RESULTS AND DISCUSSION

EXPERIMENT-I : Preweaning undernutrition.

Severe undernutrition and malnutrition are formidable problems affecting a depressingly large proportion of the world's population. Undernutrition has many medical consequences, among which those arising from the vulnerability of the immature brain to metabolic imbalance loom large.

Marasmus is found to be the commonest form of malnutrition. Marasmus is prevalent in urban areas and is getting more common because of increasing urbanisation and decreasing feeding (Waterlow and Payne, 1975). It is reported that breast milk fails to cover the energy needs of many infants in the developing countries after 3 months of age (Kim and Pollitt, 1987).

Suckling period is the time when rat brain is developing rapidly and is highly vulnerable to undernutrition (Dobbing, 1981). The present experiment was carried out with a view to investigate the possible effect of calorie undernutrition in the postnatal period on the energy metabolism of brain. Various experimental

procedures have been suggested to induce postnatal undernutrition in the animals. They all act through the same mechanism of restricting the quantity of milk available to the litter without affecting its quality (Wiggins, 1982; Gabr, 1987). Increasing the litter size as in the present experiment do restrict the litters to the quantum of milk available. This seems to have more relevance to human conditions. Human starvation typically results from a lack of all major food sources. Often the diet is essentially adequate in composition, but greatly insufficient in quantity. This is supported by human studies (Gabr, 1987) as well as studies on laboratory animals (Grigor et al, 1987; Grimble and Mansray, 1987). Marasmus seems to be a disease primarily confined to infancy (McLaren, 1973).

However, human studies leave little room of doubt, that humans also follow what has already been demonstrated with laboratory animals (Winick, 1969; Rosso et al, 1970; Dobbing and Smart, 1973; Wiggins, 1982; Galler, 1984). A large share of human infants are undernourished. There seems to be a link between postnatal undernutrition and the retardation of human mental development (Chase, 1973; Winick, 1979; Gabr, 1981).

TABLE-20 : Effect of preweaning undernutrition on body and brain weight.

	Control 8 to litter	Undernourished 16 to litter
Body weight (g) :		
Initial	6.0 ± 0.13	5.9 ± 0.4
Final	24.0 ± 0.39	14.9 ± 0.36***
Percentage of control	100	62
Weight gain (g)	18	9.0
Brain weight	1.0 ± 0.014	0.96 ± 0.024***
Percentage of control	100	87
$\frac{\text{Brain weight}}{\text{Body weight}} \times 100$	4.58	6.44

Period of treatment 14 days
8 animals were used in each experiment
Values marked with asterisk are significantly different from control.
'p' less than 0.001 for ***.

Undernutrition, early in life produces neurological (Morand et al, 1982) as well as behavioral alterations (Levitsky et al, 1979; Chamove, 1983; Galler, 1984). Postnatal undernutrition may cause structural, neurochemical and developmental alterations in rats (Balazs et al, 1979; Winick, 1979). An early undernutrition in the postnatal period impairs generation of glial cells and myelin formation more than neuronal generation (Reddy and Horrocks, 1982; Subbarao and Subbarao, 1982; Wiggins, 1982) retards synaptogenesis and consequently the development of neuronal contacts (Salas et al, 1974; Cravioto et al, 1976; Dyson and Jones, 1976). Preweaning undernutrition is found to manifest memory and learning disabilities in adult (Vendite et al, 1985).

Undernutrition from birth to 14th day postnatal

Undernutrition was induced from the day of birth by increasing the number of litters to a dam as described in methods. Control dam had only 8 litters, while in the undernourished group there were 16 litters to a dam.

Data on Table-20 gives the body and brain weight. The undernourished had body and brain weights 62% and 87% respectively of the control. This is in agreement with the earlier result (Rajalakshmi and Nakhasi, 1974).

TABLE-21 : Effect of preweaning undernutrition on enzymes of glycolysis and HMP shunt in brain.

Enzyme Units/g	Control (8 to a litter)	Undernourished (16 to a litter)
Hexokinase	6.9 ± 0.25	6.3 ± 0.28
Glucose-6-phosphate dihydrogenase	3.62 ± 0.07	3.41 ± 0.11
Phosphofructokinase	9.2 ± 0.16	8.8 ± 0.18
Fructose-16-diphosphate aldolase	4.9 ± 0.21	4.4 ± 0.18
Pyruvate kinase	74.2 ± 1.92	64.1 ± 1.90**
Lactate dehydrogenase	48 ± 2.73	43 ± 2.28

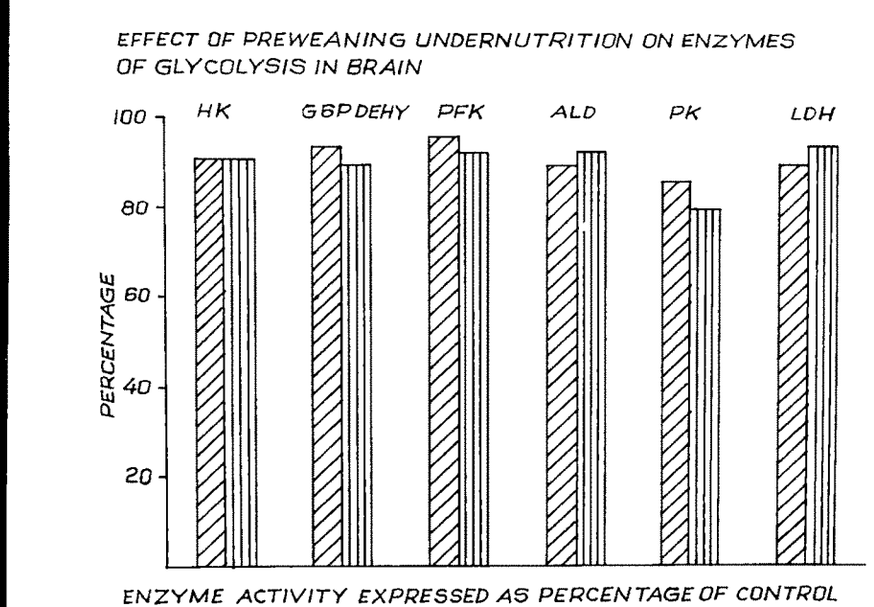
Period of treatment - 14 days.

8 animals were used in each experiment.

Values marked with asterisk are significantly different from control.

'p' less than 0.01 for **.

Fig.1 : Effect of preweaning undernutrition on enzymes of glycolysis in brain.



HK	=	Hexokinase;
G6P DEHY	=	Glucose 6 phosphate dehydrogenase
PFK	=	Phosphofructokinase
ALD	=	Aldolase. PK = Pyruvate kinase
LDH	=	Lactate dehydrogenase.

Among the enzymes of glycolysis in brain except pyruvate kinase all the enzymes studied showed a comparable activity with that of the control group. The activity of pyruvate kinase (Table-21) was significantly decreased in the brain of undernourished animal. There was approximately 14% decrease in the activity of the enzyme compared to the control animals (Figure 1). Though not statistically significant, decreased specific activity of pyruvate kinase in brain of 10 day old undernourished rat has been reported (Chase et al, 1976). Pyruvate kinase is one of the key enzymes of glycolysis in brain. Along with hexokinase and phosphofructokinase it forms a regulatory system of glycolysis in brain (McIlwain and Bachelard, 1971). A decreased activity of pyruvate kinase may decrease the glycolytic rate in the undernourished brain. Decreased rate of glycolysis is reported in undernutrition (Patel and Balazs, 1975; Dyson and Jones, 1976a).

Though there was a decreased glycolysis, the activity of glucose-6-phosphate dehydrogenase, a representative of hexosemonophosphate pathway was not affected by postnatal undernutrition (Table-21). It is well known that this pathway provides enough reduced coenzyme NADPH for the synthesis of lipids (Baquer et al, 1977).

TABLE-22 : Effect of preweaning undernutrition on enzymes of Krebs cycle in brain.

Enzyme Units/g	Control (8 to a litter)	Undernourished (16 to a litter)
Pyruvate dehydrogenase	0.32 ± 0.015	0.29 ± 0.013
Isocitrate dehydrogenase - - NADP.	4.7 ± 0.11	4.4 ± 0.10
Succinate dehydrogenase	0.56 ± 0.032	0.53 ± 0.027
Malate dehydrogenase - NADP (Malic enzyme)	1.79 ± 0.071	1.55 ± 0.106

Period of treatment - 14 days.

8 animals were used in each experiment.

Besides, the other two enzymes, NADP-isocitrate dehydrogenase and malic enzyme which also provide the reduced coenzyme (Patel, 1973; Hawkins and Mans, 1983) were found to have registered comparable activity with that of the control animals on 14th day of postnatal undernutrition. This may perhaps be the reason why the total lipid concentration was not found to be reduced in the brain of undernourished animal on 14th postnatal day (Rajalakshmi and Nakhasi, 1974; Reddy and Horrocks,1982). However, Chase et al. (1976) reported a decreased incorporation of U-^{14}C glucose into brain lipids in postnatal undernutrition.

It is interesting to note that none of the enzymes of energy metabolism in brain (Table-22) showed any significant change from the control in the postnatal undernutrition during the first 14 days. This may be because the enzymes of energy metabolism of brain develops in the latter half of postnatal growth of rat brain (Balazs and Patel, 1973). Besides, pyruvate dehydrogenase, the key enzyme of glucose oxidation develops only at a later period (Land et al, 1977; Booth et al, 1980). This is quite evident as there is a three fold increase in the glucose utilization by brain between second and third postnatal week (Miller and Corddy, 1981). However, brain may be able to adapt to undernutrition. This is

TABLE-23 : Effect of preweaning undernutritiom on body and brain weight.

	Control (8 to a litter)	Undernourished (16 to a litter)
Body weight (g) :		
Initial	6.1 ± 0.11	6.0 ± 0.14
Final	40.5 ± 0.53	24.3 ± 0.68***
Percentage of control	100	60
Weight gain	34.4	18.3
Brain weight (g) :	1.32 ± 0.01	1.17 ± 0.016***
Percentage of control	100	88.6
$\frac{\text{Brain weight}}{\text{Body weight}} \times 100$	3.25	4.81

Period of treatment - 21 days.

8 animals were used in each experiment.

Values marked with asterisk significantly different from control.
'p' less than 0.001 for ***.

achieved by an augmented utilization of ketone bodies by undernourished rat brain (Shambaugh et al, 1984).

Undernutrition from birth to 21 day postnatal

Undernutrition was induced in the postnatal period by increasing the litter size as described above. The possible effects of pre- and postnatal malnutrition on the growth and development of the brain are gaining increased attention. There is increasing evidence which shows that malnutrition per se alters the central nervous system by limiting its metabolic, structural and functional capabilities and performance. Anatomically brain weight, cell number, cell size, cellular organization and myelin formation has been found to be decreased by moderate to severe malnutrition occuring early in development. Various biochemical parameters like RNA, DNA, proteins and enzymes are reduced (Vindite et al, 1985).

Data on Table-23 shows a significant decrease in body and brain weights of animals undernourished during the first 21 postnatal days. The undernourished registered 60% of the body weight of the control while they had a brain weight approximately 89% of the control.

TABLE-24 : Preweaning undernutrition on enzymes of glycolysis and HMP shunt in brain.

Enzyme Units/g	Control (8 to a litter)	Undernourished (16 to a litter)
Hexokinase	8.8 ± 0.48	8.0 ± 0.34
Glucose-6-phosphate dehydrogenase	3.06 ± 0.15	2.76 ± 0.11
Phosphofructokinase	12.3 ± 0.55	11.4 ± 0.42
Fructose 16 diphosphate aldolase	8.9 ± 0.29	8.3 ± 0.23
Pyruvate kinase	111.6 ± 3.42	89.2 ± 3.06***
Lactate dehydrogenase	80 ± 2.51	75 ± 1.98

Period of treatment - 21 days.

8 animals were used in each experiment.

Values marked with asterisk are significantly different from control.
'p' less than 0.001 for ***.

These results are comparable with those reported earlier (Rajalakshmi and Nakhasi, 1974; Reddy and Horrocks,1982). Data on Table-24 shows that activity of pyruvate kinase was significantly reduced in the brain of undernourished animals while none of the other glycolytic enzymes were affected. There was approximately 20% reduction in the activity of pyruvate kinase (Figure 1). However, Chase et al. (1976) found no change in the specific activity of pyruvate kinase in the brain of postnatally undernourished. Perhaps this could be attributed to a change in the expression of the enzyme activity in the present experiment based on brain weight. However, Tyzbir et al. (1977) had recommended the advantage of expressing enzyme activity per unit brain weight over that of protein. Most of the glycolytic enzymes attain the adult pattern of activity during the third postnatal week. Retarded development of pyruvate kinase can lead to a retarded glycolytic flux in the brain. Retarded glycolytic flux in the brain of undernourished rats have been reported (Balazs et al, 1979; Shambaugh et al,1984).

Although, Adlard and Dobbing (1970) reported a significant reduction in the activity of aldolase in the brain of severely undernourished rat, there was no change in the present experiment. This is because the undernutrition was comparatively more severe in their experiment,

TABLE-25 : Effect of preweaning undernutrition on enzymes of Krebs cycle in brain.

Enzyme (units/g)	Control 8 to 2 litter	Undernourished 16 to a litter
Pyruvate dehydrogenase	0.86 ± 0.029	0.70 ± 0.025***
Isocitrate dehydrogenase - NADP	3.8 ± 0.1	3.4 ± 0.09**
Succinate dehydrogenase	0.96 ± 0.025	0.79 ± 0.038**
Malic enzyme (NADP)	2.20 ± 0.059	2.10 ± 0.015

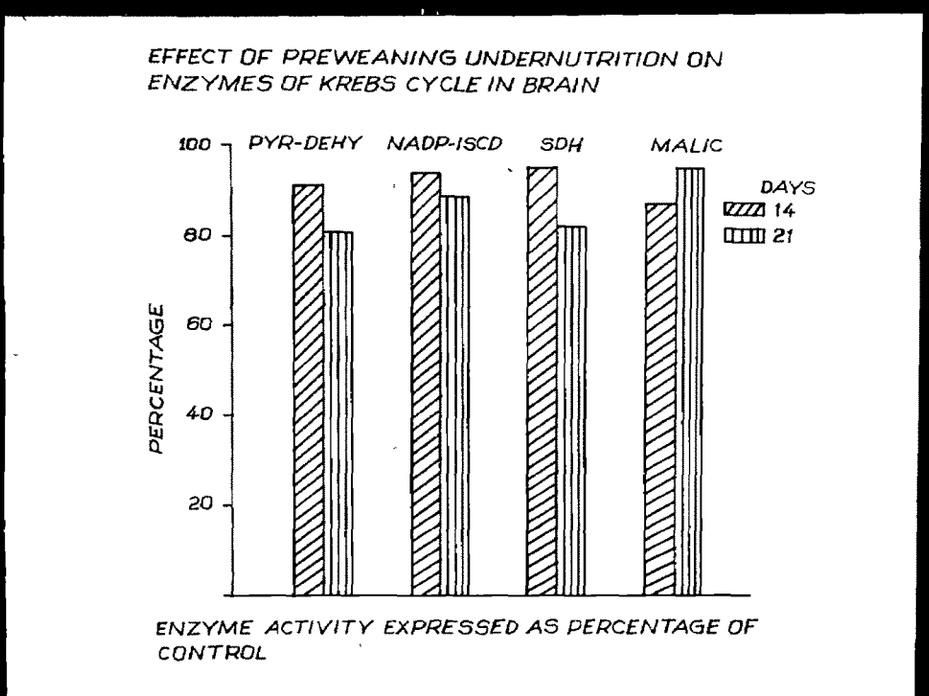
Period of treatment 21 days

8 animals were used in each experiment

Values marked with asterisk are significantly different from control.

'p' less than 0.01 for ** and 0.001 for ***.

Fig.2 : Effect of preweaning undernutrition on enzymes of Krebs cycle in brain.



PYR DEHY = Pyruvate dehydrogenase
NADP-ISCD = Isocitrate dehydrogenase - NADP
SDH = Succinate dehydrogenase
MALIC = Malic enzyme - NADP
Control - 8 litters per dam; Undernourished 16 litters per dam

as it was spread over both gestation and lactation. There was no change in the activity of glucose-6-phosphate dehydrogenase, a representative enzyme of hexosamonophosphate shunt and phosphofructokinase, one of the key regulatory enzymes of glycolysis, in the brain of undernourished rats in comparison with control animals. Similar observation was reported in the activities of these enzymes in postnatal protein deficiency (Coupain et al, 1977).

Data on Table-25 shows the activity of representative enzymes of energy metabolism. Pyruvate dehydrogenase was significantly reduced in the undernourished group. There was approximately 19% (Figure 2) reduction in the activity of pyruvate dehydrogenase. This may imply a marked retardation in the development of this key enzyme. Since 90% of respiration in brain is accounted by complete oxidation of glucose, pyruvate oxidation in the mitochondria plays a key role in energy metabolism of the brain (Deshmukh et al, 1980). Besides the development of pyruvate dehydrogenase is found to be linked with the development of neurological competence (Booth et al, 1980). Perhaps this may be relevant in the neurological abnormality observed in postnatally malnourished children (Wiggins, 1982). It is observed

that there is a substantial increase in the concentration of ketone body in brain in postnatal undernutrition and decrease in glucose concentration in brain (Patel and Balazs, 1975). Although ketone bodies provide energy to the brain (Shambaugh et al, 1984) they are potent inhibitors of pyruvate dehydrogenase complex of brain (Booth and Clark, 1981). There is a direct relationship between the rate of glucose oxidation and the synthesis of acetylcholine in brain (Gibson and Blass, 1976; Kalaria and Prince, 1985). Undernutrition in the early life retarded development of neurotransmitter system involving acetylcholine and GABA in different regions of the brain (Patel et al, 1978).

Data on Table-25 gives an account of the activity of the enzymes of energy metabolism of brain. NADP-isocitrate dehydrogenase was found to be decreased significantly. There was approximately a deficit of 11% in the activity of the enzyme in the brain of undernourished animals. Isocitrate occupies a central position in the intermediary metabolism and is the substrate for a variety of synthetic and energy yielding pathways. The NADP-isocitrate dehydrogenase may serve as an important link between Krebs cycle intermediates and amino acid metabolism particularly active in

immature brain (Loverde and Lehrer, 1973). Indeed, the conversion of glucose carbon into amino acids derived through Krebs cycle is significantly retarded in postnatal undernutrition (Patel and Balazs, 1975; Patel et al, 1978; Lim and Davison, 1980; Shambaugh et al, 1984). It is all the more significant to note that this rapid conversion of glucose carbon into amino acids takes place during the period of functional maturation of the brain (Balazs et al, 1979). One of the reasons for a retardation in the conversion of glucose carbon into amino acids in undernourished brain is the poor glycolytic rate (Balazs et al, 1975). Undernutrition had profound influence on brain glucose metabolism. Undernutrition in the neonatal period retarded the development of metabolic compartmentation. There was a substantial reduction in the brain glucose level in preweaning undernutrition (Patel and Balazs, 1975).

Succinate dehydrogenase, one of the key enzymes of energy metabolism was found to be significantly reduced in the brain of undernourished animals (Table-25). A significant reduction in the activity of succinate dehydrogenase in the brain of animals undernourished pre and postnatally has been reported (Adlard and Dobbing, 1970; Tyzbir et al, 1977). A fall in the activity of

succinate dehydrogenase approximately 18% (Figure 2) may be indicative of a depressed oxidative metabolism in the brain of undernourished animals. Though, as discussed earlier ketone bodies can be a supplementary source of energy, it cannot effectively replace glucose as the source of cerebral energy. Glucose is an obligatory source of energy for the brain (Williamson, 1987). Hence an impairment of glucose metabolism may have deleterious effect on brain metabolism. It is indeed worth recalling that energy metabolism of brain is coupled with physiological function (Sokoloff,1977). This is all the more evident from the fact that many of the CNS depressants decrease glucose oxidation. CNS depression is associated with a selective reduction in energy production in neuronal metabolic compartment (Mohler et al, 1975). Areas which are more active physiologically have a higher consumption of glucose (Turek et al, 1986). However, it is unfortunate that little attention is paid to the energy requirement of the developing brain. Adequate calories must be provided to maintain the high energy phosphate levels for the synthesis of proteins and lipids. (Lim and Davison,1980).

Preweaning period in rat is a time of enormous changes within the brain and a time when brain is most sensitive to detrimental effects of undernutrition (Winick, 1970a). A high proportion of oxidative metabolism of cerebral cortex occurs in dendritic areas which are rich in synapses (Diamond & Fishman, 1973a). It seems that synapse plays an active role in the process of learning (Dyson and Jones, 1976a). Hence it may be tempting to suggest a correlation between synaptic energy metabolism and learning. It is presumed that there is an increased demand for energy for performing intellectual work (Von Muralt, 1976). The functional activity of brain is related to the activity of Na^+K^+ ATPase which is involved in the maintenance and restoration of neuronal membrane potential. A major share of energy produced in cerebral tissue is utilized for ion transport mechanisms important in maintaining the physiological function of the tissue (Bachelard and McIlwain, 1970). Approximately half of the total cerebral energy cycle turnover takes place in nerve endings (Salganicoff, 1968).

Studies on children (Kumar et al, 1977) showed a decreased nerve conduction velocity in marasmus and kwashiorkor. Similarly developmental protein malnutrition in rats registered an impaired functional capability of neurone (Stern et al, 1983). Thus an impairment of

energy metabolism of brain during undernutrition in the postnatal period may underlie the genesis of impaired memory and learning processes as well as behavioral abnormalities, as already mentioned.

EXPERIMENT-II : Moderate dietary protein deficiency in the postweaning period.

A large section of the worlds population is subsisting on low protein intake. Animals adjust to a low protein intake by an economic use of amino acid by its recycling and decreased excretion (Waterlow, 1968).

Moderate protein deficiency in the postweaning period was found to affect enzymes of glutamate metabolism in the brain (Rajalakshmi et al, 1974a). There are earlier reports of reduced DNA and protein in the cerebrum of rat in protein deficiency during the postweaning period (Mehta and Chakravarti, 1973). The present experiment was carried out to find out whether a moderate protein deficiency during the postweaning period do exert any adverse effect on the vital energy metabolism of brain.

Data on Table-26 shows a considerable decrease in the body weight (38% of control) and brain weight (83% of control) of protein deficient animals. This is in agreement with the earlier reported values of Rajalakshmi et al. (1974a). The present values are slightly higher than reported by Rajalakshmi et al.(1974d).

TABLE-26 : Effect of postweaning protein deficiency on body and brain weight.

	LP	HP
Body weight (g) :		
Initial	44 ± 0.43	44 ± 0.45
Final	81*** ± 0.73	212 ± 2.71
Percentage of control	38	100
Total body weight gain (g)	37	167
Weight gain per week	3.7	16.7
Brain weight	1.30 ± 0.078**	1.56 ± 0.013
$\frac{\text{Brain weight}}{\text{Body weight}} \times 100$	1.60	0.74

Period of treatment 10 weeks.

LP & HP animals were fed 5% and 20% protein diet ad libitum respectively.

8 animals were used in each group.

Values marked with asterisk are significantly different from control, 'p' less than 0.01 for **.

TABLE-27 : Effect of postweaning protein deficiency on food intake and its utilization.

	LP	HP
Terminal body weight (g)	81 ± 0.73	212 ± 2.71
Food intake (g)		
g/day/week	5.2 ± 0.084	8.4 ± 0.39
g/100g body weight	8.2	6.7
Weight gain (g)		
Per g food	0.101	0.284
Per 100 K.Cal	2.65	7.46

LP = Low Protein fed animals (5%)

HP = High Protein fed animals (20%)

This perhaps may be due to the fact that they fed 4% protein diet for the experimental animals and also the short term nature of the experiment. However, both brain weight and body weights were significantly reduced. In all the instances body growth is more severely retarded.

Food intake was comparatively much less in the low protein fed animals (Table-27). Poor food intake among the low protein fed animals is a widely observed phenomenon (Rajalakshmi et al, 1974c; Kwong and Barnes, 1977; Peter and Harper, 1985). It is believed that the depressed food intake in low protein fed animals is due to decreased level of plasma and brain essential amino acids (Peng et al, 1974).

However, it is evident from Table-27 that in relation to body weight, food intake was much higher among the low protein fed animals. Nevertheless, weight gain in relation to food and energy consumption was much less in low protein fed animals. This suggests that a moderate protein deficiency apparently imposes a wasteful metabolism of calories (Hegsted, 1974). The impaired utilization of food for growth with low levels of dietary protein is well documented (McCracken, 1976; Danto, 1987). Protein restricted animals are

found to show decreased efficiency to utilize energy (Crist et al, 1980; Hillgartner and Romsos, 1987). This perhaps may be due to certain hormonal mechanism. In low protein fed animals serum T_3 level is elevated and has a permissive role in reducing efficiency of energy utilization (Hillgartner and Romsos, 1987).

Many children adapt to low protein intakes by growing less rapidly, without developing cellular or hormonal signs of protein deficiency. This suggests that protein intake may determine how energy is utilised and ultimately the rate of growth. An understanding of the inefficient use of energy when protein intake is inadequate is of practical clinical important (McLean and Graham, 1979; Fischer and Canolty, 1983).

Shunting of growth is an adaptive mechanism to low protein diet resorted by man and animals. It may make the animal or man to be more viable for nutritional adaptation in a low protein diet. The requirements of protein and calories can be scaled down by shunting mechanism of adaptation (Waterlow, 1968). This may be the reason for the low body weight observed in low protein fed animals. Adult humans are found to adapt excellently

in acute protein deficiency by juidicious utilization of endogenous amino acids (Steffe et al, 1976).

However, 5% dietary protein is inadequate for weaned rats to promote optimum growth and maintenance. The optimum diet for a weaned rat should contain 15% protein as casein and for adult rat it is approximately 10% casein diet (Krajcovicova and Dibak, 1980). It is reported that 4% dietary crude protein is required to satisfy maintenance needs for nitrogen among monogastric animals. However, this can be brought down to an astonishingly low level of 0.25% crude protein under an adequate energy and sterile intestinal conditions by freeing the intestine from harbouring microorganisms by the use of antibiotics. It is perhaps these microorganisms in the intestine which consume a large share of dietary free amino acids (Nipper et al, 1987).

Data on activities of enzymes in brain representing glycolysis (hexokinase, phosphofructokinase and pyruvate kinase) hexosemonophosphate shunt (glucose-6-phosphate dehydrogenase), energy metabolism (pyruvate dehydrogenase and succinate dehydrogenase) as well as NADP-isocitrate dehydrogenase and NADP-malate

TABLE-28 : Effect of postweaning protein deficiency on enzymes of glycolysis and HMP shunt in brain.

Enzyme (units/g)	LP	HP
Hexokinase	8.8 ± 0.37	9.7 ± 0.45
Glucose-6-phosphate dehydrogenase	2.74 ± 0.10	2.84 ± 0.10
Phosphofructokinase	15.2 ± 0.55	14.6 ± 0.65
Fructose-1,6-diphosphate aldolase	9.6 ± 0.37	10.1 ± 0.28
Pyruvate kinase	156 ± 6.94	165 ± 5.20
Lactate dehydrogenase	90 ± 2.80	94 ± 3.34

LP = Low Protein fed animals (5%)

HP = High Protein fed animals (20%)

8 animals were used in each group.

Period of treatment - 10 weeks.

TABLE-29 : Effect of postweaning protein deficiency on enzymes of Krebs cycle in brain.

Enzyme (units/g)	LP	HP
Pyruvate dehydrogenase	1.54 ± 0.048	1.61 ± 0.049
Isocitrate dehydrogenase - NADP	2.54 ± 0.06	2.45 ± 0.07
Succinate dehydrogenase	1.34 ± 0.053	1.48 ± 0.052
Malate dehydrogenase - NADP (Malic enzyme)	1.29 ± 0.061	1.41 ± 0.075

LP = Low Protein fed animals (5%)

HP = High Protein fed animals (20%)

8 animals were used in each group.

Period of treatment 10 weeks.

dehydrogenase representing carbon dioxide fixing enzymes in brain are given in Tables-28 & 29. The values of the activities of hexokinase (E.C.2.7.1.1) (Wilson, 1972; Kaur et al, 1983). Glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49) (Leong et al, 1981); phosphofructokinase (E.C. 2.7.1.11) (Leong et al,1981); aldolase (E.C. 4.1.2.13) (McDonnel and Greengard,1974; Toble et al, 1976); pyruvate kinase (E.C. 2.7.1.40) (McDonnel and Greengard, 1974); lactate dehydrogenase (E.C. 1.1.1.27) (Wilson, 1972); pyruvate dehydrogenase (E.C. 1.2.4.1) (Bonnet et al, 1966; Land et al, 1977; Leong et al, 1981); NADP-isocitrate dehydrogenase (E.C. 1.1.1.42) (Swaiman et al, 1970; Leong et al,1981); succinate dehydrogenase (E.C. 1.3.99.1) (DeRobertis et al, 1962) and malic enzyme (E.C. 1.1.1.40) (Leong et al, 1981) are comparable with reported values as indicated.

Despite a significant decrease in brain weight in the low protein fed animals, none of the above mentioned enzymes were affected by protein deficiency. This is in contrast with earlier observation where enzymes of glutamate metabolism were significantly decreased in moderate protein deficiency (Rajalakshmi et al, 1974a). Since brain relies on glucose carbon

for the synthesis of many non-essential amino acids a stable amino acid composition in brain in moderate protein deficiency (Gustafson et al, 1986) may be an indirect evidence for the uninterrupted operation of glycolysis and Kreb's cycle in the brain of protein deficient animals. However, later with a different strain of the animals these differences in low protein fed animals were found to be abolished. There was significant decrease in the activity of glutamate dehydrogenase in the brain of low protein fed animals provided these animals were malnourished postnatally (Rajalakshmi, 1981). Nevertheless, studies on lipid composition of brain in moderate protein deficiency in the postweaning period come as supporting evidence for the present result (Dickerson et al, 1972; Rajalakshmi et al, 1974d).

Postweaning protein deficiency caused a significant reduction in the acetylcholine content of brain (Rajalakshmi et al, 1974e). This perhaps might be due to a decreased brain weight and thus a decreased brain size in the low protein fed animals. Besides, an increased acetylcholine esterase activity is reported in the brain of low protein fed animals (Coupain et al, 1977).

Moderate protein deficiency during the postweaning period was found to have no effect on brain lipids (Guthrie and Brown, 1968; Dickerson et al, 1972; Smart et al, 1973; Rajalakshmi et al, 1974a). Studies conducted on the lipid composition of different regions of the brain once again gave ample testimony to the earlier observation that brain is resistant to moderate protein deficiency in the postweaning period (Rajalakshmi and Nakhasi, 1974a). The lack of any effect on the energy metabolism of brain due to moderate protein deficiency might be indicative of an adaptive mechanism of brain to long term nutritional stress (Coupain et al, 1977). Adaptation of brain development to long term nutritional stress spanning both preweaning and postweaning period has been sounded by West and Kemper (1976).

The observation that a moderate protein deficiency which results in retardation of brain growth do not necessarily result in an impaired metabolic activity is encouraging from the point of practical nutrition. About a third of the children in the rural area of this country are found to show mild deficiency of protein as judged by clinical

and biochemical criteria (Rajalakshmi et al, 1973). The present result atleast gives room for hope for these children that the future may not be grim for them. This does not minimize the importance of ensuring adequate nutrition for all.

TABLE-30 : Effect of postweaning calorie restriction on body and brain growth.

	Control	50% restricted	67% restricted
Body weight (g) :			
Initial	44 ± 0.55	43 ± 0.51	43 ± 0.53
Final	201 ± 1.61	96 ± 0.85***	56 ± 1.08***
Percentage of control		47.7	27.8
Total weight gain (g)	157	53	13
Weight gain per week	15.7	5.3	1.3
Brain weight (g)	1.59 ± 0.017	1.43 ± 0.015***	1.32 ± 0.016***
Percentage of control		90	83
Brain weight / Body weight x 100	0.79	1.48	2.35

Period of treatment 10 weeks.

All animals were fed 20% casein diet. Control animals were fed ad libitum, while other animals were fed restricted amounts of diet as indicated.

8 animals were used in each group.

Values marked with astrick are significantly different from control, 'p' less than 0.001 for ***.

(50% restricted) were weighing 48% of the control while severely undernourished animals (33% restricted) had only 28% of the body weight of the control. However, achieving and maintaining a low body weight is the key to long term adaptation to undernutrition (Waterlow, 1986). Brain weight also were significantly reduced (Table-30) in undernourished animals. These results are in agreement with earlier reports (Rajalakshmi et al, 1974a; 1974d). There was 17% decrease in the brain weight in the severely undernourished animals. Can this be a mode of adaptation? Perhaps may be. Since in animals such as rat, brain energy consumption is high (Norberg and Seisjo, 1974) a reduction in brain weight might constitute a long term adaptive device. The growth of the body was more severely retarded in both moderately and severely undernourished animals. Structural composition of brain is known to be maintained and defended by adult animals in protein and calorie deficiencies (Lytle et al, 1984).

However, a picture that emerges from these studies is the capacity of the animals to continue to grow in spite of severe undernutrition. If we assume that in rat, as in other species, basal calorie requirement constitutes 50% of the total calorie

TABLE-31 : Effect of postweaning calorie restriction on food intake and its utilization.

	Control	50% restricted	67% restricted
Terminal body weight (g)	201 ± 1.61	96 ± 0.85	56 ± 1.08
Food intake (g)			
g/day/week	9.0 ± 0.64	4.5 ± 0.10	3.0 ± 0.22
g/100g body weight	7.3	6.3	5.7
Weight gain (g)			
per g food	0.249	0.168	0.062
per 100 K.Cal	6.54	4.42	1.63

Period of treatment 10 weeks.

All animals were fed 20% casein diet. Control animals were fed ad libitum, while other animals were given restricted amount of food as indicated.

8 animals were used in each group.

consumed (Evans and Miller, 1968), restriction of food intake to 50% or 33% of ad libitum food intake of weight matched controls should result in cessation of growth or induce loss of weight. Yet, this is seldom a found to be the case. The present observation suggests the capacity of the undernourished animals to adapt to the situation presumably by a reduction in the basal metabolic rate. It has been observed in humans as well as in rats, that they adapt to restricted intake by reducing the basal metabolic rate.(Forsum et al, 1981; Hill et al, 1985). A comparison of the data with that of low protein fed animals (Table-27 Vs. 31) reveals that for a unit body weight the food intake is much higher in the low protein fed animals than the moderately undernourished animals (50% restricted). Yet, the weight gain is much higher in the calorie restricted animals than in the moderately low protein fed animals. Thus utilization of energy is much better when the protein requirement is satisfied.

Indeed, this is not true in the case of severely undernourished (33% restricted) animals. These animals were found to grow much less in comparison with low protein fed animals. Thus growing animals may

TABLE-32: Effect of postweaning calorie restriction on enzymes of glycolysis in brain.

	Control	50% rescricted	67% restricted
Hexokinase	9.4 ± 0.31	8.6 ± 0.23	9.1 ± 0.38
Phosphofructokinase	14.5 ± 0.42	13.4 ± 0-61	15.3 ± 0.69
Fructose-16-diphosphate aldolase	9.6 ± 0.36	9.0 ± 0.34	8.7 ± 0.28
Pyruvate kinase	165 ± 3.59	171 ± 3.23	160 ± 3.89
Lactate dehydrogenase	95 ± 3.69	91 ± 1.48	89 ± 2.54

Period of treatment 10 weeks.

8 animals were used in each group.

All animals were fed 20% casein diet.

Control animals were fed ad libitum; while other animals were given restricted amount as indicated.

TABLE-33 : Effect of postweaning calorie restriction on enzymes of Krebs cycle in brain.

	Control	50% restricted	67% restricted
Pyruvate dehydrogenase	1.56 ± 0.043	1.51 ± 0.062	1.47 ± 0.053
Isocitrate dehydrogenase - NADP	2.51 ± 0.056	2.56 ± 0.074	2.38 ± 0.067
Succinate dehydrogenase	1.48 ± 0.068	1.59 ± 0.051	1.52 ± 0.075
Malate dehydrogenase - - NADP (Malic enzyme)	1.53 ± 0.078	1.41 ± 0.057	1.48 ± 0.084

Period of treatment 10 weeks.

8 animals were used in each group.

All animals were fed 20% casein diet.

Control animals were fed ad libitum; while other animals were given restricted amount as indicated.

able to utilize protein only in conditions of adequate energy intake (Waterlow, 1986). The effect of energy intake on nitrogen retention and weight gain in human is well established. When energy intakes are inadequate, nitrogen utilization is less efficient and in growing organism weight gain slows down (Fischer and Canolty, 1983). It is reported that food restricted animals showed considerable energy conservation and decline in energy expenditure. The decrease in energy expenditure can be explained by a concomittant decline in lean body mass, which is the most metabolitically active tissue (Hill et al, 1986). Recently it was shown on human volunteers that energy restricted diet had no effect on nitrogen balance. Thus when energy intake is severely restricted whole body nitrogen turnover, protein synthesis and protein breakdown are maintained provided an adequate protein supply is assured (Garlic et al, 1978; Pencharz et al, 1980). This may perhaps be the reason for the continued growth of animals observed in spite of severe calorie restriction.

Activities of representative enzymes of glycolysis and Kreb's cycle are given on Tables-32 & 33, respectively. There was no significant difference in the activity of any of the enzymes of glycolysis or

Krebs cycle in the brain of undernourished animals. Thus unlike in the preweaning period, brain is resistant to a postweaning undernutrition. The adaptive capability of adult brain should be greatly appreciated in the context that unlike other species, due to the large brain to body size, human brain requires a much larger proportion of whole body energy (Hawkins and Mans, 1983).

Various compensatory mechanism come into operation throughout the body when food supply is restricted or unbalanced. In the liver, the rate of protein synthesis and catabolism of amino acids are greatly reduced. There is an enhanced breakdown of proteins in organs such as pancreas, intestinal mucosa and muscle which produces endogenous supply of amino acids. As a result certain organs such as brain are relatively little affected by food deprivation in the adult (Balazs et al, 1979). This is further reaffirmed by the finding of Lehr and Gayet (1967) that during dietary restriction of adult rats there is an increased specific activity of brain aspartate and glutamate after subcutaneous injection of U-^{14}C glucose. This perhaps may be due to an augmented operation of the Krebs cycle in the undernourished adult brain as aspartate and glutamate are derived from Krebs cycle intermediates.

Though, undernutrition during gestation and lactation markedly decreased activity of some of the enzymes like 3-oxoacid CoA transferase and acetoacetyl CoA thiolase in the brain during suckling period, a continued undernutrition extending in the postweaning period reverted the activity of these enzymes to the control values. This may explain a 'catch up' phenomena in postweaning period and thus a compensatory mechanism of the brain to reverse the detrimental effects of undernutrition during more critical periods of brain development (Escriva et al, 1985).

Another novel compensatory mechanism resorted by brain during starvation is the use of ketone bodies for energy purpose (Owen et al, 1967; Seisjo and Agardh, 1983). However, ketone bodies can only partially replace glucose as a substrate (Sokoloff et al, 1977b). A recent report confirms that ketone bodies provide only a modest amount of brain fuel during starvation (Hawkins et al, 1986)

The present observation that brain is resistant to postweaning energy restriction is further supported by the observation on the metabolism of putative neurotransmitter glutamate (Rajalakshmi et al, 1974a) and on lipid composition on brain during severe undernutrition

in the postweaning period (Rajalakshmi et al, 1974d), even on the face a significant reduction in body and brain weights. Compared to other organs, adult brain is relatively immune to any change in weight or biochemical composition as a result of starvation (Young and Scrimshaw, 1971; Wiggins, 1982). Postweaning undernutrition was found to have no effect on brain cholesterol (Dobbing, 1968) or phospholipid and other fractions of brain lipids (Dobbing and Widdowson, 1965; Rajalakshmi et al, 1974d).

Pyruvate dehydrogenase plays a key role in carbohydrate metabolism upon which brain normally relies. The properties of pyruvate dehydrogenase in liver, kidney, heart and brain are very similar (Jope and Blars, 1976). Studies on man and experimental animals suggest that the metabolism of glucose by skeletal muscle is depressed during starvation. It is observed that glucose metabolism in skeletal muscle is inhibited during starvation at the step of pyruvate oxidation (Ruderman et al, 1977). However, it has been observed that during starvation the active form of pyruvate dehydrogenase is decreased in liver, heart and kidney, but not in brain (Wieland et al, 1971). This might perhaps give credence to the observation that adult brain can adapt to undernutrition better than

many other organs. Continued undernutrition extending from preweaning period to postweaning period had no influence on specific activities of some of the enzymes of carbohydrate metabolism such as adolase and NADP isocitrate dehydrogenase in the brain (Chase et al, 1976).

This may further reinforce the present observation that brain glycolysis and energy metabolism were spared during postweaning undernutrition, though there were significant decrease in both brain and body weights. Thus brain function is spared even in severe undernutrition if the undernutrition is restricted only to the postweaning period (Dyson and Jones, 1976; Karlsson and Svennerholm, 1978; Resnick and Morgane, 1984). This assumes much significance in the light of the observation that restriction of energy intake may considerably extend the lifespan of humans (Nutr. Rev., 1982; Harper, 1982).

EXPERIMENT-IV : Severe protein deficiency in the postweaning period.

In the earlier experiment it was found that a moderate protein deficiency in the postweaning period failed to elicit any adverse effects on energy metabolism of brain. The present study is undertaken to investigate the effect of very severe dietary protein deficiency on energy metabolism of brain in the immediate postweaning period.

Kwashiorkor is specifically a protein deficiency syndrome, which occurs after weaning when the infant no longer receives a nutritionally balanced supply of maternal milk and no supplementary balanced diet is provided (Dyson and Jones, 1976a). In many countries of the world infants are raised on milk alone only for a few months. During the second half of the first year of life the milk supply may be reduced and some other food may be introduced into the diet. It is at this time that protein malnutrition generally makes its appearance (Bhattacharya, 1986). This is because the vegetable foods utilized in so many of the developing

countries provide a poor source of protein than does milk. The inferior quality and poor digestibility of the protein seems to be responsible for the genesis of protein energy malnutrition (Dodge et al, 1975a).

Epidemological data suggest that the diets of children at risk of kwashiorkor or marasmus are poor both in energy and protein. According to some authors energy deficiency is more important than protein deficiency in the diet of preschool children who are at risk of developing protein energy malnutriton (Srikantia, 1969; McLaren, 1974). The dietary differentiation between kwashiorkor and marasmus is therefore far from clear. The more common form of protein energy malnutrition is the one where protein malnutrition is combined with energy deficiency (Bhattacharya, 1986).

Some of the clinical and biochemical manifestations of kwashiorkor and marasmus have been reproduced in animals such as monkey and rats. Use of such experimental models does clarify the pathophysiology of kwashiorkor and marasmus. But these models may not be strictly comparable to human disease. The dietary factor has got all attention, but the important environmental factors have been ignored (Bhattacharya, 1986).

Though the more common form of protein energy malnutrition is the one where protein malnutrition is combined with energy deficiency. Incidentally it has been viewed that the major bottleneck in the diet of an Indian preschool child is energy rather than protein (Bhattacharya, 1986). Casava (Manihot Esculenta) is an important agricultural product of tropical Africa and Brazil. It is an important cheap source of energy for the millions. Consumption of casava has been associated with kwashiorkor because of the very low protein content (Rutkowski et al, 1985; Morales and Graham, 1987).

Weaned rats were given diet containing different levels of protein. They are protein free diet i.e. 0%, 2%, 5%, 10% and 20% protein diets. Severe deficiency in the dietary protein causes increased body protein catabolism, resulting in negative growth. It was already reported that a diet severely deficient in protein induces accelerated catabolism of body protein. This results in a negative growth rate in animals in the immediate postweaning period (Rajalakshmi et al, 1974c). However, 4% protein in the diet is found to be adequate for maintaining the body weight (Miller and Payne, 1961).

TABLE-34 : Effect of different degrees of postweaning protein deficiency on d body and brain weight.

	Percentage of protein in the diet				
	0	2	5	10	20
Body weight (g)					
Initial	44 ± 0.53	43 ± 0.71	44 ± 0.65	44 ± 0.52	43 ± 0.65
Final	30 ± 0.80***	32 ± 0.74***	60 ± 0.91***	93 ± 1.14***	121 ± 0.89
Percentage of control	24.7	26.4	49.5	76.8	100
Total weight gain or less	- 14	- 11	16	49	78
Weight gain or loss per week	- 2.8	- 2.2	3.2	9.8	15.6
Brain weight (g)	1.25***±0.011	1.33***±0.012	1.46**±0.050	1.59±0.010	1.61±0.017
Percentage of control	77.6	82.6	90.6	98.7	100
$\frac{\text{Brain weight}}{\text{Body weight}} \times 100$	4.16	4.15	2.43	1.70	1.33

Period of treatment 5 weeks.
8 animals were used in each group.
Values marked with asterisk are significantly different from control,
'p' less than 0.02 for ** and 0.001 for ***.

As expected, at the end of the experiment animals fed protein free diet registered a negative growth and had a body weight approximately 25% of the control animals. Further analysis of the data on Table-34 reveals that even 2% protein fed animals registered a negative growth rate and had only 26% of the body weight of the control at the end of the experiment. Animals fed 5% protein diet approximately had 50% body weight of the control. Apparently there is some difference in the body weight of 5% protein fed animals between the present experiment and experiment-II when expressed as percentage of control. However, in absolute terms it is much less. This may be because of a difference in the duration of these two experiments and slight difference in the growth rate of control animals in the two experiments. The experiment could not be prolonged beyond 5 weeks as animals of the severely protein deficient group started dyeing.

Brain weight was found to be significantly reduced in the severely protein malnourished animals. Thus animals fed protein free diet registered a 22% decrease in brain weight and 2% protein fed animals had a decreased brain weight by 17% while 5% protein

TABLE-35 : Effect of different degrees of postweaning protein deficiency on food intake and its utilization.

	Percentage of dietary protein				
	0	2	5	10	20
Terminal body weight(g)	30 ± 0.80	32 ± 0.74	60 ± 0.91	93 ± 1.14	121 ± 0.89
Food intake (g)					
g/day/week	3.4 ± 0.28	3.9 ± 0.18	5.8 ± 0.21	7.2 ± 0.44	7.9 ± 0.85
g/100 body wt.	10.8	10.2	9.8	9.2	8.1
Weight gain or loss					
per g food	- 0.117	- 0.080	0.078	0.194	0.282
per 100 K.Cal	- 3.07	- 2.10	2.05	5.10	7.42

Period of treatment 5 weeks

8 animals were used in each group.

TABLE-36 : Effect of different degrees of postweaning protein deficiency on enzymes of glycolysis in brain.

Enzyme (units/g)	Percentage of dietary protein				
	0	2	5	10	20
Hexokinase	8.9 ± 0.22	9.1 ± 0.39	8.6 ± 0.37	9.7 ± 0.43	9.3 ± 0.47
Phosphofructokinase	13.8 ± 0.49	14.8 ± 0.48	14.4 ± 0.66	15.4 ± 0.68	15.1 ± 0.71
Fructose 16 diphosphate aldolase	8.5 ± 0.23	8.3 ± 0.32	8.7 ± 0.35	9.0 ± 0.43	9.2 ± 0.43
Pyruvate kinase	164 ± 3.97	161 ± 4.14	168 ± 4.28	167 ± 4.44	171 ± 3.45
Lactate dehydrogenase	100 ± 3.94	98 ± 2.74	92 ± 1.88	95 ± 4.15	93 ± 2.67

Period of treatment 5 weeks.

8 animals were used in each group.

TABLE-37 : Effect of different degrees of postweaning protein deficiency on enzymes of Krebs cycle in brain.

Enzyme (units/g)	Percentage of dietary protein				
	0	2	5	10	20
Pyruvate dehydrogenase	1.13 ± 0.038***	1.61 ± 0.048	1.56 ± 0.049	1.45 ± 0.061	1.62 ± 0.065
Isocitrate dehydrogenase (NADP)	1.97*** ± 0.070	2.18 ± 0.078**	2.56 ± 0.075	2.47 ± 0.50	2.52 ± 0.069
Succinate dehydrogenase	1.06 ± 0.041***	1.23 ± 0.071**	1.39 ± 0.063	1.48 ± 0.058	1.54 ± 0.057
Malic enzyme (NADP)	1.27 ± 0.036**	1.47 ± 0.055	1.61 ± 0.069	1.51 ± 0.083	1.56 ± 0.070

Period of treatment 5 weeks.

8 animals were used in each experiment.

Values marked with asterisk is significantly different from control.

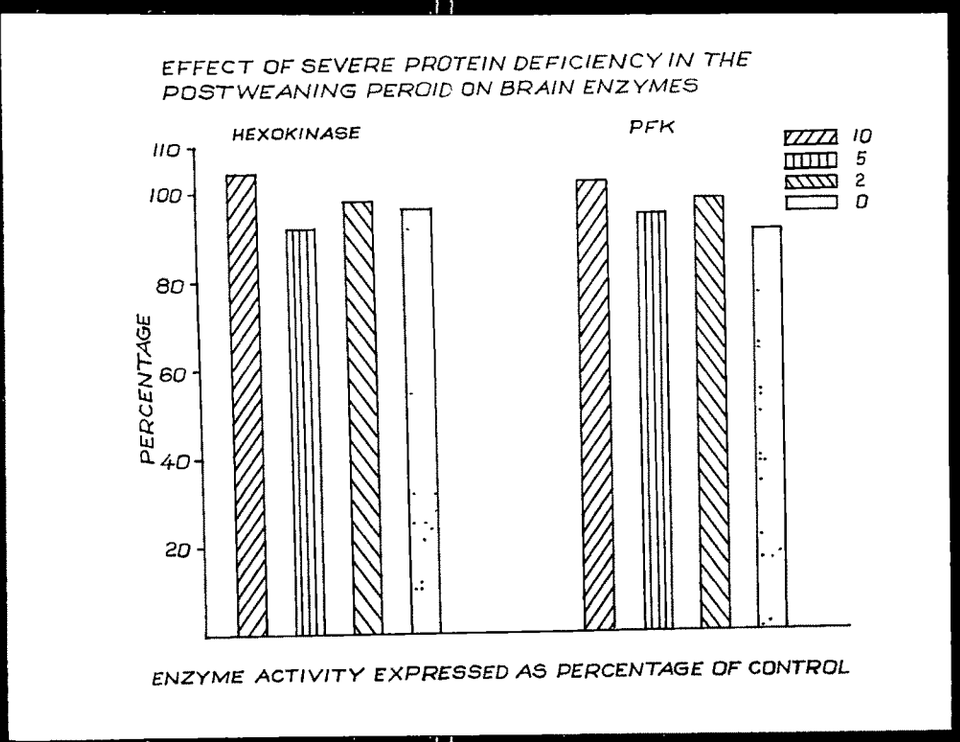
'p' less than 0.01 for ** and 0.001 for ***.

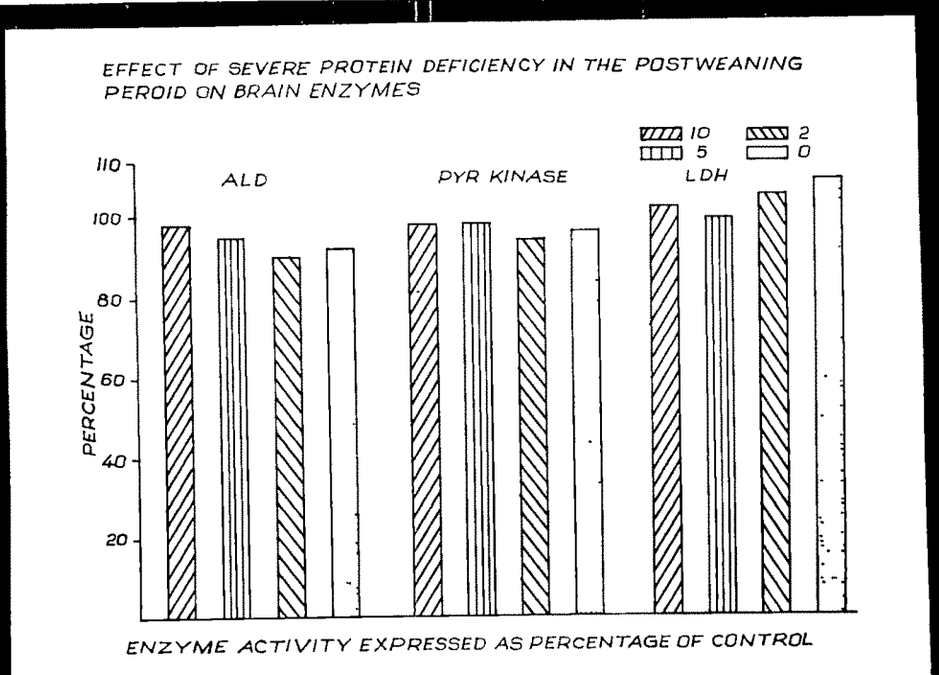
fed animals showed only 10% decrease in brain weight (Table-34). It is worth recalling that Brown (1966) and Engsner et al. (1974) reported significantly low brain weight in malnourished Ugandan children.

It is interesting to note that the brain weight of animals fed 10% protein diet was in par with the brain weight of control animals. Thus 10% protein diet might be sufficient to prevent retardation of brain growth. Food intake (Table-35) when expressed per unit body weight was found to be much higher in severely protein deficient animals. This once again confirms the earlier observation that energy is poorly utilized in protein deficiency (Crist et al, 1980; Donato, 1987). The apparent value for energy cost for protein deposition increases with reduction in dietary protein content and at low energy intakes (Coyer et al, 1985). Hence the rate of growth of low protein fed animals shall be much lower compared to control animals.

Data on activities of enzymes of glycolysis and Krebs cycle are given in Table-36 & 37 respectively. It is surprising to note that none of the enzymes of glycolysis were affected by protein deprivation or in severe protein deficiency in the diet. On the contrary,

Fig.3 : Effect of severe protein deficiency in the postweaning period on brain enzymes (enzymes of glycolysis).





Hexokinase; PFK = Phosphofructokinase;

ALD = Aldolase; Pyr. Kinase = Pyruvate Kinase.

LDH = Lactate dehydrogenase

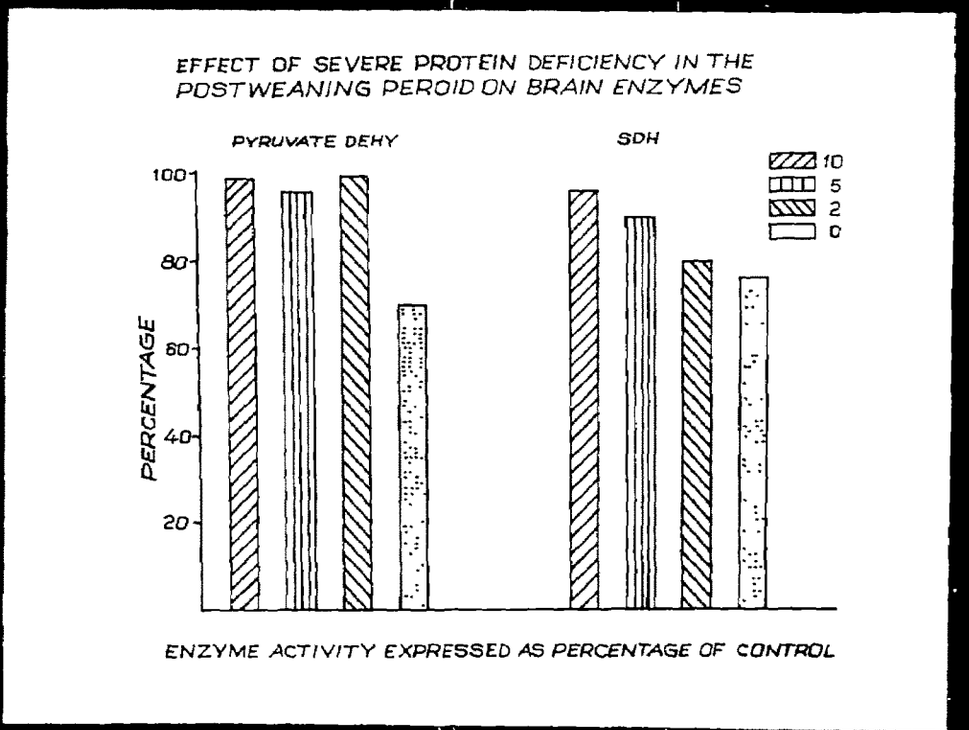
Figures represent the percentage of protein in the diet.

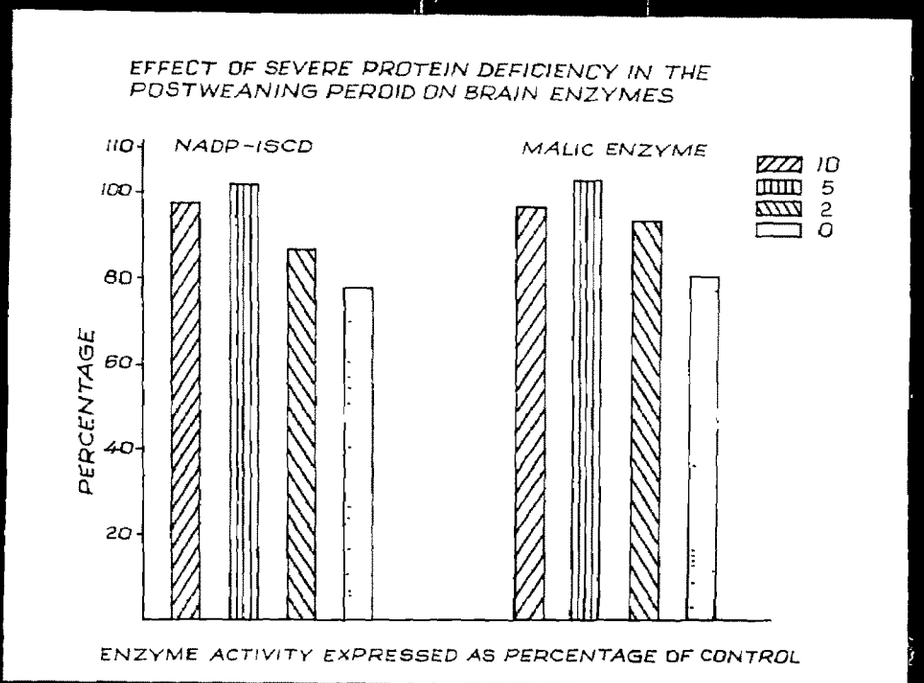
'0' means that the animals belonging to this group were fed a

though not significant, there was an increase in the activity of lactate dehydrogenase approximately (Fig. 3) by 8% in the protein deprived group. This may be indicative of an increased lactic acid production in the brain of protein deprived animals. Lactic acid can function as a source of energy for the brain (Williamson, 1987).

Hypoglycemia is often associated with kwashiorkor and other forms of severe malnutrition in children (Wapnir and Lifshitz, 1977). Hypoglycemia in kwashiorkor perhaps may be due to low circulating alanine or glucagon level (Buchanan et al, 1976). Besides, intestinal atrophy often observed in kwashiorkor diminishes the capacity to absorb carbohydrates (Zeman and Fratzke, 1977). However, blood glucose level cannot be considered as an index of brain function. The turnover of glucose in brain is influenced by the functional and physiological state of animal. Anesthesia, decreases glucose utilization throughout the brain, without lowering blood glucose level and therefore also tissue level of glucose (Savaki et al, 1980). Similarly Thurston (1976) observed dangerously low glucose level in brain in experimental analogus situation to anoxia and salicylate poisoning despite a normal blood glucose level.

Fig. 4 : Effect of severe protein deficiency in the postweaning period on brain enzymes. (enzyme of Krebs cycle).





Pyr. Dehy.	=	Pyruvate dehydrogenase.
SDH	=	Succinate dehydrogenase
NADP-ISCD	=	Isocitrate dehydrogenase (NADP)
Malic enzyme	=	Malic enzyme - (NADP)

Figures represent the percentage of protein in the diet.

There was a significant reduction in the activity of pyruvate dehydrogenase and succinate dehydrogenase in the brain of protein malnourished animals (0% and 2% protein diet). A substantial reduction in the activity of pyruvate dehydrogenase (30%) and succinate dehydrogenase (24%) (Fig. 4) may be an indication of decreased rate of oxidative metabolism in the brain of animals fed a protein free diet. This is in agreement with the observation of Mehta et al. (1977) on the metabolism of brain in kwashiorkor children. An uninterrupted glycolysis may be an adaptive mechanism of the brain in order to satisfy the energy needs for more fundamental functions of brain. Decreased energy metabolism indicates a substantial reduction in the synthesis of high energy compounds. As neuronal transmission demands a large share of ATP synthesized in the brain (Yarowsky and Ingwar, 1981), a decreased oxidative metabolism might perhaps reduce the rate of neuronal transmission in protein calorie malnutrition. This perhaps may be one of the basis for the poor memory and learning abilities observed in protein calorie malnutrition (Cravioto et al, 1976; Gabr, 1987). A decreased neuronal function is observed in developmental protein deficiency (Stern et al, 1983).

Though pyruvate dehydrogenase catalyses the flux generating step in Krebs cycle, it is surprising to note that its activity in brain is considerably lower than that of any of the glycolytic enzymes (Hawkins and Mans, 1983). Brain metabolism is almost totally dependent on glucose, and pyruvate dehydrogenase activity in brain appears to be only marginally sufficient to maintain normal pyruvate flux through oxidative pathway (Browning et al, 1981; Reding et al, 1982).

Potassium ion exert profound influence on brain metabolism. A major effect is to increase velocity of formation of acetyl CoA from pyruvate (Kini, 1959). It is worth mentioning that there is severe loss of body potassium in kwashiorkor, a large share of which being from brain (Garrow, 1976). The availability of acetyl CoA from glucose is an important factor which might affect synthesis of acetyl choline in cholinergic nerve terminals (Jope, 1979; Ghajak et al, 1985; Kalria and Prince, 1985). In view of these, it may not be possible to rule out a decreased synthesis of acetyl choline in brain of severely protein deficient animals due to significant reduction in the activity of pyruvate dehydrogenase. However, in Alzhaimers disease there is no correlation between glucose metabolism and decreased

acetyl choline level (Sims et al, 1980). The direct inhibition of pyruvate dehydrogenase complex by α-keto acids is an important mechanism leading to brain damage in phenylketoneuria (Blass and Lewis, 1973).

Increased level of biogenic amines are reported in the brain of protein malnourished animals. The abnormal behaviour of animals in severe protein deficiency may be due to the accumulation of these amines (Sobotka et al, 1974; Stern et al, 1975). One such amine is histamine whose concentration in brain is elevated in protein malnutrition (Enwonwu and Okolie , 1983). As most of the neurotransmitter substances are nitrogenous substances derived from dietary protein or amino acids, dietary protein has an influence on the synthesis of these substances (Lovenberg, 1986). Several observations suggest that deficiencies of protein and calories have different effects on brain development (Simons and Johnston, 1976).

Indeed it may be so. Brain could adapt to severe calorie restriction in the postweaning period, while in severe protein deficiency energy metabolism of brain was found to be deranged. A significant reduction of succinate dehydrogenase denotes a reduced mitochondrial energy metabolism.

NADP-isocitrate dehydrogenase and NADP-malate dehydrogenase (malic enzyme) are involved in the CO_2 fixation reaction in the brain. However, under physiological conditions these enzymes contribute very little to the total CO_2 fixation in the brain (Hawkins and Mans, 1983). They are more important sources of the reduced coenzymes - NADPH. It is significant from the fact that there is low turnover of hexosemonophosphate shunt in adult brain (Hawkins and Mans, 1983).

NADP-isocitrate dehydrogenase is associated with the conversion of glucose carbon into amino acids in cytosol and hence in protein synthesis (Loverde and Lehrer, 1973). It is observed that protein deficiency leads to reduced protein synthesis through a reduction in the brain levels of key amino acids (Nowak and Munro, 1977). Both the these enzymes are responsible for NADPH generation in the adult brain. They were significantly decreased in the brain of protein derived animals. However, in severe protein deficient animals (2% protein diet) only NADP-isocitrate dehydrogenase was significantly reduced. Thus the activity of NADP-isocitrate dehydrogenase was decreased by 22% and 13% in the brain of animals fed 0% and 2% protein diets respectively while malic enzyme was reduced by 19% in animals fed protein free diet (Fig. 4).

It is recognized that a child experiences more from mothers responses. One of the first effects of malnutrition is a reduction in the childs responsiveness to stimulation and the emergence of various degree of apathy. This apathy, can have consequences on stimulation, learning and interpersonal relations and the end result being poor quality of performance on later more complex learning tasks. It is apparent that children who survive a severe episode of malnutrition of sufficient duration early in life are handicapped in learning some of the more fundamental academic skills and are therefore less able to profit from school. The child who lags in the performance of basic mechanism related to fundamental skills such as reading and writing will be ill prepared for the learning tasks required by him when he enters school (Cravioto and Delicardie, 1973).

The role of nutrition in brain function is amply demonstrated recently by Lytle et al. (1984). Malnutrition constitutes a costly drain on national economy. These are direct cost for treatment and rehabilitation, while indirect costs due to poor physical performance, learning abilities, behavioral changes and negative effect on family planning

(Gabr, 1987). It is to be taken into account that the promotion of good intelligence and emotional development does not depend on providing good nutrition alone, but many environmental factors which encourage or inhibit the childs advancement (Dobbing, 1986).

EXPERIMENT-V : Chronic protein deficiency in the adult.

It was observed in the earlier experiment that matured brain is capable of successfully resisting a moderate protein deficiency. This experiment was designed in an apparent bid to evaluate the effect of chronic protein deficiency in adult age. There is considerable increase in the proportion of the aged in the general population (Harper, 1982). Unfortunately nutritional studies on the aged are much limited, eventhough signs of nutritional inadequacy are more common in the elderly. The aging process involves progressive changes in various organs leading to decreased functional ability (Young et al, 1976).

Data on body and brain weights are given in Table-38. In comparison to the rate of body growth of the animals in the postweaning experiment (Table-26) growth rate is much less in the adult animals. Though the rate of body growth is less, the difference between moderately low protein and high protein diet still persists, even in the adult age. In contrast to postweaning protein deficiency (Table-26) moderate

TABLE-38 : Effect of chronic protein deficiency in adult age on body and brain weight.

	LP	HP
Body weight (g)		
Initial	199 ± 4.37	206 ± 3.07
Final	225 ± 3.37***	389 ± 4.07
Percentage of control	57.8	
Total body weight gain (g)	26 ± 2.07	183 ± 2.96
Weight gain per week	0.74	5.2
Brain weight (g)	1.54 ± 0.06	1.63 ± 0.06
Percentage of control	94.4	100
$\frac{\text{Brain weight}}{\text{Body weight}} \times 100$	0.68	0.41

LP & HP animals were fed a diet containing 5% and 20% protein respectively.

Period of treatment 35 weeks.

8 animals were used in each group.

TABLE-39 : Effect of chronic protein deficiency in adult age-on food intake and its utilization.

	LP	HP
Terminal body weight (g)	225 ± 3.37	389 ± 4.07
Food intake (g)		
g/day/week	8.6 ± 0.39	12.4 ± 0.38
g/100 body weight	3.82	3.18
Weight gain (g)		
per g food	0.012	0.060
per 100 K.Cal.	0.32	1.57

LP = Low Protein fed animal 5%

HP = High Protein fed animal 20%

Period of treatment 35 weeks.

8 animals were used in each group.

protein deficiency during adult age had no effect on brain weight. Low protein fed animals had a brain weight as much as 94% of the control (Table-38). This perhaps may be because the brain has almost attained its maximum growth at the time when the experiment started. However, body growth still continue, though at a slow pace.

In comparison with animals of the postweaning period, food intake is much less in the adult animals when expressed per unit body weight (Table-39 Vs Table-27). One of the most important body function that control calorie intake is basal metabolic rate. Basal metabolic rate is found to be reduced with age (Barrows and Kokkonen, 1977). Thus a low energy requirement in an advancing age (Buskirk and Mendez, 1980) may explain decreased food intake of animals in the present experiment.

As age advances the total body protein mass declines with age and also the net body protein synthesis (Young et al, 1976; Clifford, 1980). This explains the basis for a possible decline in protein and amino acid requirements of the body as age advances. Though a moderately protein deficient

diet retard the body growth in adult age, the retardation is not as much as that observed in the postweaning protein deficiency (Table-38 Vs. Table-26). The low protein fed animals weigh as much as 58% of the control though after a prolonged period of protein deficiency. While in moderate protein deficiency in the postweaning period, the protein deficient animals weighed only 38% of the control. This dispropionate effect in the gain in body weight in experimental animals may be due to a low protein requirement in the adult age.

It has been shown that dietary protein requirement decrease with aging. There was no difference in the rate of liver protein synthesis between a low protein fed (5% casein) and high protein fed (25% casein) animal. However, ^{14}C-orotic acid incorporation into liver nuclear RNA was less in 3.5% casein fed rats. A comparison of the result with young rats the 3.5% casein fed old rats produced biochemical changes in liver that are qualitatively and quantitatively similar to ones observed in young adult rats fed 6% casein diet which allows a more direct comparison of the effect of protein deficiency in the aged rats (Rozovski and Temkin, 1984). Adult humans are found to successfully combat acute dietary protein deficiency

TABLE-40 : Effect of chronic protein deficiency in adult age on enzymes of glycolysis in brain.

Enzyme (units/g)	LP	HP
Hexokinase	8.7 ± 0.31	9.3 ± 0.41
Phosphofructokinase	13.9 ± 0.10	13.0 ± 0.38
Fructose-16-diphosphate aldolase	8.3 ± 0.26	7.8 ± 0.19
Pyruvate kinase	162.9 ± 4.19	172.8 ± 3.70
Lactate dehydrogenase	76 ± 3.27	80 ± 3.37

LP = Low Protein fed animals 5%

HP = High Protein fed animals 20%

Period of treatment 35 weeks

8 animals were used in each group.

TABLE-41 : Effect of chronic protein deficiency in adult age on enzymes of Krebs cycle in brain.

	LP	HP
Pyruvate dehydrogenase	1.67 ± 0.070	1.57 ± 0.063
Isocitrate dehydrogenase - NADP	2.63 ± 0.063	2.22 ± 0.048
Succinate dehydrogenase	NE	NE
Malate dehydrogenase - NADP (Malic enzyme)	1.45 ± 0.080	1.36 ± 0.072

LP = Low Protein fed animals 5%

HP = High protein fed animals 20%

Period of treatment 35 weeks

8 animals weere used in each group.

by an efficient reutilization of endogenous amino acids (Steffe et al, 1976).

Unlike that of the immediate postweaning protein deficiency, adult age protein deficiency though chronic in nature did not make any effect on brain weight. Analysis of the data on Table-40 & 41 shows that a chronic protein deficiency in the adult age did not make any dent in the activities of the enzymes of glycolysis and Krebs cycle. However, a careful analysis shows that (Table-40) all enzymes except hexokinase have registered 10-15% decrease in the activity. Age related changes in the metabolism of brain is well known. Zubairu et al.(1983) have reported 20% decrease in the formation of CO_2 through glycolysis and Krebs cycle in the brain of older rats. However, among the enzymes of energy cycle pyruvate dehydrogenase remained unchanged. This is supported by the finding of Zubairu et al. (1983).

Growing evidence indicate a decrease in brain energy metabolism with age. Evidence based on biochemical study clearly indicate a progressive decline in cerebral adaptability to metabolic demand

with age (Sylvia and Rosenthal, 1979). Age related changes in cerebral glucose metabolism was found to be regionally selective. Some of the regions such as striatum and inferior colliculus showed significant reduction in glucose oxidation with age, while structures like thalamus and hypothalamus did not show any change with age (London et al, 1981). Similarly rate of protein synthesis in brain also decreased with age (Ingwar et al, 1985).

During the growth and development, the pattern of glucose metabolism changes both in terms of enzyme profile (Elhasan et al, 1981; Leong et al, 1981) and the proportioning of glucose among the different pathways of its metabolism (Baquer et al, 1977). Age related changes have been reported to occur in the rate of glucose utilization and oxygen consumption (Smith et al, 1980). However, there is a small decline in the activity of malic enzyme and NADP-isocitrate dehydrogenase which may be hinting decreased production of NADPH. This is important from the point of a markedly low hexose monophosphate shunt in adult brain (Zubairu et al, 1983). These authors also observed a decline in NAD-isocitrate dehydrogenase with advancing age with a decline in the glucose influx. All these changes may point to the triggering of senile regression of old age.

EXPERIMENT-VI : Postweaning deficiency of vitamin A.

Hypovitaminosis A has been considered as a widespread public health problem among children of socio-economically deprived families in many parts of the world (Favaro et al, 1986). An important mode of transfer of vitamin A from mother to offspring is through breastmilk. Poor vitamin A content in the maternal milk is found among the malnourished mothers of the developing countries (Gabr, 1981; Davila et al, 1985). Low birth weight infants are found to have low liver storage of the vitamin (Woodruff et al, 1986). Low birth weight is more among the poor of the developing countries (Rajalakshmi, 1981). Hence there is more likelihood that vitamin A deficiency may be more among the underprivileged children in the poor communities of the developing countries (Nutr. Rev., 1985). The vulnerability of young children to the vitamin deficiency shortly after weaning is widespread in many of the Asian countries (Cohen et al, 1987).

Though in smaller quantity, vitamin A is transported through the blood brain barrier (Padrige et al, 1984). The presence of cellular binding protein

TABLE-42 : Effect of postweaning vitamin A deficiency on body and brain weight.

	Percentage protein in the diet			
	LP + Vit A	LP - Vit A	HP + Vit A	HP - Vit A
Body weight (g)				
Initial	43 ± 0.46	43 ± 0.46	43 ± 0.69	42 ± 0.44
Final	75 ± 1.02	65 ± 1.04***	162 ± 1.65	88 ± 0.99***
Percentage of control	100	86.6	100	54.3
Total weight gain (g)	32	22	119	46
Weight gain per week	3.5	2.4	13.2	5.1
Brain weight (g)	1.49 ± 0.02	1.47 ± 0.021	1.68 ± 0.022	1.48 ± 0.025***
Percentage of control		98		88
$\frac{\text{Brain weight}}{\text{Body weight}} \times 100$	1.98	2.26	1.93	1.68
Liver vitamin A	72.5 ± 2.93	28.2 ± 1.87***	48.6 ± 1.92	3.8 ± 0.63***

LP + Vit A = Low protein diet with vitamin A
HP + Vit A = High protein diet with vitamin A
LP - Vit A = Low protein diet without vitamin A
HP - Vit A = High Protein diet without vitamin A

Period of treatment 9 weeks

8 animals were used in each group.
*** for $p' < 0.001$.

which is responsible for the transport of the vitamin into the cell in brain is reported by various authors (Adachi et al, 1981; Nutr. Rev., 1985a, Kato et al, 1985). The vitamin plays an important role in the maintenance of structural and functional integrity of biological membranes (Roles, 1969; Ram and Misra, 1978). The vitamin has a strong affinity to membrane and may be acting as cross-linking agent between lipid and protein of the membrane. Vitamin A in membrane can alter the surface charge of the membrane and change the confirmation of membrane bound enzymes. This in turn may affect transport of nutrients, ions and water through membrane and can influence cellular metabolism. The vitamin has an important role in supporting the catalytically active confirmation of membrane bound enzymes (Mack et al, 1972). However, it is unfortunate that the mechanism of action of the vitamin other than on vision has yet to be identified (Sklan, 1987).

Body and brain weight of animals fed low protein and high protein diets with and without supplementing the vitamin are given in Table-42. Though the rate of growth is decreased in the deprivation of vitamin A in both protein deficiency and sufficiency, the degree of arrest of growth in vitamin deficiency

is more among the high protein fed animals. One of the most important physiological function of vitamin A is to promote growth. One of the symptom of its deficiency is retarded growth (Hayes, 1971; Wolf, 1980). There is a close relationship between vitamin A and protein in the diet. A high protein diet increases rate of growth and hence the greater demand for the vitamin. This was amply demonstrated earlier (McLaren, 1959; Srikantia,1975). Perhaps this may be the reason for the severe retardation of growth observed in the high protein fed animals. Thus vitamin A deficient high protein fed animals weighed only 54% of the control while its counterpart in the low protein fed group weighed almost 87% of the control (Table-42). Above all the inefficient utilization of dietary protein in vitamin A deficiency is yet another factor for the retarded growth of animals (Hayes,1971).

Data on brain weight (Table-42) shows that there is no difference in the brain weight in the vitamin deficiency in the low protein fed animals. However, there was a significant decrease in brain weight in the vitamin A deficient animals among the high protein fed animals. The deficiency symptom of the vitamin appear much early in the high protein fed animals due to greater demand for the same. There was 12% decrease in

brain weight of animals fed vitamin A deficient high protein diet compared to the control animals. Significant decrease in brain weight has been observed earlier (Bhatt and Rama Rao, 1978; Sharma and Misra, 1986) in maternal vitamin A deficiency.

As liver represents a larger share of the vitamin store in the body, liver vitamin A store is a better index to assess the vitamin A status of the body (Underwood et al, 1979; Goodman and Blaner, 1984; Amedee-Manesme et al, 1987). As expected, vitamin A deficiency in the high protein fed animals caused a virtual depletion of the vitamin from liver (Table-42). The present result follows the pattern of liver vitamin A in children by Zaklama et al. (1972) and the pattern discussed by Wolf (1980) and Young and Mitchell (1986). The poor liver storage very well correlates with the clinical symptoms of the deficiency observed in these animals fed high protein diet. However, the animals fed low protein diet free of vitamin A still had substantial amount of liver storage of the vitamin. This may be the reason why these animals did not show any clinical symptoms of the vitamin deficiency. Owing to a poor growth rate their vitamin requirement will be much less in comparison to that of high protein

TABLE-43 : Effect of postweaning vitamin A deficiency on food intake and its utilization.

	LP + Vit A	LP - Vit A	HP + Vit A	HP - Vit A
Terminal body weight	75 ± 1.02	65 ± 1.04	162 ± 1.65	88 ± 0.99
Food intake (g)				
g/day/week	5.7 ± 0.19	5.4 ± 0.16	9.0 ± 0.72	6.3 ± 0.55
per 100g body weight	7.8	8.9	6.8	7.3
Weight gain (g)				
per g food	0.087	0.063	0.21	0.12
per 100 K.Cal.	2.30	1.66	5.52	3.15

LP + Vit A = Low protein diet with vitamin A
HP + Vit A = High protein diet with vitamin A
LP - Vit A = Low protein diet without vitamin A
HP - Vit A = High protein diet without vitamin A

Period of treatment 9 weeks

8 animals were used in each group.

TABLE-44 : Effect of postweaning vitamin A deficiency on enzymes of glycolysis and HMP shunt in brain.

Enzymes (units/g)	LP + Vit.A	LP-Vit.A	HP + Vit.A	HP - Vit.A
Hexokinase	8.8 ± 0.31	9.0 ± 0.38	9.5 ± 0.37	9.1 ± 0.31
Glucose-6-phosphate dehydrogenase	2.77 ± 0.102	2.90 ± 0.14	2.84 ± 0.10	2.57 ± 0.12
Phosphofructokinase	14.2 ± 0.55	13.5 ± 0.53	14.7 ± 0.60	10.6 ± 0.32***
Fructose-1,6-diphosphate aldolase	8.8 ± 0.28	9.0 ± 0.30	9.6 ± 0.45	7.5 ± 0.21***
Pyruvate kinase	159 ± 7.53	156 ± 5.33	168 ± 5.23	161 ± 4.41
Lactate dehydrogenase	92 ± 2.64	90 ± 3.16	98 ± 3.88	88 ± 2.96

Period of treatment 9 weeks.

8 animals were used in each experiment.

Values marked with asterisk are significantly different from control.

'p' less than 0.001 for ***.

LP + Vit.A = Low Protein Diet with Vitamin A

HP + Vit.A = High Protein Diet with Vitamin A

LP - Vit.A = Low Protein Diet without Vitamin A

HP - Vit.A = High Protein Diet without Vitamin A.

TABLE-45 : Effect of postweaning vitamin A deficiency on enzymes of Krebs cycle in brain.

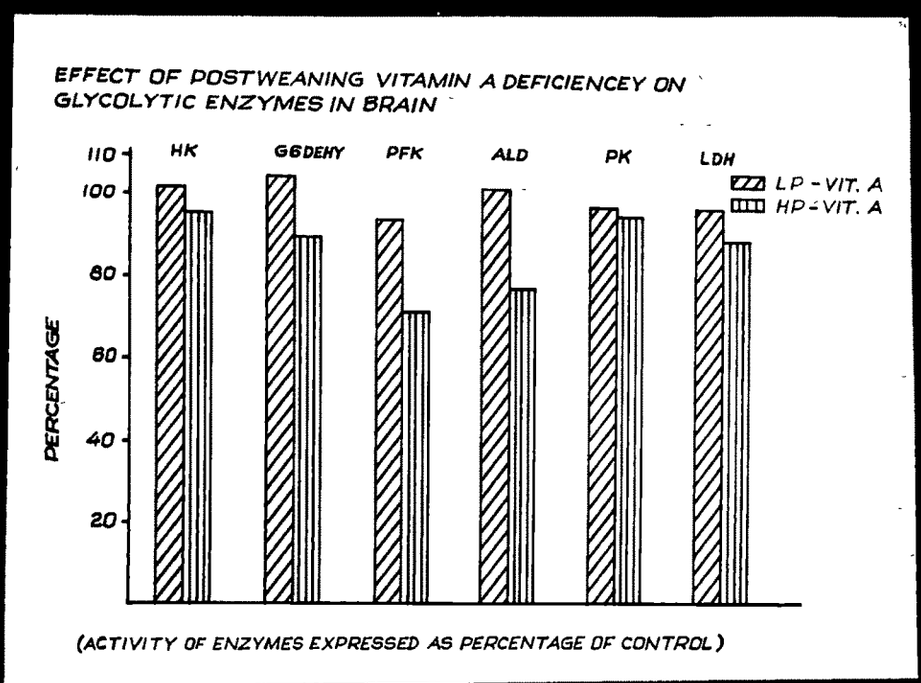
Enzymes (units/g)	LP + Vit A	LP - Vit A	HP + Vit A	HP - Vit A
Pyruvate dehydrogenase	1.45 ± 0.058	1.52 ± 0.044	1.61 ± 0.055	1.57 ± 0.037
Isocitrate dehydrogenase - NADP	2.57 ± 0.078	2.46 ± 0.085	2.52 ± 0.059	2.66 ± 0.068
Succinate dehydrogenase	1.38 ± 0.048	1.43 ± 0.060	1.34 ± 0.050	1.44 ± 0.052
Malate dehydrogenase - NADP (Malic enzyme)	1.55 ± 0.085	1.47 ± 0.070	1.62 ± 0.078	1.44 ± 0.064

LP + Vit A = Low protein diet with vitamin A
HP + Vit A = High protein diet with vitamin A
LP - Vit A = Low protein diet without vitamin A
HP - Vit A = High protein diet without vitamin A

Period of treatment 9 weeks.

8 animals were used in each experiment.

Fig. 5 : Effect of postweaning vitamin A deficiency on glycolytic enzymes in brain.



HK = Hexokinase; G6P DEHY = Glucose-6-phosphate dehydrogenase.

PFK = Phosphofructokinase

ALD = Aldolase; PK = Pyruvate Kinase.

LDH = Lactate dehydrogenase.

LP-Vit A = low protein diet (5% protein) free of vitamin A.

HP-Vit A = High protein diet (20% protein) free of vitamin A.

fed animals. This is in agreement with the field studies reported by Srikantia (1975) and Wolf (1980). High protein fed vitamin deficient animals started loosing weight and showed ocular symptoms of the vitamin deficiency towards the end of the experiment. It may be worth recalling that many proteins in the body are stabilised by the vitamin and deteriorates in its absence (Arroyave, 1969).

Data on brain enzymes of glycolysis and energy metabolism are presented in Tables-44 & 45, respectively. Among the enzymes of glycolysis, activities of phosphofructokinase and aldolase were significantly decreased in the high protein fed vitamin deprived animals. Brain of the vitamin deficient animal registered 28% decrease in phosphofructokinase and 22% decrease in aldolase (Fig. 5). Other enzymes of glycolysis such as hexokinase, pyruvate kinase and lactate dehydrogenase did not show any significant change. However, there was no change in any of the glycolytic enzymes in the brain of low protein fed vitamin deprived animals. A near normal activity of glycolytic enzymes in the brain of low protein fed vitamin deficient animals should not be interpreted that a low protein diet prevent the onset of symptoms of vitamin A deficiency. It only delays the process of deficiency symptoms. It should be

remembered that a vast majority of children who show clinical symptoms of the vitamin are protein malnourished too.

Though there were no pair-fed control for the vitamin A deficient animals, it was observed in the earlier experiment (Experiment-IV) that severe calorie restriction has no effect on enzymes of glycolysis (Table-32). Thus, a low food intake (Table-43) among the vitamin A deficient animals cannot be a contributing factor for the decreased activity of the enzymes observed.

Contrary to enzymes of glycolysis, enzymes of energy metabolism was spared in vitamin A deficiency (Table-45). Lead poisoning was found to decrease aldolase activity in the brain of protein deficient rats (Wapnir et al, 1979). Vitamin A deficient was found to inhibit glycolysis in the liver (Phillips and Nockels, 1970). Phosphofructokinase is one of the regulatory enzymes of glycolysis in brain (Lajtha et al, 1981). It seems that under physiological conditions brain glucose utilization is limited by glucose flux through phosphofructokinase (Dwyer and Wasterlain, 1985). Glucose-6-phosphate dehydrogenase, a representative enzyme of hexosemonophosphate shunt was not

affected by vitamin A deficiency. Aldolase holds a rather unique position in the glycolytic pathway of brain. This enzyme has substantially lower maximal catalytic capacity, a characteristic generally associated with rate limiting enzymes (Coupain et al, 1977).

Vitamin A deficiency causes lesions of central nervous system (Wolf, 1980). The present result is indicative of an impaired glycolytic pathway in brain during vitamin A deficiency. This may perhaps explain a retarded deposition of myelin lipids observed in vitamin A deficiency (Bhatt and Rama Rao, 1978; Joshi et al, 1982). Vitamin A deficiency has been reported to change the chemical (Dodge et al, 1975c) as well as anatomical (Hayes et al, 1971; Wolf, 1980) make up of brain. Similarly Nakhasi et al. (1977) observed a significantly decreased activity of 2',3'-cyclic nucleotide-3-phosphohydrolase (CNP) in the brain of vitamin A deficient rats. Cyclic nucleotide phosphohydrolase is a marker for the myelin formation in the developing brain (Norton, 1981; Wiggins, 1982). Thus in the absence of any change in the enzymes such as Glucose-6-phosphate dehydrogenase, NADP-isocitrate dehydrogenase and malic enzyme, the formation of the

reduced coenzyme NADP in brain might be normal even in the vitamin deficient animals. Hence the reported abnormality in the synthesis of myelin lipids in brain in vitamin A deficiency might be due to a block in the glycolytic pathway. However, further studies using $U^{14}C$-glucose and its incorporation into lipids in brain in the vitamin deficient animal is necessary to substantiate this claim.
