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# METABOLISM AND FUNCTION OF *myo*-INOSITOL AND INOSITOL PHOSPHOLIPIDS

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## INTRODUCTION

Inositol (*myo*-inositol) and its various biochemical derivatives are broadly distributed in mammalian tissues and cells, higher plants, fungi, and some bacteria where they provide important biological functions. The metabolism of *myo*-inositol has been extensively studied in mammalian cells, particularly in relation to the biosynthesis and degradation of the *myo*-inositol-containing phospholipids associated with biological membranes. The formation and function of the 1-stearoyl 2-arachidonoyl molecular species that greatly predominates in the membrane phosphoinositides of most mammalian cells has been a topic for active research.

*myo*-Inositol is an essential growth factor for many cells in tissue culture (47) and is capable of promoting the growth of young rats in a manner dependent on the diet composition (125). *myo*-Inositol is a required nutrient in the diet of the female gerbil (85) and other animals under certain conditions. Much of the nutritionally related work on dietary *myo*-inositol in the past has been directed toward a documentation of its role as a lipotropic factor for various animal species. More recently, the influence of dietary *myo*-inositol on the levels of free *myo*-inositol and its phospholipid derivatives in mammalian cells and the metabolic basis for the accumulation of triacylglycerol in the liver or intestine resulting from a *myo*-inositol deficiency have been of considerable interest. One of the most exciting developments in the field of *myo*-inositol functions in the cell has arisen very recently with the recognition of a dynamic role for membrane phosphoinositides in providing for the release of the second messengers 1,2-diacylglycerol and inositol trisphosphate in stimulated cells. These latter events may well play a central role in signal transmission for various hormones, neurotransmitters, growth factors, etc. Abnormalities in the metabolism of *myo*-inositol and/or inositol phospholipids have been documented and implicated in various disease states, including diabetes, renal disorders, and cancer.

This review highlights in an abbreviated manner many of those recent advances in the metabolism and function of *myo*-inositol and inositol phospholipids that are likely to be of interest to individuals with a combined concern for the nutritional, biochemical, and clinical aspects.

## DIETARY SOURCES AND BIOLOGICAL FORMS

*myo*-Inositol is found in the mixed diets of humans (diets that include animal and plant food sources) in its free form, as inositol-containing phospholipid, and as phytic acid (inositol hexaphosphate). The cyclitols include the inositols, of which there are nine possible isomers of hexahydroxycyclohexane. Of the latter, *myo*-inositol greatly predominates in mammalian tissues and cells and is

the form of primary nutritional and metabolic interest. Articles are available that discuss the appropriate nomenclature and structures for inositol and the inositol-containing phospholipids (2, 118, 215). In this relatively brief review, the *myo*-isomer is the one considered unless otherwise indicated in the text and, for simplicity, the term "inositol" is used in place of *myo*-inositol.

It has been estimated that a mixed North American diet provides the human adult with approximately 1 g of total inositol per day (65). Inositol is a common constituent of plant foodstuffs, where a considerable portion of it is present as phytic acid. In the seeds of some cereals, inositol hexaphosphate can represent a major source of the total phosphorus present (183). Considerable data is available on the phytic acid contents of various plant products such as seeds, cereal grains, fruits, and vegetables (164, 184). In animal products such as fish, poultry, meats, and dairy products, inositol is present (143, 184) both in its free form and as inositol-containing phospholipid (primarily phosphatidylinositol, PI). The concentration in cow's milk of *myo*-inositol is approximately 30–80 mg/l (143); inositol is added to some infant formulas at a level of 0.01%. This cyclitol has also been given GRAS status, which indicates that no evidence currently implicates it as a dietary hazard to the public when used at current levels.

The organs of the male reproductive tract are particularly rich in free inositol (50). High concentrations have been confirmed in the testis, epididymal, vesicular, and prostatic fluids of the rat (63, 134, 210). Mammalian semen is a rich source of free inositol, with the seminal plasma having concentrations several-fold greater than in blood. Unbound inositol levels in the brain, cerebrospinal fluid, and choroid plexus are also higher than in plasma (194). Burton et al (23) reported the levels of free inositol in rat plasma (72-day-old animals) to be 50 $\mu$M when animals were fed a control diet containing inositol. The plasma concentration of free inositol in normal human subjects is approximately 30 $\mu$M (99). The concentration of inositol is about 0.6 mM at 3–7 months of lactation in human breast milk (24). Interestingly, Naccarato & Wells (157) reported the presence of a disaccharide form of inositol, 6-$\beta$-galactinol, in human and rat milk in addition to rat mammary gland. This latter sugar was found to represent approximately 17% of the total nonlipid inositol in rat milk on the 18th day of lactation (156). In contrast to the liver where lipid-bound inositol predominates, the levels of free inositol in the small intestine, kidney, and cerebrum from a 72-day-old rat were greater than those of inositol-containing phospholipid (23). Chu & Geyer (32) reported a significant predominance of free inositol over lipid-bound inositol (mainly PI) in the brain, kidney, and lung of both male and female gerbils fed a stock diet containing 0.3% total inositol. A predominance of inositol phospholipid was found in the pancreas, heart, liver, and muscle, whereas nearly equal amounts of free and lipid-bound inositol were found in the plasma and intestine.

In mammalian tissues and cells, inositol exists primarily in its free form and bound covalently to phospholipid as phosphatidylinositol. Much lower concentrations of the polyphosphoinositides (PI 4-phosphate and PI 4,5,-bisphosphate) also exist. The structures for free *myo*-inositol and the three phospholipid forms are given in Figure 1. In view of their metabolic lability, considerable degradation of PI 4-phosphate (PIP) and PI 4,5-bisphosphate ($PIP_2$) can occur during tissue handling prior to lipid extraction and analysis (79). PI represents 2–12% of the total phospholipid in various mammalian tissues (216). Concentrations of the polyphosphoinositides tend to be higher in nervous tissue; quantitation of inositol phospholipids of rat forebrain obtained by a freeze-blowing method revealed the levels of PIP and $PIP_2$ to be 30 and 70%, respectively, of that for PI (161). PI represents 3% of the total phospholipid in rat brain (8). In the human platelet, PI, PIP, and $PIP_2$ represent approximately 5, 1, and 0.3%, respectively, of the total membrane phospholipid (145, 149).

Inositol pentaphosphate is a predominant organic phosphate in the erythrocytes of most avian species (121). In contrast, the major organic phosphate in the erythrocyte of the adult ostrich is inositol tetraphosphate (116). Inositol diphosphate has been isolated and identified in erythrocytes from lungfish (117) and inositol pentaphosphate in the red blood cells of elasmobranch fishes (21). These compounds may play a role in regulating the oxygen affinity of the hemoglobins.



## METABOLISM OF INOSITOL AND INOSITOL PHOSPHOLIPIDS

### *Digestion and Absorption*

Phytic acid present in plant foodstuffs is hydrolyzed in the gut of monogastric animals by the enzyme phytase. This enzyme has been found in plant material and also in the intestinal mucosa of various animals (97, 164) where it is capable of releasing free inositol, orthophosphate, and intermediary products including the mono-, di-, tri-, tetra-, and pentaphosphate esters of inositol. Several investigators have reported that dietary phytic acid can reduce the bioavailability and utilization of both calcium and zinc. Nahapetian & Young (158) compared the effect of low and high dietary calcium intakes on the in vivo metabolic fate of oral doses of [$^{14}$C]phytate or [$^{14}$C]inositol in the rat. The high-calcium diet was found to increase significantly the loss of radioactivity in the feces and to reduce the appearance of radioactivity in expired air and in body tissues following [$^{14}$C]phytate administration; however, the high calcium intake did not affect the fate of [$^{14}$C]inositol. The authors further suggested that phytate or derivatives are almost quantitatively absorbed when the calcium intake is low. House et al (111) reported the respective values for apparent and

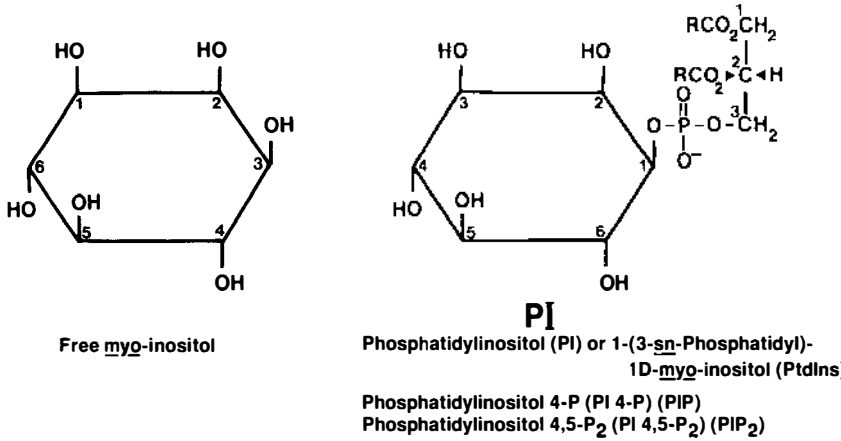


*Figure 1* Structures of free *myo*-inositol and the phosphoinositides.

true absorption of zinc to be approximately 30% lower in rats fed a diet containing phytate as compared to those fed a basal diet.

Research on the mode of absorption of free inositol in segments of hamster small intestine (29) has revealed that inositol is actively transported. Uptake and accumulation occur against a concentration gradient in a $Na^+$- and energy-dependent manner. The pathway by which inositol crossed the brush border membrane appeared not to be identical to the D-glucose pathway since phlorizin interacted competitively with the inositol binding site with an affinity considerably less than that for the common glucose binding site. Despite the fact that a considerable portion of the ingested inositol is consumed in the form of PI, little attention has been paid to the mode of digestion and absorption of this lipid form of dietary inositol. If the pathway for PI digestion should be analogous to that for dietary phosphatidylcholine (133), dietary PI may be hydrolyzed by a pancreatic phospholipase A in the intestinal lumen. The resulting lyso PI may then be reacylated via acyltransferase activity upon entering the intestinal cell or further hydrolyzed with the release of glycerylphosphorylinositol. Free inositol is transported in human blood plasma at a concentration of approximately 30 $\mu$M in normal subjects (99). PI is also present in small but significant amounts in association with the circulating serum lipoproteins (22, 30).

## *Biosynthesis and Catabolism of Inositol*

The pioneering work of Eisenberg & Bolden (49) as well as of Hauser & Finelli (80) indicated a biosynthetic capacity of rat testis, brain, kidney, and liver to synthesize inositol from glucose. Clements & Diethelm (36) attempted to assess the in vivo rate of inositol formation in the human kidney. The rate of endogenous synthesis from one normal human kidney was estimated to approach 2 g/day, thereby providing for 4 g of newly synthesized inositol per

day in the binephric human; this is significantly above the daily dietary intake. Extrarenal tissues can also contribute to the endogenous production of inositol in the human and in experimental animals. Spector & Lorenzo (194) estimated that approximately one half of the unbound inositol in rabbit brain is synthesized from glucose in situ, with the remainder being transported into the brain from the blood. The conversion of glucose 6-phosphate to inositol 1-phosphate by the inositol 1-phosphate synthase, followed by a dephosphorylation reaction catalyzed by inositol 1-phosphatase activity (48), provides for the enzymatic biosynthesis of inositol. The stereospecificity of the synthase for nicotinamide adenine dinucleotide has been investigated in detail (28). Recently, Wong & Sherman (221) found the testicular inositol 1-phosphate synthase to have at least a 5-fold preference for the $\beta$-anomer of its natural substrate D-glucose 6-phosphate. Maeda & Eisenberg (152) have purified the inositol 1-phosphate synthase to homogeneity.

The early work of Howard & Anderson (112) led to the conclusion that the kidney is the only organ of importance in inositol catabolism, since [2-$^{14}$C]inositol was not degraded to respiratory $^{14}CO_2$ if rats were nephrectomized. In confirmation and extension of this early work, Lewin and colleagues (137) observed that bilaterally nephrectomized rats were essentially unable to convert inositol into $CO_2$ as compared to controls, which catabolized 16% of the injected [2-$^{14}$C]inositol to $^{14}CO_2$ in 5 hr. Since less than 1% of the administered radioactive inositol was released into the urine over the same time period, the catabolism of inositol by the kidney was of much greater significance than its excretion in the urine.

In animals, the initial committed step in the metabolism of inositol occurs exclusively in the kidney and involves cleavage of the ring to yield D-glucuronic acid (112). Through subsequent metabolic steps, D-xylulose 5-phosphate is produced and enters the pentose phosphate cycle. Reddy and colleagues (177, 178) purified the inositol oxygenase to homogeneity from hog kidney and found the enzyme to be specific for *myo*-inositol as a substrate, with some analogs being inhibitory. In human subjects, urinary excretion was found to account for only a small fraction of the disposal of inositol by the kidney (36). The kidney appears to be an important regulator of plasma inositol concentrations in human subjects. Interestingly, Fliesler et al (55) have suggested that inositol can be catabolized systemically to precursors utilized for glycerol lipid biosynthesis in the frog retina.

## *Cellular Uptake of Inositol*

The metabolic fate of radioactive inositol has been studied (137) in mature male rats following intraperitoneal injection. Within a few hours after injecting [2-$^{14}$C]inositol to control animals, the spleen, liver, pituitary gland, kidney,

and notably the thyroid glands were found to concentrate the labelled cyclitol from the blood actively. Despite having high levels of endogenous inositol, the testes did not concentrate radioactive inositol from the blood. Muscle tissues (diaphragm and heart) concentrated little inositol, and no significant concentration was observed in the brain and epididymal fat pad. The vas deferens, epididymis, coagulating gland, seminal vesicle, and prostate had radioactivity levels that were approximately 10- to 30-fold those in blood serum. Most of the radioactivity was found in the aqueous trichloroactic acid extract—largely as free inositol in most organs with the exception of the liver, where the lipid fraction contained the majority of the radiolabelled inositol. The majority of the hepatic radioactivity was found to be associated with membrane fractions, including microsomal and mitochondrial (136).

The uptake of inositol by kidney slices appears to occur against a concentration gradient by means of a $Na^+$- and energy-dependent active transport (78, 203). A specific inositol transport system has been demonstrated in a plasma membrane fraction from rat kidney that contained brush border membranes (202). Inositol uptake by the membrane exhibited similarities to that by slices and was temperature dependent, pH sensitive, stereospecific, and inhibited by phlorizin. Both the binding and transport of inositol by brush border membranes of rat kidney were dependent on $Na^+$ (204).

The choroid plexus has been implicated as a locus of inositol transport from plasma to the cerebrospinal fluid. Spector (193), using isolated brain slices, characterized a saturable uptake system for inositol that was considered to be an active transport system and potentially capable of explaining the large inositol concentration differential between brain and cerebrospinal fluid. Warfield et al (211) obtained contrasting results in rat brain synaptosomes, where inositol was taken up via an unsaturable process that did not provide a concentration gradient indicative of active transport. Furthermore, the latter workers suggested that the uptake system in rabbit brain slices may reflect a species difference or uptake by a component of the slice other than neuron.

Recently, Segal et al (185) found the uptake of radiolabelled inositol by Schwann cells isolated from the sciatic nerve of very young rats to occur by a saturable, $Na^+$-dependent, phlorizin-inhibited mechanism with an apparent $K_m$ of 30 $\mu$M. In isolated rat liver parenchymal cells, it was concluded that inositol uptake is nonactive and occurs by a carrier-mediated process different from that for glucose (175). The intracellular concentration of inositol never exceeded the extracellular concentration, and uptake was not affected by inhibitors of mitochondrial ATP synthesis. Very recently, Auchus et al (6) have found the 5-hydroxyl of inositol to be essential for uptake into mouse fibrosarcoma cells. The 5-deoxy-inositol analog could not replace inositol as an essential growth factor and was not incorporated into the cellular phospholipid or accumulated in the cytoplasm of these cells.

## *Incorporation of Inositol into Phospholipid*

Two established biochemical mechanisms can provide for the entry of radiolabelled free inositol directly into cellular PI (Figure 2). The de novo biosynthesis of PI (167) involves the reaction of inositol with the liponucleotide, CDP-diacylglycerol, in the presence of the enzyme CDP-diacylglycerol: inositol phosphatidyltransferase (PI synthetase). This enzyme, which resides mainly in the microsomal fraction, has been solubilized and purified from rat brain and liver (176,200). Recently, Ghalayini & Eichberg (64) purified the PI synthetase from rat brain homogenate by approximately 250-fold and found the enzyme to exhibit a $K_m$ of 4.6 mM for inositol.

Alternatively, free inositol can react with endogenous phospholipid and enter PI by a $Mn^{2+}$-stimulated exchange reaction found in microsomes (93, 167). When rat liver microsomes were prelabelled with [$^{14}$C]choline in phosphatidylcholine, [$^{14}$C]ethanolamine in phosphatidylethanolamine, or [$^{3}$H]inositol in PI and chased with cold inositol under conditions that would activate the exchange reaction, support was forthcoming that PI is the preferred substrate for this reaction (94). Studies on the partially purified exchange enzyme from rat liver have also indicated (201) that choline- and ethanolamine-containing phospholipids do not play a role as acceptors for the exchange enzyme. The apparent $K_m$ for the $Mn^{2+}$-stimulated incorporation of free inositol into PI in rat liver microsomes was found to be 0.024 mM for the exchange reaction (93), which was generally similar to the $K_m$ value of 0.04 mM reported (201) for the partially purified exchange enzyme.

In the presence of CMP, the PI synthetase can operate in the reverse direction, which forms CDP-diacylglycerol and inositol from PI (7, 19, 92). The subsequent formation of PIP and $PIP_2$ from PI is catalyzed by the ATP: PI

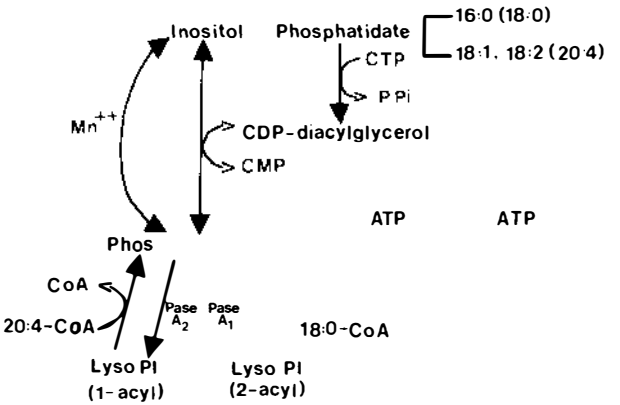


*Figure 2* Pathways for the entry of *myo*-inositol into the phosphoinositides and the formation of the 1-stearoyl 2-arachidonoyl molecular species.

4-phosphotransferase (PI kinase) and ATP:PIP 5-phosphotransferase (PIP kinase), respectively (40). The PI and PIP kinases are present on the cytosolic surface of the human erythrocyte membrane (58). Cellular phosphomonoesterases (phosphatases) have been reported (44, 144, 192) that can hydrolyze $PIP_2$ and PIP to yield PIP and PI, respectively, by sequential dephosphorylation.

## *Formation of 1-Stearoyl 2-Arachidonoyl Phosphatidylinositol*

The fatty acid compositions and distributions in PI, PIP, and $PIP_2$ are rather unique as compared to other phospholipids in most mammalian tissues and cells in that these phospholipids tend to be most enriched in stearic and arachidonic acids (96, 105). Stearate is found predominantely in the *sn*-1-position and arachidonate in the *sn*-2-position of the *sn*-glycero(3) backbone in the phosphoinositides (105, 205). Extensive molecular species analyses have revealed that the 1-stearoyl 2-arachidonoyl species predominates in PI from sources such as rat liver (102), bovine brain (105), human platelets (145), and also in the PIP and $PIP_2$ (105) from bovine brain (see Table 1). It is apparent that the PI from rat liver and human platelets is much more enriched in the 1-stearoyl 2-arachidonoyl species as compared to phosphatidylcholine, phosphatidylethanolamine, or phosphatidylserine isolated from the corresponding sources (104, 145). Tetraenoic species (mainly stearoyl arachidonoyl) also predominate (82% of total PI) in pig lymphocytes (197). Some exceptions to this predominance of the 1-stearoyl 2-arachidonoyl species in the inositol-containing phospholipids do exist; for example, monoenoic and not tetraenoic species predominate in lamb liver PI (141). Interestingly, the preferential pairing of arachidonate in the 2-position with stearate (over palmitate) in the 1-position has been found to be much more restrictive than when oleate resides in the 2-position of PI, PIP, or $PIP_2$ (102, 105).

**Table 1** Levels of 1-stearoyl 2-arachidonoyl molecular species in inositol-containing phospholipids

| Phospholipid | Source | Mol % of phospholipid | Ref. |
|---|---|---|---|
| Phosphatidylinositol | rat liver | 77 | 102 |
| Phosphatidylcholine | rat liver | 21 | 102 |
| Phosphatidylethanolamine | rat liver | 35 | 102 |
| Phosphatidylinositol | bovine brain | 60 | 105 |
| Phosphatidylinositol 4-phosphate | bovine brain | 43 | 105 |
| Phosphatidylinositol 4,5-bisphosphate | bovine brain | 42 | 105 |
| Phosphatidylinositol | human platelets | 71 | 145 |
| Phosphatidylcholine | human platelets | 10 | 145 |
| Phosphatidylethanolamine | human platelets | 47 | 145 |
| Phosphatidylserine | human platelets | 41 | 145 |

Tracer studies in vivo using radioactive glycerol, orthophosphate, and inositol suggested (96, 103) that the distributions of radioactivity among the newly synthesized species of rat liver PI could not account for the preponderance of tetraenoic molecular species in this phospholipid. Over later times following injection of these isotopes, evidence was provided for the transfer of radioactivity from monoenoic plus dienoic molecular species formed via de novo synthesis to tetraenoic species by way of deacylation-reacylation pathways. The in vivo results with radioactive inositol suggested that the reaction of free inositol with endogenous arachidonoyl CDP-diacylglycerol catalyzed by PI synthetase could account for approximately one half of the natural abundance of tetraenoic PI in rat liver (96), whereas the other half may originate by a retailoring cycle involving lyso PI intermediates (as outlined in Figure 2). Endogenous rat liver phosphatidate is enriched in palmitate in the 1-position and in oleate and linoleate in the 2-position (172) and is limited with respect to stearic and arachidonate in the 1- and 2-positions, respectively.

In vitro studies (106) have indicated that the CDP-diacylglycerol formed from the phosphatidate derived from the acylation of glycero(3)phosphate in rat liver microsomes is not enriched in arachidonate. Thompson & MacDonald (206) reported that rat liver CDP-diacylglycerol has levels of stearic and arachidonic acids that are intermediary between phosphatidic acid and PI. It is possible that a portion of the stearoyl arachidonoyl species in CDP-diacylglycerol may be derived via the back reaction catalyzed by the PI synthetase (and thereby derived indirectly via deacylation-reacylation reactions at the level of PI itself). The existence of phospholipases $A_1$ and $A_2$, which can deacylate PI in mammalian tissues and generate the intermediary lyso derivatives of PI depicted in Figure 2, have been reported (66, 89, 151, 189).

The predominant formation of tetraenoic species of PI from radioactive lyso(1-acyl) PI in the presence of ATP, CoA, and $Mg^{2+}$ has been demonstrated in rat liver homogenates and microsomal preparations (95) as has the existence of microsomal acyltransferases with affinities for arachidonoyl-CoA (95) and stearoyl-CoA (108) as acyl donors with lyso(1-acyl) PI and lyso(2-acyl) PI as acyl acceptors, respectively. The acyl-CoA : lyso(1-acyl) PI acyltransferase in rat brain (9) and human platelet microsomes (124) exhibits a selectivity toward arachidonoyl-CoA. Subcellular distribution studies (170) revealed that the lyso(1-acyl) PI acyltransferase activity in rat brain resides mainly in the endoplasmic reticulum.

## *Phosphatidylinositol Transfer Proteins*

Since PI biosynthesis is localized in the endoplasmic reticulum of eukaryotic cells, much research has been devoted recently to the mechanisms by which PI is transported at the intracellular level. There is increasing evidence to suggest that PI exchange proteins found in the cytosol of several tissues and cells might

provide this function. Early work in this area revealed a net transfer of PI from microsomes and mitochondria to liposomes as catalyzed by transfer proteins isolated from beef brain and rat liver (73, 224). Helmkamp (87) discussed in some detail the PI transfer proteins with respect to their structure, catalytic activity, and possible physiological functions. These proteins may play key roles in the transfer of PI from the endoplasmic reticulum to other cellular fractions that apparently lack the potential for PI synthesis, such as the mitochondria and plasma membrane. Laffont and colleagues (132) have described the presence of a PI transfer protein in the cytosol of porcine platelets; George & Helmkamp (60) recently reported upon the purification and some characteristics of such a transfer protein from human platelets. The protein preferentially transferred PI, with phosphatidylcholine and phosphatidylglycerol being transferred to a lesser extent and no transfer being apparent for phosphatidylethanolamine.

It will be of considerable interest in the near future to determine the potential role of such PI transfer proteins in PI function at the membrane level. Furthermore, the characterization of transfer proteins with affinities for PIP and possibly also $PIP_2$ will likely be topics for future research. It will be of additional interest to compare the relative suitability of the 1-stearoyl 2-arachidonyl relative to other molecular species of PI as substrates for the PI transfer proteins in mammalian cells.

## EFFECTS OF DIETARY INOSITOL

### *Alteration of Cellular Levels of Inositol and Phosphatidylinositol*

The effect on tissue inositol levels of feeding inositol-depleted and -supplemented diets to the neonatal and developing rat was extensively investigated by Burton et al (23). The level of free inositol in all tissues studied (testis, liver, plasma, heart, lens, lung, kidney, and small intestine) with the exception of the cerebrum and cerebellum was significantly reduced when inositol-depleted diets were fed as compared to controls. The liver was the only tissue studied in which the inositol-containing phospholipid was lowered along with the free inositol levels.

The effect of inositol deprivation in pregnant and lactating rats on the inositol status in the circulation and in selected tissues was studied by Burton & Wells (25) in fetal and postnatal offspring. In these experiments, the pups were fed the corresponding diets after weaning until three months of age. A close correlation between the free inositol content in the diet and in the milk was obtained. At day 8 of lactation, the levels of free inositol in mammary gland and milk, and also of the 6-$\beta$-galactinol derivative in milk, were several-fold higher in animals receiving the supplemented diet as compared to those fed the inositol-deficient

diet. The levels of free inositol in plasma, liver, kidney, and intestine of pups at all ages studied increased significantly with dietary inositol supplementation.

Using the female gerbil as an experimental animal model, Chu & Geyer (33) reported that animals fed an inositol-deficient diet for three weeks showed significantly depressed levels of both free and lipid-bound inositol in the intestine and liver, but only a depression in free inositol in the kidney and pancreas, relative to control animals receiving a diet containing inositol at the level of 0.1%; the levels of free and lipid-bound inositol were not significantly different in the brain of control or deficient gerbils. The hepatic level of PI was 68% lower (101) in rainbow trout fed an inositol-deficient diet for eight weeks relative to control animals receiving a supplement of dietary inositol (500 mg/kg diet); absolute liver weights were not significantly different in the two groups.

Inositol-deficient diets have also been found to reduce the level of free inositol in the urine of male rats (188). Oral doses of supplementary inositol can significantly elevate plasma inositol concentrations in human subjects (37) despite the capacity for certain tissues to synthesize this cyclitol. The fatty acid patterns of cellular PI can be significantly altered by dietary change (97); for example, the level of eicosapentaenoic acid in human platelet PI increased (from 0.1 to 0.5 mol %) when this fatty acid was ingested (100). Unfortunately, little information is available to date on the potential for dietary inositol levels and fatty acids to influence the concentrations and molecular species compositions of the polyphosphoinositides (PIP and $PIP_2$) in mammalian tissues and cells and their turnover upon cell stimulation.

## *Dietary Inositol Deficiency and Triacylglycerol Accumulation in Liver*

The pioneering work of Gavin & McHenry (59) indicated that inositol could serve as a lipotropic factor. Furthermore, the development of the fatty liver in inositol-deficient rats was particularly evident when the diet was devoid of choline. Many of the nutritional experiments used in early experiments to produce an inositol-dependent response in the rat utilized the feeding of a low-protein depletion diet free of B vitamins and fat before administrating the B vitamins with or without supplementary inositol (128). Subsequently, Hayashi and colleagues (82) reported upon an inositol-deficient experimental diet containing phthalylsulfathiazole that resulted in an inositol-responsive accumulation of triacylglycerol in rat liver when highly saturated fats were fed. The drug was added to inhibit the growth of intestinal bacteria that can synthesize inositol. After only 7 days of dietary treatment, rats fed a basal diet containing 10% by weight of hydrogenated cottonseed oil without inositol had liver triacylglycerol concentrations that were 158% higher than those in animals fed the control diet containing 0.5% inositol.

Subsequent work has revealed (3) that in the rat the type of dietary triglyceride can influence the function of dietary inositol as a lipotrope in a manner not simply related to the degree of saturation of the fat. Dietary conditions have been reported in the young rat (5) that can give rise to a significant elevation in hepatic triglyceride concentrations with the exclusion of inositol from the basal diet even when the regimen contains sufficient quantities of all the essential nutrients (including protein, B vitamins, choline, and essential fatty acids) and does not contain a sulfathiazole drug. Fatty acid analyses have revealed that the weight percentage of linoleic acid is reduced in the phospholipid while the relative abundance of palmitoleic tends to be elevated in the triacylglycerol when inositol-deficient diets producing an accumulation of rat liver triacylglycerol are fed (3, 4). Using lactating rats as the experimental animal model, Burton & Wells (25) reported that inositol-deprived dams developed severe fatty livers that were improved by dietary inositol supplementation or by terminating lactation. Triacylglycerol and esterified cholesterol levels were greatly elevated in inositol-deficient dams after 14 days of lactation, although liver free cholesterol and phospholipid levels (particularly PI) were significantly decreased (26). An increase in the size and number of fat droplets in the livers of the deficient dams was observed upon electron microscopy.

It has been of continued interest during the past several years to elucidate the metabolic basis for the accumulation of liver triacylglycerols in experimental animals under conditions of inositol deficiency. The early work of Hasan and colleagues (74, 75) suggested that the transport of lipoproteins containing triacylglycerol from liver into plasma is impeded when inositol-deficient diets are consumed since inositol was found to promote the synthesis of PI, which increases the synthesis of lipoprotein in liver and its secretion. Hepatic triacylglycerol secretion rates have been measured in vivo in rats in relation to the dietary inositol status following the injection of Triton, which coats very-low-density lipoproteins (159) so as to prevent their degradation by lipoprotein lipase. Using this technique, secretion rates were found to be significantly lower in inositol-deficient gerbils as compared to controls (109), which thereby implicates an inadequate release of lipoprotein as a causative factor in the hepatic lipid accumulation. In further support of this concept, a depression in the levels of total plasma lipoprotein lipid, very-low-density lipoprotein, high-density lipoprotein, total phospholipid, and plasma PI in inositol-deprived dams during lactation has been observed (26). In addition, lactating rats supplemented with inositol exhibited a greater loss of radioactivity from liver triacylglycerol (27) concomitant with a more rapid appearance in serum triacylglycerols following the intravenous injection of labelled palmitic acid as compared to animals given an inositol-deficient diet.

As an alternative explanation for the hepatic triacylglycerol accumulation in inositol deficiency, Hayashi and colleagues (83) have concluded that an in-

creased mobilization of fatty acid from adipose depots to the liver in the inositol-deficient rat may be an important causative factor. In support of this concept, they found the transfer of radioactivity from epididymal fat pads prelabelled with radioactive palmitic acid to the liver lipids to be almost 3-fold higher in the inositol-deficient animals as compared to controls. Subsequent work by these investigators (81) suggested that the increased lipolysis associated with an inositol-deficient state may result from an activation of hormone-sensitive lipase in adipose tissue. The concentration of plasma epinephrine, a potential activator of the lipase, was higher in the inositol-deficient animals. The enhanced lipolysis was attributed to an excitation of the sympathetic nerve terminals innervating the adipose tissues, not to an elevation of serum epinephrine released from the adrenals, since adrenalectomy did not influence the hepatic triacylglycerol accumulation caused by inositol deficiency.

The elevation of serum free fatty acids and liver triacylglycerol levels in the inositol-deficient condition was inhibited by treatment with sympathetic nervous blockers such as bupranolol and hexamethonium. This led to the suggestion that the central autonomic discharge to the adipose tissue may be increased in the deficient rat. These investigators also suggested that the decreased levels of inositol in the brain and especially of the hypothalamus in the deficient animal may link an excitation of this region to certain metabolic alterations associated with a dietary deficiency of inositol.

Since the lipogenic enzymes of rat liver undergo large alterations in activity with dietary alterations, Beach & Flick (11) studied the effect of feeding inositol-sufficient and -deficient diets as described by Hayashi et al (82) on the activities of fatty acid synthetase and acetyl-CoA carboxylase. The specific activities of these two enzymes were elevated approximately 2-fold in the inositol-deficient group over controls within 3–4 days of feeding the experimental diets. The rates of fatty acid synthetase synthesis in the inositol-deficient animals were significantly higher than controls after 12–18 hr of feeding, as measured by [$^3$H]leucine incorporation into immunoprecipitable fatty acid synthetase polypeptide, and then declined to control levels by one day. These authors have suggested that the hepatic lipodystrophy observed during inositol deficiency in rats may be partly due to an elevation in the levels of the lipogenic enzymes during the early stages of the developing deficiency. The relative quantitative contribution of the various biochemical mechanisms to the hepatic triacylglycerol accumulation in inositol deficiency remains to be further investigated.

## *Dietary Inositol Deficiency and Intestinal Lipodystrophy*

Hegsted et al (86) documented the high sensitivity of female gerbils toward the development of a pronounced intestinal lipodystrophy (characterized by the accumulation of fat in the intestinal mucosal cells) when fed a diet containing

coconut oil. The syndrome was prevented by the inclusion of inositol in the diet. In the chronic condition, there was a progressive loss of body weight associated with alopecia, which was further complicated by an exudative dermatitis and inanition. The small intestine from the inositol-deficient gerbils was greatly enlarged and the serosal surface was unusually white, with the exposed mucosa appearing swollen, corrugated, and equally whitened at necroscopy. Kroes et al (130) subsequently observed that dietary triacylglycerols rich in lauric, capric, or myristic acids produced the maximum accumulation of gut lipid upon feeding inositol-deficient diets. Additional research led to the concept that dietary inositol deprivation restricts the transport of saturated fat by the mucosal cells more than that of unsaturated lipid (213).

The intestinal lipodystrophy induced by dietary fat in female gerbils was reversed to normal (31) by inositol given in the diet or by injection within 1–4 days. Plasma chylomicron and lipid levels increased before the rapid disappearance of accumulated fat from the intestine. In addition, dietary inositol promoted an increase in triacylglycerol release from everted gut sacs. These and subsequent results by Chu & Geyer (32) led to the suggestion that a reduced biosynthesis of PI owing to a limited inositol availability may well be responsible for the impaired chylomicron assembly and secretion and intestinal lipid clearance in the gerbil consuming an inositol-depleted diet. A comparative study (34) using *M. unguiculatus* and *M. libycus* revealed that both species of gerbils developed an intestinal lipodystrophy due to a dietary deficiency of inositol.

The lower susceptibility of male gerbils to inositol deficiency may be due to the contribution of inositol biosynthesis in the testis, as shown by differences between intact and castrated animals (33). Like the rat, a gerbil testis contains high activities of both inositol 1-phosphate synthase and phosphatase. The activities of the two inositol biosynthetic enzymes were not significantly different in the liver, intestinal mucosa, kidney, and brain of male versus female animals, nor was the activity of the inositol degrading enzyme, inositol oxygenase, located in the kidney. Furthermore, the various enzyme activities were not significantly modified by dietary inositol supplementation.

## *Dietary Inositol and the Lung*

Since extracellularly derived inositol influences the metabolism of type II cells from the lung, Hallman (71) has studied the ability of inositol to promote differentiation and growth. Dietary inositol doubled the already high fetal serum inositol concentrations between fetal days 26 and 28 but had no detectable effects on the lung. The administration of inositol together with glucocorticoid decreased the glucocorticoid-induced decrease in lung dry weight when compared to controls. Inositol also potentiated a glucocorticoid-induced increase in the lavageable surfactant phospholipids. In an organ culture system,

the addition of inositol increased the incorporation of glucose and acetate into the fatty acid moiety of surfactant phosphatidylcholine. Hallman (71) has proposed that the high level of extracellular inositol in immature fetuses provides an environment that promotes both the hormone-stimulated lung differentiation and growth.

## CELLULAR FUNCTIONS OF PHOSPHATIDYLINOSITOL AND POLYPHOSPHOINOSITIDES IN MAMMALIAN CELLS

### *Membrane Structure and Function*



In view of their intimate association with biological membranes, a plethora of membrane functions related to the inositol-containing phospholipids (PI, PIP, and $PIP_2$) have been documented in mammalian cells and tissues. These functions may be related to the preponderance of 1-stearoyl 2-arachidonoyl species that commonly reside in these three phospholipids, discussed earlier in this review. As seen in Table 2, the PI, PIP, and $PIP_2$ residing in purified plasma membrane from human platelets are greatly enriched in stearic acid (41–43 mol % of total) and arachidonic acid (42–46 mol % of total). It has been reported that most of the PI pool is localized on the luminal side of the endoplasmic reticulum (160), although results from specific hydrolysis of PI confirmed an essentially symmetric distribution across the liver microsomal and the Golgi vesicle membranes (198). It has been proposed that the pattern of phospholipid asymmetry observed in the erythrocyte membrane is not a general feature of biological membranes (181). Perret et al (168) found PI to be situated mainly at the internal face of the plasma membrane from human platelets. Recently, Herbette et al (88) found 88% of the PI to be localized in the inner monolayer of the sarcoplasmic reticulum lipid bilayer.

A function for PI in regulating enzyme activity and transport processes is supported by evidence of specific interactions between this phospholipid and protein. Mandersloot and colleagues (148) have shown PI to be the endogenous activator of the $(Na^+ + K^+)$-adenosine trisphosphatase (ATPase) in microsomes isolated from rabbit kidney. The enzymes alkaline phosphatase and 5'-nucleotidase appeared to exhibit a specific association with PI in the plasma membrane (140). Rothman (180) has proposed that specific electrostatic interactions between the polar head group of PI and protein could provide for the formation of complexes that are important for the passage of proteins through membranes. The nonpolar aliphatic fatty acyl chains of the phospholipid may provide a hydrophobic microenvironment for the proteins. Interestingly, PI has been reported as an essential constituent of the acetyl-CoA carboxylase from rat liver (84); PI was found to have a controlling influence on the kinetic properties of citrate activation of the enzyme. PI has also been found to have potent effects

**Table 2** Fatty acid composition of phosphoinositides from plasma membrane of human platelets[a]

| Fatty Acid | Phosphatidylinositol | Phosphatidylinositol 4-phosphate | Phosphatidylinositol 4,5-bisphosphate |
|---|---|---|---|
| 16:0 | 2.6±0.8 | 2.9±0.2 | <1.0 |
| 18:0 | 41.3±0.9 | 41.5±1.8 | 43.4±0.4 |
| 18:1 | 6.7±0.2 | 6.4±0.9 | 7.9±0.4 |
| 18:2 | 1.2±0.7 | 0.6±0.2 | 2.6±1.1 |
| 20:4 | 46.0±0.9 | 43.3±1.8 | 41.9±1.0 |

[a]Unpublished data of Holub, Mahadevappa, and Belkhode. Values are given in mol % of total fatty acids and represent means ± S.E. for three separate experiments. Other minor fatty acids have been omitted from the table.



on tyrosine hydroxylase, an enzyme that catalyzes the rate-limiting step in the biosynthesis of catecholamines, dopamine, and norepinephrine (139). Very recently, Futerman and colleagues (57) provided evidence to support the concept that PI is involved in anchoring acetylcholinesterase to the plasma membrane of *Torpedo*.

## *Source of Free Arachidonic Acid for Eicosanoid Production*

The release of arachidonic acid from membrane 1-stearoyl 2-arachidonoyl PI is of considerable importance in controlling the formation of the eicosanoids (via cyclooxygenase/lipoxygenase activities) including prostaglandins, thromboxanes, leukotrienes, and other metabolites in mammalian tissues and cells. Arachidonic acid is also found in the choline-, ethanolamine-, and serine-containing phospholipids, and the former two, in particular, can also be of considerable significance in providing for the release of free arachidonic acid. There are a number of metabolic pathways that may contribute to the release of free arachidonic acid from the turnover of membrane PI in both resting and agonist-stimulated cells (98). As discussed above, PI can be directly deacylated via phospholipase $A_2$ activity with the release of arachidonic acid; alternatively, deacylation via phospholipase $A_1$ activity and subsequent hydrolysis of the lyso(2-acyl) PI by lysophospholipase activity (115) can also provide for the release of the eicosanoid precursor. Cellular PI can be degraded directly via a phospholipase C type cleavage (by phosphodiesterase activity) (42, 114) or by its conversion to the polyphosphoinositides, which can be hydrolyzed also to 1,2-diacylglycerols via phosphodiesterase activity (126). The intermediary 1,2-diacylglycerol (enriched in stearic and arachidonic acids) can be hydrolyzed by diacylglycerol lipase with the formation of 2-monoacylglycerol (containing arachidonic acid); the latter lipid can then be cleaved by monoacylglycerol lipase to release free arachidonic acid (12, 146). Alternatively, the transient diacylglycerol can be phosphorylated via diacylglycerol kinase to form phosphatidic acid enriched in arachidonate at the 2-position (107). Billah

et al (17) reported a particulate phospholipase $A_2$ enzyme that appears to act selectively on phosphatidic acid to release arachidonic acid.

## *Involvement in Mediation of Cellular Responses to External Stimuli*

The pioneering work of Hokin & Hokin (91), which demonstrated that acetylcholine stimulates the incorporation of radioactive inorganic orthophosphate into the inositol phospholipid of pancreas slices, suggested a possible physiological function for this accelerated metabolism. Considerable interest in this area has arisen from the discussions of Michell (153, 154) and accompanying experimental evidence suggesting that an accelerated metabolism and degradation of membrane PI by phospholipase C (phosphodiesterase) activity may be intimately related to the regulation of calcium fluxes that accompany cellular responses to external stimuli such as hormones and neurotransmitters.

An enhancement of PI metabolism in appropriate target tissues may occur, with stimuli having the ability to produce rapid physiological responses (muscarinic cholinergic, $\alpha$-adrenergic, etc) as well as those that bring about longer-term stimulation of cell proliferation (phytohemagglutinin and other mitogens, etc). This "PI effect" appeared to involve an initial degradation of membrane PI (154) and control cell surface $Ca^{2+}$ permeability, thereby giving rise to an elevation in intracellular $Ca^{2+}$ concentration (119). Kirk and colleagues (127) provided evidence for a relationship between enhanced PI metabolism and the activation of glycogen phosphorylase in hepatocytes exposed to vasopressin and related peptides. A role for PI turnover in stimulus-secretion coupling has also been provided with respect to $Ca^{2+}$-mediated histamine secretion in antigen-sensitized rat peritoneal mast cells stimulated with a number of ligands (38). Pickard & Hawthorne (169) have implicated the possible role of PI turnover in transmitter release by showing that electrical stimulation of synaptosomes labelled with radioactive phosphate in vivo caused a loss of radioactivity from the PI associated with the synaptic vessicles. A role for stimulated PI turnover in secretagogue-stimulated secretion was also revealed by in vitro work with rat pancreatic tissues (191).

There is now increasing evidence to suggest that direct PI hydrolysis by phospholipase C during agonist action may not be responsible for the initiation of calcium mobilization. The work of Abdel-Latif et al (1), which demonstrated the stimulatory effect of acetylcholine on the breakdown of prelabelled $PIP_2$ in iris smooth muscle, and subsequent investigations suggested that alterations in the polyphosphoinositides may be associated with receptor activation. According to this concept, receptor occupancy would exert primary control over the hydrolysis of $PIP_2$ via phosphodiesterase activity at the plasma membrane level, such that the disappearance of PI would be a consequence of its utilization for the replenishment of $PIP_2$ (16). A number of thoughtful reviews have been

written very recently on the involvement of $PIP_2$ in relation to PI and PIP in mediating cellular responses to various agonists (for example, 16, 51, 54, 90, 147, and 155). A most timely and important conference was recently convened in Dallas by Drs. Bleasdale, Eichberg, and Hauser and reported upon (18).

A brief summarization of the possible involvement of PI and the polyphosphoinositides (particularly $PIP_2$) in receptor-mediated cellular responses is shown in Figure 3. Although the PI greatly predominates in quantity over PIP and $PIP_2$ in mammalian cells, and also in the very few studies on plasma membrane preparations characterized to date, research on stimulated blowfly salivary glands and other cells (14, 15) indicates that the accumulation of inositol trisphosphate precedes that of inositol 1-phosphate. This suggests that the degradation of $PIP_2$ and not PI may well be an initial event occurring upon receptor occupancy. Based on work in human platelets, Vickers et al (209) have suggested that the formation of diacylglycerol and inositol phosphates, and not $PIP_2$ decreases, may be the important change in platelet activation by thrombin. In vitro enzyme studies indicate that the hydrolysis of all three inositol-containing phospholipids is stimulated by $Ca^{2+}$, although only the polyphosphoinositides were hydrolyzed in the presence of EGTA (218). These results support the concept that the hydrolysis of $PIP_2$ is favored at low $Ca^{2+}$ concentrations (prior to $Ca^{2+}$ flux) such that the elevation in cytoplasmic $Ca^{2+}$ provides for the $Ca^{2+}$-dependent hydrolysis of PI by phospholipase C (14, 146, 218). Quantitative analysis revealed a significant decrease in the mass of stearoyl arachidonoyl PI from the plasma membrane of thrombin-stimulated human platelets (190), which may be of importance in mediating cellular

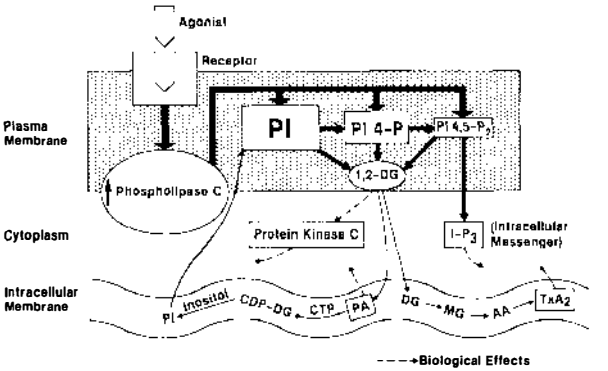


*Figure 3* Proposed mechanisms for receptor-mediated cellular responses involving phosphaudylinositol. In addition to providing for the formation of inositol trisphosphate ($I\text{-}P_3$), 1,2-diacylglycerol (1,2-DG), and phosphatidic acid (PA), the accelerated phosphoinositide metabolism can provide for the release of free arachidonic acid and its active metabolites [e.g. thromboxane $A_2$ ($TxA_2$) is a potentiator of platelet aggregation and a vasoconstrictor]. As discussed in the text, the action of phospholipase $A_2$ on PI and PA can also release free arachidonic acid.

responses to external stimuli including the formation of arachidonoyl diacylglycerol.

## *Source of the Second Messenger 1,2-Diacylglycerol and Phosphatidate*

In addition to providing a source of arachidonic acid for eicosanoid production (discussed in the previous section), the released diacylglycerol in stimulated cells may play an important messenger role in the cellular responses. Although the relative direct contribution of phospholipase C hydrolysis of $PIP_2$, PIP, and PI to the 1,2-diacylglycerol pool that accumulates at various times following receptor occupation has not been elucidated in various mammalian cells, Wilson et al (220) have concluded that the bulk of the PI breakdown in thrombin-stimulated platelets occurs via the direct hydrolysis of PI by phospholipase C to release diacylglycerol. Haslam & Davidson (77) have suggested that a GTP-binding protein may play a role in receptor-induced diacylglycerol formation based on work in permeabilized platelets. There is supporting evidence to suggest that the diacylglycerol that is transiently produced from the inositol phospholipids upon cellular stimulation may have an important second messenger function in activating protein kinase C to phosphorylate specific proteins (162). This function of diacylglycerol as a signal for the transmembrane control of protein phosphorylation is a complex process requiring both calcium and phosphatidylserine as cofactors, and it may mediate various physiological processes such as secretion and proliferation. For example, this metabolic sequence appears to be associated with 40 kDa protein phosphorylation and the release reaction in platelets (162). Cooper et al (41) observed that a 16-kDa protein present in a purified rat liver plasma membrane fraction and also in the cytosol can be phosphorylated by the endogenously activated protein kinase C.

Interestingly, tumor-promoting phorbol esters may substitute for diacylglycerol and permanently activate protein kinase C when they are intercalated into the cell membrane (163). The phosphorylation of diacylglycerol to phosphatidic acid by diacylglycerol kinase activity can provide for the marked elevations in cellular phosphatidate levels that often accompany cellular activation (52). Phosphatidate is able to complex with calcium and transport this cation as an ionophore (207). For example, Gerrard et al (61, 62) have suggested that the formation of phosphatidate and its monoacyl derivative may serve to initiate an intracellular flux of calcium by releasing the cation from an intracellular store and may contribute to the elevation in cytoplasmic calcium concentrations associated with platelet responses such as aggregation. In addition, the phosphatidate can be reconverted back to PI via de novo synthesis in the intracellular membrane. The PI transport proteins (see earlier section) may be important for the transfer of the newly synthesized PI to the plasma membrane site to replenish the degraded PI.

## *Source of the Second Messenger Inositol Trisphosphate*

There is now considerable evidence to indicate that the mobilization of calcium from intracellular stores is an early and important event following the extracellular stimulation of various mammalian cells. Considerable excitement has been generated in the field of inositol functioning at the cellular level with the mounting evidence to support the concept that the formation and function of inositol trisphosphate (1,4,5-trisphosphate), as derived from $PIP_2$, may link receptor occupancy to the mobilization of calcium from internal stores (15, 16). Berridge & Irvine (16) have described a number of tissues that might well respond to various external stimuli by such a mechanism. There is experimental evidence from stimulated salivary glands and other cells to indicate that the rapid appearance of inositol trisphosphate precedes calcium mobilization (16). In this way, inositol trisphosphate functions as an intracellular messenger in mammalian cells that are activated by calcium-mobilizing stimuli such as hormones, secretagogues, and other agonists. Berridge (15) discussed the potential synergistic interactions between the diacylglycerol and calcium signal pathways.

Using permeabilized pancreatic acinar cells, Streb et al (196) found that micromolar concentrations of inositol trisphosphate cause the release of calcium from a nonmitochondrial storage site. These latter findings have been confirmed in a number of other cellular systems (123, 174). In vitro studies have demonstrated that addition of inositol trisphosphate can induce the release of calcium from microsomal fractions of rat liver and insulinoma (43, 173). In the case of rat insulinoma, no release of calcium from mitochondria or secretory granules was found in response to inositol trisphosphate (173). Interestingly, inositol 1,3,4-trisphosphate was released in carbachol-stimulated rat parotid glands after an initial lag period; in addition, the level of inositol 1,4,5-trisphosphate was increased early (113). Potential sources and functions for the inositol 1,3,4-trisphosphate remain to be investigated. Very recently, Wilson and colleagues (219) demonstrated that the water-soluble products of polyphosphoinositide cleavage by purified ram seminal vesicle phospholipase C enzymes also contain cyclic phosphates, which may play a role in phosphoinositide-derived signal transduction.

## *Degradation of Inositol Trisphosphate*

Because inositol trisphosphate may play an important role as a second messenger for the mobilization of intracellular calcium, interest has arisen regarding the biochemical steps responsible for degrading this intermediate as potential sites in metabolic regulation. Seyfred et al (186) found the specific activity of the D-inositol 1,4,5-trisphosphate phosphatase to be highest in the plasma membrane fraction from rat liver, whereas the D-inositol 1,4-bisphosphate phosphatase activity was highest in the cytosolic and microsomal fractions.

These findings have been supported by other investigators (122). Storey et al (195) have also characterized the stepwise enzymatic dephosphorylation of inositol 1,4,5-trisphosphate to inositol in liver. Very recently, a phosphomonoesterase has been isolated from human platelets (39) that specifically hydrolyzes the 5-phosphate of inositol 1,4,5-trisphosphate. Other water-soluble inositol phosphates as well as phosphorylated sugars were not hydrolyzed; the only inositol-containing phospholipid cleaved was $PIP_2$ and at a rate less than 1% that for inositol trisphosphate. Research is needed on the control of inositol trisphosphate degradation in relation to the accelerated hydrolysis of $PIP_2$ upon receptor occupation in target cells.



## ABNORMAL METABOLISM OF INOSITOL AND INOSITOL PHOSPHOLIPIDS IN DISEASE STATES

### *Diabetes*

Diabetics are known to exhibit decreased peripheral motor and sensory nerve conduction velocities without evidence of polyneuropathy. The relationship of inositol to impaired functioning of the peripheral nervous system in rats with acute streptozotocin-induced diabetes has been studied by Greene and colleagues (67). Experimental diabetes rendered these animals unable to maintain normal concentrations of free inositol in the peripheral nerve, which was related to a decreased motor nerve conduction velocity. The free inositol levels in the nerve diminished despite the fact that circulating levels of free inositol were similar in normal and diabetic rats. Insulin treatment prevented the decrease in nerve inositol levels and the impaired nerve conduction velocity in the diabetic rats. Dietary inositol supplementation, which elevates plasma inositol levels, has proven beneficial in ameliorating the motor nerve conduction velocity in the acute streptozotocin-diabetic rat (70). Interestingly, an excessive elevation in plasma inositol levels induced by feeding a diet containing 3% (rather than 1%) inositol decreased the motor nerve conduction velocity in both normal and diabetic animals (67).

Structural changes in nerve membrane in diabetes and their reversal by inositol and insulin administration have been revealed by electron microscopy (56). The specific activity of the inositol 1-phosphate synthase involved in inositol biosynthesis was found to be lower in the testis but not in the sciatic nerve of diabetic rats relative to controls (217). Clements & Reynertson (37) suggested that hyperglycemia in the untreated human diabetic may impede inositol transport, thereby resulting in a widespread intracellular deficiency in human subjects. The inositoluria observed in human diabetes may arise from the inhibitory effect of glucose on renal tubular reabsorption of inositol. Consequently, urinary inositol excretion can account for a significant fraction of the dietary inositol intake of the untreated diabetic; the urinary excretion is lowered toward normal levels with insulin treatment. The activity of the kidney

inositol oxygenase that degrades inositol was markedly decreased in experimental diabetes. This may contribute to the elevated concentrations of inositol in the diabetic kidney and the increased clearance of inositol (166, 217).

Greene & Lattimer (68) studied the phospholipid-dependent membrane-bound ($Na^+ + K^+$)-ATPase in an attempt to provide a potential mechanism that would link defects in diabetic peripheral nerve to the abnormal inositol metabolism. The enzyme activity was found to be reduced in homogenates of sciatic nerve in experimental diabetes. This reduction in enzyme activity was selectively prevented by 1% inositol supplementation, which restored normal nerve conduction. These authors suggested that dietary inositol may correct diabetic nerve conduction by changing enzyme activity, and this may be mediated via alterations in inositol-containing phospholipids. Subsequent work demonstrated that sorbinil treatment, which preserves normal nerve inositol contents (53), prevents the fall in nerve ($Na^+ + K^+$)-ATPase activity that has been linked to conduction slowing in the diabetic rat (69).

During the past few years, attention has been directed toward the levels and metabolism of inositol-containing phospholipids in relation to diabetes. The rate of labelled inositol entry into PI falls in intact nerve segments from diabetic rats but not in broken cell preparations (110). This suggests a depression in inositol transport occurring in diabetes. Palmano et al (166) reported a lowered concentration of lipid-bound inositol in the nerves of acutely diabetic animals. Biochemical analyses on sciatic nerves removed postmortem from diabetic patients and normal subjects have revealed the concentrations of both inositol phospholipid and free inositol to be significantly lower in nerves from the diabetics (150). Bell and colleagues (13) observed an increased turnover of $PIP_2$ in sciatic nerve from streptozotocin-diabetic rats that appears relatively early and persists throughout the course of the disease. These authors suggested that this metabolic alteration may be related to a primary defect responsible for the accompanying deficient peripheral nerve function. Further research is needed on the enzyme-catalyzed steps associated with the turnover of the phosphoinositides in diabetic as compared to normal animals. Clements & Reynertson (37) demonstrated that a 3-g oral load of inositol could significantly elevate plasma inositol concentrations in human subjects, with diabetics showing a greater response. These investigators suggested that oral inositol supplementation might be of benefit in the prevention and treatment of certain complications associated with human diabetes mellitus. The effect of inositol on neurophysiological measurements in diabetic patients has been investigated by Salway et al (182). The administration of a 0.5-g oral dose of inositol twice a day for two weeks increased the amplitude of the evoked action potentials of the median, sural, and popliteal nerves by an average of 76, 160, and 40% respectively. Their results suggested a therapeutic role for inositol in diabetic neuropathy.

## *Renal Disorders*

Significant abnormalities in inositol metabolism accompanying renal failure have been well documented in human patients and are manifested in a dramatic hyperinositolemia (35, 135, 171). For example, patients with chronic renal failure have been reported to have circulating inositol levels 7-fold greater than those for controls (99) (240 versus 33 $\mu$M). It has been suggested that an impaired renal oxidation of inositol to D-glucuronate may contribute to the abnormally elevated plasma inositol levels in uremic patients (35) since the kidney is the major site for inositol catabolism in the body. A decreased glomerular filtration rate and a disturbed inositol reabsorption are also present in advanced forms of glomerulonephritis (171). Consequently, the estimation of serum and urinary inositol may have advantages in the evaluation of kidney function. Plasma inositol levels show a moderate decrease during hemodialysis in patients with chronic renal failure but to a lesser extent than the plasma urea nitrogen. Clements & Diethelm (36) reported that the half-life of inositol disappearance, which was prolonged in patients with chronic renal failure, was obviated following successful renal transplantation based on experiments in which [$^3$H]inositol was injected into an antecubital vein of fasting subjects.

Research has been conducted on the potential toxic effects of abnormally high levels of circulating inositol in both experimental animals and human subjects. Large doses of inositol given to rats increase PI levels in the endoplasmic reticulum of liver with no obvious deleterious effects (222). Doses of inositol did not produce any appreciable change in the liver or kidney of male and female rats when studied morphologically by light microscopy (76). The sciatic nerve motor neuron conduction velocity markedly decreased when normal male rats were placed on a diet enriched in inositol for one week (35). The condition improved when the animals were restored to a normal diet. These and subsequent experiments have supported the idea that hyperinositolemia may contribute to the pathogenesis of the uremic polyneuropathy in subjects with chronic renal failure (46).

Reznek and colleagues (179) showed that rises in plasma inositol concentrations were related to a depression of sural nerve conduction velocity based on experiments with uremic patients, but a relationship with clinically evident neuropathy was not a established. Liveson et al (138) observed the development of cytoplasmic abnormalities following the exposure of dorsal root ganglion cells to inositol. Neurotoxicity was revealed at an inositol concentration as low as 109 $\mu$M, which is considerably below the levels found in many uremic patients (99). Other work in this area will be greatly enhanced by the application of suitable animal models for studying the interrelationship betwee abnormalities in inositol and inositol phospholipid metabolism and chroni kidney disorders. Interestingly, measurement of the levels of free inositol in t early effluent perfusate appears to offer an accurate method for evaluating t

viability of preserved kidneys and predicting graft functioning after renal transplantation (131).

## *Other Disease States*

Alterations in inositol and inositol phospholipid metabolism have also been implicated in various other disease states, although, in most cases, no definitive associations have been established. Shastri and colleagues (187) reported that only the PI (as a percentage of total phospholipid) was significantly higher in the blood platelets from type II hypercholesterolemic patients relative to normal subjects. The absolute concentration of arachidonoyl PI ($\mu$mol per $10^9$ platelets) was also found to be elevated in the hypercholesterolemic gerbil and human platelets, primarily because of the higher amounts of this phospholipid and not because of differences in the percentage contribution of arachidonate to the total fatty acids in PI (100). These findings may be relevant to the hypersensitivity of platelets from hypercholesterolemic human subjects to pro-aggregating agents, which may contribute to their increased risk for arterial thrombosis. Cholesterol feeding has been reported to influence the rapid metabolism of PI in the pig and monkey aorta (20, 45).

Galactitol concentration is elevated and free and lipid-bound inositol levels are depressed in the brains of galactosemic infants or animals subjected to experimental galactose toxicity (214). The PI response to acetylcholine is impaired in synaptosomes from galactose-fed rats, which suggests that these animals may be deficient in the number of acetylcholine receptors or have a defect in the step between receptor-neurotransmitter interaction and PI breakdown (212). Analysis of a biopsy specimen from the liver of an adult patient with hepatosplenomegaly and hyperlipidemia revealed a marked elevation of PI (223). Since an immunologic abnormality appears to be involved in the pathogenesis of multiple sclerosis, it is of interest that the lymphocytes from such patients incorporate less labelled inositol into PI than those from control patients when stimulated by phytohemagglutinin (165). Hallman and colleagues (72) have reported that the abnormality in surfactant phospholipids in adult respiratory distress syndrome is usually associated with low PI and low plasma inositol levels. The metabolism of inositol lipids has also been implicated in cell proliferation and cancer. PI turnover has been proposed to be regulated by the oncogene protein kinases either directly by acting as PI kinases or indirectly as tyrosine kinases (142).

Reduced inositol levels have been reported in cerebrospinal fluid from patients with affective disorders (10), but fluid levels of inositol in schizophrenic patients were not significantly different from healthy controls (199). The defective phosphoinositide metabolism observed in the erythrocyte membrane of the spontaneously hypertensive rat was not a consequence of the blood pressure elevation and may be related to the pathogenesis of hypertension

(129). Significant differences have also been reported in the fatty acid composition of PI from total and subcellular fractions of liver as compared to hepatoma (208). Interestingly, soybean PI (enriched in linoleic acid in the 2-position) but not animal PI (enriched in arachidonic acid in the 2-position) can exert cytotoxicity toward tumor cells from cultured cell lines without affecting normal cell lines (120).

It will be of considerable interest in the future to elucidate the abnormalities in the metabolism of $PIP_2$, PIP, and PI in various disease states. Since the concentrations of these cellular inositol phospholipids and their various component molecular species are subject to significant nutritional modifications, it will also be of interest to determine whether restoring the inositol lipid patterns to normal by dietary or other intervention may be used clinically to prevent and treat disease states involving abnormalities in inositol phospholipid-mediated physiological responses.



## SUMMARY AND CONCLUSIONS

Alterations in the level of dietary inositol can significantly influence the concentration of free inositol and inositol-containing phospholipid in the circulation and in selected mammalian tissues and cells. The 1-stearoyl 2-arachidonoyl molecular species that commonly predominates in cellular phosphoinositides may be of considerable importance for the functioning of these phospholipids in biological membranes. Retailoring reactions subsequent to the de novo biosynthesis of PI involving the acylation of lyso(1-acyl) PI allow for the preferential enrichment of this phospholipid in arachidonic acid. The impaired release of plasma lipoprotein, increased fatty acid mobilization from adipose tissue, and enhanced fatty acid synthesis in liver have all been implicated as causative factors in the hepatic triacylglycerol accumulation occurring with experimental inositol deficiency. The severe intestinal lipodystrophy that develops in female gerbils consuming inositol-deficient diets is likely mediated by a reduced synthesis of PI and the associated impairment of chylomicron assembly and secretion.

Membrane PI can potentially regulate enzyme activities and transport processes as well as providing a source of free arachidonic acid for production of the eicosanoids. There has been mounting evidence recently to indicate that an accelerated turnover of the phosphoinositides may play a key role in mediating cellular responses to external stimuli. The transient rise of phosphoinositide-derived 1,2-diacylglycerol in stimulated cells may serve as a signal for the transmembrane control of protein phosphorylation by activating protein kinase C. Receptor occupancy also elicits the phosphodiesterase-catalyzed release of the second messenger inositol 1,4,5-trisphosphate, which appears to provide for the mobilization of calcium from internal stores.

Subnormal levels of free inositol and inositol phospholipid, as found in the nerves of animals with experimental diabetes and in sciatic nerves removed postmortem from diabetic patients, have been implicated in the impaired nerve conduction of human diabetics. Patients with renal failure exhibit a dramatic hyperinositolemia that may have clinical significance. Nutritional intervention may offer an approach for counteracting abnormalities in inositol and inositol phospholipid profiles and associated physiological responses in certain disease states.

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