RESULTS AND DISCUSSION

EXPERIMENT - I

EXPERIMENT - I

Ia CHANGES IN THE CONCENTRATION OF POLYPI AT DIFFERENT TIMES POST-MORTEM

It is well known that several biochemical changes occur in brain post-mortem. Glycolytic changes (Friede and Van Houten, 1961; Mark et al., 1968), increased GABA (Yoshino and Elliott, 1970) and 5-HT levels (Sloviter and Connor, 1977) and a substantial decline in several neurotransmitter metabolizing enzymes (Fahn and Cote, 1976) in the brain have been reported. Considerable evidence exists to show that among the lipids PolyPI are rapidly lost post-mortem in rat brain (Dawson and Eichberg,1965; Eichberg and Hauser, 1967; Sheltawy and Dawson, 1969; Hauser et al., 1971a; Gonzalez-Sastre et al., 1971). Eichberg and Hauser (1967) have shown that the activity of PolyPI phosphohydrolases however, do not change for at least 120 min after death. Studies using microwave irradiation have shown improved recoveries of endogenous PtdIns(4,5)P_2 in rat (Soukup et al., 1978a) and mouse (Nishihara and Keenan, 1983) brain. Based on the above, several authors have suggested that PolyPI may exist in the form of two pools in brain, one that is readily susceptible to hydrolytic attack and disappears rapidly post-mortem and the other that is hydrolysed at a slower rate. Special interest lies in the rapidly disappearing pool of PolyPI which may have a functional role in CNS and other

membranes. Further, post-mortem changes in PolyPI levels have also been reported to be dependent on the age of the animals (Sheltawy and Dawson, 1969). Studies were therefore carried out first to investigate the concentration of PolyPI at different times post-mortem in rats of two ages (21 and 56 days old). The heads of rats were immersed in liquid N_2 at different times after decapitation viz. 2sec, 1, 10, 20, 30, 40 and 70 min and the brains from the frozen heads were used for extraction and analysis of PolyPI (see Materials and Methods, pp-139).

The data obtained on losses in PtdIns(4,5)P_2 and PtdIns4P at different times post-mortem in rat brain (21 and 56 days old) are given in Table 26 and Fig. 12. Substantial losses were observed in PtdIns(4,5)P_2 (63% - 21 days; 71%- 56 days) and PtdIns4P (71% - 21 days; 44% - 56 days) during the 70 min post-mortem period. The maximum decline occurred in the first min (26-27% - PtdIns(4,5)P_2; 22-30% - PtdIns4P) followed by a steady decline to 70 min. Eichberg and Hauser (1967) reported that the levels of PolyPI decrease by about 46% within 10 min after death and thereafter the depletion is very slow for the next 110 min. In this study the brains of rats were frozen in liquid N_2 after decapitation and thus the levels of PolyPI lost during the first minute could not be determined.

The rate of decrease (nmoles/min) in the levels of PolyPI during different time intervals post-mortem are given in

Table 27. The rate of depletion of PtdIns(4,5)P_2 during different time intervals post-mortem is found to be generally higher at 56 than at 21 days of age. These results are at variance with those of Sheltawy and Dawson (1969) where the rate and magnitude of the fall in PtdIns(4,5)P_2 was shown to be rapid and more extensive in the younger rats. However, the PtdIns4P fraction showed no correlation with age in their studies as well as in the present study.

It is evident from Table 27 that the rate of decrease (nmoles of PolyPI lost/min) is highest between 0-1 min and gradually drops with increasing time post-mortem. The decreases in the levels of PtdIns(4,5)P_2 and PtdIns4P during 0-1, 1-10 and 10-70 min post-mortem time intervals are significant in both 21 and 56 day old brains. The changes observed after 10 min post-mortem i.e. between 10-20, 20-30, 30-40 and 40-70 min post-mortem time intervals are however not significant. These results suggest that PolyPI may exist as two pools, one, a metabolically active pool and the other a relatively stable pool. Studies carried out on PolyPI in myelin (Deshmukh et al., 1980), have shown that PtdIns(4,5)P_2 and PtdIns4P in myelin isolated from 24 day old rat brains represent 55% and 44% respectively of the total homogenate levels. In the present study 10 min post-mortem levels of PtdIns(4,5)P_2 and PtdIns4P at 21 days of age are in close correlation with those

reported for whole myelin while the 1 min post-mortem levels are appreciably higher. This suggests that PolyPI levels at 1 min post-mortem are not totally representative of a stable pool, all of it being located in myelin, but a fraction of the same (lost during the next 10 min) is also present in non-myelinated structures. The fact that considerable amounts of PolyPI are lost even after 10 min post-mortem suggests that the relatively stable pool presumably localized in myelin is also further degraded and this pool may have a functional role in myelin metabolism. In this connection, it is interesting to note that the enzymes responsible for the synthesis and catabolism of PolyPI are also partially localized in myelin (Deshmukh et al., 1978; 1982).

PolyPi which presumably have a role in the rapid conduction of nerve impulses along axons (Kai and Hawthorne, 1969) and across synapses (Griffin and Hawthorne, 1978) would constitute an active fraction of the total PolyPI. The sharp decline in the first x minute post-mortem followed by a steady fall suggests that PolyPI lost during the first minute may be related to such events.

Based on the above discussion, certain speculations on the designation, localization and role of different pools of PolyPI have been made which are summarized in Fig. 13 and

Table 28. The assumption, that heads frozen in liquid N_2 immediately following decapitation would contain the absolute or maximum levels of PolyPI, may be far from true since the set of decapitation itself may lead to losses in PolyPI levels. Mark et al (1968) reported that brains of rats where whole animals, heads and brains were frozen in liquid N_2 contained 0.91, 0.36 and 0.22 umoles of glucose/g respectively. This indicates that 60% of the glucose in brain is metabolized by the act of decapitation and only 16% more is labile in the 1 minute interval that follows it. Soukap et al (1978) have shown the levels of PtdIns(4,5)P_2 and PtdIns4P to be 460 nmoles/g and 149 nmoles/g wet wt respectively in microwave irradiated 34 day old rat brains. A 17% loss in PtdIns(4,5)P_2 was observed in brains where the heads of the animals were frozen in liquid N_2. However, in the present study, it was rather difficult to carry out such precise determinations due to the existing limitations in this laboratory on the technique employed for tissue fixation though attempts were made along this direction. In effect, the results of the present study may not reflect the true in vivo concentrations of PolyPI pools but give us an idea of the pattern of changes that occur post-mortem and provide a base for studies on PolyPI in the developing rat brain and the effects of nutritional deficiencies

on the same during different periods of development. The above could be extended using improved techniques to inactivate the brain efficiently.

The question remains, whether the rapid loss part-mortem of PolyPI is due to a phosphodiesteratic or phosphomonoesteratic cleavage, or a combination of both activities. A rise in the levels of intracellular Ca^{2+} ions post-mortem could regulate the hydrolysis of PolyPI since both enzymes are known to be activated by this cation (Table 13). Nijjar and Hawthorne (1977) have suggested that the amount of phosphohydrolase activity present in the brain is sufficient to hydrolyze all the PolyPI within short intervals after death. Since considerable amounts remain several minutes after death, it is speculated that PolyPI exist in two forms, one attacked rapidly by the phosphohydrolases (lost immediately post-mortem) which may be present in combination with proteins and ions like Ca^{2+} and Mg^{2+} and the non-complexed form attacked at a slower rate (lost gradually post-mortem). PolyPI have in fact been considered to occur as Ca^{2+} or Mg^{2+} complexes (Kerr et al., 1964; Hendrickson and Ballou, 1964; Eichberg and Dawson, 1965) bound through ionic linkages to protein. Studies on the cholinergic proteolipid receptor fraction isolated from the cerebral cortex have shown that it contains PtdIns$(4,5)P_2$ which may function as a binding component of the nicotinic cholinergic receptor and thereby have a role in

synaptic events (Wu et al., 1977; Cho et al., 1978). The precise localization, metabolic properties and specific functions of these pools need further investigations.

Ib CHANGES IN THE CONCENTRATION OF POLYPI IN RAT BRAIN DURING DEVELOPMENT

Several studies have been carried out on the PolyPI composition of developing brain of rat (Rossiter and Gardiner, 1966; Wells and Dittmer, 1967; Eichberg and Hauser, 1967; Sheltawy and Dawson, 1969; Keough and Thompson, 1970), guinea-pig (Sheltawy and Dawson, 1969) and chick (Shaikh and Palmer, 1976). As mentioned earlier the methods used for tissue fixation to preserve these compounds varied widely thereby leading to a wide range of values in literature (Table 6). No systematic study has focused on determining the levels of PolyPI pools during the development of rat brain. Since PolyPI pools are presumably located in different cell structures of the brain (Eichberg et al., 1971; Hauser et al., 1971a) which mature during different stages of development (Benjamins and McKhann, 1981) the levels of PolyPI pools during development were investigated. Results of the present study have recently appeared (Uma and Ramakrishnan, 1983a).

Studies were therefore carried out to estimate the concentration of PolyPI and their post-mortem losses in rat brain at different ages. Groups of rats were killed at 0, 7, 14, 21, 34

and 63 days of age and the concentrations of PolyPI were determined both at "0 min" and 1 min post-mortem at each age. These two time points were chosen based on the results of preliminary study (i.e. experiment Ia).

Data obtained on body and brain weights are presented in Table 29. The body and brain weights were comparable with several literature reports (Wells and Dittmer, 1967; DeSouza and Horrocks, 1979; Reddy et al., 1983). The weight of the brain reached 83% of the reference value (i.e. 9 weeks) by 3 weeks of age, while the corresponding value for body weight was only 27%. The brain and body weights were 18% and 4% at birth and reached 44% and 9% at one week and 71% and 15% at two weeks after birth. The percent increment in brain weight during the pre- and post-weaning periods were 300% and 20% respectively, while the body weight increases were many fold higher. The pattern is consistent with the well-known priority enjoyed by the brain during development.

The data on the concentration of PolyPI at "0 min" and 1 min post-mortem are presented in Table 30 and Figure 14. The results on PtdIns4P at 1 min post-mortem need to be interpreted with caution since it is an intermediate in the biosynthetic and degradative pathways of PtdIns(4,5)P_2.

The concentrations of total PtdIns(4,5)P_2 and PtdIns4P increased from birth to 63 and 34 days, respectively. The values for PtdIns4P were higher than those reported by Eichberg and Hauser (1967) and Soukup et al (1978a). This increase could be attributed to the inclusion of $CaCl_2$ in the neutral solvent extraction step as shown in the rat (Hauser and Eichberg, 1973) and chick (Shaikh and Palmer, 1976) brain. The improved recoveries are probably a result of reduced losses of PolyPI into the initial neutral solvent extracts due to enhanced binding of these lipids to tissue proteins.

Although PolyPI levels increased both during pre- and post-weaning periods, PtdIns(4,5)P_2 showed a peak increase between 21 and 34 days (61%) and PtdIns4P between 14 and 21 days of age (56%). Developmental studies on the kinases have shown that PtdIns kinase increases in activity well before myelination while PtdIns4P kinase increases rapidly during myelination (Salway et al., 1968; Eichberg and Hauser, 1969; Shaikh and Palmer, 1977a). The PtdIns(4,5)P_2 phosphomonoesterase (Salway et al., 1968; Shaikh and Palmer, 1977b) and PtdIns(4,5)P_2 phosphodiesterase (Keough and Thompson, 1970; Shaikh and Palmer, 1977b) have also been shown to increase rapidly during myelination implying some co-ordination of control of the enzymes and substrates, especially PtdIns(4,5)P_2. Preliminary studies carried out in this laboratory on inositol phosphatases hydrolysing Ins1P, Ins(1,4)P_2 and Ins(1,4,5)P_3 (Muralidharan et al., unpublished) have shown the activities of these enzymes to

increase rapidly during the peak period of myelination. Several myelin-specific lipids, namely galactolipids and ethanolamine plasmalogens also show rapid increases during this period (Wells and Dittmer, 1967; Reddy et al., 1983). Thus, there seems to be a close correlation between the deposition of PtdIns(4,5)P_2, myelinogenesis and development of the biosynthetic and hydrolytic enzymes active against PtdIns(4,5)P_2 in the rat brain. The metabolism of PtdIns4P appears to be more related to neuronal structures rather than myelin.

PolyPI levels as percent of reference value (i.e. 63 day old) in the developing rat brain are given in Table 31. Results show that substantial amounts of PolyPI are present in rat brain shortly after birth (22%-PtdIns(4,5)P_2 and 38% PtdIns4P of maximum adult value). This agrees with several earlier reports (Rossiter and Gardiner, 1966; Eichberg and Hauser, 1967; Wells and Dittmer, 1967; Sheltawy and Dawson, 1969) and also confirms the findings that PolyPI are indeed present in cellular membranes other than myelin (Eichberg et al., 1971; Hauser et al., 1971b; Eichberg and Hauser, 1973). Significant amounts of the metabolically relatively stable pool (1 min) of PolyPI (26% - PtdIns(4,5)P_2 and 50% - PtdIns4P of maximum adult value) were present at birth, presumably located in structures including those which later elaborate myelin. In this connection, it is interesting to note that significant amounts of these lipids are present in the myelin-rich P_2A

fraction of 7 day-old rat brains although no profile characteristic of compacted myelin were seen in this fraction (Kichberg and Hauser, 1973). The myelin like material in this fraction has been proposed to be a modified form of oligodendroglial plasma membrane (Agrawal et al., 1970). Alternatively, this pool of PolyPI present at birth may represent the fraction C (lost during 1-40 min post-mortem, refer pp 189-190) presumably located in non-myelin membranes.

The changes in the relative proportions of the metabolically highly active pool (0-1 min post-mortem values - pool B) and the relatively inert pool (1 min post-mortem values - pools A_1 + C) in the developing rat brain are given in Fig. 15. At all ages the pools (A_1 + C) represented a greater fraction than pool B of PolyPI. Since the differences between "0 min" and 1 min values are not significant at birth, the question arises if the metabolically highly active pool of PolyPI are formed only after birth. This active pool of PtdIns(4,5)P_2 seems to appear at 7 days and that of PtdIns4P at 14 days of age.

The percent increments in brain weight and the content of PolyPI during different stages of development are given in Table 32. The increments in brain weight were highest during the first week after birth and gradually dropped with increasing

age. A similar pattern of changes was observed in the content of PolyPI at "0 min" and 1 min post-mortem although the increments were relatively higher than that observed for brain weight. This indicates that the increments in the levels of PolyPI are not merely due to an increase in the tissue weight of the animal. Further, significant increases in the content of PtdIns(4,5)P_2 occurred between 5 and 9 weeks of age although the brain weight showed no changes during this period of development. This suggests that PtdIns(4,5)P_2 may have an important role to play in the post-weaning period.

The concentrations of PtdIns(4,5)P_2 and PtdIns4P at 1 min post-mortem (pool A_1 + C) increased from birth to 63 and 34 days of age, respectively, indicating that the pools A_1 and C are deposited both during prê- and post-weaning periods. Since the levels of PolyPI at 10 min post-mortem have not been determined, the actual levels of pool C lost between 1 and 10 min post-mortem could not be calculated. However, the results suggest that the metabolically relatively inert pool of PolyPI may be important in the maintenance of the structural integrity of neuronal, glial and myelin membranes. This pool of PtdIns(4,5)P_2 alone exhibited a significant increase of 53% (Table 32) between 5 and 9 weeks of age. Hauser et al (1971a) have shown that significant amounts of PtdIns(4,5)P_2 are deposited in the brain stem between 34 and 60 days of age. Thus, the data obtained in the present study provide supportive evidence to

the hypothesis that the 1 min post-mortem levels represent a major fraction of PolyPI located in white matter structure like the brain stem.

The metabolically highly active pool of PtdIns(4,5)P_2 seems to appear at 7 days while that of PtdIns4P at 14 days of age (Table 30). Maximum deposition of the active pool of PtdIns(4,5)P_2 occurred after weaning (3-5 weeks - Table 32) during the period of glial cell proliferation and continued myelination. In this connection it is interesting to note that a metabolically active pool of PolyPI exists even in myelin and that the necessary biosynthetic and hydrolysing enzymes are present in the "heavy" myelin fraction (Deshmukh et al., 1978; 1982). On the other hand, rapid increases in this pool of PtdIns4P occurred before weaning (2-3 weeks) during the period of active synaptogenesis. These changes in PtdIns(4,5)P_2 may be related to the role of neuronal, glial and myelin membranes while that of PtdIns4P mainly to neuronal and to synaptic membranes.

In summary, the results suggest that PolyPI in brain exist in the form of at least two pools, namely, the metabolically highly active pool (pool B) that is readily susceptible to hydrolytic attack and the relatively inert pool (pool A_1 + C) that is hydrolysed at a slower rate. The

proportion of the two pools appear to vary at different times post-mortem, the active pool disappearing gradually with increasing time. Results also suggest the existence of multiple metabolic pools - each pool being relatively more stable or relatively more active than the other. The metabolically highly active pool (pool B) of PolyPI could not be detected at birth indicating that they begin to appear at the onset of neuronal and synaptic maturation and continue to be deposited during the period of active myelination and glial cell proliferation. On the other hand, considerable amounts of the metabolically relatively inert pool of PolyPI are present at birth probably located in neurons or in structures that later elaborate myelin. The metabolically highly active pool as PtdIns$(4,5)P_2$ is deposited rapidly after weaning during the period of glial cell maturation and continued myelination. It therefore seems to be more important in glial and myelin metabolism. As rapid deposition of this pool of PtdIns4P occurs before weaning during the period of active synaptogenesis and may be important in neuronal metabolism. On the other hand the relatively inert pool of both lipids are deposited during pre- and post-weaning periods indicating their importance in glial and myelin metabolism apart from their role in neurons. However, it is necessary to carry out further studies on subcellular fractions of the brain or on neuronal and glial cells cultured in vitro to obtain precise knowledge on the role of PolyPI pools in different types of nerve cells.

TABLE 26 : CHANGES IN THE CONCENTRATION OF POLYPHOSPHOINOSITIDES AT DIFFERENT TIMES POST-MORTEM IN 21 AND 56 DAY OLD RAT BRAINS.

Dissection begun post-mortem (min)	No. of observations	PtdIns(4,5)P_2				PtdIns4P			
		21 days		56 days		21 days		56 days	
		nmoles / g wet wt	% of "0 min" value	nmoles / g wet wt	% of "0 min" value	nmoles / g wet wt	% of "0 min" value	nmoles / g wet wt	% of "0 min" value
		(Mean ± S.D.)							
0*	n_{21}=5 n_{56}= 3	250±28		426±22		192±20		321±18	
1	n_{21}= 5 n_{56}= 3	182±9	73	315±21	74	150±20	78	226±9	70
10	n_{21}= 3 n_{56}= 2	147±7	59	214 249	54	118±13	61	222 249	73
20	n_{21}= 2 n_{56}= 2	120 125	50	214 222	51	86 102	49	221 236	71
30	n_{21}= 2 n_{56}= 2	102 120	44	152 176	38	94 90	45	201 230	67
40	n_{21}= 2 n_{56}= 2	102 98	40	138 150	34	87 69	41	177 213	61
70	n_{21}= 2 n_{56}= 2	86 99	37	132 118	29	60 52	29	165 185	56

* PolyPI levels at "0 min" represent 2 sec post-mortem value.

n_{21}= number of observations at 21 days.

n_{56}= number of observations at 56 days.

Fig. 12 : PolyPI levels at different times post-mortem.

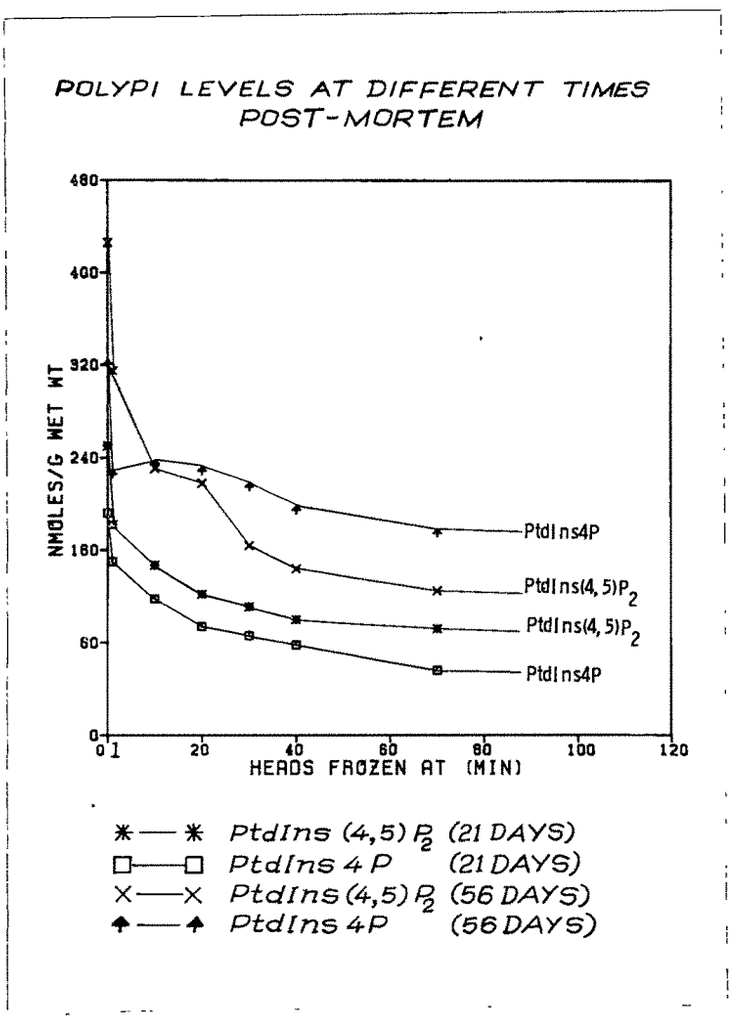


TABLE 27 : RATE OF DECREASE (nmoles/min) IN THE LEVELS OF POLY-PHOSPHOINOSITIDES DURING DIFFERENT TIME-INTERVALS POST-MORTEM IN RAT BRAIN (21 and 56 days old).

Post-mortem time interval (min)	0-1	1-10	10-20	20-30	30-40	40-70
21 DAYS						
PtdIns(4,5)P_2	68***	3.8***	2.4	1.2	1.1	0.23
PtdIns4P	42**	3.5**	2.4	0.2	1.4	0.73
56 DAYS						
PtdIns(4,5)P_2	111***	9.2**	1.4	5.4	2.0	0.63
PtdIns4P	95***	-	0.7	1.3	2.1	0.66

*** Values represent significant decreases during the time interval specified $P < 0.005$.

** Values represent significant decreases during the time interval specified $P < 0.02$ and 0.05

Fig. 13 : Schematic representation of PolyPI pools.

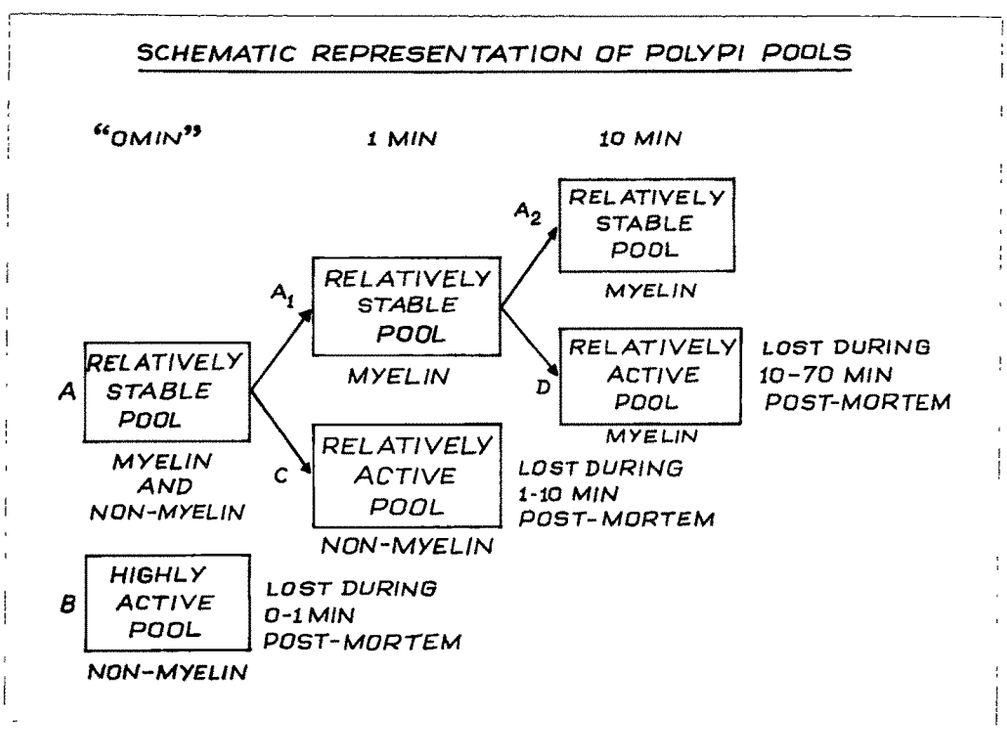


TABLE 28 : POSTULATED DESIGNATION, LOCALIZATION AND ROLE OF POLYPI POOLS IN RAT BRAIN.

		Designation	Localization	Role
"0 min"	(A+B)	Total levels of PolyPI	Neuronal, glial and myelin membranes	Structural and functional role in neuronal, glial and myelin membranes
1 min	A_1	1,Metabolically relatively stable pool of PolyPI	Myelin membrane	Structural and functional role in myelin membrane
	C	2. Metabolically relatively active pool of PolyPI	Non-myelin membrane	Functional role in non-myelin membrane
(0-1) min	B	Metabolically highly active pool of PolyPI	Non-myelin membrane	Functional role in non-myelin membrane
10 min	A_2	Metabolically relatively stable pool of PolyPI	Myelin membrane	Structural and functional role in myelin membrane
	D	Metabolically relatively pool of PolyPI	Myelin membrane	Functional role in meylin membrane
(1-10)min	C	Metabolically relatively active pool of PolyPI	Non-myelin membrane	Functional role in non-myelin membranes
(10-70) min	D	Metabolically relatively active pool of PolyPI	Myelin membrane	Functional role in myelin membrane

TABLE 29 : BODY AND BRAIN WEIGHTS AT DIFFERENT AGES IN THE RAT.

Age (weeks)	0 n=16	1 n=32	2 n=13	3 n=18	5 n=14	9 n=8
			mean ± s.d.			
Body weight (g)	6.2 ± 0.8	14.6 ± 1.4	25.8 ± 1.1	45.0 ± 3.5	70.5 ± 7.6	167.0 ± 20.0
Brain weight (g)	0.30 ±0.05	0.71 ±0.13	1.17 ±0.06	1.36 ±0.08	1.54 ±0.10	1.64 ±0.12
$\frac{\text{Brain weight}}{\text{Body weight}}$ x 100	4.8	4.9	4.5	3.0	2.2	0.9
% of adult weight (i.e., 63 days old)						
Body weight	3.7	8.7	15.4	27.0	42.2	100
Brain weight	18.1	43.6	71.3	83.3	93.3	100

n = No of observations for determination of body and brain weight at all ages except brain weight at birth (n = 4) and first week (n = 8) where brains of four rats were pooled.

TABLE 30 : POLYPHOSPHOINOSITIDES IN DEVELOPING RAT BRAIN.

Age (weeks)	0	1	2	3	5	9
	$n_0 = 2$	$n_0 = 4$	$n_0 = 8$	$n_0 = 9$	$n_0 = 7$	$n_0 = 4$
	$n_1 = 2$	$n_1 = 4$	$n_1 = 5$	$n_1 = 9$	$n_1 = 7$	$n_1 = 4$
	nmoles/g wet wt.; mean ± s.d.					
PtdIns(4,5)P_2						
"0 min" (Q)*	108 ± 7	164 ± 42	213 ± 15	269 ± 49	433 ± 15	476 ± 18
1 min (R)	95 ± 4	102 ± 22	156 ± 29	199 ± 23	262 ± 28	368 ± 60
Q - R	13	62	57	70	171	108
Significance between (Q) & (R) P <	N.S.	0.01	0.01	0.01	0.001	0.05
PtdIns4P						
"0 min" (S)*	84 ± 18	105 ± 9	132 ± 31	206 ± 38	234 ± 18	222 ± 42
1 min (T)	83 ± 5	101 ± 33	105 ± 23	171 ± 35	203 ± 12	166 ± 20
S - T	1	4	27	35	31	56
Significance between (S) & (T) P <	N.S.	N.S.	N.S.	0.1	0.01	0.1

Brains of four rats were pooled for each sample at 0 and 7 days of age.
At all other ages only one brain was used for each sample.
n_0 - Number of samples used for determination of PolyPI levels at "0 min".
n_1 - Number of samples used for determination of PolyPI levels at 1 min.
* Time to cut and drop the head in liquid N_2 was 2 sec.
N.S. - not significant.

Fig. 14 : PolyPI levels in developing rat brain.

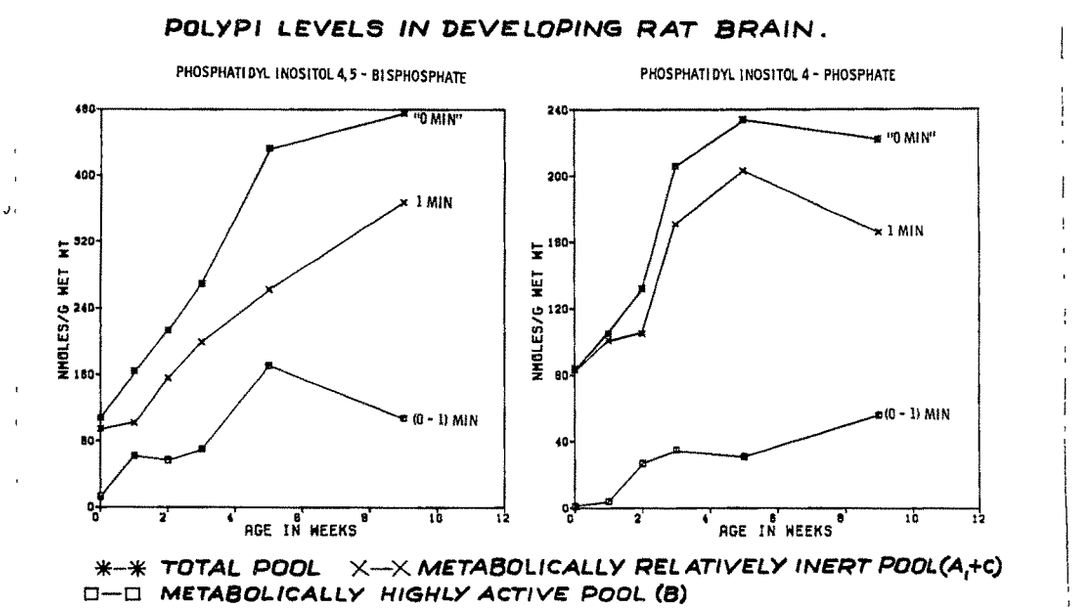


TABLE 31 : POLYPHOSPHOINOSITIDE COMPOSITION OF DEVELOPING RAT BRAIN AS PERCENT OF REFERENCE VALUE (i.e., 63-DAY-OLD RATS)*.

	Age (weeks)				
	0	1	2	3	5
PtdIns(4,5)P_2					
"0 min" (Q)	22	34	45	56	91
1 min (R)	26	28	42	54	71
(Q - R)	12	57	53	65	158
PtdIns4P					
"0 min (S)	38	47	59	93	105
1 min (T)	50	60	63	103	122
(S - T)	2	7	48	62	55

* Values calculated from mean ± s.d. values of Table 30.

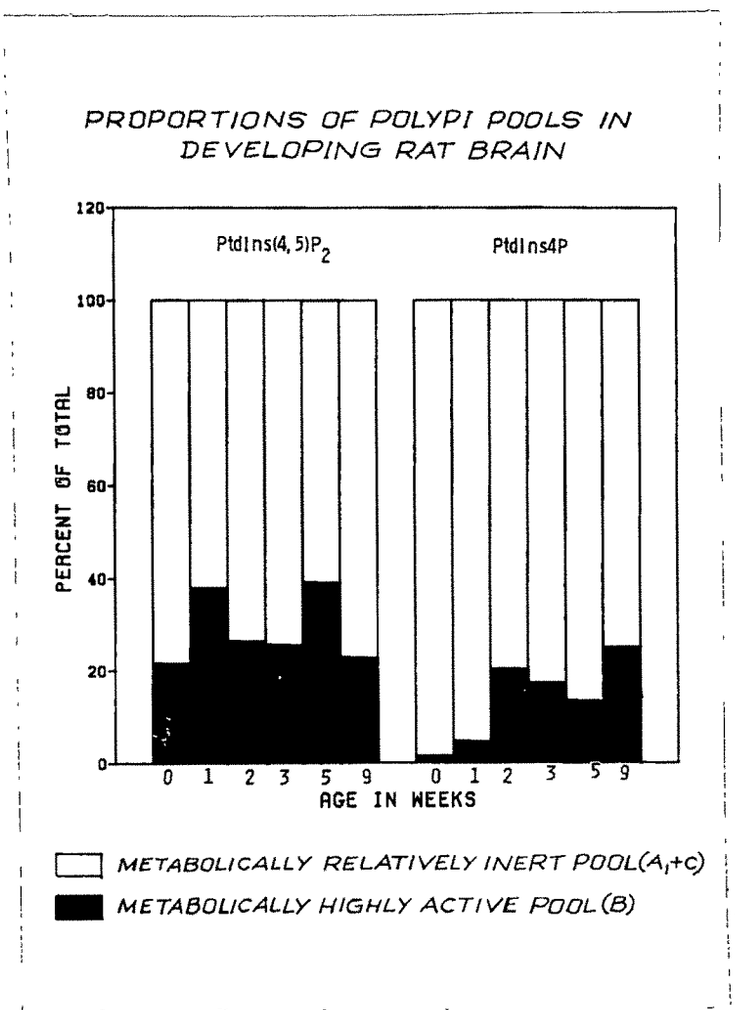
TABLE 32 : PERCENT INCREMENTS IN THE CONTENT OF POLYPHOSPHOINOSITIDE POOLS DURING DIFFERENT PERIODS OF DEVELOPMENT IN RAT BRAIN.

Age (weeks)	0-1	1-2	2-3	3-5	5-9
Brain wt	137***	65***	16***	13***	6
PtdIns(4,5)P_2					
"0 min" (Q)	211***	115***	50***	76***	17**
1 min (R)	216**	140***	44***	51***	53**
Q - R	200	55	75	150	-
PtdIns4P					
"0 min" (S)	150***	114***	91***	20**	1
1 min (T)	268	57	91***	33***	12
S - T	-	-	87	-	225

*** Values represent significant increases during the age interval specified $P < 0.001$ and 0.005.

** Values represent significant increases during the age interval specified $P < 0.01$, 0.02 and 0.05.

Fig. 15 : Proportions of PolyPI pools in developing rat brain.



E X P E R I M E N T - II

E X P E R I M E N T

II

EXPERIMENT - II

EFFECTS OF NUTRITIONAL ALTERATIONS DURING PRE- AND POST-WEANING PERIODS ON POLYPI POOLS IN RAT BRAIN.

Undernutrition instituted at any time during the entire programme of myelination and synaptogenesis in the rat brain is known to affect different aspects of development. The adverse effects of nutritional stress during the suckling period on the maturation of neurons (Cragg, 1972; Gambetti et al., 1974; Burns et al., 1975; Shoemaker and Bloom, 1977), glia (Robain and Ponsot, 1978; Sikes et al., 1981) and myelin (Wiggins, 1982) are well documented. The concentrations of different lipids are significantly reduced in whole brain (Rajalakshmi and Nakhasi, 1974a; Krigman and Hogan, 1976; Reddy and Sastry, 1978), gray and white matter (Reddy and Horrocks, 1982), myelin (Nakhasi et al., 1975; Wiggins and Fuller, 1978; Reddy et al., 1979) and other subcellular membranes (Pasquini et al., 1981) of neonatally undernourished rats.

Nutritional rehabilitation subsequent to deprivation in early life is not found to correct fully the deficits in lipids of the whole brain (Geison and Waisman, 1970; Rajalakshmi et al., 1974b; Reddy and Sastry, 1978) myelin (Simons and Johnston, 1976; Reddy et al., 1979; Yusuf et al., 1981; Fuller et al., 1984) and white matter (Reddy et al., 1982).

Protein deficiency after weaning has not been investigated in detail. Scattered reports indicate no changes in lipid levels (Guthrie and Brown, 1968; Rajalakshmi et al., 1974b) and decrease in the activities of glutamate decarboxylase and glutamate dehydrogenase (Rajalakshmi et al., 1974c) in the rat brain. A recent study on protein deficiency instituted from 60-240 days of age has shown that long-term low protein intake could affect the lipid composition of the rat brain during adulthood (Vrbaski, 1983). Further, some studies suggest that effects of nutritional deficiencies during the post-weaning period depend on the previous dietary history of the animal (Reddy and Ramakrishnan, 1982).

Diminished levels of PtdIns have been reported in studies on deprivation of myo-inositol (Burton and Wells, 1976) during lactation in rats. Levels of PolyPI are considerably lowered in brains of quaking mutant mice, which are characterized by inadequate myelination (Hauser et al., 1971b). The effects of nutritional insufficiency on the classical "PtdIns effect" in response to several neurotransmitters has been studied in different brain regions (Reddy and Sastry, 1979). Results indicate that the cholinergic, adrenergic and serotonergic synapses are affected by pre-weaning undernutrition in the brain stem but not in the cerebral cortex while in the cerebellum only the serotonergic systems are altered. Investigations on brain gangliosides, a minor component of total lipids

like PolyPI, which predominate in nerve endings and are considered to be functional lipids (Kasarskis et al., 1981) suggest an adverse effect due to undernutrition (Reddy and Sastry, 1978; Pasquini et al., 1981; Reddy et al., 1982). However, no studies have been carried out on the effects of protein deficiency during the pre- and post-weaning stages of development on PolyPI pools in rat brain.

Observations on the developmental pattern of PolyPI pools (experiment - Ib) suggested that the metabolically relatively inert pool (pools A_1 + C) may have an important role in the structure of neuronal, glial and myelin membranes as this pool is detected even at birth and is found to increase during pre- and post-weaning stages of development. The metabolically highly active pool (pool B) of PtdIns(4,5)P_2 showed maximum deposition after weaning (3-5 weeks) and that of PtdIns4P before weaning (2-3 weeks) suggesting the importance of the former in neuronal, glial and myelin membranes and the latter mainly in neuronal and synaptic membranes.

Based on the above observations several questions arose :

(1) If there is a metabolically highly active pool of PtdIns(4,5)P_2 deposited rapidly after weaning when glial cells continue to mature and continued myelinogenesis occurs, would there be any influence of nutritional deprivations on this pool by (a) post-weaning protein deficiency alone? (b) pre-weaning undernutrition superimposed by post-weaning protein deficiency?

(2) If there is a metabolically highly active pool of PtdIns4P deposited before weaning during the active period of synaptogenesis, would there be any damage to this pool by pre-weaning undernutrition?

(3) What would be the influence of nutritional alterations during pre- and post-weaning periods on the metabolically relatively inert pools of PolyPI?

In an attempt to provide answers to the above questions studies were carried out to see the effects of pre- and post-weaning undernutrition on the concentration of PolyPI pools in rat brain. Additional studies were made on the continuation of pre-weaning undernutrition during the post-weaning period and of the reversibility of the effects observed at weaning with dietary rehabilitation after weaning. After the appropriate nutritional regimen (refer Materials and Methods, p 140) PolyPI levels were determined in rat brains at two time points after decapitation, namely in tissues removed from heads of rats frozen either immediately or after standing at room temperature for 1 min. Reports of the findings have appeared recently (Uma and Ramakrishnan, 1983b).

Body and brain weights

Neonatal undernutrition caused significant reductions in the body (72%) and brain (24%) weights of rats (Table 33). The results are comparable to several reports where the same method

of feeding a low protein (4-5%) diet to the mothers during the suckling period has been employed for inducing undernutrition (Reddy et al., 1982; Reddy and Horrocks, 1982).

Protein deficiency during the post-weaning period affected the body (66%) and brain (15%) weights to a smaller extent (Table 34). Rajalakshmi et al (1974a) observed similar deficits using a 4% protein diet. The same authors reported lower deficits (52%-body wt and 10% brain wt) when a 5% protein diet was used (Rajalakshmi and Nakhasi, 1974b).

Nutritional rehabilitation during the post-weaning period improved the body and brain weights considerably but left them still significantly different from controls (Table 35). The extent of "catch up" was similar to those observed in previous studies (Reddy and Sastry, 1978; Reddy et al., 1982).

When undernutrition during the suckling period was superimposed by post-weaning protein deficiency, the percent deficits in body (86%) and brain (33%) weights were increased further as compared to 21 day L^- animals (Table 36). Similar observations have been made by Reddy and Ramakrishnan (1982) using the same animal paradigm.

PolyPI levels

Effect of neonatal undernutrition on PolyPI levels in whole brain

The concentrations of PolyPI in whole brain of 21 day old control (L^+) and undernourished(L^-) rats are shown in Table 33

and Fig. 16. Levels of PtdIns(4,5)P_2 and PtdIns4P were decreased by 41% and 34%, respectively, in the L^- "o min" samples. Deficits in the 1 min L^- samples were found to be 59% and 37% indicating that the higher phosphorylated derivative is more sensitive to nutritional deprivation. As regards the pool lost between 0 and 1 minute post-mortem marginal effects were observed only in the case of PtdIns4P (23%) but there was no appropriate method to determine if this decrease was statistically significant.

Effect of post-weaning protein deficiency on PolyPI levels in whole brain

The concentration of PolyPI in whole brain of 63 day old control (L^+P^+) and protein deficient (L^+P^-) rats are shown in Table 34 and Fig. 17. In contrast to the effects of pre-weaning undernutrition, levels of PtdIns(4,5)P_2 and PtdIns4P were not affected in the L^+P^- "0 min" and 1 min samples. Significant losses in PtdIns(4,5)P_2 (123 nmoles/g wet wt) during the first minute post-mortem were observed in the control (L^+P^+) brains while those in the protein deficient group (L^+P^-) were considerably lower (56 nmoles/g wet wt). This observation did not appear to be true in the case of PtdIns4P.

Effect of nutritional rehabilitation on PolyPI levels in whole brain

The concentrations of PolyPI in whole brain of control (L^+P^+) and rehabilitated (L^-P^+) group of animals are shown in

Table 35 and Fig. 18. When neonatally undernourished rats were nutritionally rehabilitated for 6 weeks by feeding a 20% protein diet during the post-weaning period, the levels of PtdIns(4,5)P_2 and PtdIns4P at "0 min" and 1 min post-mortem returned to normal. In fact levels of PtdIns4P in (0-1) min samples were higher than controls. As stated earlier the data on 1 min and (0-1) min samples for PtdIns4P should be discussed with reservations, since the 1 min value may also represent some PtdIns4P formed by the degradation of PtdIns(4,5)P_2.

Effect of continuation of pre-weaning undernutrition during the post-weaning period on PolyPI levels in whole brain.

Table 36 and Fig. 19 give the concentrations of PolyPI pools in the continued (L^-P^-) group of animals with respect to controls (L^+P^+) at 63 days of age. Levels of PtdIns(4,5)P_2 and PtdIns4P were decreased by 69% and 36% respectively in the L^-P^- "0 min" samples. Deficits in the 1 min L^-P^- samples were 62% (PtdIns(4,5)P_2) and 34% (PtdIns4P) for the two lipids. The higher phosphorylated derivative was again affected to a greater degree as in the case of L^- brains. The pool of PtdIns(4,5)P_2 lost between 0 and 1 minute post-mortem was affected severely (92%) while the same remained unaffected during the pre-weaning period.

A comparative analysis of the effect of varying nutritional conditions during the pre- and post-weaning periods on PolyPI pools as well as the myelin yield (Harjit and Ramakrishnan, unpublished) in rat brain is given in Table 37.

In the major portion of this study contain assumptions have been made based on observations of the previous experiment on post-mortem changes in PolyPI levels with time, the results of which led us to hypothesize that 1 minute post-mortem values represent a major fraction of PolyPI located in myelinated structures and the (0-1) minute values represent those located in non-myelinated structures of the brain, In light of this hypothesis, the results of the present experiment have been discussed.

Figs 20A and 20b represent the levels of metabolically highly active and relatively inert pools of PolyPI as percent of total in different groups of animals. The metabolically relatively inert pool of PolyPI (pools A_1 + C) represented a greater fraction than the active pool (pool B) in all the six groups of animals (i.e. L^+, L^-, L^+P^+, L^+P^-, L^-P^+, L^-P^-) irrespective of the nutritional status.

The effects of undernutrition on the rapidly disappearing pool (0-1 min post-mortem - pool B) are rather difficult to interpret as there is no appropriate method to determine if the observed changes are statistically significant. The present studies, however, indicate that this pool increases in control animals by 54% between 21 and 63 days of age. Protein deficiency during this period not only prevents this increase but results in a small depletion (20%) of this pool. Interestingly,

if such deficiency was superimposed over neonatal under-nutrition (L^-P^-) there was a drastic reduction in this pool and the final value was only 8% of control (L^+P^+). Thus it appears that the nutritional status during the post-weaning period is very important for the levels of this pool of PtdIns(4,5)P_2 in rat brain since similar effects were not apparent on the 0-1 min pool of PtdIns4P. The factors responsible for the decreased hydrolysis during the first minute post-mortem in L^-P^- and L^+P^- brains are unknown. It is well known that the activities of PolyPI phosphohydrolases are regulated by Ca^{2+} and Mg^{2+} ions (Table 13). Nijjar and Hawthorne (1977) pointed out that an increase in the levels of Ca^{2+} ions on post-mortem may be responsible for the rapid post-mortem degradation of these compounds. The levels of Ca^{2+} ions could thus be one of the possible regulatory factors leading to decreased hydrolysis and in effect decreased levels of the metabolically highly active pool of PolyPI in L^-P^- and L^+P^- brains.

The severe effects of nutritional insufficiency after weaning on the rapidly disappearing pools of PtdIns(4,5)P_2 suggests an aborration in the functional role of the metabolically active pool of this lipid presumably located in glial and myelin membranes which continue to nature during the post-weaning period. An active pool of PolyPI and the necessary enzymes responsible for its turnover have been shown to be

located in "heavy" myelin (Deshmukh et al., 1978; 1980; 1982). It is interesting to note that several neurotransmitters like ACh (Rajalakshmi et al., 1974a), NE and DA (Bhave et al., unpublished) and enzymes of GABA metabolism like glutamate dehydrogenase and glutamate decarboxylase are reduced (Rajalakshmi et al., 1974) by post-weaning protein deprivation. This suggests a possible relationship in the metabolism of neurotransmitters and the rapidly disappearing pool of PolyPI, both being involved in functional events of nerve cell membranes.

The relatively inert pool of PolyPI (1 min post-mortem pools A_1 + C) is severely affected in L^- brains. The effects are carried over on continuation of protein deficiency (L^-P^-) but reversed on nutritional rehabilitation (L^-P^+) during the post-weaning period. In contrast to the effects on the rapidly disappearing pool, this pool of PtdIns(4,5)P_2 appears to be more affected prior to weaning rather than after weaning. As far as the inert pool of PtdIns4P is concerned the effects of pre-weaning undernutrition are not further increased by post-weaning protein undernutrition. This is consistent with observations made on the developmental pattern of PtdIns4P where no increases have been observed in the inert pool of PtdIns4P after weaning. Protein deficiency during the post-weaning period alone does not have any effect on this pool of both the lipids.

This severe effect on the relatively inert pool of PolyPI (probably pool A_1) located in white matter structures during the suckling period, is consistent with the deficits observed in the lipid composition of white matter (Reddy et al., 1982) and of myelin (Nakhasi, et al., 1975; Wiggins and Fuller, 1978; Reddy et al., 1979). Nutritional rehabilitation subsequent to deprivation in early life does not fully correct the deficits in myelin and myelin-specific lipids (Simons and Johnston, 1976; Reddy et al., 1979; Yusuf et al., 1981; Fuller et al., 1984) nor those in white matter content and its lipid composition (Reddy et al., 1982). In contrast, the present study shows that PolyPI located in white matter structures are restored on nutritional rehabilitation, indicating that the behavior of these lipids differs from that of the other lipids.

Quantitative comparisons on the content of myelin (Harjit and Ramakrishnan, unpublished) and 1 min post-mortem levels of PolyPI under different nutritional conditions have been made in Table 37. Pre-weaning undernutrition decreases the yield of myelin by 20% while the deficits in the 1 min post-mortem levels of PolyPI are considerably higher (PtdIns(4,5)P_2 - 59% and PtdIns4P - 37%). This is also found to be true in the case of rats undernourished during the pre- and post-weaning periods. However, protein deficiency after weaning has no effect on the yield of myelin as well as the 1 min post-mortem levels of PolyPI in brain. Since the deficits observed in myelin yield

and 1 min post-mortem levels of PolyPI in L^- and L^-P^- brains are not similar this confirms earlier suggestions that the 1 min post-mortem levels of PolyPI are not totally representative of the pool localized in myelin thereby indicating that a portion of it is also present in non-myelinated structures.

Thus it appears from these studies that the relatively inert pool of PolyPI which is presumed to play an important role in the structure of neurons, glia and myelin is affected both by pre-weaning undernutrition and continued post-weaning protein deficiency. The metabolically highly active pool of PtdIns(4,5)P_2 deposited rapidly after weaning is severely affected by protein deficiency during the post-weaning period suggesting an aberration in the functional maturation of glial cells and myelin. Post-weaning nutritional rehabilitation reverses the effects of neonatal undernutrition on the two pools of PolyPI.

Although the present investigations do not directly deal with different cell types or subcellular membranes, they underline the importance of nutritional status on PolyPI pools and it would be interesting to extend these studies to different cell types in the nervous tissue. Further studies are needed to investigate the effects using microwave irradiation for arresting the degradation of PolyPI rapidly. Since phosphoinositide metabolism and neurotransmitter associated events

are closely interrelated, it would be also worthwhile to study the interaction of the two with respect to undernutrition.

TABLE 33 : EFFECT OF PRE-WEANING UNDERNUTRITION ON (a) THE BODY AND BRAIN WEIGHTS OF RATS (b) THE CONCENTRATION OF POLYPI POOLS IN RAT BRAIN (21 DAYS OLD).

	L^+ - I n_o = 9 n_1 = 9	L^- - II n_o = 7 n_1 = 5	Significance between (I) and (II) p
	mean ± s.d.		
Body weight (g)+	45.0± 3.5	12.7± 1.0 (28)	0.001
Brain weight (g)+	1.36±0.08	1.04±0.05 (76)	0.001
	nmoles/g wet wt; mean ± s.d.		
PtdIns(4,5)P_2			
"0 min" (Q)*	269 ± 49	158 ± 32	0.001
1 min (R)	199 ± 23	82 ± 8	0.001
Q - R	70	76	
% Decrease	26	48	
Significance between (Q) and (R) p	0.01	0.001	
PtdIns4P			
"0 min" (S)*	206 ± 38	135 ± 24	0.001
1 min (T)	171 ± 35	108 ± 18	0.001
S - T	35	27	
% Decrease	17	20	
Significance between (S) and (T) p	0.1	0.05	

n_o = Number of samples used for determination of PolyPI levels at "0 min".

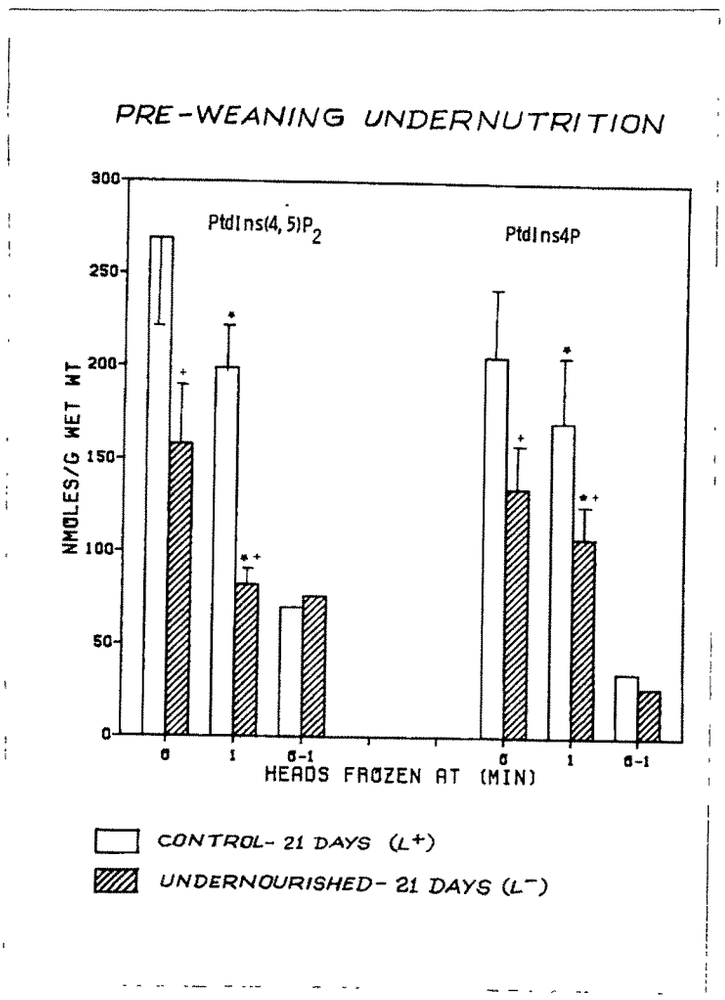
n_1 = Number of samples used for determination of PolyPI levels at 1 min.

* Time taken to cut the head and drop it in liquid N_2 was 2 sec.

\+ Number of animals used for calculating mean body and brain weights were - L^+ = 18; L^- = 12.

Numbers in parentheses denote % of control values.

Fig. 16 : Effect of pre-weaning undernutrition on PolyPI pools in rat brain.



Values are expressed as means $\pm$ s.d. * Values significantly different from "0min" ($p < 0.1$) + Values significantly different from control (L^+) ($p < 0.001$). X axis labels refer to the time at which heads of the animals were frozen in liquid N_2 after decapitation.

TABLE 34 : EFFECT OF POST-WEANING PROTEIN DEFICIENCY ON (a) THE BODY AND BRAIN WEIGHTS OF RATS (b) THE CONCENTRATION OF POLYPI POOLS IN RAT BRAIN '63 DAYS OLD).

	L^+P^+ - I $n_o = 4$ $n_1 = 4$	L^+P^- - II $n_o = 4$ $n_1 = 4$	Significance between (I) and (II) p<
	mean ± s.d.		
Body weight (g)+	141 ± 19	34.5±5	0.001
Brain weight (g)+	1.60 ± 0.05	1.65±0.06	0.001
	nmoles/g wet wt; mean ± s.d.		
PtdIns(4,5)P_2			
"0 min" (Q)*	424 ± 46	378 ± 36	N.S.
1 min (R)	301 ± 25	322 ± 66	N.S.
Q - R	123	54	
% Decrease	29	15	
Significance between (Q) and (R) p<	0.005	N.S.	
PtdIns4P			
"0 min" (Q)*	253 ± 36	253 ± 9	N.S.
1 min (R)	198 ± 36	218 ± 35	N.S.
Q - R	55	35	
% Decrease	22	14	
Significance between (Q) and (R) p<	0.1	N.S.	

n_o - Number of samples used for determination of PolyPI levels at "0 min".

n_1 - Number of samples used for determination of PolyPI levels at 1 min.

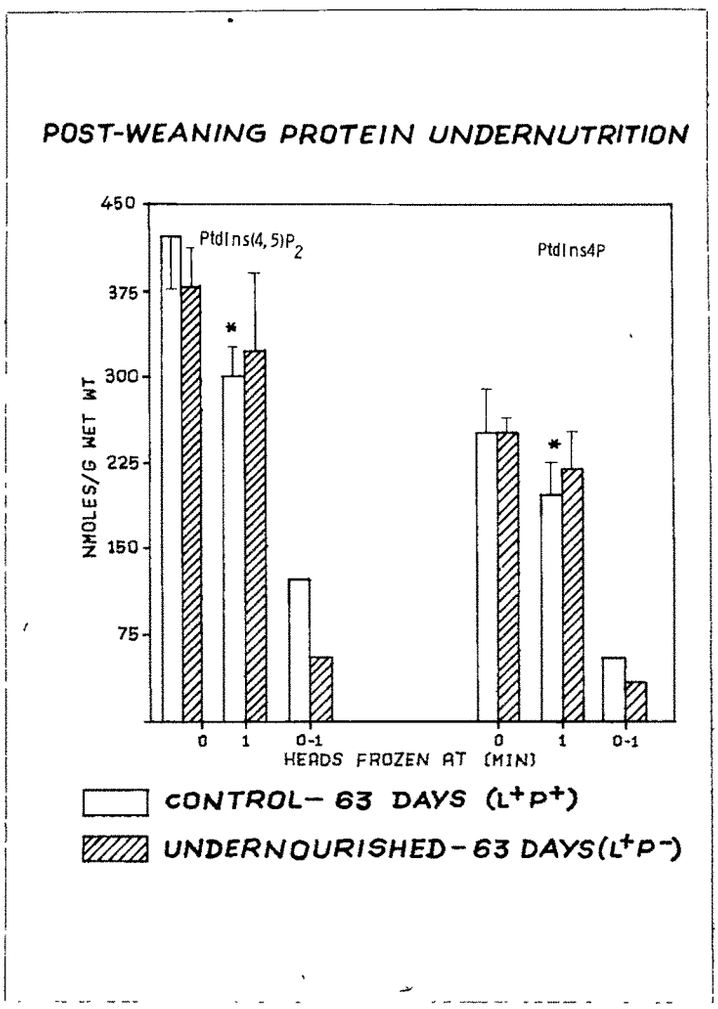
* - Time taken to cut the head and drop it in liquid N_2 was 2 sec.

\+ - Number of animals used for calculating mean body and brain weights were $L^+P^+ = 9$; $L^+P^- = 8$.

N.S. - Not significant.

Numbers in parentheses denote % of control values.

Fig. 17 : Effect of post-weaning protein undernutrition on PolyPI pools in rat brain.



Values are expressed as mean ± s.d. * Values significantly different from "0 min". ($p < 0.1$). X axis labels refer to the time at which heads of the animals were frozen in liquid N_2 after decapitation.

TABLE 35 : EFFECT OF POST-WEANING NUTRITIONAL REHABILITATION ON (a) THE BODY AND BRAIN WEIGHTS OF RATS (b) THE CONCENTRATION OF POLYPI POOLS IN BRAINS OF RATS UNDERNOURISHED PRIOR TO WEANING (63 DAYS OLD).

	L^+P^+ - I $n_o = 4$ $n_1 = 4$	L^-P^+ - II $n_o = 4$ $n_1 = 4$	Significance between (I) and (II) p <
	mean ± s.d.		
Body weight (g)+	167 ± 20	130 ± 11 (78)	0.001
Brain weight (g)+	1.64 ± 0.12	1.43 ± 0.09 (87)	0.01
	nmoles/g wet wt; mean ± s.d.		
PtdIns(4,5)P_2			
"0 min" (Q)*	476 ± 18	425 ± 81	N.S.
1 min (Q)	368 ± 60	350 ± 39	N.S.
Q - R	108	75	
% Decrease	23	18	
Significance between (Q) and (R) p <	0.05	0.1	
PtdIns4P			
"0 min" (S)*	222 ± 42	239 ± 43	N.S.
1 min (T)	166 ± 20	143 ± 39	N.S.
S - T	56	96	
% Decrease	25	40	
Significance between (S) and (T) p <	0.1	0.001	

n_o - Number of samples used for determination of PolyPI levels at "0 min".

n_1 - Number of samples used for determination of PolyPI levels at 1 min.

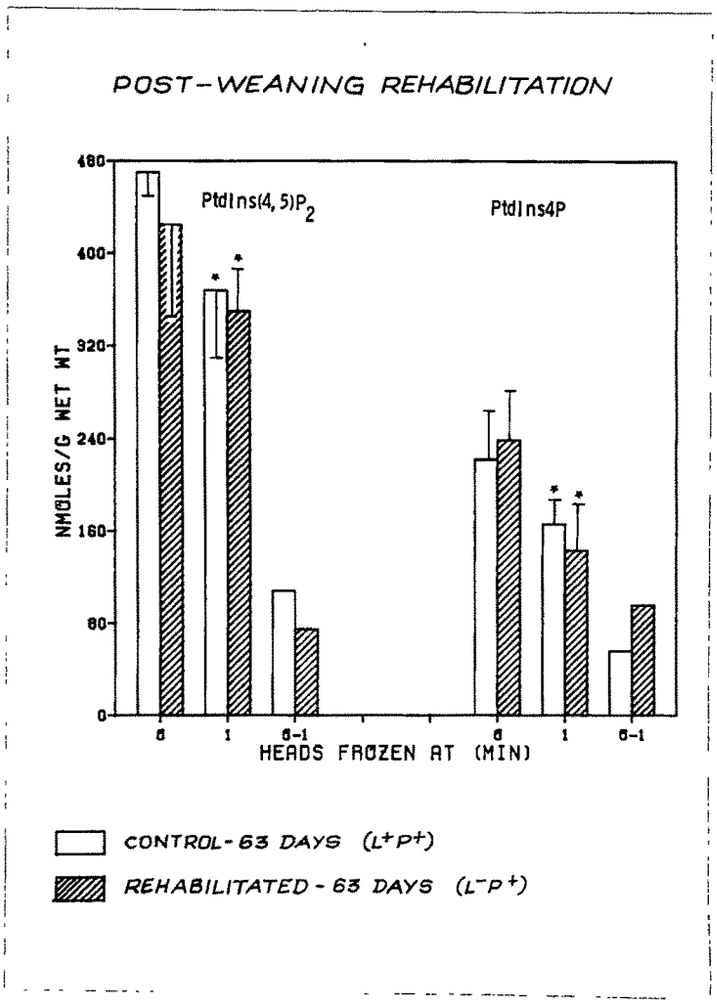
* - Time taken to cut the head and drop it in liquid N_2 was 2 sec.

\+ - Number of animals used for calculating mean body and brain weights were $L^+P^+ = 8$; $L^-P^+ = 8$.

N.S. - Not significant.

Numbers in parentheses denote % of control values.

Fig. 18 : Effect of post-weaning nutritional rehabilitation on PolyPI pools in rat brain.



Values are expressed as means ± s.d. * Values significantly different from "0 min" ($p < 0.1$). X axis labels refer to the time at which heads of the animals were frozen in liquid N_2 after decapitation.

TABLE 36 : EFFECTS OF PRE- AND POST-WEANING PROTEIN UNDERNUTRITION ON (a) THE BODY AND BRAIN WEIGHTS OF RATS (b) THE CONCENTRATION OF POLYPI POOLS IN RAT BRAIN (63 DAYS OLD).

	L^+P^+ - I n_o = 4 n_1 = 4	L^-P^- - II n_o = 6 n_1 = 5	Significance between (I) and (II) p
	mean ± s.d.		
Body weight (g)+	167 ± 20	23 ± 7 (14)	0.001
Brain weight (g)+	1.64±0.12	1.09±0.06 (67)	0.001
	nmoles/g wet wt; mean ± s.d.		
PtdIns(4,5)P_2			
"0 min" (Q)*	470 ± 18	147 ± 24	0.001
1 min (R)	368 ± 60	138 ± 29	0.001
Q - R	108	9	
% Decrease	23	6	
Significance between (Q) and (R) p	0.05	N.S.	
PtdIns4P			
"0 min" (S)*	222 ± 42	143 ± 27	0.01
1 min (T)	166 ± 20	109 ± 24	0.01
S - T	56	34	
% Decrease	25	24	
Significance between (S) and (T) p	0.1	0.1	

n_o - Number of samples used for determination of PolyPI levels at "0 min".

n_1 - Number of samples used for determination of PolyPI levels at 1 min.

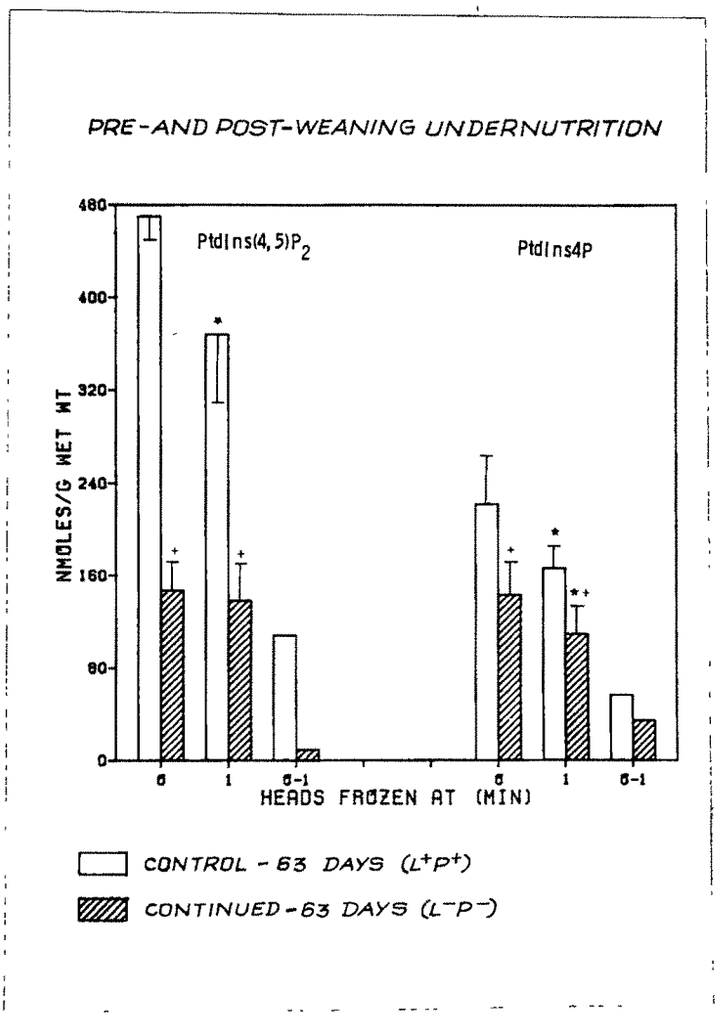
* - Time taken to cut the head and drop it in liquid N_2 was 2 sec.

\+ - Number of animals used for calculating mean body and brain weights were L^+P^+ = 9; L^-P^- = 11.

N.S. - Not significant.

Numbers in parentheses denote % of control values.

Fig. 19 : Effect of continued post-weaning protein under-nutrition on PolyPI pools in rat brain.



Values are expressed as means $\pm$ s.d. * Values significantly different from "0 min" ($p < 0.1$). + Values significantly different from control (L^+P^+) ($p < 0.01$). X axis labels refer to the time at which heads of the animals were frozen in liquid N_2 after decapitation.

TABLE 37 : COMPARATIVE EFFECTS OF NUTRITIONAL ALTERATIONS DURING THE PRE- AND POST-WEANING PERIODS ON THE CONCENTRATION OF POLYPI IN RAT BRAIN.

	L^-	L^+P^-	L^-P^+	L^-P^-
	% of control (L^+ or L^+P^+)			
PtdIns(4,5)P_2				
"0 min"	59***	89	89	31***
≥ 1 min	41***	107	95	38***
(0 - 1) min	109	46	69	8
PtdIns4P				
"0 min"	66***	100	108	64**
1 min	63***	110	86	66**
(0 - 1) min	77	64	171	67
Myelin+ (mg/g)	80**	98	88*	79*

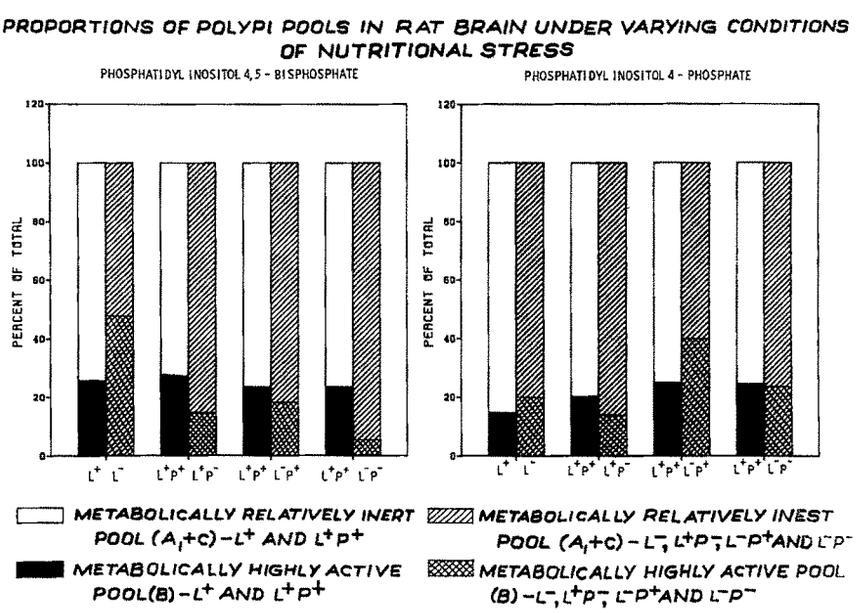
*** Values significantly different from control p 0.001.

** Values significantly different from control p 0.01.

* Values significantly different from control p 0.05.

+ Values for myelin yield have been taken from studies carried out in this laboratory (Harjit and Ramakrishnan, unpublished).

Fig. 20 : Proportions of PolyPI pools under varying conditions of nutritional stress.



EXPERIMENT - III

EXPERIMENT

III

EXPERIMENT - III

EFFECTS OF PRE-WEANING UNDERNUTRITION AND POST-WEANING PROTEIN DEFICIENCY ON THE INCORPORATION OF LABELLED $^{32}P_i$ INTO POLYPI POOLS OF RAT BRAIN

A good deal of evidence exists to show that phospho-inositides exhibit a high turnover rate of their monoester phosphate groups inspite of being a minor component of cell membranes. A rapid incorporation of $^{32}P_i$ into phosphoinositides of rat whole brain (Friedel and Schanberg, 1971), brain regions (Gonzalez-Sastre et al., 1971; Soukup et al., 1978a), brain subcellular fractions (Kai and Hawthorne, 1966; Mandel and Nussbaum, 1966), neuronal and glial cells (Freysz et al., 1969), brain myelin (Eichberg and Dawson, 1965), and subfractions of myelin (Deshmukh et al., 1981) have been reported. Further, a selective increase in the incorporation of $^{32}P_i$ into PtdA, PtdIns and PolyPI in response to a wide variety of stimuli in several tissues has been well documented (Michell, 1975; 1982; Abdel-Latif, 1983).

The incorporation in vivo of (^{14}C) glucose into different phospholipids has been shown to be significantly reduced in neonatally undernourished rat brains (Agrawal et al., 1971; Chase et al., 1976). Similarly 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 myelin isolated from

rat brain. Incorporation of $^{32}P_i$ in total phospholipids and its fractions have been shown to be increased under conditions of nutritional stress (Jailkhani and Subrahmanyam, 1977; Reddy and Sastry, 1978) although PolyPI have not been investigated in these studies. However, the ability to incorporate $^{32}P_i$ *in vivo* into PolyPI is substantially reduced in quaking mutant mice, probably as a result of an yet unknown biochemical lesion related to normal myelination (Hauser *et al.*, 1971b).

Results on PolyPI levels (experiment - II) showed that the metabolically relatively inert pool (1 min post-mortem value - pools A_1 + C) is affected by undernutrition during the pre-weaning period while the metabolically highly active pool (0-4 min post-mortem value - pool B) of PtdIns(4,5)P_2 is preferentially affected during the post-weaning period. In order to establish whether the decreased levels of PolyPI are accompanied by altered metabolism the incorporation of intraperitoneally injected $^{32}P_i$ into these compounds was studied.

Studies were therefore carried out to see the effects of pre-weaning undernutrition and post-weaning protein deficiency on the incorporation of $^{32}P_i$ into PolyPI pools of rat brain. For this study the peak time of incorporation at 3 and 9 weeks of age was first determined in the control animals. Later incorporation of $^{32}P_i$ into PolyPI was determined in the control (L^+, L^+P^+) and experimental (L^-, L^+P^-) rat brains at 3 and 9 weeks of age at two time points after death, namely in tissues

removed from heads of rats frozen either immediately or after standing at room temperature for 1 min (see Materials and Methods, pp 142-143). Losts of incorporation during the first minute was studied, since rapid losses in the levels have been observed during this time interval in previous experiments and the same would also be likely to reflect processes related to neuronal and glial functions.

Incorporation of labelled $^{32}P_i$ into PolyPI of 21 and 63 day old rat brains at different times after intraperitoneal injection.

The time course of incorporation of $^{32}P_i$ in PolyPI of 21 and 63 day old control rat brains are given in Figs. 21a and 21b respectively. Radioactivity on the ordinate is expressed as CPM/g wet wt which represents a measure of the total incorporation at each time point determined. During the time-course of labeling, peak incorporation was observed at two time points for both lipids at both ages. At 21 days of age the first peak was at 4 hr for both lipids and the second at 12 and 8 hr for PtdIns(4,5)P_2 and PtdIns4P respectively. At 63 days of age the first peak was at 2 hr for both lipids and a second peak was observed only in the case of PtdIns4P at 8 hr after injection of the label. Incorporation in PtdIns(4,5)P_2 showed a steady rise from 5-24 hr reaching a level almost equal to that at the first peak time (i.e. 2 hr). At all time points and at both ages, the total incorporation was found to be higher

in PtdIns(4,5)P_2 than PtdIns4P. Based on the results of this preliminary experiment the time-period chosen for equilibration of the label was 4 hr for the study on pre-weaning undernutrition and 2 hr for the post-weaning protein deficiency experiment.

Effect of pre-weaning undernutrition on the incorporation of $^{32}P_1$ into PolyPI of whole brain.

The incorporation of $^{32}P_1$ into PolyPI of 21 day old control (L^+) and undernourished (L^-) rat brains are given in Table 38 and Fig. 22a. The total incorporation, expressed as CPM/g wet wt. in PtdIns(4,5)P_2 was not found to be affected in the L^- "0 min" and 1 min samples while in PtdIns4P it was significantly reduced (74% at "0 min" and 71% at 1 min).

Data are expressed as specific radioactivity in Table 39 and Fig. 22b. The total incorporation, expressed as CPM/umole of PolyPI-P, in PtdIns(4,5)P_2 was found to be significantly increased in the L^- "0 min" (127%) and 1 min samples (103%). In contrast, the rate of incorporation was significantly reduced in PtdIns4P at both time points (60% at "0 min" and 53% at 1 min).

Effect of post-weaning protein deficiency on the incorporation of $^{32}P_1$ into PolyPI of whole brain

The incorporation of $^{32}P_1$ into PolyPI of 63 day old control (L^+P^+) and protein deficiency (L^+P^-) brains are given in

Table 40 and Fig. 23a. The total incorporation, expressed as CPM/g wet wt. was found to be significantly decreased by 31% and 26% in PtdIns(4,5)P_2 and PtdIns4P respectively in the L^+P^- "0 min" samples. In the L^+P^- 1 min samples a decrease was observed only in PtdIns(4,5)P_2 (17%).

Data are expressed as specific radioactivity in Table 41 and Fig. 23b. The total incorporation, expressed as CPM/umole of PolyPI-P, in both lipids was found to be significantly decreased only in the L^+P^- "0 min" samples. A decreasing trend was also observed in the 1 min samples but this decrease was not significant.

The biosynthetic potential of a tissue is more realistically assessed by comparing specific radioactivities rather than the total incorporation. The former expression helps in meaningful interpretation of data in most cases especially when the pool size of the component under study differs in different tissue samples and a direct precursor is used for labeling the compound. A comparative analysis of the radioactivity and concentration data on the effects of pre-weaning undernutrition and post-weaning protein deficiency on the metabolically relatively inert pool (1 min post-mortem value - pool A_1 + C) and the highly active pool (0-1 min post-mortem value - pool B) are given in Table 42. As mentioned in previous experiments, the effects of undernutrition on the incorporation of $^{32}P_1$ into the rapidly disappearing pool (0-1 min post-mortem) are difficult to

interpret as there is no appropriate method to determine if the observed changes are statistically significant.

Results on PolyPI levels show that neonatal undernutrition significantly reduces the size of the metabolically relatively inert pool and has marginal or no effects on the metabolically highly active pool of both lipids at 21 days of age. However, a significant increase in the specific radioactivity of PtdIns(4,5)P_2 and a decrease in PtdIns4P in both the pools has been observed in the present study. These changes are expressed diagrammatically in Fig. 24. The fact that the specific radioactivity of the metabolically relatively inert pool (Pools A + C) of PtdIns4P is reduced in L$^-$ brains suggests a decrease in the activity of PtdIns kinase which would lead to decreased levels of PtdIns4P in neonatally undernourished rat brains. However, an increase in the specific radioactivity and decreased levels of PtdIns(4,5)P_2 suggests that the catabolism of this lipid by the phosphodiesterase pathway is probably increased. In the case of metabolically highly active pool (Pool B), decreased specific radioactivity and no change in the levels of PtdIns4P sgggsts that its catabolism is also reduced, possibly to a larger extent than the synthesis. Similarly, an increase in the specific radioactivity and no change in the levels of PtdIns(4,5)P_2 suggest an increased catabolism, possibly to a larger extent than the synthesis.

Thus, pre-weaning undernutrition appears to increase the metabolic activity of both pools of PtdIns(4,5)P_2 and decrease that of PtdIns4P. It would be interesting to study the incorporation at 10 min post-mortem in order to get an idea of the metabolic activity of the pool lost between 1 and 10 min (pool C) post-mortem. Increased incorporation of $^{32}P_1$ into phospholipids of neonatally undernourished animals has been reported (Jailkhani and Subrahmanyam, 1977; Reddy and Sastry, 1978). The specific radioactivity in PolyPI isolated from quaking mouse brains has also been reported to be higher than that in normal brains (Hauser et al., 1971b). Sharma et al (1980) reported an increased incorporation of intraperitoneally injected $^{32}P_1$ in PtdIns(4,5)P_2 and PtdIns4P of neonatally undernourished rat brains. In their study, PolyPI were extracted and analysed from brains frozen in ice-cold sucrose for 10 min after decapitation of the animal. Bell et al (1982) have shown a specific and significant increase in $^{32}P_1$ incorporation in brain PtdIns(4,5)P_2 of rats which had been diabetic for over 20 weeks. These results only go to suggest that enhanced phospholipid and especially PtdIns(4,5)P_2 metabolism is a common feature observed under stress or diseased conditions.

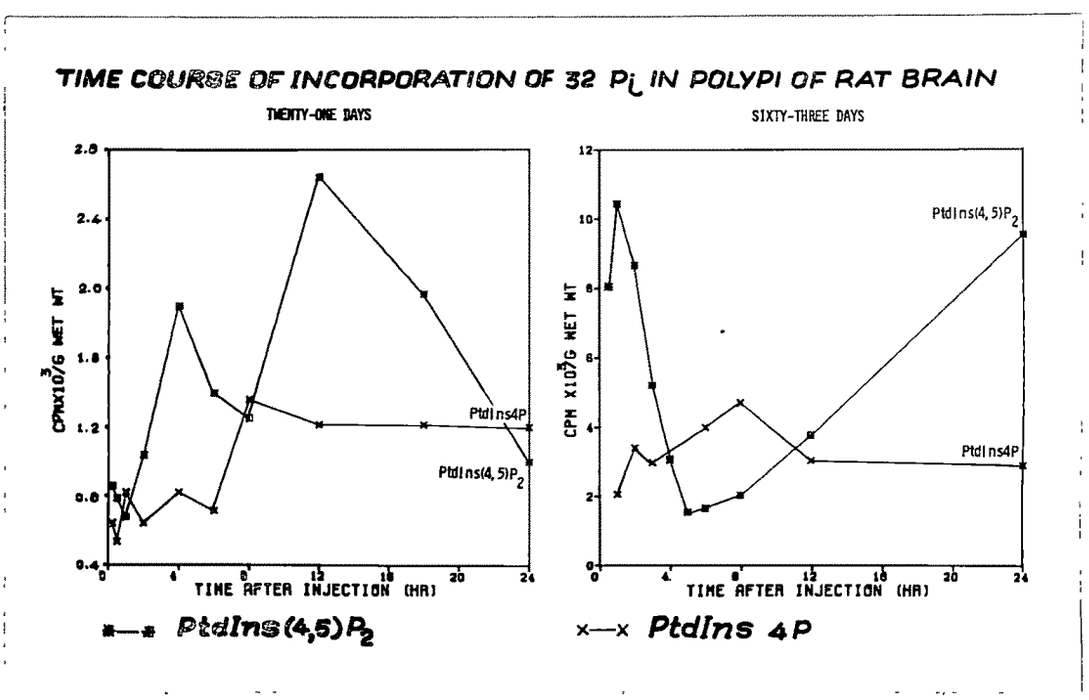
Post-weaning protein deficiency does not have any effect on either the levels or the specific radioactivity of the metabolically relatively inert pool (pools A_1 + C) of both

lipids suggesting that the metabolic activity of this pool remains unaffected in contrast to the effects produced by pre-weaning undernutrition. On the other hand, large reductions in the levels of the metabolically highly active pool (pool B) of PtdIns(4,5)P_2 accompanied by no changes in the specific radioactivities of PtdIns(4,5)P_2 and Gibson and Brammer (1981) have shown that Ca^{2+} ions inhibit the synthesis of PtdIns in oligodendroglial cells of rat brain. Based on the earlier suggestions (experiment - II) that this highly active pool of PtdIns(4,5)P_2 is localised in glial cells and myelin, it is possible that increased intracellular Ca^{2+} levels inhibit the synthesis of PtdIns thereby leading to decreased levels of PtdIns(4,5)P_2 in the protein deficient brains. Alternatively, the PtdIns(4,5)P_2 phosphodiesterase may also be activated by high levels of Ca^{2+} ions as discussed in experiment - II. In effect, both possibilities would lead to significant reductions in the levels of PtdIns(4,5)P_2 in the protein deficient.

The possible mechanisms operating for the metabolically relatively inert pool and the highly active pool of PolyPI in neonatally undernourished (L^-) and post-weaning protein deficient (L^+P^-) brains are summarized in Fig. 24. The physiological significance of these results remain unknown. Since several enzymatic reactions of phosphoinositide metabolism are regulated by Ca^{2+} ions, the availability of the ion may be a

critical factor responsible for the metabolic activity of different pools of PolyPI localized in different cellular membranes. Ca^{2+} ions are known to be potential inhibitors of CDP-diacylglycerol inositol phosphatidyl transferase amd PtdIns kinase in the brain. However, the PolyPI phosphomonoesterase and phosphodiesterase enzymes are activated by this ion (refer Table 13). Detailed studies on the synthesizing and hydrolying enzymes under different conditions of nutritional stress are necessary to get a clear picture on the metabolic activity of different pools of PolyPI.

Fig. 21 : Time course of incorporation of $^{32}P_i$ into PolyPI of rat brains.



Rats were decapitated at different times after intraperitoneal injection of 200 µci of $NaH_3{}^{32}PO_4$/100 g body wt.

TABLE 38 : EFFECT OF PRE-WEANING UNDERNUTRITION ON THE INCORPORATION OF $^{32}P_1$ INTO POLYPI OF RAT BRAIN (21 DAYS OLD).

	L^+ - I n_0 = 4 n_1 = 4	L^- - II n_o = 3 n_1 = 3	Significance between (I) and (II) p <
	CPM/g wet wt; mean ± s.d.		
PtdIns(4,5)P_2			
"0 min" (Q)*	4,012 ± 569	5,344 ± 719 (133)	N.S.
1 Min (R)	3,134 ± 857	2,625 ± 272 (84)	N.S.
(0-1) (Q-R)	878	2,719 (310)	
% change	-22	-51	
Significance between Q and R p <	0.1	0.01	
PtdIns4P			
"0 Min" (S)*	2,207 ± 254	572 ± 130 (26)	0.001
1 Min (T)	1,919 ± 234	560 ± 30 (29)	0.001
(0-1) (S-T)	288	12 (4)	
% change	-13	-2	
Significance between S and T p <	0.1	N.S.	

Time period of equlibration of the label after injection-4 hr.
* Time taken to cut the head and drop it in liquid N_2 was 2 sec
n_0 - Number of samples used for determination of PolyPI levels at "0 min".
n_1 - Number of samples used for determination of PolyPI levels at 1 min.
Number in parentheses denote % of control values.

TABLE 39 : EFFECT OF PRE-WEANING UNDERNUTRITION ON THE INCORPORATION OF $^{32}P_i$ INTO POLYPI OF RAT BRAIN (21 DAYS OLD).

	L^+ - I $n_0 = 4$ $n_1 = 4$	L^- - II $n_0 = 3$ $n_1 = 3$	Significance between (I) and (II) $p <$
	CPM/umole of PolyPI-P* ; mean ± s.d.		
PtdIns(4,5)P_2			
"0 Min"	14,916 ± 2,116	33,825 ± 4,551 (227)	0.01
1 Min	15,750 ± 4,310	32,016 ± 3,315 (303)	0.005
0-1 Min	12,543	35,776 (285)	
PtdIns4P			
"0 Min"	10,712 ± 1,235	4,239 ± 960 (40)	0.001
1 Min	11,222 ± 1,367	5,182 ± 285 (47)	0.001
0-1 Min	8,228	444 (5)	

Time period of equilibration of the label after injection-4 hr

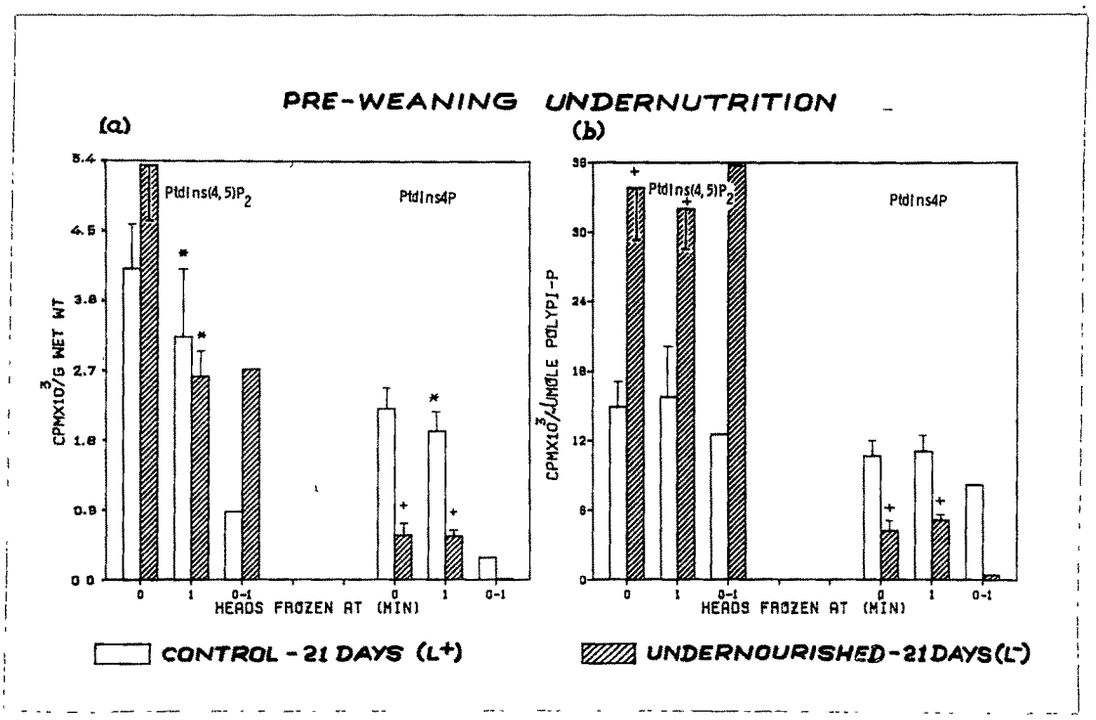
* PolyPI values for L^+ and L^- groups have been taken from experiment II (Table 33) for calculation of specific radioactivity.

n_0 - Number of samples used for determination of PolyPI levels at "0 min".

n_1 - Number of samples used for determination of PolyPI levels at 1 min.

Numbers in parentheses denote % of control values.

Fig. 22 : Effect of pre-weaning undernutrition on the labeling of $^{32}P_i$ in PolyPI. of rat brain.



Values are expressed as means ± s.d.

*Values significantly different from "0 Min" ($p < 0.1$).

\+ Values significantly different from control (L^+) ($p < 0.001$ for 22a and $p < 0.01$ for 22b).

x-Axis labels refer to the time at which heads of the animals were frozen in liquid N_2 after decapitation.

Time period of equilibration of the label after injection - 4 hr

TABLE 40 : EFFECT OF POST-WEANING PROTEIN DEFICIENCY ON THE INCORPORATION OF $^{32}P_i$ INTO POLYPI OF RAT BRAIN (63 DAYS OLD).

	L^+P^+ - I $n_0 = 4$ $n_1 = 4$	L^+P^- - II $n_0 = 4$ $n_1 = 4$	Significance between (I) and (II) $p <$
	CPM/g wet wt; mean ± s.e.		
PtdIns(4,5)P_2			
"0 Min" (Q)*	7,195 ± 458	4,967 ± 328 (69)	0.001
1 Min (R)	4,426 ± 408	3,662 ± 406 (83)	0.05
0-1 (Q-R)	2,769	1,305 (47)	
% change	-38.5	-26.0	
Significance between Q and R $p <$	0.001	0.001	
PtdIns4P			
"0 Min" (S)*	3,143 ± 232	2,327 ± 294 (74)	0.001
1 Min (T)	2,117 ± 150	1,925 ± 276 (90)	N.S.
0-1 (S-T)	1,026	402 (39)	
% change	-33.0	-17.0	
Significance between S and T $p <$	0.001	0.1	

Time period of equilibration of the label after injection-2 hr.
* Time taken to cut the head and drop it in liquid N_2 was 2 sec.
n_0 - Number of samples used for determination of PolyPI levels at "0 min".
n_1 - Number of samples used for determination of PolyPI levels at 1 min.
N.S. - Not significant.
Numbers in parentheses denote % of control values.

TABLE 41 : EFFECT OF POST-WEANING PROTEIN UNDERNUTRITION ON THE INCORPORATION OF $^{32}P_1$ INTO POLYPI OF RAT BRAIN (63 DAYS OLD).

	L^+P^+ - I $n_0 = 4$ $n_1 = 4$	L^+P^- - II $n_0 = 4$ $n_1 = 4$	Significance between (I) and (II) p
	CPM/umole of PolyPI-P* ; mean ± s.d.		
PtdIns(4,5)P_2			
"0 Min"	17,065 ± 1,268	13,249 ± 1,512 (78)	0.02
1 Min	14,756 ± 1,658	11,751 ± 2,705 (80)	N.S.
0 - 1 Min	22,512	23,304 (104)	
PtdIns4P			
"0 Min"	12,533 ± 1,007	9,249 ± 1,469 (74)	0.02
1 Min	10,909 ± 2,196	9,131 ± 2,688 (84)	N.S.
0 - 1 Min	18,655	11,486 (62)	

Time period of equilibration of the label after injection-2 hr.

* PolyPI values for L^+P^+ and L^+P^- groups have been taken from experiment II (Table 34) for calculation of specific radioactivity.

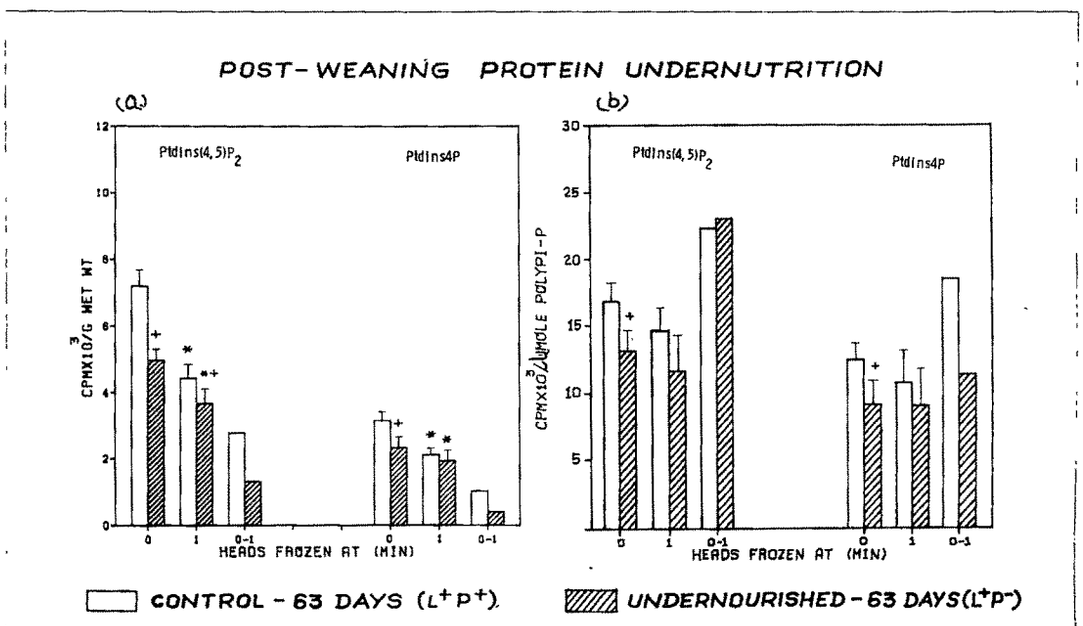
n_0 - Number of samples used for determination of PolyPI levels at "0 Min".

n_1 - Number of samples used for determination of PolyPI levels at 1 min.

Numbers in parentheses denote % of control values.

N.S. - Not significant.

Fig. 23 : Effect of post-weaning protein undernutrition on the labeling of $^{32}P_i$ in PolyPĪ of rat brain.



Values are expressed as means ± s.d.

* Values significantly different from "0 Min" ($p < 0.1$).

\+ Values significantly different from control (L^+P^+) ($p < 0.05$ for 23a and $p < 0.02$ for 23b).

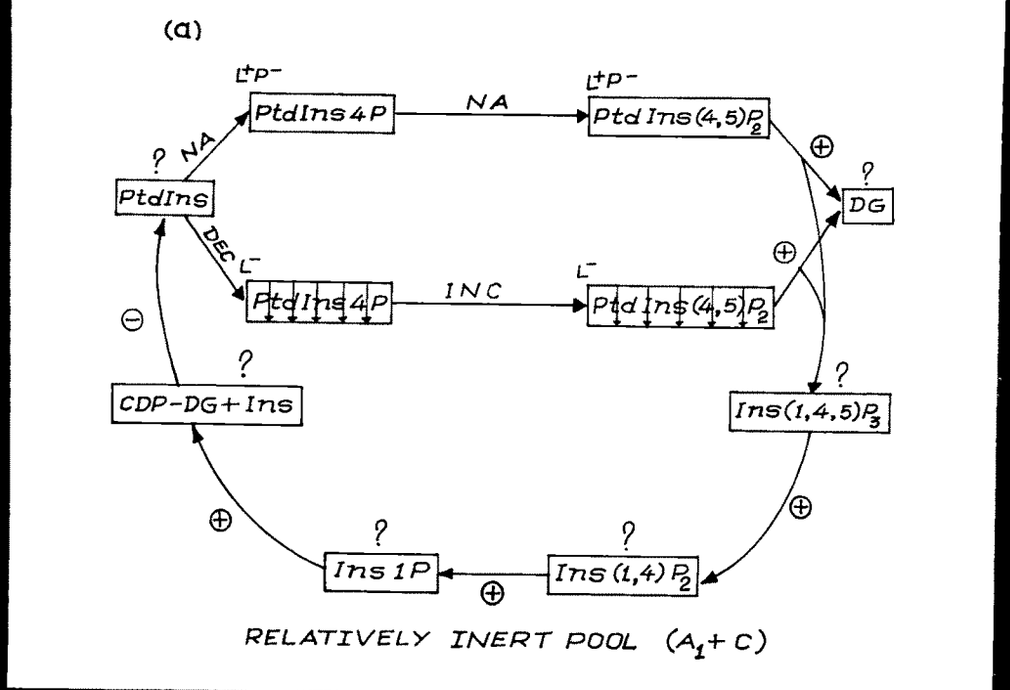
x - axis labels refer to the time at which heads of the animals were frozen in liquid N_2 after decapitation.

Time period of equilibration of the label after injection - 2 hr.

TABLE 42 : COMPARATIVE EFFECTS OF PRE-WEANING UNDERNUTRITION AND POST-WEANING PROTEIN DEFICIENCY ON THE LEVELS AND INCORPORATION OF $^{32}P_i$ INTO POLYPI POOLS OF RAT BRAIN.

	PtdIns(4,5)P_2				PtdIns4P			
	L^+	L^-	L^+P^+	L^+P^-	L^+	L^-	L^+P^+	L^+P^-
1 MIN								
Concentration (nmoles/g wet wt)	199	82***	301	322	171	108***	198	218
Incorporation (CPM/g wet wt)	3,134	2,625	4,426	3,662**	1,919	560***	2,117	1,925
(CPM/µmole of PolyPI-P)	15,750	32,016***	14,756	10,909	11,108	5,182***	11,751	9,131
0-1 MIN								
Concentration (nmoles/g wet wt)	70	76	123	56	35	27	55	35
Incorporation (CPM/g wet wt)	878	2,719	2,769	1,305	288	12	1,026	402
(CPM/µmole of PolyPI-P)	12,543	35,776	22,512	23,304	8,228	444	18,655	11,486

*** Values significantly different from control (L^+) $P < 0.001$ and 0.005


(a)
L+P−
PtdIns4P
NA
L+P−
PtdIns(4,5)P2
?
NA
PtdIns
DEC
L−
PtdIns4P
INC
L−
PtdIns(4,5)P2
DG
CDP-DG+Ins
Ins(1,4,5)P3
Ins1P
Ins(1,4)P2
RELATIVELY INERT POOL (A1+C)

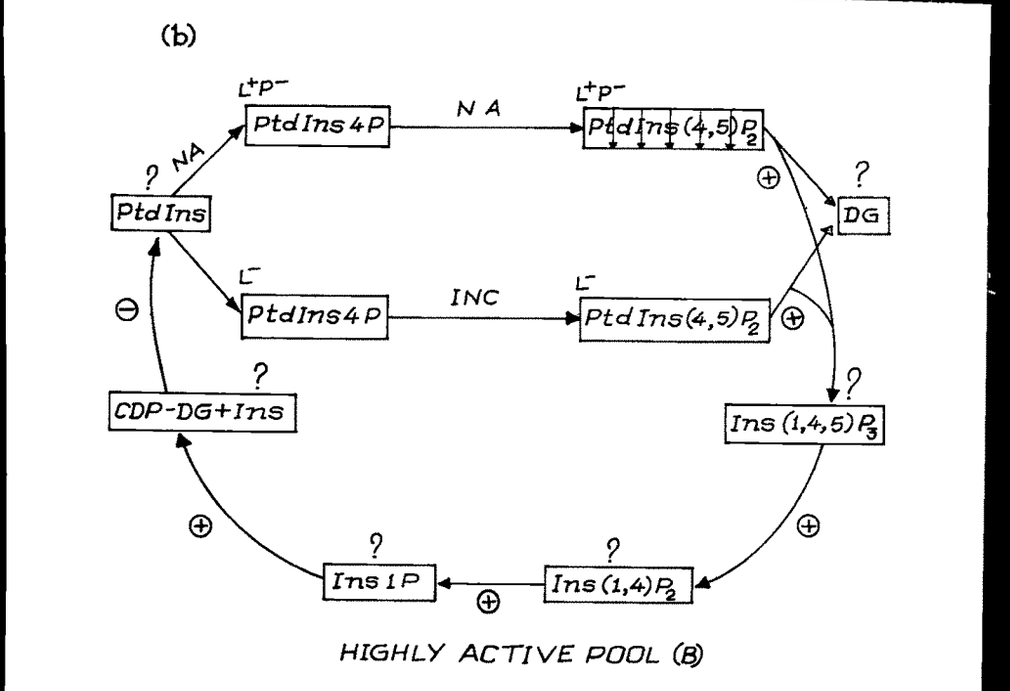

(b)
L+P−
PtdIns4P
NA
L+P−
PtdIns(4,5)P2
?
NA
PtdIns
L−
PtdIns4P
INC
L−
PtdIns(4,5)P2
DG
CDP-DG+Ins
Ins(1,4,5)P3
Ins1P
Ins(1,4)P2
HIGHLY ACTIVE POOL (B)

Fig. 24 : Metabolism of PolyPI pools in undernourished rat brain.

↓↓↓ - Decreased levels of PtdIns(4,5)P_2/PtdIns4P

□ - No change in levels of PtdIns(4,5)P_2/PtdIns4P

? - Fate of levels unknown.

DEC - Decreased incorporation.

INC - Increased incorporation.

NA - Incorporation not altered

(-) - Inhibition by Ca^{2+} ions

(+) - Activation by Ca^{2+} ions

EXPERIMENT - IV

EXPERIMENT

IV

EXPERIMENT - IV

EFFECTS OF NUTRITIONAL ALTERATIONS DURING PRE- AND POST-WEANING PERIODS ON POLYPI POOLS IN RAT BRAIN REGIONS

Brain regions vary considerably in weight, period of maturation, structure, composition and function. It is therefore reasonable to assume that undernutrition has variable effects on different components in different brain regions. This has indeed been shown to be true with regard to lipid levels (Dickerson and Jarvis, 1970; Ghittoni and DeRaveglia, 1972; Rajalakshmi and Nakhasi, 1974b) and levels of different neurotransmitters and their metabolizing enzymes (Shoemaker and Wurtman, 1971; Rajalakshmi et al., 1974c; Sobotka et al., 1974) in rat brain. Comparative effects of nutritional stress on gray and white matter lipids in rats have been reported (Reddy et al., 1982). Lipid levels in the developing cerebrum, cerebellum and brain stem of normal and undernourished children have also been investigated (Martinez, 1982).

Only limited information on the regional levels of PolyPI is available (Sheltawy and Dawson, 1969; Birnberger and Eliasson, 1970; Hauser et al., 1971a; Eichberg et al., 1971), although the lipids are implicated in several functional processes (Downes and Michell, 1982; Abdel-Latif, 1983). The response to the injection of neurotransmitters on the incorporation of $^{32}P_i$ into

PolyPI has been studied in several brain regions using microwave irradiation techniques (Soukup et al., 1978b). This neurotransmitter stimulated phenomenon has also been studied during neonatal undernutrition in different brain regions but the lipids investigated were PtdIns and PtdA (Reddy and Sastry, 1979). No report exists on changes in the regional distribution of PolyPI following nutritional deprivation until date.

Studies on whole brain showed that neonatal undernutrition reduces the concentration of total and metabolically relatively stable pools of PtdIns(4,5)P_2 and PtdIns4P significantly while the more active pools of the two lipids are not affected (experiment - II). These effects were reversed by post-weaning nutritional rehabilitation. Protein undernutrition during the post-weaning period alone as also continued feeding of a low protein diet after weaning decreased the metabolically highly active pool of PtdIns(4,5)P_2 suggesting a role of this component in the functional development of glial and myelin membranes which continues actively after weaning.

To provide further insight into the fate of the metabolic pools of PolyPI the nutritional studies on whole brain have been extended to discrete brain regions. After the appropriate nutritional regimen, PolyPI levels were determined in regions enriched in neuronal cell bodies (cerebral cortex and cerebellum) or myelin (brain stem) at two time points after death, namely in

tissues dissected and frozen either rapidly or after standing at room temperature for 10 min. Reports of the findings have appeared (Hauser and Swaminathan, 1982; Hauser and Ananth, 1983).

Body, brainstem and cerebellum weights

Neonatal undernutrition (L^-) caused a significant reduction in body weight of the animals (58%). The weights of brainstem and cerebellum were also decreased by 40% and 35%, respectively (Table 43). Post-weaning protein deficiency (L^+P^-) affected the body weight drastically, reduction being 82%. Brain stem and cerebellar weights were reduced much less, the deficits being of the order of 25% (Table 44). Rehabilitation (L^-P^+) improved the body and brain region weights but left them still significantly lower than controls (L^+P^+) (Table 43).

The deficits in body weights were smaller at 21 days of age (L^-) than those observed in the previous experiment on whole brain (experiment - II) and by other investigators using the same model (Nakhasi et al., 1975; Reddy et al., 1982). However, the deficits were comparable with those reported by Rajalakshmi et al. (1974a) where undernutrition was induced by increasing the litter size being reared by the mothers fed a high protein diet. Protein deficiency during the post-weaning period (L^+P^-) caused a greater reduction in the body weight (82%) as compared to whole brain studies (experiment - II) and

earlier reports of Rajalakshmi et al (1974a; 1974b) and Rajalakshmi and Nakhasi (1974a), who used diets containing higher amounts of protein (4-5%) for the experimental animals. The "catch-up" in the body weights of nutritionally rehabilitated rats ($L^{-}P^{+}$) was more complete than that observed in the present study (experiment - II) and some others (Reddy and Sastry, 1978; Reddy et al., 1982). These differences in the deficits observed in body weights at 21 and 63 days of age in the experimental groups could be largely due to differences in the strain of rats used for the two experiments. However, the possibility of environmental factors contributing to the variation in experimental results must also be considered.

Cerebroside levels

The levels of cerebrosides in brain regions (Table 43, 44, 45 and 46) were determined in order to evaluate the nutritional effects and the reliability of the dissection procedure. The values for cerebrosides served as an index of the degree of myelination. Cerebroside analysis was carried out on all samples of cerebral cortex in order to be able to check for white matter contamination, if any. Not all cerebellum and brain stem samples from each group were analysed. The constancy of cerebroside values in the cerebral cortex samples from all five groups of animals (Tables 43, 44, 45 and 46) indicated that the method of dissection was reproducible and the cerebrosides detected were not due to inclusion of subcortical white matter in the samples.

Small amounts of cerebrosides are present in gray matter structures well before the onset of myelination and the determination of cerebroside levels in 7-day-old rat cerebral cortex, brain stem and whole brain gave 0.088, 0.712 and 0.206 umoles/g wet wt, respectively.

The concentrations of total cerebrosides in the cerebral cortex were significantly reduced (35%) in neonatally undernourished rats (L^-) and this decrease was reflected in both NFA and HFA cerebrosides (Table 43). However, their concentration in brain stem and cerebellum was not affected. Krigman and Hogan (1976) have reported a decrease in the concentration of NFA-cerebrosides in 30-day-old undernourished rat brain.

Post-weaning protein deficiency (L^+P^-) caused no changes in cerebroside levels in the three regions examined (Table 44) and the effects on cerebral cortex at weaning were reversed by post-weaning nutritional rehabilitation (L^+P^+) (Table 45).

Table 46 gives the distribution of NFA and HFA-cerebrosides in different brain regions under varying nutritional conditions. At 21 days NFA and HFA-cerebrosides contributed about equally to the total in cerebral cortex and cerebellum while the brain stem contained a higher proportion of HFA-cerebrosides. This was not found to be affected in the undernourished animals (L^-). The proportion of HFA-cerebrosides increased appreciably in cerebral cortex between 21 and 63 days of age while no change was observed in brain stem and cerebellum. The pattern of cerebrosides in

post-weaning protein deficient (L^+P^-) and rehabilitated (L^-P^+) animals was similar to that of the controls (L^+P^+) at 63 days in all the three regions.

PolyPI Levels

Effect of neonatal undernutrition on PolyPI levels in brain regions

The concentrations of PolyPI in the three brain regions of 21 day old control (L^+) and undernourished (L^-) rats are shown in Table 47 and Figs. 25a and 25b. PtdIns4P is the PolyPI in each region accounting for 60-65% of total PolyPI in both control and undernourished animals. Levels of PtdIns4P and PtdIns(4,5)P_2 ("0" min samples) were decreased by 40% and 70%, respectively in the cerebral cortex of the undernourished (L^-)rats when compared to controls (L^+). Such deficits in brain stem and cerebellum were of the order of 45-50%. In the 10 min L^- samples the deficits were found to be 20-30% in all three regions, indicating the presence of a portion of these lipids which is affected considerably less by nutritional deficiency. The PolyPI pool lost between 1 and 10 min post-mortem was practically absent in the L^- brain regions, except in the case of PtdIns4P in cerebral cortex where a 58% decrease was observed.

Effect of post-weaning protein deficiency on PolyPI levels in brain regions

The concentrations of PolyPI in the three brain regions of 63 day old control (L^+P^+) and protein deficient (L^+P^-) rats

are shown in Table 48 and Figs. 26a and 26b. Concentration of PtdIns4P was higher than Ptd Ins(4,5)P_2 in the three regions studied. However, there is a further deposition of PtdIns(4,5)P_2 between 21 and 63 days age so that the contribution of this lipid increases to 40-45% when compared to 35-40% at 21 days of age. Levels of PtdIns4P and PtdIns(4,5)P_2 in "0 min" and 10 min samples from all three regions remained unaffected by the nutritional deficiency.

Effect of nutritional rehabilitation on PolyPI levels in brain regions

The concentrations of PolyPI in the three brain regions of control (L^+P^+) and rehabilitated (L^-P^+) group of animals are shown in Table 49 and Figs. 27a and 27b. As in the previous groups PtdIns4P is the predominant PolyPI in L^-P^+brain regions. Levels of PolyPI were partially restored in the "0 min" samples of cerebral cortex from L^-P^+ animals, PtdIns4P being 72% and PtdIns(4,5)P_2 77% of the controls. In brain stem and cerebellum, levels were 62-70% of the controls. In the 10 min samples, which represented that portion of both lipids which was affected to a smaller extent by protein deficiency at weaning than the more labile pool, there was a complete reversal in all three regions. However, the PolyPI pool lost between 1 and 11 min port-mortem (absent in L^- brain regions) was not regenerated during nutritional rehabilitation.

A minor section of this experiment, constituting the analysis of cerebroside levels, was carried out mainly to check for white matter content in all cerebral cortex samples. This seemed essential since gray matter was dissected as quickly as possible from the chilled hemispheres where visual discrimination of gray and white matter was difficult. Cerebrosides are well documented to be myelin indicators and serve as a quantitative histochemical referent for myelinated fibers in nervous tissue (Lewin and Hess, 1965; Norton, 1981; Chao and Rumsby, 1981). The dissection procedure appeared to be quite reproducible, so that it did not seem necessary to make any corrections for sub-cortical white matter inclusion in the cerebral cortex samples.

In the main portion of this experiment, (PolyPI determination) the time required for dissection and freezing of brain regions was 0.75-1 min, so that the results at "0 min" are comparable only with 1 min post-mortem levels of PolyPI from whole brain, but not with those obtained from heads immersed in liquid N_2 within 2 sec after decapitation (experiment - II). Further, since separation of brain regions, especially gray matter, from heads frozen in liquid N_2 was not possible, the metabolically highly active pool, which is lost during the first minute, in different brain regions could not be determined. However, the levels of PolyPI at 10 min post-mortem in the brain regions were estimated. This time point was chosen based on the results of experiment Ia (refer Table 26 p 186). Calculations

made on the rate of decrease of PolyPI in whole brain (nmoles/min) during different time intervals post-mortem indicated significant losses during 0-1 and 1-10 min post-mortem. Further, 10 min post-mortem values were comparable to the levels reported for myelin isolated from rat whole brain (Deshmukh et al., 1980). Previous reports (Eichberg et al., 1971; Hauser et al., 1971a) have also shown this time period (1-10 min post-mortem) to reflect substantial changes in the PolyPI levels of different brain regions and have led to speculations on the localization, properties and role of different metabolic pools of PolyPI in the nervous system. It was therefore thought that determination of PolyPI levels at 1 and 10 min post-mortem would give an idea of the effects of nutritional stress on two pools of PolyPI in different brain regions - namely, the metabolically relatively inert pool (10 min post-mortem value - pool A_1) presumably located in myelin and the active pool (1-10 min post-mortem value - pool C) presumably located in non-myelin membranes. (Refer Fig. 13, p. 189). On the other hand, studies on whole brain reflected the effects of undernutrition on the metabolically highly active pool (0-1 min post-mortem value - pool B) and the relatively inert pool active pool (1 min post-mortem value - pool A_1 + C) present in the whole brain.

PtdIns4P was the major PolyPI in most brain tissue samples analysed. This observation is at variance with earlier studies where PtdIns(4,5)P_2 was shown to be the major PolyPI (Hauser

et al., 1971a; Soukup et al., 1978a). This is consistent with the results on whole brain (experiment I and II) where improved recoveries of PtdIns4P were observed and this was attributed to the inclusion of $CaCl_2$ in the neutral solvent extraction step (Hauser and Eichberg, 1973).

The data on PolyPI levels in the "0 min" samples from all six groups followed the order; brain stem cerebellum cerebral cortex, which confirm and extend previous observations that total PolyPI are enriched in white matter structures, mainly in the myelin sheath (Hauser et al., 1971a; Eichberg et al., 1971).

Comparative effects of nutritional alterations during the pre- and post-weaning periods on the post-mortem depletion of PolyPI in brain regions are given in Table 50. Post-mortem losses of PolyPI (loss during 1-10 min) were relatively higher in the cerebral cortex when compared to cerebellum and brain stem in all groups of animals. This provides additional evidence to the earlier suggestion that the pool of PolyPI lost rapidly after death is located largely in gray matter structures with in neuronal cells, and the relatively loss labile pool is located in white matter structures rich in glial cells and myelin (Hauser et al., 1971a; Eichberg et al., 1971). Rapid depletion of PolyPI (27%) in rat whole brain occurs in the first minute after death followed by a steady decline to 70 min (experiment Ia). As mentioned earlier, this metabolically highly active pool which is lost in the first minute could not be determined in different brain regions due to technical difficulties.

Comparative effects of nutritional alterations during the pre- and post-weaning periods on the 1 min post-mortem levels of PolyPI (pools A_1 + C) in whole brain (from experiment - II, Table 37) and brain regions as well as the myelin yield from whole brain (Harjit and Ramakrishnan, unpublished) are given in Table 51. A high proportion of myelinated structures being present in the brain stem, it would be logical to expect the changes in this relatively inert pool of PolyPI in whole brain to be reflected in this region. This is found to be indeed true in 5 out of 6 measures (Table 51) thereby strengthening the results on whole brain. In addition to the significant deficits observed in L^- brain stem samples, deficits were observed in cerebral cortex and cerebellum as well, suggesting that a portion of this pool is also located in non-myelinated structures. In the earlier experiments on whole brain the deficits in the 1 min post-mortem levels of PolyPI were found to be considerably higher than the deficits observed in the yield of myelin (Harjit and Ramakrishnan, unpublished) and this difference was attributed to the partial localization of this pool in non-myelinated structures. The data obtained on brain regions therefore provides further supportive evidence to the earlier suggestions.

Comparative effects of nutritional alterations during the pre- and post-weaning periods on the 10 min post-mortem levels of PolyPI (pool A_1) in brain regions as well as the myelin yield (Harjit and Ramakrishnan, unpublished) from whole brain

are given in Table 52. It is evident from the table that this pool of PtdIns(4,5)P_2 is moderately affected in brain stem and cerebellum and that of PtdIns4P in cerebral cortex and cerebellum. However, these effects were restored to normal upon subsequent rehabilitation during the post-weaning period. Protein deficiency after weaning does not affect this pool in any of the regions examined.

The effects on the 10 min post-mortem levels of PolyPI in brain regions being moderate, are comparable to the similar deficits observed in the myelin yield from L^- brains. Also, morphological studies have revealed relatively moderate effects on the number of oligodendroglial cells in most areas of brain (Sikes et al., 1981) with the exception of corpus callosum which shows relatively larger deficits (Robain and Ponsot, 1978). The results thus suggest that the 10 min post-mortem values may represent the metabolically inert pool of PolyPI bearing a closer relationship to myelin and glial cells. It would be necessary to determine the levels of this pool in glialcells and myelin at different times post-mortem in order to get further insight into this metabolic relationship.

The pool of PolyPI lost between 1 and 10 min post-mortem (pool C) was virtually eliminated in L^- brain regions and was not restored when the animals were nutritionally rehabilitated (Figs. 25, 26 and 27). However, protein deficiency after weaning did not affect this pool in any of the regions examined.

In contrast, studies on whole brain (experiment - II) showed that the metabolically highly active pool (pool B) lost between 0 and 1 min post-mortem was not affected in L^- whole brain but was drastically decreased in L^+P^- (mainly PtdIns(4,5)P_2) brains. This differential effect of undernutrition on the PolyPI pool lost between 0 and 1 min post-mortem (whole brain studies) and that lost between 1 and 10 min post-mortem (regional studies) suggest the existence of more than one metabolically active pool. It is possible that one pool might readily become exposed to the PolyPI phosphohydrolases so as to be promptly hydrolysed while the other would initially be protected against hydrolytic cleavage.

The severe effects of nutritional insufficiency at weaning on the pool of PolyPI lost between 1 and 10 min post-mortem (pool C) which is also presumably located largely in gray matter structures, is consistent with morphological changes such as retarded neuropil development (Cragg, 1972), decreased dendritic arborization (Cordoro et al., 1976; Pysh et al., 1979; Hammer, 1981), diminished size and density of presynaptic endings (Gambetti et al., 1974) and decreased number of synapses per unit area (Sheemaker and Bloom, 1977) as well as the changes in functionally important neuronal lipids like gray matter gangliosides (Reddy et al., 1982). However, the partial persistence of the effects of weaning on this pool of PolyPI in nutritionally rehabilitated brain regions does not agree with the complete

"catch-up" observed in morphology (Pysh et al., 1979) and the weight and lipid composition of gray matter (Reddy et al., 1982). The effects observed after weaning are consistent with studies on the lipid levels in brain regions (Rajalakshmi and Nakhasi, 1974b) where no changes were observed during post-weaning protein deprivation.

In attempting to compare the results at 1 and 10 min post-mortem in whole brain and brain regions the differences in the severity of undernutrition induced in the two studies as judged by the body weights (13 g and 18 g in Baroda and Harvard respectively) as also the differences in the strain of rats used (Charles Foster strain at Baroda and Sprague Dawley strain at Harvard) raised doubts that required clarification. For this purpose undernutrition was induced by increasing the litter size of the rats at Baroda (Charles Foster strain) from 8 to 16 in order to be able to achieve body weights comparable to those obtained for the 5% protein fed 21 day old undernourished rats (Sprague Dawley strain at Harvard). The PolyPI levels were determined in the cerebral cortex, brain stem and cerebellum of samples frozen at 1 and 10 min. after decapitation of 21 day old control and undernourished animals. In general, results obtained were comparable in a qualitative way indicating that the mode of producing undernutrition and the strain of rats did not influence the results.

A summary of the effects of nutritional stress on PolyPI pools in whole brain (experiment - II) and brain regions is given in Table 53. The findings that emerge from the neurochemical data suggest the possible existence of multiple metabolic pools with differential localization, orientation to hydrolyzing enzymes, susceptibility to stress conditions, properties and roles in the nervous system.

TABLE 43 : EFFECT OF PRE-WEANING UNDERNUTRITION ON (a) THE BODY, BRAIN, STEM AND CEREBELLAR WEIGHTS OF RATS (b) THE CONCENTRATION OF TOTAL, NFA AND HFA CEREBROSIDES IN RAT BRAIN REGIONS (21 DAYS OLD).

	Cerebral cortex		Significance between (I) and (II) p <	Brain stem		Significance between (I) and (II) p <	Cerebellum		Significance between (I) and (II) p <
	L^+-(I) n = 12	L^--(II) n = 8		L^+-(I) n = 4	L^--(II) n = 4		L^+-(I) n = 3	L^--(II) n = 3	
	mean ± s.d.								
Body wt (g)	43.0 ± 3.0	18.0 ± 1.0 (42)	0.001						
Tissue wt (g)	-	-	-	0.140 ±0.017	0.084 ±0.009 (60)	0.001	0.187 ±0.024	0.122 ±0.013 (65)	0.001
	mg/g wet wt; mean ± s.d.								
Total cerebroside	0.37 ±0.09	0.24 ±0.07 (65)	0.001	12.3 ± 2.6	10.8 ± 2.6 (88)	N.S.	1.9 ± 0.2	1.7 ± 0.3 (89)	N.S.
NFA cerebroside	0.17 ±0.04	0.11 ±0.04 (65)	0.01	4.8 ± 1.2	4.3 ± 1.3 (90)	N.S.	0.8 ± 0.1	0.7 ± 0.1 (88)	N.S.
HFA cerebroside	0.21 ±0.05	0.13 ±0.04 (62)	0.005	7.5 ±1.4	6.5 ±1.3 (87)	N.S.	1.2 ± 0.1	1.0 ± 0.2 (91)	N.S.

Numbers in parentheses denote % of control values.
n = Number of samples used for determination of cerebroside levels.
Numbers of observations for (1) body weight - L^+=36; L^-=44
(2) stem weight - L^+=12; L^-= 8
(3) cerebellar weight - L^+=12; L^-=8

N.S. - not significant

TABLE 44 : EFFECT OF POST-WEANING PROTEIN UNDERNUTRITION ON (a) THE BODY, BRAIN STEM AND CEREBELLAR WEIGHTS OF RATS (b) THE CONCENTRATION OF TOTAL, NFA AND HFA CEREBROSIDES IN RAT BRAIN REGIONS (63 DAYS OLD).

	Cerebral cortex		Significance between (I) and (II) p <	Brain stem		Significance between (I) and (II) p <	Cerebellum		Significance between (I) and (II) p <
	L^+P^+ (I) n = 8	L^+P^- (II) n = 8		L^+P^+ (I) n = 3	L^+P^- (II) n = 3		L^+P^+ (I) n = 2	L^+P^- (II) n = 3	
	mean ± s.d.								
Body wt (g)	280 ±37	50±6 (18)	0.001						
Tissue wt (g)	-	-	-	0.225 ±0.036	0.169 ±0.021 (75)	0.005	0.259 ±0.019	0.189 ±0.018 (73)	0.001
	mg/g wet wt; mean ± s.d.								
Total cerebroside	1.71 ±0.3	1.8 ± 0.2 (106)	N.S.	28.2 ± 4.0	25.2 ± 2.9 (89)	N.S.	9.8 ± 1.1	8.1 ± 0.7 (83)	N.S.
NFA cerebroside	0.49 ±0.08	0.51 ±0.07 (104)	N.S.	11.3 ± 1.6	10.2 ± 0.5 (90)	N.S.	3.7 ± 0.5	2.9 ± 0.3 (78)	N.S.
HFA cerebroside	1.24 ±0.23	1.24 ±0.14 (100)	N.S.	16.9 ± 2.5	14.9 ± 2.5 (88)	N.S.	6.1 ± 0.6	5.1 ± 0.4 (84)	N.S.

Numbers in parentheses denote % of control values.
n = Number of samples used for determination of cerebroside levels.
Numbers of observations for (1) body weight - L^+P^+=16; L^+P^-=29
(2) stem weight - L^+P^+=8 ; L^+P^-=8
(3) cerebellar weight - L^+P^+=8; L^+P^-=8

N.S. - Not significant.

TABLE 45 : EFFECT OF POST-WEANING NUTRITIONAL REHABILITATION ON (a) THE BODY, BRAIN STEM AND CEREBELLAR WEIGHTS OF RATS (b) THE CONCENTRATION OF TOTAL, NFA AND HFA CEREBROSIDES IN RAT BRAIN REGIONS (63 DAYS OLD).

	Cerebral cortex		Signi-ficance between (I) and (II) p <	Brain stem		Signi-ficance between (I) and (II) p <	Cerebellum		Signi-ficance between (I) and (II) p <
	L^+P^+- (I) n = 8	L^-P^+-(II) n = 8		L^+P^+- (I) n = 3	L^-P^+-(II) n = 3		L^+P^+- (I) n = 2	L^-P^+-(II) n = 3	
				mean ± s.d.					
Body wt (g)	280±37	240±37 (86)	0.01						
Tissue wt (g)	-	-	-	0.225 ±0.036	0.209 ±0.021 (93)	N.S.	0.259 ±0.019	0.226 ±0.014 (87)	0.005
				mg/g wet wt; mean ± s.d.					
Total cerebro-side	1.71 ± 0.3	2.2 ± 0.5 (129)	0.1	28.2 ± 4.0	31.9 ± 5.1 (113)	N.S.	9.8 ± 1.1	9.9 ± 2.0 (101)	N.S.
NFA cerebro-side	0.49 ±0.08	0.52 ±0.15 (106)	N.S.	11.3 ± 1.6	11.2 ± 1.7 (99)	N.S.	3.7 ± 0.5	3.3 ± 0.7 (89)	N.S.
HFA cerebro-side	1.24 ±0.23	1.65 ±0.39 (133)	0.02	16.9 ± 2.5	20.7 ± 3.7 (122)	N.S.	6.1 ± 0.6	6.7 ± 1.4 (110)	N.S.

Numbers in parentheses denote % of control values.
n = Number of samples used for determination of cerebroside levels.
Numbers of observations for (1) body weight - L^+P^+=16; L^-P^+=16.
(2) stem weight - L^+P^+= 8; L^-P^+= 8
(3) cerebellar weight - L^+P^+=8; L^-P^+=8

N.S.- Not significant

TABLE 46 : COMPARATIVE EFFECTS OF NUTRITIONAL ALTERATIONS DURING THE PRE- AND POST-WEANING PERIODS ON THE DISTRIBUTION OF NFA AND HFA CEREBROSIDES IN RAT BRAIN REGIONS.*

		L^+	L^-	L^+P^+	L^+P^-	L^-P^+
Cerebral cortex	NFA-Cerebroside	45.9	45.8	28.8	28.3	23.6
	HFA-Cerebroside	54.1	54.2	71.2	71.7	76.4
Brain stem	NFA-Cerebroside	39.0	39.8	40.0	40.4	35.1
	HFA-Cerebroside	61.0	60.2	60.0	59.6	64.9
Cerebellum	NFA-Cerebroside	42.1	41.1	37.7	35.8	33.3
	HFA-Cerebroside	57.9	58.9	62.3	64.2	66.7

*Values expressed as % of total cerebroside levels.

TABLE 47 : EFFECT OF PRE-WEANING UNDERNUTRITION ON THE CONCENTRATION OF POLYPI IN RAT BRAIN REGION (21 DAYS OLD).

Region		PtdIns(4,5)P_2		Significance between (I) & (II) p	PtdIns4P		Significance between (III) & (IV) p
		L^+ - (I)	L^- - (II)		L^+ - (III)	L^- - (IV)	
1	2	3	4	5	6	7	8
		nmoles/g wet wt; mean ± s.d.			nmoles/g wet wt; mean ± s.d.		
Cerebral cortex	"0 Min (Q)*	114.4 ±21.4	34.3 ± 8.7 (30)	0.001	200.1 ±26.7	117.6 ± 20.6 (59)	0.001
	10 Min (R)*	44.8 ± 9.2	35.6 ± 5.1 (80)	N.S.	63.9 ±10.1	49.4 ± 6.4 (77)	0.025
	% Decrease in 10 min	61	-	-	68	58	-
	Significance between (Q) and (R) p	0.001	-	-	0.001	0.001	-

contd...

TABLE 47 : contd.

1	2	3	4	5	6	7	8
Brain stem	"0 Min" (Q)*	257.8 ±57.5	144.1 ±26.4 (56)	0.01	469.9 ±77.4	246.3 ±55.4 (52)	0.005
	10 Min (R)*	213.9 ±19.9	150.1 ±32.6 (70)	0.005	328.1 ±44.8	283.9 ±31.8 (86)	N.S.
	% Decrease in 10 min	17	-	-	30	-	-
	Significance between (Q) and (R) p	N.S.	-	-	0.005	-	-
Cerebellum	"0 Min" (Q)*	140.8 ±14.9	77.4 ± 3.9 (55)	0.001	220.3 ±27.4	106.9 ±14.0 (47)	0.001
	10 Min (R)*	114.3 ±11.6	90.3 ± 9.3 (79)	0.01	168.7 ±30.5	122.7 ±13.4 (73)	0.02
	% Decrease in 10 min	19	-	-	25	-	-
	Significance between (Q) and (R) p	0.01	-	-	0.001	-	-

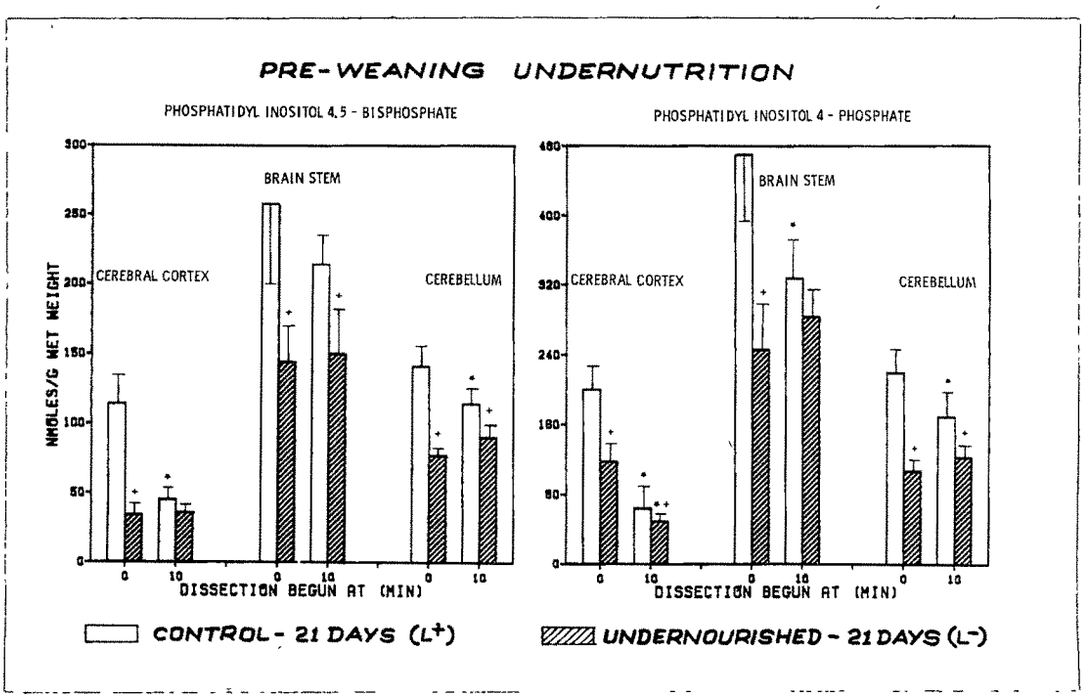
* Time taken to separate the brain regions after decapitation and drop them in liquid N_2 was approximately 60 sec. "0 min" and 10 min post-mortem value represent 1 min and 11 min value in brain regions.

Number of samples analysed at each time point (0 and 10 min) for controls (L^+) were six and experimentals (L^-) four.

Numbers in parentheses denote % of control values.

N.S. - Not significant.

Fig. 25 :- Effect of pre-weaning undernutrition on PolyPI pools in rat brain regions.



Values are expressed as means ± s.d.

* Values significantly different from "0 Min" ($p < 0.01$).

\+ Values significantly different from control (L^+) ($p < 0.025$).

x-axis labels refer to the time at which dissection of brain regions was begun following decapitation of the animals and dipping of the brain in liquid N_2.

TABLE 48 : EFFECT OF POST-WEANING PROTEIN UNDERNUTRITION ON THE CONCENTRATION OF POLYPI IN RAT BRAIN REGIONS (63 DAYS OLD).

Region		PtdIns(4,5)P_2		Significance between (I) & (II) p	PtdIns4P		Significance between (III)& (IV) p
		L^+P^+ - (I)	L^+P^--(II)		L^+P^+-(III)	L^+P^--(IV)	
1	2	3	4	5	6	7	8
		nmoles/g wet wt; mean ± s.d.			nmoles/g wet wt; mean ± s.d.		
Cerebral cortex	"0 Min" (Q)*	118.9 ±13.0	106.7 ±25.8 (90)	N.S.	226.7 ±43.5	172.5 ±29.4 (76)	0.01
	10 Min (R)*	65.2 ± 8.0	58.5 ±12.3 (90)	N.S.	90.1 ±12.4	90.6 ± 5.3 (101)	N.S.
	% Decrease in 10 min	45	45	-	60	48	-
	Significance between (Q) and (R) p	0.001	0.02	-	0.001	0.005	-

contd...

TABLE 48 : contd.

1	2	3	4	5	6	7	8
Brain stem	"0 Min" (Q)*	386.1 ±146.6	442.3 ± 88.2 (115)	N.S.	523.2 ±123.3	533.7 ± 52.3 (102)	N.S.
	10 Min (R)*	331.5 ±135.5	403.5 ± 87.4 (122)	N.S.	428.1 ±105.4	473.2 ± 38.6 (111)	N.S.
	% Decrease in 10 min	14	9	-	18	11	-
	Significance between (Q) and (R) p	N.S.	N.S.	-	N.S.	N.S.	-
Cerebellum	"0 Min" (Q)*	203.6 ± 76.8	225.6 ± 32.6 (111)	N.S.	292.0 ± 88.5	302.9 ± 46.8 (104)	N.S.
	10 Min (R)*	144.9 ± 45.4	154.6 ± 39.5 (107)	N.S.	184.2 ± 43.5	202.1 ± 26.8 (110)	N.S.
	% Decrease in 10 min	29	32	-	37	33	-
	Significance between (Q) and (R) p	N.S.	0.05	-	0.1	0.01	-

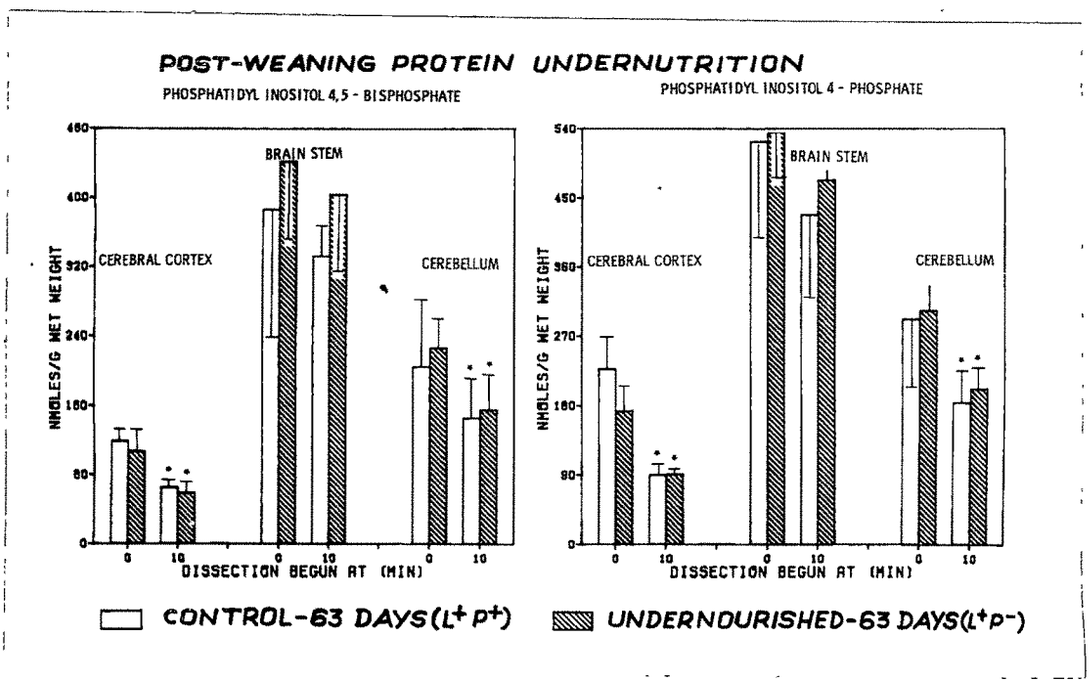
Number of samples analysed at each time point (0 and 10 min) for both controls (L^+P^+) and experimentals (L^+P^-) were four.

* Time taken to separate the brain regions after decapitation and drop them in liquid N_2 was approximately 60 sec. "0 min" and 10 min post-mortem value therefore represents 1 min and 11 min value in brain regions.

N.S. - Not significant.

Numbers in parentheses denote % of control values.

Fig. 26 : Effect of post-weaning protein undernutrition on PolyPI pools in rat brain regions.



Values are expressed as means ± s.d.

* Values significantly different from "0 min". ($p < 0.1$)

\+ Values significantly different from control (L^+P^+) ($p < 0.01$).

x - axis labels refer to the time at which dissection of brain regions was begun following decapitation of the animals and dipping of the brain in liquid N_2.

TABLE 49 : EFFECT OF POST-WEANING NUTRITIONAL REHABILITATION ON THE CONCENTRATION OF POLYPI IN BRAIN REGIONS OF RATS UNDERNOURISHED PRIOR TO WEANING (63 DAYS OLD).

Region		PtdIns(4,5)P_2		Significance between (I) & (II) p	PtdIns4P		Significance between (III) & (IV) p
		L^+P^+- (I)	L^-P^+-(II)		L^+P^+-(III)	L^-P^+-(IV)	
1	2	3	4	5	6	7	8
		nmoles/g wet wt; mean ± s.d.			nmoles/g wet wt; mean ± s.d.		
Cerebral cortex	"0 Min" (Q)*	118.9 ± 13.0	85.6 ±21.2 (72)	0.05	226.7 ± 43.5	174.9 ± 20.4 (77)	0.1
	10 Min (R)*	65.2 ± 8.0	78.6 ±11.0 (121)	N.S.	90.1 ± 12.4	119.3 ± 13.2 (132)	N.S.
	% Decrease in 10 min	45	8	-	60	32	-
	Significance between (Q) and (R) p	0.001	N.S.	-	0.001	0.005	-

contd...

TABLE 49 : contd.

1	2	3	4	5	6	7	8
Brain stem	"0 Min" (Q)*	386.1 ±146.6	357.5 ±132.3 (93)	N.S.	523.2 ±123.3	330.8 ± 54.5 (63)	0.05
	10 Min (R)*	331.5 ±135.5	400.1 ± 42.2 (121)	N.S.	428.1 ±105.4	431.4 ± 59.1 (101)	N.S.
	% Decrease in 10 min	14	-	-	18	-	-
	Significance between (Q) and (R) p	N.S.	-	-	N.S.	-	-
Cerebellum	"0 Min"(Q)*	203.6 ± 76.8	141.1 ± 32.9 (69)	N.S.	292.0 ± 88.5	181.8 ± 35.4 (62)	N.S.
	10 Min (R)*	144.9 ± 45.4	127.9 ± 22.7 (88)	N.S.	184.2 ± 43.5	174.0 ± 25.2 (95)	N.S.
	% Decrease in 10 min	29	9	-	37	4	-
	Significance between (Q) and (R) p	N.S.	N.S.	-	0.1	N.S.	-

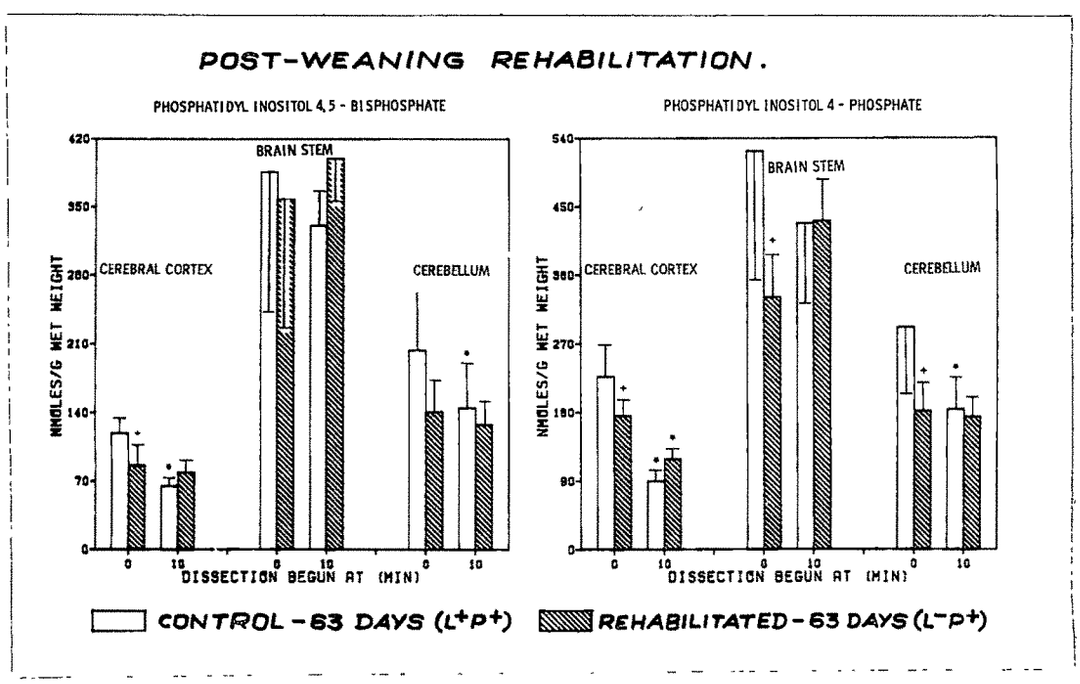
Number of samples analysed at each time point (0 and 10 min) for both controls (L^+P^+) and rehabilitated (L^-P^+) group of animals were four.

* Time taken to separate the brain regions after decapitation and drop them in liquid N_2 was approximately 60 sec. "0 Min" and 10 min post mortem value therefore represents 1 min and 11 min value in brain regions.

N.S. - Not significant.

Numbers in parentheses denote % of control values.

Fig. 27 : Effect of post-weaning nutritional rehabilitation on PolyPI pools in rat brain regions.



Values are expressed as means ± s.d.

* Values significantly different from "0 min" ($p < 0.1$).

\+ Values significantly different from control, (L^+P^+) ($p < 0.1$)

x - axis labels refer to the time at which dissection of brain regions was begun following decapitation of the animals and dipping of the brain in liquid N_2.

TABLE 50 : COMPARATIVE EFFECTS OF NUTRITIONAL ALTERATIONS DURING THE PRE- AND POST-WEANING PERIODS ON THE POST-MORTEM DEPLETION OF POLYPI IN RAT BRAIN REGIONS.

	PtdIns(4,5)P_2					PtdIns4P				
	L^+	L^-	L^+P^+	L^+P^-	L^-P^+	L^+	L^-	L^+P^+	L^+P^-	L^-P^+
Cerebral cortex	-61***	+4	-45***	-45**	-8	-68***	-58***	-60***	-48***	-32***
Brain stem	-17	+4	-14	-9	+12	-30***	+15	-18	-11	+30
Cerebellum	-19**	+17	-29	-32**	-9	-25***	+15	-37	-33**	-4

Values expressed as % change in 10 min samples with respect to "0 min".

\- Indicates decrease in 10 min samples with respect to "0 min".

\+ Indicates increase in 10 min samples with respect to "0 min".

*** Values significantly different from "0min" levels $p < 0.001$ and 0.005

** Values significantly different from "0 min" levels $p < 0.01$, 0.02 and 0.05

* Values significantly different from "0 min" levels $p < 0.1$.

TABLE 51 : COMPARATIVE EFFECTS OF NUTRITIONAL ALTERATIONS DURING THE PRE- AND POST-WEANING PERIODS ON THE 1 MIN POST-MORTEM LEVELS OF POLYPI IN RAT WHOLE BRAIN AND BRAIN REGIONS.

	PtdIns(4,5)P_2			PtdIns4P		
	L^-	L^+P^-	L^-P^+	L^-	L^+P^-	L^-P^+
	as % of control (L^+ or L^+P^+)					
Whole brain	41***	107	95	63***	110	86
Cerebral cortex	30***	90	72**	59***	76**	77*
Brain stem	56**	115	93	52***	102	63**
Cerebellum	55***	111	69	47***	104	62*
Myelin@ (mg/g)	80**	98	88**			

*** Values significantly different from control $p <$ 0.001 and 0.005.

** Values significantly different from control $p <$ 0.01, 0.02 and 0.05.

* Values significantly different from control $p <$ 0.1

@ Values for myelin yield have been taken from studies in our laboratory (Harjit and Ramakrishnan, unpublished).

TABLE 52 : COMPARATIVE EFFECTS OF NUTRITIONAL ALTERATIONS DURING THE PRE- AND POST-WEANING PERIODS ON THE CONCENTRATION OF POLYPI AT 10 MIN POST-MORTEM IN RAT BRAIN REGIONS.

	PtdIns(4,5)P_2			PtdIns4P		
	L^-	$L^+P^\pm$	L^-P^+	L^-	L^+P^-	L^-P^+
	as % of control (L^+ or L^+P^+)					
Myelin@ (mg/g)	80**	98	88**			
Cerebral cortex	80	90	121	77**	101	132
Brain stem	70***	122	121	86	111	101
Cerebellum	79**	107	88	73**	110	95

*** Values significantly different from control $p < 0.001$ and 0.005

** Values significantly different from control $p < 0.01$, 0.02 and 0.05

@ Values for myelin yield have been taken from studies in thislaboratory (Harjit and Ramakrishnan, unpublished).

TABLE 53 : SUMMARY OF EXPERIMENTS II AND IV (WHOLE BRAIN AND BRAIN REGIONS).

PolyPI pool	Tissue	PtdIns(4,5)P_2				PtdIns4P			
		L^-	L^+P^-	L^-P^+	L^-P^-	L^-	L^+P^-	L^-P^+	L^-P^-
1	2	3	4	5	6	7	8	9	10
"0 Min" (A + B)	Whole brain	DEC	NE	RES	DEC	DEC	NE	RES	DEC
Total pool	The total levels of PtdIns(4,5)P_2 and PtdIns4P are decreased by pre-weaning undernutrition but not by post-weaning protein deficiency. The effects at weaning are reversed on nutritional rehabilitation and further decreased on continuation of protein deficiency during the post-weaning period.								
1 Min (A_1 + C)	Whole brain	DEC	NE	RES	DEC	DEC	NE	RES	DEC
Metabolically relatively inert pool + relatively active pool	Cerebral cortex	DEC	NE	RES-P	-	DEC	DEC	RES-P	-
	Brain stem	DEC	NE	RES	-	DEC	NE	RES-P	-
	Cerebellum	DEC*	NE	RES	-	DEC	NE	RES-P	-
	The metabolically active (C) and inert (A1) pools of PtdIns(4,5)P_2 and PtdIns4P are significantly reduced in the whole brain and brain regions by pre-weaning undernutrition but not by post-weaning protein deficiency (exception - cerebral cortex PtdIns4P).								

contd...

Table 53 : contd.

1	2	3	4	5	6	7	8	9	10
	The effects at weaning on both the pools are reversed only partially on nutritional rehabilitation. However, which pool is less reversible is not clear.								
	1 min post-mortem levels of PtdIns(4,5)P_2 and PtdIns4P are not totally representative of the metabolically inert pool in myelinated structures but a portion of it is also located in non-myelinated structures.								
10 Min (A_1)	Cerebral cortex	NE	NE	NE	-	DEC	NE	RES	-
Metabolically relatively inert pool	Brain stem	DEC	NE	RES	-	NE	NE	NE	-
	Cerebellum	DEC	NE	RES	-	DEC	NE	RES	-
	In the cerebral cortex, levels of PtdIns4P and probably PtdIns(4,5)P_2 are decreased by pre-weaning undernutrition and restored on nutritional rehabilitation.								
	In the brain stem levels of PtdIns(4,5)P_2 are decreased by pre-weaning undernutrition and restored on nutritional rehabilitation.								
	In the cerebellum levels of PtdIns(4,5)P_2 and PtdIns4P are decreased by pre-weaning undernutrition and restored on nutritional rehabilitation.								
	Post-weaning protein deficiency has no effect on both lipids in any of the three brain regions.								
0-1 Min	Whole brain	NE	DEC	NE	DEC	NE	NE	NE	NE
Metabolically highly active pool	The metabolically highly active pool of PtdIns(4,5)P_2 and PtdIns4P is not affected by pre-weaning undernutrition.								

contd...

Table 53 : contd.

1	2	3	4	5	6	7	8	9	10
	Protein deficiency during the post-weaning period drastically decreases the metabolically highly active pool of PtdIns(4,5)P_2 suggesting an aberration in myelin and glial cell maturation.								
1-10 Min (C)	Cerebral cortex	ELM	NE	Not RES	-	DEC	DEC	Not-RES	-
Metabolically relatively active pool	Brain stem	ND	ND	ND	-	ND	ND	ND	-
	Cerebellum	ELM	NE	Not-RES	-	ELM	NE	Not-RES	-
	The metabolically active pool (relatively less active than the pool lost during the 1st minute post-mortem) is virtually eliminated in the cerebral cortex and cerebellum by pre-weaning undernutrition and these effects are not reversed on nutritional rehabilitation. Post-weaning protein deficiency decreases this pool of PtdIns4P in the cerebral cortex alone.								
	The differential effects on the 0-1 min and 1-10 min pool suggest the existence of more than two pools. It is possible that one pool might become readily exposed to the enzyme so as to be promptly hydrolysed while the other would be initially protected against hydrolytic cleavage.								

contd...

Table 53 : contd.

DEC = Decreased

NE = Not effect

ND = Not detected

\- = Not determined

RES = Restored

RES-P = Restored partially

Not-RES = Not restored

ELM = Eliminated

EXPERIMENT - V.

EXPERIMENT - V

EFFECTS OF NUTRITIONAL ALTERATIONS DURING PRE- AND POST-WEANING PERIODS ON POLYPI POOLS IN RAT KIDNEY.

As mentioned earlier, the effects of undernutrition on organs of the animal body other than the brain are relatively severe (Widdowson and McCance, 1960; Winick and Noble, 1966). With regard to lipid composition, extensive studies have been carried out on the intestine under conditions of nutritional stress during pre-natal, neonatal and post-weaning stages of development (Arockiadoss, 1982). Phospholipid composition has been shown to be altered in several tissues like the liver, spleen, kidney, lung, heart and testes during neonatal under-nutrition (Khanna and Reddy, 1983; Reddy and Khanna, 1983).

It is well known that like the brain, rat kidney is also endowed with the ability to synthesize myo-inositol and its phospholipid derivatives i.e. PolyPI. Although concentrations of PolyPI are by far the highest in brain, kidney contains substantial quantities of these compounds (Dittmer and Douglas, 1969; Tou et al., 1972; Hauser and Eichberg, 1973). Further, 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., 1973). Several authors suggest that PolyPI in kidney may play a role as important functional

components of the tubule membranes during secretion and reabsorption of solutes from the lumen of the tubule (Farese et al., 1980; 1981; Bidot-Lopez et al., 1981; Benabe et al., 1982; Hruska et al., 1983).

The kidney of an infant, although perfectly adequate for normal purposes, is less adaptable than that of an adult to various insults. A gradual loss of nephrons and reduction in renal functional capacity with age has been reported (Zeman, 1968; Zeman and Stanbrough, 1969). With special reference to inositol phospholipid metabolism, it is interesting to note that patients with chronic renal failure exhibit dramatic elevations in serum levels of free myo-inositol which, in turn, may contribute to the pathogenesis of uremic polyneuropathy (Clements et al., 1973; Pitkanen, 1976). A decreased glomerular filtration rate and disturbed inositol reabsorption have also been shown to be present in advanced forms of glomerulonephritis. In view of the above, levels of PolyPI pools in the kidney were also analysed for possible differential effects of nutritional modifications in neural versus non-neural tissues.

For this study animals used in experiment IV were taken and PolyPI levels determined in the kidney at two time points after death, namely in tissues dissected and frozen either rapidly or after standing at room temperature for 10 min. (See Materials and Methods p. 144).

Effect of neonatal undernutrition on PolyPI levels in kidney

The concentrations of PolyPI in kidney of 21 day old control (L^+) and undernourished (L^-) rats are shown in Table 54 and Fig. 28. Levels of PtdIns(4,5)P_2 and PtdIns4P were decreased by 18% and 34% respectively in the L^- "0 min" samples. In the 10 min L^- samples deficits were observed only in PtdIns4P (38%) indicating that the lower phosphorylated derivative is more sensitive to nutritional deprivation. The PolyPI pool lost between 1 and 10 min post-mortem, however, did not seem to be affected.

Effect of post-weaning protein deficiency on PolyPI levels in kidney

The concentrations of PolyPI in kidney of 63 day old control (L^+P^+) and protein deficient (L^+P^-) rats are given in Table 55 and Fig. 29. It is evident that levels of PtdIns(4,5)P_2 and PtdIns4P are not altered at both time points ("0 min" and 10 min) by this nutritional regimen.

Effect of post-weaning nutritional rehabilitation on PolyPI levels in kidney

The concentrations of PolyPI in kidney of 63 day old control (L^+P^+) and rehabilitated (L^-P^+) rats are given in Table 56 and Fig. 30. Deficits observed in the "0 min" levels of PtdIns(4,5)P_2 at weaning were restored completely while a

partial catch up was observed in PtdIns4P. In the 10 min samples the decreases observed in PtdIns4P at weaning continued to persist during subsequent rehabilitation in the post-weaning period.

As in the previous experiment, the time required for dissection and freezing of kidneys was 0.75-1 min, so that the results at "0 min" actually represent 1 min post-mortem levels of PolyPI but not the 2 sec levels as in the case of whole brain where heads were frozen in liquid N_2 after decapitation. Since freezing of the whole animal and removal of the kidney from bodies frozen in liquid N_2 was difficult, the metabolically highly active pool lost during the first minute could not be determined in this tissue. However, PolyPI levels at 10 min post-mortem were also estimated. Significant losses (25-40%) during this time period (1-10 min post-mortem) could be detected only in PtdIns(4,5)P_2 in all the five (L^+L^-, L^+P^+, L^+P^- and L^-P^+) groups of animals. As in the case of brain it is not known if the lability post-mortem of these lipids in kidney is due to a diesteratic or monoesteratic cleavage or a combination of both enzymes. The activities of the hydrolases are however higher in the brain when compared to kidney (Table 11). Further, the extent of losses post-mortem in PolyPI levels would also depend on the Ca^{2+} ion concentration which regulates the activities of these phosphohydrolases (Table 13) and also the accessibility of the different pools to these enzymes in the membrane.

The effects of varying nutritional conditions on the 1 min post-mortem levels of PolyPI in brain and kidney and 10 min post-mortem levels in kidney are given in Table 57. Brain and kidney show similar amount of deficits in the 1 min post-mortem levels of PtdIns4P at weaning. These deficits continue to persist in the kidney while there is a partial reversal in the case of brain on subsequent rehabilitation during the post-weaning period. Deficits observed in PtdIns(4,5)P_2 are lower than PtdIns4P in the kidney while the reverse is true for the brain, The preferential effects on PtdIns4P in kidney indicates that the lower phosphorylated derivative is more sensitive to nutritional deprivation in this tissue. This is also true in the 10 min post-mortem levels of PolyPI in kidney where PtdIns4P is preferentially reduced at weaning and the deficits continue to persist on subsequent rehabilitation.

A preferential decrease of PtdIns4P as compared with PtdIns(4,5)P_2 suggests that this lipid is not merely an intermediate in the synthesis and catabolism of PtdIns(4,5)P_2. It is interesting to note that a selective effect on PtdIns4P metabolism in response to c-AMP is observed in rabbit kidney cortex (Baricos et al., 1979). Separate enzymes mediating the phosphodiesteratic cleavage of PtdIns4P and PtdIns(4,5)P_2 have been demonstrated in kidney cortex (Tou et al., 1973), while a single enzyme appears to attack both substrates in the

brain (Keough and Thompson, 1970). The existence of separate enzymes could partially explain the preferential decrease of PtdIns4P as well as the different effects of nutritional deprivation on the two lipids observed in kidney as compared with brain.

Several authors have studied the influence of hormones on PolyPI metabolism and shown that PTH which regulates a variety of renal functions rapidly increase the concentrations of PtdIns(4,5)P_2 and PtdIns4P in rabbit kidney cortex and this effect is abolished by pretreatment with cycloheximide which also inhibits certain renal functions - phosphaturia and amine acid transport (Farese et al., 1981; Bidot Lopez et al., 1981). However, the functional importance of the PolyPI effect in PTH action is presently unknown. Khanna and Reddy (1983) observed no major changes in the concentration of different phospholipids from the kidney during neonatal undernutrition. However, these authors did not report PolyPI levels, since their extraction procedure did not permit the isolation of these lipids. Tou et al (1972) reported no changes in kidney PolyPI levels of rats starved for 48 h, whereas an increase in PolyPI specific radioactivity was observed after injection of ^{32}P. What relation does the influence of nutritional status on PolyPI metabolism has to renal functions is not known.

In sum, these preliminary studies indicate that protein deficiency may have different effects on PolyPI pools in

neural (brain and non-neural (kidney) tissues. The factors regulating these effects in different tissues remain to be investigated.

TABLE 54 : EFFECT OF PRE-WEANING UNDERNUTRITION ON (a) THE BODY AND KIDNEY WEIGHTS OF RATS (b) THE CONCENTRATION OF POLYPI POOLS IN RAT KIDNEY (21 DAYS OLD).

	L^+ - I n_o = 4 n_{10} = 4	L^- - II n_o = 4 n_{10} = 4	Significance between (I) and (II) p
	mean ± s.d.		
Body weight (g)+	43.0 ± 3.0	18.0 ± 1.0	0.001
Kidney weight (g)+	0.50 ± 0.04	0.26 ± 0.02	0.001
	nmoles/g wet wt; mean ± s.d.		
PtdIns(4,5)P_2			
"0 Min" (Q)*	39.4 ± 3.9	32.3 ± 3.6	0.05
10 Min (R)	26.7 ± 7.7	20.0 ± 3.5	N.S.
% Decrease in 10 min	32	38	
Significance between (Q) and (R) p	0.05	0.005	
PtdIns4P			
"0 Min" (S)*	74.1 ± 7.8	48.9 ± 2.1	0.001
10 Min (T)	63.6 ± 16.9	39.4 ± 4.1	0.05
% Decrease in 10 min	14	19	
Significance between (S) and (T) p	N.S.	0.01	

n_o - Number of samples used for determination of PolyPI levels at "0 min".

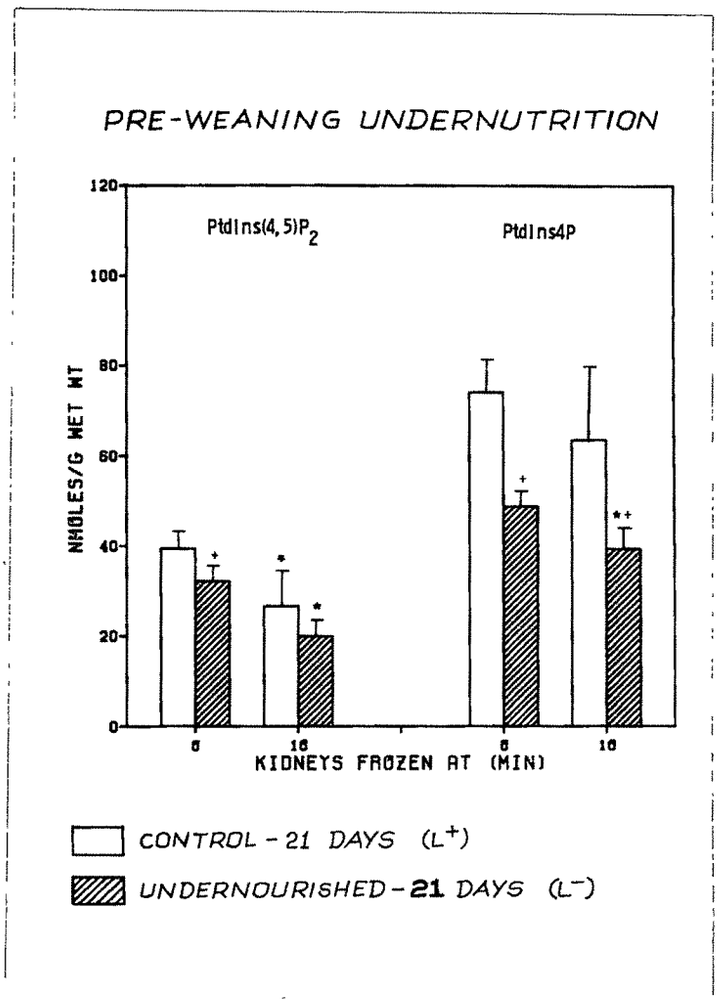
n_{10} - Number of samples used for determination of PolyPI levels at 10 min.

* Time taken to remove the kidneys after decapitation and drop them in liquid N_2 was approximately 60 sec. "0 min" post-mortem value therefore represents 1 min value in kidney.

\+ Number of observations for (1) body weight L^+ = 36; L^- = 44
(2) kidney weight L^+ = 8; L^- = 8.

NS - Not significant.

Fig. 28 : Effect of pre-weaning undernutrition on PolyPI pools in rat kidney.



Values are expressed as means ± s.d.

* Values significantly different from "0 min" ($p < 0.05$).

\+ Values significantly different from control (L^+) ($p < 0.05$).

x - axis labels refer to the time at which dissection of kidney was begun following decapitation of the animals.

TABLE 55 : EFFECT OF POST-WEANING PROTEIN DEFICIENCY ON (a) THE BODY AND KIDNEY WEIGHTS OF RATS (b) THE CONCENTRATION OF POLYPI POOLS IN RAT KIDNEY (63 DAYS OLD).

	L^+P^+ - I n_o = 2 n_{10} = 2	L^+P^- - II n_o = 4 n_{10} = 4	Significance between (I) and (II) p
	mean ± s.d.		
Body weight (g)+	280 ± 37	50 ± 6	0.001
Kidney weight (g)+	2.35 ± 0.31	0.57 ± 0.07	0.001
	nmoles/g wet wt; mean ± s.d.		
PtdIns(4,5)P_2			
"0 min" (Q)*	99.4 ± 2.8	86.5 ± 10.2	N.S.
10 Min (R)	60.0 ± 11.0	54.4 ± 6.5	N.S.
% Decrease in 10 min	40	37	
Significance between (Q) and (R) p	-	0.001	
PtdIns4P			
"0 Min" (S)*	108.4± 9.8	115.1 ± 13.8	N.S.
10 Min (T)	112.4± 5.9	107.3 ± 9.4	N.S.
% Decrease in 10 min	-	7	
Significance between (S) and (T) p	-	N.S.	

n_o - Number of samples used for determination of PolyPI levels at "0 min".

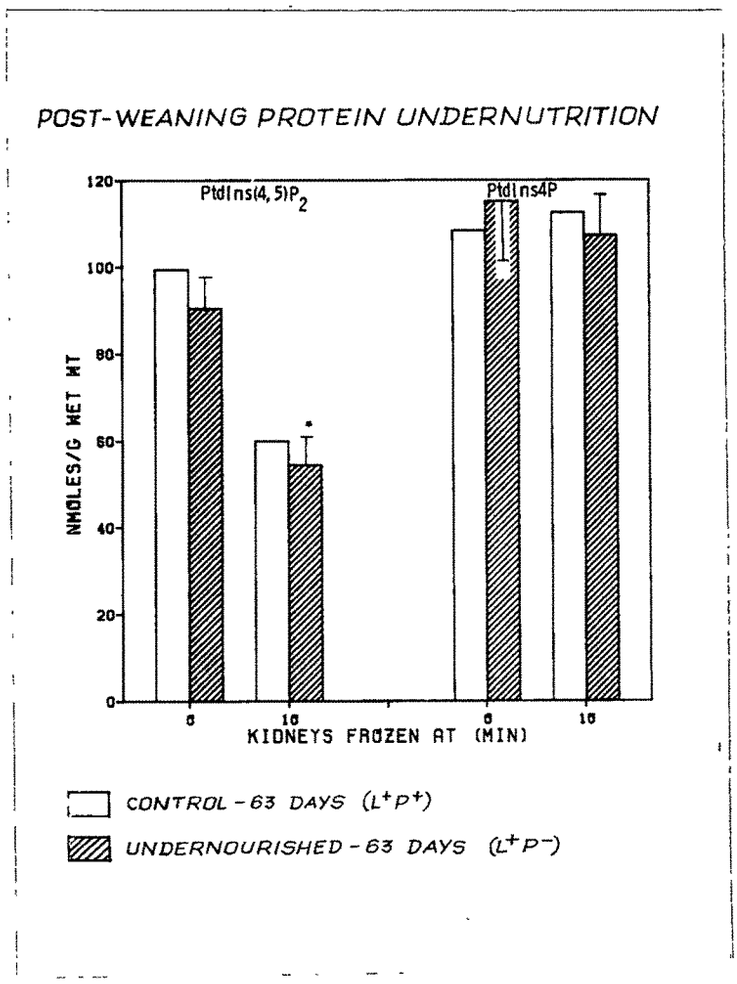
n_{10} - Number of samples used for determination of PolyPI levels at 10 min.

* - Time taken to remove the kidneys after decapitation and drop them in liquid N_2 was approximately 60 sec. "0 min" post-mortem value therefore represents 1 min value in kidney.

\+ - Number of observations for (1) body weight L^+P^+=16; L^+P^-=29
(2) kidney weight L^+P^+=8; L^+P^-=8

N.S. - Not significant.

Fig. 29 : Effect of post-weaning protein undernutrition on PolyPI pools in rat kidney.



Values are expressed as means ± s.d.

* Values significantly different from "0 min". ($p < 0.001$).

TABLE 56 : EFFECT OF POST-WEANING NUTRITIONAL REHABILITATION ON (a) THE BODY AND KIDNEY WEIGHTS OF RATS (b) THE CONCENTRATION OF POLYPI POOLS IN KIDNEYS OF RATS UNDERNOURISHED PRIOR TO WEANING (63 DAYS OLD).

	L^+P^+ - I n_0 = 2 n_{10} = 2	L^-P^+ - II n_0 = 4 n_{10} = 4	Significance between (I) and (II) p
	mean ± s.d.		
Body weight (g)+	280 ± 37	240 ± 37	0.01
Kidney weight (g)+	2.35 ± 0.31	1.91 ± 0.08	0.005
	nmoles/g wet wt; mean ± s.d.		
PtdIns(4,5)P_2			
"0 Min" (Q)*	99.4 ± 2.8	108.2 ± 26.1	N.S.
10 Min (R)	60.0 ± 11.0	40.9 ± 7.8	N.S.
% Decrease in 10 min	40	62	
Significance between (Q) and (R) p	-	0.005	
PtdIns4P			
"0 Min" (S)*	108.4 ± 9.8	80.8 ± 9.4	0.05
10 Min (T)	112.4 ± 5.9	71.9 ± 19.7	0.05
% Decrease in 10 min	-	11	
Significance between (S) and (T) p	-	N.S.	

n_0 - Number of samples used for determination of PolyPI levels at "0 min".

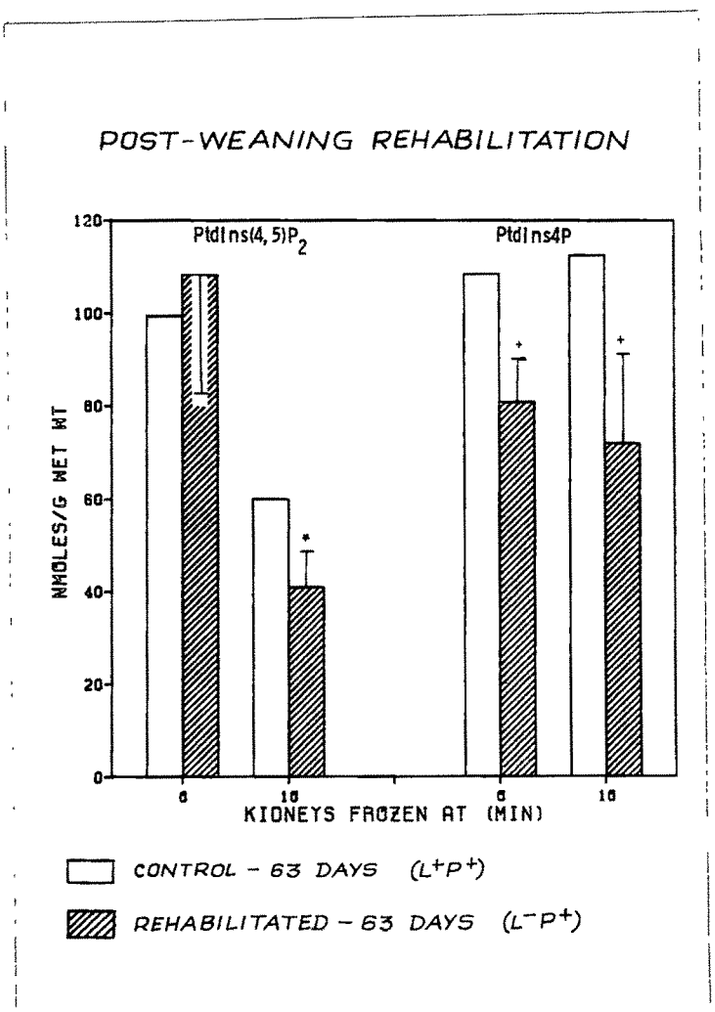
n_{10} - Number of samples used for determination of PolyPI levels at 10 min.

* - Time taken to remove the kidneys after decapitation and drop them in liquid N_2 was approximately 60 sec. "0 Min" post-mortem value therefore represents 1 min value in kidney.

\+ - Number of observations for (1) body weight L^+P^+=16; L^-P^+=16
(2) kidney weight L^+P^+=8; L^-P^+=8

N.S. - Not significant.

Fig. 30 : Effect of post-weaning nutritional rehabilitation on PolyPI pools in rat kidney.



Values are expressed as means ± s.d.

* Values significantly different from "0 min" ($p < 0.005$).

\+ Values significantly different from control (L^+P^+) ($p < 0.05$).

TABLE 57 : EFFECT OF VARYING NUTRITIONAL CONDITIONS ON THE 1 MIN POST-MORTEM LEVELS OF POLYPI IN BRAIN AND KIDNEY AND 10 MIN POST-MORTEM LEVELS OF POLYPI IN KIDNEY.

	PtdIns(4,5)P_2			PtdIns4P		
	L^-	L^+P^-	L^-P^+	L^-	L^+P^-	L^-P^+
	as % of control (L^+ or L^+P^+)					
1 Min						
Brain	41***	107	95	63***	110	86
Kidney	82**	87	109	66***	106	75**
10 Min						
Kidney	75	104	68	62**	95	64**

*** Values significantly different from control $p < 0.001$

** Values significantly different from control $p < 0.05$