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The Role of Trace Elements in Foetal and Neonatal Development

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The roles of trace elements in foetal and neonatal development

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Manganese, zinc and copper are essential for normal prenatal and neonatal development. Manganese deficiency causes skeletal abnormalities, congenital ataxia due to abnormal inner ear development, and abnormal brain function. Depression of mucopolysaccharide synthesis and manganese superoxide dismutase activity may be fundamental to ultrastructural and other defects. In copper deficiency, neurological and skeletal abnormalities are due to impairment of phospholipid synthesis and collagen crosslinking, and possibly to low activity of copper metalloenzymes. The fundamental defect leading to the extremely teratogenic effects of zinc deficiency is related to depressed synthesis of DNA. In the neonatal period, poor survival and growth and depressed function of the immune system are salient features.

Developmental patterns of trace element concentrations in various tissues suggest that important changes in metabolic regulation of trace elements may occur during the neonatal period. This hypothesis is being investigated by studies of molecular localization of trace elements in certain neonatal tissues, in conjunction with similar observations in milk.

Introduction

It has been well established that the essential trace elements are required for normal development both in prenatal and postnatal life (Underwood 1977; Hurley 1980). To review all present knowledge of the manifestations of trace element deficiencies during crucial periods of development, as well as their underlying biochemical and physiological mechanisms, would clearly be impossible in the space available to me. I shall therefore discuss two elements, manganese and zinc, in relation to prenatal development, attempting to relate the defects resulting from their deficiency during prenatal life to knowledge of their biochemical function. (This topic, including teratogenic effects of copper deficiency, has recently been reviewed (Hurley 1981).) I shall then discuss trace elements in the suckling period, using zinc and copper as examples.

Manganese

Manganese deficiency results in abnormalities of the skeleton that may be seen both prenatally and postnatally. Nutritional chondrodystrophy occurs in the embryos of hens fed a manganese-deficient diet (Lyons & Insko 1937) as well as in the offspring of manganese-deficient rats. There is severe shortening of the long bones of the skeleton in proportion to body length (Hurley *et al.* 1961*a*) as well as disproportionate growth of the skull, which is significantly shorter, wider and higher in relation to skull length than in normal rats (Hurley *et al.* 1961*b*). Delayed and anomalous ossification of the inner ear also occurs in prenatal manganese deficiency (Hurley *et al.* 1960).

The most dramatic effect of prenatal manganese deficiency is congenital irreversible ataxia characterized by lack of coordination, lack of equilibrium, and retraction of the head (Hurley

et al. 1958; Hurley & Everson 1963). This is most dramatically demonstrated by the lack of swimming ability manifested by these animals. The ataxia is caused by abnormal development of the inner ear. Specifically, the otoliths, the calcified structures in the vestibular portion of the inner ear required for normal body righting reflexes, develop abnormally (Erway *et al.* 1966, 1970).

The developmental defect of otolith morphology, as well as the abnormal skeletal development, may be ascribed to defective synthesis of mucopolysaccharides or glycosaminoglycans. In the skeleton this was demonstrated by depressed uptake of radioactive sulphate in foetal cartilage from manganese-deficient foetuses (Hurley *et al.* 1968), as well as in depressed activity of glycosyl transferase in cartilage from growing chicks (Leach & Lilburn 1978). In the inner ear, the otoliths are composed of otoconia, small crystalline structures embedded in an amorphous, apparently mucopolysaccharide-rich matrix. In the otolithic matrix, and in both cells and matrix of the otic cartilage of manganese-deficient mice, there is evidence that mucopolysaccharide synthesis is depressed (Shrader *et al.* 1973). The function of manganese as a cofactor of the glycosyl transferase enzymes (Leach & Lilburn 1978) may thus be related to two important manifestations of prenatal manganese deficiency, namely chondrodystrophy and ataxia.

Manganese deficiency also produces ultrastructural abnormalities (Bell & Hurley 1973). Mitochondria from manganese-deficient animals show elongated stacked cristae parallel to the outer membrane instead of perpendicular to it, and membrane integrity is also affected. In some mitochondria the outer mitochondrial membrane is missing. In addition, isolated mitochondria from manganese-deficient animals show reduced oxygen uptake (Hurley *et al.* 1970).

One of the biochemical functions of manganese is its role as a component of the metalloenzyme manganese-superoxide dismutase (MnSOD) (Utter 1976). We have shown that in liver, brain, heart and lung, the MnSOD activity is significantly lower in manganese-deficient mice than in controls (de Rosa *et al.* 1980). Similarly, in chickens given a manganese-deficient diet from hatching, the MnSOD activity of the liver was significantly lower than in controls by day 7, and its activity could be restored to normal by the addition of manganese to the diet. Concomitantly, copper–zinc-superoxide dismutase (CuZnSOD) in the liver was higher than normal in the manganese-deficient animals. Paynter (1980) has recently shown that manganese-deficient rats also demonstrated a lower than normal activity of MnSOD in heart muscle, and the activity of the enzyme was correlated with the manganese concentration of the diet.

The SOD enzymes are thought to protect cells from the deleterious effects of the superoxide free radical (McCord *et al.* 1971). It is thus possible that the changes in mitochondrial structure produced by manganese deficiency are related to the role of this enzyme in protecting cells. Furthermore, mitochondrial abnormalities similar to those that we have shown in manganese-deficient mice have also been reported in animals exposed to excessive amounts of oxygen. The ultrastructural changes that I have described resulting from manganese deficiency, as well as the depression of SOD activity, were observed in animals after birth rather than in foetuses, but it is possible that similar effects of a milder type occur in foetuses as well, and that they may be related at a fundamental level to some of the congenital abnormalities produced by deficiency of this essential metal.

Another effect of manganese deficiency is on brain function. Manganese deficiency increases the susceptibility of rats to convulsions. Manganese-deficient rats exhibit a lower than normal electro-shock threshold (Hurley *et al.* 1961*c*). Manganese deficient rats also show electroencephalograms similar to those of epileptics. In addition, the death and convulsions resulting

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from hydralazine injections in normal rats can be prevented or diminished by prior injection of manganese (Hurley *et al.* 1963). It therefore appears that a normal level of manganese is required for normal brain function.

Recently these animal experiments have been applied in studies of people with convulsive disorders. Because of the experiments on convulsions in manganese-deficient rats, as well as other evidence that manganese is involved in the metabolism of biogenic amines (Papavasiliou *et al.* 1968; Papavasiliou 1981), Tanaka and his associates in Montreal (Tanaka 1978) measured manganese concentration in the whole blood of children with convulsive disorders of unknown cause. They found that in one-third of these children the blood manganese concentration was more than two standard deviations below normal. Papavasiliou *et al.* (1979) confirmed a relation between blood manganese concentration and convulsive disorders. They measured the manganese concentration of whole blood in more than 50 adult epileptics and found it to be significantly lower than in controls; it was also demonstrated that blood manganese concentration was not correlated with the blood levels of the anticonvulsive drugs taken by these patients.

The significance of these findings in terms of manganese nutrition or metabolism or their relation to prenatal or postnatal development is unknown at present. The relation of these observations to the fundamental biochemical functions of manganese is also not clear. The role of manganese in brain function may be related to the metabolism of the biogenic amines, to superoxide dismutase activity, to glycosyl transferase function, or, indeed, to all of these. These possibilities certainly provide an important and potentially fruitful area for future research.

Zinc

Zinc deficiency during prenatal life produces very rapid effects on embryonic and foetal development. When normal female rats are mated and given a zinc-deficient diet throughout pregnancy, the number of foetuses at term is much less than normal, the body mass is about half that of normal full term foetuses and about 90% of them show one or more malformations (Hurley & Swenerton 1966; Hurley *et al.* 1971). Even shorter periods of zinc deficiency during pregnancy produce teratogenic effects. For example, if the diet is deficient in zinc for the first 10 days of gestation, 22% of the foetuses are malformed at term, while zinc deficiency from day 6 to day 14 of pregnancy caused approximately half of the foetuses to be malformed. The types of malformation produced by zinc deficiency are varied and involve every organ system of the body. In one study, approximately 40% of foetuses had cleft palates, 47% had brain abnormalities, and the soft tissues were also affected.

It should also be noted that the degree of teratogenesis resulting from zinc deficiency, i.e. the percentage of malformed foetuses, is related to the amount of zinc in the environment as well as in the diet. In addition, the type of diet influences the bioavailability of dietary zinc. Such factors as the protein source or the dietary content of fibre, phytate or other mineral elements, affect the absorption of even the small amounts of zinc present in deficient diets (Underwood 1977). Thus, because of these factors, as well as genetic variation (Hurley & Bell 1974), the frequency of malformations produced by maternal dietary zinc deficiency may not be exactly the same in different laboratories. Nevertheless, the production of congenital malformations resulting from zinc deficiency in mammals has been reported from a number of laboratories in different parts of the world.

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In addition, biochemical abnormalities are also apparent in rat foetuses. In the lung, for example, there is depressed synthesis of the pulmonary surfactant phospholipids (Vojnik & Hurley 1977). In the normal course of biochemical maturation of lung function, the surfactant phospholipids increase in the foetal lung and they are extremely important for normal respiratory function of the newborn animal. In the normal rat foetus, there is a large increase in the concentration of one of the major components of lung surfactant, lecithin, from day 20 to day 21, but in the zinc-deficient foetus it was lower than normal on both of these days. Thus, biochemical maturation as well as morphological development was disturbed by maternal zinc deficiency.

Similarly, biochemical development and enzymic differentiation of the pancreas were also abnormal as a result of zinc deficiency (Robinson & Hurley 1981*a*, *b*). Two enzymes, carboxypeptidase A, a zinc metalloenzyme, and chymotrypsin A, which is not a zinc-containing enzyme, are produced by the pancreas during foetal life. In zinc-deficient foetuses on day 19 of gestation, unlike those from pair-fed controls, the amounts of both of these enzymes were lower than normal. Similarly, amounts of two of the hormones produced by the foetal pancreas, glucagon and insulin, were also lower than normal in the pancreas of zinc-deficient rat foetuses. Since biochemical differentiation of the pancreas occurs rather late in prenatal life, it is unlikely that the abnormal biochemical maturation of this organ produced by maternal zinc deprivation plays a fundamental role in most of the congenital malformations observed. Nevertheless, it may influence some of the later aspects of prenatal development, and would undoubtedly affect the ability of the organism to cope with neonatal life.

The exact biochemical mechanisms responsible for these perturbations of prenatal morphological and biochemical development cannot be described in detail. However, we believe that the fundamental defect responsible for abnormal embryonic development in zinc-deficient animals results from the role of zinc in the synthesis of nucleic acids. Embryos from zinc-deficient rats showed a lower than normal uptake of tritiated thymidine both into the whole body (Swenerton *et al.* 1969) and into DNA itself, and this was reversed by prior injection of zinc (Eckhert & Hurley 1977). In addition, the activity of thymidine kinase in rat embryos as early as day 9 of gestation was lower than normal in zinc deficiency (Duncan & Hurley 1978). Similarly, DNA polymerase activity was low (Duncan & Hurley 1978). DNA polymerase is a zinc metalloenzyme, and thymidine kinase is especially important in rapidly proliferating tissues such as the embryo. Our hypothesis is therefore that depression of thymidine kinase activity results in the similarly depressed synthesis of DNA, which, in turn, may alter relative rates of cell proliferation and lead to abnormalities in differential growth rates. These alterations in the normally tightly synchronized events of morphogenesis could result in the abnormal development characteristic of this deficiency. In addition, zinc-deficient rats, both pregnant females and foetuses, show chromosomal aberrations that may also be related to the effect of zinc on nucleic acids (Bell *et al.* 1975).

Although most of the work on prenatal effects of zinc deficiency has been conducted with animals, there is some evidence that zinc may be related to abnormal development in man (Hambidge *et al.* 1975). Jameson (1976) recently studied serum zinc concentrations in early human pregnancy and found that low concentrations of serum zinc were related to abnormal labour, premature delivery, postmature delivery, immature infants and congenital malformations.

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Trace elements in the suckling period

Zinc deficiency during the suckling period has very dramatic effects on growth and development. A zinc-deficient diet fed to lactating rats caused depressed development of mammary glands (Mutch & Hurley 1980), depressed milk production and poor neonatal survival (Mutch & Hurley 1974). The offspring showed clear signs of zinc deficiency and were permanently stunted. Neonatal mice are also susceptible to zinc deficiency (Beach *et al.* 1980*a*). Lactating mice were given, from the day of parturition, diets that contained zinc at 2.5, 5, 9, or 100 μg/g. One group was given the control diet (100 μg/g) and pair-fed to the 5 μg/g group. The growth of the pups was markedly affected by the zinc concentration of the mothers' diet. In addition, zinc-deficient offspring showed severe alopecia. The masses of the organs were normal relative to body mass as a whole, except for the spleen and the thymus. In zinc-deficient offspring, the thymus actually regressed relative to body mass. In addition, immune function in these animals was severely impaired (Beach *et al.* 1979). Mitogen responsiveness was very low in zinc-deficient mice, and serum immunoglobulins were abnormal in amount. Immunoglobulin A was not detectable in any of the zinc-deficient groups. Immunoglobulins M and IgG_{2a} were not detectable in the 2.5 and 5 μg/g groups. In contrast, IgG_1 was higher in amount in the zinc-deficient mice than in controls (Beach *et al.* 1980*b*).

Zinc deficiency in human infants is most dramatic in the genetic disorder acrodermatitis enteropathica. For many years it was recognized that the onset of this disease usually occurred when infants were weaned from breast milk to cows' milk (Danbolt 1948). Furthermore, the symptoms of the disorder could be alleviated by giving human milk to such children. When it became apparent that the manifestations of this disease were caused by zinc deficiency, we developed the hypothesis that the availability of zinc from human milk was higher than that from cows' milk because of a difference in the molecular localization or zinc binding. We tested this hypothesis by separating human milk and cows' milk by gel filtration (Eckhert *et al.* 1977). One of the problems with gel filtration chromatography has been that these gels have surface charges that produce anomalous elution behaviour. We, as well as others, have been misled by the method and, as a result, drew some erroneous conclusions. Now, however, we treat all the gels with sodium borohydride, a method developed by Lönnerdal (1980) in our laboratory which removes the surface charges from the gel and gives zinc recoveries of approximately 100 %, even with as little as 0.1 mg applied to the column.

Under these conditions, the zinc from human milk elutes on Sephadex G-50 as two peaks, a high molecular mass peak above $M_r = 70000$ and a low molecular mass peak, with $M_r \approx 650$ (Lönnerdal *et al.* 1980*c*). In contrast, the zinc in cows' milk is found almost entirely in the high molecular mass fraction. Thus, in human milk, the low molecular mass zinc peak contains a higher proportion of the total zinc than is found in cows' milk. By infrared and nuclear magnetic resonance spectroscopy we identified the low molecular mass ligand as citrate. Measurements of citrate by a specific enzymatic assay, as well as of zinc, showed that the zinc in the low molecular mass fraction and the citrate co-eluted from human milk in both gel filtration and ion exchange chromatography. In contrast, the citrate in cows' milk bound only a very small proportion of the zinc. Thus it was established that the form of zinc binding in human milk and in cows' milk are quite different and that citrate, although present in cows' milk, binds only a small proportion of its zinc, whereas in human milk considerably more zinc is bound to citrate.

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Evans & Johnson (1980) have reported identification of the low molecular mass zinc-binding ligand in human milk as another compound, namely picolinic acid. We tested this material and found that it did not elute from gel filtration columns in the same fraction as the low molecular mass zinc-containing fraction from human milk (Hurley & Lönnerdal 1981). Using the same column as for human milk, we found that picolinic acid plus zinc eluted in a position quite different from that shown by zinc in human milk. Furthermore, when picolinic acid plus zinc was added to human milk, it did not elute in the same position as the low molecular mass zinc complex of human milk. These observations, as well as other considerations discussed elsewhere (Hurley & Lönnerdal 1980*a*, *b*, 1981) lead us to conclude that picolinic acid is not an important zinc-binding ligand in human milk.

Copper in human milk, in contrast to zinc, is not bound to a low molecular mass component (Lönnerdal *et al.* 1980*a*).

Investigations on the influence of low molecular mass ligands on zinc absorption, as well as our interest in developmental changes in trace element metabolism, led us to study zinc binding in rat intestinal mucosa. Under conditions of cold, zinc in mucosa eluted from Sephadex G-50 columns in two peaks on day 1 after birth: a high molecular mass peak ($M_r \geqslant 30000$) (I) and a peak of intermediate molecular mass (II). By day 10, both of these peaks were considerably smaller and remained so to day 60.

However, if the intestinal homogenates were incubated for 30 min before gel filtration, the elution patterns were quite different, with the second peak shifted to a relative molecular mass of about 1500 (III). Again, both peaks were significantly smaller by day 10, and by day 30, III was no longer visible, while II was seen. These results (Lönnerdal *et al.* 1981) suggest that low molecular mass ($M_r \leqslant 1500$) zinc complexes observed in rat intestinal homogenates are artefacts, probably due to autolysis of metallothionein, as Cousins *et al.* (1978) have reported, and which we have confirmed (Lönnerdal *et al.* 1980*b*). These results also demonstrate developmental changes in molecular localization of zinc during the first 30 days of postnatal life.

Copper binding in rat intestine also shows dramatic changes in the first 30 days after birth (Keen *et al.* 1980). Copper appeared in the same peaks as zinc on days 1 and 10, but by day 30 no bound copper was seen.

Similarly, developmental changes are seen in zinc and copper binding in liver. Zinc in liver is bound in a high molecular mass peak ($M_r \geqslant 30000$) (II) and in a peak of intermediate molecular mass ($M_r \approx 8000$)(II). By day 10, a shift occurs, with I decreasing and II increasing. By day 30, I has increased further still, while II is no longer apparent. Similar changes were seen in liver.

The reasons for these developmental changes and their significance are unknown. Whether they represent alterations in mechanisms of intestinal transport and absorption or differences in the metal complexes presented to the intestinal lumen from the food of the young animal remains to be investigated. For example, the shift from colostrum, high in zinc and copper, to mature milk may play a role.

In summary, we have shown that prenatal and neonatal deficiencies of certain trace elements produce deleterious effects on the offspring, including congenital malformations and other abnormalities, impairment of biochemical differentiation and function, and depression of immunological development. Trace element nutrition and metabolism in the neonatal period, with its high nutrient requirements, may be related to the molecular localization of these metals in milk, the food of the newborn, and may be reflected in their tissues.

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References (Hurley)

Beach, R. S., Gershwin, M. E. & Hurley, L. S. 1979 Altered thymic structure and mitogen responsiveness in postnatally zinc deprived mice. *Devl comp. Immunol.* **3**, 725–738.

Beach, R. S., Gershwin, M. E. & Hurley, L. S. 1980*a* Growth and development in postnatally zinc deprived mice. *J. Nutr.* **110**, 201–211.

Beach, R. S., Gershwin, M. E. & Hurley, L. S. 1980*b* Impaired immunologic ontogeny in postnatal zinc deprivation. *J. Nutr.* **110**, 805–815.

Bell, L. T. & Hurley, L. S. 1973 Ultrastructural effects of manganese deficiency in liver, heart, kidney and pancreas of mice. *Lab. Invest.* **29**, 723–736.

Bell, L. T., Branstrator, M., Roux, C. & Hurley, L. S. 1975 Chromosomal abnormalities in maternal and fetal tissues of magnesium or zinc deficient rats. *Teratology* **12**, 221–226.

Cousins, R. J., Smith, R. T., Failla, M. L. & Markowitz, L. A. 1978 Origin of low molecular weight ligands in rat intestine. *Life Sci.* **23**, 1819–1826.

Danbolt, N. 1948 Acrodermatitis enteropathica. *Acta derm. vener.* **28**, 532–543.

de Rosa, G., Keen, C. L., Leach, R. M. & Hurley, L. S. 1980 Regulation of superoxide dismutase activity by dietary manganese. *J. Nutr.* **110**, 795–804.

Duncan, J. R. & Hurley, L. S. 1978 Thymidine kinase and DNA polymerase activity in normal and zinc deficient developing rat embryos. *Proc. Soc. exp. Biol. Med.* **159**, 39–43.

Eckhert, C. D. & Hurley, L. S. 1977 Reduced DNA synthesis in zinc deficiency: Regional differences in embryonic rats. *J. Nutr.* **107**, 855–861.

Eckhert, C. D., Sloan, M., Duncan, J. & Hurley, L. S. 1977 Zinc binding: a difference between human and bovine milk. *Science, N.Y.* **195**, 789–790.

Erway, L., Hurley, L. S. & Fraser, A. 1966 Neurological defect: manganese in phenocopy and prevention of a genetic abnormality of inner ear. *Science, N.Y.* **152**, 1766–1768.

Erway, L., Hurley, L. S. & Fraser, A. 1970 Congenital ataxia and otolith defects due to manganese deficiency in mice. *J. Nutr.* **100**, 643–654.

Evans, G. W. & Johnson, P. E. 1980 Characterization and quantitation of a zinc-binding ligand in human milk. *Pediat. Res.* **14**, 876.

Hambidge, K. M., Neldner, K. H. & Walravens, P. A. 1975 Zinc, acrodermatitis enteropathica, and congenital malformations. *Lancet* i, 577–578.

Hurley, L. S. 1980 *Developmental nutrition.* Englewood Cliffs, N.J.: Prentice-Hall.

Hurley, L. S. 1981 Teratogenic aspects of manganese, zinc, and copper nutrition. *Physiol. Rev.* (In the press.)

Hurley, L. S. & Bell, L. T. 1974 Genetic influence on response to dietary manganese deficiency. *J. Nutr.* **104**, 133–137.

Hurley, L. S. & Everson, G. J. 1963 Influence of timing of short-term supplementation during gestation on congenital abnormalities of manganese-deficient rats. *J. Nutr.* **79**, 23–27.

Hurley, L. S. & Lönnerdal, B. 1980*a* Letter to the editor: zinc absorption and human milk. *Nutr. Rev.* **38**, 395.

Hurley, L. S. & Lönnerdal, B. 1980*b* Tryptophan, picolinic acid and zinc absorption: an unconvincing case. *J. Nutr.* **110**, 2536–2538.

Hurley, L. S. & Lönnerdal, B. 1981 Picolinic acid as a zinc binding ligand in human milk: an unconvincing case. *Pediat. Res.* **15**, 166–167.

Hurley, L. S. & Swenerton, H. 1966 Congenital malformations resulting from zinc deficiency in rats. *Proc. Soc. exp. Biol. Med.* **123**, 692–697.

Hurley, L. S., Everson, G. J. & Geiger, J. F. 1958 Manganese deficiency in rats: congenital nature of ataxia. *J. Nutr.* **66**, 309–320.

Hurley, L. S., Wooten, E., Everson, G. J. & Asling, C. W. 1960 Anomalous development of ossification in the inner ear of offspring of manganese-deficient rats. *J. Nutr.* **71**, 15–19.

Hurley, L. S., Everson, G. J., Wooten, E. & Asling, C. W. 1961*a* Disproportionate growth in offspring of manganese-deficient rats. I. The long bones. *J. Nutr.* **74**, 274–281.

Hurley, L. S., Wooten, E. & Everson, G. J. 1961*b* Disproportionate growth in offspring of manganese-deficient rats. II. Skull, brain, and cerebrospinal fluid pressure. *J. Nutr.* **74**, 282–288.

Hurley, L. S., Woolley, D. E. & Timiras, P. S. 1961*c* Threshold and pattern of electroshock seizures in ataxic manganese-deficient rats. *Proc. Soc. exp. Biol. Med.* **106**, 343–346.

Hurley, L. S., Woolley, D. E., Rosenthal, E. & Timiras, P. S. 1963 Influence of manganese on susceptibility of rats to convulsions. *Am. J. Physiol.* **204**, 493–496.

Hurley, L. S., Gowan, J. & Shrader, R. 1968 Genetic–nutritional interaction in relation to manganese and calcification. In *Les tissus calcifiés* (5e *Symposium Européen, Société d'Enseignement Supérieur*), Paris, pp. 101–104.

Hurley, L. S., Theriault, L. & Dreosti, I. E. 1970 Liver mitochondria from manganese-deficient and pallid mice: function and ultrastructure. *Science, N.Y.* **170**, 1316–1318.

Hurley, L. S., Gowan, J. & Swenerton, H. 1971 Teratogenic effects of short-term and transitory zinc deficiency in rats. *Teratology* **4**, 199–204.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Jameson, S. 1976 Effects of zinc deficiency in human reproduction. *Acta med. scand. Suppl.* no. 593.
Keen, C. L., Lönnerdal, B. & Hurley, L. S. 1980 Developmental changes in the molecular distribution of copper and zinc in rat tissues. *Fedn Proc. Fedn Am. Soc. exp. Biol.* **39**, 1749.
Leach, R. M. & Lilburn, M. S. 1978 Manganese metabolism and its function. *Wld Rev. Nutr. Diet* **32**, 123–134.
Lönnerdal, B. 1980 Chemical modification of dextran gels for gel filtration of trace element ligands. In *Trace element analytical chemistry in medicine and biology* (*Proc. First International Workshop*) (ed. P. Schramel), pp. 439–446.
Lönnerdal, B., Keen, C. L., Hoffman, B. & Hurley, L. S. 1980*a* Copper ligands in human milk – a vehicle for copper supplementation in the treatment of Menkes' disease? *Am. J. Dis. Child.* **134**, 802–803.
Lönnerdal, B., Keen, C. L., Sloan, M. V. & Hurley, L. S. 1980*b* Molecular localization of zinc in rat milk and neonatal intestine. *J. Nutr.* **110**, 2414–2418.
Lönnerdal, B., Stanislowski, A. G. & Hurley, L. S. 1980*c* Isolation of a low molecular weight zinc binding ligand from human milk. *J. inorg. Biochem.* **12**, 71–78.
Lyons, M. & Insko, W. M. 1937 Chondrodystrophy in the chick embryo produced by manganese deficiency in the diet of the hen. *Ky agric. Exp. Stn Bull.* no. 371.
McCord, J. M., Keele, B. B., Jr & Fridovich, I. 1971 An enzyme-based theory of obligate anaerobiosis: the physiological function of superoxide dismutase. *Proc. natn. Acad. Sci. U.S.A.* **68**, 1024–1027.
Mutch, P. B. & Hurley, L. S. 1974 Effect of zinc deficiency during lactation on postnatal growth and development. *J. Nutr.* **104**, 828–842.
Mutch, P. B. & Hurley, L. S. 1980 Mammary gland function and development: effect of zinc deficiency in rat. *Am. J. Physiol.* **238**, E26–E31.
Papavasiliou, P. S. 1981 Manganese and the extrapyramidal system. In *Electrolytes and neuropsychiatric disorders* (ed. P. Alexander). New York: Spectrum Publications. (In the press.)
Papavasiliou, P. S., Miller, S. T. & Cotzias, G. C. 1968 Functional interactions between biogenic amines 3′,5′-cyclic AMP and manganese. *Nature, Lond.* **220**, 74–75.
Papavasiliou, P. S., Kutt, H., Miller, S. T., Rosal, V., Wang, Y. Y. & Aronson, R. B. 1979 Seizure disorders and trace metals: manganese tissue levels in treated epileptics. *Neurology* **29**, 1466–1473.
Paynter, D. J. 1980 Changes in activity of the manganese superoxide dismutase enzyme in tissues of the rat with changes in dietary manganese. *J. Nutr.* **110**, 437–447.
Robinson, L. K. & Hurley, L. S. 1981*a* Effect of maternal zinc deficiency or food restriction on rat fetal pancreas. I. Procarboxypeptidase A and chymotrypsinogen. *J. Nutr.* **111**, 858–868.
Robinson, L. K. & Hurley, L. S. 1981*b* Effect of maternal zinc deficiency or food restriction on rat fetal pancreas. II. Insulin and glucagon. *J. Nutr.* **111**, 869–877.
Shrader, R. E., Erway, L. & Hurley, L. S. 1973 Mucopolysaccharide synthesis in the developing inner ear of manganese-deficient and pallid mutant mice. *Teratology* **8**, 257–266.
Swenerton, H., Shrader, R. & Hurley, L. S. 1969 Zinc-deficient embryos: reduced thymidine incorporation. *Science, N.Y.* **166**, 1014–1015.
Tanaka, Y. 1978 Manganese: its neurological and teratological significance in man. Presented at American Chemical Society National Meeting, Chicago.
Underwood, E. J. 1977 *Trace elements in human and animal nutrition*, 4th edn. New York: Academic Press.
Utter, M. F. 1976 The biochemistry of manganese. In *The biochemistry of manganese* (ed. R. Burch), pp. 713–727. (*Med. Clins N. Am.* **60**, 713–727.)
Vojnik, C. & Hurley, L. S. 1977 Abnormal prenatal lung development resulting from maternal zinc deficiency in rats. *J. Nutr.* **107**, 862–872.

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