

History of Zinc as Related to Brain Function¹

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ABSTRACT Zinc (Zn) is essential for synthesis of coenzymes that mediate biogenic-amine synthesis and metabolism. Zn from vesicles in presynaptic terminals of certain glutaminergic neurons modulates postsynaptic N-methyl-D-aspartate (NMDA) receptors for glutamate. Large amounts of Zn released from vesicles by seizures or ischemia can kill postsynaptic neurons. Acute Zn deficiency impairs brain function of experimental animals and humans. Zn deficiency in experimental animals during early brain development causes malformations, whereas deficiency later in brain development causes microscopic abnormalities and impairs subsequent function. A limited number of studies suggest that similar phenomena can occur in humans. *J. Nutr.* 130: 496S–502S, 2000.

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Knowledge of the relationship of zinc nutrition to brain development and function has come from research in several disciplines. Many advances occurred in parallel with limited cross-disciplinary communication. In this review we attempt “bridge the gap” and provide a coherent story.

Zinc in brain tissue

Sheline et al. (1943) first reported ⁶⁵Zn uptake by brain in dogs and mice. Uptake was slower and the amount retained was less than that in other tissues. A decade later, Maske (1955) serendipitously discovered that diphenylthiocarbazine (dithizone) stains a pool of Zn that is strikingly localized. Staining of hippocampal mossy fibers was intense. Subsequently, Hu and Friede (1968) measured Zn in 24 regions of human brain by atomic absorption spectroscopy. Concentrations in hippocampus were highest, but gray matter of the cortex was nearly as rich. White matter had the lowest concentrations. Concentrations of Zn in newborn brain were lower than in adults.

Soon after Maske, McLardy (1960) found Zn in mossy-fiber “giant” boutons. Later, he reported several other Zn-containing fiber systems (McLardy 1970). After McLardy (1960), von Euler (1962) found that bathing the surface of the hippocampus with H₂S-saturated saline removed Zn and changed the

evoked potential response after electrical stimulation. He noted that the H₂S caused a variety of changes and therefore was cautious in concluding that removal of Zn caused the changes.

Nearly simultaneously with the above anatomical studies, Zeigler et al. (1964) measured the effect of Zn deficiency on the kinetics of Zn in chick brain. Zn deficiency increased the ⁶⁵Zn uptake but had no apparent effect on the concentration of stable Zn. Cox et al. (1969) used rats to confirm that Zn deficiency had little effect on the concentration of Zn in brain. He also showed that high intakes of Zn increased the concentration of Zn in brain. About a decade later, Wallwork et al. (1983) used weanling rats to confirm that Zn deficiency has little effect on brain Zn, with the exception of a decreased concentration of Zn in the olfactory bulb. In addition, he found that brain copper was increased by Zn deficiency.

Studies by Haug (1967) built on the work of McLardy (1960). With the use of electron microscopy and a modified silver-sulfide stain (Timm 1958), Haug showed electron-dense silver particles that were located within the mossy-fiber giant boutons, evenly distributed, and not in mitochondria. Later, Haug et al. (1971) showed that transection of mossy-fiber axons caused a rapid disappearance of Zn from the vesicles in the presynaptic boutons (terminals).

Nearly two decades after von Euler, Hesse et al. (1979) confirmed that Zn status can affect synaptic responses in the hippocampus. Using Zn-deprived rats, he showed decreases in evoked responses after repeated low frequency stimulation of the dentate gyrus. In contrast, repeated stimulation of commissural axons did not result in decreased evoked responses. Hesse suggested that his findings were caused by a decrease in vesicle Zn. More recent findings suggest that this is unlikely. Commissural axon terminals were shown to contain as much vesicle Zn as mossy-fiber terminals (Frederickson et al. 1992, Long et al. 1995).

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Shortly after Hesse (1979), Frederickson et al. (1982 and 1983), with the use of stable-isotope dilution mass spectrometry, found that ~8% of Zn in the hippocampus is in vesicles. Soon after, three groups showed that Zn is released from axon terminals during electrophysiologic activity. Howell et al. (1984) showed that electrical stimulation *in vitro* caused uptake of ⁶⁵Zn tracer by presynaptic terminals of mossy-fiber axons, and that previously incorporated ⁶⁵Zn was released. Assaf and Chung (1984) reported similar findings on the basis of the chemical analysis of poststimulation superfusate, and Sloviter (1985) showed by electron microscopy and modified silver stain (Timm 1958) that electrical stimulation decreased vesicle Zn in mossy-fiber axon terminals. About the same time Perez-Clausell and Danscher (1985) showed by electron microscopy and modified silver stain (Timm 1958) that Zn is present in ~10% of the clear round vesicles of Gray's Type I (excitatory) synaptic boutons. These authors subsequently showed (Perez-Clausell and Danscher 1986) by *in vivo* sulfide binding that Zn released from vesicles can move from the synaptic cleft to the extracellular space.

Peters et al. (1987) and Westbrook et al. (1987) showed that vesicle Zn that is released into the synaptic cleft during neurotransmission modulates *N*-methyl-D-aspartate (NMDA)⁴-specific postsynaptic receptors for glutamate in a rapid, dose-dependent and reversible manner. Consistent with Zn having a modulator role, Fukahori et al. (1988) found lower Zn concentrations in the dentate area of the hippocampus of a strain of mice with a high propensity for seizures. Zn deficiency decreased hippocampal Zn and increased seizures, whereas high intakes of Zn increased hippocampal Zn and decreased seizures (Fukahori and Itoh 1990). Mitchell et al. (1990) confirmed that Zn status can affect seizure susceptibility. *In vivo* chelation of Zn with dithizone increased the sensitivity of rats to kainic acid-induced seizures. Morton et al. (1990) also found that Zn status affected seizure threshold. Subcutaneous administration of Zn decreased noise-induced seizures in DBA/2J mice, but had no effect on seizures caused by kainic acid.

Findings of Frederickson et al. (1990) were consistent with vesicle Zn affecting cognition. Reversible chelation of Zn *in vivo* "produced a time-locked and selective disruption of hippocampal-dependent spatial-working memory." Subsequently, Browning et al. (1994 and 1995) found in Guinea pigs that Zn deficiency decreased the concentration of postsynaptic NMDA-specific glutamate-mediated calcium channels in cortical synaptosomes.

Palmiter (1996a and 1996b) and Palmiter and Findley (1995) reported specific Zn-transporter (Zn-T) membrane proteins. ZnT-1 facilitates Zn efflux from cells; ZnT-2 facilitates Zn uptake by endosomal vesicles; and ZnT-3 facilitates Zn uptake by the Zn-containing vesicles of axon terminals of glutaminergic neurons.

In vitro studies showed that oxidation of metallothionein (MT) by glutathione disulfide (GSSG) released Zn to specific ligands (Maret 1994 and 1995). This suggests that one function of MT is to serve as a store for Zn. Induction of liver MT by Zn was described nearly three decades ago (Bremner and Davies 1975, Richards and Cousins 1975, 1976a and 1976b, Winge et al. 1975). Soon after, Cherian (1977) showed that Zn bound to liver MT can be released to other ligands. Subsequently, Sas and Pethes (1981) showed that Zn defi-

ciency decreases incorporation of ⁶⁵Zn into brain MT, and Brady (1983) found that the MT concentration in brain of suckling rats is similar to that in kidney and greater than that in heart, lung, spleen and thymus. Ebadi and Swanson (1987) characterized brain MT in rats and Gulati et al. (1987) showed that MT in monkey brain is not inducible by Cd. Later, Hao et al. (1994) reported high concentrations of metallothionein-I (MT-I) mRNA in cerebellum, hippocampus and the ventricles. The same year, Gasull et al. (1994) confirmed that Zn status influences MT concentrations and that there are substantial differences in MT-I and MT-II concentrations among different regions of brain. In addition, Masters et al. (1994) showed that the mRNA for isoform MT-III, a metallothionein unique to brain, is present in glutaminergic neurons that have Zn-containing vesicles. They also showed that MT-III in cultured cells stimulates Zn uptake. Later, Erickson et al. (1997) showed that mice lacking the MT-III gene had low Zn concentrations in hippocampus, whereas, at the same time, their histochemically reactive Zn in presynaptic vesicles appeared similar to that of controls. The MT-III-deficient mice were highly susceptible to kainic acid-induced seizures and postsynaptic neuron injury (like other zinc-deficient rodents). In contrast, mice with the extra MT-III gene were resistant to seizures and postsynaptic neuron injury. These findings suggest that MT-III might influence the release of vesicle Zn into the synaptic cleft.

Extension of the *in vitro* studies, cited above, of the oxidation of MT by GSSG (Maret 1994) revealed that certain selenium compounds also release Zn from MT (Jacob et al. 1999). In addition glutathione (GSH) (Jiang et al. 1998b) and ATP (Jiang et al. 1998a) facilitate Zn release by GSSG. In addition, oxidation of certain Zn-binding ligands by GSH releases Zn to thionein (Maret et al. 1999).

Churchich et al. (1989) reported that Zn-ATP is required by pyridoxal (PL) kinase for the formation of pyridoxal-5-phosphate (PLP). Subsequently, Yamada et al. (1990) and Nakano and McCormick (1991) found that Zn-ATP is also required by flavokinase for synthesis of flavin mononucleotide (FMN), the precursor of FAD. PLP and FAD are coenzymes for biogenic-amine synthesis (Dakshinamurti et al. 1990) and monoamine oxidase (MAO) metabolism, respectively (Hsu et al. 1988). The susceptibility of these processes to Zn deficiency is unknown.

High concentrations of extracellular Zn can kill neurons. Yokoyama et al. (1986) found that 30 μmol/L or more of Zn in tissue culture killed neurons. Soon after, Frederickson et al. (1988 and 1989) reported the toxicity of Zn for neurons *in vivo*. With the use of a quinoline fluorescence technique, they found that kainic acid-induced seizures caused loss of Zn from the presynaptic axon terminals of hippocampal mossy fibers and that, coincidentally, the postsynaptic neurons showed intense fluorescence for Zn and signs of degeneration. Soon after, Tonder et al. (1990) found similar abnormalities in rats that had been subjected to cerebral ischemia. Recently, Choi (1996), Sensi et al. (1997), Yin and Weiss (1995) and Yin et al. (1998) suggested mechanisms whereby Zn enters postsynaptic neurons. They include passage through voltage-gated calcium channels, transporter-mediated exchange with intracellular sodium, passage through NMDA receptor-gated channels and penetration through calcium-permeable α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA)- or kainate receptor-gated channels.

Other *in vitro* evidence of the toxicity of Zn was provided by Bush et al. (1993, 1994a, 1994b and 1994c). At physiologic concentrations and pH, Zn complexed with "amyloid protein precursor," and "A-β-1-40," a component of cerebral amyloid

⁴ Abbreviations used: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate; EEG, electroencephalogram; FMN, flavin mononucleotide; GSH, glutathione; GSSG, glutathione disulfide; MAO, monoamine oxidase; MT, metallothionein; NMDA, *N*-methyl-D-aspartate; NPY, neuropeptide Y; PL, pyridoxal; PLP, pyridoxal-5-phosphate; ZnT, zinc transporter.

that is present in spinal fluid. A- β -1-40 solubility was decreased and resistance of the resulting amyloid to tryptic digestion was increased. Bush suggested that a similar *in vivo* phenomenon might contribute to dementia.

Brain development

Hurley and Swenerton (1966) first reported that severe Zn deprivation of rats during organogenesis causes brain malformations. They also found decreased DNA synthesis in embryonic brain tissue (Swenerton et al. 1969). Later, McKenzie et al. (1975) showed that maternal Zn deprivation during the last third of gestation decreased brain DNA. Sandstead et al. (1972) found low ^3H -thymidine incorporation into DNA and ^{35}S into protein in Zn-deficient neonatal rats on postnatal d 11. Later, Fosmire et al. (1975) found a decrease in brain polysomes and protein per cell in Zn-deprived pups on postnatal d 5. Consistent with these findings, Duerre et al. (1977) discovered that Zn deficiency impaired the incorporation of ^3H -leucine into brain histone- and nonhistone-proteins on postnatal d 10, and Buell et al. (1977) showed that Zn deficiency decreased brain growth, DNA, RNA and protein concentrations in pups, aged 21 d. In addition, division and migration of external granular cells of the cerebellum were retarded. Dvergsten (Dvergsten 1984, Dvergsten et al. 1983, 1984a and 1984b) described the histologic effects of severe Zn deficiency on cerebellum of rat pups, aged 21 d. Granule cell number relative to Purkinje cells was decreased $\sim 60\%$. Dendrite growth of Purkinje, basket and stellate cells was decreased and the height of the Purkinje cells dendrite arbor and its branching were severely decreased. Consistent with immaturity, ribosomes were clustered in the basal cytoplasm of Purkinje cells. In addition, asymmetric synapses between parallel fibers (axons of granule cells) and dendrites of the Purkinje, basket and stellate cells were decreased $\sim 40\%$.

Brain function in animals

Williams and Mills (1970) and Chesters and Quarterman (1970) reported cyclic feeding in Zn-deficient rats. Wallwork et al. (1981) and Wallwork and Sandstead (1983) showed that plasma Zn concentrations were inversely related to the cycle and that concentrations of glucose and amino acids in plasma, and amino acids in brain, did not appear related to the cycle. Subsequently, Reeves and O'Dell (1984) found that dietary restriction of tyrosine decreased the concentrations of tyrosine and catecholamines in the hypothalamus and increased the appetite of Zn-deficient rats. More recently, Selvais et al. (1997) found that Zn-deficient Wistar rats had galanin mRNA and increased neuropeptide Y (NPY) mRNA in hypothalamus. NPY in the suprachiasmatic nuclei of the geniculohypothalamic tract was inversely related to the appetite cycle. Zn repletion decreased NPY mRNA toward normal. In contrast, in Zucker rats, which have high basal NPY, Zn deficiency had no effect on NPY.

Macapinlac et al. (1967) first noted that Zn-deficient squirrel monkeys were apathetic. Subsequently, Caldwell et al. (1970) found that Zn-deficient rats were more hesitant and made more errors in a simple water maze than the pair-fed control rats. Hesse et al. (1979) confirmed their findings. Subsequently, Gordon et al. (1982) showed that severe Zn deficiency caused less activity and grooming in aged rats (300 d old); Massaro (1982) reported that moderate Zn deprivation impaired complex behaviors; and Valdes et al. (1982) found an association between lateralization of Zn in the brain and spatial preference in rats.

Golub et al. (1994 and 1996) measured effects of "moderate" Zn deprivation on behavior of prepubertal and adolescent nonhuman primates. Fifteen weeks of Zn deprivation in prepubertal animals decreased plasma Zn but had no apparent effect on growth. "Spontaneous motor activity was lower and performance of a visual-attention task and short-term-memory task were impaired." In adolescent females, "moderate" Zn deficiency retarded the adolescent growth spurt, and decreased daytime activity and attention.

Halas (Halas et al. 1977a, 1976 and 1980, Halas and Eberhardt 1975, 1977b, 1979, 1983, 1986 and 1987, Halas and Sandstead 1975 and 1980, Lokken et al. 1973) first measured the effects of developmental Zn deprivation in rats. The first experiment found that Zn deprivation of dams throughout lactation (birth to postnatal d 21) caused errors of choice during running of a "Tolman Honzig" maze without affecting the running time of offspring, aged 60-80 d (Lokken et al. 1973). The second experiment found that Zn deprivation on d 15-20 of gestation impaired avoidance of shock by young adult male offspring but had no similar effect on female offspring (Halas et al. 1976, Halas and Sandstead 1975). The third experiment found that intrauterine Zn deprivation increased shock-induced aggression in nutritionally rehabilitated 75-d-old female offspring but not in males (Halas et al. 1975 and 1977b). The last experiment differed from all others in that Halas measured the effects of mild maternal Zn deficiency (10 $\mu\text{g/g}$ diet) throughout gestation and lactation on subsequent performance of adult offspring (Halas et al. 1986). Pups and dams showed no overt signs of Zn deficiency other than mild growth deficit in pups. After weaning, the pups were fed a complete diet that was adequate in Zn. When tested at age 100 d, the previously Zn-deprived rats made many more errors in an open 17-arm radial maze than did controls. Penland and Sawler (1987) measured the electroencephalogram (EEG) of rats from Halas' last experiment. In addition to changes in EEG activity in the Zn-deprived group, brain zinc/copper ratios were positively correlated with left-minus-right hemisphere asymmetries in the EEG.

Developmental Zn deprivation was also studied in nonhuman primates. Early studies (Sandstead et al. 1978, Strobel and Sandstead 1984) in a small number of animals found that maternal Zn deprivation in the last third of pregnancy changed maternal-infant interactions and impaired later ability to solve complex problems at about age 2 y; by age 3 y, problem-solving ability was similar to that of controls. More recently, Golub et al. (1995) found that "marginal" Zn deprivation of dams throughout gestation caused a syndrome of lethargy, apathy and hypoactivity in offspring.

Findings in humans

Zn deficiency from dietary inadequacy was first described among poor Iranian farm boys by Prasad et al. (1961). Subsequently, the condition was identified among poor Egyptian farm boys who displayed dwarfism, hypogonadism, iron deficiency, hookworm and schistosomiasis (Prasad et al. 1963a and 1963b, Sandstead et al. 1967). These patients were similar in appearance to those with severe hookworm that were described in the first decade of this century by Dock and Bass (1910). Abnormal behaviors occurred in some. In the second decade of this century, the International Health Board of the Rockefeller Foundation (1919) reported an association between hookworm infection and low cognitive performance in U.S. Army recruits and in children from South-Eastern mill towns. The same year Waite and Nelson (1919) found a direct association between the severity of hookworm infection and

impaired mental development in children from North Queensland, Australia. One suspects that Zn deficiency contributed to the cognitive abnormalities described.

Twenty-five years ago Henkin et al. (1975) discovered that severe Zn deficiency impaired neuromotor and cognitive performance of adults. He induced Zn deficiency by administration of large doses of histidine, which caused high urinary excretion of Zn. All subjects developed abnormal taste and smell acuity. Some were ataxic, some were depressed, some hallucinated and some developed paranoia. Soon after Henkin's report Moynahan (1976) described abnormal behavior in a patient with acrodermatitis enteropathica, and Kay et al. (1976) found abnormal behaviors in patients with Zn deficiency as a result of inadequate parenteral feeding.

Hambidge et al. (1975) reviewed the effects of inadequately treated maternal acrodermatitis enteropathica on offspring. Some infants had brain malformations. Related to these observations, reports from Turkey suggested that low maternal Zn nutriture increased the occurrence of fetal anencephaly (Çavdar et al. 1983 and 1988).

Relevant to human fetal development and postnatal risk of behavioral deficits, nearly three decades ago, Jameson (1976) found significantly higher maternal serum Zn concentrations among women who normally delivered mature infants than he found among women who had abnormal deliveries and/or abnormally developed infants. In the latter group, eight infants had congenital malformations. In addition, women with dysmature infants had significantly lower serum Zn concentrations than women who had uncomplicated deliveries of mature infants. Subsequently, Meadows et al. (1983 and 1981) found that low Zn concentrations in maternal and newborn leukocytes were associated with fetal growth stunting. A subsequent double-blind randomized placebo-controlled Zn repletion trial by Cherry et al. (1989) found significant decreases in premature delivery and a highly significant decrease in the need for respiratory assistance among newborn infants of normal-weight low income black teen-age girls. More recently, Goldenberg et al. (1995) found higher birth weight and larger head size among infants of Zn-repleted low income mothers. Kirksey et al. (1991 and 1994) first reported relationships between the maternal diet during pregnancy and postnatal behavior of infants. Mother-baby pairs were studied in an Egyptian village. Maternal consumption of foods derived from animals that were rich in Zn was positively associated with higher neonatal attention scores on the Brazelton Neonatal Development Assessment Scale. At 6 mo of age, motor performance scores on the Bayley Scales of Infant Development were inversely associated with maternal intakes of Zn from plants, dietary phytate and fiber during pregnancy.

Effects of postnatal Zn nutriture on infant development were reported by Friel et al. (1993). Linear growth and motor development were higher in newborns <1500 g that were given 11 mg Zn/L of formula from birth to 6 mo compared with infants given 6.7 mg Zn/L. Later, Sazawal et al. (1996) reported that repletion with 10 mg Zn/d simultaneously with potentially limiting vitamins increased activity and energy expenditure of low income urban Indian children, aged 12–23 mo. Similarly, Bentley et al. (1997) found that Guatemalan infants given 10 mg Zn/d for 7 mo sat up and played more than infants given placebo. Ashworth et al. (1998) also found that Zn repletion improved behavioral ratings. His subjects were low-birth-weight Brazilian infants, aged 12 mo, who were given 5 mg Zn/d 6 d/wk during the first 8 postnatal weeks. Controls given 1 mg Zn/d lagged behind.

In children Thatcher et al. (1984) found a direct association between an index of Zn status (hair Zn concentration)

and reading performance on a standardized test. In addition, coherence of the frontal lobe EEG was related directly to the concentration of Zn in hair. Consistent with Thatcher, Wachs et al. (1995) found that certain preadolescent behaviors of Egyptian children were associated with the consumption of foods that were derived from animals and are rich in Zn.

Sandstead et al. (1998) and Penland (Penland 1999, Penland et al. 1997, 1999a and 1999b) found in three groups of children that repletion of Zn nutriture, in the context of repletion of other potentially limiting micronutrients (Ronaghy et al. 1974), improved neuropsychological function. The subjects were low income urban ($n = 740$) and rural ($n = 540$) Chinese, aged 6–9 y, and low income urban U.S. Mexican-Americans, aged 6–9 y ($n = 240$). They participated in 10-wk double-blind, randomized, controlled treatment trials. Neuropsychological function was assessed by a computerized task set that was configured by Penland (1994) for testing of many facets of neuropsychological function. All studies found that repletion with 20 mg Zn simultaneously with other potentially limiting micronutrients caused the greatest improvement in performance of a complex reasoning task, compared with controls. The Chinese subjects also showed improvement in other dimensions of neuropsychological function. Before these studies Gibson et al. (1989) and Cavan et al. (1993) found no improvement in cognition of low income children, aged 6–7 y, who were repleted with 10 mg Zn/d. The assessment tool measured global indices of cognition. We suspect the tool was insensitive.

In adults, Henrotte et al. (1977) found that low concentrations of Zn in RBC were associated with lower frequency of the EEG during hyperventilation. Later he reported an association between Type A personality, high resting RBC Zn concentration and low urinary Zn concentration, as contrasted with Type B personality (Henrotte et al. 1985). When Type A subjects were exposed to stress, they excreted more Zn in their urine than did type B subjects. Goldstein and Pfeiffer (1978) reported that treatment of schizophrenic patients with Zn was followed by a decrease in EEG amplitude (toward normal), in contrast to the effect of placebo. The change was consistent with a decrease in cortical excitability. Subsequently, Tang (1991) reported lower concentrations of Zn in hair from female epileptic patients than from controls. In addition, the occurrence of seizures was associated with low plasma Zn concentrations during the past year.

Three pilot studies suggested that mild Zn deficiency might decrease cognition of adults. Tucker and Sandstead (1984) found decreased memory for digits and decreases in several perceptual tasks in men who were fed diets that provided ~3.5 mg Zn/d while they were living in a highly controlled environment. Darnell and Sandstead (1991) found in 11 ambulatory women with serum ferritin concentrations < 20 $\mu\text{g/L}$, that 8 wk of repletion with 30 mg Zn/d simultaneously with other potentially limiting micronutrients improved short-term visual memory (Wechsler 1981). In contrast, six similar women who were given only micronutrients showed no change in short-term visual memory. Penland (1991) found decreased neuropsychological function in 11 men, aged 21–38 y, who were experimentally deprived of Zn. In random and double-blind trials, they were fed diets that provided 1, 2, 3 or 4 mg Zn/2000 kcal, each for intervals of 35 d (Johnson et al. 1993). The subjects were repleted with 10 mg Zn/d for 35 d at the end of the study. The low Zn diets decreased function similarly. Two psychomotor tasks (tracking and connect-the-dots), two attention tasks (orienting and misdirection), one perceptual task (search-count), three memory tasks (letter,

shape and cube recognition) and one spatial task (maze) were impaired.

Relevant to Zn nutriture of the elderly, Burnet (1981) suggested that low Zn nutriture increases the risk of dementia. He based his thesis on the requirement of Zn for DNA synthesis and repair (Lieberman and Ove 1962, Lieberman et al. 1963). The more recent findings of Tully et al. (1995) appear to support Burnet's idea. They found a negative association between the serum Zn concentration 1 y before death and the frequency of "senile" and "diffuse" plaques in the brains of 12 elderly women who were examined postmortem.

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