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Humps on young human and rabbit articular cartilage

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It would appear that the ridges and undulations described by various authors (McCall, 1968; Gardner & Woodward, 1969; Walker *et al.* 1969; Redler & Zimny, 1970; Gardner, 1972; Mow, Lai & Redler, 1974; Redler, 1974) as a constant and characteristic feature of the normal articular surface (as seen with the scanning electron microscope) are in fact either artefactual, atypical or pathological, for such features are not seen on cartilage attached to subchondral bone but are readily produced if cartilage is detached from subchondral bone, or if it is cut or injured in various ways (Clarke, 1973*a*; Ghadially *et al.* 1976).

Indeed the only constant feature seen with the scanning electron microscope on the surface of both normal human (Clarke, 1971*a*, *b*, *c*; 1973*a*, *b*) and rabbit cartilage (Ghadially, Ailsby & Oryschak, 1974; Ghadially & Ghadially, 1975; Ghadially *et al.* 1976) attached to subchondral bone is the occurrence of numerous shallow pits or depressions which are thought to represent the presence of underlying chondrocytes.

Mound-like elevations (referred to as humps) were seen by Redler (1974) on human cartilage and he thought that this feature was of more frequent occurrence on cartilage from juveniles. However, the fact that this author considers ridges to be a constant feature of the normal articular surface, and often his reports leave one wondering whether cartilage attached to or detached from bone was studied, detracts from such a thesis, particularly when one takes into account the fact that humps have been seen on injured rabbit cartilage (Ghadially *et al.* 1976) and on the articular cartilage of dogs that from their weight and external appearance were regarded as mature or even quite old (Ghadially *et al.* 1977).

Therefore when we recently examined the articular cartilage from a 7 years old boy we were surprised to find that the surface was not covered by the ubiquitous pits we expected to see but instead by numerous humps (Figs. 1 and 2). This suggested that the surface topography of the articular surface may indeed be different in young individuals, as suggested by Redler (1974). In order to test this hypothesis we examined the articular cartilage of young rabbits. The purpose of this paper is to present further evidence supporting the thesis that ridges and undulations are in fact shrinkage artefacts, and also record our new findings regarding the nature of humps and the surface topography of young articular cartilage.

MATERIAL AND METHODS

Human articular cartilage

This was obtained at autopsy performed approximately 2 hours after the death of a 7 years old boy from acute meningitis. A piece (approximately $7 \times 5 \times 3$ cm thick) of the medial femoral condyle was removed, with the aid of a vibrating electric saw,

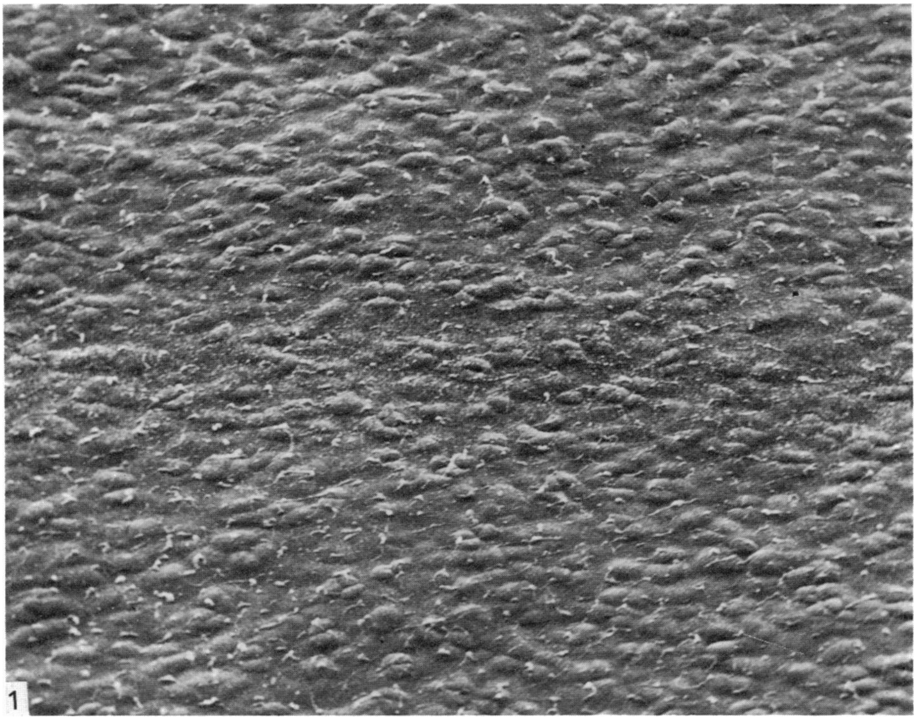

Fig. 1. Surface of articular cartilage from a 7 years old boy showing numerous humps and a flocculent precipitate of synovial fluid. ×230.

from the left knee joint, which appeared perfectly normal. The specimen was then washed in normal saline and fixed and processed as described later.

Rabbit cartilage

Three, 1 month old rabbits and three 2 months old rabbits were used in this study. The animals were killed by an intracardiac injection of nembutal and the lower ends of the right and left femora were dissected out, taking care that the articular surface was not touched or damaged. After removing attached muscle and the joint capsule, the specimen, which now comprised femoral condyles and about 1 cm of attached shaft, was washed in normal saline and processed as described below.

Processing of tissues

Human and rabbit tissues were fixed in 3% glutaraldehyde in 0·1 M cacodylate buffer (pH 7·3) for 2 weeks and dehydrated in increasing concentrations of ethanol over the course of another week. Pieces of cartilage (about 1 cm^2) with attached subchondral bone were dissected off from the human specimen using a fine fretsaw. After rinsing with alcohol these pieces were allowed to dry in air.

In the case of the rabbit specimens the condyles were dissected off the femora in a similar manner. Half of these were air-dried while the others were transferred to amyl acetate and dried by the critical point method using CO_2. Specimens were mounted with the aid of Electrodag on specially prepared large low aluminium stubs (1·5 cm in diameter and 2 mm thick) so as to compensate for the rather thick (1–1·5 cm) human specimens comprising cartilage plus a substantial piece of subchondral bone which were used in this study.

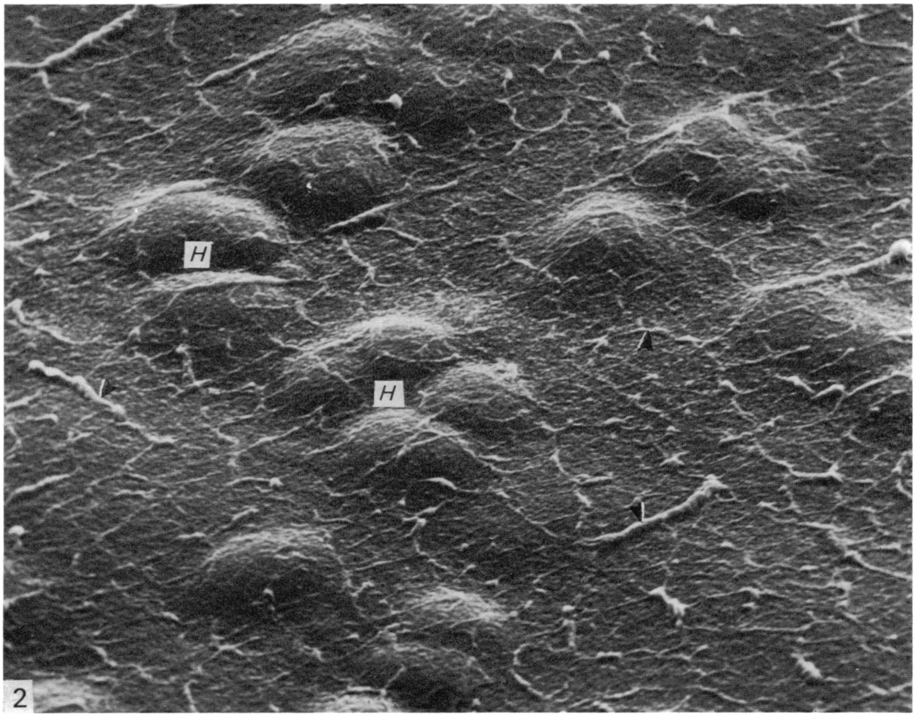

Fig. 2. A high power view from the specimen shown in Fig. 1. Note groups of humps (*H*) and the reticulum of precipitated synovial fluid (arrowheads) on the articular surface. × 1250.

Specimens were then vacuum coated with gold in a sputtering device and examined with the scanning electron microscope (Cambridge Stereoscan) using an accelerating voltage of 20 KeV.

RESULTS

Human cartilage

The washing procedure had failed to remove completely the synovial fluid, so the surface was covered by a reticulum of precipitated material. However, this did not materially hinder the visualizing of the articular surface (Figs. 1 and 2) which appeared to be populated by innumerable humps. No pits as found in adult human or rabbit cartilage (Clarke, 1973*a*; Ghadially *et al.* 1974, 1976) were detected in this specimen from a 7 years old boy. The humps were often seen in groups of two to four. Ridges and undulations were absent from the articular surface except adjacent to the cut edge of the specimen where they were quite prominent (Fig. 3).

One month old rabbit cartilage

The articular surface of air-dried condyles was remarkably rough-looking. Here one could identify humps, ridges and undulations (Fig. 4). By comparison the condyles dried by the critical point method were rather smooth, the only conspicuous surface asperity being the occurrence of numerous humps (Fig. 5). Such humps usually had a thin moat around them, which suggested that these humps had developed in the floor of pits (Fig. 6).

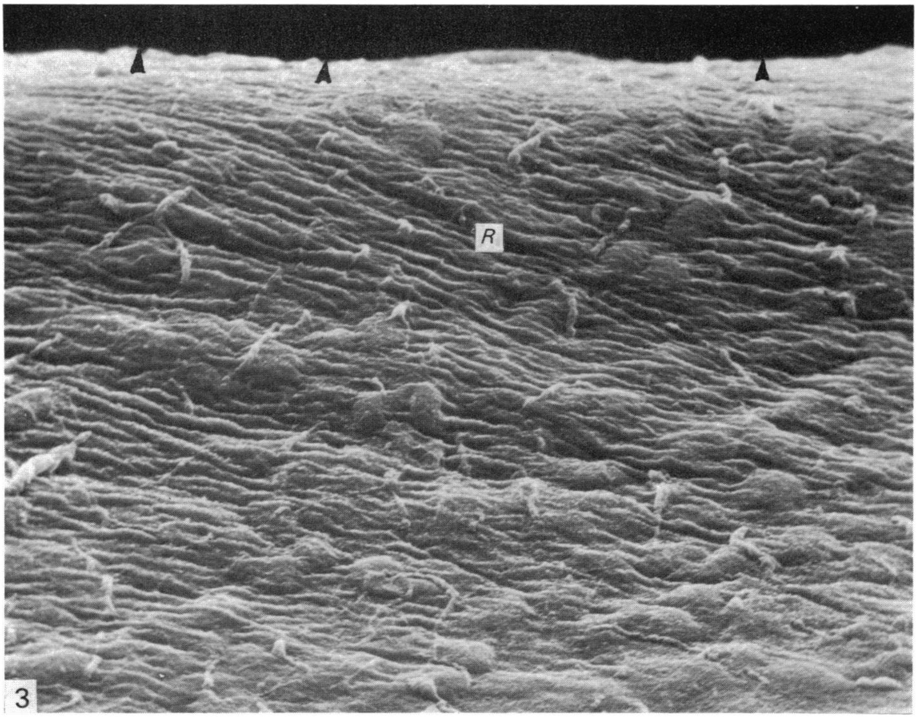

Fig. 3. Ridges (*R*) found adjacent to the cut edge (arrowheads) of the specimen shown in Fig. 1. ×580.

Two months old rabbit cartilage

The surface of these specimens was characterized by the presence of numerous shallow pits. These pits were extremely shallow and difficult to detect or record photographically in critical-point-dried specimens, but they were slightly more prominent and hence just recordable in air-dried specimens (Fig. 7). Humps were seen arising from the floor of some of the pits (Fig. 8).

DISCUSSION

Effect of tissue fixation and dehydration

It is important to keep constantly in mind the alterations in morphology produced by methods of tissue preparation. Indeed it is our belief that the pits and humps seen on the articular surface are largely, perhaps entirely, due to the shrinkage and distortion engendered by such manoeuvres. This is particularly true in the case of tissues prepared for the scanning electron microscope, for in contrast to tissues prepared for light or transmission electron microscopy, quite large pieces of tissue are fixed and dehydrated without substitution (i.e. without replacement of tissue water by embedding medium) so that more marked alteration of morphology can be expected to occur.

The need to use quite large pieces of tissue also necessitates a rather prolonged fixation period (10–14 days) and a slower more extended period of dehydration (about 7 days). Preliminary and continuing studies by us (Ghadially *et al.* 1976; Ghadially, unpublished) have shown that shorter periods of fixation (e.g. 2–4 days)

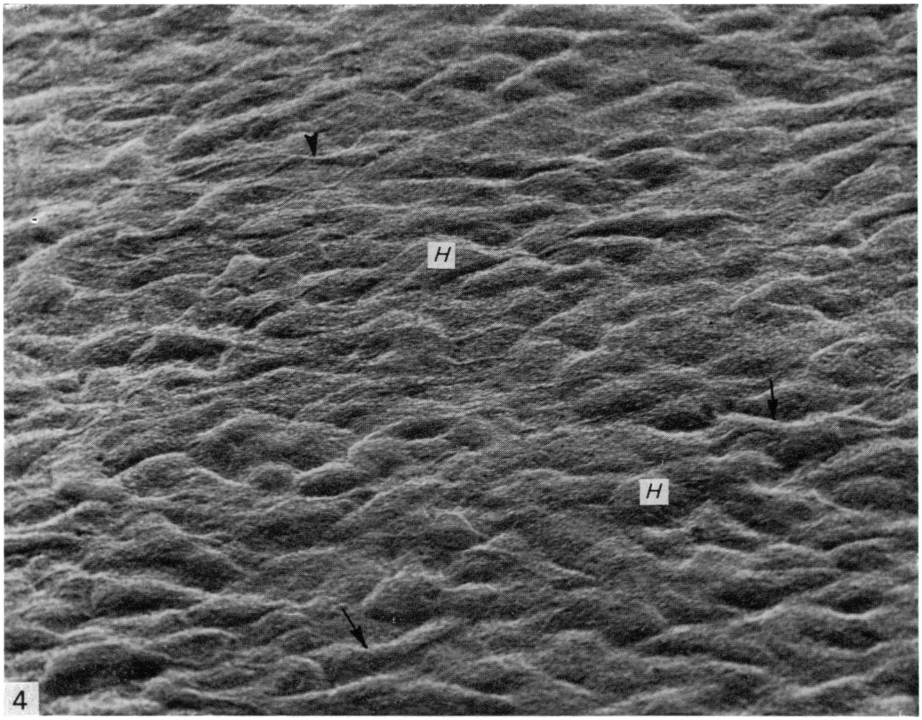

Fig. 4. Air-dried, 1 month old rabbit articular cartilage. Humps (*H*) and coarse (arrow) and fine (arrowhead) ridges are seen on the surface. ×770.

yield a specimen which is easily damaged in the electron beam, so that multiple fine fissures and contamination marks develop as one is studying such specimens in the scanning electron microscope. However, even in such specimens characteristic surface markings such as pits and humps can be visualized before the above mentioned changes develop, making the specimen useless for photographic recording.

On the other hand more prolonged fixation (3–4 weeks) tends to produce a very brittle specimen in which quite large cracks may develop in the articular cartilage either spontaneously, or when the specimen is handled during the sawing of the bony support and mounting. Again, even in such specimens pits and humps can be demonstrated, as also can ridges and undulations that develop adjacent to the cracks.

Thus it seems to us that pits and humps are very reproducible and constant features, and neither varying periods of fixation nor quite drastic variation in the concentration or osmolarity of the fixative (Ghadially *et al.* 1977) dictate whether humps or pits will be seen on the surface. Hence one must seek the explanation of such differences in the tissue itself; that is to say in the differences between juvenile and adult cartilage.

Undulations and ridges

The results of the present study provide further evidence to support the idea that undulations and ridges are not constant features of the surface of normal articular cartilage. The erroneous notion that they are stems mainly from examination of cartilage detached from its bony support, allowing excessive shrinkage and curling of the specimen to occur (Ghadially *et al.* 1976).

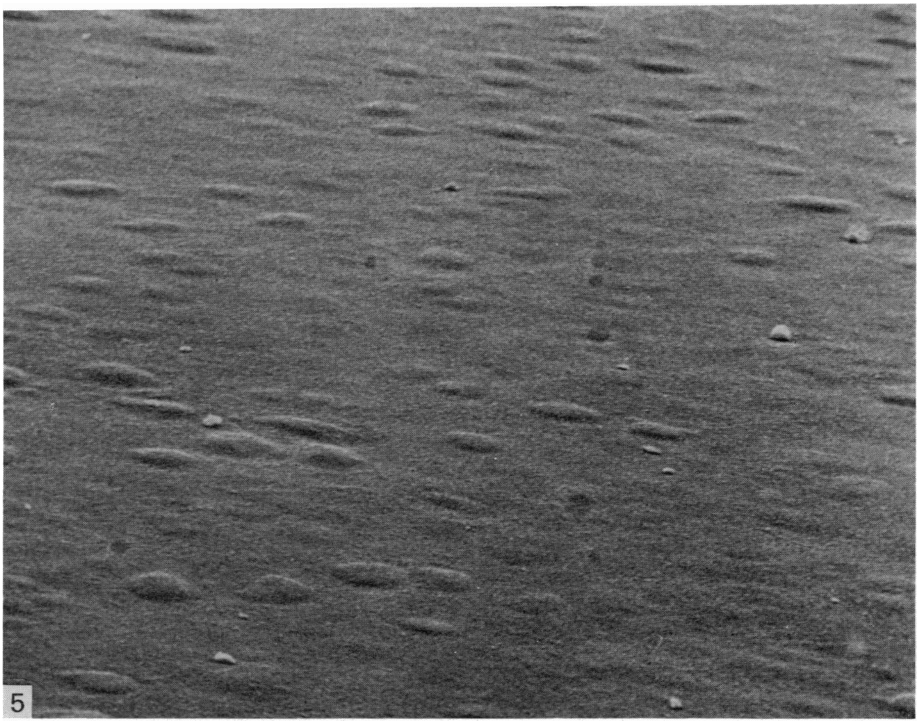

Fig. 5. Critical-point-dried, 1 month old rabbit cartilage. Numerous humps are seen on the articular surface. × 620.

The fact that in the present study the articular surface of air-dried cartilage (attached to bone) from 1 month old rabbits showed ridges and undulations does not militate against the above-mentioned concept, for one can argue that, at this age, neither a calcified zone (zone 1V) nor an adequately firm bony support capable of restraining the cartilage from irregular shrinkage and distortion are fully developed. Such a contention is also supported by the fact that when shrinkage and distortion of the specimen were minimized by the critical-point-drying method undulations and ridges did not develop on the 1 month old articular surface.

The occurrence of ridges and undulations adjacent to the cut edge of the juvenile human specimen described in this paper is also in keeping with the hypothesis promulgated above, and with (1) the results of previous studies by Clarke (1971*c*) who noted ridges adjacent to the fractured edge of adult human cartilage and with (2) the fact that in both rabbit (Ghadially *et al.* 1976) and dog (Ghadially *et al.* 1977) articular cartilage ridges and undulations coursing in various directions are produced when cartilage is detached from the bone or cut or injured in various ways.

Humps and pits

According to Clarke (1973*a*) the only constant and characteristic feature of the surface of adult human cartilage attached to bone is the occurrence of numerous pits. The same situation has been shown to prevail in the articular cartilage of rabbits over 3 months old (Ghadially *et al.* 1971; 1976). However, the results of the present study clearly show that the surface topography of the articular surface of very young rabbits and of children may be quite different, for both in a 7 years old boy and in

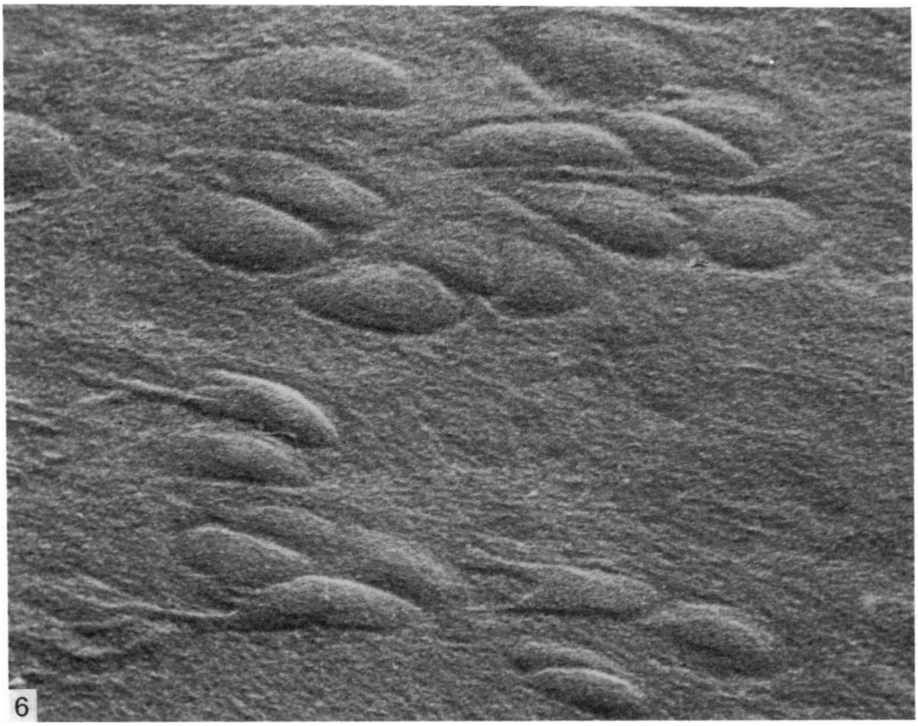

Fig. 6. High power view from the specimen shown in Fig. 5. The humps have a moat around them. × 1150.

1 month old rabbits the surface was found to be covered by humps, and not pits as seen in adults of these species. In the 2 months old rabbit an intermediate situation seems to prevail, for here the pits are very shallow and on some occasions humps are seen arising from the floor of these pits.

Neither the significance nor the nature of the pits and humps seen on the articular surface with the scanning electron microscope are clear, and there are grave doubts as to whether such features occur *in vivo*. Regarding pits, the currently held view (Clarke, 1973*a*; Ghadially *et al.* 1976) is that they reflect the presence of underlying chondrocytes.

A plausible explanation would be that the method of specimen preparation leads to shrinkage and collapse of many of the superficially placed chondrocytes and lacunae so that the surface 'caves in' at these sites.

Similarly, since the morphology of the humps and their arrangement in groups is reminiscent of chondrocyte formations, we may surmise that humps indicate superficially placed chondrocytes that are elevated above the general articular surface. Whether such formations occur *in vivo*, or whether they are produced during specimen preparation, remain matters for conjecture.

Light microscopic studies aimed at resolving this dilemma are singularly difficult to interpret. The most extensive studies on this point are by Gardner and his colleagues who at first (see review by Gardner, 1972) described systems of undulations of various dimensions on the surface of human articular cartilage, but in a later study (Longmore & Gardner, 1975) they talk about 'quarternary ridges' and

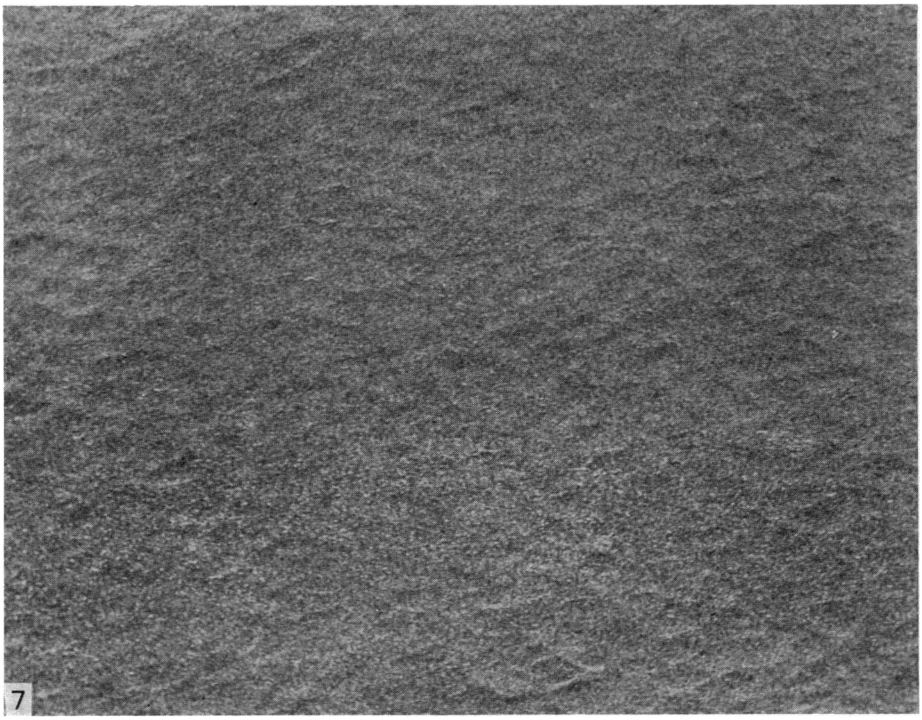

Fig. 7. Air-dried 2 months old rabbit cartilage. The presence of numerous shallow pits gives the surface a beaten copper, or golf ball-like appearance. ×210.

'tertiary hollows' (presumably the same as our pits): but one is left unconvinced that such features occur *in vivo*.

Pits or illusions of pits, and also occasional or probable humps, have been noted by others (Clarke, 1973 *a*; Meachim, 1972) but they have been quite cautious in interpreting such phenomena. Regarding this matter, Meachim & Stockwell (1974) state: "'Surface markings' of similar shape and size to those observed by Clarke with the scanning electron microscope can also be demonstrated by light microscopy (Clarke, 1971 *a*). They are seen when the surface of adult human articular cartilage is examined in incident light, and when transmitted light is directed through a thin tangential slice cut from the surface (Figs. 1, 10). Such markings represent the site of the cells in the superficial matrix (Clarke, 1971 *a*; Meachin, 1972 *b*). The question then arises whether they are simply a consequence of the differing optical properties of the lacunae and intercellular matrix, or whether actual depressions of the surface are present over the cells."

Besides such optical problems one has to recall that often such reports deal with human cartilage collected at autopsy (e.g. Clarke, 1973 *a*; Longmore & Gardner, 1975) and one is left wondering whether post-mortem shrinkage of chondrocytes and lacunae occurs, giving rise to pits. Be that as it may, in our view light microscopic studies of this kind cannot possibly answer the question as to whether pits are actually present during life, for even if one were, for the sake of argument, to concede that genuine depressions have been seen with light microscopy, one could still argue that exposing and handling the cartilage lead to a certain amount of fluid loss with resultant production of pits.

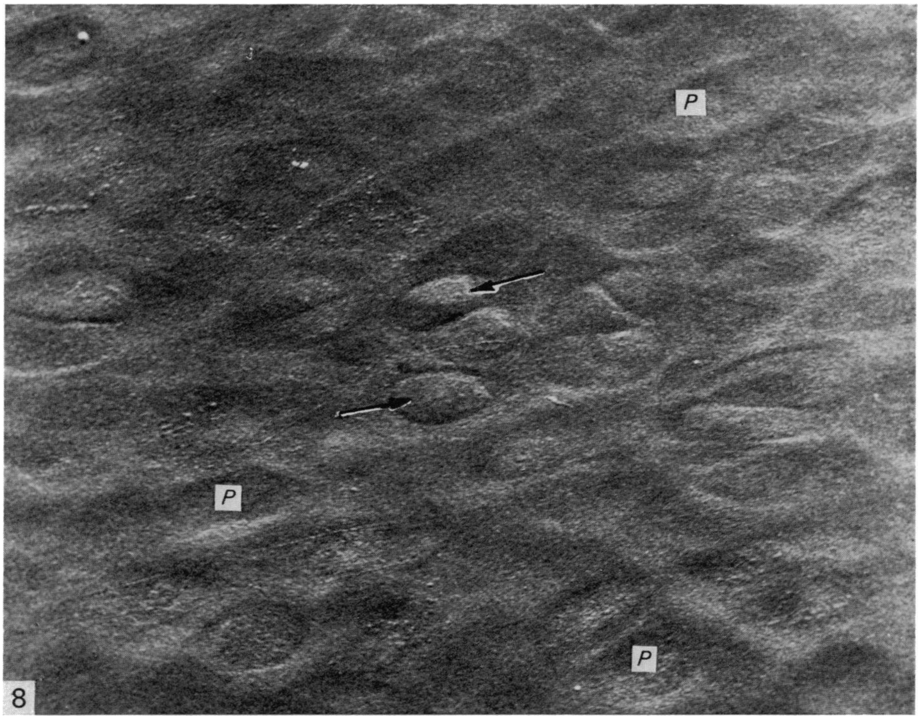

Fig. 8. High power view from the specimen shown in Fig. 7. Note the shallow pits (*P*) and humps (arrows) arising from the floor of some of the pits. ×700.

The reasons why the presence of underlying chondrocytes is reflected by pits in adult articular cartilage but by humps in young articular cartilage must now be considered. Clearly, anatomical differences must be sought. The significant difference may well be that the superficial zone (Zone 1) of very young cartilage is much more cellular than in the adult, and there may also be physico-chemical differences in the matrix. Hence one may speculate that either humps occur *in vivo* or that the overall shrinkage of the tissue pushes up the superficial chondrocytes and produces humps.

In the case of rabbit cartilage, where injury leads to the production of humps, one may speculate that the curling and shrinking of cartilage push up some of the most superficially placed chondrocytes, thus producing humps. The occurrence of humps on the articular surface of apparently mature or old dogs (exact age not known) suggests that in some species very superficially placed chondrocytes persist longer than in others. We (Ghadially, unpublished observation) have recently looked at cartilage from the femoral condyle of a 20 months old cat (with fused epiphysis) and here also quite a few humps were detectable, although the major feature was the presence of numerous pits.

Thus the collective evidence suggests that in many animal species the surface of *young* articular cartilage is populated by humps, and pits do not occur. As animals mature a mixture of humps, humps in pits and pits are seen: in some species at least such formations are eventually totally replaced by pits. It seems likely that this change occurs more quickly and completely in some species than in others. The possibility that there may also be variations in this respect between the different joints of the same animal awaits investigation.

Finally, it is difficult to support the idea that pits are essential for joint lubrication by helping to trap and retain synovial fluid between the articulating surfaces when one finds that humps instead of pits are present on the surface of young articular cartilage; for surely there are no good reasons for believing that the joints of young or juvenile individuals are poorly lubricated and functionally inferior to those of adults!

SUMMARY

Scanning electron microscopic studies revealed numerous humps on the surface of articular cartilage from a 7 years old boy and from 1 month old rabbits. This shows that the surface topography of articular cartilage in young individuals is different from that of older individuals (human and rabbit) where the surface is beset by numerous pits. In 2 months old rabbits an intermediate situation was witnessed, for both shallow pits and occasional humps were present. Ridges and undulations were not seen on the articular surface of the 7 years old boy, except near the cut edge of the specimen. They were, however, found on air-dried specimens of 1 month old rabbit cartilage, but were absent from specimens dried by the critical-point method. The collective evidence supports the idea that ridges and undulations are not a constant feature of the normal articular surface, but that such features are artefactual, a typical or pathological.

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REFERENCES

Clarke, I. C. (1971*a*). Human articular surface contours and related surface depression frequency studies. *Annals of the Rheumatic Diseases* **30**, 15–23.

Clarke, I. C. (1971*b*). A method for the replication of articular cartilage surfaces suitable for the scanning electron microscope. *Journal of Microscopy* **93**, 67–71.

Clarke, L. C. (1971*c*). Surface characteristics of human articular cartilage – a scanning electron microscope study. *Journal of Anatomy* **108**, 23–30.

Clarke, I. C. (1973*a*). Correlation of S.E.M. replication and light microscopy studies of the bearing surfaces in human joints. *Scanning Electron Microscopy* (Part 3). *Proceedings of the Workshop on Scanning Electron Microscopy in Pathology* (ed. O. Johari and I. Corvin.), pp. 659–666. Chicago: Illinois Research Institute.

Clarke, I. C. (1973*b*). Quantitative measurement of human articular surface topography '*in vitro*' by profile recorder and stereomicroscopy techniques. *Journal of Microscopy* **97**, 309–314.

Gardner, D. L. (1972). The influence of microscopic technology on knowledge of cartilage surface structure. *Annals of the Rheumatic Diseases* **31**, 235–258.

Gardner, D. L. & Woodward, D. (1969). Scanning electron microscopy and replica studies of articular surfaces of guinea-pig synovial joints. *Annals of the Rheumatic Diseases* **28**, 379–391.

Ghadially, F. N., Ailsby, R. L. & Oryschak, A. F. (1974). Scanning electron microscopy of superficial defects in articular cartilage. *Annals of the Rheumatic Diseases* **44**, 327–332.

Ghadially, F. N., Ghadially, J. A., Oryschak, A. F. & Yong, N. K. (1976). Experimental production of ridges on rabbit articular cartilage: A scanning electron microscope study. *Journal of Anatomy* **121**, 119–132.

Ghadially, F. N., Ghadially, J. A., Oryschak, A. F. & Yong, N. K. (1977). The surface of dog articular cartilage: a scanning electron microscopic study. *Journal of Anatomy* **123**, 527–536.

Ghadially, J. A. & Ghadially, F. N. (1975). Evidence of cartilage flow in deep defects in articular cartilage. *Virchows Archiv. B Cell Pathology* **18**, 193–204.

Longmore, R. B. & Gardner, D. L. (1975). Development with age of human articular cartilage surface structure. A survey by interference microscopy of the lateral femoral condyle. *Annals of the Rheumatic Diseases* **34**, 26.

McCall, J. G. (1968). Scanning electron microscopy of articular surfaces. *Lancet* ii, 1194.

Meachim, G. (1972). Light microscopy of Indian ink preparations of fibrillated cartilage. *Annals of the Rheumatic Diseases* **31**, 457.

MEACHIM, G. & STOCKWELL, R. A. (1974). The matrix. In *Adult Articular Cartilage* (ed. M. A. R. Freeman). New York: Grune and Stratton.

MOW, VAN C., LAI, W. M. & REDLER, I. (1974). Some surface characteristics of articular cartilage. 1. A scanning electron microscopy study and a theoretical model for the dynamic interaction of synovial fluid and articular cartilage. *Journal of Biomechanics* **7**, 449–456.

REDLER, I. (1974). A scanning electron microscopic study of human normal and osteoarthritic articular cartilage. *Clinical Orthopaedics and Related Research* **103**, 262–268.

REDLER, I. & ZIMNY, M. L. (1970). Scanning electron microscopy of normal and abnormal articular cartilage and synovium. *Journal of Bone and Joint Surgery* **52**A, 1395–1404.

WALKER, P. S., SIKORSKI, J., DOWSON, D., LONGFIELD, M. D., WRIGHT, V. & BUCKLEY, T. (1969). Behaviour of synovial fluid on surfaces of articular cartilage. A scanning electron microscope study. *Annals of the Rheumatic Diseases* **28**, 1–14.