Trace Elements in Prenatal and Neonatal Development: Zinc and Manganese

Lucille S. Hurley

Department of Nutrition, University of California, Davis, California 95616

A comprehensive review of the role of all trace elements in fetal and neonatal development here is possible at only the most superficial level. Therefore two trace elements, zinc and manganese, are used to exemplify the concepts involved. The points to be made are, first, that maternal dietary trace element deficiencies can affect fetal and neonatal development at several levels of organization. Second, even mild zinc deficiency during pregnancy may have deleterious effects on the offspring; and third, trace element deficiencies that affect the fetus may come about not only through inadequate dietary intake, but through a number of factors that interact with trace elements.

When considering the effect of trace elements and their deficiencies on early development, it is important to recognize two principles of developmental nutrition and teratology: time specificity and agent specificity (1). The time during the developmental process at which nutritional insults occur is an important factor in determining the response of the developing organism (2). During critical periods in development, in which organs, tissues, or other components are forming, they are vulnerable to damage, so that even short periods of nutritional deficiencies may have permanent effects. Conversely, the same insult at a later time will not produce the defect. Because of agent specificity, different nutrients with their specific biochemical roles in development may have greater or lesser effects. Thus, different trace element deficiencies may have different final effects on a developing embryo, fetus, or neonate, even though the timing of the deficiency is the same.

EFFECTS OF ZINC AND MANGANESE DEFICIENCIES ON THE EMBRYO AND FETUS

Morphological Abnormalities

The most drastic effect of nutritional deficiency on an embryo is, of course, death. Severe zinc deficiency has a significant effect on the survival of rat embryos. When normal pregnant rats are given a diet severely deficient in zinc at the beginning of pregnancy, approximately half of their implantation sites are resorbed.
This is in contrast to manganese deficiency in which embryonic death is not very high in rats (3,4). Manganese deficiency in guinea pigs, however, causes stillbirths and abortions (5). In the embryos of zinc-deficient rats that survive to term, marked morphological abnormalities are apparent as early as day 3 of gestation; i.e., after 3 days of the deficient diet. By day 5, the preimplantation blastocyst stage, there is markedly abnormal morphology (6). This rapid effect of maternal dietary zinc deficiency is due to the rapid drop in plasma zinc levels that occurs under these conditions, so that within less than 24 hr the plasma zinc level is less than half its original value (7,8). The low plasma zinc level is also reflected in an abnormally low zinc concentration in the uterine fluid (9). It is of interest to note that the concentration of zinc in rat uterine fluid is significantly higher than that of the blood plasma, suggesting that the normal environment of the preimplantation fertilized egg is rich in this element. At term, gross malformations are seen, encompassing every organ system. (For review see ref. 3.)

In prenatal manganese deficiency, morphological effects are very different from those resulting from zinc deprivation. Defects occur in the fetus, but they are limited to systems involving calcification: the otoliths (the calcified structures in the vestibular portion of the inner ears) and the skeleton. Another point of difference is that the time required to produce the deficiency is short in the case of zinc and long in the case of manganese (3,10).

Function or Biochemical Abnormalities

Maternal dietary deficiency of zinc produces a variety of functional and biochemical abnormalities in rat fetuses. There are pronounced effects on the biochemical maturation of the lung. Synthesis of phospholipid components of surfactant is depressed, and there is an abnormal lecithin/sphingomyelin (L:S) ratio in amniotic fluid. In normal fetuses this ratio rises near term, whereas in zinc-deficient ones it does not. The lack of rise of the L:S ratio reflects the failure of zinc-deficient embryos to increase the lecithin concentration of their lungs (11). Biochemical maturation in the pancreas is also depressed, with both pancreatic enzymes and pancreatic hormones abnormally low in fetuses of zinc-deficient females (12,13). Furthermore, profound defects in DNA synthesis occur; activities of both thymidine kinase and DNA polymerase are depressed in fetal liver, and chromosomal aberrations are high (14,15). Studies by Vallee and Falchuk have indicated that zinc is directly involved in genetic expression (16).

Again, manganese-deficient fetuses show biochemical defects very different from those of zinc-deficient fetuses. One major effect is a depression of mucopolysaccharide synthesis, which is responsible for the failure of otolith development and the abnormal skeletal development (3). Another effect of manganese deficiency is on carbohydrate metabolism. Second-generation offspring of manganese-deficient rats show a diabetic type of response to a glucose tolerance test, which appears to result from decreased insulin output (17,18).
Postnatal Effects of Prenatal Deficiency

One of the earliest described postnatal effects of a prenatal trace element deficiency is the irreversible congenital ataxia observed in the offspring of manganese-deficient animals (4). This ataxia results from abnormal development of the inner ear (otolith development) (19,20) and is due specifically to inadequate manganese at a specific critical period during prenatal development (3).

Zinc deficiency during prenatal life also causes postnatal effects. When pregnant mice were given a diet that was marginally deficient in zinc for only the last two-thirds of their pregnancy, their offspring showed abnormal serum immunoglobin M (IgM). At 6 and 10 weeks of age, IgM was not detectable. At 6 months of age, IgM was present but at significantly lower concentrations than in controls. When these offspring were mated and their offspring were examined the same way, IgM was again significantly lower than in controls at 6 and 10 weeks of age, but not at 6 months. In the following generation, the F₂ generation, IgM was low at 6 weeks but normal at 10 weeks and 6 months, even though it was the great-grandmothers of these mice that had been deprived of zinc during the last two-thirds of their pregnancies. Postnatal survival was also depressed for two generations; it was reduced by more than 40% in the F₁ generation, and by 25% in the F₂ offspring. Thus, the effect of prenatal zinc deficiency for even a part of pregnancy had permanent effects on the offspring that persisted through two or three generations. This study was controlled for food intake and for the effects of zinc deficiency on the mammary gland (21,22).

Zinc deficiency in pregnant rats also increases neonatal mortality (23). Prenatal zinc deficiency has also been reported to have effects on postnatal behavior (24).

EFFECTS OF ZINC DEFICIENCY IN NEONATES

Zinc deficiency during the neonatal period has very important and long-lasting effects on the offspring. In rats, growth and postnatal survival of offspring of females given a zinc-deficient diet during lactation were markedly depressed (25). Mice showed an even more severe response to zinc deficiency during the suckling period. Mice suckling dams given diets with zinc content ranging from 2.5 to 9 ppm showed a marked effect of dietary zinc level on growth, and the offspring of mice fed zinc 2.5 ppm showed signs of zinc deficiency, e.g., severe alopecia (26). Zinc deficiency during the suckling period also produced drastic effects on immune function. The growth of the thymus gland and the spleen, for example, was severely depressed so that the thymus actually decreased in weight relative to the body as a whole. In addition, serum immunoglobulins were significantly affected. IgM was not detectable in groups given zinc at 2.5 and 5.0 ppm, and IgA was not detectable in the 9-ppm group as well. IgG₂A was not detectable in the 2.5- and 5.0-ppm groups, whereas IgG₁ was higher than normal in all three zinc-deficient groups (27). The effects of manganese and zinc deficiencies are summarized in Tables 1 and 2.
TABLE 1. Effects of prenatal and neonatal Mn deficiency

- Early neonatal death
- Skeletal abnormalities
  - Chondrodystrophy
  - Domed skull
  - Tibial epiphyseal dysplasia
  - Abnormal ossification of otic capsule
- Missing or abnormal otoliths in inner ear
- Congenital irreversible ataxia
- Defective synthesis of mucopolysaccharides
- Depressed activity of Mn superoxide dismutase
- Increased lipid peroxidation
- Membrane damage (ultrastructural)
- Abnormal carbohydrate metabolism
- Abnormal glucose tolerance
- Lower plasma insulin level
- Lower insulin output from pancreas

TABLE 2. Effects of prenatal zinc deficiency

- Rapid effect
- Embryonic and fetal death
- Early neonatal death
- Low birth weight
- Congenital malformations
- Hyperplasia of esophageal mucosa
- Low zinc content of tissues
- Depressed synthesis of pulmonary surfactant components
- Depressed synthesis of pancreatic proteins
- Depressed synthesis of DNA
  - Reduced thymidine kinase activity
  - Reduced DNA polymerase activity
- Persistent depressed immune function
- Chromosomal aberrations
- Postnatal behavioral abnormalities
- Depressed development and function of mammary gland in mothers

EFFECTS OF MARGINAL ZINC DEPRIVATION IN PREGNANT RHESUS MONKEYS

Effects on the Mother

At the present time we are engaged in a long-term study of the effects of marginal zinc deficiency during pregnancy in rhesus monkeys. The dietary concentration of zinc provided by the deficient purified diet containing spray-dried egg whites is 4 μg/100 g, beginning on day 0 of pregnancy. This level of dietary zinc did not produce a significant difference in plasma zinc concentration between the control group (100 μg/100 g diet) and zinc-deprived monkeys until after day 90 of gestation (average normal gestation is 165 days). Beginning in the third trimester, zinc-
deprived monkeys showed low levels of plasma zinc (less than 65 μg/100 ml). The extent of plasma zinc depression was dependent on total food intake; severely anorexic monkeys lost weight but maintained normal plasma zinc levels; monkeys that gained 20 to 30% of their body weight during pregnancy had severely depressed plasma zinc. Four of the 15 zinc-deprived animals showed severe anorexia with low food intake. These monkeys lost body weight and showed a reduction of skinfold thickness, indicating a reduction in fat stores. The anorexic monkeys did not show the severe early decline in plasma zinc levels seen in nonanorexic animals (28).

These results are consistent with the hypothesis that increased tissue catabolism, as indicated by weight loss, releases zinc into the circulation. This hypothesis was developed from earlier work, primarily with rats (3,29). The most recent studies demonstrating this effect evaluated the contribution of maternal tissue zinc to the fetus during dietary deficiency (30). We measured the deposition of zinc into the litter and compared it to the zinc intake of pregnant rats fed ad libitum either a zinc-adequate or a zinc-deficient diet. The influence of the metabolic state of the dam was studied by inclusion of another group of rats fed the zinc-deficient diet but restricted in intake. The frequency of resorptions (embryonic deaths) and of malformed fetuses was significantly lower in the pregnant rats fed the zinc-deficient diet in restricted amounts than in the zinc-deficient rats fed ad libitum. Thus restriction of food intake in zinc-deficient rats, leading to high body weight loss, produced less deleterious effects on the fetuses than did ad libitum intake of the zinc-deficient diet.

In control rats (both ad libitum and restricted intake), only 3% of maternal zinc intake was deposited in the products of conception (uterus, placenta, and fetuses). In the deficient group fed ad libitum, however, 240% of maternal zinc intake during the course of pregnancy was deposited in the products of conception, whereas in the restricted-intake deficient rats an even higher percentage, 330%, was deposited in the uterus, placenta, and fetuses. Thus, the additional zinc in the fetuses that could not have come from the dietary intake of the mother obviously came from her maternal tissues. In the restricted-intake deficient group, this amount of zinc was even higher (30). In pregnant monkeys, an early slight rise in plasma zinc was seen in both control and zinc-deficient animals (28). It is consistent with the hypothesis concerning tissue catabolism that this rise, although not very large, occurred at the same time as early anorexia of pregnancy.

Other effects of marginal zinc deprivation that were seen late in pregnancy were dermatitis, depressed activity of serum alkaline phosphatase and serum glutamate pyruvate transaminase (SGPT), low hematocrit and hemoglobin levels, a change in the serum albumin/globulin ratio, and impaired immune response.

In addition, concentration of plasma vitamin A was low in zinc-deficient monkeys. There was a positive correlation between plasma vitamin A and zinc concentrations at day 135 of pregnancy (31). At that time, there was also a positive correlation between plasma retinol-binding protein (RBP) and zinc in zinc-deprived monkeys. By 3 months postpartum, plasma zinc levels increased in all groups; a
positive correlation between zinc and vitamin A remained, but there was no correlation between zinc and RBP. At day 135 of pregnancy and at 3 months postpartum, the RBP/vitamin A ratio was higher in zinc-deficient monkeys than it was in controls. Polynomial regression of the interaction between plasma vitamin A and zinc and the RBP/vitamin A ratio and zinc indicated a curvilinear relationship between plasma zinc and each of these two parameters, suggesting that above a certain threshold level of plasma zinc vitamin A transport is not dependent primarily on plasma zinc concentration. Below this level, however, vitamin A release and transport from the liver seem to be strongly related to plasma zinc concentration. Thus, marginal zinc nutrition may alter vitamin A metabolism (31).

Pregnancy Outcome

Fetal Growth

Marginal deprivation of zinc had clear effects on the monkey fetus. As there was no depression of maternal plasma zinc until after day 90 of gestation (past the period of organogenesis), no malformations were expected or seen (32); however, total pregnancy loss (abortion, stillbirth, and neonatal death) was higher in zinc-deprived dams (33.3%) and pair-fed controls (30.7%) than in ad libitum-fed controls (11.1%). Complications of delivery were also very high. Birth weight, crown–rump length (trunk length), and femur length (long-bone growth) were significantly smaller in newborn male infants, but not in female infants of zinc-deficient monkeys, than those of control (ad libitum or pair-fed) monkeys.

Birth Weight in Relation to Maternal Plasma Zinc

When maternal plasma zinc concentrations between day 90 of gestation and delivery were averaged, there was a positive correlation with birth weight in controls; however, in zinc-deficient monkeys the correlation was negative. Furthermore, third-trimester food intake was an important negative determinant of plasma zinc in the deficient group, suggesting that in zinc-deficient animals a decline in food intake led to maternal weight loss and transfer of zinc from catabolized tissue to plasma and hence to the fetus. These results are consistent with the previously discussed hypothesis on the relationship of maternal catabolic state to fetal zinc status.

The negative correlation observed in zinc-deficient monkeys between maternal plasma zinc during pregnancy and birth weight is of special interest because of similar negative correlations reported in human populations (33–35). As control monkeys receiving adequate dietary zinc did not show a negative correlation between maternal plasma zinc concentration and infant birthweight, these results may indicate that marginal zinc deficiency was present in the human studies cited and may even be prevalent in human populations.
Other Effects on Newborns

Newborn infants of zinc-deprived monkeys also showed low levels of plasma zinc and plasma iron, whereas copper and magnesium concentrations were normal. In addition, neurobehavioral examinations revealed that muscle tonus was clearly lower in zinc-deficient infants than in controls, with forelimbs most affected. Motor and sensory reflex scores and reflex strength appeared to be normal. By 1 month of age, infants born to and suckled by zinc-deprived females showed signs of severe zinc deficiency: dermatitis and alopecia (32).

INTERACTING FACTORS LEADING TO TRACE ELEMENT DEFICIENCIES

Trace element deficiencies can be produced through primary deficiency, i.e., through inadequate intake of an element, as we have been discussing thus far. They can also occur, however, through secondary or conditioned deficiencies which may be caused by or be seen in conjunction with a number of factors (Table 3). Genetic factors may influence trace element metabolism; examples are the mutant strains of mice, e.g., pallid, in which effects of the mutant gene can be prevented by giving a high dietary intake of an element during pregnancy (36). The genetic disorder acrodermatitis enteropathica is another example; zinc can be given to compensate for this genetic defect. Genetic background, e.g., strain differences, can also influence the teratogenic response to trace element levels in diet (37–39).

Drugs can also interact with trace elements (40). Examples are the influence of dietary zinc intake on the teratogenic effects of 6-mercaptopurine (41) and acetazolamide (39). The percent of malformed fetuses produced in mice by acetazolamide was inversely proportional to the dietary zinc concentrations fed to the pregnant dams. Other examples involve the interaction of the chelating drugs D-penicillamine and triethylenetetramine with copper (42).

Trace elements can also interact with other nutrients (43). There are many examples of such interactions. One is the effect of dietary zinc deficiency on vitamin A metabolism described above in the rhesus monkey study. Dietary deficiency of zinc affected the metabolism of vitamin A and produced low levels of

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TABLE 3. Causative factors in trace element deficiencies

| Primary deficiency—inaudite dietary intake |
| Secondary ("conditioned") deficiency |
| Genetic factors |
| Mutant genes |
| Strain differences |
| Drugs or other chemicals |
| Nutritional interactions |
| Dietary binding factors |
| Plant toxins |
| Trace element interactions with other nutrients |
| Trace element—trace element interactions |
the vitamin in plasma. Finally, trace elements can interact with each other. An example is the relationship of copper and zinc: High dietary levels of one of these elements can exacerbate a deficiency of the other element in pregnant rats (44).

CONCLUDING REMARKS

When considering the possible role of trace element deficiencies in prenatal and neonatal development in humans, it should be remembered that results of animal studies suggest that even marginal deficiencies during prenatal life may have deleterious effects on fetal and neonatal development. Furthermore, deficiencies may result from the interaction of multiple factors, including genetic and dietary factors, drugs, or other environmental chemicals; even a short period of such functional deficiency, if it occurs at a critical period, can have important and permanent effects on the offspring. As most congenital defects in humans are now considered to be multifactorial in nature, research relating to the influence of trace elements and other essential nutrients and their deficiencies or excesses in pregnancy is clearly needed.

REFERENCES


DISCUSSION

Dr. Lombeck: Most our our knowledge on trace element deficiency and its influence on pregnancy derives from your work. It is still difficult to transfer experience from animal studies to human medicine, although you also used monkeys this time for your experiments. You stressed again the point that plasma zinc values alone do not give us enough information about zinc status. I shall tell you an example about the practical difficulties of monitoring zinc status in man. Last year one of the patients with acrodermatitis enteropathica became pregnant. During the last 7 years I had told her to have only a planned pregnancy. One evening she phoned: "I am pregnant in the fifth week." My answer: "Double the daily zinc dose." The plasma zinc was estimated monthly and remained normal. After 8 months of uncertainty the baby was born without complications and is healthy. What else should we have done? You reported some new data concerning glutamate pyruvate transaminase. Should I have measured this? Of course, I should not have made her anorexic.

Dr. Hurley: I do not know how to answer your question except to say that what we need is more research. Maybe that sounds like a "cop-out," but I think that we must have more information about the metabolism of these things in order to know what to look for. You are saying, what should I have looked for, and I am saying, we do not know until we can do animal studies to find out. The SGOT is a shot in the dark because this study is so expensive that we try to do everything else we possibly can. I am sure you did the only thing you could have done. I am not suggesting at this point that you should have told your patient to starve.

Dr. Bergmann: You may know the publication of Dr. Hambidge in Lancet in which he described several pregnancies in acrodermatitis enteropathica patients and observed a high prevalence of abortion and malformation.

Dr. Hurley: Yes, but this was before it was known that AE was a disorder of zinc metabolism, and those patients did not have normal serum zinc. Dr. Lombeck's patient was taking zinc beforehand, and so she was undoubtedly in a good state.

Dr. Lombeck: She got 50 mg of zinc (220 mg zinc sulfate) a day, and when she phoned me, from this point on and during lactation, she got twice that amount, i.e., a supplement of 100 mg of zinc per day.

Dr. Bergmann: Do your patients take the zinc supplement voluntarily, or do they try to avoid taking it? How is compliance?

Dr. Lombeck: Patients with acrodermatitis enteropathica always remember the severe skin lesions before zinc therapy. Therefore they take it.

Dr. Bergmann: All the patients I know took it as long as they had some rash, and as soon as the rash disappeared they discontinued it. After a couple of weeks, as the dermatitis recurred they would take it again.

Dr. Thompson: Much of what you say fits in with our own ideas. We are fortunate in being able to study an underdeveloped area of London, Lambeth, where many of the mothers eat badly. We recently confirmed that the mothers who produce more small-for-dates babies are not only zinc-depleted and have zinc-depleted babies, they also are eating less zinc. I agree that there are probably many other deficiencies as well. I suspect that zinc is just a marker, for our mothers are eating a poor diet with low zinc and protein as well. Indeed Mike Crawford at the London Zoo suggested that they also have an essential fatty-acid-deficient diet, although so far we have not been able to confirm that from our dietary assessments, which are often inaccurate. Your data show how important it is to do pair-feeding experiments, although we find they are difficult in smaller animals.
Although our data show that plasma zinc levels are not easily interpretable, the zinc in plasma must cross the placenta. We have looked at the guinea pig placenta, which is similar to the human placenta, and by elevating plasma zinc levels we have seen that more zinc can be transferred to the fetus. I think your hypothesis of maintaining plasma zinc when tissues are broken down is excellent and may explain some of the controversies over measuring white cell zinc.

**Dr. Hurley:** I might just add that we did not find any congenital malformations in these newborn monkeys, but we would not have expected to because the maternal plasma zinc was normal during the period of organogenesis.

**Dr. Volpato:** Which malformations did you find?

**Dr. Hurley:** In zinc-deficient rats and mice, all organ systems can be affected. About 40% of the fetuses have malformations of the central nervous system, about 70% have limb defects. There is also spina bifida, malformations of the lung, heart, and urogenital system, cleft palate, and cleft lip. We think that the reason so many organ systems are affected is that zinc deficiency influences nucleic acid synthesis, so everything is disrupted at a very basic level. Recent work from Vallee’s laboratory, in fact, suggests that zinc has a very direct effect on gene expression.

**Dr. Chandra:** Nine years ago we reported studies similar to yours on the intergenerational effects of nutritional deprivation; we did not have any ready explanation for the transfer of the effects from one generation to the other because even though the offspring got fed *ad libitum* we still saw the effect not only on birth weight but on growth and immune response. Do you have any idea how zinc might be doing this over generations? My second question is related to the observations which have been published showing that there are changes in essential fatty acid metabolism related to zinc and other trace element deficiencies. Do you think that may have any relevance to the pathogenesis of some of the effects you have observed?

**Dr. Hurley:** In regard to the intergenerational effect, we do not have any hypothesis as to the mechanism. We have shown in the past that zinc deficiency can produce chromosomal aberrations, but these are not the crossover type. They are gaps and fragments, so whether that has anything to do with it we do not know. It is also possible that prenatal zinc deficiency affects the mechanism for synthesis of immunoglobulins, so that this ability is influenced in some way in the future generations. We are repeating this experiment now with more studies of various biochemical aspects in the offspring to see if we can learn something about mechanisms. About the fatty acid question: we have not studied the influence of fatty acids per se, but Dr. O’Dell has suggested that some of the effects of zinc deficiency might be due to increased oxidation of fatty acids and that they might be prevented by vitamin E. We followed up on that and tested the effect of large doses of vitamin E on zinc-deficiency teratogenicity. We did not find any effect. This is not a specific answer to your question, but it is related.

**Dr. Mertz:** In your grandmother effect, do both parents have to be derived from a zinc-deficient mother, or is one enough? If so, which sex?

**Dr. Hurley:** This is the question we are trying to answer right now. In the studies I showed you, the offspring were mated brother–sister, so it is possible that there is an effect on both parents. However, I think it is unlikely that the effect is coming from the male because the influence of zinc deficiency on depressing testicular development is so pronounced that it is difficult to imagine that the testis would be functional and be able to produce sperm that were alive but abnormal.
Dr. Chandra: In our studies, zinc deprivation was carried out in the female animals who were mated with healthy males and the offspring studied. It was quite clear that the effect we saw was mediated through the mothers.

Dr. Thompson: The essential fatty acid story is interesting. We have been looking at this indirectly by examining the end product—prostaglandin production in white cells—because there is evidence of abnormal prostaglandin metabolism in the mothers of small babies, possibly through the changes of vasoactive prostaglandins in the placenta. Stephen Cunnane suggested that the pathway of essential fatty acids to prostaglandins is zinc-dependent; therefore zinc deficiency might reduce the synthesis of prostaglandins, and this might cause an ischemic placenta that does not transfer enough zinc to the fetus, thus limiting its growth.

Dr. Zlotkin: Dr. Hurley, you did not mention anything about zinc toxicity in the offspring. I recently had a letter from an obstetrician in the United States saying that he had a pregnant patient who had severe inflammatory bowel disease. He was planning to treat this patient with intravenous nutrition throughout the pregnancy and asked my opinion about the safety of TPN during pregnancy. Unfortunately, there is very little literature on the use of TPN during pregnancy, and it was difficult for me to intelligently comment. As you were speaking, it occurred to me that I had suggested that trace elements be included in the TPN formulation. It also occurred to me that the normal homeostatic mechanism (the liver) is bypassed with TPN. Do you have any experience on the toxic effects of zinc to the offspring and how high zinc levels must be in the maternal organism before toxic effects are actually seen?

Dr. Hurley: Most of my work has been with deficiency, not toxicity, but we have studied the interaction of zinc and copper in pregnant rats. We found that if the level of copper is low the copper deficiency will be exacerbated by an excess of zinc and the effects are seen in the offspring. However, I have no way of translating that into humans; a TPN expert would have to be called in for that.

Dr. Bergmann: There is a description of a hyperzincemia syndrome, and the children of the offspring of this syndrome are normal so far as I know.

Dr. Hurley: That is a genetic abnormality, and these people have abnormal zinc metabolism. That does not mean that a normal person without that biochemical aberration would respond in the same way.

Dr. Zoppi: Is there a difference between the malformations caused by pure protein-calorie malnutrition and those caused by isolated zinc deficiency?

Dr. Hurley: In the monkeys we found no malformations because the plasma zinc level of the mother was normal during the period of organogenesis. The malformations I referred to were in rats given a severely deficient diet. We did find many differences between the pair-fed monkeys and the zinc-deficient monkeys. The birth weight was normal in the pair-fed monkeys even though their food intake was the same as that of the zinc-deficient monkeys. This suggests that zinc does have a specific effect on fetal growth.

Dr. Gebre Medhin: I was very happy that you took up the issues of vitamin A and RBP. In relation to this, I should like to mention a study of fetal growth in Sweden and Ethiopia which shows some of the difficulties in interpreting trace element status in relation to RBP and vitamin A. The Swedish material gives a good picture of the fetal accumulation of vitamin A. It is clear that the Swedish fetuses are much better off in regard to their vitamin A status than the Ethiopian fetuses. However, what is really interesting about our material is the following: Among the Ethiopians the small-for-dates babies were the ones who had the highest levels of vitamin A concentrations in their liver. When we looked at the liver concentrations of iron, zinc, copper, and magnesium, there were only very small differences between the two (Swedish and Ethiopian) populations. Secondly, when we measured RBP,
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we observed only small differences between the fetuses, whereas the Ethiopian mothers had clearly lower RBP levels than their Swedish counterparts. Finally, there were no differences in the prevalence of malformations between the Ethiopian and the Swedish populations.

Dr. Eggermont: How do you explain the greater growth retardation observed in male offsprings of zinc-deficient mothers?

Dr. Hurley: A number of people, including Dr. Bergmann, have found that male infants are more affected by zinc deficiency than females. Of course, the first thing that comes to mind is that males have a higher growth rate, so that zinc may be more limiting in males than in females. However, if we take a closer look at our female babies, we see that even though the means were not statistically significant a higher proportion of individual female zinc-deficient neonates were below colony norms for the California Primate Research Center than either the pair-fed or the ad libitum-fed controls. Almost none of the controls were below the colony norms, whereas a high proportion of the female zinc-deficient infants were. So maybe zinc deficiency does have a quantitative effect on fetal growth in the females even though they are within the normal range of birth weight. Another possibility is that in female newborn monkeys there is a higher proportion of fat and a smaller proportion of muscle than in the males; because fat contains less zinc than muscle, it may be that that is an explanation for the more limiting effect of zinc deficiency on the males.
Dr. Eggermont: Did you look for malformations of the external genitalia?

Dr. Hurley: All of the infants were examined at birth by a primate veterinarian for malformations.

Dr. Cheek: Your presentation and studies on the pregnant monkey remind me of the 7 years we devoted to studies on Macaca mulatta. In one study we observed the effects of
protein and calorie-protein deprivation in the mother on the brain growth of the fetus, especially the biochemistry (Cheek et al. Am J Clin Nutr 1976;29:1149–57). The mothers received adequate vitamins and trace elements. The fetuses were taken 10 days before term (165 days), yet analyses revealed no changes in DNA, RNA, cholesterol, phospholipid, protein, or water distribution in cerebrum or cerebellum. Yet in the PEM group, the zinc concentration per gram of cerebral tissue or gram of protein was significantly elevated. In similar work, Riopelle’s group found no behavioral changes (Dev Psychobiol 1974;7:369).

What is the significance of zinc in the brain—in the hypothalamus (Ammon’s horn)? Is zinc important to learning ability? Is it important to behavior?

Dr. Hurley: Many people are getting interested in this question. The neurosciences meeting in November had a satellite symposium on zinc and nervous function. One of the people involved in our collaborative project with monkeys is doing behavioral studies on monkeys, infants as well as mothers, but these data have not been analyzed as yet.

Dr. Bergmann: Stimulated by the fascinating experimental data of Dr. Hurley, we conducted a case-control study in 17 mothers who had given birth to infants with spina bifida. Because this malformation develops in early pregnancy, we tried to get a history of zinc nutritional status by analyzing zinc in each of the 10 proximal centimeters of a hair strand collected from the right retroauricular region of the mothers soon after labor. The results are summarized in Figs. D1 and D2. In the normal controls the hair zinc concentration decreased significantly during pregnancy, whereas in spina bifida cases it did not. The difference in trends is statistically significant. In those cases there is a significant positive correlation between hair zinc of the mothers and their infants. This relationship was not present in the controls. Again the difference in trends was statistically significant.