FETAL MALNUTRITION, BRAIN GROWTH AND MENTAL DEVELOPMENT

by

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B.S., Kansas State University, 1971

A MASTER'S REPORT

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Foods and Nutrition

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1977

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TABLE OF CONTENTS

INTRODUCTION

In the United States during 1972, there were 3,258,411 live births and 41,380 fetal deaths (1). Usher (2) concluded that 30% of the fetal deaths that he reviewed could be attributed to malnutrition of the fetus. Using that figure, 12,414 of the fetal deaths in 1972 could be ascribed to fetal malnutrition. Gruenwald (3), Urrusti et al. (4) and Scott and Usher (5) estimated that 33, 40 and 50% of low-birth weight infants are not "premature" but have suffered from fetal malnutrition or fetal growth retardation. Using an average figure of 41%, 205,736 of the 501,795 low-birth weight infants presumably were infants with fetal malnutrition. Therefore the estimated total incidence in the United States of babies either dying in utero from, or born with, fetal malnutrition would be approximately 218,150 infants per year.

In the past, any infant who weighed 2500 g or less was defined as premature. However, this definition did not take into consideration gestational age of the infant. Recently, "premature" infants have been divided into two groups: a) the true premature who is the normal size for his gestational age but is born too soon, and b) the small-for-date or small-for-gestational age infant who is full-term but has not grown properly in utero.

Small-for-date infants have been further divided into those with "intrinsic" growth failure and those with "extrinsic" growth failure (figure 1). Intrinsic growth failure results from congential malformations, inborn errors of metabolism and other genetic diseases. Whatever caused the growth failure is intrinsic to the fetus and does not involve

the placenta (6). Extrinsic growth failure is caused by abnormalities in the maternal and fetal environment.

Two types of extrinsic growth failure have been described (6). Type 1 generally is caused by maternal vascular disease. There is asymmetrical growth failure in the fetus. The brain seems to be of normal size and weight, but the liver is reduced in size and depleted in glycogen. Type 2 is caused by maternal malnutrition. Fetal growth failure is symmetrical in all organs. The brain and liver are reduced in size in proportion to body size.

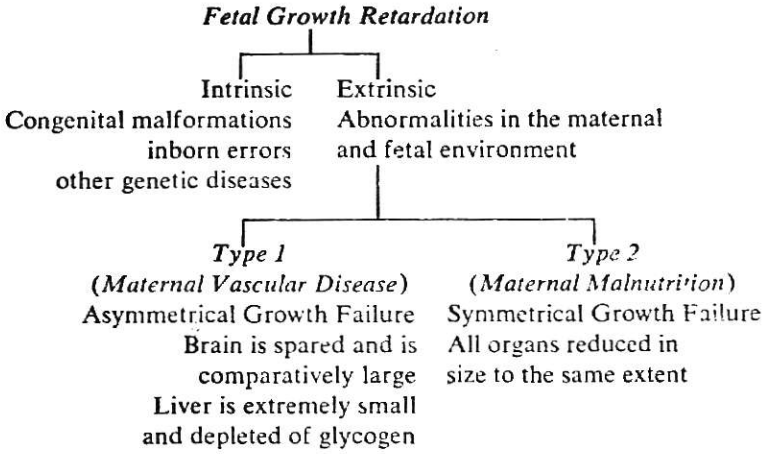

Fig. 1 Classification of fetal growth retardation (6).

Winick (6) stated that Type 1 fetal growth failure characterizes the vast majority of small-for-gestational age infants in developed societies; while Type 2 is the more prevalent form in developing countries and probably within the poorer segments of the United States. The purpose of this paper was to review current research concerning the relationship of maternal malnutrition to cellular brain growth and mental development of the offspring. The paper is divided into three main sections: normal growth and development of the brain, influence of malnutrition on brain development, and relationship of fetal malnutrition and mental development.

NORMAL DEVELOPMENT OF THE NERVOUS SYSTEM

Structural Development of the Nervous System

The human nervous system develops from the neural plate, a thickened area of embryonic ectoderm which appears during the third week of gestation. This plate becomes infolded to form a neural groove and neural folds. The neural folds fuse to form the neural tube which differentiates into the central nervous system, consisting of the brain and spinal cord. During the fourth week, the neural tube grows rapidly and forms the three primary brain vesicles: the forebrain, the midbrain and the hindbrain. The development of the adult shape of the brain is accomplished very early although the brain is still very immature. The forebrain gives rise to the cerebrum; the midbrain becomes the adult midbrain; and the hindbrain gives rise to the pons, cerebellum and medulla oblongata. The walls of the neural tube become thickened by proliferation of neuroepithelial cells which give rise to all nerve and macrogilial cells in the central nervous system. The neural crest cells differentiate into the posterior root ganglia, the sensory ganglia of the cranial nerves, autonomic ganglia and the Schwann cells. During this early period of organogenesis, the central nervous system is acquiring its general adult shape through a process of differential growth accomplished by cell division and migration within the tissue. The basic stages of nervous system development are essentially the same in all mammals (7,8,9).

Principles of Cellular Growth

Enlargement of any organ during the growing period may result from an increase in the number of cells (hyperplasia), an increase in the size of

already existing cells (hypertrophy) or the simultaneous occurrence of both. The total number of cells of an organ can be calculated by determining the total organ DNA (deoxyribonecleic acid) content and dividing by the DNA content per diploid nucleus (10). The DNA content per diploid nucleus is a constant which has been determined for several species. All diploid cells in the rat contain 6.2 pg DNA; while in the human all diploid cells contain 6.0 pg DNA. The average weight and protein and lipid content per cell can be determined by analyzing the total amount of each of these components and dividing by the number of cells. The result can be expressed chemically as weight/DNA, protein/DNA, RNA (ribonucleic acid)/DNA or lipid/DNA ratio. An increase in total organ DNA content represents an increase in the number of cells (hyperplasia). Increases in the weight/DNA or protein/DNA ratio represent an increase in cell size (hypertrophy) (11,12).

Figure 2 diagrams the three phases of organ growth. In the first phase, there is rapid cell division (hyperplasia alone), but cell size remains constant. Weight, protein and DNA all increase proportionally. In the second phase, both cell number and cell size increase as DNA and protein content rise. However, the rate of DNA synthesis begins to decrease (cell division) while protein synthesis continues at the same rate. Thus, during this second phase there is an increase in the protein/DNA ratio (cell size). In the third phase, increase in cell size results as a consequence of a further reduction or stoppage in DNA synthesis and a continued accumulation of protein. Growth finally ceases when protein synthesis and degration come into equilibrium (6).

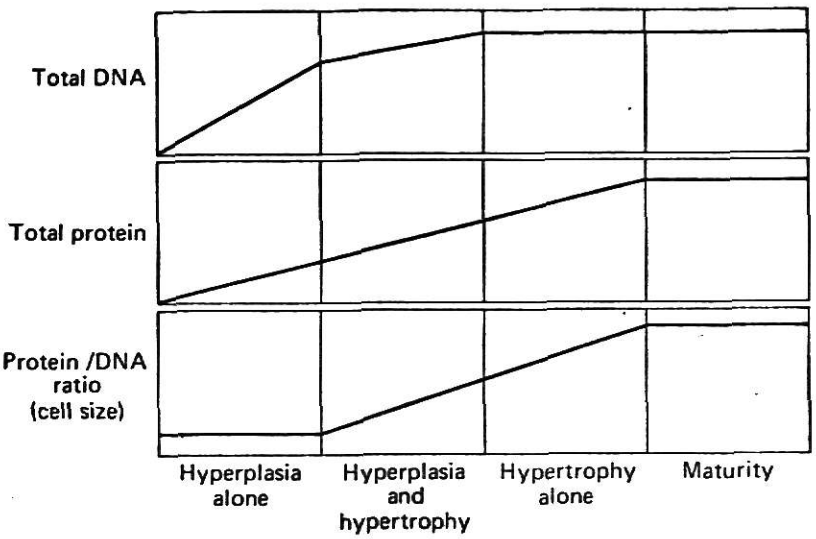

Fig. 2 Periods of cellular growth. Plotted above are the relationships between DNA and protein during the three phases of organ growth. It will be observed that DNA content crests and levels off well before organ size, as determined by protein accretion and weight gain, reaches its maximum (6).

Brain Growth In the rat, brain growth from conception to 13 postnatal days is exclusively by hyperplasia. Between 13 and 17 days it is by hyperplasia and hypertrophy and after 17 days by hypertrophy alone (11).

The basic stages of brain development are essentially the same in all mammals (9). There is a similar embryological period followed by a period of rapid neuronal cell division. Next the oligodendroglial cells multiply and synthesize myelin. The last stage is the intricate process of dendritic growth and branching and the establishment of synaptic connections. All species go through this same developmental sequence. The anatomical regions that compose the brain are similar in chemical composition and in metabolic and electrophysiological properties from one species to another (13).

The major difference (apart from the degree of complexity of the final product) is the variance in timing of these growth stages according

to species. The event of birth apparently has no significance to the structure and function of the nervous system. Rodents, rats and mice are born before the growth spurt; guinea-pigs have almost completed this stage at birth. From the point of view of growth spurt, animals can be arbitrarily divided into 'prenatal', perinatal' and 'postnatal' brain developers. Figure 3 is an attempt to classify certain animals in this manner. The shape of each curve is without significance since it is affected by the adjustments which had to be made to the time scale in order to fit several species into the same illustration. The main fact which emerges is that the timing of the brain growth spurt in relation to birth varies according to species. Therefore, such expressions as 'fetal brain' or 'neo-natal brain' or 'post-natal brain' are quite meaningless unless the species and its growth characteristics are known (9).

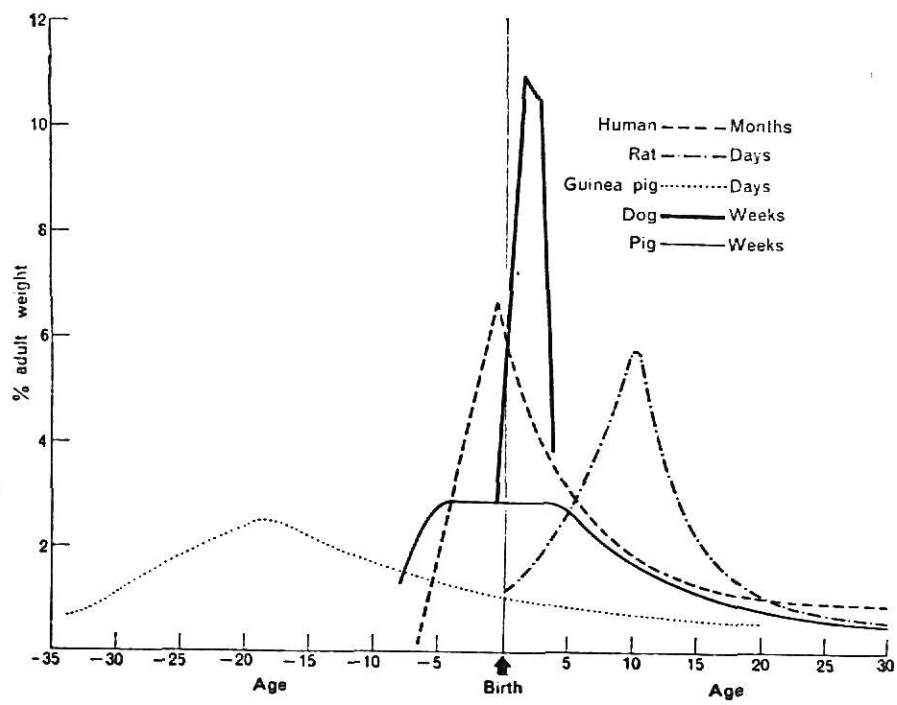

Fig. 3 Rate curves of brain growth in relation to birth in different species. Values are calculated at different time intervals for each species (9).

Weight and DNA, RNA and Protein Content of Whole Brain

The weight, and DNA, RNA and protein content of the whole rat brain have been studied (14). The weight of the whole brain increased linearly between 6 and 21 days of age. Whole brain protein content increased sharply from 49 mg at 6 days to 218 mg at 21 days (figure 4). Whole brain RNA increased from 2.18 mg to 4.91 mg and then tapered off. The whole brain DNA content increased threefold between 6 and 17 days but increased very little thereafter.

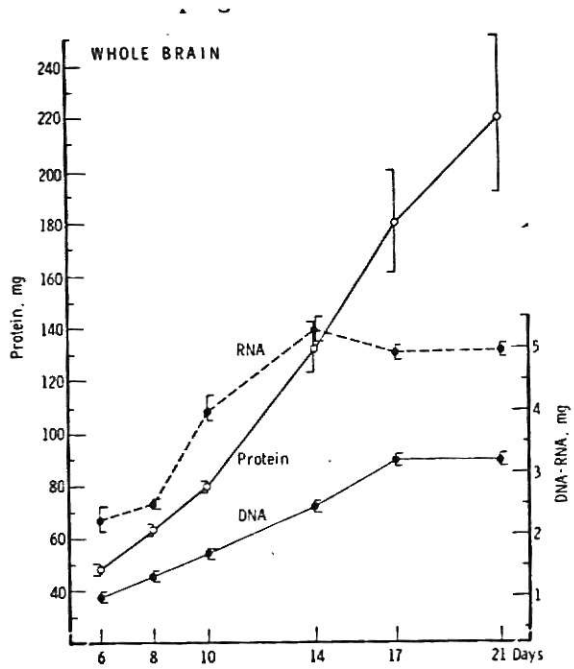

Fig. 4 DNA, RNA and protein content of whole rat brain in the developing rat. Each point represents a minimum of three animals. The ranges are indicated (14).

In the human, whole brain weight, protein and RNA increased linearly between 13 weeks gestation and 13 months of age after birth (16,17). At 13 weeks of gestation, the average brain weight was 5 g; at 13 months after birth, it reached 970 g (figure 5). During this same growth period, total brain protein increased from 193 mg to 54 g. Total RNA content increased

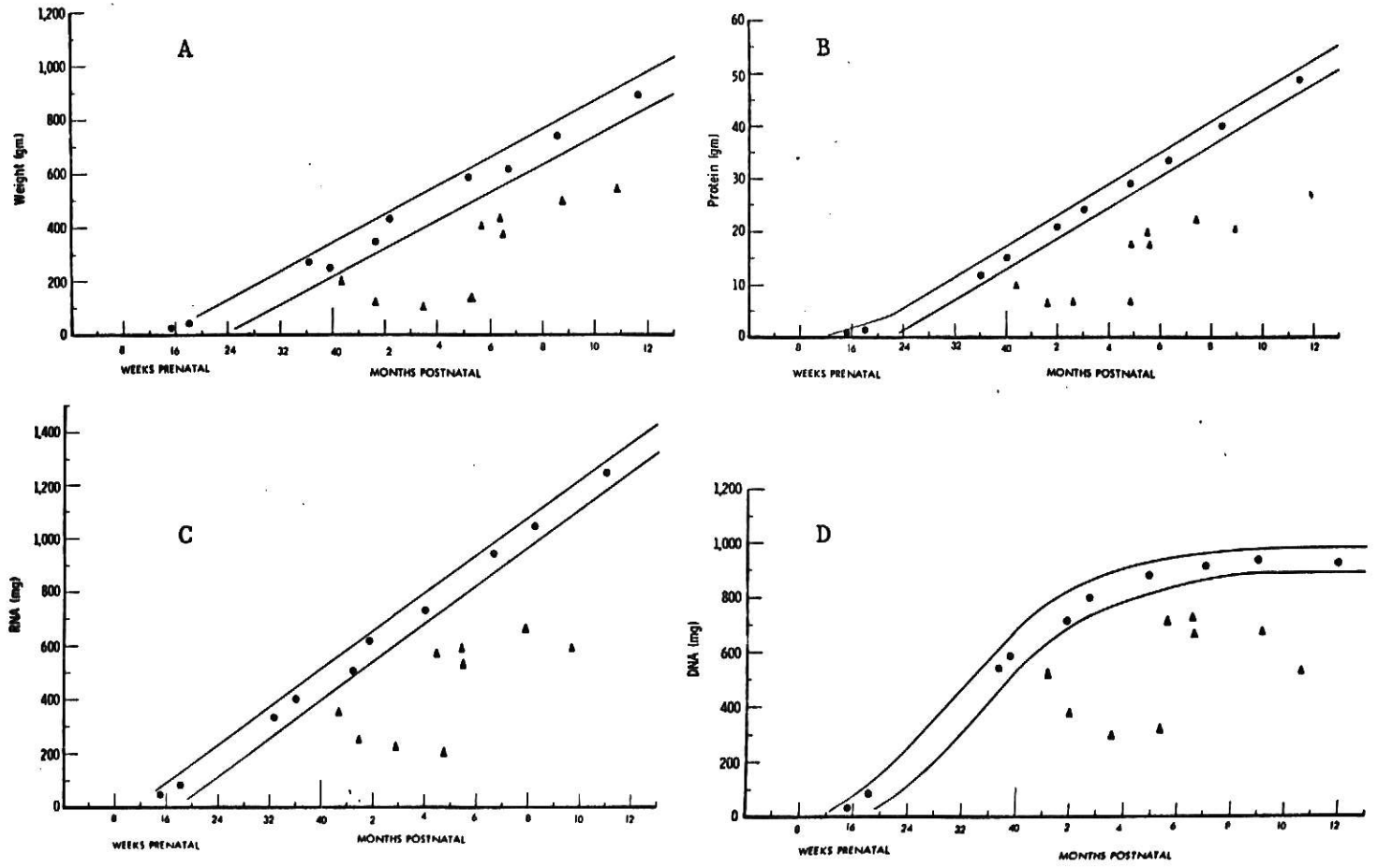

Fig. 5 Cellular growth in the brains of normal and malnourished children. Lines indicate normal range for U.S. population. o indicates normal Chilean children. Δ indicates Chilean children who died of severe malnutrition during first year of life. A. Total brain weight. B. Total protein content. C. Total RNA content. D. Total DNA content (14,15,17).

from 18 mg to 138 g. In contrast, the DNA content rose linearly from 25 mg at 13 weeks of gestation to 600 mg at birth and thereafter the slope of increase began to level off until a maximum was reached at about 5 months of age.

Winick's (15,16) data suggested that cell division in the human brain stops at 5 months of age. Dobbing and Sands (18,19) found that cell division continues into the second year of life. They showed that there are two spurts of DNA synthesis which occur normally during the development of the human brain (figures 6 and 7). The first growth spurt (primarily neuronal division) starts at about 10 to 18 weeks of gestation. The second spurt (oliogodendroglial multiplication) begins in mid-pregnancy and continues into the second postnatal year. According to their findings, 5/6 of the human brain growth spurt is postnatal.

Weight and DNA, RNA and Protein Content of Brain Regions

Not only do cellular growth rates vary among species, but individual brain regions in the same species undergo different patterns of growth. Rat In the rat, weight of the cerebrum, brainstem and hippocampus increased twofold between 6 and 21 days postnatally. During the same period, the weight of the cerebellum increased 6-fold. The weight of the cerebrum increased in a curvilinear fashion, while that of the cerebellum increased only slightly between 10 and 17 days. Weight in the brainstem increased slowly between 6 and 14 days and increased more rapidly thereafter (14).

DNA content of the cerebellum increased 8.5 times (figure 8) between 6 and 17 days. Cell division in the cerebrum progressed more slowly than in the cerebellum but continued for a longer period of time. DNA content

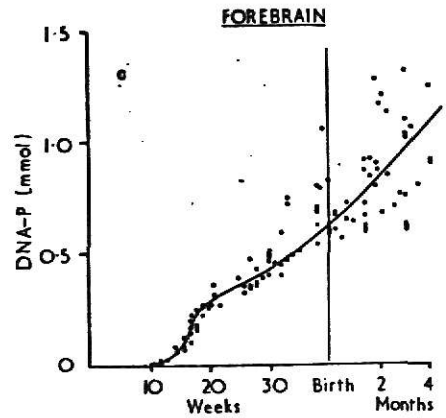

Fig. 6 The increase of whole brain DNA in developing human brain (18,19).

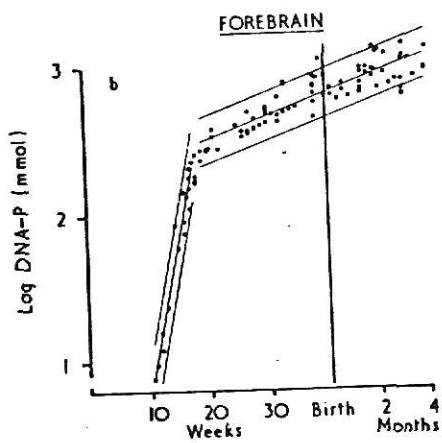

Fig. 7 A semilogarithmic plot of the data appearing in figure 6 to show the comparatively sharp separation of the two phases at 18 gestational weeks. Regression lines with 95% confidence limits are added (19).

of the cerebellum increased linearly from 0.428 mg to 1.01 mg between 6 and 21 days. DNA in the hippocampus did not increase except between days 14 and 17. This was believed to be due to a migration of cells from the lateral ventricle rather than cell division in the hippocampus (12). Most cell division in the brain stem occurred between 6 and 14 days when DNA content increased 65 percent. No further increase was seen after 14 days (12,14,16).

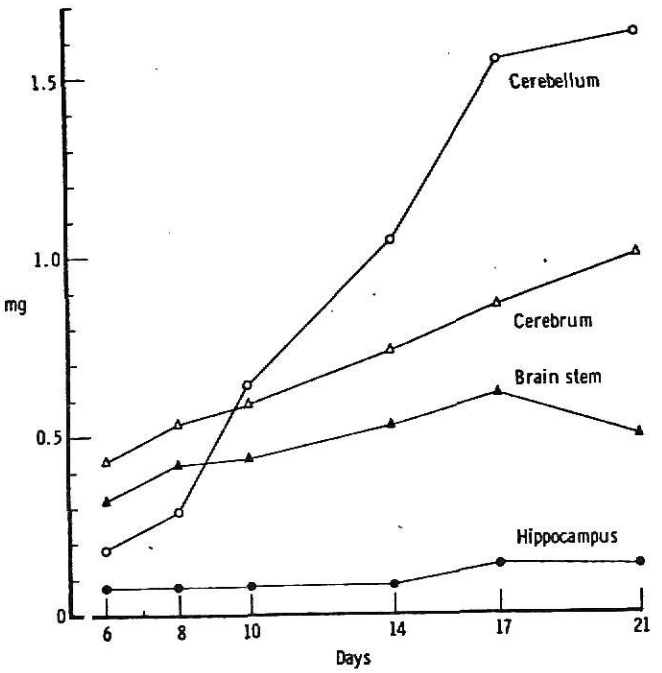

Fig. 8 Total DNA content in various regions of rat brain (16).

Cerebellar RNA increased almost fourfold between 6 and 14 days. Little increase was seen thereafter. Cerebrum RNA increased slowly from 1.09 mg to 2.36 mg between 6 and 21 days. In the hippocampus RNA increased until 14 days and then leveled off. Brain stem RNA increased sharply between 6 and 14 days and then tapered off (14).

Protein content of the rat cerebellum increased threefold between 6 and 14 days. Cerebral protein content continued to increase linearly

during the entire reference period. The exact rate of increase in protein in the hippocampus was unclear. It appears that there was a slow steady doubling between 6 and 17 days. In the brain stem, protein content increased 6-fold with the most rapid growth occurring after 10 days (14).

Human Patterns of cellular growth are not as well defined in the human brain regions as they are in the rat. Available data are based on relatively small numbers of human samples. Howard et al. (20) determined the cerebral and cerebellar weight and DNA and RNA content of 28 human fetuses following surgical interruption of pregnancy. Cerebral weight increased from 0.446 g to 178.09 g between 10 and 31 gestational weeks. The increases in DNA of cerebrum and cerebellum are plotted on logarithmic scales against gestational age in figures 9 and 10. The increase of the logarithm of cerebral DNA with respect to age is linear between 10 and 13 weeks, indicating an exponential rate of increase during this period. Thereafter the rate of increase declines, and the plot on an arithmetic scale is linear between 14 and 30 weeks.

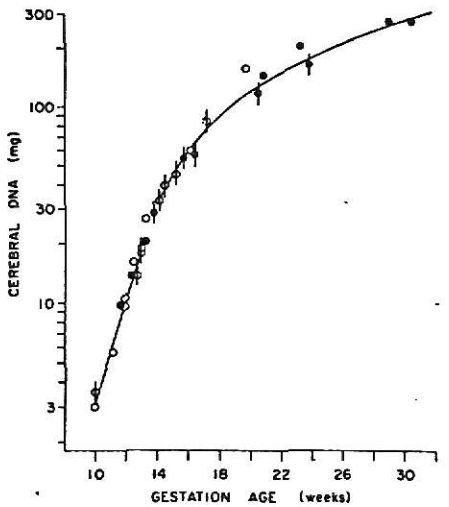

Fig. 9 The increase of total cerebral DNA during development of the human fetus. DNA in mg on a logarithmic scale. Each circle represents one brain. Solid circles, specimens from Uppsala; open circles, from Baltimore (20).

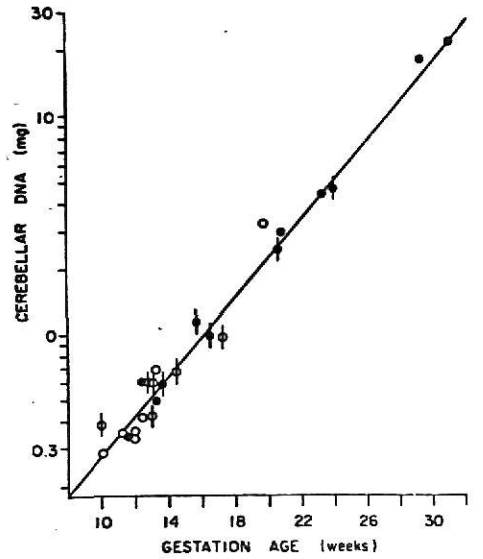

Fig. 10 The increase in total cerebellar DNA during development of the human fetus. Symbols as in figure 9 (20).

The cerebellar RNA/DNA ratio increased rapidly between 12 and 14 weeks. It continued to increase thereafter but at a slower rate (20). The slower rate of DNA increase in the cerebrum between 14 and 31 weeks suggests that cell division in some areas of the cerebrum may be terminated earlier than in the cerebellum.

Winick et al. (21) studied the cerebral and cerebellar DNA content of 12 normal children who died of accidents, poisonings or sudden death. Their data indicate that there was a progressive increase in wet weight, dry weight, total protein content and total RNA content in the cerebrum, cerebellum and brain stem during the first 2 years of life. In the cerebellum there was a 7-fold increase in wet weight, a 12-fold increase in dry weight and protein, and a 10-fold increase in RNA content between birth and 2 years. In the cerebrum, wet weight increased 3-fold, dry weight 4.5-fold, protein 9-fold and RNA 4-fold. Brain stem demonstrated a 3.6-fold rise in wet weight, a 4.5-fold rise in dry weight, an 8-fold rise in protein and a 5-fold increase in RNA content. These increases were

approximately linear in all three regions from birth to 2 years. Thus cerebrum increased in total mass and dry mass at one-half the rate of cerebellum. However, DNA content of cerebellum increased 4-fold during the first 8 to 10 months and only 0.2-fold thereafter (figure 11).

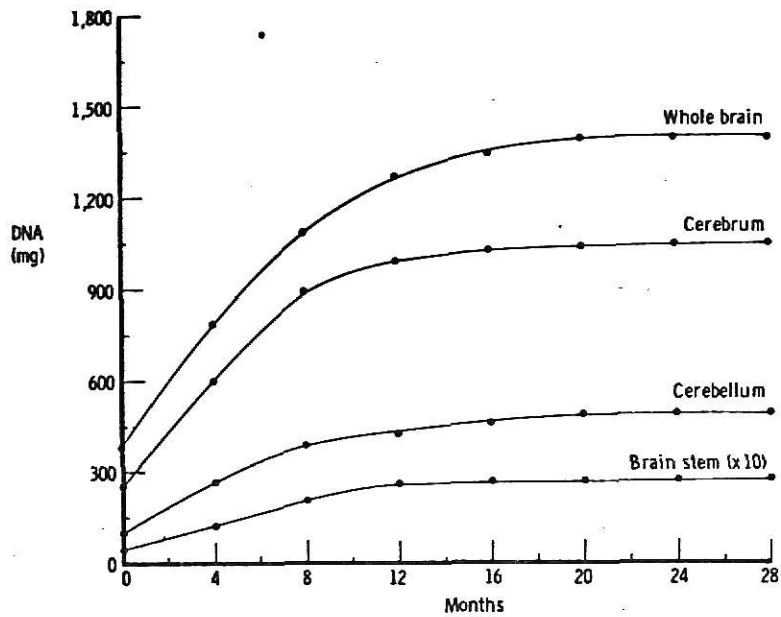

Fig. 11 DNA content in various regions of human brain during normal growth (6,21).

Cerebrum showed a 3-fold increase in cell number between birth and 6 to 8 months. Thereafter there was very little increase. Brain stem increased 2-fold to about 10 months and very little thereafter. Thus cell division proceeds at an only slightly faster rate in human cerebellum than in cerebrum (21).

Myelination

Myelination is accompanied by a progressive increase in brain function. It is always preceded by the proliferation of oligodendroglial cells. This is followed by glial cells surrounding the nerve axon in a

spiral fashion. When this "wrapping" process is completed, a progressive deposition of lipids begins within the myelin sheath. Myelin is a complex lipid made up of several components. The largest lipid component is cholesterol, but significant amounts of ethanolamine phosphatide, galactolipids, cerebrosides and cerebroside sulfates are also present (6,9).

There are at present no satisfactory morphological methods for measuring rate or degree of myelination. Analysis of component lipids or total lipids is used as a chemical index of myelination since there is very little myelin turnover within the brain (15,16). Most investigators have found a marked increase in total lipid content and concentration during early development in human, rat, dog and rabbit brain (22). Cholesterol, sphingomyelin, cerebrosides and sulfatides show a significant increase with age (23-25). Serial analysis of lipids in human brains indicated that the lipid/DNA ratio (the amount of lipid per cell) rose shortly after birth until at least 2 years of age (figure 12). This increase was reflected in a rise in both cholesterol/DNA ratio and phospholipid/DNA ratio. Postnatal lipid synthesis proceeded at a more rapid rate than DNA synthesis as a result of rapid myelination and the decrease in cell division (6,17).

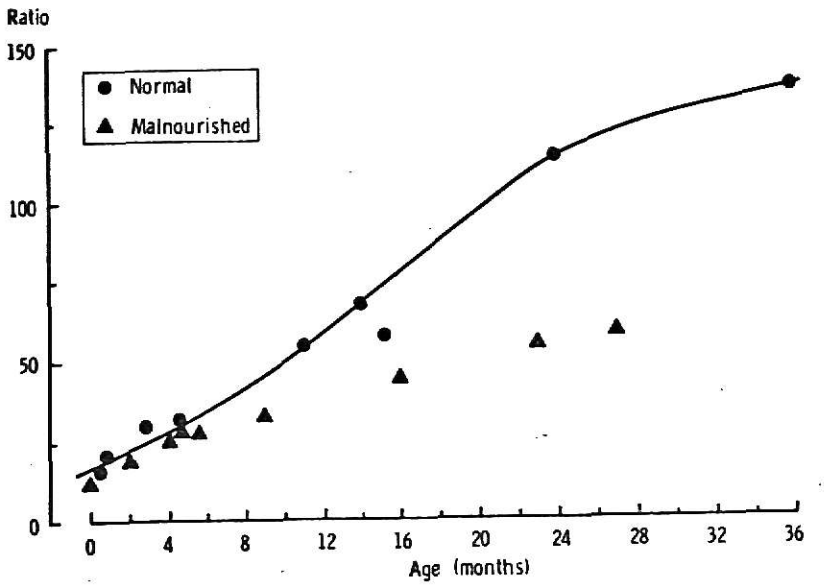

Fig. 12 Lipid/DNA ratio in normal and malnourished children at different ages (17).

MALNUTRITION AND DEVELOPMENT OF THE NERVOUS SYSTEM

Malnutrition and Cellular Growth of the Brain

Early organ growth of all nonregenerating tissues is due primarily to cell division and an increase in the number of cells. Later organ growth is due to hypertrophy with existing cells becoming larger. Winick (12,26) studied the effect of malnutrition on the stages of cellular growth in the rat. Three groups of rats were exposed to 21 days of caloric restriction at varying time intervals. The first group was malnourished from birth to 21 days, a period when all organs are growing by hyperplasia. This group showed a proportional reduction in brain weight, protein and DNA. Since the protein/DNA ratio was normal, the decrease was in the number of cells not in cell size. When refed, these animals remained with a deficit number of cells. In the second group, undernourished from 22 to 43 days of life, there was a decrease in the number of cells in all organs except the brain and lungs. These organs reached their normal cell number prior to 22 days. In the brain and lungs, cell size was reduced. When refed, the brain and lungs recovered, but the remainder of the organs were left with a deficit number of cells. In the last group, undernourished from 65 to 86 days, the normal number of cells had been reached in all body organs prior to undernourishment. This group suffered only a decrease in cell size. Cell size returned to normal in all organs upon refeeding.

Thus if malnutrition or undernutrition is imposed during the prolific phase of growth, the rate of cell division is slowed, and the ultimate number of cells is reduced. Winick's studies (12,26) indicate that the number of cells present in any organ at maturity is only

partially under genetic control. Environmental variables, such as nutrition, during the rapid phase of cellular growth affect the ultimate number of cells by altering the rate at which cell division occurs during the time prescribed by the genetic make-up of the animal. Malnutrition slows the rate of cell division, but cells continue to divide for the same period of time in the malnourished animal as in the normal animal (6).

Since fetal growth is characteristically of the prolific type, it should be particularly vulnerable to malnutrition. However, the fetus is isolated from the environment by the mother and placenta. Animal and human studies were examined to determine whether the mother or placenta provide any fetal protection (27).

Malnutrition and Brain Development in the Rat

Animal models can be useful to isolate the effects of nutrition alone on brain development. Care must be taken when extrapolating results of animal studies to humans. In studies on brain development, stages of development must be compared. Dobbing (13) hypothesized that the rat brain at birth is equivalent in developmental stage to the human fetal brain of 18 weeks gestation; the human brain at birth is developmentally equivalent to a rat brain of 5 to 7 days. The exact comparison of human and rat developmental stages has not been unanimously agreed upon; therefore, both pre- and postnatal studies of the rat were reviewed.

Body Weight Many investigators studied the effect of maternal malnutrition on the body weight of offspring. Chow and associates (28,29) reported that reduction in dietary intake by as little as 25% of the control resulted in growth stunting of the progenies. Other researchers investigated the effect of maternal protein restriction on birth weight of

offspring (30-32). Varying levels of protein restriction also resulted in decreased birth weight in offspring. Other workers (33,34) found that diets deficient in one essential amino acid retarded the growth of offspring when compared to controls.

Malnutrition and undernutrition for varying periods postnatally in the rat, pig and dog resulted in a decrease in body weight (35-38). Even after ad libitum refeeding, the animals never reached the body weight of controls.

Weight and DNA, RNA and Lipid Content of Whole Brain Many studies have been done on maternal dietary restrictions during gestation in rats. Some workers (39-42) found a reduction in total brain weight, total cell number and total protein content of the whole brain. Others (40-45) found that the cerebrum showed a greater reduction in DNA (cell number) and protein content than other regions in the rat brain.

Postnatal undernutrition in the rat may be a more accurate model for human fetal malnutrition. Culley and Lineberger (36) studied the effect of undernutrition in the rat from 5 until 11, 17 and 60 days of age on various brain parameters (table 1). Whole brain weights were significantly less in all rats on restricted diets when compared to controls. Brains from rats on the restricted feeding regimen until 11, 17 and 60 days each contained significantly less DNA and RNA than controls of the same age ($P < 0.01$). The amount of DNA in rats restricted until 17 and 60 days was not increased by ad libitum feeding until 110 days of age even though there was a greater than 50% increase in brain weight. If ad libitum feeding was begun at 11 days of age (prior to the time the brain stops accumulating DNA), some of the deficit in DNA was overcome.

TABLE 1

Effect of feed restriction on the composition of rat brain (36)

Regimen [1]	A11	A17	A60	A110	R11	R11A110	R17	R17A110	R60	R60A110
No. of rats	12	10	12	12	10	10	10	10	12	12
Body wt, g	23 ± 2	37 ± 3	247 ± 25	428 ± 54	13 ± 1	392 ± 48	16 ± 1	321 ± 45	24 ± 1	289 ± 40
Brain wt, mg	1021 ± 41	1390 ± 57	1823 ± 37	2083 ± 105	801 ± 32	1841 ± 98	1010 ± 36	1708 ± 87	1102 ± 53	1672 ± 72
DNA, mg /brain	1.77 ± 0.10 [2]	2.46 ± 0.09	2.56 ± 0.08	2.52 ± 0.11	1.35 ± 0.07	2.29 ± 0.11	2.09 ± 0.09	2.15 ± 0.09	2.12 ± 0.09	2.07 ± 0.12
DNA, % [3]	0.173 ± 0.011	0.177 ± 0.008	0.140 ± 0.005	0.121 ± 0.007	0.169 ± 0.005	0.124 ± 0.006	0.207 ± 0.010	0.126 ± 0.006	0.192 ± 0.009	0.124 ± 0.007
RNA, %	0.259 ± 0.011	0.274 ± 0.013	0.180 ± 0.008	0.162 ± 0.010	0.281 ± 0.015	0.157 ± 0.008	0.321 ± 0.019	0.163 ± 0.008	0.230 ± 0.012	0.159 ± 0.009
Lipid, %	5.8 ± 0.2	7.0 ± 0.3	10.6 ± 0.3	11.8 ± 0.3	5.3 ± 0.2	11.2 ± 0.4	6.2 ± 0.2	10.7 ± 0.3	9.4 ± 0.3	10.5 ± 0.4
Protein N, %	1.12 ± 0.06	1.30 ± 0.08	1.74 ± 0.07	1.85 ± 0.09	1.14 ± 0.07	1.87 ± 0.07	1.26 ± 0.06	1.84 ± 0.07	1.69 ± 0.08	1.82 ± 0.09

[1] A11, A17, A60 and A110 indicate animals fed ad libitum from birth until killed at 11, 17, 60 and 110 days, respectively; R11, R17 and R60 indicate animals fed limited amounts from 5 days of age until killed at 11, 17 and 60 days respectively; R11A110, R17A110 and R60A110 indicate animals fed limited amounts (similar to R11, R17 and R60) from 5 days of age until 11, 17 and 60 days of age, respectively, and then fed ad libitum until 110 days of age.

[2] Mean ± SD for five or six samples (two brains per sample).

[3] Percentage of the component in brain.

The brains of rats restricted from 5 until 11, 17 and 60 days contained significantly lower percentages, as well as total amounts, of lipid than the brains of animals fed ad libitum the entire period. After ad libitum refeeding of restricted rats until 110 days of age, the percentage and total amount of brain lipid remained significantly lower ($P < 0.01$) than noted for the control brains (36). The timing and duration of underfeeding and refeeding affected the subsequent weight and DNA, RNA and lipid content of the brain.

DNA, RNA and Protein of Brain Regions Fish and Winick (46) studied the effect of malnutrition on regional growth of the developing rat brain. There was a 35% deficit in cerebellar DNA content of malnourished animals by 6 days. This deficit remained relatively constant throughout the rest of development (figure 13). The cells in the cerebrum divide more slowly than in the cerebellum. The effects of malnutrition on cell number (DNA content) were not seen until 14 days (80% of normal). These effects became more severe as the animal aged. There was no significant difference between the DNA content of the hippocampus of the control and malnourished animals between 6 and 14 days. However, at 17 days, when the DNA content normally rises, a 20% deficit was observed in the malnourished animals.

There was a 50% reduction in total protein by 21 days in the malnourished animals when compared to the controls. Very little difference in protein content in the hippocampus was seen between the two groups. These data demonstrate that the effect of malnutrition is proportional to the rate and type of growth in the region studied. Cell division was curtailed earliest and most severely in those parts where cells were rapidly dividing (46). In summary, maternal restriction of calories, protein or both resulted in a decrease in birth weight, brain

weight and brain DNA and RNA content in the offspring. Malnutrition affected cell number in the cerebrum more than in other areas. Thus it appears that in the rat the maternal-placental barrier is not effective in protecting fetal brain from cellular effects caused by maternal food restriction. Postnatal malnutrition or undernutrition of rats resulted in a decrease in body weight, brain weight and DNA content of the brain. Some of the deficits could be regained if refeeding was begun before cell division stopped. Postnatally, the cerebellum suffered a greater reduction in cell number as a result of malnutrition than did the cerebrum and hippocampus.

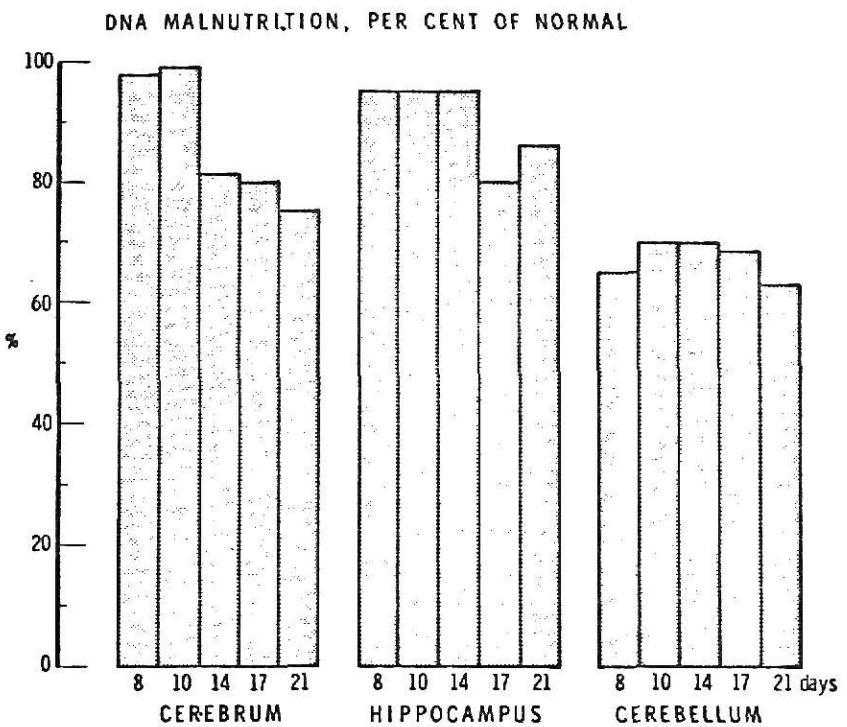

Fig. 13 Effect of malnutrition on regional growth of the rat brain (46).