CHAPTER I

INTRODUCTION

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The definition of death as central nervous death for legal purposes serves to highlight the importance of this organ and its distinctiveness from other vital organs such as the heart or kidney. It is not surprising that very early studies were concerned with the effects of nutritional stress on brain structure, composition and function (Donaldson, 1911; Jackson, 1925; Chick, Macrae, Martin and Martin, 1938; Folch-P., 1947). However, the observations that in adult animals the brain did not show any change with short term starvation and that in man intellectual capacity was not affected by prolonged starvation led to the conclusion that the brain is not affected by nutritional status and a further interest in this problem was not aroused until several decades later inspite of the dramatic effects of vitamin deficiencies on CNS function (Goldberger, 1914; Bourne, 1953; Goldsmith, 1953; Eiduson, Geller, Yuwiler and Eiduson, 1964; Coursin, 1967).

The assumption that the brain is relatively more resistant to change than other organs is not entirely unreasonable as it has to maintain a stable internal environment for efficient function and as small changes in this environment can result in gross consequences in metabolism and behaviour. For instance, the introduction of hypertonic sodium chloride into a specific region of the hypothalamus can make the animal drink

itself to death (Anderson, 1952). The maintenance of a relatively stable chemical environment demands that the entry and exit of substances and the rates at which they take place are strictly regulated. The brain has to synthesize most of its constituents as the entry of many substances into the brain is restricted by the blood brain barrier (Waelsch, 1957).

Cerebral metabolism accounts for 20-25% of basal metabolism in adult man (Kety, 1957) although the brain constitutes only 2-3% of body weight. The earlier notion regarding the metabolic inertia of the brain was reinforced by the observation that the intravenous administration of labelled amino acids was not followed by rapid incorporation in the brain (Friedberg and Greenberg, 1947). This seemed consistent with the observation that the mature central nervous system shows no sign of renewal of neurons by cell division (Leblond and Walker, 1956).

It has now been amply demonstrated that brain proteins have high turnover rates. The incorporation of labelled glucose in the brain compares with that in the liver (Gaitonde, Dahl and Elliott, 1965). The incorporation of labelled amino acids is also found to be quite rapid when injected intracisternally into the brain (Gaitonde and Richter, 1953, 1955, 1956). Such an active metabolic state implies a

continuous requirement for, and availability of, enzymes, cofactors, amino acids etc., and it is hard to believe that the supply of the same would not be affected by nutritional deficiency. Although in the initial stages of nutritional deprivation the brain may get preferential treatment, when the stocks hit the bottom of the barrel it has also to share the cut in rations.

Another factor which was ignored in the early studies was the age at which deprivation was introduced. It is now well accepted that in the young animal in which the brain is still undergoing growth and maturation and for which requirements for protein and other nutrients are greater in proportion to both body weight and energy intake nutritional deprivation may have effects different from those in the adult animal (Winick and Noble, 1966; Dobbing, 1968a).

Most of the developmental changes in the rat brain takes place during the first three weeks of postnatal life. The maximum rate of brain growth occurs in the first 10 days of life. This is represented by structural and morphological changes such as neuronal maturation, synaptogenesis and is followed by the appearance of EEG activity (Scheibel and Scheibel, 1971).

The period between 10 and 21 days of age is associated with several biochemical and functional changes in the brain as a whole. Cell division represented by DNA content reaches adult levels by about 18-21 days of age (Winick and Noble, 1965; Adams, 1966). The rapid flux of glucose carbon to amino acids (Gaitonde and Richter, 1966) and the peak period of protein synthesis (Miller, 1969) and myelination (Davison and Dobbing, 1968) occur during this period. This is accompanied by the appearance of the adult pattern of metabolism and functional maturation of the cerebral cortex. The brain may be expected to be more vulnerable to nutritional deficiencies during this period when its growth in terms of size and maturation is very rapid.

Because of the recognition that adequate nutrition may be necessary for the structural development of brain in early life, the last decade has witnessed the conduct of several studies with experimental animals on the effects of malnutrition or undernutrition in early life. Most of the studies have been concerned with parameters which reflect structural development such as brain weight, DNA, RNA, protein and cholesterol. Not many studies have been carried out on the effects of deficiency on the metabolic activity of the brain.

The use of experimental animals for the studies on the effects of nutritional deprivation on brain development raises

questions regarding the extrapolation of the results of such studies to man who is the object of our concern not only because of variations in life span between different species but also because of differences in the ontogenetic development of the brain at different stages of life. Some of these differences are shown in Table 1. It can be seen from the same that growth during the neonatal period is much more rapid in the rat and pig than in man. This may mean that the neonatal brain may be more vulnerable to malnutrition in these species than in man. Another consideration is that the stress of reproduction is also much more severe in the rat and pig (Table 2) so that nutritional deficiencies in the mother affect the offspring to a greater extent. The reasonably satisfactory gestation and lactation performance of poorly nourished women all over the world is a fairly well-documented phenomenon.

The species differences in chemical maturation indicated in Table 1 are associated with those in structural maturation as might be expected. Some of these are the completion of neuronal proliferation well before birth in man and in the immediate neonatal period in the rat. Myelination proceeds more slowly but well beyond weaning age in man whereas in the rat most of it takes place before weaning. This is also true of neuroglial proliferation.

Table 1 : Comparison of brain development in rat, pig and man*.

	rat	pig	man
body weight (kg) :			
birth	0.006	1.47	3.5
weaning	0.050	16.50	7.5
maturity	0.450	265.00	65.0
brain weight (g) :			
birth	0.25(4.20)	30(2.04)	300(8.6)
weaning	1.40(2.80)	70(0.42)	540(7.2)
maturity	2.00(0.44)	140(0.0005)	1300(2.0)
DNA (mg/brain) : (neuronal + glial)			
birth	0.69(276)	27.6(92)	366(122)
weaning	2.45(175)	41.4(59)	740(137)
maturity	2.52(126)	65.1(47)	876(67)
RNA (mg/brain) :			
birth	11.88(4750)	N.A.	318(106)
weaning	4.96(354)	N.A.	783(145)
maturity	3.24(162)	N.A.	1846(142)
protein (g/brain) :			
birth	0.014(5.6)	2.63(8.75)	17.4(5.8)
weaning	0.133(9.5)	6.50(10.00)	37.8(7.0)
maturity	0.210(10.5)	14.0(10.00)	104.0(8.0)
cholesterol (g/brain) :			
birth	0.001(0.4)	0.42(1.4)	2.1 (0.7)
weaning	0.015(1.1)	1.43(2.2)	5.4(1.0)
maturity	0.038(1.9)	4.86(3.5)	49.4(3.8)
phospholipids (g/brain) :			
birth	0.0033(1.3)	1.08(3.6)	6.6(2.2)
weaning	0.0560(4.0)	2.93(4.5)	17.8(3.3)
maturity	0.1080(5.4)	9.10(6.5)	84.5(6.5)

N.A. = Not available.

Values in parentheses give per cent of body weight in the case of brain weight and concentrations (% of brain weight) in the case of other parameters.

contd.2

contd. 2

	rat	pig	man
RNA/DNA :			
birth	16.6	N.A.	0.87
weening	2.0	N.A.	1.06
maturity	1.3	N.A.	2.11
protein/DNA :			
birth	20.3	97.2	47.5
weaning	54.3	157.0	51.1
maturity	83.3	215.1	118.7
cholesterol/DNA :			
birth	1.45	15.2	5.7
weaning	6.12	33.8	7.3
maturity	15.10	74.7	56.4
phospholipids/DNA :			
birth	4.78	39.1	18.0
weaning	22.90	70.8	24.1
maturity	42.90	140.0	96.5

* Based on data given for rats by Culley and Mertz (1964); Winick and Noble (1965,1966); Zamenhof, Marthens and Margolis (1968); Culley and Lineberger (1968); Winick, Fish and Rosso (1968); and Geison and Waisman (1970); for pigs by Dickerson and Dobbing (1967) and Dickerson, Dobbing and McCance (1967); and for man by Mandel, Rein, Hearth-Edel and Mardell (1964); Himwich (1962); Fishman, Prensky and Dodge (1969); Winick and Rosso (1969a); Ganguli, Dutta and Mukherjee (1972) and Subbarao and Janardana Sarma (1972).

Table 2 : Growth and reproduction in rat, pig and man.*

	rat	pig	man
adult body weight (kg)	0.450	265	65
birth weight (kg)	0.006	1.47	3.50
weaning weight (kg)	0.050	16.50	7.50
gestation period (weeks)	3	16	40
lactation period (weeks)	3	8	25
maternal weight at conception (kg) (a)	0.20	150	50
litter size	8	8	1
total weight of offspring (kg)			
at birth (b)	0.048	11.76	3.5
at weaning (c)	0.40	132.00	7.5
$\frac{b}{a} \times 100$	24.00	7.84	7.0
$\frac{c}{a} \times 100$	200	88	15
tissue production per day as % of maternal weight :			
during gestation	1.143	0.070	0.025
during lactation	8.40	1.43	0.045

* Based on data given by Altman and Dittmer (1972) and Mitchell (1962).

However varying reports have been made of the age at which these phenomena (cell proliferation and myelination) occur. Thus in man neuronal multiplication is reported to be achieved by 30 weeks of gestation by Robinson and Tizard, (1966) and 18 weeks by Dobbing and Sands (1973). Similarly total cell number (neuronal + glial) is reported to be achieved by 12 months after birth by Mandel et al (1964) and 8 months by Winick (1968). Myelination has been reported to go on until 4-5 years after birth by Kokrady, Mammen and Bachhawat (1971) and Dobbing and Sands (1973) although an earlier report by Davison and Dobbing (1966) suggest that it may continue upto adolescence. More definite information on these points is needed.

The manipulation of growth during the prenatal period poses problems as nutritional deprivation of the mothers may not necessarily result in fetal deprivation. However, attempts have been made to manipulate prenatal nutrition by feeding the mothers either a deficient diet or a quantitatively restricted diet. An alternative approach has been to manipulate the number of fetuses by tying one of the two intrauterine horns thereby reducing the litter size at birth (Marthens and Zamenhof, 1969). The manipulation of the diet during the postweaning period is relatively simple, but here the problem is one of separating the effects of specific

nutritional deficiencies from those of a reduction in food intake resulting from the deficiency.

Many techniques have been used to produce undernutrition in rats during the neonatal period. The most common technique is to manipulate the litter size and consequently the supply of milk to the pups (Kennedy, 1957; Widdowson and McCance, 1960). Another method consists of restricting the food intake of the mother during lactation (Chow and Lee, 1964) or feeding the mother a low protein diet (Barnes, Cunnold, Zimmerman, Simmons, MacLeod, and Krook, 1966). Restricting the access of the pups to the mother has been used by Eayrs and Horn (1955). Some investigators have manipulated maternal diet during both gestation and lactation (Simonson, Sherwin, Anilane, Yu and Chow, 1968). A combination of both approaches (increase in litter size and maternal protein restriction) has also been used to produce a more severe degree of undernutrition (Guthrie and Brown, 1968). In all these cases the effect is a decreased supply of milk but the protein content of milk is not affected. Consequently these methods do not enable us to manipulate the protein concentration of the diet.

Similarly, studies on the effects of mineral deficiencies are difficult as deficiency of a mineral such as calcium or iron in the maternal diet does not affect its concentration in milk (Karmarkar and Ramakrishnan, 1960).

Vitamin deficiencies can be induced by feeding the mother a deficient diet, but here the problem is one of isolating the effects of overall undernutrition from those due to the specific deficiency as food intake and milk production are both affected by a deficient diet.

A more rigidly controlled technique for modifying the diet of the suckling rat was reported earlier by Miller and Dymsza (1963). In this method new born pups were fed using a specially designed needle. Czajka and Miller (1968) have achieved various degrees of undernutrition by hand feeding the pups from birth with a milk formula the protein content of which was varied. They found weight gain during the neonatal period to depend on the protein content of the liquid formula as might be expected. However, this technique has not yet been extensively used for studies on the brain.

Only a few studies have been done on the effects of manipulating the plane of nutrition during the prenatal period. Pups born to rats fed a protein deficient diet during gestation had low birth weights and brain weights (Zemanhof, Marthens and Margolis, 1968; Shrader and Zeman, 1969; Zeman and Stanbrough, 1969). Brain DNA content was reduced but concentration was not affected suggesting a reduction in cell number (Zeman and Stanbrough, 1969; Zamenhof, Marthens and Grauel, 1971). Neuronal morphogenesis was found to be

considerably retarded in cerebellar explants cultivated in tissue culture in the pups of mothers fed a protein deficient diet during gestation (Allerand, 1972). An earlier study (Shrader and Zeman, 1969) showed that although the number of cells in the surface layers of the cerebral cortex was reduced they had normal enzymatic activity as measured by histochemical techniques.

Undernutrition during the neonatal period has been associated with a reduction in brain weight (Dobbing and Widdowson, 1965; Winick and Noble, 1966; Chase, Lindsley and O'Brien, 1969) and an increase in the ratio of brain weight to body weight. A decrease was found in the content of DNA (Winick and Noble, 1966; Dobbing, 1968b; Guthrie and Brown, 1968; Enwonwu and Glover, 1973), RNA (Winick and Noble, 1966; Enwonwu and Glover, 1973), protein (Winick and Noble, 1966; Gambetti, Autilio-Gambetti, Gontas, Shaffer and Stiebber, 1972; Enwonwu and Glover, 1973) and cholesterol (Dobbing, 1964; Benton, Moser, Dodge and Carr, 1966; Geison, 1967; Dobbing, 1968b; Dickerson and Jarvis, 1970).

The concentrations are not affected in the case of DNA (Guthrie and Brown, 1968; Chase et al, 1969), RNA (Guthrie and Brown, 1968) and protein (Chase et al, 1969) although a reduction in concentration as well has been reported by Winick and Noble (1966).

A reduction with neonatal undernutrition has been reported in other lipids such as phospholipids (Benton et al, 1966), gangliosides (Dickerson and Jarvis, 1970), cerebrosides (Culley and Mertz, 1964; Benton et al, 1966; Geison, 1967), proteolipids, sulfatides and plasmalogens (Geison, 1967).

Neonatal undernutrition was found to affect the concentration of the free amino acids (Reddy, Pleasants and Worstman, 1971). While the concentration of essential amino acids was increased (valine, leucine, isoleucine, histidine and arginine) that of some of nonessential amino acids was found to be reduced (glutamic acid, glutamine, alanine, serine and GABA).

Decreased myelination, impaired dendritic arborization and consequent reduction in synaptic connections have been reported (Eayrs and Horn, 1955; Dobbing, 1968b; Cragg, 1972). However, no significant change in synaptic organization was found by Gambetti et al (1972).

The cerebellum may be affected to a greater extent on the basis of some studies. Novakova, Koldvsky, Hahn and Krecek (1967) found decreased RNA in the purkinje cells of the cerebellum. Chase et al (1969) found a greater reduction in cerebellar DNA. The greater changes in cerebellum are consistent with its rapid maturation (Winick, Rosso and Brasel, 1972).

Similar or greater changes in the brain are found when the mothers are undernourished during both gestation and lactation (Dickerson and Jarvis, 1970; Enwonwu and Glover, 1973; Smart, Dobbing, Adlard, Lynch and Sands, 1973).

Undernutrition during the neonatal period has also been found to result in decreased activities of several brain enzymes including succinic dehydrogenase, aldolase, acetylcholine esterase, β-N-acetyl glucosaminidase (Adlard and Dobbing, 1971) and galactocerebroside sulfotransferase (Chase, Dorsy and Mckhann, 1967).

In contrast to undernutrition during the neonatal period postweaning undernutrition is not found to result in appreciable changes although brain weight is found to be affected (Winick and Noble, 1966; Dobbing, 1968b). Deficits were found in whole brain values but their concentrations have generally not been found to be affected.

The deficits in brain weight and brain composition due to undernutrition during the prenatal and neonatal period are not found to be fully reversed by subsequent rehabilitation in later life whereas the relatively smaller deficits in brain weight and brain composition resulting from postweaning undernutrition are found to be completely restored.

Although the rehabilitation of undernourished animals after weaning is not found to restore fully the deficits in either body weight or brain weight, the ratio of brain weight to body weight may be normal or even elevated (Winick and Noble, 1966; Guthrie and Brown, 1968). A reduction in this ratio is suggested by recent studies of Dobbing and Sands (1972) but not by the earlier studies of Dobbing (1968b).

Normal concentrations of various lipid fractions are achieved with rehabilitation (Culley and Lineberger, 1968; Guthrie and Brown, 1968) but whole brain values were less on the basis of decreased brain weights. However, Culley and Lineberger (1968) report persistent deficits in the concentrations of cholesterol, phospholipids and cerebrosides even after rehabilitation. Similar observations have been made on pigs (Dickerson, Dobbing and McCance, 1967) but in these studies the degree of undernutrition was extremely severe and prolonged and the body weights of the undernourished animals were only 3.5% of expected weights. Even so, the deficit in concentration of cholesterol was only 6% and that in brain weight, 34%.

Not many studies have been undertaken on the effects of protein deficiency during the postweaning period in rats. In pigs and dogs subjected to such deficiency the changes observed in the spinal cord included chromatolysis, increase

in the oligodendroglial cells and astroglial processes (Platt, Heard and Stewart, 1964; Platt and Stewart, 1971). The amount of white matter and the thickness of the myelin were reduced. These changes were less marked in the cerebral cortex but here the total number of cells was reduced. The changes were more pronounced in animals subjected to deficiency during the neonatal period as well, induced by feeding the mothers a protein deficient diet. The EEG rhythm was found to be abnormal but became normal on rehabilitation.

The adult brain has been found to be fairly resistant to the effects of either starvation or protein deprivation. No change was recorded in the DNA, protein and total nitrogen content in the cerebral cortex of 3-4 month old rats fed a protein free diet for a period of 100 days (Lehr and Gayet, 1963). Similarly, severely underfed adult rats did not show any reduction in either brain weight, DNA or cholesterol content (Dobbing, 1968a). No change was reported in the oxidative degradation of glutamate and alanine in the brains of 3-4 month old rats fed a protein free diet for eight weeks (Macfarlane and Vonholt, 1969).

However, some investigators have found changes even in the adult brain with severe and prolonged deprivation. For instance, short term starvation resulted in reduced rate of incorporation of labelled precursors into brain lipids

in the rat (Smith, 1965). When 3-4 month old rats were maintained on a protein free diet for six weeks the ratio of amino acid concentration to DNA concentration was reduced in some cases (aspartic acid, tyrosine, phenylalanine, lysine and taurine) and increased in others (methionine, histidine, arginine and serine) (Mandel and Mark, 1965).

Prolonged starvation in dogs has been found to result in changes in blood brain barrier as judged by the more rapid incorporation of labelled arsenic (Yaromyenka, 1969). Similar observations have also been made in rats subjected to thiamine deficiency in the postweaning period as judged by the incorporation of intravenously given labelled pyruvate into glutamic acid in the brain (Warnock and Burkhalter, 1968).

A number of studies have been made on the effects of undernutrition in early life on neuromotor development and both immediate and subsequent behaviour in rats. The former as judged by the development of reflexes such as righting, cliff avoidance, negative geotaxis, palm grasp, auditory startle, vibrissa placing, visual placing and free fall righting is found to be delayed in rats subjected to undernutrition during lactation or both gestation and lactation (Smart and Dobbing, 1971a, b). Pups born of mothers undernourished during gestation and nursed by normally nourished mothers did not show a similar retardation suggesting that

nutrition during the suckling period is more important for neuromotor development (Smart and Dobbing, 1971b). However, Simonson et al (1968) have found some developmental retardation in pups born of undernourished mothers and some behavioural effects to persist even after weaning.

Maturation of evoked cortical response to a visual or auditory stimulus has been found to be delayed in neonatally undernourished rats. These differences however disappeared at 45 days of age (Mourek, Himwich, Myslivecek and Gallison, 1967).

Interaction between the mother and the pups, environment and other stimuli may all influence the neuromotor development of the pups. Maternal behaviour has been found to be altered with malnutrition (Frankova, 1972). Similarly the interaction between pups varies when pups are reared in a small or large litter; pups in larger litters are found to be more independent and more active in the nest (Frankova, 1972).

A slow development of spontaneous exploratory activity (Frankova and Barnes, 1968a) and lower intensity of exploratory behaviour (Lat, Widdowson and McCance, 1961) was found in rats subjected to neonatal undernutrition.

Inspite of the developmental retardation noted early in life in several studies no permanent effects were observed

in undernourished and rehabilitated rats (D'Amato, 1960; Barnes et al, 1966; Guthrie, 1968). On the other hand performance was sometimes better in undernourished and rehabilitated rats compared to normally fed rats (Frankova and Barnes, 1968b). Similar observations have been made by Barnes, Moore, Reid and Pond (1968) in pigs undernourished during the postweaning period.

Many studies with rats have shown that food deprivation in early life results in persistent changes such as increased drive for food and a tendency to hoard food in the rehabilitated adult (Bronfenbrenner, 1968). In rats reared in large litters this may be due to early feeding frustration (Seitz, 1954). Increased emotionality during complex learning tasks when exposed to adverse stimuli has been found in rats subjected to nutritional stress early in life (Levitsky and Barnes, 1970).

Both neonatal undernutrition and postweaning undernutrition or protein deficiency were found to result in poor maze performance in rats (Baird, Widdowson and Cowley, 1971) although in Cowley's earlier studies postweaning protein deficieney was not reported to have any effect (Cowley and Griesel, 1963).

Severe undernutrition or protein deficiency during the postweaning period preceded by neonatal undernutrition has been found to impair psychological performance (Frankova and Barnes, 1968a) but even in this case the impairment was much greater with a protein deficient diet. Protein deficiency in the postweaning period has been found to impair performance on visual discrimination and reversal learning and water-maze performance in rats (Rajalakshmi, Govindarajan and Ramakrishnan, 1965; Rajalakshmi, [redacted] and Ramakrishnan, 1969).

Pigs subjected to protein deficiency in the postweaning period are found to show impaired performance on tasks such as electric shock avoidance (Barnes et al, 1966; 1968). They also showed an impaired ability to modify a previously acquired response no longer relevant to the changed situation. In this connection the capacity for such modification has been suggested to be related to intelligence and learning capacity (Rajalakshmi and Jeeves, 1965).

The behavioural changes observed in protein deficient dogs included lack of interest in the surroundings which disappeared by about 3 months of age. However, these animals appear less "playful" and are more "aggressive" even after rehabilitation (Platt and Stewart, 1968).

In man the peak rate of development is found in the prenatal period but the maturation of the brain continues at a progressively decreasing rate in the neonatal and postweaning period till about 3-4 years after birth as can be seen from Table 3.

It would be reasonable to expect that undernutrition during the prenatal, neonatal and postweaning periods may retard to some extent the development of the brain as well. The most dramatic instances of the effects of maternal deficiency on the offspring are cretinism and deaf-mutism due to prenatal deficiency of iodine and infantile beri-beri resulting from neonatal deficiency of thiamine.

Although by and large poorly nourished women manage to produce fairly healthy babies of normal weight, about 10% of the full term babies born of such women are found to have body weights of less than 2 kg (Rajalakshmi, 1971). Such low birth weights are associated with poor weight gain during pregnancy and low placental weight and protein content (Rajalakshmi, 1971). A reduction in birth weight was found in war-time Europe (Antonov, 1947; Smith, 1947). The postwar improvement in nutritional status has been associated with increased birth weights in Japan (Gruenwald, Funkawa, Mitani, Nishimura and Takeuchi, 1967).

Table 3 : Changes in chemical composition during brain development in man*.

Age	body weight (kg)	brain weight (g)	(values per whole brain) DNA (mg)	RNA (mg)	protein (g)	total lipids (g)	cholesterol (g)	phospholipids (g)
gestation period : (weeks)								
20	0.6	80	131	85	3.0	N.R.	0.50	1.40
30	1.0	150	203	138	5.0	2.5	0.71	2.00
40	2.7	300	366	318	17.4	8.4	2.10	6.65
lactation period : (months)								
3	5.4	440	684	N.R.	N.R.	18.5	4.05	14.50
6	7.5	620	850	900	43.4	31.0	N.R.	N.R.
postweaning period : (months)								
8	8.0	700	875	N.R.	N.R.	N.R.	N.R.	N.R.
12	9.2	940	902	1363	N.R.	57.3	13.20	33.85
24	14.0	1120	918	1602	79.5	130.0	35.85	68.30
36	15.0	1270	927	1803	99.1	190.0	47.00	82.60
			% increment per month					
gestation period : (weeks)								
20-30	27	35	22	20	27	-	17	17
30-40	68	40	32	52	99	94	78	93
birth to 3 months	33	16	29	31	25	40	31	39
3 - 5 "	13	13.6	9.1			23	25	15
6 - 12 "	3.8	8.6	1.02	8.6	4.6**	14.1		
24 - 36 "	0.6	1.1	0.08	1.0	2.1	1.9	2.6	1.8

* Based on data given by Himwich (1962); Winick and Rosso (1969a,b); Fishman, Prensky and Dodge (1969); Rosso, Hormazabal and Winick (1970); Subbarao and Janardana Sarma (1972).

** during the period 6-24 months.

N.R.= Not reported.

Studies reviewed by Knobloch and Pasamanick (1963) suggest that low birth weights are associated with increased incidence of psychological retardation in early life. But such low birth weights have been found in babies born of western women even in private nursing homes and the etiological factors may be nonnutritional although severe anorexia and nausea throughout pregnancy may result in undernutrition in some cases (Chase, personal discussion with Dr. Rajalakshmi). Gruenwald (1968) observed low birth weights more than 2 standard deviations below the mean in babies born of well-nourished mothers in a private ward. According to him such growth retarded children may suffer neonatal hypoglycemia which may cause brain damage. In this connection children with low birth weights in Ceylon and India were found to fare much better than those with similar birth weights in the West (De Silva, Fernando and Gunarante, 1962; Rajalakshmi and Ramakrishnan, 1972). This may be partly due to differences in expected weight but may also be due to differences in the cause of such low birth weights.

The few studies in this country on the development of small birth weight babies have not revealed any significant developmental deficits in these children as compared to controls (Rajalakshmi and Ramakrishnan, 1969b; Rajalakshmi and Ramakrishnan, 1972; Ghosh, Kumari, Bhargava and

Bhargava, 1972). More extensive studies on the head circumference and psychological development of such children are needed.

During the neonatal period, most Indian women of the low income group manage to maintain satisfactory lactation and the output of breast milk is of the order of 700-750 ml per day in established lactation (Venkatachalam, Susheela and Rau, 1967; Rajalakshmi, 1971). The infants double in body weight between 3-4 months of age and even low birth weight babies achieve a weight of 6-7 kg at six months of age (Rajalakshmi, 1971). However, a few children fail to grow satisfactorly even during this period and some children show only 70-90% increase in weight during the first six months instead of the expected 140%.

Small birth weight babies are seldom found in the upper class and their postnatal development is also satisfactory. In spite of the fact that lactation is, as a rule, not efficient in this group the children are given adequate quantities of animal milk or baby foods. Occasionally, however, even the upper class mother may give diluted milk or baby food for fear of indigestion. This results in diarrhœa and the mother dilutes the milk still further. Such children often show growth failure as judged by bodyweight gain although they may show normal developmental maturation.

In this connection, giving a very dilute milk formula containing 75% water even in theoretically adequate quantities as regards calories is reported to cause undernutrition in monkeys (Kerr and Waisman, 1968).

The etiology of kwashiorkor and marasmus which are prevalent in children has been described by Rajalakshmi and Ramakrishnan (1972) as follows.,

Children in the postweaning period are often fed diets poor in protein such as surplus cooking water from rice, sago, yam, banana powder and sugar-cane juice. Protein rich foods such as legumes, beans and fish, even when they are consumed by the family, are excluded from their diets. This results in kwashiorkor, a well known disease syndrome associated with protdin deficiency. The diets resulting in kwashiorkor are also generally deficient in a number of other nutrients including vitamin A.

In regions such as Gujarat, the child is allowed to eat the family foods, but because of the coarse texture of the 'roti' and highly spiced legumes and vegetables it is not able to eat enough of them thus resulting in undernutrition.

In either condition, diarrhoea may develop leading the mother to restrict the quantity or substitute starchy foods for other foods. The child may also restrict its food intake

because of the poor nutritional quality and unsuitable nature of the diet. In all these cases, the situation is aggravated by an episode of severe illness such as measles, chicken pox, diarrhoea etc.

Kwashiorkor is associated with edema, moon face, dermatitis, discolouration of hair, and often, eye symptoms. Extreme apathy is a common characteristic in kwashiorkor. Such children do not show the expected response to attractive toys and for all practical purposes they seem to have lost contact with the environment (Rajalakshmi and Ramakrishnan, 1969b).

Extreme undernutrition, due to whatever cause, on the other hand, is associated with dehydration, wasting of muscle and wrinkled skin. The children are irritable and tend to be cry babies. However, they stop crying in response to interesting stimuli even if they resume it again. They do not seem to be in the "trance" state of the kwashiorkor children.

Both kwashiorkor and marasmus are found to occur in regions such as Kerala although the dietary history does not appear to be different. But in regions such as Gujarat where starchy foods such as tapioca are not included in the diet, marasmus is more common. Whether the child develops

kwashiorkor or marasmus on a poor diet may also perhaps depend on whether he is allowed to feed himself as in Gujarat or is coaxed to eat his bellyful as in Madras. In this connection animals force-fed a diet deficient in protein or amino acids show symptoms and histological changes different from those fed ad lib. (Sidransky and Baba, 1960).

Thus, while kwashiorkor is invariably due to a diet poor in quality, marasmus could be caused by :

(a) self-restriction of food intake because of poor nutritional quality;
(b) self-restriction of food intake because of unsuitable texture and presence of spices;
(c) maternally-imposed restriction of intake of a diet also poor in quality;
(d) maternally-imposed restriction of intake of a qualitatively adequate diet such as milk or baby food.

It is obvious that conditions (a) and (c) will result in a combination of protein and calorie deficiencies whereas conditions (b) and (d) are primarily associated with a deficiency of calories. It is not surprising, therefore, that the findings on marasmus vary in contrast to the greater uniformity of findings on kwashiorkor. Some marasmic children

show wasting of muscle combined with edema at the extremities. Their etiology may presumably come under categories (a) and (c).

In this connection, as pointed out by Arroyave, Wilson, Funes and Behar (1962), serum protein and enzymes are less affected in marasmus than in kwashiorkor even after allowing for a higher concentration due to dehydration. This is also true of the excretion of 17-deoxycorticosteroids. Serum vitamin A levels are also less affected, suggesting a greater efficiency of intestinal function. Consequently disorders of the eye are found to a smaller extent in marasmus. The basal metabolism of the marasmic child may even appear to be elevated. Many clinicians have observed the greater alertness of the marasmic child. Some of the differences between the two conditions are summarized in Table 4.

In a number of studies head circumference has been used as an index of brain size. This is perhaps not unreasonable as a relation has been found between head circumference and cellular growth and brain DNA content (Winick and Rosso, 1969a). Brain weight and protein content are found to be related linearly to cranial volume calculated from head circumference. However, the use of head circumference as a measure of brain growth may be subject to certain limitations. In cases where the brain has atrophied resulting in some space

Table 4 : Some aspects of the differences between kwashiorkor and marasmus*.

	kwashiorkor	marasmus
age of maximal incidence (months)	6-18	12-48
edema	present	absent
fatty liver	"	"
intestinal absorption	severely affected	slightly affected
skin and hair changes	frequent	infrequent
psychic changes (apathy)	present	absent
blood or serum :		
hemoglobin	very low	nearly normal
total protein	" "	" "
albumin	" "	" "
A/G ratio	" "	" "
Whitehead ratio**	high	normal
hydroxy proline	very low	low
urea	low	normal
vitamin A	"	"
copper	"	"
cholesterol	"	"
lipase and amylase	"	"
lactate dehydrogenase	normal	low
leucocyte :		
fumarase	high	low
glutamate dehydrogenase	low	high
isocitrate dehydrogenase, aldolase and aconitase	normal	high

* Based on observations by Viteri et al (1964); Gopalan (1968); Pineda (1968); Whitehead (1968) and Gurson (1972).

** Ratio of glycine, serine and glutamine to branched chain amino acids.

between the cranium and the brain (Rozovski, Novoa, Abasana and Monckeberg, 1971) head circumference may over-estimate brain size. The reverse may happen in other cases where the skull is very thin because of protein and mineral deficiencies (Robinow, 1968). Incidentally head circumference may be very large in conditions such as hydrocephalus.

Severe protein calorie malnutrition has been found to be associated with brain changes such as decrease in weight (Udani, 1962; Brown, 1965; Winick and Rosso, 1969a; Udani, Mukherjee and Parekh, 1971), DNA (Rosso, Hormazabal and Winick, 1970; Winick, Rosso and Waterlow, 1970; Ganguli, Dutta and Mukherjee, 1972), RNA, protein and total lipids, (Winick and Rosso, 1969b; Ganguli et al, 1972; Subbarao and Janardana Sarma, 1972), cholesterol and phospholipids (Winick and Rosso, 1969b; Winick, Rosso and Waterlow, 1970; Subbarao and Janardana Sarma, 1972), cerebrosides and sulfatides (Fishman, Prensky and Dodge, 1969; Mokashi, Mukherjee, Ganguli and Bachhawat, 1972) and gangliosides (Mokashi et al, 1972).

The results of some of the above studies are summarized in Table 5. Bachhawat (1972) reported deficits in the concentrations of glycolipids (cerebrosides + sulfatides) and mucopolysaccharides in the whole brain and gangliosides in the white matter in malnourished children. Surprisingly, the concentration of glycolipids was more in 3 year old malnourished children as compared to controls. Although it

Table 5 : Effects of protein calorie malnutrition on chemical composition of brain in man.

	protein calorie malnutrition (Rosso et al,1970)		kwashiorkor (Subbarao and Janardana Sarma, (1972)	marasmus (Ganguli et al, 1972)
age (years)	0-0.5	1-3	2-3	1-3
	values as % of controls			
body weight	45-57	43-51	42	N.R.
brain weight	63-70	60-68	59	90
brain :				
DNA	81-85	88-100	107	68
RNA	N.R.	N.R.	41	14
protein	N.R.	N.R.	56	90
total lipids	N.R.	N.R.	38	44
cholesterol	49-75	44-48	35	N.R.
phospholipids	49-77	46-48	44	N.R.
	kwashiorkor (Bachhawat,1972) values as % of controls			
age (years)	0-1	3	4-5	8-9
cerebrosides and sulfatides	98	175	32	42
total mucopoly-saccharides	35	N.R.	N.R.	26
hyaluronic acid	123	N.R.	N.R.	187
heparin sulfate	200	N.R.	N.R.	49
low sulfated chondroitin sulfate	50	N.R.	N.R.	N.R.
chondroitin sulfate	39	N.R.	N.R.	14
gangliosides (in white matter)	100	55	41	15

N.R. = Not reported.

has been suggested that the effects of malnutrition are less evident when it occurs after the first year of life (Winick et al, 1972) no consistent trend is found in the data of either Bachhawat (1972) or Rosso et al (1970). Deficits in total lipids, glycolipids, proteolipids and plasmalogens in white matter have been found by Fishman et al (1969) in 4-12 month old malnourished children. They found an increase in neuraminic acid content (31-50%).

In man cell division stops at the same time in the cerebrum, cerebellum and brain stem (Winick et al, 1972). It is not unreasonable therefore to expect that malnutrition will affect cell number similarly in all these regions. This contrasts with the greater changes found in the cerebellum in the case of the rat in which it matures earlier and at a faster rate.

In adult man, the brain accounts for 20-25% of resting metabolism. The proportion is much higher (33-50%) in children below five years of age (Richter, 1952). In malnourished children who have a much larger brain in proportion to body weight, the share of cerebral metabolism can be expected to be even greater (Rajalakshmi, 1972) and this may account for the apparently elevated metabolism in proportion to surface area (BMR) found sometimes in marasmic children (Montgomery, 1962).

Histological changes similar to those found in malnourished pigs and dogs (Platt, Heard and Stewart, 1964) have been found in the brain of malnourished children (Udani, 1962). Davison (1967) found the myelin in the brains of malnourished children to resemble the immature myelin of malnourished animals.

Abnormalities of the EEG pattern have been found in malnourished children (Engel, 1956; Nelson, 1959; Nelson and Dean, 1959). Normal patterns evolve with nutritional rehabilitation and restoration of body weights to near normal levels (Valenzuela, Hernandez Penich and Macias, 1959). In some studies the abnormalities were found to persist (Engel, 1956).

The animal or child suffering from severe malnutrition often dies a central nervous death (Platt, 1961). An interesting observation has been made that symptoms such as tremors and convulsions occur during rehabilitation of the malnourished child (Kahn and Falke, 1956; Kahn, 1957). This was found more frequently with the very high protein diets used in earlier times (Udani, personal discussion with Dr. Rajalakshmi).

The psychological changes in the malnourished child were recognised long before the other changes and about 20 years

ago Carothers (1953) in his report to the W.H.O. mentioned the mental arrest of malnourished children.

As mentioned earlier extreme apathy is a common feature in the kwashiorkor children (Platt, 1961) and for all practical purposes they seem to have lost contact with environment (Rajalakshmi and Ramakrishnan, 1972). They stay put because of the edema but are extremely irritable when disturbed. In contrast to this marasmic children are irritable and tend to be cry babies. They are not as apathetic as the kwashiorkor children and show a fair interest in the surroundings.

A number of studies have suggested the poor psychological status of the malnourished child (Stoch and Smythe, 1963; Caback and Najadanvic, 1965; Stoch and Smythe, 1967; Monckeberg, 1968; Chase and Martin, 1970). Previously established reflexes are depressed or abolished and the elaboration of new conditioned reflexes are affected in protein malnourished children (Brozek, 1962).

The effects of early malnutrition would appear to persist to some extent on the basis of Cravioto's observations of the relation between height and intelligence in older children in a poor rural area (Cravioto, 1966). In these children height can be expected to reflect nutritional status in early life. Cravioto also found mental retardation to be less readily

reversed in kwashiorkor children hospitalised at a younger age although the effects of maternal deprivation due to separation from the mother and stay in a hospital must also be considered in such cases (Cravioto and Robles, 1965). Similar observations have been made by Stock and Smythe in longitudinal studies on undernourished children (Stock and Smythe, 1963, 1967).

In other studies kwashiorkor children did not show a deficit in subsequent performance on intelligence tests but showed a deficit in intellectual maturity as judged by a drawing test (Evans, Moodie and Hansem, 1971).

However, in many of these studies, particularly those of Stock and Smythe (1963) and Chase and Martin (1970) the social and psychological environment of the malnourished children was far from satisfactory. In this connection in studies carried out in Baroda, rural poor children have a cattell IQ of about 80 as compared to 100 in the urban poor although the two groups have a similarly poor nutritional status as judged by height, weight and composition of blood and serum (Rajalakshmi and associates, unpublished). Also, mere attendance at a play centre in a poor village improved IQ scores as much as nutritional improvement.

In the studies on urban children, growth retardation so that the body weight deficits were more than 35-40% before 2-3 years of age was not associated with deterioration in psychological performance. The children had a reasonably adequate protein status and were not lacking in affective care by the parents.

McLaren (1971) found an improvement in the psychological performance of malnourished children with good nutrition only when this was combined with environmental stimulation.

Although the brain of the adult is not vulnerable to the effects of malnutrition, psychological changes although of a transitory kind are not uncommon in severe and prolonged deprivation. This has been common observation in famine conditions and concentration camps (Smith and Woodruff, 1951; Helweg Larsen et al, 1952). The changes have been well documented in the Minnesota studies on starvation (Keys, Brozek, Henschel, Mickelsen and Taylor, 1950) and include mental confusion, apathy and inability to concentrate. These effects generally disappear quickly with dietary rehabilitation.

Most of the studies on the effects of nutritional deprivation have concentrated on the composition of the brain with regard to proteins, lipids and nucleic acids. However, the amino acid make up of the brain and the metabolism of the same also present special points of interest.

The brain contains 2% of soluble organic constituents of which a major portion is amino acid. 20% of the total nitrogen in the brain is made up of amino nitrogen 25% of which is contributed by glutamic acid and glutamine (Ansell and Richter, 1954; Waelsch, 1952; 1955; Weil-Malherbe, 1952). While the brain compares with plasma and liver in its concentrations of essential amino acids it has a much higher concentration of nonessential amino acids (Waelsch, 1957).

Glutamic acid, glutamine and gamma amino butyric acid account for a substantial portion of the nonessential amino acids. When U-^{14}C glucose was subcutaneously injected in the rat a greater proportion of labelling was found in the brain than in the liver in glutamate, glutamine and aspartate (Table 6).

The formation of glutamic acid is catalysed by the enzyme glutamate dehydrogenase. The importance of this reaction is that it occurs spontaneously i.e. without the supply of energy as soon as a sufficient concentration of ammonia is available (Krebs, Eggleston and Hems, 1948). Dewan (1938) and Von Euler, Adler, Gunther and Das (1938) established the presence of this enzyme in several mammalian tissues including the brain. The activity of glutamate dehydrogenase in the brain has not been found to be as high as that in the liver or kidney (Krebs, Eggleston and Hems,1948)

Table 6 : Relative incorporation of labelling in selected amino acids in the brain and liver in rats given U ^{14}C glucose*.

	per cent activity of total amino acids in	
	brain	liver
glutamate	37.0	5.3
GABA	4.0	0
glutamine	9.0	5.2
aspartate	9.0	2.6
alanine	2.0	3.0
total	61.0	16.1

* Data taken from Gaitonde, Dahl and Elliott (1965).

a fact which contrasts with the higher concentration of glutamic acid in the brain. This is explained by the fact that in the brain the equilibrium constant of the enzyme favours reductive amination of α-ketoglutarate and the enzyme does not normally operate in the direction of oxidation of glutamic acid (Olson and Anfinsen, 1953; Strecker, 1953; Weil-Malherbe, 1957).

In the brain, this enzyme may be expected to have a crucial role as glutamate is an important metabolite for the brain probably having an electrophysiological role. As the blood brain barrier does not permit the entry of this amino acid the glutamate in brain is locally synthesized (Strecker, 1957). The formation of glutamate also serves as an important machinery for the quick removal of ammonia which is highly toxic to the brain.

The activity of glutamate dehydrogenase is low in the neonatal rat brain and increases to its peak value which is four times its initial value by 16-23 days of age (Bayer and McMurray, 1967). The concentration of glutamic acid reaches a peak at 20-21 days (Agrawal, Davis and Himwich, 1966; Bayer and McMurray, 1967).

The role of glutamic acid in brain metabolism has elicited much speculation and interest since the observation

of Thunberg (1923) that the brain can oxidize glutamic acid *in vitro*. Many subsequent studies suggested that the mammalian brain *in vitro* can utilize glutamic acid in place of glucose (Quastel and Wheatley, 1932; Krebs, 1935a; Weil-Malherbe,1936). Extensive studies carried out by Weil-Malherbe (1936) on brain glutamic acid showed that during the oxidation of the same no ammonia is liberated. It is now known that it can be oxidised via the GABA shunt by the successive conversion of glutamic acid to GABA, succinic semialdehyde and succinate.

Glutamic acid has a high turnover rate in brain slices and is rapidly metabolized to glutamine, GABA and aspartic acid (Waelsch, 1957; Tower,1959). Enzymes involved in the metabolism of glutamic acid namely glutamine synthetase, glutamate decarboxylase, aspartate aminotransferase and alanine aminotransferase have been identified in the brain (Krebs, 1935a; Cohen and Hekhuis, 1941; Speck, 1949; Elliott, 1951; Roberts and Frankel, 1951a, b; Roberts, Harman and Frankel, 1951; Lajtha, Mela and Waelsch, 1953). It is of interest that glutamine synthetase is invariably present in the brains of all animals (Krebs, 1935b) whereas it is variably present or absent in other tissues (Wu, 1963). This enzyme also provides a local machinery for the removal of ammonia (Sapirstein, 1943). In this connection the blood brain barrier acts against glutamic acid but not against glutamine (Kamin and Handler, 1951).

Considerable excitement regarding the role of this amino acid was aroused by the earlier reports on the favourable effects of glutamic acid supplementation on the intelligence of mentally retarded children and epileptics (Zimmerman, Burgemeister and Putnam, 1949; Hoch, Albert and Waelsch, 1951). In this connection it is of interest to note that the deficits in the activities of glutamate dehydrogenase and glutamate decarboxylase observed in animals fed low protein diets were reversed when the diet was supplemented with glutamic acid (Rajalakshmi, Pillai and Ramakrishnan, 1969).

Further, it has been shown that the brain contains a protein which has a high content of glutamic acid (Moore, 1965). In other studies, a similar protein has been found to show a rapid turnover rate (Minard and Richter, 1968).

The distribution of glutamic acid and glutamine in cellular components and in different layers of the brain suggests a functional significance for both. Thus glutamic acid is distributed to a greater extent in the grey matter (Tower, 1959) which is mainly concerned with nervous activity whereas glutamine is distributed equally in grey and white matter (Krebs, Eggleston and Hems, 1949; Waelsch, 1952). The distribution of these two amino acids in the cellular particulate also shows some variation. Glutamic acid is primarily found in the mitochondrial fraction whereas glutamine

is found in the mitochondrial as well as nuclear fractions (Weil-Malherbe, 1957; Tower, 1959).

The concentrations of glutamic acid, glutamine and GABA are found to increase during fetal and postnatal development and reach ceiling values with the myelination and maturation of the neurons (Krebs, Eggleston and Hems, 1949; Baxter, Schade and Roberts, 1960; Agrawal, Davis and Himwich, 1966). In the rat the attainment of maximum levels of glutamic acid coincides with active protein synthesis associated with myelination and neuronal maturation, and with the shift to adult patterns of metabolism (Rudnick and Waelsch, 1955a, b; Tower, 1959). The postnatal increase in the cerebral glutamate level coincides also with the rapid rate of conversion of the glucose carbon into amino acids (Gaitonde and Richter, 1966). In rats these changes parallel the development of compartmentation of glutamate metabolism (Patel and Balazs, 1970) and occur between 14-21 days of age.

It has been suggested that the compartmentation of glutamic acid and GABA can be used as an index of cerebral maturation which can be used to study the effects of different influences on the development of brain during the early postnatal period (Patel and Balazs, 1970). A marked retardation in the development of this compartmentation of glutamic acid and GABA has been found with thyroid deficiency (Patel and Balazs, 1971).

GABA is uniquely present in the brain, and so is the enzyme glutamate decarboxylase involved in its formation from glutamic acid. Further, the blood brain barrier operates against this amino acid (Van Gelder and Elliott, 1958). It has also been shown that the amount of GABA present in any region of the brain varies with the activity of glutamate decarboxylase (Sisken, Roberts and Baxter, 1960). In the developing brain the concentration of GABA is found to be related to glutamate decarboxylase activity ~~(Tower, 1958;~~ (Roberts, 1960).

The enzymes of the GABA shunt, namely, glutamate decarboxylase, GABA transaminase and succinate semialdehyde dehydrogenase increase during the postnatal period in the rat brain. (Van den Berg, Van Kempen and Veldstra, 1965). The increase in GAD activity in the rat brain is maximum between 2-3 weeks of age (Van den Berg, Van Kempen and Veldstra, 1965; Bayer and McMurray, 1967). Changes in GAD activity with age in different areas of the brain are correlated highly with the rapid growth of the surface area of the dendrites while changes in GABA levels are correlated with the increase in the volume of dendrites. (Schade and Baxter, 1960; Sisken et al, 1960; Himwich, 1962; Roberts and Kuriyama, 1968). Again, it is found that the appearance of adult EEG patterns and the maturation of cell body nucleus coincide with the attainment of adult concentrations of GABA (Schade and Baxter, 1960).

Both glutamate decarboxylase (Salvador and Albers, 1959) and GABA transaminase (Albers and Brady, 1959) are found to be more in the grey matter of the central nervous system. Various regions of the same exhibit widely differing activities (Albers and Brady, 1959; Rajalakshmi, Thrivikraman and Ramakrishnan, 1971). Miller and Pitts (1967) showed that in the human brain the activity of succinate semialdehyde dehydrogenase is high in the basal ganglia, thalamus, hypothalamus and relatively low in white matter.

Reference has been made to the utilization of glutamic acid in the absence of glucose. This must proceed either through transamination with oxaloacetate or through the GABA shunt. The former mechanism seems more likely on the basis of several studies (Krebs and Bellamy, 1960; Haslam and Krebs, 1963; Balazs, 1965). However, it has been shown that a substantial portion of glucose is oxidised via the GABA shunt and estimates of the proportion so oxidised vary from 10-40% (Mckhann and Tower, 1959; 1961; Elliott, 1965). A more recent estimate obtained by following the oxidation of glucose in brain slices gave the flux through the GABA bypass as 8% of total flux through the tricarboxylic acid cycle (Patel, Balazs and Richter, 1970). However, no net change in GABA concentration is found when this is used as substrate (Elliott, 1965) although a negligible net consumption is found

in the presence of glucose (Elliott and Van Gelder, 1958). This could be because of the continued synthesis of GABA from glutamate formed during transformation and the reductive deamination of α-ketoglutarate.

Studies reviewed by Spadoni and Gaetani (1972) suggest that the formation of polysomes and protein synthetic activity in the liver depend on the free amino acid pool. The increase in the free amino acid pool in many tissues including the nervous tissue has been found to correlate with protein synthetic activity during development (Miller,1969). The rate of protein synthesis and hence the phenomenon of growth may be affected by the maintenance of balanced proportions of essential amino acids. Amino acid imbalance caused by force feeding (using tube or parenteral feeding) an imbalanced mixture of essential amino acids resulted in growth arrest and decrease in the content of DNA, RNA and protein in the brain (Sengupta, 1971). The importance of amino acids and protein metabolism in relation to the function of the brain is indicated by the experiments of Faulk and Horne (1954) who found an excess of phenylalanine to affect learning performance in rats. They also found a deficiency of this amino acid to have a similar effect. The former observation is consistent with the finding of reduced protein synthesis (Takada and Tada, 1970) and altered amino acid composition of the brain,

including a decrease in GABA found with excess phenylalanine and other amino acids (Tada, Takada and Arakawa, 1970).

Among the amino acids both glutamic acid and GABA are of special interest as the concentrations of these amino acids are high in the brain which also has the enzyme systems necessary for their metabolism. Acidic amino acids structurally related to glutamic acid depolarize neurons and are considered as excitatory substances (Curtis, 1962; Krnjevic, 1964; Curtis, 1965; Hebb, 1970) and the neutral amino acids structurally related to GABA which depress the firing of neurons are considered as inhibitory substances. Thus glutamic acid is found to have an excitatory action and GABA an inhibitory action. These two amino acids may be considered to have mutually complementary roles in the central nervous system. The elementary criteria of a transmitter substance are that it must be produced in the relevant tissue at the appropriate time, stored, released to exert an appropriate action, and be removed (Elliott, 1965). All the criteria except that of release are met by both (Elliott, 1965). Even this criterion appears to be met by GABA as in studies made on Crustacean nervous system GABA leaks from the punctured cortical surface (Otsuka, Iversen, Hall and Kravitz, 1966). The rate of release of GABA was increased by about 50% when the electroencephalographic pattern indicated arousal (Jasper, Khan and Elliott, 1965).

The powerful depressant action of GABA when applied on cortical neurons and its presence in large quantities in the brain have led to the suggestion that GABA may be a postsynaptic inhibitory transmitter substance (Krnjevic, 1964). A stereospecificity of cortical receptors for GABA has also been shown on the basis that amino acidic groups are less potent in blocking cortical neuron activity (Krnjevic, 1964). However, some investigators rule out such a role on the ground that GABA does not act on the same site as strychnine but believe that it may be involved in presynaptic inhibition (e.g. Curtis, 1963). However, there is a consensus in favour of the view that GABA does have an important electrophysiological role.

The amount of glutamate and GABA in the synaptosome is very small but may be functionally most important. Krnjevic and Whittaker (1965) obtained excitant effects with glutamate and depressant effects with GABA with the rates of release corresponding to about 700 and 350 synaptosomes per second. This is important since synaptic activation at these rates is considered to be well within the possible physiological limit (Whittaker and Sheridan, 1965).

The exact mechanism of action of GABA is not understood. From studies on the localization of enzymes of glutamate and GABA metabolism (Salganicoff and DeRobertis, 1965) GABA may be

acting in either of the two ways described below (Himwich and Agrawal, 1969). One possibility is a trans-synaptic diffusion of GABA to regulate the excitability of adjacent neurons. It has also been suggested that the GABA bound to the synaptic vesicles is released when an impulse arrives in a neuron and as a result, it crosses the synaptic cleft to exert its effects on the postsynaptic membrane.

GABA may be acting as a synaptic feed back inhibitor. According to Roberts (1966) when an excitatory transmitter affects a postsynaptic membrane and depolarization results, there is an instantaneous postsynaptic release of GABA from a bound or stored form into the extraneuronal synaptic environment and this acts as a synaptic feedback transmitter. Such an inhibitory transmitter could be bound to both presynaptic and postsynaptic membrane on the sides facing the synaptic cleft and thus accelerate the rate of return to the resting potential of all depolarised membrane sectors. Once the released GABA is taken up by the membranes it is metabolized and the energy produced is utilised for bringing back the resting condition of the membrane.

Indirect evidence regarding the role of GABA as an inhibitory transmitter is to be found from the lowered concentrations of this amino acid in epileptic seizures and

in seizures induced by convulsive agents (Roberts and Baxter, 1959).

Intravenous administration of GABA to man causes transitory paraesthesia and produces a fall in blood pressure and respiratory rate in man, dog and rabbit (Elliott and Hobbiger, 1959; Tower, 1960). This is believed to be due to the peripheral action of GABA as it does not cross the blood brain barrier (Van Gelder and Elliott, 1958).

In contrast to GABA, glutamic acid is believed to have an excitatory effect. There is no strong evidence against the possibility that L-glutamic acid may be the principal excitatory transmitter in the central nervous system (Krnjevic, 1965). Recent studies have shown a similarity between the action of glutamate on the Crustacean neuromuscular junction and that of acetylcholine on the vertebrate end-plate (Takeuchi and Takeuchi, 1964). Krnjevic and Schwartz (1967) showed that in the cat pericruciate cortex glutamic acid is more selective in its action than acetylcholine. They were also able to show by means of intracellular recording that the depolarizing action of glutamate on cortical nerve cells is accompanied by a fall in membrane resistance; the mechanism of its depolarizing action is therefore more in accordance with the expected behaviour of an excitatory transmitter than is the case for acetylcholine. Thus the concentrations of GABA

and glutamate in the central nervous system are probably very critical as any disturbance in them may change the electrophysiological status resulting in abnormal function of the brain.

The rapid flux of glucose carbon to amino acids is found only at about 10-15 days after birth, when the cerebral cortex becomes functionally mature, and is not found in the fetal or neonatal brain (Gaitonde and Richter, 1966). The supply of glucose as a precursor for amino acids becomes therefore a critical factor after 10 days of age. Therefore any condition in which glucose metabolism is hampered might be expected to affect the concentration of glutamic acid in the brain. Thus insulin induced hypoglycemia causes a reduction in glutamic acid and GABA in the central nervous system (Dawson, 1950; Cravioto, Massieu and Izquierdo, 1951; Dawson, 1953).

The learning process is believed to involve the repeated firing of groups of neurons or neuronal assemblies (Hebb, 1949). Such firing cannot be efficient if the background electrical activity is too low, in which case it may not take place, or too high in which case the sequential firing of particular groups of neurons may become disorganised. We may therefore presume that an optimum level of dendritic activity is crucial for efficient CNS function. This may involve the

maintenance of critical levels of glutamic acid and GABA (Rajalakshmi, Govindarajan and Ramakrishnan, 1965; Hebb, 1970).

Some definite correlations have been observed between the ratio of GABA to glutamic acid in the brain and some behavioural characteristics of the rats. Emotionally stable strains of rats had slightly higher levels of GABA in the brain while a more reactive strain of rats and generally in a higher state of arousal had relatively low ratios of GABA to glutamate. (Rick, Huggins and Kerkut, 1967). In another series of experiments a definite correlation was seen between choline esterase activity and GABA production in the cerebral cortex in 5 different strains of rats (Rick, Morris and Kerkut, 1968).

The above considerations led to the choice of two enzymes of glutamate metabolism as the metabolic parameters in studies carried out on the effects of nutritional deficiency in this laboratory.

Previous studies in this laboratory showed deficits in the activity of glutamate dehydrogenase (GDH) and glutamate decarboxylase (GAD) in the brain of rats fed a low protein diet, but no change in the activity of GABA transaminase (GABA-T) (Rajalakshmi et al, 1969). Diets composed of cereals

and millets of very poor protein quality such as kodri (Paspalum scorbiculatum L.) and maize (Zea mays) produced effects similar to a low protein diet. Undernutrition during the neonatal period induced by manipulating litter size also produced similar deficits in different regions of the brain (Rajalakshmi and Ramakrishnan, 1969a).

In these studies decreased enzyme levels were associated with impaired performance in behavioural measures such as performance on the water maze, the Hebb-Williams maze, visual discrimination and reversal learning, locomotion scores and tasks involving motor coordination. (Rajalakshmi and Ramakrishnan, 1969a).

The present studies were designed as an extension of these studies and sought to answer questions such as the following raised by the previous studies.

1. What is the amount of good quality protein needed in the diet to prevent the brain enzyme deficits observed in protein deficiency?
2. Is the amount of protein sufficient for preventing these deficits also sufficient for reversing the effects of previous deficiency or is a higher level of protein required for such reversal?
3. What happens when a more severe deficiency of protein is induced?

4. What is the time course of the changes observed with protein deficiency?
5. How do the effects of undernutrition compare with those of protein deficiency?
6. How does neonatal undernutrition affect the vulnerability of the brain to the effects of postweaning deficiency?

Different experiments aimed at seeking answers to the above questions were carried out. The brain enzymes studied were glutamate dehydrogenase (L-glutamate:NAD oxidoreductase, E.C., 1.4.1.2), glutamate decarboxylase (L-glutamate-1 carboxylyase, E.C., 4.1.1.15) and GABA transaminase (4-amino butyrate:2 oxoglutarate aminotransferase, E.C., 2.6.1.19). Additional studies were made on liver protein and glutathione in some experiments. The results of these studies are incorporated in this thesis.