C H A P T E R III

RESULTS AND DISCUSSION

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EXPERIMENT I

As mentioned earlier, in previous studies in this laboratory, protein deficiency was found to result in decreased activities of brain glutamate dehydrogenase and glutamate decarboxylase (Rajalakshmi, Pillai and Ramakrishnan, 1969) whereas calorie deficiency during the postweaning period had no effect. This suggested that the effects of calorie deficiency are different from those of protein deficiency and that the effects of a low protein diet are not due to differences in food intake. This suggestion was sought to be confirmed by an experiment in which animals subjected to protein deficiency were compared with those which were pair fed a high protein diet with the low protein animals and those fed a high protein diet ad libitum.

The data on food intake, weight gain and organ weights are presented in Table 8. As expected, animals fed the LP diet or the HP diet in restricted amounts showed a poor growth rate and the final body weights attained by these animals were only 82 g and 130 g as against 197 g in the controls respectively.

Table 8: Effects of protein deficiency and calorie restriction on body weight and organ weights in rats

	LP	HP	
	ad lib.	restricted*	ad lib.
food intake (g/day)	5.1	5.0	8.1
protein intake (g/day)	0.26	1.0	1.6
body weight (g)			
initial	43	43	42
terminal	82	130	197
weight gain (g)			
(a) total	39	87	155
(b) per 100 kcal consumed	2.8	6.5	7.2
weight of:			
		mean ± s.e.	
brain (g)	1.39±0.04	1.52±0.03	1.60±0.02
liver (g)	2.3±0.14	3.5±0.20	5.2±0.36
	weight (g) per 100 g of body weight		
brain	1.80	1.20	0.81
liver	3.30	2.70	2.60

period of treatment, 10 weeks; 7 animals were used in each group.
*pair fed with the LP animals.

Table 9: Effects of protein deficiency and calorie restriction on brain and liver enzymes in rats

	LP	HP	
	ad lib.	restricted*	ad lib.
	units per g tissue		
brain:	mean ± s.e.		
GDH	3.1±0.12	3.9±0.20	4.0±0.14
GAD	26±0.7	32±1.5	35±1.5
GABA-T	50±1.5	54±2.5	52±1.2
liver:			
GDH	20±1.5	21±1.2	23±1.2

period of treatment, 10 weeks; 7 animals were used in each group.
*pair fed with the LP animals.

As compared to the control group the efficiency of food utilization as judged by weight gain in relation to calorie intake was also less in the LP group but the HP restricted group did not show an impairment of the same, suggesting that moderate calorie restriction does not affect the same. Similar observations have been made in other studies in this laboratory and by other investigators (Hegsted, 1964).

The reduction in body weight was associated with a reduction in brain and liver weights, but the reduction in the former was much less as compared to that in body weight (Table 8) and the proportion of brain weight to body weight was increased. Similar observations regarding body and brain weights have been made by other investigators (e.g. Dobbing, 1968).

The activities of the enzymes, glutamate dehydrogenase (GDH) and glutamate decarboxylase (GAD) were found to be decreased in the protein deficient animals but not in the HP restricted group (Table 9). The activity of GABA-transaminase (GABA-T) was not affected in either condition, an observation consistent with previous observations (Rajalakshmi, Pillai and Ramakrishnan, 1969).

Similar observations regarding the differential effects of calorie and protein deficiencies in behavioural parameters have been made by other investigators (Barnes, Moore, Reid and Pond, 1968).

In conclusion, moderate food restriction during postweaning period was found to affect growth and to some extent brain weight but not brain enzymes. The negative effects on brain enzymes were in contrast to the findings in the case of either protein deficiency during the postweaning period or calorie deficiency prior to weaning. The results clearly demonstrate that the effects of dietary protein deficiency during the postweaning period are not due to a decrease in food intake and the associated calorie deficiency. This conclusion has been supported by recent studies in this laboratory using more severe degrees of food restriction (50 and 66%) (Rajalakshmi, Parameswaran and Ramakrishnan, unpublished). In these studies, brain enzymes were not affected even when the food restriction was so severe as to result in some loss of body weight.

In contrast to brain glutamate dehydrogenase liver glutamate dehydrogenase did not show a decrease with

protein deficiency. A similar finding has been reported by Pineda (1968), but the findings in this regard have been conflicting and a decrease in liver glutamate dehydrogenase with protein deficiency has been reported by Muramatsu and Aschida (1962). It would therefore be desirable to repeat these studies. Be that as it may, in the present studies, with the use of the same techniques, brain glutamate dehydrogenase showed a clear cut deficit whereas liver glutamate dehydrogenase did not, suggesting that the effect on the brain enzyme is not due to a generalised effect on the various glutamate dehydrogenases. Similar observations have been made of the differential effects of thiamine deficiency on brain and liver enzymes (Bennett, Jones and Nelson, 1966).

In this connection, it is of interest to note that Goldin and Frieden (1971) in their discussion on the metabolic role of glutamate dehydrogenase conclude that its role is markedly different in different tissues or under different circumstances within a given tissue.

EXPERIMENT II

Ordinary diets consumed by a large proportion of the population in this country consist mainly of cereals and millets. These diets provide 10 per cent

protein calories but are of poor protein quality. This is mainly due to the deficiency of one or more of the essential amino acids, particularly lysine.

In previous studies in this laboratory supplementation of kodri with lysine or lysine rich foods such as milk powder, bengal gram and other pulses restored the activities of brain glutamate dehydrogenase and glutamate decarboxylase to normal levels (Rajalakshmi, Pillai and Ramakrishnan, 1969). But the results were not clear cut in the case of some groups. Further, the most commonly used grains are rice, wheat, maize, jowar and bajra in that order. Studies were carried out to confirm the effects of supplementing kodri and to study the effects of such supplementation in the case of other grains namely wheat and maize.

Two experiments on the above aspects were carried out, one to study the effects of lysine supplementation to kodri. The second was designed from the standpoint of practical nutrition and bengal gram and fenugreek leaves were used as supplements rather than lysine as in diets based on plant foods, legumes and greens are

the most effective means of improving the protein quality of the diet as well as overall nutritive quality. Among other things, the supplements also increased the lysine content of the diets. As these studies were designed from the standpoint of practical nutrition the food grains were fed with only crude common salt and groundnut oil.

Lysine supplementation to kodri was found to have a beneficial effect in terms of all the parameters measured although the diets were isonitrogenous (Tables 10 and 11). As in the previous experiments it was also found to restore activities of brain glutamate dehydrogenase and glutamate decarboxylase to normal levels (Table 11).

Incidentally, in the original series of investigations in this laboratory, the high and low protein diets used contained 5 per cent and 20 per cent protein. However, in these and in previous experiments, improvement in the protein quality of kodri, a millet containing 8.3 per cent protein, without a concomitant increase in protein content was found to restore brain enzyme levels to those found in high protein fed animals. As the millet-based diet contains only 7.3 per cent protein this

Table 10: Effects of lysine supplementation to kodri (Paspalum scorbiculatum L.) on food intake, body weight and organ weights in rats

	kodri	kodri+ lysine	7.3 per cent casein diet
food intake (g/day)	4.2	7.3	7.7
protein intake (g/day)	0.31	0.53	0.56
body weight (g)			
initial	50	50	50
terminal	56	112	132
weight gain (g)			
(a) total	6	62	82
(b) per 100 kcals consumed	0.55	3.3	4.3
liver weight (g)*	2.5±0.10	4.4±0.17	4.6±0.13
brain weight (g)*	1.31±0.02	1.43±0.02	1.46±0.02
	weight (g) per 100 g body weight		
liver	4.5	3.9	3.5
brain	2.3	1.3	1.1

period of treatment, 10 weeks, 7 animals were used in each group.
*values are means ± s.e.'s.

Table 11: Effects of lysine supplementation to kodri (Paspalum scorbiculatum L.) on brain enzymes in rats

enzyme	kodri	kodri+lysine	7.3 per cent casein diet
		units per g brain	
GDH	3.1±0.17	3.9±0.20	3.7±0.13
GAD	27±0.7	32±2.0	34±1.3

period of treatment, 10 weeks; 7 animals were used in each group.
values are means ± s.e.'s.

Table 12: Effects of supplementation of bengal gram and fenugreek leaves to cereals and millets on food intake and body weight in rats

		maize	wheat	10 per cent casein diet
food intake (g/day)	A	4.7	6.1	8.2
	B	7.6	8.3	
body weight (g) (terminal)	A	65(43)	84(44)	147(43)
	B	111(44)	135(44)	
weight gain (g)				
(a) total	A	22	40	104
	B	67	91	
(b) per 100 kcal consumed	A	1.9	3.1	5.7
	B	3.9	4.9	

period of treatment, 8 weeks; 14 animals were used in each group.
values given in parentheses are initial weights of the animals.
A - basal diet composed of 150 g cereal or millet, 8.0 g oil and 4.0 g crude common salt.
B - supplemented diet composed of 120 g cereal or millet, 30 g bengal gram, 40 g fenugreek leaves, 8.0 g oil and 4.0 crude common salt.

Table 13: Effects of supplementation of bengal gram and fenugreek leaves to cereals and millets on brain enzymes in rats

		maize	wheat	10 per cent casein diet
brain weight (g)	A	1.38±0.030	1.42±0.020	1.54±0.020
	B	1.52±0.020	1.55±0.020	
brain enzymes:			units per g brain	
GDH	A	3.4±0.11	3.6±0.15	4.0±0.18
	B	4.0±0.12	3.9±0.12	
GAD	A	33±1.0	34±1.1	35±1.0
	B	35±1.0	35±0.7	

period of treatment, 8 weeks; 14 animals were used in each group.
values are means ± s.e.'s.
A - basal diet composed of 150 g cereal or millet, 8.0 g oil and 4.0 g crude common salt.
B - supplemented diet composed of 120 g cereal or millet, 30 g bengal gram, 40 g fenugreek leaves, 8.0 g oil and 4.0 g crude common salt.

suggested that 7.3 per cent of good quality protein is all that is needed to prevent the brain enzyme deficits found with protein deficiency. This suggestion was confirmed by the data obtained on the group fed a diet containing 7.3 per cent protein in the form of casein. It was also confirmed by other studies in this laboratory in which protein content was varied at 5,6,7,8,10,15 and 20 per cent (Rajalakshmi, Parameswaran and Ramakrishnan, unpublished).

When the animals were fed only wheat or maize weight gains were low as might be expected, but improved on supplementation with bengal gram and fenugreek leaves (Table 12). However only glutamate dehydrogenase but not glutamate decarboxylase was found to be affected by the poor protein quality of maize (Table 13). In the case of wheat the deficit fell short of statistical significance. The supplemented groups compared with 10 per cent casein diet.

The results in the case of kodri are to be expected as animals fed kodri gained practically no weight and thus compared with a 4 per cent casein diet which is associated with brain enzyme deficits. In the case of

maize and wheat the weight gains obtained when the grains were fed with added salts (this was not done in the present studies) compared generally with those on a 5 per cent and 7-8 per cent casein diet. On this basis one would expect deficits in both enzymes in the case of maize whereas only glutamate dehydrogenase was found to be affected. This might be because of the higher protein content of maize in spite of its poorer protein quality. The lack of clear cut deficits in the case of wheat is consistent with its expected protein value of about 7 per cent. In other studies in this laboratory a 6 per cent casein diet was clearly associated with brain enzyme deficits. Such deficits were not found with an 8 per cent casein diet and the results in the case of a 7 per cent casein diet were ambiguous. It is also possible that the high content of glutamic acid present in these grains (Naik and Das, 1972) counteracts to some extent the expected effects of the poor protein value of the grain. Further experiments on the effects of adding non-essential nitrogen to low protein diets need to be carried out. Data on the glutamic acid content of kodri as compared to that of the other grains are also needed.

In these studies the grains were fed with minimal additions of salt and oil so that the protein content was not unduly diluted. If these grains are taken with more oil, sugar and starchy foods, this may no longer be the case.

In conclusion, when kodri, maize or wheat were fed alone clear cut deficits in brain enzymes were found only with kodri. In the case of maize, only brain glutamate dehydrogenase was affected whereas even this effect was not clear cut in the case of wheat. In the case of all the grains, supplementation with lysine or lysine rich food was associated with normal enzyme activities. The differences between the grains must be deemed to be due to either those in protein content or quality or the content of other amino acids such as glutamic acid. Incidentally, these studies suggest the greater susceptibility of brain glutamate dehydrogenase as compared to glutamate decarboxylase. A similar suggestion was derived from the other studies on glutamic acid supplementation and dietary rehabilitation of animals subjected to vitamin A deficiency. In these studies the response of glutamate dehydrogenase to supplementation seemed to be slower than that of glutamate decarboxylase.

On the other hand in other studies, glutamate dehydrogenase was found to be affected earlier than glutamate decarboxylase by deficiency.

EXPERIMENT III

Previous studies in this laboratory showed that the supplementation of a low protein diet with glutamic acid will reverse the effects of protein deficiency and restore brain glutamate dehydrogenase and glutamate decarboxylase to normal levels (Rajalakshmi, Pillai and Ramakrishnan, 1969). In these studies glutamic acid was added to the low protein diet so as to raise the concentration of this amino acid to that in the 20 per cent diet. This necessitated the addition of 5 per cent glutamic acid at the 5 per cent protein level.

The beneficial effect of supplementation of glutamic acid to a low protein diet raised the question whether similar effects operate at low and high levels of protein in the diet. Studies were therefore made of the effects of glutamic acid supplementation at the 5 per cent level to diets containing 3,5,8 and 20 per cent protein. The results are presented in Tables 14 and 15.

Table 14: Effects of supplementation of glutamic acid to diets differing in protein content on food intake and body weight in rats

	per cent protein in diet							
	3		5		8		20	
	control	glutamic acid added*	control	glutamic acid added*	control	glutamic acid added*	control	glutamic acid added*
no. of animals	4	4	8	8	4	4	8	8
nitrogen in diet (g per 100 g)	0.48	0.96	0.80	1.28	1.28	1.76	3.20	3.68
food intake (g/day)	4.1	3.6	4.2	3.6	6.6	6.0	7.8	6.9
nitrogen intake (g/day)	0.020	0.037	0.033	0.048	0.084	0.110	0.250	0.250
body weight (g)								
initial	50	51	50	50	50	50	50	51
terminal	35	33	80	66	116	103	186	156
weight gain (g)								
(a) total	-15	-18	30	16	66	53	136	105
(b) per 100 kcal consumed	-	-	2.7	1.7	3.8	3.2	6.7	5.5

period of treatment, 10 weeks.
*at 5 g per 100 g of diet.

Table 15: Effects of supplementation of glutamic acid to diets differing in protein content on brain enzymes in rats

	per cent protein in diet							
	3		5		8		20	
	control	glutamic acid added*	control	glutamic acid added*	control	glutamic acid added*	control	glutamic acid added*
no. of animals	4	4	8	8	4	4	8	8
brain weight (g)	1.30±0.020	1.26±0.012	1.38±0.30	1.34±0.020	1.50±0.040	1.48±0.020	1.6±0.03	1.53±0.04
brain enzymes:				units per g brain				
GDH	3.3±0.10	4.2±0.20	3.2±0.10	3.9±0.09	3.9±0.09	3.9±0.10	4.0±0.10	4.0±0.20
GAD	NE	NE	26±1.0	31±0.5	31±1.2	31±1.5	33±0.9	33±1.0

period of treatment, 10 weeks.
values are means ± s.e.'s.
*at 5 g per 100 g of diet.

It can be seen that the supplementation of glutamic acid at all levels of protein resulted in decreased food intake, body weight and efficiency of food utilization. The decreased food intake with glutamic acid supplementation appeared to be due to the poor acceptability of the diet. However in previous studies with a longer period of treatment the food intake was improved. The apparent decrease in efficiency of food utilization for weight gain may be due to the poor food intake. This finding was repeated in a subsequent experiment.

The poor acceptability of the glutamic acid supplemented diet resulting in decreased food intake, weight gain and efficiency of food utilization was observed even when the protein content of the diet was high (Table 14). The changes in body weight were associated with changes in brain weight but the differences were not statistically significant.

Glutamic acid supplementation resulted in restoring to normal levels the activities of brain glutamate dehydrogenase and glutamate decarboxylase in animals fed 3 per cent and 5 per cent protein diets, but supplementation of 8 per cent and 20 per cent protein diets was without effect (Table 15).

The lack of any effect of glutamic acid supplementation in the case of the 8 per cent or 20 per cent diet may be due to the fact that these diets contain a sufficient amount of glutamic acid even without such addition or because when the concentrations are already normal supplementation may not have any effect. This is certainly true of increases on protein content over and above the minimal level. In conclusion, the present experiment confirms previous observations of deficits in brain glutamate dehydrogenase and glutamate decarboxylase with dietary protein deficiency and the reversal of the same by glutamic acid supplementations.

Further studies were carried out to ascertain whether the beneficial effects observed could be found with smaller doses of glutamic acid. Studies were therefore made of the effects of adding glutamic acid at concentrations of 0, 1, 2, 3, 4 and 5 per cent. The results are presented in Tables 16 and 17. It can be seen from the former that the supplementation resulted in decreased food intake, body weight and efficiency of food utilization. In spite of this, supplementation at the level of 2 per cent or more was found to restore brain

Table 16: Effects of different levels of glutamic acid supplementation to low protein diets on food intake and body weight in rats

	LP						HP
	glutamic acid added (g per 100 g diet)						
	0	1	2	3	4	5	0
nitrogen in diet (g per 100 g)	0.80	0.90	0.99	1.09	1.18	1.28	3.2
food intake (g/day)	4.2	4.2	4.0	4.0	3.9	3.6	7.8
nitrogen intake (g/day)	0.033	0.037	0.040	0.043	0.046	0.048	0.25
body weight (g)							
initial	50	48	48	50	50	50	50
terminal	80	77	70	73	67	66	186
weight gain (g)							
(a) total	30	29	22	23	17	16	136
(b) per 100 kcals consumed	2.7	2.6	2.1	2.2	1.6	1.6	6.7

period of treatment, 10 weeks; 8 animals were used in each group.

Table 17: Effects of different levels of glutamic acid supplementation to low protein diets on brain enzymes in rats

	LP						HP
	glutamic acid added (g per 100 g diet)						
	0	1	2	3	4	5	0
brain weight (g)	1.38±0.030	1.38±0.020	1.38±0.020	1.39±0.040	1.37±0.030	1.34±0.020	1.60±0.030
brain enzymes:	units per g brain						
GDH	3.2±0.10	3.7±0.10	4.0±0.14	4.0±0.15	4.0±0.13	3.9±0.09	4.0±0.10
GAD	26±1.0	28±0.9	30±1.0	31±1.2	31±1.5	31±0.5	33±0.9

period of treatment, 10 weeks; 8 animals were used in each group.
values are means ± s.e.'s.

enzyme activities to normal levels. Even at the 1 per cent level partial restoration was found.

A question arises as to whether the effects are due to the nitrogen content of the glutamic acid added which might have the effect of improving the nitrogen status of the animal. This does not seem to be the explanation as the supplementation did not bring about increases in body weight or brain weight.

Incidentally, brain weight has implicitly been suggested as a criterion of brain maturation (e.g. Dobbing, 1968). It is most significant that in the present studies brain enzyme activities were found to be increased in spite of there being no change in brain weight, whereas in the experiment described previously the HP restricted group showed a deficit in brain weight but not in brain enzymes. These observations show the limitations in using brain weight as a criterion.

The observation regarding the effects of glutamic acid supplementation needs explanation as this amino acid is not known to cross the blood-brain barrier

normally but may do so at a very slow rate (Roberts, Flexner and Flexner, 1959). Also, under certain conditions the blood-brain barrier against this amino acid is broken by means of physical or chemical methods such as local freezing or ethyl chloride treatment (Purpura, Girado, Smith and Gomez, 1958). It is possible that a low protein diet affects the integrity of the cell membrane in the brain on the basis of certain observations. For instance, Platt and Stewart (1968) observed that the integrity of cell membrane in nerve cells is affected in dogs subjected to severe protein deficiency. Some changes in the membrane structure would be consistent with the observation that brain tissue slices of low protein animals use glutamic acid more efficiently for respiration than those from high protein animals, although the reverse was observed with homogenates (Rajalakshmi, Thrivikraman and Ramakrishnan, 1971).

EXPERIMENT IV

Vitamin A is necessary for the formation and maturation of the neural tube (Richter, 1965). In experimental animals, CNS changes associated with a deficiency of vitamin A have been attributed to a poor

development of the vertebrae and the skull and a resulting pressure on the nervous system (Fell, 1960). Degenerative changes also occur independently of malformation of bones (Dam and Sondergaard, 1964). As mentioned earlier blindness, incoordination and spasms have been found in pigs fed the deficient diet for 6-10 months (Hughes, Lienhardt and Aubel, 1929). Degeneration of the nerve in the optic thalamus, the optic femoral and sciatic nerves and spinal cord was found in these animals. A similar degeneration has been found in other species (Hughes, Lienhardt and Aubel, 1929).

It is well known that vitamin A deficiency affects sensitivity to light and dark adaptation. Severe deficiency may result in blindness particularly corneal in children and the same by restricting psychological stimulation may interfere with normal psychological development. In India more than 1 per cent of the population is blind and this is largely believed to be due to a deficiency of vitamin A. Very occasionally cases of congenital blindness resulting from keratomalacia have been observed (Prof. J.K. Patel, Opthalmology Department, Medical College, Baroda, personal communication

to Dr. Rajalakshmi). Electroretinograms are affected in vitamin A deficiency (Coursin, 1968).

Vitamin A is found to be essential for the utilization of protein and vice versa (Esh and Bhattacharya, 1967; Rechcigl, Berger, Loosli and Williams, 1959; 1962). Children suffering from kwashiorkor and keratomalacia are unable to benefit from administration of vitamin A alone (Arroyave, Wilson, Mendez, Behar and Scrimshaw, 1961). The conversion of carotene to vitamin A, its absorption, transport, storage and utilization are all found to be affected by the protein status both in children and experimental animals (Mahadevan, Malathi and Ganguly, 1965). On the other hand the requirement for vitamin A is more with a high protein diet and liver stores of the vitamin are more rapidly depleted in animals fed a diet deficient in vitamin A and high in protein than those fed one low in protein.

In the context of above considerations studies were carried out on the effects of vitamin A deficiency in animals fed low and high protein diets. Groups of animals were fed the LP and HP diets with or without vitamin A for 11 weeks. As the low protein animals

failed to show a deficiency of vitamin A during this period the studies with the LP diets were repeated with a longer period of treatment, namely 17 weeks. The reversibility of the effects of vitamin A deficiency was investigated in another experiment using only high protein diets.

As may be expected, vitamin A deficiency resulted in decreased food intake and weight gain and poorer utilization of food for tissue gain but these effects were found only with the high protein diets (Table 18). This might be because the LP animals had not yet developed vitamin A deficiency on account of the slower rate at which liver stores are depleted in these animals.

The high protein animals fed the deficient diet showed typical symptoms of vitamin A deficiency. The swelling of the eye, partial closure of the eye, sticky and blood stained exudate from the eye were present in all the animals. In a few animals white patches were also seen in the cornea indicating that keratinization had begun. Towards the end of the treatment they also started losing the weight. These symptoms were not found in the low protein animals.

Table 18: Effects of vitamin A deficiency on body weight and food intake of rats fed low protein (LP) and high protein (HP) diets (11 weeks of treatment)

	LP		HP	
	control	vitamin A deficient	control	vitamin A deficient
food intake (g/day)	4.2	4.3	8.5	6.4
body weight (g)				
initial	44	45	46	46
terminal	68	69	215	142
weight gain (g)				
(a) total	24	24	169	96
(b) per 100 kcals consumed	2.0	2.0	7.6	5.8

8 animals were used in each group.

Table 19: Effects of vitamin A deficiency on brain GDH and GAD (11 weeks of treatment)

	LP		HP	
	control	vitamin A deficient	control	vitamin A deficient
body weight (g)	68	69	215	142
brain weight (g)	1.36±0.020	1.35±0.040	1.58±0.030	1.51±0.030
brain enzymes:		units per g brain		
GDH	3.3±0.09	3.0±0.12	4.1±0.13	3.4±0.10
GAD	25±0.8	25±0.8	32±1.3	26±0.7

8 animals were used in each group.
values are means ± s.e.'s.

The brain weights were reduced with vitamin A deficiency in the high protein animals, but not in the low protein animals because of the differences in growth rate, plane of metabolism and utilization of vitamin A.

The most interesting observation however was the decrease in brain glutamate dehydrogenase and glutamate decarboxylase with vitamin A deficiency in animals fed the HP diets (Table 19). The deficits in these enzymes were of the same order as in protein deficient animals suggesting that the effects of vitamin A deficiency are similar to those of protein deficiency as far as these two enzymes are concerned. It remains to be investigated whether these effects are due to vitamin A deficiency *per se* or due to its involvement in protein utilization. The latter alone cannot perhaps account for the results as the weight gain in the vitamin A deficient HP group was more than in the LP groups. Further, in previous studies in this laboratory while vitamin A deficiency was found to decrease serum protein and albumin levels slightly, the decreases were no where near the low levels found in low protein animals.

Table 20: Effects of vitamin A deficiency on food intake, body weight, brain weight and brain enzymes in rats (17 weeks of treatment)

	LP	
	control	vitamin A deficient
food intake	5.4	5.0
body weight (g)		
initial	47	47
terminal	80	70
weight gain (g)		
(a) total	33	23
(b) per 100 kcals consumed	1.6	1.2
brain weight (g)*	1.38±0.050	1.33±0.040
brain enzymes*		
	Units per g brain	
GDH	3.3±0.10	3.0±0.11
GAD	26±0.6	26±0.8

8 animals were used in each group.
*values are means ± s.e.'s.

Table 21: Body weight changes in rats subjected to vitamin A depletion and repletion

HP diet	body weight (g) period of treatment (weeks) initial	2	4	6	8	10	11	13	15	17
controls	46	88	131	166	199	214	219	232	244	252
experimentals*	46	85	125	135	139	150	145	156	164	176

*given a vitamin A deficient diet for 11 weeks and a normal diets for the next six weeks.

In this connection Halder (Personal communication to Dr. Rajalakshmi) has found the effects of vitamin A deficiency in the cornea to be different from, and more long lasting than, the effects of protein deficiency.

The LP animals did not show any appreciable effect of vitamin A deficiency either at 11 weeks of treatment or at 17 weeks of treatment (Tables 18, 19 and 20). In other studies in this laboratory, however, a longer period of treatment (20 weeks) was found to have some effect.

When high protein animals were fed the deficient diet for 11 weeks and a diet adequate in vitamin A for six weeks, the weight loss was arrested and growth was restored although full catch up growth was not evident (Table 21). The clinical symptoms found in the eye disappeared completely in all the animals.

In the case of brain enzymes, however, the restoration of activity did not appear to be complete (Table 22), particularly in the case of glutamate dehydrogenase, but the differences between the controls and the experimentals fell short of statistical significance.

Table 22: Brain enzymes in rats subjected to vitamin A depletion and repletion

	HP	
	control	experimental*
food intake (g/day)		
1-6 weeks	8.1	7.0
7-11 weeks	8.5	5.8
12-17 weeks	8.4	7.0
body weight (g)		
initial	46	46
terminal	252	176
weight gain (g)		
(a) total		
1-6 weeks	120	89
7-11 weeks	53	11
12-17 weeks	33	30
(b) per 100 kcals consumed		
1-6 weeks	8.8	7.6
7-11 weeks	4.2	1.2
12-17 weeks	2.6	2.3
brain weight (g)**	1.62±0.03	1.60±0.04
brain enzymes**	units per g brain	
GDH	3.9±0.10	3.5±0.20
GAD	31±1.0	28±1.0

*fed the vitamin A deficient diet for 11 weeks and the normal diet for 6 weeks.
**values are means ± s.e.'s.

As mentioned earlier, similar observations have been made by Halder with regard to changes in the corneal epithelium with vitamin A deficiency. It is also interesting to note that in other studies in this laboratory the effects of protein deficiency on brain enzymes were fully reversed with a similar period of treatment.

In conclusion, vitamin A deficiency was found to produce brain enzyme deficits comparable to those found in protein deficiency. These effects were found to depend on the protein status of the animal. The effects of vitamin A deficiency appeared to be less easily reversed than were those of protein deficiency in other studies. It would also appear on the basis of other studies in this laboratory that the effects must at least in part be due to vitamin A deficiency *per se* apart from any effect arising out of impaired utilization of protein in this condition.