EFFECTS OF PRE- AND POST-NATAL MALNUTRITION ON BRAIN PHYSIOLOGY

by

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INTRODUCTION

In recent years scientists, politicians and journalists have shown increasing interest in the incidence of malnutrition in infants and children and its effect on brain development. Estimates of the number of children suffering from some form of undernutrition are staggering. Some ten million children are severely undernourished, 80 million are moderately undernourished and 130-160 million are experiencing mild undernutrition (1).

Studies attempting to show a cause and effect relationship between the effects of pre- and post-natal malnutrition on brain development and subsequent mental development have received widely publicized attention. Whether there may be a correlation here as pointed out by Winick (2) or just one parameter operating in a poor environment (1, 3), the issue is one that requires continued investigation.

MALNUTRITION, BRAIN GROWTH AND DEVELOPMENT AND MENTAL CAPACITY

Effects on Brain Growth and Development in Experimental Animals

Studies investigating the effect of malnutrition on brain growth and development have produced varying results. This has been due in part to the animal studied and the duration and timing of malnutrition. Animal species differ regarding the pre- and post-natal stage of brain development. Figure 1 represents the timing of birth in relation to the brain "growth spurt," the most rapid phase of brain growth (4).

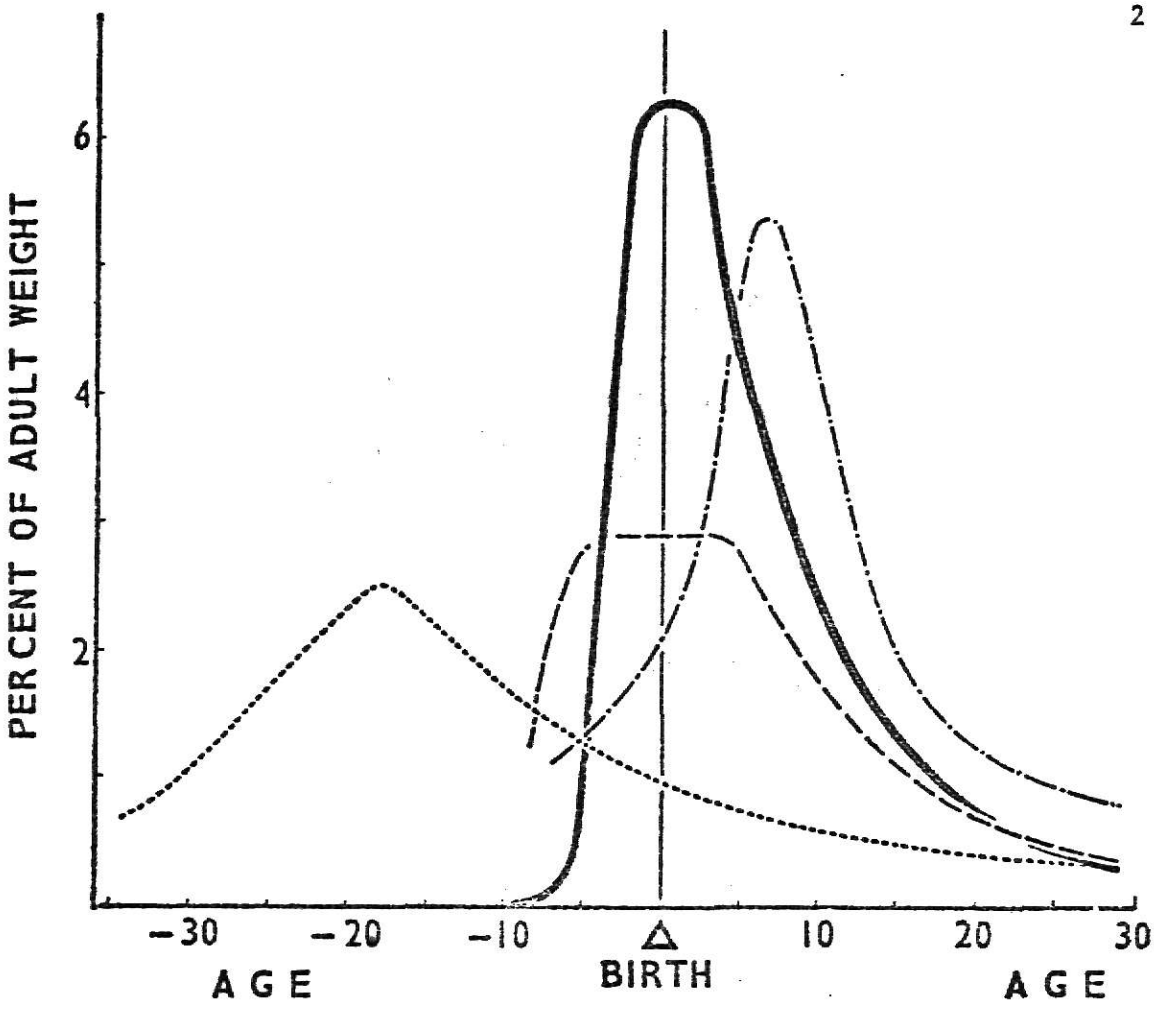

Figure 1. Velocity of human brain growth (wet weight) compared with that in other species. Prenatal and postnatal age expressed as follows: human _____ in months; guinea pig ----- in days; pig _ _ _ _ in weeks; rat__.__.__. in days (4).

The concept of heightened sensitivity of the brain to the effects of malnutrition is related to this period of rapid brain growth. The effects of malnutrition induced post-natally on the brain of the guinea pig would be expected to be different from the effects of malnutrition imposed at the same time on the brain of the rat.

Within the same spcies the effect of malnutrition at various stages of pre- and post-natal development would be different. If malnutrition occurred during the period of hyperplasia (growth by increase

in cell number), there would be a decrease in cell number. Malnutrition during the hypertrophy phase (growth by increase in cell size) would decrease cell size.

Decreased brain weight as a consequence of malnutrition has been reported (5, 6, 7). Castellano and Oliveria (7), working with mouse pups protein-calorie malnourished post-natally, observed brain weights at 16 to 24 days that were 30 percent lower than the controls. The mice were weaned at 21 days of age and given unlimited access to a stock diet. During this rehabilitation period of 30 to 60 days the deficit in brain weight was not reversed.

Total organ DNA content is a reflection of cell number. By determining total organ DNA content and dividing by the DNA content per cell one arrives at the number of cells. Dividing the weight or total protein content of the organ by cell number yields a figure for weight per cell or protein content per cell. This represents a measure of individual cell size.

Winick (2), in his studies on brain growth in rat pups protein-calorie restricted during the first 21 days of life, noted a decrease in total DNA content in cerebellum and cerebrum. This decrease in DNA content occurred earlier and was greater in the cerebellum than in the cerebrum. Cell division was most rapid in the area of the cerebellum and showed the greatest effects of malnutrition. DNA content was slightly more decreased in the cerebellum than in the cerebrum of rat pups undernourished by pre- and post-natal quantitative restriction of the dam's diet (5).

Complete protein deprivation for short periods of time during gestation resulted in fetuses with significant decreases in cerebral DNA and cerebral protein (6). Decreases tended to be larger if the period of deprivation was prolonged and if it occurred later in pregnancy. Zamenhoff et al. (8), working with pre- and post-natal protein restricted F_1 and F_2 generation rat pups, observed significant decreases in all measured parameters, ie. body weight, cerebral weight, DNA and protein. At the age of 30 days those deficiencies remained significant for F_1 but not for F_2 generations. Hill (9), in his work with rhesus monkey fetuses restricted by placental insufficiency, reported cerebral DNA unchanged while cerebellum DNA was significantly lower.

Rats fed thiamin-free diets post-natally showed depressed DNA that increased with the extent and duration of thiamin deprivation (10). The coenzyme thiamin pyrophosphate (TPP) functions in the oxidative decarboxylation of α-keto acids to carboxylic acids in the Krebs cycle. If TPP is not available then energy production from glucose metabolism is reduced which reduces the amount of available energy for brain synthesis. There was a rapid increase in DNA synthesis when thiamin was administered to the deficient rats.

The type of cell affected depends on what cell types are dividing at the time of the insult. During the first 21 days of post-natal life, the neurons of the rat cerebral cortex are not dividing and are not affected. The glial cells are dividing, however, and are reduced in number by protein-calorie malnutrition (2). In the cerebellum all of the cell types are retarded in their cell division during post-natal malnutrition.

Total number of Purkinje cells and Bergman cells were significantly decreased in the brains of 35 day old rat pups protein-calorie malnourished from gestation through weaning. The number of basket cells and astrocytes were decreased while the number of Golgi cells and stellate cells were not changed. The decrease in glial cells was 44 percent compared to a 13 percent decrease in neurons (5).

There is evidence that malnutrition delays the maturation of cells. Clos et al. (5) observed immature Purkinje cell bodies ten days post-natally in rat pups protein-calorie restricted from gestation through weaning. At later stages the cell bodies were smaller by 19 percent. Johnson and Yoesle (11) found no effects of pre- and post-natal protein restriction on neuron size in rat pups. An increase was observed in the relative number of small and medium size neurons/sq. mm, indicating a decrease in the number of neurons reaching maturity.

Disruption in the development of neuronal processes may be an even more important effect of malnutrition on the developing brain than all of the previously discussed parameters. The increase in dendritic complexity and the establishment of synaptic connections is fundamental to brain function (4). Reduction in both amplitude and number of dendrites in the pyramidal cell basilar dendritic tree was observed in the occipital cortex of post-natal protein-calorie restricted rat pups (12). Sima and Persson (13), studying pre- and post-natal protein-calorie undernutrition in rat pups, recorded a delay in the outgrowth of the apical dendrite in Purkinje cells until the ninth day. Differences in apical dendrite growth were recorded up to the eleventh day. Dendritic thickness appeared slightly reduced in the visual cortex of pre- and

post-natal protein restricted rat pups (14).

Spine density was reduced by nine percent to 22 percent on dendritic processes measured and reductions in dendritic length were found in the cerebellum for all cell types measured (14). Observations of brains from post-natal protein restricted rat pups sacrificed at 7, 19, 12 and 15 days of age showed the number of spines, the basilar dendritic density and the dendritic thickness all significantly reduced (15). These findings suggest an altered pattern of interconnections between neurons or a decrease in synaptic connectivity. There is evidence to suggest that the number of synaptic vesicles per terminal unit area is decreased (1). If this decrease in synaptic vesicles affects a decreased level in neurotransmitters then the neurotransmitter process could be altered.

Effects on Brain Growth and Development in Humans

Information regarding the effect of malnutrition on brain growth and development in children is difficult to obtain and at best is sparse. Human subjects cannot be used in the controlled experiments in which laboratory animals are placed. Reported data on various measurements from children suffering from kwashiorkor or marasmus has provided some information. The remainder comes from laboratory experiments on test animals but this information is subject to careful scrutiny before any comparisons can be made.

Measurement of head circumference, brain weight and transillumination of the skull are parameters that have been studied in determining the effects of malnutrition on brain growth and development in humans. In a study of 53 Ethiopian infants diagnosed as suffering from

kwashiorkor or marasmus-kwashiorkor, the head circumference was equal to or below the 50th percentile of Swedish standards and the heads showed increased transillumination (16, 17). A study of over 1000 autopsies in Ugandan children noted that the mean brain weights were significantly lower in children diagnosed as malnourished (18).

Studies on a group of nine children from Santiago, Chile, who died with severe malnutrition, showed reduced brain weights and decreased amounts of brain DNA, RNA and protein (19). The reduction in protein RNA was proportional to the reduction in DNA. This is consistent with the findings of reduced cell number in brains of malnourished infants.

The data suggest that severe malnutrition in infancy results in a reduction in brain size due to reduced brain cellularity.

Effects on Mental Performance in Experimental Animals

Investigators have taken their research one step further to note any changes in behavior or mental performance.

Castellano and Oliverio (7), working with post-natal protein-calorie malnourished mouse pups, reported several changes. Some reflex activities such as rooting and cliff aversion were not affected while others, such as righting, placing or grasping, clearly were retarded. Malnutrition exerted a negative effect on the development of motor and sensory behavioral activities. Exploratory activity in large litters was twice as high as in the small litters probably due to heightened excitability. Mice from small litters learned the avoidance response faster and reached a higher performance than those from large litters.

Correlation between decreased levels of cerebellar DNA and locomotor activity has been reported by Rich and Weichsel (20) who studied

post-natal protein-calorie restricted rat pups. No correlation was reported, however, for decreased cerebral DNA levels.

Hyden and Range (21) reported aberrant behavior in pre- and post-natal protein-calorie restricted rat pups. In reversal learning, the malnourished rats are significantly superior in attainment. This is possibly due to their higher motivation, being hungrier than the controls. In re-reversal learning, the malnourished rats have a drop in performance and low number of correct responses.

Effects on Mental Performance in Humans

Information on the effect of restricted food intake on human mental capacity has been more easily obtained than information of the effect of restricted food intake on brain development. Mental performance tests can be administered to subjects who were or are malnourished.

Stein and co-workers (22) studied the offspring of pregnant women who received only 450 calories per day during the German occupation of Holland. Results of intelligence test scores indicated that starvation during pregnancy had no effect on adult mental performance of the surviving male offspring.

A study of Aborigine school children whose diet was deficient during infancy showed that their school marks were significantly reduced (23). Other workers have reported retardation in perceptual and abstract abilities, intersensory organization and intelligence levels (24).

Cravioto and Robles (18), working with Mexican children recovering from kwashiorkor, reported lower than expected scores in four fields of behavior (motor, adaptive, language and personal-social). The

children, who were over six months of age at the time of their illness, showed markedly improved test scores after rehabilitation while those children under six months of age improved relatively little.

At the Nutrition Research Laboratories in Hyderabad, India, children eight to eleven years of age who at one time had been treated for kwashiorkor showed markedly lower test scores (18).

Kugelmass et al. (18) reported results from a study of malnourished children in New York. Two groups of children were tested, those malnourished and those not. The first test was administered while one group was still malnourished. The second test was administered when both groups were well nourished. Among the previously malnourished group, test scores improved by a mean of 18 points while in the originally well nourished group no change in test scores was recorded.

Ellis and Hill (25) compared two groups of children with cystic fibrosis; one group had been severely malnourished in the first year of life and the second group had not been malnourished (25). No significant differences in mean scores between groups were observed as measured by the Weschler Intelligence Scale (WISC).

A conclusion of a cause and effect relationship between malnutrition and mental capacity would be premature. Research does indicate that malnutrition affects brain development. The extent of the effect of the insult varies within the same species, among different species, and according to timing, severity and duration.

Results from tests assessing behavior and mental capacity in malnourished subjects are varied and inconclusive. The insult of malnutrition on behavior and mental capacity may be only one of several

operant factors.

More at the root of the problem might be to identify what brain functions are altered or distorted by malnutrition. The purpose of this paper is to review current research on the relationship of malnutrition to various parameters of brain function.

NORMAL BRAIN GROWTH AND DEVELOPMENT AND FUNCTION

Growth and Development in Rats and Humans

Three distinct phases of growth account for the overall enlargement of the brain (Figure 2). In the first stage, growth is due to an increase in cell number, hyperplasia. There is a proportional increase in weight, protein and DNA content. The end of this phase begins when DNA synthesis begins to slow and weight and protein content continues to rise, a phase of hyperplasia and hypertrophy. The third phase is marked by cessation in DNA synthesis and continued increase in weight and protein content, hypertrophy.

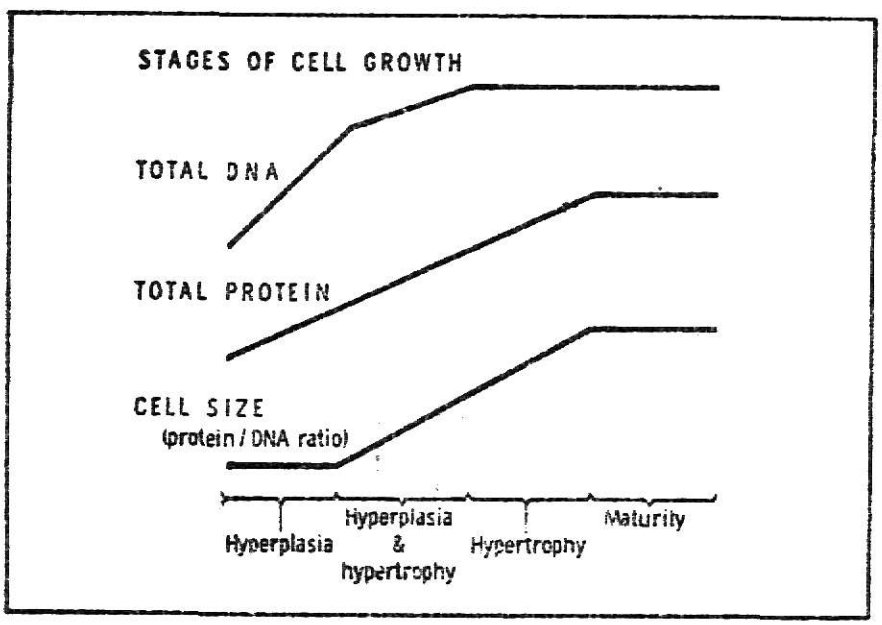

Figure 2. Stages of cell growth (2).

The division of cells destined to become neurons ceases early in neurogenesis. These neuroblasts undergo a series of migrations followed by an outgrowth of the neuronal process. The growth spurt in mammalian species commences once neuronal multiplication has ceased, and encompasses the elaboration of neuronal connections, glial multiplication and myelination. These events are depicted in Figure 3 (1).

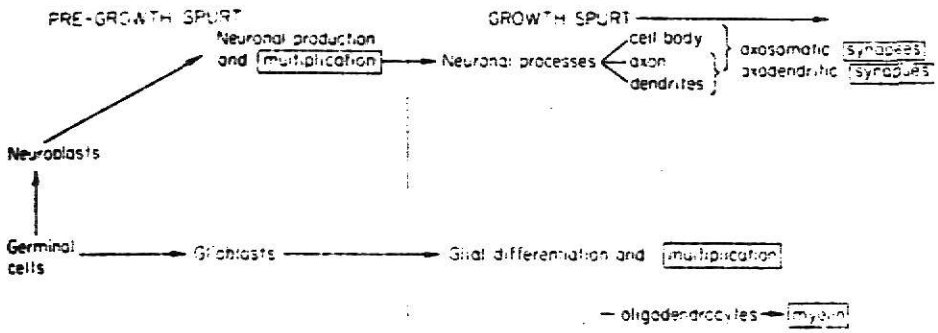

Figure 3. The principal constituents of the brain growth spurt, based on experimental work with laboratory animals (principally the rat) (1).

The development of neuronal processes (axons and dendrites), the establishment of the synaptic connections between these neuronal processes, and the beginning of myelin production by oligodendrocytes takes place during this growth spurt (1, 25, 26).

Concommitantly, there is an increase in concentration of components of nerve tissue. Included are the glial specific S-100 proteins, the neurol-specific 14.3.2 proteins and many enzymes associated with neurotransmitter metabolism including acetylcholinesterase, choline

acetylase, monoamine oxidase and glutamic acid decarboxylase. Also, an increase is observed in the concentration of neurotransmitters such as acetylcholine, norepinephrine and dopamine (26).

In the rat brain DNA synthesis ceases 20 days after birth (Figure 4) (2). Net protein synthesis continues until about 99 days after birth. Examining normal cellular growth in rat brain reveals that different regions undergo a different pattern of growth (Figure 5) (2). In cerebellum, DNA synthesis continues until 17 days post-natally. In contrast, DNA synthesis in the cerebrum proceeds more slowly but lasts until 21 days post-natally. Brain stem DNA increases until the fourteenth day post-natally, then levels off. Total protein content in the cerebellum decreases with growth while in the cerebrum protein content continues until around 99 days of age (26, 27).

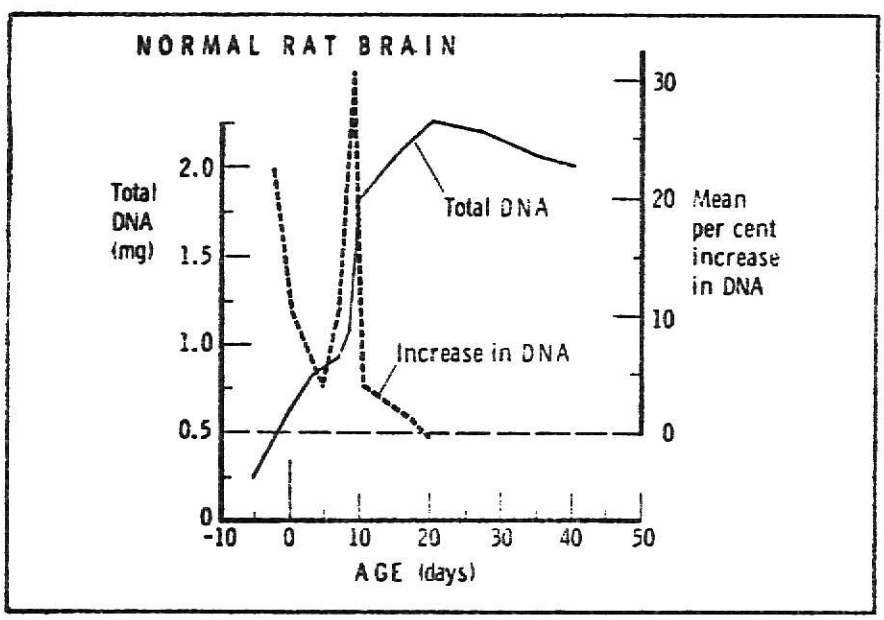

Figure 4. DNA synthesis in normal rat brain (2).

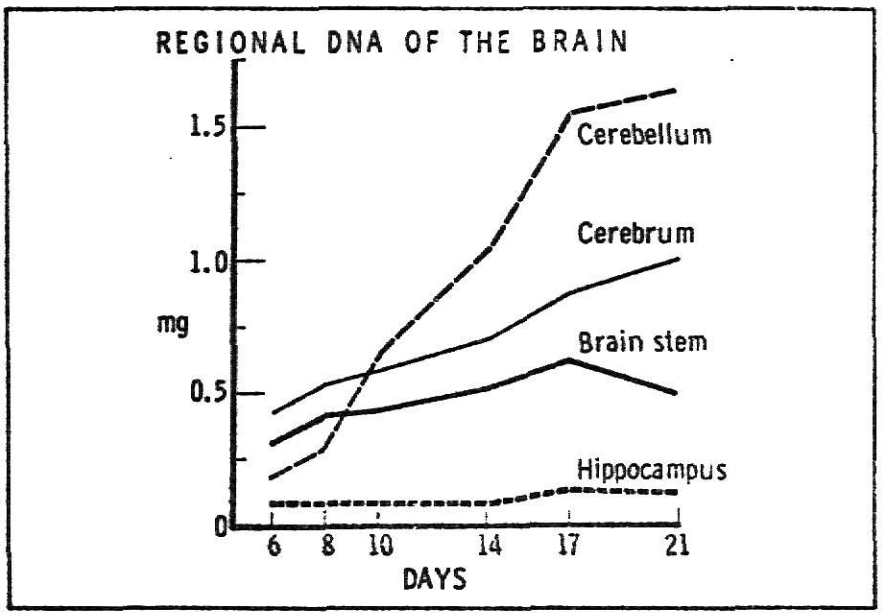

Figure 5. Normal cellular growth in regions of the rat brain (2).

Studies of the human brain indicate that DNA synthesis which is linear pre-natally, begins to slow down shortly after birth. A maximum DNA content is reached around 8 or 12 months of age (Figure 6) (26). Recent evidence suggests there may be some cell division in the human brain until 18 months. Moreover, two peaks of cell division have been observed in the human brain. One peak occurs at about 26 weeks of gestation and is ascribed to neuronal cell division. The second peak occurs shortly after birth and is ascribed to glial cell division (Figure 7) (26). Data on cellular growth of various regions of human brain is limited. The available data suggests that the rate of cell division post-natally is about the same in cerebrum and cerebellum and stops at about the same time in both areas. In the brain stem, DNA synthesis continues until at least one year of age (26, 27).

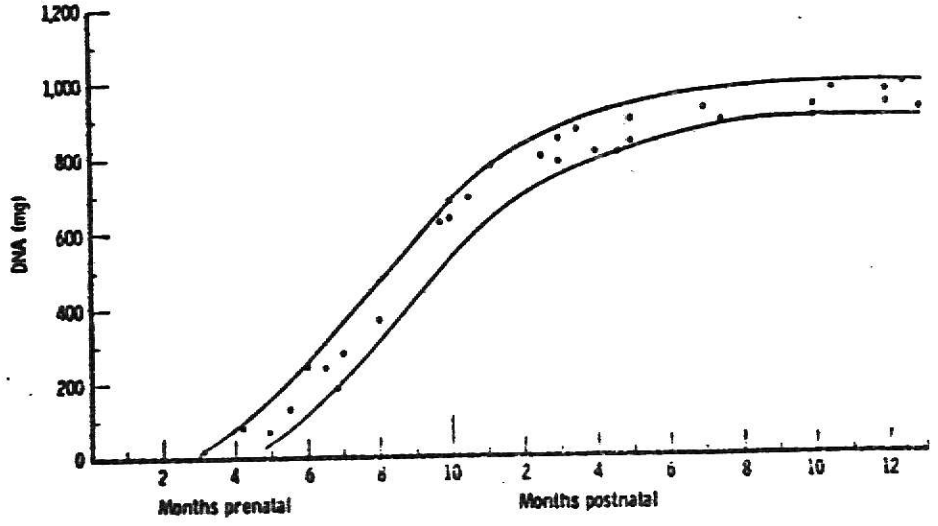

Figure 6. Total DNA content of human brain derived from 31 cases of therapeutic abortions, crib deaths, accidental deaths, and poisonings (26).

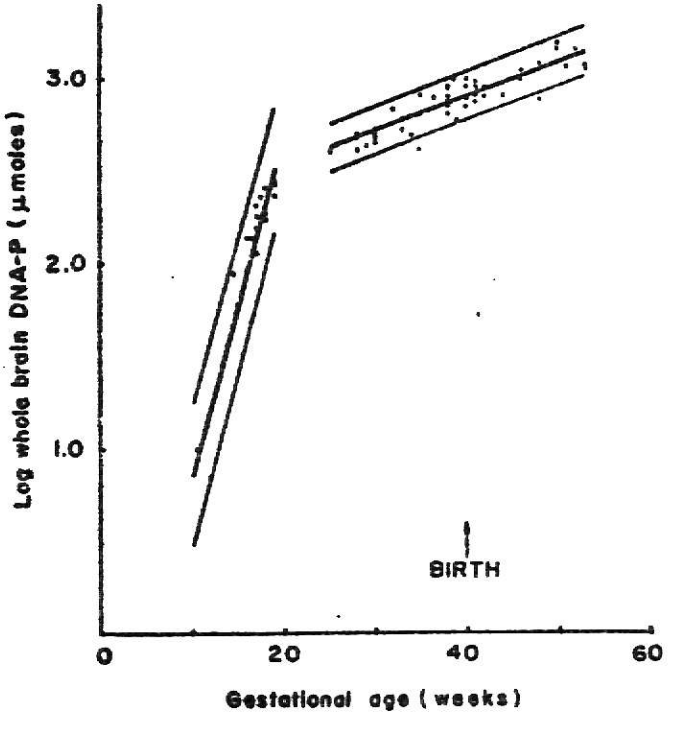

Figure 7. The logarithm of whole brain DNA in developing human brain (26).

During fetal life the brain undergoes a series of biochemical changes. Glycolysis is present during the second month, oxidative mechanisms appear the third month, and activity and localization of a number of enzymes reaches a mature pattern during the seventh month of gestation. In addition, there is evidence that the presence of acetylcholinesterase indicates tissue excitability (26, 27). The lipid to DNA ratio rises shortly after birth until at least two years of age. Thus, post-natal lipid synthesis is occurring at a more rapid rate than DNA synthesis which is related to the period of rapid myelination (26).

Synaptogenesis

Because the normal functioning of the brain is heavily dependent upon the development and branching of the axons and dendrites and the synaptic junction, this area of ultrastructure is being considered in research studies.

The synapse is the critical point of contact between connecting nerve cells and occurs most commonly between an axon and a dendrite. Synaptic contacts increase in number during the early maturation of the brain. In the rat brain there is a gradual increase in synaptic numbers throughout the first three post-natal weeks. Acceleration takes place the second week with the number of synapses doubling each day between days 4 and 11.

Alteration in the ultrastructure of individual synapses can be divided into three principal stages (Figure 8a) (1). In stage one only axodendritic synapses are recognizable. These synapses contain few vesicles and have undifferentiated or symmetrically thickened membranes. Stage two is characterized by an increase in number of synaptic vesicles,

asymmetric membrane thickenings and the appearance of axosomatic synapses. The development of dendritic spines also occur during this stage. Stage three is characterized by a marked increase in the percentage of junctions displaying adult features (1).

The thickness of the pre- and post-synaptic densities and also the width of the cleft increase as maturation progresses. The height of the dense projections increases with development while their base width decreases (Figure 8b) (1).

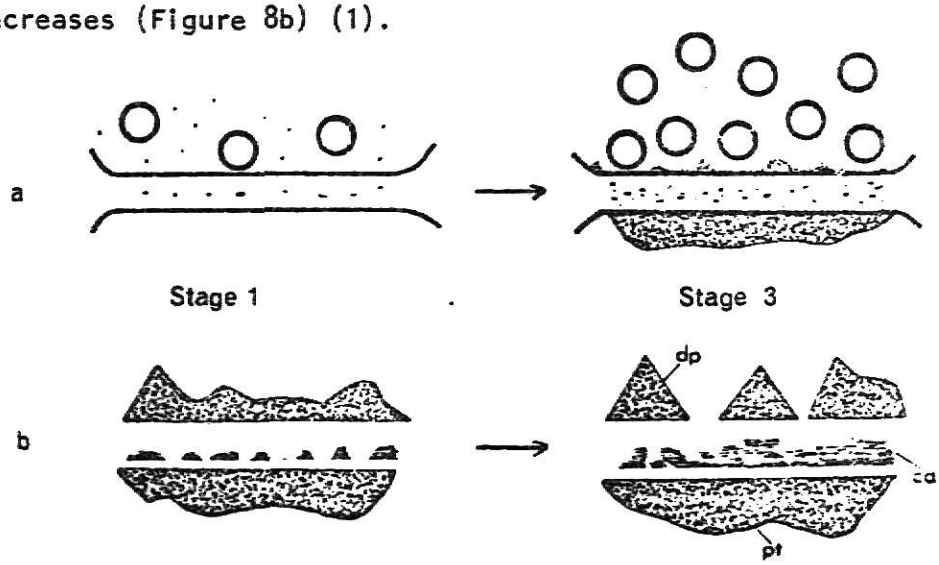

Figure 8. Schematic diagram depicting the maturation of synaptic junctions in (a) osmicated and (b) E-PTA stained rat cerebral cortex. The increase in vesicle numbers as well as the increasing asymmetry of the synaptic membranes with development are evident in (a). The increase in pre- and postsynaptic thickness and the emergence of discrete dense projections is brought out in (b). cd, Cleft densities; dp, dense projections; pt, postsynaptic thickening (1).

Function

The nervous system along with the endocrine system provides much of the control functions for the body. Three major functional levels of the nervous system include the spinal cord level, the lower brain level, and the higher brain level. The spinal cord level controls

automatic motor responses or reflexes. The lower brain level composed of the medulla, pons, mesencephalon hypothalamus, thalamus, cerebellum and basal ganglia, controls subconscious activities such as blood pressure, respiration, equilibrium, anger and excitement. The higher brain or cortical level contains approximately three-quarters of all the neuronal cell bodies of the entire nervous system and these are located in the cerebral cortex. The cerebral cortex is primarily an information storage area necessary to the thought processes (30).

Information is transmitted in the central nervous system through a succession of neurons. A synapse is a specialized junction between two neurons and usually occurs between an axon and a dendrite. An impulse is transmitted chemically from the axon to the dendrite (26).

Synapses have three regions, the presynaptic terminal region, the synaptic cleft, and the postsynaptic receptor region. The presynaptic region is distinguished by the presence of vesicles which contain neurotransmitters. The synaptic cleft separates the pre- and postsynaptic regions. The postsynaptic region is the receptor area for neurotransmitters (30).

In neurotransmission an electrical impulse travels down the axon to the presynaptic region where the terminal membrane is depolarized. The depolarization results in the release of neurotransmitters into the synaptic cleft. These diffuse across the cleft and interact with specific receptor sites on the postsynaptic membrane (27).

All of the known neurotransmitters are amines and generally may be classified as excitatory or inhibitory according to their postsynaptic effect.

MALNUTRITION AND EFFECTS ON CENTRAL NERVOUS SYSTEM FUNCTION

Synthesis of Brain Lipids and Myelination

Undernutrition during the growth spurt of the brain has been shown to not only affect cell multiplication and growth but also myelination. Research studies have been undertaken to detect changes produced by malnutrition on the different lipids related to both myelin and to other membrane structures.

Rat pups protein-calorie malnourished the first ten days of life had significant decreases of cerebrosides in the cerebral cortex and proteolipids in the cerebellum (31, 32). After a period of rehabilitation from 10 to 20 days of age, the levels of cerebrosides remained below the normal values, but the proteolipids in the cerebellum recovered to the control level.

Rat pups protein-calorie malnourished between 10 and 20 days of age had a significant reduction in gangliosides and cerebrosides in the cerebral cortex and the cerebellum (31, 32). Protein-calorie restriction during the first 20 days of life decreased almost all lipids in the cerebral cortex and cerebellum.

Cerebroside is a lipid located primarily in the myelin sheath. Reduction of cerebroside content might be an indication of an alteration in myelination.

There is evidence that gangliosides are associated with proliferation of axons and dendrites and neurotransmitters in the synapse (33). Decreased values for ganglioside content suggest an interruption in axodendritic proliferation.

Schain and Watanabe (34) reported cortical mantle cholesterol accumulations significantly depressed in post-natal protein-calorie restricted rabbit pups. Cholesterol is located in the myelin sheath as well as other membrane structures in the central nervous system.

Guinea pigs undernourished in utero showed markedly decreased levels of cerebroside, sulfatide and cholesterol (35). Guinea pigs protein-calorie undernourished in utero but fed normal diets after birth for 100 days showed cerebroside and sulfatide levels similar to control animals. Since the formation of the myelin lipids cerebroside and sulfatide takes place primarily after birth in guinea pigs, this catch-up is not surprising. Cholesterol levels remained lower after rehabilitation. This could indicate an alteration in the make-up of the myelin lipids or a decrease in lipid content in other brain membranous structures.

The human brain exhibits similar post-natal myelin accumulation. This catch-up in myelin lipid formation during rehabilitation in guinea pigs suggests optimism for rehabilitation in myelin lipid development in humans.

Two studies with rhesus monkeys reported differences in lipid content related to the timing of the insult. Check et al. (36) observed no significant changes in cholesterol and phospholipid levels in monkeys protein-calorie undernourished pre-natally. Kerr and Helmuth (37) reported significant reductions in content of most cerebral lipids, varying from 13 to 28 percent below normal values for monkeys fed low protein diets post-natally. The decrease was most marked in cerebrosides. This might be expected since the formation of myelin is incomplete

for the first six months of extrauterine life.

Rajalakshmi and Nakhasi (38), reporting the results of pre- and post-natal protein restriction in rat pups, noted no difference in concentration of total lipids at birth. However, at 21 days of age, undernutrition was associated with decreased concentration of total lipid. Lipid content in brains of rat pups fed a protein deficient diet beginning at four weeks of age until eight weeks of age showed no significant variation from the control (39). The timing of this insult was imposed later than in all previously cited reports and may have occurred after the spurt of myelination.

Studies have investigated the change in myelin composition and development associated with malnutrition. Pre- and post-natal protein restriction in rat pups resulted in myelin of immature composition at 25 and 50 days of age (40). The immature composition was indicated by low plasmalogen content at 25 days of age, and by high phospholipid, and low galactolipid and plasmalogen contents at 50 days of age.

Ahmad and Rahman (41) reported the process of myelination was lowered in brains of rat pups protein restricted during fetal life, suckling and after weaning. Cholesterol content was significantly lower but phospholipid, phosphatidylcholine, phosphatidylethanolamine and sphingomyelin contents of the brain remained the same as controls.

Fishman et al. (42) noted the amount of myelin was decreased in rat pups protein-calorie undernourished after birth but there was little change in chemical composition or interference in maturation of the membrane. Krigman and Hogan (43) also noted decreased myelin content in post-natal protein-calorie restricted rat pups. Although the composition

of myelin was relatively unchanged there was a delay in the initiation of myelinogenesis and there were significantly fewer lamellae comprising the myelin sheath.

Synthesis of myelin proteins in the brain of rat pups protein-calorie undernourished post-natally was significantly decreased (44). This could be attributed to a decrease in myelination or a delay in myelinogenesis.

The effects of specific nutrients on the developing central nervous system has been given some consideration by researchers. Pyridoxine is a co-factor in the synthesis of sphingosine, a building block essential to the synthesis of sphingolipids. Kurtz et al. (45) reported sphingomyelin and ganglioside levels were 50 to 60 percent of controls in rat pups suckled on dams fed a B_6-deficient diet. Cerebroside and sulfatide content was 60 to 70 percent of normal. Rat pups suckled by dams fed pyridoxine deficient diets during gestation and lactation had brain cerebroside and sulfatide levels that were delayed or retarded (46). The effects were reversed when the dams' diet was supplemented with pyridoxine beginning at five days post-partum. Stephens and Dakshinamurti (47) reported significantly decreased total lipid content, excluding gangliosides, in rat pups fed pyridoxine deficient diets.

No changes in brain lipids which were distinct from the effects of undernutrition were observed in rat pups exposed to a thiamin-deficient diet from the fourteenth day of gestation (48). Brains of rats fed diets deficient in essential fatty acids had lipid levels that did not differ significantly from controls (49). Sun and Sun (50) reported variances in the fatty acid composition of synaptosomal membranes in

rats fed fatty acid deficient diets. Rat pups suckled by dams fed low protein diets from day three to sixteen had retarded attainment of activity in the enzymes controlling ganglioside and glycoprotein synthesis (51). Glycoproteins and gangliosides are localized in dendritic and axonal membranes and the decrease in synthesis suggests decreased development of axons and dendrites.

Synthesis and Metabolism of Brain Proteins

The brain is an area of high metabolic rate and rapid protein turnover. Inadequate dietary intake of amino acids is likely to affect amino acid metabolism and protein synthesis. Research studies have reported varying results of increased and decreased levels of specific brain free amino acids and protein.

Badger and Tumbleson (52) reported altered patterns of amino acid concentrations in piglets fed protein and protein-calorie deficient diets after weaning. The time of initiation of malnutrition determined which amino acids were altered, the manner in which the concentrations varied and the area of the brain affected. Although the number of brain free amino acids that were altered were about the same in the protein and protein calorie malnourished piglets, different amino acids were affected.

Guinea pigs and rats fed low-protein diets post-natally had marked elevations in brain histamine concentrations (53). Occurring at the same time were increases in brain concentrations of histidine. The authors concluded that increases in the concentration of histamine were due mainly to increases in concentration of the precursor amino acid, histidine.

Rat pups suckled by dams fed protein-calorie deficient diets during gestation and lactation showed complex alterations in brain free amino acids (54). Post-natal depressions in concentrations of alanine, glycine, phosphoethanolamine, taurine and ethanolamine were not as prominent in the malnourished rats as in the controls. Ethanolamine phosphate is essential for the formation of some phospholipids and the less marked drop in ethanolamine may be an expression of reduced myelin formation.

Brain concentrations of free amino acids were reduced in rats fed a high tyrosine, low protein diet (55). Incorporation of ^{14}C-leucine into brain was reduced significantly indicating that the protein-synthesizing mechanism is susceptible to inhibition by an excessive amount of an individual amino acid. Bhagavan and Coursin (56) reported decreased incorporation of ^{14}C-leucine into brain protein in post-weanling rats fed a biotin deficient diet. Stern, et al. (57) reported incorporation of ^{14}C-leucine into brain protein at birth increased by 90 percent in rat pups born to dams fed a low protein diet during gestation and lactation. However, on days 5, 11, and 21 there was no significant effect of diet on incorporation of ^{14}C-leucine. The pyridoxal phosphate contents of brains of rat pups fed a diet low in pyridoxine pre- and post-natally were significantly lower than controls (58). Pyridoxal phosphate plays a role in protein synthesis and the DNA, RNA and protein levels in the brains of these rats were reduced also.

Dallman and Spirito (59) suggest the conservation of brain protein through the utilization of amino acids from degradation of protein elsewhere in the body. Intraperitoneal injections of ^{3}H-leucine were

administered to rat pups during the period of rapid brain growth. ^{14}C-leucine was injected intraperitoneally during the period when brain growth is diminishing but body weight gain is rapidly continuing. In rats receiving a 26 percent protein diet the ^{3}H : ^{14}C ratio remained essentially unchanged in the cerebrum, cerebellum and brain stem. However, in rats fed a 3.4 percent protein diet the ^{3}H : ^{14}C ratios in the brain decreased approaching those of skeletal muscle.

Several amino acids are precursors of neurotransmitters and are involved directly in brain function (see Figures 9, 10, 11) (26, 60). The four compounds acetylcholine, serotonin, dopamine and norepinephrine are labeled by most neurobiologists as neurotransmitters. Gamma-amino butyric acid, epinephrine, glycine and others are often included by many neurobiologists on the list of neurotransmitters. Alterations in the amounts of neurotransmitter amines might underlie some of the behavioral aberrations seen in malnourished animals.

Rats undernourished from birth to four weeks did not have a decreased concentration of acetylcholine (61). Post-weaning restrictions of both protein deficiency and severe caloric restriction resulted in significantly lower concentrations of acetylcholine. Reynolds and Blass (62) reported normal levels of acetylcholine in brains of thiamin-deficient adult rats. Cerebellar acetylcholinesterase activity was depressed in rat pups suckled by dams fed a protein-calorie deficient diet (63). Acetylcholine is inactivated by acetylcholinesterase which hydrolyzes it into choline and acetate. The choline is taken up by the neuron and resynthesized into the neurotransmitter. Depressed levels of acetylcholinesterase would decrease the amount of choline available for resynthesizing.

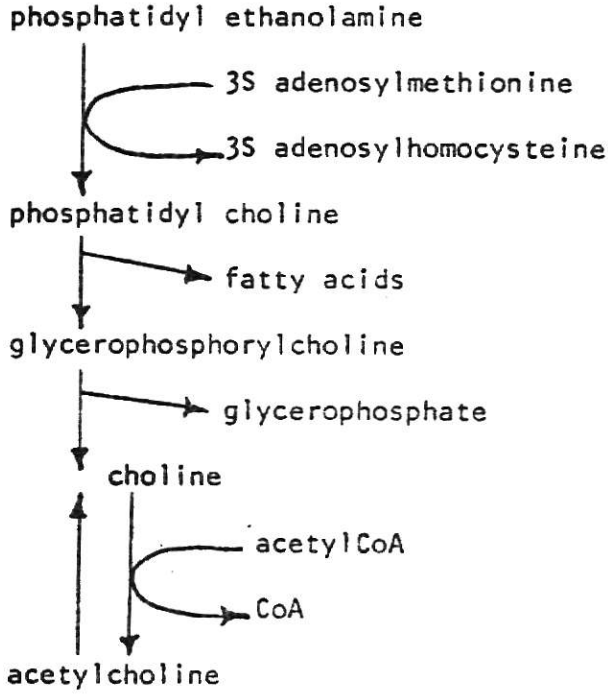

Figure 9. Synthesis of acetylcholine (26).

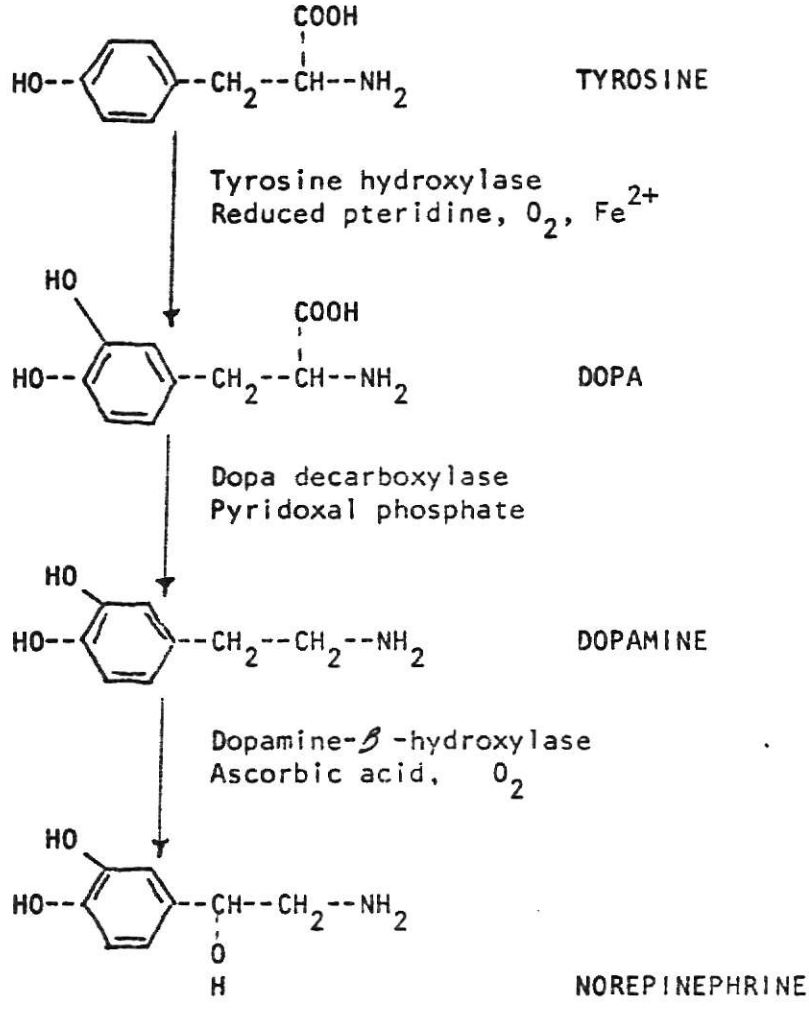

Figure 10 . Synthesis of Norepinephrine (60).

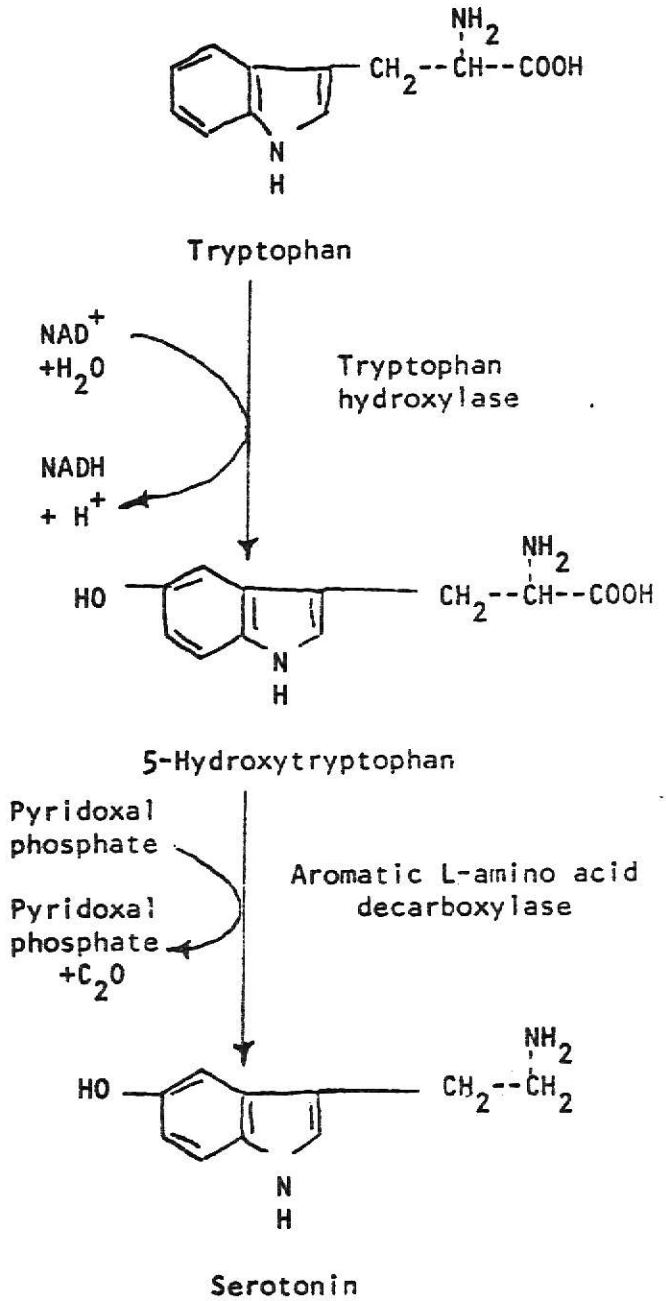

Figure 11. Synthesis of Serotonin (60).

Ramanamurthy (64) noted that maternal protein-calorie malnutrition in rats did not significantly alter the levels of serotonin, norepinephrine and dopamine in the brains of fetuses either in utero or up to the seventh day after birth. Fourteen days after birth they were found to be significantly lower and this continued up to the thirty-fifth day. In contrast, Stern, et al. (65) reported significantly elevated levels of serotonin and norepinephrine in brains of protein malnourished rats from birth to 300 days. These elevated levels in serotonin were present even when the low protein diet was deficient in the precursor amino acid, tryptophan. Brains of rat pups nursed by mothers fed low protein diets during gestation and lactation showed norepinephrine content that was 70 percent, and dopamine content that was 80 percent of that of well nourished rats (66). These decreases in brain catecholamines were not a reflection of biosynthesizing enzymes or precursor. Tyrosine hydroxylase levels were increased and tyrosine concentrations were identical to well-nourished rats. Lee (67) and Lee and Dulos (68) reported brain contents of dopamine and norepinephrine were depressed in rat pups protein-calorie malnourished pre- and post-natally. Included was a study of the norepinephrine-binding protein and dopamine-binding protein involved in catecholamine formation. In the malnourished group the total norepinephrine-binding protein was reduced but the dopamine-binding protein was not altered significantly.

Kennedy (69) measured the rapid axonal transport of proteins in rabbit vagus nerves. He reported that severe protein-calorie undernutrition in preweaned rabbits did not affect the normal rate of axonal transport.

One of the functions of Na^+-K^+-stimulated ATPase activity is in maintaining the ionic gradients essential for nerve impulse conduction. Kissane and Hawrylewicz (70) noted impaired development of this enzyme in rats protein malnourished pre- and post-natally.

Driskell and Foshee (58) studying the effects of vitamin B-6 deficiency on behavioral patterns reported no significant differences in general activity scores although the scores tended to be lower in the deficient animals. Curiosity scores from the deficient rats were significantly lower. Deficient rats tended to have fewer correct responses to tone discrimination. These rats tapped the lever more frequently whether or not the tone was sounded.

Utilization of Glucose

The mechanisms involved in the alterations of lipid and protein synthesis in malnutrition are unknown. Glucose is used by the brain as a source of energy and is a substrate in many brain intermediates. Reduced glucose utilization could affect brain synthesis of myelin and protein.

Rat pups protein-calorie malnourished post-natally showed a decrease in incorporation of U-^{14}C glucose in brain lipids and amino acids (71). This decrease was significant in ten day and 17 day old rat pups but no significant difference was found in 90 day old rat pups. Glucose levels in the brain and blood glucose concentrations were lower in the malnourished rat pups. After the initiation of rehabilitation blood glucose concentrations in the malnourished rat pups were similar to the controls. Gaitonde et al. (72) reported the utilization of U-^{14}C

glucose into amino acids decreased in the brains of post-natal thiamin restricted rat pups. Glucose uptake in the brain stem was depressed in post-natal thiamin deficient rat pups (73).

Cerebral blood flow and carbohydrate metabolism were studied in normal and protein-calorie malnourished children under the age of 40 months (74). In severe protein-calorie malnutrition the proportion of glucose undergoing aerobic oxidation is reduced. A decrease in energy production would be concommitant with a decrease in the proportion of glucose undergoing aerobic oxidation. There would follow a decrease in the amount of energy available for brain synthesizing mechanisms.

SUMMARY

Studies on animals have indicated that undernutrition in early life, when cell division is occurring, has irreversible effects on later brain development. Evidence in animals indicates that malnutrition during the period when cell division is occurring in brain causes persistent changes in animal behavior. In humans, there is some suggestion of a relationship of early nutrition to mental development.

Protein-calorie and protein malnutrition has its greatest effect on brain development during the brain growth spurt. Total organ DNA, RNA and protein levels are decreased reflecting a decrease in cell number and cell size. In experimental animals, those decreases are most pronounced in the cerebellum. The cerebellar function of muscle coordination and the cerebral function of learned behavior are affected by

protein-calorie and protein malnutrition in experimental animals. Reports of the effect of protein-calorie and protein malnutrition on learned behavior in humans are inconsistent. Some researchers report no significant differences in intelligence test scores of malnourished and normally nourished children. Others show significantly reduced scores of the malnourished children.

Distortions in nerve myelination, protein synthesis and glucose utilization accompany gross brain deficits in DNA, RNA and protein. Protein-calorie and protein restriction during the period of myelination resulted in decreased synthesis of myelin. In addition variance in the composition of myelin was observed. Alterations in levels of brain free amino acids, proteins and neurotransmitters are noted in protein-calorie and protein restriction. Those alterations were not dependent on the timing of the restriction. Decreased glucose utilization is reported in post-natal protein-calorie malnutrition.

The assumption that variation in brain size or brain cellularity has a significant influence on intelligence is probably erroneous. Brain cell number, when measured by DNA content, is a reflection of total cell mass. It does not account for the different cell types. Neuronal multiplication in humans occurs at a time when the brain is highly protected from the insult of malnutrition. Reduction in brain cell number is more likely a reduction in supportive cells, i.e., glial cells rather than neurons.

The effect of malnutrition during the period of myelination does not appear to have a significant influence on intelligence. Reduction in the number of lamellae in the myelin sheath is probably without

effect on impulse conduction. Distortions in the lipid composition of myelin may have more of an effect on nerve impulse transmission than retarded myelination. Alterations in synapse connections and neurotransmitters are probably of greater significance. Nerve impulses are transmitted across the synapse by means of the neurotransmitters. Decreases in synaptic connectivity would be expected to interfere with learning potential. Brain synthesizing mechanisms depend on available energy. A reduction in available energy, i.e., glucose, would result in reduced synthesis of brain proteins, neurotransmitters and lipids.

Continued research should be given to the changes in synapse connections, levels of neurotransmitters and distortions in cerebellum and cerebral cell types. Caution should be exercised in evaluating decreases in human mental performance by comparison of test scores to a standard. Standards should be for the population being considered and not from those of another distinctly different population.

If a cause and effect relationship could be established between early malnutrition and mental performance, then a vicious circle of events could be attributed to poverty and lack of adequate nutrition. However, nutrition is probably only one factor in a complex interaction of genetic, social, economic and environmental factors. Providing preventive health care and adequate food supplies for the pregnant mother and infant through childhood and social and educational stimulation for the child would be a step forward in reducing the effects of malnutrition on brain development.

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And to my Lord who brought me here and gave me love, strength and peace, I commit my life to be an instrument of His glory.

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EFFECTS OF PRE- AND POST-NATAL MALNUTRITION ON BRAIN PHYSIOLOGY

by

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A review of the literature related to the effects of pre- and post-natal malnutrition on brain physiology is presented. Pre- and post-natal protein-calorie and protein malnutrition is correlated with alterations in brain growth and development. Brain DNA, RNA and protein levels are reduced. Nerve myelination is distorted. Levels of brain free amino acids, proteins and neurotransmitters are altered. Glucose utilization is decreased. Evidence in animals indicates that these aberrations are reflected in changes in animal behavior. There is some suggestion of the same effect in humans.