Title

SKELETAL CHANGES IN SEVERE PHOSPHORUS DEFICIENCY IN THE RAT I. TIBIA, METACARPAL, COSTOCHONDRAL JUNCTION, CAUDAL VERTEBRA

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R. D. Coleman, H. Becks,
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February 20, 1950

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Considerable interest has been shown in the past in the effects of phosphorus and vitamin D deficiencies on inducing rachitic changes in the skeletal system of the rat. Since the earlier experimental work of McCollum et al (15), Sherman and Pappenheimer (16), and Steenbock and Black (18), rickets was believed to be due to a disproportion of calcium and phosphorus intake in the absence of vitamin D. This resulted in a disturbance of the normal process of calcification in the active growing period. It should be noted that in these earlier experiments the effects may have been complicated by vitamin deficiencies not understood at that time. Furthermore, most of the histological studies were limited for the main part to only one bone -- either the rib, femur or tibia.

Some of the essential data on the early experimental rickets are given in Table I. In 1921 Sherman and Pappenheimer (16), described a rachitogenic diet which was deficient in phosphorus and in vitamins A and D. At the same time McCollum et al (15) reported the experimental production of rickets in rats with a similar diet (McCollum Diet #3127). The phosphorus content of this diet was slightly higher than that used by Sherman and Pappenheimer. The McCollum diet #3133 and the diet of Steenbock and Black (18)* contained more butterfat and this provided a small amount of vitamin A which prevented the development of vitamin A

*Steenbock and Black's diet later became the U.S.P. Rachitogenic Test Diet.

deficiency symptoms. Their rats also lived longer and developed a more intense rickets. A more severe rickets was also produced when more calcium was added to the diet (McCollum Diet #3143). It was concluded that the ratio of calcium to phosphorus, within certain limits, was of greater importance than the absolute amount of these minerals in the diet.

Shohl and collaborators (17) showed that the growth of rats fed the Steenbock and Black diet was curtailed by 30 to 40 percent. The addition of vitamins A and D did not promote growth but did prevent the development of rickets. They believed that the limiting factor was the inadequate amount of phosphorus in the diet. Lilly, Pierce and Hall (14) reported mineralization of the rachitic rat skeleton when phosphate was added to their high calcium-low phosphorus diet.

Since the amount of nutritionally available phosphorus was not given for these earlier experimental diets, the calculated calcium-phosphorus ratios as given in Table I are only estimates. No numerical values for the amounts of vitamins A and D were given and the diets were simply described as being "decidedly low", "not sufficient", and "deficient" in vitamins A and D.

Day and McCollum in their recent investigations (6) were the first to report the amounts of vitamins A and D in the diets. The phosphorus content was extremely low (.017 percent) with a calcium-phosphorus ratio of 23.6:1. In spite of the presence of vitamins A and D, normal bone did not form. Follis, Day and McCollum (10) reported that in these rats the histologic bone changes were qualitatively similar to the classical high calcium and low phosphorus rickets as described by McCollum et al (15), Sherman and Pappenheimer (16) and Dodds and Cameron (7).

Table I

Data of Experimental Rickets

Experimental Diets	Author of Histolog. Analysis	Year	No. of Animals Used	Age at Beginning of Exp. (Days)	Duration of Exp. (Days)	Age at Autopsy (Days)	Composition of Diets					Bones Studied
							Ca %	P %	Ca:P Ratio	Vitamin A Per Kg. of Diet	Vitamin D Per Kg. of Diet	
Sherman & Pappenheimer Diet #84	Sherman & Pappenheimer	1921	30	28 - 29	30 - 32	58 - 60	.550	.087	6.3:1	"Deficient"	"Deficient"	Rib
McCollum et al Diet #3127	McCollum et al	1921-1922	5	25	24 - 39	49 - 64	0.8	0.209	3.8:1	"Deficient"	"Deficient"	Femur
McCollum et al Diet #3133	McCollum et al	1921-1922	6	16	35 - 98	51 -114	0.8	0.209	3.8:1	"Not Sufficient"	"Deficient"	Femur
McCollum et al Diet #3143	McCollum et al	1921-1922	9	28 - 50	43 - 76	88 -116	1.22	.301	4.8:1	"Decidedly low"	"Deficient"	Femur
Steenbock & Black, Diet #2965	Dodds & Cameron	1925	32	28	7 - 56	35 - 84	1.25	.250	5.0:1	"Low Amount"	"Deficient"	Tibia
Day & McCollum Diet #16	Follis, Day and McCollum	1939	73	21	48 - 67	69 - 88	.40	.017	23.6:1	15,000 U.S.P. Units	3800 I.U. of D2**	Tibia & Femur
Group I ≠	Present Studies	1949	10	21	24	45	.46	.013	35:1	20,000 U.S.P. Units	8000 I.U. D3*	Tibia, Third Metacarpal, Costochondral Junction, 9th Caudal vertabra, Incisors, Molars Temporomandibular Joint
Group II ≠		1949	15	21	32	53	.43	.015	28:1	20,000 U.S.P. Units	8000 I.U. D3*	
Group III ≠		1949	10	21	41	62	.43	.005	78:1	20,000 U.S.P. Units	8000 I.U. D3*	
Group IV ≠		1949	14	21	49	70	.43	.013	31:1	20,000 U.S.P. Units	8000 I.U. D3*	

≠ For additional information on diets see Table III
** D2 Activated Plant Sterol (Ergosterol)
* D3 Activated Animal Sterol (7 Dehydro-cholesterol)

Mineral balance studies revealed that a significant amount of phosphorus was mobilized from the bones. Their work indicated that the low phosphorus content of the diet was of greater importance than the presence of vitamins A and D. The addition of 4.5 percent of H_3PO_4 to the diet was sufficient to restore the process of normal ossification.

The following study was undertaken to test even more severe phosphorus deficiencies than those studied by Day and McCollum and to investigate their effects on the skeleton of the rat. Since normal standards for histogenesis of various bones of the rat of the Long-Evans strain had been established in this laboratory for different age groups (2, 3, 4, 5) it was felt that a histologic study of several bones including the tibia, third metacarpal, costochondral junction and 9th caudal vertebra would be a distinct advantage over previous histologic reports.*

Material and Experimental Arrangement (Table II):

Forty-nine female rats of the Long-Evans strain, weighing 45-50 grams each, were weaned at 21 days of age. Twenty-one rats were fed phosphorus deficient diets, twenty were pair-fed a phosphate supplemented diet, and eight were offered this control diet ad libitum. Food consumption and weight were recorded regularly. The experimental animals were arranged into four groups according to age and duration of the deficiency.

Group I were sacrificed at 45 days, Group II at 53 days, Group III at 62 days and Group IV at 70 days of age after an experimental period of 24, 32, 41 and 49 days respectively. The protein of the diet consisted of 20.0 percent washed beef blood fibrin which Jones (12) has

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* The skulls and teeth will be described in a second paper.

Table II

Experimental Arrangement

Group	Treatment	Number of Animals	Average Daily Diet Consumed (Grams)	Age at Beginning Exp. Diet (Days)	Duration of Exp.	Age at Autopsy	Composition of Diet				
							% Ca	% P	Ca:P Ratio	Average Daily Intake of Vitamin A (U.S.P.)	Average Daily Intake of Vitamin D_3 (I.U.)
I	Phosphorus Deficient	5	6.5	21	24	45	.46	.013	35:1	130	52
	Pair-Fed	5	6.5	21	24	45	.46	.475	98:1	130	52
II	Phosphorus Deficient	5	6.0	21	32	53	.43	.015	28:1	120	48
	Pair-Fed	5	6.0	21	32	53	.43	.475	.90:1	120	48
	Ad Libitum	5	15.0	21	32	53	.43	.475	.90:1	300	120
III	Phosphorus Deficient	5	5.2	21	41	62	.43	.005	78:1	104	41
	Pair-Fed	5	5.2	21	41	62	.43	.53	.81:1	104	41
IV	Phosphorus Deficient	6	6.0	21	49	70	.43	.013	31:1	120	48
	Pair-Fed	5	6.0	21	49	70	.43	.50	.86:1	120	48
	Ad Libitum	3	15.0	21	49	70	.43	.50	.86:1	300	120

shown to compare favorably with casein for nutrition. The very low phosphorus content was attained by employing isoelectrically precipitated fibrin for protein, and purified phosphorus-free components for the balance of the diet. The phosphorus content of the diets is shown in Table II. All were decidedly lower than any previously reported diets. The formula of the deficient diet is given in Table III.

The experimental as well as control rats received 8 I.U. of vitamin D and 20 U.S.P. units of vitamin A per gram of diet. The phosphorus deficient and the pair-fed control rats consumed between 5.2 and 6.5 grams of diet per day. They therefore injested 41 to 52 I.U. of vitamin D and 104 to 130 U.S.P. units of vitamin A daily. The rats fed ad libitum consumed approximately 15 grams per day with an intake of 120 I.U. of vitamin D and 300 U.S.P. units of vitamin A. Rats restricted to the phosphorus deficient diets gained less than 10 grams of weight during the first three weeks. They then gradually lost weight and died after 7 to 8 weeks. Tibia, metacarpal, costochondral junction and caudal vertebra were selected for histological study. The tibia was chosen because of its use as a rachitic standard and late closure of its proximal epiphyseal cartilage. The metacarpal provided a bone with an early closing epiphyseal cartilage with the additional advantage of being a small bone which could be sectioned in its entirety. The costochondral junction is of interest because of the importance of the rachitic rosary in rickets and the caudal vertebra is used as a standard in growth hormone studies (11).

The bones were fixed in 10 percent neutral formol, roentgenographed and measured. They were then decalcified in 5 percent nitric acid, embedded in nitrocellulose, sectioned and stained with hematoxylin

Table III

Composition of the Experimental Diets[1]	
Purified beef blood fibrin	20.0%
Sucrose	60.0%
Hydrogenated cottonseed oil (Crisco)	10.0%
Fish liver oil (400 I.U. vit. D and 1,000 U.S.P. Units vit. A per gram)	2.0%
Sodium chloride	1.0%
Potassium Chloride	1.5%
Magnesium Sulfate .7H_2O	0.3%
Ferric citrate	0.1%
Calcium Carbonate	1.0%
Vitamin Mixture*	1.0%
Trace Element Mixture**	1.0%

*Vitamin Mixture (per 100 g diet)

Thiamin Chloride	1.0 mg.
Riboflavin	1.0 mg.
Pyridoxin	0.5 mg.
P-Aminobenzoic acid	1.0 mg.
Nicotinic acid	5.0 mg.
Ca-pantothenate	5.0 mg.
I-Inositol	20.0 mg.
Choline Chloride	100.0 mg.
Sucrose	866.5 mg.
Total	1.0 gram

**Trace Element Mixture (per 100 g diet)

$CuSO_4.5H_2O$	13.0 mg.
$MnSO_4$	4.0 mg.
$ZnSO_4.7H_2O$	2.0 mg.
Co-acetate	1.0 mg.
KI	0.8 mg.
Sucrose	979.2 mg.
Total	1.0 gram

[1] Diet given is phosphate deficient. In the control diet, the 1.0% NaCl is replaced with 2.4% Na_2 HP04.

and eosin. Measurements of the epiphyseal cartilage width were made with an ocular micrometer.

Results:

A. Roentgenograms

The roentgenograms of the carcasses of the phosphorus deficient rats revealed an extreme lack of mineralization (Fig. 1A). The only well mineralized structures observed in this roentgenogram were the teeth which were already calcified prior to the beginning of the experiment. Growth was severely stunted.

Figs. 1B and C are reproductions of roentgenograms of a pair-fed control and an ad libitum rat and show normal mineralization.

In each of the four groups the average tibia length of the phosphorus deficient rats (Table IV) was less than that of their pair-fed and ad libitum controls. Roentgenographic reproductions of the tibia twice natural size are given in Fig. 2 for comparison. The diameter of the shaft of the deficient tibia (Fig. 2A) at its midpoint and the width of the cortical bone is less than that observed in the pair-fed rats. The former disclose a club-shaped epiphysis and an abrupt junction with the diaphysis which is characteristic of severe rickets. In contrast to this, the epiphysis of the pair-fed and ad libitum rats taper gradually into the diaphysis. The epiphyseal cartilage is very wide and in the metaphysis the trabeculae are poorly mineralized. In the tibias of the pair-fed and ad libitum controls (Fig. 2B and C) the width of the epiphyseal cartilage is narrow and the trabeculae are well ossified and distinctly seen.

A comparison of the roentgenograms of the forepaws of the phosphorus deficient rats (Fig. 3A) with those of their controls (Figs. 3B and C) show similar changes, viz. a lack of mineralization, shorter

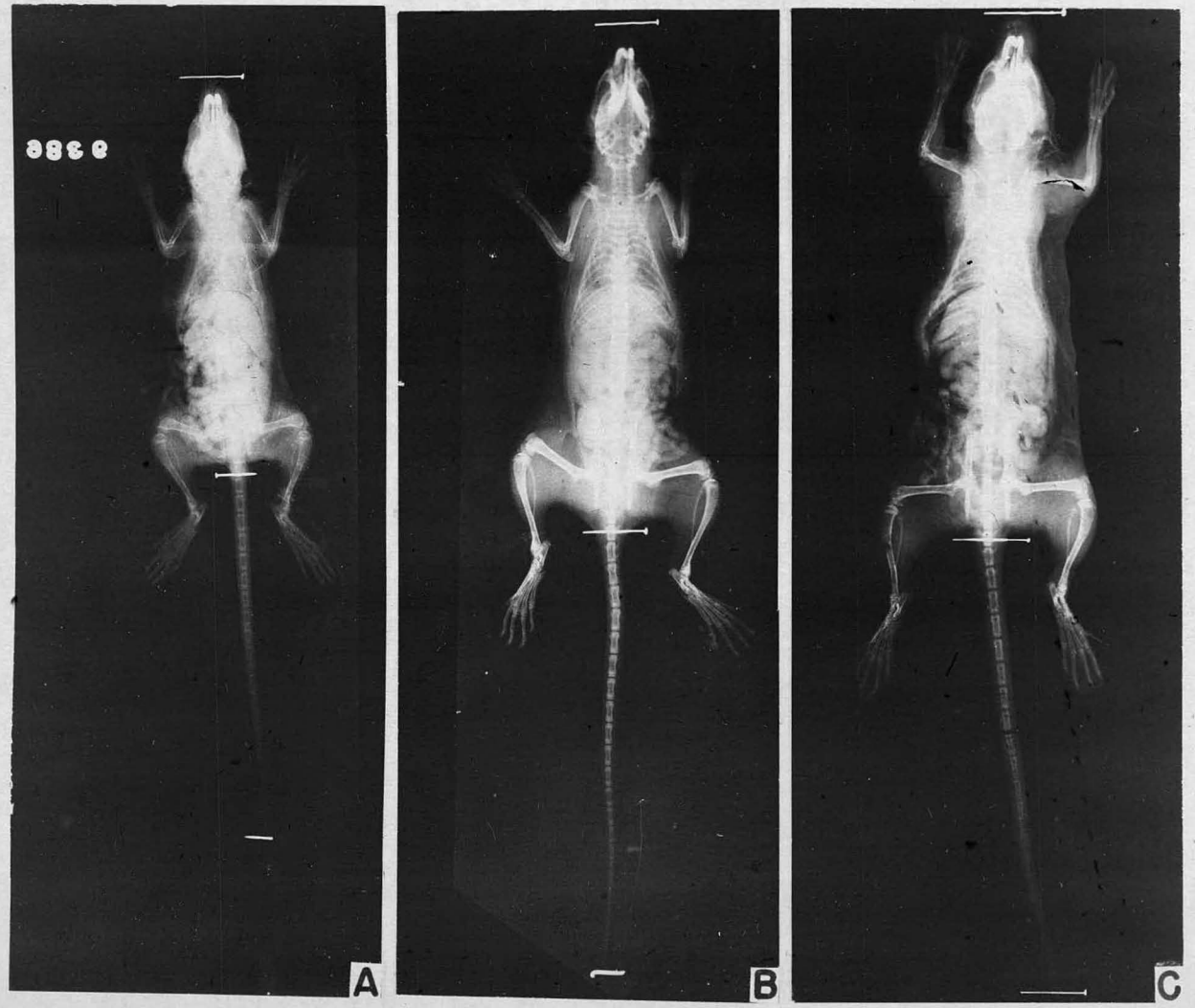

FIG. 1

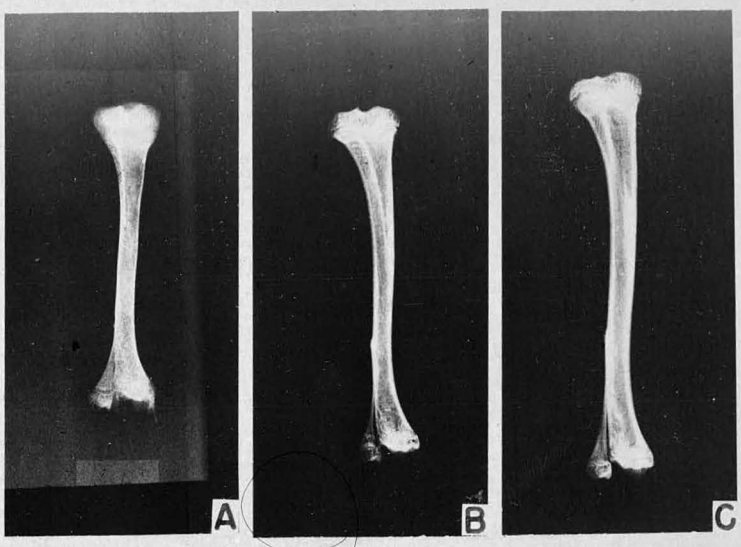

FIG. 2

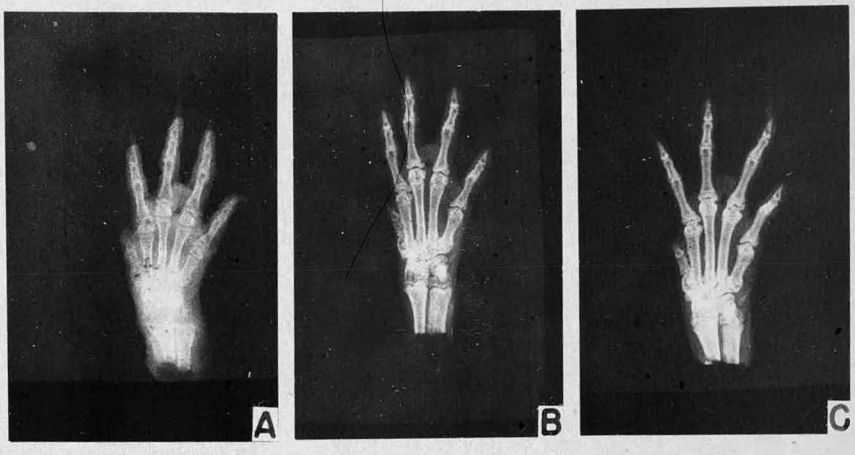

FIG. 3

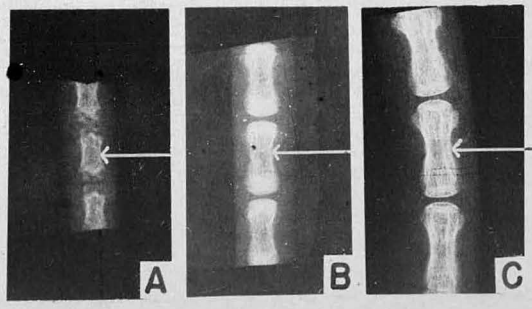

FIG. 4

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and narrower bones, and an increase in the width of the non-mineralized epiphyseal cartilage and metaphysis. The forepaws of the ad libitum and pair-fed control rats are well developed and mineralized and the width of the epiphyseal cartilage appears normal.

A roentgenogram of the ninth caudal vertebra of a phosphorus deficient rat (Fig. 4A) shows the characteristic roentgenographic appearance of rickets. As compared with the pair-fed and ad libitum controls, the maturation of the vertebra is severely retarded. The secondary ossification centers in both the proximal and distal epiphyses appear as two small radiopaque areas. In the pair-fed and ad libitum controls these centers have united and form the completed epiphysis (Figs. 4B and C). The lack of growth of the ninth caudal vertebra of the deficient rats is marked. The vertebra of the pair-fed control rats are smaller than the ad libitum controls because of the limited food intake.

B. Histologic Findings

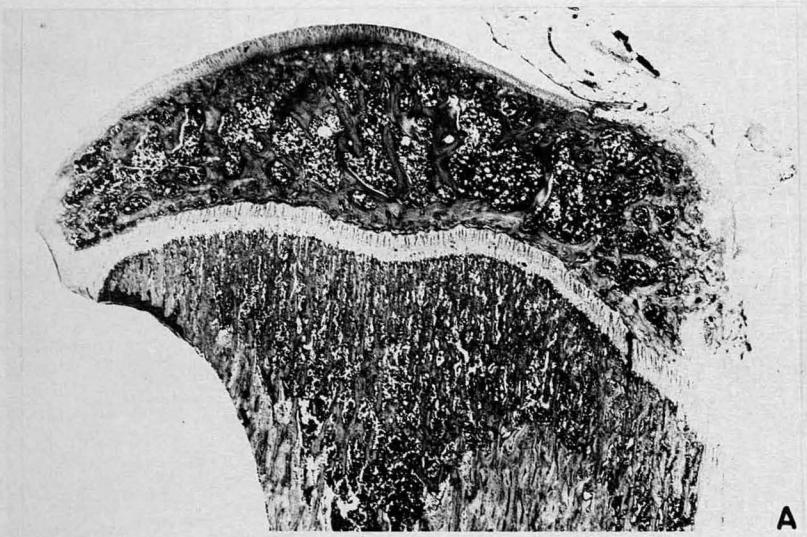
The histologic aspects of all bones of the ad libitum control groups, compare favorably with the standards established in this laboratory for the Long-Evans strain rats at various age levels (2, 3, 4, 5).

The proximal epiphyseal region of the tibia of rats fed ad libitum is shown histologically in Figs. 5A and B. The epiphyseal cartilage has an average width of 2.4 μ and 186 μ in Groups II and IV respectively (Table IV). The cells of the vesicular zone are large; numerous long, delicate and parallel trabeculae consisting almost entirely of lamellar bone are observed in the primary spongiosa. Many osteoblasts indicative of very active osteogenesis are seen on the surfaces of the

Table IV

Comparison of the Average Skeletal Measurements

Group	Treatment	Ca:P Ratio of Food	Phosphorus Deficient Diet (Days)	Age at Autopsy (Days)	Average Length			Average Width of Epiphyseal Cartilage			
					Tibia mm.	Meta-carpal mm.	10th Caudal Vertebra mm.	Tibia	3rd Meta-carpal	10th Caudal Vertebra	
										Mesial	Distal
I	Phosphorus Deficient	35:1	24	45	25.6	6.1	5.0	558	198	268	266
	Pair-Fed	0.98:1	24	45	28.6	6.9	5.8	256	162	174	165
II	Phosphorus Deficient	28:1	32	53	25.2	5.8	4.9	534	191	278	267
	Pair-Fed	0.90:1	32	53	29.3	6.7	6.1	230	127	176	165
	Ad Libitum	0.90:1	32	53	30.6	6.9	6.5	240	174	186	166
III	Phosphorus Deficient	78:1	41	62	26.8	6.1	5.0	502	188	264	260
	Pair-Fed	0.86:1	41	62	29.0	6.9	5.7	185	132	175	173
IV	Phosphorus Deficient	31:1	49	70	24.9	5.8	4.5	370	182	266	238
	Pair-Fed	0.86:1	49	70	29.7	6.9	5.9	149	107	158	132
	Ad Libitum	0.86:1	49	70	33.1	6.8	7.1	186	110	160	134



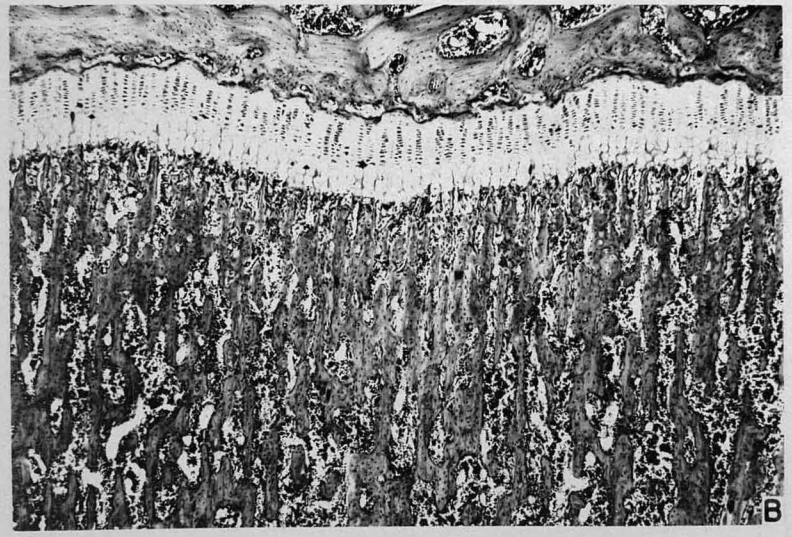
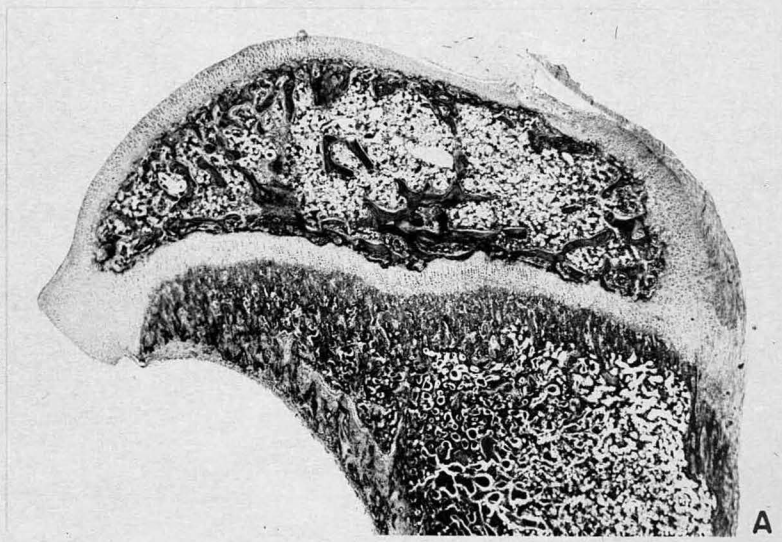


FIG. 5



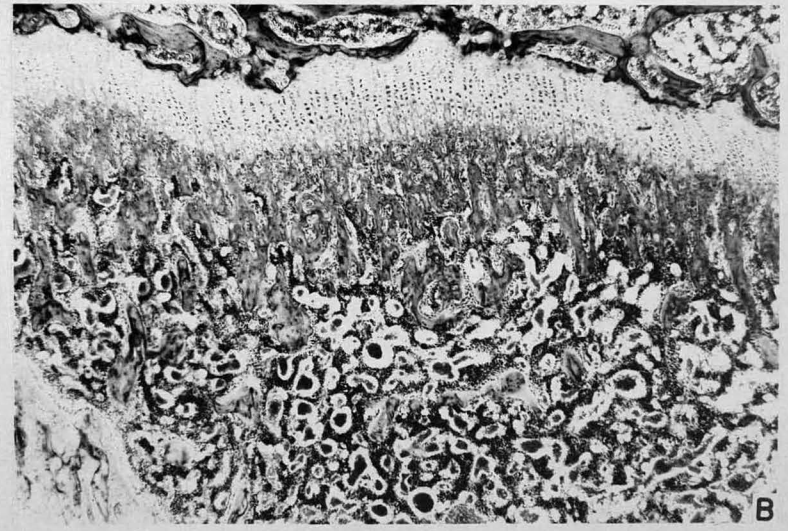


FIG. 6

trabeculae. Only a small amount of adipose tissue is visible. The cortical bone of the shaft appears calcified except for a narrow border of osteoid on the endosteal surface of the diaphysis.

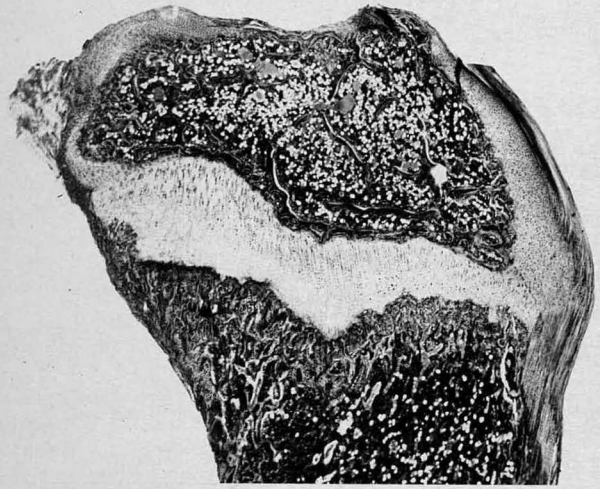
The bones of the pair-fed control rats (Figs. 6A and B) conform to almost the same standards as the ad libitum rats. They were, however, slightly smaller in size and the histologic variations may be attributed to the limited food intake. The average width of the epiphyseal cartilage in all bones was slightly less as compared to that of the ad libitum rats.

Histologically, the decrease in width is principally the result of a reduction in the size of the cells and the number of cell layers in the basophilic and vesicular zones. The intercartilaginous matrix is increased in the basophilic zone. The cells of the vesicular zone are polygonal in shape, indicating the approaching end of the growth phase. Osteogenesis is reduced and there is a decrease in the number of capillaries at the line of erosion. The trabeculae adjacent to the epiphyseal cartilage are short and coarse and lateral anastomoses are prevalent. The cortical bone of the shaft is calcified except for a narrow preosseous layer on the endosteal surface.

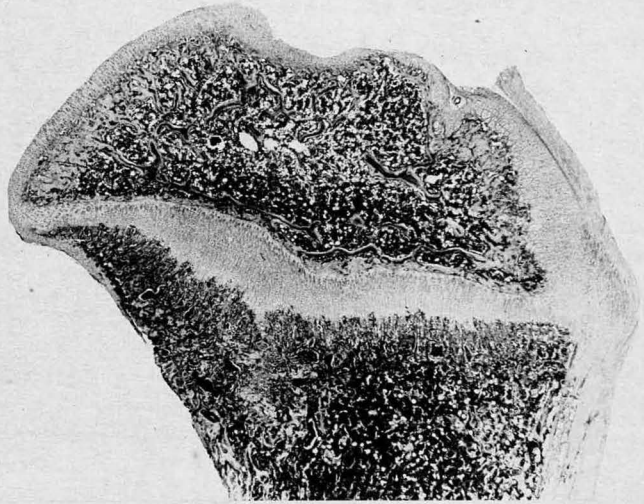
The tibial epiphyseal cartilage of the phosphorus deficient rats (Figs. 7 - 10) is markedly wider in all instances than that of the pair-fed and ad libitum fed rats of the same age. The width is not uniform but broad in the center and narrow toward the periphery. Abundant osteoid tissue is seen. Since there were significant differences in the histogenesis of endochondral ossification between the first two groups and Group III and Group IV, only the tibias of Groups I, III and IV are illustrated.

Figures 7A and 7B illustrate the irregular proximal epiphyseal cartilage in the tibia of a phosphorus deficient rat of Group I after an experimental period of 24 days. The zone of enlarged cells is extremely wide and accounts for the greatest increase in width of the entire cartilage. This zone was broad because of failure of the capillaries to invade the uncalcified cartilage. Although the width of the zone of the enlarged cells has increased, the individual cells have decreased in their vertical diameter and become shorter and flatter. The intercartilaginous matrix as well as cartilage cells upon which osteoid has been deposited act as a barrier to further resorption of cartilage cells. These observations coincide with those of Dodds and Cameron (8). In some cases the cartilage cells seem to have been infiltrated with osteoid following the opening of the lacuna. Osteoid is deposited about the chondrocytes and at the same time the cartilage cells appear to assume the histological appearance of osteocytes. The failure of the cartilage to calcify and the resistance of osteoid tissue to resorption leads to this characteristic picture. There is no decrease in the number of osteoclasts even though a lack of remodeling resorption was evident. The trabeculae are composed predominantly of osteoid tissue with cores of premorbid bone. Polygonal osteoblasts line the endosteal surface of the shaft and a wide layer of osteoid matrix is formed. Similar observations have been noted by Weinmann and Sicher (19) and Dodds (9).

Figures 8A and B demonstrate the epiphyseal region of the tibia after an experimental period of 41 days (Group III). This group was fed the diet with the lowest phosphorus content and the most extreme calcium-phosphorus ratio (78:1). The epiphyseal cartilage has decreased



A



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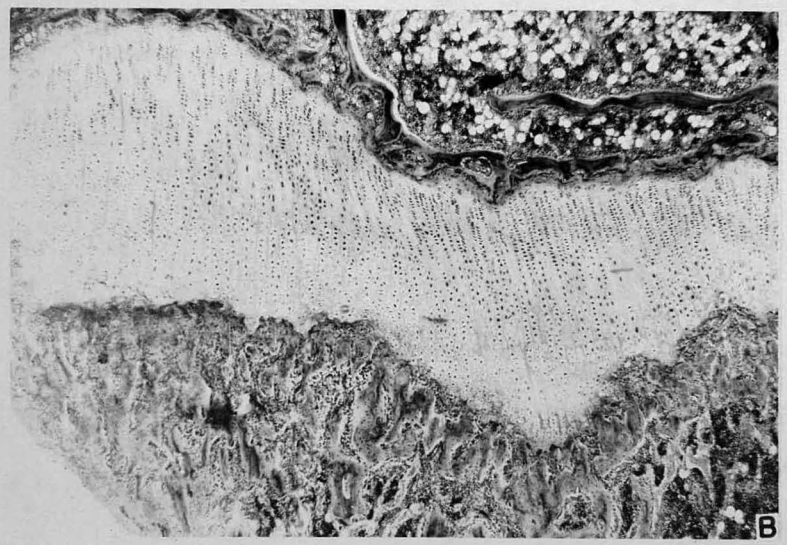
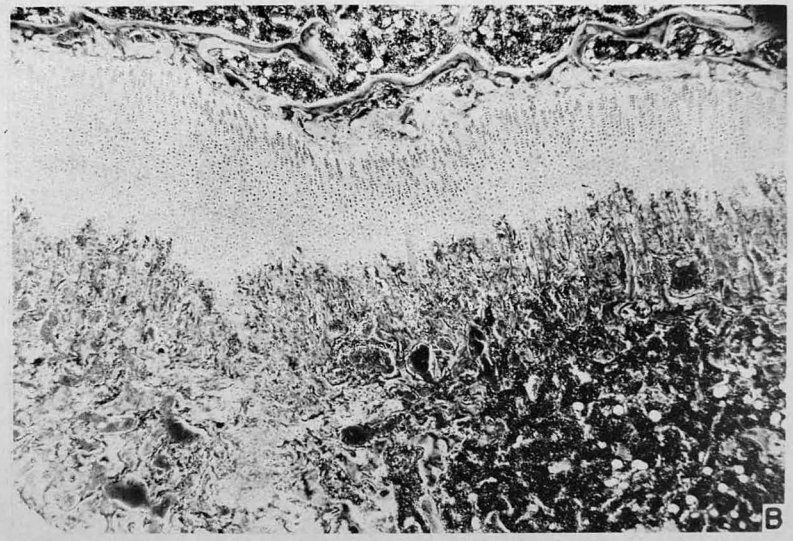


B



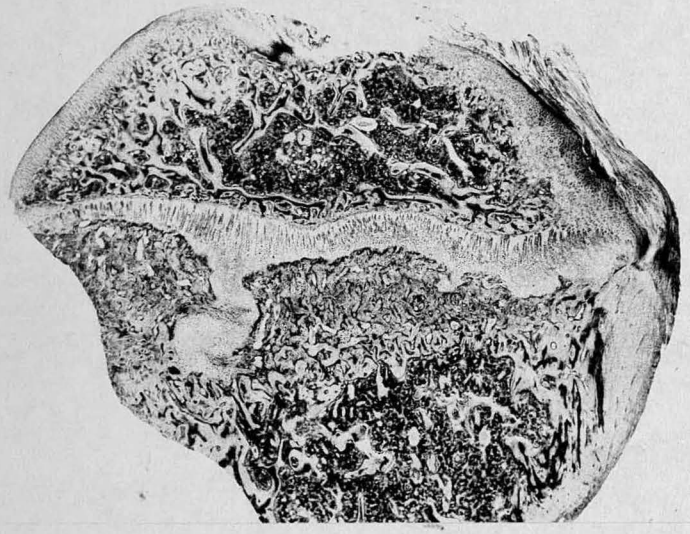
B

FIG. 7

FIG. 8

in width. Active invasion of the epiphyseal cartilage by capillaries is taking place even though there is no provisional zone of calcification. Osteoid tissue is seen on both the endosteal and periosteal surfaces. It appears to be deposited to a lesser degree on the periosteal surface. A dark basophilic zone extends longitudinally through the center of the cortex of the shaft. This zone is commonly recognized as normally mineralized bone. Numerous osteoblasts are visible on the endosteal surface of the shaft.

The rats of Group IV were sacrificed at 70 days of age after they had been on the phosphorus deficient diet for a period of 49 days. This was the longest experimental period studied. The head of the tibia (Figs. 9 and 10) is club-shaped. The epiphyseal cartilage has further decreased in width and this appears to be the result of a resumption of the invasion of the uncalcified cartilage. The removal is more pronounced at the periphery, possibly due to the larger number of blood vessels entering the marrow cavity through Volkmann's canals beneath the epiphyseal cartilage. Large numbers of capillaries are penetrating into the cartilage and producing masses of diversiformed osteoid trabeculae (Fig. 9B). The peninsula of cartilage which extends toward the shaft represents a remnant which was left in the process of cartilage removal. This renewed cartilage removal has been described by Dodds and Cameron (7) and Baillie and Irving (1). The cells on the diaphyseal aspect of the peninsula of cartilage are flattened while those near the epiphysis are more cuboidal. The bent rows of cartilage cells are probably caused by the forces of compression (Fig. 9B). Numerous osteoclasts are seen both at the line of erosion and on the periosteal surface of the



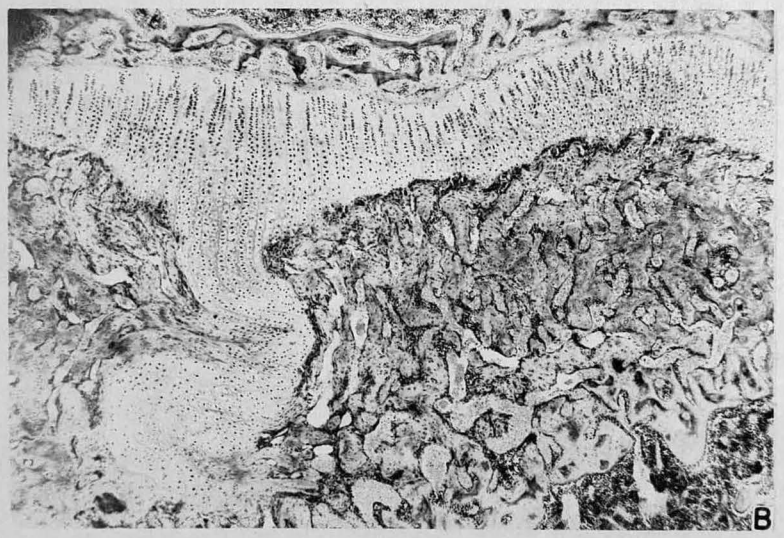
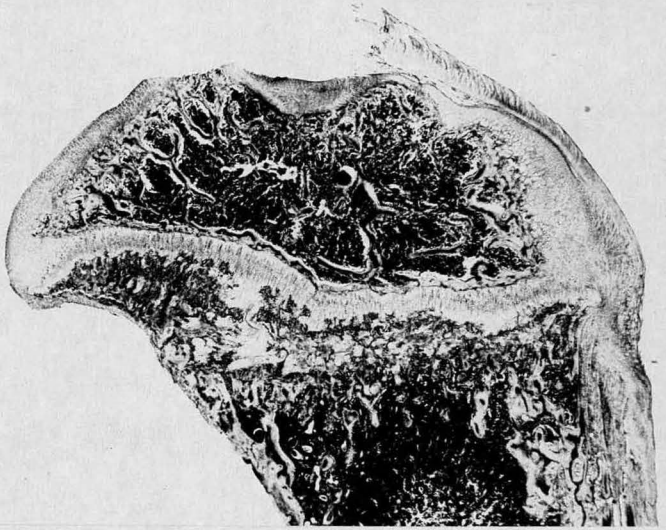


FIG. 9



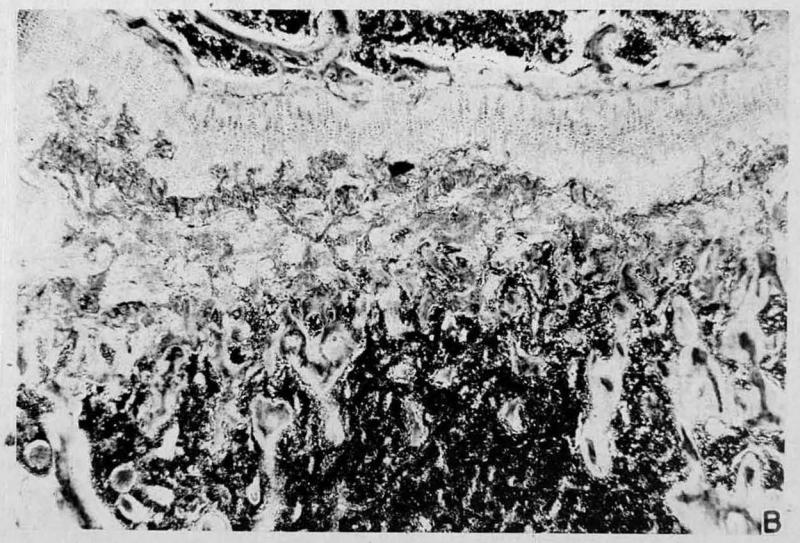


FIG. 10

metaphysis. Osteoid tissue is not resorbed and osteoclasts do not appear to perform their remodeling function. Only a small core of calcified bone is visible in the shaft at this age.

Figs. 10A and B are photomicrographs of a tibia of a rat which survived until 82 days of age. Large brush-like capillaries are seen penetrating the epiphyseal cartilage in all directions. The narrow epiphyseal cartilage is the result of renewed cartilage removal which was resumed between 62 (Group III) and 70 (Group IV) days of age. The resumed invasion seems to occur in the older age groups and later stages of the deficiency and confirms the observations of Dodds and Cameron (7) and others.

Metacarpal

In the pair-fed controls at 62 days of age (Fig. 11) resorption and fusion of the trabeculae of the third metacarpal has increased the size of the marrow cavities. The trabeculae of the metaphysis are short, thick and anastomosed, and continue in thickness to the epiphyseal cartilage. The blood elements in the marrow have been replaced to a large extent by adipose tissue.

Fig. 12 shows the changes which have occurred in the metacarpal of the phosphorus deficient rats. The cortical bone of the epiphysis is narrow. The trabeculae are more numerous, short and joined with the cortex by osteoid projections. The many remaining osteoid trabeculae in the epiphysis are indicative of the lack of resorption. The epiphyseal cartilage is only a little wider than that of the pair-fed controls; very probably the maximum growth of the metacarpal has already been achieved at 62 days of age while the tibia is still in the active growing

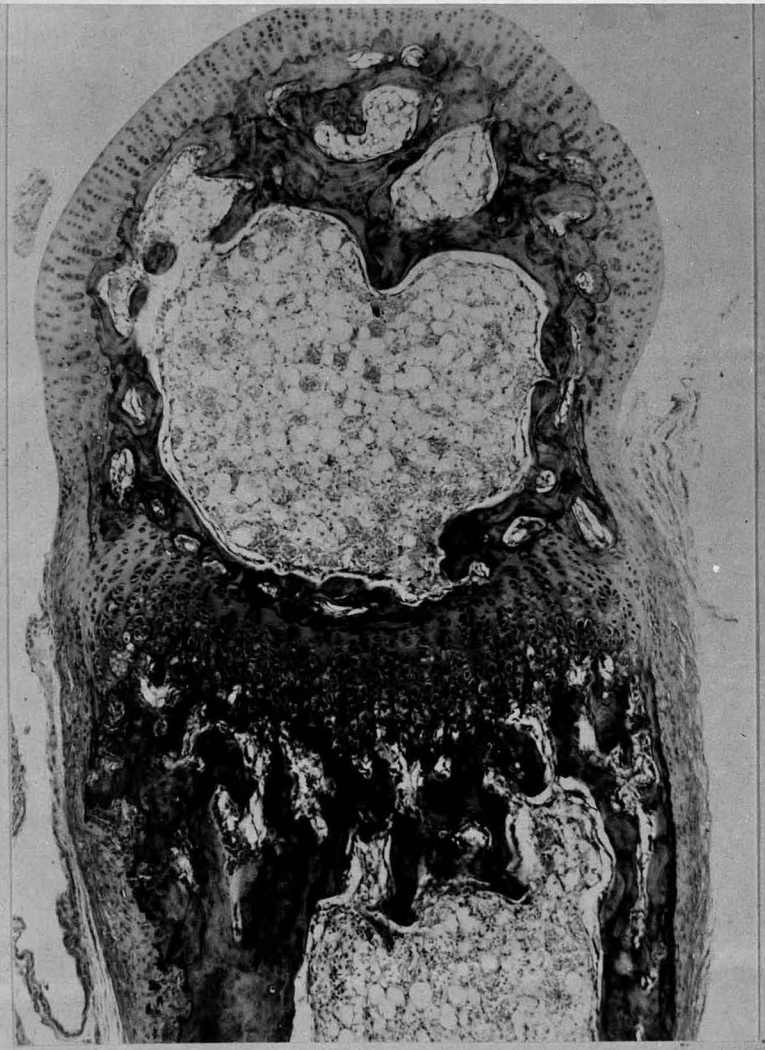


FIG. 11

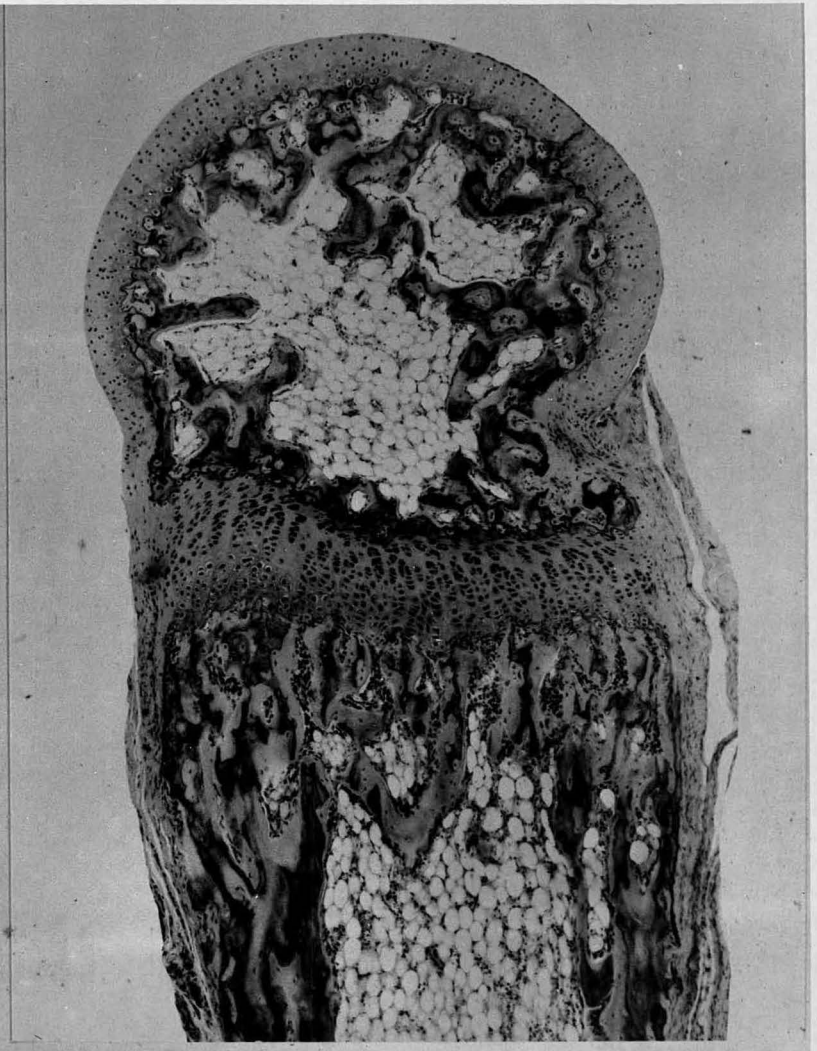


FIG. 12

stage. The width of the basophilic zone is increased over that in the pair-fed controls. There is no evidence of a zone of provisional calcification. In contrast to the tibia the zone of enlarged cells is extremely narrow. The diaphyseal trabeculae are thick and broad and osteoid-bordered throughout. These wide osteoid layers enclose a deep staining core which presumably calcified before the experimental diet was begun.

Costochondral Junction

The costochondral junction of an ad libitum rat of Group IV is shown in Fig. 13. Undifferentiated hyaline cartilage composed of small round cell nests is embedded in an abundant matrix. The basophilic zone lies immediately below the hyaline cartilage and consists of a zone of flattened cells arranged in columns, or several columns in the form of a fascicle. In contrast to the tibia, the vesicular zone is narrow. The trabeculae of the primary spongiosa are numerous, long, and continuous with the calcified cartilage matrix.

The costochondral junction of a pair-fed control rat of Group IV (Fig. 14) reveals a reduction in osteogenic activity at the line of erosion. The costochondral junction is significantly smaller than that of the ad libitum control. The basophilic zone of the epiphyseal cartilage is narrow in width, and the cells are packed closely together. The vesicular zone is almost completely absent in contrast to the wide zone in the tibia. Only a few short trabeculae are observed adjacent to the epiphyseal cartilage. These changes are associated with the restricted food intake and limited growth of these pair-fed animals.

The costochondral junctions of the phosphorus deficient rats of Group IV present the most severe changes in the series (Fig. 15). They

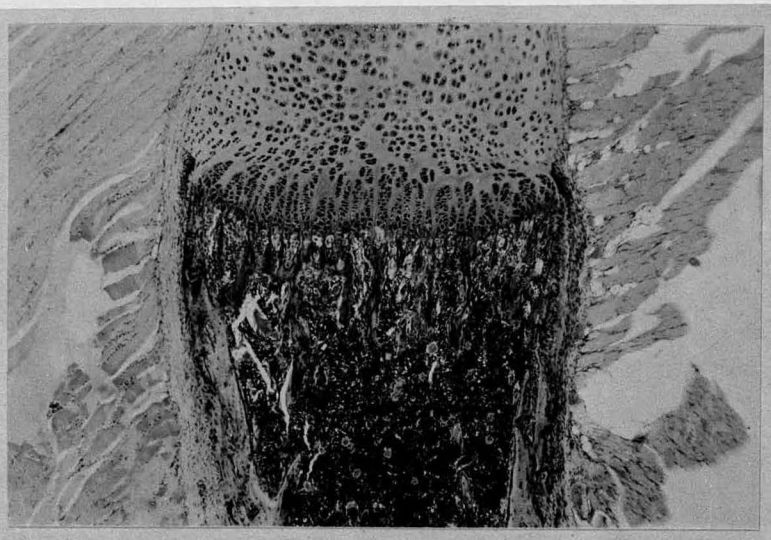


FIG. 13

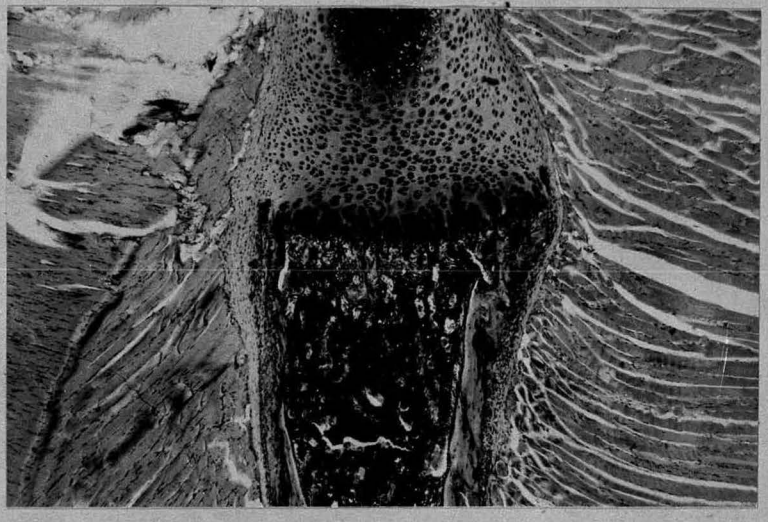


FIG. 14

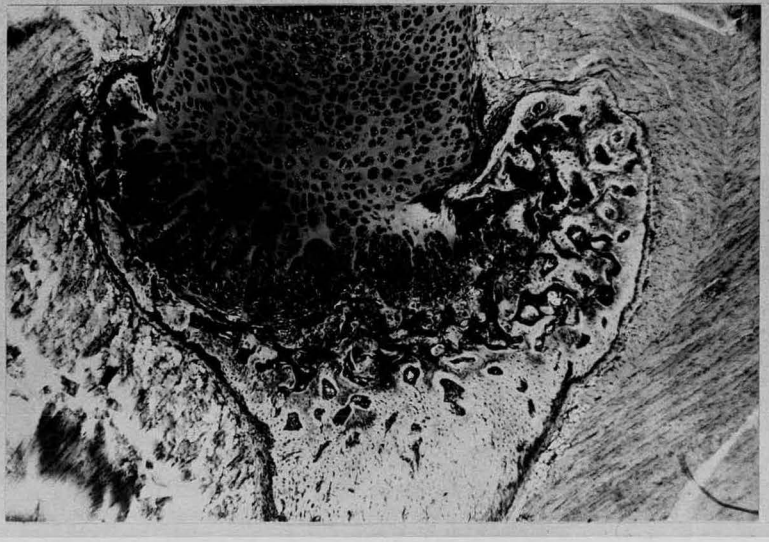


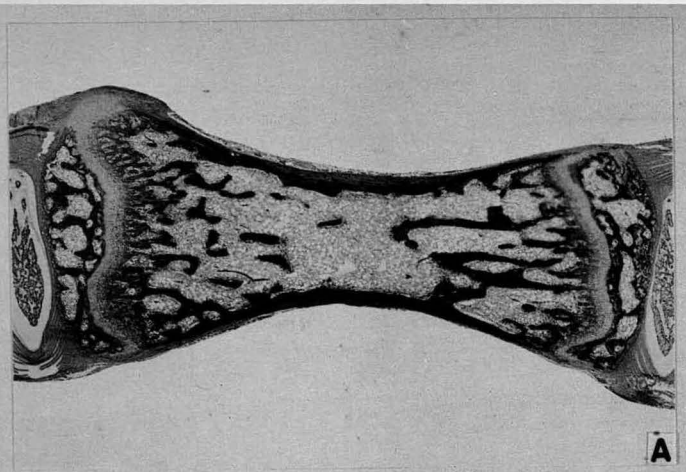
FIG. 15

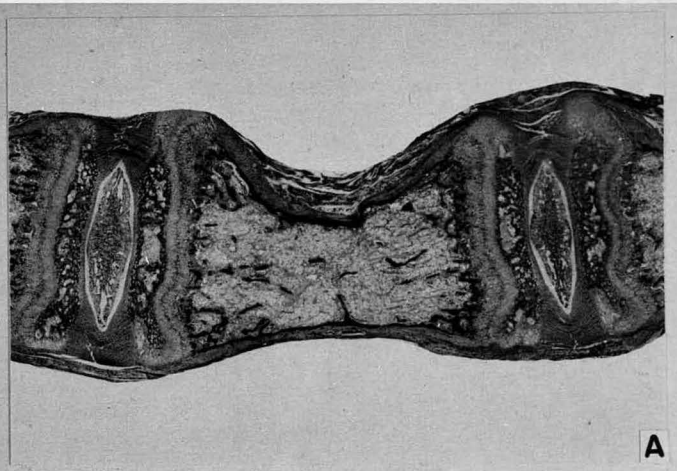
show the classical picture of rachitic rosary with the length of the columns of cells in the basophilic zone greatly increased. The chondrocytes of the vesicular zone are distorted and compressed. The rib proper consists of a mass of osteoid, containing islands of marrow and large capillaries at the cartilage junction. The perichondral osteoid has spread over the lateral margins of the epiphyseal and hyaline cartilage producing a bulbous effect. The normal growth in the length of the rib occurs at the costochondral junction by means of endochondral bone formation. Appositional growth occurs at the periphery with subsequent remodeling resorption to keep the rib uniform in width. The formation of osteoid at the periphery continues and because of lack of mineralization, remodeling resorption does not take place. The surrounding tissues mold the pliable osteoid back against the hyaline cartilage. The lack of endochondral bone formation and inability of osteoid to be resorbed are therefore responsible for these grotesque changes.

Caudal Vertebra

The ninth caudal vertebra of a pair-fed control rat of Group III is illustrated in Figs. 16A and B. Secondary ossification centers have formed in both the proximal and distal ends of the body of the vertebra. The marrow cavities contain considerable adipose tissue. The zone of provisional calcification is seen in Fig. 16B. Both the proximal and distal epiphyseal cartilages are narrow at 62 days of age with an average width of 175 micra. The trabeculae of the primary spongiosa are short, coarse, and show considerable anastomosis.

In the phosphorus deficient rats (Fig. 17A and B) the ninth caudal vertebra is considerably smaller than in the pair-fed control.





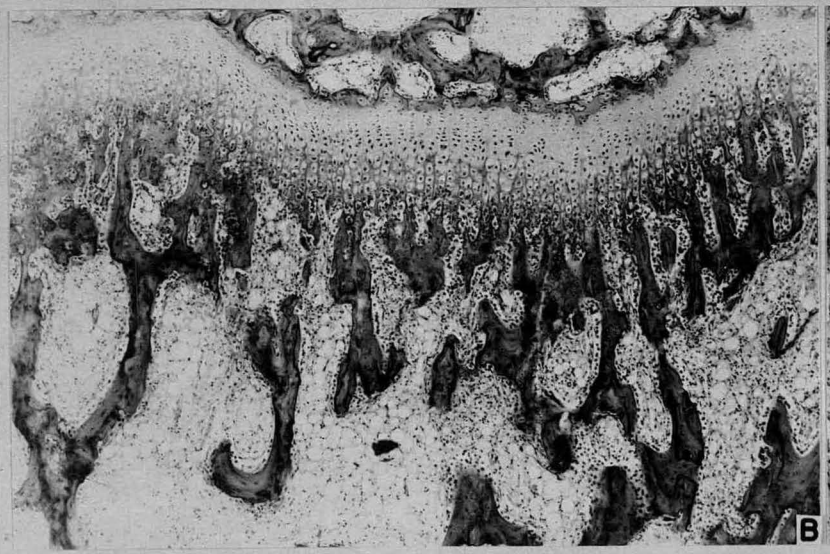


FIG. 16

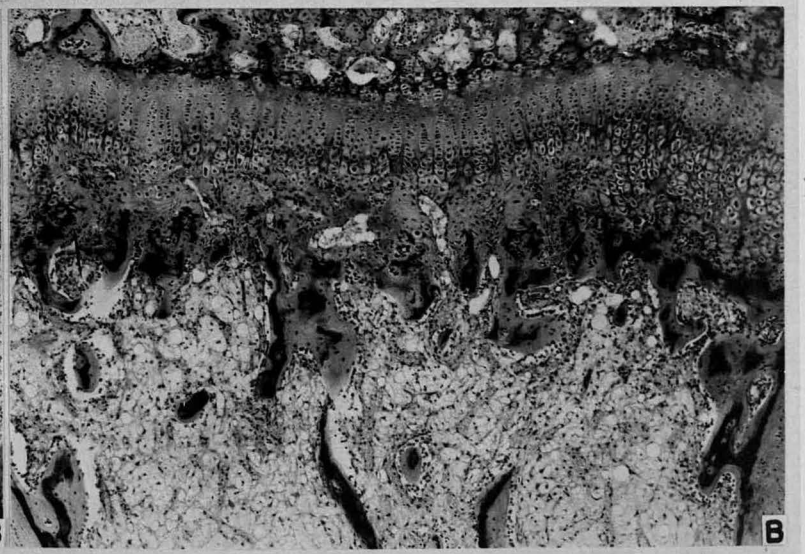


FIG. 17

There are almost no bony trabeculae in the epiphyses. Only spicules of calcified intercartilaginous matrix are observed with narrow osteoid borders. The small secondary ossification centers have formed very recently and this confirms the roentgenographic evidence of retarded maturation. The largest amount of osteoid tissue is on the periosteal surface of the shaft. The width of the epiphyseal cartilage (Fig. 17B) is broader than that of the pair-fed controls. The average proximal and distal epiphyseal cartilage width is 264 micra. As the cartilage cells approach the line of erosion they become distorted and compressed. Numerous convoluted osteoid trabeculae are visible containing blue-staining mineralized cores.

Discussion

The above experiments were planned to study the effects of severe phosphorus deficiency upon several bones of the rat skeleton. In spite of the presence of adequate amounts of vitamins A and D, severe rickets was produced as seen roentgenographically and histologically. It must therefore be assumed that the phosphorus deficiency was the most important factor in the production of rickets.

Furthermore the degree of severity of the rachitic disturbance depended upon the age of the animals and the length of the experimental period. Roentgenograms of the bones studied revealed a lack of mineralization and stunted growth compared with the pair-fed and ad libitum controls. They also showed a retardation in skeletal maturation. The first pathological change noted in all the bones was the failure of the intercartilaginous matrix of the zone of enlarged cells to calcify. This failure to mineralize may be associated with the low level of

inorganic blood phosphate in the deficient animals. The invasion of the uncalcified cartilage by the capillaries and connective tissue is not only retarded but also irregular. Cell multiplication of the chondrocytes was not immediately impeded, but failure of calcification and subsequent resorption resulted in a larger number of cells, with an increase in the width of the epiphyseal cartilage. The capillary invasion may have been inhibited by the lack of calcification or by the phosphorus deficiency per se. The result of these changes in cartilage was a retardation of that phase of bone growth which is dependent upon endochondral ossification.

After 41 days on the phosphorus deficient diet there was observed a partial invasion and erosion of the uncalcified cartilage. This was evidenced by large capillaries which appeared at the line of erosion. The animals sacrificed after 49 days on the diet showed these capillaries invading the uncalcified cartilage. The renewed invasion of cartilage may be related to the genetic and endocrine changes which occur at about 70 days in the rat. It is known that invasion of cartilage and healing occurs in rickets after a period of starvation. No evidence of calcification of the vesicular zone was detected before the cartilage removal began. It has been shown (2) that at approximately the same age as the resumed cartilage manifests itself, there is a slowing of the growth processes in the rat. The growth of cartilage fails to keep pace with the removal of these cells at the line of erosion and the epiphyseal cartilage becomes narrowed in width.

The formation of the organic matrix by the osteoblasts appears undisturbed but the second phase, the calcification of the matrix, does

not occur. The rate of formation of the bony matrix, however, does not appear to be increased. It is probably the failure of the osteoid tissue to be resorbed which prevents the remodeling of the various bones as they grow in length. This lack of resorption would account for the gross deformities seen in the tibia and the costochondral junction of the rib. It is interesting to observe that the histological severity of the rickets was not increased appreciably by an increase of the Ca:P ratio from 28:1 to 78:1, but did appear to increase with the age of the animals and the time during which they had been restricted to the low phosphorus diet.

Metabolic balance studies (13) on the deficient rats revealed a negative phosphorus balance, and loss of bone ash. It appears that phosphorus may be removed from bone to provide for the essential phosphorus needs of the soft tissues (6). There was a positive phosphorus balance in the case of both pair-fed and ad libitum control rats, and their bones were well mineralized.

Summary and Conclusions

1. Severe rickets was produced in growing rats fed diets very low in phosphorus (0.005 percent to 0.015 percent) despite the presence of presumably adequate amounts of vitamins A (25 U.S.P. units per gram) and D (8 I.U. per gram).

2. Roentgenograms showed a marked lack of mineral in the skeleton of the deficient animals, and a delay in skeletal maturation in the vertebra.

3. Histological studies were made of tibia, metacarpal, caudal vertebra and costochondral junction. Signs of severe rickets were evident

in all four bones. The following changes were observed in the epiphyseal region:

(a) An increase in width of the epiphyseal cartilage.

(b) Lack of a provisional zone of calcification.

(c) Failure of epiphyseal cartilage to be resorbed.

(d) Compression of chondrocytes.

(e) Formation of osteoid tissue.

(f) Lack of remodeling resorption of osteoid matrix.

(g) Invasion of the cartilage by blood vessels, and replacement with osteoid matrix occurring at approximately 60-70 days of age.

4. While an increase in the Ca:P ratio from 28:1 to 78:1 did not appear to intensify the severity of rickets in these animals definite changes were observed with increasing age and time on the low phosphorus diet.

5. The gross deformities of the bones in rickets may be attributed to the failure of the intercolumnar matrix and osteoid matrix to calcify. Remodeling resorption also fails to take place and the volume of osteoid is increased.

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Information Division
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Legends

Fig. 1 Reproductions of roentgenograms of rat carcasses (half natural size), 32 days on experimental diet; 53 days of age at autopsy.

A. Phosphorus deficient rat, Spec. 9386, Pl. B460
B. Pair-fed control, Spec. 9381, Pl. B478
C. Ad libitum rat, Spec. 9377, Pl. B477

Fig. 2 Reproductions of roentgenograms of rat tibias (twice natural size), 49 days on experimental diet; 70 days of age at autopsy.

A. Phosphorus deficient rat, Spec. 9456, Pl. 383
B. Pair-fed control, Spec. 9459, Pl. 382
C. Ad libitum control, Spec. 9450, Pl. 381

Fig. 3 Reproductions of roentgenograms of rat metacarpals (twice natural size), 49 days on experimental diet, 70 days of age at autopsy.

A. Phosphorus deficient rat, Spec. 9455, Pl. B399
B. Pair-fed control, Spec. 9462, Pl. B400
C. Ad libitum control, Spec. 9451, Pl. B398

Fig. 4 Reproductions of roentgenograms of rat caudal vertebrae (twice natural size) 49 days on experimental diet; 70 days of age at autopsy.

A. Phosphorus deficient rat, Spec. 9452, Pl. B393
B. Pair-fed control, Spec. 9460, Pl. B394
C. Ad libitum control, Spec. 9450, Pl. B392

Fig. 5 Proximal tibial epiphyseal cartilage of a female rat, 70 days of age at autopsy; central sagittal section. H and E stain.

A. Ad libitum control, X25, Spec. 6161, Pl. B365
B. Ad libitum control, X68, Spec. 6161, Pl. B239

Fig. 6 Proximal tibial epiphyseal cartilage of a female rat, 62 days of age at autopsy, central sagittal section. H and E stain.

A. Pair-fed normal control, X25, Spec. 9539, Pl. B189
B. Pair-fed normal control, X68, Spec. 9539, Pl. B204

Fig. 7 Proximal tibial epiphyseal cartilage of a female rat, 24 days on experimental diet, 45 days of age at autopsy; central sagittal section. H and E stain.

A. Phosphorus deficient rat, X25, Spec. 9366, Pl. B177
B. Phosphorus deficient rat, X68, Spec. 9366, Pl. B198

Fig. 8 Proximal tibial epiphyseal cartilage of a female rat, 41 days on experimental diet, 62 days of age at autopsy, central sagittal section. H and E stain.

A. Phosphorus deficient rat, X25, Spec. 9545, Pl. 468
B. Phosphorus deficient rat, X68, Spec. 9545, Pl. 466

Fig. 9 Proximal tibial epiphyseal cartilage of a female rat, 49 days on experimental diet, 62 days of age at autopsy, central sagittal section. H and E stain.

A. Phosphorus deficient rat, X25, Spec. 9455, Pl. B173
B. Phosphorus deficient rat, X68, Spec. 9455, Pl. B193

Fig. 10 Proximal tibial epiphyseal cartilage of a female rat, 61 days on experimental diet, 61 days of age at autopsy, central sagittal section. H and E stain.

A. Phosphorus deficient rat, X25, Spec. 9535, Pl. B190
B. Phosphorus deficient rat, X68, Spec. 9535, Pl. B195

Photomicrographs of central sagittal sections of the third metacarpal of female rats. H and E stain.

Fig. 11 Pair-fed control, 62 days of age at autopsy; X90, Spec. 9539, Pl. B341

Fig. 12 Phosphorus deficient rats, 70 days of age at autopsy, 49 days on experimental diet, 70 days of age at autopsy, X90, Spec. 9453, Pl. B339

Photomicrographs of central sagittal sections of the costochondral junction of the third rib of female rats. H and E stain.

Fig. 13 Ad libitum control, 70 days of age at autopsy; X68, Spec. 9449, Pl. B367

Fig. 14 Pair-fed control, 70 days of age at autopsy; X68, Spec. 9458, Pl. B368

Fig. 15 Phosphorus deficient rats, 70 days of age at autopsy; 49 days on experimental diet, X68, Spec. 9454, Pl. B366

Fig. 16 Photomicrographs of central sagittal sections of the ninth caudal vertebra of female rats, 41 days on the experimental diet; 62 days of age at autopsy.

A. Pair-fed control, X20, Spec. 9538, Pl. B480
B. Pair-fed control, X90, Spec. 9538, Pl. B336

Fig. 17 Photomicrographs of central sagittal sections of the ninth caudal vertebra of female rats, 61 days on the experimental diet; 82 days of age at autopsy.

A. Pair-fed control, X20, Spec. 9535, Pl. B479
B. Phosphorus deficient rat, X90, Spec. 9535, Pl. B337