Congenital Malformations Resulting from Zinc Deficiency in Rats.* (31578)

LUCILLE S. HURLEY AND HELENE SWENERTON
Department of Nutrition, University of California, Davis

The relationship of environmental factors, including essential nutrients, to congenital malformations in mammals has recently received increased attention(1,2,3). Although the influence of various vitamin deficiencies during pregnancy on the development of the young has been studied to considerable extent, relatively little is known about the role of trace elements in development. Previous work from this laboratory reported studies on the effects of manganese deficiency during gestation in experimental mammals. The present study is concerned with zinc.

Methods. To study the effects of zinc deficiency on embryonic development in rats, it was necessary first to establish that a specific deficiency state could be produced. This was accomplished by the use of a diet containing soybean protein, which increases the dietary requirement for zinc(4,5,6), and by stringent elimination of sources of zinc contamination from the environment.†

Female rats of the Sprague-Dawley strain were maintained from weaning on a ration with the following composition, in percent: soybean protein, 30.0; sucrose, 57.3; corn oil, 8.0; salt mix,‡ 4.0; DL-methionine, 0.7. The soybean protein was treated with ethylenediamine-tetraacetic acid (EDTA) to lower its zinc content(7). Crystalline vitamins were given separately.§ This ration contained 0 ppm of zinc, as determined by X-ray fluorescence analysis, with an error in the method of ± 2 ppm. The controls received the same diet except that zinc was added, giving a total content of 60 ppm of zinc.

Under these conditions, the animals receiving the zinc-free diet showed almost no growth, although the growth rate of the zinc-supplemented animals was not significantly different from that of rats fed a stock diet.‖ In addition, the experimental animals showed other signs of severe zinc deficiency. When these extremely abnormal animals were supplemented with zinc, their growth rate immediately rose and quickly reached that of the controls. In addition, all outward signs of zinc deficiency disappeared, and the animals became normal in appearance. This immediate and complete response to supplementation with zinc established that the sole missing factor was this essential trace element, and showed, furthermore, that the experimental conditions were controlled and standardized. This was especially important because of the ubiquitous nature of zinc, making it possible, in uncontrolled conditions, for experimental animals to derive more zinc from their environment than from their ration.

Under these extreme conditions of zinc deficiency, reproduction was not possible. The

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† For example, rubber stoppers (which have a high zinc content) were replaced with vinyl plastic stoppers on the watering devices; stainless steel, plastic, or glass equipment was used whenever possible; reagents used for salt mixes were selected for low heavy metal content; male rats were placed with females overnight only when females were in estrus in order to minimize coprophagy.

‡ Composition of basal salt mix, in grams: $CaCO_3$, 600; $Ca(H_2PO_4)_2 \cdot H_2O$, 220; K_2HPO_4, 650; NaCl, 336; $MgSO_4 \cdot 7H_2O$, 250; $FeSO_4 \cdot 7H_2O$, 50; $MnSO_4 \cdot H_2O$, 4.6; KI, 1.6; $CuSO_4 \cdot 5H_2O$, 0.6. Zinc-supplemented salt mix included the addition of 6.2 g $ZnCO_3$.

§ A mixture of crystalline vitamins in glucose, to which was added cod liver oil and α-tocopherol, was given 3 times each week in small glass dishes in amounts to provide the following intake in micrograms, per day: Ca-pantothenate, 500; p-aminobenzoic acid and riboflavin, each 100; thiamine · HCl, pyridoxine, and nicotinic acid, each 300; menadione, 250; folic acid, 6; biotin, 2.5; vitamin B_{12}, 0.3; and choline chloride, 10 mg; inositol, 5 mg; α-tocopherol and ascorbic acid, each 1 mg; vitamin A, 150, and vitamin D, 15 I.U. each. During pregnancy the vitamin supplement was doubled.

‖ Commercial rat chow (Wayne LAB-BLOX) and powdered whole milk.

TABLE I. Reproduction in Zinc-Deficient Rats.

No. rats	Net body wt change during gestation, g	Rats with living young (day 21) No.	%	Living young day 21 Total No.	Avg No. per litter	Body wt, g	Grossly malformed No.	%
				Stock				
5	+98	5	100	59	11.8	5.6	0	0
				Zn-supplemented controls				
21	+59	21	100	217	10.3	5.3	0	0
				Zn-deficient				
38	−21	19	50	128	6.7	2.7	125	98

TABLE II. Implantation Sites in Zinc-Deficient Rats.

Group	Implantation sites Total No.	No. resorbed	No. abnormal fetuses	Total affected No.	%
Stock	60	1	0	1	2
+Zn controls	226	9	0	9	4
Zn-deficient	280	152	125	277	99

females showed severe disruption of the estrous cycles; in most cases no mating took place. Therefore, in order to study the effect of zinc deficiency on prenatal development, it was necessary to induce a less severe state of deficiency which would permit reproduction to occur. This was successfully accomplished by the following procedure. Female rats were maintained from weaning until maturity on a marginally-deficient diet, containing 9 ppm of zinc. This ration differed from the extremely deficient one only in that the soybean protein was not treated with EDTA. At maturity, the females were mated with normal stock-fed males, and some were given the extremely deficient ration containing 0 ppm zinc. The control animals were also given the marginally-deficient diet until maturity, and then given a zinc-supplemented diet containing 60 ppm zinc during pregnancy. This procedure of maintaining the animals on the marginally-deficient ration from weaning to maturity had only a slightly depressing effect on their growth, and produced no outward signs of zinc deficiency.

Matings were carefully timed by observation of sperm in the daily vaginal smear; the day of finding sperm was considered day zero of gestation. On day 21 of gestation, the fetuses were delivered by Caesarean section in order to prevent the eating of defective young by the mother, thus allowing examination of every fetus. The fetuses were fixed in Bouin's solution for 48 hours and stored in 70% ethanol. After gross examination, representative specimens were cleared and stained with alizarin red for examination of the skeletons. Others were frozen for subsequent analysis of their zinc content. All implantation sites were counted in the uteri.

Results and discussion. The effects of this treatment on reproduction are summarized in Table I. The rats that received the zinc-deficient diet lost rather than gained weight during pregnancy, and less than half of them had living young at term. The deficient females also had a smaller number of young per litter than did the controls, and the young were less than half the normal body weight. In addition, 98% of the young showed gross congenital malformations.

Table II summarizes the effects of the deficiency on the total number of implantations. In the deficient females, all but 3 of 280 implantation sites were affected. That is, the implantation site either showed a resorbed conceptus or gave rise to a malformed fetus. Thus, 99% of the implantation sites in deficient females were affected as compared with 2 to 4% in females receiving adequate zinc.

Fig. 1 shows the appearance of these fetuses with misshapen heads, clubbed feet, fused or

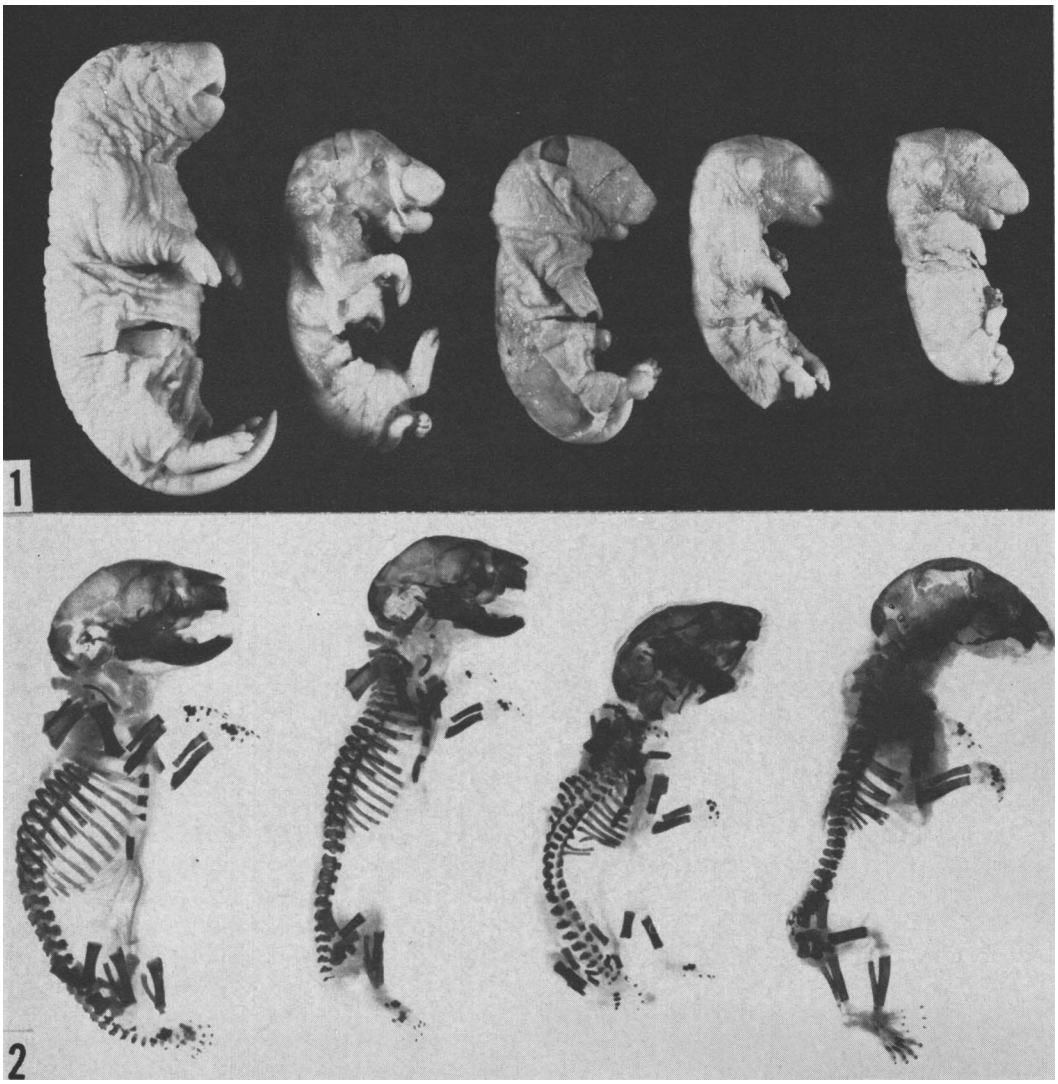

FIG. 1. Fetuses at term from rats fed zinc-supplemented ration (on far left) or zinc-deficient ration. Note small size, abnormally shaped heads, clubbed feet, fused or missing digits, short lower jaw, and short or absent tail. (This picture will also appear in a review article, Hurley, J. Nutrition, in press.)

FIG. 2. Alizarin-stained specimens of full-term rat fetuses showing control on extreme left and 3 fetuses from zinc-deficient females on right. A variety of skeletal defects, including missing vertebrae, fused ribs, scoliosis, micrognathia and agnathia, syndactyly (fused or missing digits), short limbs, and absent tail can be seen.

missing digits, and short lower jaw. Other skeletal defects which were seen in alizarin-stained specimens (Fig. 2) include fusion of the ribs, pronounced curvatures of the spinal column, missing vertebrae in the tail, and incomplete or retarded ossification in both ribs and vertebrae. The long bones were often short and, in some cases, seemed to be entirely missing. There were missing ossification centers in the digits. Doming of the skull and poor ossification of the cranial bones were noted. Examples of other defects are shown in Figs. 3 and 4.

The congenital malformations produced by zinc deficiency were varied and occurred in high incidence (Table III). A large number of

TABLE III. Types and Incidence of Gross Congenital Malformations in Zinc-Deficient Fetuses.*

Malformation	%
Cleft palate	34
Short or missing mandible	28
Scoliosis or kyphosis	47
Clubbed forefeet	34
Clubbed hindfeet	48
Fused or missing digits	80
Curly or stubby tail	83
Hydrocephalus or hydranencephalus	65
Small or missing eyes	32
Herniations	17
Heart abnormalities	13
Lung abnormalities	42
Urogenital abnormalities	49

* 128 fetuses examined.

skeletal defects were seen, in incidences ranging from 28% for short or missing lower jaw to 83% for curly or stubby tail. There was also a high incidence of soft tissue malformations, including brain defects, small or missing eyes, hernias, and heart, lung, and urogenital abnormalities.

The occurrence of skeletal defects in chick embryos from zinc-deficient hens has previously been reported(8,9). The present paper represents the first time to our knowledge that congenital malformations resulting from zinc deficiency have been produced in any mammalian species. The high incidence of these malformations and the large number of organ systems which they involve are especially noteworthy.

The zinc analyses of fetuses are summarized in Table IV. No difference was seen in the ash content of the fetuses. The zinc content of the fetuses, however, was lower in those from deficient mothers on all bases—on the basis of ash, of whole body, and of body weight. In an earlier study(10), in which the zinc deficiency was milder, no changes in zinc content of the fetuses occurred, but neither were there congenital malformations. This suggests that the congenital malformations occur because of a direct effect of lack of zinc in the fetal tissues, and not because of an indirect effect of the maternal metabolism on fetal development.

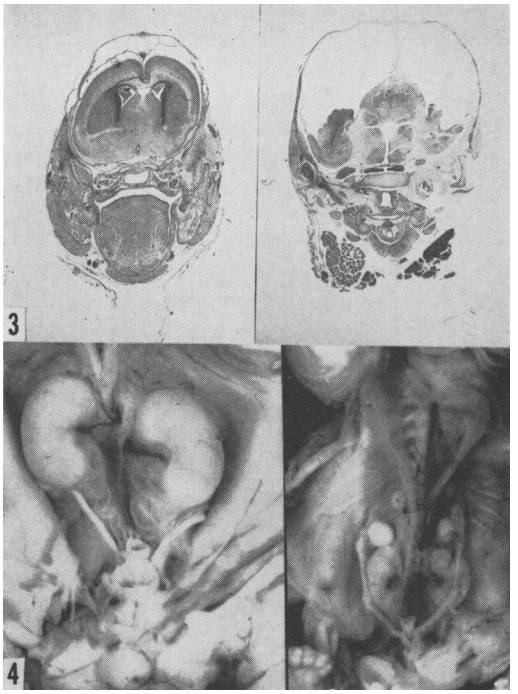

FIG. 3. Coronal sections of brain from full-term rat fetuses. Control on left, specimen from zinc-deficient mother on right, illustrating hydranencephaly, in which there is enlargement of the ventricles, and an almost complete lack of development of the cerebral cortex.

FIG. 4. Urogenital abnormalities in full-term fetus from zinc-deficient rat. In control on left, note well-developed kidney, with adrenal above it. Behind it can be seen the ovary connected to the uterus. In fetus from zinc-deficient rat, on right, the adrenal is far anterior to the kidney, and between them lies the as yet undifferentiated gonad.

Summary. A mild but specific zinc deficiency was produced in female rats by the use of a purified diet lacking the element and by stringent elimination of sources of zinc contamination from the environment. Almost all

TABLE IV. Zinc Content of Fetuses at Term.*

Group	No. fetuses	Ash, % wet wt	Zinc		
			per mg ash	per whole body	per g body wt
			μg		
+Zn controls	12	1.46 ± .03	1.28 ± .04	97 ± 3	18.6 ± .4
Zn-deficient	14	1.42 ± .06	.90 ± .05†	40 ± 3†	12.8 ± .6†

* Means ± standard error.

† $p < .001$ as compared with control.

of the full-term fetuses produced under such conditions showed gross congenital malformations encompassing a wide variety of organ systems, including skeletal, brain, eye, heart, lung, and urogenital defects. The fetuses from zinc-deficient females contained less zinc than did their controls, suggesting that the congenital anomalies resulted from a direct effect of lack of zinc in the fetal tissues.

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Observations on Antigenic Variants of Echovirus Type 11.* (31579)

NATHALIE J. SCHMIDT, EDWIN H. LENNETTE, AND HELEN H. HO
Viral and Rickettsial Disease Laboratory, California State Department of Public Health, Berkeley

Within certain echovirus immunotypes there occur antigenic variants designated "prime" strains. Such strains are neutralized to low titer or not at all by immune sera to the prototype virus strain, but immune serum prepared against a "prime" strain has high neutralizing capacity for both itself and the prototype strain. Thus, "prime" strains have a broader antigenic spectrum than does the prototype strain. Antigenic differences between "prime" and prototype strains are not so apparent in hemagglutination inhibition (HI) and complement fixation (CF) tests as in neutralization tests. To date "prime" strains have been described for echovirus types 1, 3, 4, 5, 6, 9, 29 and 30(1-3).

In the past few years we have isolated a number of strains of echovirus type 11 which are neutralized to low titer or not at all by immune serum to the prototype (Gregory) strain of echovirus type 11, but immune sera prepared against some of these strains neutralize the prototype echovirus type 11 strain. The first such strain was isolated in 1962, and in successive years the majority of echovirus type 11 strains isolated in this laboratory have been found to be more closely related to a representative "prime" strain (Silva) than to the prototype strain.

This report describes the recovery and serologic reactions of antigenic variants of echovirus type 11 and also the antibody responses of patients infected with these viral strains.

Materials and methods. Isolation and identification of viral strains. Virus was recovered from stool suspensions or throat washings in cultures of rhesus monkey kidney (MK) cells and/or human fetal diploid kidney (HFDK) cells by procedures which have been described elsewhere(4). Isolates were tested against immune sera to the polioviruses and group B coxsackieviruses in a colorimetric neutralization system(4), and in neutralization tests against immune sera to the echoviruses and

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