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Secretory Territories and Rate of Matrix Production of Osteoblasts

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The secretory territories of rat osteoblasts on the parietal bone were measured directly using scanning electron microscopy. The mean territory of 4620 cells in 19 fields was 154 μm^2 per osteoblast. The range for the fields was 136 to 177 μm^2 per osteoblast. Four hundred cells were measured individually—for these the mean value per osteoblast was 143 μm^2 with a standard deviation of 33. The daily rate of apposition over an 8 day period was 3.12 μm (standard deviation 0.22) measured by tetracycline marking of the mineral front. This gave a daily matrix production rate of approximately 470 μm^3 per osteoblast.

Key words: Osteoblast — Matrix — Bone — Scanning electron microscope.

Les territoires sécrétoires des ostéoblastes d'os pariétal de rats sont déterminées en utilisant la microscopie électronique à balayage. Le territoire moyen de 4.620 cellules, dans 19 territoires, est de 154 μm^2 par ostéoblaste. Les valeurs extrêmes par champ varient de 136 à 177 μm^2 par ostéoblaste. Quatre cent cellules sont mesurées individuellement; la valeur moyenne par ostéoblaste est de 143 μm^3 avec une déviation standard de 33. Le taux d'apposition journalier, mesuré par la tétracycline pendant 8 jours, est de 3.12 μm (déviation standard 0.22). Ce qui correspond à une production matricielle journalière d'environ 470 μm^3 par ostéoblaste.

Die Ausscheidungsbereiche von Ratten-Osteoblasten des Scheitelbeines wurden mit dem Raster-Elektronenmikroskop direkt gemessen. Der durchschnittliche Bereich von 4620 Zellen in 19 Gesichtsfeldern war 154 μm^2 per Osteoblast. Der Streubereich lag in den verschiedenen Gesichtsfeldern zwischen 136 und 177 μm^2 per Osteoblast. 400 Zellen wurden einzeln gemessen. Bei diesen war der Durchschnittswert per Osteoblast 143 μm^2, mit einer Standard-Abweichung von 33. Die tägliche Anlagerungsrate während einer Periode von 8 Tagen war 3,12 μm (Standard-Abweichung 0,22); sie wurde mittels Tetracyclinmarkierung der Mineralisierungsfront gemessen. Dies ergab eine tägliche Produktionsrate der Matrix von etwa 470 μm^3 per Osteoblast.

Introduction

Estimation of the secretory territories and rates of matrix production of osteoblasts have previously been based on sectioned material (Owen, 1963; Schen *et al.*, 1965). This study was designed to measure the area of the secretory territories of osteoblasts directly using scanning electron microscopy, and to determine the rate of matrix production of the cells by combining these values with the appositional rate.

Materials and Methods

Five 70–75 g Albino Wistar rats were used to obtain measurements of the secretory territories of the osteoblasts on the inner surface of the parietal bone. This site was selected

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because it is always an appositional surface in rats at this stage of growth (Jones and Boyde, 1970). Three of the rats had been subjected to injections of distilled water administered subcutaneously but to no other medication. The other two rats were given two subcutaneous injections of 0.5 ml of 5 mg/ml tetracycline HCl with an interval of eight days between injections. Of these, one rat was killed one half hour after the last injection; the other was killed one hour after the last injection.

Immediately upon the death of the rat one parietal bone was excised and the fibrous layer removed from its inner aspect. This dissection was done whilst the specimen was immersed in a 0.15 M cacodylate buffer at 37°. The bone was then fixed at once in a 3% glutaraldehyde solution in the same buffer. The specimens were washed with water and then dehydrated through graded ethanols to 100% ethanol. Freon 113 was substituted for the alcohol using 25%, 50%, 75% Freon 113 in ethanol and finally two changes of 100% Freon 113. The specimens were then dried using the critical point drying technique after replacing the Freon 113 with liquid carbon dioxide. Conducting coats of carbon and gold were applied *in vacuo*.

The secretory territories were determined in two ways. The first was selected in order to determine the average secretory territory of a large sample of cells. The second enabled a smaller number of cells to be measured individually and thus the range and distribution of the areas could be obtained.

The specimens were aligned in the Cambridge Stereoscan scanning electron microscope (operated at 10 kV) so that the surface to be examined was normal to the beam. Photographs were recorded at a nominal magnification of $\times 500$ for the first measurement exercise and $\times 1000$ for the second. Focussing was performed only by adjusting the vertical height of the specimen using the 'z' control, leaving the electron lens controls untouched, so that a constant magnification was maintained throughout the experiment. The magnification of the microscope was calibrated at the same settings by photographing a SIRA grid with 19.7 lines/mm. All the photographs were printed on the same batch of paper under identical conditions and kept together so that any changes of humidity would be experienced by all of them. The micrographs were enlarged 2.5 times the CRT size to improve the accuracy of the measurements.

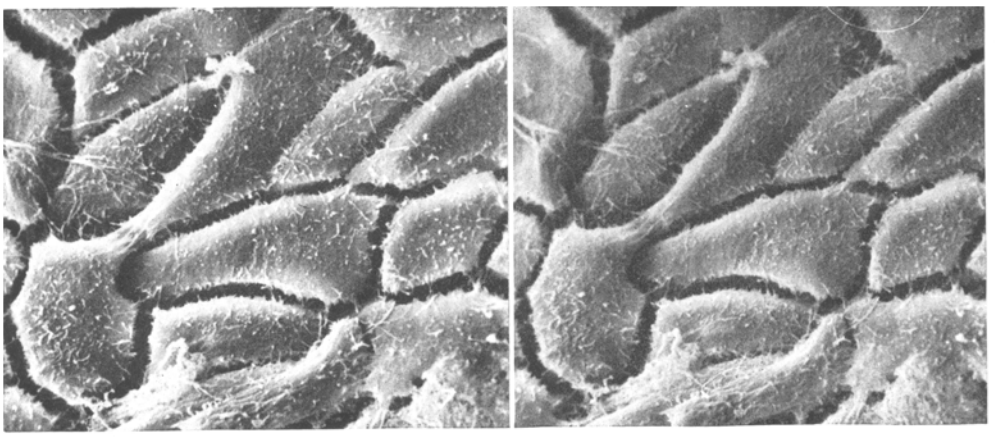
The mean secretory territory was obtained using a method developed for ameloblasts (Boyde, 1969). A line was drawn on the photograph encompassing all the whole osteoblasts. This was considered to be preferable to assigning an arbitrary fraction value to those cells at the edge of the field that were only partly included. The number of cells within the boundary was counted using an automatic counter and marking each cell counted with a brightly coloured ink. The photographs were checked twice to ensure that no cell remained uncounted. The second check was made with the photograph rotated through 180°. The area counted was then cut out and weighed and the area in square micrometers computed from the SIRA grid values obtained in a similar way.

Individual values were obtained using a planimeter and computing the surface area by measuring the known area on the SIRA grid photograph. Three readings were made for each cell and the average value recorded. The measurements from the rats which had received tetracycline were kept separate so that a check could be made that they were similar in value to the other animals.

The measurement of the territories of the osteoblasts lining the inner surface of the rat parietal was simple because, for the most part, the cells there do not overlap. However, small lateral extensions of the cell body may override another cell especially at a junction of three or four cells (Figs. 1 and 2). Numerous cell processes extend to, and are in contact with, the adjacent cell bodies: some of these pass over the adjacent osteoblast and end on the next one. The small gaps between the cells were helpful in defining some boundaries in the first exercise (Fig. 2).

Fig. 1. Cell surface detail of rat osteoblasts on the inner aspect of the parietal bone. Stereopair. Field heights 50 μm

Fig. 2. Typical field of rat osteoblasts on the inner surface of the parietal bone used for obtaining the mean secretory territory of a large number of cells by the first method. Field aligned perpendicular to the electron beam resulting in reduced topographical contrast and more "noisy" image. Field width 190 μm



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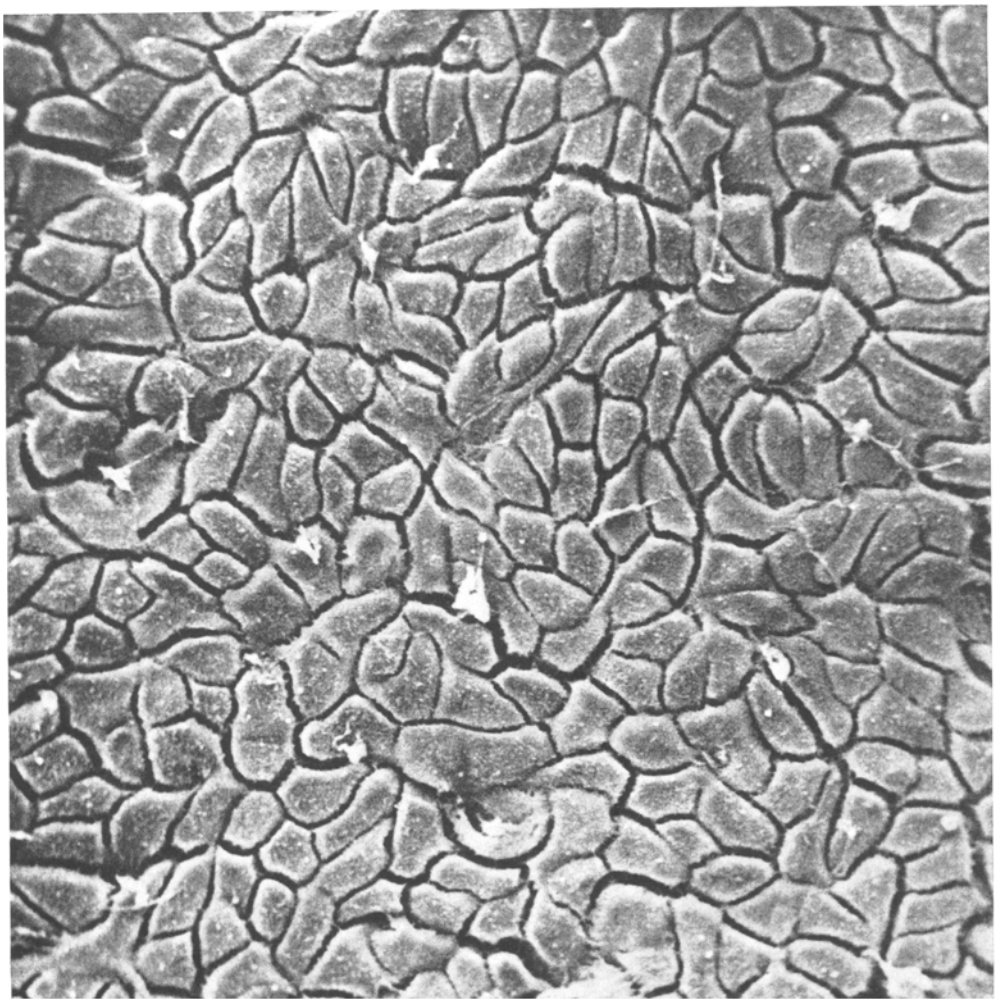

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Figs. 1 and 2

Table 1. Area of matrix surface covered by one osteoblast (μm^2)

Field	Number of cells	Area of cells	Area per cell
1	242	37661.58	156
2	288	42762.06	148
3	223	37206.18	167
4	223	35156.88	158
5	247	35885.52	145
6	269	38116.98	142
7	243	36705.24	151
8	224	39574.26	177
9	234	37616.04	161
10	271	36887.40	136
11	251	37524.96	149
12	224	36249.84	162
13	263	37798.20	144
14	228	37524.96	165
15	224	37251.72	166
16	230	36477.54	159
17	233	35612.28	153
18	225	36295.38	161
19	278	39209.94	141
Total	4620	711516.96	154

The other parietal bone of the tetracycline-treated rats was removed and sectioned frozen at ten micrometers. The interval between the two fluorescent lines which were observed under ultraviolet light was measured against a standard slide using an eyepiece graticule.

The other parietal bone of the other three rats was divided into two; one half made anorganic with a 7% sodium hypochlorite solution. This was also examined by scanning electron microscopy to check that a forming mineral surface was present over the whole surface (Jones and Boyde, 1970).

Results

19 fields of cells were counted and weighed to obtain the territory of the "average" osteoblast. This included 4620 cells in a total area of 711516.96 μm^2, giving a mean territory of 154 μm^2 per osteoblast. The value obtained from each field was also calculated and is shown in the Table 1. The range was 136 to 177 μm^2.

400 cells were measured to find the range of sizes of the secretory territories. The results are shown in Fig. 3. In this sample a mean value of 143 μm^2 per osteoblast was obtained with a standard deviation of 33. A mean value of 150 μm^2 was obtained from the tetracycline-treated animals when their results were abstracted.

16 measurements were made of the depth of bone laid down on the inner aspect of the parietal bone between the two doses of tetracycline as reflected by the change in position of the mineralizing front. Measurements were taken from different sections to make sure that the sections were perpendicular to the bone surface. Areas incorporating blood vessels were avoided but were also infrequent.

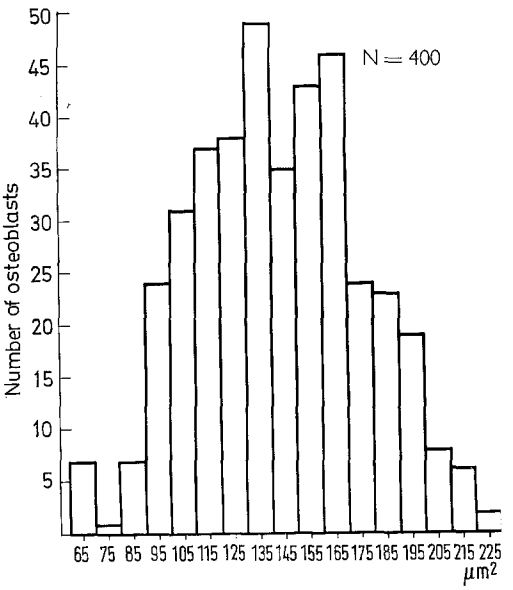

Fig. 3. Histogram showing secretory territories of osteoblasts

The mean daily rate of apposition was 3.12 μm with a standard deviation of 0.22. The range was 2.68 μm to 3.41 μm.

The mean value for the secretory territories of the osteoblasts in the tetracycline treated rats (150 μm^2) when multiplied by the mean appositional rate (3.12 μm) gives an approximate value of 470 μm^3 for the volume of bone matrix produced by each of these osteoblasts in one day.

Discussion

Published values for the rate at which cells can produce a mineralizable organic matrix are few. Human ameloblasts have a secretory territory of approximately 40 μm^2 (Boyde, 1969) which, assuming an appositional rate of 5 μm per day, would give a daily matrix production of 200 μm^3. Enamel matrix is, however, very different from that of bony tissues. Schen *et al.* (1965) estimated from stereological techniques on histological sections that there were 4550 osteoblasts per square millimetre of osteoid seam in human rib cortical bone: this would be equivalent to a mean secretory territory of 220 μm^2 for each osteoblast. The average appositional rate for human rib cortical bone based on tetracycline labelling is 1 μm per day (Landeros and Frost, 1964; Frost, 1969): thus the osteoblasts would be producing matrix at the rate of 220 μm^3 per day. The range of numbers of osteoblasts in a square millimetre was large (2770 to 7950) and equal to a range of secretory territories of 126 to 361 μm^2. Owen (1963) calculated the daily matrix production rate of young rabbit osteoblasts from sections and arrived at a value of 2860 μm^3: this is high when the volume of the cell is considered. Indeed, Owen gave a value for matrix production of 2 to 3 times the

volume of the cell in three days based on an osteoblast shaped like a cube with 15 μm sides. These methods for finding the daily matrix production and secretory territories of osteoblasts have the same problem—only two dimensions are known. The advantage of the methods used in this present study is that all three dimensions are known because the secretory territories can be measured directly using scanning electron microscopy.

Green and Goldberg (1965) used a totally different procedure to obtain data for the collagen synthesis by cultured fibroblasts. From their graph, 10^7 cells were producing 0.015 μmols of hydroxyproline each day (taking figures for the most productive cell line). One can calculate that the daily rate of matrix production of these cells was approximately 900 μm^3, using 300000 for the molecular weight of collagen, a factor of 3 for conversion of dry to wet weight, and using a value for the density of matrix of 1.46 (i.e. equal to that of osteoid: Herring, 1972) rather than that of connective tissue in general in order to relate the production of the fibroblasts to that of bone forming cells. The value obtained is about twice that of the mean rate of matrix production of the rat osteoblasts in this present study.

Although the impression is gained that actively producing osteoblasts are taller with respect to the bone surface (Pritchard, 1972) and, therefore, presumably have a smaller secretory territory to contribute to, quantitative data are lacking. It is still debatable whether alterations in the appositional rate are due to variations in the secretory territories of cells which are producing bone matrix at a fairly constant rate, or to variations in the volume of matrix produced by cells with a constant secretory territory, or whether (as is perhaps more likely) both parameters alter at once. The amount of matrix an osteoblast is capable of producing in a day may also be dependant upon its cytoplasmic volume. The rate of matrix production of explanted bone is known to vary with the age of the animal (Flanagan and Nichols, 1969) but how this relates to the dimensions or the matrix generation rate of individual osteoblasts is not clear.

Frost (1972) emphasizes that "basic multicellular units" of bone modelling are of greater importance than individual osteoblasts: this is undoubtedly true for the animal as a whole. Nevertheless, the responsiveness of bone to alterations in, for example, hormonal levels lies in the capabilities of osteoblasts, osteocytes and osteoclasts at the cellular level.

It should now be possible to determine how the secretory territories of osteoblasts alter with different rates of bone formation under normal physiological conditions, and what effects pathologies affecting bone may have on this parameter and on matrix production rates.

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