INTRODUCTION

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"Solomon still has a few nuggets of gold for us in that rather world-weary document 'Ecclesiastes'. One of them reads -- I know that whatever God does endures forever; nothing can be added to it, nor anything taken from it.....

I suppose that Cyclitols and Phosphoinositides are part of what God does. We can add more than the facts will really support when we spin our theories. We can also leave out some of the things nature is telling us. Pursued deeply enough, perhaps even Cyclitols and Phosphoinositides could be a mirror to the whole universe".

Prof. J.N. Hawthorne.

Man is a relative newcomer to the earth, but in this short time he has established himself as the most successful and dominant of species. His special place in the world is due in large measure to his mental powers based on the evolutionary development of the vast, intricate and complex communication net work called the NERVOUS SYSTEM. The system regulates and co-ordinates all body activities and is the seat of human consciousness, memory, intelligence and all emotional responses.

The nervous system is broadly made up of the central nervous system (CNS) and the peripheral nervous system (PNS)

The CNS comprises of the brain and spinal cord. The countless branches arising from it and penetrating to every other outlying region of the body constitutes the PNS. The central nervous system is mainly made up of major cell types like neurons, astrocytes and oligodendroglia and minor cell types like microneurons and microglia.

Neurons are the computers of the nervous system which carry out functions of RECEPTION, INTEGRATION, TRANSFORMATION and onward TRANSMISSION of coded information. They have all the morphological counterparts of other cell types and specialised fibrillar processes called the dendrites (short ones) or the axons (long ones) which end in junctional complexes known as the synapse. The dendrites due to their vast area can act as receptor areas of the cell and the axon by modifying its environment distributes the activity of the neuron to other neurons or effector cells.

Conduction of nerve impulses along the axon and transmission across the synapse is basically the modulation of voltage across the membrane of a neuron which occurs due to the redistribution of ions and hence of the electrical charge between the interior and exterior of the cell. Cell membranes possess different channels for different ions (Na^+, K^+ and Ca^{2+}) and each type of channel is lined with specific proteins and has its own kinetics (Edwards, 1982). Lipids which form

an integral part of neural membranes play an important role in controlling the ion movements across these channels by regulating the activities of several enzymes alongside the membrane (Quinn, 1980).

For many years glial cells were in general regarded as structural elements in the nervous system, a sort of packing material around the more delicate instrument of impulse conduction-the neuron. In recent times, speculations on the metabolic roles for glial cells have diversified in many directions. Evidence for uptake into glial lines of choline, GABA, GLUT, TAU and other putative neurotransmitters has been obtained. Glial cells may supply a variety of as yet unknown nutritive and regulatory substances and may help to define neuronal organization. Astrocytes are known to isolate or segregate synaptic complexes and the bodies of neurons ensuring that the terminals act in a discrete and localized manner. They also play an important role in repair and regeneration processes. Microglial cells, the most enigmatic cells of the central nervous system, though normally inactive, become very mobile, active macrophages in times of need. In several diseased states microglia are stimulated and migrate to the area of injury where they phagocytose the debris (Pfeiffer et al., 1977)

Extensive communication from neurons to glia as well as from glia to neurons has been shown to occur in the nervous system. This could occur both electrically, ionically and biochemically and could in some cases involve cell-cell contact. One of the principal communication links postulated to exist between neurons and glia involves the ability of glia to concentrate K^+ ions. There is active uptake of K^+ into mammalian glial cells (Henn et al., 1972) and substantial activity of Na^+/K^+ -ATPase has been demonstrated in glial cell fractions (Medzihradsky et al., 1972). Neurotransmitters released as a result of synaptic activity can stimulate β-receptors on the surface of the surrounding glia and the resultant increase in c-AMP has been shown to mediate metabolic processes in glial cells which can modify neuronal-glial interactions (Gilman and Nirenberg,1971). NE produced as a result of neuronal activity has been shown to activate glycogenolysis in glial cells and release glucose or lactic acid into the extracellular space which may enter the neighbouring neuronal cells (Newburgh and Rosenbergh, 1972). C-6 glial cell lines have been shown to make NGF which influences neuronal growth and differentiation (Murphy et al., 1977). Thus, glial cells have equally important roles to play like the neurons and the two appear to be interdependent for proper functioning of the nervous system.

A summary of the events occurring at a multiple synaptic contact where different synapses release different neurotransmitters (Ach, NE, DA, 5-HT, GABA, GLUT) and each neurotransmitter binds to different types of receptors in the post synaptic membrane are represented in Fig. 1. The incoming stimulus activates an all-or-none action potential in a spiking axon by depolarising its transmembrane potential which propagates unattenuated to the nerve terminal where ion fluxes activate a mobilization process leading to transmitter secretion and "transmission" to the post-synaptic cell. The effects on the post synaptic cells cause either excitatory or inhibitory post synaptic potentials, depending on the nature of the post synaptic cell's receptor for the particular neurotransmitter. If sufficient excitatory post synaptic potentials summate temporally from various inputs onto the cell, the post synaptic cell will integrate these potentials and give off its own all-or-none action potential, which is then transmitted to each of its own axon terminals, and the process continues (Bloom, 1980).

Cell surface receptors in the nervous system employ a variety of different mechanisms which involve the generation of specific intracellular signals to translate the information encoded in neurotransmitters into an appropriate cellular response. These signals, which include changes in the levels

SYNAPTIC TRANSMISSION

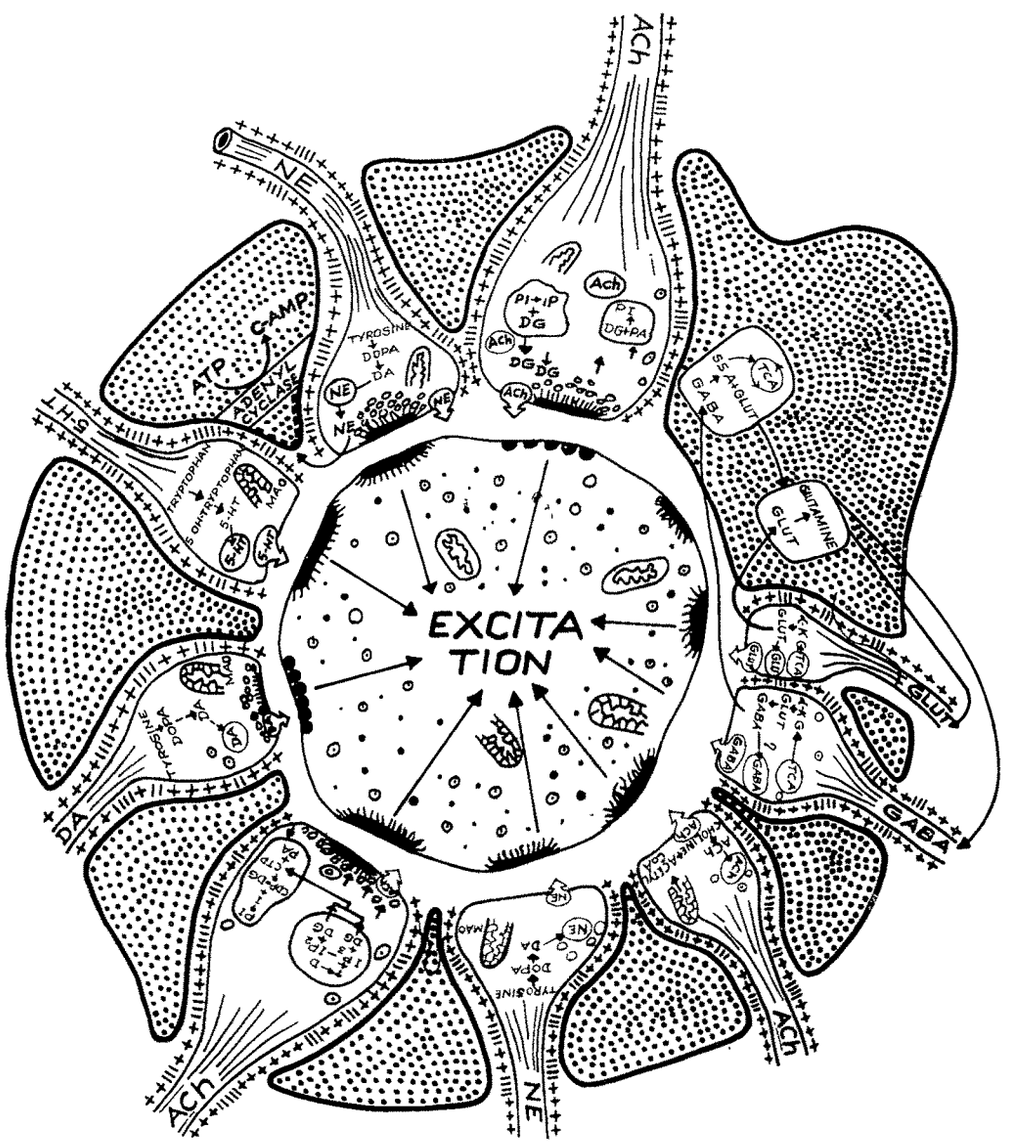


Fig. 1 : A specialised multiple group of synaptic contacts: the whole complex is surrounded by a glial capsule : Direction of transmission is indicated by arrows. Synthesis of each neurotransmitter is indicated in the particular synapse : Neuronal - glial communication is indicated for neurotransmitters like GABA, GLUT and NE.

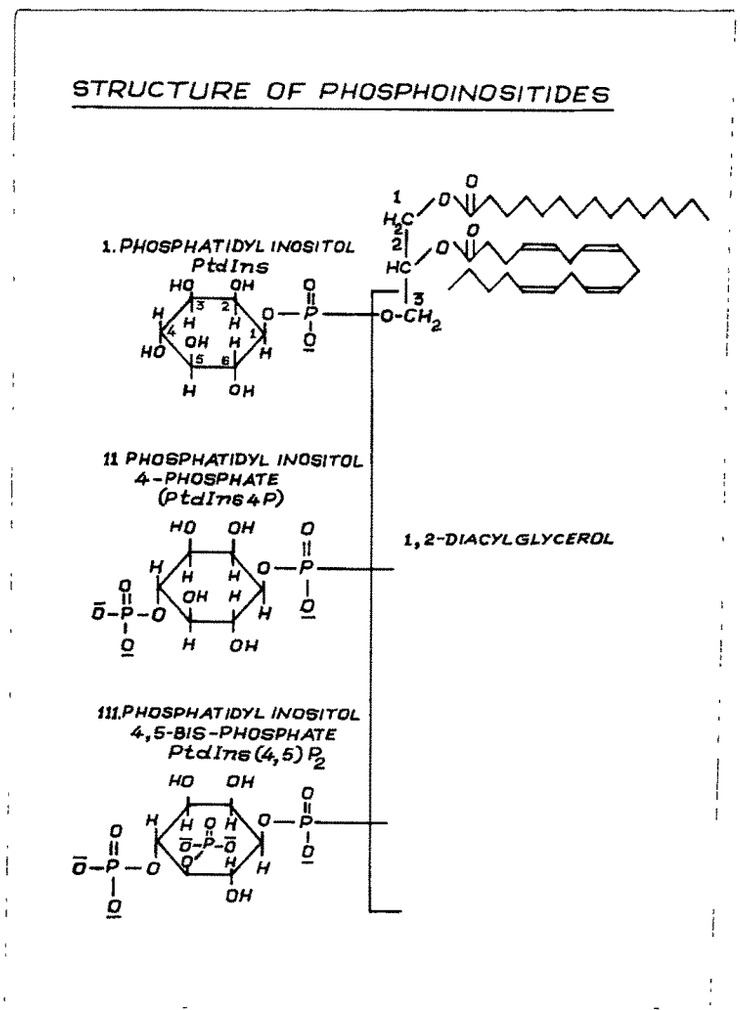
ACh - Acetylcholine; NE - Norepinephrine;
DA - Dopamine; 5-HT - 5-hydroxy tryptamine;
GABA - γ-aminobutyric acid; GLUT - Glutamic acid;
T - Triphosphoinositide; D - Diphosphoinositide;
IP_3 - Inositol(1,4,5)trisphosphate; IP_2 - Inositol (1,4) bisphosphate; DG - Diacylglycerol;
IP - Inositol-1-phosphate; PI - Phosphatidyl inositol;
PA - Phosphatidic acid; S.S.A. - Succinic semialdehyde.

of cyclic nucleotides and/or Ca^{2+}, then initiate a cascade of events that serves to amplify the primary signal, providing a sensitive means of transmitting information between cells (Downes, 1983). Several neurotransmitters involved in intercellular communication within the nervous system exert some of their effects by causing the breakdown of phospholipids. A minor component of animal phospholipids containing myo-inositol, namely, "Phosphoinositides" have long been implicated in these phenomena.

Folch and Woolley (1942) demonstrated over 40 years ago that the crude cephalin fraction of brain contains in addition to phosphatidyl ethanolamine (PtdEtn), phosphatidyl serine (PtdSer) and phosphatidyl inositol (PtdIns). He also found that brain tissue is rich in polyphosphoinositides (PolyPI), which are structurally related to PtdIns (Folch,1949). The term "Phosphoinositide" has been used to describe the three major inositol lipids - phosphatidyl inositol (PtdIns), phosphatidyl inositol 4-phosphate (PtdIns4P) and phosphatidyl inositol 4,5-bisphosphate ($PtdIns(4,5)P_2$) - Fig. 2. The structures of the higher phosphorylated derivatives were established by Brockerhoff and Ballou (1962).

Thirty years ago Hokin and Hokin (1955; 1958) demonstrated that slices of pigeon pancreas secreted amylase when treated with ACh. If the incubation medium contained $^{32}P_i$, secretion

Fig. 2 : Structure of Phosphoinositides.



The nomenclature is as recommended by the IUPAC-IUB Commission (1978) on Biochemical Nomenclature. In the preferred chair form of the ring, the hydroxyl on C-2 has the axial configuration while the remaining five are equatorial. The C-2 hydroxyl takes part in cyclic ester formation when PtdIns is hydrolysed. Fatty acids are esterified to the glycerol backbone at positions 1 and 2. Arachidonic acid with four double bonds is found at position 2.

was accompanied by increased labeling in phospholipids which were identified as PtdIns and PtdA. This phenomenon by which stimuli enhance radioactive labeling in PtdIns has been generally termed as the "Phospholipid effect" or more specifically the "PtdIns effect". Since then a variety of stimuli, including neurotransmitters, hormones, certain neuropharmacological agents, and electrical pulses have been shown to elicit this "PtdIns Effect" in a variety of tissues (Table 1). Although an upto-date list of the agents that stimulate PtdIns metabolism would include ligands acting on at least 25 different types of receptors there are still a number untested stimuli that produce Ca^{2+}-mediated physiological responses.

A widespread acceptance of the idea that breakdown of PtdIns is the primary event in stimulated cells existed for several years. However, during the past few years several laboratories have resurrected this idea and shown that the initiating reaction may be a phosphodiesterase attack at the plasma membrane on PtdIns(4,5)P_2 (and/or PtdIns4P). PolyPI received considerably less investigative attention earlier in neural and secretory membrane processes primarily due to the high lability post-mortem of these lipids. That, consistent alterations in brain PolyPI metabolism can be achieved by stimulation with neurotransmitters was demonstrated by Soukup et al (1978b). The success of this group of experimenters has been due to their use of microwave irradiation

TABLE 1 : "PHOSPHATIDYLINOSITOL EFFECT" IN DIFFERENT TISSUES.

Tissue or cell type	Stimulus	Reference
1	2	3
NERVOUS TISSUE :		
Whole brain	Muscarinic cholinergic	Abdel-Latif (1983)
	α-adrenergic	
Cerebral cortex	Muscarinic cholinergic	"
	α-adrenergic	
	5-HT	Michell (1975)
	Dopamine	
	Electrical stimulation	
	High K^+	
Superior cervical sympathetic ganglion	Muscarinic Cholinergic	"
	Electrical stimulation	
	High K^+	
Most brain regions	Muscarinic Cholinergic	"
	α-adrenergic	
	5-HT	
	Dopamine	
	H_1 - Histamine	Downes (1982)

contd...

TABLE 1 : contd.

1	2	3
Hypothalamus	Substance P Neurotensin	Downes (1982)
Hippocampus	V_1-vasopressin	"
Synaptosomes	Muscarinic Cholinergic α-adrenergic Electrical stimulation High K^+	Michell (1975)
EXOCRINE GLANDS :		
Pancreas	Muscarinic Cholinergic Pancreozymin Cholecystokinin	"
Parotid gland	Muscarinic Cholinergic α-adrenergic	"
Avian salt gland	Muscarinic Cholinergic	Abdel-Latif (1983)
Blowfly salivary gland	5-HT	"
ENDOCRINE GLANDS :		
Thyroid	Thyroid stimulating hormone	Michell (1975)
Adrenal medulla	Cholinergic	"

contd...

TABLE 1 : contd.

1	2	3
Pineal	∝-adrenergic	Michell (1975)
	5-HT	
Anterior pituitary	Corticotrophin-releasing hormone	"
OTHERS :		
Iris smooth muscle	ACh	Takenawa (1982)
	NE	
	5-HT	
	DOPA	
	Epinephrine	
	Histamine	
Ileum smooth muscle	ACh	"
	5-HT	
	Histamine	
	Carbachol	
	High K^+	
Vas deferens	ACh	"
	NE	
	Epinephrine	
Heart	∝-adrenergic	Michell (1975)
Liver	∝-adrenergic	"
Adipose	"	"
Platelets	∝-adrenergic	"
	Thrombin	
	ADP	
	Collagen	

contd...

TABLE - 1 : contd.

1	2	3
Lymphocytes (T?)	Phytohaemagglutinin Concanavalin A Soyabean lectin	Michell (1975)
Fibroblasts	Low cell density High serum concentration SV_{40} Virus transformation	Michell (1979)

Data indicate increased labelling of, or loss of label from PtdIns, PtdA. or DG or changes in the concentration of these on application of a stimulus. Reference is made to reviews or other articles wherein original references can be found.

to overcome post-mortem losses of these labile phospholipids. A second series of experiments in which stimuli produced clear, if ill-understood, effects were the studies in which Jolles and colleagues showed a transient decrease in labeling of both PtdIns and PolyPI in pre-labelled synaptosomal fractions from the limbic system (Jolles et al., 1979). Over the years, various ill-defined effects on the metabolism of PolyPI have been reported in neural as well as non-neural cells stimulated by hormones or neurotransmitters : these are briefly summarized in Table 2.

Suggestions have been made that phosphoinositides may also have important roles to play in the conduction of nerve impulses along axons, synaptic transmission, cell proliferation and differentiation, attachment and activation of several enzyme systems, shape transformation in erythrocytes, secretion and reabsorption of solutes from the lumen of renal tubule membranes and prostaglandin biosynthesis. A few of the important roles especially those related to the nervous tissue have been detailed later.

"WHY ARE THESE PHOSPHOLIPIDS UNIQUELY SUITED TO A CENTRAL ROLE IN RECEPTOR FUNCTION IN A WIDE VARIETY OF TISSUES? WHAT ARE THE SPECIAL PROPERTIES THESE LIPIDS POSSESS THAT ENABLE THEM TO PARTICIPATE IN THE ACTIONS OF SUCH A BROAD GROUP OF RECEPTORS? WHERE ARE THEY LOCALIZED AND HOW ARE THEY METABOLIZED UNDER NORMAL AND STRESS CONDITIONS"?

TABLE 2 : "POLYPHOSPHOINOSITIDE EFFECT" IN DIFFERENT TISSUES.

Tissue or cell type	Stimulus	Response	Reference
1	2	3	4
NERVOUS TISSUE			
Whole brain	Muscarinic-cholinergic	$^{32}P_i$ incorporation into PtdIns(4,5)P_2 and PtdIns4P increased. Levels of PolyPI remain unaffected.	Soukup et al (1978b)
Cerebral cortex	"	"	"
Brain stem	"	"	"
Cerebellum	"	"	"
Cerebral cortex	5-HT CCK-octa-peptide	Phospholipase C breakdown of PolyPI leading to accumulation of Ins1P.	Downes (1982)
Several brain regions	Histamine	"	"
Hypothalamus/ Striatum	Substance P	"	"
	Neurotensin	"	"
Hippocampus	Vasopressin	"	"
Mid brain microsomal fraction	Morphine	$^{32}P_i$ incorporation into PtdIns(4,5)P_2 increased	"
	β-endorphine Naloxone	"	"
EXOCRINE GLANDS			
Parotid gland	α-adrenergic	$^{32}P_i$ incorporation into PtdIns(4,5)P_2 increased	"
Avian salt gland	Muscarinic-cholinergic	$^{32}P_i$ incorporation into PtdIns4P increased	Santiago-Calvo et al (1964)

contd...

Table 2 : contd.

1	2	3	4
Blowfly salivary gland	5-HT	Rapid disappearance of (^{3}H) PtdIns(4,5)P_2 and (^{3}H) PtdIns4P. Rapid release of Ins(1,4,5)P_3 and Ins(1,4)P_2	Berridge (1983)
ENDOCRINE GLANDS			
Adrenals	ACTH	Levels of PtdIns(4,5)P_2 and PtdIns4P increased. Net increase in the de novo synthesis.	Farese et al (1980)
OTHERS			
Iris smooth muscle	Muscarinic cholinergic	$^{32}P_1$ incorporation into PtdIns(4,5)P_2 and PtdIns4P decreased. Levels of both lipids were decreased.	Abdel-Latif et al (1978b)
	∝-adrenorgic	"	Akhtar and Abdel-Latif (1982)
Kidney cortex tubules	PTH	Levels of PolyPI increased	Bidot-Lopez et al (1981)
Repatocytes	V_1-vasopressin, Angiotensin ∝-adrenorgic ATP	$^{32}P_1$ incorporation into PtdIns(4,5)P_2 and PtdIns4P decreased.	Creba et al (1983)
Blood platelets	ADP, Thrombin, Platelet activating factor	Phospholipase C mediated hydrolysis of PtdIns(4,5)P_2	Berridge and Irvine, (1984)

contd...

Table 2 : contd.

1	2	3	4
Neutrophils	f-methionyl-leucyl-phenylalanine	Phospholipase C mediated hydrolysis of Ptd Ins(4,5)P_2	Berridge and Irvine (1984)
Leukocytes	Pseudomonal leukocidin	"	"
T-lympho-blastoid cells	Phytochaema-gglutinin	"	"
Leukaemic basophils	Antigen	"	"
Swiss 3T3 cells	PDGF Vasopressin	"	"
Neuroblastoma-glioma hybrid NG 108-15	Bradykinin	"	"
Photoreceptors	Photons	"	"

Interest thus was kindled to carry out a literature survey on the characteristics, distribution, metabolism and role of phosphoinositides, myo-inositol and their important metabolites (inositol phosphates) in normal and stress conditions. The following introductory chapter is a summary of this literature survey.

PHOSPHOINOSITIDES :

Phosphoinositides possess unique physical and chemical properties which distinguish them from other phospholipids. They disperse readily in water forming micelles which are smaller than those formed by phosphatidyl choline (PtdCho), for instance. Hendrickson (1969) quotes a micellar weight of 78,100 for PtdIns(4,5)P_2 while that for PtdCho is well over a million. PtdCho is likely to form extended lamellar structures while PtdIns(4,5)P_2 micelles are probably spherical. PtdIns forms micellar aggregates of lower size than PtdIns(4,5)P_2. Na^+ or K^+ salts of phosphoinositides are readily dispersed in water but salts with divalent cations such as Mg^{2+} or Ca^{2+} are insoluble in water but readily dissolve in chloroform. PolyPI do not form liposomes but ox brain PtdIns forms regular liposomes after sonication for 45 min (Papahadjopoulos and Miller, 1967). PtdIns liposomes consisted of only one or two lamellae, 300-400 A^o in diameter, while PtdCho liposomes were multilamellar and 10 times larger.

Although the bulk of membrane phospholipids are switterionic, phosphoinositides are anionic at physiological pH with PtdIns. PtdIns4P and PtdIns(4,5)P_2 carrying one, three and five net negative charges respectively (Fig. 2). Their ion-binding properties have been studied in great detail (Hendrickson and Fullington, 1965; Hendrickson and Reinortson, 1969, 1971; Hauser and Dawson, 1967; Dawson and Hauser, 1970; Triggle, 1972; Papahadjopoulos et al., 1974). PolyPI, particularly PtdIns(4,5)P_2 bind divalent cations avidly, but without great specificity for individual ionic species. The binding of Ca^{2+} ions to phosphoinositides varies with the pH of the medium (Richard and Charles, 1967; Hauser and Dawson, 1968), PtdIns binding at low pH values and PolyPI in the range of 6.5-8.5. In this connection, it is interesting to note that isolated PolyPI fractions of brain contain considerable amounts of bound divalent cations (Kerr et al., 1964; Hendrickson and Ballou, 1964; Eichberg and Dawson, 1965). This property coupled with the fact that those lipids are rapidly dephosphorylated and rephosphorylated, suggest that they may be important in Ca^{2+} binding and permeability changes related to axonal conduction (discussed later).

Evidence exists to show that PolyPI complex readily with a variety of proteins. LeBaron and Folch (1956) first reported inositol containing "Phosphatidepeptide" in brain. In vitro studies carried out by Hendrickson (1969) indicate that

PtdIns(4,5)P_2, binds to a variety of proteins with varying degrees of specificity. The nature of the bond between the protein or peptide and the inositide is not known but the strength indicates that it may be ionic in nature. Several authors have reported that glycophorin preparations isolated from erythrocytes contain appreciable quantities of PolyPI (Armitage et al., 1977; Buckley, 1978). The cholinergic proteolipid receptor fraction isolated from cerebral cortex has been shown to contain PtdIns(4,5)P_2 which may function as a binding component of the nicotinic cholinergic receptor (Wu et al., 1977; Cho et al., 1978). However, studies by DeRobertis et al (1980) indicate that PtdIns(4,5)P_2 is absent in the chloroform-methanol extract of cerebral cortex containing the cholinergic proteolipid.

The high lability of PolyPI post-mortem has been well documented in the whole brain of rat (Dawson and Eichberg, 1965; Eichberg and Hauser, 1967; 1969), mice (Nishihara and Keenan, 1983), and guinea-pigs (Sheltawy and Dawson, 1969) as well as in different regions of the rat brain (Hauser et al., 1971; Gonzalez-Sastre et al., 1971). Maximum depletion is found to occur in the first 10 min followed by a steady decline. The depletion is found to be higher in younger animals and decreases with age (Sheltawy and Dawson, 1969). Studies using $^{32}P_i$ indicate a significant loss in specific radioactivity with time, in whole brain (Sheltway and Dawson,

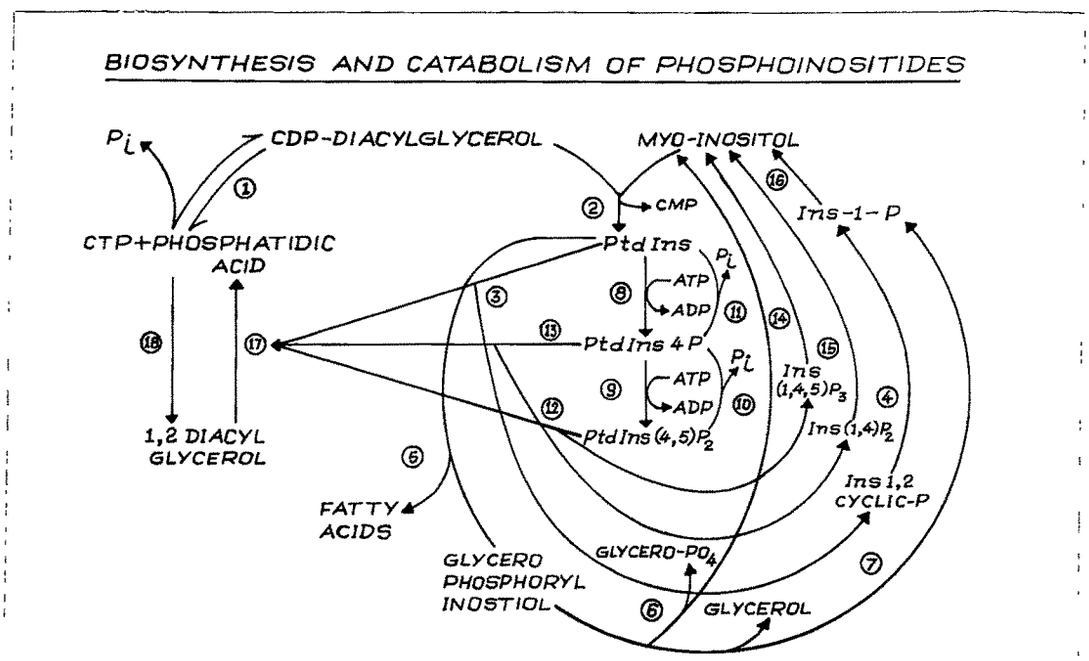
1969) and in brain regions (Gonzalez-Sastre et al., 1971) after decapitation of the animals. Interestingly, Eichberg and Hauser (1967) have shown that the activity of PolyPI phosphohydrolases do not change for at least 120 min after death. Nijjar and Hawthorne (1977) suggest that an increase in the levels of intracellular Ca^{2+} on post-mortem might be a cause for the high rate of depletion of these compounds.

Brain inositol lipids are rich in arachidonic acid which constitutes 20-30% of total fatty acid by weight, while the other phospholipids contain lower amounts eg. PtdCho 1-5%; PtdEtn, 8-13% (White,1973). Over 27 molecular species of phosphoinositides have been identified but the 1-stearate-2-arachidonate derivative constitutes more than 40% of the total. (Holub et al., 1970). There is now accumulating evidence that one of the important sources of arachidonic acid for prostaglandin biosynthesis is PtdIns in a variety of tissues, including platelets (Prescott and Majerus,1981), rabbit neutrophils (Rubin et al., 1981), cat adrenocortical cells (Schrey and Rubin, 1979), mouse fibrosarcoma (Bell et al., 1980), mouse pancreas (Marshall et al., 1980; 1981), and smooth muscle (Coburn et al., 1981). This release of arachidonic acid from phospholipids as a result of phospholipase A_2 action, followed by conversion to prostaglandins, is an important step in the regulation of a variety of physiological functions.

Phosphoinositides exhibit a high turnover rate of the monoester phosphate groups as compared to other phospholipids (Wagner et al., 1962; Brockerhoff and Ballou, 1962; Eichberg and Dawson, 1965; Santiago-Calvo et al., 1964; Kai and Hawthorne, 1966; Mandel and Nussbaum, 1966; Freysz et al., 1969; Friedel and Schanberg, 1971). The half life ($t\frac{1}{2}$)for PtdIns using $^{32}P_i$ has been shown to be 12.5, 7 and 9.5 days in whole brain, neuronal and glial cells, respectively (Freysz et al., 1969). Smith and Eng (1965) reported $t\frac{1}{2}$ values of 5 weeks and 5 days in the myelin and mitochondrial fractions of rat brain respectively, using (^{14}C) acetate. The extraction procedure in these experiments did not allow recovery of PolyPI. The $t\frac{1}{2}$ for the major phospholipids was considerably higher. Further, as mentioned earlier a selective increase in the incorporation of $^{32}P_i$ into PtdIns and PtdA in several tissues in response to a wide variety of stimuli including neurotransmitters, hormones and electrical pulses has been shown (Table 1). In recent years, PolyPI have also come into focus in this receptor response coupling phenomenon in a variety of cell membranes (Table 2).

The pathways for the synthesis and hydrolysis of phosphoinositides are given in Fig. 3. A detailed survey of literature on the enzymes involved is given later.

Fig. 3 : Biosynthesis and catabolism of phosphoinositides.



ENZYMES INVOLVED IN PHOSPHOINOSITIDE CYCLE

1. CTP : Phosphatidic acid cytidylyl transferase
2. CDP-diacylglycerol : inositol phosphatidyl transferase
3. PtdIns phosphodiesterase (phospholipase C-type)
4. Inositol 1,2-cyclic phosphate 2-phosphohydrolase.
5. Phosphatidyl inositol phosphodiesterase (phospholipase A-type)
6. 3-Sn-glycero phosphoinositol glycero phosphohydrolase
7. 3-Sn-glycero phosphoinositol inositol phosphohydrolase
8. PtdIns kinase
9. PtdIns4P kinase
10. PtdIns(4,5)P_2 phosphomonoesterase
11. PtdIns4P phosphomonoesterase
12. PolyPI phosphodiesterase
13. Ins(1,4,5)P_3ase
14. Ins(1,4)P_2ase
15. Inositol-1-phosphatase
16. Diacylglycerol kinase
17. Phosphatidate phosphohydrolase

Phospholipids constitute 60% of total lipids and phosphoinositides over 2-12% of total phospholipids in mammalian brain. Table 3 gives the concentration of the three major inositol lipids -- PtdIns, PtdIns4P and PtdIns(4,5)P_2 in different tissues of the rat. PtdIns is virtually the only phosphoinositide present in most tissues while its higher phosphorylated forms are present in trace quantities. Quantitative extraction of PolyPI from tissues presents a number of difficulties (Downes and Michell, 1982) so that most published statements on their levels must be regarded as minimum figures. The richest sources of PolyPI are brain, kidney and iris smooth muscle, although improved extraction procedures (Hauser and Eichberg, 1973) have not been applied widely to the remaining tissues. PolyPI have been detected in yeast (Steiner and Lester, 1972) and crithida fasciculata (Palmer, 1973) and PtdIns4P is probably present in higher plants (rice grains) and cress seedlings (Asada et al., 1969).

Table 4 gives the incorporation (expressed as specific radioactivity) of $^{32}P_i$ in vitro into phosphoinositides of different tissues of the rat which follows the order, brain> kidney>liver>pancreas>heart for PolyPI and liver>brain>kidney> pancreas>heart for PtdIns. Among the three inositol lipids incorporation is in the order PtdIns(4,5)P_2> PtdIns4P>PtdIns for brain and kidney while PtdIns is higher than PolyPI in the remaining tissues.

TABLE 3 : DISTRIBUTION OF PHOSPHOINOSITIDES IN DIFFERENT TISSUES OF THE RAT.

Tissue	PtdIns	PtdIns4P	PtdIns(4,5)P_2
	nmoles/g wet wt		
Brain[a]	2220	250	400
Kidney[a]	2620	40	30
Liver[a]	3320	20	30
Pancreas[a]	3070	30	60
Heart[a]	1130	-	-
Intestine[a]	1380	40	40
Spleen[a]	1140	10	-
Iris smooth muscle[b]	520	200	250
Adrenals[c]	870	27	3
Retina[d] (rabbit)	-	30	5.5

- not determined

(a) Dittmer and Douglas (1969).
(b) Abdel-Latif et al (1978b).
(c) Farese et al (1979).
(d) Alberghina et al (1982).

TABLE 4 : INCORPORATION OF $^{32}P_i$ INTO PHOSPHOINOSITIDES OF DIFFERENT TISSUES.

Tissue	PtdIns	PtdIns4P	PtdIns(4,5)P_2
	CPM/100 mg tissue*		
	CPM/µg acid labile nucleotide-P x 10^5		
Brain	19,400	31,400	37,400
Kidney	14,500	14,700	21,900
Liver	23,400	5,500	4,370
Pancreas	7,300	2,540	4,190
Heart	3,620	980	2,190

* Radioactivities have been corrected to a constant specific activity for the acid-labile nucleotide-P for comparative purposes since different tissues exhibit different rates of incorporation of $^{32}P_i$ into ATP.

All tissue slices were incubated for 2 hr with $^{32}P_i$ *in vitro*.

Santiago-Calvo *et al* (1964).

Apart from brain, kidney tissue has attracted attention of several scientists. PolyPI were first isolated by Huggins and Cohn (1959) from kidney cortex of different species. Values obtained by different workers in the subsequent years are given in Table 5. The quantitative variation could be attributed to the differences in the extraction procedures used by different workers.

The metabolism of PolyPI in kidney is unique in that there is rapid labeling of these compounds with $^{32}P_i$ *in vivo* followed by a rapid decline, which has not been observed in other organs examined (Tou *et al.*, 1972). This emphasizes the rapid metabolism of phosphomonoester groups of rat kidney PolyPI but its physiological significance remains unknown. PtdIns does not show this metabolic pattern.

It is possible that these molecules participate in some important functional component of the tubule membranes during secretion and reabsorption of solutes from the lumen of the tubule. Evidence is now accumulating to resolve this important role of inositides in the functional characteristics of the tubular membrane (Farese *et al.*, 1980; 1981; Bidot-Lopez *et al.*, 1981; Benabe *et al.*, 1982; Hruska *et al.*, 1983).

Changes in the levels of PolyPI with age has been studied in the whole brain of rats (Eichberg and Hauser, 1967; Wells and Dittmer,1967; Sheltawy and Dawson, 1969; Keough and

TABLE 5 : PHOSPHOINOSITIDE CONTENT OF RAT/RABBIT KIDNEY.

PtdIns	PtdIns4P	PtdIns(4,5)P_2	Reference
	nmoles/g wet wt		
2,720	50	30	Wagner et al (1963)
-	50-90	40-70	Dawson and Eichberg (1965)
2,620	40	30	Dittmer and Douglas (1969)
1,350	153	163	Tou et al (1972)
-	67	130	Hauser and Eichberg (1973)
1,752	81	29	Farese et al (1980)*
1,217	44	14	Bidot-Lopez et al (1981)*

- not determined.

* Levels in rabbit kidney cortex.

Thompson, 1970; Soukup et al., 1978a), guinea-pigs (Sheltawy and Dawson, 1969) and chicks (Shaikh and Palmer, 1976). Results indicate that maximum deposition of PtdIns4P occurs in the pre-weaning period while that of PtdIns(4,5)P_2 occurs both during pre- and post-weaning periods. PtdIns(4,5)P_2 increases more rapidly than PtdIns4P during the period of maximal myelination, indicating that a larger fraction of the former is located in myelin than the latter. Comparison of reported values is difficult since extraction procedures and tissue fixation methods for halting degradation of PolyPI after decapitation of the animal vary considerably (Table 6). No systematic study using improved methods of extraction (Hauser and Eichberg,1973) has been carried out on the deposition of PolyPI with age in rat brain.

The activities of PtdIns and PtdIns4P kinases in the developing post-natal rat (Salway et al., 1968; Eichberg and Hauser, 1969) and chick (Shaikh and Palmer, 1977a) brain have been well documented. Salway et al (1968) reported PtdIns kinase to increase about 3-fold from birth to 6 days of age, well before the onset of myelination in the rat brain. One experimental series showed the activity to increase during the period of active myelination (14-24 days) and remain high thereafter while the second indicated a decrease during and after myelination. The latter observation was supported by the work of Eichberg and Hauser (1969) who found PtdIns kinase

TABLE 6 : POLYPHOSPHOINOSITIDE LEVELS IN DEVELOPING RAT BRAIN - A COMPARISON OF REPORTED VALUES.

AGE	PtdIns(4,5)P_2				PtdIns4P			
	a	b	c	d	a	b	c	d
Days	nmoles/g wet wt.							
2	91	-	64	-	-	-	32	-
3	-	30	-	-	-	10	-	-
4	-	-	75	-	-	-	32	-
5	-	-	-	76	-	-	-	56
6	-	40	-	-	-	50	-	-
7	98	-	-	-	43	-	-	-
10	131	-	118	116	53	-	32	79
12	-	50	-	-	-	50	-	-
14	-	-	161	-	-	-	48	-
16	-	-	-	102	-	-	-	121
17	209	-	-	-	92	-	-	-
18	-	140	-	-	-	160	-	-
20	-	-	355	145	-	-	129	147
24	-	170	376	-	-	150	-	-
28	-	-	419	-	-	-	193	-
30	-	-	-	233	-	-	-	239
34	447	-	-	-	140	-	-	-
40	-	-	-	226	-	-	-	198
42	-	240	452	-	-	190	193	-

contd...

TABLE 6 : contd.

Age	PtdIns(4,5)P_2				PtdIns4P			
	a	b	c	d	a	b	c	d
100	548	-	-	-	179	-	-	-
180	-	410	430	-	-	210	193	-
330	-	390	-	-	-	200	-	-

\- not determined.

(a) Heads were frozen in liquid N_2; acid hydrolysis products of PolyPI were separated by high voltage electrophoresis and P determined (Eichberg and Hauser, 1967).

(b) Brains were frozen in dry ice; deacylated products of PolyPI were separated by ion exchange chromatography and P determined (Wells and Dittér, 1967).

(c) Heads were frozen in liquid N_2; acid hydrolysis products of PolyPI were separated on formaldehyde-treated paper and P determined (Sheltawy and Dawson, 1969).

(d) Brains were frozen in liquid N_2; as described in (c) (Keough and Thomson, 1970).

Levels of PolyPI using microwave irradiation technique have been determined only at three ages (Soukup et al., 1978). Values as nmoles/g wet wt: 28 days - 441 and 162; 35 days - 460 and 149; 45 days - 550 and 125 for PtdIns(4,5)P_2 and PtdIns4P respectively.

activity to decrease steadily after birth. The appearance of PtdIns kinase activity in the developing chick brain was different from that in rat brain in three respects. The increase in activity was greater (4-5 fold), was co-incident with the onset of myelination and the activity remained high during subsequent development.

The PtdIns4P kinase activity also showed a dramatic (4-fold) increase during the period of most active myelination and thereafter remained high in the chick brain. Salway et al (1968) observed a similar developmental pattern for this enzyme in post-natal rat brain. However, Eichberg and Hauser (1969) reported the increase in activity to occur during later stages of myelination. Thus the enzymatic activities appear to be correlated more with the deposition of PtdIns(4,5)P_2 in brain during myelination.

Developmental studies on PolyPI phosphohydrolases further strengthen the association of PtdIns(4,5)P_2 with myelin (Salway et al., 1968; Keough and Thompson, 1970; Shaikh and Palmer, 1977b). PtdIns(4,5)P_2 phosphomonoesterase activity increases most rapidly during myelination in the rat (Salway et al., 1968) and chick (Shaikh and Palmer, 1977b) brain. On the other hand, the development of phosphodiesterase activity does not specifically correlate with the period of myelination.

Studies on brain regions indicate obvious differences in the concentration and lability post-mortem of PolyPI (Hauser et al., 1971$_a$). The levels in gray matter are lower than in white matter. White matter is enriched in PtdIns(4,5)P_2 which is concentrated in myelin and the degradation post-mortem is considerable for both lipids in gray but not in white matter (Table 7). With increasing age there is further deposition of PtdIns(4,5)P_2 in white matter while the levels in other regions remain unaltered. A similar distribution pattern has also been shown in guinea-pig gray and white matter (Sheltawy and Dawson, 1969). Gonzalez-Sastre et al (1971) have further extended the studies using ^{32}P-orthophosphate. The specific radioactivity of PtdIns(4,5)P_2 in brain stem was not affected in the 10 min post-mortem samples while in the cerebrum and cerebellum they were considerably lower than the "0 min" post-mortem samples. Thus, PolyPI may exist as two pools in the cerebrum and cerebellum one which is hydrolysed rapidly post-mortem and the other at a slower rate. In the brain stem there appears to be a single pool that is not affected post-mortem. It can be therefore concluded that a metabolically active pool of these compounds is associated with gray matter structures and a relatively stable pool with white matter structures in the nervous system.

Table 8 gives the PolyPI levels among a few mammalian species studied. The concentration of PtdIns(4,5)P_2 does not

TABLE 7 : DISTRIBUTION OF POLYPHOSPHOINOSITIDES IN RAT BRAIN REGIONS.

Region	Dissection begun post mortem (min)	PtdIns(4,5)P_2 (nmoles/g wet wt)	Change (%)	PtdIns4P (nmoles/g wet wt)	Change (%)
Whole brain	0	318.3	-30.1	162.9	-53.5
	10	222.6		75.8	
Forebrain	0	163.7	-42.3	112.3	-79.9
	10	94.4		22.6	
Olfactory lobes	0	118.3	-39.0	77.4	-
	10	73.0		79.9	
Cortical gray matter	0	103.2	-43.7	112.9	-68.6
	10	58.1		35.5	
Thalamus + hypothalamus	0	175.3	-35.6	150.0	-50.0
	10	112.9		74.2	
Cerebellum	0	248.4	-36.8	179.0	-50.5
	10	157.0		88.7	
Brain stem + Medulla	0	549.5	- 3.7	235.5	-13.7
	10	529.0		203.2	

"0 min" refers to the time at which brains were frozen in liquid N_2 after decapitation which was approximately 30-45 sec.

Hauser et al (1971a).

TABLE 8 : POLYPHOSPHOINOSITIDES IN THE CENTRAL AND PERIPHERAL NERVOUS SYSTEM OF DIFFERENT SPECIES.

	PtdIns	PtdIns4P	PtdIns(4,5)P_2
		nmoles/g wet wt.	
CNS			
Guinea-pig[a]	-	177	585
Cat[b]	-	61	271
Rat[a]	-	197	349
Ox[a]	-	45	615
Human[c]	-	100	80
PNS[d]			
Crab - claw	226	N.D.	11*
leg	322	N.D.	11*
Lobster - claw	193	8	43
leg	97	2	32
Cow - splenic	484	N.D.	2
Rabbit - sciatic	710	32	537
Sheep - sciatic	1,613	145	1,000
Monkey - sciatic	290	97	569
Hen - sciatic	452	21	258

* Detected in one animal only.

ND - not detected

\- not determined.

(a) Dawson and Eichberg (1965)

(b) Palmer and Rossiter (1965)

(c) Kerr et al (1964)

(d) Sheltawy and Dawson (1966).

appear to have any correlation with the extent of myelination although it is considered to be predominantly localized in myelin (Eichberg and Dawson, 1965). However, these data are not reliable, since the methods used for extraction as well as the technique used for tissue fixation vary widely for the different species examined.

As mentioned earlier, brain PolyPI are reported to be predominantly localized in the myelin fraction (Eichberg and Dawson, 1965; Eichberg and Hauser, 1973; Shaikh and Palmer, 1976). There is little published information on the localization of phosphoinositides within biological membranes. In erythrocyte membranes, choline-containing phospholipids are located mainly in the outer layer and amino phospholipids and PtdIns are preferentially located in the inner leaflet (Opdenkamp, 1979). As for PolyPI, Garrett and Redman (1975) prepared inside-out and right-side-out vesicles from human erythrocytes that were impermeable to γ-$^{32}P_i$-ATP. Synthesis of PolyPI occurs only with the inside-out vesicles, suggesting that the phosphoinositide kinases are on the cytoplasmic side of the plasma membrane. Although the above studies suggest that PtdIns and PolyPI are probably located at the cytoplasmic side of cell membranes, it must be emphasized that the data on phospholipid asymmetry in biological membranes are still incomplete and, in many cases, controversial.

Because of the slow turnover of myelin lipids and proteins, it has been suggested that the highly reactive PolyPI and the necessary enzymes might be located in myelin appurtenances such as the internal or external mesaxon or the membrane loops at the nodes of Ranvier. Deshmukh et al (1978) carried out in vitro studies on the biosynthesis of PtdIns4P and PtdIns(4,5)P_2 in three myelin subfractions, namely, "heavy", "medium" and "light" myelin. The most active incorporation of $^{32}P_i$ from γ-$^{32}P_i$-ATP into PtdIns4P and PtdIns(4,5)P_2 occurred in "heavy" myelin, indicating the association of kinases with the myelin appurtenant structures. However, the medium and light myelin also showed appreciable activity, but gave specific activities lower than those of the heavy fraction. Furthermore, the analysis of the distribution of PolyPI showed that they were concentrated in "light" myelin - Table 9 (Deshmukh et al., 1980). This suggested that there is rapid metabolism of phosphoinositides in the tightly packed myelin lamellae and that this region contains the necessary phosphorylating and dephosphorylating enzymes together with their substrates. However, since these findings were obtained from prefractionated myelin fractions in vitro, they do not necessarily reflect in vivo turnover rates -- for example, "light" myelin in situ might be quite inaccessible to ATP and therefore exhibit a slow turnover of PolyPI. To investigate this possibility Deshmukh et al (1981) studied the incorporation

TABLE 9 : DISTRIBUTION OF PHOSPHOINOSITIDES AND THE POLYPI PHOSPHOHYDROLASES IN SUB-FRACTIONS OF RAT BRAIN MYELIN.

Fractions	Concentration			Enzyme Activity	
	PtdIns[a]	PtdIns4P[a]	PtdIns(4,5)P_2[a]	PolyPI phosphomonoesterase[b]	PolyPI phosphodiesterase[b]
	nmoles/g wet wt. of brain			nmoles lipid hydrolysed/mg protein/hr	
Homogenate	1,260	160	267		
Whole myelin	270	70	147	271	288
Light myelin	49	18	24	223	290
Medium myelin	96	19	41	265	400
Heavy myelin	65	5	22	258	475
Pellet	14	2	3	151	156
Recovery (%)*	79	63	61	61	89

* Percent of whole myelin phosphoinositides and its hydrolysing enzyme activities recovered from all subfractions of myelin.

(a) Deshmukh et al (1980).

(b) Deshmukh et al (1982).

in vivo of intracerebrally injected $^{32}P_i$ into phosphoinositides of myelin subfractions. There was rapid incorporation of $^{32}P_i$ into PolyPI, which contained 50-70% of the radioactivity in total brain lipids and more than 70% among myelin lipids. The order of relative specific radioactivities in the myelin fractions was heavy>light>medium. These results support the possibility of association of the highly reactive PolyPI with the myelin appurtenant regions e.g. in the paranodal loops, where they may be involved in transmembrane cation flux. Attempts have also been made to purify the myelin bound PolyPI phosphohydrolases and study their characteristics (Hwan *et al.*, 1981; Deshmukh *et al.*, 1982). Recently Deshmukh *et al* (1984) have examined the mutual stimulation by myelin basic protein (MBP) and PtdIns4P of their phosphorylation by detergent solubilized myelin kinases. Results suggest that the phosphate turnover of MBP and PtdIns4P may be coupled *in vivo*.

PolyPI are also present in structures of nervous tissue other than myelin (Sheltawy and Dawson, 1969; Eichberg *et al.*, 1971; Eichberg and Hauser, 1973). Of the neuroglial cell types present in brain, oligodendrocytes are presumed to contain PolyPI, although no evidence exists to date. Eichberg *et al* (1971) showed that PolyPI are constituents of normal newborn hamster astrocytes in dispersed cell culture, as well as of an adult rat astrocytoma cell line, suggesting that these lipids are components of immature astrocytes in brain. Such a

fraction might constitute a metabolically active pool of these substances distinct from the bulk of PolyPI located in myelin. The high metabolic activity of these lipids in cultured astrocytes incubated under arbitrary conditions indicates that they may play a significant role in the still unknown function of these cells in central nervous system.

In summary, the above studies show that PolyPI, although present in trace quantities, are functionally important components by virtue of their unique properties viz., binding to cations and proteins, high lability post-mortem, high content of arachidonic acid and high turnover rates. Studies in the nervous tissue indicate that PolyPI may exist as two pools in regions like the cerebrum and cerebellum (rich in gray matter) and as a single pool in brain stem (rich in white matter). The precise localization, properties, role and metabolism of these pools is not yet known. A strong correlation may exist between the turnover of PolyPI pools and inositol phosphates and the enzymes involved in their synthesis and hydrolysis during the development and maturation of brain cells. Future investigations on inositol phosphates, phospholipase activities, PolyPI metabolism in neuronal and glial cultures and subcellular distribution of PolyPI pools are essential to clarify the function of these compounds and to delineate the location and role of discrete pools with different metabolic activities.

The following is a concise summary of the studies carried out on the enzymes concerned with the metabolism of phosphoinositides. The various tissues in which the different enzymes have been detected are given in Table 10. The activities of the major synthesizing and hydrolysing enzymes, their subcellular localization and the effect of inorganic cations (Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}) on their activities in brain and kidney are given in Tables 11, 12, and 13 respectively.

CTP : Phosphatidic acid cytidylyl transferase

One of the key features of PtdIns biosynthesis is the prominent involvement of the liponucleotide CDP-diacylglycerol which is formed from CTP and PtdA by the action of CTP:phosphatidic acid cytidylyl transferase.

$$\text{PtdA} + \text{CTP} \longrightarrow \text{CDP-diacylglycerol} + P_i \qquad (1)$$

The enzyme has been studied in rat brain preparations and several other tissues (Table 10). In brain, it is mainly located in the microsomal fraction with small but significant activity in the mitochondrial fraction (Table 12).

The concentrations of CDP-diacylglycerol in mammalian tissues are extremely small, and thus, very little work has been done on its characterisation. The concentration in brain ranges from 9.2 - 15.5 μmol/kg of tissue, which corresponds to about 1% of the level of PtdA. Analysis of fatty acid

TABLE 10 : ENZYMES OF PHOSPHOINOSITIDE METABOLISM IN DIFFERENT TISSUES.

Enzyme	Tissue	Reference
CTP:PtdA cytidylyl transferase	Brain Liver Lung Small intestine Kidney Heart Diaphragm Skeletal muscle	Bishop and Strickland (1976)
CDP-diacylglycerol: inositol phosphatidyl transferase	Brain Liver Kidney Heart Lung Spleen (Guinea pig)	Benjamins and Agranoff (1969)
PtdIns phosphodiesterase (Phospholipase C-type)	Brain (Ox)	Thompson and Dawson (1964)
	Kidney	Speziale et al (1982)
	Pancreas	Dawson (1959)
	Liver	Kemp et al (1961)
	Intestine	Atherton et al (1966)
	Thyroid	Jungalwala et al (1971)
	Kidney, Spleen Adrenals, Intestinal, Mucosa	Atherton and Hawthorne (1968)
	Mouse fibroblasts	Koch and Diringer (1973)
	Brain (Rat)	Hirasawa et al (1982a)
3-sn-glycero-phosphoinositol glycero phosphohydrolase	Brain Kidney Liver Pancreas Spleen Int. mucosa	Dawson et al (1979)
PtdIns kinase	Brain, Kidney, Liver, Pancreas, Spleen, Testis, Lung, Heart and Skeletal muscle	Harwood and Hawthorne (1969a).

contd...

TABLE : 10 contd.

Enzyme	Tissue	Reference
	Adrenal chromaffin granules	Muller and Kirshner (1975)
	Brain-P	Bostwick and Eichberg (1981)
PtdIns4P kinase	Brain-P	Salway et al (1968)
	Kidney cortex	Tou et al (1970)
	Erythrocytes	Peterson and Kirshner (1970)
	Platelets	Cohen et al (1971)
PolyPI-phospho-monoesterase	Brain-P	Nijjar and Hawthorne (1977)
	Brain (Ox)-P	Dawson and Thompson (1964)
	Kidney-P	Lee and Huggins (1968a) Lapetina et al (1975)
	Erythrocytes	Garrett et al (1976) Roach and Palmer (1981)
	Iris smooth-muscle	Akhtar and Abdel-Latif (1978)
PolyPI-Phospho-diesterase	Brain	Keough and Thompson (1972)
	Brain (Ox)-P	Thompson and Dawson (1964)
	Kidney	Tou et al (1973) Lapetina et al (1975)
	Intestine	Atherton and Hawthorne (1968)
	Thyroid	Jungalwala et al (1971)
	Iris smooth-muscle	Akhtar and Abdel-Latif (1978)
Ins1Pase	Brain (Bovine)-P	Hallcher and Sherman (1980)
	Brain	Wells et al (1969)
	Intestine	Rao et al (unpublished)
	Brain	Merlyn et al (unpublished)
Ins(1,4,5)P_3ase +	Intestine	Merlyn et al (unpublished)
Ins(1,4)P_2ase	Brain	Rao et al (unpublished)

P - Indicates that the enzyme has been purified. All studies refer to enzyme activities in rat tissues unless otherwise specified.

TABLE -11 : ACTIVITIES OF MAJOR SYNTHESIZING AND CATABOLIZING ENZYMES OF PHOSPHOINOSITIDE METABOLISM IN RAT BRAIN AND KIDNEY.

Enzyme	Enzyme activity expressed as	Enzyme activity in	
		Brain	Kidney
CTP : PtdA Cytidylyl transferase	nmoles of CTP incorp/min/mg protein	1.87[a]	0.69[a]
CDP-diacylglycerol inositol phosphatidyl transferase	nmoles of CMP released/min/mg protein	0.82[b]	0.52[b]
PtdIns phosphodiesterase (Phospholipase-C type)	nmoles of P_i released/min/g wet wt	2016[c]	-
PtdIns kinase	nmoles of PtdIns4P formed/min/g wet wt	43[d]	19.5[d]
PtdIns4P kinase	nmoles of PtdIns(4,5)P_2 formed/min/g wet wt	2.4 ([d]	1.45[d]
PolyPI phosphomonoesterase	nmoles of P_i released/min/g wet wt	7500[e]	1472[f]
PolyPI phosphodiesterase	nmoles of P_i released/min/g wet wt		5101[h]
Ins1Pase	nmoles of Pi released/min/g wet wt	4800[i]	-
Ins(1,4)P_2ase	nmoles of P_i released/min/g wet wt	1130[i]	-
Ins(1,4,5)P_3ase	nmoles of P_i released/min/g wet wt	1330[i]	-

contd.

TABLE -11: contd.

\- Not determined

(a) Bishop and Strickland (1976).

(b) Benjamins and Agranoff (1969).

(c) Irvine and Dawson (1978).

(d) Huggins et al (1969).

(e) Salway et al (1968).

(f) Lee and Huggins (1968a).

(g) Keough and Thompson (1970).

(h) Tou et al (1973).

(i) Anjali et al, unpublished.

TABLE 12 : SUBCELLULAR LOCALIZATION OF ENZYMES OF PHOSPHOINOSITIDE METABOLISM IN RAT BRAIN AND KIDNEY.

Enzyme	Brain	Reference	Kidney	Reference
CTP:PtdA cytidylyl transferase	ER	Petzold and Agranoff (1967)	-	-
CDP-diacylglycerol : inositol phosphatidyl transferase	MIC	Benjamins and Agranoff (1969)	-	-
PtdIns-phosphodiesterase (phospholipase-C type)	SOL (Ca^{2+}-dep)	Irvine and Dawson (1978)	SOL	Atherton and Hawthorne (1968)
	Lyso(Ca^{2+}-indep)	Irvine et al (1978)		
	SOL & PM	Leu and Sun (1983)		
PtdIns-phosphodiesterase (phospholipase-A type)	MIC & SN	Shum et al (1979)	-	-
3-sn-glycero-phosphoryl inositol inositol phosphohydrolase	-	-	Brush border of proximal tubules	Dawson and Hemington (1977)
Inositol(1,2)-cyclic phosphate-2-phospho-hydrolase	PM	Dawson and Clarke (1972)	PM	Dawson and Clarke (1972)
Ins1Pase	SOL	Hallcher and Sherman (1980)	-	-
	SOL (GM) SOL & PART (WM)	Anjali et al (unpublished)		

TABLE 12 : contd.

Enzyme	Brain	Reference	Kidney	Reference
PtdIns kinase	PM	Harwood and Hawthorne (1969a)	MIC	Tou et al (1969)
PtdIns4P kinase	SOL	Kai et al (1966) Kai et al (1968)	PM	Tou et al (1970)
	PNS myelin	Iacobelli (1969)		
	CNS myelin	Deshmukh et al (1983)		
PolyPI phosphomono-esterase	SOL & PART	Salway et al (1967)	MIC	Lee and Huggins (1968a)
	SOL	Nijjar and Hawthorne (1977)		
	CNS myelin and it's sub-fractions	Deshmukh et al (1982)	MIC	Cooper and Hawthorne (1975)
PolyPI phosphodiesterase	SOL & PART	Keough and Thompson (1972)	SOL	Lapetina et al (1975)
	CNS myelin and its sub-fractions	Deshmukh et al (1982)	SOL	Tou et al (1973)
Ins(1,4,5)P_3ase + Ins(1,4)P_2ase GM	PART	Anjali et al unpublished	-	-
Ins(1,4,5)P_3ase + Ins(1,4)P_2ase WM	PART			

PM - plasma membrane; ER - endoplasmic reticulum; SOL- soluble; PART - particulate; MIC - microsomes; GM - gray matter; WM - white matter.

- not determined.

Table 13 : Effect of cations on enzymes of phosphoinositide metabolism in rat brain and kidney.

Enzyme	Brain				Reference
	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	
CTP: PtdA cytidylyl transferase	I	A	-	-	Gibson and Brammer (1981)
CDP-diacylglycerol inositol phosphatidyl transferase	I	A	-	-	Gibson and Brammer (1981)
PtdIns phospho-diesterase (phospholipase-C type)	A	I	-	I	Irvine et al (1979)
PtdIns kinase	I	A	I	I	Kai et al (1966a)
PtdIns$_4$P kinase	A	A	I	I	Kai et al (1966b)
PolyPI phospho-monoesterase	A	A	I	I	Nijjar and Hawthorne (1977)
PolyPI phospho-diesterase	A	-	-	-	Keough and Thompson (1972)
InslPase	NE	NE	NE	NE	Meryln et al (unpublished)
Ins(1,4)P_2 + Ins(1,4,5)P_3ase	NE	I	I	I	Merylyn et al (unpublished)

I : Inhibitor

A : Activator

- : Not determined

NE: No effect

contdd.

Table 13 : contd..

Enzyme	Kidney				Reference
	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	
CTP: PtdA cytidylyl transferase	-	-	-	-	-
CDP-diacylglycerol inositol phosphatidyl transferase	-	-	-	-	-
PtdIns phospho-diesterase (phospholipase-C type)	-	-	-	-	-
PtdIns kinase	I	A	NE	NE	Tou et al (1969)
$PtdIns_4P$ kinase	A (partially)	A	I	I	Tou et al (1970)
PolyPI phospho-monoesterase	I	-	-	-	Lee & Huggins (1968b)
PolyPI phospho-diesterase	I	I	-	-	Tou et al (1973)
InslPase	-	-	-	-	-
$Ins(1,4)P_2$ + $Ins(1,4,5)P_3$ase	-	-	-	-	-

I : Inhibitor

A : Activator

\- : Not determined

NE : No effect

composition showed that 1-stearoyl, 2 arachidonyl is the major species of brain CDP-diacylglycerol suggesting that arachidonyl-rich PtdIns can be derived from the liponucleotide. CDP-diacyl-glycerol is split quantitatively into PtdA and CMP by a membrane bound phosphohydrolase from E-coli that is specific for the liponucleotide (Rittenhouse, 1981).

CDP-diacylglycerol inositol phosphatidyl transferase

Various mammalian systems are capable of incorporating myo-inositol into PtdIns by two routes, which can be distinguished by their cytidine nucleotide and divalent cation requirements. One is the de novo synthesis of PtdIns from CDP-diacylglycerol and free myo-inositol (equation 2); in the other, myo-inositol is incorporated into PtdIns in the presence of Mn^{2+} as a result of an enzymatic exchange reaction with endogenous PtdIns. In the latter, myo-inositol incorporation is not mediated by CDP-diacylglycerol.

CDP-diacylglycerol + myo-inositol ---> PtdIns + CMP (2)

To assess the possible quantitative significance of the Mn^{2+} stimulated CTP-independent pathway Holub (1974) injected rats intraperitoneally with (^{3}H)-inositol and determined the molecular species of PtdIns produced in liver microsomes. Radioactive PtdIns formed in vitro was 82% tetraenoic and 4% mono +dienoic species while in vivo it was 52% tetraenoic and

33% mono + dienoic species. He concluded that a major proportion of the free inositol enters PtdIns by the de novo biosynthetic pathway of these lipids. The PtdIns-myo-inositol exchange enzyme has been purified and characterized from rat liver microsomes (Takenawa and Egawa,1980).

Gibson and Brammer (1981) investigated these two routes of PtdIns biosynthesis in isolated bovine oligodendrocytes. The CTP-dependent route has a higher velocity of inositol incorporation, can utilise either Mn^{2+} or Mg^{2+} as a divalent ion co-factor, and is strongly inhibited by Ca^{2+} ions at concentrations less than 1 mM. The primary site of inhibitory action appears to be the enzyme CDP-diacylglycerol:inositol phosphatidyl transferase though synthesis of CDP-diacylglycerol is also inhibited by endogenous Ca^{2+} ions present in the oligodendrocyte homogenate. In contrast, the CTP-independent route is stimulated only by Mn^{2+} while Ca^{2+} does not inhibit the reaction at least at concentrations up to 1 mM. It therefore appears that a potentially important factor which may affect oligodendroglial PtdIns synthesis is the level of intracellular Ca^{2+} ions. Similar observations have been made in brain cortex synaptosomal and microsomal fractions which actively catalyze the incorporation of (^{3}H)-inositol into endogenous PtdIns (Strosznajder, 1983).

Besides the above mentioned pathways a direct acylation system is present in brain for the biosynthesis of PtdIns. In

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the presence of ATP, Mg^{2+} and CoA, synaptosomes are capable of transferring arachidonate to Lyso-PtdIns. Activity of the synaptosomal transferase is localized mainly in the synaptic vesicles and plasma membranes. This direct acylation system in brain synaptic membranes may provide a rapid means of replenishing PtdIns in the deacylation-reacylation cycle operating during cellular hyperactivity. Secondly it may also replenish PtdIns required for the formation of PtdIns(4,5)P_2 and PtdIns4P during synaptic activity. In addition, it may be useful for protecting membranes from deleterious effects caused by the accumulation of lyso compounds (Sun et al., 1978).

The CDP-diacylglycerol : inositol phosphatidyl transferase which is responsible for the de novo biosynthesis of PtdIns (equation 2) has been investigated in several mammalian tissues (Table 10). Eichberg and Ghalayini (1981) have succeeded in purifying the enzyme by affinity chromatography and studies are in progress to investigate its role in the stimulated PtdIns turnover and receptor-mediated cellular responses.

The catabolism of PtdIns in animal tissues is markedly different from other major phospholipids. (1) The predominant route of degradation is mediated by a PtdIns-specific phosphodiesterase (phospholipase C-type) located in the cytosol, that cleaves the phospholipid into DG and a mixture of Ins1,2cyclicP and Ins1P (Dawson, 1959; Kemp et al., 1961). The latter arises

from the hydrolysis of the initial cleavage product (Ins1,-2cyclic-P) which is then converted to Ins1P (Dawson et al., 1971; Dawson and Clarke, 1972; Lapetina and Michell, 1973). (2) In some tissues, the predominant water-soluble product is found to be glycerophosphoinositol implying that deacylation is a major route for catabolism (Seamark et al., 1968).

1. PtdIns specific phosphodiesterase (phospholipase C-type)

$$\text{PtdIns} + H_2O \longrightarrow \text{DG} + \text{Ins1,2cyclic-P} + \text{Ins1P} \qquad (3)$$

This is a phospholipase C-type of enzyme widely distributed in animal tissues (Table 10), mainly localized in the cytosolic fraction and has an absolute requirement for Ca^{2+} ions. The enzyme present in the thyroid and intestine attacks all three inositol lipids while in the other tissues only PtdIns is hydrolysed. Reports on the localization of this enzyme in rat brain are conflicting. It was earlier shown that brain has considerable membrane bound activity but Irvine and Dawson (1978) have confirmed that the enzyme is purely a cytoplasmic one. Recently Leu and Sun (1983) have shown that the enzyme is both cytosolic and membranous and is present in all brain subcellular fractions except myelin. The order of activity was reported to be cytosol > synaptosomes > synaptic plasma membrane > synaptic vesicles = microsomes > myelin. Among the brain regions examined, hypothalamus showed the highest activity. Several phospholipids (PtdSer, PtdEtn and PtdA)

are potent stimulators of the enzyme while Mg^{2+} ions, positively charged proteins such as histones and PtdCho are inhibitory (Irvine et al., 1979a). Furthermore, treatment of the brain extract with exogenous proteinases causes the appearance of new forms of the enzyme with a Ca^{2+}-sensitivity increased by three orders of magnitude over the original forms (Hirasawa et al., 1982b). Whether these Ca^{2+}-sensitive forms of the enzyme exist in resting tissues, or whether proteolytic cleavage is actually part of the PtdIns phosphodiesterase regulation mechanism is not known. Polyamines like putrescine and cadaverine stimulate the activity of this enzyme, while spermine and spermidine do so at low concentrations but inhibit at higher concentrations (Eichberg et al., 1981). Polyamines are known to be present in myelin (Shaw, 1979) and in synaptic membranes (Seiler and DecKardt, 1976). It is speculated that these compounds may regulate PtdIns turnover during synaptic activity.

Recently Hirasawa et al (1982a)have examined the heterogeneity of this enzyme by standard isoelectric focusing and a new chromatofocusing technique in rat brain. The enzyme exhibited considerable heterogeneity, both with respect to pH optima of activity, and its isoelectric properties. When the cytosolic fraction was analysed by isoelectric focusing on acrylamide gels, and the enzyme assayed at pH 5.5, four peaks were found at pH ranges 7.4, 6.2, 6.0 - 5.8, 4.8 - 4.4, and 4.2 - 3.8. These observations have also been extended to the

sheep pancreas supernatant fraction (Dawson et al., 1982) and evidence has also been obtained to show that a similar situation exists in the rat liver and kidney. The chemical nature of the heterogeneity (i.e. if they are true isoenzymes) is still unclear.

A second PtdIns phosphodiesterase, distinct from the Ca^{2+}-sensitive enzyme, has been reported to be present in the lysosomes of rat brain and liver (Irvine et al., 1978). This enzyme is inhibited by Ca^{2+} and is exclusively localized in the lysosomal fraction. The enzyme functions primarily as a cyclizing phosphotransferase by using part of the substrate molecule as an acceptor and catalyses the release of Ins1,2cyclic-P from PtdIns. The formation of the cyclic ester has now been confirmed in several tissues. A specific phosphodiesterase that converts the cyclic ester to Ins1P has been reported in the kidney, where it is selectively localized in the brush border of the proximal tubules (Dawson and Clarke, 1972).

The enzyme has been shown to be present in mouse fibroblasts and is apparently more active in normal growing cells than in either confluent cells or in SV 40 transformed cells. It is suggested that the cleavage of PtdIns may be linked in some manner to the regulation of cell growth (Diringer, 1978). The activity of this Ca^{2+}-independent lysosomal enzyme may be a candidate for the increased breakdown of PtdIns observed in many states of cellular hyperactivity, where the response of

PtdIns to excitatory agents is sometimes independent of a rise in intracelular levels of Ca^{2+} ions. The accumulation of PtdIns in lysosomes that occurs after chronic treatment with some cationic amphiphilic drugs (CAD) could be caused in part by their inhibition of PtdIns degradation in the lysosomes by this enzyme. However, the exact physiological role of the enzyme remains unknown.

2. PtdIns phosphodiesterase (phospholipase A-type)

PtdIns --> Fatty acid + Lyso-PtdIns

↓

glycerophosphoryl-inositol + Fatty acid

This deacylation of PtdIns at the 2-position to give lyso-PtdIns and free fatty acids was demonstrated in the 12,000-106,000g pellet and supernatant prepared from rat brain homogenate (Shum et al., 1979). As mentioned earlier this lyso-PtdIns formed could be reacylated to PtdIns by the direct acylation system present in brain synaptic membranes (Sun et al., 1978). Alternatively the action of lysophospholipase could release free fatty acid and glycerophosphoryl inositol. Incubation of rat brain synaptosomes with 1-acyl-2(^{14}C)-arachidonyl sn-glycerophosphoinositol and sodium deoxycholate has been shown to yield diacylglycerol and free arachidonic acid (Der and Sun, 1981). Diacylglycerol formation could be attributed to hydrolysis by the PtdIns specific phospholipase-C and FFA release to hydrolysis by phospholipase A_2. Labelled FFA may

have also been derived from phospholipase A_2-lysophospholipase action (Der and Sun, 1981) or by the action of phospholipase C-DG lipase (Cabot and Gatt, 1976; 1977; 1978). Unsaturated fatty acids have been shown to stimulate PtdIns-specific phospholipase-C (Irvine et al., 1979b) suggesting that this deacylation reaction could further stimulate PtdIns hydrolysis. It is possible that the two hydrolytic processes are inter-related by some unknown mechanism. A phospholipase A_2 which shows selectivity towards PtdIns has been purified from bovine brain microsomes. The enzyme has appropriate properties required for functioning in a deacylation-reacylation cycle for PtdIns (Gray and Strickland, 1982).

The glycerophosphorylinositol formed by the deacylating enzyme could be hydrolyzed either to glycerol and phosphoryl inositol or glycerophosphate and inositol.

a. 3-sn-glycerophosphorylinositol glycero phosphohydrolase:

glycero(3)phosphorylinositol ----> glycero-PO_4 + myo-inositol (6)

This enzyme is reported to be present in a variety of tissues in the rat namely, liver, pancreas, brain, spleen, kidney and intestinal mucosa (Dawson et al., 1979). Although the richest sources of the enzyme in rat are kidney and intestinal mucosa, pancreas was used to study the characteristics of the enzyme since it is known to contain an active deacylating

system for forming glycerophosphorylinositol from PtdIns.

b. 3-sn-glycerophosphorylinositol inositol phosphohydrolase

glycero(3)phosphoinositol --> glycerol + Ins1P (7)

This enzyme was first detected in insect larvae (Hildenbrandt and Bieber, 1972) and later in the kidney cortex of rat (Dawson and Hemington, 1977). The enzyme is active at alkaline pH and is present in the brush border of proximal tubules where it may have some role in filtration or reabsorption processes. There are indications that this enzyme also occurs in other tissues and if coupled with a lysosomal phospholipase deacylating PtdIns this would represent a different route for the catabolism of PtdIns in mammalian tissues. It remains an open question as to which pathway is of quantitative importance both in the basic turnover of PtdIns and the increased turnover in stimulated cells.

PtdIns and PtdIns4P kinases

Biosynthesis of PolyPI proceeds by sequential kinase reactions from PtdIns :

PtdIns + ATP ----> PtdIns4P + ADP (8)

PtdIns4P + ATP ----> PtdIns(4,5)P_2 + ADP (9)

These reactions were first demonstrated by incorporation of radioactive phospholipid precursors, e.g., $^{32}P_i$, (^{14}C)-glycerol, and (^{3}H)-inositol, *in vivo* and *in vitro* in brain

slices (Hawthorne and Kai, 1970). These studies have shown that whereas (^{14}C)-glycerol and (^{3}H)-inositol are incorporated in the order PtdIns > PtdIns4P > PtdIns(4,5)P_2, $^{32}P_i$ is incorporated in the order PtdIns(4,5)P_2 > PtdIns4P > PtdIns. Phosphorylation of PtdIns by ATP produces PolyPI in which the phosphodiester originates from PtdA and the phosphomonoester directly from ATP. The kinases add phosphates to the 4 and 5 position of inositol in PtdIns; the structure of the latter remains intact during these interconversions.

PtdIns kinase is unequivocally a particulate enzyme in most tissues while PtdIns4P kinase is cytoplasmic in rat brain and membraneus in kidney cortex (Table 12). Both kinases appear to be localized on the cytoplasmic side of the erythrocyte membrane (Garrett and Redman, 1975). They are Mg^{2+} dependent enzymes and inhibited by Ca^{2+}, when Mg^{2+} is present, both in brain and kidney. Na^{+} and K^{+} are inhibitory to PtdIns kinase in brain and PtdIns4P kinase in brain and kidney (Table 13). Bostwick and Eichberg (1981) have attempted the purification of detergent-solubilized PtdIns kinase and examined its properties from rat brain microsomes. The role of these kinases (mainly PtdIns4P kinase) in synaptic events is described later.

Enzymes hydrolysing PolyPI are highly active and probably play a significant role in the turnover of these compounds *in vivo*. Their activities are about 100 times higher (Table 11)

than those of the biosynthetic enzymes suggesting a strict regulation of the former *in vivo*.

PolyPI are rapidly catabolized either to PtdIns and P_i by monoesteratic cleavage of the phosphate groups by PolyPI monoesterase or to DG and their respective inositol phosphates by a PolyPI phosphodiesterase.

$$PtdIns(4,5)P_2 + H_2O \longrightarrow PtdIns4P + P_i \quad (10)$$

$$PtdIns4P + H_2O \longrightarrow PtdIns + P_i \quad (11)$$

$$PtdIns(4,5)P_2 + H_2O \longrightarrow DG + Ins(1,4,5)P_3 \quad (12)$$

$$PtdIns4P + H_2O \longrightarrow DG + Ins(1,4)P_2 \quad (13)$$

A brief account of the various aspects studied on PolyPI-phosphomonoesterase and PolyPI-phosphodiesterase in the past 25 years in selected tissues are summarized in Tables 14 and 15, respectively.

PolyPI-phosphomonoesterase

This enzyme has been submitted to considerably more study than the phosphodiesterase(s). Its subcellular distribution has not been definitely established and may well differ in different tissues. Harwood and Hawthorne (1969b) compared the subcellular distribution of $PtdIns(4,5)P_2$ phosphomonoesterase and phosphodiesterase in guinea-pig synaptosomes, and showed that considerably more phosphomonoesterase than diesterase

TABLE 14 : HISTORY OF POLYPHOSPHOINOSITIDE PHOSPHOMONOESTERASE

Aspect studied	Reference
BRAIN	
Partial purification from ox brain and its kinetic properties	Dawson and Thompson (1964)
Subcellular localization in rat brain	Salway et al (1967)
Developmental pattern in rat brain	Salway et al (1968)
Subcellular localization in guinea-pig brain	Sheltawy et al (1972)
Developmental pattern in chick brain and sciatic nerve	Shaikh and Palmer (1977b)
Purification from rat brain and its kinetic properties	Nijjar and Hawthorne (1977)
Purification from rat brain myelin and its kinetic properties	Hwan et al (1981)
Distribution in rat brain myelin subfractions	Deshmukh et al (1982)
KIDNEY	
Subcellular localization in rat kidney-cortex	Lee and Huggins (1968a)
Partial purification (50-fold) from rat kidney-cortex and its kinetic properties	Lee and Huggins (1968b)
Subcellular localization and general properties	Cooper and Hawthorne (1975)
RBC MEMBRANE	
Localization in human RBC membrane	Garrett and Redman (1975)

contd...

TABLE 14 : contd.

Aspect studied	Reference
Role in shape transformation of erythrocytes	Garrett et al (1976)
Partial purification from human erythrocytes and its kinetic properties	Roach and Palmer (1981)
IRIS SMOOTH MUSCLE	
General properties in rabbit iris smooth muscle (1st report)	Akhtar and Abdel-Latif (1978)

activity was present in the membranous fractions. Furthermore, Sheltawy et al (1972) have suggested that the levels of particulate activity in such studies are underestimated. This is probably due to a factor (a protein?) in the pH 5 supernatant of brain tissue which stimulates the activity of the enzyme in the particulate fractions and has been shown to be true in the case of iris smooth muscle (Akhtar and Abdel-Latif, 1978) and kidney (Cooper and Hawthorne, 1975). Recently Deshmukh et al (1982) have shown that the enzyme is equally distributed in myelin subfractions. The enzyme thus appears to be bimodal in its distribution (i.e., partly soluble and partly membranous).

The purified brain enzyme is activated by low concentrations of Ca^{2+} ions while relatively higher concentrations of Mg^{2+} were required to elicit comparable activation (Table 13). In contrast the erythrocyte enzyme was inhibited by Ca^{2+} (Roach and Palmer, 1981). Dialysis against EDTA of the brain enzyme caused inhibition and the dialyzed enzyme was completely dependent on Mg^{2+} (Salway et al., 1967). This suggested that the enzyme contains a tenaciously-bound Mg^{2+}, though it should be noted that Akhtar and Abdel-Latif (1978) found no inhibition of the rabbit iris muscle phosphomonoesterase by dialysis against 1 mM EDTA.

It is not yet certain, whether separate enzymes exist for the hydrolysis of PtdIns4P and PtdIns(4,5)P_2 or a single enzyme attacks both substrates. Nijjar and Hawthorne (1977)

found that a highly purified enzyme from rat brain prefers PtdIns(4,5)P_2 but also readily hydrolyses PtdIns4P. On the other hand, the enzyme purified from human erythrocytes (Roach and Palmer, 1981) was inactive against PtdIns4P. Cooper and Hawthorne, (1975) observed several differences in the optimal conditions for hydrolysis of the two substrates by kidney tissue fractions; these differences were complex and the authors concluded that although one enzyme seems likely, two cannot be excluded.

PolyPI phosphomonoesterase was first purified from ox brain (Dawson and Thompson, 1964) and later from rat brain cytosol (Nijjar and Hawthorne, 1977). Preliminary studies using ammonium sulfate fractionation of solubilized myelin-bound PtdIns(4,5)P_2 phosphomonoesterase show that cytosol and myelin enzymes have different sedimentation properties (Hwan et al., 1981). Further studies on purified enzyme preparations may clarify the question of their identity.

PolyPI phosphodiesterase

Like the phosphomonoesterases, PolyPI-phosphodiesterases are partly soluble and partly associated with membranes. Keough and Thompson (1972) investigated extensively the distribution of the three phosphodiesterases (for PtdIns, PtdIns4P and PtdIns(4,5)P_2) and showed that they were essentially similar

TABLE 15 : HISTORY OF POLYPHOSPHOINOSITIDE PHOSPHODIESTERASE

Aspect studied	Reference
BRAIN	
Partial purification from ox brain and its kinetic properties	Thompson and Dawson (1964)
Developmental pattern and subcellular localization in rat brain	Keough and Thompson (1970)
General properties in soluble and particulate fractions of rat brain	Keough and Thompson (1972)
Developmental pattern in chick brain and sciatic nerve	Shaikh and Palmer (1977b)
Purification from rat brain myelin and its kinetic properties	Hwan et al (1981)
Distribution in subfractions of rat brain myelin	Deshmukh et al (1982)
KIDNEY	
Partial purification from rat kidney-cortex (7-fold) and its kinetic properties	Tou et al (1973)
Degradation of $^{32}P_i$ labeled inositides by soluble kidney enzyme	Lapetina et al (1975)
RBC MEMBRANE	
Calcium-activated phospho-diesteratic breakdown of PolyPI in human and rabbit erythrocyte membrane	Allan and Michell (1978)
Method for the assay of PolyPI phosphodiesterase	Downes and Michell (1980)

contd...

TABLE 15 : contd.

Aspect studied	Reference
INTESTINE	
Enzyme activity in intestinal mucosa and some of its properties	Atherton *et al* (1966)
Subcellular localization and its properties	Atherton and Hawthorne (1968)
General properties in rabbit iris smooth muscle	Akhtar and Abdel-Latif (1978)

in the rat brain. They have been recently studied in myelin subfractions (Deshmukh et al., 1982). The enzyme is activated by Ca^{2+} in brain and inhibited in the kidney (Table 13). Data on brain (Thompson and Dawson, 1964) and erythrocytes (Downes and Michell, 1981) are both consistent with one phosphodiesterase activity hydrolysing both PtdIns4P and PtdIns(4,5)P_2, but this has not been definitely established. Several authors have recently shown that the phosphodiesteratic cleavage of PolyPI is perhaps a more significant consequence of ligand-receptor interactions in stimulated cells (Van Rooijen et al., 1983; Berridge et al., 1983). The enzyme could, therefore, play an important role in stimulated "PolyPI turnover" occurring in cells exposed to stimuli.

De novo biosynthesis of phospholipids, including PtdIns, occurs at the endoplasmic reticulum, and therefore some mechanism must exist by which they are transported to other cellular membranes. Several phospholipid exchange proteins have been investigated in a variety of animal tissues (Wirtz, 1974) that catalyze the transfer of phospholipids from a donor such as endoplasmic reticulum to an acceptor such as mitochondria, nerve terminals or myelin.

PtdIns transfer activity has been demonstrated in rat cerebral hemispheres (Brophy and Aitken, 1979) showing a peak increase between 14 and 18 days during the stages of most

active myelination. Exchange of PtdIns and PtdCho between microsomal and myelin membranes has been demonstrated (Ruenwongsa et al., 1979). This exchange is reversible and is catalysed by soluble proteins from the brain homogenate precipitated at pH 5.1. Recently Wirtz et al (1983) have shown that synaptosomes from rat brain and axoplasm from squid giant axon have relatively high levels of PtdIns transfer activity. The exchange proteins may thus have a role in facilitating the transfer in vivo of PtdIns from the site of synthesis in the microsomes to myelin and also in the membrane-associated "PtdIns turnover" for replenishment of PtdIns degraded on stimulation.

In summary, the biosynthesis of PtdIns from CDP-diacylglycerol and myo-inositol occurs at the endoplasmic reticulum, whereas its phosphorylation by ATP in the presence of specific kinases to PtdIns4P and PtdIns(4,5)P_2 takes place at the plasma membrane and membranes of other cellular organelles. The breakdown of phosphoinositides to 1,2-diacylglycerol and water-soluble inositol phosphates by the phosphodiesterases occurs in both the soluble and particulate fractions. Removal of the phosphomonoesters from PolyPI by the phosphomonoesterases also takes place in soluble and particulate fractions, the ratio of soluble to particulate being dependent on the tissue. The subcellular distribution of most enzymes are similar in brain and kidney with a few exceptions (Table 12). PtdIns4P kinase is a soluble enzyme in brain while in kidney it appears to be

membrane bound. PolyPI phosphodiesterase is purely soluble in kidney while the distribution in brain appears to be partly soluble and partly membranous. The physiological significance, if any, of these differences in the distribution of the enzymes is unclear.

The effect of ions on phosphoinositide-metabolising enzymes have been studied in great detail. Table 13 indicates that Ca^{2+} ions inhibit the synthesis of PtdIns and activate its breakdown in both brain and kidney. Synthesis of PtdIns4P and PtdIns$(4,5)P_2$ is also inhibited by Ca^{2+} in both tissues. However, the breakdown of both lipids by the mono and diphosphoesterases is activated in brain and inhibited in kidney. Mg^{2+} ions act as an activator for the enzymes synthesizing PtdIns and PolyPI in most tissues, kidney and brain being no exception to the rule. The effects of Na^+ and K^+ have not been investigated in detail. Both ions inhibit the synthesis of PtdIns4P and PtdIns$(4,5)P_2$ in brain and PtdIns$(4,5)P_2$ in kidney. Hydrolysis of PolyPI by the phosphomonoesterases is also inhibited by Na^+ and K^+ in the brain. Their effects on the kidney enzyme have not been investigated.

Thus, the above studies show that several enzymes are involved both in the basic and stimulated turnover of PolyPI. There is still a large need to establish their precise subcellular localization and study their characteristics on

purified enzymes in order to understand their role and factors which regulate their function.

Several roles postulated for these compounds have been mentioned earlier, The following is a brief extension of a few functionally important ones.

In the last decade a large amount of literature has appeared on the role of polyphosphoinositides in receptor function which includes a number of excellent reviews (Michell, 1975; 1979; 1983a; Hawthorne and White, 1975; Wells and Eisenberg, 1978; Hawthorne and Pickard, 1979; Berridge, 1981; 1982; 1984; Downes and Michell, 1982; Downes, 1982; 1983; Abdel-Latif, 1983; Agranoff, 1983; Farese, 1983). Receptors that provoke inositol phospholipid hydrolysis (Tables 1 and 2) in peripheral tissues appear to have one thing in common : the physiological responses to receptor activation are all initiated by a rise in cytosolic Ca^{2+} concentration. Ca^{2+} can be released from membrane-bound stores and can flood into the cell down a steep concentration gradient, presumably through specific channels in the membrane. The involvement of Ca^{2+} ions in the "PtdIns Effect" is unclear and highly controversial. In the early 1970's, it was shown that in most systems, enhanced PtdIns turnover is insensitive to omission of Ca^{2+} from the incubation medium. It was reported that Ca^{2+} is not required for PtdIns and/or PtdA turnover in response to various stimuli in adrenal medulla

(Trifaro, 1969), parotid (Jones and Michell, 1976), guinea-pig ileum (Jafferji and Michell, 1976) synaptosomes (Yagihara et al, 1973) liver, mast cells, blood platelets and leukaemic basophil (Berridge, 1984). In blowfly salivary glands, the breakdown of $^{32}P_1$-labelled PtdIns was not increased by ionophore A23187, and the increase in response to 5-HT was unaffected by the absence of Ca^{2+} from the bathing medium (Fain and Berridge, 1979).

On the other hand, in several tissues, "PtdIns Effect" was found to be Ca^{2+}-dependent. Stimulation of rabbit neutrophils by the synthetic peptide f-Met-Leu-Phe (Cockroft et al., 1980), dispersed pancreatic fragments by ionophore A23187 and carbachol (Farese et al., 1982), rat pancreatic islets by glucose (Clements et al., 1981), rat hepatocytes by vasopressin and epinophrine (Prpic et al., 1982) and iris smooth muscle by ACh (Abdel-Latif, 1976) were dependent on Ca^{2+} ions. In addition Ca^{2+} has been reported to be required for maximum PtdIns and/or PtdA turnover in response to TSH in the thyroid (Zor et al., 1968), electrical stimulation of synaptosomes (Hawthorne and Bleasdale, 1975) and thrombin activation in the platelets (Lapetina, 1983).

Thus, on the basis of the requirement for Ca^{2+}, one can discern two categories of tissues but it remains to be established whether the molecular mechanisms underlying these effects in both types of tissues are different from each other (Michell, 1982; Hawthorne, 1982).

What is the likely relationship among these different processes - receptor activation, disappearance of PtdIns, PtdIns4P and PtdIns(4,5)P_2 and mobilization of Ca^{2+} in the cytosol that accompany one another in so many different stimulated cells? Berridge (1983) has put forward two mechanisms whereby agonists might induce a large decrease in the levels of PolyPI (Fig. 4). One possible mechanism (accepted earlier) would depend upon the action of phospho-monoesterases that sequentially remove phosphate from the 5- and 4- position of the myo-inositol ring to give PtdIns. In this mechanism, the primary substrate used by the receptor would be PtdIns, which is degraded by a phosphodiesterase to yield diacylglycerol and a mixture of Ins1P and Ins1,2-cyclic-P when studied *in vitro*. The alternative mechanism (currently accepted) which could also lead to a decrease in the levels of PolyPI, involves a phosphodiesterase that acts directly on PtdIns(4,5)P_2 to form diacylglycerol and Ins(1,4,5)P_3. The latter would then be degraded sequentially through a series of phosphatases to Ins(1,4)P_2, Ins1P and free inositol. The situation regarding PtdIns4P is a little more complicated in that it could either be hydrolysed directly by a similar phospho-diesterase to give diacylglycerol and Ins(1,4)P_2 or it could first be converted to PtdIns(4,5)P_2 before hydrolysis as depicted in Fig. 4. In this mechanism, PtdIns functions indirectly as a reservoir of the precursor molecules that are

Fig. 4 : Two alternative mechanisms for the agonist (A) - dependent hydrolysis of membrane phosphoinositides by Ca^{2+} mobilizing receptors.

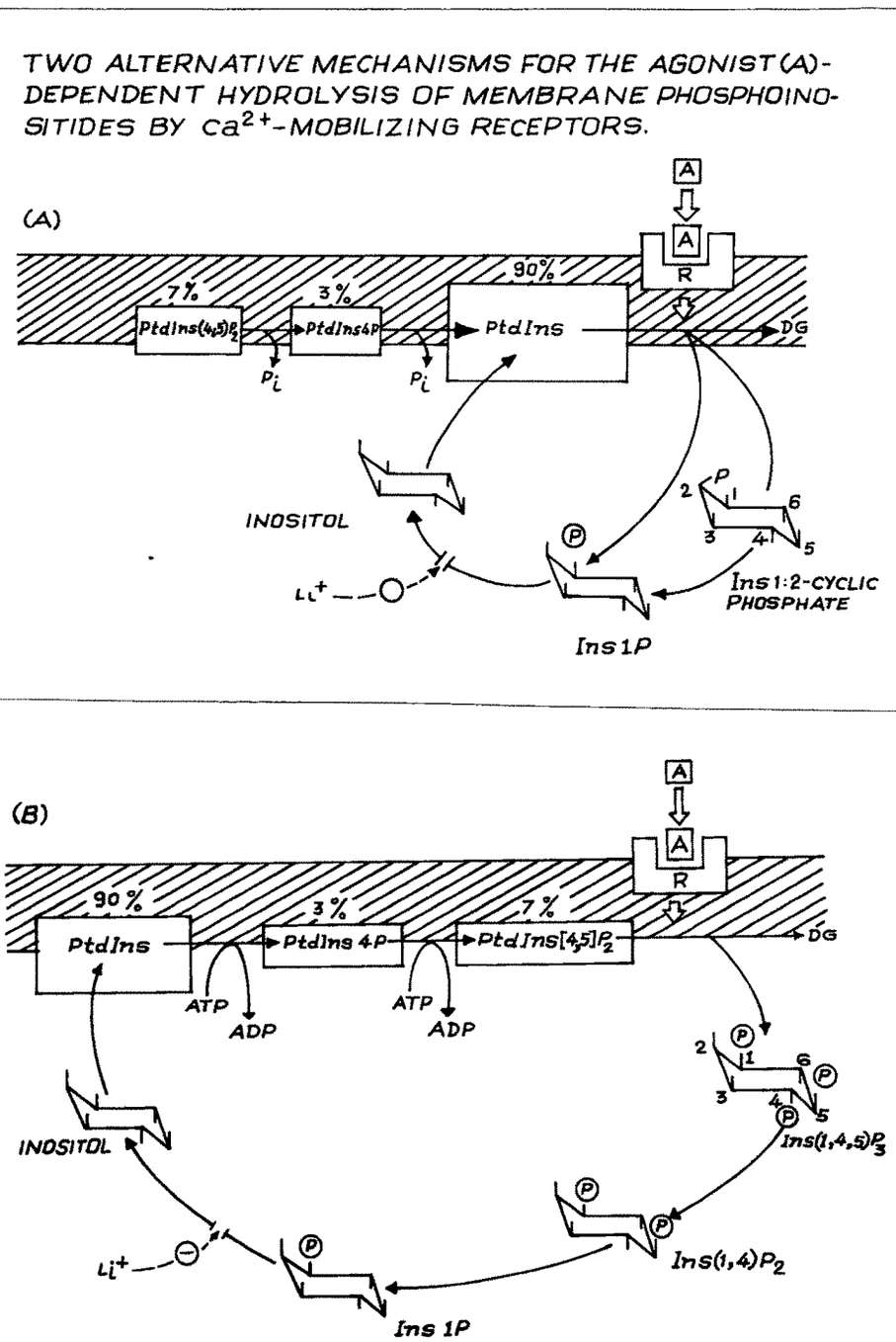


Fig. 4 (a) The primary substrate for the receptor (R) mechanism is PtdIns that is hydrolysed by a phosphodiesterase to yield DG and a mixture of Ins 1,2 cyclic-P and Ins1P. PtdIns(4,5)P_2 and PtdIns4P could also be used by the receptor mechanism once they have been converted into PtdIns by dephosphorylation.

(b) The primary substrate for the receptor (R) mechanism is PtdIns(4,5)P_2 that is hydrolysed to DG and Ins(1,4,5)P_3 which then enters an inositol phosphate cycle during which the three phosphates are removed sequentially to liberate the inositol required to resynthesize PtdIns. The final step of this cycle, the dephosphorylation of Ins1P to inositol is inhibited by lithium. PtdIns serves as a reservoir to supply precursors that can be phosphorylated to maintain the small hormone-sensitive PtdIns(4,5)P_2 pool. Berridge (1983).

(c) Model to illustrate the proposed role of phosphoinositides in the action of mitogenic signals included on this Figure is the contribution of various oncogene products to this inositol lipid signalling system. Abbreviations used: DG, diacylglycerol; EGF, epidermal growth factor; PDGF, platelet-derived growth factor.

phosphorylated to form PolyPI used by the receptor transducing mechanism.

Supportive evidence for the second mechanism comes from several recent studies (Table 2) where a phosphodiesteratic cleavage of PtdIns(4,5)P_2 and rapid accumulation of Ins(1,4,5)P_3 has been shown to occur very rapidly on stimulation in nerve ending membranes (VanRooijen et al., 1983), synaptosomal fractions (Griffin and Hawthorne, 1978), iris smooth-muscle (Akhtar and Abdel-Latif, 1980), rat parotid fragments, blowfly salivary gland and brain slices (Berridge et al., 1983), hepatocytes (Creba et al., 1983), superior cervical sympathetic ganglia (Michell et al., 1983) and several others (Berridge, 1984). It is speculated that inositol phosphates released might be the long-sought after "secondary messengers" in the receptor transducing mechanism that functions to mobilize and thus regulate the intracellular level of Ca^{2+} ions.

Recent studies have shown that the two products of PtdIns(4,5)P_2 hydrolysis i.e. Ins(1,4,5)P_3 and diacylglycerol could function as "secondary messengers" to activate two independent but parallel signal pathways that may also be responsible for releasing arachidonic acid and for activating guanylate cyclase. Diacylglycerol functions within the plane of the membrane to increase protein phosphorylation by activating C-kinase. It is either phosphorylated to phosphatidic acid by a

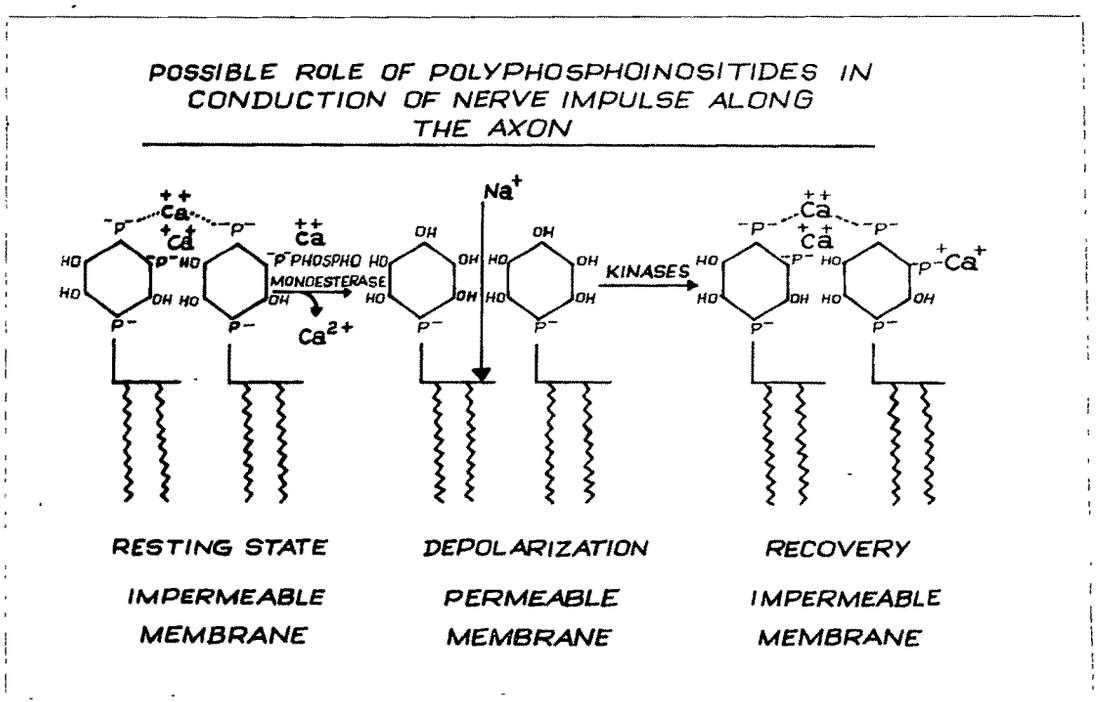
kinase or hydrolysed to monoacylglycerol and arachidonic acid by the diacylglycerol lipase. $Ins(1,4,5)P_3$ is released into the cytosol to function as a second messenger to mobilize calcium from intracellular stores. These two signal pathways appear to function in a synergistic manner to stimulate a wide variety of cellular protesses (Berridge, 1984). Fig. 4c.

Phosphoinositides appear to play an important role in modulating enzyme activities in cellular membranes. The enzymic characteristics of Na^+/K^+ dependent ATPase and the transport of various solutes have been shown to be severely impaired in inositol-deficient mammalian cells (Charalampous, 1971). PtdIns has been shown to regulate the activity of several enzymes like tyrosine hydroxylase in rabbit adrenal glands (Lloyd, 1979), Na^+/K^+ ATPase in microsomes of rabbit kidney (Mandersloot et al., 1978), alkaline phosphatase in microsomes of pig kidney (Low and Zilversait, 1980), acetyl-CoA carboxylase in rat liver (Blyatt and Kim, 1982), and acetyl-cholinesterase and arylacylamidase in sheep basal ganglia (Majumdar and Balasubramanian, 1982). The activities of adenylate cyclase (Hebdon et al., 1981) and galactosyl transferase (Ratnam et al., 1980) in plasma membranes of mammalian cells are dependent on their interactions with anionic lipids, particularly PtdIns and PtdSer. Alkaline phosphatase, acetylcholinesterase and 5'-nucleotidase are

released from a wide variety of tissues by bacterial PtdIns phosphodiesterase (Low and Zilversmit, 1980) suggesting that PtdIns may be responsible for the attachment of these enzymes to cellular membranes. The molecular mechanism(s) underlying the activation and interaction of PtdIns with enzymes is unclear and contraversial. It is speculated that they could create a negative charge near the active site, be components of the active site of itself, provide ion-specific sites, or serve in some kind of recognition role to insure insertion of the protein both in the required part of the membrane and in the necessary aligment.

With special focus on the nervous tissue phosphoinositides appear to be functionally important in the conduction of nerve impulses along axons and transmission across the synapse. A possible role for phosphoinositides in the conduction of nerve impulse along the axon was put forward by Kai and Hawthorne (1969). The authors hypothesized that Ca^{2+} bound to two PtdIns4P or PtdIns(4,5)P_2 molecules might bridge and obstruct an ion channel through the axonal membrane and that dephosphorylation to yield Ptd Ins would release Ca^{2+} ions and open the channel. The crucial interconversion was envisaged as between PolyPI (with a high affinity for Ca^{2+}) and PtdIns (with a lower affinity for Ca^{2+}) - Fig. 5.

Fig. 5 : Possible role of Polyphosphoinositides in conduction of nerve impulse along the axon.



Several experimental observations strongly support this model. Effect of ions on the activities of the phosphorylating and dephosphorylating enzymes are consistent with the changes in the local ionic environment at the inner face of the axonal membrane which are likely to occur during membrane depolarization. (Salway et al., 1967). Determination of the metal complex stability constants and acid dissociation constants of deacylated PolyPI indicated that a conversion of trisphosphate to bisphosphate would release 70% of bound Ca^{2+} and cause a 25% decrease in ligand charge, which could conceivably bring about a reorganization of the membrane with a resulting change in Na^{+} and K^{+} permeability (Hendrickson and Reinertsen, 1971). The effects of electrical stimulation were reported to be almost negligible by Salway and Hughes (1972), indicating that PtdIns(4,5)P_2 involved in any one neuronal event may represent a very small proportion of a large pool. However, White and Larrabee (1973) observed that labeling of PtdIns(4,5)P_2 was greatest in vagus nerves (non-myelinated) and considerably lower in phrenic nerve (myelinated ones) when stimulated electrically. Tretjak et al (1977) found that a decrease in the resting potential value of crab nerve fibres was accompanied by a reduction in PtdIns(4,5)P_2 content and an increase in the membrane permeability for K^{+} ions suggesting that PolyPI participate in the control of K^{+} channel permeability. Proscrini (inhibitor or acetylcholinesterase) protected the nerve fibres

against the action of ACh suggesting that ACh produces PolyPI hydrolysis via nerve fibre acetylcholinesterase activation. It is possible that this enzyme activity is connected with PolyPI phosphomonoesterase in crab nerve fibres. However, firm evidence for a role of PolyPI in axonal conduction is now emerging.

The role of phosphoinositides in synaptic events although has not been well documented, they have been implicated in these processes. In the late sixties Durell et al (1969) were the first to suggest the involvement of phosphoinositides in the mechanism of action of ACh in synaptosomes. However, the experimental evidence reported by several investigators subsequently has been confusing and in many instances contradictory. Yagihara and Hawthorne (1972) found no effect of ACh on PtdIns(4,5)P_2 labeling in synaptosomes and although Schacht and Agranoff (1972) observed some reduction in labeling, the same was not atropine-sensitive. Studies using cerebral cortex slices showed increased $^{32}P_1$ labeling in PtdA and PtdIns by ACh. NE and other neurotransmitters, to be widely distributed among subcellular fractions. Synaptosomes showed a marked effect, but stimulation was also seen in microsomal, mitochondrial and nuclear fraction. Glial cells as well as neuronal cells exhibited increases (Abdel-Latif et al., 1974).

Törda (1972; 1974) demonstrated that post-synaptic membrane depolarization induced by ACh is the result of conversion of PtdIns(4,5)P_2 to PtdIns4P. This conversion is controlled by PtdIns(4,5)P_2 phosphomonoesterase which is made up of catalytic and regulatory subunits. The binding of ACh to the regulatory subunit is believed to change the conformation of the enzyme leading to its activation and dephosphorylation of PtdIns(4,5)P_2. She also demonstrated that transformation of PtdIns4P back into PtdIns(4,5)P_2 leads to hyperpolarization of the post-synaptic membrane. PtdIns4P kinase has also been shown to consist of regulatory and catalytic subunits and binding of c-AMP to the regulatory subunit causes activation of the enzyme leading to rephosphorylation of PtdIns4P. This work, however, has never been substantiated or followed up in the subsequent years.

Strong evidence for a role of PtdIns in synaptic transmission came from the studies of Pickard and Hawthorne (1978) on the effects of electrical stimulation on PtdIns metabolism in prelabelled synaptosomes. $^{32}P_1$ was injected intracerebrally into guinea-pigs and synaptosomes were prepared from the cerebral cortex. Specific radioactivity in PtdA and PtdIns were at a maximum 2 hr after the injection and 3-8 times higher than in the cerebral cortex. Electrical stimulation produced a drop in the specific radioactivity of PtdIns and PtdA. Study of the sub-synaptosomal fractions obtained after osmotic rupture of synaptosomes revealed that highly labelled PtdIns was in the

synaptic vesicle fraction and PtdA in the microsomal fraction. Electrical stimulation caused losses in radioactivity from the respective fractions. These authors speculated that depolarization of the synaptosomal plasma membrane leads to phosphodiesteratic cleavage of PtdIns in the vesicle membrane, possibly mediated by the entry of Ca^{2+} ions. Diacylglycerol thus formed might promote fusion of vesicle with the pre-synaptic plasma membrane for exocytosis of the neurotransmitter (Fig. 1). Allan and Michell (1975) have shown that diacylglycerol induces membrane fusion and formation of microvesicles in the erythrocyte. After discharge of the vesicle contents the membrane is retrieved by mechanisms involving the pinching off of large vesicles, often seen as coated vesicles, where resynthesis occurs. Loss of labelled phosphatidate from the microsomal fraction may reflect resynthesis of PtdIns via CDP-diacylglycerol on the membranes of the endoplasmic reticulum. This loss was accompanied by increased labeling of CDP-diacylglycerol as would be expected during resynthesis. Transfer of PtdIns between membranes can be facilitated by the exchange protein reported to be present in synaptosomes (Wirtz et al., 1976).

Evidence for a role of PolyPI in synaptic transmission came from the work on iris smooth muscle where ACh and NE were found to stimulate the breakdown of PtdIns(4,5)P_2 and these effects were blocked by atropine and phentolamine, respectively (Abdel-Latif et al., 1977; 1978a). Fisher and Agranoff (1981)

reported a PolyPI effect in synaptosomes that was also blocked by atropine indicating that the effect is mediated by muscarinic receptors. The effects of neomycin on PolyPI metabolism in intact synaptosomes have also been investigated (Griffin et al., 1980).

Griffin and Hawthorne (1978) studied the Ca^{2+} -activated hydrolysis of PolyPI in guinea-pig synaptosomes using the Ca^{2+} ionophore A23187. The addition of Ca^{2+} ionophore to synaptosomes isolated from guinea-pig cortex labelled with $^{32}P_1$ caused loss of radioactivity and decrease in the concentration of PtdIns(4,5)P_2 as well as PtdIns4P while no changes were observed in other phospholipids except PtdA which showed a significant increase in labeling. Addition of ionophore to synaptosomes labelled with (H^3)-inositol caused loss of label from PtdIns(4,5)P_2 and PtdIns4P and no change in PtdIns. Incorporation was found to be increased in Ins1P and Ins(1,4)P_2 while no significant production of Ins(1,4,5)P_3 occurred, perhaps due to its breakdown by Ins(1,4,5)P_3 phosphatase. Recently Van Rooijen et al (1983) have provided the first direct evidence for the presence of membrane-bound, Ca^{2+}-stimulated phosphodiesterase activity acting on endogenous PolyPI in nerve ending membranes. The results suggest that Ca^{2+} probably activates PtdIns(4,5)P_2 phosphodiesterase releasing Ins(1,4,5)P_3 and diacylglycerol. The diacylglycerol formed promotes fusion of vesicle membrane and release of neurotransmitter as described

earlier (Fig. 1). Synaptosomes contain an active diacylglycerol kinase (Lapetina and Hawthorne, 1971; Kanoh et al., 1983) which converts DG to PtdA and then resynthesis occurs.

The cascade of events initiated by inositol phospholipid breakdown in synaptosomes appears to involve activation of protein kinases which amplify the response by catalysing the phosphorylation of functional proteins within the cell. One of them is protein kinase-C activated by Ca^{2+} and phospholipids. Diacylglycerol derived from the receptor linked inositol phospholipid breakdown dramatically increases the affinity of protein kinase-C for Ca^{2+} and thereby renders this enzyme fully active leading to phosphorylation of functional proteins. Protein phosphorylation and Ca^{2+} mobilization appear to act synergistically to elicit a final physiological response (Michell, 1983b).

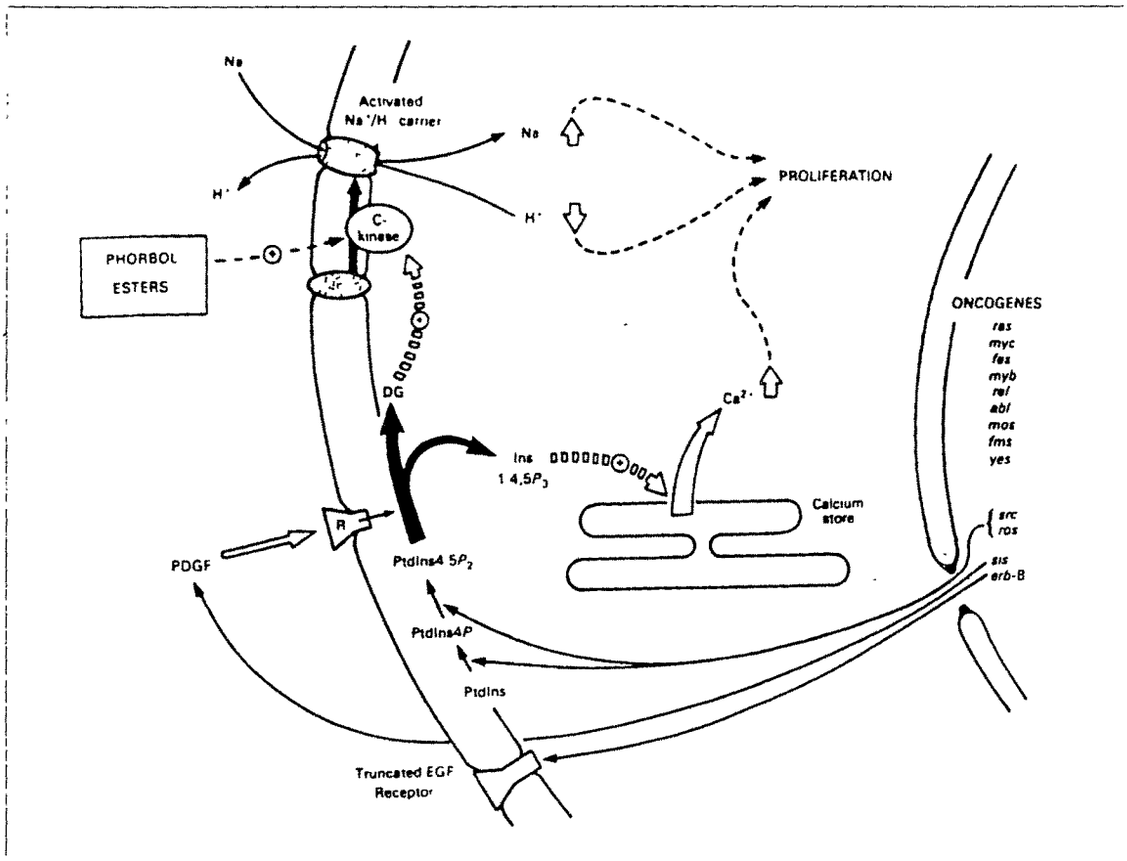
Studies by Gispen (1983) suggests that a peptide-sensitive multifunctional enzyme complex that contains a protein kinase, a substrate protein and PtdIns4P kinase exists in the pre-synaptic plasma membranes. This protein kinase appears to be similar to protein kinase-C and is a Ca^{2+}/calmodulin sensitive, cyclic nucleotide-independent enzyme that is inhibited by ACTH. One of its substrate proteins is the nervous tissue-specific protein B-50, which is predominantly localized in membranes of the pre-synaptic region of neurons. The degree of phosphorylation of the substrate protein seems to regulate the sensitivity

of PtdIns4P kinase and thereby the conversion of PtdIns4P to PtdIns(4,5)P_2 and synaptic transmission across neurons.

One of the most intriguing aspects of the polyphosphoinositide system is its involvement in the regulation of cell division. A model illustrating how the phosphoinositide response might contribute to the onset of proliferation is shown in Fig. 6. Mitogenic signals like platelet derived growth factor (PDGF) may initiate the breakdown of PtdIns(4,5)P_2 to give the two second messengers Ins(1,4,5)P_3 and diacylglycerol. Ins(1,4,5)P_3 may induce a Ca^{2+} signal by mobilizing intracellular calcium while diacylglycerol may be responsible for regulating sodium entry and hydrogen extrusion. Diacylglycerol stimulates protein kinase C which is probably the site of action of tumour promoting phorbol esters. As phorbol esters can activate the Na^+/H^+ exchange carrier it has been proposed that diacylglycerol may act in a similar way to increase cytoplasmic pH. These two signal pathways appear to function in a synergistic manner to stimulate cell proliferation (Berridge, 1984).

Recent studies by two groups of investigators (Lewis Cantley of Harward and Ian Macara of University of Rochester School of Medicine) have shown that this polyphosphoinositide signalling system may be connected to uncontrollable growth and other aberrant effects of oncogenes - genes that cause the

Fig. 6 : Proposed role of phosphoinositides in the action of mitogenic signals.



A large number of cellular processes may be switched on through the combined action of these two parallel signal pathways that begin with the against-dependent hydrolysis of PtdIns4,5P_2 to give Ins1,4,5P_3 and diacylglycerol. The former is removed by a trisphosphatase, whereas diacylglycerol is converted to either phosphatidic acid or monoacylglycerol. Ins1,4,5P_3 acts to mobilize intracellular calcium, whereas diacylglycerol stimulates the same C-kinase that can be activated by phorbol esters. Abbreviations used: PDE, phosphodiesterase; CaM, calmodulin.

malignant transformation of cells. The primary evidence for both groups is that certain oncogene products can act as inositol lipid kinases. Sugimoto et al (1984) show that purified $pp60^{v-src}$ the tyrosine kinase coded by the src oncogene of Rous sarcoma virus (RSV), can phosphorylate PtdIns, PtdIns4P and 1,2-diacylglycerol. Macara et al (in press) demonstrate that $p68^{v-ros}$, the tyrosine kinase coded by the ros oncogene of the avian sarcoma virus UR2, can phosphorylate PtdIns. The final product of the pathway is PtdIns(4,5)P_2 which is hydrolysed when receptors are activated. This phosphorylation of PtdIns appears to be a specific effect of tyrosine kinase.

The question is, how is the receptor activated signal at the cellular membrane transmitted to the nucleus where cell division occurs. One possibility is that activation of the polyphosphoinositide system affects cellular ion concentration, increasing those of calcium and sodium ions and decreasing those of hydrogen ions. Changes such as these might influence the activities of enzymes throughout the cell. Berridge speculates that Ins(1,3,4)P_3 that appears much more slowly and builds up to a maximum about 30 minutes after an appropriate hormone binds to its receptor may help produce the long-term effects of receptor activation, perhaps including transmission of signals to the nucleus, with Ins(1,4,5)P_3 affecting the short-term actions.

So far, two oncogenes have already been linked to growth factors. Part of the sis gene codes for a protein in platelet derived growth factor and the erb B gene codes for a segment of the receptor for epidermal growth factor. If ros and src transform by virtue of their actions on the polyphosphoinositide system, then the effect could be a third example of an intersection with growth factor activity. Much more work will be needed to determine whether this is the case.

MYO-INOSITOL

Most of the classical growth factors described in and before the early years of this century have since received "formal" recognition as vitamins and subsequently have been shown to fulfil some vital role as cofactors for prosthetic groups of enzymes. Myo-inositol is one of the few that have not had their essential nature explained in any satisfactory way and although it is generally included among the vitamins (Williams et al., 1940; Woolley, 1941; Eagle et al., 1957; Hegsted et al., 1974), its status in this category is not completely secure since it can be synthesized from glucose. Myo-inositol continues to be a biochemical enigma even though considerable amount of work has been done to find its physiological role.

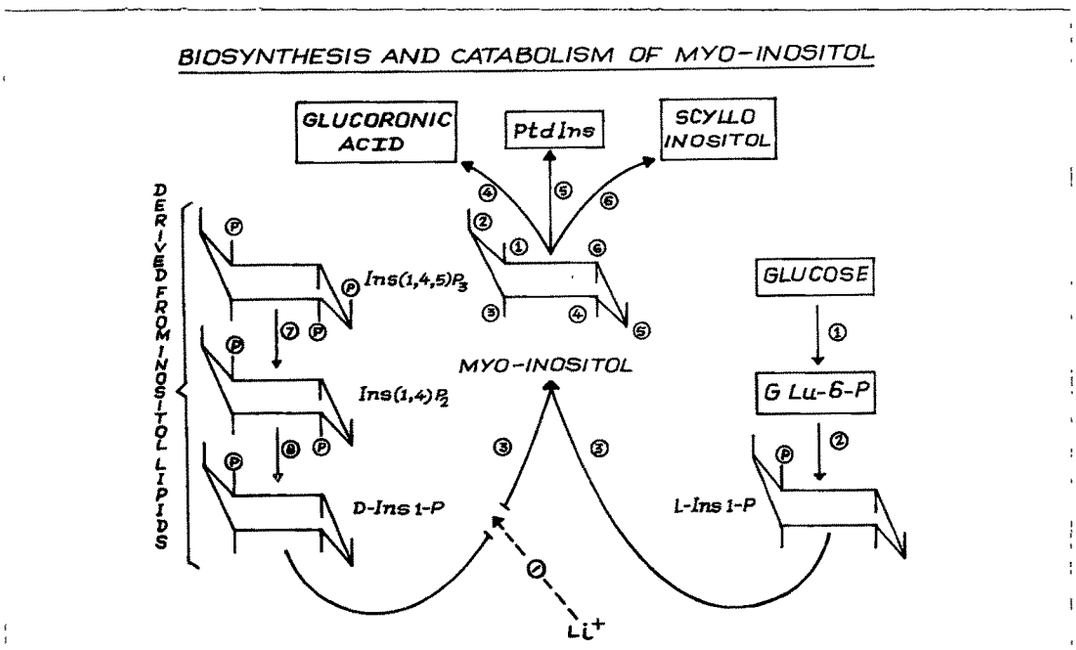
Myo-inositol, one of the nine possibe isomers of hexahydroxycyclohexane is the most prevalent isomer in nature. When viewed in its predominant chair form, five of myo-inositol's hydroxyls are seen to be in equatorial positions while the remaining hydroxyl at position 2 in the accepted system of numbering is axial (refer Fig. 2). The following is a brief summary of its distribution, metabolism and role in mammalian tissues. The presence and role of scyllo-inositol has also been mentioned. The importance of myo-inositol from a nutritional viewpoint is discussed later.

Myo-inositol in eukaryotic cells exists largely in combined form in the phospholipids of biomembranes. However, high concentrations of free inositol in rat organs indicate that this compound may play an important role in the function of the cell other than as a component of phosphoinositides.

The biosynthesis of inositol is carried out in four steps: Glu-6-PO_4 undergoes oxidation to yield 5-keto glu-6-PO_4 which undergoes cyclization to form the intermediate product Inosose-2 1-PO_4. This in turn is reduced to Ins1P and further dephosphorylated to form inositol (Eisenberg, 1978; Maeda and Eisenberg, 1980; Eisenberg and Maeda,1984). In addition to this de novo synthesis inositol also appears to be supplied to various tissues by transport from blood (Hauser, 1969a; Caspary and Crane, 1970). At present, there are three known metabolic reactions for which myo-inositol serves as a substrate: epimerization to scyllo-inositol (Posternak et al., 1963), oxidation to glucuronic acid (Howard and Anderson, 1967) and incorporation into PtdIns (Fig. 7) (Eagle et al., 1957; Dawson and Clarke, 1972).

Inositol is a common component of foodstuffs, being present mainly as phytate ie., inositol hexaphosphate (Sebrell and Harris, 1967). Free myo-inositol is present in high concentrations in animal tissues, particularly in the brain, peripheral nerves and in glandular organs. Table 16 summarizes the concentrations of free and lipid-bound myo-inositol in

Fig. 7 : Biosynthesis and catabolism of myo-inositol.



1. Glucokinase
2. L-myoinositol 1-phosphate synthase
3. Ins1Pase
4. Myoinositol oxidase
5. CDP:diacylglycerol inositol phosphatidyl transferase
6. Myoinositol epimerase
7. Ins(1,4,5)P_3ase
8. Ins(1,4)P_2ase

TABLE 16 : MYO-INOSITOL AND SCYLLO-INOSITOL LEVELS IN DIFFERENT TISSUES OF THE RAT.

Tissue	Myo-inositol		Scyllo-inositol
	Free	Lipid-bound	
	nmoles/g wet wt		
Brain	6.62[e]	1.52[e]	0.16[a]
Cerebrum	4.90[d]	1.90[d]	-
Cerebellum	5.80[d]	-	-
Gray matter	6.35[b]	-	0.12[b]
White matter	7.77[b]	-	0.20[b]
Spinal cord	7.40[a]	-	0.18[a]
Sciatic nerve	3.10[e]	1.89[e]	0.25[b]
Kidney	4.51[e]	1.07[e]	0.45[a]
Thyroid	6.73[c]	-	-
Small intestine	2.20[d]	1.00[d]	-
Testis	2.85[f]	-	-
Spleen	1.22[c]	-	-
Lung	1.10[d]	-	-
Heart	0.55[d]	-	-

- not determined
(a) Sherman et al (1968)
(b) Stewart et al (1968)
(c) Dawson and Freinkel (1961)
(d) Burton et al (1976)
(e) Palmano et al (1977)
(f) Eisenberg and Bolden (1964)

different tissues of the rat. The levels of scyllo-inositol have also been given wherever detected. Free myo-inositol comprises a major portion of the total levels in tissues where both free and lipid-bound myo-inositol levels have been determined. Brain, spinal cord, kidney and thyroid contain high amounts of free myo-inositol while low amounts are present in the liver, lung and heart. Organs of the male reproductive tract are rich in free inositol, mammalian semen being one of the richest sources, with the concentration in seminal plasma being several fold higher than blood (Ghafoorunissa, 1976).

Free myo-inositol levels in gray matter are not appreciably different from those in white matter (Stewart et al., 1968) although PolyPI concentrations differ considerably (Hauser et al., 1971a). However, marked differences exist in the concentrations of free myo-inositol in different types of nerve cells. Stewart et al (1969) made comparisons of myo-inositol levels in layers of cerebellar and cerebral cortex and showed that most of the brain inositol (free) is contained in glial cells. Concentrations of myo-inositol (mmol/kg dry wt) in molecular, granular and medullary layers of the rat in cerebellum were found to be 28.2, 14.1 and 17.6 respectively (Allison et al., 1980). Sherman et al (1977) measured the levels of myo-inositol in single cells from defined areas of the nervous system using ion monitoring techniques. Neuronal inositol levels in Deiter's nucleus do not differ significantly from levels in the

surrounding neuropil while those in the anterior horn of the spinal cord are significantly greater than in the surrounding neuropil. Inositol levels in the rat hypothalamic medial preoptic nucleus are more than twice those in the lateral preoptic nucleus. Kusama and Stewart (1970) have shown that axons contain lower concentrations of myo-inositol than Schwann cells. These variations may reflect some functional differences which remain obscure.

Recently Hawthorne et al (1983) have reported the levels of free inositol in normal human brain to decrease steadily from 6.5 μmol/g wet wt at 20 years of age to 2.7 μmol/g wet wt at age 93. Lipid bound inositol fell from 2.1 μmol/g wet wt at 20 years to 0.9 μmol/g wet wt at age 90. Two similar brain samples from patients which Alzheimer's disease had somewhat reduced lipid-bound inositol but greatly increased free inositol when compared with age-matched controls. It is too soon however, to conclude that these results are typical of Alzheimer brains.

Free myo-inositol levels in brain tissue are considerably higher than in cerebrospinal fluid (CSF), which are, in turn, higher than in plasma (Nixon, 1959; Sherman et al., 1968; Lewin et al., 1973; Spector and Lorenzo, 1975a; 1975b). The large concentration differential between brain, CSF and blood led earlier investigators to postulate that myo-inositol is

actively transported from blood to brain although the mammalian brain is able to synthesize at least some of its own myo-inositol (Hauser and Finelli, 1963) and therefore should not be entirely dependent on entry of myo-inositol from blood.

The amount of myo-inositol in the CNS derived from synthesis in situ versus transport from blood varies among different species. In rabbit brain approximately 50% of the unbound myo-inositol is synthesized from glucose and 50% is transported from blood (Spector and Lorenzo, 1975a). The transport from blood is regulated by a saturable transport system, the locus of which is probably the choroid plexus (Spector and Lorenzo, 1975b). The rate of entry of myo-inositol from blood into CNS in the rat is very low indicating that a major portion originates in the brain (Lewin et al., 1976; Barkai, 1981). Margolis et al (1971) working with barbiturate-anesthetized dogs presented results indicating that there is no significant transport of myo-inositol from blood to brain. The reason for the apparent species variation is not understood.

Rat synaptosomes maintain a high concentration of inositol relative to that in plasma or CSF although synaptosomal synthesis of inositol from glucose is low (Warfield and Segal, 1978). Warfield et al (1978) showed that a saturable transport system for inositol in rat brain synaptosomes does not exist and the mechanism by which synaptosomes maintain their high concentration

of inositol relative to CSF is not clear. The low capacity, saturable inositol uptake system observed in rabbit brain slices may reflect a species difference or uptake by elements of the slice other than neuronal membranes (Spector, 1976b). In contrast to myo-inositol, scyllo-inositol in brain comes mainly from blood (Spector, 1976a; 1978) and is not synthesized from glucose via myo-inositol (Sherman et al., 1968; Hipps and Sherman, 1976).

In view of the lack of a known function for scyllo-inositol in mammalian tissues, it is possible that scyllo-inositol in brain is an example of a substance that enters brain predominantly from blood because it fortuitously has a high affinity for the transport system that transports myo-inositol.

Like the brain, rat kidney is also endowed with the ability to synthesize myo-inositol (Hauser and Finelli, 1963). Kidney represents a special situation, since it is the organ responsible for conservation of inositol through reabsorption from the glomerular filtrate, presumably by the proximal tubules. Urinary elimination of inositol, is found to be only 2% of the filtered load (Perles et al., 1960). Helleu (1957) showed that scyllo-inositol is excreted in the urine by humans following an oral administration of myo-inositol and he suggested that this excretion occurred by the action of a dehydrogenase followed by reduction to scyllo-inositol. Active

transport systems for inositol have been clearly delineated in rat kidney cortex slices (Hauser, 1969a; 1969b) and the system has been well defined in isolated brush border membranes of rat kidney (Takenawa et al., 1977). The transport is an energy and Na^+-dependent, stereospecific uphill process which can be inhibited by analogues of myo-inositol, scyllo-inositol and inosose-2.

The significance of high concentration of free myo-inositol in most mammalian tissues is unknown. Several studies indicate that myo-inositol is essential for normal growth in cell cultures (Eagle et al., 1957), for protection of microbial cells from dehydration (Webb, 1963), for maintaining structure of cell membranes as a constituent of inositol lipids (Hawthorne, 1964), in controlling the rates of K^+ influx and Na^+ efflux across membranes (Charalampous, 1971), as a lipotropic agent (Gavin and McHenry, 1941; Hegsted et al., 1973; Hayashi et al., 1974a) and as a therapeutic agent in the treatment of diabetic neuropathy (Clements, 1973; Greene et al., 1975; Salway et al., 1978).

Simmons et al (1982) studied the role of myo-inositol in the regulation of nerve metabolism. In endoneurium preparations from rabbit tibial nerve approximately 25% of the resting energy utilization was inhibited by a medium containing defatted albumin and selectively restored by arachidonic acid but unaffected by indomethacin or nordihydroguaiaretic acid. The

same component of energy utilization was inhibited by small decreases in endoneurial myo-inositol, which decreases incorporation of (^{14}C)-labelled arachidonic acid into PtdIns. Thus, normal myo-inositol concentration in nerve endoneurium appears to be required to prevent lowering of PtdIns synthetase activity at sites of rapid PtdIns turnover involved in metabolic regulation.

The importance of free inositol in reproduction has been indicated by several workers. Since the epididymis maintains a higher concentration of this cyclitol than the testis, Eisenberg and Bolden (1964) proposed that inositol may play a role in the maturaion of spermatozoa suggesting the involvement of inositol in spermatogenesis. Robinson and Fritz (1979) have suggested that inositol may be one of the components synthesized by Sertoli cells which are important in establishing the unique microenvironment in the seminiferous tubule required for germinal cell development.

The relatively high intracellular levels of free inositol in nervous and secretory tissues, enriched in microtubules, may reflect a role for this cyclitol in controlling the functional states of microtubules. Kirazov and Lagnado (1977) observed a preferential binding of inositol to assembly-competent tubulin oligomers and a protection by inositol of microtubules against cold and Ca^{2+} induced depolymerization. Thus, inositol

served to stabilize both microtubules and the intermediate aggregate species of tubulin with which they are in dynamic equilibrium.

The requirement for myo-inositol is remarkably specific and no inositol isomer or cogener has proved capable of substituting for it. Thus, any role proposed must tell as what the vitamin function of inositol is in chemical terms and also reveal why only the 5 equatorial/1 axial configuration, present in just one of the nine possible isomers of hexahydroxy cyclohexane is singularly suited for this purpose.

INOSITOL PHOSPHATES

In addition to the existence of free inositol, PtdIns, PtdIns4P and PtdIns(4,5)P_2, mammalian tissues contain low concentrations of the metabolites of these compounds such as inositol-1-phosphate - Ins1P, inositol 1:2-cyclic phosphate - Ins1,2cyclicP, inositol bisphosphate - Ins(1,4)P_2 and inositol trisphosphate - Ins(1,4,5)P_3 (Dawson et al., 1971; Koch and Diringer, 1974; Griffin and Hawthorne, 1978). Inositol pentaphosphate has long been recognized as a predominant organic phosphate in erythrocytes of most avian species (Johnson and Tate, 1969) with, the exception of the adult ostrich which contains inositol tetraphosphate (Issacks et al., 1977). Hubscher and Hawthorne (1957) have detected Ins1P in several tissues although the levels have been quantitated only in the

ox liver (40-50 moles/kg wet wt.). However, no direct evidence exists to show the presence of Ins(1,4,5)P_3 and Ins(1,4)P_2 as free compounds in animal tissues.

Ins1P can be synthesized from glucose which leads to the formation of the L-isomer of this compound. Alternatively it can also be derived by the phosphodiesteratic cleavage of PtdIns which forms the D-isomer.

Glucose ----> Glu-6-PO_4 ----------> L-Ins1-P

PtdIns -----> DG + Ins1,2cyclic-P -----> D-Ins1-P

Ins(1,4,5)P_3 and Ins(1,4)P_2 are derived by the phosphodiesteratic cleavage of PtdIns(4,5)P_2 and PtdIns4P respectively.

PtdIns(4,5)P_2 + H_2O ---------> DG + Ins(1,4,5)P_3

PtdIns4P + H_2O ----------> DG + Ins(1,4)P_2

Inositol kinases being absent in animal tissues the only source of inositol phosphates appears to be the breakdown products of their lipid-bound forms (i.e. phosphoinositides). As mentioned earlier rapid increases in the levels of Ins(1,4,5)P_3 and Ins(1,4)P_2 have been demonstrated in guinea-pig synaptosomes, rabbit iris smooth-muscle, hepatocytes, nerve-ending membranes, and in blow-fly salivary gland in response to a variety of stimuli (Table 2). Their levels however have not been quantitated in these studies.

The water soluble inositol phosphates are degraded by inositol phosphatases into free myo-inositol and inorganic phosphate :

$$Ins(1,4,5)P_3 \longrightarrow Ins(1,4)P_2 + P_i$$

$$Ins(1,4)P_2 \longrightarrow Ins1\text{-}P + P_i$$

$$Ins1\text{-}P \longrightarrow \text{myo-inositol} + P_i$$

The dephosphorylation of Ins1P has been studied in several tissues (Table 10) as an intermediate in inositol biosynthesis. The enzyme has been purified from rat brain and its kinetic properties determined (Hallcher and Sherman, 1980). It does not seem to differentiate between the L and D isomers of Ins1P at least in the case of the bovine brain. The dephosphorylation of the higher phosphorylated derivatives --$Ins(1,4,5)P_3$ and $Ins(1,4)P_2$ have been detected in vitro. Dawson and Thompson (1964) showed that $PtdIns(4,5)P_2$ phosphomonoesterase was active against $Ins(1,4,5)P_3$. Lapetina et al (1975) showed that rat kidney fractions actively degrade $Ins(1,4,5)P_3$ and $Ins(1,4)P_2$. Indirect evidence for the presence of these phosphatases comes from the studies of Griffin and Hawthorne (1978) in guinea-pig synaptosomes.

Apart from the phosphatases mentioned above, the other possible enzymes capable of dephosphorylating inositol phosphates include phytase, acid phosphatase and alkaline

phosphatase. Studies are in progress in this laboratory to see if a specific enzyme system responsible for the dephosphorylation of inositol phosphates exists in brain and if so, is it different from phytase and alkaline phosphatase. Preliminary observations on the crude brain extract indicated that the inositol phosphatase hydrolysing Ins1P is different from the one hydrolysing Ins(1,4)P_2 and Ins(1,4,5)P_3. These phosphatases were shown to be higher in gray than white matter suggesting that they may be more concerned with synaptosomal function as suggested by Griffin and Hawthorne (1978). The activity in whole brain increased with age reaching a maximum at 3 weeks and then either remained constant or decreased slightly. Ins1Pase activity was present mainly in the soluble fraction of gray matter but distributed equally between the soluble and particulate fractions of white matter. Ins(1,4,5)P_3ase and Ins(1,4)P_2ase were however concentrated in the particulate fraction of gray and white matter in rat brain Table 12 (Anjali et al., unpublished). Among the different regions studied activity of Ins1-Pase followed the order - hypothalamus > cerebellum > cerebral cortex > brain stem and that of Ins(1,4)P_2ase + Ins(1,4,5)P_3ase hypothalamus > cerebral cortex > brain stem > cerebellum. Catecholamine turnover being highest in the hypothalamus (Holzbawn and Sharman, 1972), results indicate that these enzymes may have a role to play in NE stimulated PtdIns turnover observed in the brain (Merlyn et al., unpublished). The enzymes are currently being purified and their kinetic

properties are being studied (Rao and Ramakrishnan, unpublished).

A reduction in myo-inositol levels, elevation in the levels of Ins1P and inhibition of Ins1-Pase have been observed in the brains of lithium-treated rats (Allison, 1978; Sherman et al., 1981). This effect is more confined to the layers of the cerebral cortex while the cerebellum is totally unreactive (Allison et al., 1980). Accumulation of Ins1P by lithium inhibition has been a useful tool in the development of a sensitive assay for receptor-stimulated phospholipase-C activity and the detection in brain slice preparations of large responses to a variety of neurotransmitters (Downes, 1982).

Although the phosphorylated derivatives of inositol represent minor forms of this cyclitol in animal tissues, there is experimental evidence indicating that some of these compounds may have important cellular functions. It has been recognized from in vitro experiments that various organic phosphates, and particularly inositol hexaphosphate, can decrease the affinity of hemoglobin for oxygen by forming a very tight complex with deoxyhemoglobin (Edalji et al., 1976). Inositol pentaphosphate is effective in causing a shift of the oxygen equilibrium curve of fetal and adult type duck hemoglobins (Borgese and Nagel,1977). It has been suggested that Ins1,2cyclic-P which is derived from the hydrolysis of PtdIns,

may play a role as an intracellular "secondary messenger" (Hawthorne and Pickard, 1979). Recent studies however, indicate that Ins(1,4,5)P_3 released by the phosphodiesteratic cleavage might be the long sought after second messenger that functions to mobolize and thus regulate the intracellular level of Ca^{2+} ions. An important experimental result obtained by Streb et al. (1983) showed that exogenous Ins(1,4,5)P_3 released Ca^{2+} from exocrine pancreatic cells that had been manipulated to make them leaky to small molecules. Since this initial report, a number of publications have appeared showing that Ins(1,4,5)P_3 can cause Ca^{2+} release from wide variety of cell types. Ca^{2+} release induced by Ins(1,4,5)P_3 is rapid, being detectable within 2 sec and complete within 15-60 sec. This is certainly fast enough to account for the increase in $(Ca^{2+})_i$ observed after receptor occupation. Secondly, the response is obtained with low concentrations of Ins(1,4,5)P_3 ($< 1\ \mu M$) and is specific for this compound; other derivatives such as inositol 1-phosphate or inositol 1,4-bisphosphate are ineffective.

Another aspect under investigation is the intracellular origin of Ca^{2+} released by Ins(1,4,5)P_3. Experimentally,

this has been approached by including in the incubation medium, inhibitors, which selectively block uptake of Ca^{2+} into the mitochondria or the endoplasmic reticulum (ER) of the permeabilized cells. The results obtained in the different cell types are again in general agreement and show that Ins(1,4,5)P_3 releases Ca^{2+} from the ER but not the mitochondria. Ca^{2+} sequestered within the ER is released by Ins(1,4,5)P_3 even when vanadate is added to inhibit Ca^{2+}-ATPase, the enzyme which serves to transport Ca^{2+} into the lumen of ER (Prentki et al., 1984). This suggests that Ca^{2+} uptake and Ins(1,4,5)P_3 -induced Ca^{2+} release occur by separate mechanisms.

A particularly interesting system, which may also involve Ins(1,4,5)P_3 as an intracellular mediator, is visual transduction. In this case, the absorption of light by molecules of rhodopsin embedded in specialized membrane regions of the photoreceptor cell produces (through an unknown sequence of events) a change in the plasma membrane potential. Since a single photon can change the conductance of several hundred ion channels in the plasma membrane, it has been assumed that diffusible messenger molecules must be involved in visual excitation and subsequent adaptation. Recently two groups of researchers, working on the photoreceptor of the horse-shoe

crab have found that microinjection of Ins(1,4,5)P_3 into the cell, in the dark, produces changes in the plasma membrane potential identical to those produced by a flash of light (Brown et al., in press; Fein et al., in press), Steady illumination causes a fall in PtdIns(4,5)P_2 and increases the Ins(1,4,5)P_3 content of the horse-shoe crab eye. It remains to be determined whether Ins(1,4,5)P_3 exerts its effect within the photoreceptor by mobilizing Ca^{2+} or through some other mechanism. The conclusion reached by both groups of workers is that Ins(1,4,5)P_3 may be diffusible messenger involved in the complex cascade of reactions that underly visual excitation.

The ever-growing list of cell types found to be responsive to Ins(1,4,5)P_3 suggests that this molecule has fundamental importance in how cells respond to Ca^{2+} -mobilizing stimuli. Perhaps other metabolites produced when inositol-lipid breakdown is stimulated, have regulatory roles that remain to be identified. Of particular interest is 1,2-diacylglycerol which activates a specific protein kinase. The picture which is emerging, at least in some cell types, is that stimulation of the phospholipid-dependent protein kinase and mobilization of Ca^{2+} are both essential for full cellular response (Berridge, 1984; Rasmussen and Barrett, 1984).

NUTRITIONAL INSUFFICIENCY-EFFECT ON BRAIN STRUCTURE AND LIPIDS

Human starvation typically results from a lack of all major food sources. Often the diet is essentially adequate in composition, but greatly insufficient in quantity. These conditions lead to starvation from undernourishment. Controlled laboratory models aimed at mimicking this human condition typically involve (1) depriving pregnant and lactating dams of normal amounts of food or protein, resulting in the subnormal production of milk or (2) limiting the offspring's access to milk by separating pups from the mother for about 2 hr on the second postnatal day and increasing to a maximum of 12 hr by the second week and (3) increasing the litter size thereby reducing the quantity of milk available. Each model has certain advantages and disadvantages in comparison with others (Wiggins, 1979; Crnic and Chase, 1980). In particular, no single model can control for all possible variables of environment that might conceivably contribute to the experimental result.

Nutritional stress induced during different stages of development appears to have differential effects on the proliferation of neuronal and glial cells and the maturation of neurons, glia, synapse and myelin. Furthermore different regions of the brain exhibit differential sensitivity to several parameters measured. Table 17 summarizes the effects of undernutrition during the pre-natal, neonatal and post-weaning stages of development on the whole brain, brain regions, cell types and associated structures.

Significant deficits have been reported in the body, brain and cord weights of newborn pups undernourished during the pre-natal period. The concentrations of DNA, RNA and protein in the brain were not affected (Zamenhof et al., 1968; Envonwu and Glover, 1973) though the content of DNA was lower signifying a reduction in the number of neuronal cells with no change in cell size. Specific cell types may also be affected as Shrader and Zeman (1969) found deficits in the number of multipolar neurons in the brain and spinal cord. The concentration of lipids is not affected either in the brain (Rajalakshmi and Nakhasi, 1974a; Karlsson and Svennerholm, 1978) or in the spinal cord (Sharma, 1979) - Table 17. Pre-natal undernutrition does not seem to have any lasting effect in terms of biochemical maturation if adequate nutrition is provided during the neonatal and post-weaning periods (Zamenhof et al., 1973).

In the rat, rapid phase of maturation takes place during the neonatal period and undernutrition during this period has been found to have adverse effects (Table 17). The DNA content is decreased (Winick and Noble, 1966) although the concentrations of DNA, RNA and protein are unaffected. The ratio of RNA to DNA and protein to DNA is decreased suggesting a decrease in cell size (Gambetti et al., 1972). Since neurons and glioblasts appear largely during early fetal development neonatal undernutrition has been shown not to affect the generation of brain cells. The maturation of cell types appears to be more

vulnerable especially in late maturing structures. Subnormal dendritic arborization (Cragg, 1972; Cordero et al., 1976; Pysh et al., 1979; Hammer, 1981) and synaptic development (Gambetti et al., 1974; Shoemaker and Bloom, 1977; Hillman and Chen, 1981b) have been reported. Variable reductions in the number of granule cells (11-37%) have been reported in the cerebellum (Clos et al., 1977; McConnell and Berry, 1978; Hillman and Chen, 1981a). Purkinje cell number has been shown to be only mildly reduced (West and Kemper, 1976). Hillman and Chen (1981a) reported that the ratio of granule cells to Purkinje cells was selectively altered. This led to alterations in the synaptic relationships with an increase in the size of synaptic contact areas accompanied by reduction in the total number of synapses on purkinje cells (Hillman and Chen, 1981b).

The effect of undernutrition on glial maturation has not been extensively investigated. In early studies the proliferation of glial cells as also their maturation was shown to be affected. For instance, Siassi and Siassi (1973) reported a reduction in the non-neuronal cell count in cerebral cortex and Bass et al (1970) showed a failure of glial cells to migrate to the appropriate layers in the cerebral cortex. In the cerebellar molecular layer glial cell processes were found to be reduced (Clos et al., 1973). The more recent studies have revealed relatively moderate effects on the number of oligodendroglial cells in most areas of the brain (Sikes et al.,

1981). In fact the density of glia has been shown to be increased in several brain regions (Sturrock et al., 1976;1977; Sikes et al., 1981) which probably results from a reduction in neuron dendrite branching (McConnell and Berry, 1978; Pysh et al., 1979) or a decrease in average cell size.
As exceptions to this trend, the proportion of oligodendroglia in the corpus callosum and subcortical white matter is found to be reduced in rats nutritionally deprived during the neonatal period (Robain and Ponsot, 1978; Lai et al., 1980; Sikes et al., 1981). Robain and Ponsot (1978) reported the density of glial cells to be reduced by 50% in the corpus callosum of neonatally undernourished rats. The majority of cells in this structure still had a glioblastic appearance, while in normal rats the majority of glial cells were oligodendrocytes. The relative vulnerability of oligodendroglia in the corpus callosum may be related to the late maturation of this structure.

Not all membranes are affected to the same degree by postnatal nutritional deprivation. Myelin is known to be severely impaired while nerve endings and other membranes are spared to a great extent. One of the important effects on myelin development appears to be a failure to initiate myelination of a substantial number of nerve fibers, resulting in partial amyelination of late maturing white matter tracts. In corpus callosum and pyramidal tract, for example, the proportion of myelinated fibers in a constant area of tissue

does not increase as rapidly as in normal rats (Krigman and Hogan, 1976; Lai and Lewis, 1980; Delaney et al., 1981). Robain and Ponsot (1978) compared the number of myelinated fibers in photomicrographs of 3 spinal cord tracts of rats from 5 to 25 days of age. The number of myelinated fibers was essentially unaffected in the fasciculus cuneatus, which is relatively mature by the end of the first postnatal week. In the fasciculus gracilis and fasciculus corticospinalis, which mature later during the second and third postnatal weeks, the number of myelinated fibers ranged from only 10-50% of normal.

In contrast, the effects of postnatal nutritional deprivation on myelin ultrastructure are remarkably slight. Krigman and Hogan (1976) observed that on average the number of myelin lamellae/axon was reduced largely as a result of an increase in the proportion of promyelinating fibers (having only 1-2 lamellae) in 30-day old undernourished rats. A frequency distribution analysis showing the proportion of axons having specific numbers of lamellae reveals that there is a small shift toward more fibers having comparatively low numbers of lamellae in brain, spinal cord and sciatic nerve of undernourished rats (Lai and Lewis, 1980; Delaney et al., 1981).

The weight of freeze dried myelin has been shown to be reduced in the brains of postnatally undernourished rats (Fishman et al., 1971; Wiggins et al., 1976; Reddy et al., 1979; Harjit and Rajalakshmi, unpublished observations). The

reduction in myelin yield could result from a delay in the on-set of myelination, a decrease in the synthesis or an increase in turnover.

Comparisons of myelin synthesis in postnatally undernourished and control rats have been made by comparing the relative rate of isotope utilization during membrane assembly of myelin proteins and lipids (Wiggins, 1979; Wiggins and Fuller, 1979; Wiggins et al., 1976; 1979). Results show that the synthesis of myelin fraction is greatly depressed as compared with the crude nuclear, synaptosomal, mitochondrial and microsomal fractions. Possible regional differences in the effect of postnatal undernourishment on myelin synthesis have been examined in 7 different brain regions viz., cerebral cortex, cerebellum, midbrain, hypothalamus, hippocampus, striatum, and medulla oblongata (Wiggins and Fuller,1979). Results indicate that myelin synthesis is depressed to about the same extent throughout the brain.

The sensitivity of different regions of the brain to nutritional atress varies greatly. Undernutrition curtailed the increase in cell numbeer by 6 days of age in the cerebellum, beyond 14 days in the cerebrum and hippocampus while the brain stem remained unaffected (Fish and Winick, 1969). Among the different brain regions cerebellum has been studied extensively with respect to undernutrition. As mentioned earlier, neuronal cell number is reduced mainly in the cerebellum while reduced

proportions of oligodendroglia are observed in the corpus callosum and subcortical white matter. Myelin synthesis is depressed to the same extent in most brain regions examined. A differential sensitivity has been shown with regard to lipid levels in different regions. Ghittoni and DeRaveglia (1972) reported deficits in the concentrations of cerebrosides, cholesterol, inositol phospholipids, sphingomyelin and total gangliosides in neonatally undernourished rat brain cerebral and cerebellar cortex. However, the pattern of individual gangliosides was affected only in the cerebellum. Martinez (1982) reported the levels of plasmalogens and galactolipids to be more affected in the cerebrum than in the cerebellum of malnourished children. The reasons for the different effects on rat and human brains remain unclear.

The several changes observed in the morphology of brain cells and related structures as described above are associated with changes in the concentration of lipids which constitute the important structural and functional components of neuronal, glial and myelin membranes. Table 18 gives the comparative data of the effects of undernutrition during the neonatal period on the lipid composition of rat whole brain, gray matter, white matter, brain myelin, spinal cord and cord myelin.

Undernutrition during the neonatal period is known to significantly reduce the concentrations of cholesterol, galactolipids, phospholipids and gangliosides in rat whole brain

(Dobbing,1964; Culley and Lineberger, 1968; Geison and Waisman, 1970; Ghittoni and deRaveglia, 1972; Rajalakshmi and Nakhasi, 1974a; Krigman and Hogan,1976; Reddy and Sastry, 1978). Rao (1979) reported altered fatty acid profiles of cerebrosides and phospholipids in neonatally undernourished rat brains. Among the polyunsaturated fatty acids of phospholipids the contribution of arachidonic acid (20:4) to the total fatty acids was lower in 10 and 21 day old rats. Altered fatty acid pattern might be the result of impaired myelination of brain due to nutritional stress.

Neonatal undernutrition decreased the content of gray and white matter, the effects being more severe in the latter. The lipid composition of gray matter was relatively unaffected, a deficit being found only with regard to galactolipids and gangliosides while significant deficits were found in the levels of cholesterol, galactolipids and phospholipids such as ethanolamine and choline plasmalogens (Reddy et al., 1982) - Table 18.

The principal effects of postnatal undernourishment on the lipid composition of brain myelin appear as a delay in the rate of its maturation. The overall rate of myelin lipid formation measured about 13% of normal at 18 days, 40% of normal at 20 days, and 55% of normal at 26 days in undernourished rats (Wiggins et al., 1976). Fishman et al (1971) first observed that the myelin lipid composition was slightly altered as a

result of postnatal undernourishment, and Simons and Johnston (1976) noted that the myelin lipid composition of young protein malnourished rats appeared immature and that certain long-term maturational changes characteristic of normal myelin lipids failed to occur. The composition of cerebroside fatty acids was found to be increased in C24h:1 and C24:1. Protein undernutrition of lactating rats caused comparatively large changes in the molar ratio of lipid classes, resulting in increased proportions of cholesterol and phospholipid relative to galactolipid (Nakhasi et al., 1975; Simons and Johnston, 1976; Yusuf et al., 1981; Fuller et al., 1984). The plasmalogen content of brain myelin (Simons and Johnston, 1976) is reduced and the compositions of phospholipids and gangliosides (Yusuf et al., 1981) are altered. As regards other membranes studies are few and scattered. Pasquini et al (1981) reported decreases in cholesterol levels in the mitochondrial membrane and galactolipids in the microsomal membrane of neonatally undernourished rats. Total gangliosides were decreased in myelin and nerve-endings to values around 50% of normal controls. Total phospholipids were normal in microsomal and nerve ending membranes. However, mitochondria exhibited significant reductions in Sph PtdCho and PtdEtn. Hitzemann (1981) has shown the composition of acyl-linked fatty acids in phospholipids of rat cortical synaptic membranes to be markedly affected. The functional significance of these changes remain unknown.

In contrast to the effects of neonatal undernutrition, post-weaning undernutrition and/or protein deficiency affects growth but not the concentration of lipids in the whole brain (Dobbing and Widdowson, 1965; Rajalakshmi et al., 1974b), brain regions (Dickerson et al., 1972; Rajalakshmi and Nakhasi, 1974b) or the spinal cord (Dickerson and Walmsley, 1967). Recently Vrbaski (1983) have reported that protein deficiency if continued for very long periods (60-240 days) lipid levels are altered in the rat brain. In this study while the total ganglioside content remained unaffected, the composition of ganglioside fractions was altered significantly. Cerebrosides with non-hydroxy fatty acids were also found to be lowered while the phospholipids remained unaffected with the exception of Sph. In sum, any stress appears to have most deleterious effects when induced during the rapid phase of development, which in the case of rat brain, occurs during the neonatal period and only small increments in lipids occur in the post-weaning period.

The potential for rehabilitation through nutritional therapy has been investigated extensively. With regard to brain lipids, however, the findings are conflicting and the reversibility perhaps depends on the size of the initial deficit as well as the extent of rehabilitation. In some studies deficits are reported to persist even after rehabilitation in the levels of brain cholesterol (Culley and Lineberger,

1968; Dobbing, 1968; Geison and Waisman, 1970; Dickerson and Jarvis, 1970; Smart et al., 1973; Rajalakshmi et al., 1974b; Reddy and Sastry, 1978), galactolipids (Culley and Lineberger, 1968; Geison and Waisman, 1970; Reddy and Sastry, 1978), gangliosides (Dickerson and Jarvis, 1970), phospholipids (Culley and Lineberger, 1968; Rajalakshmi et al., 1974b; Reddy and Sastry, 1978) and plasmalogens (Geison and Waisman, 1970; Reddy and Sastry, 1978). Some reports indicate a complete catch-up with respect to lipid composition in the whole brain. (Benton et al., 1966; Guthrie and Brown, 1968).

Nutritional rehabilitation during the post-weaning period abolished the effects on gray matter at weaning but the deficits in weight and lipid content of white matter persisted (Reddy et al;, 1982).

In studies on myelin synthesis in normal, undernourished and rehabilitated rats (Wiggins et al., 1976), results show that feeding ad libitum for 6 days following 3 weeks of post-natal undernourishment increased the rate of myelin protein and lipid synthesis over the rate in undernourished rats. The stimulated rate was intermediate between that of normal and undernourished rats at the same age. Thus, nutritional rehabilitation cannot fully restore the normal tissue concentration of myelin when the early deprivation is severe. Studies on myelin yield in young adult rats, following a period

of early undernourishment (Wiggins and Fuller, 1978; Reddy et al., 1979) directly confirm this prediction. Evidence is also available to show that nutritional rehabilitation does not restore the brain myelin lipids to their normal composition (Yusuf et al., 1981). The results indicate that hypomyelination resultant from early undernutrition persists in the adult animals.

Experiments with labelled (^{14}C)-glucose have shown decreased incorporation into brain cholesterol, phospholipids and galactolipids (Agrawal et al.,1971; Chase et al., 1976; Jaikhani and Subrahmanyan, 1977). Wiggins et al.(1976) reported the incorporation of (^{3}H) and (^{14}C)-acetate, choline and glycerol to be reduced by about 60% in lipids of isolated myelin from whole brain. This decrease was also observed in myelin isolated from different brain regions (Wiggins and Fuller, 1979). However, incorporation of labelled $^{32}P_i$ into phospholipids was found to be increased (Reddy and Sastry, 1978). PolyPI have not been investigated in any of the above studies. However, in quaking mutant mice characterized by myelination deficits, the ability to incorporate $^{32}P_i$ in vivo into PolyPI has been shown to be substantially reduced (Hauser et al., 1971b).

The effects of undernutrition on other organs of the animal body are relatively severe when compared to the brain

(Widdowson and McCance, 1960; Platt et al., 1964; Winick and Noble, 1966). To date the studies on tissues other than the brain with regard to lipid composition are scarce and mainly concerned with the post-weaning period (Gerson,1974; Krause and Beamer, 1974). Recent studies have shown that the phospholipid composition of several tissues like liver, spleen, kidney, lung, heart and testes are altered by protein deficiency during the neonatal period (Khanna and Reddy, 1983; Reddy and Khanna, 1983). Extensive studies have been carried out on the lipid composition of the intestine under conditions of nutritional stress during the prenatal, neonatal and post-weaning stages of development (Arockiadoss, 1982). Levels of PtdIns(4,5)P_2 have also been reported to be diminished in the intestine of neonatally undernourished rats (Bhandari et al., 1981).

With special reference to kidney, the progeny of rats fed a low protein diet during pregnancy show definite morphological, histochemical and functional differences when compared with controls. Renal immaturity in the protein-deficient young is indicated by loss of cell cytoplasm in the proximal convulated tubules, loops of Henle and collecting tubules, increased quantities of mesenchymal-like connective tissue, fewer identifiable glomeruli and a large proportion of immature glomeruli. Glomerular filtration rate is severely depressed and excretion of urine is reduced during both water diuresis and osmotic diuresis, (Zeman, 1968; Hall and Zeman, 1968).

The effects of nutritional deprivation on human brain development are largely determined by inference, as the socio-economic conditions prevailing in locations where infant death from starvation is common sefve to minimize opportunities for significant neurochemical study. Very little is actually known about the neurochemical pathology of starved human infants. Fishman et al (1969) found that white matter concentrations of proteolipid protein, cerebroside and plasmalogens are reduced in brains of malnourished Puerto Rican infants compared with normal ones. Similarly Chase et al (1974) observed that the brain concentration of cerebroside and sulfatide were below normal in Guatemalan infants who had died of malnutrition. The lipid composition of isolated myelin was relatively normal (Fox et al., 1972), as in animal studies. Infant starvation caused a specific relative reduction in the synthesis and accumulation of myelin membrane, although myelin has not been quantitatively isolated. Furthermore, Kumar et al(1977) found that both marasmus and kwashiorkor contributed to a decrease in nerve conduction velocities in children. Martinez (1982) reported the levels of plasmalogens to be more affected in the cerebrum than in the cerebellum of malnourished children. Galactolipids, which are more myelin-specific, were only decreased in the cerebrum. This is in contrast to the data obtained on rats (Wiggins and Fuller, 1979) which show a myelin deficit quite similar in all brain regions. It may be suggested

TABLE 17 : EFFECTS OF NUTRITIONAL STRESS DURING DIFFERENT STAGES OF BRAIN DEVELOPMENT.

Effect observed	Reference
I. NUTRITIONAL STRESS DURING THE PRE-NATAL PERIOD	
WHOLE BRAIN :	
Smaller fetal brains associated with reduction in DNA content	Zamenhof et al (1968) Zeman and Stanbrough (1969)
Concentration of DNA, RNA, protein and lipids is not affected.	Zamenhof et al (1968) Envonwu and Glover (1973) Rajalakshmi and Nakhasi (1974a) Karlsson and Svennerholm (1978)
BRAIN CELLS :	
Reduced generation of neuronal cells	Zamenhof et al (1968)
Apparent loss of large multipolar neurons in brain and spinal cord at term.	Shrader and Zeman (1969)
BRAIN REGIONS :	
Reduced cell number and protein content of cerebellum. Cerebrum not affected when placental blood vessels ligated during the last third trimester of gestation in rhesus monkey.	Hill et al (1971)
Larger deficits in cerebellar DNA (20%) than in cerebral cortex (12%) when vascular supply intercepted during the third trimester in rabbits.	Van Marthens et al (1975)

contd...

TABLE : 17 contd.

Effect observed	Reference
II. NUTRITIONAL STRESS DURING THE NEONATAL PERIOD	
WHOLE BRAIN :	
Reduction in brain weight and DNA content. RNA and protein concentrations remain unaffected.	Winick and Noble (1966) Culley and Lineberger (1968) Subba Rao (1982)
Deficits in brain cholesterol, galactolipids, phospholipids and gangliosides.	Dobbing (1964) Culley and Lineberger (1968) Geison and Waisman (1970) Ghittoni and DeRaveglia (1972) Rajalakshmi and Nakhasi (1974a) Krigman and Hogan (1976) Reddy and Sastry (1978)
Deficits in the concentration of lipids in gray and white matter of brain.	Reddy and Horrocks (1982) Reddy et al (1982)
Altered fatty acid profiles of phospholipids and cerebrosides.	Fishman et al (1971) Rao (1979)
Lowered incorporation of several precursors into brain lipids	Agrawal et al (1971) Chase et al (1976) Jailkhani and Subrahmanyan (1977) Reddy and Sastry (1978)
NEURONS AND SYNAPSE :	
No nerve degeneration but neuronal cell density was increased and the dendritic development reduced.	Eayrs and Horn (1955)
Reduced dry weight and RNA content in spinal motor neurons.	Haltia (1970)

contd...

TABLE 17 : contd.

Effect observed	Reference
Subnormal dendritic arborization	Cragg (1972) Cordero et al (1976) Pysh et al (1979) Hammer (1981)
Subnormal synaptic development with reduction in the number of axonal terminals and diminished density of pre-synaptic endings.	Gambetti et al (1974) Shoemaker and Bloom (1977) Hillman and Chen (1981)
GLIA :	
Decrease in glial proliferation and migration in cerebrum	Bass et al (1970)
Fewer glial cell processes in cerebellar molecular layer	Clos et al (1973)
Increased density of glia in several brain regions	Sturrock et al (1976;1977) Sikes et al (1981)
Reduced proportion of oligodendroglia in rat corpus callosum and subcortical white matter	Robain and Ponsot (1978) Lai et al (1980) Sikes et al (1981)
Retardation in the differentiation of glioblasts to mature oligodendroglia in corpus callosum.	Robain and Ponsot (1978)
Lowered yield of myelin in preparations from the brains of undernourished animals	Fishman et al (1971) Wiggins et al (1976) Reddy et al (1979) Harjit and Rajalakshmi (unpublished)
Reduction in the proportion of myelinated nerve fibres in certain structures of brain and spinal cord	Krigman and Hogan (1976) Robain and Ponsot (1978) Lai and Lewis (1980) Delaney et al (1981)
Reduction in the specific activity of CNPase in brain myelin.	Nakhasi et al (1975) Reddy et al (1979)

contd...

TABLE 17 : contd.

Effect observed	Reference
No gross differences in the lipid composition of myelin from rat brain. However, the composition was altered with regard to cerebroside fatty acids, plasmalogens, type of gangliosides present. Proportions of cholesterol and phospholipid relative to galactolipid was increased.	Fishman et al (1971) Simons and Johnston (1976) Yusuf et al (1981) Fuller et al (1984)
Specific reduction in the incorporation of isotopes during membrane assembly of proteins and lipids.	Wiggins et al (1976;1979) Wiggins (1979) Wiggins and Fuller (1979)
Reduced proportions of basic and proteolipid protein in myelin.	Wiggins et al (1976) Reddy et al (1979)
CEREBRAL CORTEX :	
Reduced volume and greater cortical thickness.	Sugita (1918)
Reduced cell counts in cerebral cortex; predominantly non-neuronal cells.	Siassi and Siassi (1973)
Reduced mitotic rate	Patel et al (1973)
Increased density of neuronal cell bodies in visual and frontal cortex owing to retarded neuropil development	Cragg (1972)
Reduced number of spines, dendritic density and thickness in pyramidal cells of layer V in frontal and occipetal cortex.	Salas et al (1974)

contd...

TABLE 17 : contd.

Effect observed	Reference
Deficits in the concentration of cholesterol cerebrosides, PtdIns, Sph and total gangliosides.	Ghittoni and deRaveglia (1972)
CEREBELLUM :	
Reduced DNA content	Fish and Winick (1969)
Reduction in granule cell number (11-37%)	Clos et al (1977) McConnell and Berry (1978) Hillman and Chen (1981a)
Mild reduction in purkinje cell number	West and Kemper (1976)
Reduced cell number in germinal external granular layer and internal granular layer. Reduced number of stellate, basket, granule and purkinje cells.	Barnes and Altman (1973)
Retarded outgrowth of apical dendritic tree of cerebellar purkinje cells.	Sima and Persson (1975)
Decreased purkinje cell packing density in cerebellar vermis. 20% loss of dendritic field area of purkinje cells.	Pysh and Perkins (1975)
Deficits in the number of synapses in molecular layers of cerebellum.	Shoemaker and Bloom (1977)
Ratio of granule to purkinje cells selectively altered leading to alterations in synaptic relationships.	Hillman and Chen (1981a; 1981b)
Pattern of gangliosides altered.	Ghittoni and deRaveglia (1972).

contd...

TABLE 17 : contd.

Effect observed	Reference
III. NUTRITIONAL STRESS DURING THE POST-WEANING PERIOD	
WHOLE BRAIN :	
Reduction in brain weight	Rajalakshmi et al (1974a) Rajalakshmi and Nakhasi (1974b)
Lipid levels unaffected	Guthrie and Brown (1968) Rajalakshmi et al (1974b)
Reduced levels of ACh	Rajalakshmi et al (1974a)
Decrease in the activities of glutamate dehydrogenase and glutamate decarboxylase	Rajalakshmi et al (1974c)
BRAIN CELLS :	
Loss of chromatin from motor neurons	Platt et al (1964)
Lamellar whirls and aggregation of synaptic vesicles, believed to be a sign of degeneration in pre-synaptic elements.	Yu et al (1974)
Reduction in the number and span of dendritic basillar processes of layer V cortical pyramidal neurons.	Cordero et al (1976)
Glia cell number increased	Platt et al (1964)
BRAIN MYELIN :	
Concentration of myelin reduced by 20 % in the optic tract.	Buchanan and Roberts (1948)
Swollen myelin sheaths in pig spinal cord	Platt et al (1964)
Chemical composition of myelin unaffected.	Fishman et al (1971)

TABLE 18 : EFFECT OF PRE-WEANING UNDERNUTRITION ON THE LIPID COMPOSITION OF CENTRAL NERVOUS SYSTEM.

	Whole brain				Spinal cord	
	Whole tissue[a]	Gray matter[a]	White matter[a]	Myelin[b]	Whole tissue[b]	Myelin[b]
	% of control					
Wt. of the component	77*	82*	53*	81*	63*	61*
Total lipids	85*	96*	86*	100	-	-
Cholesterol	87*	101	90	100	61*	100
Galactolipid	82*	86*	80*	100	77	101
Phospholipid	87*	95	85*	100	84*	99
Gangliosides	-	92*	98	-	106	-
PtdEtn	85*	84	82*	100	86*	104
PtdCho	92	102	88	104	87	97
Sph	70	118	92	76*	92	97
PtdSer + PtdIns	88*	100	101	85	84	106

* Values significantly different from control.

(a) Reddy et al (1982).

(b) Harjit and Rajalakshmi (unpublished).

that the developmental differences between species are wider than is commonly thought, and thus also the response of the brain in each case to undernutrition. However, additional comparative data on cellular development of the human and rat brain are required to extrapolate the results on the postnatal vulnerability of the developing rat brain to potential risks in the human population.

ALTERATIONS UNDER STRESS-MYO-INOSITOL AND PHOSPHOINOSITIDES

The importance of myo-inositol and phosphoinositides from a nutritional viewpoint has been significantly underestimated. The National Research Council does not list myo-inositol as a dietary requirement for various laboratory animals including the rat, which indicates that it is not yet considered necessary for the optimal performance of biological functions (National Academy of Sciences, 1972). However, recent advances in myo-inositol research have resulted in a renewed consideration of its nutritional importance.

Many of the nutritional conditions used in early work to produce an inositol-dependent response in the rat have employed feeding a low protein diet free of B vitamins and fat before administration of the B vitamins with or without myo-inositol. Gavin and McHenry (1941) were the first to demonstrate that myo-inositol acts as a lipotropic agent reducing the amount of fat in the liver of rats fed under certain dietary conditions.

This observation has been confirmed in subsequent studies (Handler, 1946; Hasan et al., 1970). Hayashi et al (1974a) showed that a well balanced diet can also produce a significant increase in the level of hepatic triacylglycerols and cholesterol provided phthalylsulfathiazole is added to inhibit the growth of intestinal bacteria which are known to synthesize myo-inositol. The lipotrppic function of myo-inositol is dependent on the type of dietary fat used, saturated fats producing more lipodystrophy than unsaturated ones (Hegsted et al., 1973; 1974; Kroes et al., 1973; Anderson and Holub, 1976).

Burton and Wells (1976) demonstrated that under the stress of lactation, myo-inositol deficiency leads to the formation of fatty liver even when the diet is made up of unsaturated fat. Most of the tissues examined from rats fed the myo-inositol-deficient diet had lower free myo-inositol levels than the controls with the exception of cerebrum and cerebellum, while lipid-bound myo-inositol was normal with the exception of liver. Despite reduced free and lipid-bound myo-inositol in the liver, there was no evidence of fatty liver in the young rats at any age. However, the mothers developed a marked fatty liver in response to lactation, which was characterised by diminished PtdIns (50%) and total phospholipid (57%) levels when compared to controls. The increase in lipid was predominantly in the triglyceride fraction. A similar effect has also been noted in laying hens (Reed et al., 1968). Thus, the physiological

stress of transport of certain fats, lactation and of laying may greatly exceed the requirements of myo-inositol characteristic of the normal function of a tissue.

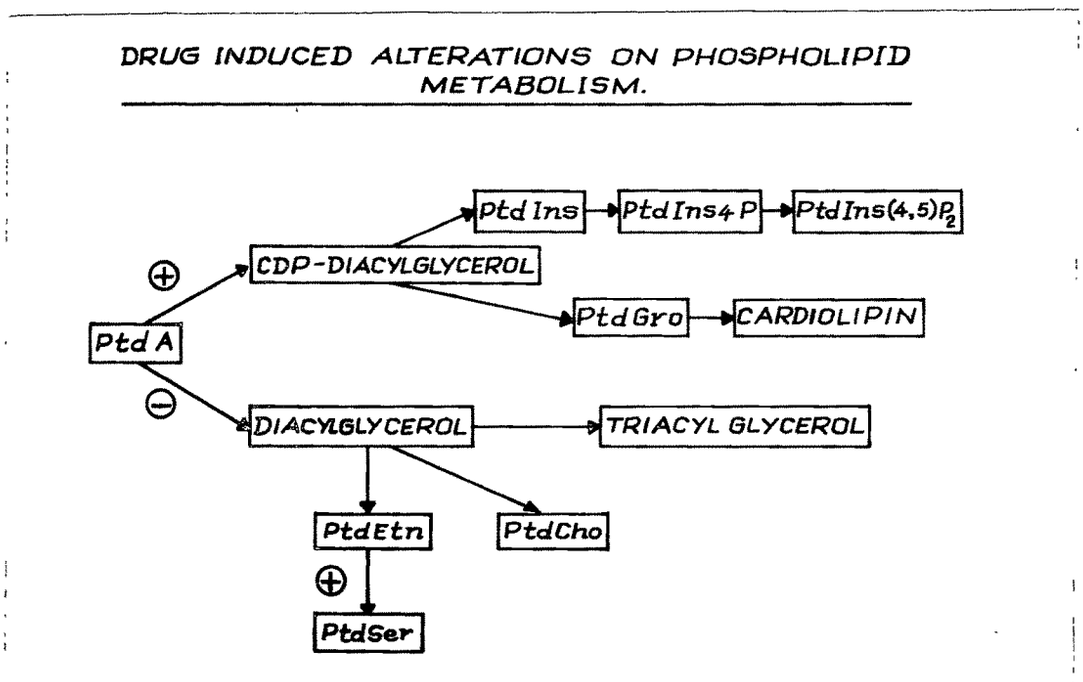
Experimental conditions that are nutritionally more acceptable and relevant than the previous ones have been devised (Anderson and Holub, 1980a) for studying myo-inositol deficiency in the young rat. The exclusion of myo-inositol from these diets that met or exceeded the NRC requirements for all nutrients, including choline, gave rise to mean hepatic triglyceride concentrations that were higher by 230% and 90% in males and females, respectively, as compared to corresponding rats fed control diets supplemented with 0.1% myo-inositol. A greater sensitivity of younger rats to dietary myo-inositol (Anderson and Holub, 1980b) was observed which may reflect a greater nutritional requirement or a lower capacity for endogenous biosynthesis and/or higher capacity for catabolism of this cyclitol. In this regard it is interesting to note that organs of the young rat appear to be better able to convert glucose to myo-inositol than those of the fully mature animal.

Anderson and Holub (1980b) have also studied the relative response of hepatic lipids in the rat to graded levels of dietary myo-inositol and other lipotropes. In these experiments, myo-inositol and choline were found to have an equivalent lipotropic potency when compared at a dietary level equal to the NRC requirement for choline. Fatty acid analyses have revealed

that the weight percentage of 18:2 is reduced in the phospholipid, and the relative abundance of 16:1 tends to be elevated in the triglyceride when inositol-deficient diets are fed. Hayashi et al (1974b) observed that an increased mobilisation of non-esterified fatty acids from adipose tissue to liver was a major cause of the hepatic accumulation of triacylglycerols in the myo-inositol deficient rat. Burton and Wells (1977) have observed a depression in the levels of total plasma lipoprotein lipid, very-low-density lipoprotein, high-density lipoprotein, total phospholipid, and plasma PtdIns in inositol-deprived dams during lactation, thereby suggesting a block in hepatic lipoprotein secretion. The exact mechanism of lipid accumulation in the liver or intestine of animals subjected to myo-inositol deficiency remains unknown.

A large variety of CADs, including chlorpromazine and other phenothiazine tranquilizers, morphine, nicotine, fenfluramine and its derivatives and propranolol markedly alter phosphoinositide metabolism in a wide variety of tissues. In general, these drugs interact with lipids, especially the acidic phospholipids, the tissue concentrations of which are markedly elevated by the drug treatment. As indicated in Fig.8 they bring about a redirection of phospholipid metabolism which consists of increased synthesis of acidic phospholipids (phosphoinositides and cardiolipin), and inhibition of neutral

Fig. 8 : Drug induced alterations on phosphoinositide metabolism.



(+) - Activation

(-) - Inhibition

phospholipids (PtdCho and PtdEtn) and glyceride formation (Abdel-Latif,1983).

The stimulation of phosphoinositide metabolism by CADs involves increased de novo synthesis, rather than merely enhanced turnover as in the receptor mediated "PtdIns Effect" described earlier. Thus CADs increase the incorporation of both $^{32}P_i$ and (^{14}C)-glycerol into phosphoinositides while an increased incorporation of $^{32}P_i$ and ^{3}H inositol but not of ^{14}C-glycerol is observed in the latter phenomenon. Further, the phospholipid class affected appears to depend on the metabolic capabilities of the individual tissues. In cerebral cortex mince preparations, the most prominent changes observed with propranolol were an increase in the incorporation of $^{32}P_i$ into PtdA and PolyPI and a decrease in PtdCho (Pappu and Hauser, 1981). These authors also showed similar stimulation in minces from other brain areas (brain stem, hypothalamus), but no effects in peripheral tissues (kidney, liver and lung), where a much smaller percentage of the incorporated radioactivity appeared in PolyPI. The bulk of the incorporation of $^{32}P_i$ in rat kidney cortex mince was found in PtdCho and propranolol addition resulted in increased labeling into PtdIns while the radioactivity in PtdCho was reduced by one-thirds. In liver mince, on the other hand, the two neutral phospholipids, PtdCho and PtdEtn, were labelled equally and the decrease in both brought about by propranolol was substantial. A fourfold

increase in PtdA labeling was also reported in this tissue. Eichberg et al (1978) showed that in rat pineal glands, propranolol and several tertiary amine local anesthetics enhance the incorporation of $^{32}P_i$ into PtdIns and PtdGro, causing accumulation of phosphatidyl-CMP and depress the labeling in PtdCho and triacylglycerol. The reason for these differences remain unknown.

The alterations in phospholipid metabolism may be brought about through interaction of CAD's with one or more pertinent enzymes or their substrates. In particular, cytosolic phosphatidate phosphohydrolase has been implicated and found to be inhibited by CADs (Ide and Nakazawa, 1980), especially propranolol (Pappu and Hauser, 1981). This could, to a large extent, account for the lowered neutral phospholipid synthesis through reduced DG availability. In addition to phosphatidate phosphohydrolase, inhibition of other phospholipases has been considered as possibly participating in the mechanism of CAD action, particularly in lysosomes (Matsuzawa and Hostetler, 1980; Hostetler and Matsuzawa, 1981).

When lysosomal phospholipases from liver were tested against PtdCho for inhibition by propranolol, the concentration which resulted in 50% inhibition was 0.25mM for phospholipase-A and 0.38mM for phospholipase-C (Hostetler and Matsuzawa, 1981). Other CADs also exerted this effect at similar concentrations.

Cytidylyl transferase activity has been claimed to be stimulated in the liver (Sturton and Brindley, 1977). Abdel-Latif et al., (unpublished) have observed that propranolol and mepacrine exert multiple effects on the enzymes in iris muscle, by stimulating the activities of phosphatidate phosphohydrolases, diacylglycerol kinase, PtdIns kinase and PtdIns4P kinase and inhibiting the activity of cholinephosphotransferase. Whether CADs exert a direct effect on the enzyme or complex with its substrate, thus preventing enzyme access is uncertain. The competition of CADs with membrane-bound Ca^{2+} may increase the intracellular Ca^{2+} concentration and be a critical feature of the observed effect. The relative importance of the isolated effects of CADs - inhibition or stimulation of enzyme activities, complex formation with lipid substrates, alteration of Ca^{2+} pools, changes in membrane permeability - in bringing about the observed changes in lipid metabolism and their clinical implications, all remain to be investigated. A detailed study could provide an understanding of the origin of the side effects seen with certain clinically prevalent CADs and thereby lead to means for their prevention.

There has recently been a great surge of interest in the relationship of inositol and phosphoinositides to various diseases and associated abnormalities. Altered metabolism has been documented in patients with diabetes mellitus, chronic renal failure, galactosemia, hereditary ataxia and multiple sclerosis. The following is a brief survey of literature on

each of these diseased states as related to the metabolism of inositol and phosphoinositides.

Changes in the metabolism of myo-inositol, phosphoinositides and other phospholipids have been demonstrated in peripheral nerves in experimental diabetes induced in rats by intraperitoneal injection of streptozotocin. A reduction in the inositol content of sciatic nerve in rats with streptozotocin induced diabetes was first observed by Greene et al (1975) and has since been amply confirmed (Palmano et al., 1977; Clements, 1979; Hawthorne et al., 1984). The distribution space of the cyclitol within the nerve has been reported to be decreased (Clements and Stockard, 1980), a finding which suggests that an abnormality of myo-inositol transport or of its accessibility to nerve structures may be involved. Recently Greene and Lattimer (1983) have shown that inositol may be associated with Na^+/K^+ ATPase function and this enzyme in microsomal fractions from rat sciatic nerve is found to be less active in diabetic animals. The levels of lipid-bound inositol (PtdIns and PolyPI) in peripheral nerves has been reported to be depressed in acute but not chronic streptozotocin-induced diabetes (Palmano et al., 1977; Eichberg et al., 1984). Natarajan et al (1981) reported a 7% decrease in total phospholipid and a relative decrease in PtdIns of sciatic nerves of diabetic rats. Incubations of isolated sciatic nerves of diabetic rats in a medium containing

$^{32}P_i$ gave decreased labeling in PtdIns and PolyPI as compared to controls. The incorporation of (^{3}H)-inositol into sciatic nerve lipids has also been reported to be decreased both in vivo and in vitro under the same conditions (Clements and Stockard, 1980).

In contrast, Bell et al (1982) have shown that the levels of PtdIns(4,5)P_2 in the sciatic nerves of normal animals was indistinguishable from that in animals which had been diabetic for over 20 weeks and, a specific and significant increase from 48 to 67% in the amount of $^{32}P_i$ incorporated in PtdIns(4,5)P_2 occurred. Nerve conduction velocities for both motor and sensory components of rat sciatic and caudal nerves were decreased by 20% in the 10 week and 20 week old diabetic animals as compared to controls. Ca^{2+} depletion of normal nerves increased the incorporation of $^{32}P_i$ in PtdIns(4,5)P_2 to the same extent as seen in diabetic nerves incubated in a complete medium. Since several synthesizing and hydrolysing enzymes of phosphoinositide metabolism either require or are affected by Ca^{2+} (Table 13) the availability of the ion might somehow he altered in nerves from diabetic animals. It would be desirable to determine whether the activity of PtdIns(4,5)P_2 phosphodiesterase, a Ca^{2+} requiring enzyme, is reduced in diabetic nerves, since this would tend to cause an increase in labelled substrate. Marginal decreases in PtdIns4P kinase activity (Ca^{2+}-inhibited) and a substantial decline in the activity of CDP-diacylglycerol:inositol phosphatidyl transferase (Ca^{2+}-inhibited) have been reported in diabetic nerves,

(Whiting et al., 1977; 1979; Kumara-Siri and Gould, 1980). Further, incorporation of $^{32}P_i$ into PtdIns(4,5)P_2 in purified sciatic nerve myelin was found to be similar in normal and diabetic state suggesting that alterations occur in a non-myelin structure, possibly axolemma and are therefore associated with adverse functional changes in peripheral nerves (Eichberg et al., 1984). Future investigations employing this model may be useful in ascertaining whether PolyPI in fact play a role in axonal conduction.

A dramatic elevation in serum levels of free myo-inositol has been documented in human subjects with chronic renal failure (Clements et al., 1973; Pitkanen, 1976). In advanced forms of glomerulonephritis, a decreased glomerular filtration rate and disturbed myo-inositol reabsorption are also present. Clements and Diethelm (1979) have observed that the halftime of inositol disappearance, which was prolonged in patients with chronic renal failure, was restored to normal following successful renal transplantation. The adverse effects of raised plasma myo-inositol levels on peripheral nervous function have been examined in normal male rats by placing them on a diet enriched in myo-inositol for one week (Clements et al., 1973). A striking decrease in sciatic nerve conduction velocity developed in these animals which improved when they were restored to a normal diet. Hyperinositolemia may thus contribute to the

pathogenesis of uremic polyneuropathy in subjects with chronic renal failure. Liveson et al (1977) reported the development of cytoplasmic abnormalities within several days following exposure of dorsal root ganglion cells to levels of inositol analogues at the serum concentration found in uremic patients. It remains to be established whether a reduction in plasma myo-inositol levels by dietary modification might prove to be beneficial in patients with chronic renal failure.

Here dietary ataxia in rabbits progressively involves lateral brainstem and deep cerebellar nuclei. Eliasson et al (1967) found decreased levels of phosphoinositides in the brainstem of ataxic rabbits; incorporation of inositol into these lipids was also found to be decreased. This decrease resulted from increased breakdown of CDP-diacylglycerol, presumably by CDP-diacylglycerol hydrolase (Eliasson et al., 1972). The activity of the latter has been demonstrated in brain (Rittenhouse et al., 1981). It has been reported that an elevation in galactitol concentration and a depression in free and lipid-bound inositol levels develops in the brains of galactosemic infants or animals subjected to experimental galactose toxicity (Wells and Wells, 1967). Evidence indicates that the PtdIns response to ACh is impaired in synaptosomes from galactose-fed rats, which suggests that these animals may be deficient in the number of ACh receptors or have a defect in a step between receptor-neurotransmitter interaction and PtdIns breakdown (Warfield and Segal, 1978). Lymphocytes from multiple

sclerosis patients exhibit a lower incorporation of (^{3}H)-inositol into PtdIns than those from control patients when stimulated by phytoehemagglutinin (Offner et al.,1974), since on immunologic abnormality appears to be involved in the pathogenesis of multiple sclerosis. A further characterization of various diseases with the aid of appropriate animal models may be helpful in delineating a role for inositol and phosphoinositides in their prevention and treatment.

The literature thus reviewed shows that PolyPI, although present in trace quantities, are functionally important components by virtue of their several unique properties. They may exist in the form of two or more pools each one having different properties, role, localization and metabolism in different cell membranes. Studies on undernutrition have been mainly concerned with the developmental changes and the effects of nutritional stress on the major lipids (namely cholesterol, galactolipids and phospholipids) of whole brain, gray and white matter and isolated myelin. Though altered metabolism of PolyPI has been documented in several diseased states, no investigations have been apparently carried out on these metabolically active and functionally important lipids in relation to the nutritional status during different stages of development. One of the main difficulties for not studying these aspects has probably been the high lability post-mortem of these lipids and the minute amounts present in most tissues. With the advent

of microwave irradiation techniques and modified extraction procedures for halting the rapid degradation of these compounds it has become possible to elucidate the functional roles of these lipids in nerve cell membranes. Hence, as a part of long range studies on the role of nutrition on CNS lipids studies were carried out to understand the role of nutrition on PolyPI in rat brain.

Preliminary studies were first carried out on the post-mortem changes with time in young and adult rat brain PolyPI levels. Further, studies were extended to estimate PolyPI levels at different stages of the developing rat brain at two time points ("0 min" and 1 min representing 2 sec and 60 sec post-mortem values respectively). Studies were also carried to find out the effects of pre- and/or post-weaning under-nutrition and post-weaning nutritional rehabilitation on PolyPI levels at "0 min" and 1 min after decapitation of the animal. Since the candidate got an opportunity to go and work in Prof. Hauser's laboratory at Harvard it was possible to carry out preliminary studies on the effects of pre- and post-weaning undernutrition and post-weaning nutritional rehabilitation on PolyPI levels in brain regions enriched either in neuronal structures (cerebral cortex and cerebellum) or in glial cells and myelin (brain stem) as well as non-neural tissues like the kidney. The data obtained in these investigations are incorporated in this thesis.