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Comparative patterns of cell division in epiphyseal cartilage plates in the rabbit

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INTRODUCTION

The results of cell kinetic investigations of many of the cartilage plates in the bones of the hind limb and pelvis of rats have been published (Kember, 1972; 1973). Based on these comparative data, a hypothesis was proposed that in a given animal the division rate of proliferating chondrocytes in all cartilage plates is constant, and that variations in overall growth produced by the various plates are largely due to changes in the numbers of cells in proliferation zones. Differences in the mean sizes of hypertrophic cells also make some contribution to changes in growth rates between plates.

Not all the results in these studies were consistent with this hypothesis, and the work of Roberts (1979), who showed a correlation between labelling index and proliferation zone size in the cartilage plates of the rat skull, has thrown some further doubt on the ideas. In order to make further investigation, it was decided to examine the comparative cell kinetics of a series of cartilage plates in another animal, the rabbit. This paper describes the results of that study, which was combined with measurements of overall growth of a number of the bones in the hind limbs of the rabbits used for the cell kinetic studies.

MATERIALS AND METHODS

Three rabbits of the New Zealand White strain were used in this study. These animals were bred in the Medical College and were 8 weeks of age and 2 kg in weight, on average, at the start of the experiments.

The first stage was to take radiographs of the hind limbs. The animals were lightly anaesthetised with Sagatal and radiographs were taken with the rabbits prone and supine (80 kV, 60 cm focus-film distance, Kodirex film). In each case, care was taken to achieve a uniform position with the tibiae parallel to film (prone view) and the feet parallel (supine view). The radiographs were repeated after ten days.

Calculations of bone growth during the ten days interval were made from measurements on the radiographs using dividers and a steel rule. Distances between corresponding features on the bones in the pairs of radiographs were recorded for the tibiae, metatarsals, proximal phalanges and middle phalanges. In some radiographs of the middle phalanges the features were not distinct enough to make measurements. Again, in certain pairs of radiographs, the junction of the fibula and tibia was a clear feature and it was possible to separate the growth contribution of the proximal and distal cartilage plates of the tibia. Measurements of the femora were not made because these bones were not parallel to the film in either of the views employed.

Immediately after the second series of radiographs had been taken the animals were injected with 1 mCi tritiated thymidine (Amersham International 5 mCi/mmole) through an ear vein. One hour later the animals were killed and the bones of the hind limbs dissected out. These were fixed in formol saline, decalcified in EDTA and blocked in wax. Longitudinal sections were cut at a thickness of 5 μm. Four sets of sections were used for autoradiography, one set having been previously stained with Feulgen reagent. The slides were coated with K 5 emulsion using the dipping technique. The other sets were stained with haematoxylin and eosin after development.

In general, the cell kinetic techniques used in the earlier studies were followed. On the sections stained with haematoxylin and eosin, the positions of 100 labelled cells were recorded in terms of the counted position of each labelled cell down the cartilage columns. The decision about which cell at the epiphyseal border should be designated cell number one introduced a large degree of subjectivity into these counts. The numbers of hypertrophic cells per column at the metaphyseal border of the plate were also recorded. Labelling indices were measured on the Feulgen stained auto-radiographs where the nuclei, both labelled and unlabelled, could be readily recognised. Counts of labelled nuclei in three consecutive groups of 100 nuclei were taken from each of two sections per cartilage plate. This again was a subjective procedure in that counts were limited to the zone of apparently 'actively' proliferating cells within the cartilage columns.

Finally an IBAS computer based image analyser was used to find the heights in the direction of growth of 50 consecutive hypertrophic cells along the metaphyseal border of each cartilage plate.

For all these measurements, regions of the cartilage plate close to the centre of the bones were selected to avoid possible 'edge' effects near the perichondrium.

RESULTS

The growth rates for the bones measured over the ten days interval are presented in Table 1. This Table shows the number of bones measured, the growth rate in micrometres per day and the standard deviation of the growth rates.

Data are given for the overall growth of the tibia and for the separate contributions of the proximal and distal growth plates with measurements on the fibula. In the case of these latter data, it was assumed that the junction of the tibia and the fibula remained a reliable 'fixed' marker for the measurement of these separate growth rate contributions.

For the radiographs the bones were within 2 cm distance of the film with a focus-film distance of 60 cm, so that the magnification could have been no larger than 3 %.

The results of the major cell kinetic study are summarised in Table 2 where data from each of the three rabbits are presented. The labelling indices were calculated from counts on 600 nuclei per epiphyseal plate so that a standard error of between 1·5 and 2 % would be expected for labelling indices of 12 % and 25 % respectively. The differences between the majority of labelling index values for the same plate for the three rabbits lay at about this expected degree of variability with two exceptions (proximal end of femur for rabbit 3 and middle phalanx for rabbit 1). There could be no doubt, however, that the hypothesis that the labelling indices in all the growth plates were the same had to be rejected.

The second set of data in Table 2 gives the estimated values for the number of cells in the proliferation zone of each plate. Those estimates were based on the labelling

Table 1. *Radiographic measurements of bone growth rate*

	Whole tibia	Proximal tibia	Distal tibia	Fibula	Meta-tarsal	Proximal phalanx	Middle phalanx	Calcaneum
Samples	6	4	4	4	12	10	2	2
Growth (μm/day)	670	390	290	410	220	110	80	75
S.D.	40	30	60	15	30	20	—	—

Table 2. *Summary of cell kinetic data for rabbits* 1, 2 *and* 3

	Labelling index %			Proliferation zone			Hypertrophic cell size (μm)		
	1	2	3	1	2	3	1	2	3
Proximal femur	13	13	17	13	13	13	19	24	22
Distal femur	20	19	21	21	18	14	26	28	27
Proximal tibia	23	21	25	16	18	17	27	28	24
Distal tibia	21	22	23	18	18	16	25	28	25
Fibula	24	24	26	16	19	21	28	28	22
Metatarsal	19	18	22	18	20	16	21	22	21
Proximal phalanx	12	15	12	12	17	13	20	23	18
Middle phalanx	9	13	12	14	14	10	17	19	20
Calcaneum	—	—	12	—	—	8	—	—	21

Table 3. *Comparison of measured and calculated growth rates* (μm/day)

	Measured	Calculated*
Tibia	670	610
Proximal tibia	390	310
Fibula	410	360
Distal tibia	290	300
Metatarsal	220	230
Proximal phalanx	110	120
Middle phalanx	(80)	80
Calcaneum	(75)	60

* Assuming T_s = 8 hours.

profiles, which are shown in Figure 1. The variation from animal to animal could not readily be treated to statistical analysis but again, with a few exceptions, the proliferation zone sizes for corresponding plates were comparable from rabbit to rabbit but showed some variation from plate to plate, the larger zones being the distal end of the femur, proximal and distal ends of the tibia, fibula and metatarsal. The phalanges and the proximal femur had shorter proliferation zones. It is not sensible to comment on the calcaneum with only one specimen available.

The overall shapes of the labelling profiles in Figure 1 show one similarity in that the labelling frequencies in the top few positions in cell columns were low. Also, as expected, the profiles for the proximal tibia and fibula were nearly equivalent. It is to be noted that the longer columns had a much increased zone of maturing cells, i.e. between the proliferation zone and the hypertrophic cell zone. The longer

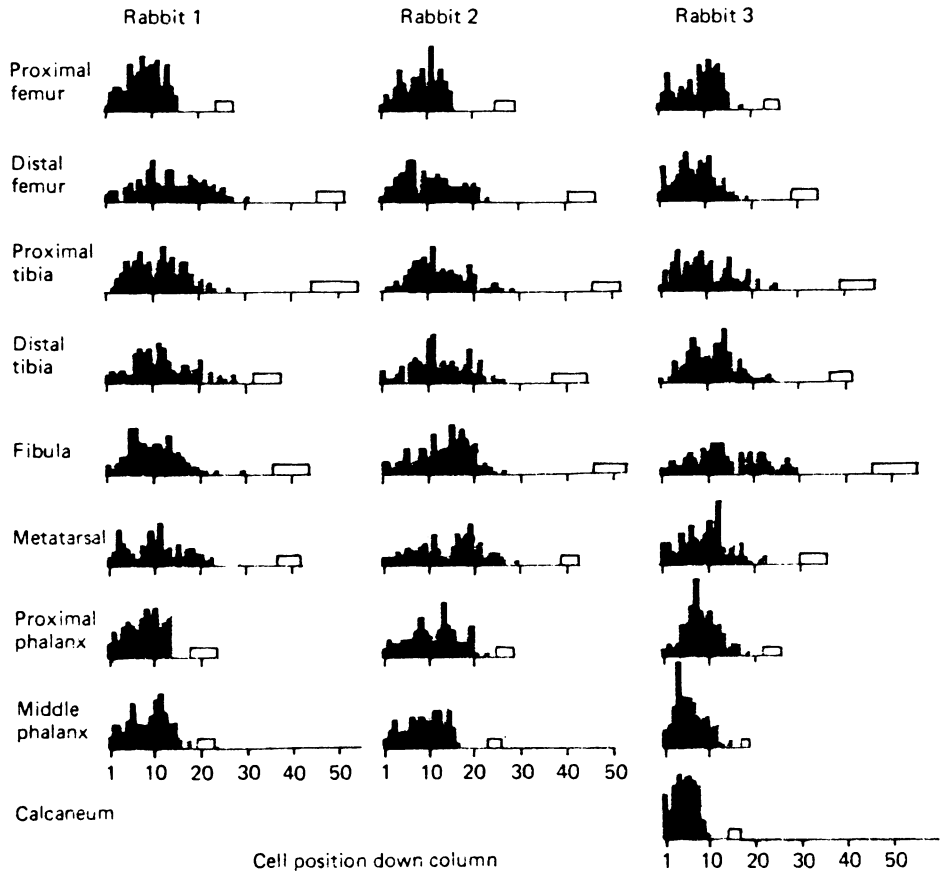

Fig. 1. The black histograms show the numbers of labelled nuclei at counted positions down the cartilage columns in these epiphyseal plates in three rabbits. The positions of 100 labelled nuclei were recorded for each histogram. The open rectangles show the position and numbers of hypertrophic cells in each column.

maturation time was, perhaps, reflected in the increased size of the corresponding hypertrophic cells. These sizes are also tabulated in Table 2.

The values given for hypertrophic cell sizes are the means of 50 measurements. Standard deviations varied between 4 and 9 μm, with a mean of just over 6 μm. This gave a standard error of 0·9 μm for these data. With the exception of the distal femur (rabbit 1) and fibula (rabbit 3) the values lay within the expected limits for variation between animals.

DISCUSSION

An attempt to relate the radiographic measurements with the cell kinetics is shown in Table 3. The growth rate calculated from the autoradiographic analysis is compared with the overall growth rate of the bones and, where possible, with the separate contributions of specific growth plates.

The degree of comparability is surprising considering the subjective nature of the cell kinetic analysis and the omission of any allowance for factors such as the shrinkage that occurs in histological processing. The calculated growth rates are

based on an estimate of 8 hours from the DNA synthesis time (T_s) of these chondrocytes. The cell cycle time T_c can then be estimated from the equation

$$\frac{T_s}{T_c} \simeq \frac{L.I.}{100}.$$

The use of this basic equation, that does not include a factor to account for the age distribution of proliferating cells, tends to overestimate the length of T_c. The division rate for proliferating chondrocytes in cells/day is given by the inverse of T_c. Then growth rate = number of cells in proliferation zone × division rate × average height of hypertrophied cells. The growth rate will be in micrometres per day.

The main aim of the experiment was to study relationships between the cell kinetic variables that determine bone growth rate. It is evident that the simple hypothesis that the controls on proliferation rate of chondrocytes are independent of the controls on proliferation zone size is probably not true. Higher labelling indices are linked with longer proliferation zones in this series of bones.

The abandonment of the separate controls concept does not make for simpler theories of growth control mechanisms. For example, suppose that dividing chondrocytes produce a growth promoting substance that diffuses slowly out of the growth plate in both the epiphyseal and metaphyseal directions. Further, let it be supposed that cell division can only occur when the local concentration of such promoter is high enough. Under these conditions rapidly dividing cells would produce higher levels of promoter and thus a wider proliferation zone. This poses several problems. Firstly, there would need to be a limit on the maximum division rate, or else division rate would accelerate continuously in ever expanding proliferation zones. Secondly, how is the width of a proliferation zone set up originally? Thirdly, how would this promoter interact with all the other factors which are known to affect bone growth rate (Kember, 1978)? These data are offered, then, as grist for the theorists' mill, but they must be incorporated into the final product.

The process of data collection revealed evidence of a variation of mean hypertrophic cell size across the width of the cartilage plate. This accords with the general finding that the pattern of columns changes towards the edges of the plate. Such observations deserve further investigation since they point to the subtlety of the bone growth process.

SUMMARY

The growth rates of the bones in the hind limbs of three young rabbits have been measured radiographically and compared with cell kinetic studies of the corresponding cartilage growth plates. The measured growth rates agreed well with the values calculated from the cell kinetic data.

The proportion of nuclei labelling with tritiated thymidine, the number of cells in the proliferation zone of the columns and the sizes of hypertrophied cells were measured for each cartilage plate. It was found that the plates in the distal femur, proximal and distal tibia and fibula had the highest values for all three quantities. The plates of the proximal femur and metatarsals had intermediate values and the phalanges had the lowest values for all three measurements.

The consequences of this correlation between the three variables that affect bone growth are discussed in relation to possible control mechanisms.

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