doses (25 mg/day/4 days) as previously described for the experiments using commercial quercetin, no cataracts were found in a group of 6 rats after 3 months following the administration of quercetin.

The mixture of impurities separated from the quercetin on the silicic acid column was eluted as a group and fed to 2 rats. Within 2 weeks, 1 of these 2 rats already exhibited the beginning of cataract development.

Work is now in progress on separation of the 8 or more individual compounds present as impurities in the commercial quercetin and on testing the effect of each compound on development of cataracts.

Summary. When 20 mg or 100 mg of commercial quercetin suspended in U.S.P. syrup was put into the stomach of a rat, either in a single dose or in 4 daily doses of 5 mg or 25 mg respectively, 50% of the animals so treated developed a cataract in one eye within 10 weeks following receipt of the quercetin. No bilateral cataracts were observed. Chromatographically pure quercetin did not cause cataracts under similar conditions.

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Connective Tissue Defect in the Chick Resulting from Copper Deficiency.* (26951)

B. L. O'DELL, B. C. HARDWICK, GENEVIEVE REYNOLDS AND J. E. SAVAGE
Departments of Agricultural Chemistry and Poultry Husbandry, University of Missouri, Columbia

Since the observations of Hart *et al.* (1), the primary physiological role of copper has been assumed to be concerned with hemopoiesis. A function in connective tissue metabolism has been suggested by bone deformities that occur in dogs (2) pigs (3) and chickens (4), and by the subcutaneous hemorrhage and abdominal hernias observed in newborn rats (5) when these animals were deprived of dietary copper. Gallagher (4) was unable to produce copper-deficiency anemia in chicks but recently Hill and Matrone (6) have produced anemia in presence of adequate iron. The purpose of the present study was to investigate other aspects of the pathology of copper deficiency in chicks.

Methods. Crossbred Vantress X White Rock chicks were kept in battery brooders which had been coated with an epoxy resin. Eight 4-week trials were run using 10 or 20 chicks per group. The basal diet had the following composition: Non-fat milk solids 55.0 g, sucrose 35.3 g, soybean oil 5.0 g, glycine 1.5 g, L-arginine HCl 1.0 g, DL-methionine 0.5 g, $CaHPO_4$ 1.0 g, NaCl 0.5 g, choline Cl 0.2 g, $MnSO_4 \cdot H_2O$ 31 mg, $FeSO_4 \cdot 7H_2O$ 25 mg, KI 0.3 mg. A vitamin

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TABLE I. Gross Pathology of Copper Deficiency.

Observations	Basal	Basal + 5 ppm Cu
Avg wt, g	244 ± 6*(86)†	388 ± 7 (77)
Mortality, %	23 (112)	1.3 (78)
Packed red cell vol, %	28.5 ± .5 (60)	31.5 ± .5 (52)
Leg weakness, %	31 (90)	2 (60)
Bone deformity or perosis, %	21 (90)	2 (60)
Subcutaneous hemorrhage, %	47 (90)	2 (60)
Internal hemorrhage at necropsy, %	78 (40)	0

* Stand. error of mean. † No. of chicks upon which observation was based.

supplement (7) was added.† By analysis this diet contained about 0.8 ppm of copper and the control diet was supplemented with 20 mg of $CuSO_4 \cdot 5H_2O$ per kg. Feed in resin-coated troughs and distilled water in glass fountains were supplied *ad libitum*. At the end of the fourth week blood was drawn from the ulnar vein for hematocrit determination and tissues were saved for histological examination. In some cases tissue was taken immediately after death that resulted from internal hemorrhage. Aortae were fixed in neutral formalin, embedded, sectioned and stained according to standard procedures (8).

Results and discussion. A summary of the gross pathology observed in the copper deficient chicks is presented in Table 1. Although the severity of depletion varied from trial to trial, on the average chicks that consumed the copper deficient diet weighed significantly ($P < 0.01$) less at 4 weeks of age. There was only a 10% decrease in average hematocrit value, but the difference was statistically significant ($P < 0.01$).

One of the early symptoms of the deficiency was a tendency for the chicks to squat or to exhibit an unsteady gait. Although it was difficult to make a clear distinction at all stages between this leg weakness and the condition commonly described as perosis, it was evident that severely depleted chicks frequently became completely paralyzed without showing either slipped tendon or bone deformities. Approximately 30% of the copper-deficient chicks exhibited some degree of leg weakness or paralysis. Occasionally there was associated with these symptoms a bone deformity characterized by sharp bending at the proximal end of the metatarsus. In general, the bones were more fragile than normal.

The most spectacular symptom was the widespread occurrence of both subcutaneous and internal hemorrhage. Subcutaneous hemorrhages became evident in a high percentage of the birds by the end of the third week and was most commonly observed on the thighs and breast. Essentially no hemorrhages occurred in chicks that consumed the copper-supplemented diet.

There was a rather high mortality rate (23%) among the copper deficient chicks and a large proportion of the deaths could be attributed to internal hemorrhage. Of 40 chicks necropsied up to 6 weeks of age 78% showed massive blood clots in the pericardial sac or in the peritoneal cavity, providing evidence for a defect in the atria, aorta and other major vessels.

The aorta was chosen for histological study and photomicrographs of selected sections are shown in Figs. 1-6. The section shown in Fig. 1 was taken from a copper deficient chick soon after death. There was a massive hemorrhage in the peritoneal cavity and the aorta was discolored from hemorrhage within the vessel wall. The walls of the aorta were greatly thickened, the lumen was extremely small, and dissect-

†Folacin was supplied by American Cyanamid Co., biotin by Hoffmann-LaRoche, the other vitamins by Merck and Co., methionine by Dow Chemical Co., arginine by General Mills, Inc. and glycine by Monsanto Chemical Co.

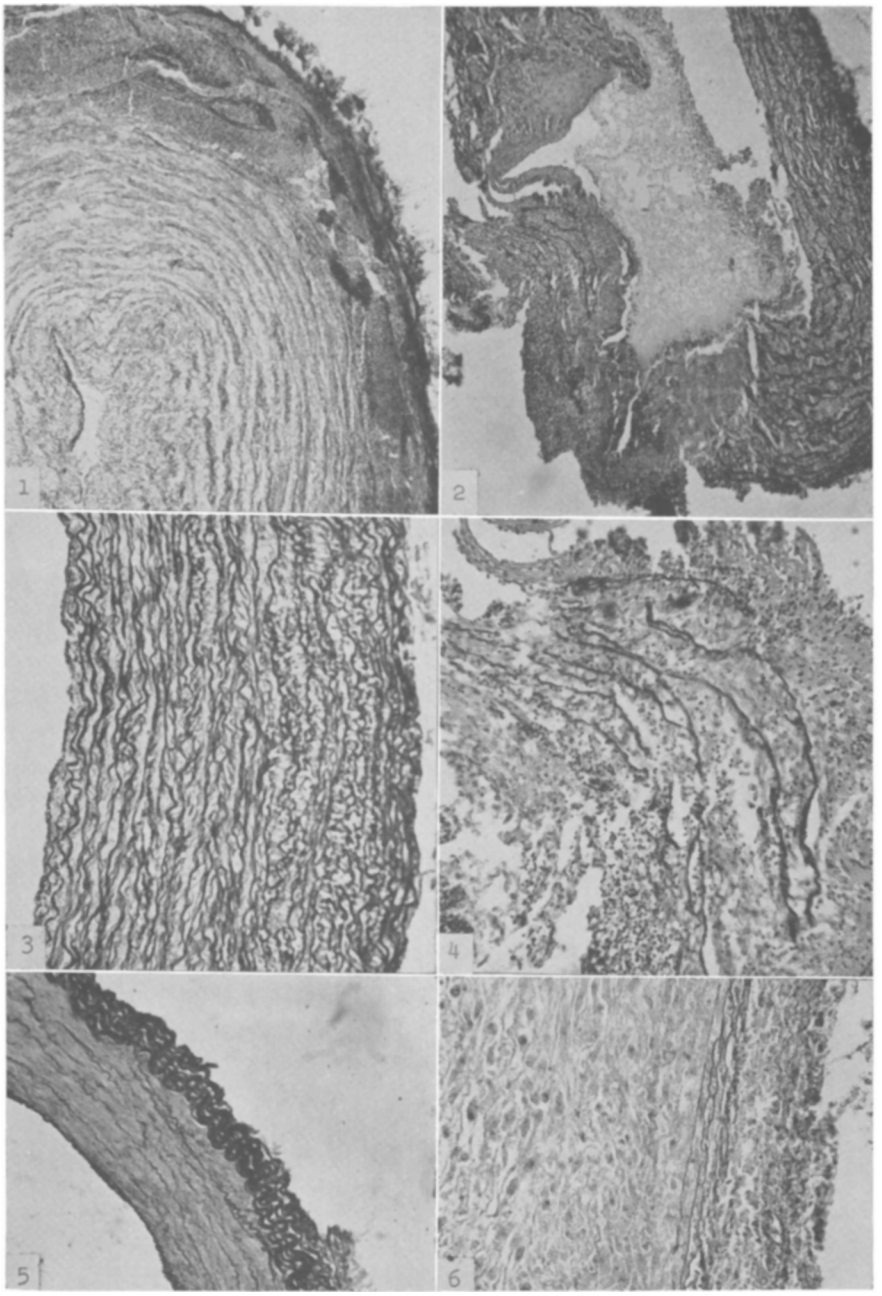

FIG. 1. Cross-section of aorta from copper-deficient chick showing dissecting aneurysm and thickening of the medial wall. Weigert × 45.

FIG. 2. Cross-section of deficient aorta showing rupture of the wall just distal to the arch and attempted repair of a previous break. Weigert × 45.

FIG. 3. Cross-section of control aorta showing normal elastic fibers in an area near arch. Weigert × 150.

FIG. 4. Cross-section of deficient aorta. Note fragmentation and focal breaks in elastic fibers and wide separation of laminae. Weigert × 150.

FIG. 5. Cross-section of abdominal aorta from control chick. Weigert × 150.

FIG. 6. Cross-section of abdominal aorta from deficient chick. Note sparsity of elastic fibers and accumulation of non-elastic tissue in media. Weigert × 150.

ing aneurysm was clearly evident. The aortic section shown in Fig. 2 was taken from a chick that died from pericardial hemorrhage. Grossly the aorta was enlarged and dark in color. The histologic section taken just distal to the aortic arch showed essentially complete rupture of the vessel wall. The elastic fibers were disrupted and attempted repair of the damage at one point was evident.

A section of a control aorta just distal to the arch (Fig. 3) shows numerous elastic fibers throughout the media. In contrast, the elastic laminae in the deficient aorta (Fig. 4) were fragmented, showed focal breaks and were widely separated by material that stained green by the Gomori trichrome method and was probably collagen or a collagen-like material. The amount of this material varied with degree of damage and was not present in all copper deficient aortae observed. Figs. 5 and 6 show abdominal aortae of control and deficient chicks. In this area the elastic fibers in the control aorta were concentrated in the outer portion of the vessel wall. The wall of the deficient aorta was greatly thickened and the elastic fibers were only barely visible.

The lesions described here suggest that copper plays a role in connective tissue metabolism. The exact nature of the derangement in copper deficiency is not known but the histologic observations suggest a defect in elastic tissue and an accumulation of an unidentified material in the interlaminar spaces.

Inasmuch as mortality from internal hemorrhage was high whereas the anemia produced was rather mild, it is evident that in this species the role of copper in connective tissue metabolism is more important for survival than its role in hemopoiesis. Hemorrhage appears to be related to the integrity of the vessel walls rather than to a coagulation defect. Although a controlled study of coagulation was not made, grossly the blood clotted in a normal manner. Whether or not the leg weakness was due to a connective tissue defect is not clear, but the deformities of the bones were no doubt related to the connective tissue defect.

Aortic lesions similar to those described above have been observed in copper deficient swine (9). A high proportion of the animals died of aortic rupture and sections of the aorta showed dissecting aneurysm and focal breaks in the elastic laminae.

Summary. Chicks reared for 4 weeks on a modified non-fat milk diet deficient in copper developed mild anemia, leg weakness, bone deformities and a high incidence of subcutaneous hemorrhage. Mortality amounted to 23% and most deaths resulted from internal hemorrhage associated with rupture of major vessels. Histologic studies suggest a derangement of connective tissue metabolism with the major defect in elastic tissue.

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