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Effect of Zinc Deficiency in Hens on Hatchability and Embryonic Development.*† (25784)

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Following demonstration of zinc deficiency in chicks(1,2) and poults(3), an investigation of the effect of low zinc intake by the laying hen was undertaken in this laboratory. A preliminary study revealed that hens receiving a purified diet containing 6 ppm zinc laid eggs which hatched considerably poorer than those of hens fed a similar diet to which 96 ppm zinc had been added(4). Subsequently, Turk *et al.*(5) reported that hatchability was decreased when hens were fed a soy protein diet of low zinc content (8-9 ppm) and that the chicks which hatched were weak, feathered poorly and usually died within a few days.

Methods. Babcock strain White Leghorn pullets were housed in individual stainless steel cages and fed the basal diet described in Table I, estimated to contain 6 ppm zinc. Feed and distilled water were provided *ad libitum* in stainless steel containers. Fresh diet was prepared at 3-week intervals. Following a 6-week preliminary feeding period, 12 hens were fed the basal diet with 120 ppm zinc added as zinc chloride and 12 hens were continued on the unsupplemented basal diet. The hens were inseminated at weekly intervals with 0.1 cc of pooled semen from a group of flightless strain males which were maintained

TABLE I. Composition of Basal Ration.

	%		%
Isolated soybean protein*	21.7	Corn oil	4.6
		Antioxidant†	.02
Sucrose	61.63	Choline chloride	.15
DL-methionine	.6	Mineral mixture‡	10.45
Glycine	.5	Vitamin " §"	.35

* ADM assay protein (Archer Daniels-Midland Co.) washed 3 times with distilled water and dried at approximately 55°C.

† 1,2-dihydro-6-ethoxy, 2,2,4-trimethylquinoline (Santoquin).

‡ C.P. or reagent grade, supplied (% of diet): 1.53 $CaCO_3$, 6.80 $Ca_3(PO_4)_2$, 0.80 K_2HPO_4, 0.27 KCl, 0.30 $MgSO_4 \cdot 7H_2O$, 0.65 NaCl, 0.05 $FeSO_4 \cdot 7H_2O$, 0.002 $CuSO_4 \cdot 5H_2O$, 0.03 $MnSO_4 \cdot H_2O$, 0.0002 $CoSO_4 \cdot 7H_2O$, 0.001 KI, 0.01 $AlK(SO_4)_2 \cdot 12H_2O$, 0.001 $Na_2MoO_4 \cdot 2H_2O$, 0.005 $Na_2SiO_3 \cdot 9H_2O$, 0.001 H_3BO_3, 0.00007 Na_2SeO_3.

§ Supplied/100 g of diet: mg, 6.6 alpha-tocopheryl acetate, .003 cyanocobalamin, 1.0 menadione, 100 inositol, .5 para-aminobenzoic acid, .08 biotin, .2 folacin, 1.8 pyridoxine HCl, 10.0 niacin, 3.4 calcium pantothenate, 2.0 riboflavin, 2.0 thiamine HCl, 3.0 ascorbic acid; also 1170 I.U. vit. A and 150 I.C.U. vit. D_3.

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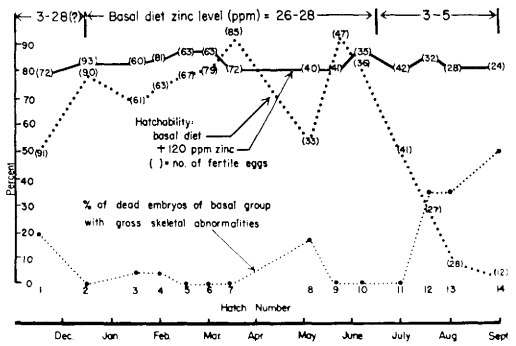

FIG. 1. Effect of zinc level of hen diet on (1) hatchability and (2) incidence of embryos with characteristic trunk and limb abnormalities. These skeletal abnormalities were never observed in embryos from hens fed the zinc-supplemented diet.

on a practical type ration containing 42 ppm zinc. Eggs were collected for 2-week periods (storage temperature 50°F) and incubated. Fertility was determined on 7th day of incubation and at this time, and periodically thereafter, all dead embryos were examined for gross anomalies. Some of the 18-19 day embryos found to be obviously malformed upon candling were examined while still living. Zinc determinations were made by the one-color dithizone procedure described by Benne(6). Certain of the terms describing embryonic malformations (anouria and ectrosomia) were taken from Ancel(7).

Results. The percentage of fertile eggs which hatched (hatchability) and the percentage of dead embryos which were characteristically malformed are shown in Fig. 1 for 14 hatches. Hatchability of eggs from the hens fed the zinc-supplemented diet was relatively uniform throughout the experiment, varying between 79% and 89%. No consistent type of malformation was seen in the embryos from hens receiving the supplemented diet in any of the 14 hatches.

Hatchability of eggs laid by hens receiving the unsupplemented diet was erratic during the first 10 hatches, but generally lower than that of eggs from the zinc-supplemented hens. During this period (see Fig. 1) the basal diet was found by analysis to contain approximately 28 ppm zinc rather than 6 ppm as has been presumed. The contamination was eventually traced to the $CaCO_3$ being used, which, though purportedly of reagent grade, contained 1400 ppm zinc. When this product was replaced with one of suitable quality (3 ppm zinc), zinc content of basal diet was reduced to approximately 4 ppm, and hatchability rapidly decreased to zero (hatches 11-14). Decrease in hatchability was paralleled by a decrease in zinc content of the yolk of the basal group eggs from 26-38 ppm to 7.5-11 ppm(8).

In all instances, except hatch 11, whenever hatchability was lowered, gross malformations of a characteristic type were encountered in the dead embryos of the basal group eggs, incidence of these abnormalities being directly related to level of embryonic mortality (inversely related to hatchability, Fig. 1). The characterizing feature of these malformations was evidence of some degree of faulty trunk and limb development. The caudal-most part of the vertebrae was always absent (anouria) (Fig. 3) and, in some instances, a larger portion of the trunk appeared to be missing (ectrosomia) or shortened (Figs. 2,4). Dorsal curvature of the spine (lordosis) was also found (Fig. 3, embryo B). In some cases portions of the limbs (Fig. 3, embryo C), or the limbs in their entirety (ectromelia) were missing (Fig. 2 and certain embryos in Figs. 3 and 4). A common occurrence was a short leg with only 2 toes (ectrodactyly) (Fig. 3, embryo C, Fig. 4, embryo B). Occasionally, an apparent fusion of the lower limbs was observed (symmelia) (Fig. 2, embryo E).

Embryos living to develop head structures comparable to normal 6-7 day embryos frequently had abnormal brains and visceral arches, and small eyes (microphthalmia) (Fig. 3, 4), as well as ectromelia and apparent ectrosomia (Fig. 2). Exposed viscera (celosomia), though not common in 6-7 day embryos, was almost invariably associated with skeletal malformations in embryos developing beyond 7 days (Figs. 3, 4). Abnormalities of the beak and other external head structures were observed (Fig. 4). Malformed embryos living to 18-20 days were usually well-feathered but sometimes had extreme skin edema (Fig. 4, embryo A). Viscera of embryos which developed 9-19 days appeared surprisingly normal under a dissecting microscope, with heart, kidneys, gonads, lungs and intestinal tract well developed.

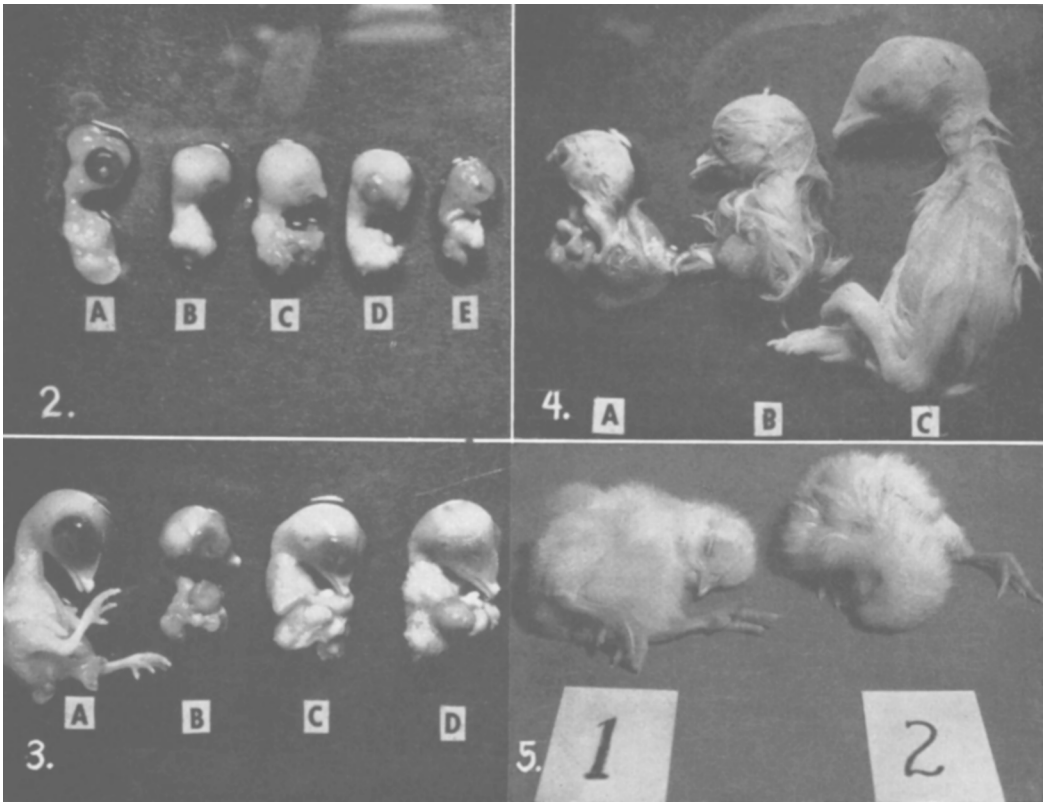

FIG. 2. A, normal chick embryo incubated 6 days. B, C, D and E, dead embryos from zinc-deficient hens developed to 6-7 days incubation. B, C, and D have ectrosomia and ectromelia. E has symmelia, lordosis, and ectromelia of wing buds. C and E have microphthalmia.

FIG. 3. A, normal chick embryo incubated 12 days. B, C, and D, dead embryos from zinc-deficient hens developed to about 12 days. Each has celosomia, anouria, and degrees of microphthalmia. B and D have complete ectromelia, while C shows ectrodactyly and micromelia. B has lordosis.

FIG. 4. C, normal chick embryo incubated 18 days. A and B, living embryos from zinc-deficient hens incubated 18 days. Both have celosomia, microphthalmia, ectromelia, and beak deformities. A has skin edema and complete ectromelia, while B has partial ectromelia, ectrodactyly, and micromelia.

FIG. 5. Chicks hatched from hens fed the unsupplemented basal diet.

In hatches 1 and 12-14, particularly, a considerable portion of the basal group chicks which started to hatch were unable to emerge from the shell. Others exhibited varying degrees of weakness after hatching, some being entirely unable to stand, and others showing a disinclination to walk and a tendency to squat (Fig. 5).

Discussion. The need for an adequate zinc intake by the breeding hen to insure normal embryonic development was confirmed in this experiment. It was indicated, furthermore, that a sufficiently restricted zinc intake can be expected to result in total loss of hatchability.

Absence of trunk and limb abnormalities in the zinc-supplemented group embryos and appearance of these anomalies in increasing numbers as hatchability of the basal group eggs decreased suggests that zinc-dependent metabolic processes are of particular importance in development of skeletal mesoderm. It is recognized, however, that histological studies might reveal equally significant, though less obvious, effects of zinc deficiency on other types of embryonic tissue.

Summary. Evidence is presented indicating that deficiency of zinc in the diet of breeding hen, results in (1) lowered hatchability (2) gross embryonic anomalies characterized by impaired skeletal development and (3) vary-

ing degrees of weakness in chicks which hatch.

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Lung Tumors in Hamsters Produced by Polyoma Virus. (25785)

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Ease of transmission of polyoma infection in mouse colonies suggests that virus is spread by way of the respiratory tract(1). Rowe *et al.* reported that mice and hamsters can be infected with polyoma virus administered intranasally, although they did not describe the tumors(2). Eddy and associates described development of fibrosarcomas and angiomatous lesions in hamsters following subcutaneous injection of polyoma virus(3). Only a few animals in their study had lung neoplasms. The present report is part of study on pathogenesis of polyoma infection in the hamster and compares virus administration routes on development of lung tumors.

Materials and methods. Tissue Cultures. Embryos were removed from pregnant mice of ICR strain on 14th or 15th day of gestation. Trypsinized cultures of embryonic cells were prepared and cultivated as described by Stewart *et al.*(4). Cells were grown in 4 oz. prescription bottles and in roller tubes. Cultures were grown in 2% calf serum in medium 199 containing penicillin and streptomycin and maintained in 1% calf serum in medium 199 by weekly changes of fluids. The strain of virus in these studies was received from Dr. Bernice Eddy. Virus was carried through 7 passages in cultures of mouse embryo cells. Harvests for passage were usually collected 14 days after inoculation. Sixth and seventh passage viruses were used. For certain tests, virus was passed through Selas .03 filters. Syrian hamsters were obtained from Lakeview Hamster Colony, Lakeview, N. J. Pregnant mothers were received 3 or 4 days before parturition. Litters of hamsters less than 24 hours old were pooled, randomized and redistributed to dams. Virus or control fluids were instilled intranasally (IN) by placing a drop over noses and forcing inhalation by closing the mouth. For subcutaneous (SC) and intraperitoneal (IP) injections, each animal received 0.1 ml of undiluted culture fluids. For intracerebral (IC) 0.02 ml was injected. Preliminary tests showed that uninfected mouse cell suspensions were not tumorigenic by inoculation routes employed.

Results. Virus injected subcutaneously resulted in gross tumors in subcutis, liver, kidney, heart and, in one instance only, in lungs. Subcutaneous tumors often occurred at multiple sites. On occasion they reached massive proportions accounting for 25% of body weight. Growth of these tumors varied and was often rapid. In one animal a tumor weighing 21 g at necropsy was palpated a week earlier as 2-3 mm nodule. The neoplasms resembled sarcomas described by Eddy *et al.*(3). Those seen in other tissues except the liver were similar in appearance. Histologically the growths had the structure of fibrosarcoma. Microscopic tumors were found in certain tissues, including brain, not detected by gross examination. Liver involvement was angiomatous with lesions occasion-