EXPERIMENTAL DETERMINATION OF RATE OF LONGITUDINAL BONE GROWTH

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In 1727, Steven Hales demonstrated experimentally, by measuring the leg-bone of a chick where 2 months earlier he had drilled holes to act as markers, that long bones grow by the addition of new tissues at the ends of the shaft, and not by general interstitial expansion. Duhamel (1742) and John Hunter (in about 1770) also performed this type of experiment† and Hunter, in the bones of young pigs which had been fed with madder, confirmed his conclusions by observing the terminal location of the new tissue formed under these circumstances. Later, the use of the microscope (Goodsir, 1845; Koelliker, 1852; Virchow, 1860) revealed that proliferation of cartilage, and the subsequent replacement of this tissue by bone, were the cellular processes on which longitudinal growth depends.

In a mammalian bone, the growing zone comprises the epiphysial cartilage plate and the bony tissue adjacent to its metaphysial surface. The general microscopic anatomy of this 'endochondral ossification' is now well known (Lacroix, 1949; Ham, 1950), but among different species of mammals there is wide variation in the detailed structure of the growing zone. Even within one species marked differences of histological pattern are found, according to the bone concerned and its stage of development. Although quantitative data have not so far been available, some, at least, of this structural variation is attributable to differences in growth rate. For this reason the purpose of the present investigation has been to determine directly the growth rate of the growing zone of the epiphysial plate at the lower end of the femur in the rat and the rabbit, and to use this information in interpreting the microscopic structure in the two species.

METHOD

Using Himalayan rabbits and rats of the Sprague-Dawley strain, data were collected on the growth occurring at the epiphysial plate at the lower end of the femur. After inserting a small metal marker into the femoral shaft, the increase in length of the distal part of the diaphysis was determined radiographically, pictures being taken every few days throughout the period studied (See Pl. 1, fig. 1).

The rabbits received a stock laboratory diet, including generous amounts of green vegetables. The rats received a commercial pellet diet‡ which contained vitamin and mineral supplements and which had a protein content of 21 %.

* Part of this work was carried out during the tenure of a research fellowship in the Department of Pathology, Northwestern University Medical School, Chicago, Illinois, U.S.A.

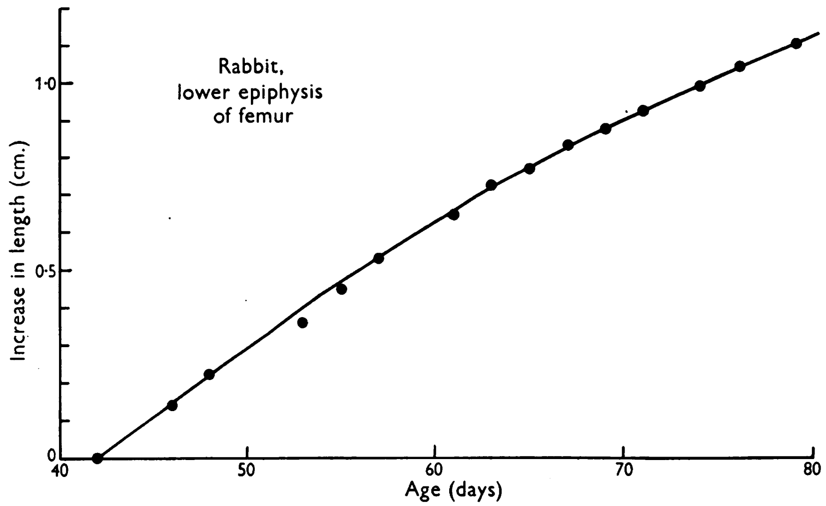
† Hunter's experimental specimen has been preserved, because of its historical interest, in the museum of the Royal College of Surgeons (See Dobson, 1948).

‡ 'Rockland' rat diet; from Rockland Farms, New City, New York (U.S.A.).

The radiographs were taken with the anaesthetized animal held in a standard position, its pelvis and legs strapped to a specially constructed frame. Fine-detail X-ray film was used, and the distance between the film and the X-ray tube was 60 in.*

Measurements on the films were made with dividers. It was convenient to measure the distance between the point of the marker and the surface of one of the femoral condyles, and then, by a second measurement on the same film, to correct this for any increase in size of the epiphysis itself.

The measurements for any individual animal usually fell on a smooth curve (Text-fig. 1) and such a record permits the calculation of the growth rate of the epiphysial plate during the period of the experiment.



Text-fig. 1. Rabbit. Longitudinal growth of the lower epiphysial plate of the femur.

RESULTS

Text-Fig. 1 shows the growth curve for the epiphysial plate at the lower end of the femur of a young male rabbit, approximately 6 weeks of age at the commencement of the experiment. The points plotted represent individual X-ray measurements. The original length of the measured segment of the femur is regarded as zero, only its subsequent increase being plotted.

Over the 34 days of the experiment, the rate of growth diminishes from 0·34 to 0·24 mm. per day, with an average value of 0·31 mm. per day.

Similar information for the rat is provided in Table 1, and represented graphically in Text-fig. 3. Data from six male rats of the Sprague-Dawley strain are tabulated, the graph being a smooth curve fitting the averaged values for these rats. At the commencement of the experiment the rats were 5 weeks old, and each weighed approximately 70 g. All rats were measured over a period of 5 weeks, and in three

* In a group of ten animals killed immediately after being X-rayed in this way, there was no 'parallax' error. The dimensions of the bones (direct measurement) and of their radiographs were identical.

of them measurements were continued for a further 6 weeks. Over the initial 5-week period the average gain in weight for the rats was 5 g. per day. For the initial 5-week period the growth rate is 0·18 mm. per day, again being greater at the beginning than at the end of this time. Growth rates for the individual rats are 0·19, 0·18, 0·22, 0·16, 0·19 and 0·16 mm. per day respectively. The curve flattens considerably for the three rats allowed to survive longer, the growth rate over the subsequent 6-week period being approximately 0·04 mm. per day.

Table 1

Age (days)	Length of measured segment of femur (in cm.)					
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6
35 (0)	1·11 (0·00)	1·22 (0·00)	1·33 (0·00)	1·04 (0·00)	1·05 (0·00)	1·01 (0·00)
39 (4)	1·22 (0·11)	1·34 (0·12)	1·43 (0·10)	— —	— —	— —
42 (7)	— —	— —	— —	1·22 (0·18)	1·35 (0·30)	1·17 (0·16)
43 (8)	1·30 (0·19)	1·43 (0·21)	1·59 (0·26)	— —	— —	— —
48 (13)	1·43 (0·32)	1·48 (0·26)	1·68 (0·35)	— —	— —	— —
50 (15)	— —	— —	— —	1·37 (0·33)	1·48 (0·43)	1·33 (0·32)
52 (17)	1·51 (0·40)	1·56 (0·34)	1·79 (0·46)	— —	— —	— —
57 (22)	1·54 (0·43)	1·60 (0·38)	1·87 (0·54)	1·48 (0·44)	1·60 (0·55)	1·44 (0·43)
62 (27)	1·67 (0·56)	1·66 (0·54)	1·96 (0·63)	— —	— —	— —
69 (34)	1·76 (0·65)	1·83 (0·61)	2·07 (0·74)	— —	— —	— —
71 (36)	— —	— —	— —	1·62 (0·58)	1·74 (0·69)	1·57 (0·56)
88 (53)	— —	— —	— —	1·75 (0·71)	1·82 (0·77)	1·64 (0·63)
102 (67)	— —	— —	— —	1·82 (0·78)	1·93 (0·88)	1·71 (0·70)
116 (81)	— —	— —	— —	1·84 (0·80)	1·98 (0·93)	1·75 (0·74)

The figures in parentheses represent the time from the start of the experiment, and the increase in length of the measured segment in this period.

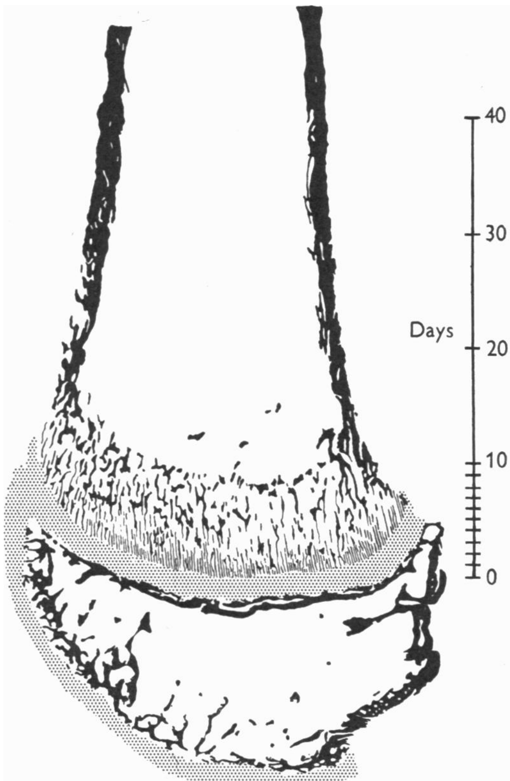
MICROSCOPIC STRUCTURE OF GROWTH ZONE

When the epiphysial plate at the lower end of the femur is examined microscopically in rats and rabbits of the age at which the above growth determinations were carried out, a considerable variation in structure is seen among the individuals of each group. The thickness of the epiphysial cartilage plate, and the number and arrangement of the metaphysial trabeculae, differ from animal to animal, whether early or late in the period studied. In contrast, measurements of growth rates for such a series of animals are relatively uniform, particularly when determined over periods longer than a few days. This histological variation suggests the possibility of some fairly rapid fluctuation in growth rate—and thus in structure—for any individual animal.

Because of this variation in histological structure the following descriptions and illustrations relate to bones which represent the 'mean' of a considerable number studied histologically at approximately the same age. It is obvious that the general appearance of the growing region of a bone in a microscopic section will depend on the plane in which the section has been cut. In the present study sections were cut in the sagittal plane, and passed through the longitudinal axis of the femoral shaft. If a section happened to deviate from this plane, it was not considered representative.

Rabbit

The general structure of the growing region of the lower femoral epiphysial plate in a rabbit 9 weeks old—corresponding to the middle of the period for which the growth determinations were made—is seen in Text-fig. 2.



Text-fig. 2. Rabbit ($\times$ 5). View of the lower end of the femur, with time scale calculated from the rate of growth.

The initial phase of the growth process consists of a proliferation and enlargement of the longitudinally arranged cells of the cartilage plate. The 'hypertrophic' cartilage is constantly eroded by vascular connective tissue from its metaphysial surface, and proliferating osteoblasts lay down bone on the surfaces of the remaining cartilage scaffolding.

Under conditions of uniform growth these processes are co-ordinated, and the rate of advance of the 'front' of vascularization and osteoblastic bone formation is the same as the growth rate of the cartilage of the epiphysial plate. Thus the thickness of the 'growing' cartilage plate remains constant, and the new bone laid down in its metaphysial surface is a measure of the rate of growth of the growing zone as a whole. In the present experiment this was found to be approximately 0·3 mm. per day.

Those bone trabeculae actually in process of formation are situated immediately adjacent to the surface of the epiphysial cartilage plate, while older trabeculae are left progressively farther away from this surface as growth continues. Assuming a uniform rate of growth, the 'age' of any bony structure can therefore be determined by measuring its distance from the advancing 'front'. In Text-fig. 2 and Pl. 1, fig. 2, the 'time-scale' at the side of the growing bone tissue shows this distance, the unit of measurement being the thickness of metaphysial bone laid down in 1 day (i.e. 0·3 mm.). Not only does the scale indicate the 'age' of bone trabeculae at its level, but it indicates the site of the growing surface of the epiphysial plate at the time of formation of these trabeculae.

By following histological changes in the bone trabeculae through the regions covered by the time scale, the approximate timing of the various cellular processes affecting them can be estimated.

In the zone of tissue 0·5 mm. wide adjacent to the advancing 'front'—that is for the first 36 hr. of their existence—the bone trabeculae are covered with proliferating osteoblasts and show deposition of lamellar bone on their surfaces (Pl. 1, fig. 3). Farther away from the 'front', continued osteoblastic activity increases the thickness of individual trabeculae, while extensive remodelling reduces their number and changes their originally longitudinal direction and parallel arrangement. In this zone, from about 0·5 to 2·5 mm. from the 'front' and thus covering a period of about $8\frac{1}{2}$ days, there is evidence of both osteoblastic and osteoclastic activity, and the pattern of the reconstructed bone trabeculae presumably results from a balanced equilibrium between these two opposed processes.

Farther still, at a distance of about 2·5 mm. from the 'front', the metaphysial trabeculae terminate, osteoclasts being conspicuous at their ends, and presumably being the agents by which they are destroyed. This final dissolution usually takes place quite abruptly, a network of moderately thick trabeculae suddenly giving way to bone marrow which is almost entirely devoid of trabeculae. The narrowness of the zone of conspicuous osteoclasis, which is no more than 0·2–0·3 mm. in width, shows that this is a rapid process, no more than 16–24 hr. being taken for the complete destruction of bone trabeculae as they enter this region. In addition to the osteoclasts engaged in the erosion of bone trabeculae, the zone of destruction contains additional cells of this type which are not related to bone trabeculae and which appear to have persisted after the disappearance of these structures.

From this description, it can be seen that the average 'life-history' for an individual bone trabecula of the growing region is as follows: an initial period of approximately 36 hr., when predominantly osteoblastic activity occurs; then progressive remodelling involving balanced osteoblastic and osteoclastic activity, continuing for about 8–9 days; finally abrupt osteoclastic destruction, attaining completion in less than 24 hr. This description is put forward as an 'average' for the femoral epiphysis of a rabbit at the age of 9 weeks, and individual animals may show some transient departure from it.

In the present investigation, the proliferation and maturation of cells in the cartilage plate have not been considered. It should be remembered that, although the cartilage plate has the same overall rate of growth as the bone tissue, the time-scale shown for the bone cannot be applied to the interpretation of distances within the growing cartilage.

Rat

The general sequence of events in the growth of the lower femoral epiphysial plate of the rat is similar to that described for the rabbit, although there are considerable differences in details. Text-fig. 4 shows the general appearance of this region in a rat 50 days of age, at the centre of the initial 5-week period for which growth determinations are shown in Text-fig. 3, and therefore assumed to be growing at approximately 0·18 mm. per day. Some rats show a thicker proliferating zone of the epiphysial cartilage and a more regular pattern of newly formed bone trabeculae adjacent to it, and this 'optimal' appearance is the one usually illustrated and described in the literature.

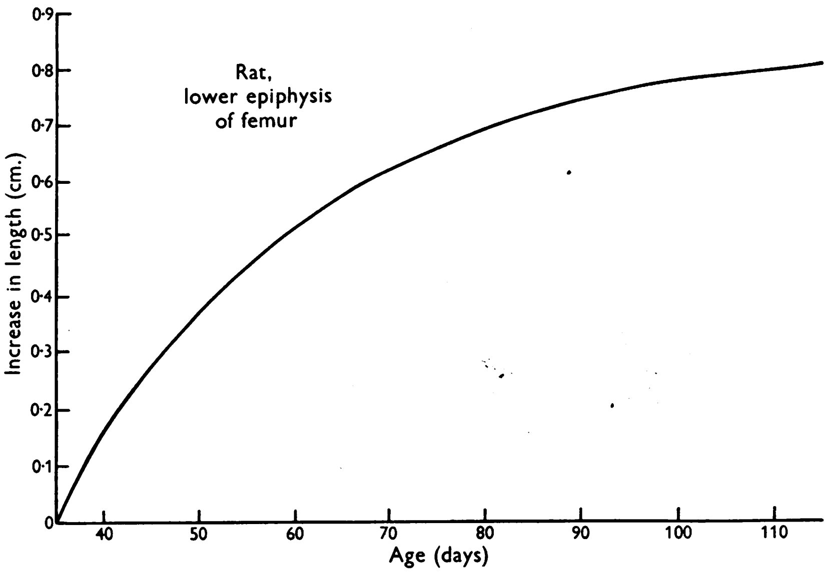
The apices of the metaphysial trabeculae show osteoblastic proliferation and lamellar bone deposition, but, in the rat femur illustrated (Pl. 1, fig. 4) evidence of remodelling of the trabeculae is present almost from the start, and is active at 0·2 mm. from the 'front'—i.e. about 24 hr. after the initial formation of the trabeculae. In most of the rat femora studied, remodelling of metaphysial trabeculae changes gradually to osteoclastic resorption, the abrupt line of dissolution described in the rabbit being absent. At a distance of 0·7 mm. (equivalent to 4 days) from the 'front', trabeculae are reduced in thickness and in number, but not until a distance of 3·4 mm. (i.e. 19 days) are they completely absent. In accord with this finding, osteoclasts are not concentrated in a narrow zone, but are fairly evenly scattered over the surfaces of bone trabeculae in the broader area of their dissolution.

DISCUSSION

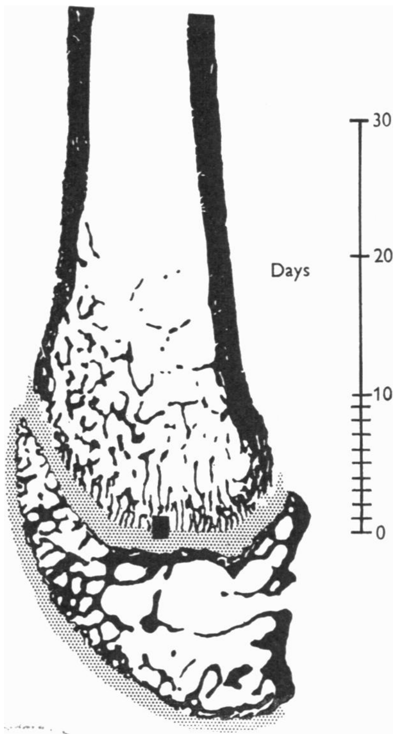
The literature contains occasional items of information from which the rate of longitudinal bone growth in various species can be estimated, but no comparisons have been made between such growth rates and the microscopic structure of the corresponding bones.

In the rabbit, for instance, some experiments of Dubreuil (1913) are available, where the tibia was X-rayed at intervals after the insertion of metallic markers into the shaft. Measurements of this author's illustrations give a value of approximately 0·4 mm. per day for the rate of growth of the proximal epiphysial plate of the tibia during the early period of active growth. No figures for the femur are given.

In the rat, from measurements made on bones at various stages in their development (Donaldson, 1924) an approximate figure of 0·2 mm. per day can be obtained for the rate of longitudinal growth of the whole femur (i.e. the combined rates for the upper and lower ends) between the ages of 50 and 90 days. Similar figures provided by Ray, Simpson, Li, Asling & Evans (1950) for the femur length in normal rats at 30 and 70 days of age, give a figure of 0·17–0·19 mm. per day for the



Text-fig. 3. Rat. Longitudinal growth of the lower epiphysial plate of the femur.



Text-fig. 4. Rat (× 10). View of the lower end of femur, with time scale calculated from the rate of growth. The black area indicates the location of Pl. 1, fig. 4.

growth rate of the whole bone over this period. In their studies with 'bone-seeking' radioactive isotopes, Leblond, Wilkinson, Belanger & Robichon (1950), using autoradiographs of bones from growing animals killed at varying intervals after the injection of such isotopes, were able to identify—from the region of most intense radioactivity—the site of the zone of calcification of the epiphysial plate at the time of injection. The depth of new bone formed subsequently could thus be determined, and in young rats measurements suggested a growth rate of approximately 0·15 mm. per day for the upper end of the tibia, and 0·10 mm. per day for the upper end of the humerus. No figures for the femur were reported.

In the pig, the data of Payton (1932), who experimented with madder feeding, allow the growth rates of a variety of epiphysial plates to be estimated. Values range from about 0·2 to 0·5 mm. per day at 80 days of age, and from 0·05 to 0·18 mm. per day at 150 days. In the goat, Bisgard & Bisgard (1935) inserted metallic bone markers in order to determine the relative amount each epiphysial plate contributes to the length of a bone. Their figures give a rate of 0·36 mm. per day for the lower epiphysial plate of the ulna between 4 and 32 days of age, and of 0·1 mm. per day for the same structure at 180 days.

In man, the growth rates of the various epiphysial plates of the skeleton have been studied in some detail, although again there has been no attempt to correlate known growth rates with the histological structure of the tissues concerned. Especially in the lower limb, a knowledge of epiphysial growth rates is of clinical importance in the prediction of the relative limb-shortening that will follow the operative arrest of growth of any particular epiphysial plate (Blount & Zeier, 1952; Green & Anderson, 1947; Gill & Abbott, 1942). From the radiographic data of Gill & Abbott (1942) the rate of growth of the lower femoral epiphysial plate between the ages of 5 and 13 years is estimated at approximately 1·25 cm. per year (i.e. as little as 0·035 mm. per day). The great difference between this rate of growth for an epiphysial plate in man, on the one hand, and those rates determined in small laboratory animals, on the other, should be remembered when the histological structures of the corresponding growing regions are compared.

SUMMARY

Using young rabbits and rats, the rate of growth of the epiphysial cartilage plate at the lower end of the femur has been determined radiographically. A figure of 0·31 mm. per day was obtained for the rabbit (age 9 weeks), and 0·18 mm. per day for the rat (age 50 days).

This information is used in interpreting the microscopic structure of the growing region of the bone in each species, and in determining the duration of each of the cellular processes concerned in the formation and maturation of bone trabeculae in this situation.

The relative duration of the various cellular processes varies from species to species, and this factor, in addition to variation in absolute growth rates, is responsible for the differences in the microscopic structure of the growing region in the two species studied.

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EXPLANATION OF PLATE

Fig. 1. X-rays of rabbit femur, before and after an interval of 10 days. The marker allows the longitudinal growth of the lower epiphysial plate during this period to be determined.

Fig. 2. Rabbit (× 30). View of the epiphysial plate and metaphysial bone trabeculae shown in Text-fig. 2. The time scale indicates the 'age' of any bone trabeculae, as measured from the growing surface of the epiphysial plate.

Fig. 3. Rabbit (× 122). Growing cartilage and newly formed bone trabeculae from Pl. 1, fig. 2.

Fig. 4. Rat (× 123). Growing cartilage and newly formed bone trabeculae from the area indicated in Text-fig. 4.


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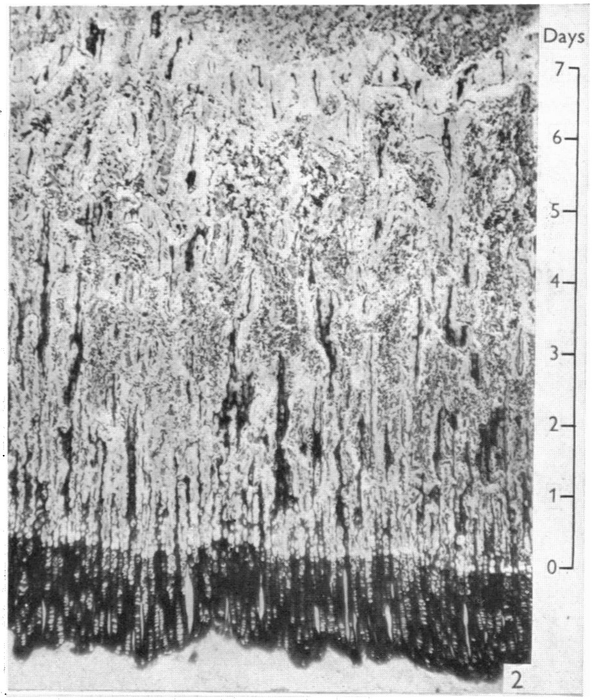

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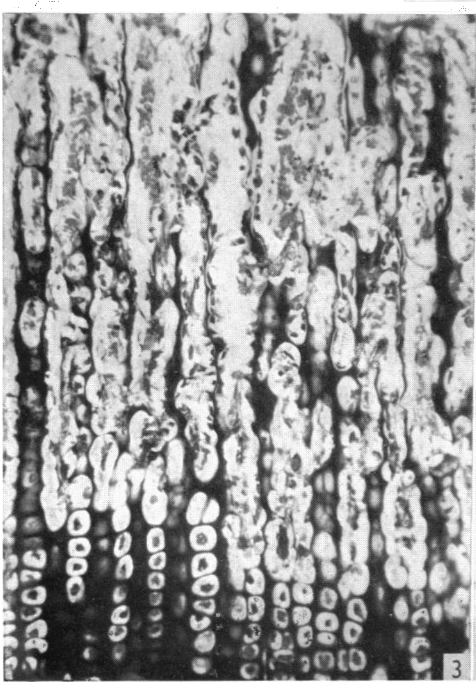
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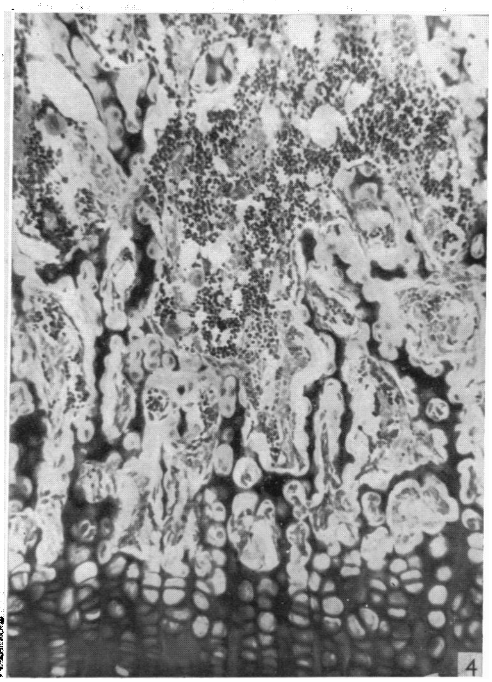
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SISSONS—Experimental determination of rate of longitudinal bone growth