15608

# Inhibition of Endochondral Ossification in Pantothenic Acid Deficiency.

BARNET M. LEVY AND MARTIN SILBERBERG.

*From the Department of Oral Pathology, Washington University School of Dentistry, and the Snodgras Laboratory, City Hospital of St. Louis, St. Louis, Mo.*

Growing mice and rats kept on a diet deficient in pantothenic acid lose or fail to gain weight;[1] in young rats this deficiency causes hypoplasia of the growing cartilage.[2] To our knowledge, however, no detailed studies have been made of the histological changes occurring in the skeleton under this condition.

*Material and Methods.* Thirty-seven mice of the closely inbred strain C57 black were used. Thirty animals were 4 to 5 weeks old, and 7 were 10 to 12 months old at the beginning of the experiments. The mice were fed a synthetic diet deficient in pantothenic acid *ad libitum* for varying periods of time. Water was available at all times. The ration consisted of

| | | |
|---|---|---|
| Sucrose | 68 | % |
| Vitamin test casein | 18 | " |
| Vegetable oil | 10 | " |
| U.S.P. salt mixture No. 2 | 4 | " |
| Cod liver oil | 1 | " |
| Riboflavin | 0.5 mg | " |
| Thiamin | 0.2 " | " |
| Nicotinic acid | 1.0 " | " |
| Pyridoxine | 0.2 " | " |
| Choline chloride | 1.0 " | " |
| Inositol | 100.0 " | "* |

Nine growing animals were kept on the deficient diet for 4 to 5 weeks, after which period they were fed Purina Dog Chow, and repair was allowed to take place for varying periods of time. Details concerning the number of animals, their age, sex and time of observation in each group are given in Table I.

Additional mice of the same strain and sexes, but fed the complete synthetic diet, as well as animals kept on the standard diet, served as controls. Ten mg % calcium pantothenate was added to the deficient diet for synthetic control diet.

At the end of the periods, the experimental and control mice were sacrificed; the tibia and femur were removed as a whole, decalcified in 45% formic acid, embedded in paraffin, sectioned and stained with hematoxylin and eosin. The studies were made at the growth zones of the upper tibia and lower femur.

*Observations. Gross.* After about one week on the deficient diet, the animals began to lose hair, and smooth bald areas appeared on the back or over the rump. This hair loss increased markedly during the experimental period, and the skin became dry and scaly. The weights of the animals are to be found in Column 6 of the table. The mice kept on the deficient diet showed considerable loss of weight. Upon being fed the repair diet, their weight increase was rapid and amounted in some animals to as much as 7 g in 5 days.

*Histological Examination.* A description of the histological appearance of the growth zones in normal mice (Fig. 1), has been given elsewhere.[3] In growing male mice kept on the deficient diet for one week (Fig. 2), the epiphyseal disk was only half as wide as in the controls. The cartilage cell rows were regular, but they contained only from 6 to 4 inactive columnar and from 3 to one hypertrophic cartilage cells instead of the usual number of 10 columnar and 4 hypertrophic cells. Both cell types were distinctly smaller than ordinarily. The ground substance was more abundant than in the controls. Calcification of the provisional cartilage proceeded at a regular rate. The metaphysis contained few and collapsed capillaries. The primary trabeculae were

[1] Morris, H. P., *J. Nat. Cancer Inst.*, 1944, **5**, 115.

[2] Ashburn, L. L., *Pub. Health Rep.*, 1940, **55**, 1337.

* We are indebted to Merck and Company for the liberal supply of vitamins used in this study.

[3] Silberberg, M., and Silberberg, R., *Am. J. Anat.*, 1941, **68**, 691.

TABLE I.

| No. of animals | Sex ♂ | Sex ♀ | Age at beginning of exp. | Time fed deficient diet | Time fed repair diet | Mean wt (g) Initial | Mean wt (g) Final |
|---|---|---|---|---|---|---|---|
| 6 | 3 | 3 | 4-4½ wk. | 1 wk. | — | 13 ± 4 | 13 ± 2 |
| 6 | 2 | 4 | 4-4½ " | 2 " | — | 14 ± 3 | 12 ± 2 |
| 9 | 5 | 4 | 4-4½ " | 4 " | — | 13 ± 4 | 11 ± 2 |
| 3 | 2 | 1 | 4-4½ " | 4½ " | 5 days | 14 ± 3 | 21 ± 3 |
| 2 | 1 | 1 | 4-4½ " | 4½ " | 2 wk. | 15 ± 3 | 23 ± 1 |
| 2 | 1 | 1 | 4-4½ " | 4½ " | 3 " | 15 ± 2 | 24 ± 1 |
| 2 | 1 | 1 | 4-4½ " | 4½ " | 4 " | 16 | 25 |
| 2 | | 2 | 10-12 mo. | 1 " | — | 36 ± 3 | 32 ± 2 |
| 1 | | 1 | 10-12 " | 2 " | — | 36 | 36 |
| 1 | | 1 | 10-12 " | 4 " | — | 36 | 35 |
| 1 | | 1 | 10-12 " | 7 " | — | 37 | 35 |
| 2 | | 2 | 10-12 " | 9 " | — | 34 ± 1 | 32 ± 2 |

much shorter and scarcer than in the controls; they were covered by a discontinuous layer of small and flat spindle cells instead of the usual osteoblastic cell layer. The shaft consisted of small osteocytes; osteoblasts at the inner and outer layers were scanty, and vascularization was less marked than usual.

In the corresponding females (Fig. 3), the changes were less accentuated than in the males. The growth zones were about two-thirds as wide as those of the control female fed the complete synthetic diet. In the single cartilage cell row, 8 or 7 columnar and 3 or 2 hypertrophic cells were counted. They were smaller than in the normal controls, but larger than in the corresponding deficient males. The amount of matrix present was intermediate between the normal and that seen in the deficient males. Similarly, the bony spicules were fewer, shorter and thinner than usual, but they were thicker, longer and more numerous than in the deficient males, and more osteoblasts were found.

In males kept on the deficient diet for 2 weeks, the cartilage cell rows were composed of 5 small columnar cells and one small hypertrophic cell. The matrix had further increased in amount and density, whereas metaphysis and shaft had not changed as compared with the previous stage. After 4 weeks, the structure of the cartilage was not different from that seen after 2 weeks. However, the trabeculae had become still shorter, and they showed some transverse bony links (Fig. 4), indicating a cessation of growth. In healthy untreated animals of this strain, this condition is not seen before the age of 4 to 5 months. Females fed the deficient diet for 2 weeks showed more marked changes than those deficient for one week. The cartilage cell rows were shorter and were composed of 7 or 6 columnar and 3 or 2 hypertrophic cells. After 4 weeks, the single row contained 5 columnar cells, whereas the number of hypertrophic cells remained 3 or 2.

In the animals kept on the deficient diet for 4½ weeks and returned to the standard diet, processes of repair took place rapidly. After only 5 days of refeeding (Fig. 6), the growth zones were as wide and, in some instances, even wider than in the controls; a single row containing from 12 to 10 mitotically proliferating large columnar and 4 large hypertrophic cartilage cells. The matrix was less abundant; calcification of the cartilage and replacement by bone were in good progress. The vascularization of metaphysis and shaft was good. The number and thickness of spicules were the same as seen in young healthy controls, and osteoblasts were plentiful everywhere. The histological pattern resembled that of a rapidly growing control animal 4 to 5 weeks of age. However, at the later stages, in spite of active growth of the epiphyseal cartilage, intensified hyalinization of the matrix indicated some previous damage to the cartilage.

In the adult mice fed the deficient diet, the epiphyseal cartilage was inactive, and in some places epiphyseodiaphyseal union was about to occur. No effect of the defi-

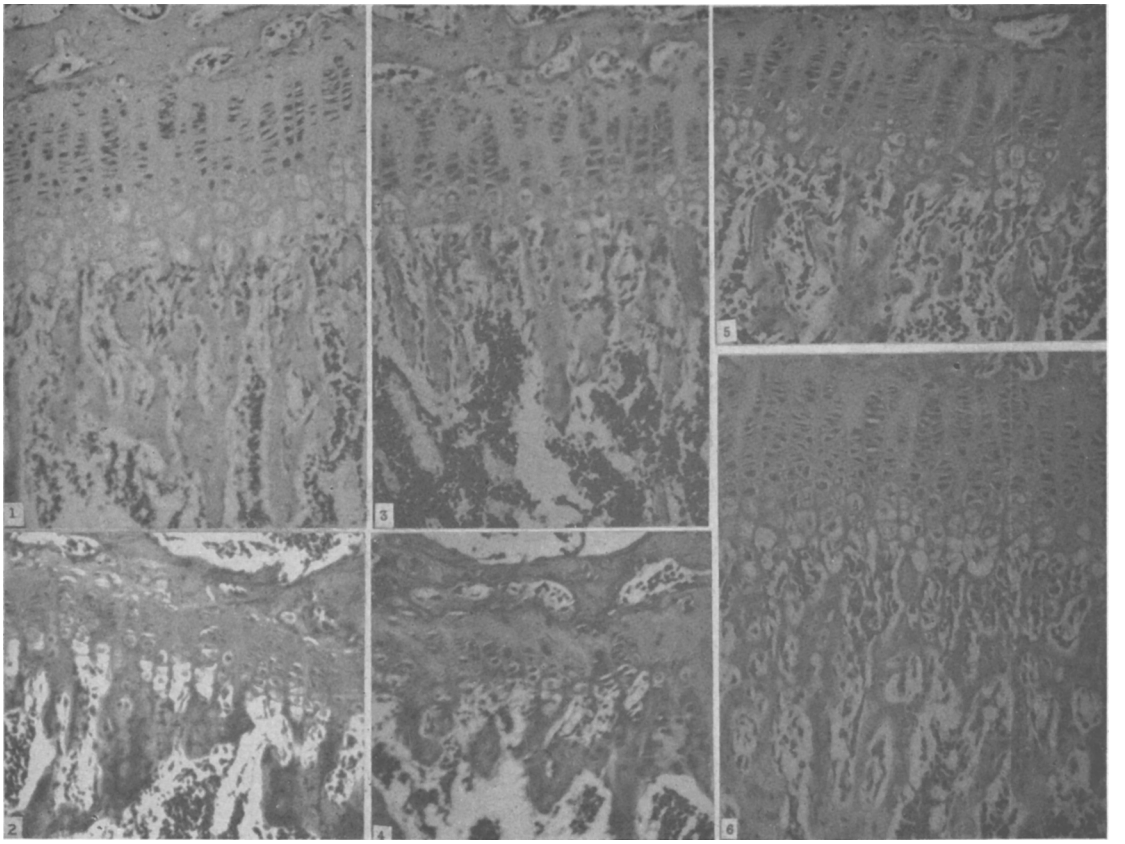

FIG. 1.—Section through the growth zone of the upper tibia of a 6-week-old female mouse of Strain C 57 black fed the synthetic control diet since the age of 4 weeks. Magnification 100×.

The epiphyseal disc is wide and shows regular configuration. The metaphysis contains numerous long trabeculæ separated by bone marrow.

FIG. 2.—Section through the growth zone of the upper tibia of a 6-week-old male mouse of Strain C 57 black fed the deficient diet for one week. Magnification 100×.

The epiphyseal disc is narrowed as compared with Fig. 1, and the subepiphyseal spicules are fewer and shorter than in the control.

FIG. 3.—Section through the growth zone through the upper tibia of a 6-week-old female mouse of Strain C 57 black fed the deficient diet for one week. Magnification 100×.

The epiphyseal disc is narrower than in the control (Fig. 1) but wider than in the deficient male (Fig. 2). The subepiphyseal spicules are decreased in number as compared with the normal control (Fig. 1).

FIG. 4.—Section through the growth zone of the upper tibia of an 8-week-old male mouse of Strain C 57 black fed the deficient diet for 4 weeks. Magnification 100×.

The epiphyseal disc is markedly narrowed in width. The subepiphyseal trabeculæ show transverse bony links.

FIG. 5.—Section through the growth zone of the upper tibia of a 3-week-old female mouse of Strain C 57 black fed the deficient diet for 4 weeks. Magnification 100×.

The epiphyseal plate is wider than in the corresponding male (Fig. 4). There is a suggestion of early transverse bony links uniting the subepiphyseal spicules.

FIG. 6.—Section through the growth zone of the upper tibia of an 8-week-old male mouse of Strain C 57 black fed the deficient diet for 4½ weeks, and subsequently refed the stock diet for 5 days. Magnification 100×.

The epiphyseal disc resembles that of the 6-week-old control animal (Fig. 1).

cient diet was noted in either cartilage, metaphysis, bone marrow or shaft.

*Comment.* In growing mice of strain C57 black, pantothenic acid deficiency caused an inhibition of endochondral ossification. Proliferation as well as hypertrophy of the cartilage were markedly decreased or, at later stages, completely arrested. Associated

with the inhibition of growth was intensified hyalinization of the matrix, but no disturbances in calcification were noted. In the metaphysis, there was deficient formation of osteoblasts leading to a decrease in the number and size of primary spicules. Vascularization and ossification of the shaft were likewise decreased. The decreased growth processes in the epiphyseal cartilage are comparable to those observed in growing rats kept on pantothenic acid-deficient diet;[2] they are compatible with and account for the reduced growth rate of mice thus treated.[1] The skeletal changes observed in this series of mice are different from those seen in animals kept on a quantitatively reduced but qualitatively adequate diet. In the latter, growth processes proceed at a decreased rate;[4] ossification may be increased in spite of the decreased growth rate, and the bone marrow undergoes serous atrophy. In pantothenic acid deficiency, ossification ceased with the proliferation and development of the cartilage, and no changes in the bone marrow occurred.

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

In the male, these changes were accentuated after only one week, whereas in the female the growth inhibition reached a similar degree after 2 weeks. After 4 weeks, animals of both sexes showed about the same changes in the growth zones. Since, at this age, the normal growth rate in males is greater than that of the females, it is not surprising that the male responded more readily to the vitamin deficiency than the female. The skeletal tissues of the adult mouse are not as dependent on this vitamin as those of the growing animal.

*Summary.* In growing mice, pantothenic acid is essential for the growth of the skeleton. Deficiency of this vitamin causes inhibition of growth and endochondral ossification. The male reacts more quickly to lack of pantothenic acid than the female. On feeding an adequate stock diet subsequent to a prolonged deficiency, growth of cartilage and ossification are resumed rapidly and may surpass in intensity the processes seen in normal nondeficient animals. In the adult mouse, skeletal changes were not observed under the influence of pantothenic acid deficiency.

## 15609

## Tests for Hepatic Dysfunction of Mice.*

J. CASALS AND PETER K. OLITSKY.

*From the Laboratories of The Rockefeller Institute for Medical Research, New York City.*

Attempts to transmit infectious hepatitis and homologous serum jaundice to laboratory animals have been made by several investigators with results that are negative or inconclusive. In this laboratory we have tried to infect mice with human material derived either from spontaneous cases of infectious hepatitis or from artificially-induced serum jaundice. These experiments are still in progress.

* We thank Dr. H. A. Schneider for his help in the studies here reported on *S. enteritidis* and dietary influences. We are also indebted to Dr. R. E. Shank for his cooperation.

In the course of the latter work it was thought that mice may develop experimentally an inapparent disease with hepatic damage, without showing objective signs. In such a case it would be advantageous (a) to recognize the condition and (b) to follow the course of the damaged liver without sacrificing the animal to observe the lesions. Also, if blind passages were attempted, the knowledge of the state of the hepatic function might give an indication as to the best time to carry out such passages. For these reasons we undertook an investigation of tests of hepatic dysfunction in Swiss mice,