STUDIES ON COPPER METABOLISM

XXXII. Cardiovascular Lesions in Copper-Deficient Swine

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During the course of our investigations on copper deficiency in swine,[1-4] it was observed that hemopericardium due to rupture of the aorta or heart was frequently present on necropsy examination.[5,6] The purposes of this paper are to describe the pathologic lesions in such animals, to present evidence that the cardiovascular lesions are a consequence of a deficiency of copper and to report preliminary studies on mechanical properties of the aortas.

Although hematologic,[7] neurologic,[8] and osseous abnormalities[9-11] had been described in association with a dietary deprivation of copper, this was, to the best of our knowledge, the first evidence to be presented that copper is essential for the integrity of the vascular tissue. Of possible interest, however, is the disorder known as "falling disease" which has been described in cattle grazing on copper-deficient pastures in western Australia[12] and in the United States.[13] This disorder is so named because the cattle, usually after mild exercise and excitement, suddenly "fall over dead." Atrophy and fibrosis of the myocardium have been reported in such animals, and it has been suggested that death is the consequence of acute heart failure. Lesions in the aorta and other large vessels have not been described although the pathologic features have not been studied extensively. While this report was in preparation, the occurrence of hemopericardium due to aortic rupture in chicks[14] and lesions of the aorta in turkey poults[15] reared on a copper-deficient diet were reported.

Experimental

Animals and Diet

Fifty-eight swine (Yorkshire, Duroc Jersey and Chester White cross breeds) from 8 different litters were weaned at 2 to 7 days of age and placed in individual pens constructed of galvanized iron fitted with stainless steel feed troughs. The basal diet, which has been described in detail previously,[1] consisted of a standard commercial

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brand of canned evaporated milk diluted 1:1 with tap water to which sodium sulfide had been added. The diet was fed in an amount of 230 ml. (152 calories) per kg. of body weight per pig per day. All batches of milk were analyzed for copper and were found to contain 0.08 to 0.13 mg. of copper per liter of undiluted milk. Reduced iron, spectroscopically free of copper, was dissolved in a minimum quantity of hydrochloric acid, diluted, and added daily to the diet of certain of the animals, as noted below, in an amount such that the animals received 30 mg. of iron per kg. of body weight. An aqueous solution of copper sulfate was added daily to the diet of the control and iron-deficient pigs in an amount such that the animals received 0.5 mg. of copper per kg. of body weight.

Design of the Experiments

Group I (Control Group). The diet of the 17 animals in this group was supplemented with both iron and copper in the amounts given above. All animals developed normally, grew well and were sacrificed at the ages noted in Table I. Measurements of serum copper were made in all of the animals (Table II). The serum of 3 of the pigs was analyzed for iron. The values for copper and for iron were within the limits reported previously for control animals.[1]

Group II (Copper-Deficient). The diet of the 26 animals in this group was supplemented with iron but not with copper. The animals gained weight at a rate comparable to group I but developed hypocupremia, hypoferremia (Table II), anemia (Table I) and the skeletal abnormalities described previously.[11] Twenty-three of the animals died during the experimental period. The other 3 animals (#15–58, 15–63 and 15–69)

TABLE I

DATA ON AGE, BODY WEIGHT AND VOLUME OF PACKED RED CELLS AT TERMINATION OF THE EXPERIMENT

Group	Description	No. pigs	Age at termination (days)	Body weight (kg.)	V.P.R.C.* (ml./100 ml.)
I	Control	17	109 ± 22.2 † (70 — 143)	21 ± 6.7 (11.4 — 32.4)	42 ± 8.0 (36 — 50)
II	Copper-deficient	26	98 ± 22.5 (55 — 127)	16 ± 5.3 (8.8 — 28.2)	24 ± 9.6 (10 — 42) ‡
III	Copper-deficient, supplemented	7	90 ± 13.3 (68 — 99)	15 ± 3.5 (9.6 — 23.0)	15 ± 3.9 (9 — 27)
IV	Iron-deficient	8	91 ± 14.6 (62 — 107)	19 ± 3.6 (10.6 — 26.8)	10 ± 4.0 (6 — 20)

* V.P.R.C. refers to volume of packed red cells.

† The mean values ± one standard deviation are given; the values in parentheses refer to range.

‡ The high value is from a pig that died at 55 days before deficiency was established.

were sacrificed at 77, 120, and 120 days of age, respectively, in order to examine the cardiovascular system histologically before death occurred as a consequence of the experimental conditions. Only one pig (#15–49) died before deficiency was established, at 55 days of age.

To control the influence of total dietary intake on the development of the cardiovascular lesions, 3 pairs of litter mate pigs were matched as to initial weight. The diet of one member of each pair was supplemented with iron and with copper. The other member of each pair was fed a diet supplemented with iron only. The volume of diet consumed each day by the copper-deficient member of each pair was measured and recorded. The following day the corresponding control animal was fed that vol-

ume of diet consumed by his partner the previous day. The control member of each pair was sacrificed within 2 days following the death of his partner. Data concerning the initial and final body weights and the volume of packed red cells at the time of the termination of the experiment are presented in Table III.

TABLE II

SERUM COPPER AND IRON VALUES

Group	Description	Serum copper		Serum iron	
		No. pigs	μg./100 ml.	No. pigs	μg./100 ml.
I	Control	17	172 ± 27.5 * (119 — 206)	3	274 (249 — 307)
II	Copper-deficient	25	13 ± 12.6 (1 — 44)	3	41 (37 — 46)
III	Copper-deficient, supplemented	7	10 ± 7.6 (1 — 22)		
IV	Iron-deficient	8	190 ± 18.3 (180 — 208)	8	40 ± 15.4 (24 — 69)

* The mean values ± one standard deviation are given; the values in parentheses refer to range.

TABLE III

DATA ON PAIR-FED ANIMALS

Pair	Initial weight (kg.)	Final weight (kg.)	Final V.P.R.C.* (ml.)	Final age (days)
Control	3.2	11.4	37	99
Copper-deficient	3.2	11.6	12	97
Control	2.6	14.4	41	103
Copper-deficient	2.6	13.4	22	102
Control	3.0	15.6	41	100
Copper-deficient	3.0	15.0	10	100

* V.P.R.C. refers to volume of packed red cells.

Group III (Copper-Deficient, Supplemented). To determine if vitamins or minerals other than copper in any way influenced the development of cardiovascular lesions, 7 pigs were fed the copper-deficient diet used for group II, supplemented with 7 trace elements, 12 water-soluble and 4 fat-soluble vitamins. The mineral supplement was as follows (gm. per liter): manganese chloride ($MnCl_2 \cdot 4H_2O$), 1.8; aluminum sulfate ($Al_2(SO_4)_3 \cdot 18H_2O$), 0.6; sodium fluoride (NaF), 3.1; potassium iodide (KI), 3.1; zinc sulfate ($ZnSO_4 \cdot 7H_2O$), 1.8; cobalt nitrate ($Co(NO_3)_2 \cdot 6H_2O$), 1.8; and nickel acetate ($Ni(C_2H_3O_2)_2 \cdot 4H_2O$), 2.8. This was added daily to the diet in an amount of 0.2 ml. per kg. of body weight. The water-soluble vitamin supplement was as follows (mg. per kg. of body weight per pig per day): thiamine hydrochloride, 0.25; riboflavin, 0.12; nicotinic acid, 1.2; pyridoxine hydrochloride, 0.2; calcium pantothenate, 0.5; inositol, 0.2; para-aminobenzoic acid, 0.1; biotin, 0.1; pteroylglutamic acid, 0.1; cobalamine, 0.01; choline chloride, 10.0; and ascorbic acid, 15.0. Three thousand units of vitamin A, 600 units of vitamin D, 1 mg. of vitamin E, and 1 mg. of vitamin K per kg. of body weight were given once a week.

The rate of weight gain was comparable to the weight gain observed in group II (Table I). Six of the animals died during the experimental period. The seventh pig (#15–90) was killed at 68 days of age for the purpose of examining the cardiovascular system histologically in an early stage of the deficiency.

Group IV (Iron-Deficient). To determine if the development of cardiovascular lesions in the copper-deficient pigs could be a consequence of tissue anoxia secondary to the development of severe anemia, anemia of comparable severity was produced in 8 pigs by omitting iron from the milk diet and adding only the copper sulfate supplement. Phlebotomy for the removal of 100 to 400 ml. of blood was performed in order to accentuate the anemia. Four of the animals were maintained on the iron-deficient diet until death occurred. The other 4 animals (#15–94, 15–95, 15–98 and 16–00) were sacrificed at 107, 72, 98 and 94 days of age, respectively. All of the animals developed severe anemia (Table I) and hypoferremia (Table II).

Methods

Serum copper was determined by the method of Gubler, Lahey, Ashenbrucker, Cartwright and Wintrobe[16]; dietary copper by the method of Markowitz, Shields, Klassen, Cartwright and Wintrobe[17]; and serum iron by the method of Hamilton, Gubler, Cartwright and Wintrobe.[18]

The pigs were sacrificed by exsanguination under sodium pentobarbital anesthesia. Necropsies were performed immediately or as soon as death was discovered. Segments of fresh aorta were transferred immediately to ice-cold physiologic saline and stored in the refrigerator until mechanical tests were performed. Transverse rings 2 to 3 mm. wide were mounted on the instrument described by Rigby, Hirai, Spikes and Eyring.[19] (We are indebted to Dr. John D. Spikes for allowing us to use the apparatus.) Diameter, thickness and length were measured at 1 gm. loads with a telemicroscope. Preliminary experiments demonstrated that the breaking stress of the specimens was reproducible only under carefully controlled conditions. Therefore, the segments of aorta were elongated in stepwise increments of 5 to 10 per cent of the original length, with time allowed at each step for plastic phenomena to come to equilibrium before subsequent elongation. The force developed during elongation was measured with a Statham strain gauge and Brown strip-chart recorder calibrated with known weights. Tissues were fixed in Helly's fluid and in 10 per cent formalin and embedded in paraffin for histologic examination. In addition to hematoxylin and eosin stained sections, the vessels were examined with elastic tissue (Verhoeff, Weigert or orcein) and van Gieson stains. Selected sections were also stained by the Masson trichrome, toluidine blue and periodic acid-Schiff (PAS) methods.

Necropsy Observations

The principal findings at necropsy are summarized in Table IV.

TABLE IV

INCIDENCE OF PRINCIPAL NECROPSY FINDINGS

	Group I Control	Group II Copper-deficient	Group III Copper-deficient, supplemented	Group IV Iron-deficient
Number of pigs	17	26	7	8
Number of pigs died	0	23	6	4
Gross cardiovascular lesions *	0	25 (96%)†	6 (86%)	0
Hemopericardium	0	18 (69%)	2 (29%)	0
Serous effusions	0	2 (8%)	4 (57%)	1 (12%)

* Exclusive of cardiac hypertrophy (see Table V).

† Figures in parentheses are percentages of pigs in that group.

Copper-Deficient Group

The cause of death in 18 of the copper-deficient pigs (group II) at ages 61 to 127 days was recent hemorrhage due to rupture of the aorta, heart, or the coronary or pulmonary arteries. In all instances, this resulted in hemopericardium amounting to 25 to 400 ml. Two other pigs, that died at 105 and 107 days, had fluid in the serous cavities, evidently from heart failure. One of these had a ruptured papillary muscle. Three pigs that died at 76 to 96 days had markedly hypertrophied hearts but no evidence of congestive heart failure and no cardiovascular rupture; one had an organizing pericarditis. The cause of death in these 3 pigs was not evident. The 3 pigs sacrificed at 77 to 120 days had hypertrophied hearts, and 1 had a myocardial infarct and marked hydropericardium. The lesions responsible for the cardiovascular ruptures, myocardial infarcts and heart failure will be described below. With one exception, every pig in the copper-deficient group had distinctive lesions in the aorta, and lesions were frequently encountered in other vessels also.

Copper-Deficient, Mineral and Vitamin-Supplemented Group

Six pigs of group III died at ages 88 to 99 days. Two of these had hemopericardium due to rupture of the pulmonary artery in 1 and to rupture of the right atrium in the other. Four pigs of this group, including the 2 with hemopericardium, had ruptured papillary muscles. All had hypertrophied hearts, 4 had serous effusions and 1 had pulmonary edema. Only 1 pig of this group, sacrificed at 68 days of age, failed to show cardiovascular lesions similar to those in group II.

Iron-Deficient Group

Four pigs in group IV died at ages 62 to 102 days. Only 1 of these had a serous effusion (500 ml. ascites), but all had markedly hypertrophied hearts. Four pigs sacrificed at ages 72 to 107 days also had hypertrophied hearts. None of the pigs of group IV had other gross cardiovascular lesions like those of groups II and III.

Control Group

None of the 17 pigs of group I died. One had a cardiac weight 2.9 S.D. above the normal mean. None had gross cardiovascular lesions like those in groups II and III.

The Gross Cardiovascular Lesions

The principal types of cardiovascular lesion are summarized in Table V.

TABLE V

INCIDENCE OF GROSS CARDIOVASCULAR LESIONS

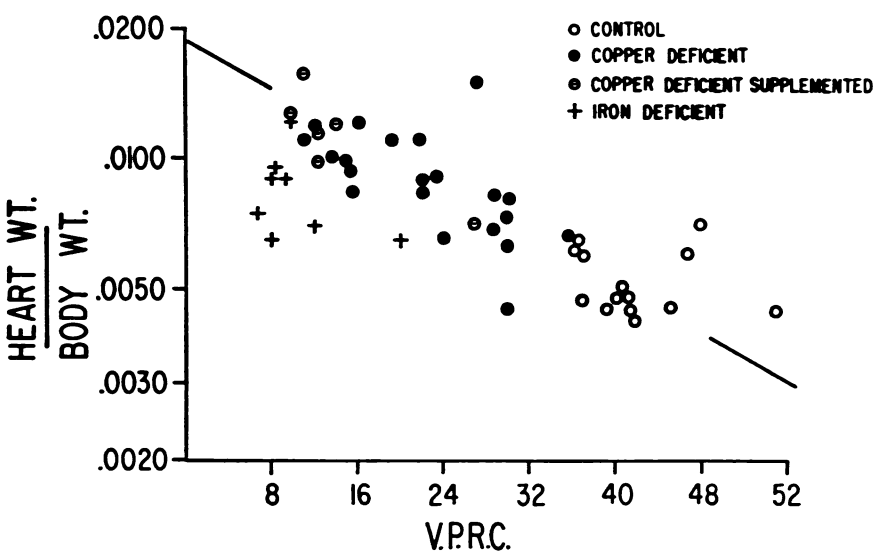
	Group I Control	Group II Copper-deficient	Group III Copper-deficient, supplemented	Group IV Iron-deficient
Number of pigs	17	26	7	8
Cardiac hypertrophy *	0	24 (92%)†	6 (86%)	8 (100%)
Other cardiac lesions	0	11 (42%)	5 (71%)	0
Aortic lesions	0	24 (92%)	6 (86%)	0
Other vascular lesions	0	7 (27%)	3 (43%)	0

* Heart weight greater than 3 S.D. above the normal mean.

† Figures in parentheses are percentages of pigs in that group.

Cardiac Hypertrophy

The heart weights (HW) of 17 control pigs (group I), weighing 0.7 to 29.6 kg., were plotted against body weight (BW), and the line relating these 2 measurements was found to be described by the equation $HW = 0.0391\ BW^{0.785}$. Within the range of body weights between 10 and 30 kg., the ratio of heart weight/body weight in 15 control pigs was $4.98\ (\pm 0.803) \times 10^{-3}$. This ratio was markedly elevated in groups II, III and IV. Irrespective of the type of deficiency, the heart weight was directly correlated with the anemia down to a volume of packed red cells of about 10 ml. per hundred ml. (Text-fig. 1). As the volume of packed red cells fell below this value, there was a fall in relative heart weight.



TEXT-FIGURE 1. Relationship of anemia to relative cardiac weight. Ordinate is the heart weight/body weight on a logarithmic scale. Abscissa is the volume of packed red cells in ml. per hundred ml. of blood.

Other Cardiac Lesions

Rupture of the heart in the copper-deficient groups resulted from 3 mechanisms. In 1 pig it was due to perforation of a recent myocardial infarct. In 3 pigs it was due to perforation of an intramural hemorrhage. In 2 pigs perforation of the atrium followed rupture of a papillary muscle. In addition, in 4 instances leakage of large subepicardial hemorrhages not associated with myocardial infarction had occurred.

Myocardial infarction occurred in 3 pigs (Fig. 1). In each instance it was the result of stenosis of a large coronary artery by intramural hemorrhage (see below). Large intramural left ventricular hemorrhages occurred in 4 pigs. Ruptures of papillary muscles were found in the right ventricle in 1 pig and in the left in 5 (Fig. 2). Large subepicardial hemorrhages in 4 pigs were the result of rupture of superficial coronary vessels (Fig. 3).

Manual examination of the hearts gave the distinct impression of abnormal myocardial friability. No means of accurately measuring this was found and its incidence cannot be stated exactly, but the friability was repeatedly marked by two independent observers and was detectable before and after fixation of the hearts.

Aortic Lesions

Rupture of the aorta occurred invariably in the ascending segment or first part of the arch. This varied in location and extent but most frequently it resulted from an oblique linear split in the intima and media that extended from just above a sinus of Valsalva to the beginning of the arch (Fig. 4). In a few instances the ruptures were nearly transverse to the long axis and extended directly through the media. Nevertheless, extensive dissection of intramural hemorrhage comparable to that in human dissecting aneurysms never occurred. The tract always penetrated to the adventitia after a short distance. In 9 pigs there were similar splits that had not completely penetrated the wall and ruptured.

The descending thoracic aorta was the least seriously affected segment. Here there were a few small intramural hemorrhages and linear cracks that invariably occurred about the ostia of intercostal arteries (Fig. 5).

The abdominal aorta was the most regularly affected by gross lesions. These were found in 91 per cent of the pigs in groups II and III in which this segment was examined. The lesions differed from those in the ascending aorta and, in spite of their extent, they never resulted in rupture. They consisted of short longitudinal cracks and fissures that extended directly part way or completely through the media, seldom obliquely and never dissected extensively within the media (Fig. 6).

A curious thickening of the aortic media was detected in the copper-deficient pigs. This was most noticeable in the thoracic portion. It is reflected in the comparative aortic weights in Table VI. The ratio of

TABLE VI

RELATIVE AORTIC WEIGHTS

Group	No. pigs	Aortic weight/ body weight (gm./kg.)
I Control	9	0.803 ± 0.133 *
II Copper-deficient	8	1.16 ± 0.032
III Copper-deficient, supplemented	7	1.005 ± 0.079
IV Iron-deficient	7	0.853 ± 0.110

* The mean values ± one standard deviation are given.

aortic weight to body weight in both copper-deficient groups was significantly higher than that of the controls. The relative aortic weight of the iron-deficient group was not significantly different from that of the controls.

Other Vascular Lesions

Rupture of the main pulmonary artery in 2 pigs resulted from lesions exactly like those described in the ascending aorta. Altogether, 5 pigs had similar splits, cracks and limited intramural hemorrhages in the major branches of the pulmonary artery. In 6 pigs gross intramural dissecting hemorrhages were found in large coronary arteries. It has been stated above that these produced myocardial infarcts in 3 pigs. Similar hemorrhages were seen in brachiocephalic, left brachial, and carotid arteries in a few instances, but since these were not as regularly or carefully examined as the other vessels mentioned, the comparative incidence of the lesions is not known. No vascular lesions were seen in the other viscera.

HISTOLOGIC EXAMINATION

Sections of the hearts and of 3 segments of aorta (ascending, descending thoracic and abdominal) were examined in all pigs in addition to representative samples of all gross lesions encountered.

In only those instances in which gross myocardial infarcts were recognized could a pre-existing histologic lesion of the myocardium be identified as a basis for the ruptures. The infarcts were recent. No myocardial necrosis was found in other instances of cardiac rupture excepting the few cells at the edge of the defect. The latter were condensed, acidophilic and lacking in striations (Fig. 7). Fresh hemorrhage in the myocardium and epicardium separated apparently normal fibers and other structures.

In the aortas, on the other hand, many histologic defects were found in sites not involved by gross lesions. The commonest of these defects was an accumulation of amorphous material between the elastic laminae of the media (Fig. 8). This was detectable in 26 (79 per cent) of the copper-deficient aortas (groups II and III). In 3 it was so copious as to produce microscopic cysts. The staining of the material varied from a very pale, faintly basophilic quality to a deep eosinophilia. The pale-staining material was strongly metachromatic with toluidine blue. The more eosinophilic deposits frequently stained selectively with the elastic stains (Fig. 9). Neither material stained with the PAS reaction. A focal excess of similar pale-staining metachromatic material was seen in the region of the arch of the aorta in 2 controls (12 per cent) and in 1 iron-deficient pig (12 per cent). Rupture or abnormal fenestration of elastic laminae was observed in 13 (40 per cent) of the aortas from the copper-deficient groups (Fig. 10). This usually occurred in areas of the greatest interlaminar deposit, and it was invariably seen where these deposits produced spaces of cystic proportions. This was never observed in control pigs, but it was associated with the focal excess of interlaminar material in a single pig of the iron-deficient group referred to above.

Dissecting medial hemorrhage was observed in sections of 20 (60 per cent) of the aortas of the copper-deficient groups. This is not a reliable indication that microscopic hemorrhage always preceded the rupture since the sections were often made to illustrate the gross hemorrhages, and the sampling was biased. However, microscopic hemorrhages did occur in association with the elastic defects referred to above in the absence of gross hemorrhage and rupture. None were found in the control or iron-deficient groups.

Small laminar medial necroses were found in the aortas of 7 pigs in group II. These were invariably on the margins of dissecting hemorrhages and may have been a consequence of the interruption of vasa vasorum by the splits in the media. Collagenous repair of the medial defects was frequently evident in the abdominal aorta where the cracks and fissures were bordered by proliferating fibroblasts (Fig. 10). This was almost entirely lacking in the thoracic aortic defects.

Examination of other arteries was not performed systematically. Sections were made of all gross lesions encountered in them, and the pulmonary arteries were sampled in additional instances. Lesions were found in the pulmonary arteries exactly like those in the thoracic aorta. The occlusive lesions of coronary arteries were found to be dissecting hemorrhages. These penetrated the media and dissected between it and the adventitia (Fig. 11). Intramural hemorrhages of the same basic type were seen in the other muscular arteries enumerated above. None

were encountered in the lung, liver, pancreas or kidney of the deficient animals examined. A systematic study of the visceral arteries is planned in experiments to follow.

Mechanical Properties of the Aorta

Excised segments of the descending thoracic aorta were carefully cut to loops of comparable width, and only those loops free from both gross lesions and ostia of intercostal arteries were selected for mechanical stress analysis. The selected loops were stretched in a reproducible fashion to the breaking point while the tension developed within the loop was continuously monitored. The tensile strength was calculated as the maximum force per square cm. of cross section area (Table VII). The strength of the loops of aorta from the copper-deficient pigs was markedly reduced in comparison to those of the controls.

Table VII

Tensile strength of descending thoracic aorta

Group	No. pigs	Tensile strength (kg./cm.^{2})
I Control	8	21.6 ± 4.3 *
II Copper-deficient	5	8.6 ± 6.8

* The mean values ± one standard deviation are given.

Discussion

The good correlation between the degree of anemia and the relative heart weights in these experiments is consistent with the hypothesis that the cardiac hypertrophy was a response to increased cardiac output consequent upon anemia. Cardiac hypertrophy in copper-deficient swine has been reported previously, and a greater degree of hypertrophy has been noted in copper-deficient than in iron-deficient animals[4] with comparable degrees of anemia. The lesser degree of hypertrophy in the iron-deficient animals is unexplained. The serous effusions were probably due to cardiac failure. These occurred in the copper-deficient and in the iron-deficient groups alike.

Cardiac rupture occurred only in the copper-deficient groups. In some instances this was a consequence of coronary vascular lesions leading to myocardial infarction. In 6 instances there was rupture of papillary muscles that could not be attributed to vascular lesions. Rupture of the atria in 2 instances was almost certainly coincident with a marked regurgitation through atrioventricular valves following rupture of papillary muscles. These events occurred in some of the largest hearts. Dilatation and increased stroke volume may have placed an exceptional strain upon the papillary muscles. However, the unusual friability of the myo-

cardium, demonstrable at necropsy, is believed to indicate an underlying defect in muscular or connective tissue structure. The lack of a histologically demonstrable antecedent lesion leaves the exact site of the defect undisclosed at present. The myocardial fine structure is under investigation.

Histologic study of the aorta and other arteries has come closer to providing an explanation of the vascular ruptures, but even this is somewhat less than satisfactory. That significant histologic defects exist in the vessels prior to rupture has been amply demonstrated, and a deficit in mechanical strength has been established. The most conspicuous structural defects appeared in the elastic tissue. A decrease in insoluble elastin residue of affected aortas[20,21] and alterations in the mechanical properties of elastin isolated from affected aortas[21] suggest that the basis for the histologic defects may reside in this component. However, it is not clear whether these abnormalities are primary or whether a rupture of elastic laminae is the consequence of altered mechanical properties of other supportive components of the vascular wall. An effort is being made to determine whether such alterations exist in the muscle or collagen. No appreciable change in the amount or properties of the collagen has been found histologically or by chemical extraction,[20] but an increase of extractable hexosamine[20] may be correlated with the interlaminar accumulation of metachromatic material described above.

The evidence that the cardiovascular lesions were due to copper deficiency may be summarized as follows: (1) The lesions were present in the animals fed a diet deficient in copper; (2) control animals fed the same diet but supplemented with copper did not develop such lesions; (3) restriction of the dietary intake of the control animals to the amount ingested by the copper-deficient animals did not result in the appearance of lesions in the cardiovascular system of the control pigs; (4) the addition of 7 trace elements other than copper, 12 water-soluble vitamins and 4 fat-soluble vitamins to the diet of the copper-deficient pigs did not prevent the development of lesions; and (5) these lesions, excluding cardiac hypertrophy, were not observed in iron-deficient pigs with anemia comparable in severity and duration to the anemia in copper-deficient pigs. Studies on the reversibility of the lesions with copper were not made because no method could be devised to follow the progress of the lesions in a given pig after the administration of copper. However, the therapeutic effects of the administration of copper to such animals after the deficiency was established, at least insofar as growth, hematologic response, general appearance and survival are concerned, have been described in detail elsewhere.[1] Thus, it seems reasonable to conclude that the vascular lesions resulted from a dietary deficiency of copper.

The manner in which copper deficiency produced the lesions is unknown. One is led to seek an explanation of the deficiency of a trace element in the impairment of some enzyme system. Copper is reported to be a constituent of cytochrome oxidase[22-24] and other oxidative enzymes.[25] Cytochrome oxidase activity is markedly reduced in the myocardium of copper-deficient swine,[4] and copper content has been correlated with cytochrome oxidase activity in the tissues of swayback lambs.[26] Aortic tissue is reported to have an appreciable cytochrome oxidase activity also.[27] The role of this enzyme in the biosynthesis and maintenance of connective tissues is not known, but it would be expected to play a vital role in the metabolism of muscle cells. It has been suggested that copper may be necessary for the synthesis of heme *a*,[28] and Lemberg found a lack of this porphyrin in the myocardium of one of the deficient pigs of our experimental group II reported here.[29] In relation to the biosynthesis of collagen, it may be significant not only that copper is reported to be a constituent of ascorbic acid oxidase but that copper ion and its complexes may catalyze the oxidation of ascorbate.[30]

No satisfactory understanding of the pathogenesis of the cardiovascular lesions of copper-deficient swine has been gained by reference to the several well-known examples of aortic rupture in man and other animals. Somewhat similar histologic lesions of the aorta occur irregularly in human cases of dissecting aneurysm,[31] in Marfan's syndrome[32] and in lathyrism of various species. The vascular lesions in these conditions are not identical to those in copper-deficient swine, however, and the cardiac lesions are peculiar to copper deficiency. The collagenous abnormality reported in lathyrism[33,34] has not been detected in copper-deficient swine.[20] However, the elastic defects reported in the lathyritic rat aorta[35] and the impairment of formation of elastin in the tissues of lathyritic rat fetuses[36] suggest that one common pathway may be involved in lathyrism and in copper deficiency. It may be pertinent that copper is an effective inhibitor of elastases.[37] However, it has been reported that dietary copper supplements do not prevent the vascular lesions of lathyrism in chicks.[15]

Summary

Thirty-three pigs, reared from birth on a copper-deficient skimmed milk diet supplemented with iron, developed hypocupremia, anemia, and cardiac hypertrophy. Twenty of these died at ages 61 to 127 days with hemopericardium due to rupture of the heart, aorta, coronary or pulmonary arteries. One or more of a series of distinctive lesions, consisting of aortic intimal splits and fissures, ruptured papillary muscles, dissecting coronary hemorrhages, and histologic defects of the vascular

elastic tissue with interlaminar deposits of material staining variably like acid mucopolysaccharide or elastin, were found in all but 2 of the deficient animals.

A supplement of other minerals and vitamins given to 7 of these animals did not prevent these lesions. Seventeen controls given the same diet with a supplement of copper failed to show any of these abnormalities when sacrificed at 70 to 143 days of age. Eight controls on the milk diet, supplemented with copper but lacking iron, developed hypoferremia with anemia and cardiac hypertrophy comparable to the copper-deficient pigs. The iron-deficient pigs failed to show any of the distinctive lesions described, except some localized interlaminar deposit of metachromatic material in one animal; however, 4 of them died, probably from cardiac failure.

The tensile strength of the aorta was markedly reduced in the copper-deficient animals compared to the controls, and there was abnormal friability of the myocardium in the deficient animals. It is concluded that copper is needed for the maintenance of the structural elements of the heart and arteries of swine.

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[*Illustrations follow*]

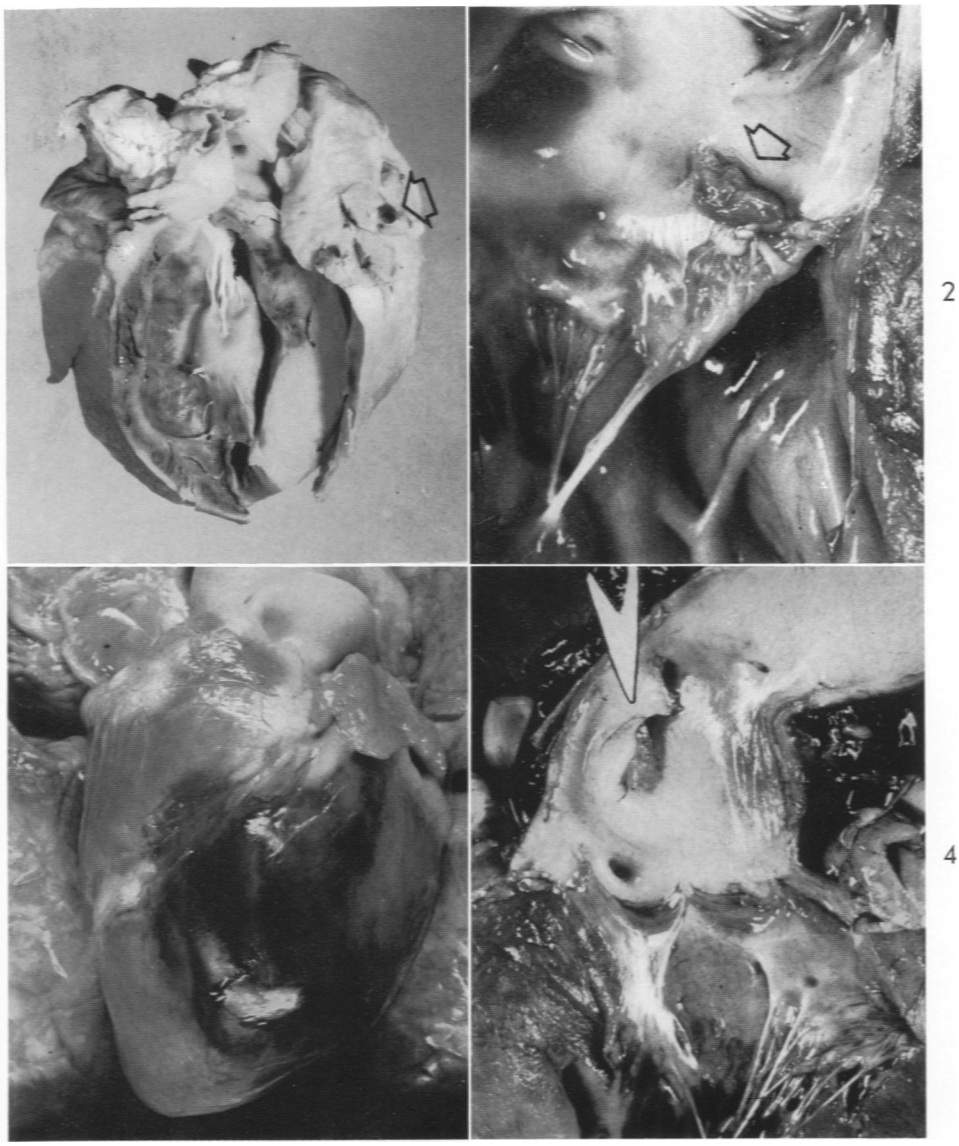
LEGENDS FOR FIGURES

FIG. 1. Myocardial infarct. A coronary artery occluded by dissecting hemorrhage is indicated by arrow.

FIG. 2. Ruptured papillary muscle. The ruptured apex of the papillary muscle is shown attached to the reflected posterior mitral leaflet.

FIG. 3. Subepicardial hemorrhage. The hemorrhage, covering the anterior surface of the left ventricle, arose from a ruptured left coronary artery.

FIG. 4. Ruptured aorta. The irregular defect is shown in the ascending aorta (arrow). The hemorrhage that surrounds the aorta ruptured into the pericardial sac.



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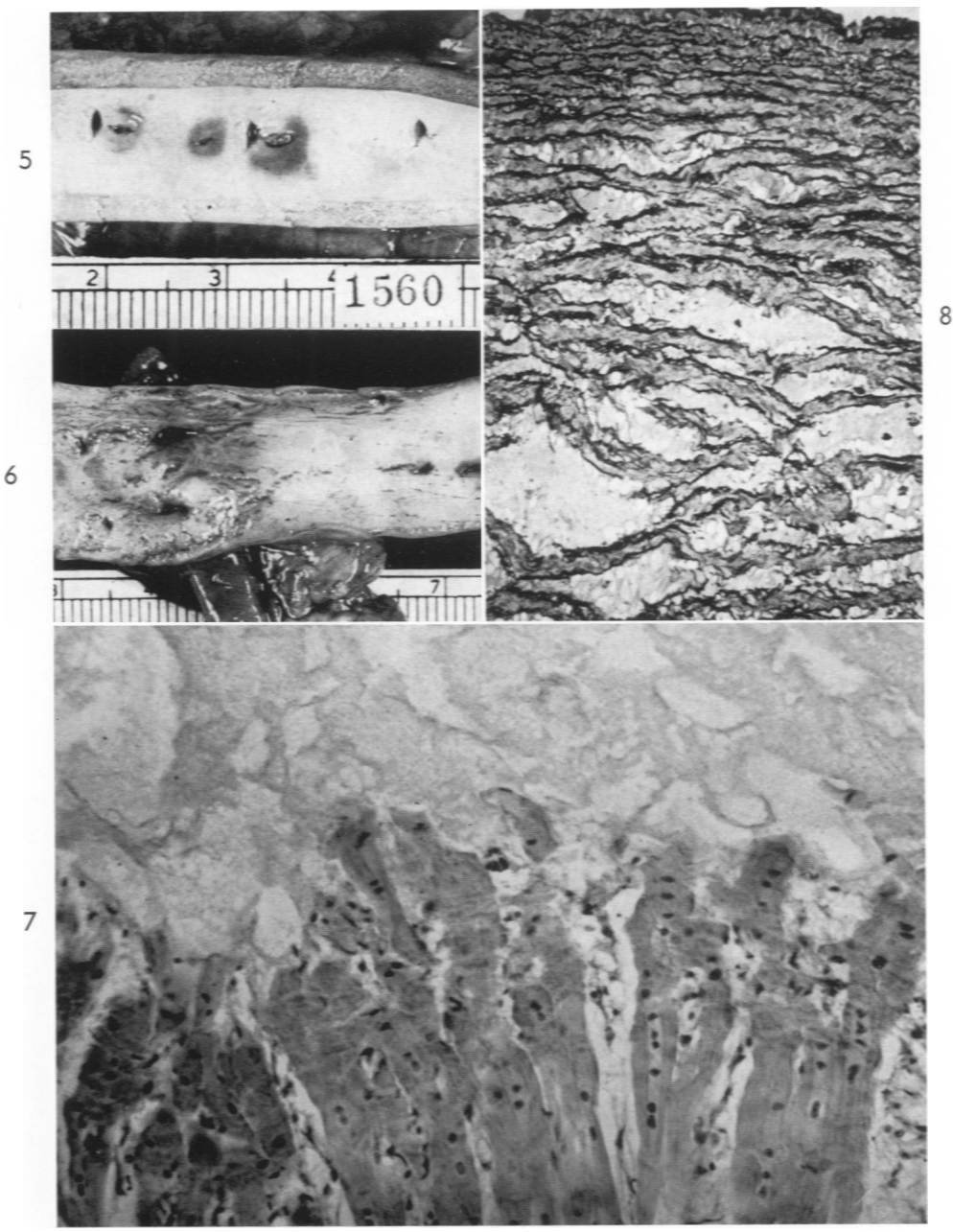

FIG. 5. Intramural hemorrhage in the descending thoracic aorta. The cracks and hemorrhages surround the ostia of intercostal arteries. Note the cut edge of the thickened aortic wall.

FIG. 6. Fissures in abdominal aorta. Many of the lesions penetrate the media deeply.

FIG. 7. Ruptured edge of papillary muscle. Histologic section shows ruptured fibers, without evidence of prior necrosis, covered by fresh fibrin. Hematoxylin and eosin stain. × 250.

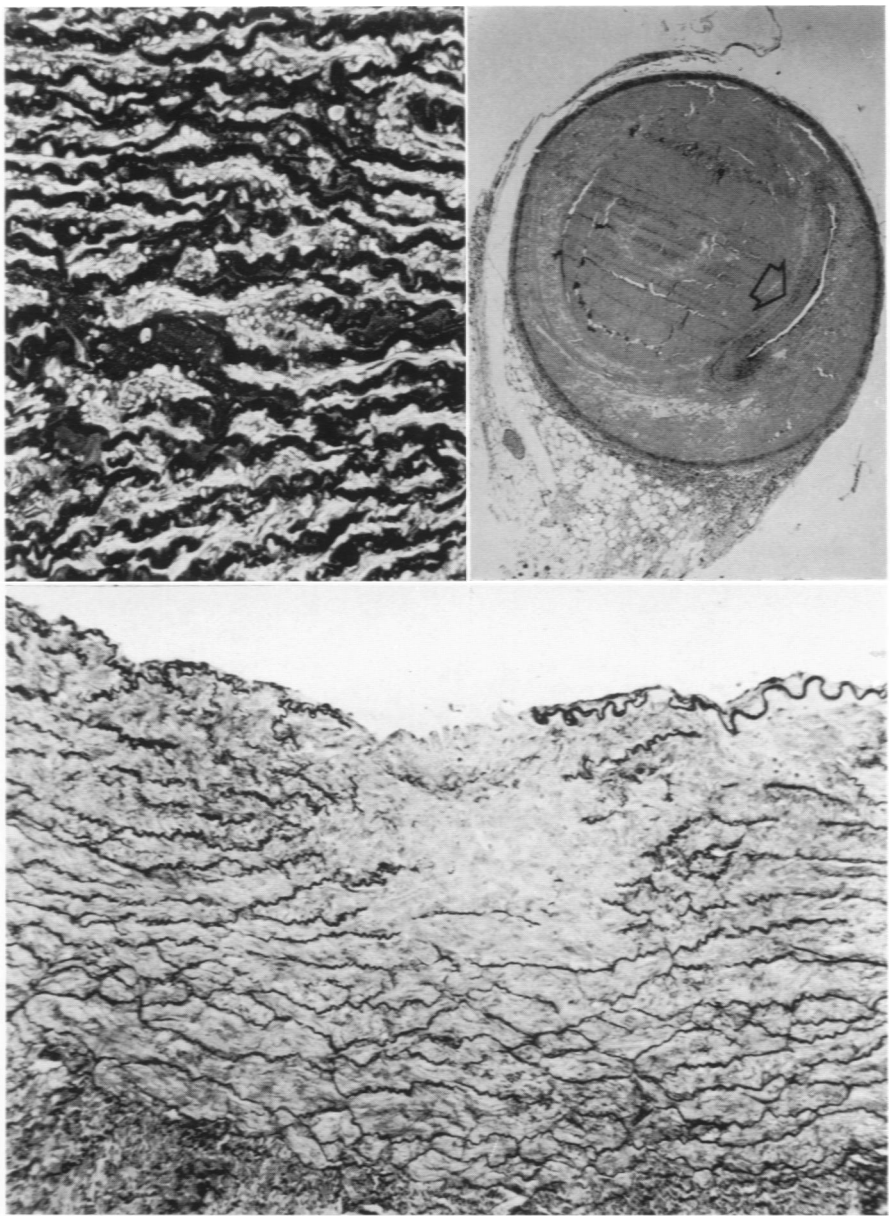

FIG. 8. Thoracic aorta. Histologic section shows separation of elastic laminae by pale-staining amorphous material. Verhoeff elastic tissue stain. × 100.

FIG. 9. Thoracic aorta. Interlaminar accumulations of deeply staining amorphous material take the resorcin-fuchsin stain. Weigert elastic and van Gieson stains. × 100.

FIG. 10. Abdominal aorta. Ruptured elastic laminae in a healing fissure. Verhoeff elastic tissue stain. × 175.

FIG. 11. Coronary artery. The lumen is collapsed (arrow) by dissecting hemorrhage that separates the media and adventitia. Verhoeff elastic tissue and van Gieson stain. × 16.