Effect of Cortisone on Growing Bones of the Rat. (18607)

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Because of the profound changes which may be encountered in the skeleton in cases of Cushing's syndrome, particularly in the infant(1), we have felt it was desirable to determine whether such lesions could be reproduced experimentally with adrenal cortical hormone. Wells and Kendall(2) have reported a retardation of growth in rats treated with Compound E. Winter *et al.*(3) have administered 3 mg cortisone daily to rats weighing 200 g. Sections of the ribs and tibiae of such animals revealed a reduction in the width of the epiphyseal cartilage but no rarefaction of cortical or cancellous bone.

Experimental. Cortisone acetate* was administered subcutaneously each day to male rats (McCollum strain) weighing 40-50 g at the beginning of the experiment. The following amounts of cortisone in mg per kilo body weight per day were employed: 0.25, 2.5, 10.0, 20.0, 40.0, 50.0, 75.0, and 100.0. There were 6 to 8 rats in each group. To control those groups which failed to gain the food intake of normal rats was restricted so as to give comparable growth curves. At varying intervals animals were sacrificed; the ribs and long bones were fixed in 80% alcohol or neutral 4% formalin, dehydrated, embedded in paraffin or nitrocellulose and stained with hematoxylin-eosin or eosin-toluidine blue.

Results. When daily doses of cortisone amounting to 0.25, 2.5, 10.0 and 20.0 mg per kilo were administered to young rats there was no appreciable effect on their growth over a period of 3 weeks as compared with control animals of the same weight. However, when 40 or 50 mg per kilo were administered, after an initial gain for 1 or 2 days the growth curves flattened out and remained so during a 3 to 4 week interval. Amounts of cortisone greater than this led to a prompt plateauing of the growth curve followed by a gradual loss of weight.

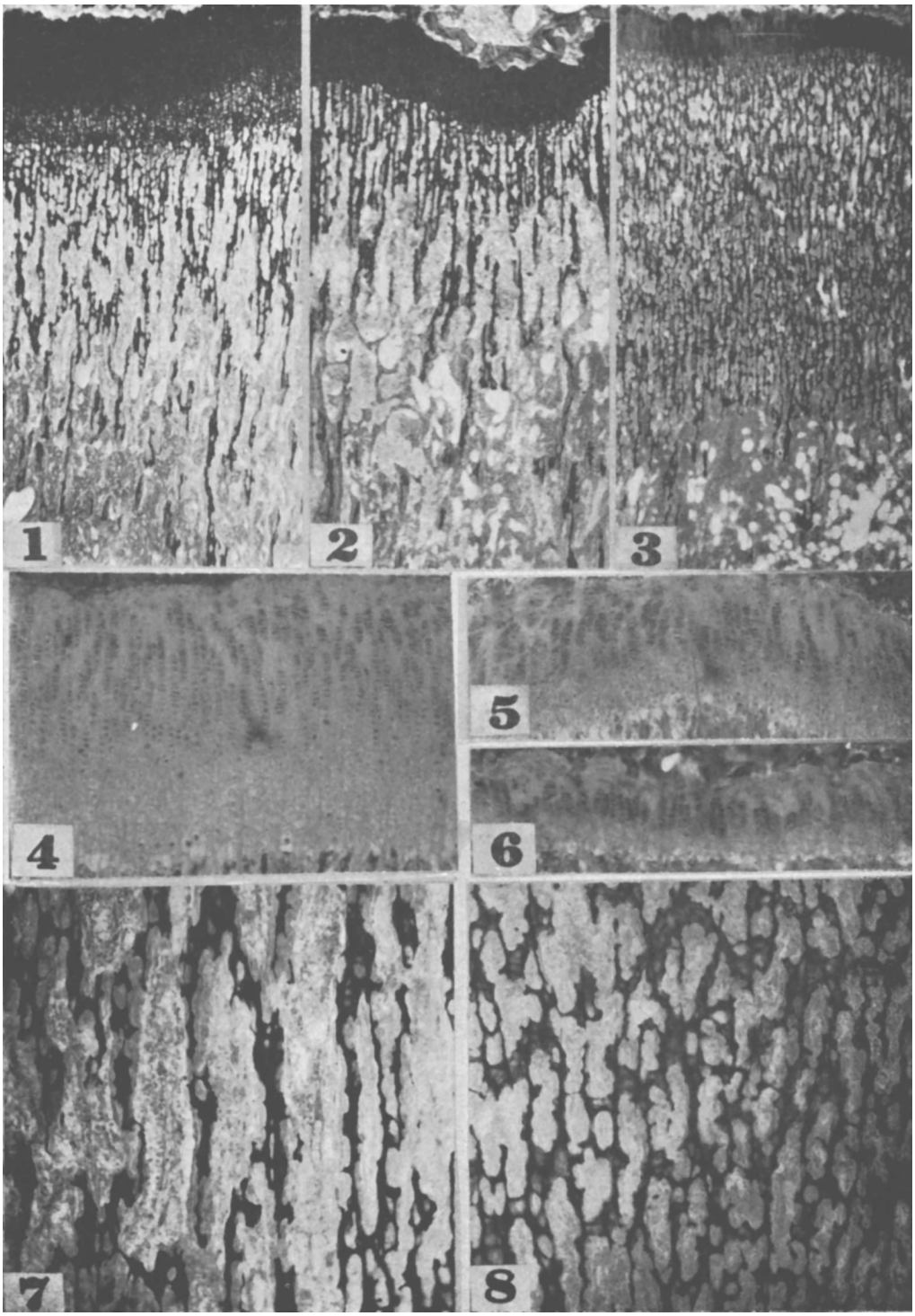
Examination of the skeletal tissues revealed no change in the groups receiving .25 to 20 mg per kilo, *i.e.* those whose growth was unaffected. In the bones of the rats receiving 40 or 50 mg per kilo profound alterations were found. After being treated for 3 days there was a definite narrowing of the epiphyseal cartilage due in large part to a reduction in the number of hypertrophic cells, but also as a result of retardation in the proliferation of undifferentiated cells. More prominent was the presence of an increased amount of calcified cartilagenous matrix encased in bone beneath the epiphyseal cartilage. As the duration of treatment continued this zone increased in width so that after 3 weeks there was a broad zone of dense interlacing trabeculae each with a central core of cartilage matrix encased in bone. There did not appear to be any excess number of osteoblasts nor were osteoclasts present in any conspicuous form. There was no increased activity of the periosteal cells nor was there any evidence of increased or decreased endosteal bone formation in the cortex of the shaft. In comparison with the changes noted above at the cartilage-shaft junction in rats whose growth was inhibited by caloric restriction, although the width of the epiphyseal cartilage plate was reduced, there was rarefaction of the underlying spongiosa. When rats to which cortisone had been administered for 2 weeks were then allowed to go untreated there was a resumption of normal growth activity of the cartilage. The mass of cartilagenous matrix encased in bone moved away from the cartilage as the latter proliferated and was then slowly destroyed. Animals receiving 75 or

1. Follis, R. H., Jr., *Bull. Johns Hopkins Hosp.*, 1951, v88, 440.

2. Wells, B. B., and Kendall, E., *Proc. Staff Meetings Mayo Clin.*, 1940, v15, 324.

3. Winter, C. A., Silber, R. H., and Stoerk, H. C., *Endocrinol.*, 1950, v47, 60.

* The cortisone acetate (Cortone) was furnished through the kindness of Merck and Co., Rahway, N. J.



All sections stained with eosin-toluidine blue.

FIG. 1. Normal tibia of young rat (×32).

FIG. 2. Tibia of rat whose diet had been restricted calorically so weight gain was similar

to rat shown in Fig. 3. Note great reduction in width of epiphyseal cartilage and fewer trabeculae in shaft (×32).

FIG. 3. Rat treated with 50 mg cortisone for 3 weeks. Note great narrowing of cartilage and increased density of zone made up of cores of calcified matrix encased in bone. Fig. 1, 2, and 3 are all of same magnification (×32).

FIG. 4. Higher power of epiphyseal cartilage of normal rat (×64).

FIG. 5. Higher power (same magnification as Fig. 4) of epiphyseal cartilage of rat shown in Fig. 2 (×64).

FIG. 6. Higher power (same magnification as Fig. 4 and 5) of same cortisone treated animal shows in Fig. 3 (×64).

FIG. 7. High power view of spongiosa of normal rat (×64).

FIG. 8. Higher power view (same magnification of rat shown in Fig. 7) to show increased amount of persisting calcified matrix encased in bone (×64).

100 mg cortisone daily per kilo whose growth curves showed a drop did not exhibit the above change, due apparently to complete cessation of growth activity.

Discussion. There would appear to be two possible ways to explain the pathogenesis of the changes noted above: increased osteoblastic activity or decreased osteolytic activity or a combination of both. As far as the former is concerned there does not appear to be an increased number of osteoblasts about the trabeculae of the spongiosa beneath the atrophic epiphyseal cartilage. There is no excessive formation of cortical bone, either periosteal or endosteal. The width of the zone of increased density corresponds with the interval in which the animal was treated with cortisone so that one must assume that normal osteoblastic growth sequences have taken place. On the other hand the persistence of large amounts of calcified cartilagenous matrix encased in bone is certainly most abnormal so that the conclusion seems unescapable that there is an inhibition or retardation in normal osteolytic sequences. Such changes are, of course, not those which may be encountered as a result of caloric restriction or deficiency of one or more of the forty-odd essential nutrients. Under such circumstances there is narrowing of the epiphyseal cartilage together with rarefaction of the spongiosa beneath(4).

The change produced in these bones by cortisone is most reminiscent of alterations which may be encountered in the growing rat when large amounts of estrogen are administered. Some years ago the conclusion was reached(5) that the increased density at the growing ends of the bones of estrogen treated rats was due to some derangement in destructive activity. This interpretation has been upheld by others(6). The mechanism is, of course, unknown. In view of the peculiar effect of estrogen on the overproduction of endosteal bone in the mouse it will be most interesting to study the effects of cortisone in this species.

Summary. When cortisone acetate in appropriate doses is administered to growing rats, a dense zone composed of spicules of calcified cartilagenous matrix encased in bone appears at the growing ends of the bone. The width of this zone may be correlated with the duration of administration of the hormone. It is concluded that this area of increased density results from a disturbance in normal osteolytic activity.

4. Follis, R. H., Jr., *J. Mount Sinai Hosp.*, 1949, v16, 1.

5. Day, H. G., and Follis, R. H., Jr., *Endocrinol.*, 1941, v28, 83.

6. Urist, M. R., Budy, A. M., and McLean, F. C., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 324.

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