injection. The animals died at 222 and 261 days respectively. A similar group of mice treated similarly with 1:2:5:6 dibenzanthracene resulted in spindle cell sarcomas at the site of injection in 8 animals. The latter died in 150 to 200 days.

11743

Effect of Elementary Phosphorus Upon the Epiphysis of the Albino Rat.*

EDWARD A. GALL, JOSEPH S. BARR AND JAMES R. LINGLEY.
(Introduced by L. Dienes.)

From the Departments of Pathology, Orthopedics and Roentgenology of the Massachusetts General Hospital, Boston.

Wegner described the development of a sclerotic zone in the vicinity of growing epiphyseal cartilage following the administration of small amounts of elementary phosphorus.[1] Since the process was subsequently found to be opaque to roentgen rays[2, 3, 4] it occurred to one of us (J.S.B.) that this phosphorus line might offer an *in vivo* radiographic marker of the varied growth rate in the epiphyseal region. No clear conception of the developmental mechanism of the line was available and this study was therefore performed in order to investigate its histogenesis.

Material and Methods. Subjects were albino rats 4 to 8 months of age sustained upon the BMS regimen recommended by Russell.[5] There were 28 experimental animals, 6 of which were utilized as controls. To the remainder a daily dose of 2.0 cc of elementary phosphorus in oil of peach kernels was administered by gavage. The solution contained 0.05 mg of elementary yellow phosphorus per cubic centimeter. Roentgenograms were taken of the limbs at intervals in order to detect the development of the opaque area of osteosclerosis.

Animals were sacrificed at periods up to 68 days after the initial administration of phosphorus. Both femurs and tibias were sub-

* The expenses of this study were defrayed by a grant from the Infantile Paralysis Research Fund of the National Foundation for Infantile Paralysis, Inc.

1 Wegner, G., *Virch. Arch.*, 1872, **55**, 11.

2 Park, E. A., Jackson, D., Goodwin, T. C., and Kadji, L., *J. Ped.*, 1933, **3**, 265.

3 Phemister, D. B., *J. A. M. A.*, 1918, **70**, 1737.

4 Phemister, D. B., Miller, E. M., and Bonar, B. E., *J. A. M. A.*, 1921, **76**, 850.

5 Russell, W. C., *J. Nutrition*, 1932, **5**, 347.

jected to investigation. Intact tibias were fixed in 90% alcohol and using the Ingalls modification of the Gomorri silver stain[6] the degree of mineralization in the epiphyseal region was determined. The lower end of each femur was split longitudinally, fixed in Zenker's fluid with 5% acetic acid, sectioned in paraffin, and stained with phloxine-methylene blue.

In the normal rat, calcification occurs in that portion of the epiphyseal cartilage adjacent to the metaphysis.[7] Erosion of the lacunae and removal of effete chondroblasts leaves only thin longitudinal strands of calcified matrix constituting the primary trabeculae. Physiologic factors thereupon cause the resorption of the great majority of these, and the remainder, following the deposition of bone, comprise the secondary or true metaphyseal trabeculae. (Fig. 2.) Among the marrow constituents (osteoblasts, hematopoietic elements, etc.) an occasional multinucleated osteoclast is apparent.

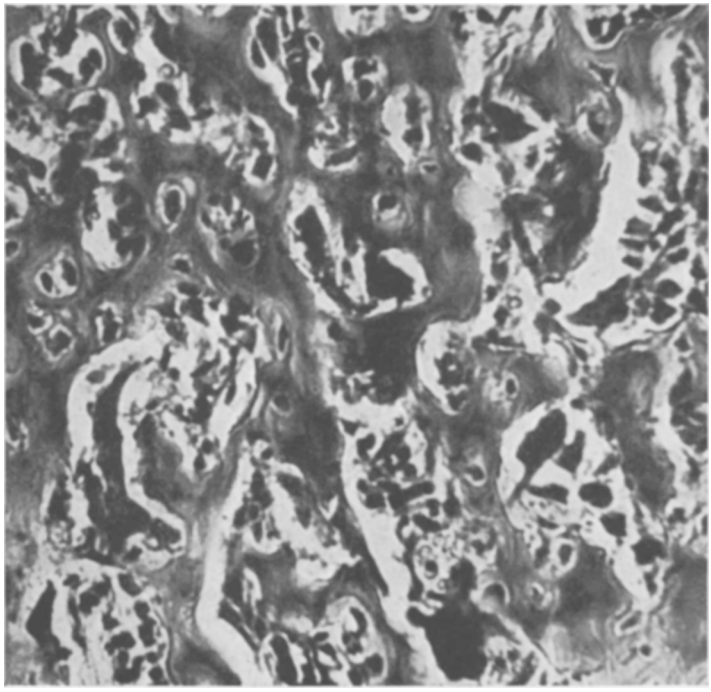

FIG. 1.

The appearance of the metaphyseal marrow seven days after the administration of elementary phosphorus. Seven large dark staining irregular osteoclasts are evident. × 400.

[6] Ingalls, T. H., and Merrill, O. E., *J. Nutrition*, in press.

[7] Dodds, G. S., and Cameron, H. C., *Am. J. Anat.*, 1934, **55**, 135.

FIG. 2 (left).
A silver stain of the subepiphyseal region in the tibia of a normal albino rat. Mineralized cartilage and bone stain black. Primary spicules are much more numerous than the secondary trabeculæ. × 150.

FIG. 3 (right).
A similar area in the tibia of a rat 28 days after the initial administration of elementary phosphorus. Primary trabeculæ are not resorbed and the number of secondary trabeculæ is therefore increased. × 150.

Within 3 to 9 days after the institution of the experimental phosphorus regime there was an increase in the number of osteoclasts present, particularly in the marrow immediately proximate to the primary trabeculae. (Fig. 1.) This increase was progressive and simultaneously it became evident that the area of primary ossification contained more trabeculae than were normally present. The appearance of individual trabeculae was unchanged but it was apparent that there were many more persisting through to the stage of ossification. The secondary trabeculae were not only more numerous than those in the controls but they were also considerably longer. Metaphyseal cancellous bone extended for a distance of 2 or 3 mm into the shaft instead of a fraction of a millimeter as was normally

the case. Intervening marrow content was lessened in amount as a concomitant of the increased osseous substance. Despite this fact, however, osteoclasts were as much as three times more numerous than usual and occasionally assumed giant proportions with bizarre stellate configuration.

The epiphyseal cartilage and primary trabeculae, therefore, differed morphologically in no essential manner from the normal. The fundamental variation appeared to be solely an increased number of secondary trabeculae. (Fig. 3.) Since these resulted from the deposition of osteoid by osteoblasts upon such framework as was available, it is reasonable to infer that the basic process in the development of the phosphorus line might simply be the persistence of an unusual number of primary trabeculae. Park, Jackson, Goodwin and Kadji[2] postulate that the deposition of lead in mineralizing cartilage impedes its resorption and therefore contributes to the production of the dense line in lead poisoning. It is our belief that elementary phosphorus affords similar interference with the resorbability of mineralized cartilage. This thesis was originally propounded by Kassowitz.[8] The presence of markedly increased numbers of osteoclasts implies an exaggerated attempt to overcome this induced resistance to absorption. Whatever the chemical background of the process, it is obvious that the persistence in excess of the primary trabeculae offered an overabundant framework for the deposition of bone. There was no evidence to support the contention that osteoblasts alone were stimulated to hyperfunction.[2] The appearance of the lesion suggested rather an increased activity of these cells in proportion only to the excess amount of superstructure available to them.

Roentgenograms exhibited evidence of increased radio-opacity in the subepiphyseal regions but there was lacking a sharpness of detail. It is possible that this resulted either from an inadequate dosage of phosphorus or from the fact that this element was administered up to the day of death and therefore insufficient time elapsed to permit separation of the phosphorus line from the epiphyseal cartilage. The sharply contrasting clear zone ordinarily present was thus obscured.

The changes observed consisted of a slight to marked increase in the density of the metaphyseal region increasing in width with the continued feeding of phosphorus. Minimal changes occasionally required comparison with films taken prior to the experimental period in order to establish conclusively the distinction of the line.

[8] Kassowitz, M., *Z. f. Klin. Med.*, 1883, **7**, 93.

The degree of ossification was in these cases insufficient to produce obvious density. Histologic changes, however, were apparent in all animals receiving phosphorus for at least one week.

Summary and Conclusions. Twenty-two albino rats were given small amounts of elementary phosphorus supplementary to an adequate diet. Roentgenograms taken before and during the experimental period demonstrated the development of an area of increased density in the subepiphyseal region.

Histologic studies revealed a failure of resorption of the usual number of primary epiphyseal trabeculae. Since bone was deposited upon these persistent spicules in an otherwise normal fashion an apparent sclerosis was produced. Although the degree of ossification was distinctly greater than normal there was no evidence of direct stimulation of osteoblastic activity. The lesion was dependent entirely upon the unusual preservation of mineralized cartilage. It was thought that the persistence of these primary trabeculae was related to the influence of elementary phosphorus deposited in this region.

11744 P

Experimental Atherosclerosis and High Protein Diets.*

DOROTHY R. MEEKER AND HOMER D. KESTEN. (Introduced by J. W. Jobling.)

From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.

The relationship of protein and experimental atherosclerosis has been summarized by Duff.[1] No clear cut experimental evidence was available that the feeding of proteins in conjunction with cholesterol promotes experimental atherosclerosis. Nor was there any indication that a sterol-free protein diet can produce atherosclerotic lesions.

In the course of a study on the possible effects of a high protein diet on cholesterol-induced arteriosclerosis, young adult chinchilla rabbits were fed one of 3 diets:

(A) A normal stock diet (15% digestible protein, dry weight) composed of white flour, alfalfa leaf meal, linseed oil meal, salt mixture, ground carrots, and water.

* The earlier portion of this work has been aided by a grant from the Josiah Macy, Jr., Foundation.

[1] Duff, G. L., *Arch. Path.*, 1935, **20**, 81, 259.