C H A P T E R III

RESULTS AND DISCUSSION

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Experiment I :

Prenatal undernutrition :

As mentioned earlier, for the studies on prenatal undernutrition young virgin females were given a low protein diet for one month before mating and continued on this diet till partus. Although some adverse effects were found on the reproductive performance of these animals, viable pups were produced. The effects observed were a greater number of still births and increased mortality during the postnatal period (Table 6). However, in several studies carried out in this laboratory over the last decade, these effects showed considerable variation from experiment to experiment.

Maternal protein deficiency during gestation has been found to be associated with poor maternal weight gain, increased incidence of still births, but gestational age and average litter size were not found to be affected (Nelson and Evans, 1953; Venkatachalam and Ramanathan, 1964; Siassi and Siassi, 1973). Similar observations have been made with energy restriction (Barnes and Altman, 1973; Smart and Dobbing, 1971a; Balázs and Patel, 1973). A decrease in litter size with such restriction has been found by Chow and Lee (1964). Maternal protein deficiency during lactation had more serious consequences than that during pregnancy on the

Table 6 : Effects of a low protein diet@ on reproductive performance.

		% protein in diet	
		20	5
1.	no. of females kept for breeding	16	51
2.	no. of pregnancies	14	32
3.	% fertility	87	62
4.	average litter size	8.0	7.5
5.	no. of pups still born	1	5
6.	no. of live births	107	204
7.	no. of deaths in the neonatal period	8	84
8.	% mortality	7	41
9.	average birth weight (g)	6.0	4.0
10.	average weaning weight (g)@@	42	12

@ fed from one month before mating till 3 weeks after partus.

@@ 3rd week weight.

performance of the pups. The mortality of pups was 41% compared to 7% in controls. Most of this occurred during the first week of life.

Some differences in maternal behaviour were also noticed. The control mothers usually huddled close to the pups. In contrast, the pups of protein deficient mothers were scattered all over the cage. Frankova (1972,1974) who has made a more detailed study of maternal behaviour in protein deficiency has made similar observations.

Pups born of the protein deficient mothers were found to have significantly smaller body and brain weights as compared to controls (Table 7). Similar observations have been made by other investigators (Zamenhof, Marthens and Margolis, 1968; Envonwu and Glover, 1973; Siassi and Siassi, 1973; Clark, Zamenhof and Marthens, 1973). In contrast no significant decrease in brain weight was found with 50% restriction in the amount of food available to the mothers (Kumar and Sanger, 1970; Balázs and Patel, 1973).

The protein concentration of the brain was not found to be altered, a finding consistent with that of several other investigators (Zamenhof et al, 1968; Zeman and Stanbrough, 1969; Balázs and Patel, 1973; Envonwu and Glover, 1973).

Table 7 : Effects of maternal protein deficiency during gestation on prenatal development (Day 1).

	% protein in maternal diet		$\frac{LP}{HP}$ X 100
	20 (HP)	5 (LP)	
no. of pups@	22	18	
body weight (g)	6.5 ± 0.084	4.0 ± 0.03***	64
brain weight (g)	0.280 ± 0.001	0.220 ± 0.003***	78
values per (g) brain :			
protein (mg)	58 ± 0.82	59 ± 0.30	105
units of :			
GDH	0.58 ± 0.033	0.59 ± 0.033	105
GAD	3.5 ± 0.19	3.5 ± 0.27	100

@ Three brains were pooled for each determination.

Values marked with asterisk significantly different from control values. $P < 0.001$ for ***.

The activities of brain glutamate dehydrogenase and decarboxylase in the brain were not affected in the progeny of the deficient mothers. Similar observations have been made by Adlard and Dobbing (1971) with regard to enzymes such as succinic dehydrogenase, aldolase, acetylcholine esterase and β-N-acetyl glucosaminidase in pups born of food restricted mothers. However, in other studies in this laboratory a deficit in acetylcholine esterase was found in congenitally malnourished pups and this was associated with a delayed appearance of reflex activities such as righting, negative geotaxis, cliff avoidance which are found in normal animals within the first three days after birth. Thus the effects of prenatal deficiency may depend on the parameter investigated.

Experiment IIa :

Effects of neonatal undernutrition at different ages :

As mentioned earlier, attempts were made to extend the studies previously carried out on the effects of neonatal undernutrition to a study of these effects in relation to age and severity of undernutrition. The present experiment was concerned with the effects of undernutrition at different ages.

Neonatal undernutrition was induced by manipulating litter size soon after partus. Pups reared in standard or large littérs of 8 or 16 were killed at 4 weeks after birth and assayed for the activities of glutamate dehydrogenase and decarboxylase.

The results are presented in Table 8. It can be seen from the same that undernutrition during the neonatal period resulted in significant deficits in brain GDH and GAD. This is possibly because of the fact that the maturation of both these enzymes takes place during the neonatal period. A question arose as to whether the effects of undernutrition vary with the stage of development and therefore with age. To study the effects of neonatal undernutrition at different ages pups reared in standard or large litters were killed at 1, 2 and 3 weeks of age. Also some pups were killed at birth for comparison. The results are presented in Table 9. As can be seen from the same, neonatal undernutrition was found to result in a progressive retardation in body growth right from the first week of life. A similar retardation in brain weight was not observed. Similar observations are found by Swaiman <u>et al</u> (1970).

Eventhough the body weight and brain weight deficits were apparent from the first week of life the enzymes GDH and GAD were affected only from 2 weeks of age. Moreover, deficits were higher at 2 weeks of age than at 3 or 4 weeks. These results suggest that at the age of about 2 weeks the brain is more vulnerable to nutritional deficiency.

Table 8 : Effects of neonatal undernutrition on brain weight and brain enzymes (28 days).

	litter size		UN/C X 100
	8 C	16 UN	
no. of animals	8	16	
	mean ± s.e.		
body weight (g) at :			
birth	4.7	4.7	100
28 days	46.0±0.73	25.0±0.20	54
brain weight (g)	1.26±0.02	1.13±0.02***	90
	units per g. brain tissue		
GDH	3.8±0.10	3.2±0.02***	84
GAD	33.0±0.90	27.0±0.44***	82

Values marked with asterisk significantly different from control values. P $<$ 0.001 for ***.

Table 9 : Effects of neonatal undernutrition on body weight, brain weight and brain enzymes at different ages in strain A.

	body weight (g)		brain weight (g)		units per g brain			
					GDH		GAD	
litter size[@]	8	16	8	16	8	16	8	16
				mean ± s.e.				
age (days)								
0	4.7±0.11		0.20±0.005		0.58±0.014		5.3±0.25	
7	11.0 ±0.05	7.3(65) ±0.10	0.60 ±0.01	0.49(82)*** ±0.03	1.5 ±0.05	1.4(95) ±0.02	8.7 ±0.14	8.5(97) ±0.25
14	18.4 ±0.22	12.0(65) ±0.38	1.01 ±0.03	0.90(89)*** ±0.02	2.5 ±0.09	1.7(68)*** ±0.05	18.0 ±0.30	12.0(67)*** ±0.40
21	30.0 ±0.78	18.0(60) ±0.44	1.15 ±0.03	1.04(90)*** ±0.02	3.5 ±0.10	2.9(83)*** ±0.02	22.0 ±0.70	17.0(75)*** ±0.30
28	46.0 ±0.73	25.0(54) ±0.20	1.26 ±0.02	1.13(90)*** ±0.02	3.8 ±0.10	3.2(84)*** ±0.02	33.0 ±0.90	27.0(82)*** ±0.44

@ Results based on all the animals in each litter.

Figures in parentheses indicate values as % of control values.

Values marked with asterisk significantly different from control values $P < 0.001$ for ***.

The data on weight and brain enzymes at different ages are presented in Table 11. It can be seen from the same that the rate of increase in body and brain weight and brain GDH activity were maximum during the first week of life, whereas GAD activity showed a maximum increase during the second week. A similar pattern is found with regard to body weight and brain weight in the data of Adlard and Dobbing (1971); Envonwu and Glover (1973); Siassi and Siassi (1973) and with regard to brain GDH in the values reported by Bayer and McMurry (1967). The pattern with regard to GAD is also consistent with other studies (Van den berg et al, 1964; Bayer and McMurry, 1967; Sims and Pitts, 1970).

A comparison of the increments with those in the undernourished animals suggests that undernutrition slowed down enzyme maturation appreciably during the second week when body and brain growth which slowed down during the first week did not show a further decline.

Experiment IIb :

As mentioned earlier the older slow-growing strain in this laboratory has been more or less replaced by a fast growing strain. The above experiment was repeated with the new strain in order to investigate strain differences if any. As evident from Table 10, these results are consistent with those on the slow growing strain. Protein concentration was

Table 10 : Effects of neonatal undernutrition on body weight, brain weight and brain enzymes at different ages in strain B.@

	body weight (g)		brain weight (g)		Protein (mg/g)		units per g brain			
							GDH		GAD	
litter size@	8	16	8	16	8	16	8	16	8	16
	mean ± s.e.									
age (days)										
0	5.7 ± 0.085		0.23 ± 0.002		57.0 ± 2.5		0.58 ± 0.038		3.8 ± 0.22	
7	16.0 ±0.53	10.0 ±0.21 (62)	0.68 ±0.017	0.58*** ±0.020 (85)	57.0 ± 1.0	56.0 ±0.84 (98)	1.50 ±0.095	1.45 ±0.093 (97)	8.2 ±0.60	7.8 ±0.69 (96)
14	25.0 ±0.33	14.0 ±0.40 (56)	1.15 ±0.023	0.980*** ±0.012 (86)	76.0 ±0.81	78.0 ± 1.1 (102)	3.12 ±0.03	2.46*** ±0.10 (80)	19.3 ±0.73	14.6*** ±0.64 (75)
21	44.0 ±1.75	22.0 ±0.63 (50)	1.40 ±0.012	1.19*** ±0.015 (85)	93.0 ± 1.5	90.0 ± 1.3 (96)	4.46 ±0.095	3.79*** ±0.067 (85)	24.7 ±0.80	20.3*** ±0.37 (82)

@ Values based on 11 animals in each group at 21 days and 7 animals each in the other groups.

Figures in parentheses indicate values as % of control values.

Values marked with asterisk significantly different from control values, $P < 0.001$ for ***.

Table 11 : Percentage increments in control and undernourished animals of strains A and B during different ages.

age interval (days)		Body weight		brain weight		protein per g tissue		values per g tissue			
								GDH		GAD	
		8	16	8	16	8	16	8	16	8	16
0 - 7											
	A	134	55	200	145	-	-	158	141	64	60
	B	186	79	196	153	0	0	159	150	116	108
8 - 14											
	A	67	64	68	84	-	-	69	21	107	41
	B	56	40	70	68	31	34	107	72	135	85
15 - 21											
	A	63	50	14	16	-	-	40	71	22	42
	B	76	57	22	21	22	14	43	52	28	45
22 - 28											
	A	53	39	10	10	-	-	9	14	50	59
0 - 28											
	A	880	432	530	465	-	-	555	469	522	416
0 - 21											
	B	786	393	508	417	63	58	769	653	640	534

not found to be affected at any of the ages studied. Similar observations have been made by Swaiman _et al_ (1970); Envonwu and Glover (1973), and Sobotka _et al_ (1974).

As in the previous experiment enzyme deficits were apparent only at 2 weeks of age and also greater at this point. This strain appears to have higher activities of GDH than the old strain (A) but GAD activity was found to be more or less similar in both. The pattern of increments in body and brain weights and in brain enzyme activity was similar in both cases. Protein concentration was found to increase from the first week onwards. Similar observations have been made by Cheek _et al_ (1969), Envonwu and Glover (1973) and Baláźs and Patel (1973).

Experiment III :

Effects of different degrees of undernutrition during the neonatal period :

In the experiments just described neonatal undernutrition was found to affect brain enzyme activity at 14 and 21 days of age but not at 7 days. These observations raised a question as to whether the effects of deficiency vary with its severity and more specifically, whether the picture at 7 days would be affected by a more severe degree of undernutrition and whether the size of the deficits observed varies at other ages.

Studies were therefore made of different degrees of undernutrition induced by (a) increasing litter size (group II), (b) feeding the mothers a low protein diet during lactation (group III) and (c) feeding the mothers a low protein diet during gestation and lactation (group IV). A control group of mothers (group I) with standard litter size was fed a high protein diet (Stock diet providing 18% protein) throughout.

[As soon as an animal in group IV delivered, mothers which had delivered on the same day in the stock colony were assigned to group I, II and III with litter size adjusted to 8, 16 and 8. In the case of group IV, an adequate number of females were kept on the low protein diet and bred so that more than one female delivered about the same time and it was possible to adjust litter size in this group also to 6-8 in the event of these mothers producing small litters. The pups from all the groups were killed at 7, 14 and 21 days of age.]

As expected, the severity of undernutrition was greater with maternal protein deficiency than with increased litter size and somewhat greater when maternal protein deficiency was induced during both gestation and lactation than during only lactation (Table 12).

Table 12 : Effects of different degrees of undernutrition on body weight at different ages.

group	body weight (g) mean ± s.e.			
	I control (C)	II large litter (LL)	III	IV
			maternal protein diet during	
			lactation ($G^+ L^-$)	gestation + lactation ($G^- L^-$)
age (days)				
7	16 ± 0.50	10.0 ± 0.20	10.0 ± 0.90	7.0 ± 0.21
14	26 ± 0.53	14.0 ± 0.40	11.0 ± 0.94	7.0 ± 0.34
21	42 ± 0.67	22.0 ± 0.63	14.0 ± 0.49	12.0 ± 0.14

Effects of different degrees of undernutrition from birth to 7 days of age.

The results of these studies are presented in Table 13. Body weight was found to be affected in all the three groups but more so in the case of pups from mothers fed a low protein diet during both gestation and lactation. Brain weight followed a similar pattern. Protein concentration as well as enzyme activities were not found to be affected in any of the groups. Similar observations have been made with regard to GAD with maternal B_6 deficiency by Bayoumi and Smith (1971) and with regard to succinate dehydrogenase, acetyl choline esterase and aldolase with maternal food restriction by Adlard and Dobbing (1971). In both these cases the body weight deficits were comparable to those obtained in the present study with maternal protein deficiency.

The lack of any effects at seven days of age even with a more severe degree of undernutrition is perhaps explicable when we consider the growth retardation was much less severe at this age and this was also true of brain weight deficits. It also appears that in the early neonatal period brain growth retardation resulting from undernutrition is much less than body growth retardation on the basis of the data presented in Table 15. This might also be true of brain enzyme maturation which is maintained even in the face of a deficit in brain weight in the case of the prenatally undernourished animals.

Table 13 : Effects of different degrees of nutritional deficiency at 7 days of age.

	I C	II LL	III $G^+ L^-$	IV $G^- L^-$
		mean ± s.e.		
no. of animals investigated@	9	7	10	7
body weight (g)	16±0.50	10.0±0.20***	10.0±0.90***	7.0±0.21***
brain weight (g)	0.71±0.019	0.58±0.020***	0.64±0.020**	0.49±0.017***
brain weight : body weight	0.044±0.001	0.058±0.001***	0.064±0.002***	0.070±0.001***
		values per g brain		
protein (mg)	57.0±0.088	56.0±0.084	55.0±0.088	56.0±0.090
GDH (units)	1.57±0.085	1.45±0.093	1.67±0.067	1.40±0.083
GAD (units)	8.1±0.46	7.9±0.69	7.5±0.50	7.8±0.32

@ Representative animals taken from different litters.
values marked with asterisk significantly different from control values.
P $<$ 0.01 for ** and P $<$ 0.001 for ***.

Effects of different degrees of undernutrition from birth to 14 and 21 days of age :

Table 14 presents the results for studies on the effects of different degrees of undernutrition from birth to 14 and 21 days. The body weight deficits in the undernourished groups were greater at 14 days than at 7 days and greater in pups born of the protein deficient animals than those reared in large size litters. This is not surprising as the mother nursing a large litter can compensate to some extent by increasing food intake which is reduced with protein deficiency. The food intake of mothers with standard and large litters was 34 g and 45 g as against 9-12 g in mothers fed a low protein diet. During this period brain weight deficits persisted in the group reared in large litters and increased further in pups reared by low protein mothers.

As in the previous experiment protein concentration was not affected in any of the groups and enzyme activities were significantly decreased in all the undernourished groups as compared to controls but the deficits in brain enzyme activities were unrelated to the degree of undernutrition. This is pe rhaps not surprising in the light of other studies in this laboratory in which animals fed a 5% protein diet were found to show similar deficits but deficits were not increased by increasing the severity of deficiency by feeding

Table 14 : Effects of different degrees of nutritional deficiency from birth to 14 and 21 days of age.

group	I C		II LL		III $G^+ L^-$		IV $G^- L^-$	
age (days)	14	21	14	21	14	21	14	21
no. of animals investigated	11	21	7	11	14	13	13	12
				mean ± s.e.				
body weight (g)	26.0 ±0.56	42.0 ±0.67	14.0 ±0.40	22.0 ±0.63	11.0 ±0.94	14.0 ±0.49	7.0 ±0.34	12.0 ±0.14
brain weight (g)	1.20 ±0.019	1.38 ±0.012	0.98*** ±0.012	1.17*** ±0.019	0.90*** ±0.014	1.10*** ±0.012	0.70*** ±0.010	1.03*** ±0.14
brain weight / body weight :	0.046 ±0.006	0.033 ±0.001	0.070 ±0.004	0.053*** ±0.004	0.082*** ±0.002	0.078*** ±0.002	0.100*** ±0.003	0.086*** ±0.002
				values per g brain				
protein (mg)	77.6 ±0.75	95.0 ± 1.2	78.0 ± 1.1	90.0 ± 1.3	78.0 ± 1.5	96.0 ± 1.2	76.0 ±0.92	94.0 ± 0.90
GDH (units)	3.13 ±0.050	4.38 ±0.049	2.49*** ±0.10	3.79*** ±0.067	2.45*** ±0.066	3.76*** ±0.055	2.36*** ±0.069	3.80*** ±0.059
GAD (units)	19.1 ±0.45	24.2 ±0.52	14.6*** ±0.64	20.3*** ±0.38	14.5*** ±0.69	19.3*** ±0.47	12.7*** ±0.69	19.7*** ±0.88

values amarked with asterisk significantly different from control values, $P < 0.001$ for ***.

the animals diets containing 2, 3 or 4% protein although such low levels of protein resulted in some loss of weight (Rajalakshmi, Parameswaran and Ramakrishnan, 1974). On the other hand, in studies on the effects of neonatal undernutrition on brain lipids, deficits in the concentrations of cholesterol, phospholipids and galactolipids were found to increase with more severe degrees of undernutrition (Rajalakshmi and Nakhasi, 1974). These findings suggest that the effects of deficiencies on metabolic activity may present a picture different from that on chemical composition although the two are interrelated. Such a suggestion is also supported by a rather contrasting observation, namely, that protein deficiency in the immediate post-weaning period produced deficits in brain enzymes (Rajalakshmi et al, 1965) but did not influence the concentration of lipids (Rajalakshmi, Nakhasi and Ramakrishnan, 1974).

The above deficits persisted at 21 days but the percentage deficits did not increase (Table 15). Rather, the data suggest some decrease in the size of these deficits. This may be due to operation of an adaptive mechanism whereby brain growth and maturation are maintainedd at the expense of body growth. The ratio of body weight to brain weight was greater in the undernourished animals, the differences between controls and undernourished animals becoming more evident with the progress of undernutrition (Table 15).

Table 15 : Effects of undernutrition in relation to age and severity of treatment.

	values as % of control values								
group	II (LL)			III (G^+ L^-)			IV (G^- L^-)		
age (days)	7	14	21	7	14	21	7	14	21
body weight	62*	54*	52*	62*	42*	33*	44*	27*	29*
brain weight	82*	81*	85*	90*	75*	80*	70*	59*	75*
brain weight / body weight	130*	152*	160*	145*	180*	236*	160*	240*	260*
	values per g brain								
Protein	98	100*	94	96	100	100	98	100	100
brain enzymes									
GDH	92	80*	94*	106	78*	80*	89	74*	81*
GAD	98	80*	88*	93	77*	84*	96	72*	84*

Similar adaptive mechanisms are evident in the differential effects of undernutrition on different aspects of body growth. In human children, increments in height are achieved even at the expense of weight. Similarly, the length of the trunk is less affected than that of the long limbs so that sitting height is less affected than standing height, an adaptation of obvious value to the organism.

Experiment IV :

Effects of neonatal undernutrition confined to different ages on brain enzymes :

In the experiments just described, deficits in brain GDH and GAD were found with neonatal undernutrition induced from birth to 2 or 3 weeks of age but not at one week. ~~This raised~~ the question as to whether adequate nutrition during the second and third weeks is more critical than during the first week and as to whether the lack of a perceptible effect at 7 days is due to the shorter period of treatment or because of decreased vulnerability during the first week of life. Studies were therefore undertaken on the effects of varying the period of undernutrition on the deficits observed. Undernutrition was induced during only the first week or third week or during the first two or last two weeks or during all three weeks of the neonatal period by transferring the pups to mothers fed a low protein diet at the specified ages. All the pups were killed at 21 days of age.

It can be seen from the data presented in Tables 16 and 17 that animals subjected to undernutrition only during the first week of life did not differ from controls with regard to even body or brain weight suggesting a complete catch up growth.

Undernutrition confined to the last week resulted in significant deficits in body and brain weight and also in the activity of GDH but neither protein concentration nor GAD activity were affected. In this connection GDH activity seems to be relatively more sensitive to nutritional deficiencies than GAD, on the basis of other experiments to be reported subsequently in this thesis as well as other studies in this laboratory (Parameswaran, 1974).

Undernutrition confined to the last two weeks produced deficits in body weight, brain weight and brain GDH activity comparable to those resulting from three weeks of undernutrition, but GAD was not affected, again suggesting the greater sensitivity of GDH to nutritional deficiency. The percentage deficits with undernutrition from 7-21 days of age compared with those obtained with the same induced from 0-14 days of age except in the case of GAD. This is perhaps

Table 16 5 : Effects of undernutrition confined to different ages on body weight, brain weight and brain protein.

group	no.of animals	nutritional status[a] during days			body weight (g)	brain weight (g)	protein mg/g
		0-7	8-14	15-21			
I	8	N	N	N	42 ± 0.83	1.41 ± 0.016	92.0 ± 0.95
II	7	UN	N	N	44 ± 1.6 (105)	1.35 ± 0.015 (95)	92.0 ± 0.95
III	9	UN	UN	N	22 ± 0.5*** (50)	1.17 ± 0.010*** (83)	93.0 ± 0.83
IV	9	N	N	UN	24 ± 0.70*** (57)	1.26 ± 0.017*** (89)	92.0 ± 1.2
V	12	N	UN	UN	15 ± 0.43*** (36)	1.11 ± 0.019*** (79)	90.0 ± 2.1
VI	5	UN	UN	UN	14 ± 1.0*** (33)	1.09 ± 0.027*** (77)	90.0 ± 0.56

*** Values significantly different from control values, $P < 0.001$ for ***.

Values in parentheses represent percentage of control.

a N - normal, UN - undernourished.

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Table 17 : Effects of undernutrition confined to different ages on brain enzymes at 21 days of age.

group	nutritional status during days			no. of animals	units per g brain	
	0 - 7	8 - 14	15 - 21		GDH	GAD
					mean ± s.e.	
I	N	N	N	8	4.59±0.043	24.0±0.64
II	UN	N	N	7	4.56±0.10 (99)	24.4±0.34 (101)
III	UN	UN	N	9	4.12±0.084 (90) ***	24.3±0.58 (101)
IV	N	N	UN	9	4.23±0.072 (92) ***	24.8±0.98 (103)
V	N	UN	UN	12	3.80±0.087 (83) ***	23.5±0.89 (98)
VI	UN	UN	UN	5	3.78±0.11 (82) ***	20.4±0.67 (85) ***

Values marked with asterisk significantly different from control values. $P < 0.001$ for ***

Values in parentheses are percentages of control values.

consistent with the appreciable increment in GAD activity during the first two weeks, the percentage increase during this period being 116 and 135 as compared to 22 during the last week on the basis of values obtained for control animals at different ages in the previous experiment (Table 11).

Although a similar pattern is evident in the case of GDH also, the percentage increment in GDH activity (43) during the third week of life was greater than that in the case of GAD (22). However, we are unable to find a satisfactory explanation for the differential effects on GDH and GAD.

Animals undernourished during the first two weeks after birth and normally nourished during the third week had not achieved catch up growth as judged by body or brain weight. Again it is interesting to note that inspite of these persisting deficits in body and brain weights GAD activity did not differ from control values whereas GDH activity was found to be decreased.

In summary these results suggest that the effects of undernutrition vary not only with the period of treatment but also the age at which they are introduced and that the latter may influence the outcome even at comparable levels of deficits in body and brain weights.

Experiment V :

Effects of postweaning deficiencies and subsequent rehabilitation on brain enzymes in neonatally undernourished rats :

In the preceding experiment the effects of undernutrition from birth to 2 weeks of age were not fully reversed by rehabilitation for a period of one week, raising a question regarding the reversibility of the effects of early undernutrition. In earlier studies in this laboratory on using a slow-growing strain, protein deficiency in the postweaning period was found to affect these brain enzymes whereas undernutrition was without a similar effect. However, the effects of postweaning undernutrition were found to be influenced by the plane of nutrition prior to weaning (Rajalakshmi et al, 1974a).

In this connection, Barnes and his associates (1968a,b) have found a combination of neonatal undernutrition followed by protein deficiency or undernutrition during the postweaning period to produce irreversible changes in behavior. The present studies were undertaken in this context on the effects of nutritional deficiencies confined to or continued beyond the neonatal period. As mentioned earlier, animals undernourished till weaning were given a low or high protein diet or the latter in restricted amounts for 6 weeks. The animals in all the groups were then fed a high protein diet for 6 weeks. Batches of animals from each group were killed at each stage i.e. at 3, 9 and 15 weeks of age.

The results of these studies are presented in Table 18. A 4% protein diet was used in these studies as this was considered just enough to maintain body weight in previous studies with another strain (Parameswaran, 1974). However, a small weight gain was obtained with this level in the present studies presumably because of either strain differences or variations in the quality of casein. The food intake in the restricted group was sought to be adjusted to 2-2.5 g so as to result in body weights comparable to those of low protein group.

As was expected the animals reared in large litters had smaller body and brain weights at weaning and decreased activities of brain enzymes. Animals subjected to under-nutrition in early life and rehabilitated on the high protein diet for 6 weeks (2b) had not achieved a complete catch-up with regard to brain and body weights, but had normal levels of enzyme activity. In previous studies the brain weight of the undernourished and rehabilitated animals did not differ (Rajalakshmi _et al_, 1974a) from control values. That this was not the case in the present studies might have been because of the greater body and brain weight deficits at weaning.

Table 18 : Effects of postweaning deficiencies and subsequent rehabilitation in relation to plane of nutrition during neonatal period on brain enzymes.

group	Ia	Ib	IIa	IIb	IIc	IId	IIIa	IIIb	IIIc	IIId
duration (weeks)	phase I (0-3)		phase II (3-9)				phase III (9-15)			
diet/ nutrition-al status phase I	N	UN	N	UN	UN	UN	N	UN	UN	UN
phase II	-	-	HP	HP	LP	HP-R	HP	HP	LP	HP-R
phase III	-	-	-	-	-	-	HP	HP	HP	HP
					mean ± s.e.					
no. of animals	8	8	10	6	7	6	7	6	7	7
average food intake (g/day)	-	-	9.3	6.3	3.1	2.0-2.5	11.5	9.8	5.6	7.3
body weight (g)	43.0 ±0.72 ([illegible])	19.0 ± 1.8 (44)	166 ±1.8	111 ±2.1 (67)	25.0 ± 2.2 (15)	27.0 ± 1.7 (15)	246 ±17.0	180 ±7.0 (76)	125 ±8.0 (54)	148 ±4.6 (65)
brain weight(g)	1.39 ±0.022	1.13*** ±0.012 (82)	1.64 ±0.020	1.48*** ±0.013 (90)	1.18*** ±0.019 (71)	1.20*** ±0.017 (73)	1.73 ±0.012	1.62*** ±0.023 (93)	1.52*** ±0.011 (88)	1.54*** ±0.08 (89)

The percentage increments in body weights during phase II and phase III were greater in the undernourished and rehabilitated animals (group II) than in controls (group I) (Table 19) an observation which is widespread. These increments, however, declined during phase III. Eventhough animals fed the 4% protein diet or the 20% diet in restricted amounts had comparable weights before rehabilitation, the latter were much more active in the cage than the former and showed a better response to subsequent rehabilitation.

As in previous studies, protein concentration was not found to be affected by either undernutrition or protein deficiency during any phase of the experiment. Neonatally undernourished animals when rehabilitated during phase II had normal activities of the brain enzymes studied in spite of persisting deficits in body and brain weights whereas deficits in GDH persisted when fed either the 4% protein diet or high protein diet in restricted amounts. The activity was not further lowered with a low protein diet, an observation consistent with previous findings (Rajalakshmi _et al_, 1974a). GAD activity was lower in both these groups but the values were not significantly different from controls. This is at variance with the results of previous studies using a different strain (Rajalakshmi _et al_, 1974a) and we can only attribute this to strain differences. In any case, it is

Table 19 : Percentage increments in body and brain weight in different groups.

Group	treatment during phase			body weight		brain weight	
	I	II	III	Phase II	Phase III	Phase II	Phase III
a	N	N	N	300	48	18	5
b	UN	N	N	480	63	31	9
c	UN	LP	N	31	400	4	29
d	UN	UN	N	42	450	6	28

interesting to note again that GDH shows greater sensitivity to the effects of nutritional deprivation than GAD.

The animals subjected to nutritional deficiencies during phase I and II and rehabilitated during phase III had normal activities of the brain enzymes studied in spite of the persisting deficits in body and brain weights. This contrasts with the apparently irreversible affects on behavior noted by Barnes and his associates (1966, 1968b)and similar irreversible deficits on brain lipids noted in other studies in this laboratory (Nakhasi, unpublished). These studies underline the differential effects of prolonged and severe undernutrition on differential aspects of brain structure and function.

Experiment VI :

Effects of neonatal undernutrition on different regions of rat brain :

The brain is far from a homogeneous organ and the different regions of the same vary considerably in weight, period of maturation, structure, composition and function. It is therefore to be expected that they would also vary in their susceptibility to the effects of nutrition. Such has indeed been found to be the case in studies on the effects of undernutrition in rats during the neonatal period on parameters such as DNA, RNA, protein, lipids and enzymes (Table 20).

Table 20 : Findings on the effects of neonatal undernutrition@ in different brain regions of rat.

reference	age of under-nutrition (days)	% body weight deficit	regions studied	parameters measured	findings
Fish and Winick (1969)	21	-	cerebrum, cerebellum, hippocampus, stem	DNA and protein content	deficits found in DNA content and protein/DNA in cerebellum, cerebrum and hippocampus. maximum deficits in cerebellum.
Chase, Lindsley and O'Brien (1969)	18	50	cerebrum, cerebellum	DNA and protein content	maximum deficits in cerebellum.
Dickerson and Jarvis (1970)	21	66	forebrain, cerebellum stem	DNA content and concentration of cholesterol, gangliosides Ach E	DNA content and cholesterol concentration were most deficient in the cerebellum. gangliosides and Ach E in brain-stem.
Adlard and Dobbing (1972)	21	64	forebrain, cerebellum, stem, olfactory lobes	Ach E	deficits found in all regions greatest in olfactory lobes

Similar differences have been observed in the effects of protein deficiency during the immediate postweaning period on several brain enzymes including GDH and GAD (Rajalakshmi, Thrivikraman and Ramakrishnan, 1971). Studies were therefore carried out on the effects of neonatal undernutrition on these enzymes as well as DNA and protein content in different regions of the brain, namely, the cerebrum, cerebellum and brain stem. The period of undernutrition was 14 or 21 days.

The results of these studies are presented in Tables 21 and 22 and 23. All the regions had decreased weights but the deficit was maximum in the case of cerebellum (Table 23). The percentage deficits were more or less the same at 14 and 21 days. DNA content was found to be increased between 14 and 21 days of age in all the regions as might be expected. Although the undernourished animals also showed appreciable increase, the DNA content of the cerebrum and cerebellum was less in this group at 14 days of age and in the cerebellum at 21 days of age suggesting some compensatory increase in cell number in the cerebrum during the third week. At both periods the deficit was greater in the case of the cerebellum, an observation consistent with others findings (Fish and Winick, 1969; Sobotka *et al*, 1974). Protein concentration increased between 14 and 21 days in both groups and was not affected by undernutrition in any of the regions. Similar findings have been made by other investigators (Patel *et al*,

Table 21 : Effects of neonatal undernutrition on the growth, protein and enzymes of different brain regions at 14 days of age.

	cerebrum		cerebellum		stem	
	C	UN	C	UN	C	UN
			mean ± s.e.[a]			
body weight (g)			24.0±0.37	10.0±0.10		
weight of the region (g)	1.015±0.006	0.766±0.003***	0.136±0.0012	0.087±0.001***	0.070±0.0012	0.055±0.0017***
DNA (mg/region)	1.13±0.014	0.96±0.011***	0.90±0.027	0.60±0.017***	0.090±0.012	0.085±0.014
DNA (mg/g)	1.11±0.017	1.25±0.019***	6.68±0.030	6.90±0.039***	1.29±0.031	1.54±0.034**
Protein (mg/g)	71.0±0.96	71.0±1.3	86.0± 1.4	83.0± 1.0	68.0±0.95	68.0± 1.3
Protein/DNA	65.0±1.5	53.0± 1.7***	13.0±0.80	12.0±0.45	53.0±1.7	45.0±1.7***
units per g :						
GDH	3.37±0.076	2.66±0.12***	2.25±0.17	1.80±0.14**	3.66±0.11	3.37±0.17
GAD	18.6±0.47	15.2±0.55***	9.2± 0.5	9.0 ±0.56	17.1±0.91	17.5±1.13

[a] based on 7–8 estimations, each estimation involving 4–5 animals in each case.

C – control, UN – undernourished – Undernutrition was induced by feeding mothers protein deficient diet from partus till period specified.

Values marked with asterisk significantly different from control values, $P < 0.01$ for **, $P < 0.001$ for ***.

Table 22 : Effects of neonatal undernutrition on the growth, protein and enzymex of different brain regions at 21 days of age.

	cerebrum		cerebellum		stem	
	C	UN	C	UN	C	UN
			mean ± s.e.[a]			
body weight (g)	-	-	45.0 ± 0.51	13.0 ± 0.33		
weight of the region (g)	1.112 ± 0.0083	0.855*** ± 0.0083	0.172 ± 0.0027	0.119*** ± 0.0025	0.090 ± 0.0015	0.070*** ± 0.0014
DNA (mg/region)	1.26 ± 0.029	1.21 ± 0.025	1.10 ± 0.020	0.81*** ± 0.019	0.12 ± 0.014	0.11 ± 0.012
DNA (mg/g)	1.12 ± 0.019	1.42*** ± 0.018	6.43 ± 0.027	6.80 ± 0.029	1.48 ± 0.016	1.46 ± 0.021
protein (mg/g)	90.0 ± 1.3	91.0 ± 2.1	108.0 ± 3.0	105.0 ± 1.3	78.0 ± 1.1	78.0 ± 1.1
protein/DNA	80.3 ± 1.0	62.7 ± 2.2	16.8 ± 0.92	15.4 ± 0.6	52.9 ± 1.1	53.4 ± 1.6
units per g						
GDH	4.61 ± 0.049	4.09*** ± 0.025	3.00 ± 0.082	2.35*** ± 0.13	5.04 ± 0.11	4.95 ± 0.091
(b)	4.5		3.6		5.4	
GAD	26.7 ± 0.ø95	22.5** ± 0.79	18.6 ± 0.36	16.1*** ± 0.21	17.5 ± 0.57	18.2 ± 0.74
(b)	36.0		21.0		15.4	

a based on 7-8 estimations, each estimation involving 4-5 animals in each case.
C - controls, UN - undernourished. (b) values for adult control rats (2 months).
values marked with asterisk significantly different from control values, P $<$ 0.01 for **, P $<$ 0.001 for ***.

Table 23 : Values for undernourished animals as % of control values.

	cerebrum		cerebellum		stem	
age (days)	14	21	14	21	14	21
weight	75*	77*	63*	63*	79*	77*
DNA total	85*	96	67*	74*	94	92
DNA per g brain	113*	126*	103	105	119*	100
protein per g brain	100	101	96	97	100	100
$\frac{\text{protein}}{\text{DNA}}$	82*	78*	93	92	83*	100
units/g						
GDH	79*	86*	80*	78*	92	96
GAD	81*	84*	97	84*	102	104

values marked with asterisk significantly different from control values.

1973; Sobotka _et al_, 1974). Protein/DNA seemed to be significantly decreased in the cerebrum and stem at 14 days and in the cerebrum at 21 days. Maximum deficits were found in the cerebrum.

GDH was found to be affected at 14 and 21 days of age in the cerebrum and cerebellum. The deficits were of the same magnitude in both the regions at 14 days but were higher in the cerebellum at 21 days. The brain stem was not affected. In this connection the stem seems to be less susceptible to undernutrition. GAD activity was affected only in the cerebrum at 14 days, and in both the cerebrum and cerebellum at 21 days, the deficits in both regions being similar. Again the stem remained unaffected. It is interesting to note that the stem seems to attain adult values at 14 days of age whereas in both the cerebrum and cerebellum the activity of this enzyme continues to increase well after this period (Table 22). This is consistant with the findings of Bayoumi and Smith (1973).

In summary these studies suggest the greater susceptibility of the cerebellum to the effects of nutritional deficiency, the reverse being true of the brain stem. The former is consistent with the findings of Fish and Winick (1969), Chase, Lindsley and O'Brien (1969), Dickerson and Jarvis (1970), Patel, Balázs and Johnson (1973), Sobotka _et al_ (1974) whereas the latter is consistent with the findings of Fish and Winick (1969) and Sobotka et al (1974).