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REVIEW

Vitamins and Brain Development

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Summary

Effects of deficiency of vitamins on early development of brain have been reviewed. Unusual developmental problems in neurogenesis specific for the brain and impairment of its functional capacities due to vitamin deficiency have been discussed. The species-specific "critical periods" in development of various systems have been mentioned. Indices such as reflex activity, locomotion, special senses, cognition and adaptive behavior were used for assessing brain maturation in experimental models and humans. Significant examples include brain anomalies in humans and other mammals caused by retinoid excess or deficit; increase in calbindin D28K, a vitamin D dependent calcium-binding protein during postnatal period in rat; hydrocephalus and exencephaly in prenatal rats and subarachnoidal or intracerebral hemorrhage in infants caused by vitamin E deficiency. Peripheral neuropathic lesions leading to infantile beriberi is caused by thiamine deficiency. Impaired growth in retinal layers leading to delay in maturation of electroretinogram and depth-perception in postnatal rats occur due to pyridoxine deficiency. Infants of severely vitamin B12 deficient mothers show abnormalities in behavior involving basal ganglia and pyramidal tract. Folic acid deficiency results in delayed maturation of the basic electroencepalographic patterns. In addition, vitamin-interactions leading to developmental errors have been pointed out. Vitamin B6 deficiency impairs vitamin B12 absorption and biotin deficiency may be aggravated by pantothenic acid deficiency. Vitamin C deficiency resulting in impaired metabolism may produce symptoms of deficiency of folic acid. Another characteristic examples is that iron absorption from dietary sources is dependent on ascorbic acid.

Key words

Ontogeny • Brain • Neurogenesis • Behavior • Malnutrition • Vitamin E • Vitamin D • Vitamins B •Thiamine • Pyridoxine • Riboflavin • Vitamin B12 • Vitamin C • Pantothenic acid • Folic acid

One of the important reasons for the increasing interest in developmental studies during the last several decades, referred to as ontogeny, is the realization of its significance in clinical situations. The highest mortality rate of all age groups still occurs in the perinatal period. Neonatology, dealing with problems of development, prematurity, dysmaturity and postnatal adaptation draws heavily from experimental as well as clinical studies.

Development of the brain

It must be recognized that there are certain unusual developmental problems specific for the brain,

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which have been exhaustively discussed by Dodge *et al.* (1975). Though the events related to neurogenesis pass through an orderly succession of stages such as cell birth, migration, differentiation, maturation and cell death, neurons in a given region do not all differentiate, migrate and achieve maturation at the same time. For example, in the cerebellar cortex, granule cells migrate to their final positions long after Purkinje cells. Moreover, their functional capacities continue to be expressed throughout life under either normal conditions or in response to injury, aging or insult such as the deficiency/excess of vitamins.

The indices used to assess growth of the brain and development of its external configuration include division and migration of neurons and glial cells, maturation of neurons and growth of the neuropil, development of synapses and formation of the myelin membrane. Different regions and also different pathways in the same region vary considerably in the times of myelination which may continue for considerable periods of time during late embryonic life or early in postnatal life and into childhood as in the case of humans (Shepherd 1983). Besides, biochemical determinations can provide direct estimates of the structural components of the brain. DNA is a reliable measure of the cell number as a constant amount of DNA is present in each diploid cell of a given species (Leslie 1955, Gray and DeLuca 1956). RNA is considered representative of the biosynthetic potential (Pope *et al.* 1964) or of the surface area of the cell (Edstrom and Pigon 1958). The other chemical markers of development include brain specific proteins such as S-100 and 14-3-2 for glial cells and neurons, respectively (Cicero *et al.* 1970). As some enzymes change their major site of activity in different subcellular fractions with age, the assessment of enzymatic activity as a marker must be viewed with caution. The concept of "critical period's in development"still remains valid in this context (Flexner 1952). The times when various critical conditions or stimuli are effective are concentrated in the same, relatively short period after hatching in birds or birth of mammals, the species in which these critical periods have been studied extensively. A number of effects or events only occur during a relatively brief critical period. For example, the effect of monocular deprivation on the ocular dominance of visual cortical neurons in cats and monkeys (Wiesel and Hubel 1963), imprinting in birds (Ewert 1980) and the learning of song in some species of birds (DeVoogd and Nottebohm 1981) Although the critical periods for various systems and conditions overlap in time, it is unlikely that they all share the same underlying mechanisms. In general, however, the critical period is fairly specific for each form of deprivation in a particular species. Thus, in the cat, the critical period for development of binocular vision, for which congruent input from both eyes is required, extends from 3-16 weeks of age. After this, the vulnerability to monocular visual deprivation or strabismus is greatly reduced. The ability of the system to recover from deprivation decreases with age and this capacity for recovery declines sharply after the critical period. For example, the cerebral lateralization of functions such as language is already present at birth in humans, but recovery of function occurs after removal of or damage to the dominant hemisphere, provided it occurs before 3-4 years of age. A brief description of some examples of critical periods in various systems is given below.

Sexual organization and behavior

Estradiol is the primary determinant of brain sexual organization in birds as well as mammals (Arnold and Gorski 1984). However, during a critical period of development, estrogen masculinizes the mammalian brain whereas it feminizes the avian brain. In the quail, injection of estradiol into the egg on E10 does not change sexual behavior in females, but causes complete reversal in males. The critical period for feminization of copulatory behavior of males by estrogen occurs prior to the 12th day of an 18-day incubation period in the quail (Adkins-Regan 1987).

Muscle spindle denervation atrophy

There is a critical period of up to 2 weeks after birth during which muscle spindles depend on their sensory nerves for development and survival. With increasing age, muscle spindles in the rat hindlimb have a progressively decreasing dependence on their nerve supply (Zelená 1964). In newborn rats, sensory deprivation results in atrophy and degeneration of muscle spindles within 10 days. Denervation in rats 20 days after birth, when the muscle spindles are fully differentiated, merely results in slight atrophy of the intrafusal fibres 10 days later. There is thus a critical period during which muscle spindles will degenerate if deprived of their innervation, but will survive if they are denervated after that period. In the gastrocnemius of the rat, the spindle needs its sensory innervation until 6-8 days postnatally, after which it survives if denervated (Werner 1973).

Malnutrition in humans

Several studies have supported the concept of a critical period from birth until 2 years of age, during which the nervous system is most vulnerable to malnutrition and most responsive to nutritional treatment. Winick and coworkers (1975) found that the IQ and scholastic performance between 6-12 years of age were normal in malnourished Korean children adopted by American families before the age of 2 years, but were significantly below normal in children adopted after 2 years of age.

Several indices exist for assessing brain maturation. Smaller molecules such as amino acids are of limited value as indices, as the concentration of various amino acids in the brain changes due to several factors about which little is known as yet. For example, we have no information about the exact biochemical pathways which are instrumental in increasing glutamate levels during early postnatal life, despite extensive knowledge about glutamic acid metabolism in the brain (Dodge *et al.* 1975). The localization of transmitter amines obtained through specific histochemical and histofluorescence techniques has been used extensively to study their changes during maturation of the brain. However, not only multiple pools of these transmitters do exist in the brain, but their affinity for binding with synaptic membranes also varies (Fonnum 1973, Dahlstrom 1973).

The ontogeny of electrocortical activity has also been used for evaluating brain maturation (Bernhard *et al.* 1967). Ellingson and coworkers have extensively studied the ontogenesis of evoked potentials and EEG activity in infants (Rose and Ellingson 1968). Development of sleep spindles during the first year of life has also been studied in normal full-term, premature infants and in children with Down's syndrome (Peters *et al.* 1981, Ellingson 1982). However, there are considerable species variations in the temporal aspects of the ontogeny of synaptic activity (Purpura *et al.* 1967). The maturational pattern of evoked cortical potentials was another widely used index (Hunt and Goldring 1951). As might be expected, species differences do exist and must be considered while studying these physiological processes in relation to development (Rose and Ellingson 1968). Changes in reflex activity, elaboration of locomotion, development of special senses, cognition and highly organized adaptive behavior as correlates of psychological development consequent to and concomitant with the growth of the brain were used especially in higher organisms and humans (Dodge *et al.* 1975).

Inadequate nutrition

Mayer (1976) estimated that about one eighth of the human population is poorly nourished or undernourished. Statistics about the average food consumption may, however, be misleading as some sections of the population such as young children and pregnant and lactating women are far more severely affected than others. Increased mortality rate is only one of the results of inadequate nutrition. Yet another serious problem involving social costs relates to the distinct possibility of permanent physical damage to the growing individual that may be wrought by quantitatively inadequate food intake, or by diets qualitatively deficient in some specific factors such as vitamins. The brain, besides the immune system is among the more vulnerable targets (Reinis and Goldman 1980).

This review examines the effects of deficiency of vitamins on early development of the brain.

Vitamin A

Excess and deficiency of vitamin A in the mother's diet during pregnancy have been shown to cause malformations of the fetal brain and hydrocephalus (Millen *et al.* 1954). Howell and Thompson (1967) reported that there was obstruction to CSF circulation in vitamin A deficient chickens. This was traced to increased periosteal bone formation and must therefore be regarded as an indirect or secondary effect of the deficiency leading to impairment of brain growth. Gyorgy (1968) reported lower IQ in vitamin A deficient children. When newborn rats were deprived of vitamin A, this resulted in a defect of myelination without gross destruction or deformity of brain structure (Clausen 1969). When mothers were deprived of vitamin A early in pregnancy, their surviving offspring exhibited gross deformities of the visual system (Warkany 1971). Corey and Hayes (1972) reported that, in most species studied, deficiency of vitamin A brought about a rise in cerebrospinal fluid pressure. Among different forms of vitamin A, it was reported that monosyl retinyl phosphate (MRP) and galactosyl retinyl phosphate (Gal RP) could be of great importance in the biosynthesis of glycoproteins for cell surfaces, which are essential for adhesion properties of cells (Wolf *et al.* 1979). Further, glycoprotein such as serum alpha-macroglobulin (OC-G) in the rat was found to be defective under vitamin A deficiency, as a result of low glycosylation (Navia and Harris 1980).

Recently, Sharma and Misra (1990) reported that maternal vitamin A restriction caused altered brain development in the offspring in terms of tissue weight,

DNA, RNA and protein levels and biosynthesis of DNA and protein (estimated from thymidine and leucine incorporation, respectively). They demonstrated a dose-dependent effect of maternal vitamin A restriction on the metabolism of DNA, RNA and protein in the developing brain of the offspring.

It has been shown conclusively that vitamin A (retinoid) is an essential nutrient for fetal and subsequent mammalian development, is involved in gene expression, cell differentiation, proliferation, migration and death. Retinoic acid (RA), the morphogenic derivative of vitamin A, is highly teratogenic. In humans, retinoid excess or deficit can result in brain anomalies and psychosis. An excellent review on these aspects was written by Goodman (1995).

Vitamin D

There is increasing evidence indicating that vitamin D metabolites may be required for optimal cerebellar development. In an investigation into the influence of vitamin D status on the activity of creatine kinase in the brain during postnatal development, it was shown that specific activities of cerebral and cerebellar creatine kinases increase. Vitamin D depleted rats failed to exhibit this normal process. The developing cerebellum, but not the cerebrum, in both vitamin D depleted rats and in normally fed animals responded sequentially to a single injection of a vitamin D metabolite by displaying increased specific activity of creatine kinase. Similar sequential responsiveness to vitamin D metabolites was shown in the cerebellum of the rabbit, a "perinatal brain developer" at an earlier age compared to the rat, a "postnatal brain developer" (Binderman *et al.* 1988). Large increases occur in brain calbindin-D28KmRNA between one and two weeks of age in the rat, during which major synapse formation takes place. In as much as calbindin D28K is a vitamin D dependent calcium binding protein, this observation by Varghese *et al.* (1988) may signify the importance of vitamin D for brain development.

Vitamin E

Verma and Weiking (1967) reported certain disorders of the developing nervous system of vitamin E deficient rats. In their study, rats were made deficient in vitamin E before mating and until the 10th day of gestation, at which a single dose of vitamin E was given to prevent resorption of the embryos. Morphological abnormalities in embryos obtained from day 11 and 21 of gestation included hydrocephalus, exencephaly, reduction of neurons, defective development of the choroid plexus, gliosis and decreased synthesis of acetylcholinesterase which were correlated with a decrease in Nissl substance in neurons. Kruk and Enesco (1981) reported that alpha-tocopherol reduces the levels of the fluorescent age pigment (fluorometrically measured lipofuscin) in the brain of young mice, as compared to untreated control mice at 3 and 5 months of age.

While reporting on the effect of age on vitamin E concentrations and on the uptake of radioactive vitamin E in various regions of the rat brain, Vattassery *et al.* (1984) observed that concentrations of alpha-tocopherol increased significantly with age in the medulla and spinal cord, whereas no such changes could be detected in other brain areas. The uptake pattern of radioactive alpha-tocopherol from the serum by various areas of the brain was similar and unrelated to age. However, since the authors used 3, 14 and 30-month-old rats, it is difficult to assess the significance of this study in relation to development of the brain strictly within the parameters defined earlier. Meydani *et al.* (1986) studied the influence of dietary vitamin E and age on regional distribution of alpha-tocopherol in the rat brain. They reported that only the cerebellum of young and old rats showed a marked increase of alpha-tocopherol with vitamin E supplementation. They also observed that a relatively shorter period of dietary vitamin E deficiency would suffice to deplete plasma alpha-tocopherol and depress glutathione peroxidase (GSH-Px) activity significantly in young rats. However, as the study was restricted to rats between 1 and 15 months of age, it is not clear what these observations signify in terms of brain development.

It is known that dietary fatty acids modify phospholipid fatty acids in the brain and liver of growing chickens after hatching. The role of vitamin E deficiency in this process in relation to the pathogenesis of chick nutritional encephalomalacia (NE) was investigated by Fuhrmann and Sallmann (1996). According to this study, NE-producing dietary conditions were not accompanied by specific alterations in cerebellar phospholipid fatty acids due to the alpha-tocopherol content of the diet. On the contrary, alterations of membrane fatty acids in the liver appeared to play an important role in the pathogenesis of NE. Fuhrmann *et al.* (1996) also showed that intensified lipid peroxidation did not occur in the cerebellum of the chickens fed with the NE-producing

diet. It is the liver which seems to be most affected by the oxidative stress.

Vitamin K

Among the different forms of vitamin K, K2 is synthesized by bacterial flora of the intestinal tract. Since the intestinal bacteria, are not yet well established during the first few days of life, hypoprothrombinemia may be present in 0.1-0.5 % of full-term infants. This may lead to subarachnoidal or intracerebral hemorrhage in some cases without supplementation of this vitamin (Vietti *et al.* 1960). This is an example of the vitamin deficiency affecting the developing brain indirectly.

Thiamine

Spillane (1947) reported that thiamine deficiency leads to infantile beriberi, involving peripheral neuropathic lesions. Thanangkul and Whitaker (1966) considered infantile beriberi as a mixed deficiency syndrome, although seizures, mental symptoms and opisthotonus, when present, respond rapidly to pure thiamine supplementation suggesting a specific deficiency of thiamine. There were reasons for this assumption, firstly multiple oral B vitamins have often been given to the mother after thiamine had been administered parenterally and secondly, seizures and irritability are characteristic of pure pyridoxine deficiency. Victor et al. (1971) went so far as to suggest that as in adults, peripheral neuropathic lesions found in nutritional deficiency states including infantile beriberi are most likely related to some factor other than thiamine. There is, however, no doubt that developing rats are much more susceptible to thiamine deficiency than adults (Geel and Dreyfus 1974). Maternal thiamine deficiency in the rat was shown to reduce the thickness of the retina postnatally in the offspring. The attainment of the same level of threshold intensity of the electroretinogram (ERG) as in adults was shown to be delayed by 4 days and depth perception by one day postnatally in the offspring of dams which had been made deficient in thiamine during pregnancy and lactation (Mukkadan and Ramakrishna 1982, 1983, Mukkadan 1984).

Sanjeeva and Ramakrishnan (1983) observed that maternal thiamine deficiency and undernutrition cause significant deficits in the weight of the spinal cord of the offspring at 21 days of age. In an important study on postnatal development of thiamine metabolism in the rat brain, Matsuda *et al.* (1989) reported that microsomal thiamine triphosphatase activity in the cerebral cortex and cerebellum increased from birth to 3 weeks of age, whereas that in the liver did not change during early postnatal development.

Microsomal thiamine diphosphatase activity in the cerebral cortex showed a transient increase at 1-2 weeks. Thiamine pyrophosphokinase in the cerebellum increased from birth to 3 weeks and then decreased. The content of thiamine and its phosphate esters was enhanced during synaptogenesis confirming the hypothesis that thiamine may be involved in nerve conduction. Furthermore, the effects of thiamine deficiency on thiamine-dependent enzymes in regions of the brain of pregnant rats and their offspring have recently been reported by Fournier and Butterworth (1990). The activities of three thiamine-dependent enzymes, pyruvate dehydrogenase complex (PDHC), alpha-keto-glutarate dehydrogenase (alpha-KGDH) and transketolase (TK), were significantly reduced in the cerebral cortex of offspring of thiamine-deficient mothers 13 days postnatally. Thiamine-dependent enzymes are important for the establishment of adult patterns of cerebral energy metabolism and also for myelin synthesis. Maternal thiamine deficiency resulting in diminished activities of these enzymes during a vulnerable period in brain development may therefore have serious metabolic consequences leading to permanent neurological perturbations in the offspring. Brain acetylcholine levels were found to be significantly decreased on the 21st and 28th days in pups of dams fed thiamine-deficient diets during gestation and lactation, whereas the same was decreased on the 28th day in pups of dams fed a thiamine-deficient diet during lactation. Subsequent dietary rehabilitation was found to reverse the deficits in brain acetylcholine levels. Activities of cholinergic enzymes, however, remained unaltered in the deficient groups (Kulkarni and Gaitonde 1983).

Ba *et al.* (1996) have reported that the most prevalent mechanism contributing to ethanol-induced thiamine deficiency in chronic alcoholics could be due to alterations of thiamine metabolism, and particularly to reduced conversion of the vitamin to its metabolically active form TPP (thiamine pyrophosphate). Exposing rat pups to ethanol during pregnancy and lactation showed significant impairment of neurobehavioral development, more cornered pyramidal cells altered in shape in the hippocampal subfield CA3, reduced cell number and cell size pointing to long-lasting effects of maternal alcohol exposure leading to thiamine deficiency in the offspring.

Riboflavin

Fordyce and Judy (1975) studied the effect of riboflavin depletion in rats at various stages of development by means of biochemical and behavioral parameters. They concluded that riboflavin restriction during gestation and lactation, but not gestation alone, appeared to produce permanent alterations in general activity scores and the content of brain nucleic acids and proteins in male rat progeny. However, a deficiency of riboflavin did not bring about perceptible impairment of retinal development in rat pups, when the dams were made deficient during gestation and lactation (Mukkadan and Ramakrishna 1982, 1983, Mukkadan 1984)

Ogunleye and Odutuga (1989) reported that after 21-day-old weanling rats had been maintained on diets deficient in riboflavin, their brain weight was by 19.8 % lower than those of rats on control diets. Myelin lipids, cerebrosides and sphingomyelin as well as phosphatidylethanolamine, a significant component of the myelin membrane, underwent a proportional reduction. Since riboflavin plays a role in the metabolism of essential fatty acids in brain lipids, its deficiency has been considered similar to that of essential fatty acid deficiency, causing an impairment in brain development and maturation.

Pyridoxine

The tragic instance of infants fed a liquid canned milk formula devoid of vitamin B6, who suffered from a widespread epidemic of nervousness, irritability and seizures is widely known (Snyderman *et al.* 1953). The marked EEG abnormalities also noted in these infants, were abolished within minutes after an injection of pyridoxine (Coursin 1954, Moloney and Parmelee 1954). Dakshinamurthy and Stephens (1969) reported that growth and development of the brain was severely affected by pyridoxine deficiency in neonatal rat. Bayoumi and Smith (1972) reported that severe vitamin B6 deficiency in pregnant rats during the last two weeks of gestation reduced the weight of the brain in newborn rats. When this deficiency was extended into the lactation period, body and brain weights were severely retarded. Kurtz *et al.* (1972) studied the coenzyme levels in brains of suckling rats, the progeny of dams fed with a vitamin B6 deficient diet, after parturition and reported that they were reduced by about one third of the controls. In the pups surviving 21 days, the brain weight was only 80% of the controls. Cerebral sphingolipids and GABA were reduced to 30-50 %, whereas cystathionine, glycine, citrulline, taurine and the branched chain amino acids were all somewhat elevated. The clinical correlates of these apparently metabolic disturbances brought about by pyridoxine deficiency are not known.

Alton-Mackey and Walker (1973) showed that infant rats born to vitamin B6 deprived mothers had lower body weight and impaired neuromotor development. The residual effect of pyridoxine deficiency during gestation on the offspring persisted in spite of improvement resulting from the introduction of normal foster mothers.

Weanling rats maintained on a B6-deficient diet were discovered to have lower brain RNA concentrations (Moon and Kirksey 1973). However, the DNA content in these rats was not reduced. Stewart *et al.* (1973) observed that maternal or neonatal pyridoxine deficiency retards brain development. They reported a marked reduction in the pyridoxal-5-phosphate content, cell number and size, the norepinephrine and serotonin content and *in vivo* incorporation of labeled amino acids and glucose into proteins. Their study clearly established that permanent irreversible deficits in CNS function could be seen in such rats during the post-weanling period. The study of Driskell and Foshee (1974) on the behavioral patterns, brain nucleic acid and pyridoxal phosphate contents of male rat progeny from vitamin B6-depleted dams supports this contention.

In a study on the susceptibility to thiosemicarbazide (an antagonist of vitamin B6) and ontogenetic development of the brain, Yamashita (1974) and Thomas and Kirksey (1976a) studied the postnatal patterns of fatty acids in the brains of progeny from vitamin B6 deficient rats before and after pyridoxine supplementation. Fatty acids C18:2, C20:4 and C22:6 in the cerebellum were significantly lower in the brains of 15-day-old pups from unsupplemented deficient dams compared to values from pups of control dams. Significant reductions in the omega 6 fatty acids (C18:2, C20:4 and C22:4) were evident in the cerebella of 15-day-old progeny of unsupplemented deficient dams. The supplementation of deficient dams with vitamin B6 at 5 and 10 days *post partum*, prevented the reduction of omega 6 fatty acids found in deficient progeny. Thomas and Kirksey (1976b) studied the postnatal patterns of brain lipids in the progeny of vitamin B6 deficient rats before and after pyridoxine supplementation. They reported that the postnatal development of cerebroside and ganglioside levels was delayed or retarded in the brains of pups from unsupplemented deficient dams. Supplementation of dams fed a low level of pyridoxine

(1.2 mg/kg diet) with this vitamin beginning at 5 days *post partum* reversed all the observed effects of low vitamin intake on brain lipids in progeny.

Aycock and Kirksey (1976) reported that the brain weight and alanine aminotransferase (ALAT) activity (initial and following the *in vitro* addition of pyridoxalphosphate) were significantly reduced in the brains of 12-day-old pups of dams fed the lowest level of pyridoxine compared to other treatments. The study of Morre *et al.* (1978) on the effects of vitamin B6 deficiency on the developing CNS of the rat indicated that brain development, particularly myelination, was affected by a deficiency of vitamin B6 prior to and including the period of rapid myelination.

Krishna and Ramakrishna (1984) reported that the ontogeny of exploratory behavior was delayed in B6 deficient pups, presumably due to impaired or delayed neuromuscular development. Mukkadan and Ramakrishna (1982, 1983) and Mukkadan (1984) showed that in the case of vitamin B6 deficient pups there was a significant reduction in the thickness of the retina, particularly that of the ganglion cell layer. These authors reported that the appearance of 'b' wave in the ERG was delayed by two days in this group. It is well known that the development of inner synapses precedes the appearance of the 'b' wave and it is therefore reasonable to assume that synapse formation is delayed in the retina of the developing rat, owing to the deficiency of vitamin B6. These authors also reported that the same level of threshold intensity of ERG as in the case of adults was delayed by 6 days in the case of B6 deficient rat pups. A delay in the acquisition of depth perception by 2 days was also observed in the deficient group.

In an elegant study on the effects of maternal vitamin B6 deficiency on specific regions of the developing rat brain, the extrapyramidal motor system, Wasynczuk *et al.* (1983) demonstrated that in the caudate or putamen, where some synapses had been postulated to arise from GABAergic striatal inter-neurons, fewer synapses were present in the progeny of rats deficient in vitamin B6. Furthermore, the content of GABA was decreased in this region. This may result in a dysfunction of the circuitry of the caudate nucleus or putamen under vitamin B6 deficiency, leading to gross neurological symptoms.

Guilarte (1989) reported that dietary restriction of vitamin B6 during gestation and lactation produces spontaneous seizures in neonatal rats. The concentrations of neuroactive amino acids, glutamic acid and gama-aminobutyric acid were significantly lower and those of glycine significantly higher in selected brain regions of vitamin B6 restricted 14-day-old rat compared to the controls. Remarkably, these changes were unique for 10 and 14 days of age where spontaneous seizures were observed, but were not present at the age of 28 and 56 days, when seizures were absent.

More recently, Pilachowski and Guilarte (1993) showed that vitamin B6 deficiency significantly reduced the potency of GABA to enhance the [^{3}H]-flunitrazepam binding to cortical membranes prepared from 14-day-old rats. These results suggest that an uncoupling of the $GABA_A$/benzodiazepine receptor occurs at a developmental period when the animals are most susceptible to spontaneous seizures. Recent findings from human and animal studies on vitamin B6 and cognitive development have been reviewed by Guilarte (1993). These studies have indicated that vitamin B6 deficiency during gestation and lactation alters the function of N-methyl-D-aspartate receptors, a subtype of receptors of the glutamatergic neurotransmitter system, considered to play an important role in learning and memory.

Kirksey *et al.* (1990) demonstrated in their anatomical study that deficits of vitamin B6 imposed *in utero* and up to 30 days postnatally, decreased the number of neurons in the neocortex. Higher order dendrites were reduced and the synaptic density was found to be diminished. In the cerebellum, molecular and granular areas were reduced. The organization of Purkinje cells was disrupted, and myelinated axons were reduced in number. While this study has thus confirmed earlier observations regarding the critical role of pyridoxine in neurogenesis, the pathological changes in neuronal differentiation and synaptogenesis, coupled with decreased myelination, must affect the rate and extent of transmission of nerve impulses.

Cobalamin

Vitamin B12 deficiency is known to produce abnormalities in the CNS of developing chick embryos (Alexander 1957). Jadhav *et al.* (1962) reported that infants of severely vitamin B12 deficient mothers develop involuntary movements suggestive of basal ganglia involvement and also spasticity and extensor plantar responses suggestive of involvement of the pyramidal tract. Furthermore, the EEG showed a generalized slowing down in one infant studied.

Stollhoff and Schutte (1987) reported a case of a 18-month-old boy with megaloblastic anemia and a progressive neurological disorder clinically resembling leukodystrophy. The anemia disappeared after vitamin

B12 therapy and the neurological condition dramatically improved. One effect of the deficiency of methyl cobalamin and of the associated failure of the methionine synthase reaction has been shown to be associated with impaired myelination of the brain of newborns. It has been made clear that a normally functioning cobalamin-dependent methyl transferase is essential for the development and function of the human brain (Hall 1990). While studying the long-term neurological consequences of nutritional vitamin B12 deficiency in infants, Graham *et al.* (1992) found a consistent clinical pattern in deficient infants; irritability, anorexia and failure to thrive, associated with marked developmental regression and poor brain growth.

Folic acid

It has been shown that folic acid supplied in the periconceptual period can lower the occurrence of neural tube defects. These defects which can be prevented by administration of folic acid are thought to be due to the twin influences of hyperhomocystinemia and a genetic predisposition due to the mutation of the methylenetetrahydrofolate-reductase gene (Eskes 1997). Early studies in rats indicate that folate deprivation of mothers from an early stage of pregnancy results in congenital abnormalities of various organs in 95 % of litters, including the nervous system (Nelson *et al.* 1952). Stempak (1965) observed that a folic acid-free diet given during gestation results in the resorption of fetuses in the rat. It is possible, therefore, that extreme folic acid deficiency during pregnancy might produce alterations in the structure and function of the nervous system of the fetus. Arakawa *et al.* (1967) produced microcephaly and dilatation of cerebral ventricles in the offspring by administering 6-mercaptopurine to the dams on day 11 of gestation in rats

Arakawa *et al.* (1969a) induced folic acid deficiency in rats early in infancy which resulted in delayed maturation of the basic EEG pattern of rats at 5, 6 and 7 weeks of age. Arakawa and his associates (1969b) have also reported that there is definite delay in the maturational patterns of EEG of infants, breast fed by folate-deficient mothers.

These earlier studies were later confirmed by Reinis and Goldman (1980). According to these authors, folic acid is probably required for the development of the brain since maternal nutritional deficiency that further lowers the folate level in breast milk delays the development of the neonatal EEG.

Nicotinic acid

6-aminonicotinamide (6-AN) is a nicotinic acid (B3) antagonist which has a profound influence on brain development by inhibiting the enzyme ornithine decarboxylase involved in cellular replication and differentiation (Morris *et al.* 1985). Cerebellar morphology of the treated rats indicated an early adverse effect of 6-AN on granule cell division, resulting in eventual disruption of the characteristic laminar structure of this brain region.

Ascorbic acid

Zalani *et al.* (1989) reported higher concentrations of ascorbic acid in the brains of human fetuses when compared to the adrenal gland at all gestational ages. Though the authors suggested that the ascorbic acid may therefore be important for brain development, it is not clear as to how the vitamin C influences development, although it has been speculated that ascorbic acid and its oxidized forms are regulators of cell division (Edgar 1970).

Vitamin interactions

Vitamin interactions are of great importance in understanding developmental errors, caused by deficiency of various vitamins. Such interactions are more pronounced and best understood in the case of B vitamins. Vitamin B6 deficiency has been reported to impair vitamin B12 absorption in the rat which lowers vitamin B12 levels in the serum and reduces vitamin B12 stores in the liver (Yeh and Chow 1959, Ranke *et al.* 1960). In riboflavin deficiency, pyridoxine may not be properly utilized which can cause a deficiency of vitamin B6 (Krishnaswamy 1971). Biotin deficiency may be aggravated by simultaneous pantothenic acid deficiency. The addition of biotin to the diet not only protects the animals from a biotin deficiency but also reduces the severity of the symptoms of pantothenic acid deficiency. Vitamin C deficiency is known to affect folate metabolism and may therefore produce the symptoms of dietary deficiency of folic acid (May *et al.* 1950, Stokes *et al.* 1975). Ascorbic acid also enhances the reaction which transforms pyridoxine to pyridoxic acid. The question whether the intake of large doses of ascorbic acid increases the requirement for vitamin B6 or not has not yet been resolved.

In vitro synthesis of ascorbic acid is reduced in livers of vitamin E deficient rats (Carpenter *et al.* 1959, McCay *et al.* 1959). DNA synthesis is impaired in the

anemia observed in vitamin E deficient monkeys, similar to that resulting from vitamin B12 deficiency (Dinning 1962).

It is no less important to understand the interactions between vitamins and certain trace elements. Women deficient in vitamin D exhibit marked osteopenia during pregnancy which persists after delivery until vitamin D is administered (De Luca 1980). Enhancement of iron absorption from vegetable sources is directly proportional to the amount of ascorbic acid present. In fact, anemia from nutritional iron deficiency can be combated by using ascorbic acid (Lynch and Cook 1980). Besides, such interactions among trace elements and certain environmental toxicants must also be reckoned with. Sickle cell anemic children appear to be more susceptible to lead-induced neuropathy than are normal children. They have depressed plasma and red blood cell vitamin E levels and have elevated levels of lead in their hair (Erenberg *et al.* 1974). As people are constantly exposed to multiple nutritional and/or toxic effects, justification for multifactorial studies is increasing and should improve the understanding of the relationship between vitamins and brain development.

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