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The distribution of nuclei in imprints of feline osteoclasts

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INTRODUCTION

The numbers of nuclei in multinucleated giant cells vary greatly. However, the significance of this variability is not known although it has been suggested for odontoclasts, which are multinucleated giant cells similar to oesteoclasts, that there may be an optimum size for maximum efficiency of such cells during resorption of mineralized tissues (Addison, 1976). In that study intact human odontoclasts in smear preparations had between 2 and 72 nuclei.

Few studies of the variation in the number of nuclei in osteoclasts have been made, but Hancox (1956) observed in sections that human osteoclasts have from 2 to 200 nuclei. He also noted a species difference, with feline osteoclasts being larger than human osteoclasts which were, in turn, larger than rodent osteoclasts. Other studies have recorded an average of 4·1 nuclei per osteoclast in rat tibias (Miller, Webster & Jee, 1975), up to 125 nuclei in porcine osteoclasts (Arey, 1919) and 2–110 nuclei in thick osteoclasts in hanging drop cultures of frontal bone (Hancox, 1946).

This paper reports the distribution of nuclei within a normal osteoclast population in kittens, using whole cell preparations.

METHODS

Four 18 weeks old kittens were killed by nitrogen inhalation and bone imprints prepared from the femoral metaphyses. The distal ends of the femurs were dissected and freed of soft tissue, especially fat, which could cause smudging of the imprints. The bones were immersed in ice cold tissue culture medium (TC20, Wellcome Ltd.) and used within one hour. The combined articular cartilage and epiphysis of each bone was snapped off, a clean separation occurring at the epiphysial–metaphyseal boundary. The exposed metaphysis was pared away with a sharp razor blade to produce a flat surface. The spongy trabecular bone was blotted against filter paper and imprints prepared on clean glass slides by a gentle stamping motion. The bone was repeatedly trimmed to produce a fresh surface.

The imprints were first used in a previously reported histochemical investigation of succinate dehydrogenase, beta-hydroxy butyrate dehydrogenase, malate dehydrogenase and glutamate dehydrogenase in kitten osteoclasts (Addison, 1976). However the stained preparations from this investigation were found very suitable for making nuclear counts in individual osteoclasts, particularly in preparations where the intensity of the cytoplasmic staining contrasted strongly with the unstained nuclei. One imprint stained by each of the enzyme techniques was therefore selected for nuclear counting.

Nuclei were counted by scanning the slides systematically at a magnification of

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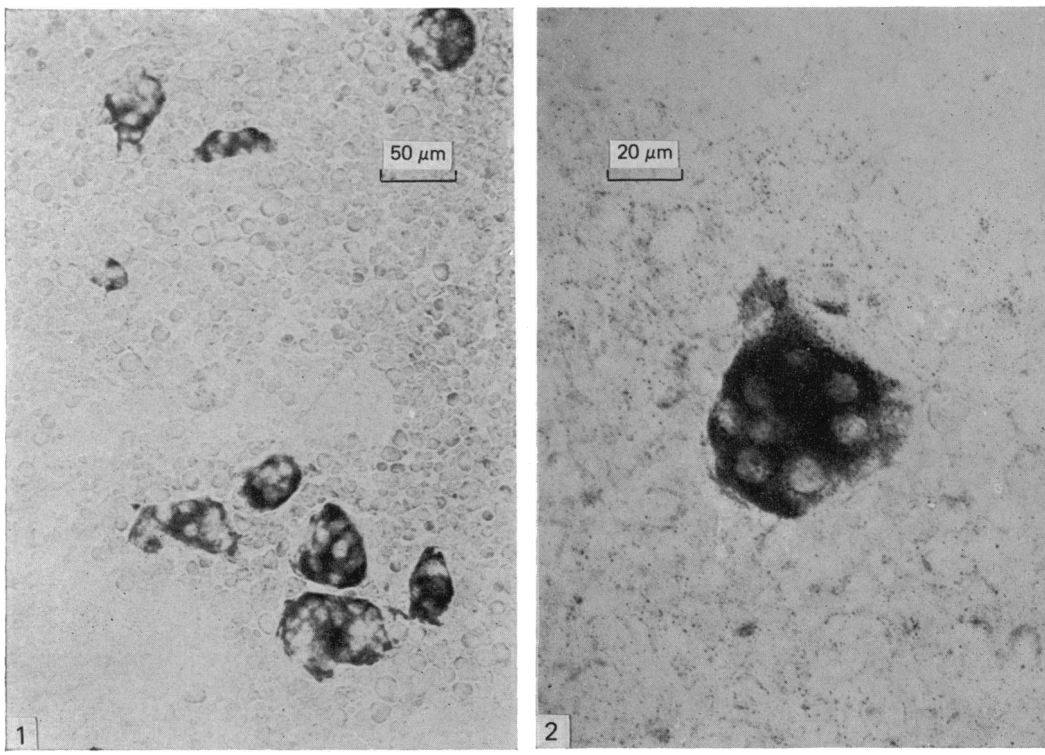

Fig. 1. Osteoclast imprint. Succinate dehydrogenase reaction after 30 minutes incubation. No counterstain. Illustrates strong staining of osteoclasts compared with other bone marrow cells, and shows that large numbers of osteoclasts are seen in imprints. × 200.

Fig. 2. Osteoclast imprint illustrating relative absence of reaction product overlying the nuclei in a densely stained cell. Succinate dehydrogenase, 30 minutes incubation. × 500.

× 125 using a Leitz optical microscope. Lateral traverses were made, and the slide advanced by one field width at a time in a direction at right angles to the traverses. Thus counting of cells more than once was avoided. Only cells having two or more nuclei were regarded as osteoclasts.

Tally counts of the numbers of osteoclasts having a particular number of nuclei were made and the totals integrated. The relative frequencies of osteoclasts having particular numbers of nuclei, and cumulative frequencies, were calculated as a percentage of the total number of cells. The results for the four animals were compared by χ^2 analysis.

RESULTS

The osteoclasts, in general, were stained more heavily than the other cells present, so that both their outlines and their nuclei were easily discerned at the magnification used (Figs. 1, 2).

Table 1 shows the tally totals of osteoclasts with different numbers of nuclei in the four kittens, and also the combined totals. A total of 1683 cells was counted, the number of cells of any one slide ranging from 77 to 142. A total of 9176 nuclei was counted, the mean number per cell being 5·5 (S.D. ± 2·6, S.E. ± 0·064), and the median 4·6 nuclei per cell.

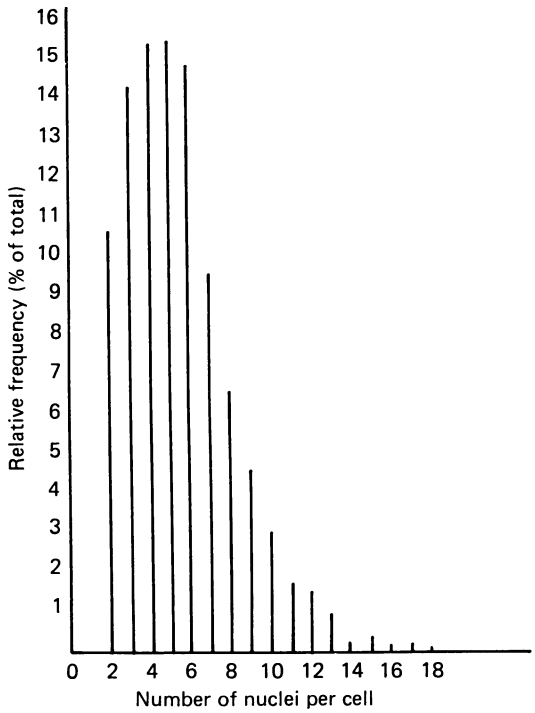

Fig. 3.

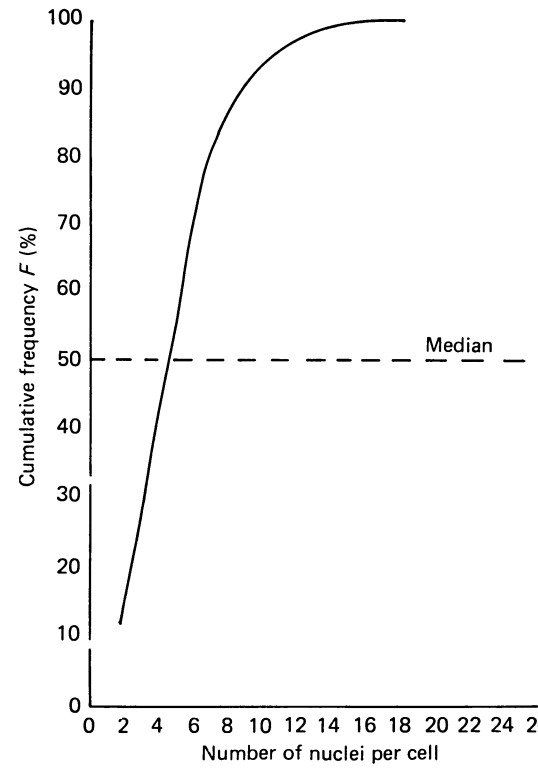

Fig. 4.

Fig. 3. Histogram of relative frequency of osteoclasts having different numbers of nuclei.

Fig. 4. Cumulative frequency curve showing the distribution of osteoclasts of different size.

A histogram of relative frequency against numbers of nuclei showed the distribution to be asymmetric with a positive skew (Fig. 3). With such a distribution the mean is much influenced by the presence of isolated high values. The median, which is a more stable measure of the central tendency, was read directly from a graph of cumulative frequency plotted against numbers of nuclei (Fig. 4). The curve approximated to a straight line over much of its course, tailing off as cells with larger numbers of nuclei were reached. 81 % of cells had between 2 and 7 nuclei while only 5 % had more than 10 nuclei per cell.

Although the trend for the data in individual animals was similar to the overall trend, a χ^2 analysis revealed that the differences between animals were statistically significant ($\chi^2 = 61$; D.F. $= 30$; $P < 0{\cdot}001$).

DISCUSSION

The present study has established base-line data for kitten osteoclasts which may be of value in determining the functional significance of the variability in size of individual osteoclasts. The advantages of counting nuclei of multinucleate giant cells in imprints of whole cells, rather than in sections, have previously been pointed out (Addison, 1976). Although it is possible that some osteoclasts were

Table 1. *Frequency distribution of feline osteoclasts having varying numbers of nuclei*

No. of nuclei per cell	Total				Total	%
	1	2	3	4		
2	57	25	56	44	182	10·81
3	75	56	57	55	243	14·44
4	79	73	52	58	262	15·57
5	81	60	63	60	264	15·69
6	65	61	60	67	253	15·03
7	37	48	32	44	161	9·57
8	25	35	22	29	111	6·60
9	18	27	16	16	77	4·58
10	10	16	11	10	50	2·97
11	5	6	12	4	28	1·66
12	5	7	9	6	25	1·49
13	1	1	6	4	15	0·89
14	—	1	1	1	3	0·18
15	—	—	4	—	4	0·24
16	1	—	—	1	2	0·12
17	—	—	2	—	2	0·12
18	—	—	1	—	1	0·06
Total	459	417	404	399	1683	100

damaged during the bone trimming, the regularity of the frequency distribution curve and the small standard error of the mean nuclear count suggest that the results accurately reflect the distribution of nuclei in intact kitten osteoclasts.

There are few data with which to compare the present results, although Miller *et al.* (1975) found an average of 4·1 nuclei per cell in rat tibial osteoclasts. The present finding of 5·5 nuclei per osteoclast thus supports the view of Hancox (1956) that feline osteoclasts are larger than those in rodents, and of similar size to those in chicks (Hancox, 1946). However, the distribution pattern of cells having different numbers of nuclei has not previously been reported for osteoclasts, and the present results have shown the importance of the median as a more stable measure of the central tendency, so the median is clearly the value which should be used for comparative purposes. In a similar study of intact human odontoclasts a median number of 6·4 nuclei per cell was reported (Addison, 1976), indicating that they are larger than kitten osteoclasts. While in the present investigation the largest number of nuclei in a kitten osteoclast was 31, Hancox (1956) has reported up to 200 nuclei in human osteoclasts, and up to 110 nuclei in chick osteoclasts. The figures for the maximum number of nuclei for pig osteoclasts (125) and for human osteoclasts (60), found by Arey (1919) and Kölliker (1873) respectively, also illustrate the large size which osteoclasts can reach in these species.

The significance of size in osteoclasts has received little attention, but in a comparative study of odontoclasts and osteoclasts it has been suggested that size may be an important parameter of resorptive activity (Addison, 1976). It was postulated that there may be an optimum size for the maximum efficiency of odontoclasts, which may be related to the functioning of the ruffled border. The optimum size may vary under different functional circumstances, since there is evidence that the size of both odontoclasts and osteoclasts in kittens increases following treatment with parathyroid hormone *in vivo* (Addison, 1976). More recently Rowe & Hausmann (1977),

while agreeing that numbers of nuclei may be an important measure of resorptive activity in osteoclasts, reported that the number of nuclei in cultured rat osteoclasts did not increase following treatment with lipopolysaccharide.

It is probable that increases in the size of osteoclasts occur by fusion of precursor cells both to each other and to existing multinucleated cells. The distribution pattern of nuclei in kitten osteoclasts can be accounted for in this way. Indirect evidence that osteoclasts arise by fusion of precursors comes from the studies of Parodi *et al.* (1970, 1973). They used orthodontic forces to produce tooth movement and osteoclastic activity in the periodontal ligament of rats. Following total body irradiation the effect of the orthodontic forces on the numbers of osteoclasts was unchanged; with and without irradiation an increase in numbers of osteoclasts occurred. Fusion of cells might well be less affected by irradiation than mitosis. In addition, Ubios (1972) has reported that osteoclasts do not synthesise DNA. Investigations of changes in the size of osteoclasts may enable a better understanding of the origin and fate of these cells. Intriguing is the possibility that there may be a stable population of long living cells or 'osteoclast units' which are both capable of coming together to form few but large cells or of parting to form more numerous but smaller cells. Splitting up of living osteoclasts to form two smaller cells has been observed by microcinephotography (Hancox, 1972).

SUMMARY

Fresh imprints of metaphyseal bone from the femurs of four kittens aged 18 weeks were stained by histochemical methods for succinate, malate, beta-hydroxy butyrate, and glutamate dehydrogenases. In these preparations the unstained nuclei contrasted sharply with the background stained cytoplasm, making possible accurate nucleus counts in intact osteoclasts. The nuclei in 1683 osteoclasts were counted and the data revealed an asymmetric distribution of cells having different numbers of nuclei. The method may be of value in determining the precise significance of osteoclast size in relation to function.

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