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ANALYSIS OF DIURNAL RATE OF LONGITUDINAL GROWTH OF BONES

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In an investigation of the effects of oxygen breathing on the growth in length from the epiphyseal growth plate with oxytetracycline labelling it proved necessary to ascertain whether such growth has any diurnal periodicity. The possibility of such a rhythm has been suggested by Simmons. Utilizing the colchicine method in growing mice he found the number of mitotic figures in the epiphyseal plates of the distal femur and proximal tibia to be 26.8 per cent respectively 23.3 per cent larger in a night group than in a day group (*Simmons* 1962). In an other study utilizing glycine C-14 incorporation (*Simmons* 1966) he established that collagen synthesis and new bone formation had a corresponding rhythm with the greatest activity in the environmental light period 09.00–21.00, although the rats feed most actively at night. He does not say whether this implies a corresponding variation of the growth in length of the metaphysis or an increase in the height of the epiphyseal plate or simply a variation in the activity without any effect on the metric growth in length of the bone. He felt that the day-night differences reflected the diurnal variation of the secretory activity of the parathyroid gland and the adrenal cortex. This diurnal periodicity is reflected by a wide variety of tissues and has also been demonstrated with tritium-thymidine in growing bone (*Messier & Leblond* 1960). The purpose of the present study was to ascertain whether allowance must be made for any such variation in the longitudinal growth rate of the metaphysis of the bone in young rabbits as measured by oxytetracycline fluorescence labelling during short time variation of the oxygen content of the air inhaled.

METHOD

The growth in length of the bone was measured by the technic of *Hansson* (1967). Oxytetracycline in a dose of 1 mg/kg bodyweight was injected *i. v.* into 4 to 6 weeks old rabbits. Such doses do not influence the growth (*Hansson* 1967). Repeated injections at exactly 12 hour intervals mark the position of the zone of calcification on each occasion; in sections placed parallel to the metaphyseal trabeculae these appear in the ultraviolet microscope as parallel lines spaced at intervals corresponding exactly to the growth in length of the metaphysis during each twelve-hour period. According to this method, the proximal part of the tibia is dissected out, fixed for two days in pure ethanol, and sectioned with a razor blade without previous decalcification. The sections are treated for five minutes in xylol and mounted in DePeX. The distance between the lines was measured in 5 to 10 sections from each animal and the means were calculated. The error of the method in the hands of the author has been determined by simultaneous preparations of the left tibia and the right independently and the mean difference was 3.7 per cent (*Persson* 1967).

MATERIAL

The material consisted of 28 white rabbits, aged 4-5 weeks, from five litters. The animals were kept together with their mothers in hutches in an in-door stable and were given pellets and water *ad lib.* The stable was illuminated by daylight from 5 a. m. to 7 p. m. Each animal received all together 5 injections of oxytetracycline, the interval between consecutive injections being exactly 12 hours to allow 4 semi-diurnal recordings in each animal. The animals were divided into two groups, two litters of totalling 13 animals were given the injections at 13.40 hours and 01.40 hours respectively, (in Table 1 called p.m./a.m.) and 15 rabbits belonging to the remaining three litters were given injections at 07.40 and 19.40 hours (in Table 2 called day/night). The use of this 6 hour difference between the times of the injections in the two groups was intended to reveal any variation possibly concealed by being equally large before and after the time of the injection. By judging from four semi-diurnal observations in each animal it was possible to see if any variation found was repeated in both the consecutive days, thereby giving support to a true diurnal rhythm.

RESULTS AND DISCUSSION

The mean values show in Table 2 that the distance was about 1 per cent more during the daytime than at night and in Table 1 about 1 per cent greater in the afternoon than during the morning hours. This tendency was noted in both days in both groups, but the differences were too small to be statistically significant using the t-test. The values noted on the second day were also somewhat lower than those recorded on the first day, which is the normal decrease at this age (*Hansson* 1967). Since the 12-hour periods in the two groups overlapped one another by 50 per cent, it was possible also to evaluate the

Table 1. Growth distances in microns per each of four consecutive periods of 12 hours and mean values. P.m. is 13.40-01.40, a.m. is 01.40-13.40.

Rabbit No.	p.m.	a.m.	p.m.	a.m.
1	230	210	230	220
2	250	250	260	250
3	220	230	230	230
4	260	240	240	240
5	260	260	250	250
6	270	250	260	240
7	230	240	230	230
8	300	310	300	300
9	260	260	260	240
10	290	290	290	290
11	290	280	300	290
12	290	280	280	290
13	300	310	300	300
Mean value	265	262	264	259

Table 2. Growth distances in microns per each of four consecutive periods of 12 hours and mean values. Day is 07.40-19.40, night is 19.40-07.40.

Rabbit No.	Night	Day	Night	Day
14	280	280	280	290
15	220	240	190	200
16	280	270	290	300
17	270	280	260	270
18	270	270	260	280
19	260	260	260	270
20	270	270	280	270
21	270	280	260	280
22	300	310	300	290
23	260	260	260	260
24	280	280	290	250
25	300	300	300	300
26	300	300	300	290
27	300	320	300	300
28	300	300	300	300
Mean value	277	281	275	277

growth during 6-hour periods. If these 6-hour periods be called a, b, c and d , with period a beginning at 01.40 hours, the mean value of $b+c$ will be larger than that of $a+d$, and that of $c+d$ larger than that of $a+b$. Addition of these inequalities will show that $c>a$, that is, growth

is greater during 13.40-19.40 than during 01.40-07.40. Normalisation of the means of the observations ($a + b + c + d = 1$) will show finally that $d > b$ i.e. growth is greater during 19.40-01.40 than during 07.40-13.40. But since the differences found were about 1 per cent only and statistically insignificant they warrant no final conclusions. It would appear that the growth in length from the epiphyseal growth plate in young rabbits does not vary to any considerable extent from one 6-hour period to another, unless diphasic variation occurs within periods of less than 6 hours, a possibility that seems unlikely. Even if such a short diphasic variation did occur, it would in no way influence the value of this tetracycline method for studying growth during consecutive 12-hour periods.

SUMMARY

A diurnal rhythm in the growth of bones has been reported by Simmons. To see whether this rhythm is found in the metric growth in length of the metaphysis in rabbits, oxytetracycline labelling was used by repeated injections at 12 hour intervals. The variation found was statistically insignificant, the mean differences being about 1 per cent with greater values for the afternoon. Allowance for a diurnal rhythm is not necessary in the use of this method.

RESUME

Un rythme diurne de la croissance des os a été rapporté par Simmons. Pour constater si ce rythme se trouve dans la croissance métrique en longueur de la métaphyse chez les lapins, on a utilisé une coloration à l'oxytétracycline par injections répétées à 12 heures d'intervalle. La variation constatée est insignifiante statistiquement, la différence essentielle étant d'environ 1 per cent avec des données plus élevées dans l'après-midi. La reconnaissance d'un rythme diurne n'est pas nécessaire si l'on utilise cette méthode.

ZUSAMMENFASSUNG

Ein täglicher Rhythmus des Knochenwachstums ist von Simmons berichtet worden. Um zu sehen ob dieser Rhythmus im metrischen Längenwachstum der Metaphyse von Kaninchen gefunden werden kann, wurde Oxytetracyclinmarkierung mittels wiederholter Injektion in 12 stündlichen Intervallen verwendet. Die gefundenen Verschiedenheiten

waren statistisch nicht bezeichnend, indem die Hauptunterschiede ungefähr 1 Prozent mit grösseren Werten am Nachmittag, waren. Berücksichtigung eines täglichen Rhythmus ist nicht nötig bei der Anwendung dieser Methode.

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