ers in perosis due to the above nutrient deficiencies. Thus it is possible that osteodystrophy in chicks following hemin administration may be associated with interruption of some biochemical pathway(s) in common with the anomalies produced by these deficiencies and Sedormid.

Summary. An investigation of certain physical and biochemical aspects of osteodystrophy in chicks treated with hemin revealed a significant decrease in serum and bone alkaline phosphatase activity. In addition, feathering was abnormal. No change in per cent bone ash was observed. Possible relationship to known nutrient deficiency symptoms is discussed.

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Received August 27, 1962. P.S.E.B.M., 1963, v112.

Histology of Osteodystrophy in Hemin Treated Chicks.* (28082)

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Abnormal bone development, poor growth and high mortality in chicks after porphyrin administration has been demonstrated in this laboratory(1,2). Certain physical and biochemical changes associated with hemin treatment have also been reported(3). The present study was designed to describe the histology of these dystrophic bones and possibly relate these findings to bone pathology reported in zinc(4,5), manganese and choline (6) deficiency perosis.

Materials and methods. Day-old White Rock male chicks (Arbor Acres) were used. Housing and feeding of chicks and preparation and administration of solution were essentially as previously reported(2). In this study 5 mg level of hemin (Eastman Kodak Co.) was used and treatment was started on 2nd day. The distal epiphysis of the tibiotarsus and the proximal epiphysis of the tarsometatarsus of 20 chicks (10 control, 10

* Work supported in part by NIH grant. Article No. A1002, Contribution No. 3390, of Maryland Agric. Exp. Sta., Dept. of Poultry Sci.

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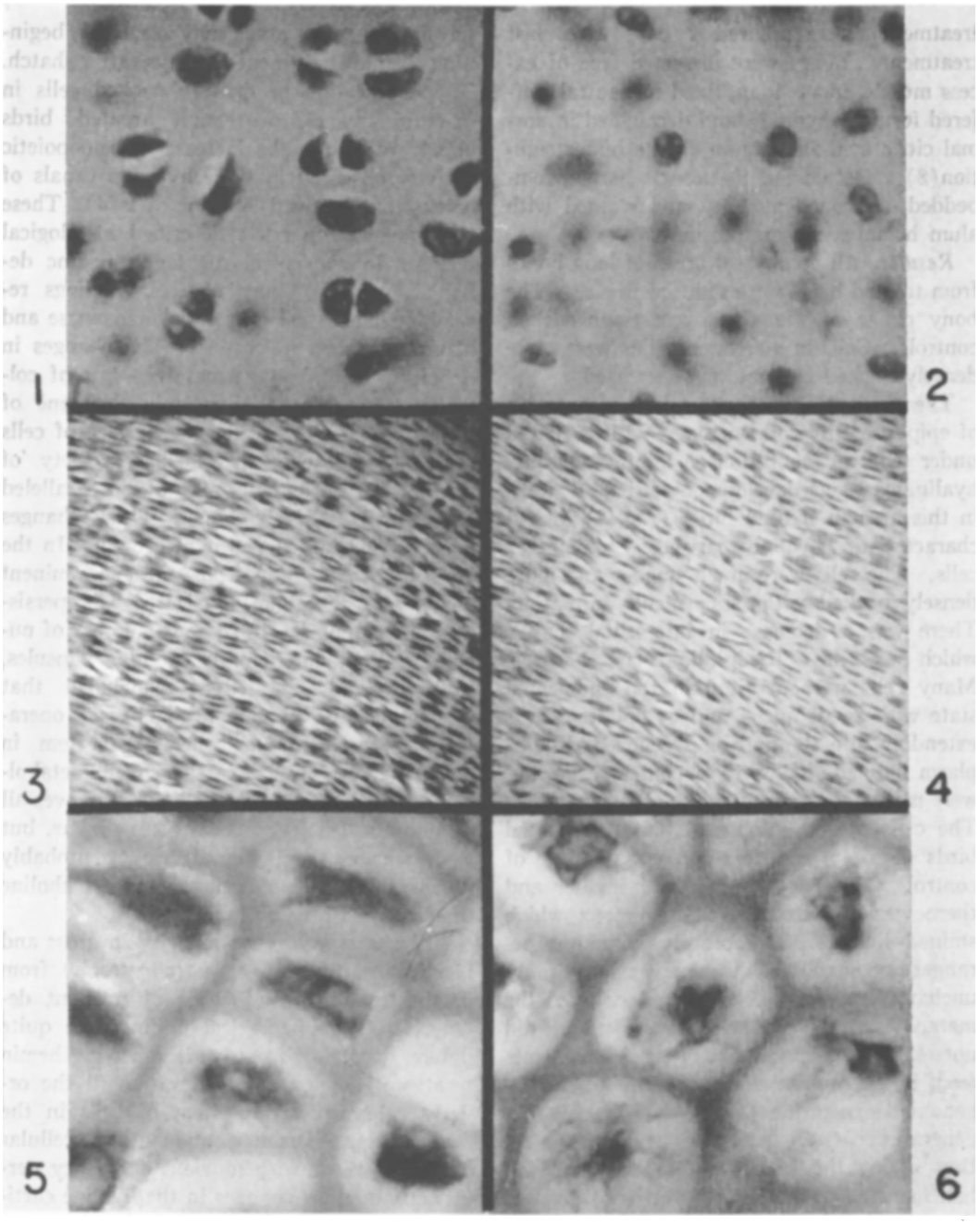

FIG. 1. Epiphyseal hyaline cartilage of proximal tarsometatarsus of 8-day-old control chick. H & E (approx. 320 ×).

FIG. 2. Similar area as Fig. 1. 8-day-old chick, 2 days following hemin treatment. H & E (approx. 320 ×).

FIG. 3. Epiphyseal plate of proximal tarsometatarsus of 8-day-old control chick. H & E (approx. 120 ×).

FIG. 4. Similar area as Fig. 3. 8-day-old chick, 2 days following hemin treatment. H & E (approx. 120 ×).

FIG. 5. Zone of maturation of metaphysis of proximal tarsometatarsus of 8-day-old control chick. H & E (approx. 1700 ×).

FIG. 6. Similar area as Fig. 5. 8-day-old chick, 2 days following hemin treatment. H & E (approx. 1700 ×).

treatment) were secured 2 days after last treatment. Bones were dissected free of excess muscle and tendon, fixed in neutral buffered formaldehyde(7) and decalcified in normal citric acid-ammonium citrate buffer solution(8). Decalcified tissue was paraffin embedded, sectioned at 10 μ and stained with alum hematoxylin and eosin.

Results. All epiphyseal areas of long bones from treated birds were reduced in size. The bony collar of the shaft was thinner than controls. Cells in marrow cavities were more densely packed and fat was decreased.

The major defect appeared in maturation of epiphyseal hyaline cartilage. Birds reared under control conditions exhibited normal hyaline cartilage development (Fig. 1). Cells in this area in treated birds (Fig. 2) lacked characteristic hyaline cartilage "daughter" cells. The cells were much smaller and more densely packed with less capsule formation. There was a decrease in interstitial matrix which was less basophilic than controls. Many cells were arrested in the embryonal state with small nuclei and cytoplasmic tails extending into the matrix. Nuclei and cytoplasm stained lighter than control cells and were more homogeneous rather than granular. The cells of the epiphyseal plate of treated birds (Fig. 4) were less dense than those of controls (Fig. 3). Nuclei were smaller and there was an increase in the matrix which stained lighter. The orderly columnar arrangement of cells in this zone was essentially unchanged. The zone of hypertrophy of the metaphysis was extremely narrow and did not show the normal continual sequences from hypertrophy and vacuolation to cartilagenous degeneration (Fig. 6). However, degeneration of the cartilage cells was seen, thus giving the impression that cells which had matured prior to treatment continued to degenerate normally and become calcified.

Discussion. The general histological picture suggests that hemin administration results in cellular arrest characteristic of the stage at which treatment began. The normal epiphysis at the time of hatch is a flattened cap of cartilage with an extremely dense matrix, and cells are barely beginning to enlarge (9). Early developmental arrest in the epiphysis and other areas may occur by beginning hemin treatment 2 days after hatch. The observation of densely packed cells in marrow cavites of hemin treated birds closely resembles the increase in hemopoietic activity observed in the Haversian canals of chicks that received low-zinc diets(4). These authors and others(5) described histological changes in epiphyseal cartilages in zinc deficient chicks. Many of their findings resemble changes in bones from manganese and choline deficient chicks(6). The changes in deficiency perosis were mainly a loss of columnar arrangement of cells in the zone of proliferation with an increase in size of cells and matrix. Cells assumed a variety of shapes and their long axis often paralleled the long axis of the bone. These changes were carried into the zone of growth. In the zone of maturation, the most prominent change was the abnormal size of cells, persistence of cytoplasm, increase in number of nuclei per cell, and densely staining capsules. Wolbach and Hegsted(6) concluded that manganese and choline probably are operative in a common biochemical system in chicks for epiphyseal cartilage cell metabolism. Young *et al.*(5) noted that the over-all findings in deficiency perosis are similar, but that changes from zinc deficiency probably differ from those of manganese and choline in some presently obscure way.

Although there is a similarity in gross and biochemical findings of osteodystrophy from hemin treatment and those of nutrient deficiencies, the histological findings are quite different. Bones from birds following hemin treatment do not show alteration of the orderly columnar arrangement of cells in the zone of proliferation and other cellular changes. Birds with nutrient deficiency perosis do not show changes in the hyaline cartilage of the epiphysis as seen in hemin osteodystrophy. While it is possible that some mechanism(s) in common with that of zinc, manganese and choline deficiencies is interrupted with hemin treatment, its histological expression is different.

Summary. Investigation of histological changes in osteodystrophy in chicks treated with hemin revealed that the major defect

was in maturation of epiphyseal hyaline cartilage. Cells in this area appeared to be arrested in development. These findings are compared with cellular changes found in certain nutrient deficiency perosis.

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Received August 27, 1962. P.S.E.B.M., 1963, v112.

Influence of Potassium and Calcium on Behavior of Isolated Rat Atria.* (28083)

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The effects of various drugs and environmental conditions on the membrane potentials and contractility of the rat atrium have been studied in this laboratory(1-6). Correct interpretation of the results requires a knowledge of the influence of the medium which is in contact with the atria during the experimental procedure. This paper is an attempt to supply such information as well as to determine the effects of changes in 2 of the components of that solution, the potassium and calcium ions, on various parameters of atrial function.

Methods. The atrial preparation and apparatus. Rats weighing from 205 to 365 g were killed by decapitation and the hearts were removed and placed in a beaker containing chilled oxygenated Krebs-Ringer bicarbonate medium of the following composition (mM): NaCl 120, KCl 6, $CaCl_2$ 1.22, $MgSO_4$ 1.34, NaH_2PO_4 1.21, $NaHCO_3$ 25.3, glucose 5.55. This solution will be called normal in the text. The atria were dissected from the ventricles and mounted as described previously(1). Electrical stimulation, when necessary, was at a rate of 200/minute. The temperature of the bath was 30°C and the medium was gassed throughout the experiment with 95% O_2-5% CO_2. A strain gauge and Dynograph were used to record the semi-isometric contractions. The resting tension was adjusted to 750 mg at 5 minute intervals throughout the experiment by means of a micrometer attached to the muscle holder.

Blotting procedure preparatory to chemical determinations. After dissection in Krebs-Ringer solution, atria were blotted gently on filter paper between the fingertips either 10, 20 or 30 times and weighed. The blotting and weighing procedures took 2, 3 and 3½ minutes respectively. The atria were then dried to constant weight at 110°C for at least 24 hours and reweighed. The per cent water was calculated to be 80.7 after 10 blottings, 77.8 after 20 blottings and 77.6 after 30 blottings. A significant difference was found between the per cent water at 10 and 20 blottings ($p<.001$ "t" test). No significant difference was found between the per cent water at 20 and 30 blottings ($p>.5$ "t" test). It is concluded that 10 blottings are not sufficient to remove superficial fluid adhering to tissue prior to analysis. Twenty blottings removed as much water as 30 blottings and was, therefore, sufficient.

* This investigation was supported by a post-doctoral fellowship from Nat. Heart Inst., P.H.S.

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