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APPOSITIONAL GROWTH RATE IN RAT BONES USING THE TETRACYCLINE LABELLING METHOD

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In 1957 it was first observed by *Milch* and others that the tetracycline antibiotics became localised in areas of new bone formation and since then, a large number of reports have been published on the tetracycline labelling technique for the study of bone growth in human material as well as animals such as dogs and cats (*Frost et al.* 1960, *Lee et al.* 1965, *Manson & Waters* 1965). However, quantitative studies on bone growth in rats have been few, though the rat is a commonly used laboratory animal. This may be due to difficulties in making serial sections of undecalcified rat bones, it being known that the osteoblastic activity is irregular in rat bones and a true illustration of the amount of osteoblastic activity requires examination of a large number of serial sections. A rapid method for producing serial sections of undecalcified rat bones was recently described (*Raman* 1966b), and this method was used in conjunction with the tetracycline labelling technique for the study of appositional bone growth in rats.

MATERIALS AND METHODS

Male Wistar rats of known age were chosen and grouped according to age so that there were seven groups of 10 animals each, ranging in age from 4 weeks to 16 weeks. They were fed rat pellets and water. On the first day of the experiment, the rats were given an intra-peritoneal injection of Terramycin (Pfizer) 40 mg/kg body weight in normal saline and the drug was repeated in the same dosage on the eleventh day. The animals were killed with chloroform forty-eight hours after the second injection and the femurs and tibiae immediately removed and dehydrated in absolut alcohol. After embedding in 'Tensol' cement (*Raman* 1966a) serial sections of the diaphyses of the bones were cut by the method previously described. The sections were ground to a thickness of approximately 50 μ and mounted in D.P.X.

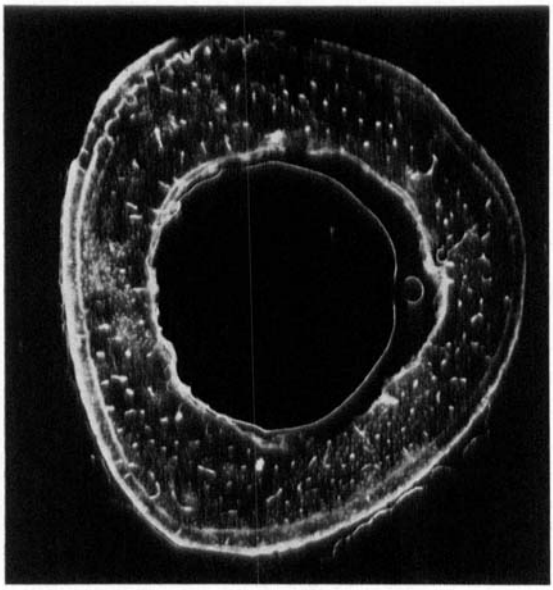

Figure 1. Photomicrograph of a section of tibia showing the two complete rings of tetracycline label. (Unstained, ground section in ultra-violet light, × 120).

and examined in transmitted ultra-violet light with a Leitz fluorescent microscope. On the average, it was possible to obtain 40 sections from the femurs and 30 sections from the tibiae.

Although most of the sections showed two complete rings of tetracycline labels (Figure 1), there were a few sections, especially from the animals in the younger age group, in which the rings were incomplete. Whether the rings were complete or not, each section was examined and the distance between the rings of tetracycline labels on the periosteal side were measured to the nearest micron using a Leitz screw micrometer eyepiece. Eight random readings were taken for each section at different points and averaged and the process was repeated for all the sections from each bone and the mean appositional growth rate on the periosteal aspect was calculated.

RESULTS

The results are given in Tables 1 and 2.

DISCUSSION

The rate of appositional growth was greatest in the younger animals and decreased with increasing age of the animals (Figure 2). The femur grew more rapidly than the tibia in the younger animal, and as the animal got older, the rate of growth in the two bones tended to become uniform. Comparison of the percentage increase in body weight with the growth rates in the bones showed a similar pattern. These results

Table 1. Appositional growth rate in femur.

Group	Age	Mean distance between tetracycline labels in μ*	App. growth rate/day in μ	S.E. ±
I	4 weeks	105	10.5	± 0.17
II	6 weeks	76	7.6	± 0.11
III	8 weeks	60	6.0	± 0.13
IV	10 weeks	46	4.6	± 0.10
V	12 weeks	36	3.6	± 0.13
VI	14 weeks	27	2.7	± 0.07
VII	16 weeks	24	2.4	± 0.10

* Average of 10 animals.

Table 2. Appositional growth rate of tibia.

Group	Age	Mean distance between tetracycline labels in μ*	App. growth rate/day in μ	S.E. ±
I	4 weeks	83	8.3	± 0.10
II	6 weeks	63	6.3	± 0.10
III	8 weeks	48	4.8	± 0.08
IV	10 weeks	40	4.0	± 0.11
V	12 weeks	29	2.9	± 0.09
VI	14 weeks	22	2.2	± 0.06
VII	16 weeks	19	1.9	± 0.06

* Average of 10 animals.

are in agreement with those of *Tapp* (1966) who studied the rate of increase in the cross sectional area of tibiae of rats with age.

Whereas most of the sections from the older animals showed two clear rings of tetracycline labels, in sections of bones from animals in the younger groups the rings were incomplete. This was due to the extensive remodelling that takes place in younger bones. *Frost et al.* (1961) do not consider it justifiable to include sections which lack two complete rings because they feel that bone forming in these areas does so under the protection of different control and triggering mechanisms than that formed in the making of concentric bands. On the other hand, if sections consisting of two complete rings are the only ones measured, it will not be truly representative of the osteoblastic activity of the bone as a whole because the osteoblastic activity in rat bones varies from one part to another of the same bone. It is therefore felt that if a

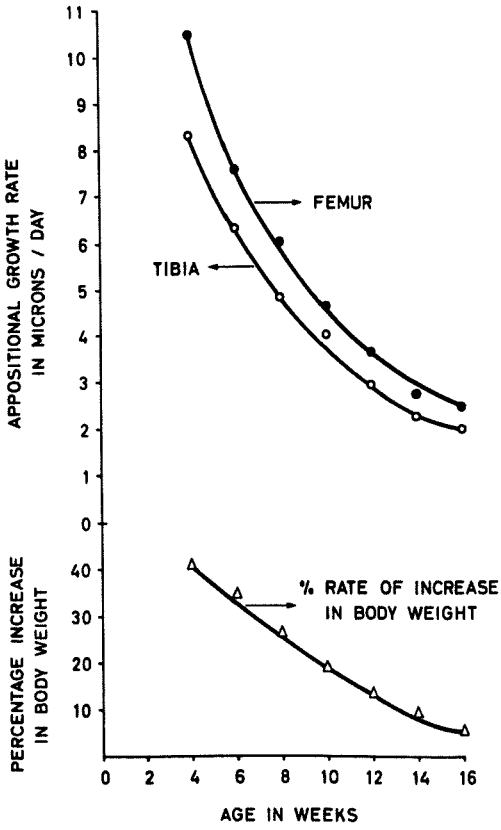

Figure 2. The variation in the appositional growth rates of the femur and tibia and the percentage increase in body weight plotted against the age of the animals.

large number of serial sections from each bone are examined and measured, a more accurate picture of the growth rate could be obtained.

The method is useful for measuring the periosteal appositional growth rates in the diaphysis, and it is possible to measure endosteal growth rate in a similar way. Further, the method could be used to study rates of bone growth under various conditions such as the influence of hormones and drugs and these could be compared with growth under physiological conditions.

SUMMARY

The periosteal appositional growth rates in the femurs and tibiae of rats of different ages were measured using the tetracycline labelling technique. It was seen that the growth rates declined as the animals became older, and it is suggested how a study like this could give information on bone growth rates under different experimental conditions.

RESUME

Les taux de la croissance appositionelle proximale du fémur et du tibia chez des rats de différents âges ont été mesurés au moyen de la technique de la coloration à la tétracycline. On a constaté que les taux de crossance tombent au fur et à mesure que les animaux deviennent plus âgés. On suggère qu'une étude de ce genre est susceptible de donner des informations sur les taux de croissance des os dans différentes conditions expérimentales.

ZUSAMMENFASSUNG

Die periostale, appositionelle Wachstumgeschwindigkeit an Femuren und Tibiæ von Ratten verschiedenen Alters wurde mittels der Tetracykline Markierungstechnik gemessen. Man sah, dass die Wachstumsgeschwindigkeit mit zunehmenden Alter der Tiere abnahm und manmeint, dass eine gleichartige Studie Aufschluss über Knochenwachstumsgeschwindigkeit unter verschiedenen experimentellen Bedingungen geben könnte.

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