

One of the challenges of this century will be to develop sufficient diagnostic tools to assess copper status in humans. This has been a real limitation that has hindered not only the proper management of copper nutrition but also the credibility of this essential nutrient in public-health and agricultural decisions. For without a proper status indicator, it is very difficult to set recommended intakes of a nutrient.

Could there be permanent changes to the developing infant if copper is not adequate in the diet of the mothers and to what extent might this occur in the human population? A number of factors could have an impact on this scenario. 1) Infants born prematurely and those not having sufficient time to accumulate adequate copper even if it were able to be delivered by the pregnant mother are at risk. 2) Teen pregnancies, where the mother's own copper requirement is high for her own development in addition to delivering copper to the developing fetus, are an issue. 3) Limiting copper in the diet is an issue. Many studies have shown that the intake of copper in the US diet is lower than 1 mg of copper per day. This is well below the current Estimated Safe and Adequate Daily Intake for copper. 4) Excessive use of supplements that interfere with copper, in particular zinc supplements, which are becoming ever more popular for treating, for example, the common cold, are an issue.

CONCLUDING REMARKS

The long-term impact of inappropriate copper during perinatal development could be quite serious if extrapolation of data from iron to copper is considered. Full cognitive capacity of infants may be denied if adequate copper nutrition is not present during the developmental period of the brain. There is a critical need to identify good biochemical markers of copper status, especially during pregnancy and early development, to be able to detect and treat potentially copper-deficient subjects. There is little risk in

recommending a supplement of 1 mg of copper per day during pregnancy and lactation. This level would reverse problems of copper deficiency in infants and potentially could block the impact of low dietary copper on development of our children. This would have far-reaching consequences in allowing full cognitive development of our future generations.

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Nutrient Deprivation and Brain Function: Iron

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NEUROCHEMICAL CONSEQUENCES OF NUTRITIONAL IRON DEFICIENCY

It is well recognized that nutritional iron deficiency (NID) is the most prevalent nutritional deficiency in the world. It can affect more than 400 million individuals (according to the World Health Organization) and is most prevalent in infants and young children. The importance of iron in systemic cellular biochemistry, where it is used in the synthesis of DNA and proteins and is involved as cofactor for numerous enzymes, structural protein, and physiologic responses, is well recognized. The vast literature that has been published on its various functions and dysfunctions in systemic organs as a consequence of its deficiency is too numerous to

summarize in this editorial. However, what is not known and not readily recognized is that early iron deficiency (ID) can have a profound long-term effect on brain function, with possible irreversible brain damage at the cellular and neuronal levels. Even so, until 1974, little or no attention was paid to brain iron metabolism and brain function. Since then, there has been an active interest in brain iron metabolism, not only as a consequence of its deficiency, with an effect on learning and cognitive processes, but also the role of excess brain iron accumulation and its involvement in neurodegeneration and progressive neurodegenerative diseases (Parkinson's disease, Alzheimer's disease, Huntington's chorea, Haller-Voren-Spatz disease, etc.).¹⁻⁴ To appreciate the effect of NID on the brain, one must consider its distribution.

The first studies of iron in human brain were those by Pollitt et al.⁵ and Hallgren and Sourander⁶ who showed that adult brain iron was unevenly distributed and some brain regions, namely the extrapyramidal regions (globus pallidus, substantia nigra, thalamus, ventral thalamus, red nucleus, intrapenduncular nucleus,

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dentate gyrus, cingula nucleus), had the highest concentrations, and in some cases there was more iron per gram of wet weight in these regions than in the liver. Ferritin is similarly distributed. These data have consistently been confirmed for human, monkey, dog, and rat brains.

However, there is no correlation between distributions of iron-ferritin concentration and those of transferrin-transferrin receptors. The highest concentration of the latter is found in the hippocampal and cortical regions, which have relatively low iron and ferritin contents.⁷⁻¹⁰ By contrast, at birth in rat brain, most of the iron is found in cortical and hippocampal areas. Studies involving brain ⁵⁹Fe uptake in newborn rats with limited blood-brain barrier (BBB) have shown two crucial aspects of brain iron metabolism.¹ The major portion of ⁵⁹Fe is found in the globus pallidus, substantia nigra, intrapeduncular nucleus, dentate gyrus, etc.² More than 90% of the iron (⁵⁹Fe) present in newborn rats 24 h after its injection was still in the brain of the adult animal, which clearly indicates that iron is highly conserved in the brain and has a very slow turnover.^{11,12} Although iron transport into the brain is through transferrin receptors residing in capillary endothelial cells, the mechanism by which iron is transported from one brain site to another is not known. The most puzzling question, which cannot be discussed at length in this editorial, is why brain regions such as the globus pallidus, substantia nigra, dentate gyrus, and caudate nucleus, accumulate the highest concentration of iron in the brain. More recent studies have pointed to iron-induced oxidative stress in the neurodegenerative processes in these regions and the consequence is some of the most devastating neurodegenerative diseases (Parkinson's disease, Alzheimer's disease, Huntington's chorea, Haller-Voreden-Spatz disease). There have been several reviews dealing with brain iron metabolism, brain function in ID,^{1,13-15} and iron in neurodegenerative diseases.^{3,4} This editorial concentrates on the most recent findings of ID on brain function.

NUTRITIONAL IRON DEFICIENCY AND BRAIN IRON

The first studies that dealt with the effects of NID on the brain were those by Werkman et al.,¹⁶ Webb and Oski,¹⁷ and Dallman and others.^{18,19} These investigators reported that NID in children induced behavioral abnormalities including reduction of learning abilities (cognitive impairments) and that NID in rats fed an ID diet results in the reduction of brain iron, although not as much as that in the liver. These reports together with the earlier studies^{5,6} on the uneven distribution of iron in human brain prompted my colleagues and I to investigate the brain biochemical, pharmacologic, physiologic, and behavioral effects of NID in rats as a model for the human condition.^{20,21} Our original hypothesis was based on the notion that the behavioral changes noted in ID children by Webb and Oski¹⁷ were related to changes in the metabolism of aminergic neurotransmitters in the central nervous system, i.e., dopamine, serotonin, and noradrenaline, because a significant body of evidence has implicated these neurotransmitters in behavior and learning processes. There was good reason for this hypothesis because the rate-limiting enzymes for the synthesis (tyrosine and tryptophan hydroxylases) and catabolizing enzymes (monoamine oxidase) of the aminergic neurotransmitters were shown to be dependent on iron for their full enzymatic activity. Thus, any changes in the activities of these enzymes may alter the brain level of these neurotransmitters at specific sites (hypothalamus, striatum, raphe nucleus, frontal cortex, and hippocampus) in the brain and alter the physiology of their respective neurons as a consequence of over- or underproduction of the neurotransmitters. We demonstrated that, whereas NID induced in rats by feeding them a diet low in iron (5 parts per million) results in the reduction of iron-dependent enzymes (monoamine oxidase, phenylalanine hydroxylase, succinic dehydrogenase, cytochrome oxidase to mention a few) in peripheral tissues, none of the brain enzymes containing iron (including tyrosine hydroxylase, tryptophan hy-

TABLE I.

EFFECT ON IRON DEFICIENCY ON BRAIN ENZYMES*	
Phenylalanine hydroxylase	Unchanged
Tyrosine hydroxylase	Unchanged
Tryptophan hydroxylase	Unchanged
Monoamine oxidase	Unchanged
Aldehyde dehydrogenase	Unchanged
Cytochrome oxidase C	Unchanged
Succinic dehydrogenase	Unchanged
Aminobutyric acid transaminase	Decreased
Glutamate decarboxylase	Decreased

* Adult (aged 48 d) male rats were made nutritionally iron deficient by feeding them a semisynthetic diet deficient in iron. Control animals received the same diet, to which iron sulphate had been added. In both groups the animals were pair fed to maintain similar weight. There was a decrease in liver, heart, and adrenal glands.

droxylase, and monoamine oxidase) as a cofactor was changed (Table I),^{20,21} despite the fact that adult (48 d old) rat brain iron was reduced by 30% to 40% in the striatum, hippocampus, cortex, and raphe nucleus. The effect was region dependent. It is now apparent that the effect of NID on tissue (brain and liver) iron is also dependent on the age of the animal and time of the NID diet. Whereas adult rat (47 d old) liver iron and ferritin stores can be reduced relatively fast (within 2 wk by 80–90%), brain iron is hardly changed until 3–5 wk on the ID diet. Newborn pups (10 d old) were more readily made ID than young (21 d old) animals.^{12,18-21}

The unchanged activities of the brain neurotransmitter enzymes in ID rats are complemented by unaltered brain (striatum, caudate nucleus, and raphe nucleus) levels and turnover of dopamine, noradrenaline, and serotonin (Table II).^{20,21} Nevertheless, our animal behavioral studies with functional activities of the neurotransmitters dopamine and serotonin indicated a highly significant degree of deficit, which for the first time complemented the "behavioral" deficits reported by Webb and Oski¹⁷ in children with NID. The neurochemical explanation for the behavioral deficits was not easily forthcoming, and we suggested that brain ID may result in an alteration in receptor number and function for any of these neurotransmitters.²⁰ However, the ability to measure various brain neurotransmitter receptors (B_{max} , receptor number) and K_a by employing radioligands did not become available until 1976. As shown in Table III, the only neurotransmitter receptors that were affected were those of dopamine.²¹ By employing radioligand analysis of dopamine D1 and D2 receptors, we observed an increase in K_a of dopamine D1 and a decrease of dopamine D2 B_{max} in the striatum of NID rats, which complemented the significant diminution of dopamine-dependent behaviors, as elicited by the

TABLE II.

NEUROTRANSMITTERS AND THEIR PRECURSOR LEVELS IN BRAINS OF IRON-DEFICIENT RATS		
	Concentration	Turnover
Serotonin	Decreased	Unchanged
Dopamine	Unchanged	Increased
Noradrenaline	Unchanged	Unchanged
Tryptophan	Unchanged	—
Tyrosine	Unchanged	—
5-Hydroxyindole acetic acid	Decreased	—

TABLE III.

EFFECT OF IRON DEFICIENCY ON BRAIN NEUROTRANSMITTER RECEPTORS AS IDENTIFIED BY SPECIFIC RADIOLIGANDS		
Receptors	K _a *	B _{max}
γ-Adrenoceptor (³ H-WBA101)	Unchanged	Unchanged
β-Adrenoceptor (³ H-DHA)	Unchanged	Unchanged
Muscarinic-cholinergic receptor (³ H-ONB)	Unchanged	Unchanged
Dopamine D ₂ receptor (³ H-spiperone)	Unchanged	Decreased
Dopamine D ₁ receptor	Unchanged	Unchanged
5-HT ₂ receptor (³ H-serotonin)	Unchanged	Unchanged
γ-Aminobutyric acid receptor (³ H-musimol)	Unchanged	Increased
Benzodiazepine	?	?

* Affinity constant for the receptor.

treatments of rats with the dopamine agonist, apomorphine. This was the first time that a neurochemical change could be associated with the behavioral effects observed in NID. Our numerous behavioral and neurochemical investigations related to reduction of dopamine D₂ receptor B_{max} clearly indicates a subsensitivity of this receptor with the initiation of NID (Table IV).²¹ There was a direct parallel between time-dependent reduction of brain (striatum) iron, dopamine D₂ receptor B_{max}, and apomorphine-elicited dopamine-dependent behavior.^{12,21} This effect was related to iron deficiency and not to the anemia resulting from it because hemolytic anemia induced by chronic treatment of rats with phenylhydrazine does not alter brain dopamine D₂ receptor number or their behavioral responses.¹² Furthermore, supplementation of ID rats with an iron-enriched diet (control) can result in restoration of brain iron dopamine receptor B_{max} and behavioral responses. This was an age-dependent phenomenon and has clear clinical implications for children with NID. One obvious but extremely important finding in our studies was the handling of iron by brain versus liver during iron supplementation. Whereas rat liver iron could be restored with 1 or 2 wk, brain iron increased very gradually,

TABLE IV.

BIOCHEMICAL AND BEHAVIORAL CONSEQUENCES OF REDUCED DOPAMINE D ₂ RECEPTOR IN THE BRAINS OF IRON-DEFICIENT RATS	
	Response
Monoamine oxidase inhibitor plus tryptophan	Decreased
Monoamine oxidase inhibitor plus L-dopa	Decreased
Monoamine oxidase inhibitor plus 5-hydroxy-tryptophan	Decreased
5-methoxy- <i>N,N</i> -dimethyltryptamine	Decreased
D-amphetamine	Decreased
Apomorphine	Decreased
Learning processes	Decreased
Thyrotropin-releasing hormone and its analog	Unchanged
Phenobarbitone sleeping time	Increased
Serum prolactin	Increased
Serum testosterone	Increased
Liver prolactin receptor	Increased
Antinoceptive response to β-endorphin and enkephalins	Increased
Dynorphin and met- and leu-enkephalin concentrations in the globus pallidus, substantia nigra, caudate nucleus, and central gray	Increased

reaching its preiron deficiency level within 3–4 wk. Continuing iron supplementation for some 6 mo resulted in liver iron being increased by some 20-fold, whereas brain iron remained constant. This discrepancy between iron handling by the liver and brain indicates that iron transport in the brain is handled differently and may be related to the BBB because in adult rats serum iron has no access to the brain. Furthermore, the turnover of brain iron is significantly much slower than the turnover in the liver, and almost all the iron that is present in the brain is conserved throughout life.

Examination of BBB in NID rats indicated selective alteration, and we suggested that this alteration could be at the level of the gap or tight junctions of capillary endothelial cells that constitute the BBB.²² However Taylor et al.²³ also provided evidence for an upregulation of transferrin and transferrin receptors during NID and a downregulation when brain iron is restored. It is possible that both mechanisms are involved, and clearly more work needs to be done to clarify the differences between liver and brain handling of iron. Certainly the roles of the recently described iron regulatory proteins 1 and 2 during iron deficiency and repletion in the brain need clarification.^{24,25}

CONSEQUENCES OF EARLY IRON DEFICIENCY ON BRAIN FUNCTION AND BEHAVIOR

It is during the first decade of a child's life (the first 4 y) that ID becomes evident.¹⁴ This is apparently the most crucial period of brain development, when DNA and protein synthesis and neuronal growth and differentiation take place and maturation of enzymes occurs. In this period, myelination of neurons is at its peak, and there is an essential role for iron in myelin deposition by oligodendrocytes.²⁶

ID significantly interferes with myelination of the neurons.²⁷ Numerous studies on rat brain development have confirmed this observation. The obvious question would be, What are the long-term consequences of ID in this period on brain development and function? Dallman and colleagues^{18,19} reported persistent deficiency of brain iron during short-term deprivation in young rats after iron supplementation. They attributed this persistence to the very slow turnover of iron in the brain as opposed to that in the liver.

We reexamined this finding in rats of different ages (newborn, young, and adult) made NID. Not only did we confirm the findings of Dallman et al. in newborn rats, but we also showed this feature to be age dependent.¹² Whereas young and adult rats could recover their brain iron, newborn rats could not after iron repletion.

Furthermore, newborn rats had unrecoverable behavioral deficit, which was related to the deficiency of striatal dopamine D₂ receptor, and brain iron could not be restored even after 6 mo of iron therapy, despite the normalization of their hematologic indices. These findings point to long-term irreversible consequences of early ID on brain function and may support what Lozzof et al. and others^{28–31} have consistently reported in ID infants and young children with impaired attentional problems where long-term iron therapy was ineffective. Whether our animal models reflect the human condition may be a matter of debate, and all animal models have their drawbacks. Nevertheless, early ID does impair brain biochemistry and function, and its consequences need to be appreciated, considering the susceptibility of human brain to undernourishment in the first decade of life^{32–34} when the major portion (80%) of iron found in the adult brain is deposited and interaction of iron with other metals may be crucial.^{33,34}

Although our original behavioral studies were related to dopamine neurotransmission deficit in ID, we have extended our work to investigate learning parameters in the Morris water maze. Young ID rats were poorer performers (longer time and more trials) in finding the platform in the Morris maze and had lower activity,^{35–37} and 4 wk of iron therapy (repletion) did not alter the

learning performance of these rats. Confirmation of this result has recently come from Felt and Lozzoff.³¹ These results raise the concern that ID during the course of early brain development, both prenatally and postnatally, are damaging. Thus, it is crucial to maintain normal brain iron concentration^{32–34,38,39} because neuronal iron uptake is age dependent and iron deficiency can occur as a consequence of iron's dependence on the transferrin receptor.⁴⁰ The one puzzling factor in comparing the effects of NID in newborn and adult rat brain iron is that adult but not newborn rat brain recovers its iron, which suggests some other unidentified, underlying, and irreversible cause.

INTERACTION OF DOPAMINE AND OPIOIDS IN IRON DEFICIENCY AND THE LEARNING PROCESSES

The role of dopamine in learning and cognitive processes has been discussed at length by Yehuda and myself.^{36,37} Consideration also has to be given to other mechanisms and changes that occur in the brain during ID as a consequence of reduction in dopamine neurotransmission or other processes. For example, we showed that ID alters several brain proteins as identified by two-dimensional electrophoresis; among these was a protein with a molecular weight identical to that of dopamine D2 receptor that decreased, whereas the other proteins increased.⁴¹ We were not able to identify many of the protein changes. What roles these protein alterations have in brain function clearly need thorough investigation.

The brain areas known to have the highest concentration of iron (globus pallidus, substantia nigra, dentate gyrus, caudate nucleus, thalamus, putamen, ventral tegmentum) are innervated with the densest population of opioid peptides (enkephalins, endorphins, and dynorphin B). The importance of endogenous opioid peptides alone and their interaction with dopamine in learning processes has been investigated, and it is now evident that they are closely involved in learning and cognition processes. On several occasions, it has been reported that administration of the opioid antagonists (e.g., naloxone, MIF) improves learning,^{42–43} and this may be dependent on dopamine transmission functional activity and learning processes. As a consequence of dopamine D2 receptor subsensitivity, we investigated the function of central opiates in these animals. ID rats showed a highly significant antinociception that was further exaggerated when treated intraperitoneally with the opiates morphine, met-enkephalin, leu-enkephalin, and endorphin. Naloxone and MIF^{44–46} could block these effects. Normal rats do not show antinociception to opioid peptides because these peptides do not cross the BBB and are rapidly metabolized by metalloproteinase in the systemic organs. Thus, in NID newborn rats, metabolism of opioid peptides and their brain transport is affected. BBB studies have clearly shown an uptake of the opioid peptide β -endorphin in ID but not control rats.²² The latter results may indicate the reason ID but not control rats exhibit antinociception in response to opioid peptides (met-enkephalin and dynorphin B) as measured in the globus pallidus, caudate nucleus, substantia nigra, midbrain tegmentum, and central gray.^{42–46} The mechanism whereby NID brings about increased brain levels of opioid peptides is not well understood. It is well established that dopamine is inhibitory to opiates,^{42,43,47} and antagonism of dopamine receptors with dopamine D2 receptor antagonists (haloperidol, chlorpromazine) results in brain elevation of opioid peptides similar to that shown in ID rats.^{47–50} The explanation for this may be found in studies where it has been demonstrated that dopamine D2 receptor antagonists induce proenkephalin mRNA.^{47–50} Whether NID brings about the same changes in opiate mRNAs and opiate antagonists can reverse the diminished learning processes remains to be investigated.

CONCLUSION

The effect of ID on brain function is a relatively new subject. Nevertheless, it is clear from animal studies that ID can profoundly affect the structural components, neurons, neurotransmitter metabolism, and function of the central nervous system. This may not be unexpected because iron plays a crucial role in many physiologic processes.

Further, iron metabolism is one of the most tightly regulated events within the cells because both its deficiency and excess can affect many enzymatic and structural proteins. Thus, iron homeostasis is crucial for brain function. The abnormality of iron in brain metabolism and function has not received as much attention as this abnormality in the systemic organs, which may be related to the notion that the brain is impervious to such changes. For example, it is now well recognized that there are a number of very important neurodegenerative diseases (Freidrich's ataxia, Parkinson's disease, Alzheimer's disease, Haller-Vorden-Spatz disease, Wilson's disease) where iron has access to the brain by accumulating in specific neurons and may be involved in the neurodegenerative processes in these diseases.³

This editorial has dealt with the effects of ID on the dopamine opiate system, mainly because little attention has been paid to other brain neurotransmitter systems (e.g., γ -aminobutyric acid, glutamate, nitric oxide) that could also be affected.⁵¹ Because the interaction between dopamine and opiates plays such a crucial central role in brain neurotransmission and affects other neuronal systems, it is most likely that the effects of NID on brain are more complex than previously thought.

More recent work from our own laboratory has indicated that brain ID is a two-edged sword. Although I have demonstrated throughout this editorial that ID can profoundly affect brain biochemistry and function, there is now evidence that in certain circumstances ID can be protective to the adult brain. Thus, rats made ID are less susceptible to brain neurodegeneration in response to neurotoxins (such as kainate and 6-hydroxydopamine) used as tools for animal models of epilepsy and Parkinson's disease.⁵² The mechanism of neurotoxin-dependent neurodegeneration has been attributed to the ability of these neurotoxins to release ferritin-dependent chelatable iron and the participation of the metal in redox generation of oxygen radical species, with the consequential onset of oxidative stress.

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