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SECTION MEETINGS

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16270

Skeletal Growth in Pyridoxine Deficient Mice.*

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The present investigation deals with the histological changes in the long bones associated with the arrest of skeletal growth in pyridoxine deficient mice.

Material and Methods. Twenty-eight male and female mice of strain C57 black, 28 days old, were used. Fourteen animals were placed on a synthetic diet complete except for the lack of pyridoxine (Group I). Fourteen animals were placed on a synthetic diet complete except for pyridoxine but containing 54% casein (Group II). Animals of corresponding strain and age, fed a stock diet (Purina Laboratory Chow), used in previous experiments served as normal controls. The rations were composed as follows:

	Group I (Pyridoxine free)	Group II (Pyridoxine free, high protein, low sucrose)
Sucrose	68 g	30 g
Vit. test casein	18 "	54 "
Vegetable oil	10 "	10 "
U.S.P. salt mix. No. 12	4 "	4 "

* Aided by a grant of the Committee on Scientific Research of the American Medical Association and the U. S. Public Health Service.

Cod liver oil	1 cc	1 cc
Riboflavin	0.5 mg	0.5 mg
Thiamin	0.2 "	0.2 "
Nicotinic acid	1.0 "	1.0 "
Choline HCl	1.0 "	1.0 "
Inositol	100.0 "	100.0 "
Ca pantothenate	10.0 "	10.0 "
Biotin	25 μg	25 μg

The animals were kept on the deficient diets for 1, 2, 4 or 8 weeks. Of those fed the deficient diets for 4 weeks, 8 were refed the stock diet (Purina Laboratory Chow) for 1, 3, 5, 10 or 14 days. Paraffin sections of the kneejoint were prepared and stained with hematoxylin and eosin.

Group I. After 1 week's deficiency, the epiphyseal discs at the upper end of the tibia were narrowed from the normal 300 to 170 microns. The cartilage cells were decreased in size, and instead of the usual 4 hypertrophic and 10 columnar cells there were 3 hypertrophic and 7 columnar cells in the individual cartilage cell row. The intercellular matrix was somewhat increased. The metaphysis was vascular, and the trabeculae were covered by numerous osteoblasts. The cortex measured 85 microns in diameter which is about normal. After 2 weeks, the epiphyseal discs were 150 microns wide and contained 2 or 3 cells of hypertrophic and 6 of columnar type. Both cell types were markedly reduced in size. The metaphyseal spicules were shortened and linked with one another. The osteoblasts were more oblong than ordinarily. Severe changes were noted after 4 weeks' deficiency: The epiphyseal discs measured 90 microns in width, and the cartilage cells had undergone additional shrinkage; the cartilaginous matrix was more abundant and denser. The few trabeculae present were short, and here and there, interlaced. The osteoblasts in the metaphysis and along the endosteum were spindle-shaped and markedly decreased in number. Osteoclasts were scarce. The cortex of the shaft was thinned out, and particularly at the junction of the epiphyseal disc and the shaft ("Umbauzone"), there was a conspicuous decline of activity: Little bone was present, osteoblasts were scanty and small, and the precartilage usually arising from the perichondrium failed to develop. The articular cartilage showed corresponding atrophy. After 8 weeks, the epiphyseal discs as well as the peripheral areas of remodeling presented a picture of complete inactivity. The former measured 50 microns in width. The individual cartilage cell row contained 4 to 6 very small columnar and 2 shrunken cells of hypertrophic type. Hyalinization of the matrix was widespread, and in the distal third of the epiphyseal disc dense deposits of calcium were noted. Vascular erosion of the cartilage had ceased, and bony trabeculae were lacking. A very thin often membrane-like bony lamella delimited the cartilage from the marrow. The few fragments of osseous spicules present in the distal metaphysis were covered by a discontinuous layer of spindle-shaped cells. The cortex of the shaft measured in some places as little as 20 microns in diameter and was considerably thinner than the fibrous periosteum. The cartilage of the joint was atrophic.

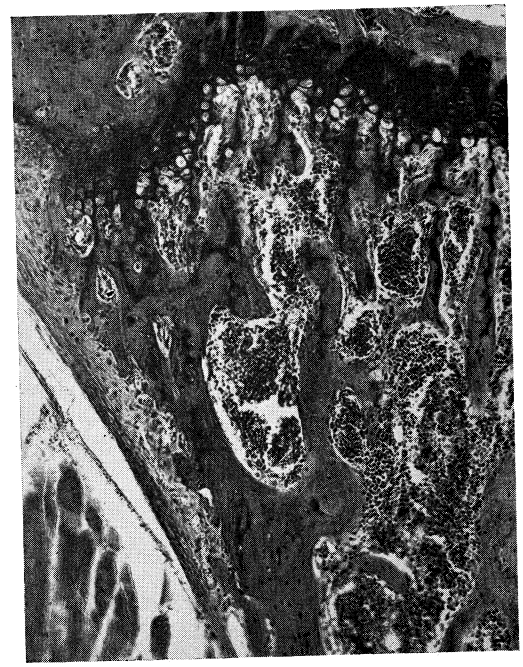

FIG. 1.

Section through the upper end of the tibia of a female mouse of strain C57, 12 weeks old, fed the stock diet. The growth zone is hyalinized and calcified. The hypertrophic cartilage cells are distinct. The bony spicules are long and thick. The shaft contains abundant bone. Medium high magnification.

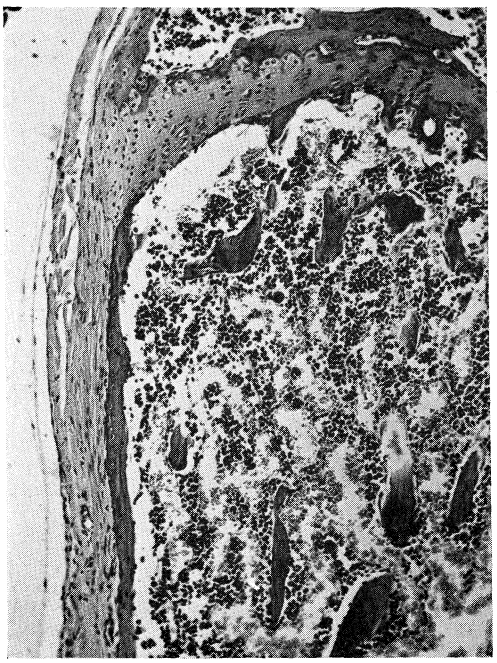

FIG. 2.
Section through the upper end of the tibia of a female mouse of strain C57, 12 weeks old, kept on a pyridoxine-deficient diet high in protein for 8 weeks. There are no hypertrophic cartilage cells, and bony trabeculae are rudimentary. A thin osseous lamella delimits the epiphyseal cartilage from the bone marrow. The shaft is markedly thinned out. Same magnification as No. 1.

There were no definite sex differences in the susceptibility to pyridoxine deficiency.

The early effects of refeeding were observed after 3 days: The epiphyseal discs at the upper end of the tibia were about 160 microns wide as compared with 90 microns after 4 weeks of deficiency. The hypertrophic cartilage cells were larger and their number had increased from 1 to 3 cells. The columnar cells likewise appeared larger and mitotic proliferation was resumed. The new formation of cartilage from the perichondrium at the periphery of the epiphyseal discs was particularly conspicuous. After 5 days of refeeding, the epiphyseal disc was 200 microns wide and thus only one-third narrower than usual. Columnar and hypertrophic cartilage cells had further increased in size, and they were present in almost normal numbers (3:9 as compared with a normal of 4:10). Osteoblasts were numerous, although they were still more oblong than ordinarily. The cortex had become thicker and measured 90 microns. During the second week of refeeding, the growth processes were restored to almost normal, and the epiphyseal discs were even somewhat wider than in normally fed animals of corresponding age. Mitoses were not infrequent in the proliferating cartilage cells. However, there were still some increase of the matrix and slight atrophy of the cartilage cells. Replacement of the hypertrophic cartilage cells by bone proceeded at a normal rate; osteoblasts were abundant and large. The cortex and articular cartilage were of usual structure.

Group II. During the first 2 weeks, the effect of the high protein on the pyridoxine deficiency was not definite. However, after 4 weeks a distinctly unfavorable influence of the addition of casein became noticeable. The growth zones at the upper end of the tibia measured 60 microns in width as com-

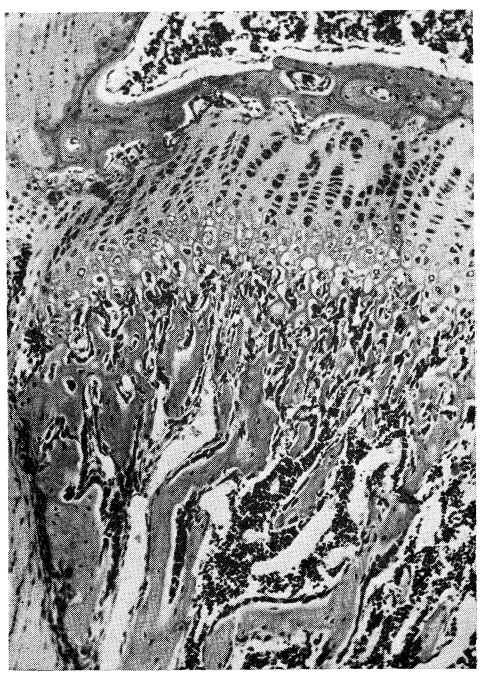

FIG. 3.
Section through the upper end of the tibia of a female mouse of strain C57, 9½ weeks old, kept on a pyridoxine-deficient diet for 4 weeks, and refed the stock diet for 10 days. The epiphyseal disk is wide, cellular and has a youthful appearance. Growth, development, and osseous replacement of cartilage are in progress. Much bone is present. Same magnification as No. 1.

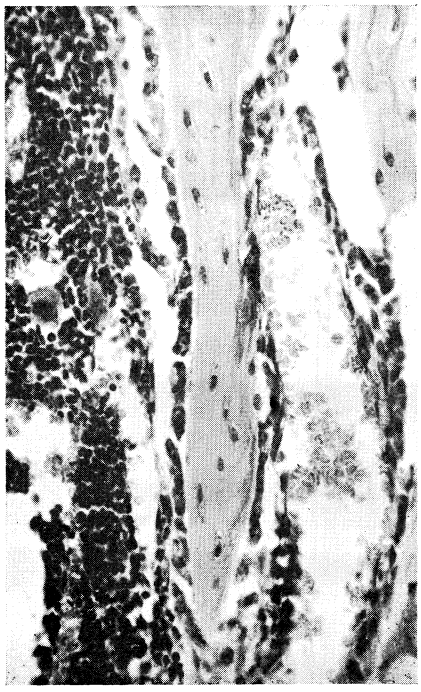

FIG. 4.
High power photograph of a bony trabecula of the same animal as in Fig. 1. The spicule is thick and is covered by a continuous layer of large osteoblasts.

pared with 90 microns in mice kept on the deficient diet with regular protein content. The cells were smaller and fewer than in the latter group, and the matrix was more hyalinized. These differences were accentuated after 8 weeks and manifested themselves chiefly in the absence of hypertrophic cartilage cells and in even more advanced hyalinization of the matrix.

Resumption of growth after refeeding was slower in the group receiving the high protein diet. A noteworthy enlargement of the epiphyseal discs was not seen until after 5 days of realimentation. Even after 10 days, the cartilage cells were still smaller than ordinarily, although the individual cartilage cell row contained the usual number of cells. Mitotic proliferation of the cartilage cells was less prominent indicating a comparatively slow rate of growth. Moreover, plugs of degenerated cartilage and hyalinization of the ground substance were still noticeable. Osteoblasts were rather scanty, and they had not yet completely regained their epithelioid shape.

Summary and Conclusions. In pyridoxine deficient young mice, growth of cartilage and formation of bone were inhibited and finally ceased. Upon refeeding the complete diet, recovery was noticeable after 3 days and was still in progress after 14 days. The growth processes were quickly restored with mitoses present in the germinal layer of the cartilage as well as in the osteoblasts.

The effects of pyridoxine deficiency on cartilage resemble those seen in pantothenic acid deficient mice,[1] whereas the disturbance of ossification was not unlike that observed in riboflavin deficiency.[2] The specificity of the skeletal changes may thus be questioned.

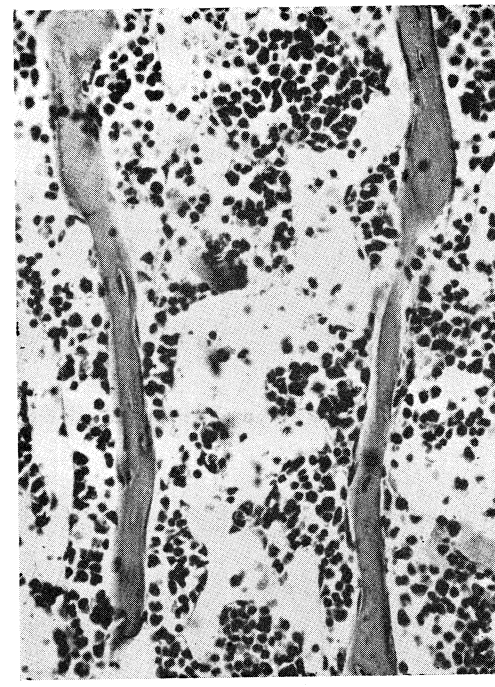

FIG. 5.
High power photograph of 2 bony trabeculae of a female mouse of strain C57, 12 weeks of age, and fed a pyridoxine-deficient diet for 8 weeks. The spicules are thin and covered only here and there by spindle-shaped cells. Osteoblasts are not seen. The bone marrow is atrophic. Same magnification as No. 4.

1 Levy, B. M., and Silberberg, M., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 380.

2 Levy, B. M., and Silberberg, R., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 355.

In young rats fed a pyridoxine deficient diet,[3] the changes in the growth zones were comparable to those of underfed young guinea pigs,[4] kept on a quantitatively restricted but adequately balanced diet. To some extent this is true for the cartilage of mice also. However, the cartilage cells as well as the osteoblasts responded more unfavorably to the pyridoxine deficiency than to general underfeeding, and the formation of bone was more reduced than in any of our previous experiments. This difference might indicate a specific effect of the pyridoxine deficiency, but it may be a manifestation of the more marked sensitivity of the mouse to the deficiency as compared to that of the rat.[5,6]

Feeding a high protein diet accentuated the effects of pyridoxine deficiency inasmuch as growth of cartilage and bone was more inhibited than in pyridoxine deficient mice receiving a diet with regular casein content. These findings are in agreement with previous observations on the greater requirements for pyridoxine in animals fed high protein diets.

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4 Silberberg, M., and Silberberg, R., *Arch. Path.*, 1940, **30**, 675.

5 Cerecedo, L. R., and Foy, J. R., *Arch. Biochem.*, 1944, **5**, 207.

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16271

Experimental Fort Bragg Fever (Pretibial Fever) in Chimpanzees.*

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The chimpanzee (*Pan Satyrus*) has been used extensively in experimental work on poliomyelitis[1,2] and to a lesser degree in work on the common cold,[3] but the use of this animal as an experimental host for the reproduction of human infectious disease is a field deserving further exploration. Particularly would this seem to apply to the virus field and to diseases which have readily discernible cutaneous manifestations. We have recently made attempts to induce in these animals 3 human virus diseases: sandfly (Phlebotomus or pappataci) fever, dengue fever, and Fort Bragg (pretibial) fever. This paper will be concerned only with a description of our experiments with the latter disease. Results with the other two diseases will be described in another paper.[4]

Fort Bragg fever was first described by Daniels and Grennan,[5] under the term *pretibial fever*. It is a disease characterized by an acute fever of approximately 5 days' duration, frontal and postorbital aching, splenomegaly, and often with a patchy erythematous rash on the pretibial regions.† Respiratory manifestations are minimal and not persistent. Cases of this disease have appeared in groups during the summer months of 1942, 1943, and 1944 on the military reservation at Fort Bragg, N. C. The manner in which it spreads among humans has not been determined.

* This investigation was conducted by the Commission on Virus and Rickettsial Diseases, Army Epidemiological Board, Office of the Surgeon General, U. S. Army, Washington, D.C.

1 Bodian, D., and Howe, H. A., *J. Exp. Med.*, 1947, **81**, 255.

2 Melnick, J. L., and Horstmann, D. M., *J. Exp. Med.*, 1947, **85**, 287.

3 Dochez, A. R., Shibley, G. S., and Mills, K. C., *J. Exp. Med.*, 1930, **52**, 701.

4 Paul, J. R. Melnick, J. L., and Sabin, A. B., to be published.

5 Daniels, W. B., and Grennan, H. A., *J.A.M.A.*, 1943, **122**, 361.

† The rash on the shins was such a prevalent feature that Daniels and Grennan[5] have designated this disease as *pretibial fever*.