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A maturation change detected in the semilunar cartilages with the scanning electron microscope

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INTRODUCTION

It is now generally accepted that the surface of adult human and animal articular cartilage attached to subchondral bone shows innumerable shallow depressions called 'pits', while such cartilage from juveniles and young animals shows mound-like elevations called 'humps' (Clarke, 1973; Ghadially *et al.* 1976, 1977*b*, 1978). This is thought to be a maturation change reflecting the abundance and relatively superficial position of the chondrocytes in young as compared with mature cartilage.

Some authors have claimed that the surface of articular cartilage is beset by a series of undulations and ridges which play an important role in joint lubrication by trapping synovial fluid between the articular surfaces (McCall, 1968; Gardner & Woodward, 1969; Walker *et al.* 1969; Redler & Zimny, 1970; Gardner, 1972; Mow, Lai & Redler, 1974; Redler, 1974), but in a series of experiments we (Ghadially *et al.* 1976, 1977*a*) have shown that ridges and undulations are not of normal occurrence but are the result of curling, shrinkage and distortion when cartilage is detached from bone or injured in various ways (e.g. when punching out a hole or making linear or cruciate cuts).

Numerous studies have been made of the surface of articular cartilage, but only one study of the surface of human semilunar cartilages has been published (Refiori, 1971). This author observed ridges on the surface, and also some humps in the case of younger individuals. Since semilunar cartilages are not firmly attached to bone the occurrence of ridges is easily understood, but why pits should be absent is not so apparent.

It seemed therefore worthwhile to study the problem in greater detail in various species. We have now studied the surface of young and mature, rabbit and human, semilunar cartilages and also looked at the semilunar cartilages of a cat and a discoid meniscus from the knee of a monkey. The results of these studies are reported in this paper.

MATERIALS AND METHODS

Animal semilunar cartilages

Animals were killed by an intracardiac injection of Nembutal and the medial and lateral semilunar cartilage from both knees of the following species were collected: (1) two 1 month old rabbits; (2) two 2 months old rabbits; (3) two 6 months old rabbits; (4) three 1 year old rabbits; (5) three 2 years old rabbits; (6) one 6 months old cat and (7) a discoid left lateral meniscus from the knee of a monkey (*Macaca fascicularis*).

Human semilunar cartilages

The left medial semilunar cartilage was collected under the following circumstances; (1) at autopsy performed 24 hours after death on a 6 days old male infant, (2) at autopsy performed 17 hours after death on a 12 years old boy and (3) at autopsy performed 5 hours after death on a 17 years old boy. Besides these we collected (1) a left lateral semilunar cartilage during menisectomy in a 26 years old man and (2) the medial and lateral semilunar cartilages from the amputated leg of a 41 years old man.

Preparation of tissues

Preliminary studies showed that when the small semilunar cartilages of animals are processed detached from the tibial plateau they suffer much shrinkage and distortion. Often they are so markedly shrunken and curled that it is either impossible to mount them flat on a specimen stub, or else when mounted they curled overnight and detached themselves from the stub. Therefore the semilunar cartilages were studied attached to the tibial plateau. In the case of the small specimens from the young rabbits the entire plateau bearing both semilunar cartilages was studied intact. In the case of the larger specimens the plateau was cut into two halves, one bearing the medial semilunar cartilage, the other bearing the lateral semilunar cartilage.

The impossibility of accommodating within the microscope the much larger human semilunar cartilages accompanied by the tibial plateau forced us to process and examine only the middle portion of the semilunar cartilage detached from the tibial plateau.

Except for the one and two months old rabbits, all specimens were prepared by the air-drying method described below. In the case of the former, half the specimens were air-dried and the other half prepared by the critical point-drying method. The manner of preparation of tissues was similar to that used by us previously to prepare articular cartilage for scanning electron microscopy (Ghadially *et al.* 1976, 1977*a,b*, 1978). Briefly this consists of (1) washing the surface with saline to remove synovial fluid and any blood that might have contaminated the surface; (2) fixation in 3 % glutaraldehyde in cacodylate buffer (pH 7·3) for 1 week in the case of small specimens and 2 weeks in the case of large ones; (3) dehydration in increasing concentrations of alcohol; followed by (4) mounting on stubs and coating with gold prior to examination in the Cambridge Stereoscan electron microscope.

RESULTS

The features described below refer to the superior surface of the semilunar cartilage. There were no noteworthy differences between the semilunar cartilages from the left and the right knee or between the medial and lateral semilunar cartilages.

Rabbit semilunar cartilage

One and 2 months old specimens

Both naked eye examination and inspection with a × 7 magnifying glass showed the surface of fresh specimens to be remarkably smooth and glistening. On the other hand, scanning electron microscopy showed (Figs. 1–4) ridges and furrows which were more marked at the outer and inner margins of the semilunar cartilages. The

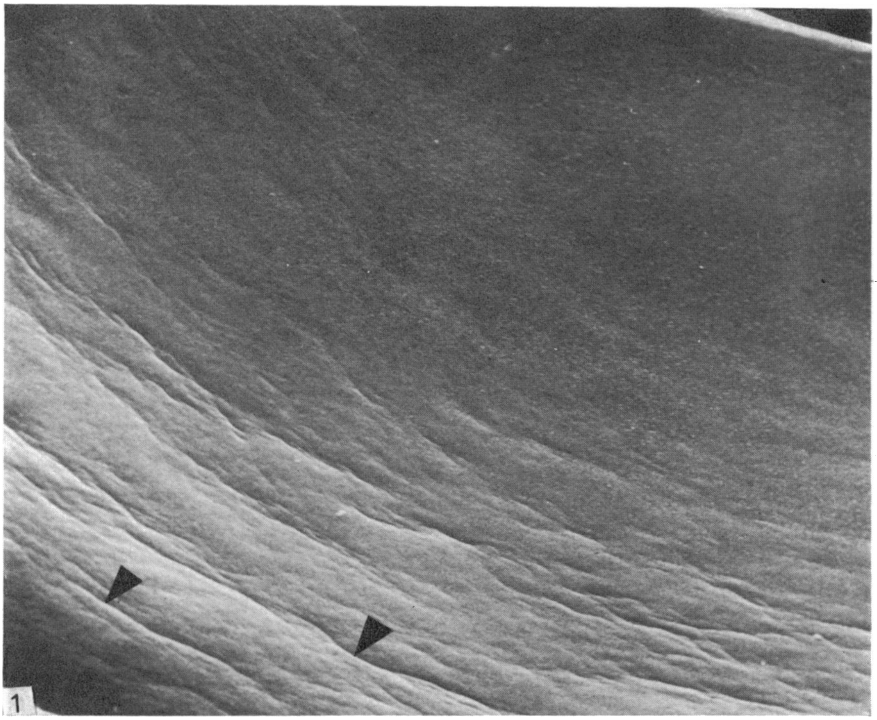

Fig. 1. Critical point-dried left medial semilunar cartilage from a 1 month old rabbit. Note undulations and furrows (arrowheads) mainly near the outer margin. × 64.

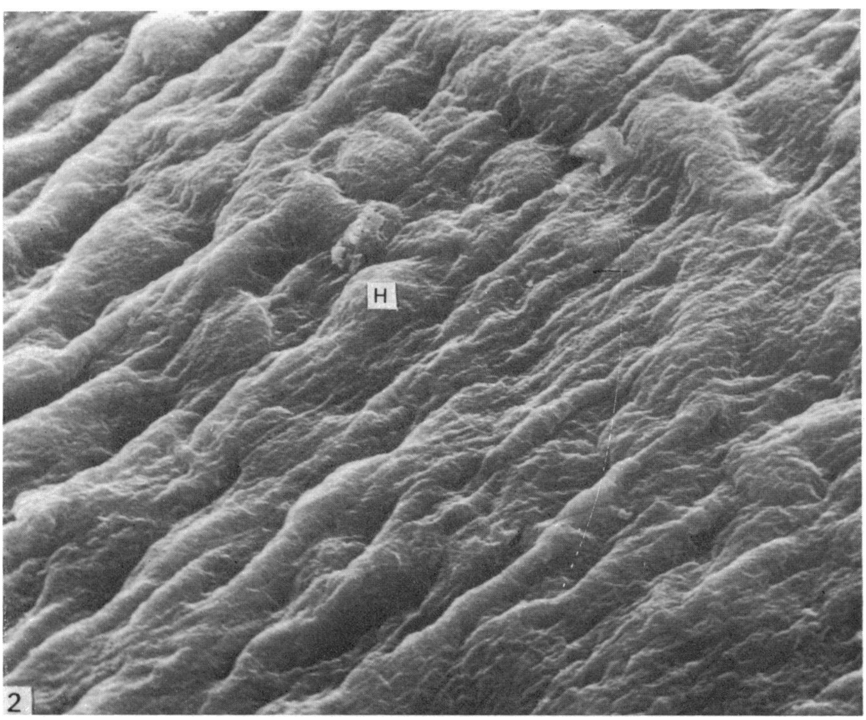

Fig. 2. Air-dried right lateral semilunar cartilage from a 1 month old rabbit showing undulations, ridges and humps (*H*). × 1400.

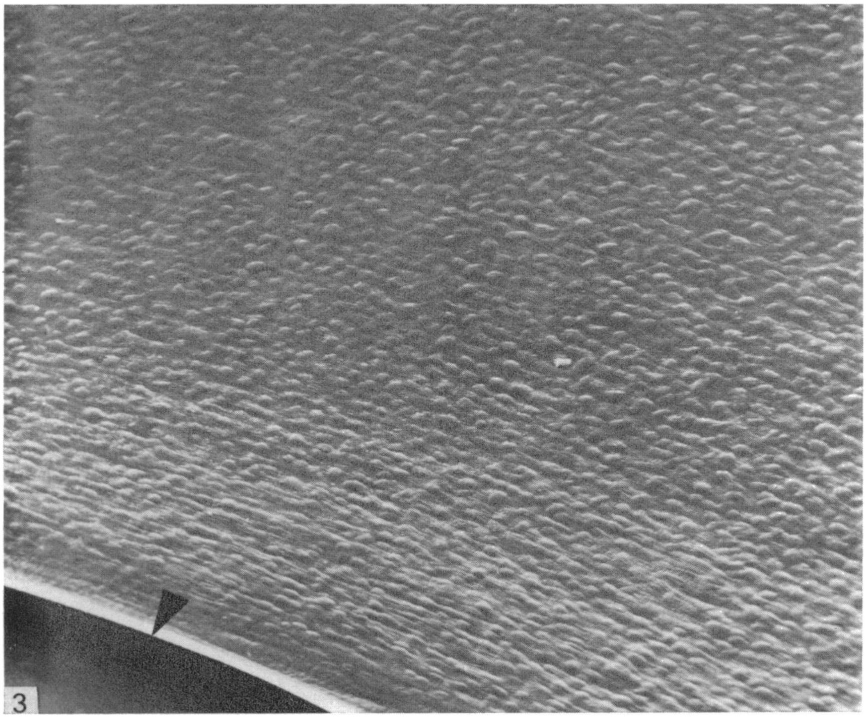

Fig. 3. Air-dried right medial semilunar cartilage from a 1 month old rabbit. Note the presence of innumerable humps and ridges parallel to the inner margin (arrowhead). × 210.

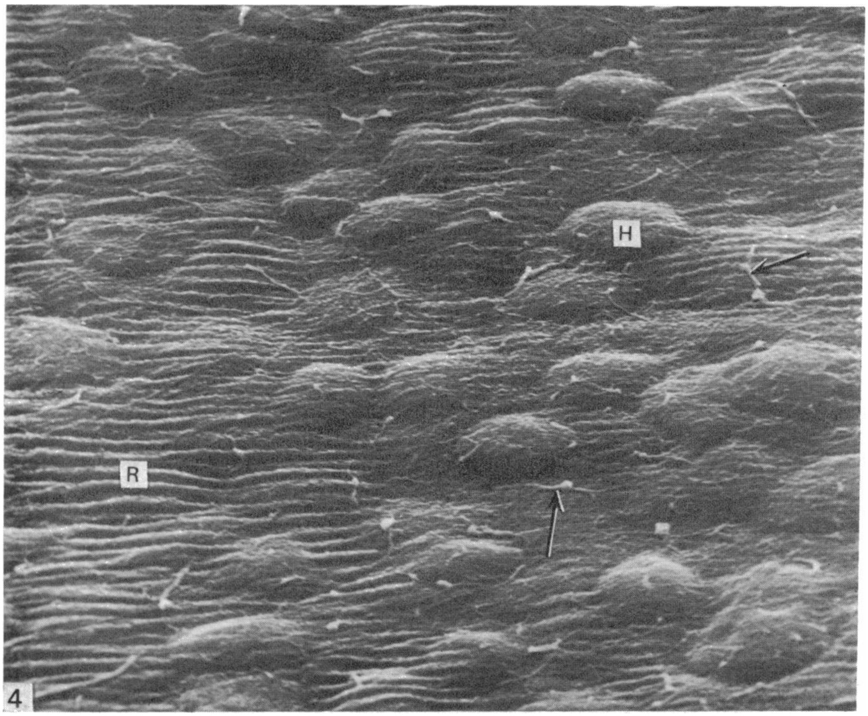

Fig. 4. Air-dried right lateral semilunar cartilage from a 1 month old rabbit showing humps (*H*), ridges (*R*) and precipitated synovial fluid (arrows). × 13500.

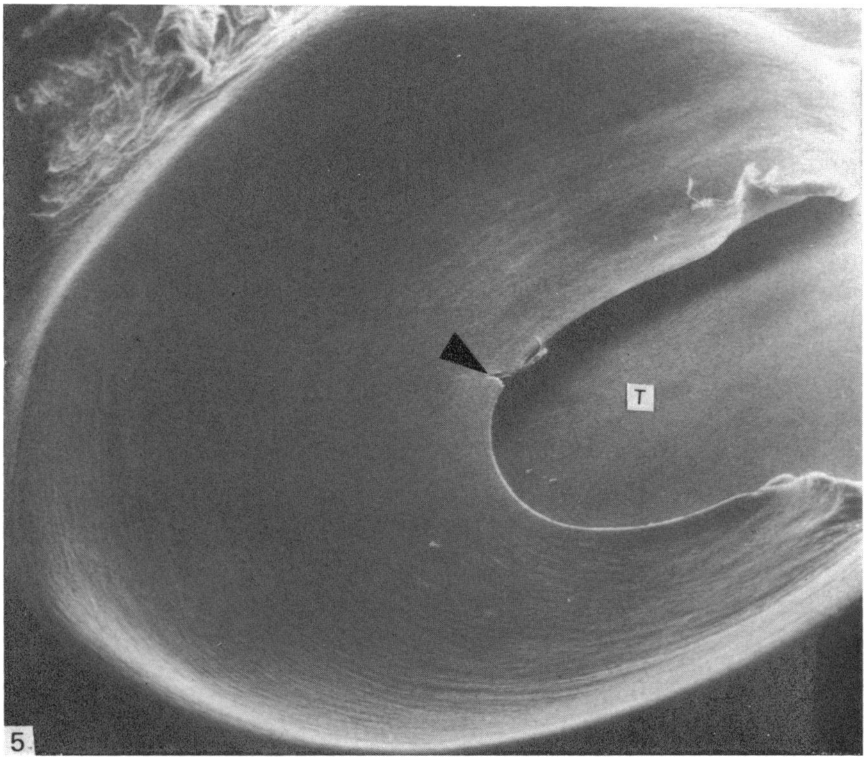

Fig. 5. Air-dried left lateral semilunar cartilage from a 6 months old rabbit showing fine ridges on the surface. A cut (arrowhead) accidentally made during specimen collection is evident. Except for the presence of one slight depression, the tibial plateau (*T*) has a remarkably smooth surface. × 23.

main feature, however, was the occurrence of numerous humps on the surface. Such humps were more pronounced and more numerous in the 1 month specimens than in the 2 months specimens, and in the air-dried specimens than in the critical point-dried specimens.

Six months specimens

Naked eye and ×7 magnifying glass inspection of fresh specimens showed the surface to be remarkably smooth and glistening, but with the scanning electron microscope numerous ridges and undulations were seen. Besides this the surface was covered by 'humps in pits' and a few simple pits (Figs 5, 6).

One and 2 years old specimens

Naked eye and ×7 magnifying glass inspection of fresh specimens revealed an essentially smooth glistening surface, save for the presence of an occasional fine ridge or furrow near the outer margin. The inner margin of the semilunar cartilage was at times somewhat irregular or frayed. Scanning electron microscopy showed (Figs. 7–10) numerous fine ridges and undulations which had a circumferential orientation, A few shallow cracks or fissures were seen in two 2 years old specimens, while another showed marked fraying of the inner edge of the semilunar cartilage. The ubiquitous feature, however, was the occurrence of numerous pits on the surface of all specimens.

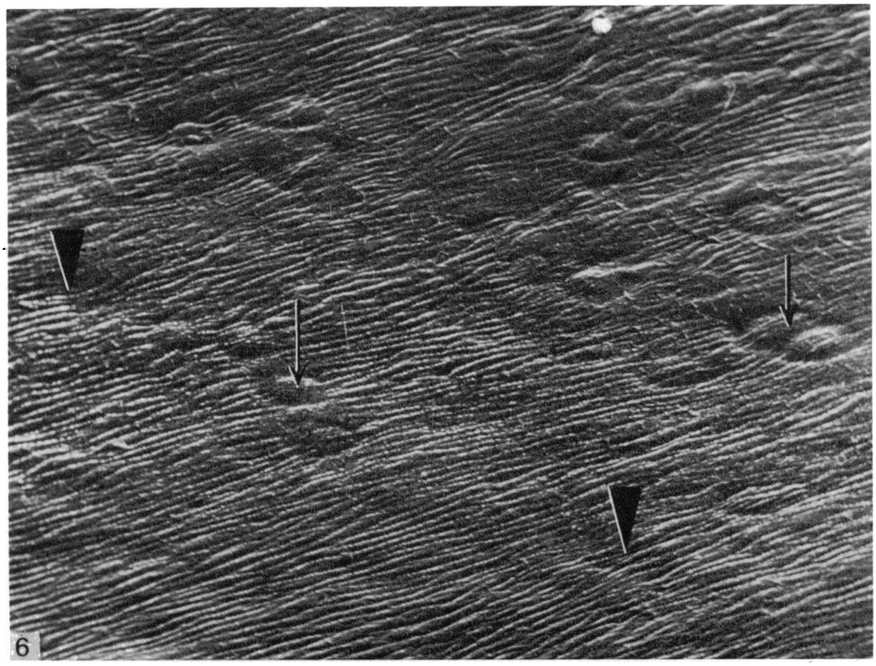

Fig. 6. Air-dried right medial semilunar cartilage from a 6 months old rabbit. Note the presence of 'humps in pits' (arrows), pits (arrowheads) and innumerable fine ridges. × 675.

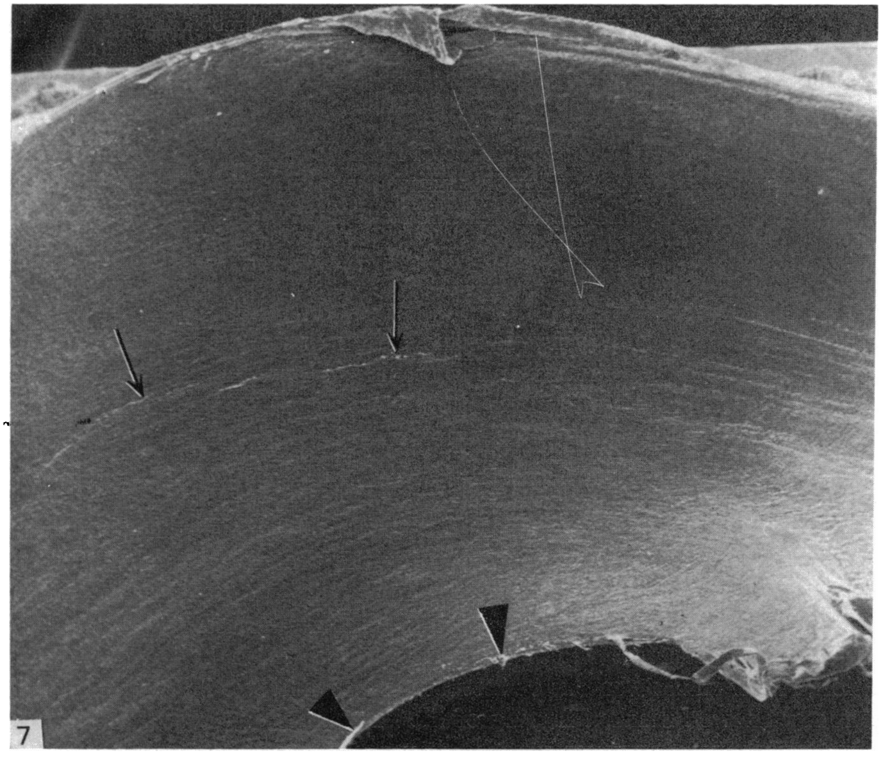

Fig. 7. Air-dried left lateral semilunar cartilage from a 2 years old rabbit. Note the ridges on the surface, a few fissures (arrows) and the slightly frayed (arrowheads) inner margin. × 27.

Fig. 8. High power view of a superficial fissure (arrowheads) shown in Fig. 7. Ridges and pits (arrow) are not clearly demonstrated at this low tilt angle, but they may just be discerned. × 260.

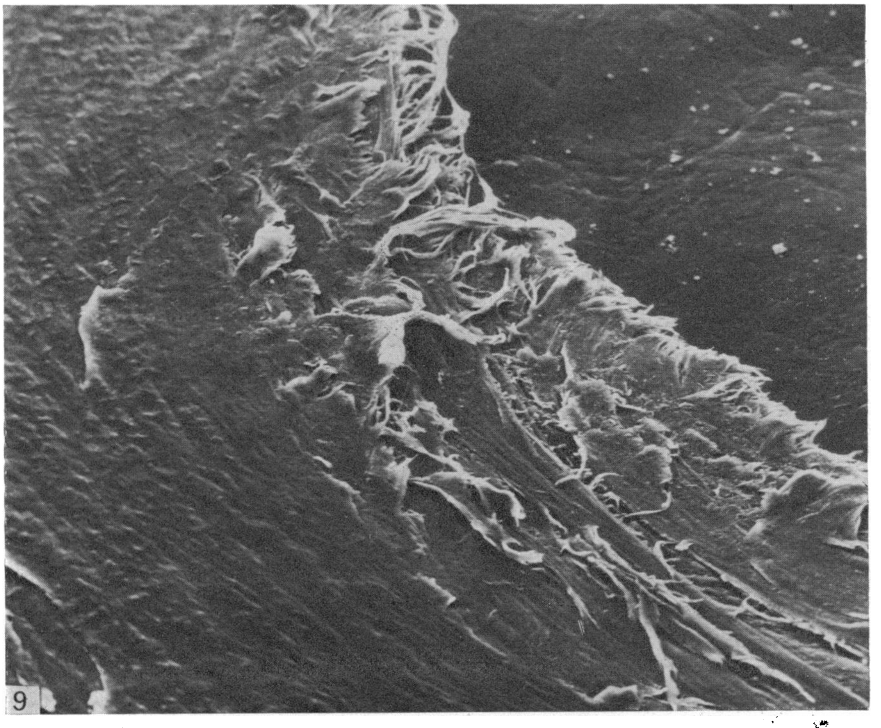

Fig. 9. Air-dried left medial semilunar cartilage from a 2 years old rabbit showing a markedly frayed inner margin. × 85.

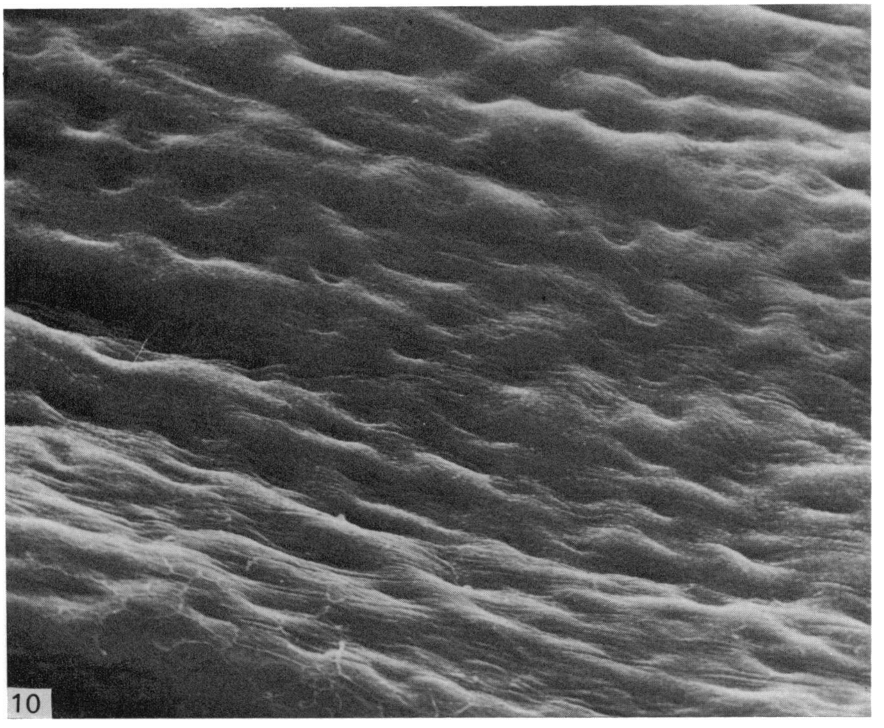

Fig. 10. Same specimen as shown in Figs. 7 and 8. By increasing the tilt angle one can now see innumerable pits set amongst the ridges and undulations. × 590.

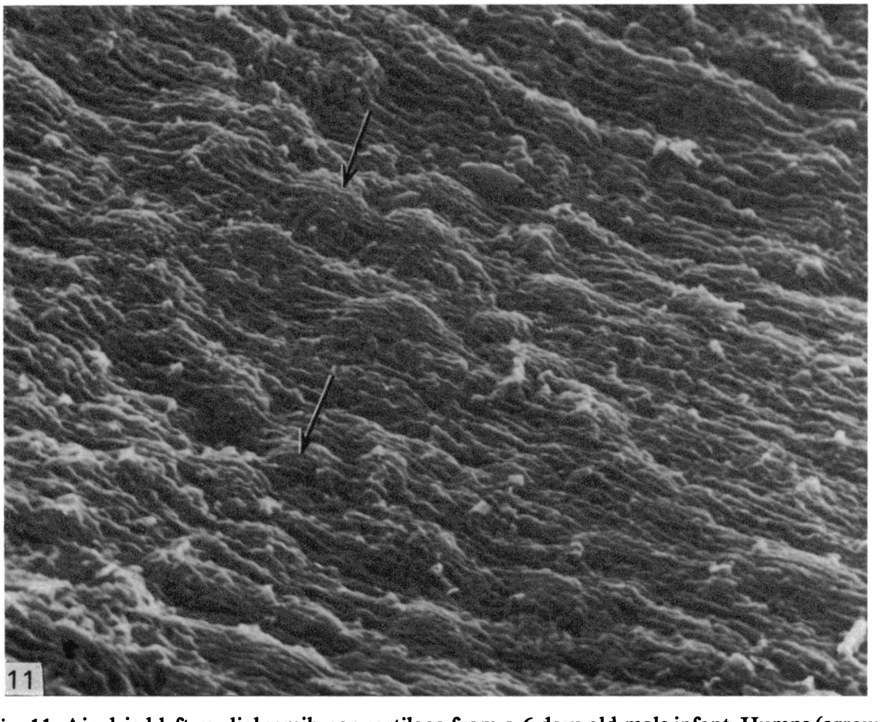

Fig. 11. Air-dried left medial semilunar cartilage from a 6 days old male infant. Humps (arrows) are clearly discerned amongst the ridges and undulations. × 105.

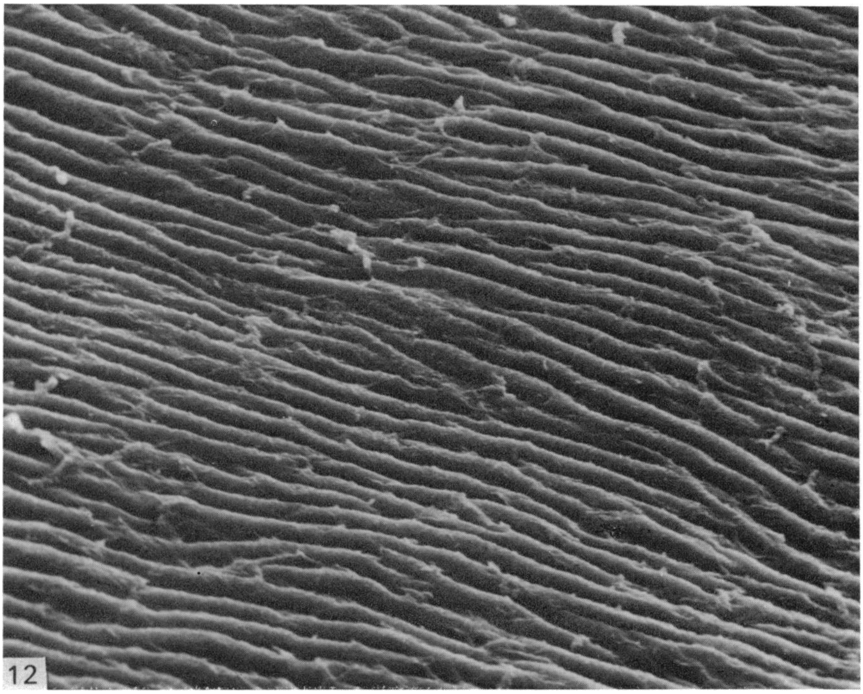

Fig. 12. Air-dried left medial semilunar cartilage from a 12 years old boy showing innumerable ridges. × 540.

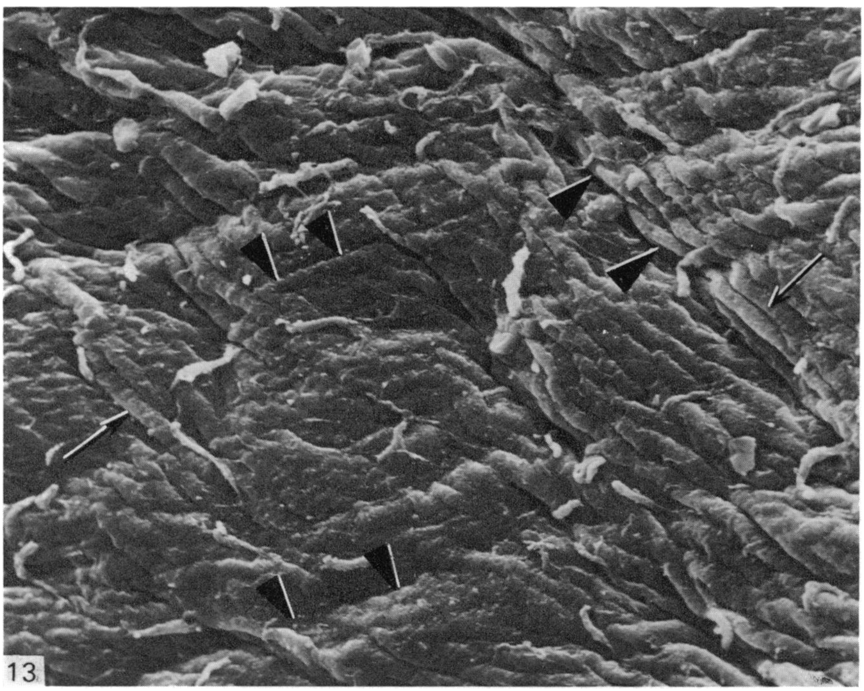

Fig. 13. Air-dried left lateral semilunar cartilage from a 26 years old man. The surface is beset by ridges (arrows) and furrows (arrowheads). × 590.

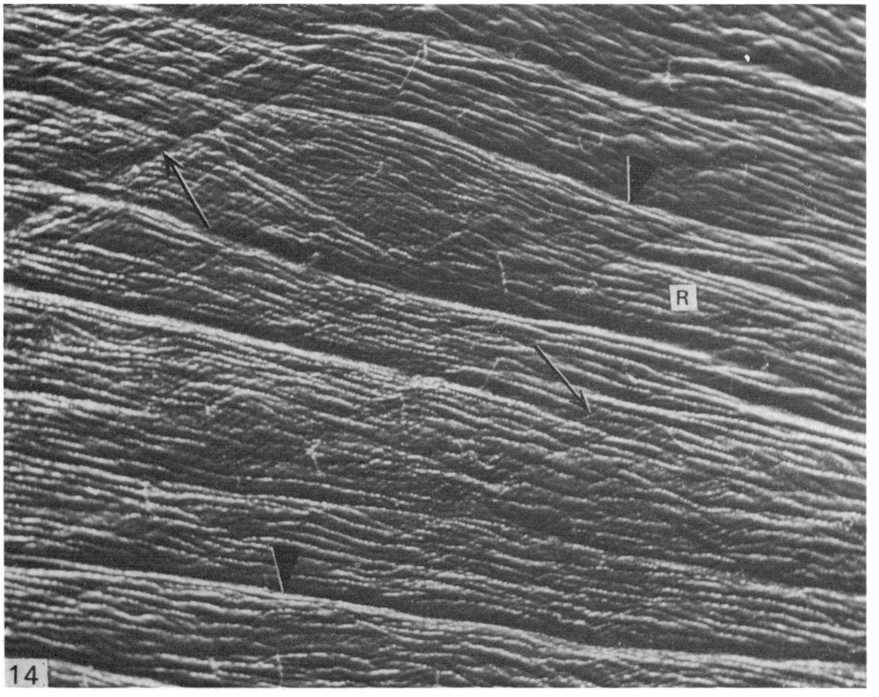

Fig. 14. Air-dried left lateral semilunar cartilage from a 41 years old man. Note parallel ridges (*R*) and deep furrows (arrowheads). Oblique to these run shallower furrows (arrows). × 235.

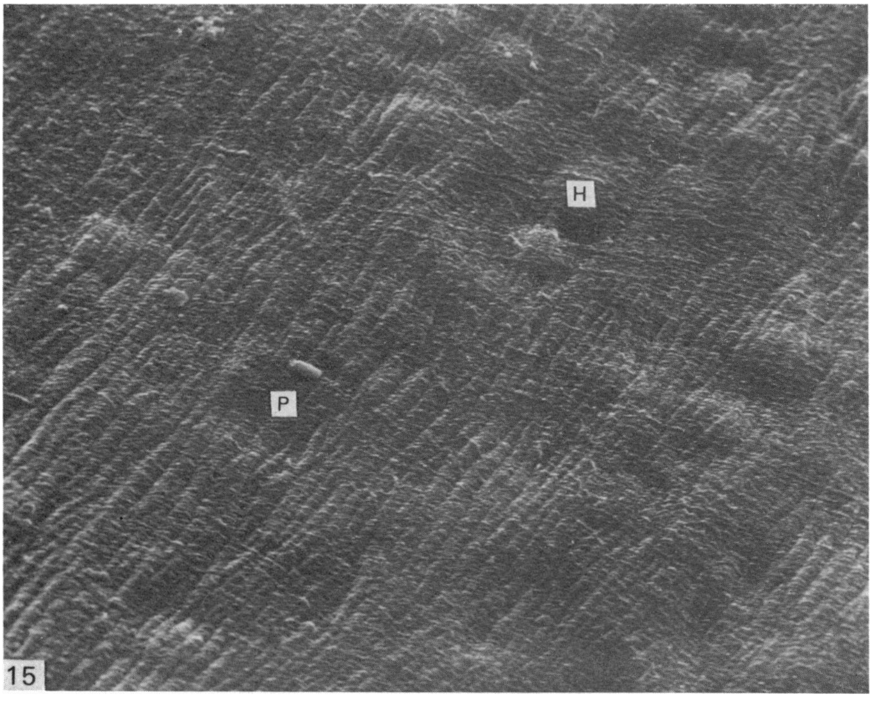

Fig. 15. Air-dried left lateral semilunar cartilage from a 6 months old cat showing ridges, undulations, humps in pits (*H*) and pits (*P*). × 13500.

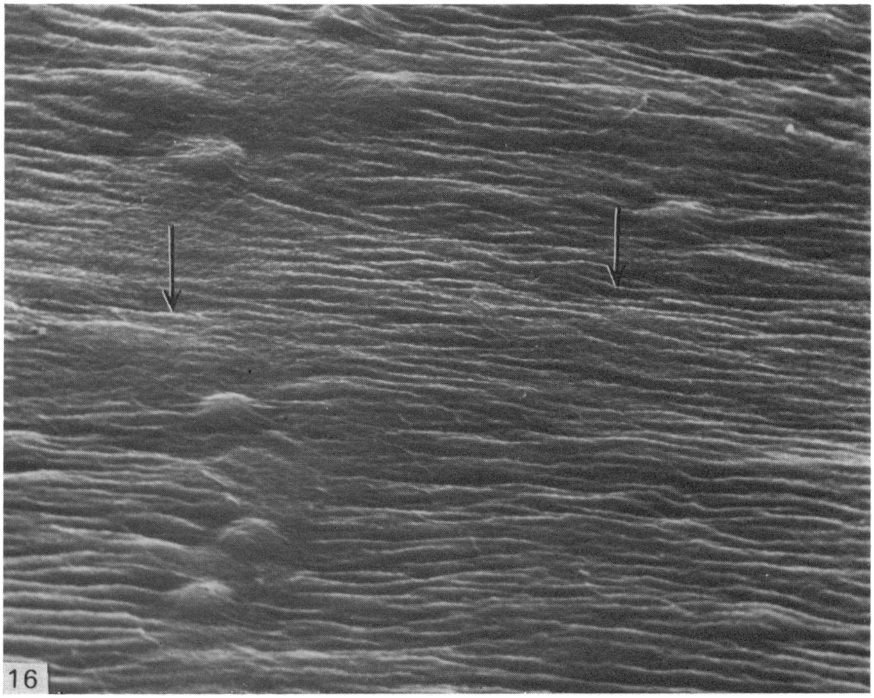

Fig. 16. Air-dried right lateral discoid meniscus from a 2 years old monkey showing ridges, humps in pits and a probable pit or two (arrows). × 11750.

Human specimens

Naked eye and ×7 magnifying glass inspection showed a smooth glistening surface, save for the presence of a few circumferential ridges, as described in the rabbit. The surgically removed semilunar cartilage had a frayed inner margin and some focal areas of roughening, but the major part of the surface appeared fairly normal. With the scanning electron microscope (Figs. 11–14) the surface of the 6 days specimen was found to be markedly shrunken and irregular. It was beset by numerous undulations, ridges and humps. The surface of the remaining human specimens showed innumerable parallel ridges, or ridges and furrows lying at right angles or oblique to each other. Neither pits nor humps could be identified with certainty in these specimens.

Cat and monkey specimens

Naked eye and ×7 magnifying glass inspection showed a smooth glistening surface, save for the presence of a few circumferential ridges, as described in the rabbit. Scanning electron microscopy showed (Figs. 15, 16) that the surface of the semilunar cartilage from both species was covered with ridges and undulations. In the case of the cat, 'humps in pits', and pits, were also clearly seen. In the specimen from the monkey 'humps in pits' were numerous, but pits were few and often difficult to discern.

DISCUSSION

Undulations and ridges

As pointed out in the Introduction, numerous studies now attest to the fact that undulations and ridges are produced by shrinkage and distortion when articular cartilage detached from bone is processed for scanning electron microscopy, and that this artefact can be eliminated by retaining a substantial portion of the subchondral bone.

The semilunar cartilages are not firmly tethered to bone like articular cartilage: they are anchored only by ligamentous and capsular connexions at their outer border. Hence it is not surprising that every specimen of semilunar cartilage we examined showed ridges, undulations and furrows. Presumably shrinkage and distortion were controlled to some extent in the case of the animal material where the semilunar cartilages were processed while tethered to the tibial plateau, but in the case of the human material, where mechanical consideration precluded such a manoeuvre, ridging was quite severe and we could detect neither pits nor humps, except in the 6 days specimen where, amongst numerous ridges, some humps were seen.

Thus until a better way of handling adult human semilunar cartilage is devised one cannot determine whether humps are converted to, or replaced by, pits as the cartilages mature.

The occurrence of ridges and undulations on semilunar cartilages adds weight to the already well established thesis that these features are artefacts of tissue preparation. Further, it demonstrates that it matters not whether the cartilage is 'naturally detached' (as in the case of semilunar cartilages) or 'detached by a scalpel' (as in the case of articular cartilage).

Pits and humps

Past studies with the scanning electron microscope have shown that the surface of young and juvenile articular cartilage in rabbit, cat and man is populated by humps, while the surface of mature articular cartilage is beset by numerous pits (Ghadially *et al.* 1977*a*, *b*). The transition from humps to pits occurs at ages which appear to be species dependent. At the stage of transition the cartilage shows both 'humps in pits' and pits. In the rabbit the age of transition is about 2 months; in the cat, 6–8 months. In the case of man, the age at which this maturation change occurs remains to be determined, but it seems likely that it lies around 10 years, for we have seen humps only in the case of a 7 years old boy (Ghadially *et al.* 1977*b*) and 'humps in pits' and pits in the case of a 10 years old boy (unpublished observation).

The results of our present study indicate that a similar maturation change occurs on the surface of animal semilunar cartilages. This was most convincingly demonstrated in the rabbit, but the age of transition appeared to be somewhat later, for the surface of the 2 months old semilunar cartilage was beset by humps only, and it was only at 6 months that 'humps in pits' and pits were seen. In the cat, however, the transition from humps to pits occurs at approximately 6 months in both the articular cartilage covering the femoral condyles (Ghadially *et al.* 1977*b*) and in the semilunar cartilages (Fig. 14).

In the case of man, technical difficulties obscure the situation, but at least we were able to determine that humps occur on young semilunar cartilages. One can here envisage various possibilities such as: (1) the humps persist, (2) the humps are replaced by pits or, (3) neither humps nor pits occur on mature human semilunar cartilages. The finding of 'humps in pits' and pits in the 2 years old monkey is consistent with the idea that we were looking at the transition phase, but data about this species are so scant that no firm conclusion is warranted.

Thus the observations made in this study lead to the tentative conclusion that, like articular cartilage, the surface of young and juvenile semilunar cartilage is covered by humps, and that in some animals at least these are converted to or replaced by pits; in others, like man, technical difficulties militate against determining the true state of affairs at present.

Regarding the nature and significance of humps, 'humps in pits' and pits in articular cartilage the reader is referred to our previous papers where these matters have been extensively discussed (Ghadially *et al.* 1976, 1977*a*, *b*, 1978). Briefly our views are that humps and pits reflect the presence of underlying chondrocytes, but there is as yet no reason to believe that such features occur *in vivo*. The maturation change from humps to pits, as revealed by the scanning electron microscope, reflects the reduced population of Zone I chondrocytes in the adult, and also, perhaps, alterations in the physicochemical nature of the matrix.

It therefore seems likely that in the semilunar cartilages also the pits and humps reflect the presence of underlying chondrocytes, and that maturation changes are engendered in a similar fashion, but further work is required to prove this.

SUMMARY

The surface of rabbit, cat, monkey and human semilunar cartilages was examined with the scanning electron microscope. A common feature was the occurrence of numerous ridges, undulations and furrows on the surface, but this was thought to be due to marked shrinkage and distortion of cartilage not firmly attached to bone. Humps were seen on the semilunar cartilages of young animals, but pits occurred in adults. This is thought to reflect a maturation change. Humps were seen in a young human semilunar cartilage, but pits were not seen in adult specimens. It is not clear whether pits are truly absent or just masked by the severe ridging produced during the preparation of large human specimens.

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