ing that has been practiced wittingly, and that only to a limited extent, has been for running activity in revolving drums.

The reduced number of fungiform papillae may be an instance of the effect of domestication which results when rats no longer have to forage for their food or to depend on finer taste differentiations in the selection of their food. Before such a conclusion could be drawn, it would be necessary to know whether domestic rats from other colonies show a similar reduction in the number of papillae. Whether or not a direct relation exists between keenness of taste and the total number of fungiform and foliate papillae (and, indirectly, the taste buds contained in them) remains unknown. Taste-threshold tests made before and after sectioning of taste nerves to the various papillae may provide an answer to this question.

Finally, attention may be called again to the opportunity offered by these findings for studies in the field of genetics. An actual count of the papillae makes it possible to express a feature of a strain of rats in numerical rather than in qualitative terms.

*Summary.* 1. The number of fungiform and foliate papillae were counted on the tongues of 100 recently trapped wild Norway rats and of 20 wild Alexandrine rats, and compared with the number on the tongues of 103 domestic Norway rats. 2. The number of fungiform papillae on the tongues of the domestic Norway rats ranged from 114 to 221, with a mean of 178.3, while for the recently trapped wild Norway ancestors it ranged from 167 to 271, with a mean of 217.9, and for the Alexandrine rats it ranged from 147 to 231, with a mean of 191.5. The mean of the domestic Norway rat thus showed a 17% reduction from that of its wild ancestor. 3. The number of foliate papillae ranged from 8 to 16 for the domestic rats, with a mean of 11.98, and ranged from 7 to 20 for the wild Norways, with a mean of 11.46. The 2 strains thus showed essentially the same number of foliate papillae, but both showed fewer than are found in Alexandrine rats, in which the number of papillae ranged from 9 to 18, and had a mean of 13.5. 4. In general, both types of papillae were more uniform in size, structure and distribution in the wild than in the domestic strains.

15600

## Effect of Riboflavin Deficiency on Endochondral Ossification of Mice.*

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The role of riboflavin in the maintenance of normal body growth has been demonstrated in rats, mice, pigs,[1] and monkeys.[2] Growth is retarded if riboflavin is omitted from the diet, and young rats are born with skeletal abnormalities[3] (especially micromelia) if the mothers are fed a riboflavin-deficient diet during pregnancy. These observations indicate a disturbance of endochondral ossification. The present investigation was undertaken in order to determine which phase of this process is dependent upon the presence of riboflavin, and to compare the find-

* Aided by the Louis M. Monheimer Memorial Fund.

1 Eddy, W. H., and Dalldorf, F., *The Avitaminoses. The Chemical, Clinical and Pathological Aspects of the Vitamin Deficiency Diseases,* 3rd Edition, The Williams & Wilkins Company, Baltimore, 1944.

2 Cooperman, J. M., Waisman, H. A., McCall, K. B., and Elvehjem, C. A., *J. Nutrition,* 1945, **30**, 45.

3 Warkany, F., and Nelson, R. C., *Arch. Path.,* 1942, **34**, 375.

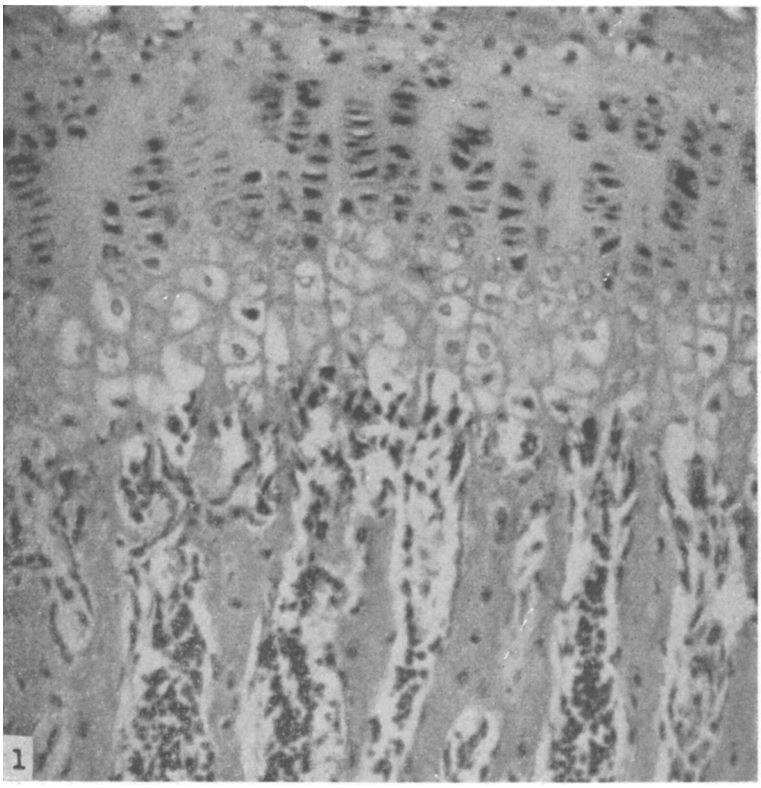

FIG. 1.
Growth zone of the upper tibia of a female mouse of Strain C57, black, 42 days of age. Mag. 200 ×.

ings with those obtained in pantothenic acid deficiency.[4]

*Material and Methods.* Twenty-four growing and 11 adult male and female mice of Strain C57, black, were fed a diet which was complete except for the lack of riboflavin.†

The ration was composed as follows:

| | |
|---|---|
| Sucrose | 68 % |
| Vitamin test casein | 18 " |
| Vegetable oil | 10 " |
| U.S.P. salt mixture No. 2 | 4 " |
| Cod liver oil | 1 " |
| Thiamin | 0.2 mg% |
| Nicotinic acid | 1.0 " |
| Pyridoxine | 0.2 " |
| Choline chloride | 1.0 " |
| Inositol | 100.0 " |
| Calcium pantothenate | 10.0 " |

The growing mice were 30 days old, and the adult mice were 9 or 14 months of age at the beginning of the experiments. The animals were kept on the deficient diet for periods of one, 2, 3 or 4 weeks and, in the adult series, for 5 weeks. After each of these periods 2 or 3 animals were killed. In addition, 4 animals fed the deficient diet for 4 weeks were returned to a normal diet of Purina Chow for periods of 6 or 10 days and were then sacrificed. At the autopsy the tibia and femur were removed together, fixed in 10% formalin, decalcified with 45% formic acid, sectioned and stained with hematoxylin and eosin. All mice kept on the deficient diet either failed to gain weight or made slight gains up to 4 g. After one week the hair appeared greasy and stringy. After 2 weeks, there was slight loss of hair especially around the mouth and on the back. As the deficiency progressed, several small ulcers appeared around the mouth,

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

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

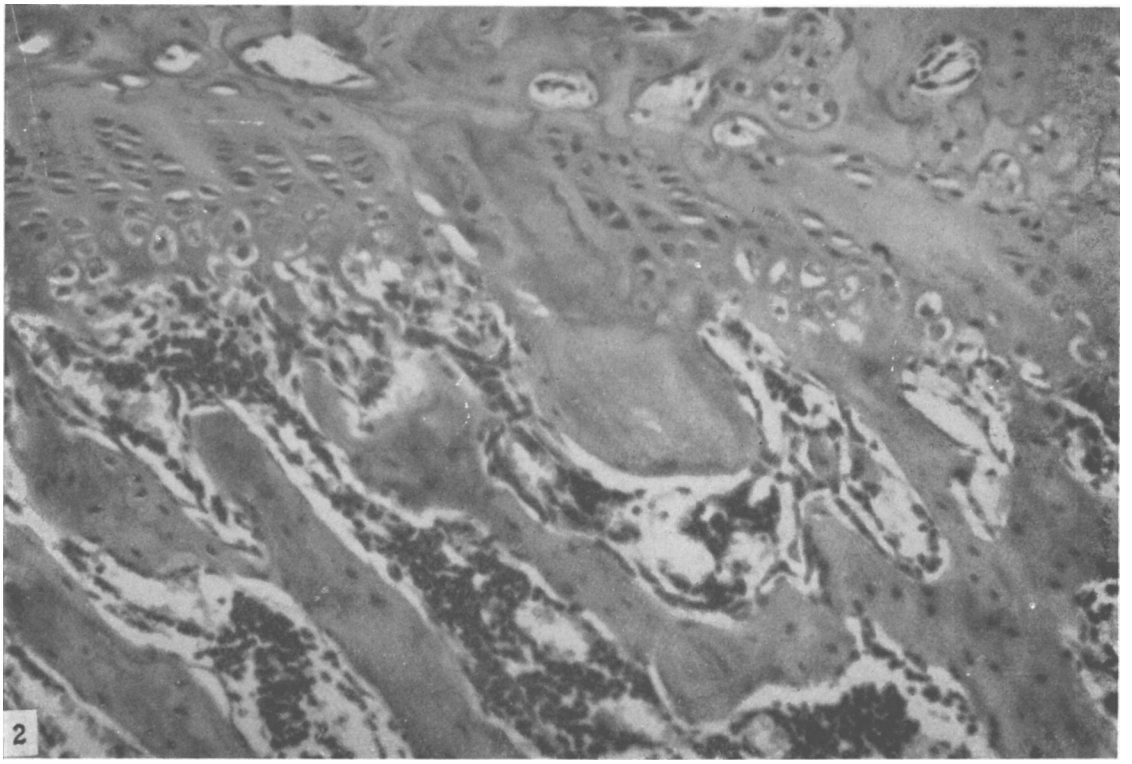


FIG. 2.
Growth zone of the upper tibia of a female mouse of Strain C57, 58 days of age and kept on a riboflavin deficient diet for 28 days. The growth zone is greatly narrowed and traversed by a thick plug of degenerated cartilage. Mag. 200 ×.

and scabs were noted on the back, ears and tail.

*Histological Findings. Growing Animals.* The epiphyseal zone of the animals kept on the deficient diet for one week was of normal width, containing 10 columnar and 4 hypertrophic cells. There was slight increase in the intercartilaginous matrix. The nuclei of the columnar cells were clumped and more irregular than usual. There was active breakdown of the hypertrophic cartilage. The subepiphyseal spicules were normal in number and thickness. The metaphyseal capillaries were well filled and accompanied by numerous osteoblasts, as is usual. The bony shafts were likewise unchanged.

In animals kept on the deficient diet for 2 weeks, the growth zone was narrower. In the single cartilage cell row, the number of columnar cells was decreased to 6 or 8 and that of the hypertrophic cells to 2 or 3. Both types of cells were smaller than normal, and the columns were irregular. There was hyalinization of the matrix and, here and there, amorphous plugs began to form, traversing the entire width of the growth zone. The spicules were fairly thick, but shorter than ordinarily. The metaphyseal capillaries were less filled than previously and partly collapsed. Osteoblasts were present in moderate numbers, but they were more oblong than usual, and clusters of osteoblasts were found in grooves at the surface of spicules without osseous substance being deposited between them. The shafts showed slightly enlarged vessels.

Feeding of the deficient diet for 3 or 4 weeks caused a marked narrowing of the growth zone (Fig. 1 and 2). There were 6 columnar and one or 2 hypertrophic cells in a single cartilage cell row, both types of cells being smaller than usual and showing irregular nuclei. The matrix was even more hyalinized, and moderately wide amorphous

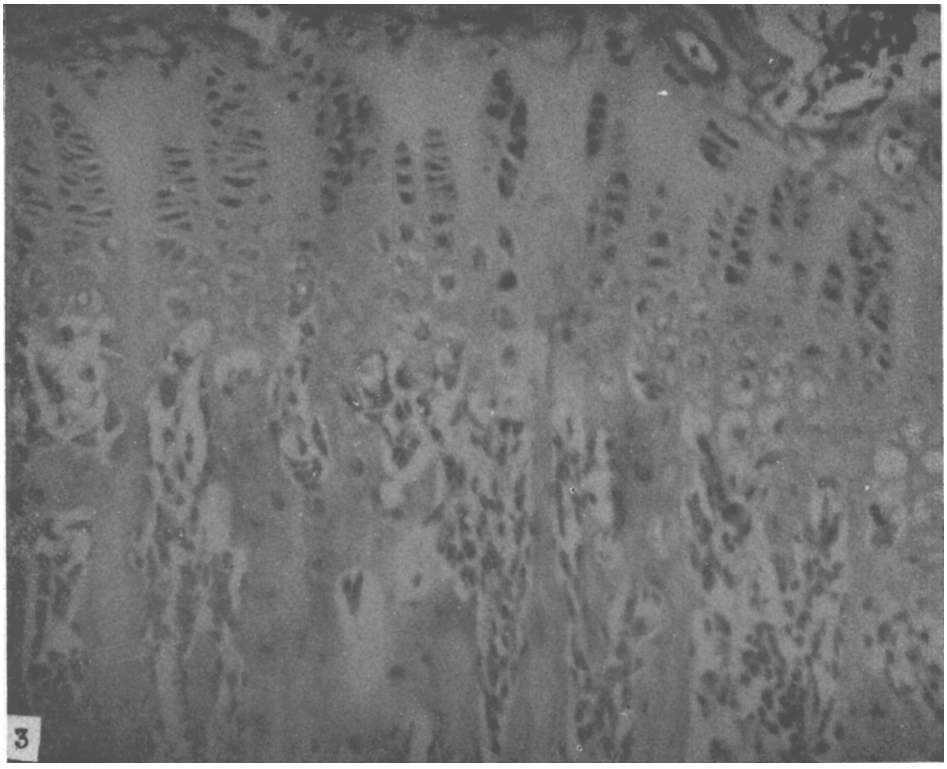


FIG. 3.
Growth zone of the upper tibia of a female mouse of Strain C57, black, 68 days old, kept on a riboflavin deficient diet for 4 weeks and refed an adequate stock diet for 6 days. The growth zone is wider than in Fig. 2. A plug of degenerated cartilage is seen in the middle of the field, being pushed toward the metaphysis. Mag. 200 ×.

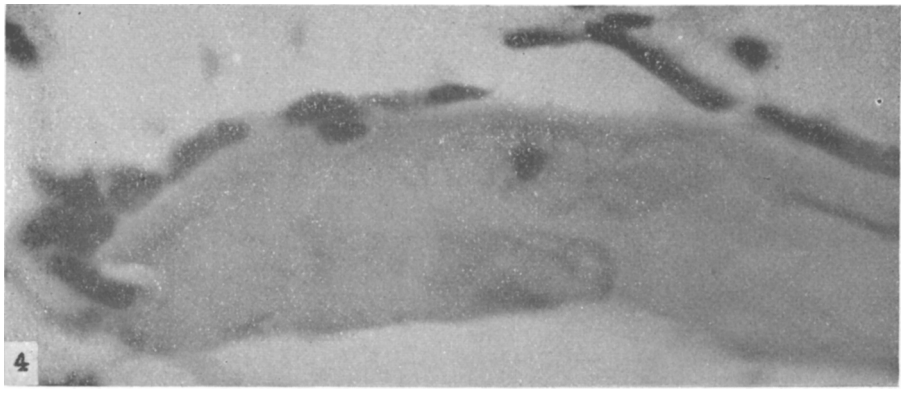


FIG. 4.
Section through the metaphysis of the same mouse as in Fig. 2. A bony spicule surrounded by a single layer of elongated spindle-shaped osteoblasts. Mag. 1275 ×.

plugs of destroyed cartilage appeared between the preserved cartilage rows. Still, the metaphyseal capillaries were breaking down the hypertrophic cartilage and in some places had advanced almost to the border of the columnar cartilage, where bone was being deposited. The trabeculae were further shortened. However, there was no trans-

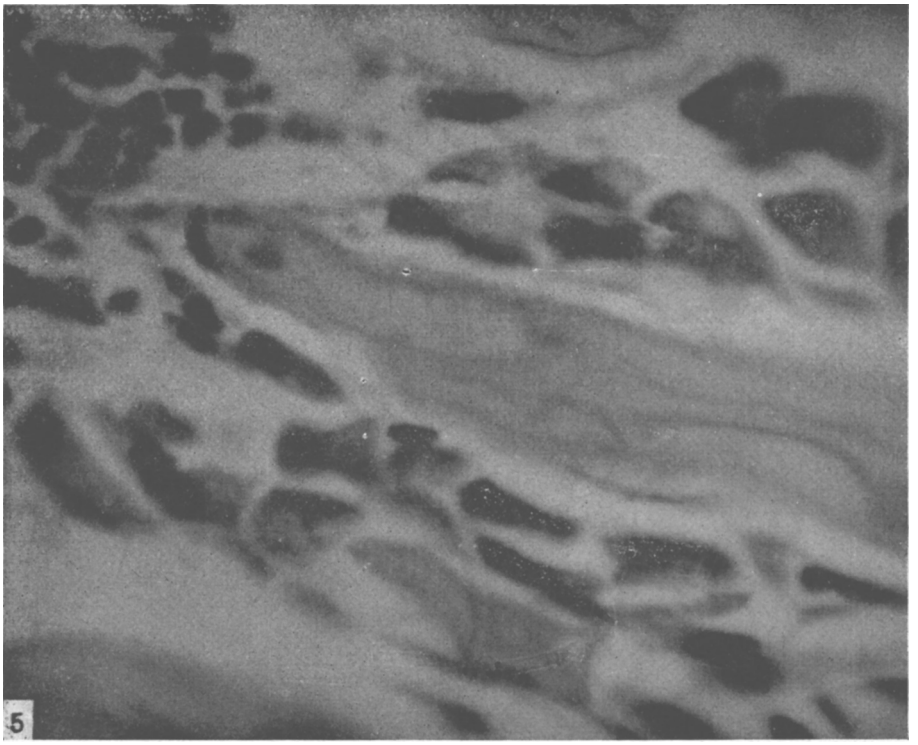

FIG. 5.
Section through the metaphysis of the same mouse as in Fig. 3, showing a bony spicule surrounded by large osteoblasts. Mag. 1275 ×.

verse bony plate, as is found in conditions associated with arrest of growth. Osteoblasts were scantier than before, and they were spindle-shaped (Fig. 4). They formed small clusters here and there, suggesting an attempt to form spicules. No osseous matrix, however, was deposited between them. The shafts were thin and showed only a scattering of spindle-shaped osteoblasts at the endosteal surface.

The above findings were present in male as well as in female mice. After one or 2 weeks, the males showed somewhat more marked changes than the females, but after 3 or 4 weeks the findings were about equal in both sexes.

Upon refeeding, there was a rapid return of the growing cartilage to normal conditions (Fig. 3). After 6 days the epiphyseal plates were enlarged, due to an increase in size as well as in the number of the cells. There were now 8 to 9 columnar and 2 to 3 hypertrophic cells. There was still increased hyalinization of the matrix, and an occasional small amorphous plug of degenerated cartilage could be found. These plugs, however, did not traverse the entire growth zone, but reached from the level of the most distal hypertrophic cartilage cells to about the middle of the width of the columnar zone. It thus seemed as if repair of the cartilage was accomplished by replacement from the undifferentiated cartilage and that the degenerated tissue was pushed down toward the metaphysis. A few eosinophilic leucocytes were noted in the vicinity of these plugs. The metaphyseal capillaries were well filled and accompanied by numerous large osteoblasts (Fig. 5), some of which were still spindle-shaped, whereas others were polygonal, as is usual. The trabeculae were somewhat longer than in the corresponding animal which was not refed.

After 10 days of refeeding, repair had made further progress. The growth zone was of normal width. There were now 10 to 12

columnar and 3 hypertrophic cells, the columnar cells being somewhat increased over the normal and indicating an energetic resumption of growth. The metaphysis was well vascularized. The spicules were of normal length and width, and they were covered by thick layers of large polygonal osteoblasts. Repair was accomplished more rapidly in females than in males.

*Adult Animals.* In control animals the epiphyseal disc was composed of a small amount of inert degenerated cartilage, sealed off on both sides by a bony plate. The bony shaft and the epiphyseal spicules were lined by large, ovoid osteoblasts. In the mice kept on the deficient diet for 4 and 5 weeks, no changes were noted in the growth zone. However, the number of osteoblasts was decreased, and the bony structures were covered by a discontinuous layer of spindle-shaped cells.

*Summary and Conclusions.* In growing mice, riboflavin deficiency decreases both the rate of growth and of ossification, but intensifies the degeneration of the cartilage. These changes represent the histological basis for the inhibition of lengthwise growth seen in riboflavin-deficient animals. As compared with the changes seen in pantothenic acid deficiency, lack of riboflavin inhibits the proliferation of the cartilage to a lesser extent, whereas it produces degeneration of the cartilage, not seen in pantothenic acid deficiency. As in pantothenic acid deficiency, males are more sensitive to the lack of the vitamin than are females.

In adult mice, the endosteal and peritrabecular osteoblasts undergo atrophy.

## 15601 P

## Infection Experiments with the Hookworm (*Bunostomum phlebotomum*) in Calves.

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The calves used in these experiments were obtained from the L. S. U. Dairy Department as soon after birth as possible, usually within 36 hours, and raised under parasite-free conditions in the animal building of the Veterinary Science Department.

Three animals 2½ to 13 months old have been inoculated with infective larvae by mouth. All had begun passing eggs by between 64 and 83 days after receiving the larvae. One animal developed a diarrhea lasting about 10 days at approximately 30 days after inoculation.

Three other calves were inoculated by placing larvae on the skin of the flank. These animals were then fastened in stanchions in such a manner as to prevent licking the larvae from the skin, in one instance for 42 days, another 67 days, and in the case of the 3rd for 97 days. Two of these calves developed a very severe bloody diarrhea 31 and 38 days after the application of the larvae, which continued for 10 days in one, and for 18 days in the other. A very marked skin irritation developed over the area of application of the larvae in all 3 animals. Two of these animals began passing eggs on the 57th and 65th days after the application of the larvae. The third calf with the most severe diarrhea and which became the most emaciated, remained negative to the end of the experiment, 133 days.

Three additional calves began passing hookworm eggs at between 60 and 115 days of age after being raised under conditions free of parasitic infection. The animal that was positive at 115 days was not examined between the 78th and 115th day and it is believed that a positive examination could have been secured earlier than the 115th day. It seems evident that these 3 animals secured their infective larvae during the few hours