THE EFFECT OF CORTISONE ON GROWING BONE IN THE RAT

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CORTISONE has a profound effect on growing bone tissue (Follis, 1951 ; Sissons and Hadfield, 1955 ; Storey, 1957, 1960 and 1961 ; Hulth and Westerborn, 1963). However, different experimental animals react in somewhat different ways. A sufficiently large dose of cortisone always causes a slowing down or a cessation of bone growth. In the majority of animals (as well as in man) cortisone also causes increased osteoclastic resorption, as well as resorption around the vascular channels in the bone. The rat differs from the other animals in that there is frequently no resorption of this kind, probably due to its adaptive calcium metabolism (Storey, 1960). In the metaphyses of the rat there arises the so-called dense bone (Follis, 1951 ; Storey, 1960), which consists, according to these authors, of chondrocytes, mineralized cartilage matrix which has not been resorbed in the ordinary way and superinduced lamellar bone. Storey was able to show that dense bone results when the rat receives a high balanced dietary level of calcium and phosphorus. On a diet with unbalanced proportions of calcium and phosphorus or containing small amounts of these substances, resorption ensues instead.

The specific reaction of rat bone to cortisone seems to deserve more detailed study. The methods which we employed in this investigation consisted of a combined technique of microradiography, tetracycline-induced fluorescence (Milch, Rall and Tobie, 1957) and micro-angiography. The questions we wished to shed light on by this technique were the degree of mineralization of the dense bone, the extent of cortisone's growth-preventing effect, the occurrence of resorption in established bone and finally the part played by the blood vessels in the cortisone-induced changes in the bone.

MATERIAL AND METHODS

Two series, each of 40 rats, were used. In series I the rats weighed about 80 g. and received cortisone in the form of Cortodrin® (Astra) in a dose of 25 mg. per kg. body weight; in series II the weight was about 130 g. and Cortodrin was administered in a dose of 50 mg. per kg. body weight. The cortisone was administered daily for up to 15 days. The animals were killed 1–25 days from the beginning of the experiment. Most of them were labelled with oxytetracycline (Terramycin®, Pfizer) in a dose of 50 mg. per kg. body weight 2 days before death (Hulth and Olerud, 1963). Ten rats in each series received tetracycline on the first day of the experiment and a second dose 1 day before death (25 mg. per kg). Micro-angiography with indian ink diluted 1 : 3 in physiological saline was performed on a group of 25 animals labelled with tetracycline 2 days before death (experimental animals and controls). The fluid was injected intermittently with an ordinary syringe in small amounts via polyethylene catheters in the abdominal aorta in the caudal and the cranial directions. The animals were stored in a freezer at $-20°$ until the whole experimental series was ready.

The bodies were then thawed in 10 per cent formalin, after which the parietal bone and both tibiae were prepared free. After fixation for a maximum of 24 hr. in 10 per cent formalin, the preparations were dehydrated in absolute alcohol for 2–3 days with several changes. Most of the preparations were embedded in methyl methacrylate. The plastic blocks were sawn into slices 0·5 mm. thick, which were then ground down to a thickness of 100 μ. A number of the sections were examined by microradiography (Hulth and Olerud, 1962*a*). They were mounted under coverslips in fluorescence-free balsam and examined in the fluorescence microscope fitted with an UV light source (Hulth and Olerud, 1962*b*). Some tibia preparations from different periods of the experiment were decalcified in formic acid and sodium citrate for ordinary histological examination. They were stained with haematoxylin and eosin.

RESULTS

Similar changes arose in both series. The large dose of cortisone, however, produced more conspicuous changes than the small. The natural increase in weight of the young animals ceased and was converted into a decrease. When the cortisone was withdrawn, the body weight rose again in both series.

Histological examination

The changes were those of similar investigations on the effect of cortisone. After 2–3 days there was a reduction in the height of the epiphyseal cartilage, which gradually diminished more and more. In particular the number of hypertrophic cells decreased and these cells did not mature normally and were not resorbed. On this account, a large number of cartilage cells remained in the metaphyseal trabeculae. Osteoblasts on these trabeculae were greatly reduced in numbers or were completely absent. By the 8th–11th day of the experiment dense metaphyseal bone had been formed, consisting of compact, long, metaphyseal trabeculae made up of unresorbed cartilage cells and matrix with only small, intervening, marrow spaces between the trabeculae (Fig. 1). When the supply of cortisone ceased, restitution appeared to take place relatively rapidly ; the hypertrophic cells seemed to mature in the normal way and undergo removal of growing capillaries.

Fluorescence microscopy

Tetracycline fluorescent micrographs yield information on the reduction of growth in the cortisone rats, compared with that of the controls. Both the appositional and the endochondral growths decline with a dose of 25 mg. of cortisone per kg. body weight but do not cease entirely. On the other hand, the series with the larger dose stopped growing completely after 4–5 days.

Appositional growth.—This was studied in tibia sections and in the parietal bone. In the middle of the tibia growth takes place mainly by periosteal apposition and in the process the tetracycline forms a fluorescent yellow line in the periphery of the section, in part also on the endosteal side. The bone newly formed after such labelling can be clearly observed lying outside the fluorescent line in a section of this kind. This growth appears still more clearly if a further dose of tetracycline is given shortly before death. The dose of tetracycline given at the start of the experiment is not affected at all by the cortisone. On the other hand, additional doses of tetracycline seem to produce a very much smaller amount of labelling or none at all. Such a second labelling may be difficult to distinguish from the first in the cortisone animals as is shown in Fig. 2*a* and *b*,

which shows a cortisone-treated rat on the 5th day of the experiment, compared with the corresponding control rat; both animals had received two doses of tetracycline. Similar conditions apply in the parietal bone. The micro-angiographs of tibia sections show that there is no dilatation of the vascular channels round the functioning blood vessels (Fig. 3*a* and *b*).

The endochondral growth in the upper epiphysis of the tibia can be excellently illustrated by fluorescence micrographs. If two labellings are given in the way mentioned above at an interval of, say, 6 days, the last dose (the day before death) will give rise to a fluorescent line immediately below the epiphyseal cartilage (approximately half a cartilage breadth below). The labelling produced by the first dose already lies well down in the metaphysis. This labelling is interrupted for long sections on account of the natural disappearance of the trabeculae by resorption. With a longer interval between the labellings the first line of fluorescence disappears down towards the open marrow cavity and can be discerned only in the cortex. When a large dose of cortisone is administered, the conditions are quite different. The last dose gives rise to a weak double line, representing, on the one hand, the upper part of the mineralized cartilage septa and, on the other, probably the site of incipient osteogenesis on the trabeculae. Below this double line there is a relatively wide area, representing the dense bone which does not fluoresce. The first dose of tetracycline produces a line at the lower boundary of the dense zone at the transition to relatively normal bone trabeculae. There is no great difference between the preparations from the 4th and the 8th days of the experiment, indicating that, with the large dose of cortisone, there is a certain amount of growth for 2–3 days (corresponding to the extension of the dense zone) but that afterwards there is a complete standstill (Fig. 4*a* and *b*). The height of the epiphyseal cartilage lamella is also greatly reduced.

With the smaller dose of cortisone, so clear a dense zone in the metaphysis is not developed, nor is growth completely retarded. There is, however, a clear retardation of growth compared with the normal animals.

With a combination of indian ink micro-angiography and a single tetracycline labelling administered 48 hr. before death, it is possible to show further characteristics of the dense zone. In the normal cases the ascending capillaries are narrow and slender and end in the mineralization zone of the cartilage. In 48 hr. the fluorescent line has moved down a fairly considerable distance into the metaphysis (Fig. 5*a*). When the cortisone changes are maximal (on the 10th day in our experiments), the blood vessels are greatly changed. They follow an irregular course and do not reach up to the mineralization zone (Fig. 5*b*). Immediately below this zone, there are instead lumpy vascular balls which fill up the cavities which have arisen in the metaphyseal trabeculae. Adjoining the low epiphyseal cartilage there are irregular lines of fluorescence corresponding to the cell boundaries. The fluorescence is mainly in that part of the trabeculae which is located immediately below the cartilage. Otherwise scarcely any of the trabeculae in the dense metaphyseal zone take up any fluorescence, indicating that there is no reactive bone tissue there. In some preparations we also find numerous penetrations of the low epiphyseal cartilage by blood vessels coming from the epiphysis which have found their way across the cartilage zone, indicating incipient closure of the epiphyseal cartilage.

Incipient restitution can be observed only 2 days after the withdrawal of

cortisone. The capillaries passing through the dense zone have all begun to " get up " and are directed up towards the degenerating hypertrophic cells. The epiphyseal cartilage has already increased in height. The tetracycline administered 48 hr. before death has moved down about half the breadth of the epiphyseal cartilage (Fig. 5*c*). Particularly strong resorption is observed to set in, especially towards the upper part of the dense zone. This is perhaps even clearer in the following procedure.

Microradiographic examination of the dense zone shows that it consists of compact, mineralized trabeculae with a high degree of mineralization. These trabeculae are straight but heavier and longer than normal (Fig. 6*a* and *b*). The epiphyseal cavities in the dense zone shown in the indian ink angiographs appear here with greater clarity. In preparations from the 7th day after the cortisone was withdrawn, the greater part of the dense zone already seems to have been resorbed (Fig. 6*c*). Below this dense zone there are bone trabeculae of relatively normal appearance down towards the open marrow cavity.

DISCUSSION

The combined investigation reported here shows clearly that the effect of cortisone on the rat consists of a retardation or (with a large dose) inhibition of both the appositional and the endochondral growths. This is a sign of damage

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EXPLANATION OF PLATES

FIG. 1.—Photomicrograph of the metaphysis in the tibia of a cortisone-treated rat (smaller dose) on the 10th day. The trabeculae consist exclusively of unresorbed cartilage cells and matrix. Growth has not stopped, which explains the very long, altered trabeculae. ×2·5.

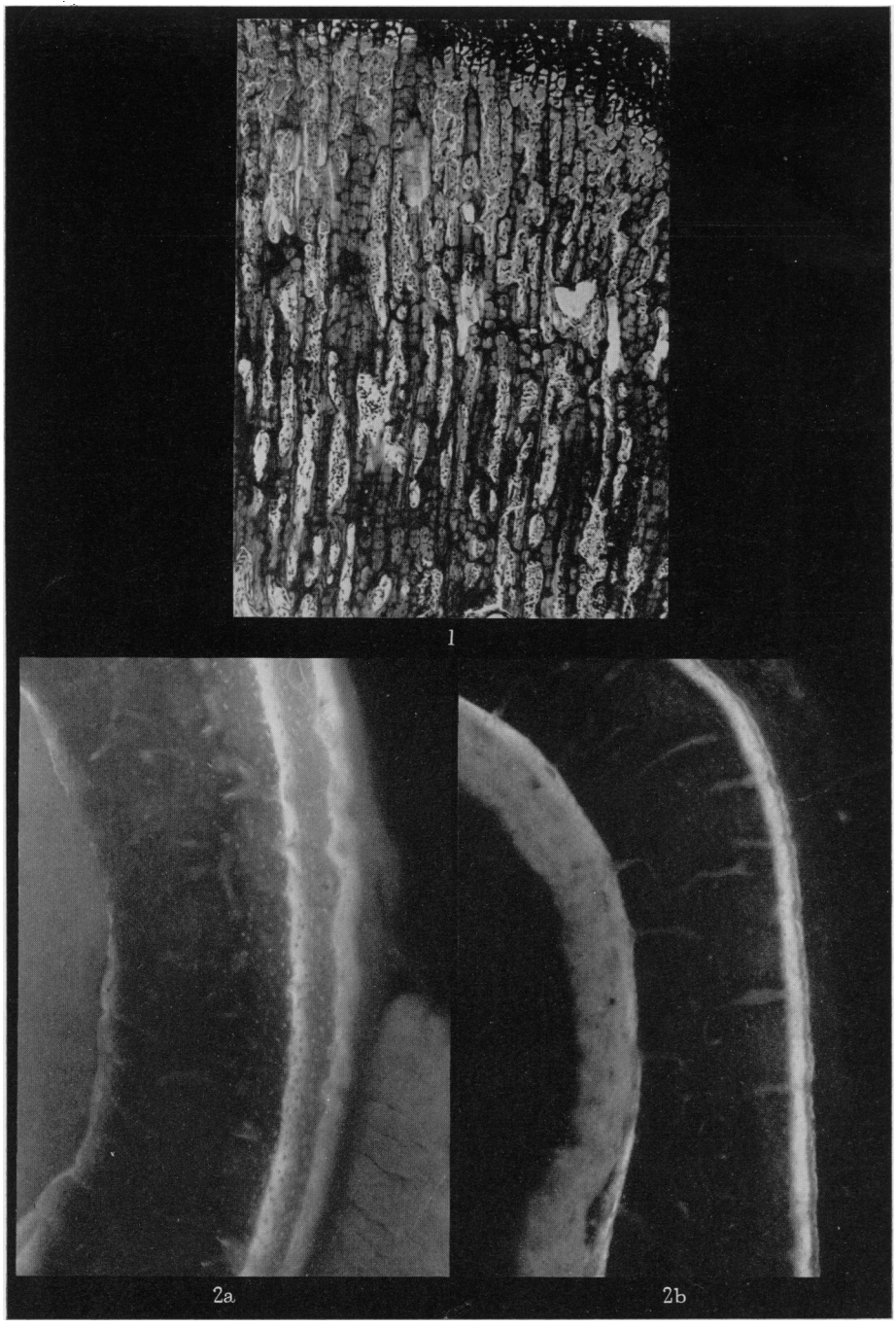
FIG. 2*a* and *b*.—Fluorescence photomicrograph of tibia section of normal rat (2*a*) and cortisone-treated rat (2*b*), which received Terramycin on the 1st day and on the day before death on the 7th day. Apposition from the periosteal side (to the right). Clear growth on the control tibia but very insignificant growth on the experimental preparation and the last dose has in this case produced a much weaker line of fluorescence. ×40.

FIG. 3*a* and *b*.—Fluorescence photomicrograph and angiomicrograph of tibia section of normal rat (3*a*) and rat treated with cortisone for 10 days (3*b*). Both received a dose of Terramycin 2 days before death. The control preparations have strong periosteal and somewhat weaker endosteal labelling. The cortisone preparations have no fluorescence at all. The vascular channels and the blood vessels have a similar appearance. ×10.

FIG. 4*a* and *b*.—Fluorescence photomicrograph of growth zone of tibia from normal and cortisone-treated rats. Duration of experiment, 6 days. Terramycin at the beginning of the experiment and on the day before death. Double labelling can be observed in the epiphyseal bone trabeculae of the normal tibia (at the top in 4*a*). In the metaphysis only the last labelling can be seen in the picture ; the first has disappeared downwards and out of sight. In the cortisone preparations there is no double labelling in the bone epiphysis. The epiphysial cartilage is very low. In the metaphysis the last labelling has produced a line at the very top in the epiphyseal cartilage of matrix and a diffuse line immediately below. The first labelling remains intact a short way down in the metaphysis, separated from the last by the non-fluorescent dense zone. Weak fluorescence of metaphysial bone trabeculae on both preparations. ×2·5.

FIG. 5*a–c*.—Fluorescence photomicrograph and angiomicrograph of epiphyseal line in tibia of normal rat (5*a*), rat treated with cortisone for 10 days (5*b*) and cortisone-treated rat on the 12th day (day after withdrawal of cortisone) (5*c*). Terramycin was administered to all the animals 2 days before death. Resorption cavities immediately below the epiphyseal cartilage in 5*b*. In 5*c* the blood vessels have begun to rise and the epiphyseal cartilage has already increased in height. ×2·5.

FIG. 6*a–c*.—Radiomicrographs of growth zones of normal rat (6*a*), rat treated with cortisone for 10 days (6*b*) and for 13 days (2 days after withdrawal of cortisone) (6*c*). The dense zone in 6*b* shows coarse, highly mineralized trabeculae with resorption cavities adjoining the epiphyseal cartilage. In 6*c* the dense zone has largely been resorbed and the metaphysis is resuming a normal appearance. ×2·5.



1

2a

2b

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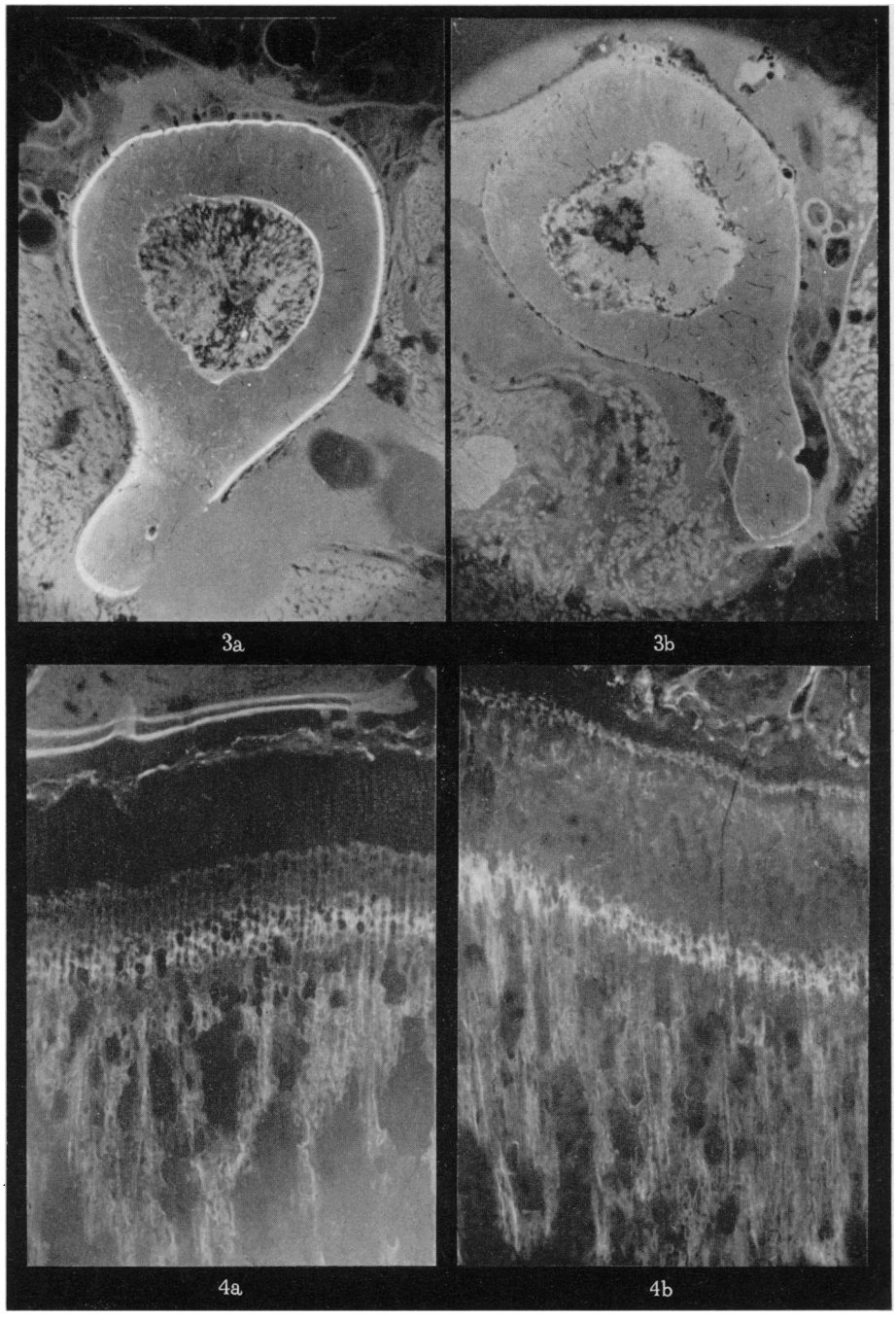

3a 3b

4a 4b

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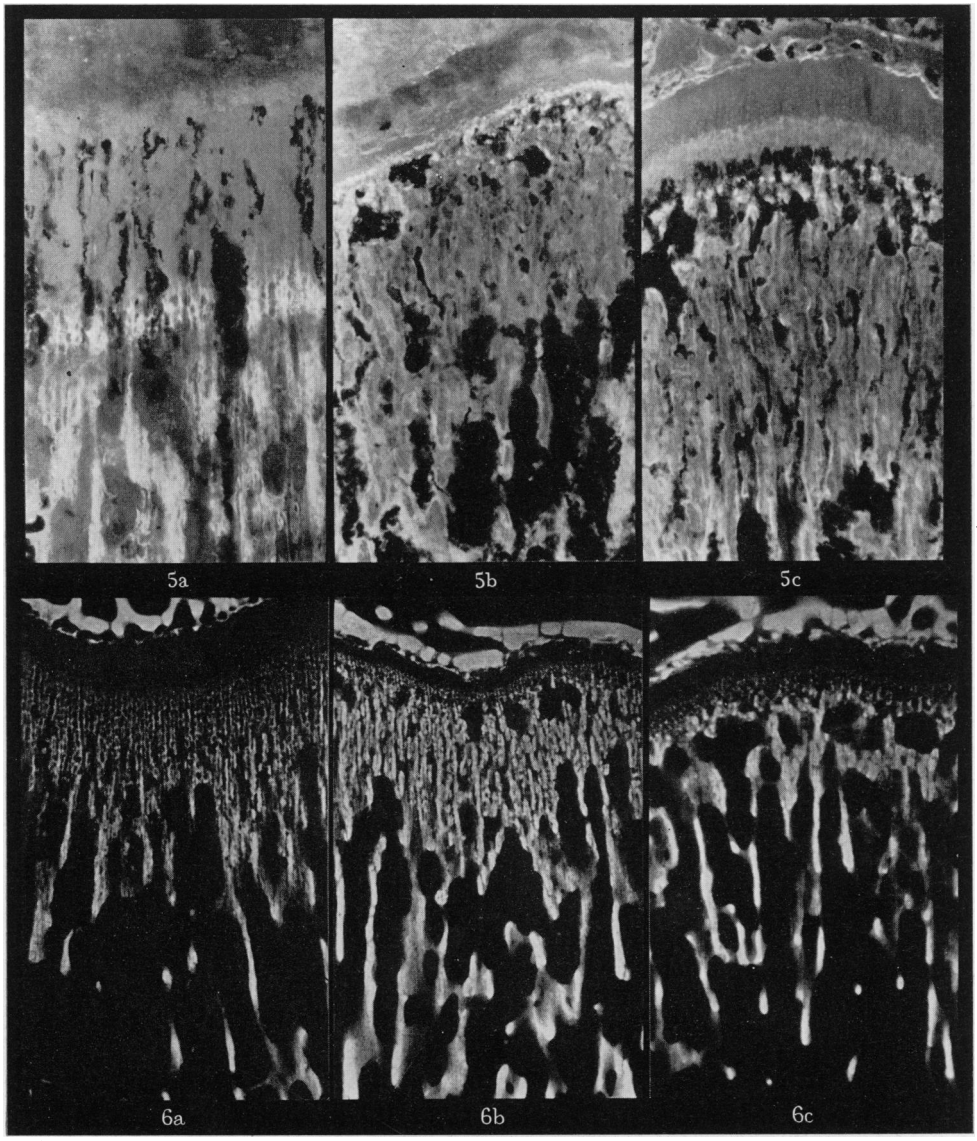
5a
5b
5c
6a
6b
6c

to the osteoblasts and the chondrocytes respectively (Hulth and Westerborn, 1963). There is poor or no fluorescence from tetracycline given after cortisone treatment has continued for 2–3 days with a large dose. There is no or reduced formation of new bone.

In the rat there is also reduced resorption. Labelling with a dose of tetracycline given at the start of the experiment is therefore not affected at all by the cortisone but remains completely intact. The reduced resorption also means that the hypertrophic cells in the epiphyseal cartilage are not removed in the normal way. Nor is there initially any resorption further down in the metaphysis, for which reason there come into existence metaphyseal trabeculae consisting of cartilage cells and calcified cartilage matrix with a high degree of mineralization. After a week, however, there may arise in the upper part of the dense zone which has thus come into existence resorption cavities filled with blood vessels. These vessels follow an irregular course. With a large dose of cortisone there is complete obstruction of growth in the epiphyseal cartilage after 3–4 days and the dense zone mentioned represents the growth which has taken place during this time. With a smaller dose the growth does not entirely cease and therefore trabeculae with cartilage cells and matrix are visible well down in the metaphysis. After withdrawal of the cortisone growth starts again very rapidly. The epiphyseal cartilage increases in height, the blood vessels rise and reach up to the degenerating cartilage cells, the cartilage trabeculae are resorbed and there come into existence ordinary bone trabeculae consisting of a core of cartilage matrix with superinduced lamellar bone.

The fact that the trabeculae in the dense metaphyseal zone do not take up any fluorescent substance must mean that they consist solely of calcified cartilage matrix with such a high degree of mineralization that they can no longer bind the tetracycline. When the effect of the cortisone is powerful, the tetracycline attaches itself only to the uppermost part of the mineralized cartilage matrix, which has a lower degree of mineralization. This area is in any case available to the blood vessels which descend from the bone epiphysis to the epiphyseal cartilage, nourishing it by diffusion. When the cortisone has done severe damage, these vessels may penetrate the epiphyseal cartilage in several places, which does not occur in normal epiphyseal cartilage. A certain labelling of the calcified cartilage in the epiphyseal line also occurs normally, but the maximal tetracycline fluorescence lies some way down, representing the site of incipent osteoblastic functioning. In the cortisone treatment of fractures there arises an abundance of cartilage callus with a low degree of mineralization, which binds a large amount of tetracycline (Hulth and Olerud, 1963). This is analogous with the above-mentioned findings concerning the epiphyseal cartilage.

The dosage of cortisone must be kept particularly high compared with that applicable in human medicine if it is to produce the effect on bone and cartilage tissue mentioned above. The rat is more resistant to cortisone than man and the rabbit. Even with so high a dose as 25 mg. per kg. body weight, the effect is not very striking. Growth continues but to a reduced extent, as can easily be ascertained by tetracycline labelling. One circumstance which may well partly explain the considerably smaller effect of 25 mg. per kg. compared with 50 mg. per kg. is that the former dose was given to younger animals with a smaller weight. Their growth is probably more difficult to retard than that of the older and heavier rats. Histologically but not microradiographically, however, a clearly observable,

dense metaphyseal zone appeared in several of the rats which received the smaller dose of cortisone.

The tetracycline-fluorescence technique is a particularly valuable method, replacing autoradiography with calcium isotopes in experimental work in which it is desired to compare the growth of different animals. The combination with micro-angiography gives, in addition, a further view of the changes in bone induced by cortisone.

SUMMARY

The effect of cortisone in growing bone in the rat was studied by various methods: histology, micro-angiography, tetracycline-induced fluorescence and microradiography. The rat differs from, for example, the rabbit in that cortisone does not provoke any great degree of resorption. Labelling by a dose of tetracycline administered at the beginning of the experiment therefore remains intact in the bone. By this means it is possible to observe how much the growth is retarded. Tetracycline administered when the animal is under the influence of a large dose of cortisone produces poor or no fluorescent labelling in the bone. In the metaphysis there is formed the so-called dense bone, which consists of unresorbed cartilage cells and cartilage matrix. These trabeculae, unlike normal trabeculae, do not take up any tetracycline, indicating that they do not contain any newly established bone. Instead fluorescence appears right up towards the epiphyseal cartilage, on the tips of the mineralized matrix spiculae, possibly in the form of a double line. The appearance and course of the blood vessels is considerably changed in the metaphysis; they become lumpy and do not reach up to the cartilage. There arise absorption cavities within the dense bone, all at the same height. When the cortisone effect ceases, a rapid restitution of these blood vessels and of the metaphyseal trabeculae takes place. The vascular channels of the cortex are not dilatated by the cortisone.

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