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The Migration of Osteoblasts

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Summary. The endocranial matrix surfaces of parietal bones of 2-week old Albino Wistar rats were partly denuded of osteoblasts and then cultured for various periods up to 24 h, in control or PTE-enriched medium. They were examined by scanning electron microscopy and evidence for cell locomotion was found. Osteoblasts traversed the denuded bone surface and cut edges of bone in either medium, and cells also migrated out from vascular channels.

Glass spicules were placed on the otherwise undisturbed osteoblast layer in similar organ cultures for 2, 3 or 5 days. Osteoblasts migrated from the bone to populate the glass, negotiating any angle. The cells in PTE-enriched media were always aligned parallel to one another and elongated, tended to align with the edges of the glass and, in time, formed a substrate of aligned fibrils whose axes were parallel to those of the cells. Osteoblasts in control medium on glass showed variable degrees of alignment and elongation and were less influenced by the edges of the glass. Non-locomotory, nearly equidiametrical cells on glass in 5d control cultures had formed a substrate of randomly oriented fibrils.

Migrating osteoblasts on bone matrix did not have leading edge ruffles; isolated, migrating ones on glass did.

Key words: Osteoblasts – Organ culture – Locomotion – PTH.

Introduction

An interest in the ability of osteoblasts to move laterally arose from studies of the habits of osteoblasts in vivo (Jones, 1974) and in vitro under different experimental conditions (Jones and Boyde, 1976a; Jones and Ness, 1977). In normal life, productive osteoblasts constitute a tesselation of confluent cells which have processes that extend into the bone matrix. It is generally assumed that their lateral

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movement is limited or negligible. In non-productive phases, however, the connections of osteoblasts with subadjacent osteocytes may be reduced or non-existent, and cell processes may withdraw from the matrix (Jones and Boyde, 1976a). The locomotion of existing, fully differentiated osteoblasts to populate cell-free bone surfaces would entail such a disconnection and also a propensity for lateral movement. This preliminary report examines the faculty of osteoblasts, maintained on their bone in organ culture, for migration.

Materials and Methods

The parietal bones from 30 anaesthetized, 2-week-old Albino Wistar rats were excised free of dura mater and periosteum in dishes containing warm (37°C) Eagles's medium (Dulbeccos' modification) to which 10% foetal calf serum and 150 μg/ml of ascorbic acid had been added. Some bones were then fixed immediately in 3% glutaraldehyde in 0.15 M cacodylate buffer, pH 7.2, at 37°C, and these served as controls. In the experimental group, the integrity of the osteoblast layer on the endocranial aspect of the bone was deliberately disrupted either at the onset of the culture period, or during it. Small areas, about 80 × 250 μm in size, and tracks, from 50–100 μm across, were cleared of cells using a blunt metal instrument. Alternatively, all the cells were removed from one half of the specimen using a wedge of silicon rubber. The specimens were placed in fresh medium in a new dish after such wounding. Part of each specimen was then cut away with a sharp scalpel blade through the cleared area and fixed immediately so that a record was obtained of the extent of cell clearance. The rest was cultured in fresh medium in new dishes for 2, 4, 6, 8, 16 or 24 h in 3 ml of medium in a plastic petri dish. In each case, bone from one half of a calvarium was cultured in the control medium above (CC cultures), and from the other half in the same medium to which was added additionally 0.5 U/ml of parathyroid extract (PTE cultures: Parathormone, Lilley).

At the end of the culture period, the specimens were fixed in 3% glutaraldehyde in 0.15 M cacodylate buffer warmed to 37°C. Following fixation, all specimens for scanning electron microscopy were washed in distilled water, dehydrated gradually to 100% ethanol, and this exchanged for Freon 113 in steps. Liquid carbon dioxide was substituted for the Freon 113 and the specimens were critical point dried. The bone specimens were mounted with the endocranial aspect uppermost and sputter-coated with gold prior to their examination using a scanning electron microscope (Stereoscan S4-10, Cambridge, England) operated at 10 kV.

Half calvaria were also cultured with small fragments of glass coverslips (0.1 mm thick) laid upon the osteoblast layer. These were left on the bone for 2, 3 or 5 days and then either removed and processed for light microscopy, or left in situ for examination by scanning electron microscopy. In some instances glass was removed from the calvarium at 2 or 3 days and a fresh piece placed on the bone, and the culture continued. Some glass fragments removed from the bone were placed on unbroken glass coverslips and the culture of these cells continued for a further few days. The maximum total culture period in these experiments was 5 days. Both control and PTE-enriched (0.5 U/ml) media, as described above, were used. The specimens were placed in fresh medium after either 2 or 3 days.

Observations

The appearances of rat endocranial osteoblasts immediately upon calvarial removal, and after culture in control medium or medium to which parathyroid extract has been added, have been described previously (Jones, 1974; Jones and Boyde, 1976a; Jones and Ness, 1977).

Disruption of the Cell Layer. The silicon rubber wedge employed to disrupt the osteoblasts in these experiments completely removed the cells (including those

partially incorporated in the bone matrix in developing lacunae) over large areas with no apparent interference with the collagenous substratum. At the periphery of the cleared area, osteoblasts in a line one or two cells wide were disordered or disturbed: they were more rounded and raised a little from the bone matrix. Rarely, a larger patch of cells had become detached and had been folded back off the bone. This allowed the observation that cells becoming osteocytes could readily be distinguished from the surrounding osteoblasts, and matched to the forming lacunae they had vacated, not only by their oval shape and different level, but also by the relative dearth of the fine cell processes, which are a feature of osteoblasts.

The lesions made with the blunt metal instrument resulted in successful removal of the cells; but in most instances some damage to the surface of the bone matrix was found in the central region. The collagen fibres were either cut and bunched up on each side of the narrow line where they were severed, or there resulted a smeared line of bone similar in nature to the smear effects we have observed on instrumented hard tissues (Jones et al., 1972). A margin of undisturbed, cell-free, matrix surface was present on each side of the traumatized zone and this was bordered by cells which were either undisturbed, or again rounded and raised slightly from the bone in the first or first and second ranks. Otherwise, the osteoblast layer appeared to be unaffected by these mechanical interferences (Fig. 1).

Migration of the Osteoblasts on Bone. Some rounded and partly detached cells still remained at the margin of the cleared bone matrix surface after two hours culture. Most cells in the first rank had several small pseudopodia which extended from the side of the osteoblast that had been freed from its neighbour. Although the pseudopodia extended along the axes of collagen fibres where these were approximately at right angles to the border of cells, they were also seen to radiate across collagen fibres lying parallel to the border. The cells behind the first rank remained closely packed; small ruffles had developed on these cells. By 4 h some pseudopodia had extended into vacated, half-formed osteocyte lacunae. Evidence of the beginning of the locomotion of the cell body was given by slightly more space between the cells of the second and third ranks compared with the remainder of the undisturbed osteoblasts. Some cell loss would be expected to occur in culture (Jones and Ness, 1977), but evenly throughout the cell layer. By 6 h the cells had travelled further, both over apparently undisturbed matrix surface and over smeared bone (Fig. 2). The first rank cells of the 8 h cultures had moved away from their neighbours so that their cell bodies were no longer approximated to other osteoblasts (Fig. 3). The cells of the next ranks had themselves become more widely separated, and were to be distinguished, apart from the spacing, by their lack or sparsity of ruffling compared with the nonlocomotory cells. Isolated migrating cells at the end of 24 h in culture were mainly elongated but with several extensions of the lamellar cytoplasm (Figs. 4, 5). Their long trailing edges extending up to 75 microns from the cell body frequently crossed those from other cells and indicated the paths the cells had followed. Some of the spaced cells of the most forward ranks had migrated with apparent disregard for the pattern of the collagen they had traversed. However, preliminary measurements of the angular discrepancy between the collagen fibre axes and the long axes of elongated cells suggest a significant overall concordance (Fig. 6). The nearly confluent, migrating cells deep to the first ranks

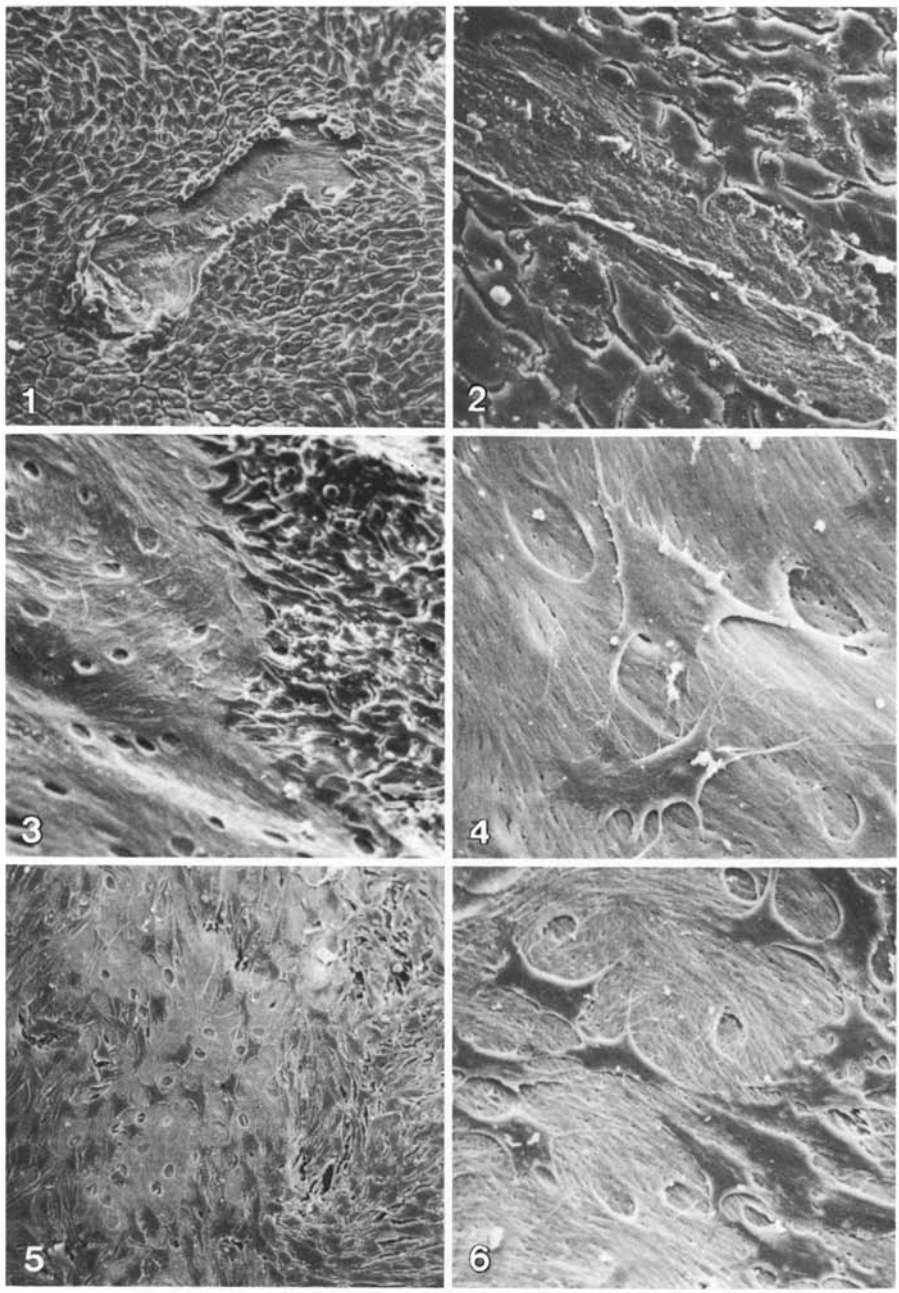


Fig. 1. Scanning electron micrograph (SEM) of rat osteoblasts on endocranial bone matrix fixed immediately after wounding the surface. The central part of the exposed bone is smeared. Cells at the edge of the wound are dislodged and rounded. Field width 410 μm

Fig. 2. Cell layer interrupted at 0 h; control culture for 6 h. Osteoblasts have migrated over the undisturbed and smeared bone matrix. SEM field width 180 μm

Fig. 3. 8 h PTE culture. Osteoblasts are migrating on to matrix that was entirely cleared of cells by a silicon rubber wedge at 0 h. SEM field width 420 μm

Fig. 4. 24 h PTE culture; half of bone matrix cleared of cells at 0 h. Migrating osteoblasts bear no ruffles. SEM field width 110 μm

Fig. 5. 24 h PTE culture; 0 h cell clearance from left-hand $^{2}/_{3}$ of field. Note the alignment of the confluent osteoblasts at the right. SEM field width 550 μm

Fig. 6. 24 h PTE culture; osteoblasts have migrated over bone cleared at 0 h. The long axes of the cells are related to the collagen fibre axes. SEM field width 160 μm

were better aligned and more likely to be parallel to the underlying collagen in the PTE cultures than in the control medium ones. Leading edge ruffles similar to those exhibited by the spaced osteoblasts on glass were not seen in cells traversing the bone matrix.

100 μm-wide tracks that had been cleared of cells at the beginning of the experiments had been repopulated completely by migrating cells by the end of 24 h. The configuration of the cells in the previously cleared area conformed to the surrounding osteoblasts in both the CC and PTE cultures. There was no discontinuity in the arrays of cells in the PTE cultures to indicate the site of clearance.

The cut edges of the bones (which were on average 60 μm thick) had also been covered by cells that were closely packed by 24 h. in culture. The leading cells must have travelled further than this distance, for cutting the bone generally resulted in cell loss for a distance of up to 20 μm from the cut edge. The cells migrating over the the edge were bent through a right angle (Fig. 7). In PTE cultures the cells on the cut edge, which was smeared, tended to be aligned both to each other, and parallel with the edges. Patterning such as this was rare in the CC cultures.

It was possible to estimate the migratory distance accomplished by the osteoblasts, in specimens cleared of cells by a silicon rubber wedge, by contrasting the cultured part with a control specimen that had been cut off at right angles to the cleared border immediately after the clearance and fixed immediately. The leading osteoblasts of both CC and PTE cultures had travelled an average of 250 μm (range 100–400 μm) during the first 24 h of culture (Figs. 9–11).

Besides the migration of osteoblasts over the denuded bone surface and the cut edge of the bone, there was a radial migration of cells extending 100 to 300 μm from the openings of vascular channels sited in the denuded area. However, the cells that migrated from vascular channels could not be positively identified as osteoblasts, and might have been a mixed population (Fig. 8).

A few cells that had previously lain in partly-formed osteocyte lacunae and had not been swept away, as was usual, from the cleared areas, had developed small ruffles in the early hours of culture, exhibited pseudopodia, and extended out over the adjacent matrix surface (Fig. 12). Thus it would appear that these cells were also capable of locomotion.

Migration of Osteoblasts from Bone to Glass. The osteoblasts cultured in both control and PTE-enriched media migrated from the bone surface and populated the glass spicules (Fig. 13). Because the endocranial aspect of the cultured bone fragments is concave and the glass fragments are flat, the osteoblasts usually only had access to the glass fragments at their corners. Thus the patterns of advance of the cell sheets could be traced from these corners (Figs. 14, 18, 19). The angle that the glass edge made with the bone was no deterrent to the migration (Figs. 16, 17): cells migrated on to both the upper and lower surfaces of the glass. Behind the advancing edge of the cell sheet on the glass the cells cultured with added PTE were always elongated, aligned parallel to one another, and had a greater tendency to be aligned parallel to the edges of the glass (Fig. 17) than the CC ones (Figs. 16, 20). In PTE cultures, the advancing cell sheets extended further along the sides of the glass fragments so that a concave advancing front was seen on the top surface (Fig. 18).

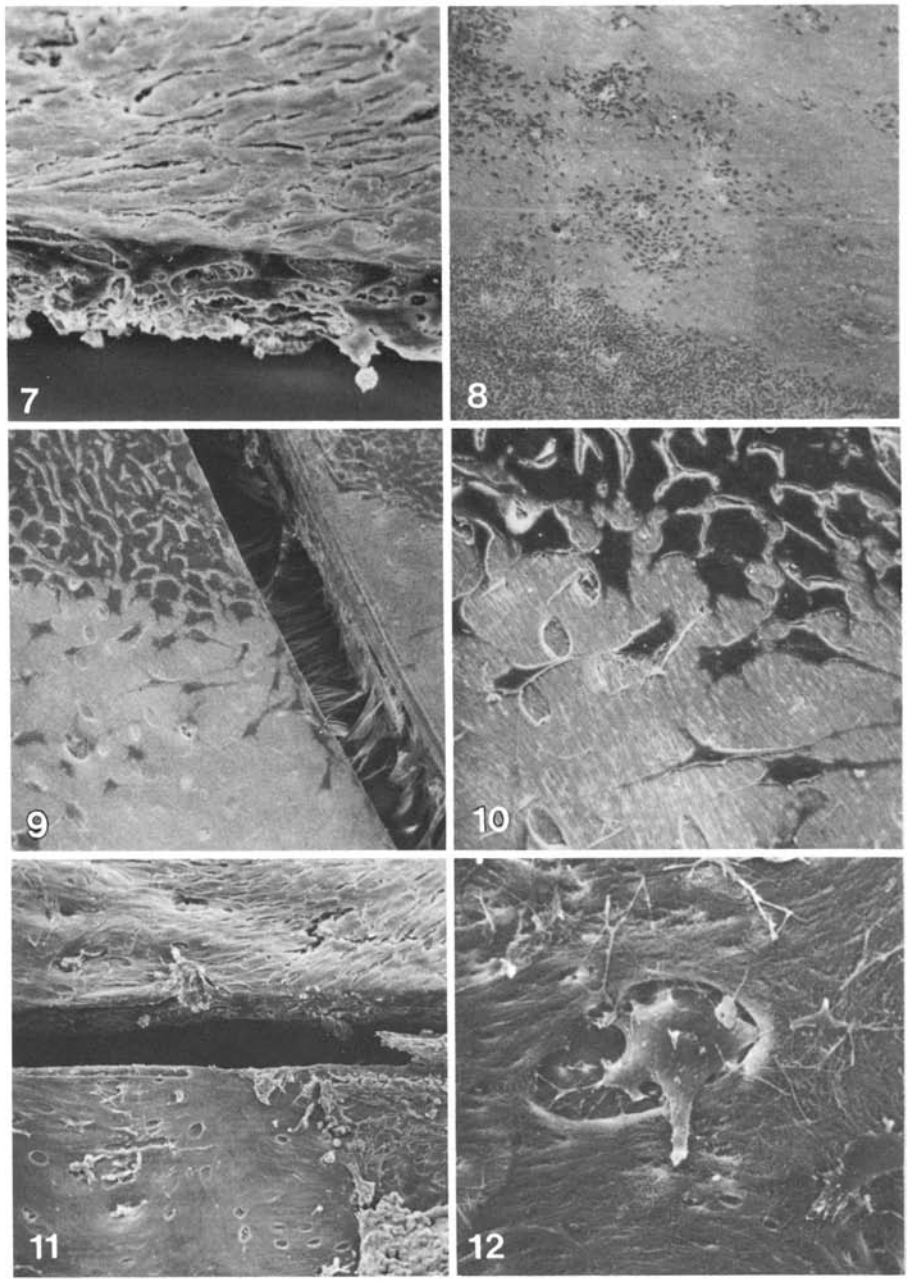


Fig. 7. Osteoblasts have migrated over the edge of the bone cut at 0 h. 24 h PTE culture: note the elongation and alignment of the cells. SEM field width 160 μm

Fig. 8. 24 h control culture. Except for the bottom of the field, the bone matrix surface was cleared of cells at 0 h. Cells have migrated from vascular canal openings, and extended from the intact osteoblastic sheet. SEM field width 2 mm

This contrasted with the CC cultures, where the advance was roughly equidistant at all points from the corners, so that the advancing front was convex (Fig. 19). In control medium, after 2 or 3 days of placing the glass on the bone, the cells behind the leading ranks were sometimes like the PTE ones. However, they also appeared in tesselations similar to those of in vivo productive or resting osteoblasts (Figs. 20, 21). After 5 days in culture, CC osteoblasts on glass sometimes showed a marked reduction in elongation and alignment similar to that found in 24 h or longer CC cultures of osteoblasts on the bone matrix.

In both the CC and PTE cultures, the well-spaced cells at the advancing edge on the glass had one or more extensive, broad, flat leading edges with ruffles (Fig. 15), and were not to be distinguished in appearance from cultured fibroblasts migrating over a plane surface (Abercrombie et al., 1970). The closely approximated cells in the first one or two ranks at the advancing edge were often very flattened and spread. The rate of extension of the advancing edge of cells on the glass spicules, measured over 2- or 3-day periods, ranged from 170–350 μm per day. The direction of movements of the widely spaced cells, as deduced from the positions of their lamellipodia and trailing processes, were random. Mitosis was particularly evident in the spaced cells on the glass, and also in the cells on the bone in the "feeder" area close to the glass.

Cells that had migrated on to glass spicules from the bone surface also migrated off the spicules on to glass coverslips when that opportunity was given to them.

A fibrous network resembling collagen could be seen between fortuitous shrinkage gaps in the cell sheet below some of the confluent cells of the glass spicules which had been left undisturbed on the bone organ cultures for 5 days (Figs. 22, 23). These cells were thought to be no longer migrating because of their shapes and positions, and the possession, particularly notable in the case of the CC cultures, of dorsal ruffles. The fibrils were fine and random below the almost equidiametrical cells of the CC cultures (Fig. 23); they were also fine, but in parallel order and with their long axes parallel to those of the cells in the PTE cultures (Fig. 22).

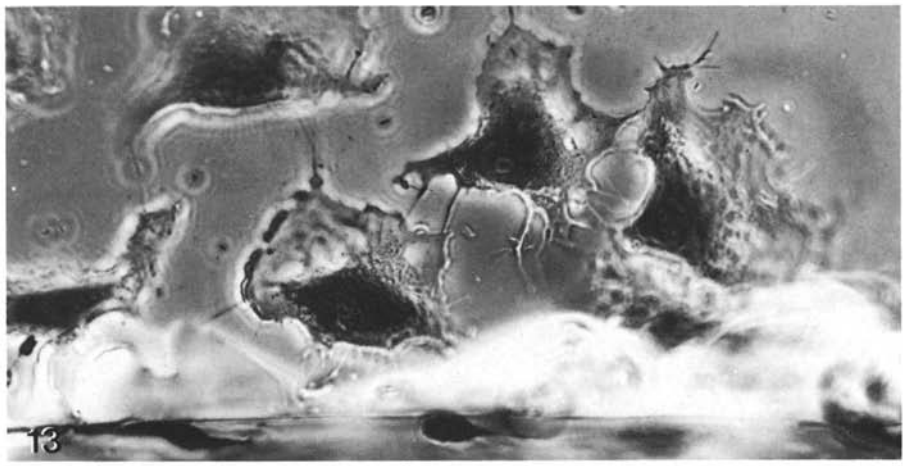
The patterns of the arrays of osteoblasts on the bone in the 5-day cultures were so striking that they could almost be discerned by eye. It was not possible to determine from surface examination, however, whether multilayering had occurred.

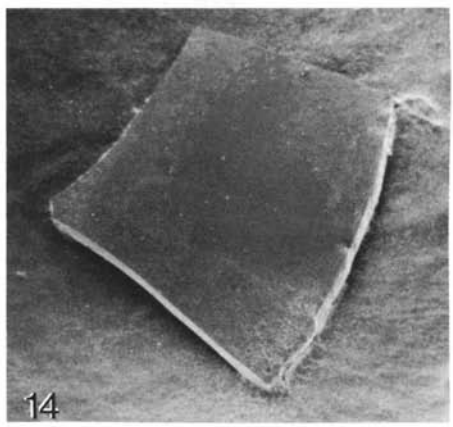
Fig. 9. 24 h control culture on left, 0 h control on right; cut edges rematched when mounted for SEM. The cells have migrated across the denuded matrix and cell areas have increased, due both to cell loss in culture and to migration into cell-free areas. SEM field width 550 μm

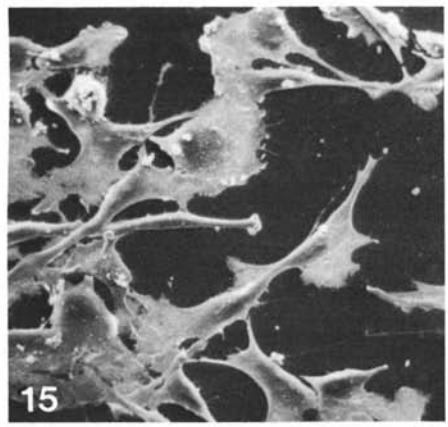
Fig. 10. Higher magnification of figure 9. The long axes of the isolated migratory cells which are not parallel to underlying collagen fibres may have been affected by the direction of the clearance strokes (from right to left of field). SEM field width 190 μm

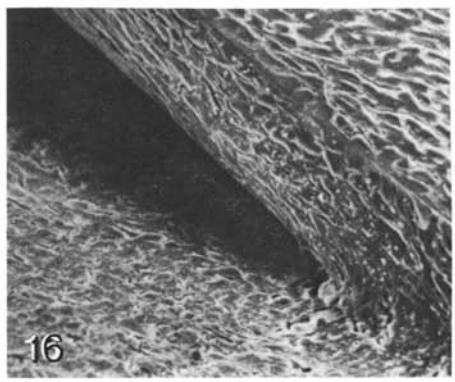
Fig. 11. 24 h PTE culture (above) rematched to control cut away at 0 h (below). The osteoblastic sheet was partly reflected in the control half, exposing the bottom surfaces of osteoblasts. PTE-treated osteoblasts have elongated and migrated over exposed bone surface and the cut edge. SEM field width 470 μm

Fig. 12. 24 h control culture osteocyte in half-formed lacuna has extended a process over the adjacent bone surface and no longer conforms to the lacunar shape. SEM field width 38 μm









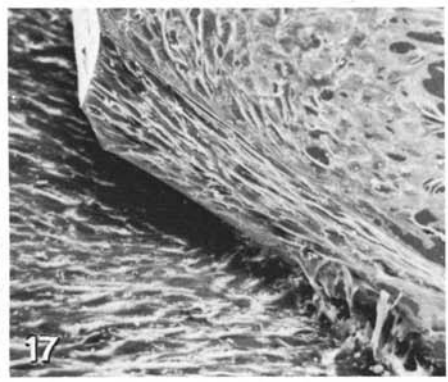


Fig. 13. 2 day control culture osteoblasts have migrated from bone on to glass fragment. Phase contrast light micrograph of balsam-mounted fixed preparation $\times 1080$

Fig. 14. Bone cultured for 5 days in control medium; glass placed on surface for last 3 days. Cells have spread on to the glass from the right-hand corners and left-hand side where the glass contacted the osteoblasts on the bone. SEM field width 4 mm

Fig. 15. Leading edge ruffles on spaced osteoblasts migrating on glass. 2 day control culture of glass placed on osteoblast layer of bone surface. SEM field width 180 μm

Fig. 16. Glass 3 days on 5 day bone control culture. Osteoblasts have populated the glass (upper right) moving from the bone (lower left), having gained access at the corner (lower right). Cells on the bone are less elongated and aligned than those in similar PTE cultures. SEM field width 470 μm

Fig. 17. Glass 3 days on bone, 5 day PTE culture. Osteoblasts gained access to the glass along its side (lower right) and are aligned parallel with the edges. Cells on the bone are elongated and aligned. SEM field width 460 μm

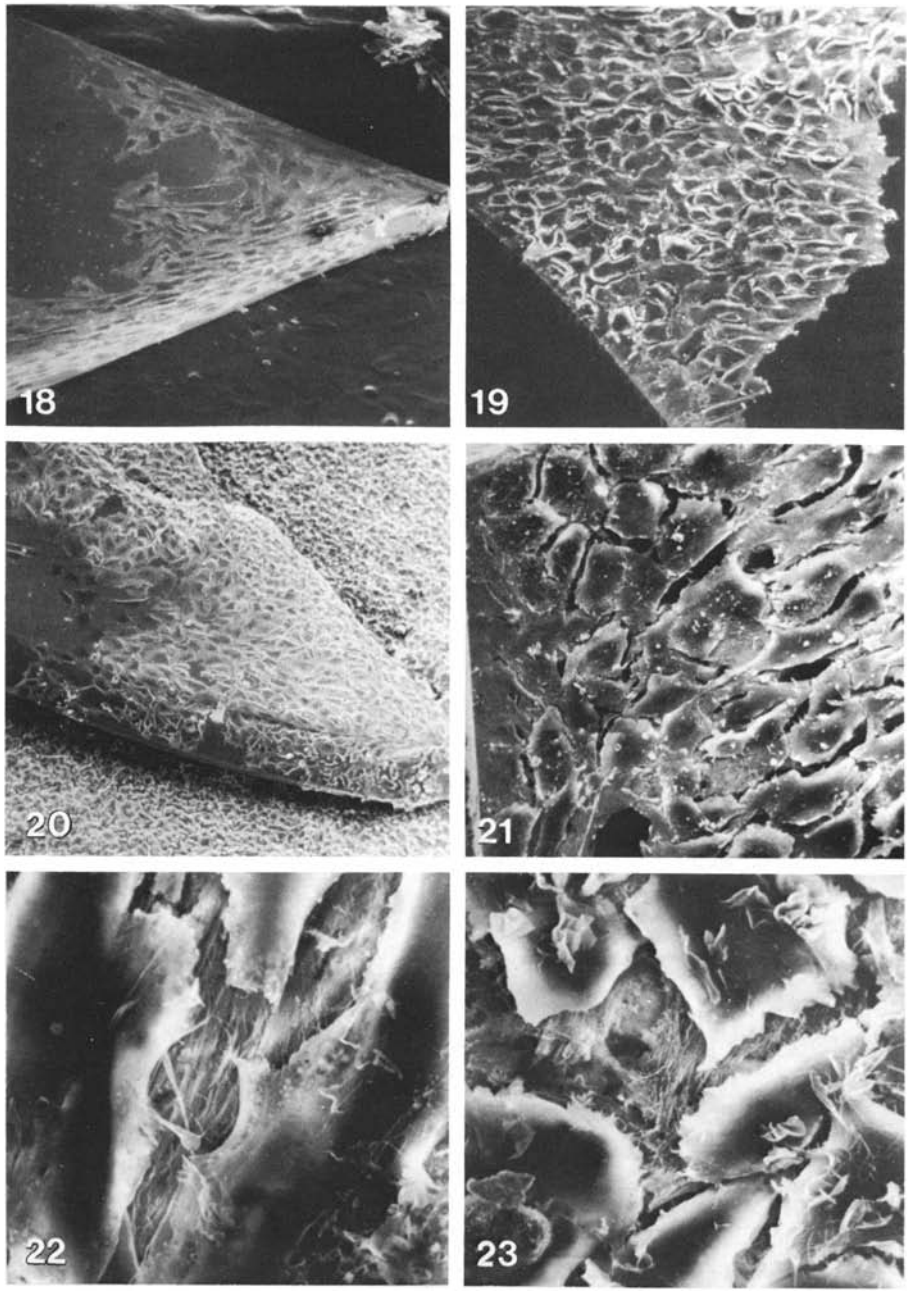


Fig. 18. Concave advancing front of osteoblastic sheet spreading from access corner at right. Glass removed from bone after 2 day PTE culture. SEM field width 920 μm

Fig. 19. Convex advancing front of osteoblastic sheet whose access to the glass was from the corner at top left. Glass 3 days on 5 day control culture bone. Note the reduced elongation and alignment of the cells. SEM field width 470 μm

Fig. 20. 2 day control culture of glass on bone. The cells are not aligned with the glass edges, nor have they extended preferentially along the edges. SEM field width 950 μm

Fig. 21. 2 day control culture. Osteoblasts on the glass fragment have made no fibrous matrix as yet, but have assumed a degree of elongation and alignment similar to that occurring in vivo on bone matrix. The gaps between cells are shrinkage artefacts. SEM field width 170 μm

Fig. 22. 5 day PTE culture. Osteoblasts that had migrated on to the glass were elongated and aligned, and had made a matrix of fibrils aligned parallel to the long axes of the cells. SEM field width 45 μm

Fig. 23. 5 day control culture osteoblasts on glass are more ruffled, less elongated and aligned and have made a fibrous substrate of more random fibrils. SEM field width 45 μm

Discussion

It is apparent that, given the opportunity provided by an adjacent surface devoid of cells, cultured osteoblasts migrate. However, the possibility that some of the cells migrating either across the bone surface or on to the glass spicules could have been derived from cells other than differentiated osteoblasts must be considered. The procedures adopted in this study have always resulted in a single layer of cells remaining on the bone, assumed to be osteoblasts from their position, surface morphology and cytology when studied by SEM and TEM (Jones, 1974; Jones and Boyde, 1976a). In order to minimise the possibility of cells in suspension settling on the cleared bone or on the glass, the specimens had been removed from the operating dishes to fresh ones containing fresh medium twice. We saw no evidence that settling from suspension had occurred. The most likely source of contamination would be from cells migrating from the vascular openings, which might include endothelial cells, or even fibroblasts, among their numbers. It is significant, however, that the cells which had migrated, whether on bone or on glass, were affected by PTE: they adopted the configurations which we have described for PTE-cultured osteoblasts left as an intact sheet exposed on the bone, forming arrays of cells which were parallel to each other within an array. This is also the format seen in confluent cultures of fibroblasts (Elsdale, 1968) and periosteal cells (Boyde et al., 1976); and in uncharacterized cells migrating from whole bone explants (Kano-Tanaka et al, 1976), and adopted by cultured osteoprogenitor cells when left undisturbed but exposed on the fibrous periosteum during culture in control medium (Jones, unpublished data). However, without PTE the confluent cells behind the advancing ones generally did not exhibit the same fibroblastic appearance. Cells that were probably not locomotory also mimicked the in vivo tessellations of osteoblasts with elongation ratios of approximately 2 or 3, or later appeared similar to the control-culture formations of undisturbed osteoblasts on bone, in which both the elongation and the alignment of the cells were markedly reduced (Jones and Ness, 1977). This latter arrangement distinguishes our putative osteoblasts from confluent fibroblasts in control medium. The adoption and maintenance of an elongated and aligned form in PTE-enriched medium would be expected to distinguish the same osteoblasts from confluent endothelial cells (Haudenschild et al., 1976).

Thus, it is highly probable that the cells which repopulated the denuded bone surfaces or populated glass surfaces in this study were mainly osteoblasts by origin, since preosteoblasts had been removed from the bone surface and we could delineate the likely extent of cells emanating from blood vessel canal openings. Osteoblasts are differentiated cells, usually derived from osteoprogenitor cells (Owen, 1963, 1973), which, on ceasing matrix formation, either become incorporated in the bone as osteocytes or line the inactive bone surface as resting osteoblasts, sometimes called "surface osteocytes" (Parfitt, 1976a). However, cells which were previously productive osteoblasts can be stimulated to divide in vitro by altering their functional status (Jones and Boyde, 1976a) or by spacing or discontinuity of the cell layer. It is entirely possible that some of the mitosing cells that are occasionally seen at the surface in adult bone in vivo have been formerly fully differentiated osteoblasts. It would be interesting to discover if multilayering

would occur with time on either the bone or glass in the media used; and also whether an osteoprogenitor multilayer could be re-established above a single-celled layer of functioning osteoblasts from the cells of osteoblastic origin.

It would be difficult to determine whether the osteoblasts' faculty for locomotion in culture is employed in vivo, but it seems unlikely that osteoblasts next to an unpopulated matrix surface would not move or spread across the bone when spaced. The nature of the surface seems not to be of paramount importance, nor does its angular inclination to the osteoblastic sheet. Cells moved on to the lower surface of the glass fragments which necessitated describing very acute angles, and up and over vertical or overhanging cliffs on to the top surface of the glass. Individual cells sometimes conformed to three planes at right angles. Cells migrating over the cut surface of the bone also bent at right angles as they moved from one surface to the other. We suggest that the lack of confluence promotes locomotion in the osteoblastic sheet. This movement is controlled by cell surface to substrate and cellular interactions.

The structure of the microfilamentous arrays in the osteoblasts which were negotiating edges and corners is not known, but new assemblies of filaments are presumably formed in the parts of the cell moving on to a different plane, just as they are in the newly extended pseudopodia of fibroblasts in culture on a flat surface (Bragina et al., 1976). An exception to this may have occurred where the corrugations caused by the collagen fibres in the bone matrix surface were parallel to the cut edge over which the cells were migrating. The long axes of the cells at the edge, and bestriding it, were then usually parallel to its line; indeed whatever their angle of approach, PTE cells tended to become more aligned with the edge. It is unnecessary in this situation to postulate the assembly of new microfilaments in the part of the cell that traverses the edge even though the angular difference between the planes is 90° or more (Dunn and Heath, 1976). Only a series of small sideways shifts are then required, without any change in the arrays of microfilaments, as the cell translates sideways over the edge. Nevertheless, particularly on the glass spicules, elongated cells were also seen bent over the right-angled edges with their long axes perpendicular to the edge, and this transit obviously involves new microfilaments.

The increased tendency for the PTE-osteoblasts to be aligned with one another on the side of the glass and parallel to its edges, compared with the CC osteoblasts of lesser elongation, and to have travelled further along the sides and stayed closer to the upper edge rather than crossing into the middle of the upper surface of the glass, may be an expression of the greater importance of contact guidance provided by the substrate form to elongated cells with parallel microfilamentous arrays, and lends support to the prediction of Dunn and Heath (1976).

The shapes of the isolated locomotory cells on glass were different from those on the bone matrix. The latter did not usually have the very thin, broad leading edges and never exhibited the extensive leading edge ruffles of the former, indicating that the adhesion of the osteoblasts to glass was not as good as to bone. The glass was a plane surface and, as the medium always contained serum, attachment would have been to a layer of serum adsorbed on to the glass (Abercrombie, et al., 1970). By contrast, the bone matrix surface was anisotropically corrugated by collagen fibrils and might not have had adsorbed serum in the same way. However, serum proteins

are a normal part of the bone fluid in vivo and are also incorporated in the calcified bone (Owen et al, 1973). The lamina limitans found below resting, but not productive, osteoblasts could be a layer of adsorbed serum proteins which accumulates on the matrix surface in vivo when matrix production ceases (Scherft, 1972; Luk et al., 1974). It is obviously necessary to examine the bone surface by transmission electron microscopy to find out whether an adsorbed layer accumulates in culture.

Thus we do not yet know the nature of the attachment of the migrating osteoblasts to the once-exposed bone matrix they are repopulating, nor, therefore, what factors could be involved in their movement on bone. However, we have found that serum is necessary in the medium for the migration of the osteoblasts across the bone, and also for the spreading (but not the attachment) of the osteoblasts on glass (Jones, unpublished data).

The question also arises as to why the osteoblasts migrate from bone, a substrate to which they appear to have a greater adhesivity after it has been bared, to glass. This seems contrary to what one would expect from the known migratory habits of fibroblasts (Maroudas, 1973, 1975). Maroudas has, however, suggested (personal communication) that cellular exudate below the cultured cells on the bone might act as an "excluding layer", reducing with time the adhesivity of that surface for the osteoblasts compared to the exudate-free, serum-coated glass, and causing the cells to transfer. This would occur when the inhibitory effect of polymer exclusion due to the exudate outweighed the stimulatory effect of polymer aggregation in the collagenous matrix (Maroudas, 1977). We have noted previously that the adaptation of the osteoblasts to their natural substrate is reduced with time in culture (Jones and Boyde, 1976a).

The shapes obtained by the cells migrating over their natural substrate would be closer to that expected for locomotion in vivo: the two disparate forms are not unlike those found with endothelial cells migrating either in culture or on their normal substrate of an isotropic collagen fibrillar network in vivo (Nelson and Revel, 1975).

Although it is unknown whether osteoblasts locomote in vivo, they are undoubtedly mobile and change shape and size with different rates of matrix production (Marotti et al, 1975). Parathyroid extract influences, either directly or indirectly, the alignment and the elongation of cultured osteoblasts on the parallel-fibred bone of young growing rats (Jones and Boyde, 1976a). Because it is known that parathyroid hormone initially supresses the synthetic activity of cultured osteoblasts (Dietrich et al., 1976), we had wondered whether the motility of such cells would be subsequently increased, perhaps as the cell-substrate adhesivity decreased (Gail and Boone, 1972), and that this might be one factor in improving the alignment of cells within an array. This alignment would be followed by an increase in elongation due to uniaxial movements (Jones and Ness, 1977).

The formation of parallel collagen-like fibrils below the aligned cells in the PTE five-day cultures indicates that an elongated form and a reduction in the expression of dorsal ruffling are not synonymous with a complete halt in matrix formation. The ruffling activity of these secretory cells in the PTE-enriched medium was greater, however, than that of their non-secretory peers. It is likely that the effect of PTE on the osteoblasts in the longer organ cultures varies from the short-term

effect, as has been reported with chronic low dosage of parathyroid hormone in vivo (Parsons and Reit, 1974) and in vitro (Flanagan and Nichols, 1965). Thus matrix production may have stopped initially in the 5-day cultures but then restarted. It has been assumed that the newly productive cells in chronic administration of PTH arise from osteoprogenitor cells, not from the original osteoblast layer (Parfitt, 1976b), but this cannot be so with our cultures. However, new collagen synthesis by the once-productive osteoblasts may, perhaps, not occur unless mitosis has intervened: this we have not investigated.

We have found that well-developed ruffling membranes occur on free dorsal surfaces of osteoblasts cultured in medium when it contains serum, but are rudimentary on those cultured without serum or in PTE-enriched medium with serum. Plasma membrane turnover and macropinocytosis generally decrease upon the omission of serum or growth-promoting factors from the medium and it is interesting to note that in glial cells (Brunk et al., 1976) as with our osteoblasts, elongation of the cells accompanies these changes. Additionally, the cells thought to be locomotory at the border with denuded bone in the CC cultures bore no ruffles; whilst those deep to the border and likely to be productive were well ruffled on their dorsal surfaces in CC cultures, and ruffled in PTE ones.

The arrangement of the fibrillar axes parallel to the long axes of our cells on glass suggests that the cells are exerting tensions, possibly as a result of uniaxial motility (Harris, 1973). We do not yet know if the equidiametrical CC osteoblasts are moving haphazardly, as do epithelial cells (Steinberg, 1973), and thereby promoting random order in the fibrils they are producing; or whether they are not moving at all and thus not furnishing tensions which could affect the collagen order, as we suspect is the situation that arises in dentine formation (Jones and Boyde, 1976b). The reason why woven bone is usually formed in vitro, whether the original bone be woven or lamellar (Parfitt, 1976a), is probably that the hormonal balance in the medium is not adjusted sensitively enough for the maintenance of both suitable (but not necessarily constant) rates of matrix formation and sufficient alignment of the osteoblasts to promote an organization of parallel collagen fibrils (Jones and Ness, 1977).

There are, undoubtedly, many factors that influence the development of collagen patterns in bone in vivo. Our in vitro experiments on unstressed bone suggest that cellular ones which increase the probability of parallel alignment of collagen fibrils include confluence, elongation and alignment in the formative cell layer, a low and fluctuating rate of matrix formation and uniaxial motility of the osteoblasts. Rapid matrix formation, discontinuity in the cell layer, cells with little or no elongation and alignment, and random or no motility should favour random collagen order. One might guess that the gradual change in orientation of collagen within a lamella in adult bone would occur during a period of matrix formation, and not in a resting period. The more abrupt change in orientation from one lamella to another could be the consequence of a prolonged resting period in the cycle of activity of the osteoblasts. This would initially enhance the alignment of the cells' long axes to the axes of subadjacent collagen fibres; then with continued or increased cell movement, and a reduction of the influence of the substrate form on an osteoblast compared with the influence of the cell population, the cell pattern would evolve (Elsdale and Wasoff, 1976) and alignment to the substrate lessen and

then be lost. The next layer of bone matrix to be formed would reflect the new arrays, and might be unrelated to the last. It is interesting in this context to consider the structure of the laminar bone of young, rapidly or continuously growing mammals. The parallel-fibred bone does not show abrupt changes in the mean order of collagen fibres from one layer to the next, and this may be correlated with a moderate rate of matrix production without long resting periods so that the osteoblasts are rarely, if ever, entirely released from the influence of their substrate. The rate of matrix formation may also affect the proportion of the glycosaminoglycan component secreted, which, at least in cartilage, can modify the form of fibrils (Kochhar et al., 1976). However, as yet too little is known of the dynamic behaviour of osteoblasts for such speculation.

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