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Experimental production of ridges on rabbit articular cartilage: a scanning electron microscope study

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INTRODUCTION

Since the advent of scanning electron microscopy numerous studies on the surface topography of articular cartilage have been published (McCall, 1968; Gardner & Woodward, 1969; Walker *et al.* 1969; Redler & Zimny, 1970; Gardner, 1972; Mow, Lai & Redler, 1974). These studies lead one to believe that a series of undulations or ridges (called fibre bundles by some) are a normal and constant feature of the articular surface and that such formations play an important role in the functioning and lubrication of joints.

In contrast to this are the series of studies on human articular cartilage by Clarke (1971*a*, *b*, *c*, 1973*a*, *b*) who has failed to find such ridges, except near the fractured edge of some specimens. It should be noted that while Clarke reports on pieces of articular cartilage attached to subchondral bone, in some of the above mentioned studies the cartilage was detached from bone, while in others one is left guessing as to whether the cartilage was examined attached to bone, or was removed from its bony support prior to study. According to Clarke (1973*a*), the only surface irregularity constantly present and detectable with the scanning electron microscope is the occurrence of numerous shallow pits or depressions. These are thought to reflect the presence of underlying chondrocytes and their lacunae.

Thus from the existing literature on the subject one is led to suspect that ridges and undulations on the articular surface may be artefacts of tissue collection and preparation and not a true feature of the articular surface worthy of consideration when thinking about joint mechanics and lubrication. It seemed to us that this problem could be more fruitfully studied in the experimental animal than in man because of the difficulties in getting adequate supplies of fresh cartilage.

In this paper we report our observations on the surface topography of the normal rabbit articular cartilage attached to bone, and on the changes that occur when the cartilage is detached from bone, or its integrity is violated, by making cuts or holes in its substance.

MATERIALS AND METHODS

Experimental procedure

Twelve rabbits of both sexes, between 3 and 4 months old, were used in this experiment. The animals were killed by an intracardiac injection of Nembutal. The knee joints were opened and the lower ends of the femora were dissected out, taking care that the articular surface was not touched or damaged. After removing attached muscle and joint capsule, the specimen, which now comprised femoral condyles and about 2 cm of attached shaft, was rinsed in four changes of normal saline. The specimen was now held in a vice and cuts and holes were made on the femoral condyles as described later. At no stage of these procedures were the femoral condyles allowed to become dry. This was ensured by an assistant whose sole task was to drop normal saline on to the articular surfaces as and when it seemed necessary.

Our experimental design provided a total of 48 femoral condyles for study. One of the condyles from each joint (24 in all) was reserved to serve as a control, depicting the appearance of normal articular cartilage. The remaining 24 condyles were treated as follows:

Longitudinal and transverse cuts

On the articular surface of 4 condyles, a longitudinal cut (sagittal plane) was made with the aid of a scalpel while on 4 others a transverse cut (coronal plane) was made. Each condyle bore one cut only and it extended along the entire length or breadth of the condyle respectively. Later studies showed that the depth of the cut varied somewhat but in most instances this was a full thickness cut which had penetrated the calcified zone and/or the subchondral bony plate, at least in some portion of its course (usually the middle).

Cruciate cuts

On the articular surface of 4 condyles a cruciate incision was made. This is equivalent to making a longitudinal and transverse incision on the same condyle in the manner described above. The two incisions were at right angles to one another, the long arm of the cross lay along the sagittal plane.

Holes

On the articular surface of 4 condyles a hole approximately 2 mm in diameter was made with the aid of a punch and reamer, as described previously in our studies on the healing of deep defects (core defect) in articular cartilage (Ghadially, Fuller & Kirkaldy-Willis, 1971). This kind of defect crosses subchondral bone and extends into the marrow spaces.

Longitudinal and transverse pieces

From the articular surface of 8 condyles a roughly rectangular piece of cartilage measuring approximately 5–8 mm long, 3–4 mm wide and less than 1 mm thick (zones I, II and III) was removed with the aid of a scalpel. Such pieces of cartilage demonstrated a strong tendency to curl, with the articular surface forming the concave face. They were therefore pinned out (4 fine pins, 1 in each corner) on a piece of cork to hold them as flat as possible. The term 'longitudinal piece' will be used to designate those pieces of cartilage (4 in number) where the long axis of the piece corresponds to the sagittal plane of the joint and similarly the term 'transverse piece' will indicate those (4 in number) where the long axis of the piece lay along the coronal plane.

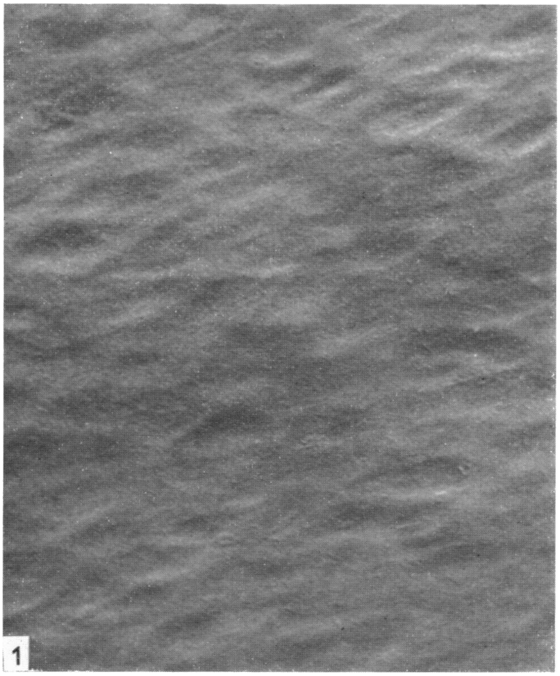

Fig. 1. Surface of control condyle showing the pitted appearance characteristic of normal articular cartilage, viewed with the scanning electron microscope. ×400.

Processing of tissues

The lower ends of the femora, treated as indicated above, were cleansed once more by squirting normal saline on them from a wash bottle. They were then fixed in 3% glutaraldehyde in cacodylate buffer (pH 7·3) for 1–2 weeks and dehydrated in increasing concentrations of ethanol over the course of another week. The major portion of each femoral condyle was then dissected off with a fine fret saw, rinsed in absolute alcohol, and mounted on specimen stubs with 'Electrodag'. Specimens were vacuum coated with gold in a sputtering device and examined in a Cambridge 'Stereoscan' electron microscope.

RESULTS

Normal articular cartilage

Every control condyle examined with the scanning electron microscope revealed the presence of numerous shallow pits on the surface of articular cartilage (Fig. 1). This gave the surface a beaten-copper or golf ball-like appearance. No ridges or undulations were seen with the scanning electron microscope. It is, however, worth mentioning that, on naked eye examination, the articular cartilage on a few freshly collected specimens showed a slightly wavy surface (two or three 'waves' over the entire condylar surface). This could be just discerned when the condyles were viewed tangential to the articular surface.

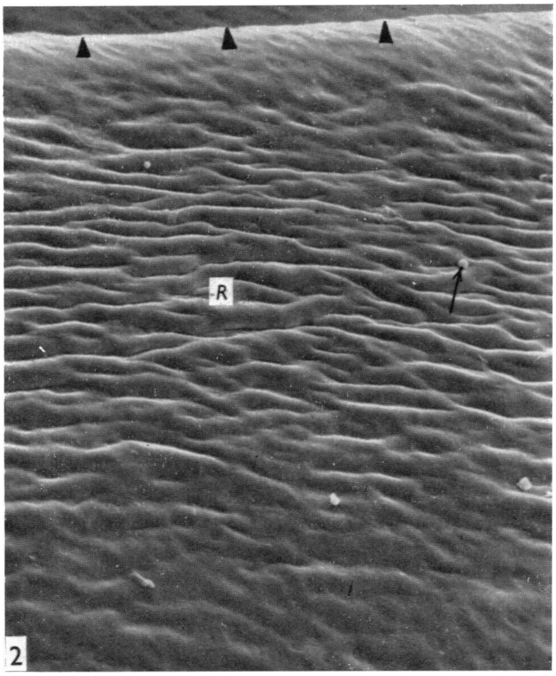

Fig. 2. Cartilage near the edge (arrowheads) of a longitudinal cut showing parallel ridges (*R*) that have developed on the articular surface. The small objects (arrow) are erythrocytes which contaminated the surface during specimen collection. × 150.

Longitudinal and transverse cuts

The changes in surface morphology that occurred were similar in both type of cuts, except that the longitudinal incision tended to gape more and the changes adjacent to it were more marked (Fig. 2). The cartilage immediately adjacent to the cut was at times slightly curled upwards or downwards, and its surface was beset by pits, although occasionally a few mound-like or dome-like elevations (referred to henceforth as 'humps') instead of pits were seen (Fig. 3). Adjacent to this area there was a zone where the cartilage had developed ridges or undulations oriented parallel to the cut edge (Fig. 2). Adjacent to the zone with ridges the articular cartilage had a somewhat irregular appearance but here again, while the pits predominated, occasional humps were present. Well away from these zones, the cartilage appeared normal, that is to say no surface irregularity was seen save the ubiquitous pits characteristic of cartilage viewed with the scanning electron microscope.

It should be noted that the above is a general description of the phenomena observed. There were also some variations. For example, the ridges were not detectable all along the cut edge nor were they equally well developed in every specimen. The humps were of quite infrequent occurrence and could not be found in every specimen.

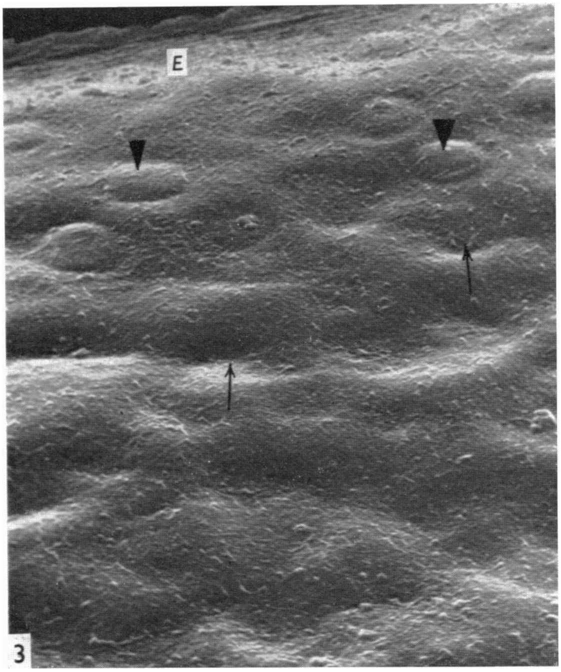

Fig. 3. Cartilage near the edge (E) of a longitudinal cut showing humps (arrowheads) and pits (arrows). The flocculent material on the surface probably derives from synovial fluid which was not washed off completely. $\times$ 750.

Cruciate cuts

The gaping cruciate cut gave the cartilage a 'hot-cross bun' appearance and divided the cartilage into what may be loosely referred to as four quadrants (Fig. 4). Here the ridge patterns were quite complex. In some quadrants the ridges that developed were more or less parallel to one cut edge and almost at right angles to another while in other instances ridges parallel to each edge were seen. However, perhaps the most intriguing pattern of ridges, seen in quite a few quadrants, is depicted in Figure 5. Here large or coarse oblique ridges surmounted by medium sized and fine irregular ridges are to be seen.

Holes

Just as in the case of linear and cruciate cuts made in articular cartilage, the cartilage at the margin of the hole (Fig. 6) may curl and pout above the surface in some segments of the edge of the defect, while in others it may lie flat or, on rare occasions, it may even curl slightly into the hole. The alterations in the surface morphology of adjacent cartilage were also similar. These comprised the formation of large or coarse ridges (Fig. 7) and fine ridges (Figs. 8, 9) which were usually oriented approximately parallel to the edge. However, in no instance were the ridges truly concentric in the sense that they extended along the entire circumference of the hole. The ridges were prominent in some places, but barely discernible, or absent in others, and in

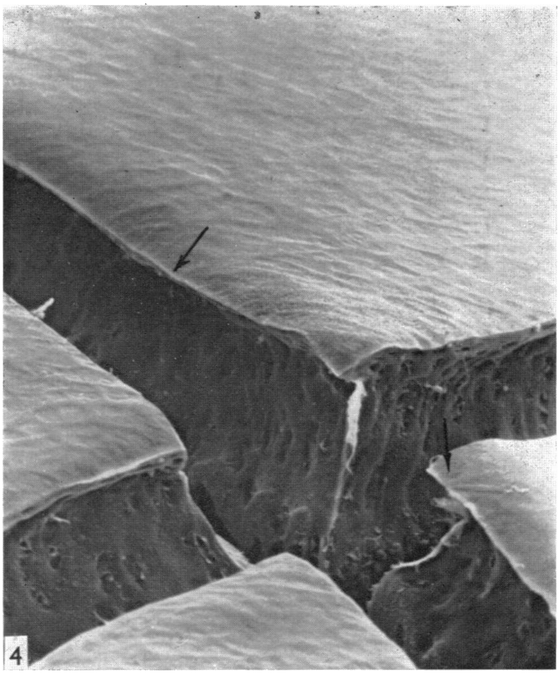

Fig. 4. A gaping cruciate cut. Note how the corners and cut edges curl (arrows) near the edge of the defect. × 140.

some segments an entirely different arrangement of ridges was seen. Here large ridges radiating from the margin of the hole were intersected by fine ridges and furrows (Fig. 10). Once again a few humps were seen in the altered cartilage adjacent to the hole (Fig. 11).

Longitudinal and transverse pieces

The patterns of ridges that developed in the longitudinal and transverse pieces of cartilage excised from the condylar surface were essentially similar. A main system of parallel ridges had developed along the long axis of the specimen (Figs. 12, 13). This central system of ridges was flanked by irregular ridges and furrows of varying orientation. Pinning the cartilage did not prevent a slight lifting and curling of the long edge of the specimen. Near such zones of curling a further set of fine ridges had developed. No humps were detected in any of the excised pieces of cartilage, but deep pits were present on some of the ridges (Fig. 13).

DISCUSSION

Pits

The results of our experiment show that the only type of surface irregularity constantly found on the articular surface of the normal rabbit femoral condyle is the occurrence of numerous shallow pits or depressions. This is in keeping with the

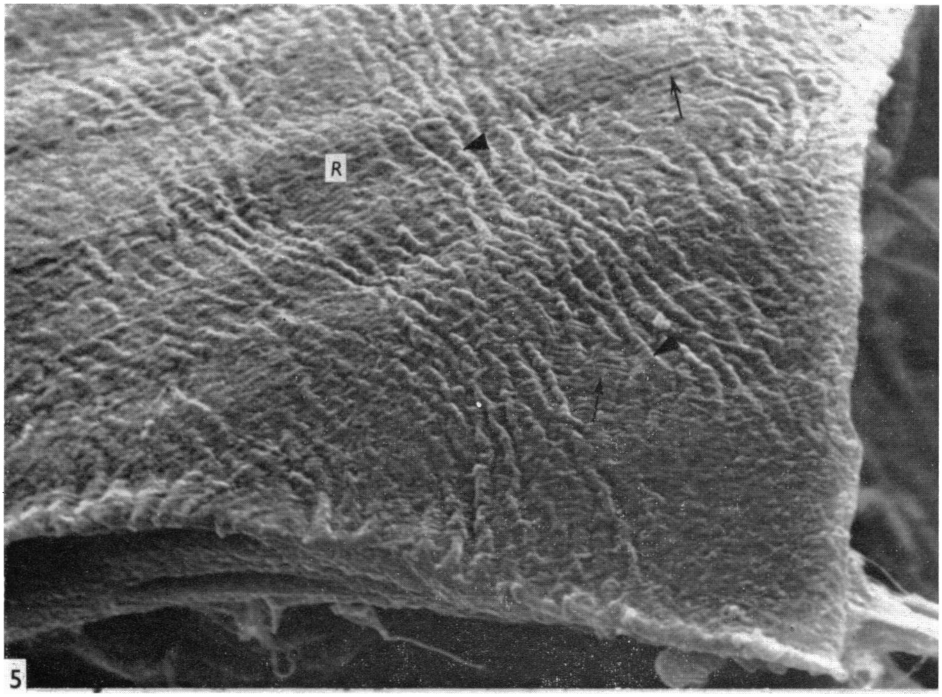

Fig. 5. High power view from a quadrant of cartilage shown in Fig. 4. Note large or coarse ridges (*R*), surmounted by medium sized (arrowheads) and fine ridges (arrows). × 1200.

results of Clarke (1973*a*) on human cartilage. Briefly, our view, which concurs with his, is that such depressions are due to underlying chondrocytes and their lacunae. Whether such depressions or pits occur *in vivo* is debatable (see discussion in Clarke, 1973*a*). It is conceivable that very shallow pits may occur *in vivo* and that their size is enlarged and exaggerated by the techniques employed in specimen preparation, or else that no such pits occur *in vivo* and that they are entirely artefacts of tissue preparation. Whatever may be the true state of affairs, it is not difficult to visualize how the method of specimen preparation would lead to a shrinkage, or even a total collapse, of many of the superficially placed lacunae, and that the surface will then 'cave in' or collapse at such sites to produce the pitted appearance so characteristic of articular cartilage viewed with the scanning electron microscope.

Ridges

Besides the specimens of normal intact rabbit cartilage that we examined with the scanning electron microscope during the course of this study, we have examined well over a hundred other specimens of rabbit cartilage during our past studies (Ghadially, Ailsby & Oryschak, 1974; Ghadially & Oryschak, 1975) and a few examples of human cartilage also. This collective experience now leads us to conclude that surface irregularities, variously referred to as ridges, undulations, furrows and fibre bundles, seen with the scanning electron microscope are not a characteristic

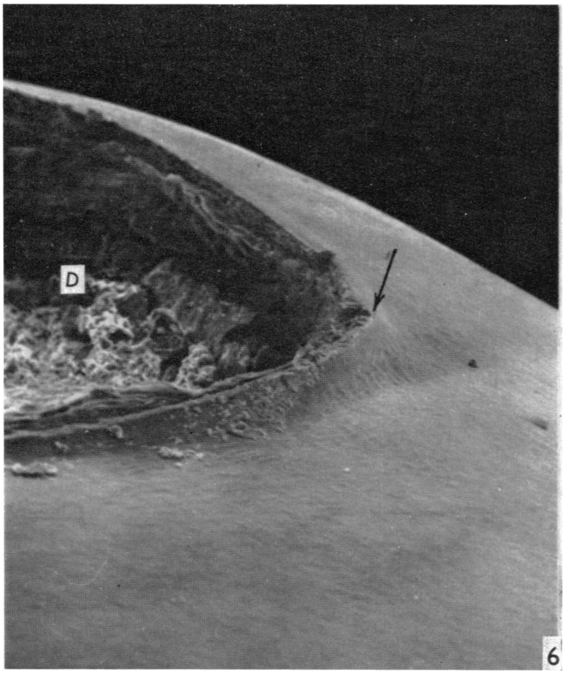

Fig. 6. Core defect (*D*) showing a segment of the edge where the cartilage stands up (arrow) above the level of the articular surface. × 70.

or constant feature of the normal articular surface as has been claimed by some authors (McCall, 1968; Gardner & Woodward, 1969; Walker *et al.* 1969; Redler & Zimny, 1970; Gardner, 1972; Mow *et al.* 1974).

However, such ridges can be readily created by producing defects in cartilage, or by detaching cartilage from its bony support. This is in keeping with the observations of Clarke (1971 *c*, 1973 *a*), who has also failed to find ridges on the surface of normal intact human cartilage, but has observed parallel ridges adjacent to the fractured edge of the specimen. Our studies, however, show that the situation is much more complex and that the ridges are not always parallel to the cut edge.

Recently Mow *et al.* (1974) reported on the occurrence of systems of ridges oriented obliquely or perpendicular to the cut or fractured edge of the specimen. They believe that such ridges must be distinguished from the artefactually produced ridges running parallel to the fractured edge as reported by Clarke (1971 *c*). However, it seems to us that such distinctions are not valid, for our present study shows that ridges of varying dimensions and virtually every conceivable orientation can be produced experimentally where none existed before. Further, since no clear statement is made by Mow *et al.* (1974) as to whether cartilage attached to bone was studied or not, we remain unconvinced that these ridges were not artefactually produced, or at best represent an atypical or pathological situation. Such suspicions are further supported by the illustrations in their paper, one of which shows copious amounts of unidenti-

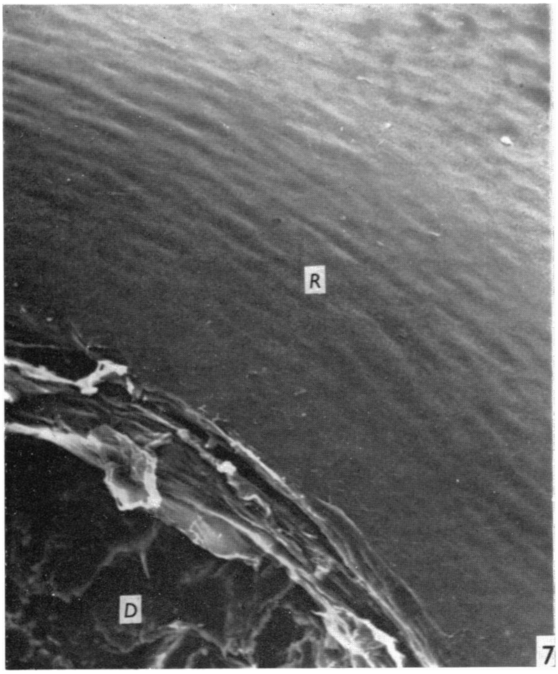

Fig. 7. Cartilage near edge of a core defect (*D*). Coarse ridges (*R*) have developed a little distance from the edge. × 140.

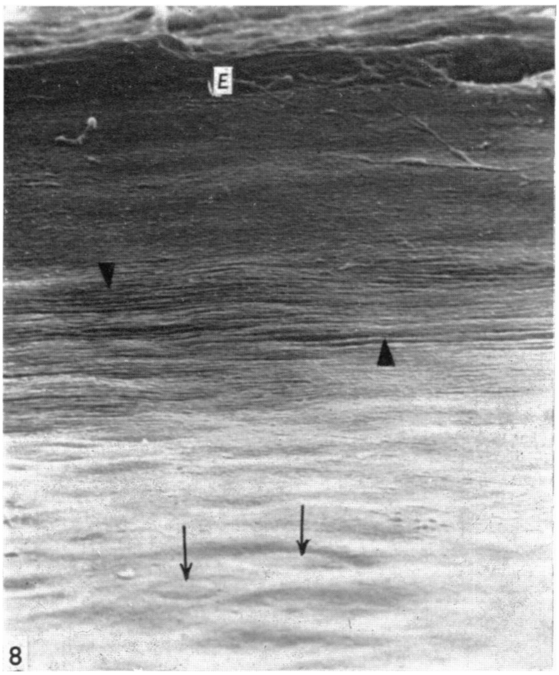

Fig. 8. Cartilage near edge (*E*) of a core defect showing fine ridges (arrowheads) and poorly developed humps in the floor of pits (arrows). × 680.

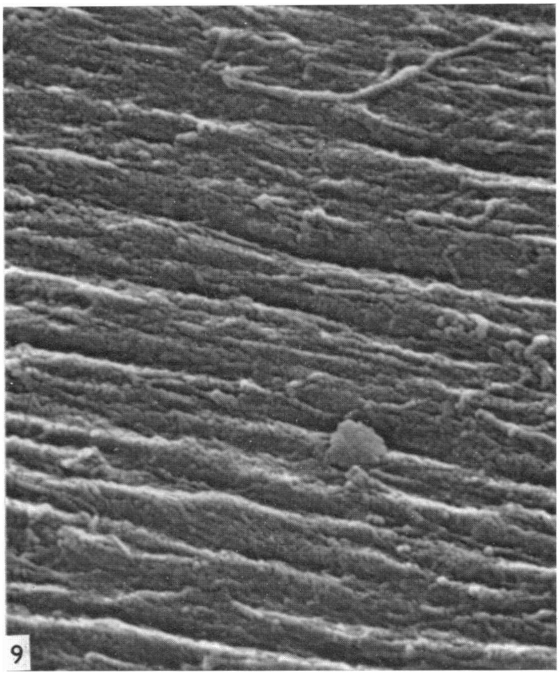

Fig. 9. High power view of fine ridges shown in Fig. 8. Note the rough fibrous texture of these ridges. ×4900.

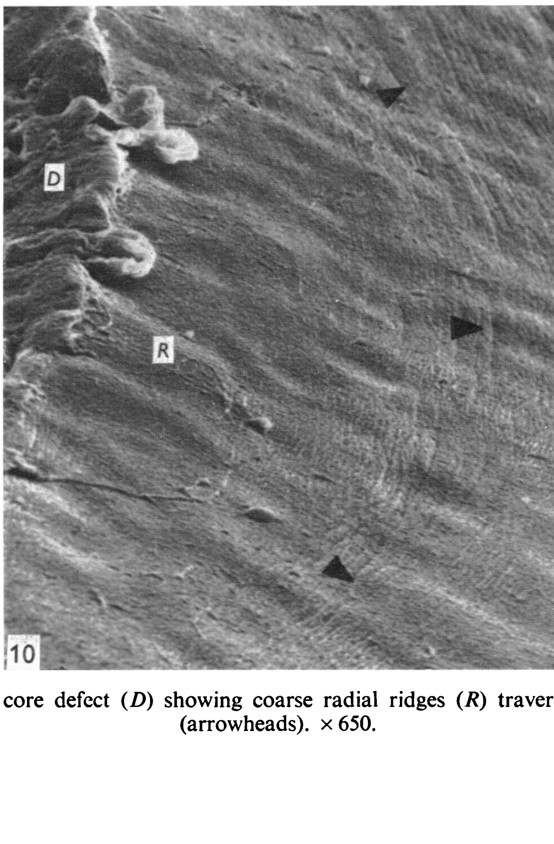

Fig. 10. Edge of core defect (*D*) showing coarse radial ridges (*R*) traversed by fine ridges (arrowheads). ×650.

Fig. 11. High power view of humps lying in a zone of fine ridges adjacent to a core defect. × 1500.

fiable amorphous debris on the surface, while others show tags and fine thread-like formations arising from the surface.

In an attempt to show ridges on the surface of 'fresh cartilage' Gardner (1972) examined 'unfixed, undried, uncoated cartilage' in the scanning electron microscope. By reducing the accelerating voltage it is possible to image unfixed, uncoated biological specimens, but how one can look upon a specimen which has been subjected to the high vacuum conditions that prevail in the scanning electron microscope as 'undried' is beyond our comprehension. It seems to us that this is just about the worst way of drying a specimen, and is calculated to produce severe disruption and distortion of the surface.

In Figure 16 of his paper Gardner (1972) has succeeded in demonstrating a system of ridges on the surface of the cartilage, but they clearly are adjacent to a zone where the cartilage has been damaged, presumably by drying and/or the electron beam. We have noted a similar phenomenon in the scanning electron microscope. At one time we used to fix cartilage for 2–3 days instead of the 1–2 week period we now employ. Such inadequately fixed cartilage often develops cracks, fissures and associated ridges while one is watching it in the scanning electron microscope. Thus, this experiment by Gardner (1972) on 'fresh cartilage', far from showing ridges on normal cartilage, demonstrates that no matter how cartilage is damaged it is likely to develop ridges. The collective evidence now suggests that ridges and undulations seen on the articular surface with the scanning electron microscope do not reflect a normal state of affairs, but that such formations are either artefactual, atypical or

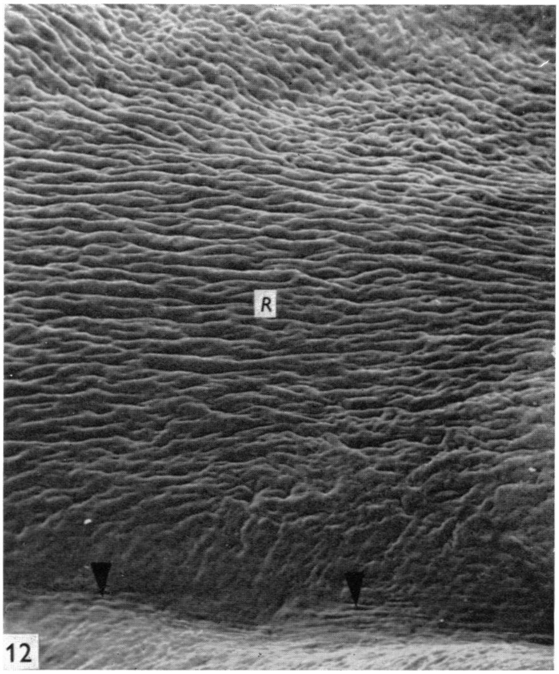

Fig. 12. Transverse piece of cartilage showing central zone of parallel ridges (*R*) flanked by irregularly deployed ridges. The lower, long edge of the specimen (cut edge not seen here) curled upwards, producing fine ridges (arrowheads). × 65.

pathological, and that they should therefore not be taken into consideration when thinking about joint mechanics and lubrication.

Humps

This term is used to describe round or oval raised areas on the articular surface, often with a circular moat around them. Some humps have a rounded top and are hence dome-like, while others have a rather flat top and are hence more like a raised plateau. Redler (1974) has described such humps, in normal human articular cartilage, which he states are 'especially common in specimens removed from children and adolescents'. He believes that this is a normal feature of the articular surface and that these humps presumably represent chondrocytes lying in shallow lacunae. Our view is that most, perhaps all of them are superficially placed lacunae and chondrocytes, or chondrocytes raised above the surface; for their size and shape is similar to the pits, and at times small humps may be seen in the floor of pits (Fig. 8).

The question that now arises is whether humps occur *in vivo* or whether they are yet another artefact of specimen preparation. The fact that we found humps adjacent to damaged cartilage, but not in intact cartilage does suggest that, at least in some cases, the humps may be artefactually produced. However, our results on this point are not too conclusive for humps were not seen adjacent to every defect in every specimen. Further, one may argue that even if some humps are artefacts of tissue

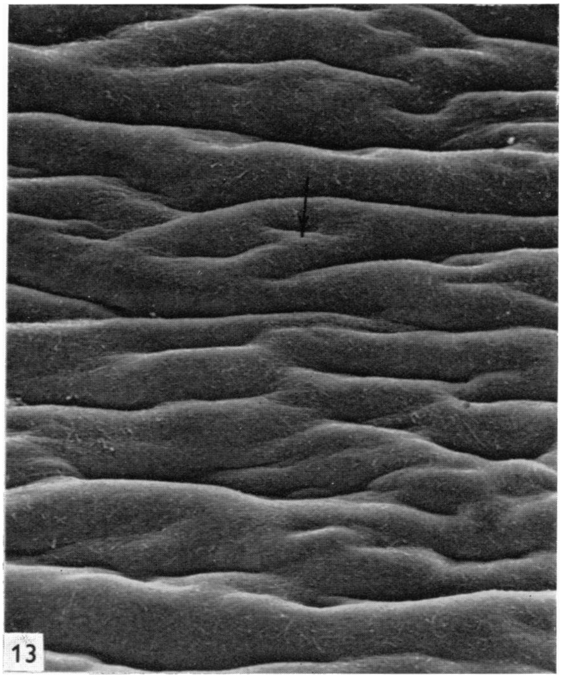

Fig. 13. High power view of central ridges shown in Fig. 12. Note deep pits (arrow) on some of the ridges. × 325.

preparation, this does not preclude the possibility that in some instances they may be an *in vivo* phenomenon.

There is some experimental evidence which suggests that chondrocytes expand in volume during exercise and shrink during rest (Ekholm & Norback, 1951). Hence Clarke (1973*a*) has suggested that during life such changes may be reflected as humps or depressions on the surface and that at an intermediate state the surface may be quite smooth. Clearly, further experiments are needed to elucidate the nature of these humps and how they are produced. Certainly humps were not found by us to be a normal feature of rabbit articular cartilage examined with the scanning electron microscope, nor have we, during the course of our past light and transmission electron microscopic studies (Ghadially & Roy, 1969), seen chondrocytes raised above the articular surface, in either rabbit or human cartilage.

SUMMARY

The surface of normal rabbit articular cartilage attached to subchondral bone has a pitted appearance when examined with the scanning electron microscope. It is thought that these pits are similar to those seen in human cartilage and that they reflect the presence of underlying chondrocytes and lacunae, shrunken by preparative procedures. Ridges or undulations were not seen on the normal articular surface with the scanning electron microscope but complex systems of coarse and fine ridges were produced when cartilage was damaged by cutting, by making a hole in it or by

detaching it from subchondral bone. Humps or mound-like elevations also developed in some instances when cartilage was damaged by cutting or by making a hole in its substance.

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