cereal grains. *Ann. N. Y. Acad. Sci.* **165**, 801–814.

Ayet, G., Burbano, C., Cuadrado, C., Pedrosa, M. M., Robredo, L. M., Muzquiz, M., de la Cuadra, C., Castaño, A. and Osagie, A. 1997. Effect of germination, under different environmental conditions, on saponins, phytic acid and tannins in lentils (*Lens culinaris*). *J. Sci. Food Agric.* **74**, 273–279.

Ballou, C. E. and Pizer, L. I. 1959. Synthesis of an optically active *myo*-inositol 1-phosphate. *J. Am. Chem. Soc.* **81**, 4745.

Bandy, B., Walter, P. B., Moon, J. and Davison, A. J. 2001. Reaction of oxygen with 6-hydroxydopamine catalyzed by Cu, Fe, Mn, and V complexes: identification of a thermodynamic window for effective metal catalysis. *Arch. Biochem. Biophys.* **389**, 22–30.

Barker, C. J., French, P. J., Moore, A. J., Nilsson, T., Berggren, P. O., Bunce, C. M., Kirk, C. J. and Michell, R. H. 1995. Inositol 1,2,3-trisphosphate and inositol 1,2- and/or 2,3-bisphosphate are normal constituents of mammalian cells. *Biochem. J.* **306**, 557–564.

Barrientos, L., Scott, J. J. and Murthy, P. P. N. 1994. Specificity of hydrolysis of phytic acid by alkaline phytase from lily pollen. *Plant Physiol.* **106**, 1489–1495.

Baten, A., Ullah, A., Tomazic, V. J. and Shamsuddin, A. M. 1989. Inositol-phosphate-induced enhancement of natural killer cell activity correlates with tumor suppression. *Carcinogenesis* **10**, 1595–1598.

Bell, D. and McDermott, B. J. 1998. D-myo inositol 1,2,6, triphosphate (alpha-trinositol, pp56): selective antagonist at neuropeptide Y (NPY) Y-receptors or selective inhibitor of phosphatidylinositol cell signaling? *Gen. Pharmacol.* **31**, 689–696.

Beno-Moualem, M. D., Naveh, L. and Jacoby, B. 1995. Responses of red beet tissue to hypertonic salt-shock: Inositol 1,4,5-trisphosphate, ATPase activation and protein phosphorylation. *Plant Physiol. Biochem.* **33**, 311–318.

Berdanier, C. D. 1992. Is inositol an essential nutrient? *Nutr. Today* **27**, 22–26.

Bergman, E. L., Fredlund, K., Reinikainen, P. and Sandberg, A. S. 1999. Hydrothermal processing of barley (cv. Blenheim): optimisation of phytate degradation and increase of free myo-inositol. *J. Cereal Sci.* **29**, 261–272.

Berka, R. M., Rey, M. W., Brown, K. M., Byun, T. and Klotz, A. V. 1998. Molecular characterization and expression of a phytase gene from the thermophilic fungus *Thermomyces lanuginosus*. *Appl. Environ. Microbiol.* **64**, 4423–4427.

Berridge, M. J. 1993. Inositol trisphosphate and calcium signalling. *Nature* **361**, 315–325.

Bos, K. D., Verbeek, C., van Eden, C. H. P., Slump, P. and Wolters, M. G. E. 1991. Improved determination of phytate by ion-exchange chromatography. *J. Agric. Food Chem.* **39**, 1770–1772.

Boucher, L., Chassaigne, M. and Ropars, C. 1996. Internalization and distribution of inositol hexakisphosphate in red blood cells. *Biotechnol. Appl. Biochem.* **24**, 73–78.

Brearley, C. A. and Hanke, D. E. 1996. Metabolic evidence for the order of addition of individual phosphate esters to the myo-inositol moiety of inositol hexakisphosphate in the duckweed *Spirodela polyrhiza* L. *Biochem. J.* **314**, 227–233.

Brearley, C. A. and Hanke, D. E. 2000. Metabolic relations of inositol 3,4,5,6-tetrakisphosphate revealed by cell permeabilization. Identification of inositol 3,4,5,6-tetrakisphosphate 1-kinase and inositol 3,4,5,6-tetrakisphosphate phosphatase activities in mesophyll cells. *Plant Physiol.* **122**, 1209–1216.

Brooks, S. P. J. and Lampi, B. J. 2001. Problems associated with measuring phytate in infant cereals. *J. Agric. Food Chem.* **49**, 564–569.

Brune, M., Rossander, H. L., Hallberg, L., Gleerup, A. and Sandberg, A. -S. 1992. Iron absorption from bread in humans: Inhibiting effects of cereal fiber, phytate and inositol phosphates with different number of phosphate groups. *J. Nutr.* **122**, 442–449.

Bullock, J. I., Duffin, P. A., Nolan, K. B. and Smith, T. K. 1995. Effect of phytate on the in-vitro solubility of Al^{3+}, Ca^{2+}, Hg^{2+} and Pb^{2+} as a function of pH at 37°C. *J. Sci. Food Agric.* **67**, 507–509.

Bunce, C. M., French, P. J., Allen, P., Mountford, J. C., Moor, B., Greaves, M. F., Michell, R. H. and Brown, G. 1993. Comparison of the levels of inositol metabolites in transformed haemopoietic cells and their normal counterparts. *Biochem. J.* **289**, 667–673.

Burbano, C., Muzquiz, M., Osagie, A., Ayet, G. and Cuadrado, C. 1995. Determination of phytate and lower inositol phosphates in Spanish legumes by HPLC methodology. *Food Chem.* **52**, 321–325.

Burford, N. T., Nahorski, S. R., Chung, S. K., Chang, Y. T. and Wilcox, R. A. 1997. Binding and activity of the nine possible regioisomers of myo-inositol tetrakisphosphate at the inositol 1,4,5-trisphosphate receptor. *Cell Calcium* **21**, 301–310.

Burgess, J. R., and Gao, F. 2002. The antioxidant effects of inositol phosphates. *In* "Food Phytates" (Reddy, N. R. and Sathe, S. K., eds.), pp. 189–197. CRC Press, Boca Raton, FL.

Caffrey, J. J., Darden, T., Wenk, M. R. and Shears, S. B. 2001. Expanding coincident signaling by PTEN through its inositol 1,3,4,5,6-pentakisphosphate 3-phosphatase activity. *FEBS Lett.* **499**, 6–10.

Campbell, W. W., Ostlund, R. E., Joseph, L. J., Farrell, P. A. and Evans, W. J. 2001. Relationships of plasma C-peptide and gender to the urinary excretion of inositols in older people. *Horm. Metab. Res.* **33**, 44–51.

Carlsson, N. -G., Bergman, E. -L., Skoglund, E., Hasselblad, K. and Sandberg, A. -S. 2001. Rapid analysis of inositol phosphates. *J. Agric. Food Chem.* **49**, 1695–1701.

Carstensen, S., Pliska Matyshak, G., Bhuvarahamurthy, N., Robbins, K. M. and Murthy, P. P. N. 1999. Biosynthesis and localization of phosphatidyl-scyllo-inositol in barley aleurone cells. *Lipids* **34**, 67–73.

Cecconi, O., Nelson, R. M., Roberts, W. G., Hanasaki, K., Mannori, G., Schultz, C., Ulich, T. R., Aruffo, A. and Bevilacqua, M. P. 1994. Inositol polyanions: Noncarbohydrate inhibitors of L- and P-selectin that block inflammation. *J. Biol. Chem.* **269**, 15060–15066.

Champagne, E. T. and Fisher, M. S. 1990. Binding differences of Zn(II) and Cu(II) ions with phytate. *J. Inorg. Biochem.* **38**, 217–223.

Champagne, E. T. and Hinojosa, O. 1987. Independent and mutual interactions of copper(II) and zinc(II) ions with phytic acid. *J. Inorg. Biochem.* **30**, 15–33.

Champagne, E. T., Fisher, M. S. and Hinojosa, O. 1990. NMR and ESR studies of interactions among divalent cations, phytic acid, and *N*-acetyl-amino acids. *J. Inorg. Biochem.* **38**, 199–215.

Chattaway, J. A., Drobak, B. K., Watkins, P. A. C., Dawson, A. P., Letcher, A. J., Stephens, L. R. and Irvine, R. F. 1992. An inositol 1,4,5-trisphosphate-6-kinase activity in pea roots. *Planta* **187**, 542–545.

Chen, N., Ma, W.-Y. and Dong, Z. 2001. Inositol hexaphosphate inhibits ultraviolet B-induced signal transduction. *Mol. Carcinog.* **31**, 139–144.

Cilliers, J. J. L. and van Niekert, P. J. 1986. LC determination of phytic acid in food by postcolumn colorimetric detection. *J. Agric. Food Chem.* **34**, 680–683.

Clements, R. S., Jr. and Darnell, B. 1980. *Myo*-inositol content of common foods: development of a high-*myo*-inositol diet. *Am. J. Clin. Nutr.* **33**, 1954–1967.

Coello, P., Maughan, J. P., Mendoza, A., Philip. R., Bollinger, D. W., Veum, T. L., Vodkin, L. O. and Polacco, J. C. 2001. Generation of low phytic acid *Arabidopsis* seeds expressing an *E. coli* phytase during embryo development. *Seed Sci. Res.* **11**, 285–291.

Cohen, S. M., Arnold, L. L., Cano, M., Ito, M., Garland, E. M. and Shaw, R. A. 2000. Calcium phosphate-containing precipitate and the carcinogenicity of sodium salts in rats. *Carcinogenesis* **21**, 783–792.

Copani, A., Raciti, G., Bruno, V., Sortino, M. A., Nicoletti, F., Canonico, P. L. and Cambria, A. 1991. Inositolhexakisphosphate stimulates calcium-45 influx in purified mitochondria from rat liver. *Ital. J. Biochem.* **40**, 289–294.

Cornforth, D. P. 2002. Potential use of phytate as an antioxidant in cooked meats. *In* "Food Phytates" (Reddy, N. R. and Sathe, S. K., eds), pp. 199–209. CRC Press, Boca Raton, FL.

Cosgrove, D. J. and Irving, G. C. J. 1980. "Inositol Phosphates: Their Chemistry, Biochemistry, and Physiology. Studies in Organic Chemistry; 4." Elsevier Scientific Pub. Co., New York.

Craxton, A., Erneux, C. and Shears, S. B. 1994. Inositol 1,4,5,6-tetrakisphosphate is phosphorylated in rat liver by a 3-kinase that is distinct from inositol 1,4,5-trisphosphate 3-kinase. *J. Biol. Chem.* **269**, 4337–4342.

Davidsson, L., Galan, P., Kastenmayer, P., Cherouvrier, F., Juillerat, M. A., Hercberg, S. and Hurrell, R. F. 1994. Iron bioavailability studies in infants: the influence of phytic acid and ascorbic acid in infant formulas based on soy isolate. *Pediatric Res.* **36**, 816–822.

de la Cuadra, C., Muzquiz, M., Burbano, C., Ayet, G., Calvo, R., Osagie, A. and Cuadrado, C. 1994. Alkaloid, α-galactoside and phytic acid changes in germinating lupin seeds. *J. Sci. Food Agric.* **66**, 357–364.

Delisle, S., Radenberg, T., Wintermantel, M. R., Tietz, C., Parys, J. B., Pittet, D., Welsh, M. J. and Mayr, G. W. 1994. Second messenger specificity of the inositol trisphosphate

receptor: Reappraisal based on novel inositol phosphates. *Am. J. Physiol.* **266**, C429–C436.

Dong, Z., Huang, C. and Ma, W. Y. 1999. PI-3 kinase in signal transduction, cell transformation, and as a target for chemoprevention of cancer. *Anticancer Res.* **19**, 3743–3747.

Efanov, A. M., Zaitsev, S. V. and Berggren, P. O. 1997. Inositol hexakisphosphate stimulates non-Ca^{2+} mediated and primes Ca^{2+} mediated exocytosis of insulin by activation of protein kinase *C. Proc. Natl Acad. Sci. USA* **94**, 4435–4439.

Eggleton, P. 1999. Effect of IP6 on human neutrophil cytokine production and cell morphology. *Anticancer Res.* **19**, 3711–3715.

Einat, H. and Belmaker, R. H. 2001. The effects of inositol treatment in animal models of psychiatric disorders. *J. Affective Disord.* **62**, 113–121.

Empson, K. L., Labuza, T. P. and Graf, E. 1991. Phytic acid as a food antioxidant. *J. Food Sci.* **56**, 560–563.

Estensen, R. D. and Wattenberg, L. W. 1993. Studies of chemopreventive effects of *myo*-inositol on benzo(a)pyrene-induced neoplasia of the lung and forestomach of female A/J mice. *Carcinogenesis* **14**, 1975–1977.

Feng, Y., Wente, S. R. and Majerus, P. W. 2001. Overexpression of the inositol phosphatase SopB in human 293 cells stimulates cellular chloride influx and inhibits nuclear mRNA export. *Proc. Natl Acad. Sci. USA* **98**, 875–879.

Ferguson, E. L., Gibson, R. S., Opare, O. C., Osei, O. F., Stephen, A. M., Lehrfeld, J. and Thompson, L. U. 1993. The zinc, calcium, copper, manganese, nonstarch polysaccharide and phytate content of seventy-eight locally grown and prepared African foods. *J. Food Compos. Anal.* **6**, 87–99.

Fleischer, B., Xie, J., Mayrleitner, M., Shears, S. B., Palmer, D. J. and Fleischer, S. 1994. Golgi coatomer binds, and forms K^+-selective channels gated by, inositol polyphosphates. *J. Biol. Chem.* **269**, 17826–17832.

Fredlund, K., Asp, N. G., Larsson, M., Marklinder, I. and Sandberg, A. -S. 1997. Phytate reduction in whole grains of wheat, rye, barley and oats after hydrothermal treatment. *J. Cereal Sci.* **25**, 83–91.

Fredrikson, M., Alminger, M. L., Carlsson, N. -G. and Sandberg, A. -S. 2001a. Phytate content and phytate degradation by endogenous phytase in pea (*Pisum sativum*). *J. Sci. Food Agric.* **81**, 1139–1144.

Fredrikson, M., Biot, P., Alminger, M. L., Carlsson, N. -G. and Sandberg, A. -S. 2001b. Production process for high-quality pea-protein isolate with low content of oligosaccharides and phytate. *J. Agric. Food Chem.* **49**, 1208–1212.

Freund, W. D., Mayr, G. W., Tietz, C. and Schultz, J. E. 1992. Metabolism of inositol phosphates in the protozoan *Paramecium*: Characterization of a novel inositol-hexakisphosphate-dephosphorylating enzyme. *Eur. J. Biochem.* **207**, 359–367.

Frossard, E., Bucher, M., Maechler, F., Mozafar, A. and Hurrell, R. 2000. Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. *J. Sci. Food Agric.* **80**, 861–879.

Fukuda, M., Aruga, J., Niinobe, M., Aimoto, S. and Mikoshiba, K. 1994. Inositol-1,3,4,5-tetrakisphosphate binding to C2B domain of IP4BP/synaptotagmin II. *J. Biol. Chem.* **269**, 29206–29211.

Gaidarov, I., Chen, Q., Falck, J. R., Reddy, K. K. and Keen, J. H. 1996. A functional phosphatidylinositol 3,4,5-trisphosphate/phosphoinositide binding domain in the clathrin adaptor AP-2 alpha subunit: Implications for the endocytic pathway. *J. Biol. Chem.* **271**, 20922–20929.

Germain, G. S. and Houghton, P. J. 1997. Phytic acid stimulates the growth of a human rhabdomyosarcoma. *In Vitro Cell. Dev. Biol. Animal* **33**, 581–583.

Gersonde, K. and Ganguly, T. 1986. Inositol phosphates as modulators of oxygen affinity in hemoglobin. In "Phytic Acid: Chemistry & Applications" (E. Graf, ed.), pp. 195–248. Pilatus Press, Minneapolis, MN.

Graf, E. and Dintzis, F. R. 1982. Determination of phytic acid in foods by high-performance liquid chromatography. *J. Agric. Food Chem.* **30**, 1094–1097.

Graf, E. and Eaton, J. W. 1990. Antioxidant functions of phytic acid. *Free Radic. Biol. Med.* **8**, 61–69.

Graf, E., Empson, K. L. and Eaton, J. W. 1987. Phytic acid. A natural antioxidant. *J. Biol. Chem.* **262**, 11647–11650.

Grases, F. and Costa-Bauzá, A. 1999. Phytate (IP6) is a powerful agent for preventing calcifications in biological fluids: Usefulness in renal lithiasis treatment. *Anticancer Res.* **19**, 3717–3722.

Grases, F., March, J. G., Prieto, R. M., Simonet, B. M., Costa, B. A., Garcia, R. A. and Conte, A. 2000. Urinary phytate in calcium oxalate stone formers and healthy people: Dietary effects on phytate excretion. *Scand. J. Urol. Nephrol.* **34**, 162–164.

Grases, F., Simonet, B. M., Prieto, R. M. and March, J. G. 2001a. Phytate levels in diverse rat tissues: influence of dietary phytate. *Brit. J. Nutr.* **86**, 225–231.

Grases, F., Simonet, B. M., Prieto, R. M. and March, J. G. 2001b. Variation of $InsP_4$, $InsP_5$ and $InsP_6$ levels in tissues and biological fluids depending on dietary phytate. *J. Nutr. Biochem.* **12**, 595–601.

Grases, F., Simonet, B. M., Vucenik, I., Prieto, R. M., Costa-Bauzá, A., March, J. G. and Shamsuddin, A. M. 2001c. Absorption and excretion of orally administered inositol hexaphosphate (IP_6 or phytate) in humans. *BioFactors* **15**, 53–61.

Grases, F., Simonet, B. M., Prieto, R. M. and March J. G. 2001d. Dietary phytate and mineral bioavailability. *J. Trace Elem. Med. Biol.* **15**, 221–228.

Greiner, R. and Alminger, M. L. 1999. Purification and characterization of a phytate degrading enzyme from germinated oat (*Avena sativa*). *J. Sci. Food Agric.* **79**, 1453–1460.

Greiner, R. and Alminger, M. L. 2001. Stereospecificity of *myo*-inositol hexakisphosphate dephosphorylation by phytate-degrading enzymes of cereals. *J. Food Biochem.* **25**, 229–248.

Greiner, R. and Konietzny, U. 1998. Endogenous phytate-degrading enzymes are responsible for phytate reduction while preparing beans (*Phaseolus vulgaris*). *J. Food Process. Preserv.* **22**, 321–331.

Greiner, R. and Konietzny, U. 1999. Improving enzymatic reduction of *myo*-inositol phosphates with inhibitory effects on mineral absorption in black beans (*Phaseolus vulgaris* var. Preto). *J. Food Process. Preserv.* **23**, 249–261.

Greiner, R., Konietzny, U. and Jany, K. D. 1993. Purification and characterization of two phytases from *Escherichia coli*. *Arch. Biochem. Biophys.* **303**, 107–113.

Greiner, R., Haller, E., Konietzny, U. and Jany, K. D. 1997. Purification and characterization of a phytase from *Klebsiella terrigena*. *Arch. Biochem. Biophys.* **341**, 201–206.

Greiner, R., Konietzny, U. and Jany, K. D. 1998. Purification and properties of a phytase from rye. *J. Food Biochem.* **22**, 143–161.

Greiner, R., Carlsson, N. and Alminger, M. L. 2000a. Stereospecificity of *myo*-inositol hexakisphosphate dephosphorylation by a phytate-degrading enzyme of *Escherichia coli*. *J. Biotechnol.* **84**, 53–62.

Greiner, R., Jany, K. D. and Larsson, A. M. 2000b. Identification and purification of *myo*-inositol hexakisphosphate phosphohydrolases (phytases) from barley (*Hordeum vulgare*). *J. Cereal Sci.* **31**, 127–139.

Greiner, R., Alminger, M. L. and Carlsson, N. -G. 2001a. Stereospecificity of *myo*-inositol hexakisphosphate dephosphorylation by a phytate-degrading enzyme of baker's yeast. *J. Agric. Food Chem.* **49**, 2228–2233.

Greiner, R., Muzquiz, M., Burbano, C., Cuadrado, C., Pedrosa, M. M. and Goyoaga, C. 2001b. Purification and characterization of a phytate-degrading enzyme from germinated faba beans (*Vicia faba* var. Alameda). *J. Agric. Food Chem.* **49**, 2234–2240.

Guse, A. H., Greiner, E., Emmrich, F. and Brand, K. 1993. Mass changes of inositol-1,3,4,5,6-pentakisphosphate and inositol hexakisphosphate during cell cycle progression in rat thymocytes. *J. Biol. Chem.* **268**, 7129–7133.

Gustafsson, E. L. and Sandberg, A. -S. 1995. Phytate reduction in brown beans (*Phaseolus vulgaris* L.). *J. Food Sci.* **60**, 149–152.

Han, O., Failla, M. L., Hill, A. D., Morris, E. R. and Smith, J. C., Jr. 1994. Inositol phosphates inhibit uptake and transport of iron and zinc by a human intestinal cell line. *J. Nutr.* **124**, 580–587.

Hanakahi, L. A., Bartlet-Jones, M., Chappell, C., Pappin, D. and West, S. C. 2000. Binding of inositol phosphate to DNA-PK and stimulation of double-strand break repair. *Cell* **102**, 721–729.

Harland, B. F. and Harland, J. 1980. Fermentative reduction of phytate in rye, white, and whole wheat breads. *Cereal Chem.* **57**, 226–229.

Harland, B. F. and Morris, E. R. 1995. Phytate: A good or a bad food component? *Nutr. Res.* **15**, 733–754.

Harland, B. F. and Narula, G. 1999. Food phytate and its hydrolysis products. *Nutr. Res.* **19**, 947–961.

Harrington, M. E., Flynn, A. and Cashman, K. D. 2001. Effects of dietary fibre extracts on calcium absorption in the rat. *Food Chem.* **73**, 263–269.

Hatzack, F., Johansen, K. S. and Rasmussen, S. K. 2000. Nutritionally relevant parameters in low-phytate barley (*Hordeum vulgare* L.) grain mutants. *J. Agric. Food Chem.* **48**, 6074–6080.

Hatzack, F., Hübel, F., Zhang, W., Hansen, P. E. and Rasmussen, S. K. 2001. Inositol phosphates from barley low-phytate grain mutants analyzed by metal-dye detection HPLC and NMR. *Biochem. J.* **354**, 473–480.

Hawkins, P. T., Poyner, D. R., Jackson, T. R., Letcher, A. J., Lander, D. A. and Irvine, R. F. 1993. Inhibition of iron-catalysed hydroxyl radical formation by inositol polyphosphates: A possible physiological function for *myo*-inositol hexakisphosphate. *Biochem. J.* **294**, 929–934.

Hayakawa, T., Toma, Y. and Igaue, I. 1989. Purification and characterization of acid phosphatases with or without phytase activity from rice bran. *Agric. Biol. Chem.* **53**, 1475–1483.

Heaney, R. P., Weaver, C. M. and Fitzsimmons, M. C. 1991. Soybean phytate content: Effect on calcium absorption. *Am. J. Clin. Nutr.* **53**, 745–747.

Hecht, S. S., Kenney, P. M. J., Wang, M., Trushin, N., Agarwal, S., Rao, A. V. and Upadhyaya, P. 1999. Evaluation of butylated hydroxyanisole, *myo*-inositol, curcumin, esculetin, resveratrol and lycopene as inhibitors of benzo(a)pyrene plus 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis in A/J mice. *Cancer Lett.* **137**, 123–130.

Hecht, S. S., Kenney, P. M. J., Wang, M. Y. and Upadhyaya, P. 2001. Dose-response study of *myo*-inositol as an inhibitor of lung tumorigenesis induced in A/J mice by benzo(a)pyrene and 4-methylnitrosamino)-1-(3-pyridyl)-1-butanone. *Cancer Lett.* **167**, 1–6.

Hermosura, M. C., Takeuchi, H., Fleig, A., Riley, A. M., Potter, B. V. L., Hirata, M. and Penner, R. 2000. $InsP_4$ facilitates store-operated calcium influx by inhibition of $InsP_3$ 5-phosphatase. *Nature* **408**, 735–740.

Hiasa, Y., Kitahori, Y., Morimoto, J., Konishi, N., Nakaoka, S. and Nishioka, H. 1992. Carcinogenicity study in rats of phytic acid 'Daiichi', a natural food additive. *Food Chem. Toxicol.* **30**, 117–125.

Hilton, J. M., Plomann, M., Ritter, B., Modregger, J., Freeman, H. N., Falck, J. R., Krishna, U. M. and Tobin, A. B. 2001. Phosphorylation of a synaptic vesicle-associated protein by an inositol hexakisphosphate-regulated protein kinase. *J. Biol. Chem.* **276**, 16341–16347.

Hirose, M., Fukushima, S., Imaida, K., Ito, N. and Shirai, T. 1999. Modifying effects of phytic acid and gamma-oryzanol on the promotion stage of rat carcinogenesis. *Anticancer Res.* **19**, 3665–3670.

Hix, D. K., Klopfenstein, C. F. and Walker, C. E. 1997. Physical and chemical attributes and consumer acceptance of sugar-snap cookies containing naturally occurring antioxidants. *Cereal Chem.* **74**, 281–283.

Ho, M. W. Y., Kaetzel, M. A., Armstrong, D. L. and Shears, S. B. 2001. Regulation of a human chloride channel. A paradigm for integrating input from calcium, type II calmodulin-dependent protein kinase, and inositol 3,4,5,6-tetrakisphosphate. *J. Biol. Chem.* **276**, 18673–18680.

Holub, B. J. 1982. The nutritional significance, metabolism, and function of *myo*-inositol and phosphatidylinositol in health and disease. *Adv. Nutr. Res.* **4**, 107–141.

Holub, B. J. 1986. Metabolism and function of *myo*-inositol and inositol phospholipids. *Ann. Rev. Nutr.* **6**, 563–597.

Hotz, C. and Gibson, R. S. 2001. Assessment of home-based processing methods to reduce the phytate content and phytate/zinc molar ratio of white maize (*Zea mays*). *J. Agric. Food Chem.* **49**, 692–698.

Huang, C., Ma, W. Y., Hecht, S. S. and Dong, Z. 1997. Inositol hexaphosphate inhibits cell transformation and activator protein 1 activation by targeting phosphatidylinositol-3′ kinase. *Cancer Res.* **57**, 2873–2878.

Huang, C. F., Voglmaier, S. M., Bembenek, M. E., Saiardi, A. and Snyder, S. H. 1998. Identification and purification of diphosphoinositol pentakisphosphate kinase, which synthesizes the inositol pyrophosphate bis(diphospho)inositol tetrakisphosphate. *Biochemistry* **37**, 14998–15004.

Hübel, F. and Beck, E. 1996. Maize root phytase. Purification, characterization, and localization of enzyme activity and its putative substrate. *Plant Physiol.* **112**, 1429–1436.

Hull, S. R., and Montgomery, R. 1995. Myo-inositol phosphates in corn steep water. *J. Agric. Food Chem.* **43**, 1516–1523.

Humbert, J. P., Matter, N., Artault, J. C., Koppler, P. and Malviya, A. N. 1996. Inositol 1,4,5-trisphosphate receptor is located to the inner nuclear membrane vindicating regulation of nuclear calcium signaling by inositol 1,4,5-trisphosphate: Discrete distribution of inositol phosphate receptors to inner and outer nuclear membranes. *J. Biol. Chem.* **271**, 478–485.

Irvine, R. F. 1990. "Methods in Inositide Research." Raven Press, New York.

Irvine, R. F. and Schell, M. J. 2001. Back in the water: The return of the inositol phosphates. *Nature Rev. Mol. Cell Biol.* **2**, 327–338.

Irving, G. C. J. and Cosgrove, D. J. 1972. Inositol phosphate phosphatases of microbiological origin: the inositol pentaphosphate products of *Aspergillus ficuum* phytases. *J. Bacteriol.* **112**, 434–438.

IUPAC-IUB 1968. Tentative rules for cyclitol nomenclature. *J. Biol. Chem.* **243**, 5809–5819.

Ismailov, I. I., Fuller, C. M., Berdiev, B. K., Shlyonsky, V. G., Benos, D. J. and Barrett, K. E. 1996. A biologic function for an 'orphan' messenger: D-*myo*-inositol 3,4,5,6-tetrakisphosphate selectively blocks epithelial calcium-activated chloride channels. *Proc. Natl Acad. Sci. USA* **93**, 10505–10509.

Jariwalla, R. J. 1999. Inositol hexaphosphate (IP6) as an anti-neoplastic and lipid-lowering agent. *Anticancer Res.* **19**, 3699–3702.

Jayaraman, T. and Marks, A. R. 2000. Calcineurin is downstream of the inositol 1,4,5-trisphosphate receptor in the apoptotic and cell growth pathways. *J. Biol. Chem.* **275**, 6417–6420.

Jenab, M. and Thompson, L. U. 1998. The influence of phytic acid in wheat bran on early biomarkers of colon carcinogenesis. *Carcinogenesis* **19**, 1087–1092.

Jenab, M. and Thompson, L. U. 2000. Phytic acid in wheat bran affects colon morphology, cell differentiation and apoptosis. *Carcinogenesis* **21**, 1547–1552.

Jenab, M. and Thompson, L. U. 2002. Role of phytic acid in cancer and other diseases. *In* "Food Phytates" (Reddy, N. R. and Sathe, S. K., eds), pp. 225–248. CRC Press, Boca Raton, FL.

Ji, H., Sandberg, K., Baukal, A. J. and Catt, K. J. 1989. Metabolism of inositol pentakisphosphate to inositol hexakisphosphate in *Xenopus laevis* oocytes. *J. Biol. Chem.* **264**, 20185–20188.

Johnson, K., Barrientos, L. G., Le, L. and Murthy, P. P. N. 1995. Application of two-dimensional total correlation spectroscopy for structure determination of individual inositol phosphates in a mixture. *Anal. Biochem.* **231**, 421–431.

Jyonouchi, H., Sun, S., Iijima, K., Wang, M. and Hecht, S. S. 1999. Effects of anti-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene on human small airway epithelial cells and the protective effects of *myo*-inositol. *Carcinogenesis* **20**, 139–145.

Kasim, A. B. and Edwards, H. M., Jr 1998. The analysis for inositol phosphate forms in feed ingredients. *J. Sci. Food Agric.* **76**, 1–9.

Katayama, T. 1999. Hypolipidemic action of phytic acid (IP6): Prevention of fatty liver. *Anticancer Res.* **19**, 3695–3698.

Katz, S. A. 1996. Are essential micronutrients toxic? *Food Test. Anal.* **2**, 19–23.

Kavran, J. M., Klein, D. E., Lee, A., Falasca, M., Isakoff, S. J., Skolnik, E. Y. and Lemmon, M. A. 1998. Specificity and promiscuity in phosphoinositide binding by pleckstrin homology domains. *J.Biol. Chem.* **273**, 30497–30508.

Kemme, P. A., Lommen, A., De Jonge, L. H., Van der Klis, J. D., Jongbloed, A. W., Mroz, Z. and Beynen, A. C. 1999. Quantification of inositol phosphates using ^{31}P nuclear magnetic resonance spectroscopy in animal nutrition. *J. Agric. Food Chem.* **47**, 5116–5121.

Kennefick, S. and Cashman, K. D. 2000. Inhibitory effect of wheat fiber extract on calcium absorption in Caco-2 cells: Evidence for a role of associated phytate rather than fiber *per se*. *Eur. J. Nutr.* **39**, 12–17.

Kerovuo, J., Lauraeus, M., Nurminen, P., Kalkkinen, N. and Apajalahti, J. 1998. Isolation, characterization molecular gene cloning, and sequencing of a novel phytase from *Bacillus subtilis*. *Appl. Environ. Microbial.* **64**, 2079–2085.

Kerovuo, J., Rouvinen, J. and Hatzack, F. 2000. Analysis of *myo*-inositol hexakisphosphate hydrolysis by *Bacillus* phytase: Indication of a novel reaction mechanism. *Biochem. J.* **352**, 623–628.

Kim, Y. O., Kim, H. K., Bae, K. S., Yu, J. H. and Oh, T. K. 1998. Purification and properties of a thermostable phytase from *Bacillus* sp. DS11. *Enzyme Microb. Technol.* **22**, 2–7.

Knuckles, B. E. 1988. Effect of phytate and other *myo*-inositol phosphate esters on lipase activity. *J. Food Sci.* **53**, 250–252.

Knuckles, B. E. and Betschart, A. A. 1987. Effect of phytate and other *myo*-inositol phosphate esters on α-amylase digestion of starch. *J. Food Sci.* **52**, 719–721.

Kofman, O., Agam, G., Shapiro, J. and Spencer, A. 1998. Chronic dietary inositol enhances locomotor activity and brain inositol levels in rats. *Psychopharmacology* **139**, 239–242.

Kofman, O., Einat, H., Cohen, H., Tenne, H. and Shoshana, C. 2000. The anxiolytic effect of chronic inositol depends on the baseline level of anxiety. *J. Neural Transm.* **107**, 241–253.

Konietzny, U., Greiner, R. and Jany, K. D. 1994. Purification and characterization of a phytase from spelt. *J. Food Biochem.* **18**, 165–183.

Koppitz, B., Vogel, F. and Mayr, G. W. 1986. Mammalian aldolases are isomer-selective high-affinity inositol polyphosphate binders. *Eur. J. Biochem.* **161**, 421–433.

Kozlowska, H., Honke, J., Sadowska, J., Frias, J. and Vidal Valverde, C. 1996. Natural fermentation of lentils: influence of time, concentration and temperature on the kinetics of hydrolysis of inositol phosphates. *J. Sci. Food Agric.* **71**, 367–375.

Laboure, A. M., Gagnon, J. and Lescure, A. M. 1993. Purification and characterization of a

phytase (*myo*-inositol-hexakisphosphate phosphohydrolase) accumulated in maize (*Zea mays*) seedlings during germination. *Biochem. J.* **295**, 413–419.

Lapp, C. and Speiss, B. 1991. Complexation studies on inositol phosphates III. Cadmium(II), lead(II), and mercury(II) complexes of D-*myo*-inositol 1,2,6-trisphosphate. *J. Inorg. Biochem.* **42**, 257–266.

Lardy, H. A. 1954. Inositols. I. Nomenclature and formula. A. Discussion of terminology. *In* "The Vitamins. Chemistry, Physiology, Pathology" (Sebrell, W. H. Jr. and Harris, R. S., eds), Vol. II, pp. 323–329. Academic Press, New York.

Larson, S. R. and Raboy, V. 1999. Linkage mapping of maize and barley *myo*-inositol 1-phosphate synthase DNA sequences: Correspondence with a *low phytic acid* mutation. *Theor. Appl. Genet.* **99**, 27–36.

Larson, S. R., Rutger, J. N., Young, K. A. and Raboy, V. 2000. Isolation and genetic mapping of a non-lethal rice (*Oryza sativa* L.) *low phytic acid 1* mutation. *Crop Sci.* **40**, 1397–1405.

Larsson, M. and Sandberg, A. S. 1991. Phytate reduction in bread containing oat flour, oat bran or rye bran. *J. Cereal Sci.* **14**, 141–149.

Larsson, M. and Sandberg, A. S. 1992. Phytate reduction in oats during malting. *J. Food Sci.* **57**, 994–997.

Larsson, M., Minekus, M. and Havenaar, R. 1997. Estimation of the bioavailability of iron and phosphorus in cereals using a dynamic in vitro gastrointestinal model. *J. Sci. Food Agric.* **74**, 99–106.

Lassen, S. F., Bech, L., Fuglsang, C. C., Breinholt, J., Ohmann, A. and Østergaard, P. R. 1998. Peniophora phytase. International Patent # WO 98/28408.

Lee, B. J. and Hendricks, D. G. 1995. Phytic acid protective effect against beef round muscle lipid peroxidation. *J. Food Sci.* **60**, 241–244.

Lee, B. J., Hendricks, D. G. and Cornforth, D. P. 1998. Antioxidant effects of carnosine and phytic acid in a model beef system. *J. Food Sci.* **63**, 394–402.

Lee, D. Y., Schroeder, J., III and Gordon, D. T. 1988. Enhancement of Cu bioavailability in the rat by phytic acid. *J. Nutr.* **118**, 712–717.

Lehrfeld, J. 1989. High-performance liquid chromatography analysis of phytic acid on a pH-stable, macroporous polymer column. *Cereal Chem.* **66**, 510–515.

Lehrfeld, J. 1994. HPLC separation and quantification of phytic acid and some inositol phosphates in foods: problems and solutions. *J. Agric. Food Chem.* **42**, 2726–2731.

Levrat-Verny, M. A., Coudray, C., Bellanger, J., Lopez, H. W., Demigne, C., Rayssiguier, Y. and Remesy, C. 1999. Wholewheat flour ensures higher mineral absorption and bioavailability than white wheat flour in rats. *Br. J. Nutr.* **82**, 17–21.

Li, M., Osaki, M., Honma, M. and Tadano, T. 1997. Purification and characterization of phytase induced in tomato roots under phosphorus-deficient conditions. *Soil Sci. Plant Nutr.* **43**, 179–190.

Lina, B. A. R., Hollanders, V. M. H. and Kuijpers, M. H. M. 1994. The role of alkalizing and neutral potassium salts in urinary bladder carcinogenesis in rats. *Carcinogenesis* **15**, 523–527.

Llinas, R., Sugimori, M., Lang, E. J., Morita, M., Fukuda, M., Niinobe, M. and Mikoshiba, K. 1994. The inositol high-polyphosphate series blocks synaptic transmission by preventing vesicular fusion: A squid giant synapse study. *Proc. Natl Acad. Sci. USA* **91**, 12990–12993.

Loewus, F. A. and Murthy, P. P. N. 2000. *myo*-Inositol metabolism in plants. *Plant Sci.* **150**, 1–19.

Lönnerdal, B., Sandberg, A. S., Sandstrom, B. and Kunz, C. 1989. Inhibitory effects of phytic acid and other inositol phosphates on zinc and calcium absorption in suckling rats. *J. Nutr.* **119**, 211–214.

Lopez, H. W., Coudray, C., Bellanger, J., Younes, H., Demigne, C. and Remesy, C. 1998. Intestinal fermentation lessens the inhibitory effects of phytic acid on mineral utilization in rats. *J. Nutr.* **128**, 1192–1198.

Lopez, H. W., Coudray, C., Bellanger, J., Levrat-Verny, M. A., Demigne, C., Rayssiguier, Y. and Remesy, C. 2000a. Resistant starch improves mineral assimilation in rats adapted to a wheat bran diet. *Nutr. Res.* **20**, 141–155.

Lopez, H. W., Vallery, F., Levrat-Verny, M. A., Coudray, C., Demigne, C. and Remesy, C. 2000b. Dietary phytic acid and wheat bran enhance mucosal phytase activity in rat small intestine. *J. Nutr.* **130**, 2020–2025.

Lucca, P., Hurrell, R. and Potrykus, I. 2001. Approaches to improving the bioavailability and level of iron in rice seeds. *J. Sci. Food Agric.* **81**, 828–834.

Luo, H. R., Saiardi, A., Nagata, E., Ye, K. Q., Yu, H. B., Jung, T. S., Luo, X. J., Jain, S., Sawa, A. and Snyder, S. H. 2001. GRAB: A physiologic guanine nucleotide exchange factor for Rab3A, which interacts with inositol hexakisphosphate kinase. *Neuron*, **31**, 439–451.

Luo, H. R., Saiardi, A., Yu, H., Nagata, E., Ye, K. and Synder, S. H. 2002. Inositol pyrophosphates are required for DNA hyperrecombination in protein kinase C1 mutant yeast. *Biochemistry* **41**, 2509–2515.

Madurawe, R. D., Lin, Z., Dryden, P. K. and Lumpkin, J. A. 1997. Stability of lactate dehydrogenase in metal-catalyzed oxidation solutions containing chelated metals. *Biotechnol. Prog.* **13**, 179–184.

Maenz, D. D. and Classen, H. L. 1998. Phytase activity in the small intestinal brush border membrane of the chicken. *Poultry Sci.* **77**, 557–563.

March, J. G., Simonet, B. M. and Grases, F. 2001. Determination of phytic acid by gas chromatography–mass spectrometry: Application to biological samples. *J. Chromatogr. B* **757**, 247–255.

Mason, A. C., Weaver, C. M., Kimmel, S. and Brown, R. K. 1993. Effect of soybean phytate content on calcium bioavailability in mature and immature rats. *J. Agric. Food Chem.* **41**, 246–249.

Mayr, G. W. 1989. Inositol 1,4-bisphosphate is an allosteric activator of muscle-type 6-phosphofructo-1-kinase. *Biochem. J.* **259**, 463–470.

Mayr, G. W. and Thieleczek, R. 1991. Masses of inositol phosphates in resting and tetanically stimulated vertebrate skeletal muscles. *Biochem. J.* **280**, 631–640.

McConnell, F. M., Shears, S. B., Lane, P. J. L., Scheibel, M. S. and Clark, E. A. 1992. Relationships between the degree of cross-linking of surface immunoglobulin and the associated inositol-1,4,5-trisphosphate and calcium signals in human B cells. *Biochem. J.* **284**, 447–455.

McKenzie-Parnell, J. M. and Guthrie, B. E. 1986. The phytate and mineral content of some cereals, cereal products, legumes, legume products, snack bars, and nuts available in New Zealand. *Biol. Trace Elem. Res.* **10**, 107–121.

McLaurin, J., Golomb, R., Jurewicz, A., Antel, J. P. and Fraser, P. E. 2000. Inositol stereoisomers stabilize an oligomeric aggregate of Alzheimer amyloid β peptide and inhibit A β-induced toxicity. *J. Biol. Chem.* **275**, 18495–18502.

Mendoza, C., Viteri F. E., Lonnerdal, B., Young K. A., Raboy, V. and Brown K. H. 1998. Effect of genetically modified, low-phytic acid maize on absorption of iron from tortillas. *Am. J. Clin. Nutr.* **68**, 1123–1127.

Mendoza, C., Viteri, F. E., Lonnerdal, B., Raboy, V., Young, K. A. and Brown, K. H. 2001. Absorption of iron from unmodified maize and genetically altered low-phytate maize fortified with ferrous sulfate or sodium iron EDTA. *Am. J. Clin. Nutr.* **73**, 80–85.

Mernissi-Arifi, Bieth, H., Schlewer, G. and Spiess, B. 1995. Complexation studies on inositol-phosphates, VI. Al^{3+} complexes of DL-*MYO*-inositol 1,4,5-triphosphate and D-*MYO*-inositol 1,2,6-triphosphate. *J. Inorg. Biochem.* **57**, 127–133.

Midorikawa, K., Murata, M., Oikawa, S., Hiraku, Y. and Kawanishi, S. 2001. Protective effect of phytic acid on oxidative DNA damage with reference to cancer prevention. *Biochem. Biophys. Res. Commun.* **288**, 552–557.

Miyamoto, S., Kuwata, G., Imai, M., Nagao, A. and Terao, J. 2000 Protective effect of phytic acid hydrolysis products on iron-induced lipid peroxidation of liposomal membranes. *Lipids* **35**, 1411–1413.

Moore, R. J. and Veum, T. L. 1983. Adaptive increase in phytate digestibility by phosphorus-deprived rats and the relationship of intestinal phytase (EC 3.1.3.8) and alkaline phosphatase (EC 3.1.3.1) to phytate utilization. *Br. J. Nutr.* **49**, 145–152.

Morris, E. R. and Hill, A. D. 1995. Inositol phosphate, calcium, magnesium, and zinc contents of selected breakfast cereals. *J. Food Compos. Anal.* **8**, 3–11.

Morris, E. R. and Hill, A. D. 1996. Inositol phosphate content of selected dry beans, peas, and lentils, raw and cooked. *J. Food Compos. Anal.* **9**, 2–12.

Morrison, B. H., Bauer, J. A., Kalvakolanu, D. V. and Lindner, D. J. 2001. Inositol hexakisphosphate kinase 2 mediates growth suppressive and apoptotic effects of interferon-β in ovarian carcinoma cells. *J. Biol. Chem.* **276**, 24965–24970.

Mullaney, E. J., Daly, C. B. and Ullah, A. H. J. 2000. Advances in phytase research. *Adv. Appl. Microbiol.* **47**, 157–199

Munnik, T., Irvine, R. F. and Musgrave, A. 1998. Phospholipid signalling in plants. *Biochim. Biophys. Acta* **1389**, 222–272.

Nakano, T., Joh, T., Tokumoto, E. and Hayakawa, T. 1999. Purification and characterization of phytase from bran of *Triticum aestivum* L. cv. Nourin #61. *Food Sci. Technol. Res.* **5**, 18–23.

Nakano, T., Joh, T., Narita, K. and Hayakawa, T. 2000. The pathway of dephosphorylation of *myo*-inositol hexakisphosphate by phytases from wheat bran of *Triticum aestivum* L. cv. Nourin #61. *Biosci. Biotechnol. Biochem.* **64**, 995–1003.

NC-IUB 1989. Numbering of atoms in *myo*-inositol. Recommendations 1988. *Biochem. J.* **258**, 1–2.

Nemets, B., Fux, M., Levine, J. and Belmaker, R.H. 2001. Combination of antidepressant drugs: The case of inositol. *Human Psychopharmacol.* **16**, 37–43.

Nickel, K. P. and Belury, M. A. 1999. Inositol hexaphosphate reduces 12-*O*-tetradecanoylphorbol-13-acetate-induced ornithine decarboxylase independent of protein kinase C isoform expression in keratinocytes. *Cancer Lett.* **140**, 105–111.

Nicoletti, F., Bruno, V., Fiore, L., Cavallaro, S. and Canonico, P. L. 1989. Inositol hexakisphosphate (phytic acid) enhances Ca^{2+} influx and D-[^{3}H]aspartate release in cultured cerebellar neurons. *J. Neurochem.* **53**, 1026–1030.

Nishino, H., Murakoshi, M., Masuda, M., Tokuda, H., Satomi, Y., Onozuka, M., Yamaguchi, S., Bu, P., Tsuruta, A., Nosaka, K., Baba, M. and Takasuka, N. 1999. Suppression of lung and liver carcinogenesis in mice by oral administration of *myo*-inositol. *Anticancer Res.* **19**, 3663–3664.

Nogimori, K., Hughes, P. J., Glennon, M. C., Hodgson, M. E., Putney, J. W. and Shears, S. B. 1991. Purification of an inositol (1,3,4,5)-tetrakisphosphate 3-phosphatase activity from rat liver and the evaluation of its substrate specificity. *J. Biol. Chem.* **266**, 16499–16506.

Nolan, K. B., Duffin, P.A. and McWeeny, D. J. 1987. Effects of phytate on mineral bioavailability. *In vitro* studies on Mg^{2+}, Ca^{2+}, Fe^{3+}, Cu^{2+}, and Zn^{2+} (also Cd^{2+}) solubilities in the presence of phytate. *J. Sci. Food Agric.* **40**, 79–85.

Norris, F. A., Ungewickell, E. and Majerus, P. W. 1995. Inositol hexakisphosphate binds to clathrin assembly protein 3 (AP-3/AP180) and inhibits clathrin cage assembly *in vitro*. *J. Biol. Chem.* **270**, 214–217.

Odom, A. R., Stahlberg, A., Wente, S. R. and York, J. D. 2000. A role for nuclear inositol 1,4,5-trisphosphate kinase in transcriptional control. *Science* **287**, 2026–2029.

O'Neill, I. K., Sargent, M. and Trimble, M. L. 1980. Determination of phytate in foods by phosphorus-31 Fourier transform nuclear magnetic resonance spectrometry. *Anal. Chem.* **52**, 1288–1291.

Ongusaha, P. P., Hughes, P. J., Davey, J. and Michell, R. H. 1998. Inositol hexakisphosphate in *Schizosaccharomyces pombe*: Synthesis from Ins(1,4,5)P_3 and osmotic regulation. *Biochem. J.* **335**, 671–679.

Ozaki, S., DeWald, D. B., Shope, J. C., Chen, J. and Prestwich, G. D. 2000. Intracellular delivery of phosphoinositides and inositol phosphates using polyamine carriers. *Proc. Natl Acad. Sci. USA.* **97**, 11286–11291.

Pallauf, J., Pietsch, M. and Rimbach, G. 1998. Dietary phytate reduces magnesium bioavailability in growing rats. *Nutr. Res.* **18**, 1029–1037.

Patel, S., Joseph, S. K. and Thomas, A. P. 1999. Molecular properties of inositol 1,4,5-trisphosphate receptors. *Cell Calcium.* **25**, 247–264.

Perera, I. Y., Heilmann, I. and Boss, W. F. 1999. Transient and sustained inceases in inositol 1,4,5-trisphosphate precede the differential growth response in gravistimulated maize pulvini. *Proc. Natl. Acad. Sci. USA* **96**, 5838–5843.

Persson, H., Türk, M., Nyman, M. and Sandberg, A. S. 1998. Binding of Cu^{2+}, Zn^{2+}, and Cd^{2+} to inositol tri-, tetra-, penta-, hexaphosphates. *J. Agric. Food Chem.* **46**, 3194–3200.

Phillippy, B. Q. 1989. Identification by two-dimensional NMR of *myo*-inositol tris- and tetrakis(phosphates) formed from phytic acid by wheat phytase. *J. Agric. Food Chem.* **37**, 1261–1265.

Phillippy, B. Q. 1998a. Identification of inositol 1,3,4-trisphosphate 5-kinase and inositol 1,3,4,5-tetrakisphosphate 6-kinase in immature soybean seeds. *Plant Physiol.* **116**, 291–297.

Phillippy, B. Q. 1998b. Purification and catalytic properties of a phytase from scallion (*Allium fistulosum* L.) leaves. *J. Agric. Food Chem.* **46**, 3491–3496.

Phillippy, B. Q. and Bland, J. M. 1988. Gradient ion chromatography of inositol phosphates. *Anal. Biochem.* **175**, 162–166.

Phillippy, B. Q. and Graf, E. 1997. Antioxidant functions of inositol 1,2,3-trisphosphate and inositol 1,2,3,6-tetrakisphosphate. *Free Radical Biol. Med.* **22**, 939–946.

Phillippy, B. Q. and Wyatt, C. J. 2001. Degradation of phytate in foods by phytases in fruit and vegetable extracts. *J. Food Sci.* **66**, 535–539.

Phillippy, B. Q., White, K. D., Johnston, M. R., Tao, S. -H. and Fox, M. R. S. 1987. Preparation of inositol phosphates from sodium phytate by enzymatic and nonenzymatic hydrolysis. *Anal. Biochem.* **162**, 115–121.

Phillippy, B. Q., Johnston, M. R., Tao, S. -H. and Fox, M. R. S. 1988. Inositol phosphates in processed foods. *J. Food Sci.* **53**, 496–499.

Phillippy, B. Q., Ullah, A. H. J. and Ehrlich, K. C. 1994. Purification and some properties of inositol 1,3,4,5,6-pentakisphosphate 2-kinase from immature soybean seeds. *J. Biol. Chem.* **269**, 28393–28399.

Pittet, D., Schlegel, W., Lew, D. P., Monod, A. and Mayr, G. W. 1989. Mass changes in inositol tetrakis- and pentakisphosphate isomers induced by chemotactic peptide stimulation in HL-60 cells. *J. Biol. Chem.* **264**, 18489–18493.

Plaami, S. and Kumpulainen, J. 1995. Inositol phosphate content of some cereal-based foods. *J. Food Compos. Anal.* **8**, 324–335.

Porres, J. M., Stahl, C. H., Cheng, W. H., Fu, Y., Roneker, K. R., Pond, W. G. and Lei, X. G. (1999). Dietary intrinsic phytate protects colon from lipid peroxidation in pigs with a moderately high dietary iron intake. *Proc. Soc. Exp. Biol. Med.* **221**, 80–86.

Posternak, T. 1965. "The Cyclitols." Holden-Day, San Francisco, CA.

Poyner, D. R., Cooke, F., Hanley, M. R., Reynolds, D. J. M. and Hawkins, P. T. 1993. Characterization of metal ion-induced [^{3}H]inositol hexakisphosphate binding to rat cerebellar membranes. *J.Biol. Chem.* **268**, 1032–1038.

Raboy, V. and Dickinson, D. B. 1987. The timing and rate of phytic acid accumulation in developing soybean seeds. *Plant Physiol.* **85**, 841–844.

Raboy, V., Gerbasi, P. F., Young, K. A., Stoneberg, S. D., Pickett, S. G., Bauman, A. T., Murthy, P. P. N., Sheridan, W. F. and Ertl, D. S. 2000. Origin and seed phenotype of maize *low phytic acid 1-1* and *low phytic acid 2-1*. *Plant Physiol.* **124**, 355–368.

Radenberg, T., Scholz, P., Bergmann, G. and Mayr, G. W. 1989. The quantitative spectrum of inositol phosphate metabolites in avian erythrocytes, analysed by proton n.m.r. and h.p.l.c. with direct isomer detection. *Biochem. J.* **264**, 323–333.

Rakba, N., Loyer, P., Gilot, D., Delcros, J. G., Glaise, D., Baret, P., Pierre, J. L., Brissot, P. and Lescoat, G. 2000. Antiproliferative and apoptotic effects of O-Trensox, a new synthetic iron chelator, on differentiated human hepatoma cell lines. *Carcinogenesis* **21**, 943–951.

Rao, R. K. and Ramakrishnan, C. V. 1985. Studies on inositolphosphatase in rat small intestine. *Enzyme* **33**, 205–215.

Razzini, G., Berrie, C. P., Vignati, S., Broggini, M., Mascetta, G., Brancaccio, A. and Falasca, M. 2000. Novel functional PI 3-kinase antagonists inhibit cell growth and tumorigenicity in human cancer cell lines. *FASEB J.* **14**, 1179–1187.

Reddy, N. R. 2002. Occurrence, distribution, content, and dietary intake of phytate. *In* "Food Phytates" (Reddy, N. R. and Sathe, S. K. eds), pp. 25–51. CRC Press, Boca Raton, FL.

Reddy, N. R., Sathe, S. K. and Salunkhe, D. K. 1982. Phytates in legumes and cereals. Nutritional significance of phytic acid. *Adv. Food Res.* **28**, 1–92.

Reddy, N. R., Pierson, M. D., Sathe, S. K. and Salunkhe, D. K. 1989. "Phytates in Cereals and Legumes." CRC Press, Boca Raton, FL.

Rickard, S. E. and Thompson, L. U. 1997. Interactions and biological effects of phytic acid. *In* "Antinutrients and Phytochemicals in Food", ACS Symposium Series 662, pp. 294–312. American Chemical Society, Washington, DC.

Rimbach, G. and Pallauf, J. 1998. Phytic acid inhibits free radical formation in vitro but does not affect liver oxidant or antioxidant status in growing rats. *J. Nutr.* **128**, 1950–1955.

Rimbach, G. and Pallauf, J. 1999. Effect of dietary phytate on magnesium bioavailability and liver oxidant status in growing rats. *Food Chem. Toxicol.* **37**, 37–45.

Rimbach, G., Walter, A., Most, E. and Pallauf, J. 1998. Effect of microbial phytase on zinc bioavailability and cadmium and lead accumulation in growing rats. *Food Chem. Toxicol.* **36**, 7–12.

Rounds, M. A. and Nielsen, S. S. 1993. Anion-exchange high-performance liquid chromatography with post-column detection for the analysis of phytic acid and other inositol phosphates. *J. Chromatogr. A* **653**, 148–152.

Saha, P. R., Weaver, C. M. and Mason, A. C. 1994. Mineral bioavailability in rats from intrinsically labeled whole wheat flour of various phytate levels. *J. Agric. Food Chem.* **42**, 2531–2535.

Saiardi, A., Caffrey, J. J., Snyder, S. H. and Shears, S. B. 2000a. Inositol polyphosphate multikinase (ArgRIII) determines nuclear mRNA export in *Saccharomyces cerevisiae*. *FEBS Lett.* **468**, 28–32.

Saiardi, A., Caffrey, J. J., Snyder, S. H. and Shears, S. B. 2000b. The inositol hexakisphosphate kinase family. Catalytic flexibility and function in yeast vacuole biogenesis. *J. Biol. Chem.* **275**, 24686–24692.

Saiardi, A., Nagata, E., Luo, H. R., Sawa, A., Luo, X., Snowman, A. M. and Snyder, S. H. 2001. Mammalian inositol polyphosphate multikinase synthesizes inositol 1,4,5-trisphosphate and an inositol pyrophosphate. *Proc. Natl. Acad. Sci. USA* **98**, 2306–2311.

Saied, I. T. and Shamsuddin, A. M. 1998. Up-regulation of the tumor suppressor gene *p53* and *WAF1* gene expression by IP6 in HT-29 human colon carcinoma cell line. *Anticancer Res.* **18**, 1479–1484.

Sakamoto, K., Vucenik, I. and Shamsuddin, A. M. 1993. Tritiated phytic acid (inositol hexaphosphate) is absorbed and distributed to various tissues in rats. *J. Nutr.* **123**, 713–720.

Sandberg, A.-S. and Ahderinne, R. 1986. HPLC method for determination of inositol tri-, tetra, penta-, and hexaphosphates in foods and intestinal contents. *J. Food Sci.* **51**, 547–550.

Sandberg, A. -S. and Andersson, H. 1988. Effect of dietary phytase on the digestion of phytate in the stomach and small intestine of humans. *J. Nutr.* **118**, 469–473.

Sandberg, A. -S. and Svanberg, U. 1991. Phytate hydrolysis by phytase in cereals; effects on in vitro estimation of iron availability. *J. Food Sci.* **56**, 1330–1333.

Sandberg, A. -S., Carlsson, N. -G. and Svanberg, U. 1989. Effects of inositol tri-, tetra-, penta-, and hexaphosphates on in vitro estimation of iron availability. *J. Food Sci.* **54**, 159–161.

Sandberg, A. -S., Hulthén, L. R. and Türk, M. 1996. Dietary *Aspergillus niger* phytase increases iron absorption in humans. *J. Nutr.* **126**, 476–480.

Sandberg, A.-S., Brune, M., Carlsson, N. -G., Hallberg, L., Skoglund, E. and Rossander-Hulthén, L. 1999. Inositol phosphates with different numbers of phosphate groups influence iron absorption in humans. *Am. J. Clin. Nutr.* **70**, 240–246.

Sandström, B. and Sandberg, A. S. 1992. Inhibitory effects of isolated inositol phosphates on zinc absorption in humans. *J. Trace Elem. Electrolytes Health Dis.* **6**, 99–103.

Sandström, B., Bugel, S., McGaw, B. A., Price, J. and Reid, M. D. 2000. A high oat-bran intake does not impair zinc absorption in humans when added to a low-fiber animal protein-based diet. *J. Nutr.* **130**, 594–599.

Sathe, S. K. and Venkatachalam, M. 2002. Influence of processing technologies on phytate and its removal. In "Food Phytates" (Reddy, N. R. and Sathe, S. K., eds), pp. 157–188. CRC Press, Boca Raton, FL.

Schell, M. J., Letcher, A. J., Brearley, C. A., Biber, J., Murer, H. and Irvine, R. F. 1999. PiUS (Pi uptake stimulator) is an inositol hexakisphosphate kinase. *FEBS Lett.* **461**, 169–172.

Seedat, S. and Stein, D. J. 1999. Inositol augmentation of serotonin reuptake inhibitors in treatment-refractory obsessive–compulsive disorder: An open trial. *Int. Clin. Psychopharmacol.* **14**, 353–356.

Segueilha, L., Lambrechts, C., Boze, H., Moulin, G. and Galzy, P. 1992. Purification and properties of the phytase from *Schwanniomyces castellii*. *J. Ferment. Bioeng.* **74**, 7–11.

Sekiguchi, Y., Matsunaga, A., Yamamoto, A. and Inoue, Y. 2000. Analysis of condensed phosphates in food products by ion chromatography with an on-line hydroxide eluent generator. *J. Chromatogr. A* **881**, 639–644.

Shamsuddin, A. M. 1995. Inositol phosphates have novel anticancer function. *J. Nutr.* **125**, 725S–732S.

Shamsuddin, A. M. 1999. Metabolism and cellular functions of IP6: A review. *Anticancer Res.* **19**, 3733–3736.

Shamsuddin, A. M., Ullah, A. and Chakravarthy, A. K. 1989. Inositol and inositol hexaphosphate suppress cell proliferation and tumor formation in CD-1 mice. *Carcinogenesis* **10**, 1461–1463.

Shears, S. B. 1989. Metabolism of the inositol phosphates produced upon receptor activation. *Biochem. J.* **260**, 313–324.

Shears, S. B. 1998. The versatility of inositol phosphates as cellular signals. *Biochim. Biophys. Acta* **1436**, 49–67.

Shears, S. B. 2001. Assessing the omnipotence of inositol hexakisphosphate. *Cell. Signal.* **13**, 151–158.

Shears, S. B., Ali, N., Craxton, A. and Bembenek, M. E. 1995. Synthesis and metabolism of bis-diphosphoinositol tetrakisphosphate *in vitro* and *in vivo*. *J. Biol. Chem.* **270**, 10489–10497.

Shen, X., Weaver, C. M., Kempa-Steczko, A., Martin, B. R., Phillippy, B. Q. and Heaney, R. P. 1998. An inositol phosphate as a calcium absorption enhancer in rats. *J. Nutr. Biochem.* **9**, 298–301.

Simpson, C. J. and Wise, A. 1990. Binding of zinc and calcium to inositol phosphates (phytate) in vitro. *Br. J. Nutr.* **64**, 225–232.

Singh, A., Singh, S. P. and Bamezai, R. 1997. Modulatory influence of arecoline on the phytic acid-altered hepatic biotransformation system enzymes, sulfhydryl content and lipid peroxidation in a murine system. *Cancer Lett.* **117**, 1–6.

Skoglund, E. and Sandberg, A. -S. 2002. Methods for analysis of phytate. In "Food Phytates." (Reddy, N. R. and Sathe, S. K., eds), pp. 127–137. CRC Press, Boca Raton, FL.

Skoglund, E., Carlsson, N. -G. and Sandberg, A. -S. 1997a. Determination of isomers of inositol mono- to hexaphosphates in selected foods and intestinal contents using high-performance ion chromatography. *J. Agric. Food Chem.* **45**, 431–436.

Skoglund, E., Carlsson, N. -G. and Sandberg, A. -S. 1997b. Analysis of inositol mono- and diphosphate isomers using high-performance ion chromatography and pulsed amperometric detection. *J. Agric. Food Chem.* **45**, 4668–4673.

Skoglund, E., Carlsson, N. -G. and Sandberg, A. -S. 1998. High-performance chromatographic separation of inositol phosphate isomers on strong anion exchange columns. *J. Agric. Food Chem.* **46**, 1877–1882.

Skoglund, E., Lönnerdal, B. and Sandberg, A. -S. 1999. Inositol phosphates influence iron uptake in Caco-2 cells. *J. Agric. Food Chem.* **47**, 1109–1113.

Smith, P. M., Harmer, A. R., Letcher, A. J. and Irvine, R. F. 2000. The effect of inositol 1,3,4,5-tetrakisphosphate on inositol trisphosphate-induced Ca^{2+} mobilization in freshly isolated and cultured mouse lacrimal acinar cells. *Biochem. J.* **347**, 77–82.

Spiers, I. D., Freeman, S., Poyner, D. R. and Schwalbe, C. H. 1995. The first synthesis and iron binding studies of the natural product, *myo*-inositol 1,2,3-trisphosphate. *Tetrahedron Lett.* **36**, 2125–2128.

Spiers, I. D., Barker, C. J., Chung, S. K., Chang, Y. T., Freeman, S., Gardiner, J. M., Hirst, P. H., Lambert, P. A., Michell, R. H., Poyner, D. R., Schwalbe, C. H., Smith, A. W. and Solomons, K. R. H. 1996. Synthesis and iron binding studies of *myo*-inositol 1,2,3-trisphosphate and (±)-*myo*-inositol 1,2-bisphosphate, and iron binding studies of all *myo*-inositol tetrakisphosphates. *Carbohydr. Res.* **282**, 81–99.

Steadman, K. J., Burgoon, M. S., Schuster, R. L., Lewis, B. A., Edwardson, S. E. and Obendorf, R. L. 2000. Fagopyritols, D-*chiro*-inositol, and other soluble carbohydrates in buckwheat seed milling fractions. *J. Agric. Food Chem.* **48**, 2843–2847.

Stephens, L. R. and Downes, C. P. 1990. Product-precursor relationships amongst inositol polyphosphates. Incorporation of $[^{32}P]P_i$ into *myo*-inositol 1,3,4,6-tetrakisphosphate, *myo*-inositol 1,3,4,5-tetrakisphosphate, *myo*-inositol 3,4,5,6-tetrakisphosphate and *myo*-inositol 1,3,4,5,6-pentakisphosphate in intact avian erythrocytes. *Biochem. J.* **265**, 435–52.

Stephens, L. R. and Irvine, R. F. 1990. Stepwise phosphorylation of *myo*-inositol leading to *myo*-inositol hexakisphosphate in *Dictyostelium*. *Nature* **346**, 580–583.

Stephens, L. R., Kay, R. R. and Irvine, R. F. 1990. A *myo*-inositol D-3 hydroxykinase activity in *Dictyostelium*. *Biochem. J.* **272**, 201–210.

Stephens, L. R., Hawkins, P. T., Stanley, A. F., Moore, T., Poyner, D. R., Morris, P. J., Hanley, M. R., Kay, R. R. and Irvine, R. F. 1991. *Myo*-inositol pentakisphosphates. Structure, biological occurrence and phosphorylation to *myo*-inositol hexakisphosphate. *Biochem. J.* **275**, 485–499.

Stevenson, J. M., Perera, I. Y., Heilmann, I., Persson, S. and Boss, W. F. 2000. Inositol signaling and plant growth. *Trends Plant Sci.* **5**, 252–258.

Streb, H., Irvine, R. F., Berridge, M. J. and Schulz, I. 1983. Release of Ca^{2+} from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate. *Nature* **306**, 67–69.

Sutardi and Buckle, K. A. 1985. Reduction in phytic acid levels in soybeans during tempeh production, storage and frying. *J. Food Sci.* **50**, 260–261, 263.

Suzuki, T., Suematsu, M. and Makino, R. 2001. Organic phosphates as a new class of soluble guanylate cyclase inhibitors. *FEBS Lett.* **507**, 49–53.

Szwergold, B. S., Graham, R. A. and Brown, T. R. 1987. Observation of inositol pentakis- and

hexakis-phosphates in mammalian tissues by ^{31}P NMR. *Biochem. Biophys. Res. Commun.* **149**, 874–881.

Talamond, P., Doulbeau, S., Rochette, I., Guyot, J. P. and Treche, S. 2000. Anion-exchange high-performance liquid chromatography with conductivity detection for the analysis of phytic acid in food. *J. Chromatogr. A* **871**, 7–12.

Tanimura, A., Tojyo, Y. and Turner, R. J. 2000. Evidence that type I, II, and III inositol 1,4,5-trisphosphate receptors can occur as integral plasma membrane proteins. *J. Biol. Chem.* **275**, 27488–27493.

Tao, S. -H., Fox, M. R. S., Phillippy, B. Q., Fry, B. E., Jr., Johnson, M. L. and Johnston, M. R. 1986. Effects of inositol phosphates on mineral utilization. *Fed. Proc.* **45**, 819.

Tarnow, P., Jonsson, A., Lindblom, L., Gustafsson, T. and Cassuto, J. 1998. Topical D-*myo*-inositol-1,2,6-trisphosphate and hexylbetaine treatment reduces albumin extravasation in experimental rat skin burn injury. *Burns* **24**, 460–463.

Tasker, P. N., Taylor, C. W. and Nixon, G. F. 2000. Expression and distribution of $InsP_3$ receptor subtypes in proliferating vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.* **273**, 907–912.

Taylor, C. W., Genazzani, A. A. and Morris, S. A. 1999. Expression of inositol trisphosphate receptors. *Cell Calcium* **26**, 237–251.

Thieleczek, R., Mayr, G. W. and Brandt, N. R. 1989. Inositol polyphosphate-mediated repartitioning of aldolase in skeletal muscle triads and myofibrils. *J. Biol. Chem.* **264**, 7349–7356.

Timerman, A. P., Mayrleitner, M. M., Lukas, T. J., Chadwick, C. C., Saito, A., Watterson, D. M., Schindler, H. and Fleischer, S. 1992. Inositol polyphosphate receptor and clathrin assembly protein AP-2 are related proteins that form potassium-selective ion channels in planar lipid bilayers. *Proc. Natl. Acad. Sci. USA.* **89**, 8976–8980.

Tomlinson, R. V. and Ballou, C. E. 1962. Myoinositol polyphosphate intermediates in the dephosphorylation of phytic acid by phytase. *Biochemistry* **1**, 166–171.

Trugo, L. C., Muzquiz, M., Pedrosa, M.M., Ayet, G., Burbano, C., Cuadrado, C. and Cavieres, E. 1999. Influence of malting on selected components of soya bean, black bean, chickpea and barley. *Food Chem.* **65**, 85–90.

Truong-Tran, A. Q., Ho, L. H., Chai, F. and Zalewski, P. D. 2000. Cellular zinc fluxes and the regulation of apoptosis/gene-directed cell death. *J. Nutr.* **130**, 1459S–1466S.

Türk, M. and Sandberg, A. -S. 1992. Phytate degradation during breadmaking: Effect of phytase addition. *J. Cereal Sci.* **15**, 281–294.

Türk, M., Carlsson, N. -G. and Sandberg, A. -S. 1996. Reduction in the levels of phytate during wholemeal bread making; effect of yeast and wheat phytases. *J. Cereal Sci.* **23**, 257–264.

Türk, M., Sandberg, A. -S., Carlsson, N. -G. and Andlid, T. 2000. Inositol hexaphosphate hydrolysis by baker's yeast. Capacity, kinetics, and degradation products. *J.Agric. Food Chem.* **48**, 100–104.

Ullah, A. H. J. and Gibson, D. M. 1987. Extracellular phytase (E.C. 3.1.3.8) from *Aspergillus ficuum* NRRL 3135: Purification and characterization. *Prep. Biochem.* **17**, 63–91.

Vajanaphanich, M., Schultz, C., Rudolf, M. T., Wasserman, M., Enyedi, P., Craxton, A., Shears, S. B., Tsien, R. Y., Barrett, K. E. and Traynor, K. A. 1994. Long-term uncoupling of chloride secretion from intracellular calcium levels by $Ins(3,4,5,6)P_4$. *Nature* **371**, 711–714.

Valencia, S., Svanberg, U., Sandberg, A. -S. and Ruales, J. 1999. Processing and quinoa (*Chenopodium quinoa*, Willd): Effects on *in vitro* iron availability and phytate hydrolysis. *Int. J. Food Sci. Nutr.* **50**, 203–211.

Vallejo, M., Jackson, T., Lightman, S. and Hanley, M. R. 1987. Occurrence and extracellular actions of inositol pentakis- and hexakisphosphate in mammalian brain. *Nature* **330**, 656–658.

Van der Kaay, J. and Van Haastert. P. J. M. 1995. Stereospecificity of inositol hexakisphosphate dephosphorylation by *Paramecium* phytase. *Biochem. J.* **312**, 907–910.

Van der Kaay, J., Wesseling, J. and Van Haastert, P. J. M. 1995. Nucleus-associated phosphorylation of Ins(1,4,5)P_3 to InsP_6 in *Dictyostelium. Biochem. J.* **312**, 911–917.

Van Dijken, P., de Haas, J. R., Craxton, A., Erneux, C., Shears, S. B. and Van Haastert, P. J. M. 1995. A novel, phospholipase C-independent pathway of inositol 1,4,5-trisphosphate formation in *Dictyostelium* and rat liver. *J. Biol. Chem.* **270**, 29724–29731.

Voglmaier, S. M., Keen, J. H., Murphy, J. E., Ferris, C. D., Prestwich, G. D., Snyder, S. H., and Theibert, A. B. 1992. Inositol hexakisphosphate receptor identified as the clathrin assembly protein AP-2. *Biochem. Biophys. Res. Commun.* **187**, 158–163.

Voglmaier, S. M., Bembenek, M. E., Kaplin, A. I., Dorman, G., Olszewski, J. D., Prestwich, G. D. and Snyder, S. H. 1996. Purified inositol hexakisphosphate kinase is an ATP synthase: Diphosphoinositol pentakisphosphate as a high-energy phosphate donor. *Proc. Natl Acad. Sci. USA* **93**, 4305–4310.

Vohra, P., Gray, G. A. and Kratzer, F. H. 1965. Phytic acid-metal complexes. *Proc. Soc. Exp. Biol. Med.* **120**, 447–449.

Vucenik, I. and Shamsuddin, A. M. 1994. [^{3}H]Inositol hexaphosphate (phytic acid) is rapidly absorbed and metabolized by murine and human malignant cells in vitro. *J. Nutr.* **124**, 861–868.

Vucenik, I., Yang, G. Y. and Shamsuddin, A. M. 1997. Comparison of pure inositol hexaphosphate and high-bran diet in the prevention of DMBA-induced rat mammary carcinogenesis. *Nutr. Cancer* **28**, 7–13.

Vucenik, I., Kalebic, T., Tantivejkul, K. and Shamsuddin, A. M. 1998. Novel anticancer function of inositol hexaphosphate: Inhibition of human rhabdomyosarcoma *in vitro* and *in vivo*. *Anticancer Res.* **18**, 1377–1384.

Vucenik, I., Podczasy, J. J. and Shamsuddin, A. M. 1999. Antiplatelet activity of inositol hexaphosphate (IP_6). *Anticancer Res.* **19**, 3689–3693.

Wattenberg, L. W., Wiedmann, T. S., Estensen, R. D., Zimmerman, C. L., Galbraith, A. R., Steele, V. E. and Kelloff, G. J. 2000. Chemoprevention of pulmonary carcinogenesis by brief exposures to aerosolized budesonide or beclomethasone dipropionate and by the combination of aerosolized budesonide and dietary *myo*-inositol. *Carcinogenesis* **21**, 179–182.

Weaver, C. M. and Kannan, S. 2002. Phytate and mineral bioavailability. *In* "Food Phytates" (Reddy, N. R. and Sathe, S. K., eds), pp. 211–223. CRC Press, Boca Raton, FL.

Weaver, C. M., Heaney, R. P., Proulx, W. R., Hinders, S. M. and Packard, P. T. 1993. Absorbability of calcium from common beans. *J. Food Sci.* **58**, 1401–1403.

Weaver, C. M., Heaney, R. P., Teegarden, D., and Hinders, S. M. 1996. Wheat bran abolishes the inverse relationship between calcium load size and absorption fraction in women. *J. Nutr.* **126**, 303–307.

Weber, G., Shen, F., Yang, H., Prajda, N. and Li, W. 1999. Regulation of signal transduction activity in normal and cancer cells. *Anticancer Res.* **19**, 3703–3709.

Weinberg, E. D. 1999. Iron loading and disease surveillance. *Emerg. Infect. Dis.* **5**, 346–352.

Welch, R. M., House, W. A., Beebe, S. and Cheng, Z. 2000. Genetic selection for enhanced bioavailable levels of iron in bean (*Phaseolus vulgaris* L.) seeds. *J. Agric. Food Chem.* **48**, 3576–3580.

Wilcox, J. R., Premachandra, G. S., Young, K. A. and Raboy, V. 2000. Isolation of high seed inorganic P, low-phytate soybean mutants. *Crop Sci.* **40**, 1601–1605.

Wilson, M. P. and Majerus, P. W. 1997. Characterization of a cDNA encoding *Arabidopsis thaliana* inositol 1,3,4-trisphosphate 5/6-kinase. *Biochem. Biophys. Res. Commun.* **232**, 678–681.

Wyss, M., Brugger, R., Kronenberger, A., Rémy, R., Kimbel, R., Oesterhelt, G., Lehmann, M. and van Loon, A. P. G. M. 1999. Biochemical characterization of fungal phytases (*myo*-

inositol hexakisphosphate phosphohydrolases): Catalytic properties. *Appl. Environ. Microbiol.* **65**, 367–373.

Xie, W., Kaetzel, M. A., Bruzik, K. S., Dedman, J. R., Shears, S. B. and Nelson, D. J. 1996. Inositol 3,4,5,6-tetrakisphosphate inhibits the calmodulin-dependent protein kinase II-activated chloride conductance in T84 colonic epithelial cells. *J. Biol. Chem.* **271**, 14092–14097.

Xu, P., Price, J. and Aggett, P. J. 1992. Recent advances in methodology for analysis of phytate and inositol phosphates in foods. *Prog. Food Nutr. Sci.* **16**, 245–262.

Yamaguchi, Y., Ikenaka, K., Niinobe, M., Yamada, H. and Mikoshiba, K. 1996. Myelin proteolipid protein (PLP), but not DM-20, is an inositol hexakisphosphate-binding protein. *J. Biol. Chem.* **271**, 27838–27846.

Yang, W. J., Matsuda, Y., Sano, S., Masutani, H. and Nakagawa, H. 1991a. Purification and characterization of phytase from rat intestinal mucosa. *Biochim. Biophys. Acta* **1075**, 75–82.

Yang, W. J., Matusda, Y., Inomata, M. and Nakagawa, H. 1991b. Developmental and dietary induction of the 90K subunit of rat intestinal phytase. *Biochim. Biophys. Acta* **1075**, 83–87.

Yang, X., Safrany, S. T. and Shears, S. B. 1999. Site-directed mutagenesis of diphosphoinositol polyphosphate phosphohydrolase, a dual specificity NUDT enzyme that attacks diadenosine polyphosphates and diphosphoinositol polyphosphates. *J. Biol. Chem.* **274**, 35434–35440.

Yang, S. -N., Yu, J., Mayr, G. W., Hofmann, F., Larsson, O. and Berggren, P. -O. 2001. Inositol hexakisphosphate increases L-type Ca^{2+} channel activity by stimulation of adenylyl cyclase. *FASEB J.* **15**, 1753–1763.

Ye, W., Ali, N., Bembenek, M. E., Shears, S. B. and Lafer, E. M. 1995. Inhibition of clathrin assembly by high affinity binding of specific inositol polyphosphates to the synapse-specific clathrin assembly protein AP-3. *J. Biol. Chem.* **270**, 1564–1568.

York, J. D., Odom, A. R., Murphy, R., Ives, E. B. and Wente, S. R. 1999. A phospholipase C-dependent inositol polyphosphate kinase pathway required for efficient messenger RNA export. *Science* **285**, 96–100.

Yoshida, K. T., Wada, T., Koyama, H., Mizobuchi-Fukuoka, R. and Naito, S. 1999. Temporal and spatial patterns of accumulation of the transcript of *myo*-inositol-1-phosphate synthase and phytin-containing particles during seed development in rice. *Plant Physiol.* **119**, 65–72.

Zhou, J. R. and Erdman, J. W., Jr. 1995. Phytic acid in health and disease. *Crit. Rev. Food Sci. Nutr.* **35**, 495–508.

Zi, X., Singh, R. P. and Agarwal, R. 2000. Impairment of erbB1 receptor and fluid-phase endocytosis and associated mitogenic signaling by inositol hexaphosphate in human prostate carcinoma DU145 cells. *Carcinogenesis* **21**, 2225–2235.