

# Functions of Trace Elements in Brain Metabolism

JOSEPH R. PROHASKA

*Department of Biochemistry, School of Medicine,  
University of Minnesota, Duluth, Minnesota*

I. Introduction .....	858
II. Copper .....	860
A. Distribution .....	860
B. Copper proteins .....	862
C. Nutritional studies .....	868
D. Genetic studies .....	872
E. Summary of functions .....	874
III. Iron .....	874
A. Distribution .....	874
B. Heme and nonheme proteins .....	875
C. Nutritional studies .....	876
D. Summary of functions .....	879
IV. Manganese .....	879
A. Distribution .....	879
B. Enzymatic functions .....	881
C. Nutritional studies .....	881
D. Pallid mice .....	883
E. Summary of functions .....	884
V. Selenium .....	884
A. Distribution and levels .....	884
B. Glutathione peroxidase .....	885
C. Nutritional studies .....	886
D. Summary of functions .....	887
VI. Zinc .....	887
A. Distribution and levels .....	887
B. Zinc proteins .....	889
C. Nutritional studies .....	889
D. Genetic mutants .....	892
E. Summary of functions .....	892
VII. Additional Trace Elements .....	892
A. Cobalt .....	893
B. Iodine .....	893
C. Molybdenum .....	894
VIII. Conclusion .....	894

## I. INTRODUCTION

The term "trace elements" is based on historical analytic experience and refers to a collective group of chemical elements present at low concentrations

in biological cells. Some of these elements perform essential functions and thus must be obtained from the environment in adequate amounts to optimize cellular metabolism. The list of essential trace elements for animals is subject to modification and under constant debate but includes the following 12 elements according to Nielsen: arsenic, chromium, cobalt, copper, iodine, iron, manganese, molybdenum, nickel, selenium, silicon, and zinc. Some evidence exists for the essentiality of five additional ultratrace elements (boron, fluorine, lead, lithium, and vanadium) and weaker evidence for three others (bromine, cadmium, and tin) (134). Not all of the essential trace elements have known neurochemical functions. This review focuses on those elements that have been shown to be required for the development and maintenance of the central nervous system (CNS), namely cobalt, copper, iodine, iron, manganese, molybdenum, selenium, and zinc.

The chemical form in which the essential trace elements exert their biological activity is quite variable. Iodine, for example, is inserted into a phenolic ring of tyrosine en route to synthesis of the hormones L-triiodothyronine ( $T_3$ ) and thyroxine ( $T_4$ ). Selenium is covalently bonded to carbon in selenocystine at the active site of the protein glutathione peroxidase, whereas copper exerts its effect in proteins by its coordination to four amino acid ligands.

The distribution of trace elements in brain is also variable. Iodine, for example, is found in high levels in the thyroid gland but in quite low levels in brain. Some elements are distributed fairly evenly in all brain regions. Selenium is an excellent example of this (151). Others, like copper and zinc, are highly enriched in certain regions and nuclei. Even within a cell there is disparity in distribution of the trace elements at the subcellular level. Thus each trace element has its own unique form(s), distribution, and functions within the CNS.

Trace elements, because of their relatively low concentrations, often function as catalysts in metabolic processes. A number of enzymes require stoichiometric quantities of a trace element to express their catalytic activity. Serving as an enzyme component constitutes the function of certain trace elements like copper, iron, manganese, molybdenum, and selenium. Iodine functions through  $T_3$  and  $T_4$ , whereas cobalt is located in the corrin ring of vitamin  $B_{12}$ . Zinc may have a special function in the hippocampus in addition to its known role in a number of zinc metalloenzymes. Many of the enzymes that require trace elements for activity are not unique to brain; however, some are, and these are usually involved in functions essential for neuronal homeostasis such as neurotransmitter metabolism.

Our current knowledge of trace elements in brain is greatly dependent on experiments in laboratory animals using chemically defined diets deficient in single nutrients such as copper, iron, manganese, selenium, or zinc. Certain experiments of nature (congenital loss of metalloenzymes or gene products, which alter trace element milieu) have also contributed to our understanding of trace element function in brain. There are a number of recent books that discuss the neurobiology of trace elements (43) or specific elements like iron

(143) or zinc (56) in some detail. This review will not attempt to duplicate those efforts but rather to focus on current work and concepts dealing with the functions of the trace elements in brain.

## II. COPPER

### A. *Distribution*

Copper is present in relatively high levels in brain and is distributed heterogeneously in two oxidation states (cupric Cu<sup>2+</sup>, cuprous Cu<sup>+</sup>). The distribution is dependent on brain region, subcellular location, and other variables such as age, species, environmental, and genetic factors. Neural distribution of copper correlates well with known neural functions of copper.

#### 1. *Regional distribution*

Shortly after sensitive methodological improvements were made in spectrophotometric and atomic absorption analysis of trace metals, it was possible to determine copper levels from specific brain regions, especially from human brain with its larger mass (Table 1). In general, levels of copper in gray matter exceed those of white matter two- to threefold. This may reflect protein distribution and concentration. Copper is also highly enriched in certain regions like the locus ceruleus and substantia nigra. Of those rat brain regions studied (Table 1), copper was highest in the hypothalamus. Levels of copper in adult rat brain are approximately one-half of those found in human brain (Table 1). Human brain averages ~0.1 μmol copper/g fresh tissue. More extensive regional information dealing with human brain is available (11, 182, 202, 207).

#### 2. *Subcellular distribution*

Subcellular fractionation of rat brain and rat brain regions has been performed, and these fractions have been analyzed for copper and protein. Relative to protein, copper is enriched in the cytosolic fraction (123) and the mitochondrial fraction (123, 161) of whole brain, whereas copper and protein constitute about the same percent in the nuclear, myelin, microsomal, and synaptosomal fractions (123). However, one study indicated that the enrichment of copper in subcellular organelles may be dependent on brain regions (161). It should be mentioned that despite enrichment of copper in certain subcellular fractions, enough copper exists in the remaining organelles and membranes to be functional. The copper that exists within a subcellular organelle or membrane may exist within several components. For example, when the cytoplasm from rodent brain is fractionated by gel permeation

TABLE 1. Concentrations of copper, iron, manganese, selenium, and zinc in selected brain regions

Region	Element Levels, $\mu\text{g/g}$ dry wt				
	Cu	Fe	Mn	Se	Zn
<i>Human brain</i>					
Centrum semiovale	13.7	136		0.31	35.0
Cerebral cortex (frontal)	24.7	316	0.81	0.71	73.5
Cerebellar cortex	33.1		1.18	0.72	74.4
Cerebellar white matter	13.5		1.22	0.78	
Corpus callosum	9.8	123	0.97	0.39	28.8
Hippocampus	21.0	244	1.09	0.73	107
Locus ceruleus	201				
Pallidum	30.3	963	1.98	0.78	76.2
Putamen	32.9	874	2.24	1.07	76.8
Substantia nigra	59.9	675	1.36	0.87	66.8
<i>Rat brain</i>					
Cerebellum	14.3	116	2.35	1.10	53.5
Cortex	15.0	102	1.80	0.93	68.9
Hippocampus	14.5	132	2.13		88.2
Hypothalamus	18.3	89.4	5.88		52.0
Medulla oblongata	11.1	80.5	1.71	0.83	36.2
Midbrain	14.6	97.0	2.42		49.4
Striatum	14.9	61.4	1.91		67.4

Values are adult averages taken from several sources: human Cu (182, 183, 202, 207), Fe (72, 202), Mn (106), Se (106), and Zn (72, 182, 202); rat Cu (40, 99, 159), Fe (99, 100), Mn (40), Se (155), and Zn (40, 99, 100).

chromatography, several peaks containing copper can be distinguished (79, 139, 150, 195). The nature of these peaks is discussed in section II B5.

### 3. Factors influencing distribution

Other factors can influence the homeostatic levels of copper in addition to the variations in partitioning between brain regions, subcellular organelles, and membranes. These factors include, but are not limited to, age, species, environment, and genetics.

Most mammalian species demonstrate an increase in brain copper concentration during early postnatal life (183). Although the extent of the increase depends on the stage in neuronal postnatal development (hyperplasia vs. hypertrophy), the rise has been observed for many regions in human brain (110, 202) and for whole brain of rat (100, 158). Age also results in changes in the subcellular copper distribution, as it has been shown that the cytosol from young rat brain contains 97% of the total copper compared with 59% from

adult brain (195). Copper levels are not constant across species of mammals. Of interest, however, is the finding that human brain contains the highest copper concentration, whereas levels in cow, mouse, pig, rabbit, rat, and sheep brain are similar to one another and about one-half that found for human brain (139, 183). Regional studies show similar trends (Table 1).

Genetics can also influence brain copper accretion. In particular, mutation of the X chromosome, Menkes' disease in humans and the mottled mice, profoundly alters brain copper levels and function. The distribution of copper in brain can also be influenced by environmental factors such as an adequate energy and protein supply or the levels of antagonistic metals such as zinc and cadmium. Perhaps the most profound influence is exerted by dietary copper levels during brain development, for undernutrition by itself has little influence on brain copper levels (110). When copper delivery to brain does not satisfy the growth requirement, the concentration of brain copper does not rise. Interestingly, the deficits were similar for a number of brain regions when studied in sheep (75), rats (159), and mice (79). The specific effect of copper deficiency on brain function is outlined in section II C.

## B. Copper Proteins

### 1. Cuproenzymes

It has been known for some time that brain contains a number of copper ligands, and these appear to be proteins. Some of these proteins have known catalytic properties, whereas the function of others remains unknown (Table 2). Some brain enzyme activities change after dietary copper deficiency, but these are indirect changes and not copper dependent in a strict sense.

### 2. Cytochrome-c oxidase

One of the earliest enzyme activities known to be copper dependent was the catalysis of cytochrome-c oxidation. Shortly after the essentiality of copper

TABLE 2. *Brain cuproproteins*

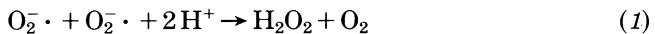
Name	Mol Wt	No. of Subunits	Cu/mol	Function
Albocuprein I	72,000	1	3	Unknown
Albocuprein II	14,000	1	3	Unknown
Copper thionein	6,000	1	12	Copper detoxification
Cytochrome oxidase	122,000	7	2	Electron transport
Dopamine $\beta$ -monoxygenase	290,000	4	8	Norepinephrine synthesis
Neurocuprein	9,000	1	1	Amine oxidation
Superoxide dismutase	32,000	2	2	O <sub>2</sub> dismutation

was established, Cohen and Elvehjem (24) showed that in brain of copper-deficient rats, the oxidase activity and a component of heme were reduced. After a somewhat argumentative period, it was shown that cytochrome oxidase did contain copper (204). Mammalian cytochrome oxidase is a complex of seven polypeptides; the sum of their weights equals 122,000 (216). The complex is the terminal oxidase of the mitochondrial electron transport system and is associated exclusively with the inner mitochondrial membrane. Cytochrome oxidase contains two molecules of heme *a*, in cytochromes *a* and *a<sub>3</sub>*, located on subunits I and II and 2 mol of copper located on subunit II (216). Both heme iron and copper are necessary for catalytic activity and are closely coupled (211).

In brain, cytochrome oxidase is an important enzyme in oxidative metabolism. Its distribution within neurons (soma vs. dendrites) reflects neuronal activity unique to that pathway (87). When an activity of 10 U cytochrome oxidase/nmol copper is assumed and the data in Reference 159 are used, the amount of copper in young adult rat brain associated with cytochrome oxidase is estimated at ~20% of the total brain copper, which indicates that this protein is one of the major cuproproteins of brain. However, an excess of mitochondrial copper exists whose function, if any, is not known. Cytochrome oxidase activity rises approximately fourfold between birth and weaning in rats (38, 158). The function of brain cytochrome oxidase is discussed in section II C1.

### 3. Superoxide dismutase

Eucaryotic cells, including neurons and glia, contain two distinct proteins that catalyze the disproportionation of superoxide ( $O_2^- \cdot$ ), the univalent reduction product of dioxygen (58)



Both are called superoxide dismutases (SOD). One is a homodimer of 32,000 mol wt. Each subunit contains 1 mol copper and zinc (CuZn-SOD). The other eucaryotic SOD contains one manganese per subunit and is a tetramer of 80,000 mol wt. The subcellular distribution of CuZn-SOD and MnSOD is unique with MnSOD located in the matrix of mitochondria, whereas CuZn-SOD is located primarily within the cytoplasm. Recently, an extracellular form of superoxide dismutase (EC-SOD) has been described (120). This protein is a tetrameric copper-containing glycoprotein and has a molecular weight of ~135,000. It may also contain zinc. It has been detected in samples of human brain at very low levels.

For the past 17 years, CuZn-SOD has been the subject of intense neurochemical research, since it was discovered that the brain protein called cerebrocuprein, which was isolated in 1957 (144), had enzymatic activity (dis-

mutation of  $O_2^- \cdot$ ). Cerebrocuprein was subsequently shown to be identical with proteins isolated from red blood cells (erythrocuprein) and liver (hepatocuprein). Copper and zinc superoxide dismutase is a major cuproprotein of brain and may account for an estimated 25% of the total copper in rat brain (139). The CNS is a highly aerobic organ consuming, in the resting state, about one-fifth of the total oxygen debt. The CNS is also sensitive of oxygen toxicity. Thus SOD activity has been assumed to be important in protecting brain from elevation of  $O_2^- \cdot$  during normal conditions and under various neuropathological states.

The brain has fairly constant levels (U/mg protein) of CuZn-SOD throughout. In rat brain the medulla appears to be slightly enriched in CuZn-SOD (196, 109), whereas in human brain (112) this is not so evident. What is clear, however, is the lack of CuZn-SOD enrichment in centers especially high in copper (Table 1). The CuZn-SOD appears to rise during postnatal development, reaching a plateau 2–3 mo after birth in rat brain (124, 158). Thereafter the activity appears to fall modestly with increasing age (33, 201). These trends do not correlate with corresponding changes in brain copper levels (122). It has been pointed out that a positive correlation exists between brain CuZn-SOD activity and life-span in long-living mammals (138).

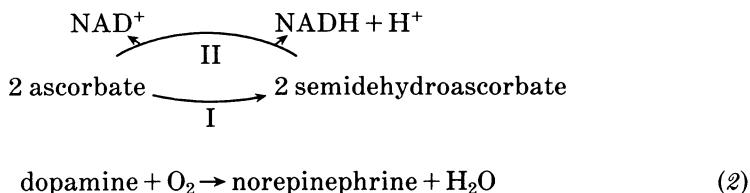
One might ask if these developmental changes in activity or others, which might occur because of genetic or environmental factors, compromise the defense mechanisms of the CNS toward oxygen metabolites. Or, more simply stated, do changes in brain CuZn-SOD activity have metabolic significance? Unless an enzyme is rate controlling in a metabolic flux, small changes in activity pose little threat to homeostatic conditions. For example, when brain CuZn-SOD activity was reduced ~25% by dietary copper deficiency in young rats, no evidence of enhanced lipid peroxidation was observed (159). However, when enzyme activity was inhibited to about the same extent (31%) in rats injected with diethyldithiocarbamate (a copper chelator), time to seizure when stressed with hyperbaric oxygen was reduced (160). This suggested that CuZn-SOD does play a role in antioxidant defense in brain. The dose of diethyldithiocarbamate used (160), however, would also have had other effects on brain, such as depletion of norepinephrine via its action of dopamine  $\beta$ -monooxygenase, inhibition of glutathione peroxidase (another antioxidant defense enzyme), and elevation of  $H_2O_2$  (179). Therefore, a tentative conclusion would be that aberrant metabolism might develop if CuZn-SOD were reduced beyond some critical point.

A number of neuropathological diseases in which brain lipid peroxidation has been implicated have been studied, and the activity of CuZn-SOD has been measured. These include chronic alcoholism, multiple sclerosis, neuronal ceroid-lipofuscinosis, and Down's syndrome. No changes in CuZn-SOD in brain were found, except in Down's syndrome. In this condition, trisomy 21, genetic amplification of CuZn-SOD results in a 50% increase in enzyme and a corresponding increase in lipid peroxidation (as measured by malondialdehyde formation *in vitro*) (15). This occurs, as suggested by the authors, because of

an increase in  $H_2O_2$  levels from excessive dismutation of  $O_2^-$ . In brain, no compensatory rise in glutathione peroxidase occurred (15). In conclusion, copper, through the CuZn-SOD molecule, exerts one of its biological functions in superoxide metabolism (see Eq. 1).

#### 4. Dopamine $\beta$ -monooxygenase

A third copper-dependent enzyme is dopamine  $\beta$ -monooxygenase (DBM). This large (mol wt 290,000) glycoprotein tetramer has interested neuropharmacologists greatly, since the enzyme is responsible for synthesis of the neurotransmitter norepinephrine



*Reaction I* is catalyzed by DBM and *reaction II* by semidehydroascorbate reductase (38). Ascorbate is believed to be the endogenous reductant for conversion of  $Cu^{2+}$  to  $Cu^+$ . Although somewhat controversial, current evidence suggests that DBM contains 8 mol copper/tetramer when fully active (3). Unlike cytochrome oxidase and SOD, DBM is a minor copper protein of brain. If 8 mol copper/mol active enzyme and a specific activity of 65 are assumed (3), it can be calculated, using data from 12-day-old mice (157), that the amount of copper in a young mouse brain associated with DBM is  $\sim 0.02\%$  of the total. This estimate likely holds for adult brain as well, since total copper and brain DBM activity change proportionately, roughly doubling in value (27, 158).

Regional distribution of DBM correlates, as it should, with noradrenergic neurons. Cell bodies of noradrenergic neurons are enriched in the locus ceruleus, brain stem, and posterior hypothalamus, and in these regions DBM is also enriched as determined enzymatically (27, 166) or immunocytochemically (22). The histochemical method has also shown DBM to be present in distal axons where, presumably, it is located near the norepinephrine storage granules. Thus, in contrast to cytochrome oxidase and CuZn-SOD, DBM has a unique and highly specialized distribution.

#### 5. Neurocuprein

When rat brain cytoplasm is fractionated by gel filtration chromatography and copper is monitored, most of the copper is associated with three

peaks (*I*, *II*, and *III*), roughly corresponding to fractions that contain proteins of molecular weights of 150,000 (*I*), 30,000 (*II*), and 10,000 (*III*) (195). A similar situation exists for mouse brain (79, 139). Additionally, a small pool associated with low-molecular-weight species may also exist (79). Although the identity of the three fractions is not known with certainty, *fraction II* may be CuZn-SOD. *Fraction III* may be a neurocuprein (176), an acidic protein isolated from bovine brain with a molecular weight of 9,000 containing a single atom of cupric copper. However, neurocuprein may not be present in rat brain; a recent review failed to list rat, but included cat, rabbit, pig, sheep, and human brain (131). Bovine neurocuprein does not possess dismutase or hydroxylase activity, but the copper can be reduced by epinephrine and norepinephrine (131). It is tenuous, at this time, to refer to neurocuprein as a cuproenzyme. However, its abundance (176) (perhaps twice as high as CuZn-SOD) emphasizes the need to learn more about this protein. In particular, the discovery of a protein similar to neurocuprein in bovine medullary chromaffin granules (131) raises the possibility of a function in catecholamine metabolism.

#### 6. Albocupreins

In 1971 a paper was published (59) that described the isolation from human brain of two new copper proteins. They were called albocuprein I and II and were pale yellow proteins of molecular weights 72,000 and 14,000, respectively. Neither protein had a described catalytic property. Unfortunately no further studies followed this initial report.

The initial study (59) did show that antibodies against albocuprein I inhibited ceruloplasmin activity. Ceruloplasmin is a serum glycoprotein of molecular weight 132,000 that contains copper. Linder and Moor (111) have shown that ceruloplasmin can be detected in rat brain. Further effort will be necessary to determine if albocuprein I is a unique neural cuproprotein or partially degraded entrapped ceruloplasmin.

Albocuprein II is not chemically similar to neurocuprein (131, 176), except both are low-molecular-weight soluble cuproproteins. Albocuprein II has no electron paramagnetic resonance spectrum and is rich in sulfhydryl groups (59). It may be similar or identical to copper thionein, since its properties resemble closely those of metallothionein.

#### 7. Copper thionein

The identity of *fraction III* (mol wt 10,000) from gel chromatography remains unknown. However, in addition to neurocuprein and albocuprein II, a third candidate exists, copper thionein. Metallothionein (MT) is a small (mol wt 6,000) cysteine-rich protein that chromatographs as though it were larger because of its unusual shape. Metallothionein will bind a number of metals, including copper and zinc. In rats, the amount of MT present in brain

varies with age (12) but usually contains bound zinc rather than copper. However, MT with copper (copper thionein) is induced when brain copper is elevated (203). The function of MT in brain copper metabolism is not known, but it may be of minor importance unless copper becomes abnormally elevated (12).

#### 8. *Lysyl oxidase*

Strictly speaking, this protein is found outside the CNS in the connective tissue associated with the cerebral vascular bed (139). Copper is required for activity, the oxidative deamination of peptidyl lysine that results in formation of cross-links in collagen and elastin. Copper functions through this protein in maintaining optimal blood flow to brain.

#### 9. *Peptidyl glycine $\alpha$ -amidating monooxygenase*

Another copper-dependent activity closely associated with the CNS is found in pituitary secretory granules (46). This enzymatic activity requires molecular oxygen and is stimulated by ascorbate to produce an  $\alpha$ -amidated peptide from a glycine-extended precursor, as shown in the example below



Copper may be reduced in peptidyl glycine  $\alpha$ -amidating monooxygenase by ascorbate in a manner similar to the mechanism for DBM; see *Equation 2*. This copper-dependent activity may be responsible for the synthesis of several bioactive peptides whose precursors contain COOH-terminal glycine such as calcitonin, vasopressin, and oxytocin. This new finding suggests a novel neuroendocrine function for copper.

#### 10. *Copper complexes*

Uncertainty still exists regarding transport of copper from blood to brain. Some believe ceruloplasmin (111), the plasma cuproprotein, is responsible; others suggest that copper-amino acid complexes are taken up. Neural copper can exist in a pool with low-molecular-weight ligands, as shown by some (79). If the ligand is appropriate, profound biological activity could ensue. For example, certain copper complexes demonstrate anticonvulsant activity (185). Copper, at high concentrations, may also influence uptake and efflux of dopamine, norepinephrine, and serotonin from presynaptic terminals (102). Also copper-amino acid complexes may influence secretion of releasing hormones from hypothalamic granules (5), further suggesting a role for copper in neuroendocrine homeostasis. This latter effect may be mediated via prostaglandin

$E_2$  (6). Copper may have neural functions distinct from the cuproenzymes; however, because of strong reactivity, cupric ions at nonphysiological doses can exert marked effects on biomolecules. Further *in vivo* work will be necessary to demonstrate convincingly some of the novel suggestions for copper complexes.

### *C. Nutritional Studies*

One of the most successful ways of establishing the functions of micronutrients such as trace elements has been the use of purified diets and single-nutrient deficiencies. Often careful examination of the resultant pathophysiology has provided a means to search for and test a hypothesis based on a single biochemical event such as reduction in an enzyme that depends on the limiting nutrient. Perhaps no other trace element exemplifies this better than copper. For example, the hypopigmentation associated with copper deficiency is caused by reduction in tyrosinase activity, a cuproenzyme contained within melanocytes that begins oxidation of tyrosine in the formation of the pigment melanin. Another example is abnormal connective tissue associated with impaired cross-linking of collagen and elastin caused by reduction in lysyl oxidase activity. Numerous investigators have studied the neurochemical effects of dietary copper deficiency using primarily experimental rodents. These studies, summarized recently elsewhere (79, 131, 139, 148), are briefly described here to illustrate how nutritional studies can be used to establish the metabolic function of trace elements.

#### *1. Brain growth and development*

The hyperplasia and hypertrophy associated with maturation of the CNS are influenced by the nutritional environment as well as endocrine status. Once neurons and glia have differentiated and the blood-brain barrier has formed, the brain is quite resistant to dietary copper deficiency. Most of the studies and field examples that describe neuropathology in copper deficiency require deprivation of copper beginning with gestational development. These situations result in greatly reduced levels of copper in brain (75, 157, 158). Associated with brains from severely deficient animals are areas of focal necrosis and smaller weight. One feature of copper deficiency and CNS function that has received much attention is myelination.

Evidence is well documented that offspring of copper-deficient rats (39), mice (157), and guinea pigs (50) exhibit hypomyelination. Whole brain analyses indicate reductions in cholesterol, sulfatide, and galactocerebroside (39). Individual phospholipid analyses show elevations in phosphatidylcholine (39). Taken together, these findings indicate a reduction in the amount of myelin and the presence of immature myelin. Copper-deficient rat (158, 223) and

mouse brains (157) exhibit decreased activity of 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP), and rat (39) and lamb brains (141) exhibit reduced activity of UDP-galactose:hydroxy-fatty acid ceramide galactosyltransferase, both indicating a reduction in maturation of the oligodendroglial cells (cells that synthesize myelin). The composition of myelin from copper-deficient rat brain, with the possible exception of fatty acids (148), is normal even though the amount of myelin per gram of brain was greatly reduced (223). Does copper have a specific role in myelin formation?

This is difficult to answer, but certainly the role of copper in myelination has been overemphasized. Some argue that reduction in cytochrome oxidase activity limits protein and lipid synthesis in developing brain of copper-deficient animals. However, ATP levels are not reduced in brain of copper-deficient rats (159) and mice (170), which exhibit hypomyelination. Another possibility centers on reduced norepinephrine levels that accompany copper deficiency. Norepinephrine, *in vitro*, can induce oligodendroglial cells to synthesize additional amounts of CNP, a myelin marker (126). If the myelination response *in vivo* is coupled to norepinephrine (NE) levels, then the copper-dependent drop in NE levels, presumably because of reduction in DBM activity, might explain hypomyelination. However, copper-deficient C3H/HeJ mice had no reduction in CNP activity despite an 80% drop in brain NE levels (157).

Not all copper-deficient species demonstrate hypomyelination. Offspring from pigs and sheep (141), for example, are characterized by demyelination (destruction of myelin) principally in the spinal cord, a region relatively unaffected in rats (19). Lamb spinal cord may also exhibit hypomyelination, however, as lipid changes similar to that seen in rat brain were observed (141). The demyelination seen in lambs with enzootic neonatal ataxia might be due in part to dysmyelination; i.e., the presence of abnormal myelin can lead to premature or accelerated catabolism of myelin (demyelination). One group, for example, reported a deficiency in one of the myelin basic proteins in myelin from ataxic lambs (141). No such abnormality was seen in myelin from copper-deficient rat brain (223). Copper may protect against demyelination via its role in CuZn-SOD. The lipids of myelin, if subjected to peroxidation by aberrant formation of oxygen radicals like hydroxyl radical ( $\text{HO}^{\cdot}$ ), could trigger demyelination. Dietary copper deficiency is known to lead to reduction in brain CuZn-SOD activity (127, 158), so one could postulate such a mechanism. However, it should be emphasized that no evidence exists for lipid peroxidation in brain from copper-deficient animals (159). Copper may have a specific function in myelination, but that function remains unknown. It is clear, however, that adequate copper is needed during brain development to ensure proper growth and maturation.

Rather than a specific neural growth requirement for copper, the development observed [hypomyelination and impaired synaptogenesis (148)] may reflect an inadequate nutrient supply due to vascular abnormalities (lysyl oxidase). Some, for example, have suggested that the lesions in copper-deficient rat brain resemble those in severe hypoxia.

## *2. Mitochondria*

A large portion of the total cellular copper pool is associated with mitochondria. Of course, a large portion of this is attached to cytochrome oxidase, but mitochondria isolated from copper-deficient brain contain copper beyond that accounted for in cytochrome oxidase (184). Does mitochondrial copper have other functions? From the excellent work of Keyhani and Keyhani (96) with yeast, it is known that copper is necessary for the conversion of porphyrin *a* to heme *a*. This explains nicely why heme *a* is lower in copper-deficient brain (24, 61) and in isolated mitochondria (159, 184). In fact, the loss of "activity" correlates with heme *a* content rather than copper content (159, 184). The molecular requirement and location of copper for heme *a* formation is not known.

In moderate copper deficiency, brain mitochondria do not demonstrate abnormalities in oxidative phosphorylation (61). Mitochondria from offspring of deficient dams, however, are enlarged in shape and impaired in function (159). One study (159) found an impairment in glutamate but not succinate oxidation, as if a defect might be site I specific. This work needs verification. Brains from copper-deficient rats (159) and mice (170) have elevated lactate levels, also suggesting mitochondrial dysfunction in regard to NADH oxidation. Functions for the non-cytochrome oxidase mitochondrial copper pool need further research for elucidation.

## *3. Neurotransmitters*

Evidence is quite clear in both domestic animals and laboratory rodents that copper deficiency alters behavior and coordination, suggesting CNS involvement. Do these observations aid us in establishing neurochemical functions for copper?

Perhaps the first recognized CNS involvement of copper was the motor incoordination of lambs suffering from enzootic ataxia or sway-back (139, 183). This might be due to demyelination of certain spinal cord tracts but might also be due to imbalance in neurotransmitters. O'Dell et al. (140) showed that brain stem levels of NE and anterior brain stem levels of dopamine (DA) were lower in brains from ataxic lambs. Dopamine levels were restored by copper treatment, but ataxia persisted (140). Serotonin levels were not changed. Earlier work in copper-deficient suckling rats established that compared with copper-adequate animals brain NE levels were lower (158); however, DA was not measured. Later work in the same lab with mice and rats confirmed the NE deficit but found no major changes in DA levels (148, 157). However, another group has found in copper-deficient rats lower DA and confirmed the NE deficit as well (53, 127). No apparent reason for these differences has been found (139). Interestingly, copper repletion corrected the NE deficit (53, 159) but not the DA loss (53). These alterations in

brain catecholamine pool size might help explain the aberrant behavior of offspring from copper-deficient dams described independently by others (19, 39). Unless copper deficiency is severe, behavior defects are difficult to demonstrate (197).

What caused the NE deficit has been a puzzling story. It was originally proposed (158) that decreased DBM activity in copper-deficient brain would result in the NE deficit. However, when measured *in vitro*, DBM activity was higher in the samples from copper-deficient animals (157). This was confirmed independently for adrenal gland and two other species (67). Further work implicated brain ascorbate in copper deficiency (154). However, a satisfactory explanation might be that when estimated *in vitro* apo-DBM is activated by extraneous copper, whereas *in vivo* DBM activity is functionally depressed and rate controlling. Four points support this idea. First, when isotopic conversion of labeled DA was monitored *in vivo* in rat heart (186) and mouse brain (80) from copper-deficient animals, decreased conversion to NE was observed. Second, apo-DBM can be rapidly activated by traces of copper (180). Third, pharmacological depletion of NE by reserpine leads to elevation in DBM activity (165). Fourth, a similar rise in activity of peptidyl glycine  $\alpha$ -amidating monooxygenase (another ascorbate-dependent monooxygenase) has been reported when enzyme activity was measured under optimal copper conditions from the neurointermediate lobe of the pituitary gland from copper-deficient rats (115). However, results from another lab appear to confuse the issue. They found with modest copper deficiency in postweanling rats a decrease in DBM activity in cortex, hippocampus, and cerebellum (213) but no change in NE levels in the same regions (212). Further effort will be required to satisfactorily elucidate the molecular basis for copper's role in NE homeostasis.

Copper may have another function in neurotransmitter metabolism in addition to its role in maintaining NE and DA pool size. Three laboratories have examined transmitter receptors in brains from copper-deficient rats in comparison to controls. The degree of deficiency and time of induction no doubt have influenced the experiments. Results are confusing and conflicting. Despite reductions in DA levels in copper-deficient rats, the corpus striatal receptor density did not rise but, in fact, fell (54). The same study showed decreased muscarinic receptor density. No changes in ligand affinities were noted in these studies. However, other workers found a decrease in muscarinic receptor density and affinity (51, 52), whereas a third group actually found an increase in muscarinic and  $\gamma$ -aminobutyric acid (GABA) receptor density (62). This latter study found no change in striatal DA receptor density. Needless to say, further effort will be required to determine the significance and mechanisms of these apparent changes in neurotransmitter receptors after copper deficiency.

Cupric ions are very reactive even at submicromolar concentrations, and it is quite apparent from *in vitro* studies that copper can influence a number of processes including neurotransmitter homeostasis. However, these obser-

vations need confirmation *in vivo*, since most of the ionic copper if administered extracerebrally would not penetrate the blood-brain barrier. Furthermore, ionic copper that did enter would likely be bound to protein ligands and thus less potent. For example, copper has been shown to facilitate binding of synaptic vesicles to plasma membrane (73) and to affect catecholamine release and uptake from rat brain synaptosomes (102). However, chronic copper dosing, although increasing brain copper levels, did not alter levels of NE, DA, or serotonin (105) nor did it influence high-affinity uptake of these same neurotransmitters (101). Another study showed that rat brain NE and DA were elevated slightly by feeding a diet containing 250 ppm copper (129). Serotonin levels were not altered despite a 2.7-fold elevation in brain copper.

The most clearly defined role of copper in neurotransmitter function is the copper-dependent regulation of NE levels. This is likely because of its role in the enzyme DBM (e.g., see *Eq. 2*). A recent study has suggested that copper may be involved in endogenous opiate homeostasis as data showed a fall in plasma enkephalin levels associated with copper deficiency and restoration after copper repletion (8). Other possible roles for copper in synthesis of other neurotransmitters, their receptors, and their uptake and release must await further elucidation.

#### *D. Genetic Studies*

A number of experiments of nature have aided in establishing the neurochemical significance of copper. Mutations in humans and mice exist, which confirm and extend the work done with domestic and laboratory animals. These mutations have been discussed elsewhere recently (139, 153).

##### *1. Humans*

In humans with Menkes' disease, neuropathological lesions that are similar to those in experimental animals have been observed. Menkes' disease results from an impairment of copper efflux from epithelial tissues, such that copper is elevated as copper thionein in kidney and intestine. Copper levels are low in liver and brain. Other trace elements are normal in concentration (136). The defect is the result of a mutation carried on the X chromosome. Neuronal degeneration, hypomyelination, cerebral vascular lesions, abnormal mitochondria, and motor dysfunction have all been described in young males with Menkes' disease (36). Biochemically, brains are characterized by low copper levels (36) and decreased cytochrome oxidase (57, 114) and DBM activity (167). Evidence for enhanced lipid peroxidation also exists (57), perhaps reflecting an impairment in CuZn-SOD activity. This mutation clearly demonstrates the importance of copper for human brain development and metabolism.

Another human mutation, this one an autosomal recessive trait, influences copper homeostasis and neuronal function and is called Wilson's disease. In this disorder, copper is not cleared from the liver, and eventually hepatic capacity is exceeded, and brain copper rises (35). Persons with Wilson's disease exhibit tremors that may be due in part to altered monoamine metabolism in brain (135). Copper may also inhibit some fundamental metabolic process such as glycolysis (104) and thus impair energy metabolism. This point is not clearly established.

## 2. Mice

A number of mutants (139, 153) have been described that exhibit abnormalities in copper homeostasis, but only the mottled mutants have consistently been shown to have altered copper metabolism in brain (Table 3). These mutants are genetic homologues of Menkes' disease, as the genetic inheritance (X linked), biochemical expression (77), and neuropathology (218) are similar. The neurochemical aspects of the mutant mice, especially the brindled allele, have been studied best by Hunt, and this work is reviewed elsewhere (79). Recently, Prohaska and co-workers have studied these mutants. Biochemically, brindled males display signs consistent with dietary copper deficiency in mice, such as reductions in concentration and activities of brain copper, NE, cytochrome oxidase, CuZn-SOD, and DBM (78, 80, 149, 157). Repletion with copper corrects, to a certain extent, these alterations (79, 152, 214). The pleiotropic effects in brain of the brindled mutation reflect a reduction in

TABLE 3. *Mutants that have altered trace element metabolism in brain*

Name	Species	Element	References	
			For	Against
Acrodermatitis enteropathica	Human	Zn	65	
Acquired vitamin B <sub>12</sub> deficiency	Human	Co	1	
Brindled ( <i>Mo<sup>br</sup></i> )	Mouse	Cu	77	
Crinkled ( <i>cr</i> )	Mouse	Cu	82	117
Menkes' disease	Human	Cu	36	
Pallid ( <i>pa</i> )	Mouse	Mn	26	
Quaking ( <i>qk</i> )	Mouse	Cu	95, 168	147
Toxic milk ( <i>tx</i> )	Mouse	Cu	163	
Wilson's disease	Human	Cu	35	

Table does not include mutations resulting in altered levels of brain metalloproteins such as Down's syndrome for CuZn-superoxide dismutase (15), cytochrome oxidase deficiency for Cu and Fe (153), and sulfite oxidase deficiency for Mo (86). In addition to pernicious anemia, congenital errors influencing intrinsic factor structure and receptors, transcobalamin I and II, and adenosylcobalamin synthesis have been identified (1). The brindled mutant was the most studied of 5 allelic mutants at the mottled locus of the X chromosome.

copper rather than a genetic block in a specific enzyme. Thus this mutation merely confirms the importance of copper in neurochemical function.

A similar situation exists for a new autosomal mutation referred to as toxic milk (163). Mutant dams retain hepatic copper and produce milk deficient in copper, such that the offspring develop tremors due to low brain copper levels. Two other murine mutants, quaking and crinkled, were reported to have abnormal copper metabolism (82, 95). Other work did not confirm this (117, 147). Although recently a paper suggested that quaking mice have altered brain copper levels (168), the data indicated that the brains from these mice were edematous, so that, based on dry weight, many neuronal components would be elevated; the components measured were copper and zinc.

#### *E. Summary of Functions*

It would be extremely useful if a mutation altering a single copper enzyme were known. Then the specific effects of loss of activity of CuZn-SOD vs. DBM vs. cytochrome oxidase could be evaluated. The nutritional and genetic tools available currently are not able to elucidate which copper-dependent function is most critical to brain development and homeostasis. Perhaps the use of specific cuproenzyme inhibitors (certainly not chelators such as diethyldithiocarbamate) would be a way to study the neurochemical function of copper.

Two main selective neurochemical functions for copper are evident. First, copper has a role in oxygen metabolism both in facilitating electron transfer in oxidative phosphorylation (cytochrome oxidase) and in regulating the levels of  $O_2^-$  and hydrogen peroxide (CuZn-SOD). Second, copper has a role in catecholamine metabolism in synthesis of NE (DBM) and perhaps in effecting catabolism (neurocuprein) and flux (ionic copper) at synapses. Other trace elements also influence these neurochemical pathways, and this will be summarized collectively in section VIII.

### III. IRON

Deprivation of iron during brain maturation may have long-term detrimental effects on cognitive behavior. This fact emphasizes the importance of iron in cerebral function. Recent reviews explore this idea in detail (142, 199). Some recent neurochemical information is summarized.

#### *A. Distribution*

Iron, like copper, is distributed heterogeneously in brain exhibiting higher concentrations in gray matter compared with white matter. In human brain, especially high levels are found in the pallidum, putamen, and substantia nigra (Table 1). The regional variability in iron levels has also been noted for

rat brain, although somewhat more modest in nature (Table 1). The subcellular distribution of brain iron has been reported (161). Large variations in the concentration of iron ( $\mu\text{g}/\text{mg}$  protein) existed between brain regions in comparable subcellular compartments as well as between subcellular compartments within a given region. Additional research will be needed to comment further on subcellular iron distribution in brain. One of the methodological obstacles that must be considered when brain iron is analyzed is blood contamination because of hemoglobin. For example, Kofod (100) reported an average brain iron value of  $12.7 \mu\text{g}/\text{g}$  wet wt for rats that were perfused before analysis. In the same study a value of  $18.2 \mu\text{g}/\text{g}$  was reported for unperfused specimens. Iron in brain can be divided into two roughly equivalent pools, one containing heme iron and the other nonheme iron. For example, a value of  $\sim 7 \mu\text{g}/\text{g}$  wet wt for rat brain nonheme iron has been reported (31, 220).

The concentration of brain iron (heme and nonheme) is influenced by several factors including species, age, and the environmental supply of iron during development. The concentration of iron in brain is higher for humans than for experimental rodents (Table 1) and for domestic animals (100). The concentration of iron is higher in adults than in infants. This has been shown for rats (31, 100) as well as for humans (118, 202). Most of the increase occurs early postnatally. Nutritional deficiency of iron during development leads to lowered brain (nonheme iron) levels in several regions (220). Deficiency in postweaning rats does little to alter brain iron pool size but, of course, has rather dramatic consequences outside the CNS (118). The effects of nutritional iron deficiency on brain is covered in section III C.

### *B. Heme and Nonheme Proteins*

When studied in tissue cultures, neuronal cells appear to take up iron via a transferrin-mediated mechanism (191); however, for this to operate in vivo diferric transferrin would have to cross the capillary endothelial cells except in the circumventricular organs, which represent only 1% of the capillary surface area. The molecular mechanism for iron uptake from blood to brain is not yet delineated fully. However, recent studies in cell culture indicate that both neuronal and glial cells take up iron via a transferrin-dependent mechanism (192). Within neuronal cells iron is bound to protein. This protein-bound iron can exist in storage pools (ferritin) or functional pools (enzymes).

#### *1. Heme proteins*

Brain has a number of proteins that contain iron bound to protoporphyrin IX. These heme-containing proteins are not unique to brain but are very important in oxidation-reduction reactions (60). They include the mitochondrial electron transport cytochromes ( $b$ ,  $c_1$ ,  $c$ ,  $a$ ,  $a_3$ ) as well as cytochromes  $b_5$

and  $P_{450}$  of the endoplasmic reticulum. In addition, brain contains a small amount of catalase located in peroxisomes, the cytosolic heme protein indolamine 2,3-dioxygenase and perhaps L-tryptophan 2,3-dioxygenase. Thus the functions of brain heme iron proteins are quite variable including oxidative phosphorylation, acyl desaturation, peroxide reduction, and amino acid catabolism.

## *2. Nonheme proteins*

In addition to the storage pool of nonheme iron as ferritin, brain contains a large number of enzymes in various subcellular compartments that contain iron not associated with the protoporphyrin ring. Some of these nonheme enzymes include mitochondrial  $\alpha$ -glycerophosphate dehydrogenase, aldehyde oxidase, succinate dehydrogenase, and various other Fe-S centers in the electron transport system. Other examples include xanthine oxidase, tyrosine 3-hydroxylase, tryptophan 5-hydroxylase, and ribonucleoside diphosphate (60). The activity of the outer mitochondrial membrane enzyme monoamine oxidase seems to be altered by iron nutrition (193), but this flavoprotein does not contain iron. Thus nonheme iron, like copper, is important in both energy metabolism and neurotransmitter homeostasis in the central nervous system. Brain may contain small amounts of other nonheme iron-containing enzymes, which are known to exist in other organs (60).

## *C. Nutritional Studies*

The limited neurochemical research on iron is somewhat surprising considering the world-wide magnitude of nutritional iron deficiency and rather good evidence in both humans and research animals of impaired neurological function accompanying iron deficiency. Many believe that the major complications of iron deficiency are secondary to anemia. Certainly, oxygen delivery to the CNS is critical. However, from many elegant transfusion studies in rats it is known that iron deficiency within organs is evident and results in altered metabolism. This is true for brain as well. An excess of iron can also lead to impaired metabolism. Although rare from pure nutritional courses, iron overload in brain along with certain genetic diseases could lead to excessive lipid peroxidation.

### *1. Deficiency*

A nutritional deficit of iron during early development (gestation or lactation) leads to significant reductions in brain of ferritin and nonheme iron pools (30). Even after repletion this deficit remains, probably because of the slow turnover of brain iron and the establishment of the blood-brain barrier

(31). This early nutritional iron insult has long-term consequences to the behavior of rats (55, 210). It was observed in both passive and active avoidance learning that early iron deficiency led to an increased responsiveness or reactivity. Biochemical mechanisms for these and other behavior changes are not known with certainty. However, dietary iron deficiency does alter neurotransmitter homeostasis. Energy metabolism (cytochrome levels and oxidative phosphorylation) does not appear to change in brain of iron-deficient rats (113).

## *2. Neurotransmitter metabolism*

Studies designed to elucidate the effects of dietary iron deficiency on neurotransmitter metabolism and levels have been disappointing and controversial. Although in peripheral organs monoamine oxidase activity is lower in iron-deficient rats (193), studies with brain have found no differences due to diet (113, 220). The activity of tyrosine 3-hydroxylase (a nonheme iron-dependent enzyme) is also not lower in brain of iron-deficient rats (186). Not surprisingly, the steady-state levels of both NE and DA are unaltered by dietary iron deficiency (220). These negative results were demonstrated in brains of rats with significant decreases in nonheme iron pools (220).

The serotonergic pathways have also been studied. One group reported a small but significant increase in brain serotonin concentration in iron-deficient rats (113). They explained this on the basis of a significant decrease in the activity of aldehyde oxidase, a nonheme iron-dependent enzyme that catalyzes formation of 5-hydroxyindole-3-acetic acid. Other workers, also studying iron deficiency in postweaning rats, found no decrease in aldehyde oxidase activity and a small decrease in serotonin concentration in iron-deficient animals (220). The latter group also reported a modest decrease in brain tryptophan 5-hydroxylase activity in the iron-deficient rats.

In general, the effects of iron deficiency on catecholamine and serotonin synthesis, catabolism, and the resultant steady-state pools are not very impressive in contrast to the reproducible behavior changes. Recent work, however, implicates a function for iron in postsynaptic binding of both DA and serotonin. Radioligand receptor binding studies indicated that iron deficiency was associated with a reduction in D<sub>2</sub>-dopaminergic receptor binding sites, whereas no differences were observed in  $\alpha$ - or  $\beta$ -adrenergic, muscarinic, serotonergic, GABAergic, or D<sub>1</sub>-dopaminergic systems (219). Other workers found a role for iron in brain serotonin storage (88), which might explain some of the altered behavioral responses to pre- and postsynaptically serotonin-acting drugs (219, 220). Iron, like copper and other trace elements, exerts unique neurochemical effects on neurotransmitter homeostasis (Fig. 1).

## *3. Iron overload*

Normally, nutritional iron excess is not a problem for the brain due to limited intestine uptake and a functional blood-brain barrier. However, in-

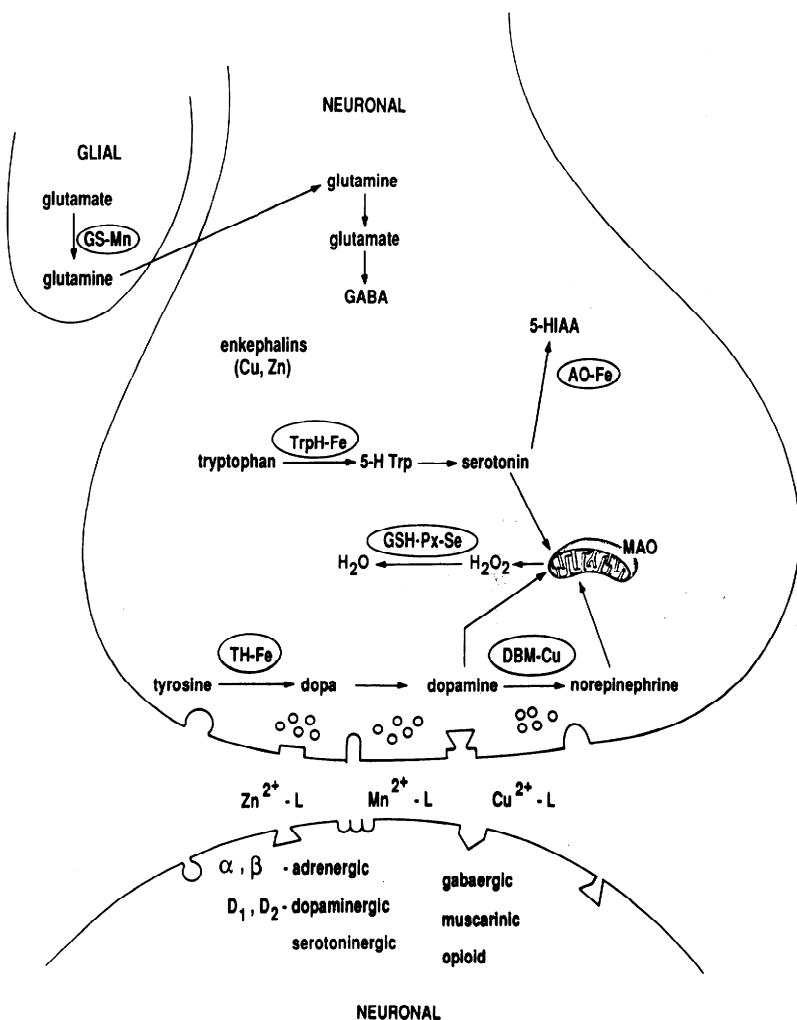
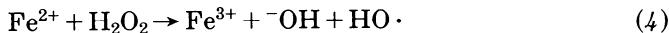


FIG. 1. Role of trace elements in neurotransmitter homeostasis. Several metal-dependent enzymes are involved including glutamine synthetase (GS), tryptophan 5-hydroxylase (TrpH), aldehyde oxidase (AO), glutathione peroxidase (GSH-Px), tyrosine 3-hydroxylase (TH), and dopamine  $\beta$ -monooxygenase (DBM). Many studies have been conducted on interactions of trace elements with ligands (L) and both pre- and postsynaptic receptors. MAO, monoamine oxidases A and B; GABA,  $\gamma$ -aminobutyric acid; 5-H Trp, 5-hydroxytryptophan; 5-HIAA, 5-hydroxyindoleacetic acid.

dividuals with disease states such as primary hemochromatosis and Hallervorden-Spatz syndrome may be at risk for neurological damage due to iron toxicity. Also epilepsy after head trauma may be caused by hemolysis of red blood cells with deposition of iron into compartments where lipid peroxidation can occur. It is known that injection of iron chloride into rat isocortex causes

transient epileptoid discharges, focal edema, and gliosis (215). This is thought to be due to formation of radicals ( $O_2^- \cdot$  and  $HO \cdot$ ) with subsequent lipid peroxidation (215). Ferrous iron can react with  $H_2O_2$  to produce  $HO \cdot$  as follows



The source of  $H_2O_2$  could come from dismutation of  $O_2^- \cdot$  (see *Eq. 1*) or from the breakdown of amines (Fig. 1). The process of lipid peroxidation might then be initiated by  $HO \cdot$ . Possibly regional rates of neuronal lipid peroxidation are related to endogenous iron levels including ferritin stores (221). Brain trace metals are involved both in production of and protection against lipid peroxidation (Fig. 2). It is the proper homeostatic control of metal metabolism that keeps the system fine tuned.

#### *D. Summary of Functions*

Iron exists in brain bound to heme- and nonheme-containing proteins. A number of enzyme functions exist in energy metabolism and neurotransmitter homeostasis (Fig. 1). Control over free iron levels or ferritin pools is important to prevent lipid peroxidation (Fig. 2) and neuronal damage.

### IV. MANGANESE

Information about the neurochemical functions of manganese is limited. The neurotoxic aspects of this metal are emphasized because of known cases of overexposure to manganese and the implication of a manganese-DA interaction. However, nutritional studies have confirmed that manganese is required for normal brain development and metabolism (81).

#### *A. Distribution*

The concentration of manganese in nervous tissue is much lower than that of copper or iron, averaging  $\sim 1\text{--}2 \mu\text{g/g}$  dry wt. For humans, these values are  $\sim 10\text{--}20$  times less than for copper and 100–500 times less than for iron (Table 1). Manganese, like copper and iron, is distributed heterogeneously in both human and rat brain (Table 1) with highest values reported for pallidum and putamen in humans and hypothalamus for rats. When rat brain regional analyses for manganese were carried out by flameless atomic absorption spectroscopy, similar results were obtained (9).

The distribution of brain manganese is affected by several factors including species, age, and diet. Although the concentration of copper and iron in human brain is greater than that for rat, the manganese level in both these species is equivalent (Table 1). Similar levels have been reported for

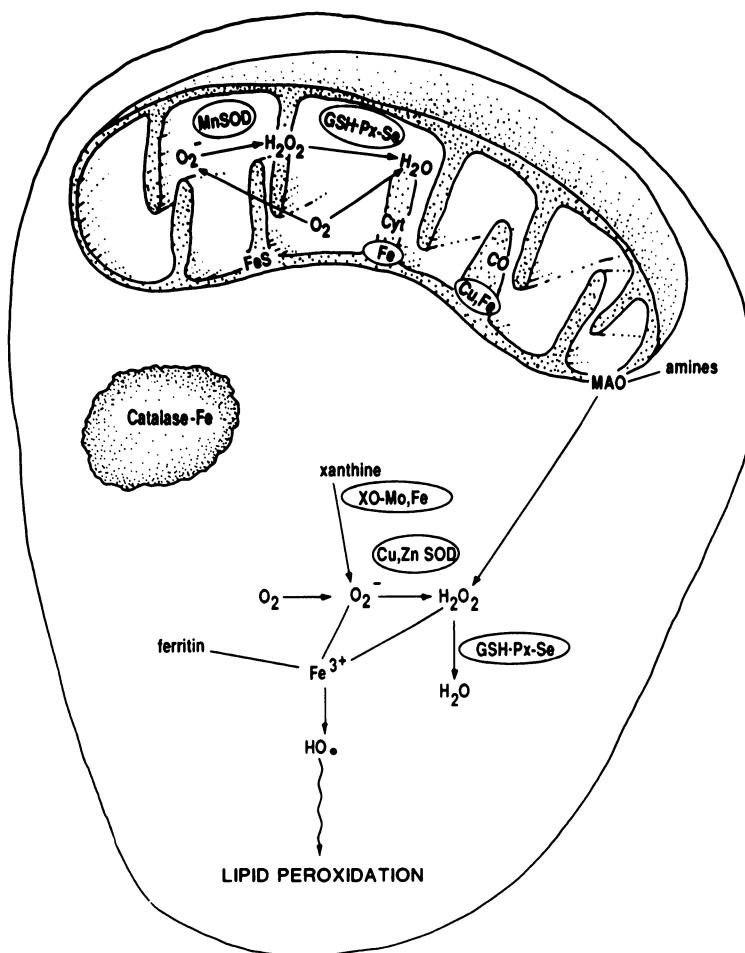


FIG. 2. Role of trace elements in oxygen metabolism of brain. Metal-dependent enzymes are involved in several reactions in several subcellular organelles. These proteins include catalase in peroxisomes; Mn-dependent superoxide dismutase (Mn-SOD), glutathione peroxidase (GSH-Px), cytochrome-c oxidase (CO), cytochromes *b*, *c*<sub>1</sub>, and *c* (Cyt), and nonheme iron proteins (FeS) in mitochondria; and xanthine oxidase (XO), CuZn-dependent superoxide dismutase (CuZn-SOD), and GSH-Px in cytoplasm. Control of free iron levels, O<sub>2</sub><sup>-</sup>, and H<sub>2</sub>O<sub>2</sub> are important in preventing formation of hydroxyl radicals (HO<sup>•</sup>) and subsequent lipid peroxidation. MAO, monoamine oxidases A and B.

cow and pig brain (217), whereas much higher levels have been published for rabbit brain manganese (66).

Human brain concentrations of manganese are greater in adults than infants below 6 mo of age (118). However, when multiple times were examined the levels did not change in a constant way during development. This may be because of variable and sometimes quite significant uptake of manganese

during fetal development. In controlled animal studies with mice brain, manganese levels rose rapidly during the first postnatal month of life (25). Studies in both chicks (47) and fetal rats (94) using  $^{54}\text{Mn}$  labeling have shown that fetal brain can take up and retain rather large proportions of administered manganese given in trace doses.

Environmental supply of manganese can also influence brain deposition, and studies of both nutritional deficiency and toxicity have been performed.

### *B. Enzymatic Functions*

Manganese, *in vitro*, is capable of activating a number of proteins, and thus confusion often exists as to what are true manganoproteins. The low cellular levels in brain ( $<10^{-5}$  M) likely precludes any *in vivo* function for free manganese activation of enzymes, although this may be possible. One enzymatic function for manganese in brain, as in other organs, is expressed by the mitochondrial protein superoxide dismutase, Mn-SOD. This protein catalyzes the same reaction (*Eq. 1*) as CuZn-SOD but is found in different cellular compartments and is the result of a different structural gene. Manganese-superoxide dismutase is an important enzyme in mitochondrial oxygen radical metabolism (Fig. 2). Another brain manganoprotein is glutamine synthetase (209). This enzyme contains four Mn ions per octamer and has been reported to account for 80% of the Mn<sup>2+</sup> in brain (208). Glutamine synthetase is especially high in glial cells where synthesis of glutamine is needed for transport to neuronal cells for conversion to the neurotransmitters glutamate and GABA. Brain may also contain other manganoproteins that require stoichiometric amounts of manganese for activity. One such candidate is calcineurin, a calmodulin-dependent phosphatase from bovine brain (63). Brain may contain other known manganese-dependent enzymes such as pyruvate carboxylase or glycosyl transferases, but this is not known. Certain brain functions depend on manganoenzymes, but our understanding of these enzymes is limited.

### *C. Nutritional Studies*

#### *1. Deficiency*

A number of studies have been carried out dealing with dietary manganese deficiency during perinatal development. Several observations have been made regarding growth and skeletal development (81). Offspring are often ataxic, but this appears to be due to malformation of the otolith within the inner ear rather than a CNS abnormality. There is some evidence for changes in brain homeostasis after manganese deficiency as evidenced by increased susceptibility of rats to convulsions (81). Although a mechanism

has yet to be proven, it is theoretically possible that limiting manganese could result in decreased brain glutamine synthetase activity. This would lead possibly to a deficit in neuronal cell glutamine, which might limit synthesis of glutamate and GABA and thus explain the convulsive disorder in manganese deficiency.

Dietary manganese does influence organ levels of manganese and, if limiting, can result in lower activity of Mn-SOD. In mice, for example, brain Mn-SOD in manganese-deficient animals was 50% of that measured in control animals (37). No correlative studies on mitochondrial morphology or lipid peroxidation were reported. Thus it is not known whether this decrease in Mn-SOD activity resulted in neuropathology. Mice used were offspring of manganese-deficient dams and were therefore subjected to manganese deprivation throughout gestation, lactation, and postweaning. A recent similar study using Swiss-Webster mice (222) instead of a hybrid strain (37) failed to confirm decreased brain Mn-SOD in mice after dietary manganese deficiency. Further research will be needed to determine the consequences, if any, of dietary manganese deficiency on neurochemical homeostasis.

## *2. Neurotransmitters*

Manganese may also influence directly neurotransmitter metabolism in addition to its catalytic role in glutamine synthetase. Chronic manganese toxicity leads to neurological signs similar to Parkinson's disease, and improvement with levodopa treatment has been observed for both conditions, implying altered DA function. The connection between manganese and monoamine metabolism has been extensively studied in many species under a variety of conditions. In many cases, however, measurement of brain manganese is not included in the data, which limits the conclusions of the studies. In general, findings are not consistent regarding manganese and neurotransmitters.

Injection of manganese dioxide into squirrel monkeys resulted in significant lowering of caudate nucleus DA levels; no changes in cerebrum or brain stem NE were noted (132). A study with rabbits confirmed the DA responses, and, in addition, decreased NE was reported (130). Oral dosing with  $MnCl_2$  in rats has also been studied. In one experiment of 7-mo duration, the concentrations of brain DA and homovanillic acid (a major DA catabolite in the CNS) were both reduced (10). However, a similar study in rats after 1 mo of manganese treatment demonstrated significant elevations of brain manganese, DA, and homovanillic acid (177). In addition, this second study reported an increased DA turnover rate in the corpus striatum of rats exposed to manganese. A study using adult Swiss albino mice and a manganese-supplemented milk diet also reported increased brain DA associated with increased brain manganese in the supplemental group (25). Neonatal rats exposed via maternal placental transfer of excess manganese showed no changes

in DA or NE levels or turnover despite significant increases in brain manganese concentrations (103). One must conclude that chronic manganese toxicity via diet or industrial exposure has variable effects on brain catecholamine homeostasis, which may depend on dose, time of exposure, and age of development. There is little doubt that if the manganese concentration is elevated to some critical level it will influence uptake and release of DA (34, 41). Whether this critical level is achieved *in vivo* under normal toxicity conditions is not known.

Limited studies have also been conducted on serotonin metabolism after chronic manganese toxicity. Published results are conflicting. When injected, MnO<sub>2</sub> decreased serotonin in the caudate nucleus of squirrel monkeys (132) but not of rabbits (130). A diet high in manganese (564 ppm) fed to male rats resulted in a significant decrease in brain serotonin level accompanied by a modest rise in brain manganese concentration (97). The author speculates, with some backup data, that the decrease in serotonin may be caused by decreased aromatic amino acid decarboxylase activity. The significance of the interactions between manganese and brain neurotransmitters including serotonin remains to be established.

### *3. Toxicity*

The toxic effects of manganese on brain are usually described in terms of its interaction with neurotransmitter metabolism as described above. However, manganese, like other transition metals, reacts with protein and as such can inhibit or activate a number of enzymes. One such example is demonstrated in studies with neuroblastoma cells where manganese is reported to inhibit adenyl cyclase and stimulate cyclic AMP phosphodiesterase activity (146). After manganese administration brain mitochondria and lysosomes appear to accumulate the bulk of excess manganese (190). Mitochondrial manganese accumulation may interfere with calcium release and thus neurotransmitter function (174).

### *D. Pallid Mice*

The normal metabolism of manganese undoubtedly depends on many gene products. One such gene, pallid, may be useful in learning more about the molecular events of manganese homeostasis (Table 3). Neurochemical aspects of mice homozygous for the mutant gene pallid (*pa/pa*) were studied. Compared with control mice, pallid mice were found to have lower brain levels of manganese (26). Brain DA and serotonin levels were equivalent in control and pallid mice. However, when levodopa or L-tryptophan were administered to these mice, basal DA and serotonin rose in control mouse brains to a much greater extent than in pallid mice. Thus these mutants appeared to have decreased brain transport of manganese, levodopa, and L-tryptophan

(26). This mouse model may be useful for further investigation of the relationship between manganese and monoamine metabolism.

#### *E. Summary of Functions*

Manganese, like other trace metals, expresses its biological activity in association with specific enzymes. For manganese known examples are Mn-SOD and glutamine synthetase. Deficiency of manganese might lead to alterations in neurotransmitter homeostasis (Fig. 1) via its influence on GABA and glutamate. It is also possible that low Mn-SOD in mitochondria could lead to lipid peroxidation (Fig. 2), which might influence calcium flux (174) and neurotransmitter release. Excessive levels of manganese may also influence monoamine homeostasis.

### V. SELENIUM

Toxicity of selenium relative to the nervous system was recognized long before its essential properties were established. The biological importance of selenium was realized formally in 1957. The neurochemical importance of selenium is based largely on animals studies still in progress. An extensive review of the neurochemical aspects of selenium was published recently (151).

#### *A. Distribution and Levels*

The concentration of selenium in human and rat brain is dependent on region but averages <1 µg/g dry wt (Table 1). This is equivalent roughly to 2.5 nmol/g fresh wt, well below the levels found for copper, iron, or manganese. The regional distribution indicates that selenium is higher in gray matter compared with white matter for human (72, 106) and rat (155) brain. The highest level was observed for putamen and lowest for centrum semiovale (Table 1). The distribution of selenium follows closely the protein distribution (155).

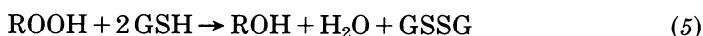
Studies with the radioactive isotope  $^{75}\text{Se}$  have been useful in probing the distribution of selenium in brain. Earlier studies in rats by Burk and colleagues (18) showed that brain retention of  $^{75}\text{Se}$ , administered as selenite ( $\text{SeO}_3^{2-}$ ), was dependent on dietary selenium. Prohaska and Ganther (155) used  $^{75}\text{Se}$  in rats consuming a diet adequate in selenium to calculate a biological half-life for brain selenium of 45 days. This same study fractionated brain  $^{75}\text{Se}$  by centrifugation and gel filtration techniques (155). Selenium followed closely protein distribution in subcellular fractions but was enriched slightly in mitochondria and diluted in cytosol. Rat brain cytosol was fractionated by gel permeation chromatography on Sephadex G-150, and  $^{75}\text{Se}$  was monitored (151, 155). Several peaks containing  $^{75}\text{Se}$  were evident, suggesting a number

of selenium-containing molecules in brain. One peak cochromatographed with enzyme activity associated with glutathione peroxidase (EC 1.11.1.9), a known selenoprotein. It was estimated, however, that only one-fifth of rat brain selenium was associated with this protein (155). No other neurochemical functions for selenium are known.

The levels of brain selenium are influenced by age and diet. For both rat (155) and human (118) brain, levels for neonates are below adult values. However, soon after birth selenium levels rise to adult values and remain constant thereafter. In rats this occurs 2 wk postnatally (155). Diet also influences brain selenium level, although rather severe selenium deficiency is needed to observe this effect. If rats are fed a diet low in selenium after weaning, only a small drop in brain selenium is observed (151). This may be due to the slow turnover of brain selenium and the early age of accretion of adult levels. A study with offspring of selenium-deficient females suggested a severe brain selenium deficit, since the activity of brain glutathione peroxidase was reduced significantly (108), whereas no such deficit was observed when the deficiency was initiated postweaning (155).

### B. Glutathione Peroxidase

The best-established biological function for selenium is its role in the protein glutathione peroxidase (GSH-Px). Glutathione peroxidase is a tetramer containing 1 gram atom of selenium per subunit. The enzyme using the reducing power of the tripeptide glutathione (GSH) catalyzes the breakdown of hydrogen peroxide and organic hydroperoxides



The oxidation of GSH is coupled with neurotransmitter (monoamines) catabolism because hydrogen peroxide is produced when monoamine oxidase reacts with, for example, DA, NE, or serotonin (116). In this way GSH-Px is used to protect neurons during neurotransmitter turnover from the deleterious effects of hydrogen peroxide (Fig. 1). Selenium via GSH-Px is also important in protecting brain unsaturated fatty acids from lipid peroxidation (Fig. 2), since removal of hydrogen peroxide generated from aerobic metabolism or superoxide disproportionation (*Eq. 1*) would block HO<sup>•</sup> formation (*Eq. 4*).

Many factors influence GSH-Px activity of brain (151). The activity of GSH-Px appears to be higher in glial vs. neuronal cells of rat brain (175) and, like selenium levels, appears to be higher in gray vs. white matter with the highest activity in the caudate putamen (14). Brain GSH-Px is found in both the cytosol and mitochondrial matrix (155). Enzyme activity per gram of brain increases during development (13, 155) to a rather stable value.

In rat brain glutathione peroxidase activity was also associated with glutathione transferases (EC 2.5.1.18), a nonselenium group of proteins (155).

This appears not to be the case for human brain, even though for human liver a large proportion of the glutathione peroxidase activity is not associated with selenium-dependent GSH-Px (20). Relative to liver, the activity of the selenoenzyme GSH-Px is low in rat (155) and human (90) brain but does fulfill an important function in aerobic metabolism and neurotransmitter homeostasis.

### C. Nutritional Studies

Several nutritional experiments have been conducted with rats using diets low in selenium (18, 108, 155). In these studies it was found that, depending on time of dietary restriction, brain selenium homeostasis was altered. However, rather severe selenium restriction was necessary. Do these studies bear a relation to neurochemical events in humans?

A number of neuropathological states have altered selenium status in blood (151). Most of these results can be explained on the basis of poor nutritional habits. One condition, Down's syndrome, deserves a few brief comments. It has been observed in patients with Down's syndrome that cells have elevated CuZn-SOD levels due to a gene-dosage effect. Originally, it was reported that GSH-Px was also elevated in red cells (151 and references therein); however, this has been challenged recently (133). Furthermore, GSH-Px is not elevated in Down's syndrome brain (16). Thus the theory that elevated GSH-Px in cells from patients with Down's syndrome is due to a compensatory mechanism because of elevated CuZn-SOD is probably not true.

One neurological disorder, not covered in an earlier review (151), that has implicated selenium is Parkinson's disease (23). This disorder is a common neurological problem in persons over 60 yr of age and has an unknown etiology. A prominent feature of Parkinson's disease is degeneration of dopaminergic neurons of the substantia nigra. Clausen (23) has suggested that lipid peroxidation may play a role in development of Parkinson's disease. In control brains, Ambani and co-workers (2) found high peroxidase activity (using o-dianisidine, not GSH) in the substantia nigra, and activity was lower in Parkinsonian brains. Recently, GSH-Px activity was shown to be lower in substantia nigra samples from patients who died of Parkinson's disease compared with matched control samples (98). Although the deficit in enzyme activity was modest, the intriguing idea that lipid peroxidation may be involved in Parkinson's disease is supported by the earlier observation of Perry et al. (142) that GSH levels were lower in substantia nigra of patients. It is not clear why GSH-Px activity should be lower in brain of persons with Parkinson's disease, since selenium analysis of a limited number of samples did not reveal any major deficits (107). Further evidence should be collected concerning the etiology of Parkinson's disease relative to selenium. Current evidence indicates that environmental exposure to selective neurotoxins like methylphenylpyridine may play a role in idiopathic Parkinson's disease (32). Whether

the homeostasis of trace elements like selenium or even copper (84) are involved demands further research.

#### *D. Summary of Functions*

Selenium is distributed heterogeneously in the CNS in close association with protein. One selenoprotein, GSH-Px, accounts for a minor fraction of the total selenium but is the only biological active form known currently. Glutathione peroxidase is an essential component of neurotransmitter turnover (Fig. 1) and aerobic metabolism (Fig. 2). Much remains to be established regarding other neurochemical roles for selenium.

### VI. ZINC

Of those trace elements known to influence neurochemical functions, zinc is clearly the most well studied yet least understood. A number of current reviews (171) deal with zinc, including entire books (56) or major portions of others (43). Many of the trace elements are needed for proper brain development (81); zinc displays perhaps the most dramatic effects, for when the supply is limited major congenital malformations of brain are observed (83). Even more impressive are the permanent adult behavior alterations that occur after a very brief period of zinc deprivation during perinatal development. These changes include impairment of long-term memory and perhaps learning and short-term memory as well (64). It has been more difficult to show neurochemical changes in brain of zinc-deficient animals (148). This review emphasizes current research dealing with the neurobiology of zinc.

#### *A. Distribution and Levels*

Like many of the other trace elements, zinc is found in variable quantities throughout the CNS. In addition to this regional heterogeneity zinc distribution is influenced by several other factors including age, species, and environment.

##### *1. Regional*

Most regions of human and rat brain contain in excess of 1  $\mu\text{mol}$  zinc/g dry wt (Table 1). Zinc levels are second only to iron in abundance. Like most trace elements, the level of zinc is higher in regions rich in gray matter compared with white matter, most likely reflecting the protein distribution. The uptake and regional distribution of zinc may be influenced by a homeostatic barrier that exists in the choroid plexus (92). The region highest in

zinc is the hippocampus (Table 1). This unique aspect of zinc neurobiology has been thoroughly reviewed (28).

Even within the hippocampus the zinc distribution is highly specific. Histochemical and atomic absorption spectrophotometric techniques have shown that zinc is enriched in the mossy fiber boutons (28). Some evidence suggests that this distribution may be related to function. Recent reports have demonstrated stimulation-induced release of zinc from hippocampal slices (4, 74).

## 2. Subcellular

Studies on the distribution of zinc among subcellular organelles of brain are limited. Adult rat brain is enriched in zinc (relative to protein) in the synaptosomal fractions compared with myelin or mitochondria (161). This is true for several brain regions. A more recent study (89) has shown that this synaptosomal enrichment is age dependent, at least for hippocampus and cerebellum. The rise in zinc in the synaptosomal fractions is accompanied by a fall in the nuclear fraction.

When whole brain cytosol was fractionated by gel filtration chromatography on Sephadex G-75, three peaks containing zinc were observed (85). The largest peak was associated with the void volume that would contain proteins whose molecular weights exceed 100,000. The other two smaller peaks eluted such that proteins in the same region would have molecular weights of 25,000 and 10,000. The low-molecular-weight peak represents metallothionein (85). In the hippocampus a smaller zinc-binding ligand was observed (173). It was suggested that this ligand might be GSH. Further work is essential to clarify and identify the subcellular forms of zinc in brain.

In contrast to several other trace elements, zinc concentration does not rise very much postnatally. This is especially evident in humans (118, 202) in whom gestational development accounts for most of the accumulation of brain zinc. In rats brain zinc rises rapidly during the lactational period; however, even here only a 50% rise was noted between birth and adulthood (156). Most of the rise in rat brain zinc occurs between the 1st and 3rd postnatal wk (100, 156).

The concentration of zinc in human and rat brain is similar. This is in contrast to copper and especially to iron (Table 1). Likewise, the zinc levels in other species like cow, pig (217), and rabbit (66) are similar to those of rats and humans. In all cases higher zinc is found in hippocampus and cerebral cortex.

Environmental factors, specifically dietary zinc, appear to influence brain zinc concentration only modestly. Since functional changes do exist in brains of zinc-deficient animals, perhaps redistribution of zinc pools does occur. There is some evidence to support this view (44, 92).

### B. Zinc Proteins

Zinc is known to be a cofactor for many different enzymes involved in intermediary metabolism and gene transcription and translation (200). Undoubtedly, many of these enzymes are found in brain. Furthermore, zinc is known to activate or inhibit many other enzymes. Some of the well-established mammalian enzymes include the following: alcohol dehydrogenase, malate dehydrogenase, and glutamate dehydrogenase; alkaline phosphatase; carbonic anhydrase; carboxypeptidases; AMP aminohydrolase;  $\delta$ -aminolevulinic acid dehydratase; leucine aminopeptidase; and CuZn-SOD.

Gel filtration of brain cytosol revealed three zinc peaks (85). One peak with molecular weight components in the 10,000 range might be metallothionein, since this has been suggested by several lines of evidence (12, 44). However, this peak might also contain other zinc-binding species such as calcium-binding proteins (7). The second peak (85) contains species of molecular weight 25,000 and might represent carbonic anhydrase and/or CuZn-SOD. The last peak in the void volume probably represents many zinc proteins. Certainly, new zinc-dependent enzymes will be described for brain. For example, the enzyme that degrades the COOH-terminal octapeptide of cholecystokinin may be zinc dependent (188).

### C. Nutritional Studies

As mentioned previously, the teratogenic and behavioral alterations that accompany zinc deficiency confirm the importance of zinc for brain development. However, numerous studies attempting to focus on the precise zinc-dependent factors responsible for this have been disappointing.

#### 1. Compositional changes

One of the first disappointing results has been the failure to demonstrate reductions in brain zinc concentration after periods of severe zinc deficiency. This is true for whole brain (156) and for specific regions. For example, in zinc-deficient suckling rats, zinc levels are not lower than controls for several regions including the hippocampus and cerebellum (42, 194, 206), two regions that become rich in zinc during development. In fact, zinc levels may be slightly greater in the deficient rat brain (194). One study indicated a slight drop in zinc levels in olfactory lobes (206). Even when subcellular distribution of cerebellar and hippocampal zinc was studied, no major changes after zinc deficiency (194) were found. Nevertheless, for rats postnatal development of both cerebellum and hippocampus was altered by zinc deficiency (17). A recent study with swine has found that cerebellar and hippocampal zinc levels were marginally lower in zinc content after induction of dietary zinc deficiency

(69). Thus zinc deficiency during perinatal development does have a modest effect on zinc levels in certain brain regions. It is interesting to note that other trace elements may change more than zinc in these animals. For example, copper levels tend to increase in brain of zinc-deficient rats (194, 206). Perhaps these secondary effects are significant in explaining neural alterations accompanying zinc deficiency.

## *2. Metabolic changes*

Despite the rather negative results of total zinc analysis some studies have shown alterations in brain metabolism after dietary zinc deficiency. The uptake and retention of zinc especially by choroid plexus was affected by dietary zinc status (92). Induction of brain metallothionein was lower in brains of zinc-deficient rats, even though total zinc levels were not lower (44).

One of the complications in assessing changes due specifically to zinc is the severe anorexia that accompanies zinc deficiency. Studies must include a pair-fed or restricted-fed group so as to separate the effects of general undernutrition from those due to zinc deficiency. For example, if DNA or protein synthesis is depressed in brain of zinc-deficient animals, one must be careful to run proper developmental controls before comparing rates. One study, for example, reported that brain carbonic anhydrase was lower in zinc-deficient rats but only compared with ad libitum fed controls (76). This same study found no change in brain glutamate dehydrogenase (GDH) activity. Another study measured cerebellar GDH and CuZn-SOD during perinatal development (156). An apparent decrease in GDH activity in cerebella of zinc-deficient rats was not observed compared with pair-fed controls. An additional study did, however, find a slight decrease in hippocampal GDH activity after a similar protocol (42). Overall one must conclude that changes in brain zinc-dependent enzymes are minimal after dietary zinc deficiency.

## *3. Myelination*

In rat brain, myelination occurs largely during the lactational period. Thus undernutrition is often reflected in delayed brain myelination. This was discussed previously for copper deficiency, for example. The myelin marker CNP has been used to follow this process in experimental zinc deficiency (42, 156). In cerebella of zinc-deficient rats the activity of CNP was shown to be lower compared with zinc-adequate rats (156); however, this apparent hypomyelination was not observed when pair-fed animals were compared. More recent studies (42), however, have shown selective decreases in cerebellar CNP and more severe reductions in the hippocampus. The myelin that is present, even though low in quantity, appears to have a normal lipid composition with the possible exception of an elevated level of phosphatidylcholine (162). This may reflect an immature myelin rather than a specific zinc-de-

pendent effect. Elevated levels of phosphatidylcholine have been reported for copper-deficient rat brain (39). The cause of zinc-dependent hypomyelination, if it is distinct from undernutrition, may be due to alterations in secondary factors such as reduced T<sub>3</sub> levels. It is known that T<sub>3</sub> levels can influence the process of myelination and, furthermore, that lower T<sub>3</sub> levels are observed in zinc deficiency (128).

#### *4. Neurotransmitters*

The abnormal eating pattern and permanent behavior changes of zinc-deficient rats have prompted many studies that have examined zinc and neurotransmitters. These studies were given further impetus by the observation of Hesse (70) that zinc-deficient rats express abnormal synaptic transmission when hippocampal mossy fiber axons are stimulated. The steady-state levels of brain NE, DA, and serotonin are not greatly influenced by dietary zinc deficiency. One study found no changes (164), whereas another found a modest rise in NE levels (205). In recent experiments it was shown that chronic zinc deficiency in rats elevated hypothalamic NE, whereas acute deficiency, while changing eating behavior, did not (93). Another study found lower levels of dynorphin, an endogenous opioid peptide, in hypothalamus but not cerebral cortex (49). This might have some relevance to the etiology of anorexia, since endogenous opioids may play a role in appetite regulation.

Several lines of evidence suggest that zinc might be involved in neurotransmitter homeostasis in addition to work on steady-state levels of neurotransmitters. In hippocampus, for example, a relationship between zinc and enkephalins has been suggested (187). Although zinc deficiency caused no changes in glutamate or aspartate binding in hippocampus, exogenous zinc influenced these processes (181). These amino acid excitatory neurotransmitters are thought to be important in hippocampal function. Zinc may also be involved in GABA homeostasis. Elevation of zinc could inhibit glutamate decarboxylase and the binding of GABA to its receptor (45).

Zinc deficiency may cause an overall decrease in neurotransmitter responsiveness rather than have a unique effect on a related pathway. Evidence for this comes from the observations that abnormal binding occurs when the opioid receptor was probed with naloxone (49) and that abnormal responses followed treatment with NE, muscimol (a GABA agonist), and bromoergocryptine (a DA agonist) (48). These generalized effects may have to do with known effects of zinc on membrane stability.

#### *5. Cell structure*

The integrity of neuronal cells might be dependent on cellular zinc levels. Evidence from brains of zinc-deficient animals support the notion that adequate zinc is necessary for membrane and cytoskeletal integrity. For example,

it has been reported that brains of zinc-deficient rats have elevated levels of malondialdehyde, an indicator of lipid peroxidation (172). This may not be the result of a zinc stabilization effect, as some have suggested, but rather the result of an elevation in unsaturated fatty acids like 20:3n-6 and 20:4n-6, which are known to be higher in brain of zinc-deficient rats (137). If elevated, these unsaturated fatty acids would increase the pool available for lipid peroxidation. The effect of zinc deficiency on lipids and thus on membranes needs further research. Another possible role for zinc in cell integrity is in microtubule formation. Hesketh (68) has shown that microtubule assembly is impaired in brain of zinc-deficient rats.

#### *D. Genetic Mutants*

Zinc metabolism is altered in the congenital condition acrodermatitis enteropathica (AE), a disease of humans (Table 3). The teratogenic effects and CNS malformations described for experimental zinc deficiency may also be expressed in offspring of women with AE (65). Humans with AE show many of the features of zinc deficiency in animals.

A neurological murine mutant, quaking, was reported to have abnormal zinc content (168). However, as stated earlier for copper, these mice have increased water content of brain, and thus the expression of the data is subject to varied interpretations.

#### *E. Summary of Functions*

Zinc is a cofactor of many proteins distributed among all enzyme classes and thus has diverse functions in brain homeostasis. Like other trace elements, it has a role in neurotransmitter homeostasis, perhaps even directly (Fig. 1). Also, zinc may be involved in brain oxidation metabolism (Fig. 2); however, this role is less well understood and may involve lipid synthesis and cytoskeletal factors, rather than direct interaction with oxygen or its reduced metabolites.

## VII. ADDITIONAL TRACE ELEMENTS

In addition to the essential elements copper, iron, manganese, selenium, and zinc discussed already, there is some neurological evidence for the essentiality of cobalt, iodine, and molybdenum. However, neurochemical information regarding these last three elements is minimal. Some trace elements impinge on neurological function because of toxic properties. Examples such as lead, mercury, cadmium, and aluminum are well known. These toxic trace elements are not discussed in this review.

#### A. Cobalt

The neurobiology of cobalt appears to be due mainly, if not totally, to its attachment in the corrin ring of vitamin B<sub>12</sub>. A good discussion of the neuropathology of vitamin B<sub>12</sub> deficiency and pernicious anemia has been compiled recently (Table 3) (1).

For most of human life cobalt levels do not change appreciably. Furthermore, levels are very low compared with the elements discussed thus far, averaging somewhat less than 10 ng/g wet wt (118). This constant level implies homeostatic control. This is fortunate and perhaps the result of evolutionary pressures, since it is well known that traces of inorganic cobalt can lead to epileptic seizures (198).

There is no real evidence of a biochemical function of cobalt other than its role in vitamin B<sub>12</sub>. When vitamin B<sub>12</sub> is deficient in tissues, the activity of vitamin B<sub>12</sub>-dependent enzymes such as methylmalonyl-CoA mutase, DL-5-methyltetrahydrofolate-homocysteine methyltransferase (methionine synthase), and leucine 2,3-aminomutase may become rate controlling in metabolism. One of the sites of neuropathology accompanying limiting vitamin B<sub>12</sub> is myelin (1). Several theories attempt to relate the dysmyelination or demyelination with vitamin B<sub>12</sub>-dependent processes. Such theories speculate as to alterations in fatty acid composition because elevated methylmalonyl CoA and propionyl CoA could lead to branched-chain and odd-chain fatty acids. Impaired methylation because of methionine imbalance is also suggested. At this point the theories are still theories, and the reader is directed elsewhere for their thorough discussion (1).

#### B. Iodine

In contrast to the wealth of literature on hypothyroidism, the data on dietary iodine deficiency and brain are limited. Hetzel and Potter (71) have recently summarized this topic. Thus only a brief review is given here. Iodine, biologically, is active in the compounds T<sub>3</sub> and T<sub>4</sub>, which are produced in the thyroid gland and act via specific receptors. Lack of dietary iodine leads to thyroid hypertrophy or goiter, growth failure, and cretinism. Effects of iodine deficiency on brain are known and, most likely, the result of T<sub>3</sub> and T<sub>4</sub> deficiency. In rats the effect is largely that of diminished hypertrophy, since DNA content is unchanged, whereas protein and cholesterol content are reduced (125). This is very similar to the delayed maturation (myelination and synaptogenesis) observed in experimental hypothyroidism. Iodine deficiency also leads to impaired brain development in other species like sheep (145) with similar results. In this case fetal iodine deficiency also leads to impaired hyperplasia.

Brain may have some unique requirements for iodine other than T<sub>3</sub> and T<sub>4</sub>, but this is not known. Brain does possess a specialized system for con-

version of  $T_4$  to  $T_3$ , suggesting some specificity (178). Another study emphasizing unique requirements for iodine in brain showed a preferential uptake of iodocompounds by myelin and synaptosomes during perinatal development in rats (29).

### C. Molybdenum

Little is known of the neurochemical functions of molybdenum. It can be inferred that molybdenum is necessary for optimal brain homeostasis, since genetic loss of the molybdoenzyme sulfite oxidase leads to brain damage and mental retardation (86). In this situation molybdenum levels are normal but the apoprotein is not. When synthesis of the molybdopterin cofactor for sulfite oxidase is impaired, similar neurological presentation is observed (169). In this case other brain molybdoenzymes would be affected as well. This suggests that the lesions are more likely the result of a loss of sulfite oxidase activity. Another brain molybdoenzyme is xanthine oxidase, a metalloflavoprotein that also contains iron. The brain enzyme, although present in small quantity, displays characteristics similar to the protein isolated from liver (119).

Limited quantitative data on molybdenum levels in human brain exist. Similar values for cerebrum and cerebellum ranging between 10 and 20 ng/g fresh wt have been reported (136). It is not known whether all brain molybdenum is associated with known molybdoenzymes such as sulfite oxidase, xanthine oxidase, and aldehyde oxidase or whether unknown molybdoproteins exist.

## VIII. CONCLUSION

Trace elements express their neurochemical activity in many ways and most often in association with some protein that serves as an enzymatic catalyst. Some rate-controlling neurochemical processes are regulated by these metalloenzymes. Some examples were summarized for neurotransmitter homeostasis (Fig. 1). Aerobic metabolism of brain also depends on trace elements for optimal operation (Fig. 2). In many cases a direct interaction between neurotransmitter and aerobic metabolism exists for trace element-dependent enzymes.

Uptake of the trace elements by brain is poorly understood. In general, uptake is rather limited and likely dependent on specific carriers such as transferrin for iron and ceruloplasmin for copper. Thus, once the blood endothelial cell barrier is established, trace element turnover is rather slow. This results in functions for trace elements quite different from the dynamic responses due to changes in elements such as sodium, potassium, calcium, and magnesium. The key for maintaining optimal neurochemical homeostasis that is dependent on trace elements is to acquire adequate levels of these

elements during brain hyperplasia and hypertrophy. Once the trace element neurochemical milieu is established it is very resistant to change.

The clerical assistance of Judy Beccetti and Audrey Comstock is appreciated.

## REFERENCES

- AGAMANOLIS, D. P., R. GREEN, AND J. W. HARRIS. The neuropathology of cobalamin deficiency. In: *Neurobiology of the Trace Elements*, edited by I. E. Dreosti and R. M. Smith. Clifton, NJ: Humana, 1983, vol. 1, p. 293-336.
- AMBANI, L. M., M. H. VAN WOERT, AND S. MURPHY. Brain peroxidase and catalase in Parkinson disease. *Arch. Neurol.* 32: 114-118, 1975.
- ASH, D. E., N. M. PAPADOPOULOS, G. COLOMBO, AND J. J. VILLAFRANCA. Kinetic and spectroscopic studies of the interaction of copper with dopamine  $\beta$ -hydroxylase. *J. Biol. Chem.* 259: 3395-3398, 1984.
- ASSAF, S. Y., AND S.-H. CHUNG. Release of endogenous Zn<sup>2+</sup> from brain tissue during activity. *Nature Lond.* 308: 734-736, 1984.
- BARNEA, A., AND G. CHO. Evidence that copper-amino acid complexes are potent stimulators of the release of luteinizing hormone-releasing hormone from isolated hypothalamic granules. *Endocrinology* 115: 936-943, 1984.
- BARNEA, A., G. CHO, AND M. COLOMBANI-VIDAL. A role for extracellular copper in modulating prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) action: facilitation of PGE<sub>2</sub> stimulation of the release of gonadotropin-releasing hormone (LHRH) from median eminence explants. *Endocrinology* 117: 415-417, 1985.
- BAUDIER, J., K. HAGLID, J. HAIECH, AND D. GÉRARD. Zinc ion binding to human brain calcium binding proteins, calmodulin and SI100b protein. *Biochem. Biophys. Res. Commun.* 114: 1138-1146, 1983.
- BATHENA, S. J., L. RECANT, N. R. VOYLES, K. I. TIMMERS, S. REISER, J. C. SMITH, JR., AND A. S. POWELL. Decreased plasma enkephalins in copper deficiency in man. *Am. J. Clin. Nutr.* 43: 42-46, 1986.
- BONILLA, E. Flameless atomic absorption spectrophotometric determination of manganese in rat brain and other tissues. *Clin. Chem.* 24: 471-474, 1978.
- BONILLA, E., AND M. DIEZ-EWALD. Effect of L-dopa on brain concentration of dopamine and homovanillic acid in rats after chronic manganese chloride administration. *J. Neurochem.* 22: 297-299, 1974.
- BONILLA, E., E. SALAZAR, J. J. VILLASMIL, R. VILLALOBOS, M. GONZALEZ, AND J. O. DAVILA. Copper distribution in the normal human brain. *Neurochem. Res.* 9: 1543-1548, 1984.
- BRADY, F. O. Metabolism of zinc and copper in the neonate: zinc thionein in developing rat brain, heart, lung, spleen, and thymus. *Life Sci.* 32: 2981-2987, 1983.
- BRANNAN, T. S., H. S. MAKER, AND C. WEISS. Developmental study of rat brain glutathione peroxidase and glutathione reductase. *Neurochem. Res.* 6: 39-43, 1981.
- BRANNAN, T. S., H. S. MAKER, C. WEISS, AND G. COHEN. Regional distribution of glutathione peroxidase in the adult rat brain. *J. Neurochem.* 35: 1013-1014, 1980.
- BROOKSBANK, B. W. L., AND R. BALAZS. Superoxide dismutase and lipoperoxidation in Down's syndrome fetal brain. *Lancet* 1: 881-882, 1983.
- BROOKSBANK, B. W. L., AND R. BALAZS. Superoxide dismutase, glutathione peroxidase and lipoperoxidation in Down's syndrome fetal brain. *Dev. Brain Res.* 16: 37-44, 1984.
- BUELL, S. J., G. J. FOSMIRE, D. A. OLLERICH, AND H. H. SANDSTEAD. Effects of postnatal zinc deficiency on cerebellar and hippocampal development in the rat. *Exp. Neurol.* 55: 199-210, 1977.
- BURK, R. F., D. G. BROWN, R. J. SEELY, AND C. C. SCAIEF III. Influence of dietary and injected selenium on whole-body retention, route of excretion, and tissue retention of <sup>75</sup>SeO<sub>4</sub><sup>2-</sup> in the rat. *J. Nutr.* 102: 1049-1056, 1972.
- CARLTON, W. W., AND W. A. KELLY. Neural lesions in the offspring of female rats fed a copper-deficient diet. *J. Nutr.* 97: 42-52, 1969.
- CARMAGNOL, F., P. M. SINET, AND H. JEROME. Selenium-dependent and non-selenium-dependent glutathione peroxidases of human tissue extracts. *Biochim. Biophys. Acta* 759: 49-57, 1983.
- CHUTKOW, J. G. Evidence for uptake of nonceruloplasminic copper in the brain: effect of ionic copper and amino acids. *Proc. Soc. Exp. Biol. Med.* 158: 113-116, 1978.
- CIMARUSTI, D. L., K. SAITO, J. E. VAUGHN, R. BARBER, E. ROBERTS, AND P. E. THOMAS. Immunocytochemical localization of dopamine- $\beta$ -hydroxylase in rat locus caeruleus and hypothalamus. *Brain Res.* 162: 55-67, 1979.
- CLAUSEN, J. Demential syndromes and the lipid metabolism. *Acta Neurol. Scand.* 70: 345-355, 1984.
- COHEN, E., AND C. A. ELVEHJEM. The relation of iron and copper to the cytochrome and oxidase content of animal tissues. *J. Biol. Chem.* 107: 97-105, 1934.
- COTZIAS, G. C., P. S. PAPAVASILIOU, I. MENA, L. C. TANG, AND S. T. MILLER. Manganese and catecholamines. *Adv. Neurol.* 5: 235-243, 1974.
- COTZIAS, G. C., L. C. TANG, S. T. MILLER, D. SLADIC-SIMIC, AND L. S. HURLEY. A mutation influencing the transportation of manganese, L-dopa, and L-tryptophan. *Science Wash. DC* 176: 410-412, 1972.
- COYLE, J. T., AND J. AXELROD. Dopamine- $\beta$ -hydroxylase in the rat brain: developmental characteristics. *J. Neurochem.* 19: 449-459, 1972.
- CRAWFORD, I. L. Zinc and the hippocampus. In: *Neurobiology of the Trace Elements*, edited by I. E. Dreosti and R. M. Smith. Clifton, NJ: Humana, 1983, vol. 1, p. 163-211.
- CRUTCHFIELD, F. L., AND M. B. DRATMAN. Early ontogeny of iodocompound-processing neural systems in rat brain. *Pediatr. Res.* 17: 8-14, 1983.
- DALLMAN, P. R., M. A. SIIMES, AND E. C. MANIES. Brain iron: persistent deficiency following short-term iron deprivation in the young rat. *Br. J. Haematol.* 31: 209-215, 1975.
- DALLMAN, P. R., AND R. A. SPIRITO. Brain iron in the rat: extremely slow turnover in normal rats may explain long-lasting effects of early iron deficiency. *J. Nutr.* 107: 1075-1081, 1977.

32. D'AMATO, R. J., Z. P. LIPMAN, AND S. H. SNYDER. Selectivity of the parkinsonian neurotoxin MPTP: toxic metabolite MPP<sup>+</sup> binds to neuromelanin. *Science Wash. DC* 231: 987-989, 1986.
33. DANH, H. C., M. S. BENEDETTI, AND P. DOSTERT. Differential changes in superoxide dismutase activity in brain and liver of old rats and mice. *J. Neurochem.* 40: 1003-1007, 1983.
34. DANIELS, A. J., K. GYSLING, AND J. ABARCA. Uptake and release of manganese by rat striatal slices. *Biochem. Pharmacol.* 30: 1833-1837, 1981.
35. DANKS, D. M. Hereditary disorders of copper metabolism in Wilson's disease and Menkes' disease. In: *The Metabolic Basis of Inherited Disease* (5th ed.), edited by J. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson, J. L. Goldstein, and M. S. Brown. New York: McGraw-Hill, 1983, p. 1251-1268.
36. DANKS, D. M., P. E. CAMPBELL, J. WALKER-SMITH, B. J. STEVENS, J. M. GILLESPIE, J. BLOOMFIELD, AND B. TURNER. Menkes' kinky-hair syndrome. *Lancet* 1: 1100-1103, 1972.
37. DE ROSA, G., C. L. KEEN, R. M. LEACH, AND L. S. HURLEY. Regulation of superoxide dismutase activity by dietary manganese. *J. Nutr.* 110: 795-804, 1980.
38. DILIBERTO, E. J., JR., AND P. L. ALLEN. Semidehydroascorbate as a product of the enzymic conversion of dopamine to norepinephrine. *Mol. Pharmacol.* 17: 421-426, 1980.
39. DIAPOLO, R. V., J. N. KANFER, AND P. M. NEWBERNE. Copper deficiency and the central nervous system. *J. Neuropathol. Exp. Neurol.* 33: 226-236, 1974.
40. DONALDSON, J., T. ST. PIERRE, J. L. MINNICH, AND A. BARBEAU. Determination of Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, and Mn<sup>2+</sup> in rat brain regions. *Can. J. Biochem.* 51: 87-92, 1973.
41. DRAPEAU, P., AND D. A. NACHSHEN. Manganese fluxes and manganese-dependent neurotransmitter release in presynaptic nerve endings isolated from rat brain. *J. Physiol. Lond.* 348: 493-510, 1984.
42. DREOSTI, I. E., S. M. MANUEL, R. A. BUCKLEY, F. J. FRASER, AND I. R. RECORD. The effect of late prenatal and/or early postnatal zinc deficiency on the development and some biochemical aspects of the cerebellum and hippocampus in rats. *Life Sci.* 28: 2133-2141, 1981.
43. DREOSTI, I. E., AND R. M. SMITH (Editors). *Neurobiology of the Trace Elements*. Clifton, NJ: Humana, 1983, vol. 1.
44. EBADI, M., AND J. C. WALLWORK. Zinc-binding proteins (ligands) in brains of severely zinc-deficient rats. *Biol. Trace Elem. Res.* 7: 129-138, 1985.
45. EBADI, M., S. WILT, R. RAMALEY, S. SWANSON, AND C. MEBUS. The role of zinc and zinc-binding proteins in regulation of glutamic acid decarboxylase in brain. *Prog. Clin. Biol. Res.* 144A: 255-275, 1984.
46. EIPPER, B. A., R. E. MAINS, AND C. C. GLEMBOTSKI. Identification in pituitary tissue of a peptide  $\alpha$ -amidation activity that acts on glycine-extended peptides and requires molecular oxygen, copper, and ascorbic acid. *Proc. Natl. Acad. Sci. USA* 80: 5144-5148, 1983.
47. ELDERS, M. J., F. E. WRIGHT, M. L. MCNATT, B. S. WINGFIELD, AND E. R. HUGHES. Brain manganese in the developing chick. *Am. J. Physiol.* 227: 31-34, 1974.
48. ESSATARA, M. B., C. J. MCCLAIN, A. S. LEVINE, AND J. E. MORLEY. Zinc deficiency and anorexia in rats: the effect of central administration of norepinephrine, muscimol and bromerogocryptine. *Physiol. Behav.* 32: 479-482, 1984.
49. ESSATARA, M. B., J. E. MORLEY, A. S. LEVINE, M. K. ELSON, R. B. SHAFER, AND C. J. MCCLAIN. The role of the endogenous opiates in zinc deficiency anorexia. *Physiol. Behav.* 32: 475-478, 1984.
50. EVERSON, G. J., R. E. SHRADER, AND T. WANG. Chemical and morphological changes in the brains of copper-deficient guinea pigs. *J. Nutr.* 96: 115-125, 1968.
51. FARRAR, J. R., AND W. HOSS. Effects of copper on the binding of agonists and antagonists to muscarinic receptors in rat brain. *Biochem. Pharmacol.* 33: 2849-2856, 1984.
52. FARRAR, J. R., W. P. HOSS, R. M. HERNDON, AND M. KUZMIAK. Characterization of muscarinic cholinergic receptors in the brains of copper-deficient rats. *J. Neurosci.* 5: 1083-1089, 1985.
53. FELLER, D. J., AND B. L. O'DELL. Dopamine and norepinephrine in discrete areas of the copper-deficient rat brain. *J. Neurochem.* 34: 1259-1263, 1980.
54. FELLER, D. J., B. L. O'DELL, AND D. B. BYLUND. Alterations in neurotransmitter receptor binding in discrete areas of the copper-deficient rat brain. *J. Neurochem.* 38: 519-524, 1982.
55. FINDLAY, E., D. T. NG, R. L. REIN, AND S. M. ARMSTRONG. The effect of iron deficiency during development on passive avoidance learning in the adult rat. *Physiol. Behav.* 27: 1089-1096, 1981.
56. FREDERICKSON, C. J., G. A. HOWELL, AND E. J. KARSKIS. *The Neurobiology of Zinc*. New York: Liss, 1984, vols. 11A and 11B.
57. FRENCH, J. H., E. S. SHERARD, H. LUBELL, M. BROTH, AND C. L. MOORE. Trichopoliodystrophy. Report of a case and biochemical studies. *Arch. Neurol.* 26: 229-244, 1972.
58. FRIDOVICH, I. Superoxide dismutases. *Annu. Rev. Biochem.* 44: 147-159, 1975.
59. FUSHIMI, H., C. R. HAMISON, AND H. A. RAVIN. Two new copper proteins from human brain isolation and properties. *J. Biochem. Tokyo* 69: 1041-1054, 1971.
60. GALAN, P., S. HERCBERG, AND Y. TOUITOU. The activity of tissue enzymes in iron-deficient rat and man: an overview. *Comp. Biochem. Physiol. B Comp. Biochem.* 77: 647-653, 1984.
61. GALLAGHER, C. H., J. D. JUDAH, AND K. R. REES. The biochemistry of copper deficiency. I. Enzymological disturbances, blood chemistry and excretion of amino-acids. *Proc. R. Soc. Lond. B Biol. Sci.* 145: 134-149, 1956.
62. GEIGER, J. D., P. K. SETH, L. M. KLEVAY, AND S. S. PARMAR. Receptor-binding changes in copper-deficient rats. *Pharmacology Basel* 28: 196-202, 1984.
63. GUPTA, R. C., R. L. KHANDELWAL, AND P. V. SULAKHE. Intrinsic phosphatase activity of bovine brain calcineurin requires a tightly bound trace metal. *FEBS Lett.* 169: 251-255, 1984.
64. HALAS, E. S. Behavioral changes accompanying zinc deficiency in animals. In: *Neurobiology of the Trace Elements*, edited by I. E. Dreosti and R. M. Smith. Clifton, NJ: Humana, 1983, vol. 1, p. 213-243.
65. HAMBIDGE, K. M., K. H. NELDNER, AND P. A. WILRAVENS. Zinc, acrodermatitis enteropathica, and congenital malformations. *Lancet* 1: 577-578, 1975.
66. HANIG, R. C., AND M. H. APRISON. Determination of calcium, copper, iron, magnesium, manganese, potassium, sodium, zinc, and chloride concentrations in several brain areas. *Anal. Biochem.* 21: 169-177, 1967.
67. HESKETH, J. E. The effect of nutritional copper deprivation on the catecholamine content and dopamine- $\beta$ -hydroxylase activity of rat and cattle adrenal glands. *Gen. Pharmacol.* 12: 445-449, 1981.

68. HESKETH, J. E. Impaired microtubule assembly in brain from zinc-deficient pigs and rats. *Int. J. Biochem.* 13: 921-926, 1981.
69. HESKETH, J., P. AGGETT, R. W. CROFTON, W. R. HUMPHRIES, AND C. F. MILLS. Effect of zinc deficiency on zinc concentrations in various regions of the pig brain: particular sensitivity of the cerebellum. *Nutr. Res.* 5: 1223-1226, 1985.
70. HESSE, G. W. Chronic zinc deficiency alters neuronal function of hippocampal mossy fibers. *Science Wash. DC* 205: 1005-1007, 1979.
71. HETZEL, B. S., AND B. J. POTTER. Iodine deficiency and the role of thyroid hormones in brain development. In: *Neurobiology of the Trace Elements*, edited by I. E. Dreosti and R. M. Smith. Clifton, NJ: Humana, 1983, vol. 1, p. 83-133.
72. HÖCK, A., U. DEMMEL, H. SCHICHA, K. KASPEREK, AND L. E. FEINENDEGEN. Trace element concentration in human brain. Activation analysis of cobalt, iron, rubidium, selenium, zinc, chromium, silver, cesium, antimony and scandium. *Brain* 98: 49-64, 1975.
73. HOSS, W., AND M. FORMANIAK. Enhancement of synaptic vesicle attachment to the plasma membrane fraction by copper. *Neurochem. Res.* 5: 795-803, 1980.
74. HOWELL, G. A., M. G. WELCH, AND C. J. FREDERICKSON. Stimulation-induced uptake and release of zinc in hippocampal slices. *Nature Lond.* 308: 736-738, 1984.
75. HOWELL, J. M., AND A. N. DAVISON. The copper content and cytochrome oxidase activity of tissues from normal and swayback lambs. *Biochem. J.* 72: 365-368, 1959.
76. HUBER, A. M., AND S. N. GERSHOFF. Effects of dietary zinc on zinc enzymes in the rat. *J. Nutr.* 103: 1175-1181, 1973.
77. HUNT, D. M. Primary defect in copper transport underlies mottled mutants in the mouse. *Nature Lond.* 249: 852-854, 1974.
78. HUNT, D. M. Catecholamine biosynthesis and the activity of a number of copper-dependent enzymes in the copper deficient mottled mouse mutants. *Comp. Biochem. Physiol. C Comp. Pharmacol.* 57: 79-83, 1977.
79. HUNT, D. M. Copper and neurological function. In: *Biological Roles of Copper*, edited by D. Evered and G. Lawrence. Amsterdam: Excerpta Med., 1980, p. 247-266.
80. HUNT, D. M., AND D. R. JOHNSON. An inherited deficiency in noradrenaline biosynthesis in the brindled mouse. *J. Neurochem.* 19: 2811-2819, 1972.
81. HURLEY, L. S. The roles of trace elements in foetal and neonatal development. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 294: 145-152, 1981.
82. HURLEY, L. S., AND L. T. BELL. Amelioration by copper supplementation of mutant gene effects in the crinkled mouse. *Proc. Soc. Exp. Biol. Med.* 149: 830-834, 1975.
83. HURLEY, L. S., AND H. SWENERTON. Congenital malformations resulting from zinc deficiency in rats. *Proc. Soc. Exp. Biol. Med.* 123: 692-696, 1966.
84. HURST, J. H., P. A. LEWITT, R. S. BURNS, N. L. FOSTER, AND W. LOVENBERG. CSF dopamine- $\beta$ -hydroxylase activity in Parkinson's disease. *Neurology* 35: 565-568, 1985.
85. ITOH, M., M. EBADI, AND S. SWANSON. The presence of zinc-binding proteins in brain. *J. Neurochem.* 41: 823-829, 1983.
86. JOHNSON, J. L., AND K. V. RAJAGOPALAN. Human sulfite oxidase deficiency. *J. Clin. Invest.* 58: 551-556, 1976.
87. KAGEYAMA, G. H., AND M. T. T. WONG-RILEY. Histoenzymatic localization of cytochrome oxidase in the hippocampus: correlation with specific neuronal types and afferent pathways. *Neuroscience* 7: 2337-2361, 1982.
88. KALADHAR, M., AND B. S. NARASINGA RAO. Effects of iron deficiency on serotonin uptake in vitro by rat brain synaptic vesicles. *J. Neurochem.* 38: 1576-1581, 1982.
89. KALINOWSKI, M., G. WOLF, AND M. MARKEFSKI. Concentration and subcellular localization of zinc in the hippocampal formation, cerebellum, and whole brain during the postnatal development of the rat. *Acta Histochem.* 73: 33-40, 1983.
90. KAPLAN, E., AND K. ANSARI. Reduction of polyunsaturated fatty acid hydroperoxides by human brain glutathione peroxidase. *Lipids* 19: 784-789, 1984.
91. KARDOS, J., J. SAMU, K. UJSZASZI, J. NAGY, I. KOVACS, J. VISY, G. MAKSAJ, AND M. SIMONYI. Cu<sup>2+</sup> is the active principle of an endogenous substance from porcine cerebral cortex which antagonizes the anticonvulsant effect of diazepam. *Neurosci. Lett.* 52: 67-72, 1984.
92. KASARSKIS, E. J. Zinc metabolism in normal and zinc-deficient rat brain. *Exp. Neurol.* 85: 114-127, 1984.
93. KASARSKIS, E. J., D. L. SPARKS, AND J. T. SLEVIN. Changes in hypothalamic noradrenergic systems during the anorexia of zinc deficiency. *Biol. Trace Elem. Res.* 9: 25-35, 1986.
94. KAUR, G., S. K. HASAN, AND R. C. SRIVASTAVA. The distribution of manganese-54 in fetal, young and adult rats. *Toxicol. Lett. Amst.* 5: 423-426, 1980.
95. KEEN, C. L., AND L. S. HURLEY. Copper supplementation in quaking mutant mice: reduced tremors and increased brain copper. *Science Wash. DC* 193: 244-246, 1976.
96. KEYHANI, E., AND J. KEYHANI. Identification of porphyrin present in apo-cytochrome c oxidase of copper-deficient yeast cells. *Biochim. Biophys. Acta* 633: 221-227, 1980.
97. KIMURA, M., N. YAGI, AND Y. ITOKAWA. Effect of subacute manganese feeding on serotonin metabolism in the rat. *J. Environ. Pathol. Toxicol.* 2: 455-461, 1978.
98. KISH, S. J., C. MORITO, AND O. HORNYKIEWICZ. Glutathione peroxidase activity in Parkinson's disease brain. *Neurosci. Lett.* 58: 343-346, 1985.
99. KISHI, R., T. IKEDA, H. MIYAKE, E. UCHINO, T. TSUZUKI, AND K. INOUE. Regional distribution of lead, zinc, iron and copper in suckling and adult rat brains. *Brain Res.* 251: 180-182, 1982.
100. KOFOD, B. Iron, copper, and zinc in rat brain. *Eur. J. Pharmacol.* 13: 40-45, 1970.
101. KOMULAINEN, H. Monoamine uptake in brain synaptosomes after administration of copper to rats. *Acta Pharmacol. Toxicol.* 53: 33-38, 1983.
102. KOMULAINEN, H., AND J. TUOMISTO. Effect of copper on the uptake and release of monoamines in rat brain synaptosomes. *Acta Pharmacol. Toxicol.* 48: 205-213, 1981.
103. KONTUR, P. J., AND L. D. FECHTER. Brain manganese, catecholamine turnover, and the development of startle in rats prenatally exposed to manganese. *Teratology* 32: 1-11, 1985.
104. LAI, J. C. K., AND J. P. BLASS. Neurotoxic effects of copper: inhibition of glycolysis and glycolytic enzymes. *Neurochem. Res.* 9: 1699-1709, 1984.
105. LAL, S., R. PAPESCHI, R. J. S. DUNCAN, AND T. L. SOURKES. Effect of copper loading on various tissue enzymes and brain monoamines in the rat. *Toxicol. Appl. Pharmacol.* 28: 395-405, 1974.
106. LARSEN, N. A., H. PAKKENBERG, E. DAMSGAARD, AND K. HEYDORN. Topographical distribution of arsenic,

- manganese, and selenium in the normal human brain. *J. Neurol. Sci.* 42: 407-416, 1979.
107. LARSEN, N. A., H. PAKKENBERG, E. DAMSGAARD, K. HEYDORN, AND S. WOLD. Distribution of arsenic, manganese, and selenium in the human brain in chronic renal insufficiency, Parkinson's disease and amyotrophic lateral sclerosis. *J. Neurol. Sci.* 51: 437-446, 1981.
  108. LAWRENCE, R. A., R. A. SUNDE, G. L. SCHWARTZ, AND W. G. HOEKSTRA. Glutathione peroxidase activity in rat lens and other tissues in relation to dietary selenium intake. *Exp. Eye Res.* 18: 563-569, 1974.
  109. LEDIG, M., R. FRIED, M. ZIESSEL, AND P. MANDEL. Regional distribution of superoxide dismutase in rat brain during postnatal development. *Dev. Brain Res.* 4: 333-337, 1982.
  110. LEIMANN, B. H., J. D. L. HANSEN, AND P. J. WARREN. The distribution of copper, zinc and manganese in various regions of the brain and in other tissues of children with protein-calorie malnutrition. *Br. J. Nutr.* 26: 197-202, 1971.
  111. LINDER, M. C., AND J. R. MOOR. Plasma ceruloplasmin evidence for its presence in and uptake by heart and other organs of the rat. *Biochim. Biophys. Acta* 499: 329-336, 1977.
  112. LOOMIS, T. C., G. YEE, AND W. L. STAHL. Regional and subcellular distribution of superoxide dismutase in brain. *Experientia* 32: 1374-1375, 1976.
  113. MACKLER, B., R. PERSON, L. R. MILLER, A. R. INAMDAR, AND C. A. FINCH. Iron deficiency in the rat: biochemical studies of brain metabolism. *Pediatr. Res.* 12: 217-220, 1978.
  114. MAEHARA, M., N. OGASAWARA, N. MIZUTANI, K. WATANABE, AND S. SUZUKI. Cytochrome c oxidase deficiency in Menkes kinky hair disease. *Brain Dev.* 5: 533-540, 1983.
  115. MAINS, R. E., A. C. MYERS, AND B. A. EIPPER. Hormonal, drug, and dietary factors affecting peptidyl glycine  $\alpha$ -amidating monooxygenase activity in various tissues of the adult male rat. *Endocrinology* 116: 2505-2515, 1985.
  116. MAKER, H. S., C. WEISS, D. J. SILIDES, AND G. COHEN. Coupling of dopamine oxidation (monoamine oxidase activity) to glutathione oxidation via the generation of hydrogen peroxide in rat brain homogenates. *J. Neurochem.* 36: 589-593, 1981.
  117. MANN, J. R., J. CAMAKARIS, J. M. GILLESPIE, B. KOELLREUTER, J.-M. MATTHIEU, P. M. ROYCE, AND D. M. DANKS. Failure to confirm abnormal copper utilization in crinkled (cr) mice. *Biol. Trace Elem. Res.* 3: 117-131, 1981.
  118. MARKESBERY, W. R., W. D. EHMANN, M. ALAUDDIN, AND T. I. M. HOSSAIN. Brain trace element concentrations in aging. *Neurobiol. Aging* 5: 19-28, 1984.
  119. MARKLEY, H. G., L. A. FAILLAUCE, AND E. MEZEY. Xanthine oxidase activity in rat brain. *Biochim. Biophys. Acta* 309: 23-31, 1973.
  120. MARKLUND, S. L. Extracellular superoxide dismutase in human tissues and human cell lines. *J. Clin. Invest.* 74: 1398-1403, 1984.
  121. MARZULLO, G., AND B. HINE. Opiate receptor function may be modulated through an oxidation-reduction mechanism. *Science Wash. DC* 208: 1171-1173, 1980.
  122. MASSIE, H. R., V. R. AIELLO, AND A. A. IODICE. Changes with age in copper and superoxide dismutase levels in brains of C57BL/6J mice. *Mech. Ageing Dev.* 10: 93-99, 1979.
  123. MATSUBA, Y., AND Y. TAKAHASHI. Spectrophotometric determination of copper with N,N,N',N'-tetraethylthiuram disulfide and an application of this method for studies of subcellular distribution of copper in rat brain. *Anal. Biochem.* 36: 182-191, 1970.
  124. MAVELLI, I., B. MONDOVI, R. FEDERICO, AND G. ROTILIO. Superoxide dimutase activity in developing rat brain. *J. Neurochem.* 31: 363-364, 1978.
  125. MCINTOSH, G. H., D. A. HOWARD, M. T. MANO, M. L. WELBY, AND B. S. HETZEL. Iodine deficiency and brain development in the rat. *Aust. J. Biol. Sci.* 34: 427-433, 1981.
  126. McMORRIS, F. A. Norepinephrine induces glial-specific enzyme activity in cultured glioma cells. *Proc. Natl. Acad. Sci. USA.* 74: 4501-4504, 1977.
  127. MORGAN, R. F., AND B. L. O'DELL. Effect of copper deficiency on the concentrations of catecholamines and related enzyme activities in the rat brain. *J. Neurochem.* 28: 207-213, 1977.
  128. MORLEY, J. E., J. GORDON, AND J. M. HERSHMAN. Zinc deficiency, chronic starvation, and hypothalamic-pituitary-thyroid function. *Am. J. Clin. Nutr.* 33: 1767-1770, 1980.
  129. MURTHY, R. C., S. LAL, D. K. SAXENA, G. S. SHUKLA, M. M. ALI, AND S. V. CHANDRA. Effect of manganese and copper interaction on behavior and biogenic amines in rats fed a 10% casein diet. *Chem. Biol. Interact.* 37: 299-308, 1981.
  130. MUSTAFA, S. J., AND S. V. CHANDRA. Levels of 5-hydroxytryptamine, dopamine and norepinephrine in whole brain of rabbits in chronic manganese toxicity. *J. Neurochem.* 18: 931-933, 1971.
  131. NALBANDYAN, R. M. Copper in brain. *Neurochem. Res.* 8: 1211-1232, 1983.
  132. NEFF, N. H., R. E. BARRETT, AND F. COSTA. Selective depletion of caudate nucleus dopamine and serotonin during chronic manganese dioxide administration to squirrel monkeys. *Experientia* 25: 1140-1141, 1969.
  133. NEVE, J., F. VERTONGEN, P. CAUCHIE, D. GNAT, AND L. MOLLE. Selenium and glutathione peroxidase in plasma and erythrocytes of Down's syndrome (trisomy 21) patients. *J. Ment. Dev. Res.* 28: 261-268, 1984.
  134. NIELSEN, F. H. Ultratrace elements in nutrition. *Annu. Rev. Nutr.* 4: 21-41, 1984.
  135. NIJEHOLT, J. L., AND J. KORF. Wilson's disease and monoamines. *Arch. Neurol.* 35: 617-618, 1978.
  136. NOOIJEN, J. L., C. J. DE GROOT, C. J. A. VAN DEN HAMER, L. A. H. MONNENS, J. WILLEMS, AND M. F. NIERMEIJER. Trace element studies in three patients and a fetus with Menkes' disease. Effect of copper therapy. *Pediatr. Res.* 15: 284-289, 1981.
  137. ODUTUGA, A. A. Effects of low-zinc status and essential fatty acid deficiency on growth and lipid composition of rat brain. *Clin. Exp. Pharmacol. Physiol.* 9: 213-221, 1982.
  138. ONO, T., AND S. OKADA. Unique increase of superoxide dismutase level in brains of long living mammals. *Exp. Gerontol.* 19: 349-354, 1984.
  139. O'DELL, B. L., AND J. R. PROHASKA. Biochemical aspects of copper deficiency in the nervous system. In: *Neurobiology of the Trace Elements*, edited by I. E. Dreosti and R. M. Smith. Clifton, NJ: Humana, 1983, vol. 1, p. 41-81.
  140. O'DELL, B. L., R. M. SMITH, AND R. A. KING. Effect of copper status on brain neurotransmitter metabolism in the lamb. *J. Neurochem.* 26: 451-455, 1976.
  141. PATTERSON, D. S. P., J. A. FOULKES, D. SWEASEY, E. M. GLANCY, AND S. TERLECKI. A neurochemical study of field cases of the delayed spinal form of swayback (enzootic ataxia) in lambs. *J. Neurochem.* 23: 1245-1253, 1974.

142. PERRY, T. L., D. V. GODIN, AND S. HANSEN. Parkinson's disease: a disorder due to nigral glutathione deficiency. *Neurosci. Lett.* 33: 305-310, 1982.
143. POLLITT, E., AND R. L. LEIBEL. *Iron Deficiency: Brain Biochemistry and Behavior*. New York: Raven, 1982.
144. PORTER, H., AND J. FOLCH. Cerebrocuprein I. A copper-containing protein isolated from brain. *J. Neurochem.* 1: 260-271, 1957.
145. POTTER, B. J., M. T. MANO, G. B. BELLING, G. H. MCINTOSH, C. HUA, B. G. CRAGG, J. MARSHALL, M. L. WELLBY, AND B. S. HETZEL. Retarded fetal brain development resulting from severe dietary iodine deficiency in sheep. *Neuropathol. Appl. Neurobiol.* 8: 303-313, 1982.
146. PRASAN, K. N. Manganese inhibits adenylyl cyclase activity and stimulates phosphodiesterase activity in neuroblastoma cells: its possible implication in manganese-poisoning. *Exp. Neurol.* 45: 554-557, 1974.
147. PROHASKA, J. R. Normal copper metabolism in quaking mice. *Life Sci.* 26: 731-735, 1980.
148. PROHASKA, J. R. Changes in brain enzymes accompanying deficiencies of the trace elements, copper, selenium, or zinc. In: *Trace Element Metabolism in Man and Animals (TEMA-4)*, edited by J. M. Howell, J. M. Gathorne, and C. L. White. Canberra: Australian Acad. Sci., 1981, p. 275-282.
149. PROHASKA, J. R. Changes in tissue growth, concentrations of copper, iron, cytochrome oxidase and superoxide dismutase subsequent to dietary or genetic copper deficiency in mice. *J. Nutr.* 113: 2048-2058, 1983.
150. PROHASKA, J. R. Comparison of copper metabolism between brindled mice and dietary copper-deficient mice using  $^{65}\text{Cu}$ . *J. Nutr.* 113: 1212-1220, 1983.
151. PROHASKA, J. R. Neurochemical aspects of selenium. In: *Neurobiology of the Trace Elements*, edited by I. E. Dreosti and R. M. Smith. Clifton, NJ: Humana, 1983, vol. 1, p. 245-268.
152. PROHASKA, J. R. Repletion of copper-deficient mice and brindled mice with copper or iron. *J. Nutr.* 114: 422-430, 1984.
153. PROHASKA, J. R. Genetic diseases of copper metabolism. *Clin. Physiol. Biochem.* 4: 87-93, 1986.
154. PROHASKA, J. R., AND D. A. COX. Decreased brain ascorbate levels in copper-deficient mice and in brindled mice. *J. Nutr.* 113: 2623-2629, 1983.
155. PROHASKA, J. R., AND H. E. GANTHER. Selenium and glutathione peroxidase in developing rat brain. *J. Neurochem.* 27: 1379-1387, 1976.
156. PROHASKA, J. R., R. W. LUECKE, AND R. JASINSKI. Effect of zinc deficiency from day 18 of gestation and/or during lactation on the development of some rat brain enzymes. *J. Nutr.* 104: 1525-1531, 1974.
157. PROHASKA, J. R., AND T. L. SMITH. Effect of dietary or genetic copper deficiency on brain catecholamines, trace metals and enzymes in mice and rats. *J. Nutr.* 112: 1706-1717, 1982.
158. PROHASKA, J. R., AND W. W. WELLS. Copper deficiency in the developing rat brain: a possible model for Menkes' steely-hair disease. *J. Neurochem.* 23: 91-98, 1974.
159. PROHASKA, J. R., AND W. W. WELLS. Copper deficiency in the developing rat brain: evidence for abnormal mitochondria. *J. Neurochem.* 25: 221-228, 1975.
160. PUGLIA, C. D., AND G. A. LOEB. Influence of rat brain superoxide dismutase inhibition by diethyldithiocarbamate upon the rate of development of central nervous system oxygen toxicity. *Toxicol. Appl. Pharmacol.* 75: 258-264, 1984.
161. RAJAN, K. S., R. W. COLBURN, AND J. M. DAVIS. Distribution of metal ions in the subcellular fractions of several rat brain areas. *Life Sci.* 18: 423-432, 1976.
162. RAMACHANDRAN, C. K., AND S. N. SHAH. Effect of feeding zinc-deficient diet to dams during lactation on brain development of the offspring in rats: lipid composition of whole brain, myelin and synaptosomes. *Biochem. Arch.* 1: 107-114, 1985.
163. RAUCH, H. Toxic milk, a new mutation affecting copper metabolism in the mouse. *J. Hered.* 74: 141-144, 1983.
164. REEVES, P. G., AND B. L. O'DELL. Short-term zinc deficiency in the rat and self-selection of dietary protein level. *J. Nutr.* 111: 375-383, 1981.
165. REIPS, D. J., T. H. JOH, AND R. A. ROSS. Effects of reserpine on activities and amounts of tyrosine hydroxylase and dopamine- $\beta$ -hydroxylase in catecholamine neuronal systems in rat brain. *J. Pharmacol. Exp. Ther.* 193: 775-784, 1975.
166. REIS, D. J., AND P. B. MOLINOFF. Brain dopamine- $\beta$ -hydroxylase: regional distribution and effects of lesions and 6-hydroxy-dopamine on activity. *J. Neurochem.* 19: 195-204, 1972.
167. ROHMER, A., J. P. KRUG, M. MENNESSON, P. MANDEL, G. MACK, AND R. ZAWISLAK. Maladie de Menkes. Etude de deux enzymes cupro-dépendantes. *Pediatrie* 32: 447-456, 1977.
168. ROSENFIELD, J., A. W. ZIMMERMAN, AND V. L. FRIEDRICH, JR. Altered brain copper and zinc content in quaking mice. *Exp. Neurol.* 82: 55-63, 1983.
169. ROTH, A., C. NOGUES, J. P. MONNET, H. OGIER, AND J. M. SAUDUBRAY. Anatomopathological findings in a case of combined deficiency of sulphite oxidase and xanthine oxidase with a defect of molybdenum cofactor. *Virchows Arch. A Pathol. Anat.* 405: 379-386, 1985.
170. RUSINKO, N., AND J. R. PROHASKA. Adenine nucleotide and lactate levels in organs from copper-deficient mice and brindled mice. *J. Nutr.* 115: 936-943, 1985.
171. SANDSTEAD, H. H. A brief history of the influence of trace elements on brain function. *Am. J. Clin. Nutr.* 43: 293-298, 1986.
172. SAS, B., AND G. PETHES. Influence of zinc deficiency on the stability of subcellular membranes and on the  $^{65}\text{Zn}$  incorporation into metallothionein. *Acta Vet. Acad. Sci. Hung.* 29: 441-450, 1981.
173. SATO, S. M., J. M. FRAZIER, AND A. M. GOLDBERG. The distribution and binding of zinc in the hippocampus. *J. Neurosci.* 4: 1662-1670, 1984.
174. SATRUSTEGUI, J., AND C. RICHTER. The role of hydroperoxides as calcium release agents in rat brain mitochondria. *Arch. Biochem. Biophys.* 233: 736-740, 1984.
175. SAVOLAINEN, H. Superoxide dismutase and glutathione peroxidase activities in rat brain. *Res. Commun. Chem. Pathol. Pharmacol.* 21: 173-176, 1978.
176. SHAROYAN, S. G., A. A. SHALJIAN, R. M. NALBANDYAN, AND H. C. BUNIATIAN. Two copper-containing proteins from white and gray matter of brain. *Biochim. Biophys. Acta* 493: 478-487, 1977.
177. SHUKLA, G. S., AND S. V. CHANDRA. Striatal dopamine turnover and L-dopa treatment after short-term exposure of rats to manganese. *Arch. Toxicol.* 47: 191-196, 1981.
178. SILVA, J. E., J. L. LEONARD, F. R. CRANTZ, AND P. R. LARSEN. Evidence for two tissue-specific pathways for *in vivo* thyroxine 5'-deiodination in the rat. *J. Clin. Invest.* 69: 1176-1184, 1982.

179. SINET, P. M., P. GARBER, AND H. JEROME.  $H_2O_2$  production, modification of the glutathione status and met-hemoglobin formation in red blood cells exposed to diethyldithiocarbamate in vitro. *Biochem. Pharmacol.* 31: 521-525, 1982.
180. SKOTLAND, T., AND T. FLATMARK. Dopamine  $\beta$ -mono-oxygenase. Binding to apoenzyme and rapid exchange in holoenzyme of  $^{64}Cu$  studied with high-performance size-exclusion gel chromatography. *Eur. J. Biochem.* 132: 171-175, 1983.
181. SLEVIN, J. T., AND E. J. KASARSKIS. Effects of zinc on markers of glutamate and aspartate neurotransmission in rat hippocampus. *Brain Res.* 334: 281-286, 1985.
182. SMEYERS-VERBEKE, J., E. DEFRISE-GUSSENHOVEN, G. EBINGER, A. LOWENTHAL, AND D. L. MASSART. Distribution of Cu and Zn in human brain tissue. *Clin. Chim. Acta* 51: 309-314, 1974.
183. SMITH, R. M. Copper in the developing brain. In: *Neurobiology of the Trace Elements*, edited by I. E. Dreosti and R. M. Smith. Clifton, NJ: Humana, 1983, vol. 1, p. 1-40.
184. SMITH, R. M., W. S. OSBORNE-WHITE, AND B. L. O'DELL. Cytochromes in brain mitochondria from lambs with enzootic ataxia. *J. Neurochem.* 26: 1145-1148, 1976.
185. SORENSEN, J. R. J., D. O. RAULS, K. RAMAKRISHNA, R. E. STULL, AND A. N. VOLDENG. Anticonvulsant activity of some copper complexes. In: *Trace Substances in Environmental Health*, edited by D. D. Hemphill. Columbia: Univ. of Missouri, 1979, vol. 13, p. 360-367.
186. SOURKES, T. L., M. QUIK, AND M. FALARDEAU. Effects of iron and copper deficiencies on monoamine metabolism. *Adv. Neurol.* 5: 253-258, 1974.
187. STENGAARD-PEDERSEN, K., K. FREEDENS, AND L.-I. LARSSON. Enkephalin and zinc in the hippocampal mossy fiber system. *Brain Res.* 21: 230-233, 1981.
188. STENGAARD-PEDERSEN, K., L.-I. LARSSON, K. FREEDENS, AND J. F. REHFELD. Modulation of chole-cystokinin concentrations in the rat hippocampus by chelation of heavy metals. *Proc. Natl. Acad. Sci. USA* 81: 5876-5880, 1984.
189. STONE, T. W., AND H. G. E. LLOYD. Effect of copper on the binding and electrophysiological actions of cyclohexyladenosine. *Brain Res.* 336: 187-189, 1985.
190. SUZUKI, H., O. WADA, K. INOUE, H. TOSAKA, AND T. ONO. Role of brain lysosomes in the development of manganese toxicity in mice. *Toxicol. Appl. Pharmacol.* 71: 422-429, 1983.
191. SWAIMAN, K. F., AND V. L. MACHEN. Iron uptake by mammalian cortical neurons. *Ann. Neurol.* 16: 66-70, 1984.
192. SWAIMAN, K. F., AND V. L. MACHEN. Chloroquine reduces neuronal and glial iron uptake. *J. Neurochem.* 46: 652-654, 1986.
193. SYMES, A. L., T. L. SOURKES, M. B. H. YOUDIM, G. GREGORIADIS, AND H. BIRNBAUM. Decreased monoamine oxidase activity in liver of iron-deficient rats. *Can. J. Biochem.* 47: 999-1002, 1969.
194. SZERDAHELYI, P., M. KOZMA, AND A. FERKE. Zinc deficiency-induced trace element concentration and localization changes in the central nervous system of albino rat during postnatal development. *Acta Histochem.* 70: 173-182, 1982.
195. TERAO, T., AND C. A. OWEN, JR. Copper metabolism in pregnant and postpartum rat and pups. *Am. J. Physiol. (Endocrinol. Metab. Gastrointest. Physiol.)* 232: E172-E179, 1977.
196. THOMAS, T. N., D. G. PRIEST, AND J. W. ZEMP. Distribution of superoxide dismutase in rat brain. *J. Neurochem.* 27: 309-310, 1976.
197. THORNE, B. M., K.-N. LIN, M. L. WEAVER, B. N. WU, AND D. M. MEDEIROS. Postweaning copper restriction and behavior in the Long-Evans rat. *Pharmacol. Biochem. Behav.* 19: 1041-1044, 1983.
198. TROTTIER, S., M. TRUCHET, AND C. LAROUDIE. Secondary ion microanalysis in the study of cobalt-induced epilepsy in the rat. *Exp. Neurol.* 76: 231-245, 1982.
199. TUCKER, D. M., R. A. SWENSON, AND H. H. SANDSTEAD. Neuropsychological effects of iron deficiency. In: *Neurobiology of the Trace Elements*, edited by I. E. Dreosti and R. M. Smith. Clifton, NJ: Humana, 1983, vol. 1, p. 269-291.
200. VALLEE, B. L. Zinc biochemistry: a perspective. *Trends Biochem. Sci.* 2: 88-91, 1976.
201. VANELLA, A., E. GEREMIA, G. D'URSO, P. TIRIOLO, K. DI SILVESTRO, R. GRIMALDI, AND R. PINTURO. Superoxide dismutase activities in aging rat brain. *Gerontology* 28: 108-113, 1982.
202. VOLKL, A., H. BERLET, AND G. ULE. Trace elements (Cu, Fe, Mg, Zn) of the brain during childhood. *Neuropaediatrics* 5: 236-242, 1974.
203. WAALKES, M. P., S. M. ROSS, C. R. CRAIG, AND J. A. THOMAS. Induction of metallothionein in the rat brain by copper implantation but not by cobalt implantation. *Toxicol. Lett. Amst.* 12: 137-142, 1982.
204. WAINIO, W. W., C. VANDER WENDE, AND N. F. SHIMP. Copper in cytochrome c oxidase. *J. Biol. Chem.* 234: 2433-2436, 1959.
205. WALLWORK, J. C., J. H. BOTNEN, AND H. H. SANDSTEAD. Influence of dietary zinc on rat brain catecholamines. *J. Nutr.* 112: 514-519, 1982.
206. WALLWORK, J. C., D. B. MILNE, R. L. SIMS, AND H. H. SANDSTEAD. Severe zinc deficiency: effects on the distribution of nine elements (potassium, phosphorus, sodium, magnesium, calcium, iron, zinc, copper and manganese) in regions of the rat brain. *J. Nutr.* 113: 1895-1905, 1983.
207. WARREN, P. J., C. J. EARL, AND R. H. S. THOMPSON. The distribution of copper in human brain. *Brain* 83: 709-717, 1960.
208. WEDLER, F. C., AND R. B. DENMAN. Glutamine synthetase: the major Mn(II) enzyme in mammalian brain. *Curr. Top. Cell. Regul.* 24: 153-169, 1984.
209. WEDLER, F. C., R. B. DENMAN, AND W. G. ROBY. Glutamine synthetase from ovine brain is a manganese(II) enzyme. *Biochemistry* 21: 6389-6396, 1982.
210. WEINBERG, J., S. LEVINE, AND P. R. DALLMAN. Long-term consequences of early iron deficiency in the rat. *Pharmacol. Biochem. Behav.* 11: 631-638, 1979.
211. WEINTRAUB, S. T., AND D. C. WHARTON. The effects of copper depletion on cytochrome c oxidase. *J. Biol. Chem.* 256: 1669-1676, 1981.
212. WENK, G. L., AND K. L. STEMMER. The influence of ingested aluminum upon norepinephrine and dopamine levels in the rat brain. *Neurotoxicology Little Rock* 2: 347-358, 1981.
213. WENK, G. L., AND K. L. STEMMER. Activity of the enzymes dopamine-beta-hydroxylase and phenylethanolamine-N-methyltransferase in discrete brain regions of the copper-zinc deficient rat following aluminum ingestion. *Neurotoxicology Little Rock* 3: 93-99, 1982.
214. WENK, G., AND K. SUZUKI. Congenital copper deficiency: copper therapy and dopamine- $\beta$ -hydroxylase activity in the mottled (brindled) mouse. *J. Neurochem.* 41: 1648-1652, 1983.

215. WILLMORE, L. J., M. HIRAMATSU, H. KOCHI, AND A. MORI. Formation of superoxide radicals after  $\text{FeCl}_3$  injection into rat isocortex. *Brain Res.* 277: 393-396, 1983.
216. WINTER, D. B., W. J. BRUYNINCKX, F. G. FOULKE, N. P. GRINICH, AND H. S. MASON. Location of heme *a* on subunits I and II and copper on subunit II of cytochrome *c* oxidase. *J. Biol. Chem.* 255: 11408-11414, 1980.
217. WONG, P. Y., AND K. FRITZE. Determination by neutron activation of copper, manganese, and zinc in the pineal body and other areas of brain tissue. *J. Neurochem.* 16: 1231-1234, 1969.
218. YAJIMA, K., AND K. SUZUKI. Neuronal degeneration in the brain of the brindled mouse. *Acta Neuropathol.* 45: 17-25, 1979.
219. YOUSIM, M. B. H., D. BEN-SHACHAR, R. ASHKENAZI, AND S. YEHUDA. Brain iron and dopamine receptor function. *Adv. Biochem. Psychopharmacol.* 37: 309-321, 1983.
220. YOUSIM, M. B. H., A. R. GREEN, M. R. BLOOMFIELD, B. D. MITCHELL, D. J. HEAL, AND D. G. GRAHAM- SMITH. The effects of iron deficiency on brain biogenic monoamine biochemistry and function in rats. *Neuropharmacology* 19: 259-267, 1980.
221. ZALESKA, M. M., AND R. A. FLOYD. Regional lipid peroxidation in rat brain in vitro: possible role of endogenous iron. *Neurochem. Res.* 10: 397-410, 1985.
222. ZIDENBERG-CHEER, S., C. L. KEEN, S. M. CASEY, AND L. S. HURLEY. Developmental changes affected by Mn deficiency. *Biol. Trace Elem. Res.* 7: 209-222, 1985.
223. ZIMMERMAN, A. W., J.-M. MATTHIEU, R. H. QUARLES, R. O. BRADY, AND J. M. HSU. Hypomyelination in copper-deficient rats. *Arch. Neurol.* 33: 111-119, 1976.