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

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INTRODUCTION

For the development of an understanding of growth control mechanisms in bone, there is a need to accumulate a basis of information about the relationship between cell division in the growth plate and overall bone growth. Since the introduction of tritiated thymidine for autoradiography in 1957 some data have become available on cell kinetics in rat and rabbit growth cartilage. The published data relate to the tibia in the rabbit (Rigal, 1962) and to the tibia (Kember, 1960), the mandible (Blackwood, 1966) and the tail vertebrae (Dixon, 1971) in the rat. The cartilage cell kinetics of the rat tibia have also been studied under a variety of experimental conditions in which the growth rate of the bone was disturbed (Kember & Walker, 1971). In the results of these latter experiments, certain patterns of cell division seemed to emerge which might give a clue to growth control mechanisms. Since these mechanisms must be effective in all long bones it is important to compare the cell kinetics of many growth plates in the same animal.

MATERIALS AND METHODS

Three 6-week-old male Wistar rats were injected intraperitoneally with 1 μCi/g of tritiated thymidine (Radiochemical Centre, 5 Ci/mmole). The animals were killed 1 hour after the injection and the bones of a hind limb, together with a 6th or 7th costochondral junction, were dissected out. After fixation in formol saline the bones were decalcified in EDTA and embedded in wax. Longitudinal sections were cut at 5 μm and sections taken from near the mid-line of the bone were selected for autoradiography by the dipping technique (Ilford K 5 emulsion). The slides were exposed for 6 weeks and were stained with haematoxylin and eosin after development.

MEASUREMENTS

After a 6 week exposure most of the labelled cells showed a high grain count (greater than 50 grains/nucleus) so that there were few nuclei whose labelling was in doubt. A minimum of 10 grains per nucleus was taken as a criterion for 'labelling' compared with backgrounds of 1 or 2 grains per nucleus. For each bone, work was carried out on two sections and the following examinations made:

(1) The percentage of labelled nuclei in the proliferation zone was estimated. This can only be an estimate, since the lower limit of the proliferation zone can only be

fixed in the observer's judgement. The labelling index, that is the percentage of labelled nuclei, must therefore always depend on some subjective factors.

(2) The positions of 100 labelled cells within the cartilage columns were found by counting the cells in each column from the epiphysis (cell number 1) down to the metaphysis. The numbered position of each labelled cell was recorded together with the total cell count per column.

(3) Using a calibrated eye-piece graticule, the heights of 25 hypertrophic cells were measured. These were 25 consecutive cells along the metaphyseal border of the epiphyseal plate. By 'height' is meant the dimension of the cells in the direction of growth of the bone.

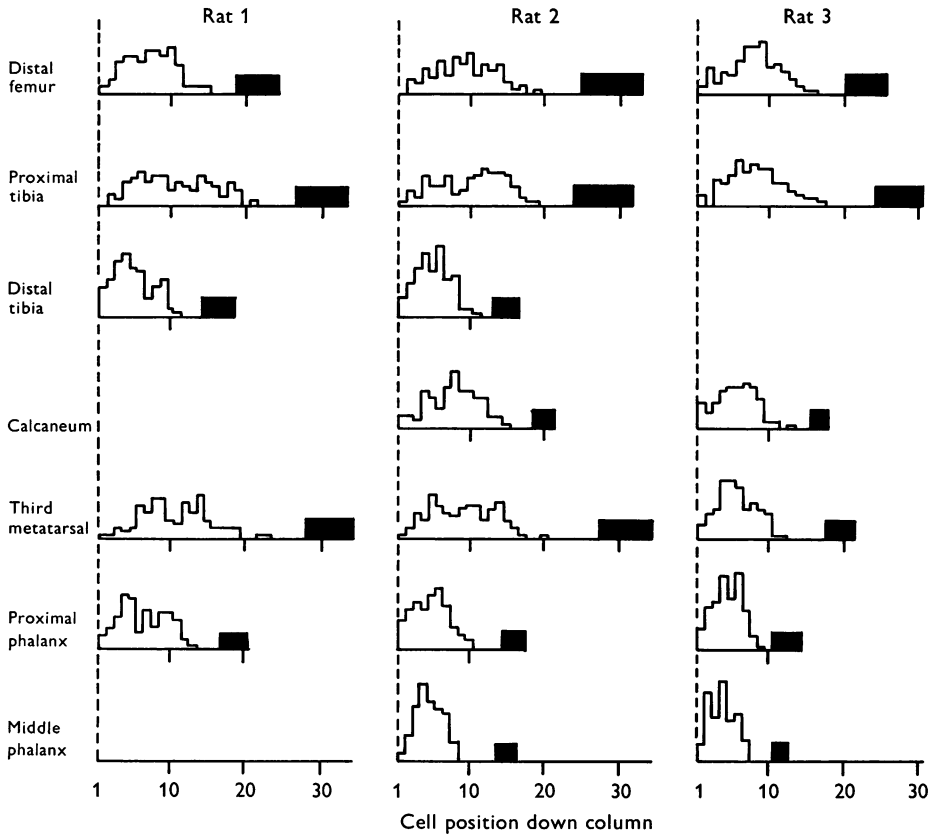

Fig. 1. Labelling profiles for epiphyseal cartilage plates in 3 rats. The profiles are frequency histograms for labelled nuclei, at 1 hour after an injection of tritiated thymidine, for each cell position down the cartilage columns, starting with position 1 adjacent to the epiphysis. The average lengths of the columns are also shown, with the approximate lengths of the hypertrophic zones (solid areas).

RESULTS

The results of the analyses of the bones are recorded in Fig. 1 and Table 1. In three instances suitable sections were not available for analysis. In Fig. 1 are presented the labelling profiles for each of the bones. These profiles show the frequency

with which labelled cells are found at each position, starting with cell number 1 adjacent to the epiphysis (for further details see Kember, 1971). Thus, referring to the first profile, which represents the distal epiphyseal plate of the first femur, out of 100 labelled cells recorded, only two were in the first position, four were in the second position down the column, nine were in the third position, ten in the fourth position, and so on.

Table 1. *Bone growth parameters for epiphyseal cartilage plates in the three rats*

	Effective cells/column in proliferation zone			Percentage labelling in proliferation zone			Hypertrophic cell height (μm)		
Rat	1	2	3	1	2	3	1	2	3
Distal femur	11	14	13	14	17	17	29	32	28
Proximal tibia	17	15	14	18	17	13	29	30	26
Distal tibia	9	8	—	10	12	—	23	25	—
Calcaneum	—	13	9	—	14	8	—	20	18
Metatarsal	15	15	10	13	14	15	24	25	20
Proximal phalanx	11	9	8	13	12	12	23	24	20
Middle phalanx	—	7	7	—	7	11	—	20	19
Rib	34	36	33	15	15	14	25	27	26

These profiles also show the average length of the cartilage columns and the approximate length of the zone of hypertrophic cells. All the plates studied appeared uniform in thickness except for the femur, which has an undulating rather than a flat 'plate' so that sections may include regions where the plate is cut obliquely.

It can be seen that all the profiles are roughly similar in *shape*. The frequency of labelling is lower in the top one or two positions, which correspond to a reserve or stem cell zone where the cells divide more slowly (Kember, 1960). Towards the metaphyseal end of the columns the frequency of labelling falls, without a sharp cut-off, to mark the differentiation of proliferating into maturing cells. The profiles show that the lengths of the proliferation zones, that is, the number of cell positions with an appreciable frequency of labelling, do vary from bone to bone in the same rat but in general there is fair agreement between the profiles for the same bone in the three animals. The obvious exception is the metatarsal plate of the third animal, which has a shorter labelling zone in comparison with the other two metatarsals.

In order to calculate the theoretical bone growth from kinetic data the number of cells per column in the proliferation zone must be counted. Any realistic estimate of the effective length of the proliferation zone must take into account the lower frequency of labelling found in cell positions at the two ends of the zone. Taking the mean frequency of each histogram as a standard, the main proliferation zone was taken to lie between the two extreme cell positions with frequencies exceeding the mean. Cell positions outside these limits were added to the proliferation zone total, but with a fractional weighting factor equal to the frequency at that position divided by the mean frequency. Thus for the top left profile in Fig. 1 the mean frequency is 6·6. The positions 3 and 11 therefore define the limits of the main proliferation zone, i.e. 9 cells long. To this is added $2/6{\cdot}6 = 0{\cdot}3$ for the first position, $4/6{\cdot}6 = 0{\cdot}6$ for the

second position, 2/6·6 = 0·3 for the 12th position, etc., making a total of 11 cells in the proliferation zone. This method, if somewhat arbitrary, provides a reasonable basis for the comparison of proliferation zones, and zone lengths calculated in this way have been entered into the appropriate columns of Table 1.

The labelling indices and the heights of hypertrophic cells are also given in Table 1. In general, there is again fair agreement for the values for the corresponding cartilage plates in the three rats. Average values have therefore been calculated for each of the three measurements and have been entered into Table 2. It was felt that the zone length and labelling index data, being estimates based on subjective observations, did not warrant statistical analysis.

Table 2. *Average values of growth parameters with calculated and measured growth rates*

	Effective cells/column in proliferation zone	Percentage labelling in proliferation zone	Hypertrophic cell height (μm)	Calculated growth rate (μm/day)	Measured growth rate (μm/day)
Distal femur	13	15	30	200	*350 ± 40
Proximal tibia	15	16	28	230	*360 ± 70
Distal tibia	9	11	24	80	
Calcaneum	11	11	19	80	90 ± 50
Metatarsal	13	14	23	140	120 ± 30
Proximal phalanx	9	12	22	80	100 ± 30
Middle phalanx	7	9	20	45	45 ± 10

* Growth rate for distal and proximal growth plates combined.

Data for the costochondral junctions are included in Table 1. These data are not strictly comparable with the values for the other plates since the structure of this growth cartilage is dissimilar from that of the long bones. There is no epiphysis in the junction, the growth cartilage being contiguous with the costal cartilage. The histology resembles that of the growth cartilage of a tibia from a week-old rat, since the cells are grouped in clones rather than columns. For this reason the cell counts to determine the positions of labelled cells were unreliable, but it is evident that the proliferation zones are much longer in the costochondral junctions than in the other plates studied. Some isolated labelled cells were found well into the costal cartilage, but the limit of the main proliferation zone appeared to coincide with the limit of the perichondral ring.

CALCULATION OF GROWTH RATES

From unpublished work in this laboratory we know that the duration of the DNA synthesis ('S') phase for growth cartilage cells of the tibia of a 6 week old rat is 7 hours. It is unlikely that it has a widely different value for the other cartilage plates. Assuming a duration of 7 hours for the 'S' phase the number of cells going through 'S' per day per column can be calculated:

$$\text{No. of 'S' cells per column per day} = \tfrac{1}{100}\text{Labelling index} \times \tfrac{24}{7} \times \text{no. of cells in proliferation zone.}$$

If all cells going through 'S' divide, this is also the number of new cells produced per column per day. If this figure is multiplied by the average height of hypertrophic cells the result is the growth contribution per day from the cartilage plate. This calculation has been carried out on the data of Table 2 and theoretical growth rates for each plate are given in the fourth column. In the last column these calculated growth rates are compared with growth rates measured from serial radiographs on five male rats of the same age and species as these experimental animals.

DISCUSSION

The data presented here indicate that there are no obviously constant factors in the cell kinetics of different growth cartilages in the same animal. All three parameters contributing to bone growth vary from bone to bone; the lengths of proliferation zones, the labelling indices and the heights of hypertrophic cells. There is a possibility that the labelling index for *dividing* cells might be constant in all the plates if there were a variation in the proportion of cells within the zones that were in active division cycle. The proportion of non-cycling cells in the tibia is very low (Kember, 1960) but Dixon (1971) has reported higher proportions in the tail vertebrae. The correlation between calculated and measured bone growth appears to be quite good. For the tibia the contributions of both proximal and distal growth plates have to be added. The proximal plate of the femur was analysed in only one bone and a contribution of approximately 80 μm per day was calculated. There are some other factors which might be included in the calculation, all of which would tend to increase the theoretical values. The first is the diurnal variation in labelling index. These three rats were injected during the morning when the labelling index is about 10% lower than the average value throughout 24 hours. Thus rather more cells divide per day than has been calculated here. Again the height measurements were made on hypertrophic cells that might have shrunk slightly in the wax embedding process. Finally there may be some increase in overall growth due to expansion of the epiphysis. This contribution is unlikely to be larger than the increase in radius of the cartilage plate, which is about 25 μm per day (radiographic measurements) for the tibia.

This survey sheds little light on possible mechanisms of growth control. The length of the proliferation zone, which was found to remain remarkably constant under a range of insults for one growth plate (Kember & Walker, 1971), varies by a factor of 2 from plate to plate within the same animal. This makes it seem unlikely that the differentiation from proliferative to maturing cell is triggered by a level in a concentration gradient. The variation in labelling indices is of the same order and would indicate some interaction between humoral factors (hormone levels, nutrition), which should be constant for all bones, and an internal control set at different levels within each cartilage plate.

It is noteworthy that the labelling index increases with the length of the proliferation zone, the longer zones having the higher labelling indices. It could be that in a thick plate the concentration of a mitotic stimulator would build up to a higher level than in a thin plate, but on the other hand the more rapidly dividing cells might result in a wider proliferation zone. The variation in the sizes of the hypertrophic cells is

a secondary effect, since the cells in a slowly growing plate will be invaded from the metaphysis before they have increased to the maximum size observed in rapidly dividing plates.

SUMMARY

Autoradiographic studies using tritiated thymidine have been used to compare the lengths of proliferation zones and the labelling indices of epiphyseal cartilage plates in the hind limbs of three rats. Measurements of the sizes of hypertrophic cells have also been tabulated.

From these data the overall growth rates of the bones have been calculated and found to agree well with radiographic measurements of growth rate.

The variations in all three growth parameters between the different growth plates in the same animal suggest that general controls of bone growth, such as hormone levels, must interact with controls on division rate and differentiation that are specific to each cartilage plate.

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