C H A P T E R III

RESULTS AND DISCUSSION

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Experiment - I

Changes in the lipid composition of the rat brain with age :

As mentioned earlier, the CNS enjoys priority in development because of its well known role as a controller of body functions. Several studies have been carried out on the lipid composition of the developing brain in several species (Brante, 1949; Wells and Dittmer, 1967; Cuzner and Davison, 1968; Dallal and Enstein, 1969; Norton and Poduslo, 1973; deSousa and Horrocks, 1979), but relatively few reports are available on the lipid composition of gray and white matter during development (Brante, 1949; O'Brein and Sampson, 1965; Vanier et al, 1971; Svennerholm and Vanier, 1972). These studies have been mainly carried out on the human brain and no systematic studies have been carried out on the rat brain although the same has served as a model for most of the neurochemical studies including those on the effects of nutritional and environmental factors.

Changes in the gray matter can be expected as a result of the maturation of the neuronal cells including axonal growth and dendritic arborization. Changes in the white matter can be expected as a result of maturation of oligodendroglial cells and increased myelination. Information on the composition of gray and white matter and the proportions of the two would, therefore, provide indices of maturation of these two components. Such information would enable us to interpret more

clearly the changes in the same resulting from nutritional and other stresses.

The present studies in the context were concerned with changes in the composition of gray and white matter in the rat brain during development. To the extent possible data were also obtained on the whole brain. From the comparative data on chemical composition of the whole brain, gray matter and white matter attempts were made to calculate the proportions of gray and white matter.

Groups of rats were killed at 0,1,2,3,4,6,9,12,20 and 52 weeks of age. Comparable numbers of males and females were used for the first seven groups of rats and only males for the last three ages. Data on body weights and brain weights are presented in Table 15. The body and brain weights ~~w~~ are comparable with those reported by others (Wells and Dittmer, 1967; Norton and Poduslo, 1973; deSousa and Horrocks , 1979). The weight of the brain reaches about 70% of the reference (52 weeks) value by 3 weeks of age, whereas the corresponding figure for the whole body weight is about 9%. The corresponding values in percentages are 55 and 5 at 2 weeks, 36 and 3 at one week and 13 and 2 at birth. Thus, the pattern is consistent with the well known priority enjoyed by the brain in ontogenic development. This also accounts for the much smaller increments in brain weights as compared to the body weight (Table 15).

Table 15 : Relative rates of growth of whole body and brain in the rat*.

	age (weeks)									
	0	1	2	3	4	6	9	12	20	52
	mean ± s.e.									
body weight (g)	6.4 ± 0.10	14.5 ± 0.28	25.0 ± 0.20	48.0 ± 1.90	72.0 ± 0.60	104.0 ± 2.2	165.0 ± 3.0	251.0 ± 4.7	286.0 ± 2.6	510.0 ± 2.6
brain weight (g)	0.26 ± 0.004	0.74 ± 0.02	1.15 ± 0.05	1.44 ± 0.01	1.49 ± 0.01	1.65 ± 0.08	1.73 ± 0.02	1.79 ± 0.02	1.94 ± 0.01	2.08 ± 0.01
$\frac{\text{brain weight}}{\text{body weight}} \times 100$	4.2	5.1	4.6	3.0	2.1	1.6	1.1	0.71	0.68	0.41
% of adult weight (i.e. 52 weeks old)										
body weight	2.3	2.8	4.9	9.3	14.1	20.4	32.4	49.2	56.1	100
brain weight	12.7	35.8	55.3	69.2	71.6	79.3	83.2	86.1	93.3	100
% increase per week										
body weight	125	72	92	50	22	20	17	1.8	2.5	
brain weight	182	55	25	3.4	5.4	1.6	1.2	1.1	0.23	

* all the values were corrected to the nearest decimal point.

Changes in moisture content and the concentrations of lipid and the nonlipid residue are shown in Table 16. With age the decrease in the moisture is more in white matter ~~and less~~ than in gray matter, the values being 86% for both gray and white matter at 2 weeks of age and76% and 70% respectively at 52 weeks of age. At all ages the nonlipid residue is found to be more than lipid ~~portion~~ component in the whole brain, gray matter as well as white matter. This contrasts with the human brain specially in the case of white matter in which the lipid ~~portion~~ component is more than the nonlipid residue (Brante, 1949). At this point it is of interest to note that the lipid content of the brain specially the white matter increases as we go up from lower vertebrates to the higher (Brante, 1949). The lipid to nonlipid ratio is more in white matter and less in gray matter at all ages, as expected.

Changes in the concentration of various lipids in whole brain, gray matter and white matter are shown in Table 17. Since gangliosides account for only a very small proportion (2%) of total lipids in the brain, the sum of chloroform: methanol (2:1) soluble lipids i.e. cholesterol, galactolipids and phospholipids has been considered to represent, for all practical purposes, the lipid content of the brain by several investigators (O'Brien and Sampson, 1965; Cuzner et al, 1968; Norton and Poduslo, 1973). In a preliminary study it was found that the sum of these lipids was comparable with the value obtained gravimetrically for total lipids, the values

Table 16 : Proportions of moisture, lipid and non-lipid component of the rat brain with development.

	age (weeks)				
	2	3	6	9	52
moisture (%)					
whole brain	86	81	79	78	74
gray matter	86	82	82	81	78
white matter	86	80	76	74	70
lipid (mg/g fresh weight)*					
whole brain	42	58	75	79	98
gray matter	38	49	59	68	62
white matter	52	85	111	113	138
non-lipid component** (mg/g fresh weight)					
whole brain	96	131	139	144	159
gray matter	99	136	126	137	157
white matter	86	118	125	150	163
lipid (% dry weight)					
whole brain	30	31	35	35	38
gray matter	28	27	32	30	28
white matter	38	42	47	44	46

contd..

Table 16 : contd.

	age (weeks)				
	2	3	6	9	52
non-lipid component (% dry weight)					
whole brain	70	69	65	65	62
gray matter	72	73	68	70	72
white matter	62	58	53	56	54
lipid/non-lipid					
whole brain	0.44	0.44	0.54	0.55	0.62
gray matter	0.38	0.36	0.47	0.42	0.40
white matter	0.61	0.72	0.89	0.75	0.85

* lipid values were taken from Table 17.

** non-lipid component is mainly protein, [illegible] moisture values were mean of 3 to 4 separate estimations. all the values were rounded up to whole figures.

Table 17 : Concentrations of various lipids in the rat brain during development.

	age (weeks)							
	2	3	4	6	9	12	20	52
	mean ± s.e. (mg/g fresh weight)							
(a) cholesterol								
whole brain	11.2 ± 0.24	15.4 ± 0.50	NE	16.9 ± 0.44	18.0 ± 0.25	NE	NE	22.6 ± 0.39
gray matter	9.4 ± 0.06	14.5 ± 0.17	14.7 ± 0.38	14.3 ± 0.33	14.7 ± 0.43	14.4 ± 0.41	14.5 ± 0.35	14.4 ± 0.18
white matter	12.4 ± 0.28	26.1 ± 0.47	26.2 ± 0.10	27.3 ± 0.82	28.1 ± 0.73	29.4 ± 1.65	30.1 ± 0.92	33.4 ± 0.85
(b) galactolipids								
whole brain	2.5 ± 0.05	6.7 ± 0.20	NE	11.4 ± 1.0	13.0 ± 0.22	NE	NE	21.0 ± 0.80
gx gray matter	1.7 ± 0.07	4.2 ± 0.20	4.5 ± 0.25	4.5 ± 0.18	4.5 ± 0.20	4.7 ± 0.17	4.5 ± 0.16	6.2 ± 0.14
white matter	7.5 ± 0.14	16.8 ± 0.23	23.2 ± 0.54	27.0 ± 1.0	28.2 ± 0.56	29.9 ± 0.33	30.9 ± 0.92	33.4 ± 0.85

contd...

Table 17 : contd.

	age (weeks)							
	2	3	4	6	9	12	20	52
	mean ± s.e. (mg/g fresh weight)							
(c) phospholipids								
whole brain	28.2 ± 0.23	35.5 ± 0.50	NE	46.9 ± 0.44	47.8 ± 0.88	NE	NE	54.7 ± 0.85
gray matter	27.0 ± 0.31	30.5 ± 0.55	41.7 ± 0.64	40.3 ± 0.29	39.1 ± 0.42	39.2 ± 0.50	39.8 ± 1.10	41.3 ± 0.90
white matter	32.5 ± 0.82	42.4 ± 0.90	53.8 ± 0.89	56.7 ± 0.77	57.0 ± 0.82	59.6 ± 1.80	63.0 ± 2.20	71.1 ± 1.70
(d) total lipid (a + b + c)*								
whole brain	42	58	NE	75	79	NE	NE	98
gray matter	38	49	61	59	58	58	59	62
white matter	52	85	103	111	113	118	124	138

* this ignores the contribution made by gangliosides but is considered valid for present purposes.

NE = not estimated

all the values were corrected to the nearest decimal points.

differing by not more than 5%. The sum of these three lipids was, therefore, considered as total lipid content.

At all the ages the concentrations of total lipids, cholesterol, galactolipids and phospholipids are more in white matter than in gray matter with intermediate values for the whole brain (Table 17). The white matter is found to contain a higher concentration of galactolipids at all stages. This is consistent with a high concentration of galactolipids in myelin which forms a major component of white matter (Norton and Autilio, 1966). A similar picture was obtained when the concentrations of different lipids in gray and white matter were expressed as ratios (Table 19).

If the values for 52 weeks were considered as reference values the total lipid concentration of gray and white matter reached 95% and 75% respectively of reference values by 4 weeks of age whereas in the whole brain the concentration was 77%, at 6 weeks of age (Table 20). A slow increase in the lipid concentration of the whole brain and white matter throughout the period of study is consistent with the slow accumulation of myelin in the rat brain during this period (Norton and Poduslo, 1973).

The concentrations of cholesterol, galactolipids and phospholipids in whole brain reached respectively 75%, 54% and 86% of reference values by 6 weeks of age. The corresponding values for gray and white matter were 99, 99 and 98 and 82, 81

Table 18 : Concentrations of various phospholipids in the rat brain during development.

	age (weeks)							
	2	3	4	6	9	12	20	52
	mean ± s.e. (mg/g fresh weight)							
(a) ethanolamine phosphoglycerides								
whole brain	9.3 ± 0.32	12.0 ± 0.39	NE	17.2 ± 1.0	18.8 ± 0.40	NE	NE	20.0 ± 1.2
gray matter	8.8 ± 0.31	9.6 ± 0.25	15.0 ± 0.29	15.0 ± 0.63	14.5 ± 0.43	14.0 ± 0.15	14.0 ± 0.42	14.4 ± 0.45
white matter	10.2 ± 0.40	16.1 ± 0.35	22.2 ± 0.39	23.3 ± 0.45	23.4 ± 0.56	24.6 ± 1.82	25.3 ± 1.1	25.3 ± 0.80
(b) choline phosphoglycerides								
whole brain	11.9 ± 0.54	13.6 ± 0.24	NE	16.8 ± 1.2	18.1 ± 0.26	NE	NE	19.2 ± 0.67
white matter	12.5 ± 0.57	14.5 ± 0.53	17.5 ± 0.90	17.5 ± 0.32	17.4 ± 0.85	17.6 ± 0.38	17.3 ± 0.78	17.6 ± 0.46
gray matter	11.4 ± 0.22	13.3 ± 0.48	16.4 ± 0.28	16.4 ± 0.47	15.3 ± 0.24	15.2 ± 0.63	15.8 ± 0.34	15.3 ± 0.37

contd..

Table 18 : contd.

	age (weeks)							
	2	3	4	6	9	12	20	52
	mean ± s.e. (mg/g fresh weight)							
(c) sphingomyelin								
whole brain	1.3 ± 0.11	2.0 ± 0.10	NE	2.4 ± 0.10	2.5 ± 0.27	NE	NE	4.7 ± 0.14
gray matter	0.7 ± 0.05	1.1 ± 0.07	1.2 ± 0.13	2.3 ± 0.15	2.4 ± 0.02	2.4 ± 0.12	2.3 ± 0.20	2.4 ± 0.13
white matter	1.9 ± 0.13	2.4 ± 0.19	2.5 ± 0.18	4.2 ± 0.37	3.9 ± 0.33	4.2 ± 0.28	3.9 ± 0.16	5.0 ± 0.16
(d) serine + inositol phosphoglycerides								
whole brain	4.8 ± 0.15	4.4 ± 0.10	-	7.3 ± 0.58	7.8 ± 0.13	NE	NE	10.2 ± 0.59
gray matter	4.2 ± 0.27	4.7 ± 0.35	5.6 ± 0.35	7.3 ± 0.30	7.0 ± 0.20	7.3 ± 0.43	7.0 ± 0.39	7.3 ± 0.22
white matter	6.1 ± 0.37	7.2 ± 0.28	8.2 ± 0.53	9.8 ± 0.21	11.1 ± 0.62	11.5 ± 0.70	12.0 ± 0.45	15.4 ± 0.33
recovery*								
whole brain	97	90	NE	93	99	NE	NE	99
gray matter	93	94	92	97	100	99	98	95
white matter	95	95	94	97	98	97	93	90

* $\frac{a + b + c + d}{\text{total Phospholipid}} \times 100$

NE = not estimated.

all the values were corrected to the nearest decimal point.

Table 19 : Ratios of white matter to gray matter lipids at different ages*.

	age (weeks)							
	2	3	4	6	9	12	20	52
total lipids	1.4	1.7	1.7	1.9	1.9	2.1	2.1	2.2
cholesterol	1.3	1.8	1.8	1.9	1.9	2.0	2.1	2.3
galactolipids	4.4	4.0	5.2	6.0	6.2	6.4	6.9	5.4
phospholipids	1.2	1.4	1.3	1.4	1.5	1.5	1.6	1.7
ethanolamine phosphoglycerides	1.2	1.7	1.5	1.6	1.7	1.8	1.8	1.8
choline phosphoglycerides	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.2
sphingomyelin	2.7	2.2	2.2	1.8	1.8	1.7	1.7	2.1
serine + inositol phosphoglycerides	1.5	1.5	1.5	1.4	1.6	1.6	1.7	2.1

* values calculated from mean values of Tables 17 and 18.

Table 20 : Lipid composition of developing rat brain as percentage of reference values (i.e. 52 weeks old rat)*.

	age (weeks)						
	2	3	4	6	9	12	20
total lipids							
whole brain	43	59	NE	77	80	NE	NE
gray matter	62	80	98	96	94	94	95
white matter	38	62	75	80	82	86	90
cholesterol							
whole brain	50	68	NE	75	80	NE	NE
gray matter	65	100	102	99	102	100	100
white matter	37	78	78	82	84	85	90
galactolipids							
whole brain	12	32	NE	54	62	NE	NE
gray matter	38	93	98	99	100	103	100
white matter	23	50	70	81	84	90	93
total phospholipids							
whole brain	52	65	NE	86	87	NE	NE
gray matter	65	74	100	98	95	96	96
white matter	46	60	76	80	80	84	89

* values calculated from mean values of Table 17.

NE = not estimated.

and 80 respectively (Table 20). Thus gray matter showed an earlier maturation with regard to lipid composition. This is consistent with the rapid increase in axonal growth and synaptic connectivity during this period in the gray matter (Benjamins and Mckhann, 1976; Vanier et al, 1971) and also the biochemical maturation of nonmyelin components during this period (De Maecioni and Caputto, 1968; Banik and Davison, 1969).

The percentage contribution of different lipid classes to total lipids varies between 2-6 weeks of age in the whole brain, as well as both gray matter and white matter with initial increases in galactolipids and cholesterol (except for whole brain cholesterol value) (Tables 21 and 22, Fig. 2a). By 4-6 weeks of age the percentage contributions of different components reached the stable values. This is also reflected in the molar ratios of the three components (Tables 21 and 22).

The data on the distribution of various phospholipids of whole brain, gray matter and white matter are shown in Table 18. It is reassuring that the sum of the different phospholipid components does not differ appreciably from the value obtained for total phospholipid, the recovery being of the order of 90-100% (Table 18). Between 2-4 weeks of life the concentration of EPG increases much more than that of CPG in whole brain, gray matter and white matter so that the ratio of EPG to CPG increases (Table 22). Both lipids reach peak concentrations by

Table 21 : Lipid composition of the rat whole brain, gray and white matter at different ages.

	age (weeks)							
	2	3	4	6	9	12	20	52
	(% of total lipids)							
cholesterol (CHL)								
whole brain	27	27	NE	22	23	NE	NE	23
gray matter	25	29	24	24	25	25	25	23
white matter	24	31	25	25	25	24	24	24
galactolipids (GL)								
whole brain	6	12	NE	15	17	NE	NE	21
gray matter	5	9	7	8	8	8	8	10
white matter	14	20	23	24	25	25	25	24
phospholipids (PL)								
whole brain	67	62	NE	62	61	NE	NE	56
gray matter	71	62	69	68	67	67	67	67
white matter	62	50	52	51	50	51	51	51
CHL : GL : PL								
whole brain	100:22:248	100:45:230	NE	100:68:282	100:74:265	NE	NE	100:91:243
gray matter	100:20:284	100:31:214	100:29:287	100:33:283	100:32:268	100:32:268	100:32:268	100:43:279
white matter	100:58:258	100:54:161	100:92:208	100:96:204	100:100:200	100:104:212	100:104:212	100:100:217

values calculated from mean values of Table 16.

NE = not estimated.

Table 22 : Phospholipid composition of rat whole brain, gray matter and white matter at different ages*.

	age (weeks)							
	2	3	4	6	9	12	20	52
	(% of total phospholipids)							
ethanolamine phosphoglycerides (EPG)								
whole brain	34	38	NE	39	40	NE	NE	37
gray matter	35	34	39	37	37	35	37	37
white matter	33	40	44	43	42	43	43	40
choline phosphoglycerides (CPG)								
whole brain	44	43	NE	38	38	NE	NE	36
gray matter	45	46	43	40	39	38	39	39
white matter	41	36	35	32	31	29	30	28
sphingomyelin								
whole brain	5	6	NE	6	5	NE	NE	9
gray matter	3	4	3	6	6	6	6	6
white matter	6	6	5	8	7	7	7	8

contd...

Table 22 : contd.

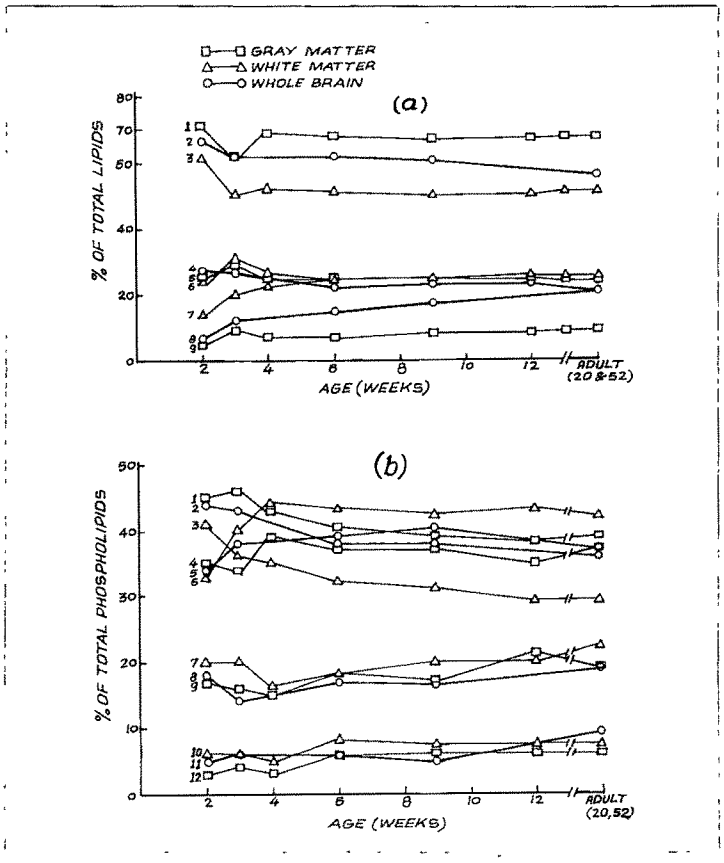
	age (weeks)							
	2	3	4	6	9	12	20	52
	(% of total phospholipids)							
serine + inositol phosphoglycerides								
whole brain	18	14	NE	17	17	NE	NE	19
gray matter	17	16	15	18	17	21	18	19
white matter	20	20	16	18	20	20	20	24
WPG to CPG ratio								
whole brain	0.77	0.88	NE	1.0	1.0	NE	NE	1.0
gray matter	0.78	0.74	0.91	0.93	0.95	0.92	0.95	0.95
white matter	0.80	1.11	1.26	1.34	1.36	1.48	1.43	1.43

* values calculated from mean values of Table 17.

NE = not estimated.

Fig. 2

Developmental changes in the lipid composition of rat brain.



(a) 1,2 and 3 : phospholipids, 4,5 and 6 : cholesterol, 7,8 and 9 : galactolipids.

(b) 1,2 and 3 : choline phosphoglycerides
4,5 and 6 : ethanolamine phosphoglycerides
7,8 and 9 : serine + inositol phosphoglycerides
10, 11 and 12 : sphingomyelin.

4-6 weeks in both gray and white matter. The concentration of sphingomyelin in these two areas increases till 6 weeks of age. This is also true of serine and inositol phosphoglycerides with regard to gray matter but the increases in whole brain and white matter continue for a much longer period (Table 18 and Fig. 2b).

The pattern of changes in the concentrations of different lipids in the whole brain with age compares with those reported by others for the rat brain (Wells and Dittmer, 1967; Cuzner and Davison, 1968; Norton and Poduslo, 1973; deSousa and Horrocks, 1979) and human brain (Brante, 1949; Rouser and Yamamoto, 1969). Similarly the pattern of changes in the concentrations of different lipids in gray and white matter with age are comparable with those reported by others for human brain (Brante, 1949; Vanier et al, 1971; Svennerholm and Vanier, 1972).

It is known that with the progress of myelination the proportion of white matter increases. However, the complexity of the brain makes it very difficult to get precise information on the proportion of white matter as it is difficult to achieve a quantitative separation of gray and white matter. An indirect approach was, therefore, made to get estimates of the proportions of gray m and white matter by comparing the composition of the whole brain with that of gray and white

matter with regard to a selected lipid component using the equation.

$$100\ r = xp + (100-x)q$$

where x represents the percentage of gray matter and p, q, and r the concentrations of the lipid respectively in gray matter, white matter and whole brain. Solving the equation for x should give us the proportions of gray and white matter.

This approach was tried with total lipids as well as galactolipids. The most consistent pattern was obtained when galactolipid concentration was used (Table 23).

To investigate further the validity of the values obtained for gray and white matter myelin content as percentage of dry white matter was calculated. Since myelin in the brain is present predominantly in the white matter, the myelin content of the whole brain reported by Norton and Poduslo (1973) was used for calculating myelin as percentage of dry white matter. As the myelin yield was reported to be only 60% by the same authors, the actual myelin content of the brain was calculated by multiplying their reported value by 5/3.

It can be seen that myelin content as percentage dry weight of white matter is 43-46 when calculated on the basis of galactolipid concentrations in whole brain, gray matter and white matter whereas it varies from 38-52 per cent when total lipid was used for calculations (Table 23). Thus the values

Table 23 : Proportions of gray and white matters of rat brain with age*.

	calculated based on									
	galactolipids					total lipids				
	age (weeks)					(age(weeks)				
	2	3	6	9	52	2	3	6	9	52
% whole brain										
gray matter	85.7	80.8	69.4	64.2	46.0	73.2	76.7	73.2	62.7	53.6
white matter	14.3	19.2	30.6	35.8	54.0	26.8	23.3	26.8	37.3	46.4
content (mg/brain)										
gray matter	986	1164	1124	1111	957	842	1105	1186	1085	1115
white matter	164	276	496	619	1123	308	335	434	645	965
dry weight (mg/brain)										
gray matter	138	209	202	211	211	118	199	214	206	245
white matter	23	56	117	163	338	43	68	102	170	290
myelin (mg/brain)**	6.0	25.5	51.6	70.2	151	6.0	25.5	51.6	70.2	151
myelin (% dry white matter)	26	46	44	43	45	14	38	51	40	52

* for calculating the proportions the mean values were taken for lipids from Table 17.

** values calculated per brain from the data of Norton and Poduslo (1973) except for 6 weeks of age taken from Reddy et al (1980).

all the myelin was assumed to be present in the white matter (Sabri and Davison, 1977).

for myelin as percentage of the dry weight of white matter came to around 45% at all the ages, except in the case of 2 weeks old rats (Table 23). It is known that myelination starts just before this period in the rat brain (Norton, 1976). These values are lower than the reported value of 50% for bovine (Norton and Autilio, 1966) and human (Brante, 1949) white matter. At this point it is of interest to note that data reported by Suzuki et al (1968) suggest a lower myelin content of white matter in the rat brain as compared to that in the bovine brain. This could be due to species differences in myelination. These observations raise a question whether the rat brain white matter contains less myelin compared to bovine brain white matter. Since the myelin as percentage of dry white matter did not show appreciable change with age it can be concluded that a pure fraction of white matter has been obtained at all ages.

To further check the validity of the values obtained for the proportions of gray and white matter, the concentrations of different lipids were recalculated for the whole brain from gray and white matter lipids and compared with the analysed values for the whole brain (Table 24 and Fig. 4). A fair measure of agreement was found between the analysed and calculated values. When the differences were expressed as percentages of analysed values, the differences did not exceed ten percent.

The proportions of gray and white matter obtained (81% and 19%) in the case of 3 weeks old rats were reasonably close to the generous estimates of 75% and 25% by Norton andPoduslo (1973). The proportions obtained in the adult rat compares with

Table 24 : Comparison of analysed and calculated values for brain lipids.

	age (weeks)														
	2			3			6			9			52		
	A	B	% diff.	A	B	% diff.	A	B	% diff.	A	B	% diff.	A	B	% diff.
	(mg/g fresh weight)														
total lipids	42.0	40.0	4.0	58.0	56.0	2.4	75.0	75.0	0	79.0	78.0	1.1	98.0	103	4.8
cholesterol	11.2	9.8	12.0	15.4	16.7	8.4	17.0	18.3	8.3	18.0	19.5	8.3	22.6	24.6	8.1
galacto-lipids	2.5	2.5	0	6.7	6.7	0	11.4	11.4	0	13.0	13.0	0	21.0	21.0	0
phospho-lipids	28.2	27.8	1.4	35.5	32.7	7.9	46.9	45.4	3.2	47.8	45.5	4.8	54.7	57.4	4.9
ethanola-mine phospho-glycerides	9.3	9.0	3.5	12.0	10.9	9.2	17.2	17.5	1.7	18.8	17.7	6.2	20.0	20.3	1.5
choline phospho-glycerides	11.9	11.6	2.5	13.6	13.6	0	16.8	16.8	0	18.1	16.1	10	19.2	16.5	14

A : analysed

B : calculated from the proportions of gray and white matter obtained by the equation using galacto-lipids

% diff. : % difference

the valuesof 50% each for gray and white matter reported for the adult human brain (Brante, 1949).

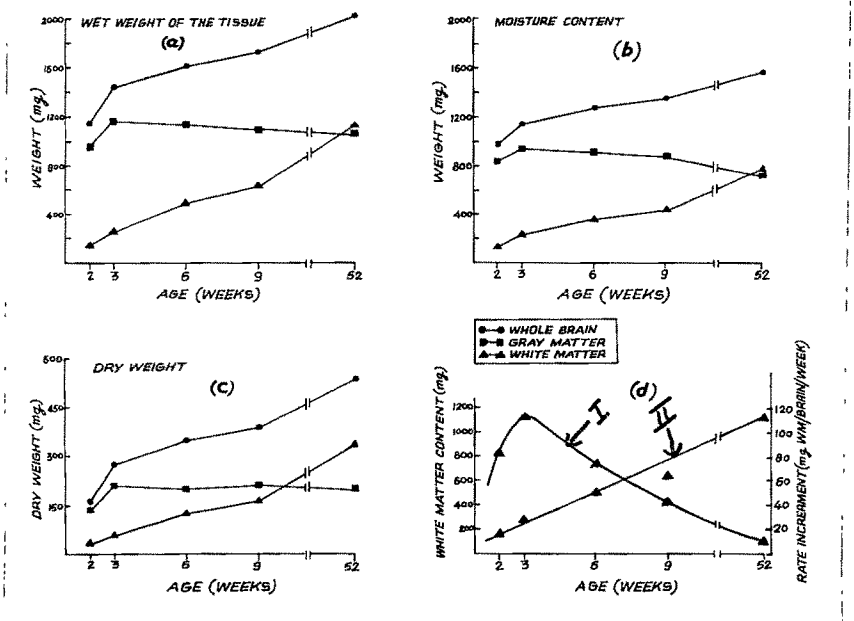
The weight of gray matter reached its maximum value by 3 weeks of age whereas the amount of white matter increased for a much longer period (Figs. 3a,b and c). The apparent decrease in the weight of gray matter is consistent with the probability that with the probability that with the progress of myelination some of the matter classified as gray at 9 weeks may tend to be classified as white at 52. The lack of a similar change in dry weight is due to the decline in moisture content between 9 and 52 weeks from 81% to 78%. The rate of increment of white matter (mg/week/brain) reached a peak value by 3 weeks of age (Fig.3d). It is reported that the rate of increment of myelin in the whole brain also reaches a peak value by 3 weeks of age (Norton and Poduslo,1973).

Changes in the contents of different lipids in whole brain and gray and white matter are shown in Fig. 4. White matter lipids showed a steady increase with age whereas the gray matter lipids reached maximum values by 6 weeks of age. The whole brain lipids also increased with age (Fig. 4). This may be due to continued increase in white matter content.

The percentage contribution of white matter lipids to whole brain increases with age whereas thatof gray matter decreases (Fig. 5). The contribution of gray matter to the whole brain comes down from 86% at 2 weeks to 46% at 52 weeks whereas the contribution of white matter goes up from 14% at 2 weeks to 54% at 52 weeks. The contribution of the total

Fig. 3

Changes in different components of rat brain during development.



3(d) I. rate increment
(mg WM/brain/week)

II. white matter content
(mg)

Fig. 4

Changes in the content of different lipids in whole brain, gray matter and white matter with age.

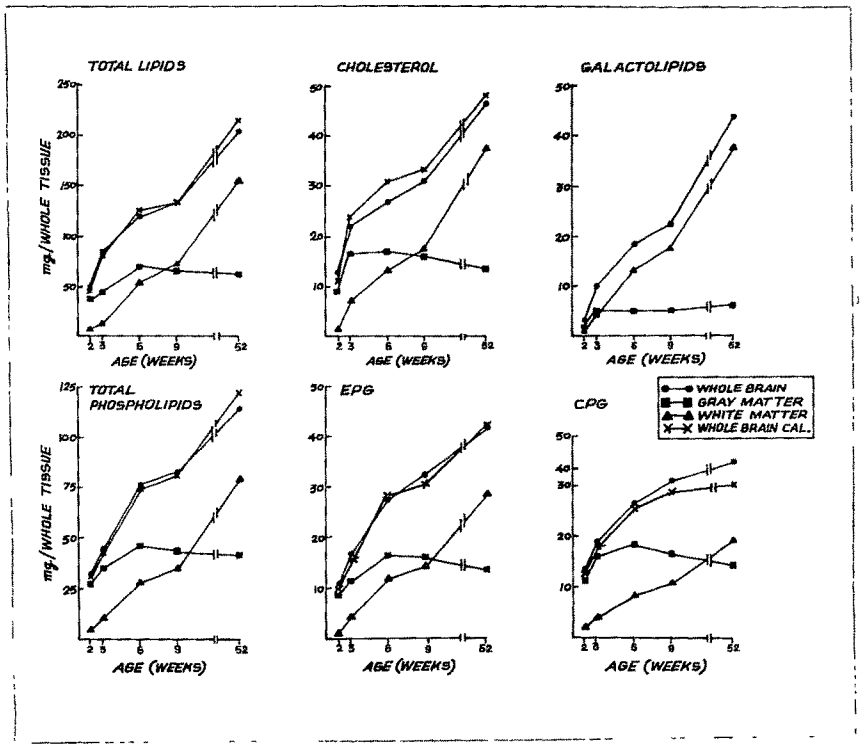
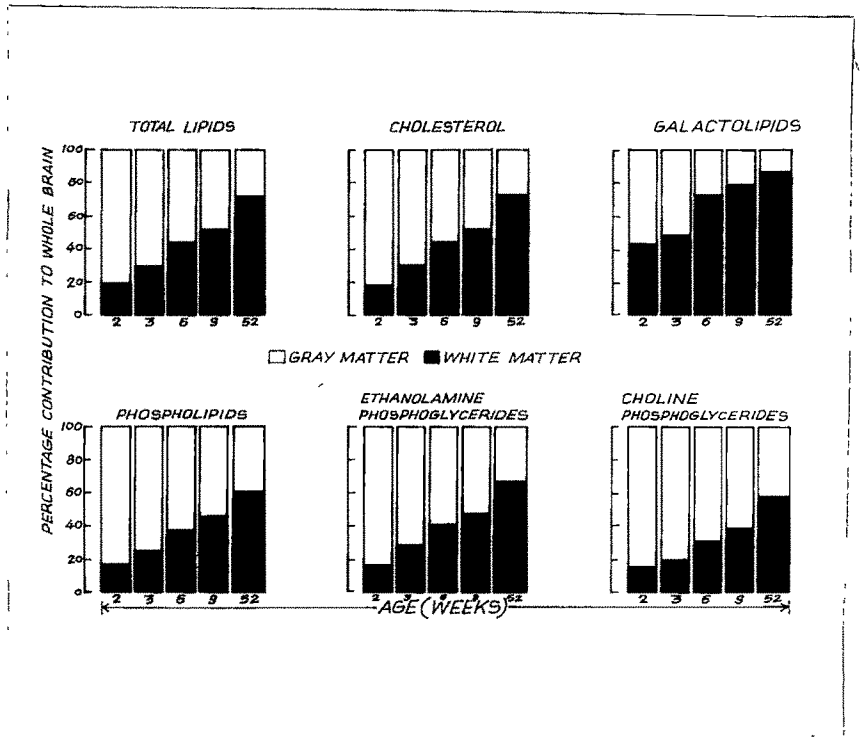


Fig. 5

Percentage contribution of different lipids by gray and white matter to the whole brain in rat during development.



lipids, cholesterol, galactolipids and phospholipids of white matter to the whole brain were greater than that of weight, the values being 19, 18, 42 and 17 per cent respectively at 2 weeks of age and 72, 70, 86 and 60 per cent at 52 weeks of age. This is consistent with the higher lipid concentration of white matter to begin with, which further increases during development.

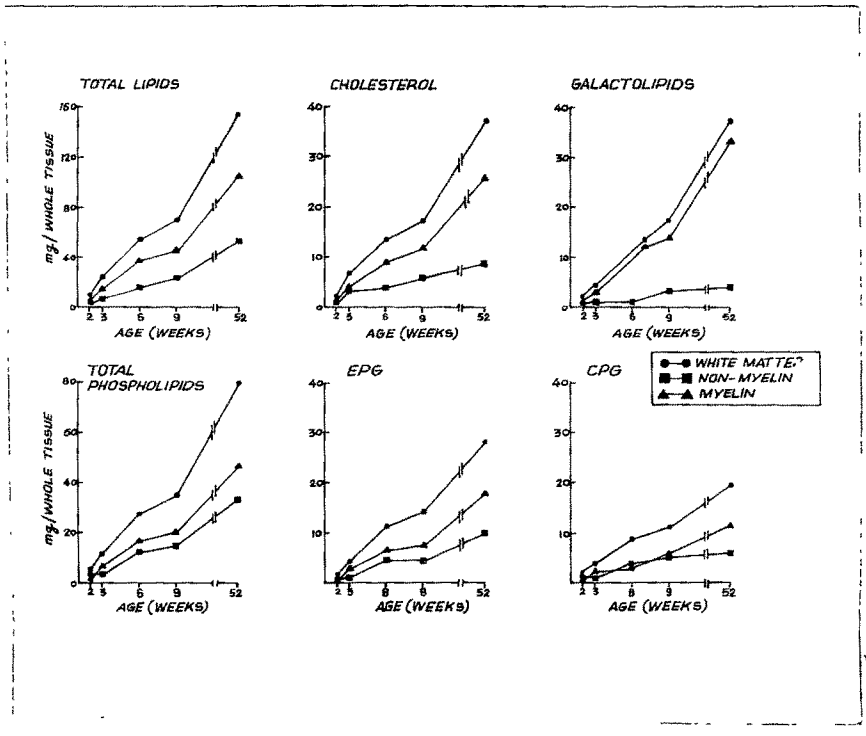
The changes in the content of different lipids in white matter, myelin and nonmyelin components of white matter are shown in Fig. 6. As expected, it is clearly seen that the content of myelin lipids is higher than that of the nonmyelin lipids. The differences between myelin and nonmyelin lipids increase with age and are maximum for galactolipids and minimum for phospholipids (Fig. 6).

The percentage contribution of myelin and nonmyelin lipids to the white matter with age is shown in Fig. 7. The contribution of myelin and nonmyelin are 65 and 35% respectively to white matter at all ages except at an early age point (2 weeks). These values are in good agreement with previously reported values for bovine white matter (Norton and Autilio, 1966). The contribution of myelin phospholipids, cholesterol and galactolipids are 43, 54 and 77% respectively at 2 weeks of age and reach 58, 73 and 88% by 52 weeks of age.

Even though the estimate derived for the contribution of the nonmyelin component white matter lipids is in good agreement

Fig. 6

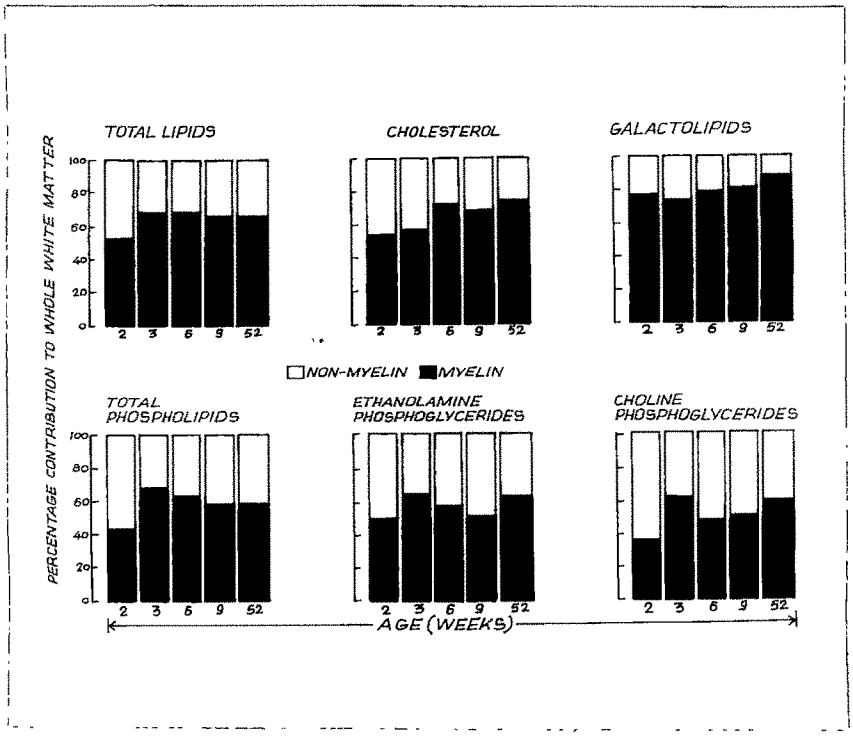
Content of different lipids in myelin and nonmyelin components of white matter with age.



Values for myelin were taken from Norton and Poduslo (1973).

Fig. 7

Percentage contribution of different lipids by myelin and nonmyelin components to the white matter with age.



with the previously reported values for bovine white matter (Norton and Autilio,1966), the pattern with regard to different lipids in nonmyelin lipids seem to be different (Table 25).

The most striking differencesx are with regard to the levels of galactolipids and cholesterol in nonmyelin lipids. Norton and Autilio (1966) have reported 28% of galactolipids and 14% of cholesterol in nonmyelin component lipids whereas the values obtained in the present study are 15% for galactolipids and 24% for cholesterol. At this point it is of interest to note that in the nonmyelin lipids of rat spinal cord cholesterol, galactolipids and phospholipids form 14, 16 and 70 per cent of total lipids respectively (Smith, 1969).

The nonmyelin component of white matter contains mainly axons, oligodendroglia and supporting astroglial cells. From Table 5 it can be seen that none of the components present in nonmyelin white matter has more than 20% of the total amount of galactolipids. The reported values for the galactolipids of nonmyelin white matter as 28.2% (Norton and Autilio, 1966) is rather surprising.

When the distribution of lipids in the different subcellular fractions of the brain (other than myelin) were examined, the contribution of galactolipids to total lipids was not more than 20% in any of the fractions (Cuzner and Davison, 1968). This further supports the lower levels of galactolipids in the nonmyelin component of white matter in

the present study. However, the lipid composition of the nonmyelin component of white matter does not show any consistent pattern with age (Table 25).

From these studies it is found that the amount of gray matter reaches adult values by 3 weeks of age whereas white matter showed a steady increase till one year of age. The concentration of different lipids seems to be more in white matter than in gray matter at all the ages studied. This is specifically true with regard to galactolipids. This is consistent with the fact that the galactolipids in the brain are mainly present in myelin which is rich in white matter. The concentrations of different lipids reaches the adult value by 4 weeks of age whereas white matter showed steady increase till one year of age. However, the composition of different lipids as percentage of total lipids reaches stable values by 4-6 weeks of age in both gray and white matter. Thus the increasing concentrations of different lipids in white matter with age were due to the slow accumulation of lipid rich myelin. The proportion of white matter in whole brain increased with age with corresponding decrease in the proportion of gray matter ~~and that of gray matter decreased.~~ Similarly the contribution of white matter lipids to whole brain lipids increased with age whereas that of gray matter lipids decreased. White matter contains around 45 and 55 per cent of myelin and nonmyelin components respectively on dry weight basis at all the ages except at 2 weeks of age. However, two thirds of white matter lipids were contributed by myelin. This is

Table 25 : Lipid composition of the non myelin component of rat brain white matter at different ages*.

	age (weeks)				
	2	3	6	9	52
	(% of total lipids)				
cholesterol	23	41	24	23	18
galactolipids	7	11	16	14	9
phospholipids	70	48	60	63	73
	(% of total phospholipids)				
ethanolamine phosphoglycerides	28	42	46	47	30
choline phospho-glycerides	40	40	41	35	21

* values calculated from non-myelin lipid values of Fig. 6.

specially true with regard to myelin specific galactolipids which form around 80% of the galactolipids in white matter.

EXPERIMENT - II

Effects of undernutrition on the developing rat brain :

As mentioned earlier, the suckling period in the rat is characterised by morphological and biochemical changes associated with the rapid maturation of the brain. This involves neuronal growth with increase in cell size, axonal growth, dendritic arborization and synaptogenesis (Davison and Dobbing, 1968; Vanier et al, 1971; Benjamins and Mckhann, 1976). It is also characterised by greater myelination and an increase in the proportion of white matter (Norton and Poduslo, 1973). Several studies have demonstrated the adverse effects of nutritional stress during this period on the development of the brain as judged by histological (Bass et al, 1970a; Siassi and Siassi, 1973; Krigman and Hogan, 1976) and biochemical studies (Culley and Mertz, 1965; Geison and Waisman, 1970; Rajalakshmi and Nakhasi, 1974; Wiggins et al, 1976; Reddy and Sastry, 1978). Some of these changes are associated with changes in gray matter (e.g. decreased dendritic arborization, axonal growth and reduced number of synapses per unit area) whereas others suggest changes in white matter (e.g. decreased number of oligodendrocytes and retarded myelination).

Studies were, therefore, carried out on the comparative effects of undernutrition on the proportions of gray and white matter and their lipid contents. Additional studies

were made of the reversibility of the effects observed with dietary rehabilitation after weaning.

Previous studies have shown that neither undernutrition nor protein deficiency in the postweaning period affects the lipid composition of the brain (Dobbing and Widdowson, 1965; Rajalakshmi and Nakhasi, 1974a). Studies were made to find out whether the results are modified by a low plane of nutrition prior to weaning as some studies suggest that the effects of nutritional stress during the postweaning period depend on the previous dietary history of the animal (Rajalakshmi and Telang, 1975).

Pups born in the stock colony on the same day were pooled together and assigned in litters of eight to mothers fed either the stock diet (18% protein (C) or a 5% casein diet (UN). Some of the animals from both groups were killed at 3 weeks of age. The remaining animals in the control group were continued on a 20% protein diet for a period of six weeks (C). The pups reared by low protein mothers were divided into 3 groups. One group received a 20% protein diet ad lib. (UN-HP), another a low protein diet (UN-LP) and the third, the high protein diet in restricted amounts (UN-HP-R).

The animals were caged individually and water was given ad lib. Body weights were recorded once a week and food intake daily.

The rats were decapitated at 9 weeks of age, the brains removed, and the gray and white matter separated and used for lipid estimations.

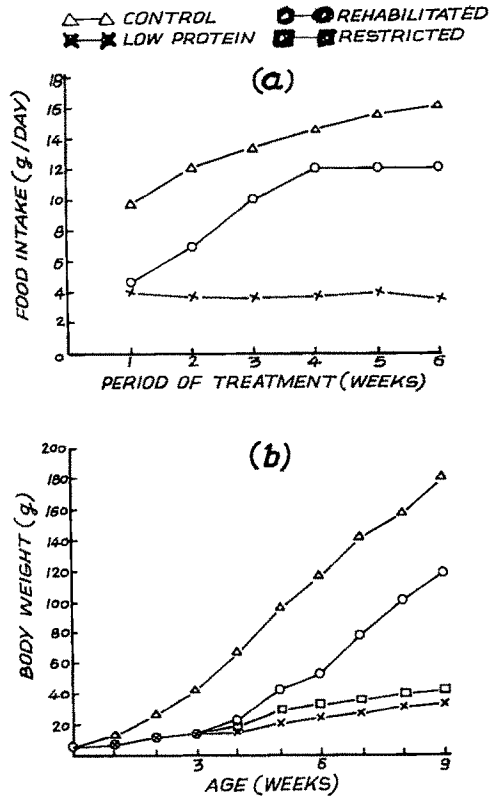
The growth rate and food intake of the rats in different groups are presented in Fig. 8. The body weights of the rehabilitated animals failed to achieve a complete reversal of growth retardation, an observation consistent with expectation on the basis of many other studies (e.g. Culley and Linenberg, 1968; Rajalakshmi et al, 1974; Reddy and Sastry, 1979). As in other studies the food intake of the rehabilitated animals was more in relation to body weight.

Effects of preweaning undernutrition :

Body and brain weights of undernourished rats were 29% and 76% of control values at the age of weaning (i.e. 3 weeks of age). Undernutrition was found to be associated with deficits in the concentrations of cholesterol, galactolipids and phospholipids in white matter whereas in gray matter only galactolipids were found to be affected (Table 26). Among the phospholipids the concentration of total plasmalogens and ethanolamine plasmalogens showed a significant decrease in the white matter followed by a moderate decrease in choline phosphoglycerides (Table 26). The concentration of phospholipid components was not affected h in gray matter. However, the composition of various lipids as percentage of total lipids was not affected during undernutrition both in gray and white matter (Table 27).

Fig. 8

Effects of undernutrition or rehabilitation on the food intake and body weights of rats.



8(a) : food intake of pups after weaning.

8(b) : body weight of pups.

Table 26 : Effects of preweaning undernutrition on the lipid composition of gray and white matter in the rat brain at 21 days of age[@].

	gray matter		white matter	
	C	UN	C	UN
1	2	3	4	5
no. of observations	8 - 9	7 - 9	8 - 9	7 - 9
		mean ± s.e.		
body weight (g)	48.0 ± 1.90	13.7 ± 0.35***		
brain weight (g)	1.44 ± 0.01	1.10 ± 0.02***		
		(mg/g fresh weight)		
lipids				
(a) cholesterol	14.2 ± 0.30	14.4 ± 0.24	27.0 ± 0.30	24.4 ± 0.29***
(b) galactolipids	4.4 ± 0.20	3.8 ± 0.13*	16.5 ± 0.26	13.2 ± 0.55***
(c) phospholipids	30.0 ± 0.91	28.6 ± 0.62	42.4 ± 0.90	35.9 ± 1.10***
gangliosides (µg/g)	NE	NE	525 ± 15	516 ± 36
total lipids (a + b + c)	48.6	46.8	25.9	73.5
phospholipid components total plasmalogens	4.7 ± 0.26	4.5 ± 0.17	9.6 ± 0.47	6.5 ± 0.34***

contd....

Table 26 : contd.

1	2	3	4	5
(d) ethanolamine plasmalogens	9.7 ± 0.54	8.1 ± 0.38	8.8 ± 0.43	5.6 ± 0.24***
(e) diacyl GPE			7.2 ± 0.22	7.5 ± 0.31
(f) choline phospho-glycerides	13.1 ± 0.66	13.4 ± 0.52	14.5 ± 0.52	12.8 ± 0.58*
(g) sphingomyelin	1.1 ± 0.07	1.3 ± 0.09	3.7 ± 0.19	3.4 ± 0.16
(h) inositol phospho-glycerides	1.2 ± 0.12	1.2 ± 0.17		
(i) serine phosphoglycerides	1.9 ± 0.15	2.0 ± 0.15	4.7 ± 0.27	5.1 ± 0.40
recovery of phospholipids $\left(\frac{d + e + f + g + h + i}{C} \times 100\right)$	90	91	92	96

C : control, UN : undernourished.

values marked with asterisk significantly different from control values, p less than 0.05 for *, 0.01 for ** and 0.001 for ***.

@ all the values were corrected to the nearest decimal point.

NE : not estimated.

Table 27 : Effects of preweaning undernutrition and postweaning undernutrition protein deficiency or rehabilitation on lipid content of gray and white matter of rat brain*.

	age (weeks)											
	3				9							
	gray matter		white matter		gray matter				white matter			
	C	UN	C	UN	C	UN-HP	UN-HP-R	UN-LP	C	UN-HP	UN-HP-R	UN-LP
1	2	3	4	5	6	7	8	9	10	11	12	13
	(% of total lipids)											
cholesterol(CHL)	29	31	31	33	25	26	25	25	25	26	25	25
galactolipids(GL)	9	8	19	18	8	8	8	8	25	24	26	25
phospholipids(PL)	62	61	49	49	67	67	66	68	50	50	49	50
CHL : GL : PL	100: 31: 211	100: 26: 199	100: 61: 157	100: 55: 148	100: 32: 268	100: 32: 272	100: 31: 254	100: 32: 272	100: 100: 200	100: 92: 192	100: 104: 196	100: 100: 200
plasmalogens	10	10	11	9	9	9	9	9	15	15	15	15
gangliosides	NE	NE	0.61	0.69	1.6	1.5	1.4	1.5	0.32	0.38	0.32	0.33
	(% of total phospholipids)											
ethanolamine plasmalogens	34	31	23	16	37	37	37	37	42	40	41	42
diacyl GPE			19	22								

contd...

Table 27 : contd.

1	2	3	4	5	6	7	8	9	10	11	12	13
choline phospho-glycerides (CPG)	50	52	37	37	39	40	40	40	31	32	32	31
sphingomyelin	4	5	10	10	6	6	6	6	7	7	8	8
inositol phospho-glycerides	5	4			17	18	17	17	20	20	18	21
serine phospho-glycerides	7	8	12	15								
EPG/CPG	0.68	0.60	1.14	1.03	0.95	0.93	0.93	0.93	1.35	1.28	1.31	1.30

E : control, UN : undernourished, UN-LP, low protein diet, UN-HP, rehabilitated with high protein diet fed ad lib.

UN-HP-R, high protein diet given in restricted amounts.

NE = not estimated.

* values calculated from mean values of Tables 26 and 28.

The lipids whose concentrations were found to be affected in white matter were also found to be affected in the whole brain during undernutrition. Culley and Mertz (1965), Rajalakshmi and Nakhasi (1974), Krigman and Hogan (1976) and Reddy and Sastry (1978) roported a significant decrease in the concentrations of cholesterol, galactolipids and total phospholipids in the whole brain, Culley et al (1966), Geison and Waisman (1970), Ghittoni and de Raneglia (1972), Reddy and Sastry (1978) and Ghittoni (1979) in plasmalogens, Ghittoni and de Raveglia (1972), Jailakhani and Subramanyam (1977) and Reddy and Sastry (1978) in ethanolamine phosphoglycerides and Reddy and Sastry (1978) in choline phosphoglycerides. Decreased concentrations of galactolipids in gray and white matter (Kokrady et al, 1972) and galactolipids and plasmalogens in the white matter (Fishman et al, 1969) have been reported in the case of malnourished children.

Effects of postweaning undernutrition or protein deficiency or rehabilitation on the lipid contents of brain gray and white matter of neonatally undernourished rat :

The results of studies on the effects of postweaning undernutrition or protein deficiency on brain lipids in neonatally undernourished rats are presented in Table 28. The deficits in body and brain weights of neonatally undernourished groups persisted even after rehabilitation for six

Table 28 : Effects of postweaning undernutrition, protein deficiency or rehabilitation on the lipid composition of brain gray and white matter of neonatally undernourished rats.

	gray matter				white matter			
	C	UN-HP	UN-HP-R	UN-LP	C	UN-HP	UN-HP-R	UN-LP
1	2	3	4	5	6	7	8	9
no. of observations	7	7	4	7	7	7	4	7
					mean ± s.e.			
body weight (g)	179 ± 10	123*** ± 10	42** ± 1.50	33** ± 1.10				
brain weight (g)	1.73 ± 0.02	1.48 ± 0.04**	1.31 ± 0.03**	1.21 ± 0.02**				
				(mg/g fresh weight)				
(a) cholesterol	14.7 ± 0.43	14.6 ± 0.28	15.6 ± 0.42	14.7 ± 0.23	28.1 ± 0.73	29.5 ± 0.26	28.2 ± 0.80	28.2 ± 0.10
(b) galactolipids	4.5 ± 0.20	4.6 ± 0.11	4.6 ± 0.10	4.4 ± 0.10	28.2 ± 0.56	27.7 ± 1.00	28.6 ± 0.78	27.1 ± 0.93
(c) phospholipids	39.1 ± 0.42	38.6 ± 0.93	39.0 ± 1.60	39.9 ± 0.65	57.0 ± 0.82	57.2 ± 0.74	55.4 ± 1.40	55.4 ± 1.10
total lipids (a + b + c)	58.3	57.8	59.2	59.0	113	114	112	111
plasmalogens	5.0 ± 0.21	5.3 ± 0.18	5.3 ± 0.28	5.0 ± 0.14	16.8 ± 0.40	17.3 ± 0.31	17.1 ± 0.19	16.3 ± 0.50
gangliosides (µg/g)	925 ± 26	819 ± 42	875 ± 63	893 ± 38	360 ± 6	366 ± 24	370 ± 9	422 ± 18*

contd...

Table 28 : contd.

1	2	3	4	5	6	7	8	9
(d) ethanolamine phospho-glycerides	14.5 ± 0.43	14.8 ± 0.34	15.0 ± 0.57	15.0 ± 0.29	23.4 ± 0.56	22.7 ± 0.62	23.5 ± 0.84	22.6 ± 0.58
(e) choline phospho-glycerides	15.3 ± 0.24	15.9 ± 0.27	16.4 ± 0.37	15.9 ± 0.15	17.4 ± 0.85	18.0 ± 0.33	17.7 ± 0.15	17.6 ± 0.26
(f) sphingomyelin	2.4 ± 0.02	2.5 ± 0.11	2.5 ± 0.05	2.5 ± 0.12	3.9 ± 0.33	4.1 ± 0.26	4.4 ± 0.39	4.3 ± 0.20
(g) serine + inositol phosphoglycerides	6.7 ± 0.20	7.1 ± 0.18	7.1 ± 0.33	6.7 ± 0.10	11.1 ± 0.62	11.0 ± 0.39	10.3 ± 0.20	11.5 ± 0.40
recovery of phospholipids $\left(\frac{d + e + f + g}{C}\right)$ X 100	100	104	105	100	98	98	100	100

C : control, UN-HP; neonatally undernourished rats were rehabilitated with high protein diet during postweaning period.
UN-HP-R : neonatally undernourished animals were given restricted high protein diet,
UN-LP : neonatally undernourished rats were given low protein diet.

values marked with asterisk significantly different from control values, p less than 0.01 for * and 0.001 for **.

weeks even though the increment in body and brain weight of the rehabilitated rats were 292% and 175% of controls respectively. Similar results were obtained in several other studies (Culley and Linenberg, 1968; Geison and Waisman, 1970; Rajalakshmi et al, 1974; Reddy and Sastry, 1978; Reddy et al, 1979). In the case of the groups which continued to be stressed nutritionally (i.e. undernourished or protein deficient diet) the percentage deficits in body and brain weights increased (Table 29).

In the case of rehabilitated rats as well as rats nutritionally stressed during postweaning period there was no change in the concentration and composition of different lipids in gray and white matter (Tables 27 and 28). This contrasts with the significant deficits reported in the concentration of whole brain lipids in rats neonatally undernourished and rehabilitated during postweaning period (Geison and Waisman, 1970; Rajalakshmi et al, 1974; Reddy and Sastry, 1978) or continued on low protein diet or restricted diet (Geison and Waisman, 1970; Krigman and Hogan, 1976; Reddy and Sastry, 1978).

The discrepancy observed in the results obtained on the whole brain and in gray and white matter could perhaps be explained on the basis of changes in the proportions of gray and white matter. In fact this led to the computations described for estimating the proportions of gray and white matter in the previous chapter.

Table 29 : Percentage increments in the body weight and brain weights of rat between 3 and 9 weeks age.

	control (A)	under nourished (B)	rehabi-litated (C)	$\frac{B}{A}$ X 100	$\frac{C}{A}$ X 100
			% increment		
body weight	273	141	798	52	292
brain weight	20	11	35	55	175
gray matter	- 5	- 3	16	-	-
white matter	135	104	165	77	122

The proportions of white and gray matter in all three groups (i.e. control, rehabilitated and protein deficient groups) were calculated using the values for galactolipids for whole brain, gray matter and white matter. Since the data on galactolipid contents of whole brain in the case of rehabilitated and protein deficient groups were not available in this laboratory, they were calculated using the data on % deficiency in galactolipids reported by Reddy and Sastry (1978) and values for control group available in this laboratory. The data on galactolipid concentration in whole brain, gray matter and white matter in normal, rehabilitated and protein deficient rats are given in Table 30. Using these values the proportions of gray and white matter were calculated using the algebraical equation mentioned in the previous experiment.

The proportion of white matter was reduced in under-nourished group at 3 weeks of age. The deficit increased in the animals subjected to postweaning protein deficiency. The question may arise whether the white matter used for the investigations was pure. Myelin as percent of dry white matter was found to be around 45% irrespective of the age and experimental group (Table 31) suggesting that a reasonably pure fraction of white matter had been isolated from all the groups.

Table 30 : Concentration of galactolipids in whole brain, gray matter and white matter of the rat.

	age (weeks)				
	3		9		
	C	UN	C	UN-LP	UN-HP
brain weight (g)	1.44	1.10	1.73	1.21	1.48
	tissue galactolipids (mg/g fresh tissue)				
whole brain	6.70	5.00	13.0	9.80	10.4
gray matter	4.43	3.83	4.53	4.44	4.56
white matter	16.5	13.2	28.2	27.1	27.7

UN-HP : neonatally undernourished rats were rehabilitated with high protein diet during postweaning period,

UN-HP-R : neonatally undernourished animals were given restricted high protein diet,

UN-LP : neonatally undernourished rats were given low protein diet during postweaning period.

Table 31 : Effects of postweaning undernutrition or rehabilitation on the contents of gray and white matter and myelin in brains of neonatally undernourished rats@.

	age (weeks)				
	3		9		
	C	UN	C	UN-HP	UN-LP
gray matter (% whole brain)	81.6	87.2	64.2	74.8	76.3
white matter (% whole brain)	18.4	12.8	35.8	25.2	23.7
content (mg/brain)					
gray matter	1172	957	1111	1106	926
white matter	264	141	619	373	288
dry weight* (mg/brain)					
gray matter	164	134	211	210	176
white matter	54	29	163	98	76
myelin yield**	22.7	13.5	75	44	32
myelin as per cent dry weight of white matter	43	47	46	45	42

C : control, UN : undernourished, UN-HP : neonatally undernourished rats fed low protein diet, UN-HP : neonatally undernourished rats rehabilitated with high protein diet ad lib.

* dry weights in the case of experimental group were calculated assuming that there were no changes in moisture content (Culley and Mertz, 1965), for control group values for moisture content were taken from expt. No. I (Table 16).

** myelin values for 3 weeks were taken from the data of Mr. Harjit Singh of this department. For 9 weeks values were taken from the data reported by Reddy et al (1979).

@ for calculating the proportions the mean values of galactolipids were taken from Table 31.

In the undernourished rat, the content of different lipids seems to be decreased in the whole brain, gray matter and white matter at 3 weeks of age, the maximum effect being observed in the case of white matter (Table 32 and Fig. 9). The deficits were persistent in all the three tissues even at 9 weeks of age when they were subjected to continued nutritional stress in the form of a low protein diet (Table 33 and Figs. 10 and 10a). On rehabilitation the content of different lipids were restored to normal values in gray matter. This is perhaps consistent with expectation as the content of gray matter as well as the concentration of lipid came back to normal. The deficits in the lipid content of whole brain are completely due to the deficits in the white matter (Table 33 and Figs. 10 and 10a).

The values for tissue weights of undernourished rats are 77, 82 and 53% of controls for whole brain, gray matter and white matter respectively at 3 weeks of age. The corresponding values at 9 weeks being 70, 83 and 47% for undernourished and 86, 100 and 60% for the rehabilitated rats (Table 34). A similar pattern of changes was observed with respect to different lipids also. The per cent contribution of different lipids by white matter to whole brain seems to be decreased in the experimental groups at 3 and 9 weeks of age (Fig. 11). The increments in the contents of different lipids in undernourished animals were less than those in the controls. But in the rehabilitated rats the increments were greater than in controls suggesting the operation of a 'catch up' phenomenon (Table 35).

Table 32 : Changes in the lipid content of whole brain gray matter and white matter at 3 weeks of age in control and undernourished rats*.

	control			undernourished		
	WB	GM	WM	WB**	GM	WM
	(mg/whole tissue)					
total lipids	82.7	57.0	22.7	55.8	44.8	10.4
cholesterol	22.1	16.6	7.1	14.8	13.8	3.4
galactolipids	9.6	5.2	4.4	5.5	3.6	1.9
phospholipids	51.0	31.2	11.2	34.9	27.4	5.1
plasmalogens	NE	5.5	2.5	NE	4.3	0.9
ethanolamine phosphoglycerides	17.2	10.2	4.2	11.2	7.8	1.9
choline phospho-glycerides	19.5	15.4	3.8	13.5	12.8	1.8
other phospholipids (SM + SPG + IPG)	9.0	5.0	2.2	5.9	4.2	1.2

WB : whole brain, GM : gray matter, WM : white matter.

* values calculated from mean values of Table 26.

** data taken from Mr. Harjit Singh of this department.

NE : not estimated.

Fig. 9

Effects of neonatal undernutrition on the content of brain lipids at 3 weeks of age.

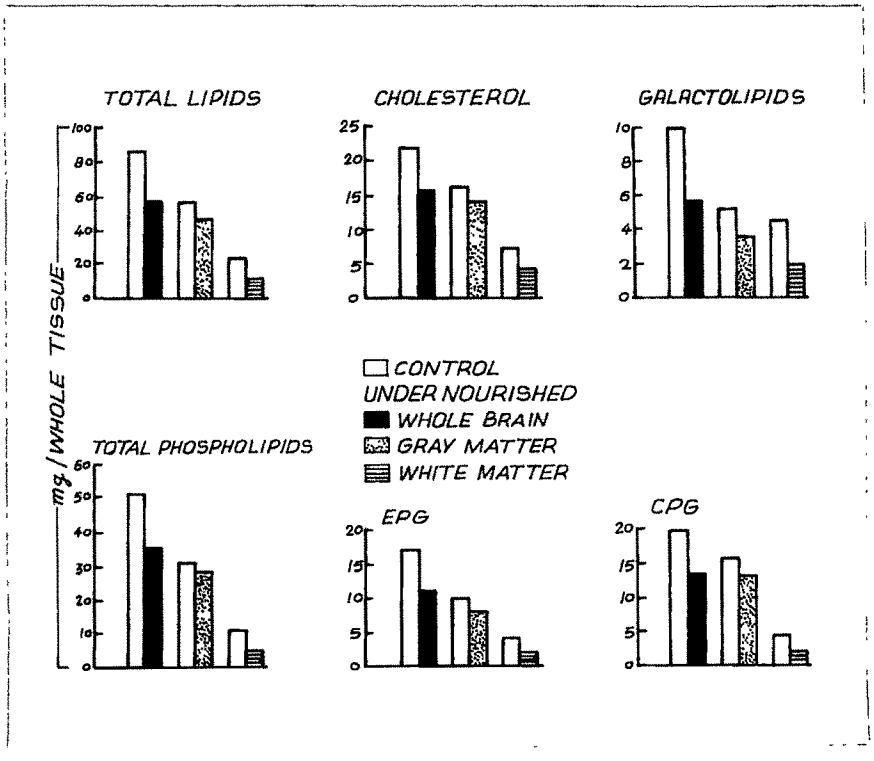


Table 33 : Effects of postweaning undernutrition and rehabilitation on the lipid content of whole brain, gray matter and white matter of neonatally undernourished rats*.

	control			UN-LP			UN-HP		
	WB	GM	WM	WB**	GM	WM	WB**	GM	WM
	(mg/whole tissue)								
total lipids	136	64.8	70.1	83.7	54.6	31.9	110	64.0	42.7
cholesterol	31.1	16.3	17.4	19.4	13.6	8.1	23.9	16.1	11.0
galactolipids	22.5	5.0	17.5	11.9	4.1	7.8	15.4	5.1	10.3
phospholipids	82.7	43.4	35.3	53.9	37.0	16.0	66.4	42.7	21.3
plasmalogens	-	5.5	10.4	-	4.7	4.7	-	5.8	6.5
gangliosides	-	1.03	0.22	-	0.83	0.12	-	0.97	0.14
ethanolamine phosphoglycerides	32.5	16.1	14.5	19.3	13.9	6.7	24.8	16.4	8.5
choline phosphoglycerides	24.4	17.0	10.7	15.7	14.7	5.0	20.1	17.6	6.7
sphingomyelin	4.3	2.7	2.4	2.8	2.3	1.1	3.6	2.7	1.5
serine and inositol phosphoglycerides	11.8	7.5	6.9	7.4	6.2	3.3	9.3	7.8	4.1

* values calculated from mean values of Table 28.

** values calculated from the data of Reddy and Sastry (1978).

WB : whole brain, GM : gray matter, WM : white matter

UN-LP : neonatally undernourished rats were given low protein diet during postweaning period,

UN-HP : neonatally undernourished rats were rehabilitated with high protein diet during postweaning period.

Fig. 10

Effects of postweaning protein deficiency or rehabilitation on the content of brain lipids at 9 weeks of age.

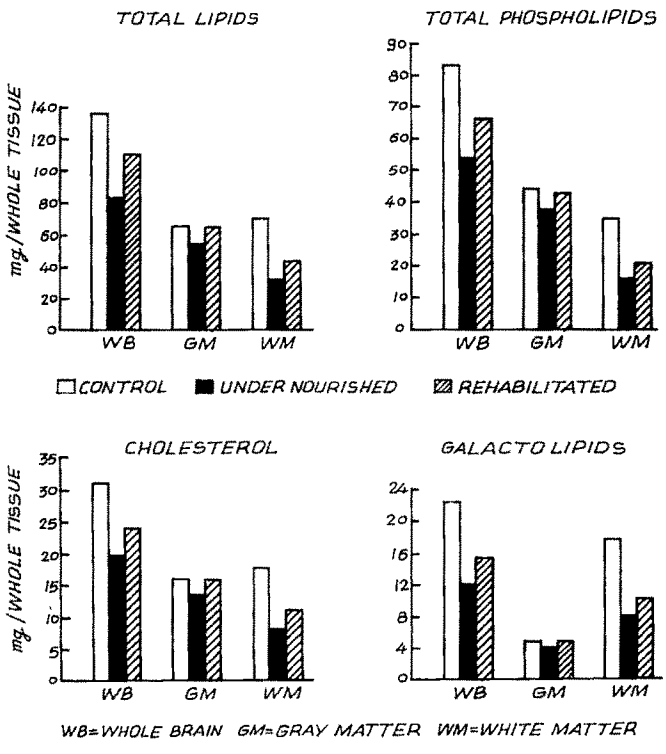


Fig. 10(a)

Effects of postweaning protein deficiency or rehabilitation on the content of different phospholipids of rat brain at 9 weeks of age.

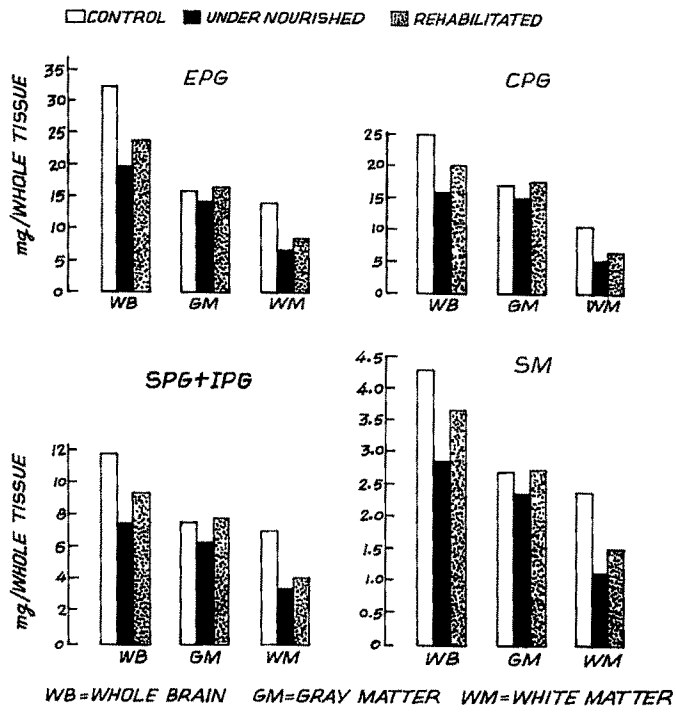


Table 34 : Effects of preweaning undernutrition and its continuation or rehabilitation during postweaning period on the content of rat brain lipids@.

	age (weeks)								
	3			9					
	undernourished			UN-LP			UN-HP		
	WB*	GM	WM	WB**	GM	WM	WB**	GM	WM
	(% of control values)								
tissue weight	77	82	53	70	83	47	86	100	60
total lipids	68	79	46	62	84	46	81	99	61
cholesterol	67	83	49	62	83	47	77	99	63
galactolipids	57	70	43	53	82	45	68	101	59
phospholipids	68	88	46	65	85	45	80	98	60
plasmalogens	-	78	36	-	84	45	-	105	62
ethanolamine phosphoglycerides	65	76	44	59	86	48	76	102	61
choline phospho-glycerides	69	83	47	64	87	47	85	104	63
sphingomyelin	65	85	54	66	87	47	85	101	63
serine and inositol phosphoglycerides				63	82	48	79	104	60

@ values calculated from mean values of Tables 26 and 28.
* values taken from Mr. Harjit Singh of this department.
** values taken from the data of Reddy and Sastry (1978).
WB : whole brain, GM : gray matter, WM : white matter.
UN-LP : neonatally undernourished rats were given low protein diet during postweaning period,
UN-HP : neonatally undernourished rats were rehabilitated with high protein during postweaning period.

Fig. 11

Effects of postweaning protein deficiency or rehabilitation on the percentage contribution of different lipids by gray and white matter to the whole brain at 3 and 9 weeks of age.

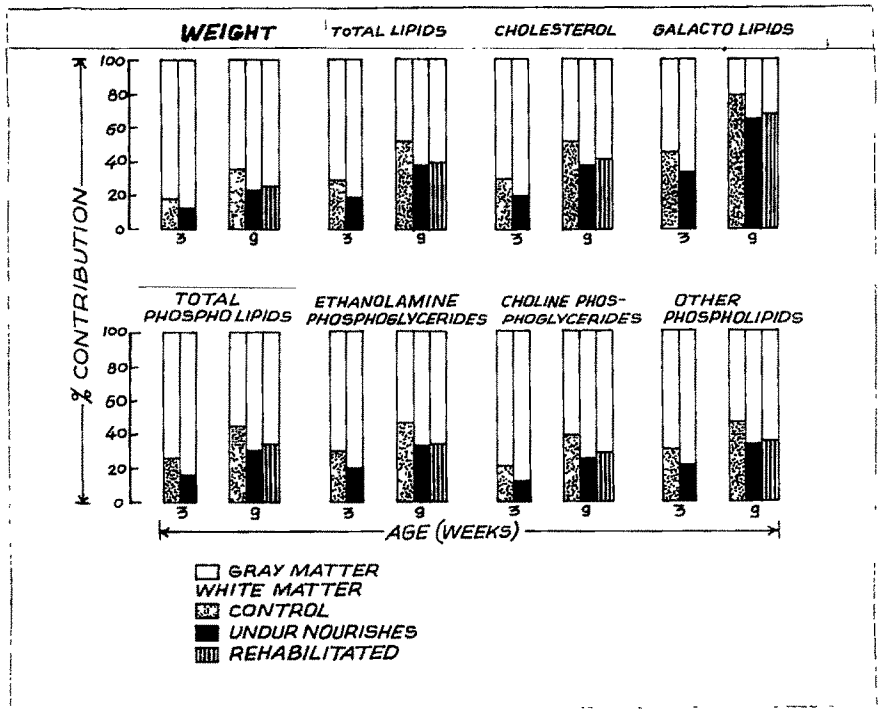


Table 35 : Per cent increment in the tissue weight and lipid content of whole brain, gray matter and white matter between the 3rd week and the 9th week in the control, undernourished and rehabilitated rats.

	control			UN-LP			UN-HP		
	WB	GM	WM	WB	GM	WM	WB	GM	WM
	% increment between 3rd and 9th weeks								
body weight	273	-	-	141	-	-	798	-	-
tissue weight	20	-5	135	11	-3	104	35	16	165
cholesterol	65	14	209	50	21	201	97	42	303
galactolipids	136	-3	301	117	13	319	181	41	454
phospholipids	62	39	215	54	35	214	90	56	318
plasmalogens	NE	0	311	NE	10	415	NE	35	609
ethanolamine phosphoglycerides	89	58	232	72	79	264	121	111	358
choline phospho-glycerides	25	10	179	16	15	178	49	38	272
other phospholipids (SM + SPG + IPG)	79	104	320	74	5	269	122	150	368

WB : whole brain, GM : gray matter, WM : white matter.

UN-LP : neonatally undernourished rats were given low protein diet during postweaning period,

UN-HP : neonatally undernourished rats were rehabilitated with high protein diet during postweaning period.

NE : not estimated.

An attempt was also made to calculate the nonmyelin lipid content of the white matter in the case of control, rehabilitated and protein deficient groups. Myelin values for the three groups were calculated from the data reported by Reddy et al (1979) making an assumption that myelin yield is only 60% (Norton and Poduslo, 1973). It is also assumed that all the myelin is present in white matter (Sabri and Davison, 1977). The decrease in the lipid content of white matter in the experimental groups was due to the decrease in lipid content of both myelin and nonmyelin fractions (Table 36). This was clearly reflected when the lipid values of experimental groups were expressed as percentage of control values (Table 37). The contribution of different lipids by myelin and nonmyelin fractions to the white matter in control and experimental groups is shown in Fig. 12. which shows that the major portion of the lipids in white matter are contributed by myelin in both control and experimental groups. It is found that the decrease in lipids is almost same in myelin and nonmyelin fraction during the preweaning undernutrition. Even though the deficits in the content of myelin and nonmyelin components were similar in the rehabilitated rats, the deficits in the nonmyelin lipids seem to be more suggesting the decreased rate of lipid synthesis in nonmyelin fraction (Table 37).

In conclusion, experimental undernutrition has differential effects on the gray and white matter lipids. The concentration of different lipids in gray matter was not affected except for

Table 36 : Effects of neonatal undernutrition and postweaning undernutrition or rehabilitation on the content of different lipids in myelin and non-myelin fractions of white matter in rat brain.

	age (weeks)														
	3						9								
	control			undernourished			control			UN-LP			UN-HP		
	WM	MY*	NMF	WM	MY*	NMF	WM	MY**	NMF	WM	MY**	NMF	WM	MY**	NMF
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	(mg/fresh tissue)														
total lipids	22.7	15.9	6.8	10.4	7.4	3.0	70.0	46.2	23.8	31.9	22.7	9.2	42.7	32.0	10.7
cholesterol	7.1	4.1	3.0	3.4	1.9	1.6	17.4	12.0	5.4	8.1	5.9	2.2	11.0	8.6	2.4
galactolipids	4.4	3.4	1.0	1.9	1.8	0.1	17.5	14.2	3.3	7.8	6.8	1.0	10.3	9.5	0.8
phospholipids	11.2	8.0	3.2	5.1	3.7	1.4	35.3	20.3	15.0	16.0	10.0	6.0	21.3	13.5	7.8
plasmalogens	2.5	2.1	0.4	0.9	1.1	-	10.4	8.1	2.4	4.7	2.3	2.4	6.5	4.0	2.5
ethanolamine phosphoglycerides	4.2	2.9	1.3	1.9	1.4	0.4	14.5	7.5	7.0	6.7	4.4	2.3	8.5	5.8	2.7

contd...

Table 36 : contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
choline phospho-glycerides	3.8	2.5	1.3	1.8	1.2	0.6	10.8	5.5	5.3	5.0	2.9	2.1	6.7	3.8	3.0
other phospho-lipids (SM+SPG+IPG)	2.2	2.2	-	1.2	1.4	-	9.3	5.9	3.4	4.4	2.7	1.7	5.6	3.7	1.9

* myelin values for 3 weeks were taken from the data of Mr. Harjit Singh of this department.

** myelin values for 9 weeks were taken from the data reported by Fishman et al (1971).

WM : white matter, MY : myelin, NMF : non myelin fraction.

UN-LP : neonatally undernourished rats were given low protein diet during postweaning period,

UN-HP : neonatally undernourished rats were rehabilitated with high protein diet during postweaning period.

Table 37 : Effects of neonatal undernutrition and postweaning undernutrition or rehabilitation on the lipid composition of myelin and non myelin fractions of white matter of rat brain.

	age (weeks)					
	3		9			
	undernourished		UN-LP		UN-HP	
	myelin	non myelin	myelin	non myelin	myelin	non myelin
	(% of control values)					
tissue weight	59	50	43	50	59	61
total lipids	46	45	49	39	69	45
cholesterol	46	52	49	40	72	44
galactolipids	52	10	48	30	67	25
phospholipids	47	43	49	40	67	52
plasmalogens	55	NA	28	NA	50	-
ethanolamine phosphoglycerides	50	31	59	33	78	38
choline phospho-glycerides	48	45	52	40	68	56
other phospholipids (SM + SPG + IPG)	64	NA	46	51	63	56

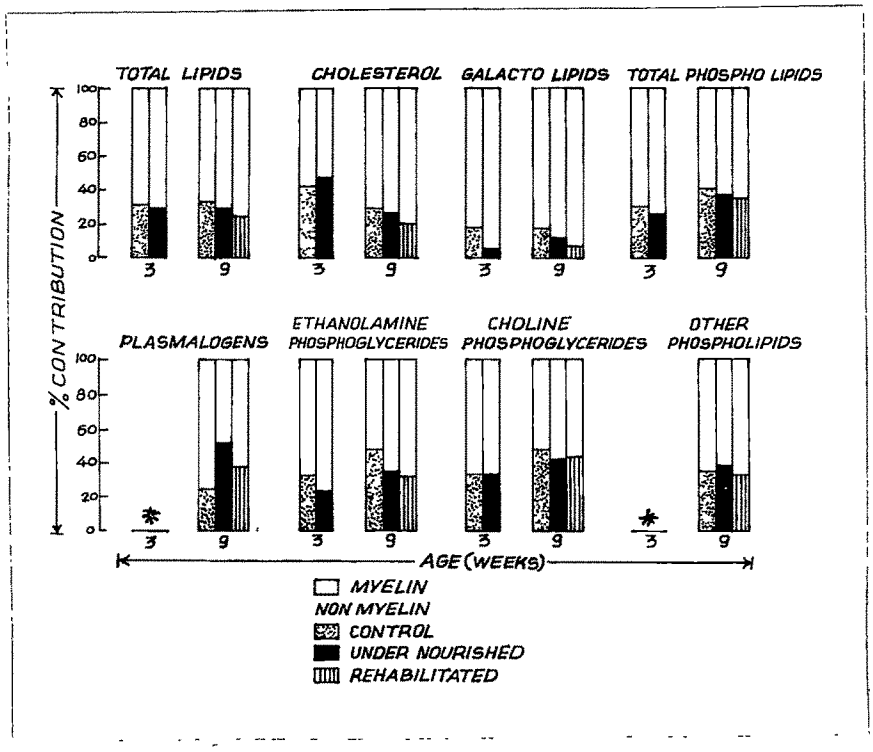
UN-LP : neonatally undernourished rats were given low protein diet during postweaning period.

UN-HP : neonatally undernourished rats were rehabilitated with high protein diet during postweaning period.

NA : not applicable.

Fig. 12

Effects of postweaning protein deficiency or rehabilitation on percentage contribution of lipids by myelin and nonmyelin components to the white matter of rat brain at 3 and 9 weeks of age.



* Values could not be calculated as the myelin values were higher than the white matter values.

galactolipids whereas the white matter showed significant deficits in most of the lipids at 3 weeks of age. At 9 weeks of age no significant differences were observed in the concentration of any of the lipids in the experimental groups both in gray and white matter suggesting that there are no qualitative differences. The changes in the concentration of different lipids at 9 weeks of age in whole brain are mainly due to the reduced amount of white matter. When the nutritional rehabilitation of neonatally undernourished rats was attempted, there was complete 'catch up' in the weight and lipid content of gray matter, but the white matter showed only 60% catch up. Thus the deficits reported in the whole brain of the rehabilitated animals are due to the deficits in the white matter only. Finally, the changes observed in the white matter with undernutrition or rehabilitation are reflected both in myelin and nonmyelin fractions of the white matter. The deffects seem to be more on the nonmyelin lipids.

EXPERIMENT - III

Effects of perinatal thiamine deficiency on the lipid composition of whole brain and spinal cord of rat.

EXPERIMENT - IV

Effects of perinatal thiamine deficiency on the lipid composition of gray and white matter of rat brain.

It is well known that a deficiency of thiamine in the maternal diet, which results in a deficiency in breast milk, is associated with infantile beriberi in areas where polished rice is the staple (Platt, 1958; Trostler et al, 1977). Thiamine deficiency causes specific CNS lesions which correlate with neurological symptoms (Dreyfus and Victor, 1961; Dreyfus,1967). CNS lesions include thinned or lost myelin segment without destruction of the axis cylinder (Denny Brown, 1958). Dreyfus (1967) and Collins (1967) have reported an altered oligodendroglial metabolism and defective myelination. Transketolase, a key enzyme of the pentose phosphate pathway is found to decrease in thiamine deficiency and this would be associated with the low production of NADPH needed for lipid synthesis (Geel and Dreyfus, 1975). The lipogenesis may also decrease due to the fact that acetyl CoA formation from pyruvate is found to decrease in thiamine deficiency (Heinrich et al, 1973). The decrease in lipid content may be related to the neurological disorders (Kanfman, 1972).

Geel and Dreyfus (1975) have reported that thiamine deficiency affects brain lipids in rat but it may be due to undernutrition produced due to low food intake. But Trostler et al(1977) have reported that thiamine deficiency produced greater deficits than undernutrition. From Table 38 it can be seen that the effects of thiamine deficiency with regard to body and brain weights as well as the lipid composition of whole brain are different in the two studies. Therefore, systematic studies were undertaken on the effects of maternal thiamine deficiency on the lipid composition of whole brain, spinal cord, gray matter and white matter of the brain in the progeny.

Female rats weighing about 200-250 g were housed with healthy young males for 2 days and then housed in separate cages. After a further period of 12 days those identified as pregnant were separated into 3 groups. The first group was fed on standard 25% protein diet (control, C) the second group the same diet with the omission of thiamine from the vitamin mixture (thiamine deficient, TD) and the third group the standard 25% protein diet in amounts matching those consumed by the thiamine deficient group (pair fed controls, PFC). After delivery the mothers were continued on respective diets until the pups were weaned (i.e. when pups were 21 days of age).

Table 38 : Effects of thiamine deficiency on the lipid composition of rat brain at weaning.

	Geel and Dreyfus (1975)		Trastler et al (1977)**		present study	
	UN	TD	UN	TD	UN	TD
			(% of control values)			
body weight (g)	25*	25*	53*	44*	42*	34*@
brain weight (g)	76*	76*	89*	87*	83*	75+@
			mg/g fresh brain			
total lipids	94*	97*	82	86	85*	88*
cholesterol	92*	102@	94	72	82*	94@
galactolipids	73*	85*	102	94	77*	73*
phospholipids	99	99	117	94	87*	89*
gangliosides	106*	114*@	-	-	98	101
EPG	99	100	-	-	92	87*
CPG	104	101	-	-	98	97
SM	100	105	-	-	89	92
PS + PI	93	95	-	-	100	100
plasmalogens	-	-	-	-	91	85*@

* values marked with asterisk significantly different from control values.

** since the values were given as mg/brain the significance of lipid values could not be calculated.

values marked with @ significantly different from UN group.

UN : undernourished by giving restricted amount of food.

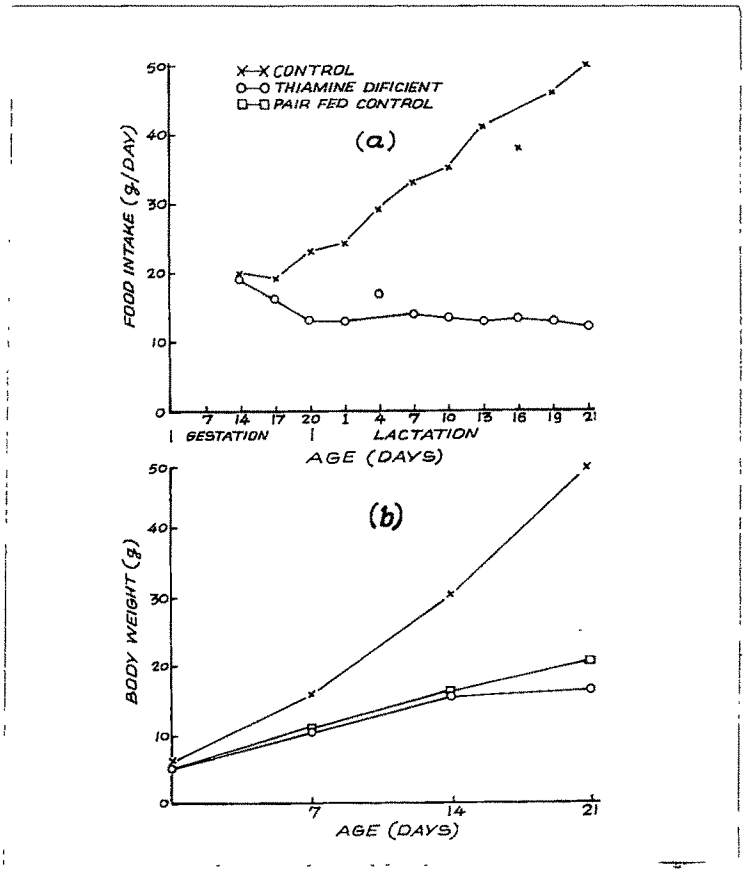
TD : thiamine deficient.

The TD pups started showing symptoms of thiamine deficiency such as abnormalities in posture, arched back and extended and stiffened hind limbs &(hind limbparalysis) from 14 days of age. Movements were restricted and the fur became coarse, oily and yellow gray in color. Similar observations were also made by Geel and Dreyfus (1974 and 1975) and Trostler et al (1977) in the case of thiamine deficient rats.

The food intake of the mothers in the thiamine deficient group was less as might be expected and this was associated with a decreased growth rate of the pups (Figs. 13a and b). The results of the effects of thiamine deficiency on pups at 3 weeks of age are shown in Table 39. The body and brain weights of the PFC group are 42% and 83% of the controls, the corresponding values for TD being 34% and 75%. Inspite of pair feeding the PFC group showed a significantly higher value for body and brain weights than the TD group (Table 39). This might be due to the poor ~~assimilation~~ utilization of the ~~food consumed by~~ nutrients in the thiamine deficient group. Similar observations have been made by the other investigators (Trostler et al, 1977; Kulkarni, 1979). The activity of transketolase was significantly less only in TD group confirming thiamine deficiency. Similar observations have been made by others (Dreyfus and Hauser, 1965; Geel and Dreyfus, 1974; Trostler et al, 1977; Prasanna, 1978).

Fig. 13

Effects of perinatal thiamine deficiency on the food intake of the mothers and body weight of the pups.



(a) : food intake of mothers.

(b) : body weight of pups.

Table 39 : Effects of perinatal thiamine deficiency on the lipid composition of brain of off springs at 21 days of age*.

	no. of observations			lipid composition of brain			% of controls		level of significance (P values less than)		
	C	PFC	TD	C	PFC	TD	PFC	TD	CVs PFC	C. vs TD	PFC vs TD
1	2	3	4	5	6	7	8	9	10	11	12
				mean ± s.e.							
body weight (g)	7	4	8	48.0	20.3	16.5	42	34	0.001	0.001	0.005
brain weight (g)	7	4	8	1.45 ± 0.02	1.20 ± 0.03	1.09 ± 0.03	83	75	0.001	0.001	0.05
transketolase activity (umoles/g/h)	7	4	8	28.2 ± 1.3	26.8 ± 1.7	12.3 ± 1.9	95	44	NS	0.001	0.001
				(mg/g fresh weight)							
total lipids	6	4	7	57.3 ± 0.7	48.8 ± 1.7	50.7 ± 1.0	85	88	0.001	0.001	NS
cholesterol	7	4	8	15.4 ± 0.5	12.6 ± 0.3	14.4 ± 0.2	82	94	0.01	NS	0.05
galactolipids	6	4	7	6.7 ± 0.2	5.1 ± 0.3	4.8 ± 0.3	77	73	0.05	0.01	NS
phospholipids	6	4	7	35.5 ± 0.5	31.0 ± 1.4	31.7 ± 0.5	87	89	0.05	0.01	NS

contd...

Table 39 : contd.

1	2	3	4	5	6	7	8	9	10	11	12
plasmalogens	7	4	8	5.5 ± 0.1	5.1 ± 0.1	4.7 ± 0.1	91	85	NS	0.01	0.05
gangliosides**	5	4	7	2.4 ± 0.1	2.4 ± 0.1	2.5 ± 0.03	98	101	NS	NS	NS
ethanolamine phosphoglycerides	7	4	8	12.0 ± 0.4	11.0 ± 0.9	10.4 ± 0.3	92	87	NS	0.05	NS
choline phospho-glycerides	7	4	8	13.6 ± 0.2	13.4 ± 0.5	13.1 ± 0.3	98	97	NS	NS	NS
sphingomyelin	7	4	8	2.0 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	89	92	NS	NS	NS
serine and inositol phospho-glycerides	7	4	8	4.4 ± 0.1	4.4 ± 0.1	4.4 ± 0.1	100	100	NS	NS	NS

* values were corrected to the nearest decimal point.

** values calculated assuming that gangliosides contain 30% NANA (Suzuki, 1976).

C : control, PFC : pair fed control, TD : thiamine deficient, NS : not significant.

The concentrations of total lipids, galactolipids and phospholipids were significantly lower in both the TD and PFC groups (Table 39). But cholesterol concentration was found to decrease significantly only in PFC group. However, the concentration of gangliosides was found to be unaffected in both PFC and TD groups. Among the phospholipids plasmalogens and EPG were found to decrease significantly only in the TD group (Table 39). The changes observed in the concentrations of whole brain total lipids, cholesterol and galactolipids in the present study are similar to the data reported by Geel and Dreyfus (1975). Inspite of severe undernutrition the concentration of phospholipids were unaffected in their study. But data reported by others show a significant decrease in the concentration of phospholipids (Culley et al, 1966; Rajalakshmi and Nakhasi, 1975; Bhat and Rama Rao, 1976). The increased concentration of gangliosides in brain of both PFC and TD groups observed by Geel and Dreyfys (1975) was not found in the present studies (Table 38).

Data obtained on the effects of thiamine deficiency on spinal cord lipids are presented in Table 40. The deficits in cord weights were more than brain weight deficits, the values being 63% and 75% of controls in TD and PFC groups respectively. This was consistent with the findings in previous studies in this laboratory (Rajalakshmi and Nakhasi, 1976; Sharma, 1979) and is accounted for by the fact that the cord grows in length

Table 40 : Effects of perinatal thiamine deficiency on the lipid composition of rat spinal cord at 21 days of age*.

	no. of observations			lipid composition of spinal cord			% of controls		level of significance (P values less than)		
	C	PFC	TD	C	PFC	TD	PFC	TD	C vs PFC	C vs TD	PFC vs TD
1	2	3	4	5	6	7	8	9	10	11	12
				mean ± s.e.							
spinal cord weight (mg)	8	4	8	169 ± 4.5	130 ± 5.4	106 ± 4.0	77	63	0.001	0.001	0.01
transketolase (umoles/g/h)	8	4	8	23.6 ± 0.9	21.9 ± 0.7	14.4 ± 0.7	97	61	NS	0.001	0.001
				(mg/g fresh weight)							
total lipids	6	4	7	112 ± 1.0	91 ± 1.7	95 ± 0.9	81	85	0.001	0.001	NS
cholesterol	7	4	8	27.2 ± 0.4	23.1 ± 0.6	26.0 ± 0.5	85	96	0.001	NS	0.01
galactolipids	6	4	7	24.7 ± 0.5	20.7 ± 0.4	20.8 ± 0.4	84	84	0.001	0.001	NS
phospholipids	7	4	8	59.6 ± 0.8	47.5 ± 1.1	47.7 ± 0.5	80	80	0.001	0.001	NS
plasmalogens	7	4	8	12.8 ± 0.3	9.1 ± 0.5	8.9 ± 0.6	71	70	0.001	0.001	NS

contdd...

Table 40 : contd.

1	2	3	4	5	6	7	8	9	10	11	12
gangliosides**	7	4	8	1.0 ± 0.03	1.1 ± 0.03	1.1 ± 0.03	106	109	NS	NS	NS
ethanolamine phosphoglycerides	7	4	8	21.6 ± 0.3	17.3 ± 1.0	16.5 ± 0.5	80	76	0.01	0.001	NS
choline phospho-glycerides	7	4	8	19.8 ± 0.4	16.8 ± 0.3	17.3 ± 0.4	85	87	0.001	0.001	NS
sphingomyelin	7	4	8	4.8 ± 0.2	4.3 ± 0.2	4.3 ± 0.2	93	89	NS	NS	NS
serine and inositol phospho-glycerides	7	4	8	8.7 ± 0.2	7.9 ± 0.4	8.0 ± 0.4	91	93	NS	NS	NS

* values were corrected to the nearest decimal point.

** values calculated assuming that gangliosides contain 30% NANA (Suzuki, 1976).

C : control, PFC : pair fed control, TD : thiamine deficient, NS : not significant.

and thickness during the postnatal period and gains weight at a faster rate than the brain (Singh and Sharma, 1980).

As in the case of the brain, transketolase activity was affected in the TD group showing that thiamine deficiency had been produced. The concentrations of all the lipids except gangliosides are higher in spinal cord than in brain (Tables 39 and 40). This is consistent with the previous findings in this laboratory (Rajalakshmi and Nakhasi, 1976; Sharma, 1979) and elsewhere (deSousa and Horrocks, 1979) and is accounted for by the fact that cord contains more amount of white matter (Zeman and Innes, 1963; Friede, 1975) which is rich in lipids. As in the case of brain, the concentrations of total lipids, galactolipids and total phospholipids were found to be significantly decreased in the TD and PFC groups (Table 40). As in the case of brain the concentration of cholesterol was not affected in thiamine deficiency but decreased during undernutrition. Among the phospholipids the concentrations of plasmalogens, EPG and CPG were found to be low in both the TD and PFC groups. The concentration of CPG was not affected in the brain.

In the brain, no changes were observed with regard to plasmalogens, EPG and CPG whereas in the cord deficits were

found with regard to all three components. Thus these studies suggest that stress produced by calorie restriction and thiamine deficiency is more in the case of spinal cord when compared to brain as far as lipids are concerned.

The composition of different lipids in the brain and spinal cord as per cent of total lipids is given in Table 41. The mole ratio of cholesterol : galactolipids : phospholipids was found to be affected in the TD group in the brain and spinal cord. Similarly, the composition of different lipids seems to be altered in the TD group (Table 41). As expected the contents of different lipids in brain and spinal cord were lower in both the TD and PFC groups (Table 42).

Since perinatal thiamine deficiency was found to affect the lipid composition of the whole brain, further studies were carried out on gray and white matter. The results of these studies are presented in Tables 43 and 44. Transketolase activity was less in both gray and white matter of the TD rats, the values being 36% and 39% of the controls.

The concentrations of galactolipids and gangliosides were found to be low in the gray matter of the PFC group whereas no

Table 41 : Effects of perinatal thiamine deficiency on the lipid composition of rat brain and spinal cord.

	brain			spinal cord		
	C	PFC	TD	C	PFC	TD
	mean ± s.e. (% of total lipids)					
cholesterol (CHL)	27 ± 0.6	26 ± 0.9	29**(a) ± 0.4	24 ± 0.4	25 ± 0.3	28***(c) ± 0.4
galactolipids (GL)	12 ± 0.5	11 ± 0.8	9***(c) ± 0.4	22 ± 0.4	23 ± 0.3	22 ± 0.3
phospholipids (PL)	62 ± 0.8	64 ± 0.6	63 ± 0.3	54 ± 0.7	52 ± 0.5	50***(b) ± 0.4
plasmalogens	10 ± 0.2	10 ± 0.3	9 ± 0.4	12 ± 0.4	10 ± 0.7	9*** ± 0.7
gangliosides	4.1 ± 0.2	4.6** ± 0.1	4.9** ± 0.1	0.9 ± 0.04	1.2 ± 0.05	1.2*** ± 0.04
mole ratio of CHL : GL : PL	44:19:100	41:17:100	46:14:100	44:41:100	48:44:100	56:44:100
	(% of total phospholipids)					
ethanolamine phosphoglycerides (EPG)	38 ± 0.9	36 ± 1.2	35 ± 0.7	39 ± 0.4	37 ± 0.7	36*** ± 0.5
choline phospho-glycerides (CPG)	42 ± 0.8	43 ± 0.8	44 ± 0.6	36 ± 0.5	36 ± 1.1	38* ± 0.6
sphingomyelin	6 ± 0.2	6 ± 0.6	6 ± 0.3	9 ± 0.3	10 ± 0.6	9 ± 0.4
serine and inositol phosphoglycerides	14 ± 0.4	15 ± 0.4	15 ± 0.4	16 ± 0.2	17 ± 0.5	17 ± 0.4
EPG/CPG	0.9	0.8	0.8	1.1	1.0	1.0

values marked with asterisk significantly different from control values, p less than 0.05 for *, 0.01 for ** and 0.001 for ***.
values marked with (a), (b) and (c) significantly different from pair fed control values, p less than 0.05, 0.01 and 0.001 respectively.
C : control, PFC : pair fed control, TD : thiamine deficient.

Table 42 : Effects of perinatal thiamine deficiency on lipid content of brain and spinal cord at 21 days of age*.

	brain					spinal cord				
	mg/brain			% control value		mg/spinal cord			% control value	
	C	PFC	TD	PFC	TD	C	PFC	TD	PFC	TD
total lipids	82.9	55.7	55.3	71	67	18.9	11.8	10.1	60	53
cholesterol	22.3	15.2	15.7	68	70	4.6	3.0	2.8	65	60
galactolipids	9.6	6.1	5.3	64	55	4.2	2.7	2.2	64	52
phospholipids	51.3	37.3	34.6	73	68	10.1	6.2	5.1	61	50
plasmalogens	8.0	6.1	5.1	76	64	2.2	1.2	1.0	55	44
gangliosides	3.5	2.8	2.7	81	76	0.17	0.14	0.12	82	69
ethanolamine phosphoglycerides	17.4	13.2	11.4	76	66	3.6	2.3	1.8	62	48
choline phospho-glycerides	19.7	16.1	14.4	82	73	3.4	2.2	1.8	65	55
sphingomyelin	2.8	2.1	2.0	74	69	0.8	0.6	0.5	68	56
serine and inositol phosphoglycerides	6.3	5.3	4.8	84	75	1.5	1.0	0.9	70	58

* values calculated from mean values of Tables 39 and 40.

Table 43 : Effects of perinatal thiamine deficiency on the lipid concentrations in rat brain gray matter at 21 days of age*.

	no. of observations			lipid composition of gray matter			% of controls		level of significance (P values less than)		
	C	PFC	TD	C	PFC	TD	PFC	TD	C vs PFC	C vs TD	PFC vs TD
1	2	3	4	5	6	7	8	9	10	11	12
					mean ± s.e.						
body weight (g)	8	4	8	49.0 ± 1.3	20.5 ± 1.0	16.4 ± 0.6	42	34	0.001	0.001	0.01
brain weight (g)	8	4	8	1.45 ± 0.02	1.22 ± 0.02	1.11 ± 0.03	84	77	0.001	0.001	0.05
transketolase (umoles/g/h)	3	2	3	18.3 ± 1.9	17.2 ±	6.5 ± 0.7	94	36	NS	0.001	-
					(mg/g fresh tissue)						
total lipids	8	4	7	48.7 ± 0.7	47.5 ± 0.6	47.8 ± 0.6	98	98	NS	NS	NS
cholesterol	8	4	7	14.4 ± 0.2	14.0 ± 0.3	14.2 ± 0.1	97	99	NS	NS	NS
galactolipids	8	4	8	4.1 ± 0.2	3.5 ± 0.1	3.8 ± 0.2	85	92	0.05	NS	NS
phospholipids	8	4	8	30.2 ± 0.6	30.0 ± 0.4	29.8 ± 0.4	100	99	NS	NS	NS
plasmalogens	8	4	8	4.4 ± 0.2	4.4 ± 0.2	4.0 ± 0.2	100	91	NS	NS	NS

contd...

Table 43 : contd.

1	2	3	4	5	6	7	8	9	10	11	12
gangliosides	8	4	7	3.5 ± 0.03	3.3 ± 0.07	3.5 ± 0.1	92	98	0.01	NS	NS
ethanolamine phosphoglycerides	7	4	7	10.6 ± 0.3	10.7 ± 0.2	10.6 ± 0.1	100	100	NS	NS	NS
choline phospho-glycerides	8	4	8	13.5 ± 0.2	13.3 ± 0.2	13.3 ± 0.3	99	99	NS	NS	NS
Sphingomyelin	6	4	8	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	95	100	NS	NS	NS
serine and inositol phosphoglycerides	7	4	7	5.0 ± 0.2	4.4 ± 0.2	4.5 ± 0.3	89	91	NS	NS	NS

* values corrected to the nearest decimal point.

** gangliosides calculated assuming that NANA forms 30% of total gangliosides (Suzuki, 1976).

C : control, PFC : pair fed control, TD : thiamine deficient, NS : not significant.

Table 44 : Effects of perinatal thiamine deficiency on the concentration of lipids in rat brain white matter at 21 days of age.

	no. of observations			lipid composition of white matter			% of controls		level of significance (P values less than)		
	C	PFC	TD	C	PFC	TD	PFC	TD	C vs PFC	C vs TD	PFC vs TD
1	2	3	4	5	6	7	8	9	10	11	12
				mean ± s.e.							
transketolase (umoles/g/h)	3	2	3	22.2 ± 0.6	21.0 ±	8.6 ± 1.4	95	39	NS	0.001	
				(mg/g fresh tissue)							
total lipids	7	4	7	87.7 ± 1.0	76.6 ± 1.9	75.7 ± 1.0	87	86	0.001	0.001	NS
cholesterol	7	4	7	27.2 ± 0.6	23.3 ± 1.0	26.3 ± 0.5	86	97	0.01	NS	0.05
galactolipids	7	4	7	17.0 ± 0.5	13.8 ± 0.7	11.1 ± 0.4	81	65	0.01	0.001	0.01
phospholipids	8	4	7	43.1 ± 0.9	39.5 ± 0.9	38.6 ± 0.6	92	90	0.05	0.001	NS
plasmalogens	7	4	8	9.7 ± 0.4	8.0 ± 0.3	6.6 ± 0.5	82	68	0.01	0.001	0.05
gangliosides	8	4	8	1.7 ± 0.03	1.6 ± 0.1	1.7 ± 0.1	94	100	NS	NS	NS
ethanolamine phosphoglycerides	6	4	7	17.4 ± 0.3	14.1 ± 0.4	14.8 ± 0.6	81	85	0.001	0.01	NS

contd...

Table 44 : contd.

1	2	3	4	5	6	7	8	9	10	11	12
choline phospho-glycerides	6	4	8	15.2 ± 0.3	14.9 ± 0.7	13.8 ± 0.7	98	91	NS	NS	NS
sphingomyelin	7	4	8	2.4 ± 0.1	2.5 ± 0.1	1.9 ± 0.1	104	82	NS	NS	NS
serine and inositol phospho-glycerides	7	4	6	6.8 ± 0.3	6.5 ± 0.5	5.9 ± 0.3	96	87	NS	NS	NS

* gangliosides calculated assuming that NANA forms 30% of total gangliosides (Suzuki, 1976).

C : control, PFC : pair fed control, TD : thiamine deficient, NS : not significant.

significant changes were found in the TD group (Table 43). In the white matter the concentrations of total lipids, galecto-lipids and phospholipids were less than in controls in both the PFC and TD groups (Table 44). Cholesterol concentration was found to be less only in the PFC group. Among the phospholipids plasmalogens and EPG were lower in both PFC and TD groups.

The low concentrations of myelin specific lipids (i.e. galactolipids and plasmalogens) in white matter (myelin rich areas) of TD rats as compared to PFC rats indicate the role of thiamine in myelination. Further studies are needed on isolated myelin to confirm this hypothesis.

The lipid composition of gray matter was found to be unaffected in both undernourished (PFC) and TD groups (Table 45). However, the lipid composition of white matter was altered in TD group. The ratio of EPG to CPG was lowered in the white matter of PFC group whereas it was not affected in TD group. The lipid composition of gray and white matter from PFC group did not differ from that of the undernourished group in experiment II in which mothers were fed low protein diet. But the concentrations were affected to a greater extent in experiment II. The decrease in amount of white and gray matter was also more in experiment II. This appears to be due to differences in the degree of undernutrition achieved.

Table 45 : Effects of perinatal thiamine deficiency on the lipid composition of rat brain gray and white matter.

	gray matter			white matter		
	C	PFC	TD	C	PFC	TD
	mean ± s.e. (% of total lipids)					
cholesterol (CHL)	30 ± 0.4	30 ± 0.3	30 ± 0.5	31 ± 0.6	31 ± 0.8	35 ± 0.4 ***(b)
galactolipids (GL)	8 ± 0.4	7 ± 0.3	8 ± 0.3	19 ± 0.5	18 ± 0.5	15 ± 0.5 ***(b)
phospholipids (PL)	62 ± 0.5	63 ± 0.3	62 ± 0.5	50 ± 1.0	51 ± 1.1	50 ± 0.9
plasmalogens	9 ± 0.2	10 ± 0.6	9 ± 0.4	11 ± 0.4	11 ± 0.6	9 ± 0.7 **(a)
gangliosides	7 ± 0.2	7 ± 0.1	7 ± 0.3	2 ± 0.1	2 ± 0.2	2 ± 0.1
mole ratio of CHL : GL : PL	48:13:100	48:11:100	48:13:100	62:38:100	61:35:100	70:30:100
	(% of total phospholipids)					
ethanolamine phosphoglycerides (EPG)	35 ± 0.8	36 ± 0.6	36 ± 0.5	42 ± 0.4	36 ± 1.1 ***	41 ± 0.7 (a)
choline phosphoglycerides (CPG)	45 ± 0.5	46 ± 0.3	45 ± 0.8	37 ± 0.8	40 ± 0.7 *	38 ± 0.6
sphingomyelin	4 ± 0.3	4 ± 0.3	4 ± 0.5	6 ± 0.4	6 ± 0.4	5 ± 0.3
serine and inositol phosphoglycerides	16 ± 0.7	15 ± 0.5	16 ± 1.1	16 ± 0.6	18 ± 0.7	16 ± 0.6
EPG/CPG	0.8	0.8	0.8	1.1	0.9	1.1

C : control, PFC : pair fed control, TD : thiamine deficient.
values marked with asterisk significantly different from control values, p less than 0.05 for *, 0.01 for ** and 0.001 for ***.
values marked with (a) and (b) significantly different from pair fed control values, p less than 0.01 for and 0.001 respectively.

The proportions of gray and white matter were calculated as described earlier. The proportions of gray and white matter were 80% and 20% for control, 84% and 16% for PFC group and 85% and 15% for TD group. In the case of undernourished rats in experiment II in which mothers were fed low protein diet the proportion was found to be 87% and 13%. The difference in the proportion of white matter obtained in the undernourished rats in the present experiment (PFC) and experiment II might be due to the differences in the degree of undernutrition achieved. While the similarity in general changes like proportions of white and gray matter in the TD and PFC groups suggest that these changes are due to calorie restriction, the changes observed in constituents such as galactolipids and plasmalogens in white matter in TD group may indicate specific effects of thiamine deficiency. In this connection changes observed in cholesterol concentration only in the PFC group may indicate the specific effect of calorie restriction on cholesterol metabolism. The fact that this is not observed in TD group which is also subjected to undernutrition indirectly is indeed very intriguing and needs further detailed investigation.

To check the validity of the values obtained for the proportions of gray and white matter, the analysed and calculated whole brain lipids were compared (Table 46). Except

Table # 46 : Comparison of analysed and calculated values for brain lipids in different groups*.

	C			PFC			TD		
	A	B	% diff.	A	B	% diff.	A	B	% diff.
	(mg/g fresh brain)								
total lipids	57.3	56.4	1.6	48.8	52.0	6.6	50.7	51.9	2.4
cholesterol	15.4	17.0	10.0	12.6	15.5	23.0	14.4	15.9	10.0
galactolipids	6.7	6.7	0.0	5.1	5.1	0.0	4.8	4.8	0.0
phospholipids	35.5	32.8	7.6	31.0	31.5	1.6	31.7	31.1	1.9
plasmalogens	5.5	5.5	0.0	5.1	5.0	2.0	4.7	4.4	6.4
gangliosides	2.4	3.2	30.0	2.4	3.0	27.0	2.5	3.2	30.0
ethanolamine phosphoglycerides	12.0	12.0	0.0	11.0	11.2	1.8	10.4	11.2	7.7
choline phospho-glycerides	13.6	13.8	1.5	13.4	13.5	0.8	13.1	13.4	2.3
sphingomyelin	2.0	1.4	30.0	1.7	1.3	27.0	1.8	1.2	30.0
serine and inositol phosphoglycerides	4.4	5.3	22.0	4.4	4.7	7.8	4.4	4.7	8.0

C : control, PFC : pair fed control, TD : thiamine deficient, A : analysed, B : calculated, % diff. : % difference between A and B.

for the gangliosides and minor phospholipids there seems to be a good correlation between the analysed and calculated values.

The weight and lipid content of gray and white matter were found to be low in PFC and TD rats (Table 47). The deficits are more in white matter than in gray matter. The PFC and TD groups had 88 and 82 per cent of control values for gray matter weight and 86 and 80 per cent values for gray matter lipid content. The corresponding values for white matter were 67 and 56 per cent and 59 and 49 per cent respectively (Table 48). In the white matter of thiamine deficient rat the values for galactolipids and plasmalogens were much lower than in PFC group. The per cent contribution of white matter lipids to the whole brain was decreased in both the PFC and TD groups (Fig. 14).

In conclusion, the data suggest that the myelin rich areas of CNS (white matter and spinal cord) are more vulnerable to thiamine deficiency or undernutrition than gray matter. Out of the two deficiencies TD seems to have a more severe effect on the white matter lipids than undernutrition induced by calorie deficiency. This might be either due to the quantitative differences achieved in the degree of undernutrition between TD and PFC groups or due to some specific effects of TD per se. Further studies are needed to confirm if thiamine has any specific role on myelination in CNS.

Table 47 : Effects of perinatal thiamine deficiency on the lipid contents of whole brain, gray matter and white matter at 21 days of age*.

	C			PFC			TD		
	WB	GM	WM	WB	GM	WM	WB	GM	WM
tissue weight (g)	1.45	1.17	0.29	1.22	1.03	0.19	1.11	0.95	0.16
				(mg/whole tissue)					
total lipids	83.3	56.8	25.1	59.5	48.8	14.7	56.4	43.5	12.2
cholesterol	22.4	16.8	7.8	15.4	14.4	4.5	16.0	13.5	4.2
galactolipids	9.7	4.8	4.9	4.2	3.6	2.7	5.4	3.6	1.8
phospholipids	51.6	35.2	12.3	37.8	30.8	7.6	35.3	28.4	6.2
plasmalogens	8.0	5.1	2.8	6.2	4.5	1.5	5.2	3.8	1.1
gangliosides	3.5	4.1	0.5	2.9	3.3	0.3	2.8	3.3	0.3
ethanolamine phosphoglycerides	17.4	12.4	5.0	13.4	11.0	2.7	11.6	10.1	2.4
choline phospho-glycerides	19.8	15.8	4.4	16.4	13.8	2.9	14.6	12.7	2.2
sphingomyelin	2.8	1.3	0.7	2.1	1.1	0.5	2.0	1.0	0.3
serine and inositol phosphoglycerides	6.3	5.8	2.0	5.4	4.5	1.3	4.9	4.3	1.0

C : control, PFC : pair fed control, TD : thiamine deficient, WB : whole brain, GM : gray matter, WM : white matter.

* values calculated from mean values of Tables 39, 43 and 44.

Table 48 : Effects of perinatal thiamine deficiency on the lipid contents of whole brain, gray matter and white matter*.

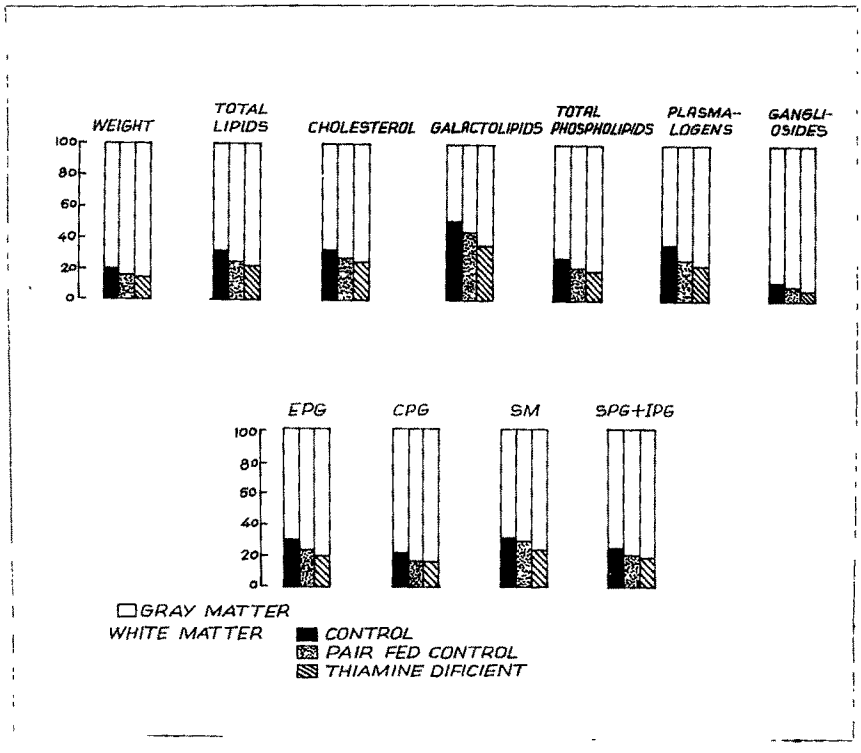
	PFC			TD		
	WB	GM	WM	WB	GM	WM
	(% of control values)					
tissue weight	84	88	67	77	82	56
total lipids	71	86	59	68	80	49
cholesterol	69	86	58	71	80	54
galactolipids	64	75	55	56	75	37
phospholipids	73	88	62	68	81	51
plasmalogens	77	88	55	65	74	38
gangliosides	82	81	62	78	80	56
ethanolamine phosphoglycerides	77	89	54	67	82	48
choline phospho-glycerides	83	87	66	74	80	51
sphingomyelin	75	84	69	70	82	46
serine and inositol phosphoglycerides	85	78	64	77	74	49

* values calculated from the mean values of Tables 39, 43 and 44.

PFC : pair fed control, TD : thiamine deficient, WB : whole brain, GM : gray matter, WM : white matter.

Fig. 14

Effects of perinatal thiamine deficiency on the percentage contribution of different lipids by gray and white matter to the whole brain at 3 weeks of age.



EXPERIMENT - V

Effects of age and nutritional deficiencies on the activity of 2',3'-cyclic nucleotide 3'-phosphohydrolase (CNP) in rat brain gray and white matter :

The enzyme, 2',3'-cyclic nucleotide 3'-phosphohydrolase (CNP), which hydrolyzes 2'-3'-cyclic nucleotides specifically to form corresponding 2'-phosphates, was first reported by Drummond and Coworkers (1962) to be rich in the central nervous system. Though the *in vivo* substrate for this enzyme is not identified and the biological role of the enzyme is yet to be precisely defined its high activity in the central nervous system has led to a number of studies on its distribution and subcellular localization in the central nervous system.

This enzyme is specially active in white matter which is found to have 4-8 fold higher specific activity than in gray matter (Kurihara and Takahashi, 1973; Deshmukh *et al*, 1974; Toews and Horrocks, 1976; Sabri and Davison, 1977). The subcellular localization of this enzyme is found to be in the myelin fraction (Kurihara and Tsukada, 1967; Kurihara *et al*, 1970). It has also been shown that the oligodendrocytes, which make myelin, contain high concentration of the enzyme (Peduslo, 1975). The activity of CNP increases during myelination. The and activity is low during defective myelination (Kurihara *et al*, 1969, 1970 and 1971). Because of its association with myelin,

CNP activity has been used as an index of myelination (Kurihara and Tsukada, 1968; Kurihara et al, 1969; 1970, 1971; Gregson and Oxberry, 1972).

In the studies previously described in this thesis the proportion of white matter in the brain changed with age and nutritional status. It is known that myelin contributes a major portion of white matter. It was considered worthwhile to find out whether CNP considered a marker enzyme for myelin is affected by age and nutritional status is influenced by these factors. The studies reported in this section were concerned with this aspect and the estimation of protein concentration.

Since this experiment was conducted in the laboratory of Prof. Horrocks Wistar rats were used for this experiment consistent with the practice in that laboratory. For the studies on aging male rats of specified ages were obtained from the commercial sources. For the studies on the effects of undernutrition, neonatal undernutrition was induced by feeding the mothers a 3.5% protein diet from partus. The control mothers were fed a diet containing 26% protein diet. Thiamine deficiency was produced in the pups as described in experiment III.

The results of studies on the variations in enzyme activity with age in gray and white matter both in terms of enzyme units/g tissue and enzyme units/mg protein are shown in Table 49. From the table it can be seen that the gray matter

Table 49 : Changes in the concentrations of protein and 2',3'-cyclic nucleotide 3'-phosphohydrolase (CNP) in rat brain gray and white matter with age*.

	age (days)						
	14	16	18	21	30	60	90
	mean ± s.e.						
no. of rats used	12	12	10	10	8	8	8
body weight (g)	35.0 ± 0.4	39.0 ± 0.6	51.0 ± 1.3	60.0 ± 2.0	127 ± 3.3.	251 ± 3.7	318 ± 4.5
brain weight (g)	1.37 ± 0.01	1.46 ± 0.01	1.48 ± 0.01	1.53 ± 0.02	1.72 ± 0.04	1.90 ± 0.02	1.94 ± 0.04
	tissue protein concentration (mg/g fresh tissue)						
gray matter (GM)	83.0 ± 1.8	93.0 ± 0.3	92.0 ± 0.7	97.C ± 4.3	99.0 ± 3.0	100 ± 0.7	104 ± 0.8
white matter (WM)	79.0 ± 1.0	81.0 ± 2.8	85.0 ± 4.0	103.0 ± 1.9	99.0 ± 2.2	95.0 ± 1.0	95.0 ± 0.6
WM/GM	0;95	0.87	0.92	1.10	1.00	0.95	0.91
	2',3'cyclic nucleotide 3'phosphohydrolase (units/mg protein)						
gray matter (GM)	3.0 ± 0.1	2.9 ± 0.1	2.7 ± 0.1	2.6 ± 0.1	3.1 ± 0.1	3.4 ± 0.1	3.4 ± 0.1
white matter (WM)	20.3 ± 0.2	19.5 ± 1.2	20.2 ± 0.4	20.9 ± 0.3	19.6 ± 0.5	20.6 ± 0.1	18.8 ± 0.4
WM/GM	6.8	6.7	7.5	8.0	6.3	6.1	5.5

contd..

Table 49 : contd.

	age (days)						
	14	16	18	21	30	60	90
	2'3'cyclic nucleotide 3'phosphohydrolase (units/g fresh tissue)						
gray matter (GM)	246 ± 10	267 ± 8	252 ± 16	259 ± 10	310 ± 6	341 ± 15	353 ± 15
white matter (WM)	1598 ± 31	1571 ± 95	1715 ± 110	2156 ± 63	1936 ± 101	1949 ± 24	1786 ± 54
WM/GM	6.5	5.9	6.8	8.3	6.3	5.7	5.1

* all the values were corrected to the nearest decimal point.

shows an increase in protein concentration till 90 days of age although much of this increase is found between 14 and 21 days of age. This increase in protein concentration correlates well with the morphological development of the gray matter such as increase in cell size, axonal growth, dendritic arborization and synaptogenesis (Benjamins and McKhann, 1976). On the other hand in the white matter the values show an abrupt increase between 18 and 21 days with some suggestions of a decline thereafter till 60 days. These changes in the protein concentration are in agreement with the peak period of myelination between 18 and 21 days and slow accumulation of lipid rich myelin thereafter (Norton and Poduslo, 1973). Similar observations have been made ~~for~~ on the concentration of protein in gray and white matter in developing human brain (Toews and Horrocks, 1976). The specific activity of CNP in white matter is 5 to 8 fold higher than in gray matter at all ages (Table 49). Similar data have been reported for the white matter of rat brain by Deshmukh et al (1974) and Sabri and Davison (1977), for rabbit brain by Kurihara and Takahashi (1973) and for human brain by Toews and Horrocks (1976). The specific activity of CNP in gray and white matter did not vary with age (Table 49). The changes observed in CNP activity with age in the whole brain is probably ~~could be~~ due to the changes in the proportions of gray and white matter (Kurihara and Tsukada, 1968; Olafson et al, 1969; Kurihara et al, 1970).

As expected, neonatal undernutrition caused a significant reduction in body and brain weights at 21 days of age (Table 50). The protein concentration in gray and white matter was not affected by undernutrition. Similar results were obtained previously in this laboratory with regard to body and brain weights (Rajalakshmi and Nakhasi, 1974 and 1975) and protein concentration in whole brain (Rajalakshmi and Telang, 1975; Telang, 1980). The specific activity of CNP was lower in both gray and white matter, the values as percentage of controls being 64 and 52 respectively. Similar observation has been made with regard to whole brain (Nakhasi et al, 1977; Reddy et al, 1979) and myelin (Nakhasi et al, 1975; Simons and Johnson, 1976; Reddy et al, 1979) in the severely undernourished rats.

The data on the effects of thiamine deficiency are given in Table 51. The concentrations of protein and specific activity of CNP were not affected by thiamine deficiency. At this point it is of interest to note that the undernutrition induced by increasing the litter size did not have any effect on the specific activity of CNP in brain tissue (Reddy et al, 1979). On the contrary when severe undernutrition was induced by maternal dietary protein deficiency there was a significant reduction in the specific activity of CNP in the brain of 21 day old pups (Nakhasi et al, 1977; Reddy et al, 1979). In the present studies also undernutrition produced by feeding the mothers a low protein diet has lowered the CNP activity in gray and white matter whereas mild undernutrition produced by pair

Table 50 : Effects of neonatal undernutrition on the protein and CNP concentrations in rat brain gray and white matter at 21 days of age.

	control (C)	under-nourished (UN)	$\frac{UN}{C}$ X 100
		mean ± s.e.	
no. of rats used	16	9	
body weight (g)	43.0 ± 0.4	13.0* ± 0.5	30
brain weight (g)	1.45 ± 0.01	1.10* ± 0.02	76
		protein, mg/g fresh tissue	
gray matter (GM)	108 ± 3.5	104 ± 1.0	96
white matter (WM)	93 ± 1.9	94 ± 2.0	101
WM/GM	0.86	0.90	
		CNP, units/mg protein	
gray matter (GM)	4.5 ± 0.4	2.9* ± 0.1	64
white matter (WM)	22.9 ± 1.4	12.1* ± 1.6	52
WM/GM	5.1	4.2	
		CNP, units/g fresh tissue	
gray matter (GM)	482 ± 6.0	297* ± 3.0	62
white matter (WM)	2130 ± 73	950* ± 130	45
WM/GM	4.4	3.2	

CNP : 2',3'-cyclic nucleotide 3'-phosphohydrolase.

values marked with asterisk significantly different from control values, p less than 0.001.

Table 51 ; Effects of perinatal thiamine deficiency on the concentration of protein and CNP in rat central nervous system.

	control (C)	thiamine deficient (TD)	pair fed control (PFC)	$\frac{TD}{C}$ X 100	$\frac{PFC}{C}$ X 100
			mean ± s.e.		
no. of rats used	16	14	8		
body weight (g)	48.0 ± 1.2	15.4* ± 0.6	18.7* ± 0.5	32	39
brain weight (g)	1.43 ± 0.01	1.08* ± 0.02	1.18* ± 0.01	76	83
spinal cord weight (mg)	170 ± 2.5	106* ± 2.2	124* ± 1.9	56	66
			protein, mg/g fresh tissue		
brain	123 ± 3.4	122 ± 2.0	117 ± 3.9	99	95
gray matter	117 ± 3.4	120 ± 1.5	117	103	100
white matter	115 ± 3.0	113 ± 2.0	112	98	97
spinal cord	114 ± 2.8	105 ± 2.3	110 ± 6.8	92	97
			CNP, units/mg protein		
brain	7.5 ± 0.1	7.3 ± 0.2	7.2 ± 0.2	98	96
gray matter	3.3 ± 0.1	3.1 ± 0.2	3.2 ± 0.3	95	98
white matter	20.8 ± 0.5	21.3 ± 0.5	19.9 ± 0.5	102	96
spinal cord	5.2 ± 0.2	5.6 ± 0.2	5.6 ± 0.3	106	106

values marked with asterisk significantly different from control values, p less than 0.001.

CNP : 2',3'-cyclic nucleotide 3'-phosphohydrolase.

feeding or thiamine deficiency do not affect the CNP activity. This points out that the effect will depend on the severity of undernutrition produced.

EXPERIMENT - VI

Effects of neonatal undernutrition on CDP-Ethanolamine: 1,2-diradyl-Sn-glycerol phosphoethanolamine transferase (PET, EC 2.7.8.1) and CDP-choline:1,2-diradyl-Sn-glycerol phosphocholine transferase (PCT, EC 2.7.8.2) in rat brain gray and white matter microsomes :

Phospholipids form a major class of lipids in the nervous system. Among the phospholipids ethanolamine and choline phosphoglycerides form around 75-80% of total phospholipids in the nervous system (Horrocks et al, 1976). Significant changes in the concentration and pattern of development of EPG and CPG were observed in the rat brain with development (Wells and Dittmer, 1967; Cuzner and Davison, 1968; Norton and Poduslo, 1973; deSousa and Horrocks, 1979). Similar changes in the pattern of EPG and CPG were observed in the developing rat brain gray and white matter (Experiment I). Neonatal undernutrition was found to lower the concentrations of EPG (Ghittoni and deRaveglia, 1972; Jailakhani and Subramanyam, 1977; Reddy and Sastry, 1978; Ghittoni, 1979) and CPG in the whole brain (Ghittoni and deRaveglia, 1972; Reddy and Sastry, 1978). But the effects were not same in the gray and white matter. Incorporation of (U-^{14}C)-glucose into these two lipids was found to be lowered in the undernourished rat brain (Agrawal et al, 1972; Jailakhani and Subramanyam, 1977) suggesting the lowered synthesis of these lipids. However, no studies have

been carried out on the enzymes involved in the synthesis of these two lipids.

The studies were, therefore, carried out to find out the effects of neonatal undernutrition on phosphoethanolamine (PET) and phosphocholine transferases (PCT), the key enzymes involved in the synthesis of these two lipids.

As in the previous experiment Wistar rats were used. Neonatal undernutrition was induced as described in previous experiment. The control and undernourished rats were decapitated, brains removed, gray and white matter separated and the homogenate was prepared. From the homogenate, microsomes were prepared and used for the estimations of phosphoethanolamine and phosphocholine transferases.

In both the groups, content of microsomes in the white matter was more than that in the gray matter. The content of microsomes was not affected by undernutrition both in gray and white matter (Table 52).

The incorporation of labelled bases into ethanolamine and choline phosphoglycerides was found to be linear between 3 and 12 µg of microsomal protein in both gray and white matter, though the incorporation was low in the case of undernourished rats (Fig. 15). For all studies 6 µg of microsomal protein was used. There was a good linearity in the incorporation with time at least upto 40 min, though again the incorporation in the

Table 52 : Effects of neonatal undernutrition on the activities of PET and PCT in rat brain gray and white matter microsomes.

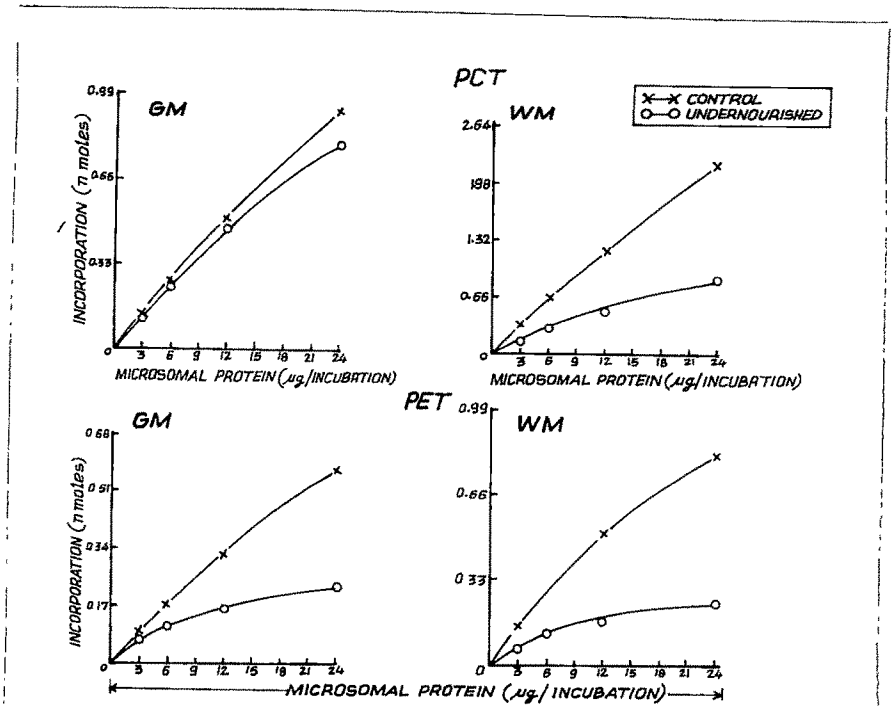
	PCT		PCT	
	GM	WM	GM	WM
	mean ± s.e.			
	microsomal protein, (mg/g wet weight)			
control	6.8 ± 0.5	10.2 ± 0.4		
undernourished	5.5 ± 0.3 (81)	10.1 ± 0.2 (99)		
	specific activity of enzyme (units/mg protein)			
control	56.0 ± 5.8	82.0 ± 2.6	139 ± 8.4	225 ± 70
undernourished	29.0** ± 1.6 (52)	36.0** ± 2.5 (44)	86.0** ± 2.0 (62)	110** ± 1.9 (49)
	enzyme concentration, (units/g wet weight)			
control	362 ± 17	874 ± 20	894 ± 28	2282 ± 30
undernourished	156** ± 4 (43)	364** ± 22 (42)	448** ± 18 (50)	1114** ± 16 (49)
	specific activity of enzyme without exogenous diglycerols (units/mg protein)			
control	15.0	18.0	10.1 ± 0.2	16.1 ± 0.1
undernourished	9.6 ± 0.5 (64)	9.0 ± 1.3 (50)	12.9 ± 1.4 (128)	11.0* ± 1.9 (68)

PET : phosphoethanolamine transferase, PCT : phosphocholine transferase, GM : gray matter, WM : white matter.

values in parantheses are percentage of controls.
values marked with asterisk significantly different from control values, p less than 0.05 for * and 0.001 for **.
body weight (g) and brain weight (g) are 53.3 ± 1.2 and 1.54 ± 0.01 for control and 13.5 ± 0.27 and 1.07 ± 0.01 for undernourished rats respectively.

Fig. 15

Effect of microsomal protein concentration on the incorporation of radioactivity from CDP-(^{14}C) choline and CDP-(^{14}C)-ethanolamine into choline and ethanolamine phosphoglycerides respectively.



GM : gray matter, WM : white matter

PCT : phosphocholine transferase

PET : phosphoethanolamine transferase.

undernourished rats was again less than in control rats (Fig. 16). Hence for all studies 20 min was used for incubating the assay mixture.

Studies carried out on the effects of concentration of diacaprin on the incorporation of radioactivity from CDP-bases into EPG and CPG showed a saturation point at 40 μM concentration (Fig. 17). Hence for all the studies this concentration was used. Fig. 18 summarizes the effects of different concentrations of CDP-(^{14}C) choline and CDP-(^{14}C) ethanolamine in the assay system on the activities of PCT and PET. From Figs. 17 and 18 it can be seen that with different concentrations of substrates the enzymes followed Michaelis-Menten kinetics.

The data on the effects of neonatal undernutrition on the activities of PET and PCT are shown in Table 52. The specific activities obtained for PET and PCT in the gray and white matter in the brain of control rats in the present study are 5-15 times higher than the previously reported values (Radominska-Pyrek and Horrocks, 1972; Radominska-Pyrek et al, 1976 and 1977). This may be due to some improvements made in the assay procedure such as the change in the protein concentration from 500 μg to 6 ug and in the amount of diglyceride from 4 mM to 40 μM. The concentration and specific activity of both the transferases were found to decrease significantly with undernutrition (Table 52).

When no exogenous diglycerols were added to the incubation medium the activities of phosphotransferases were limited by the

Fig. 16

Effect of time of incubation on the incorporation of radioactivity from CDP-(^{14}C) choline and CDP-(^{14}C)-ethanolamine into choline and ethanolamine phospho-glycerides respectively.

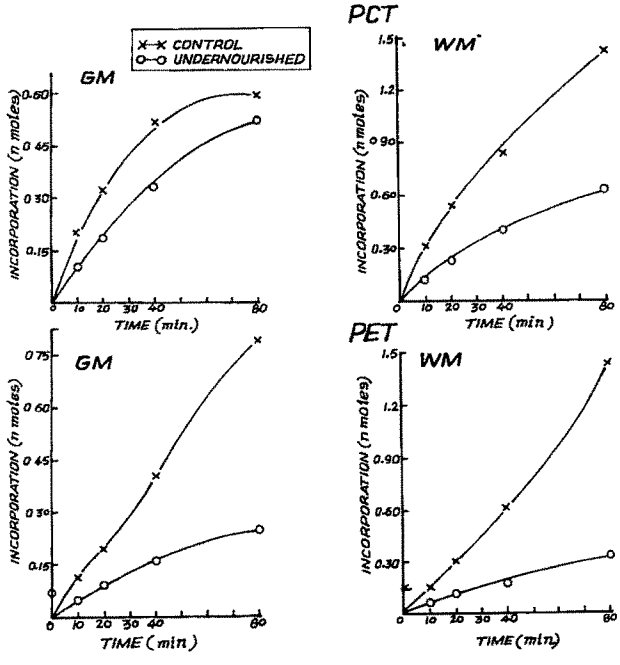


Fig. 17

Effect of dicaprin concentration on the incorporation of radioactivity from CDP-(^{14}C) choline and CDP-(^{14}C)-ethanolamine into choline and ethanolamine phospho-glycerides respectively.

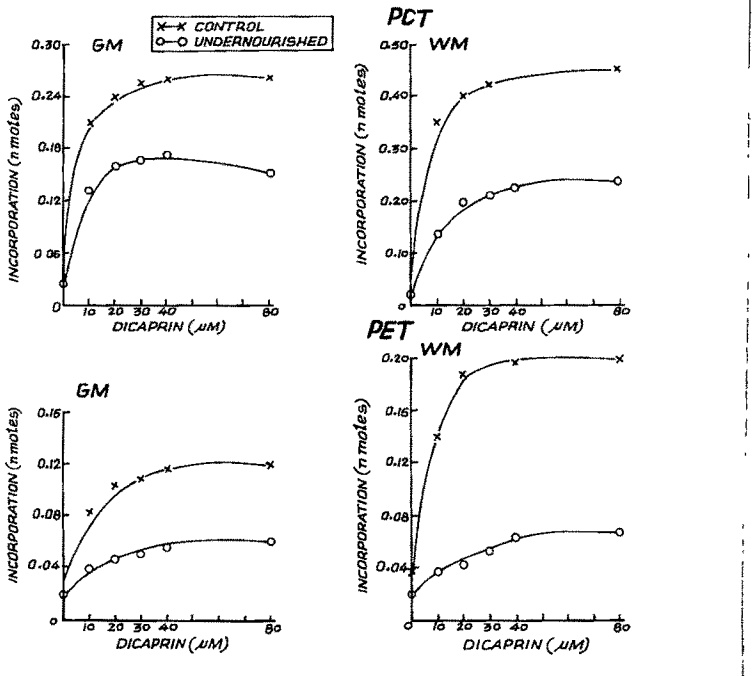


Fig. 18

Effect of CDP-(^{14}C) choline and CDP-(^{14}C) ethanolamine concentration on the incorporation of radioactivity into choline and ethanolamine phosphoglycerides respectively.

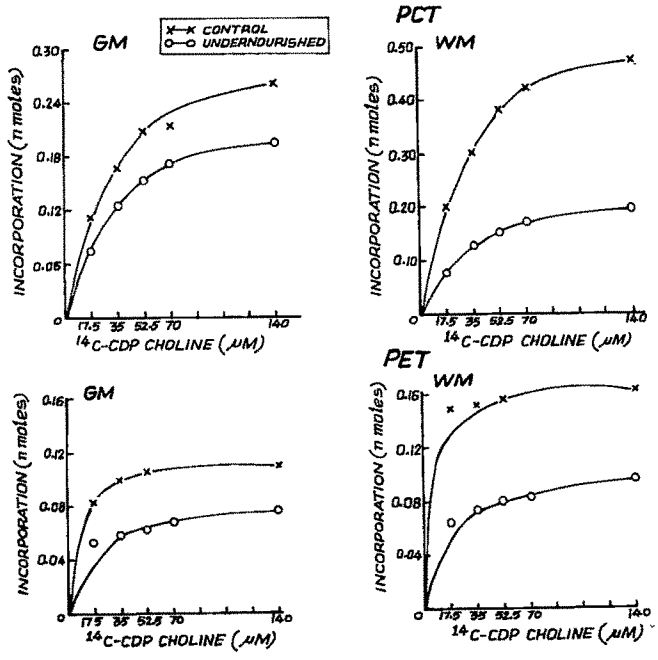
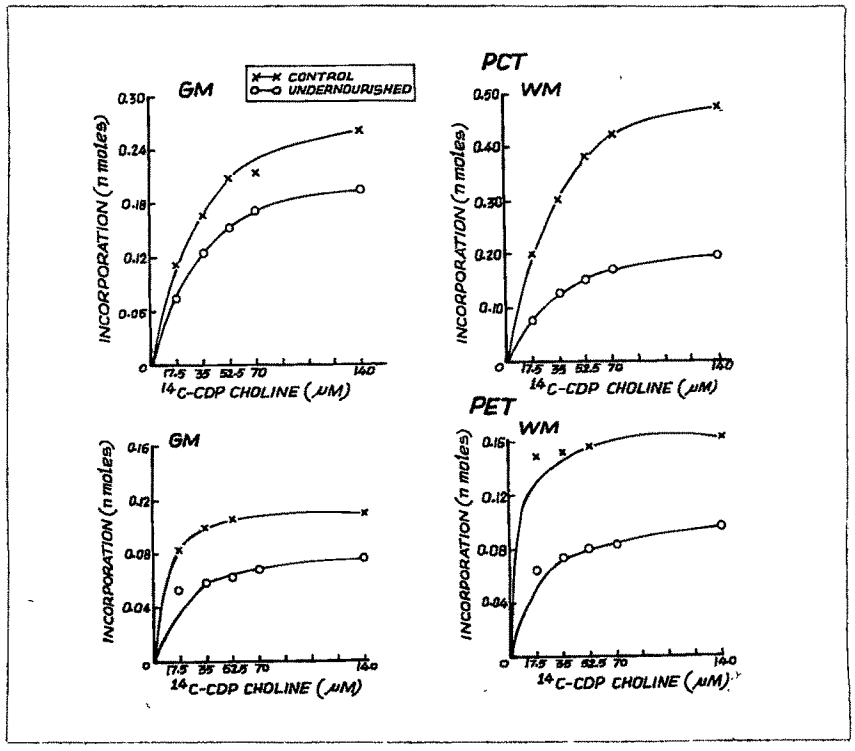


Fig. 18

Effect of CDP-(^{14}C) choline and CDP-(^{14}C) ethanolamine concentration on the incorporation of radioactivity into choline and ethanolamine phosphoglycerides respectively.



availability of diglycerols as substrates so that the enzyme activities measured gave an idea of the amount of diradylglycerols present in the microsomes. The data on the enzyme activities in the absence of added diglycerols are given in Table 52. The differences in the relative values of PCT and PET may reflect differences in accessibility of diradylglycerols to the enzymes. The availability of diradylglycerols for PET and PCT showed a significant decrease in white matter microsomes with undernutrition. Similarly the availability of diradylglycerols for PET was decreased in gray matter microsomes from undernourished rats but that of PCT was not affected (Table 52).

Km and Vmax values were determined for dicaprin and CDP-choline or CDP-ethanolamine substrates using a direct linear plot as described by Eisenthal and Cornish-Bowden (1974). This method is found to be superior to plots of reciprocal velocities versus reciprocal concentrations (Atkins and Nimmo, 1975; Markuss et al, 1976). The Km and Vmax values are given in Table 53 which shows that the Km values for both PCT and PET for dicaprin and CDP-bases were unaffected in gray matter microsomes during neonatal undernutrition. But the Km values for PCT and PET for dicaprin and CDP-ethanolamine were found to increase by two fold in white matter microsomes during neonatal undernutrition suggesting that the affinity of the enzymes towards the corresponding substrates was low during undernutrition. In all the cases the Vmax values were found to be

Table 53 : Effects of undernutrition on Km and Vmax values for phosphoethanolamine and phosphocholine transferases in microsomal fraction of the rat brain gray and white matter.

	enzyme	gray matter				white matter			
		Km (µM)		Vmax (nmoles/mg protein/h)		Km (µM)		Vmax (nmoles/mg protein/h)	
		C	UN	C	UN	C	UN	C	UN
dicaprin (diacylglycerol)	PCT	3.5	4.0	139	95	4.0	8.0	235	132
	PET	10	8.0	60	33	13	17	105	40
CDP-(^{14}C) choline	PCT	35	40	162	126	38	37	310	164
CDP-(^{14}C) ethanolamine	PET	9.0	9.0	64	40	3.6	7.0	84	45

PCT : phosphocholine transferase,

PCT : phosphoethanolamine transferase.

C : control

UN : undernourished.

decreased (Table 53). These results suggest that undernutrition might cause an alteration in the properties of these two enzymes. This needs further investigations.

The significant decrease in the activities of phosphoethanolamine and phosphocholine transferases and the decreased concentrations of endogenous diradyl-glycerols in the white matter microsomes with undernutrition well correlates with the decreased concentrations of EPG and CPG in the white matter whereas no significant changes were observed in the concentrations of EPG and CPG in gray matter with undernutrition even though a significant decrease in the activities of PET and PCT were observed. This may be because of the slow turnover of the CPG and EPG in the gray matter in the case of undernourished rats (Horrocks and Reddy, 1980). However, the activities of the enzymes measured *in vitro* need not reflect the concentrations of the tissue lipids because the different levels of substrates, inhibitors and modulators present in the *in vivo* system and their affinities towards the enzymes will determine the biosynthesis of lipids.