Use of Japanese Quail for the Study of Zinc Deficiency. (29217)

M. R. Spivey Fox and Bertha Neal Harrison
Division of Nutrition, Food and Drug Administration, Department of Health, Education and Welfare, Washington, D. C.

The production of a zinc deficiency in an experimental animal entails numerous precautions to exclude the element from the diet, the drinking water, and the environment of the animal. Since problems of contamination would be expected to be managed more easily with smaller animals, it was decided to investigate the use of Japanese quail for studies of zinc metabolism, including the production of a deficiency. These birds are much smaller than most other avian species commonly used in the laboratory.

When zinc was omitted from a purified soybean protein diet, young quail developed a severe deficiency syndrome by 4 weeks of age. The control birds fed the same diet plus zinc grew and developed normally.

Materials and methods. The quail were housed in polypropylene cages that had stainless steel wire mesh floors supported about an inch above the cage floor by hollow polyethylene stoppers. Cage covers and the rack that held the cages were painted with a clear epoxy resin paint to exclude zinc. A 25 W incandescent light bulb with an aluminum reflector was mounted about one and one-half inches above the cage. Water was supplied from a glass water bottle with a bent glass drinking tube sealed at the lower end. Access to the water was through an opening about 6 mm in diameter on the upper side of the tube near the sealed end. Food and distilled water were available *ad libitum*.

Both sexes of day-old Japanese quail of the King strain* were distributed into groups of 4-8 birds each. They received the purified diets throughout the experiment beginning on day 1. The birds were wing-banded at 7 days of age and weighed weekly; the experiments were terminated at 4 weeks. Beginning at 2 weeks of age the quality and quantity of feathers on the wings and across the back were scored on a scale of 0-10, with 10 representing normal feathering.

Zinc deficient diet Q1, which was fed to the young birds, had the following composition (g/kg): soybean protein† 350, glycine 10, DL-methionine 6, corn oil 40, Fox-Briggs Salts N(1) with zinc carbonate omitted 60, choline chloride 2, and U.S.P. dextrose 532. The following vitamins were present (mg/kg): thiamine hydrochloride 8, riboflavin 8, D-calcium pantothenate 20, nicotinic acid 100, pyridoxine hydrochloride 8, D-biotin 0.3, pteroylglutamic acid 3, vit. B_{12} 0.02, vit. A acetate 6, vit. D_3 0.02, DL-α-tocopherol acetate 25, DL-α-tocopherol 25, and 2-methyl-1,4-naphthoquinone 1. Zinc carbonate was added to the diet of the control birds to supply 90 mg zinc/kg of diet. The salts mixture was prepared from reagent grade chemicals; it supplied calcium at a level of 1.24% of the diet and phosphorus at 0.8%.

The parents of some of these birds were fed purified diet Q4. It was the same as diet Q1 except that all of the vitamins but choline chloride were doubled in concentration, 7 g dibasic calcium phosphate and 25 g calcium carbonate replaced 33 g of dextrose/kg of diet and zinc carbonate supplied 90 mg zinc/kg of diet. The remainder of the young quail were the progeny of adults fed a commercial stock diet‡ Q5. The diet as purchased was fortified with calcium carbonate (0.5% of the diet), corn oil (0.5%, carrier for the fat-soluble vitamins), 25 mg zinc/kg of diet from zinc carbonate, and all of the vitamins except choline chloride at the same levels as those in diet Q1.

The diets were analyzed for zinc several

* The original stock was obtained from the King Farm, Rixeyville, Va. The King Strain was started in 1957 from lines of *Coturnix coturnix coturnix* and *Coturnix coturnix japonica* and has been closed since that time.

† Cl soybean protein purchased from Archer-Daniels-Midland Co., Cincinnati, Ohio.

‡ Rose Bud diet purchased from Truslow Farms, Inc., Chestertown, Md.

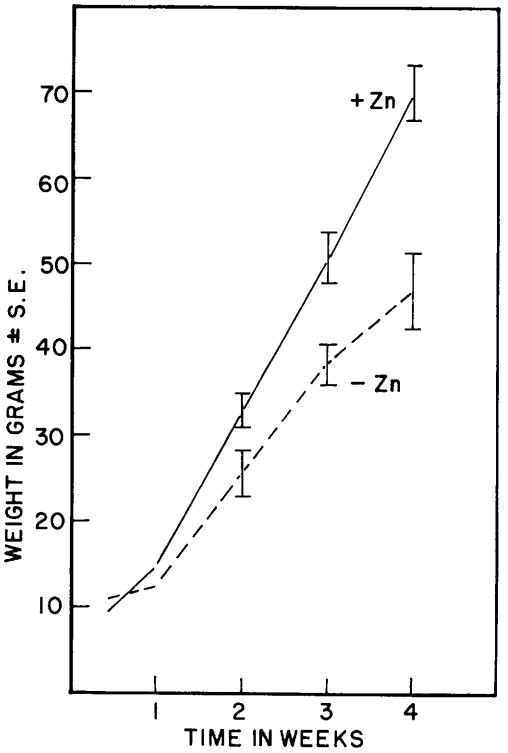

FIG. 1. Growth of zinc supplemented and zinc deficient young quail.

times during the experiments. The amounts of zinc per kg of diets Q1, Q4, and Q5 were about 10, 120 and 60 mg, respectively. The distilled water contained about 0.5 γ zinc/100 ml.

At 4 weeks of age the hematocrit and hemoglobin(2) values were determined on blood obtained by heart puncture. The birds were killed by decapitation; liver and tibias were removed and stored at −20°C prior to analysis. The bones were sectioned longitudinally and wrapped in non-woven cloth that had been rendered zinc-free by dithizone treatment. The bones were extracted for 72 hours in a Soxhlet extractor with a 2:1 mixture of ethanol:chloroform. After drying under vacuum for 18 hours at 87°C, the bones were weighed and ashed in platinum crucibles in a muffle furnace at 475°C for 18-24 hours. The ash was weighed, dissolved in 6 N hydrochloric acid and assayed for zinc colorimetrically with dithizone(3,4). Livers were homogenized in 3 volumes of water, an aliquot was ashed by the above procedure, and the zinc content of the ash was determined.

Results. Differences in growth were marked between the zinc deficient birds and the zinc supplemented birds (Fig. 1). Abnormal posture and feathering as well as the small size of the zinc deficient birds are apparent in Fig. 2. The effect of zinc deficiency upon feathering is shown more clearly in Fig. 3. In many deficient birds the feathers did little more than emerge from the follicle. When the feathers did grow somewhat longer, they were ragged and broke easily. The gross effects of the zinc deficiency were apparent by about 2 weeks of age.

The effects of the zinc deficiency on several physiological responses are presented in Table I. The parents' diet did not affect the response of the young birds and mortality was about the same for all groups. Respiration of zinc deficient birds was labored although the respiration rate was unaffected by the deficiency. Values for hemoglobin and hematocrit were not changed by the deficiency. The skin of all birds appeared normal and no perosis or hock enlargement was observed.

Significantly lower concentrations of zinc were found in the livers and tibias of zinc deficient birds as compared to the normal

FIG. 2. Zinc supplemented and zinc deficient quail, 4 wk of age.

FIG. 3. Wings from zinc supplemented (upper pair) and zinc deficient (two lower pairs) quail, 4 wk of age.

TABLE I. Effects of Zinc Deficiency on Various Physiological Parameters in the 4-Week-Old Quail (Average of 3 Experiments).

Added dietary zinc, mg/kg	Parental diet	Mortality* /Total No.	Body wt, g	Feather score†	Respirations/min	Hemoglobin, %	Hematocrit, %
0	Q4	5/18	46 ± 3.3	2.0 ±.5	98 ± 3.3	10.0 ± .72	41.8 ± 2.52
90	Q4	4/19	69 ± 2.2	8.4 ±.2	95 ± 4.5	9.2 ± .30	37.7 ± .83
0	Q5	4/11	44 ± 5.1	1.6 ±.5	93 ± 3.6	8.9 ± 1.30	36.4 ± 3.18
90	Q5	3/ 9	70 ± 3.1	8.2 ±.4	87 ± 6.5	8.4 ± 1.33	33.8 ± 3.68

Values preceded by ± are S.E.

* No. of birds that died 7-28 days/No. of birds at 7 days of age.

† Normal feathering is 10; the score reflects quality and quantity of feathers of wings and back.

TABLE II. Effects of Zinc Deficiency on Livers and Tibias.*

Added dietary zinc, mg/kg	No. samples	Liver		Tibia		
		Fresh wt, g	Zinc, γ/g†	Fat-free dry wt, g‡	Zinc, γ/g§	Ash, %§
0	9	1.46 ±.095	15.4 ± 1.9	.1277 ±.0070	120 ± 4.5	51.1 ± 1.12
90	15	2.46 ±.168	24.1 ± 2.0	.1654 ±.0056	294 ± 8.4	57.0 ± .58

Values preceded by ± are S.E.

* Progeny of adults fed diet Q4, from the same groups as those in Table I.

† Related to fresh weight.

‡ Both tibias were analyzed, but results are for one bone.

§ Related to fat-free dry weight.

controls at 4 weeks of age (Table II). The deficiency also resulted in a diminution of the percentage of ash in the tibias.

Discussion. These data demonstrate that zinc is a dietary essential for Japanese quail. It is of interest that the severe deficiency was produced without use of an excessive level of calcium, which has been used to increase the severity of a zinc deficiency with a soybean protein diet in other animals(5-8). It is probable that the young quail, like chickens(6,9), cannot effectively utilize small amounts of zinc in a soybean protein diet.

The most severe manifestations of the zinc deficiency in the quail were abnormal growth, feathering, respiration, and gait; similar defects have been reported for the chick(7,10, 11), poult(12,13), and ring-necked pheasant (14). In contrast to the other avian species, the quail did not have enlarged hocks or dermatitis; however, it appears that the feathering process is more sensitive to zinc deficiency in the quail than in the chick(7). The severe manifestations of the zinc deficiency in the quail are the subjects of present investigations on the function of zinc and its relationship to that of other nutrients. Insofar as the authors know, this is the first report of the use of a purified diet for young Japanese quail.

Summary. Day-old Japanese quail fed a zinc-low purified diet containing soybean protein were severely deficient at 4 weeks of age. The zinc deficiency was characterized by slow growth, abnormal feathering, labored respiration, an incoordinate gait, low ash content of the tibias and low concentrations of zinc in the livers and tibias. Control birds fed the same diet supplemented with zinc grew and developed normally. Successful use of the Japanese quail in this study indicates that the animal has great potential usefulness for nutrition studies.

The authors wish to thank Mr. Jesse J. Gantt for excellent technical assistance.

1. Fox, M. R. S., Briggs, G. M., *J. Nutrition,* 1960, v72, 243.

2. Drabkin, D. L., Austin, J. H., *J. Biol. Chem.,* 1932, v98, 719.

3. Vallee, B. L., Gibson, J. G., 2nd, *ibid.,* 1948, v176, 435.

4. Hoch, F. L., Vallee, B. L., *ibid.,* 1949, v181, 295.

5. O'Dell, B. L., Savage, J. E., *Poultry Sci.,* 1957, v36, 459.

6. Morrison, A. B., Sarett, H. P., *J. Nutrition,* 1958, v65, 267.
7. O'Dell, B. L., Newberne, P. M., Savage, J. E., *ibid.,* 1958, v65, 503.
8. Roberson, R. H., Schaible, P. J., *Poultry Sci.,* 1960, v39, 837.
9. O'Dell, B. L., Savage, J. E., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 304.
10. Roberson, R. H., Schaible, P. J., *Science,* 1958, v127, 875.
11. Young, R. J., Edwards, H. M., Jr., Gillis, M. B., *Poultry Sci.,* 1958, v37, 1100.
12. Supplee, W. C., Combs, G. F., Blamberg, D. L., *ibid.,* 1958, v37, 63.
13. Kratzer, F. H., Vohra, P., Allred, J. B., Davis, P. N., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 205.
14. Scott, M. L., Holm, E. R., Reynolds, R. E., *Poultry Sci.,* 1959, v38, 1344.

Received December 31, 1963. P.S.E.B.M., 1964, v116.

Effects of *in vivo* Hyperoxia on Erythrocytes 1. Hemolysis in Mice Exposed to Hyperbaric Oxygenation.* (29218)

CHARLES E. MENGEL, HERBERT E. KANN, WIRT W. SMITH AND BETTY D. HORTON
(Introduced by Ivan W. Brown, Jr.)
Department of Medicine, Duke University Medical Center, Durham, N. C.

The toxic effect of increased oxygen tensions on tissues has long been recognized (1-5). With the use of 100% O_2 environments in space capsules(6-8) and the development of hyperbaric oxygenation (OHP) for medical-surgical purposes(9,10) this effect has become of greater practical importance, emphasized by the finding that volunteers maintained in simulated space capsule environments developed hemolytic anemia(8). We have explored mechanisms of hyperoxic hemolysis by studying the effects of *in vivo* OHP on mouse erythrocytes.

Methods. Male and female, strain DBA/2, mice (all between 6 and 9 months old) were maintained on chow diets (partially vit. E deficient) or tocopherol deficient diets.† A group of the chow fed mice received 1 mg of α tocopherol acetate intraperitoneally 18 hours prior to study. From each of the 3 groups, 20 mice of comparable age and sex were used for each study. Half of these were exposed to 100% oxygen at 45 psia (lb/sq. in., absolute) and half were kept in room air at ambient pressure (15 psia). All mice were exsanguinated and studies were carried out on individual and pooled blood samples. Determinations performed were: microhematocrits and reticulocyte counts by standard methods; blood methemoglobin and plasma hemoglobin levels (determined spectrophotometrically); erythrocyte glucose-6-phosphate dehydrogenase activity (G-6-P-D)(11), reduced glutathione (GSH)(12), catalase activity(13), cholinesterase activity(14). The lytic sensitivity of erythrocytes to H_2O_2(15) was determined as follows.

All glassware was washed with concentrated HNO_3 containing a tenth volume of 30% H_2O_2, and thoroughly rinsed with deionized water. Solutions of H_2O_2 (.01%, .025%, .05%, .075%, .1%) were prepared by dilution of 30% H_2O_2 in a KH_2PO_4-NaOH buffer (250 ml · 2mKH_2PO_4 and 197 ml · 2mNaOH, diluted to 1000 ml at pH 7.4), and stored away from light at 4°C. These solutions were stable for more than 6 weeks as determined by iodometric titration. Blood from mice was heparinized and 2.5% suspensions of erythrocytes were prepared in a mixture of equal volumes of buffer and isotonic saline at pH 7.4. After incubation at 37°C for one hour, the suspensions were centrifuged and the supernatant fluid removed. The cells were washed with 10 ml of physiologic saline and 5% suspensions of erythrocytes in physiologic saline were prepared.

Samples of cell suspension (.25 ml) were placed in centrifuge tubes and equal volumes of hydrogen peroxide solution were added.

* This investigation was supported in part by a grant from National Heart Institute, USPHS.

† General Biochemicals, Chagrin Falls, Ohio, (tocopherol deficient test diet).