CHANGES IN THE SKELETAL TISSUES OF MICE FOLLOWING THE ADMINISTRATION OF THYROXIN*

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In immature guinea pigs, administration of thyroid hormone stimulates, for a certain length of time, the proliferation of the cartilage of the growth zones and of the joints. It also accelerates the differentiation of the proliferating cartilage cells into cartilage cells of the hypertrophic type, and increases the resorptive processes in the osseous tissues (Silberberg and Silberberg, 1938 and 1940). We noticed, however, that the maximum of proliferation was reached after twenty days. It seemed, therefore, desirable to investigate the effect of prolonged administration of thyroid hormone on cartilage and bone.

For this purpose, we studied the changes in the long bones and joints of mice which had received injections of thyroxin in experiments undertaken for other purposes by Leo Loeb.

MATERIAL AND METHODS

Sixteen mice, thirteen of which belonged to strain D, two to strain C57, and one to strain Old Buffalo, received subcutaneously 0.1 mg. thyroxin (Schering) on three days of the week, for periods of 2 weeks, and 1, 2, 3, 10, 11, 13 and 14 months. The animals were 6 to 8 weeks old at the beginning of the experiment. At autopsy the bones were removed and subsequently prepared for microscopic study in the same manner as in our former investigations (1941a).

MICROSCOPIC EXAMINATION

(1) *Zone of endochondral ossification*:

After two weeks of administration of thyroxin, the epiphyseal disk of the upper tibia was enlarged. Whereas, in untreated animals of

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corresponding strain and age (Fig. 1)*, the length of the cartilage cell rows begins to decrease and greater amounts of sclerosed matrix cause the columnar arrangement of the growth zones to become more irregular, in mice injected with thyroxin (Fig. 2) the cartilage cell rows were high and contained many cells. The columnar structure of the cartilage cell rows was still quite distinct, the ground substance was scarce and chondromucoid in character. The non-oriented cartilage cells were spindle-shaped and showed long plasmatic processes. In a single cartilage cell row 10 to 11 columnar cartilage cells were counted. instead of 7 to 8 as is normal for this age. These cells proliferated markedly, and frequently by way of mitotic division. They were conspicuously enlarged, took on a polyhedric and ellipsoid shape, and their cytoplasm became slightly honey-combed. The conversion of these cells into cells of hypertrophic type began to take place more proximally in the epiphyseal zone than ordinarily. However, even in the most distal parts of the epiphyseal disk they were not quite as large as the largest hypertrophic cartilage cells under normal conditions. One has the impression that the processes of both hypertrophy and breakdown of the cartilage cells were so greatly accelerated that, on the one hand, the majority of the columnar cells became hypertrophied, and, on the other hand, the hypertrophic cells broke down before they could reach their maximum size.

After continuation of the injections for a period of one month, the zone of endochondral ossification was greatly narrowed (Fig. 3). The cartilaginous matrix had increased in amount and density. More calcium salts than usual were deposited in the most distal layers of the cartilage cells, and not infrequently the cells became enclosed by acidophilic, pre-osseous ground substance and thus were converted directly into osteocytes. The cartilage columns were rather irregularly arranged and were shorter and less numerous than normally. An exact cell count could no longer be made. Simultaneously, atrophy and degenerative processes had affected individual cells as well as whole cell rows, and had converted them into amorphous material. In some of these areas, hyalinization and ossification had set in and led to the formation of numerous fairly thick bony plugs, traversing the entire width of the cartilaginous plate from the metaphysis into the epiphysis. The condition in the epiphyseal disk of these mice at the age of 10 to 12 weeks was comparable to that of normal mice of the corresponding strain 4 to 6 months of age (Silberberg and Silberberg, 1941a).

These changes became still more accentuated if thyroxin was given for periods of two months and over. After two months of injections, matrix and cells of the epiphyseal cartilage were even more intensely

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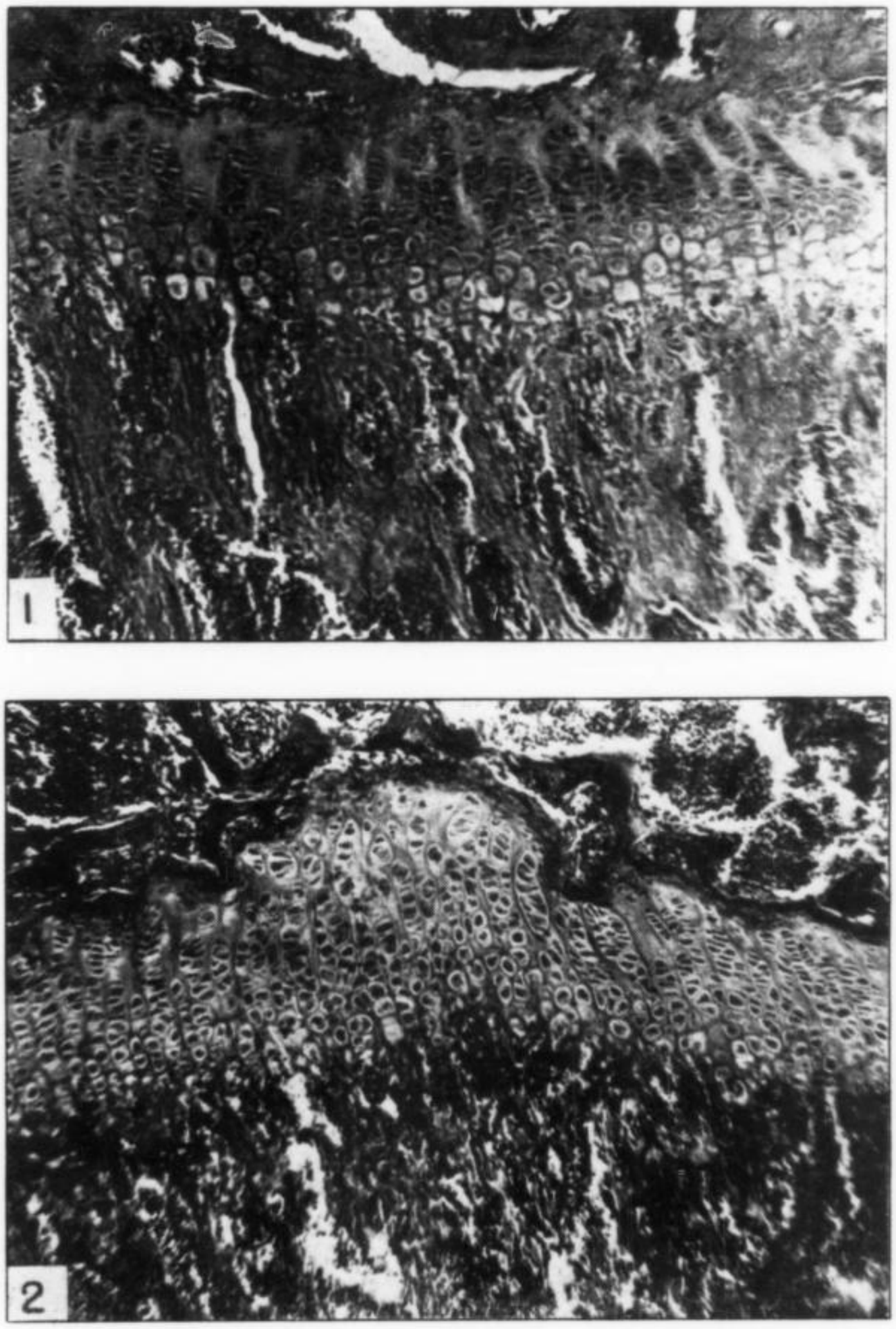

Fig. 1. Section through the epiphyseal zone of the upper tibia of a normal female D mouse two months of age. Magnification 150×.

Fig. 2. Section through the epiphyseal plate of the upper tibia of a female D mouse two months of age, which starting at the age of six weeks, had received injections of 0.1 mg of thyroxin three times weekly. Compare with Fig. 1. Epiphyseal disk somewhat enlarged, cartilage cell rows higher, columnar cartilage cells enlarged, large hypertrophic cartilage cells lacking, cartilaginous ground substance scarce, bony trabeculae thin. Same magnification as in Fig. 1.

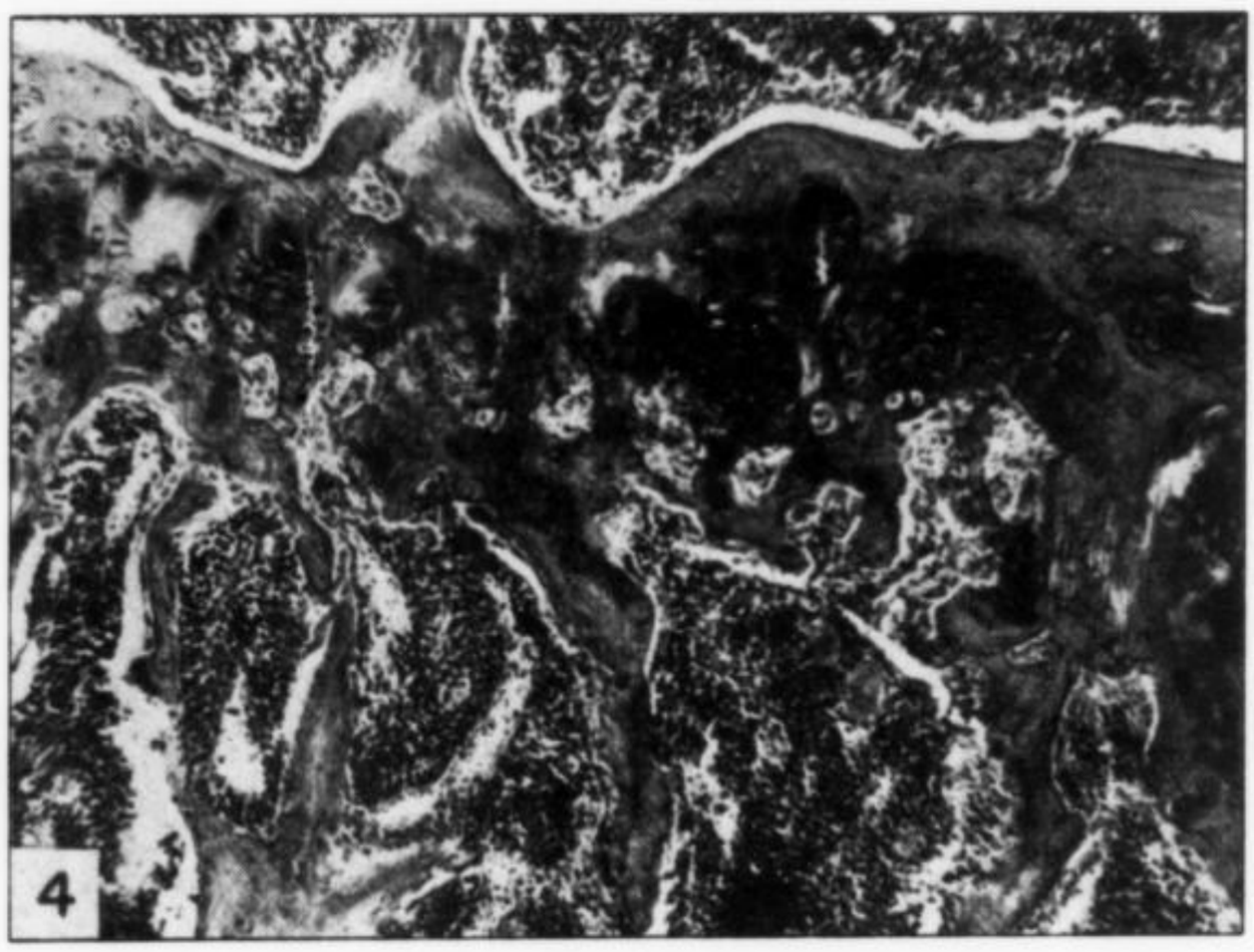

Fig. 3. Section through the epiphyseal zone of the upper tibia of a female D mouse two and a half months old, which had been injected with 0.1 mg thyroxin three times weekly for a period of one month. Epiphyseal zone narrowed and sclerosed, containing thick osseous plugs. In the subepiphyseal zone there is a beginning formation of a transverse bony lamella. Short and thin osseous spicules. Magnification 150×.

Fig. 4. Section through the epiphyseal zone of the upper tibia of a female D mouse three and a half months old, which, starting at the age of six weeks, had received injections of 0.1 mg thyroxin three times weekly. Compare with Fig. 3. The epiphyseal zone is greatly narrowed,sclerosed, calcified and ossified. The subepiphyseal osseous plate and the bony trabeculae have become thickened. Same magnification as in Fig. 3.

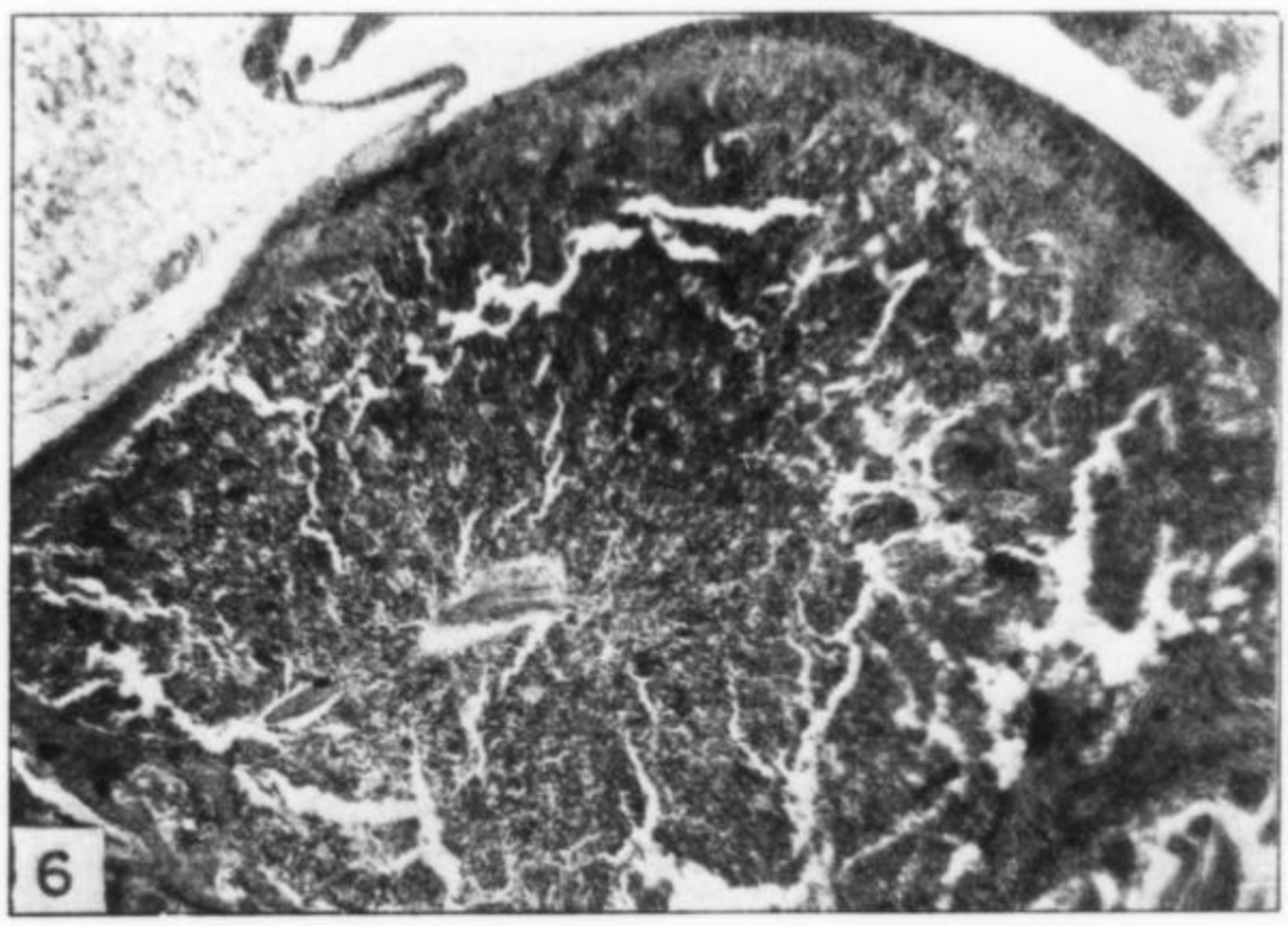

Fig. 5. Section through the epiphyseal plate of the lower femur of a female D mouse three and a half months old, which had been injected with 0.1 mg thyroxin three times weekly for a period of two months. Solution processes have led to a perforation of the epiphyseal plate. Conditions otherwise similar to those in No. 4. Same magnification as in Fig. 4.

Fig. 6. Section through the epiphyseal zone of the upper tibia of a female D mouse thirteen months old, which starting at the age of two months, had received injections of 0.1 mg thyroxin three times weekly. Complete epiphyseo-diaphyseal union has taken place. Two small remnants of bone indicate the site of the former epiphyseal plate. Magnification 120×.

calcified, sclerosed and ossified (Fig. 4). Frequently, owing to dense incrustation with calcium salts, details in the structure of the cartilage cells could no longer be detected. Centers of retrogression and ossification had become more numerous and more extensive. At the same time, capillaries of the bone marrow advanced towards and into the osseous plugs, and on account of capillary absorption and increased osteoclastic activity, the bony structures were at first perforated and later dissolved, producing increasingly widening gaps in the epiphyseal plate (Fig. 5). Thus, under the influence of thyroxin, in D mice, epiphyseo-diaphyseal union was in progress at the age of three and a half months, a condition found in non-injected mice of this strain not before the age of six to seven months. With extension of the duration of the experiment, degeneration and ossification of the epiphyseal cartilage, as well as solution of the almost completley ossified epiphyseal plate, made further advance.

After ten or more months of injections, the epiphyseal plate had been entirely dissolved (Fig. 6), and a complete epiphyseo-diaphyseal union had taken place. Only remnants of a narrow bony lamella arranged in a transverse direction indicated the site of the former epiphyseai zone. A similar stage of epiphyseo-diaphyseal union has not been observed in normal mice of any strain or age. The changes in the distal epiphyseal zone of the femur took a course similar to that seen in the proximal part of the tibia.

In the three mice belonging to strains C57 and Old Buffalo the reaction of cartilage and bone was the same in kind as in the mice of strain D. However, the processes were somewhat delayed as compared with the corresponding stages in mice belonging to strain D.

(2) *Subepiphyseal zone*:

After two weeks of injections of thyroxin, the subepiphyseal layer contained congested capillaries surrounded by closely packed connective tissue cells. These cells proliferated mitotically and were quickly converted into epithelioid osteoblasts. The osseous ground substance deposited was dense but not large in amount, and consequently the bony spicules were thin. Osteoclasis was marked and led to a shortening and thinning of the trabeculae.

After thyroxin had been administered for one month, the number of spicules was decreased because of the continued resorptive processes. However, the trabeculae which were still present were fairly long and thickened as compared with the earlier stage. Underneath the epiphyseal disk a transverse osseous plate had begun to form, which latter is normally only seen at the end of the fourth month.

After thyroxin had been injected for two or more months, the bony

trabeculae became shorter and the transverse osseous plate was more and more perforated by capillaries. After nine months or more of treatment, the subepiphyseal bony structures had been completely dissolved and longitudinal spicules were no longer seen.

(3) *Joints*:

In the articular cartilage the early changes following the administration of thyroxin consisted of an intensification of the growth processes. The cartilaginous ground substance was diminished. The cells of the uppermost, the sliding zone, and of the underlying transitional zone increased in number; the cells of the transitional zone arranged themselves in a perpendicular direction and formed short cartilage cell columns. The bony border lamella which delimits the hypertrophic cartilage cells from the bone marrow was thinned out. In some places, congested capillaries and multinucleated osteoclasts absorbed the osseous border lamella and elements of the bone marrow penetrated into the cartilaginous covering. After one month of injections, the proliferation of the cells of the various layers of the covering of the joint became less accentuated, whereas hypertrophic changes became pronounced; the resorptive processes made progress and the amount of bony tissue present was still further diminished.

After two months of treatment, the hyperplastic and hypertrophic as well as the resorptive processes receded, and, at still later periods, the opposite condition was observed. After three or four months, the cells had become even smaller than normal and a dense osseous tissue had replaced the hypertrophic and the deeper layer of the transitional zones.

After nine months and later, a slight increase in the amount of bone could be noted, but the total amount of bone formed was less than usual. Otherwise no changes were observed as compared with the earlier stages. Under normal conditions, however, towards the end of the first and during the second year of life, proliferative and retrogressive changes occur which, in many instances, lead to arthropathic lesions of varying degree, particularly in mice belonging to high tumor strains. Under the influence of prolonged administration of thyroxin, on the other hand, these age changes, if present at all, were less frequent and less severe than in non-injected animals. Of seven D mice over six months of age which had been injected with thyroxin, only one (14.3%) showed arthropathic changes, whereas in non-treated D mice of the same age the incidence of such changes had been found by the authors (1941a) to be 70%.

(4) *The bony shaft*:

Two weeks after beginning of the injections, an increased mitotic proliferation of the connective tissue cells was seen in the loosened

periosteal tissue. Some of these cells became converted into epithelioid cells, many of which, in contact with the bone, were transformed into osteoblasts, whereas others coalesced and formed multinucleated osteoclastic giant cells situated in grooves of the softened compact bone. The vascular canals of the bony cortex were enlarged.

After one or more months, the periosteal tissue and the compact bone became denser, the vascular canals narrowed and the resorptive processes were somewhat less accentuated, but the shaft remained thin, even at late stages of the experiment.

The bone marrow was cellular throughout the experimental period. In animals in the second year of life there was a tendency of the bone marrow to transform into fibrous tissue.

DISCUSSION

The effect of thyroxin on the epiphyseal growth zones consists in an intensification of the progressive and retrogressive processes involved in growth, differentiation and ageing of this tissue. Proliferation of the cartilage and conversion of the columnar into hypertrophic cartilage cells proceed at an increased rate at the early stages of the experiment. This intensification of progressive changes is followed by a likewise markedly accelerated and accentuated degeneration and ossification of the epiphyseal cartilage. Ossification may then take place directly and without a preceding destruction of the hypertrophic cartilage cells by capillaries of the bone marrow. Finally, there is also an increase in the solution processes, leading to a complete absorption of the epiphyseal plate and thus to a state of skeletal differentiation, which does not occur under normal conditions even in very old mice. Therefore, the possibility may be suggested that the failure or incompleteness of epiphyseo-diaphyseal union as seen in certain rodents, may perhaps be due to a lack of thyroid activity.

The changes in the growth zones of mice called forth by thyroxin resemble thus in some respects those which we observed after injections of bovine anterior hypophyseal extract (1941b). However, under the influence of thyroxin, both proliferative and retrogressive changes proceed at a much faster rate than under the influence of anterior pituitary extract, and the degree of epiphyseo-diaphyseal union is even more complete in the former than in the latter case. A further difference in the effects of these hormones seems to exist in that after administration of thyroxin the absorption of bony tissue is greater than under normal conditions, whereas in mice injected with anterior pituitary extract the apposition of bony tissue may be temporarily increased over normal. Similar findings have been recorded by Dott (1923) in dogs treated with thyroid and anterior pituitary hormone. This investigator observed that although skeletal growth and differentiation were equal-

ly hastened after administration of anterior pituitary hormone, in hyperthyroid animals skeletal growth was less accelerated than skeletal differentiation. Coryn (1939), however, who injected thyroxin into one young rabbit for a period of thirty days, believes that the increased growth taking place subsequent to the administration of thyroid hormone, is at a later date compensated by a premature cessation of the growth of cartilage.

The articular cartilage undergoes, under the influence of thyroxin, similar changes as the epiphyseal cartilage. But, whereas the alterations in the epiphyseal cartilage may be considered as an intensification of normal processes, a similar interpretation cannot be made as to the changes in the joints. In normal old mice we have frequently seen arthropathic lesions of varying degrees; they were severe and quite common (70%) in mice belonging to strain D. The incidence of these articular lesions in mice treated with thyroxin is so strikingly reduced (14.3%) that considerable importance must be attached to this effect. If thyroxin has the tendency to accelerate the ageing of the skeletal tissues, one should expect the age changes in the joints likewise to be intensified. Since this is not the case, and, on the contrary, even a decrease in the incidence of the arthropathic lesions is observable, it may be assumed that either thyroxin exerts a special effect on the tissues of the joint, or that the arthropathic lesions seen in normal mice are not due to old age as such, but that they are called forth by an endocrine or a metabolic disturbance, which merely coincides with old age and which may be counteracted by thyroxin. In this respect, also, the effect of thyroxin is different from that of anterior pituitary extract, under the influence of which the frequency and intensity of the articular lesions in older mice are increased.

The effect of thyroxin on the skeletal tissues of mice is thus similar to that on cartilage and bone of immature guinea pigs, which we observed previously, although the latter experiments extended over a period of only thirty days. During this short time, the increase in proliferation and the acceleration of differentiation of the epiphyseal cartilage and of the cartilage of the joints could be demonstrated, but the stage of accelerated degeneration and resorption of the epiphyseal cartilage cells had apparently not been reached at the conclusion of the experiment.

Stefanescu (1926), on the other hand, reported in growing guinea pigs, and Smith and McLean (1938) in growing rats, a decrease in the growth of the cartilage following a prolonged administration of thyroid hormone, but they did not investigate the early changes called forth by the hormone.

It might also be mentioned that the mice used in the present investigation were comparatively older than the guinea pigs formerly used and that the amount of thyroid hormone given to the mice was relatively much greater than that administered to the guinea pigs. It is conceivable that, under the influence of the large dose, the changes produced by the hormone proceed at a faster rate. Likewise, the greater age of the mice at the begining of the treatment might be responsible for the increased tendency of the cartilage to degenerate and its decreased tendency to proliferate under the influence of thyroxin, an effect which we (1939) were able to demonstrate in the case of other hormones acting on the cartilage.

SUMMARY

In growing mice, administration of thyroxin accelerates first proliferation, then ossification, and in the end resorption of the epiphyseal cartilage and of the cartilage of the joints. Resorptive processes are not only accelerated but also increased, and thus lead to a complete epiphyseo-diaphyseal union, which does not take place in normal mice of corresponding strains at any age. In general, thyroxin accelerates and intensifies therefore the age changes in the skeleton; however, it causes a decrease in the severity and incidence of certain old age changes which occur in the joints of normal mice.

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