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MICROMORPHOLOGICAL CHARACTERISATION OF NORMAL HUMAN BONE SURFACES
AS A FUNCTION OF AGE

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Introduction

Comprehensive studies have been made with the Scanning Electron Microscope of the morphologic appearances characterising the different cellular activities which occur at bone surfaces (these have been reviewed by Boyde, 1972). During the course of these surveys, in which a wide range of species were represented, bone was examined from foetal, neonate and adult humans (Boyde and Hobdel, 1969a, b). Comparisons have been made between the morphologies seen in normal and pathologically altered adult human bone (Chappard et al, 1984; Krempien, 1979; Krempien and Klimek, 1981; Sela, 1977; Sela and Boyde, 1977; Steendijk and Boyde, 1973) and a small number of case reports have documented differences between normal and diseased bone from children (Lindenfelser et al, 1972; Teitelbaum et al, 1974). However, despite these reports there is still very little information in the SEM literature concerning the appearance of human bone from normal subjects forming the part of the age scale from birth to adulthood.

In the present study the SEM was used to examine the endosteal surfaces of bone specimens from human subjects whose ages ranged from seven weeks to 87 years (Figs. 1 - 39). The purpose of the investigation was to establish whether, and in what way, the morphology of these surfaces changes with age.

Materials and Methods

All the material examined in this study (Table 1) was obtained at autopsy from Caucasian subjects who had died suddenly and for whom the post-mortem reports did not indicate likelihood of bone disease. The majority of specimens were from the mid-diaphysial regions of sixth ribs. However, bone from the tibial diaphysis of a nine year old boy and from the iliac crests of two adults was also included in the sample. The latter specimens were taken from 2 cm below and behind the anterior superior iliac spine using an 8mm internal diameter trephine. Material was stored in 70% ethanol until required.

Two principal types of preparation were examined: "organic matrix" preparations to show the organisation of the collagen matrix; and anorganic specimens to show the pattern of

Abstract

Endosteal surfaces of human bone specimens, principally from the sixth rib, from subjects ranging in age from seven weeks to 87 years were studied using the secondary electron imaging mode of the scanning electron microscope. Specimens were examined after the removal of cells only, or after the removal of cells and organic matrix.

Morphological differences made it possible to identify the age group to which a specimen belonged. The most obvious of these was the ratio of active to resting bone surfaces, which decreased with age. The organisation of the collagen matrix which was deposited at endosteal surfaces was different in the different age groups. In neonates, collagen was organised as a parallel fibred continuum. It was present in more discrete bundles in adults, although these still branched and anastomosed with one another. The bundles were parallel over limited domains which described large angles with respect to one another.

The conformation of resorbed surfaces indicated that the behaviour pattern of osteoclasts changes with age. Shallow gutters, or annular zones around resorption bays were most common in neonates. The elongation ratio of resorption tracks was greatest in infants and juveniles, indicating that osteoclast translocation was greatest during resorption at these ages. The texture of the floors of resorption bays was smooth in specimens from subjects up to 13 years, while in adults the collagen fibre bundle organisation in the resorbed tissue was often visible. This difference may reflect a more equal mineralization in the ground substance and collagen compartments of the bone matrix in children than in adults.

Key Words: Human bone, age, endosteal surfaces, collagen organisation, osteoblasts, resorption, osteoclasts, scanning electron microscope.
mineralization within the matrix. In each case endosteal surfaces were first exposed by cutting or fracturing the bone, and bone marrow was then washed away using a water jet. Organic matrix surfaces were prepared either by further, more vigorous washing to disrupt the cell layer lining the bone surfaces or by treatment of specimens in a dilute solution of enzyme detergent (Tergazyme, Alconox Inc., New York, N.Y.) for 24-72 h at 50°C. During the latter treatment, samples were periodically ultrasonicated and, at the end of the preparation period, were washed thoroughly in distilled water. They were then dehydrated through graded alcohols and either air-dried from absolute ethanol or transferred to trichlorotrifluoroethane (C₂Cl₃F₇, "Arkplone", ICI Mond Division, The Heath, Runcorn, Cheshire - American "Freon 113"), and dried in a saturated atmosphere of this solvent (Boyd, 1984; Boyd and Maconnachie, 1984).

Superficially anorganic preparations were made by immersion of specimens in a "7%" solution of sodium hypochlorite (NaOCl, BDH Chemicals Ltd., Poole, Dorset, U.K.). The stock solution was supplied at 14% available chlorine concentration and this was diluted with an equal volume of distilled water. At the end of the treatment period, specimens were thoroughly washed in distilled water, dehydrated through graded alcohols and air-dried from absolute ethanol.

Periosteal and fractured surfaces of neonate rib specimens were also prepared for examination in the SEM. They were cleaned in either enzyme detergent or NaOCl and were examined in order to assist with the interpretation of structures exposed by resorption at the endosteal surfaces of this rapidly growing bone.

**Scanning electron microscopy**

Dried specimens were mounted on aluminium stubs using carbon conductive cement and coated with gold by sputtering. They were then examined using the secondary electron (SE) and backscattered electron (BSE) imaging modes in a Cambridge Stereoscan 54-10 SEM operated at 10kV. An annular solid state detector (KE Developments, The Mount, Toft, Cambridge, UK.) fixed to the roof of the specimen chamber was used for BSE imaging and this mode was particularly valuable for viewing specimens at normal beam incidence. Stereopair micrographs were recorded with a tilt angle difference of 10° (Howell and Boyd, 1984).

Measurements were made from surface facets orientated normal to the electron beam axis and at a constant working distance. The magnification of the SEM was calibrated using a silicon standard specimen marked with 10 μm squares which was examined at the same working distance.

**Results**

A detailed account of the morphologies which characterise the different cellular activities occurring at bone surfaces has been presented by Boyd (1972). Briefly, resorption is identified by the presence of excavations which interrupt the pattern of collagen fibre bundles otherwise seen at cell-free surfaces (figure 1). Resting surfaces are those at which the collagen fibre bundles are fully mineralized and therefore retain an unaltered morphology in anorganic preparations (figure 3). Surfaces at which active bone (formation and/or) mineralization

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**Table 1.** Age, sex and cause of death of subjects.

<table>
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<tr>
<th>Age</th>
<th>Sex</th>
<th>Cause of death</th>
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</tr>
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<td>F</td>
<td>Sudden death in infancy</td>
<td>Rib</td>
</tr>
<tr>
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<td>F</td>
<td>Sudden death in infancy</td>
<td>Rib</td>
</tr>
<tr>
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<td>Rib</td>
</tr>
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<td>Rib</td>
</tr>
<tr>
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<td>M</td>
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<tr>
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<td>Rib</td>
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<tr>
<td>2.3 y</td>
<td>M</td>
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<td>Rib</td>
</tr>
<tr>
<td>9 y</td>
<td>M</td>
<td>Road traffic accident</td>
<td>Tibia</td>
</tr>
<tr>
<td>10 y</td>
<td>F</td>
<td>Misadventure</td>
<td>Rib</td>
</tr>
<tr>
<td>13 y</td>
<td>M</td>
<td>Road traffic accident</td>
<td>Rib</td>
</tr>
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<td>Rib</td>
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</tbody>
</table>

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Figure 7. 12 week male, organic matrix. Primary osteon exposed by resorption at the endosteal aspect of the lateral rib cortex. The bone surface is lined by overlapping layers of fibre bundles within which the fibrils are closely packed. Differential shrinkage has outlined the position of mineralized segments of the collagen fibres. Fieldwidth 48 µm.

Figure 8. 12 week male, organic matrix. Endosteal surface of the medial rib cortex showing a less compact arrangement of fibrils within fibre bundles than in figure 7. (OL), half-formed osteocyte lacuna. Fieldwidth 48 µm.

Figure 9. 12 week male, organic matrix. Poorly preserved osteoblasts have remained at the bone surface. These elongated cells are aligned with one another and with underlying collagen fibre bundles. Fieldwidth 140 µm.

Figure 10. 14 week female, anorganic. Primary osteon exposed by endosteal resorption. A half-formed osteocyte lacuna is seen at a mineralizing front. The floor of the lacuna is completely mineralized while small elongated nodules, which are the mineralized portions of collagen fibre bundles, lie the rest of the bone surface. Fieldwidth 50 µm.

Figure 11. 2 year 4 month male. Partial digestion of collagen by prolonged treatment with enzyme detergent has begun to expose the mineral nodules forming within the fibre bundles. Fieldwidth 48 µm.

Figure 12. 13 year male, anorganic. Mineralizing front lined by small mineral nodules at left and larger, more consolidated nodules at right. The back wall of the osteoid osteocyte is fully mineralized. Fieldwidth 20 µm.

were occurring are characterised by the loss of this fibre bundle pattern in anorganic preparations to reveal the mineral aggregates forming within the bone matrix (figures 10-14). Some of the results to be described in subsequent sections have been summarised in table 2.

Distribution of activities with age

There was a marked change with age in the amount and the distribution of different bone activity states at endosteal surfaces in the rib. In neonates all mineral surfaces presented active forming or mineralizing, rather than resting, appearances. In the youngest representatives of this age group, most endosteal surfaces had been resorbed, mineralizing fronts being confined to the lining of primary osteons exposed by this resorption. However, in the majority of cases there was a distinct asymmetry in the distribution of activities such that the endosteal surface of the medial cortex was extensively covered by a mineralizing front morphology, while the endosteal surface of the lateral cortex was resorbing or resorbed.* At 2.3 years and in the juvenile specimens, most surfaces were again covered by morphologies indicating recent bone cell activity although there were microscopic differences (described later) which distinguished them from the morphologies present in neonates. In these older cases the complete dominance of one activity type along entire bone surfaces which was seen in neonates was replaced by a mixed pattern of activities on all surfaces.

In adults there was less evidence of bone formation and resorption, many surfaces being lined by fully mineralized collagen fibre bundles characteristic of resting surfaces. Occasionally there was evidence that mineralization had extended beyond the limits of the collagen component of the bone matrix and into the ground substance. This caused the detail of fibrils at the surface of fibre bundles to be obscured and sometimes resulted in an apparent fusion of adjacent bundles (figure 4). However, this morphology, which is found at prolonged resting surfaces (Boyd and Hobdell, 1969a; Boyd, 1972), was not common.

Collagen organization

In all the material studied, collagen at endosteal bone surfaces was organised into fibre bundles. These were generally 1.5 to 3 µm in diameter and composed of fibrils 0.1-0.2 µm across. The bundles branched, and anastomosed with their neighbours, and were aligned parallel with one another over areas which varied in extent between young and adult subjects. In adults, collagen was aligned over limited territories, (the "domains" of Boyd and Hobdell, 1969a), which ranged in area from a few hundreds to thousands of square microns (figures 2 and 3). The angle between the axes of collagen orientation in overlapping domains ranged from a few degrees up to 90°, but was generally greater than 60°.

In juvenile rib specimens collagen fibre bundles were aligned over more extensive areas than in adults and were often orientated at only a small angle from the long axis of the bone. The change of orientation between overlapping layers of fibre bundles was less than that in adults (generally > 40°; Figure 5).

In neonates, the branches between collagen fibre bundles were larger than in older subjects so that the collagenous matrix at the bone surface appeared more as a continuum than as separate aligned bundles linked by small collagenous fibres (figure 6). When formation, rather than resorption, was occurring at the endosteal surface of the medial rib cortex, the collagen formed an extensive sheet and the fibres within it were generally aligned with the long axis of the bone. It was clear that underlying fibre bundles had similar orientations if superficial collagen layers were stripped from this surface during specimen preparation. This contrasted with the situation at the linings of primary osteons exposed at the endosteal surface of the lateral cortex. Here, orientation changes were evident between layers of fibrillar fibres (figure 7). The fibrils within these bundles, and also in those at trabecular bone surfaces appeared to be more compacted together than in the bundles lining the medial endosteal surface (figure 8).

The examination of specimens which had been prepared by simply fracturing open and cleaning with a water jet sometimes revealed areas in which (poorly preserved) cells remained at the bone surface. In these areas osteoblasts were clearly aligned with the collagen bundles underlying them (figure 9). This finding concurs with

*See Discussion with Reviewers.
Mineralizing fronts at the endosteal surface of the medial rib cortex. The mineral aggregates are composed of thin mineral strands which may be the mineralized parts of individual collagen fibrils. These aggregates continue to line the floor of the half-formed lacuna at centre. Fieldwidth 22 µm.

Resorption tracks aligned with the collagen fibre bundles at the bone surface. Note the smooth appearance of the floors of the resorbed areas and that the matrix surface at right is incompletely mineralized. Fieldwidth 198 µm.

Mineralizing fronts at endosteal bone surfaces showed considerable variability at all ages. Sometimes their length and breadth, which could measure as little as 5 µm, were approximately equal. However, they

Mineralizing and mineral surfaces

At all ages, mineralizing fronts at endosteal bone surfaces were represented by a pattern of elongated mineral nodules which remained following the removal of unmineralized matrix (figures 10-14). When incomplete organic matrix degradation had occurred, a result which could be achieved by prolonged treatment with enzyme detergent, it was evident that these nodules developed within the collagen fibre bundles at the bone surface (figure 11). This agrees with earlier SEM reports of mineralization in bone containing ordered collagen fibre bundles (Boyde, 1972; Boyde and Jones, 1972; Boyde and Hobdell, 1969a, b; Jones 1973).

At surfaces which were interpreted as sites of active bone formation, the mineral nodules were small, (e.g., 0.3 x 0.8 µm) and it sometimes appeared that several were growing side by side within single fibre bundles (figure 10). At more advanced stages of mineralization, the nodules gained the girth of complete collagen bundles, although it was sometimes still possible to see evidence at the surfaces of these nodules of the smaller units of which they were aggregates (figures 12 and 13).

There were differences in the apparent "vigour" of mineralizing fronts between the different age groups examined. In neonates, mineralizing fronts were usually composed of the small mineral nodules described above and it frequently appeared that mineralization was not complete in collagen layers deep to the most superficial one (figure 10). In this age group, a difference was noted between the morphology of the mineral nodules lining the surfaces of exposed primary osteons and mineralizing trabecular bone surfaces, and those found at the endosteal surface of the medial rib cortex when this was mineralising. In the former locations the nodules generally had smooth surfaces, in common with those found in bone from older subjects. However, at the medial cortex endosteal surface, the nodules did not appear as solid units of mineral, but appeared to be composed of aligned mineral strands (figure 14). These strands may represent the mineralized lengths of individual collagen fibrils and the fact that they were evident may reflect the looser packing of fibrils within the fibre bundles at this surface.

In the rib at 2.3, 10 and 13 years, and in the tibia at 9 years, mineralization was again sometimes evident as small nodules. However, this appearance was less common than in neonates and mineralizing fronts generally had a more consolidated appearance (figure 12). It is likely that at some of these surfaces active bone formation had ceased and that a slower, progressive mineralization of the remaining matrix was occurring.

In adult bone, mineralizing fronts were much less common than in younger material. Where present, they were only occasionally evident as small mineral nodules extending through more than one collagen layer. Most often, mineralization was confined to the most superficial layer and represented by large nodules, sometimes beginning to fuse end to end (figure 22).

Resorption

The size and morphology of the resorption bays at endosteal bone surfaces showed considerable variability at all ages. Sometimes their length and breadth, which could measure as little as 5 µm, were approximately equal. However, they

Figure 19. Scattergram showing the distribution of elongation ratios for the resorption bays from which the measurements presented in table 3 were made. The values on the graph represent the means of the ratios for the individual bays and show minor differences from those which can be calculated from the summed data in table 3. The 2 means at 12 weeks are for resorption at the lateral cortex endosteal surface (triangle) and at trabecular bone surfaces.
were most frequently anisotropic and shallow, with their floors not steeply curved (figure 15). These elongated furrows, or "snail tracks" (Boyd and Jones, 1979), were often aligned with their neighbours and with the axis of orientation of collagen in adjacent unresorbed areas (figures 15 and 16). The width of individual furrows did not generally exceed 25-30 µm (see table 3). Their lengths, on the other hand, were sometimes much greater and lengths in excess of 250 µm were recorded for single tracks. In some cases there was evidence of subdivisions along the length of these tracks (figure 17). Where osteoclasts were still present at resorbed areas, they were

**Table 2.** Morphologic characteristics of human endosteal bone surfaces at different ages. (N = Neonates, J = Juveniles and A = Adults). The number of plus signs is intended to show trends and not the absolute frequency of characteristics.

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<table>
<thead>
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<th>Length (µm)</th>
<th>Width (µm)</th>
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</thead>
<tbody>
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<td>25</td>
<td>52.8 (24.0)</td>
<td>25.0 (8.2)</td>
</tr>
<tr>
<td>trabec</td>
<td>15</td>
<td>68.6 (24.6)</td>
<td>17.3 (5.2)</td>
</tr>
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<td>12</td>
<td>104.8 (73.6)</td>
<td>19.3 (8.2)</td>
</tr>
<tr>
<td>10 y F</td>
<td>18</td>
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<td>18.3 (7.5)</td>
</tr>
<tr>
<td>39 y M</td>
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<td>40.9 (25.9)</td>
<td>14.1 (6.1)</td>
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<tr>
<td>43 y F</td>
<td>24</td>
<td>50.7 (38.6)</td>
<td>17.7 (7.3)</td>
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Age changes in human bone

**Figure 20.** 12 week female, partially anorganic. Elongated resorption tracks have been formed at the border of a primary osteon exposed at the endosteal surface of the lateral rib cortex. Several osteocyte lacunae have been exposed at the resorbed surfaces. Fieldwidth 198 µm.

**Figure 21.** 13 year male, anorganic. Note the anisotropy and extensive alignment of resorption tracks. Fieldwidth 324 µm.

**Figure 22.** 70 year male, anorganic. Resorption has breached a surface at which collagen was not completely mineralized. In the resorbed area the collagen orientation in lamellae at different depths has been revealed. Fieldwidth 195 µm.

**Figure 23.** 2.3 year male, anorganic. Stereopair showing resorption at left and mineralizing front at right. There is a pronounced gutter at the junction between these two morphologies. The arrow indicates an extrinsic (Sharpey) collagen fibre bundle. Fieldwidth 54 µm.

**Figure 24.** 14 week female, anorganic. Note the smooth appearance of the floors of the resorption bays and the shallow gutters, or angular zones, at the borders of some of these (arrows). Fieldwidth 100 µm.

<table>
<thead>
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<td>2. Bone structure</td>
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<tr>
<td>3. Resorption</td>
</tr>
<tr>
<td>4. Osteocyte Lacunae</td>
</tr>
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</table>

**Table 3.** Mean lengths and widths of resorption bays in five rib specimens ranging from 12 weeks to 43 years of age. All measurements were made at cortical endosteal surfaces except at 12 weeks when measurements were made at trabecular bone surfaces as well as at the lateral cortex endosteum. N = number of bays measured. Figures in brackets show standard deviations.

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<table>
<thead>
<tr>
<th>Age &amp; Sex</th>
<th>N</th>
<th>Length (µm)</th>
<th>Width (µm)</th>
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**Figure 25.** Stained specimen showing the texture of the floors of resorption bays in a bone of a 12 month old infant. There is a steady increase in the number of plus signs as the age of the specimen increases (figure 18). Other cells populating such areas showed a similar alignment.

From the examination of anorganic preparations of adult bone it was evident that resorption frequently encroached upon fully mineralized collagen fibre bundles (figure 1). However, resorption at all ages was also observed to have advanced over surfaces at which collagen was not completely mineralized (figure 22). In particular, in neonate and juvenile specimens (in which, as was noted earlier, bone surfaces were almost entirely covered by mineralizing front or resorbed morphologies) resorption of incompletely mineralized collagen was the rule (figure 23). Where such resorption had first been studied at cell-free surfaces, prior to a specimen being made anorganic, there was no evidence of superficial (unmineralized) collagen degradation in advance of the resorption front.

The microscopic texture of the floors of resorption bays was different in the bone of children and adults. In the rib up to 13 years the texture was usually smooth and revealed only faint evidence of the structure of the material which had been resorbed (figure 24). In adult bone specimens, however, a pattern of collagen fibre bundles was often observed in the floors of resorption bays; this demonstrated the orientation of fibres within lamellae at various depths (figure 25). Krempien (1979) and Krempien and...
Figure 25. 62 year male iliac crest, anorganic. Collagen fibre bundles exposed in the bases of resorption bays. This oblique view shows the fibre bundles sectioned at the edge of the resorbed area. Fieldwidth 49 µm.

Figure 26. 8 week female, anorganic. Resorption at the endosteal aspect of the lateral rib cortex has exposed areas of incompletely mineralized matrix. These are incompletely mineralized extrinsic fibre bundles (with unmineralized cores running vertically) which were included at the forming periosteal surface. Fieldwidth 107 µm.

Figure 27. 20 week female, anorganic. High magnification image showing fibril detail within the mineral nodules formed in extrinsic collagen fibre bundles. Fieldwidth 19 µm.

Figure 28. 14 week female, partially anorganic. Stereopair. The 2 resorption loci at centre have been formed in an area which has already been resorbed. However, it is not possible to tell how long the lag time between the 2 episodes of further observation would have been. There are pronounced annular zones around the 2 central loci and, also, at the borders of other resorption bays at this surface (arrow). There is a mineralizing front at left. Fieldwidth 110 µm.

Figure 29. 13 year male, anorganic. Glazing over of the detail of the mineral nodules at the boundary between resorbed and mineralizing fronts. Fieldwidth 48 µm.

Klimpel (1981) have suggested that these are exposed at the plane between successive lamellae, but further three-dimensional investigation is required to confirm or refute this.

The smooth morphology of resorption bay floors in young rib specimens was sometimes interrupted by areas of apparently incompletely mineralized matrix which had been uncovered by resorption. These areas often appeared as elongated tracts up to 8-10 µm across and were sometimes quite densely packed at the bone surface (figure 26). They were lined by elongated mineral nodules which were shown at high magnification to be incompletely mineralized extrinsic (Sharpey) collagen fibre bundles spanning the thickness of the rib cortex. These tracts were interpreted to be incompletely mineralized extrinsic collagen fibre bundles sectioned at the edge of the resorbed area. In adult bone, where resorption had exposed underlying collagen fibre bundles, there was often a sharp boundary at which the orientation of collagen at the undisturbed bone surface contrasted with that in the bases of resorption bays. Oblique views of such boundaries revealed details of the fibre bundles which had been "sectioned" by osteoclasts (figure 25) and showed that these cells frequently cut through several lamellae during a single resorptive episode: they had not removed a single lamella at a time as reported by Krempeion (1979). Where resorption loci had smooth floors, such as in younger bone, they sometimes inclined gradually up to the unresorbed bone surface, and details of the nodes of the mineralizing front across which resorption had advanced were "glossed" over (figures 21 and 29). The glossing over of the detail at these sites may be because only the bases of resorption bays in younger subjects breached mineralized bone. Pronounced gutters were occasionally seen at the border between resorption and mineralizing fronts in neonate and juvenile bone (figure 23).

Features observed particularly in the infant and juvenile rib specimens were shallow grooves, commonly 10-15 µm wide, which crossed mineralizing bone surfaces, often along the axis of collagen orientation. These grooves frequently extended from resorbed areas and sometimes linked two adjacent areas (figure 30). It is assumed that these features represent tracks made by competent, active osteoclasts in moving from one general area of activity to another. The floors of these grooves were smooth and there was a glossing over of the detail of the mineral nodules at their lateral borders (figure 31). There was little or no evidence of subdivision along their length. Fingers of more defined resorption, extending from larger complexes (figure 32), were also more commonly associated with resorption at these ages.

Osteocyte lacunae

In all the material studied, the part-formed osteocyte lacunae at endosteal bone surfaces were frequently elongated, measuring approximately 20 x 10 µm, and, as previously described (Jones, 1973; Marotti, 1981; Marotti et al, 1985a), their long axes were aligned with the surrounding collagen fibre bundles (figures 5 and 8). The floors of lacunae were the fibre bundles of underlying lamellae which were sometimes overlain by a pattern of more randomly orientated single fibrils. These floors were penetrated by the openings of a variable number of canaliculi which ranged from 5-35, but was typically in the range 15-25. This agrees with other data which has been reported in the literature (Boyde, 1972; Marotti et al 1983b).

In specimens from subjects aged 2.3 years and upwards, the collagen in the floors of half-formed lacunae was usually completely mineralized. This was not true in neonates. In this age group, anorganic preparations frequently
Figure 30. 2.3 year male, anorganic. Shallow resorbed groove (single arrow) across a mineralizing front. Part of a second groove (double arrow) can also be seen. Fieldwidth 204 µm.

Figure 31. 2.3 year male, anorganic. Stereopair showing detail of the floor of a resorption groove. The groove has a smooth floor and there is no evidence of subdivision along its length. Fieldwidth 22 µm.

Figure 32. 10 year female, anorganic. Patch of highly anisotropic osteoclasia from which a number of fingers of resorption extend. Fieldwidth 1.05 mm.

Figure 33. 14 week female, anorganic. Mineralizing front at which two osteocyte lacunae are being formed. The upper lacuna is being included at an angle to the bone surface and half of its floor remains incompletely mineralized. The lower lacuna is completely mineralized. Fieldwidth 50 µm.

Figure 34. 14 week female, anorganic. Osteocyte lacuna exposed at resorbed surface. The lacuna is lined by randomly orientated collagen fibrils and resembles the floors of some half-formed lacunae at periosteal surfaces (Figure 36). A thin resorption resistant shell remains over-hanging part of the lacuna. Fieldwidth 20 µm.

revealed lacunae which were lined by mineral nodules similar to those covering the surrounding bone surface. This finding was particularly prevalent at the endosteal surface of the medial rib cortex (Figure 14) and less common to the lacunae forming at primary osteonai and trabecular bone surfaces. Occasionally, mineralization was complete across only part of the floor of a lacuna (Figure 33). In these instances it appeared that osteocytes were being included at an angle to the forming bone surface and that fibre bundles of more than one collagen layer were contributing to their floors. This may account for the observation sometimes made of fibre bundles with different orientations at different parts of their floors and argues against lacunae being situated consistently at interlamellar planes or within lamellae having a particular fibre orientation (Kuth, 1947).

The number of lacunae being included varied over the bone surface within individual specimens. However, there did appear to be a decrease in this parameter with age. Estimates of the bone surface area associated with single osteocytes were made by counting the numbers of lacunae in fields of standard size. These measurements showed that at 2 years the "territory" of single osteocytes was 15,000 µm² (surface area of lacunar floor, 100-150 µm²). At 13 years, this figure had risen to 21,000 µm² and in adults it was 40,000 µm². Endosteal surfaces in neonates were too undulating to permit similar analysis, but by qualitative inspection the cell density was clearly higher than at 2.3 years.

The numbers and morphology of osteocyte lacunae exposed at resorbed surfaces varied with age and, in particular, there was a difference between neonates and other ages. In neonates, osteocytes were exposed in large numbers and were sometimes clustered together and even conjoined. The walls of these lacunae were sometimes incompletely mineralized. When they were mineralized they often lacked the ordered fibre bundle appearance of those exposed in older bone (Figure 34). They resembled the part-formed lacunae at periosteal bone surfaces at this age (Figure 36).

At all ages, resorption exposed osteocyte lacunae in which the walls appeared to have been protected from, or resistant to, osteoclastic activity, for they were undercut (Figure 35). This effect was sometimes more extreme in neonates when a thin "shell" remained around parts of exposed laceunae (Figure 34). Also, raised borders were sometimes found around canaliculi exposed at the bases of resorption loci and these were again more commonly found in neonates and at 2.3 years.

There was rarely evidence that mineralization in the walls of exposed lacunae had extended beyond the limits of the collagen lining them and into the ground substance to form "perilacunar bone" (Boyd, 1972). This finding agrees with previous observations (Boyd et al., 1982).

Periosteal and fractured rib surfaces in neonates

Periosteal surfaces of neonate ribs were predominantly formative. Mineralizing fronts were characterised by mineral aggregates which were round, rather than elongated, and typically had a diameter in the range 0.8-1.5 µm (Figure 36). These were not confined to the most superficial tissue layer, so that mineralizing fronts appeared to have considerable depth (Figure 37). The aggregates were composed of thin mineral strands which were aligned and were interpreted as the mineralized portions of collagen fibrils (Figure 38). Osteocyte lacunae and extrinsic collagen fibre bundles were being included at these surfaces (Figures 37 and 39). The surfaces lining spaces previously occupied by extrinsic fibres were covered by the mineral aggregates just described, as were the rear walls of many half-formed osteocyte lacunae (Figure 37).

At low magnification these growing surfaces presented the appearance of crests and troughs aligned with the long axis of the rib. The crests were produced by the formation of primary membrane bone trabeculae at the bone surface. The microscopic features of mineralizing fronts at the tops of crests and the bases of troughs were similar.

Anorganic fractured bone surfaces revealed the presence of incompletely mineralized extrinsic fibres traversing the thickness of the rib cortex. The walls of exposed osteocyte lacunae were also often incompletely mineralized and lined by mineral aggregates similar to those found at the periosteal surfaces.

Discussion

The results of this study show that the morphologic features observed at human endosteal bone surfaces change during growth and development. Some of these changes were quite predictable. The amount of apparently active
Figure 35. 2.3 year male, anorganic. Part of the wall of an osteocyte lacuna exposed by resorption. This stands proud of the surrounding bone surface, and is even undercut, indicating that it was resistant to, or protected from (perhaps by the osteocyte which occupied it), osteoclasia. Fieldwidth 54 µm. (Stereo-pair)

Figure 36. 12 week male, anorganic. Mineralizing front at the periosteal surface of the rib. Below centre is a half-formed osteocyte lacuna in which the back wall is almost completely mineralized. Collagen fibrils in this wall are randomly orientated. To the right of this lacuna are others in which the back walls are at an earlier stage of mineralization. Fieldwidth 49 µm.

Figure 37. 12 week male, anorganic. Periosteal mineralizing front showing inclusion of large numbers of osteocyte lacunae. The back walls of the half-formed lacunae are incompletely mineralized. Fieldwidth 98 µm.

Figure 38. 12 week male, anorganic, periosteal. Detail of mineral aggregates showing that they are composed of the mineralized portions of collagen fibrils. Fieldwidth 20 µm.

Figure 39. 12 week male, anorganic. Inclusion of large numbers of extrinsic collagen fibre bundles at the periosteal surface. These are inserting from top right and are seen as holes because mineralization within them lags behind that occurring in the intrinsic fibres of the bone matrix. Fieldwidth 490 µm.

The different textural morphologies seen in resorption bays in young subjects and in adults may be accounted for by a number of contributory factors. The failure to expose collagen fibre bundle patterns in the youngest subjects may be due in part to the fibre bundles being less well defined and not always organised as distinct layers. Furthermore, the resorption plane was probably often not oriented parallel to the plane of the fibre bundles in these subjects. However, this cannot be the entire explanation since lamellar bone was present in the intermediate age group and resorption tracked across surfaces lined by fibre bundles; i.e., the resorption plane was parallel with the plane of the collagen bundles. Therefore, the finding of collagen bundles only in the floors of resorption bays in adults may be related to a difference in the matrix undergoing resorption. This difference might, for example, be in the extent of the mineralization in the ground substance of the bone matrix. If this compartment were less mineralized than the collagen compartment in adults, then the latter could be more resistant to resorption and therefore "etched out" in resorbed areas.

The anisotropy of resorption at bone surfaces, which is particularly striking when comparisons are made with the morphology of resorbed areas in other mineralized tissues, has previously been described by Jones (1973); Boyde and Jones (1979) suggested that it reflects a high degree of osteoclastic translocatory activity. This suggestion is supported by the observation that resorption at periosteal bone surfaces, where cell movement may be restricted by the insertion of extrinsic fibre bundles, is more isotropic than endosteal resorption (Boyde and Jones, 1979; personal observations). The elongation ratio (length/width) for resorption loci at the buccal surface of the mandible at sites of extrinsic fibre bundle insertion (1.7) is similar to that for resorption loci in dentine (1.5). (Boyde and Jones, 1979). The present results emphasise the magnitude of the anisotropy which may occur in resorption at endosteal bone surfaces. Accepting the above hypothesis concerning cell movement, they indicate that osteoclast translocation is maximal in the rib at 2.3 years and in juveniles. The shallow resorption grooves observed crossing the bone surface at these ages provide further evidence that this is so.

The grooves just referred to have not been described previously. Their morphology suggests that they were formed by cells with continually active resorptive organs moving over the bone surface. An interesting feature of them, and also of some more frankly resorbed areas in young subjects, was the glossing over of the details of mineral modules at their borders (figures 28 and 29). The appearance of the surrounding mineralizing front sometimes indicated that mineralization had not extended as far as was
observed in the nodules bordering resorption and this suggests that some reprecipitation of mineral may have occurred at these sites during osteoclasia. This might also contribute to the very smooth appearance of the floors of some of these grooves. Reprecipitation of mineral has been documented at resorbed dentine surfaces (Boyd and Jones, 1979; Krempien, 1979; Krempien and Klimek, 1981). The hypothesis has been put forward that this is due to the interaction of osteoclasts with osteoblasts which overlie, and are aligned with, this collagen (Jones et al, 1985). Such interactions would explain why resorption at the endosteal surface of the lateral rib cortex in neonates was more isotropic than at trabecular bone surfaces at this age. In the latter situation an orientated cell layer would have been present to direct osteoclasia. At the endosteal surface of the lateral cortex on the other hand, this would only be true at the borders of exposed primary osteons and, here, increased anisotropy and alignment of resorption loci were indeed observed. This hypothesis would also explain the strong alignment of resorption over large areas which was found at 2.3 years and in juveniles. At bone surfaces at these ages, collagen, and probably, therefore, the overlying cell layer, was aligned over extensive areas.

Shallow gutters, or annular zones, (Jones et al, 1985) such as those sometimes found bordering resorption loci in the present study, have previously been observed at surfaces resorbed in vivo in a number of mammalian species and in bony fish (Jones, 1973 and Jones, unpublished work) and have also been found on resorbed surfaces of other fish (Jones, 1973). The apparently intact state of the unmineralized collagen immediately adjacent to the resorbed surfaces in bone affected by diseases such as Paget's disease in which osteoclasts are operating more slowly, if annular zones are characteristic of resorption by highly active osteoclasts, then, it is possible that they might also be found at resorbed surfaces in bone affected by diseases, such as Paget's disease in which osteoclasia is stimulated.

The demonstration of resorption in areas of incomplete matrix mineralization agrees with a number of other SEM studies of in vivo resorption (Boyd and Hobdell, 1969a; Boyd, 1972b; Jones, 1973). The apparently intact state of the unmineralized collagen immediately adjacent to the resorbed surfaces in bone affected by diseases such as Paget's disease in which osteoclasts are operating more slowly, if annular zones are characteristic of resorption by highly active osteoclasts, then, it is possible that they might also be found at resorbed surfaces in bone affected by diseases, such as Paget's disease in which osteoclasia is stimulated.

There is now substantial literature documenting the synthesis and secretion of collagen by osteoblasts and it has been suggested that these cells may play a direct role in resorption (Heath et al, 1984; Jilka and Hamilton, 1985). One role which has been suggested is the control of remodelling by the degradation of a thin surface collagen layer which, it was proposed, should be an otherwise universal feature of bone surfaces and prevent osteoclastic bone resorption (Chambers, 1985). The present result demonstrates the resorption of unmineralized collagen by osteoclasts (supporting the findings of Boyd and Hobdell, 1969a and Boyd, 1972) and clearly do not support this model. However, the question of whether osteoclast precursors can become active osteoclasts against unmineralized bone matrix surfaces remains to be answered.
The decreased frequency of inclusion of osteocytes with age may be related to the decreased fraction of bone surfaces at which active bone formation was occurring. There may also be a decrease in the rate of formation at active bone surfaces (Frost, 1969). Marotti (1977) has shown that the frequency of inclusion of osteoblasts as osteocytes during secondary osteon formation is directly related to the radial rate of closure of the Haversian canal. It seems reasonable to expect the same relationship to hold for bone formation at endosteal and trabecular bone surfaces.

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References


Discussion with Reviewers

W.A.B. Brown: Should one not anticipate that there would be marked sex differences arising from rapid bone growth taking place at puberty? Author: The earlier onset of puberty in females might produce differences in bone turnover between the sexes in this age group. However, such differences would be superimposed upon an existing picture of rapid bone turnover and would probably require the analysis of large samples if they were to be demonstrated. In the present study the specimens covering this period have been treated as a group which shared many features in common. I consider it was valid to use this group for comparison with the neonates and adult groups. However, I agree that it would be hard to make comparisons within the juvenile group.

R. Smith: This paper provides very important background information about the changes which occur in normal bone surfaces with age. As with all microscopy techniques it is difficult to be absolutely certain from static appearances exactly what events have produced them, but the alteration in patterns of resorption with age certainly suggest marked changes in osteoclast behaviour.


H.Y. Elder: The interesting observation is made that the "textural morphology" of the floors of the resorption bays differs between bone from young and adult individuals. The difference is attributed to a more even mineralization of ground substance and collagen in bone of young individuals than of adults. How consistent is this observation? I doubt the differences in ground substance and collagen in bone of young individuals. However, I agree that it would be hard to make comparisons within the juvenile group. Additionally, my data does not refute the
hypothesis that osteoclasts in adults preferentially demineralize the ground substance between collagen fibre bundles.

H.Y. Elder: From the author's stereo-pair images (or by other stereoemetric methods) is there any evidence of a difference in depth of resorption bays, which might correlate with the difference in elongation ratio which was observed between bones from individuals of different age?

Author: This question was not addressed in the present study. Should such an analysis be carried out, it is important that it be done on organic, rather than anorganic, specimens. This is because in younger subjects, where resorption was most anisotropic, osteoclasts were active at surfaces covered by an unknown thickness of osteoid.

H.Y. Elder: How common an observation was the subdivision along the snail tracks shown in Figure 17 and what significance does the author attach to it?

Author: Subdivision was a frequent finding, though sometimes very subtle. The boundaries between subdivisions are likely to demarcate the edge of the osteoclast resorptive territory during one episode in the formation of the snail track.

H.Y. Elder: It is appreciated that full specification of the proprietary enzyme detergent "Tergzyme" may not be available, but the main type(s) of enzyme present, and the dilution factor, could usefully be stated.

Author: The manufacturers of "Tergzyme" have declined to provide us with any information about this product. As regards the concentration, this was about 1% w/v.

W.A.B. Brown: In the "Distribution of activities with age" section, a diagram that would locate endosteal medial and lateral cortex would be helpful.

Author: See the diagram below.