CHAPTER III

RESULTS AND DISCUSSION

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As stated earlier the present investigations were concerned with studies on brain glutamate dehydrogenase and decarboxylase in rats. The specific objectives were :

1. to identify the levels of protein needed in the diet to prevent the effects of deficiency and to reverse the effects of previous deficiency;
2. to study the effects of more severe degrees of protein deficiency and complete protein deprivation;
3. to identify the time course of the effects of deficiency;and
4. to investigate the comparative effects of neonatal and postweaning undernutrition and of protein and calorie deficiencies during the postweaning period.

Several experiments concerned with the above aspects were carried out as indicated in the preceding section. The results of these studies will now be described.

Experiments Ia and Ib: Brain enzymes at different levels of dietary protein

As mentioned earlier this experiment arose from two previous observations. One, that weanling rats fed a 5% protein diet had lower activities of brain glutamate dehydrogenase and decarboxylase than those fed a 20% protein diet.

Secondly when the animals were fed either a diet based on kodri or the same supplemented with lysine, brain enzyme activities compared with those of a low protein diet in the former case and with those of a 20% protein diet in the latter case although both the diets were isonitrogenous and contained 7.3% protein (Rajalakshmi et al, 1969).

In the first series of experiments (Ia and Ib) animals were fed diets containing 5,8,10,15 or 20% protein for a period of 10 or 5 weeks.

The data on food intake and weight gain in animals fed different levels of protein in the first 5 weeks of treatment in both experiments are given in Table 11. As expected, food intake decreased with less protein in the diet. The depression in food intake with a low protein diet is a widely observed phenomenon and is believed to be due to the operation of homeostatic mechanisms as force-feeding of imbalanced or deficient diets is found to either induce or accelerate pathological changes (Harper, 1969). However, in relation to body weight, food intake was more with less protein in the diet. Weight gain in relation to food and energy intake decreased with decreasing levels of protein in the diet. The impaired utilization of food for growth with decreased levels of protein in the diet is well known and

Table 11 : Food intake and weight gain in rats fed different levels of protein.

experiment	food intake (g)*				weight gain (g)*			
	per day		per 100 g. body weight		per week		per 100 kcal**	
	Ia	Ib	Ia	Ib	Ia	Ib	Ia	Ib
% dietary protein								
5	4.4	5.4	7.9	8.0	2.0	4.0	1.7(1.3)	2.8(2.1)
8	5.2	7.0	6.5	7.7	7.0	8.8	5.1(2.4)	4.7(2.3)
10	6.3	7.5	6.1	7.1	11.4	12.0	6.8(2.6)	6.0(2.3)
15	7.3	8.0	5.3	6.3	18.6	16.0	9.6(2.4)	7.5(1.9)
20	7.2	8.0	5.1	6.1	19.0	17.0	9.9(1.9)	8.0(1.5)

* mean values given for the first five weeks of treatment; 8 animals used in each group.

** PER values shown in parentheses.

forms the basis for the assessment of protein quality in terms of protein efficiency ratio (PER). However, PER is not a sensitive index of the protein value of the diet when the protein content of the diet is low (Allison, Wannemacher, Middleton and Spoerlein, 1959). With variations in the protein content of the diet the ratio of weight gain to energy intake is found to give a much more consistent picture (Rajalakshmi, 1972). This is also seen in the data of the present experiment.

The weight gains in the two experiments were comparable in animals fed 10, 15 or 20% protein but differed in the case of the other groups. In studies carried out during the last several years in this laboratory, animals fed low protein diets are found to show a greater variation in weight gain (2-5 g per week) than those fed high protein diets (15-20 g per week).

Increasing the protein content of the diet above 15% did not increase weight gain suggesting that this level of protein is adequate for optimum growth for the strain of albino rats used in this laboratory. Many other studies with different levels of protein have also shown that 15-16% protein in the diet is associated with maximum weight gain in rats (Forbes, Swift, Black and Kahlenberg, 1935; Goettsch, 1960).

The data on body weight, liver weight and the protein and glutathione content of the liver are presented in Table 12. Liver weight varied with body weight as may be expected.

Liver protein was appreciably lower in the 5% protein group and moderately so in the 8% group. No differences were found between animals fed 10,15 or 20% protein. The concentration of glutathione in the liver showed a greater variation than that of protein, the values increasing progressively with increases in dietary protein content up to a level of 15%. The percentage deficits in protein with a low protein diet were much less than those in glutathione. Similar observations have been made by Srinivasan and Patwardhan (1955). The data confirm earlier impressions that liver glutathione shows a greater sensitivity to protein status than liver protein (Rajalakshmi, 1972). In human studies differences in blood glutathione between poor and wellnourished pregnant women were much greater than those in serum protein (Rajalakshmi and Ramakrishnan, 1969b), the values for serum protein in the two groups being 6.3 and 6.7 g % compared to 14.8 and 20.0 mg % for blood glutathione. These observations suggest the value of glutathione as an index of protein nutritional state although it is influenced by a number of other factors as well (Jocelyn, 1959).

Table 12 : Body weight and composition of liver in rats fed different levels of protein.

	terminal body weight (g)		liver			
			weight (g)		protein (g per 100g)	glutathione (mg/g)
experiment	Ia	Ib	Ia	Ib	Ib	Ib
			mean ± s.e.			
% dietary protein						
5	69 ± 3.8	62 ± 2.9	2.3*** ± 0.07	2.1*** ± 0.21	14.3*** ± 0.30(73)	0.53*** ± 0.03(44)
8	135 ± 5.6	91 ± 3.6	4.0*** ± 0.13	2.8*** ± 0.13	17.3*** ± 0.38(89)	0.66*** ± 0.04(55)
10	176 ± 3.8	106 ± 3.8	4.7** ± 0.31	3.3** ± 0.17	18.5 ± 0.50(95)	0.80** ± 0.06(67)
15	206 ± 8.5	127 ± 5.0	6.4 ± 0.31	4.6 ± 0.27	18.7 ± 0.53(96)	1.16 ± 0.08(97)
20	213 ± 11.1	132 ± 5.0	6.0 ± 0.35	4.65 ± 0.27	19.5 ± 0.46	1.20 ± 0.10

Period of treatment, 10 weeks in experiment Ia and 5 weeks in experiment Ib; 8 animals used in each group. Values in parentheses are per cent of those for the 20% group (controls). Values marked with asterisk significantly different from control (20% protein) values; $P < 0.01$ for **, $P < 0.001$ for ***.

The data on brain weight and brain enzymes are given in Table 13. Brain weight was found to be significantly decreased with a 5% protein in the diet but not with levels of 8% or more. The ratio of brain weight to body weight was increased appreciably with 5% protein in the diet and less so with 8 and 10%. The results for the 5% group are consistent with previous observations in this laboratory (Rajalakshmi, Malathy and Ramakrishnan, 1967).

The data on brain enzymes confirm previous observations of deficits in brain GDH and GAD with a 5% protein diet but no deficits were found with diets containing 8% or more protein. Thus although the level of dietary protein needed for optimum growth was of the order of 15%, that needed for preventing deficits in brain weight and brain enzymes was only about 8%. This is consistent with previous observations of normal enzyme levels in animals given a diet containing 7-8% of a good quality protein in the form of a millet-legume mixture (Rajalakshmi et al, 1969).

As in previous studies, GABA-T was not affected by the protein content of the diet.

It is highly significant that a level of protein low enough to result in deficits in body weight and liver concentration of protein and glutathione is not low enough to

Table 13 : Brain weight and activities of brain enzymes in rats fed different levels of protein.

experiment	brain weight (g)		Brain enzymes (enzyme units per g brain)					
			GDH		GAD		GABA-T	
	Ia	Ib	Ia	Ib	Ia	Ib	Ia	Ib
% dietary protein								
5	1.34*** ± 0.02(0.019)	1.32** ±0.03(0.021)	3.2** ± 0.11	3.2** ± 0.14	27** ± 1.1	25** ± 1.0	53 ± 3.1	56 ± 2.2
8	1.47 ± 0.02(0.011)	1.44 ±0.03(0.016)	4.0 ± 0.11	4.2 ± 0.19	31 ± 1.1	29 ± 1.2	54 ± 2.1	56 ± 2.4
10	1.51 ± 0.03(0.009)	1.43 ±0.03(0.013)	4.0 ± 0.17	4.2 ± 0.11	30 ± 0.7	30 ± 1.0	54 ± 3.1	58 ± 2.2
15	1.54 ± 0.02(0.007)	1.43 ±0.03(0.011)	4.0 ± 0.14	4.4 ± 0.10	31 ± 1.2	30 ± 1.6	55 ± 2.8	56 ± 2.0
20	1.52 ± 0.02(0.007)	1.45 ±0.02(0.011)	3.9 ± 0.13	4.2 ± 0.10	32 ± 1.6	30 ± 1.0	56 ± 2.5	58 ± 2.4

Period of treatment, 10 weeks in experiment Ia and 5 weeks in experiment Ib; 8 animals used in each group. Values in parentheses give brain weight to body weight ratio. Values marked with asterisk significantly different from control (20% protein) values; $P < 0.01$ for **, $P < 0.001$ for ***.

affect the metabolic activity of the brain and that brain enzyme deficits are found only with a more severe degree of protein deficiency. This is consistent with the general observation that the brain is less affected by nutritional deficiencies than other organs. In previous studies in this laboratory on animals fed maize (Zea mays) or the same supplemented with the deficient amino acids and niacin, animals fed the unsupplemented diet had a low concentration of niacin in the liver but not in the brain (Kurian, 1964; Peramma, 1967). Similar observations have been made with regard to other vitamins such as thiamine (Lowry, 1952).

In other studies in this laboratory the protein content of the brain was not affected with a 5% protein diet in contrast to its marked decrease in the liver (unpublished studies).

It must also be pointed out that while the brain is the organ least affected by nutritional deficiency, the liver is perhaps the organ most affected because it constitutes the major source of labile reserves for many nutrients including protein. For instance, in studies mentioned earlier on ~~the~~ maize, liver niacin was found to be more affected than not only ~~the~~ brain niacin but also kidney niacin. It is well known that liver protein and vitamin A are affected sooner than plasma protein and vitamin A with deficiency (Addis, Poo and Lew,1936;

Addis, Lee, Low and Poo, 1940; Ganguly and Krinsky, 1953; High and Wilson, 1956).

The greater sensitivity of liver glutathione as compared to that of liver protein to protein deficiency may possibly be due to its forming part of the labile nitrogen reserves in the body.

The observation that a moderate protein deficiency which results in growth retardation does not necessarily result in brain enzyme deficits is encouraging from the point of view of practical nutrition. About a third of the children in the rural areas of this country are found to show mild deficiency of protein as judged by clinical and biochemical criteria (Rajalakshmi, Sail, Shah and Ambady, 1973). A similar pattern can be assumed in other regions. About 1-2% of children are found to suffer from severe protein deficiency. The present results at least give room for hope that the outlook for the former may not be grim. This does not minimize the importance of ensuring adequate nutrition for all.

Experiment II : Minimum dietary protein needed for normal activity of brain enzymes

The results of the experiments just described suggested 8% of good quality protein in the diet to be sufficient for preventing the deficits found in brain glutamate dehydrogenase and decarboxylase. This raises the question whether this amount

is not only sufficient but also necessary. To answer this question the protein content of the diet was varied at 5,6,7,8 and 20% levels. The results of these studies are given in Table 14. Increasing the protein content of the diet to 6% was without appreciable effect on any of the parameters studied but with a further increase to 7%, weight gain and the efficiency of food utilization increased markedly.

Brain weight did not vary significantly between animals fed 8% and 7% protein but a significant decrease was found with 6% and 5%. A similar pattern was found with regard to brain GDH and GAD whose activities were significantly lower with 6% and 5% protein diets but not with 7%. However, the mean values were numerically less in the 7% group than in the 8% group and it remains to be investigated whether this represents a consistent trend or a chance difference. It would, however, seem reasonable to conclude that, in the rat, a diet containing 7-8% of good quality protein is adequate to prevent changes in the brain enzymes studied. If this is also true of other structural and metabolic parameters in the brain, we may conclude that 8% protein in the diet is certainly sufficient for man as the human requirement of protein for growth is much less than that of the rat and breast milk contains about 9-10% protein (on dry weight basis) and about 7% protein calories.

Table 14 : Food intake, weight gain, brain weight and brain enzymes in rats fed different levels of protein

	% dietary protein				
	5	6	7	8	20
	mean ± s.e.				
body weight (g) :					
initial	46 ± 1.7	45 ± 1.2	45 ± 1.2	43 ± 0.84	47 ± 0.84
terminal	62 ± 2.7	61 ± 1.3	77 ± 2.9	83 ± 2.2	130 ± 4.8
food intake (g/day)	5.2 ± 0.15	5.2 ± 0.22	5.5 ± 0.33	5.7 ± 0.13	8.3 ± 0.31
weight gain (g) :					
per week	3.3	3.3	6.2	8.0	17.0
per 100 kcal intake	2.4	2.4	4.2	5.3	7.7
brain weight (g)	1.34** ± 0.02	1.34** ± 0.02	1.37 ± 0.02	1.44 ± 0.03	1.45 ± 0.02
brain enzymes : (enzymes units per g brain)					
GDH	3.1*** ± 0.10	3.3*** ± 0.13	3.8* ± 0.12	4.1 ± 0.10	4.2 ± 0.10
GAD	24** ± 1.1	24** ± 1.3	28 ± 1.4	31 ± 1.4	30 ± 0.99

Period of treatment, 5 weeks; 8 animals used in each group. Values marked with asterisk significantly different from control (20% protein) values; $P < 0.05$ for *, $P < 0.01$ for **, $P < 0.001$ for ***.

Experiment III : Dietary protein needed for reversing the effects of protein deficiency

In previous studies in this laboratory the effects of protein deficiency on brain enzymes were fully reversed by rehabilitation with a 20% protein diet. In view of the fact that the effects of deficiency are prevented by 8% protein in the diet, the question arose as to whether this amount is also sufficient for restoring brain enzymes to normal levels in animals subjected previously to deficiency. In this connection, in the treatment of children suffering from kwashiorkor 3-5 g. of protein per kilogram of body weight are considered necessary although diets providing 2-3 g. or less of good quality protein are sufficient for preventing deficiency (Waterlow, 1961). Additional studies were therefore carried out on the amount of protein needed for the restoration of brain enzymes to normal levels in animals subjected previously to protein deficiency.

Groups of animals were fed a 5 or 20% protein for a period of 10 weeks at the end of which some animals from each group were killed. The remaining animals in the 5% group were divided further into 5 groups and fed a diet containing 5,8,10,15 or 20% protein for 10 weeks. Those in the 20% group were continued on this diet. The results are presented in Table 15. It can be seen from the same that the 20% diet effected a greater reversal of growth retardation than the other diets used.

Table 15 : Recovery from previous protein deficiency at different levels of dietary protein.

	% dietary protein							
0-10 weeks	5	26	5	5	5	5	5	20
11-20 weeks	-		5	8	10	15	20	20
	0 - 10 weeks		11 - 20 weeks					
weight gain :								
g per week	3.1	16.6	1.9	6.3	9.3	13.4	14.3	8.4
g per 100 kcal	2.3	7.0	1.1	3.0	4.6	6.0	5.7	4.1
	at 10 weeks		at 20 weeks					
terminal body weight (g)	76 ±1.5	212 ±6.8	95 ±2.5	139 ±7.6	169 ±6.3	210 ±15.0	219 ±7.6	296 ±18.4
liver :								
weight (g)	2.5*** ±0.08	5.9 ±0.66	3.1*** ±0.23	3.5*** ±0.54	4.8*** ±0.18	5.3* ±0.42	5.3* ±0.36	6.6 ±0.31
glutathione (mg/g)	0.62*** ±0.07	1.57 ±0.12	0.48*** ±0.02	0.65*** ±0.04	0.92*** ±0.07	1.15** ±0.07	1.45 ±0.08	1.58 ±0.11
brain weight (g)	1.38*** ±0.02	1.55 ±0.03	1.49*** ±0.01	1.55* ±0.03	1.57 ±0.03	1.59 ±0.02	1.61 ±0.04	1.63 ±0.02
brain enzymes (enzyme units per g brain) :								
GDH	3.2*** ±0.13	4.3 ±0.14	3.2*** ±0.10	3.7* ±0.15	4.0 ±0.11	4.0 ±0.10	3.9 ±0.09	4.3 ±0.12
GAD	26** ±0.9	31 ±1.2	26** ±0.9	27* ±1.2	33 ±1.5	31 ±1.2	30 ±1.0	32 ±1.2

Values are mean or means ± s.e.'s, 6 animals used in each group. Values marked with asterisk significantly different from control (20% protein) values; $P < 0.05$ for *, $P < 0.01$ for **, $P < 0.001$ for ***.

but the reversal was not complete even at this level.

Liver glutathione values were restored to normal levels with a 20% diet during rehabilitation but not with the other diets. The degree of reversal of liver glutathione to normal values appears to be related to the protein content of the diet reinforcing the conclusion that glutathione is a sensitive index of protein status.

Brain weights compared with control values in the case of animals rehabilitated with diets containing 10% or more of protein. Rehabilitation with diets containing 10% or more of protein was effective in restoring the activities of brain GDH and GAD to normal levels but complete restoration was not found with the 8% protein diet. The results suggest that in previously depleted animals, brain enzymes are restored to normal levels with a diet containing 10% protein in the form of casein although restoration is only partial in the case of body weight, liver weight and liver glutathione. It can be concluded therefore that the level of dietary protein required for reversing the effects of previous protein deficiency is somewhat higher than that required for preventing these effects. This is consistent with expectation.

As already pointed out, a greater amount of protein is needed for treating than for preventing severe protein deficiency in children. This is also true of many other

nutrients such as vitamin A and calcium (Bagchi, Halder and Chowdhury, 1959; Whedon, 1964).

In conclusion, the deficits in brain GDH and GAD resulting from a low protein diet could be prevented by a diet containing 8% or more of protein in the form of casein although 15% was required to achieve optimum growth. A 10% protein diet was found to be sufficient and appeared to be necessary to restore brain enzyme activities to normal levels although recoveries of body and liver weights and liver glutathione were greater with a 20% protein diet.

Experiment IV : Effect of different degrees of protein deficiency on brain enzymes

In the experiments described earlier deficits in brain glutamate dehydrogenase and decarboxylase were found with 5% protein in the diet. This raises the question as to whether these deficits would be greater with a more severe degree of protein deficiency. To answer this question groups of weanling rats were fed diets containing 0,1,2,3,4,5 or 20% protein for a period of 5 weeks and their livers and brains assayed as in previous experiments.

Data on food intake, body weight, liver weight and liver glutathione content are presented in Table 16. At levels below 4% there was definite weight loss, the same being

Table 16 : Effects of different degrees of protein deficiency on food intake, body weight and liver glutathione.

	% dietary protein						
	0	1	2	3	4	5	20
	mean ± s.e.						
food intake (g/day)	3.0 ± 0.23	3.5 ± 0.17	3.8 ± 0.20	4.0 ± 0.14	4.1 ± 0.19	5.2 ± 0.15	8.3 ± 0.31
terminal body weight (g)	30 ± 0.68	31 ± 0.60	35 ± 0.23	40 ± 1.33	46 ± 1.30	62 ± 2.70	130 ± 4.78
weight change (g/week)	- 3.2	- 3.2	- 2.0	- 1.4	0	+ 3.2	+ 16.6
liver weight (g)	1.11*** ± 0.07	1.10*** ± 0.08	1.17*** ± 0.10	1.60*** ± 0.12	1.70*** ± 0.16	2.80*** ± 0.22	4.70 ± 0.28
liver glutathione (mg/g)	0.62*** ± 0.03	0.57*** ± 0.05	0.57*** ± 0.03	0.57*** ± 0.04	0.58*** ± 0.01	0.64*** ± 0.04	1.67 ± 0.18

Period of treatment, 5 weeks; 8 animals used in each group. Values marked with asterisk significantly different from control (20% protein) values; $P < 0.001$ for ***.

progressively greater with decreasing protein content, With 4% protein (and about the same percentage of protein calories), body weights were just maintained. This is consistent with the observation that about 4% protein calories are adequate for maintenance (Miller and Payne, 1961).

The liver weight and concentration of glutathione in the liver were low in animals fed 5 per cent or less of protein. With further decrease in protein content the liver weight decreased but not the concentration of glutathione in liver. This suggests perhaps the operation of an adaptive mechanism by which critical levels of glutathione are maintained even in the face of severe deficiency. A similar phenomenon is noted with regard to concentrations of protein (Addis et al, 1940), calcium (Leitch, 1964), vitamin A (Roels, 1966) and iron (Moore, 1968).

The data on body weight, brain weight and brain enzymes are given in Table 17. Brain weight deficits increased with levels of protein below 5%. The aetivity of brain glutamate decarboxylase was low with diets providing 5% protein but did not show a further decrease with lower levels of protein in the diet.

A most surprising observation was made when the protein content of the diet was reduced to nil or negligible amounts (0.2 per cent in starch source used). Although as expected,

Table 17 : Effects of different degrees of protein deficiency on brain weight and brain enzymes.

	% dietary protein						
	0	1	2	3	4	5	20
	mean ± s.e.						
terminal body weight (g)	30 ± 0.68	31 ± 0.60	35 ± 0.23	40 ± 1.33	46 ± 1.30	62 ± 2.70	130 ± 4.78
brain weight (g)	1.22*** ± 0.04	1.20*** ± 0.02	1.21*** ± 0.02	1.26*** ± 0.02	1.26*** ± 0.02	1.34** ± 0.02	1.45 ± 0.02
brain enzymes : (enzyme units per g brain)							
GDH	4.3 ± 0.13	3.9 ± 0.17	3.4*** ± 0.10	3.3*** ± 0.14	3.2*** ± 0.14	3.1*** ± 0.10	4.2 ± 0.14
GAD	24*** ± 1.0	23*** ± 1.1	22*** ± 1.4	22*** ± 0.61	24** ± 1.13	24** ± 1.1	29 ± 0.70

Period of treatment, 5 weeks; 8 animals used in each group. Values marked with asterisk significantly different from control (20% protein) values; $P < 0.01$ for **, $P < 0.001$ for ***.

the animals lost weight (in other experiments they died if not killed within 5 to 6 weeks), no decrease in glutamate dehydrogenase was observed in these animals. These remarkable results were confirmed by repeated experimentation and await a satisfactory explanation. They certainly suggest that the effects of complete protein deprivation may not necessarily be the same as those of a low protein diet. The results are, however, consistent with the fact that the animals in the two groups were losing weight and tissue catabolism may be expected to result in the release of ammonia for the removal of which the maintenance of normal levels of glutamate dehydrogenase may be important. In this connection it has been reported that during protein starvation brain glutamate level is not altered though the levels of some other amino acids are found to be (Mandel and Mark, 1965). In this connection it is interesting to note that acute and moderate deficiencies are found to produce differential effects in the case of many nutrients such as thiamine (Swank, 1940; Hundly, 1958).

Whereas a low protein diet allows some scope for the slow adaptation of the animal to the deficient supply by effecting economies in utilization, a total deprivation in the diet presents a different situation. Particularly in the case of protein, the labile protein reserves are soon exhausted and tissue breakdown has to follow inevitably. A situation

in which the animal merely ceases to grow is quite different from the one in which the tissues undergo disintegration.

Experiment V : The temporal course of changes with protein deficiency.

In the experiments described hitherto the animals were fed the deficient diets for a period of either 5 weeks or 10 weeks and deficits in brain enzymes were not found to increase with the longer treatment period. This raised the question regarding the duration of deprivation needed for producing these deficits. Similarly, a question also arises as to whether normal levels of brain glutamate dehydrogenase are maintained throughout in rats fed protein free diets or whether they are restored to normal levels following an initial decrease. Studies were therefore carried out to identify the duration of deficiency needed for the appearance of measurable deficits in the various parameters.

Groups of animals fed diets containing 0,5, or 20% protein were killed after 1,2,3,4 and 5 weeks of treatment. The data on food intake and body weight are given in Table 18. On a protein free diet food intake fell sharply in the first week and decreased further with the progress of treatment. As mentioned earlier the animals fed a protein free diet were

Table 18 : Progressive changes with protein deficiency and deprivation in body weight and food intake.

% dietary protein	period of treatment (weeks) 1	2	3	4	5
	mean food intake (g/day)				
0	5.0 (11.6)	4.5 (11.6)	3.1 (8.6)	2.8 (8.4)	2.7 (8.7)
5	6.0 (11.7)	5.6 (10.1)	5.1 (8.6)	5.5 (8.7)	5.2 (7.6)
20	6.5 (9.4)	7.5 (9.0)	8.7 (8.5)	9.0 (7.3)	9.0 (6.4)
	body weight (g) mean ± s.e.				
0	43 ± 0.50	39 ± 1.33	36 ± 1.50	33 ± 0.91	31 ± 0.60
5	51 ± 1.30	55 ± 1.52	59 ± 1.11	63 ± 2.25	68 ± 1.98
20	69 ± 2.20	82 ± 1.83	102 ± 2.90	122 ± 5.65	139 ± 6.60

8 animals were used in each group; values in parentheses give food intake per 100 g body weight; mean body weight at start was 47 g.

unable to survive for more than 5-6 weeks and became extremely emaciated during this period. They also seemed to suffer from hind limb paralysis.

The data on brain weight and brain enzymes are shown in Table 19. It is interesting to note that the brain weights in animals fed a protein free diet appeared to show a decrease with the first two weeks of treatment. This may be due to changes such as demyelination (Cuparencu, Rosenberg and Daghie, 1959). The brain weights of the low protein animals did not show much change whereas those of the high protein animals increased progressively.

In the low protein animals significant deficits in both the brain enzymes were found with three weeks of treatment but not earlier. In the protein deprived group a similar pattern was observed with regard to GAD but normal levels of GDH were maintained throughout suggesting the operation of homeostatic mechanisms from the very early stages of protein deprivation. As three weeks of treatment are found to be needed for inducing enzyme deficits with protein deficiency, it is also possible that the operation of this mechanism is effective before the effects of deficiency become evident.

The results do not suggest a progressively greater deficit with a longer period of treatment but rather that after the initial decrease the activities are maintained at

Table 19 : Progressive changes with protein deficiency and deprivation in brain weight and brain enzymes.

	brain weight (g)			brain enzymes (enzyme units per g/brain)					
				GDH			GAD		
% dietary protein	0	5	20	0	5	20	0	5	20
Period of treatment (weeks)	mean ± s.e.								
1	1.27* ± 0.03	1.30 ± 0.02	1.37 ± 0.03	4.1 ± 0.11	4.1 ±0.11	4.3 ± 0.13	30 ± 1.2	29 ± 0.56	30 ± 0.96
2	1.22*** ± 0.02	1.31* ± 0.02	1.38 ± 0.02	4.1 ± 0.13	3.9 ± 0.10	4.3 ± 0.10	27 ± 1.31	29 ± 1.10	30 ± 1.15
3	1.21*** ± 0.01	1.32** ± 0.02	1.41 ± 0.02	4.2 ± 0.24	3.5*** ± 0.10	4.3 ± 0.13	26** ± 0.80	26** ± 1.00	30 ± 0.90
4	1.21*** ± 0.03	1.30*** ± 0.02	1.46 ± 0.03	4.1 ± 0.03	3.5*** ± 0.10	4.3 ± 0.11	24*** ± 1.00	26** ± 1.10	30 ± 0.83
5	1.21*** ± 0.01	1.30*** ± 0.02	1.45 ± 0.02	4.0 ± 0.06	3.3*** ± 0.08	4.2 ± 0.10	24*** ± 1.30	24*** ± 1.20	32 ± 1.20

8 animals used in each group; values for brain weight (g), GDH and GAD (enzyme units per g. brain) at start (weaning) were 1.31, 4.0 and 30.0 respectively.

Values marked with asterisk significantly different from control (20% protein) values at the corresponding period, $P < 0.05$ for *, $P < 0.01$ for **, $P < 0.001$ for ***.

more or less steady levels. As mentioned earlier a similar phenomenon is found in the case of other parameters in blood and liver with nutritional deficiencies.

The absence of any change in enzyme activity in the high protein group is consistent with several observations that the maturation of these enzymes is nearly completed at or about weaning. Adult levels are reported to be reached in the case of glutamate dehydrogenase by 16-23 days (Bayer and McMurray, 1967) and in the case of glutamate decarboxylase by 26-35 days according to Bayer and McMurray (1967) and 28-30 days according to Van den Berg et al (1965). and Sims and Pitts (1970).

Experiments VIa and VIb: Effects of different degrees of postweaning undernutrition.

In previous studies in this laboratory protein deficiency during the immediate postweaning period was found to produce deficits in brain glutamate dehydrogenase and decarboxylase (Rajalakshmi et al, 1969) whereas undernutrition did not have a similar effect (Rajalakshmi and Ramakrishnan, 1969a). However, in the latter studies, the degree of undernutrition was relatively mild and the undernourished animals had body weights which were about 61% of control body weights whereas the protein deficient animals had generally much smaller body weights as compared to controls. It seemed necessary to

investigate whether the effects are different with more severe undernutrition as the same is considered to result in protein deficiency (Platt, Heard, and Stewart, 1964). Studies were therefore made of the effects of different degrees of food restriction (50 and 67%) during the postweaning period on brain glutamate dehydrogenase.

Two experiments concerned with the above aspects were carried out. In both experiments, the controls were fed ad lib. The undernourished animals were fed 50% of the ad lib. food intake of controls in experiments VIa and 33% in VIb and thus the degree of restriction was 50 and 67% respectively. The experimentals were undernourished for a period of 5 weeks and half of the undernourished animals were rehabilitated for a period of 11 weeks in experiment VIa and 5 weeks in experiment VIb.

The data on food intake and weight gain in the two experiments are shown in Table 20. The food intake of the controls in the two experiments was comparable during phase I but not during phase 2.

In experiment VIa the undernourished animals had body weight deficits of 39% and 44% at the end of phase 1 and phase 2 respectively. The deficits were much greater in experiment VIb (60% and 70%) as might be expected with the more severe degree of food restriction.

Table 20 : Effects of postweaning undernutrition on food intake, body weight and weight gain in albino rats.

food restriction	--	experiment VIa** 50%		--	experiment VIb*** 67%	
	C*	UN*	R*	C*	UN*	R*
			mean ± s.e.			
body weight (g) :						
phase 1 :						
initial	45	45	45	45	45	45
at 5 weeks	135 ±5.1	83(61) ±2.2	-	131 ±7.2	52(40) ±0.5	-
phase 2 :						
at 10 weeks	189 ±7.9	116(61) ±3.1	159(84) ±5.2	187 ±16.7	57(30) ±1.6	142(76) ±3.4
at 16 weeks	236 ±12.9	133(56) ±4.1	209(89) ±6.7	-	-	-
food intake (g/day)						
phase 1	7.4(5.5)	3.7(4.4)	-	7.5(5.7)	2.5(4.8)	-
phase 2 :						
5-10 weeks	11.0(5.8)	5.5(4.7)	11.2(7.0)	8.4(4.5)	2.8(4.9)	8.5(6.0)
10-16 weeks	11.2(4.7)	5.4(4.1)	11.3(5.4)	-	-	-
weight gain (g/100 kcal)						
phase 1	8.7	7.3	-	8.2	2.0	-
phase 2 :						
5-10 weeks	3.5	4.2	4.9	4.8	1.4	7.6
10-16 weeks	2.5	1.9	2.6	-	-	-

* C = Controls fed ad lib. throughout; R = undernourished during phase 1 and fed ad lib. during phase 2; UN = undernourished throughout.
** phase 1, 5 weeks treatment; phase 2, 11 weeks treatment.
*** phase 1, 5 weeks treatment; phase 2, 5 weeks treatment.
8 animals used in each group; figures in parentheses gives the values as percentages of controls in the case of body weight and per 100 g. body weight in the case of food intake.

Food intake per 100 g. body weight was greater in the rehabilitated animals than in the controls. This is consistent with previous observations (Widdowson and McCance, 1963; Chow, Blackwell and Sherwin, 1968; Rajalakshmi and Ramakrishnan, 1969a).

However, a point that seems to have been largely ignored in such studies is the capacity of the animals to continue to grow inspite of severe undernutrition.

If we assume that in the rat, as in other species basal calories form at least 50% of total calories consumed (Evans and Miller, 1968), restriction of food intake to 50% or 33% of ad lib. intakes of weight matched controls should result in a cessation of growth or indeed in loss of weight and yet this is seldom found to be the case. In fact, the percentage of calories needed for activity in small animals such as the rat can be expected to be even less.

Some computations are made in Table 21.for control and undernourished animals at the beginning of phase 1. During this period, in the case of the controls, the available calories for basal metabolism and activity in the two experiments were 152 and 186% of calories estimated to be required for basal metabolism. In the case of the undernourished animals they were 81 and 94% respectively for

Table 21 : Calorie status of undernourished animals.

	values for first week of treatment			
food restriction	50%		67%	
	controls	under nourished	controls	under nourished
1. initial body weight (g)	45	45	45	45
2. calorie intake	22.4	11.2	26.0	10.8
3. calories for tissue gain*	4.9	1.1	4.6	0
4. calories available for BMR and activity	17.5	10.1	21.4	10.8
5. estimated basal calories**	11.5	11.5	11.5	11.5
6. 4 as % of 5	152	81	186	94
7. 5 as % of 2	51	103	44	106

* Assuming that 1 g. of tissue gain represents 2 calories.

** According to Brodie's equation $\frac{Q}{M} = a\ e^{-bM} + c$ where Q is basal heat production per day; M is body weight in kilograms, e is natural base for logarithm and a, b and c are constants (250, 0.0037 and 0 for rat)(Mitchell, 1962).

a food restriction of 50 and 67%. Estimated basal calories were 51 and 44% of available calories in the controls as against 103 and 106% in the undernourished animals in experiments VIa and VIb respectively. The latter figures suggest that basal metabolism in these animals is much less than what might be expected on the basis of body size. These observations show the capacity of the undernourished animals to adapt to the situation presumably by a reduction in basal metabolism. Similar observations have been made by other investigators (Benedict, Horst and Mendel, 1932; Will and McCay, 1943). Comparison of different species has shown the activity increment over basal metabolism to be of the order of at least 35% (Mitchell, 1962). The assumption of a similar increment in the undernourished animals would imply reduction of a similar order (30-35%) in the basal metabolism of these animals. In this connection several studies suggest an increase rather than decrease in activity in animals subjected to undernutrition (Wald and Jackson, 1944).

The data on brain weight and brain glutamate dehydrogenase are presented in Table 22. Brain weight was found to be significantly lower with undernutrition, but the deficit varied with both the period of treatment and severity of undernutrition. Brain weight as per cent of body weight was more in undernourished animals. This difference was less but not abolished in rehabilitated animals.

Table 22 : Effects of postweaning undernutrition on brain weight and brain glutamate dehydrogenase in albino rats.

	experiment VIa[1]			experiment VIb[2]				
food restriction	50%			67%				
				phase 1		phase 2		
	C[@]	UN[@]	R[@]	C[@]	UN[@]	C[@]	UN[@]	R[@]
body weight (g)	236 ± 12.9	133 ± 4.1	209 ± 6.7	131 ± 7.2	52 ± 0.5	187 ± 16.7	57 ± 1.6	142 ± 3.4
brain weight (g)	1.68 ± 0.05	1.51** ± 0.04	1.58 ± 0.04	1.48 ± 0.02	1.37** ± 0.02	1.55 ± 0.03	1.33*** ± 0.01	1.46 ± 0.06
brain weight as % of body weight	0.71	1.14	0.75	1.13	2.63	0.83	2.3	1.03
brain enzyme (enzyme unit per g. brain)								
GDH	3.8 ± 0.10	3.6 ± 0.11	3.6 ± 0.11	3.8 ± 0.14	3.8 ± 0.09	3.8 ± 0.07	3.7 ± 0.08	3.6 ± 0.10

@ C, Controls fed ad lib. throughout; R, undernourished during phase 1 and fed ad lib. during phase 2; UN, undernourished throughout.

1. phase 1, 5 weeks treatment; phase 2, 11 weeks treatment.

2. phase 1, 5 weeks treatment; phase 2, 5 weeks treatment.

8 animals used in each group.

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

Neither degree of food restriction was found to affect brain glutamate dehydrogenase although body weight at the end of 10 weeks (57g) was less than those previously reported (69g) with 10 weeks of a protein deficient diet (experiment Ia).

Brain glutamate decarboxylase was not assayed in these experiments. In recent studies in this laboratory, the enzyme was not found to be affected by severe restriction on a 10% protein diet (unpublished studies).

These observations confirm previous impressions that the effects of undernutrition may be different from those of protein deficiency during the postweaning period. This conclusion was supported by subsequent experiments with simultaneous induction of calorie and protein deficiencies (experiment VIIb).

In this connection, although protein deficiency and undernutrition result in retarded growth because of decreased food intake, for a comparable degree of growth retardation, a much greater reduction in food intake is necessary in the case of undernourished animals. Also in spite of such growth retardation the protein status of the undernourished animal is much more satisfactory than that of the protein deficient animal.

In conclusion, undernutrition during the postweaning period had no effect on brain glutamate dehydrogenase although, in previous studies in this laboratory protein deficiency during the postweaning period resulting in comparable growth deficits was found to decrease the activities of glutamate dehydrogenase and decarboxylase. The results suggest the differential effects of calorie and protein deficiencies at a comparable level of growth retardation. It is interesting to note in this connection the differences between the clinical syndromes pointed out earlier between marasmus and kwashiorkor, the former associated generally with undernutrition and the latter, with protein deficiency.

It is also relevant to consider that, whereas the situation caused by undernutrition on a qualitatively adequate diet can be conceivably met by reduced growth and body size, and perhaps activity as well specially in the case of higher animals, the situation caused by the deficiency of a specific nutrient is much more complex. The animal does adapt itself to such a situation to some extent by decreased food intake, but this cannot compensate for the decreased availability of nutrients in relation to overall energy metabolism. In this connection the requirements of most nutrients such as protein, many minerals and B-vitamins are related directly or indirectly to energy metabolism. For instance, the requirement of protein for endogenous metabolism is related to basal metabolism

(Smuts, 1935). In man even the requirements for growth are found to be so related, broadly speaking, as the basal metabolic rates are also higher during the period of growth so that the percentage of protein calories needed does not vary appreciably at different ages (Rajalakshmi and Ramakrishnan, 1969c; Rajalakshmi, 1972; Davidson, Passmore and Brock, 1973). The requirements for calcium and phosphorus for endogenous metabolism are also related to endogenous nitrogen metabolism and therefore indirectly to basal metabolism (Mitchell, 1962). Similarly the requirements of B-vitamins are related to total energy metabolism. Those of vitamins A and C are believed to be related to body weight (Mitchell, 1964) and growth rate (FAO, 1967) but both influence overall energy metabolism. A deficient supply of any nutrient in relation to total energy therefore poses a situation different from that in which a qualitatively adequate diet is available in restricted amounts.

Experiments VIIa & VIIb Effects of neonatal undernutrition.

In the experiments just described postweaning undernutrition was found to have no effect on brain glutamate dehydrogenase although protein deficiency during this period is associated with decreased activities of this enzyme.

Similar observations have been made with regard to behavioural parameters in pigs and rats by Barnes and his associates (Barnes et al, 1968; Frankova and Barnes, 1968a,b). In their earlier studies on rats, the effects of protein deficiency during the postweaning period were found to depend on the plane of nutrition during the neonatal period (Barnes, et al, 1966). It seemed worthwhile to investigate the effects of neonatal undernutrition on brain enzymes and to determine whether the effects of postweaning deficiencies on brain enzymes are influenced by nutritional status during the neonatal period.

Studies were therefore carried out on the effects of neonatal undernutrition which was induced by manipulating litter size. Groups of pups reared in standard or large litters of 8 and 16 were killed at 4 weeks after birth and their brains assayed for the activities of glutamate dehydrogenase and decarboxylase.

The results are presented in Table 23. It can be seen from the same that, in contrast to undernutrition during the postweaning period, undernutrition during the neonatal period resulted in significant deficits in brain GDH and GAD. This is consistent with the fact that the maturation of both these enzymes takes place during the neonatal period and that this might be affected by an overall nutritional deficiency.

Table 23 : Effects of neonatal undernutrition on brain weight and brain enzymes.

	litter size	
	16	8
	mean ± s.e.	
body weight (g) :		
initial (at birth)	5.5	5.5
final (at 28 days)	23.0 ± 0.65	41.0 ± 1.10
brain weight (g) :	1.12*** ± 0.02	1.30 ± 0.01
brain enzymes (enzyme units per g. brain)		
GDH	3.3*** ± 0.09	4.0 ± 0.10
GAD	24*** ± 0.84	30 ± 1.50

Number of animals is same as litter size.

Values marked with asterisk significantly different from control (8 litter size) values; $P < 0.001$ for ***

In the light of the above results additional studies were carried out to investigate (a) the reversibility of the effects of neonatal undernutrition and (b) the effects of neonatal undernutrition on vulnerability to nutritional deficiency during the postweaning period. Animals reared in large or standard litters were subjected to different dietary treatments after weaning.

In experiment VIIa on the effects of postweaning undernutrition, animals were fed a standard diet ad lib. or in restricted amounts. In experiment VIIb on the comparative effects of calorie and protein deficiencies, animals were fed a 5 or 20% protein diet or the latter in restricted amounts.

When undernutrition or protein deficiency was confined to the postweaning period, a deficit in brain enzymes was found with protein deficiency but not with undernutrition (Tables 24 and 25). However, when undernutrition during the postweaning period was preceded by neonatal undernutrition a deficit was found in both enzymes suggesting that the effects of postweaning undernutrition depend on the prior nutritional state of the animal.

A question arises regarding the body weight deficits of the animals undernourished only during the postweaning period and those undernourished during both the neonatal and postweaning periods. In the former case, body weight at the

Table 24 : Effects of postweaning undernutrition on body weight, brain weight and brain enzymes in neonatally undernourished or normally nourished rats.

neonatal	undernourished (16 to a litter)		normally nourished (8 to a litter)	
postweaning	fed ad lib.	75% restriction	fed ad lib. (control)	67% restriction
			mean ± s.e.	
food intake (g/day)	7.8	2.0	8.0	2.5
body weight (g) :				
initial	23 ± 0.45	23 ± 0.45	41 ± 0.60	41 ± 0.60
terminal	114.0 ± 4.4	32 ± 0.81	138 ± 6.3	51 ± 0.93
brain weight (g)	1.41 ± 0.02	1.11*** ± 0.03	1.44 ± 0.04	1.27** ± 0.02
brain enzymes (enzyme units per g. brain) :				
GDH	4.2 ± 0.13	3.4*** ± 0.10	4.0 ± 0.15	3.8 ± 0.12
GAD	32 ± 1.1	26*** ± 0.74	32 ± 1.2	32 ± 1.5

Period of treatment, 5 weeks; 8 animals used in each group. Values marked with asterisk significantly different from control values; $P < 0.01$ for **, $P < 0.001$ for ***.

time of sacrifice was 60g as against 34g in the latter case (Table 25). However, in other studies in this laboratory even when postweaning undernutrition was so severe as to prevent growth altogether, brain enzyme deficits were not found.

The differential effects of protein and calorie deficiencies during the postweaning period are of particular interest, as the difference was found in spite of the protein deficient animals having bigger body weights (72g) than the undernourished animals (60g) (Table 25). It is also interesting to note that the effects of postweaning undernutrition were influenced by preweaning nutrition whereas the effects of protein deficiency were not similarly influenced. Preliminary studies conducted recently also suggest that the effects of protein deficiency during postweaning period may be less evident with an enhanced plane of nutrition during the neonatal period and may also vary in different strains.

Postweaning protein deficiency produced comparable enzyme deficits in both neonatally undernourished and normally reared animals. This is consistent with the fact that brain enzyme levels vary within a narrow range and that the effects of a low protein diet are not increased by combining protein deficiency with severe undernutrition (unpublished studies) or by decreasing the protein content of the diet from 5 to 3% (Experiment IV).

Table 25 : Effects of postweaning deficiency on body weight, brain weight and brain enzymes in neonatally undernourished and normally nourished rats.

neonatal	undernourished (16 to a litter)			normally nourished (8 to a litter)		
postweaning	LP	HP		LP	HP	
mode of feeding	*ad lib.*	*ad lib.*	restricted@	*ad lib.*	*ad lib.*	restricted@
			mean ± s.e.			
food intake (g/day)	3.4	6.4	2.1	4.8	7.1	2.4
body weight (g) :						
initial	22 ± 0.68	22 ± 0.87	22 ± 1.0	50 ± 2.4	50 ± 2.0	50 ± 2.5
terminal	43 ± 4.3	157 ± 4.5	34 ± 0.3	72 ± 2.8	179 ± 5.2	60 ± 0.80
brain weight (g)	1.28** ± 0.03	1.39 ± 0.01	1.20*** ± 0.02	1.32** ± 0.01	1.40 ± 0.02	1.32** ± 0.01
brain enzymes (enzyme units per g. brain) :						
GDH	3.3** ± 0.15	4.0 ± 0.18	3.4** ± 0.10	3.1** ± 0.15	4.1 ± 0.22	3.9 ± 0.17
GAD	26** ± 1.5	33 ± 1.6	22*** ± 1.3	25** ± 1.2	32 ± 2.0	31 ± 1.7

Period of treatment, 10 weeks. 4-6 animals used in each group.

@ Fed 33.3% of the *ad lib.* intake of HP animals in each case. LP, 5% dietary protein; HP, 20% dietary protein.

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

In conclusion, these studies either confirm or affirm that :

(a) neonatal undernutrition produces brain enzyme deficits similar to those found in postweaning protein deficiency;

(b) the effects of neonatal undernutrition are reversed by dietary rehabilitation after weaning;

(c) the effects of neonatal undernutrition persist when postweaning undernutrition is superimposed on the same; and

(d) protein deficiency confined to the postweaning period produces effects different from these of undernutrition.

A question arises regarding the extrapolation of these results to man. As mentioned earlier low birth weights signifying undernutrition during the prenatal period which is roughly comparable to the neonatal period in the rat occur to some extent in man. A certain percentage of such babies are found to suffer from mental retardation. (Knobloch and Pasamanick, 1963). No information is available on their growth during the postnatal period. It is possible that some of them were undernourished during this period as well. In this connection, although low birth weight babies grow more rapidly in the first few months than those of normal weight, a few of them may continue to be undernourished so that at weaning their weights are still low (Rajalakshmi and Ramakrishnan, 1969b). Further studies are necessary from this

point of view, but the results do underline the importance of particularly ensuring the adequate nutritional care during the neonatal period of the child born with a low birth weight. The studies also raise questions regarding any relation between the susceptibility of children to postweaning deficiencies and their nutritional status at weaning. It is likely that the child with a poor nutritional status at weaning will be more seriously affected by a deficient diet in the postweaning period as well.