

# THE MORPHOLOGY AND MOTILITY OF HUMAN SYNOVIAL CELLS AND THEIR PERICELLULAR GELS: A TIME-LAPSE MICROKINEMATOGRAPHIC STUDY

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## Summary

The movements of human synovial cells and their associated pericellular gels have been studied by time-lapse microcinematography for periods up to 5 hr.

In sparse cultures, changes in cell shape are predominant, with individual cells exhibiting changes in shape common to most types of cell in culture. Cell migration occurs in short random movements in sparse cultures, but in denser cultures migration is mainly, though not entirely, oriented in the long axes of parallel bundles of the cells.

The discrepancies between the outlines of the pericellular gels and the cytoplasmic boundaries can be attributed to the relationships between cell movements and the cohesive properties of the gels. Cells move and make contact freely within the pericellular gels.

## I. INTRODUCTION

Although most of the glycosaminoglycans secreted by connective tissue cells in culture are found free in the culture medium, a small proportion is consistently recovered only after trypsin treatment of the cells (Morris and Godman 1960; Castor and Fries 1961; Daniel, Dingle, and Lucy 1961), leading to the conclusion that it is firmly bound to the cell surface (Dingle and Webb 1965).

This conclusion was confirmed morphologically when human synovial cells mixed with blood cells in culture were found to exhibit extensive but irregular investments of a pericellular gel-like material consisting mainly of hyaluronic acid (Clarris and Fraser 1968). These investments are similar to the capsules of free-living unicellular organisms in that they are extracellular and can be removed by hyaluronidase without deleterious effects, and they are difficult to stain but easily outlined by particulate matter. One puzzling difference is that the pericellular gels of cultured synovial cells do not show the definite form and boundary seen, for example, in bacterial capsules (Wilkinson 1958). They can be virtually imperceptible on one aspect of a cell and very thick on another, and can extend long distances beyond the "tails" of spindle-shaped cells.

It was suspected that the incongruity between the outlines of the cells and of their investments might arise during cell movements. A time-lapse microcinematographic study was therefore undertaken to complement these earlier observations, and also to see whether the gels had any obvious effect on the migration and apposition of synovial cells, since they appear to prevent close contact between lymphocytes and synovial cells (Fraser and Clarris 1970). Records of synovial cells

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alone were also made, since their diversity of form in culture has long been held to distinguish them from "fibroblasts" (Vaubel 1933; Murray, Stout, and Pogogeff 1944), the former presenting a high proportion of "tulip-shaped" and stellate forms rather than spindle cells.

## II. MATERIALS AND METHODS

Cell cultures were established from human synovial strains in a growth medium of 20% foetal calf serum in medium 199, and observations were begun about 24 hr after plating out the cultures in polystyrene vessels (Falcon Plastics), at densities about 40,000 cells per millilitre (Fraser and McCall 1965). In these studies the pericellular zones were outlined with diluted blood from a group O donor.

### (a) Optical System

Cultures were examined after at least 1 hr in an inverted-type model PMB biological microscope (Olympus Optical Co. Ltd., Tokyo) encased in a plastic box thermostatically held at 37°C. An Arriflex 16 mm cine-camera was coupled to the cine-photography microscope tube with an eyepiece as projector lens, and was controlled by an Arriflex time-lapse unit to take single-frame exposures at 14-sec intervals. A 6-8 V, 5A tungsten filament bulb provided illumination through a negative medium-phase contrast system and a green filter. This arrangement unfortunately limited the intensity of light, but it did not completely correct the low tonal contrast between some areas of cellular hyaloplasm and the surrounding culture medium. Ilford PAN-F film, ASA 50, was therefore selected as a compromise between the conflicting needs for high film contrast and sensitivity.

### (b) Records

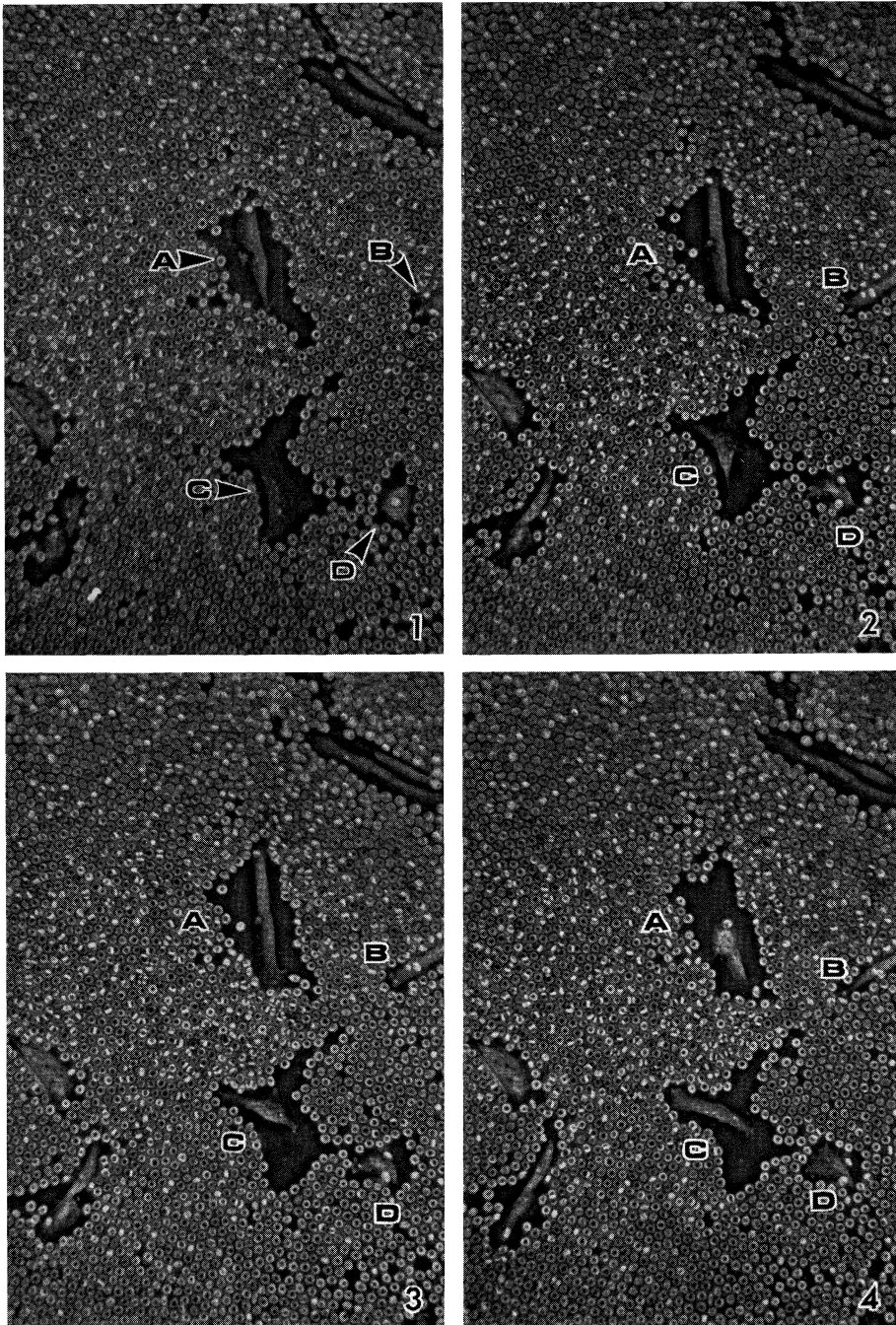
Eleven film sequences were made with different cell preparations, for periods ranging up to 5 hr. The films were examined by projection at variable speeds and fixed speeds of 2 and 16 frames per second, and representative frames selected for enlargement.

## III. RESULTS

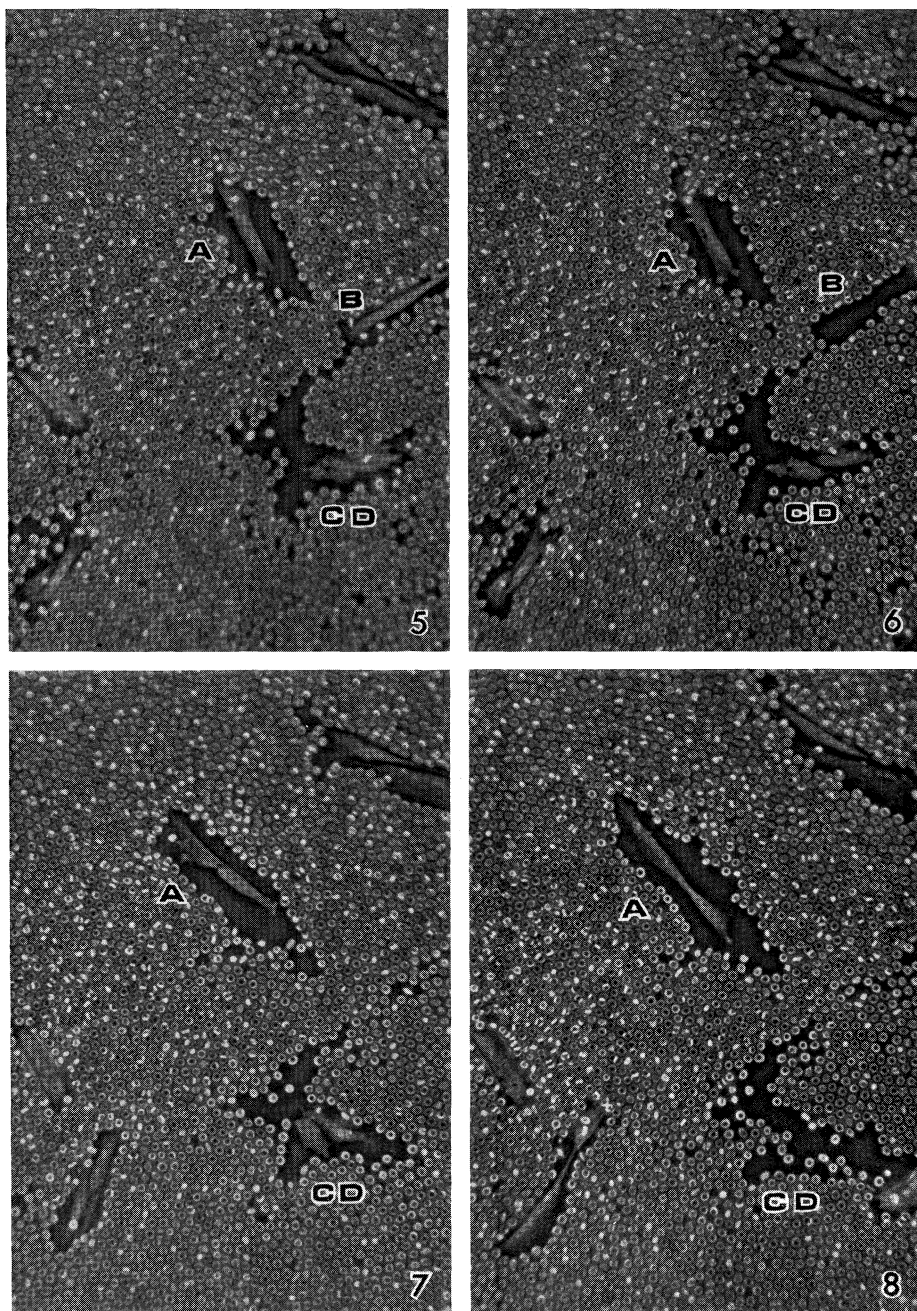
### (a) Synovial Cells Alone

Projection of the time-lapse records at 16 frames per second revealed gross changes in cell shape too slow to be analysed directly at magnifications low enough to give a wide field of vision. The changes in shape thus shown were more prominent than cell migration. The forward-moving poles of spindle-shaped cells often became spread in a fan-like manner with intense marginal undulatory movements. Cells with expansive and irregular cytoplasm might remain motionless except at the periphery, then rapidly withdraw cytoplasm to assume polar or amoeboid forms, or change from one form to another during migration.

Cell migration was often irregular in timing, and temporarily restricted by cytoplasmic filaments tethering one cell to another or to the culture vessel. These filaments stretched until their sudden release allowed the cell to move more freely. Cell migration took place mainly in either direction of the long axes of the more or less parallel groups of cells which make up the reticular patterns seen in synovial cultures of intermediate density (Fraser and McCall 1965). This behaviour is consistent with the contact-oriented movements seen in centrifugal outgrowth from explants (Abercrombie and Heaysman 1954). Occasionally cells moved into and through the main groups at wide angles, especially where smaller groups bridged the larger bundles, but the associated changes in shape made it difficult to be sure whether an angle of contact greater than 20° inhibited forward movement as is apparently the case with foetal fibroblasts (Elsdale 1968).



Figs. 1-4.—Enlargements ( $\times 120$ ) from 16 mm cine-film. Longer axes of figures represent 470 nm. Elapsed times are as follows: 1-2, 9 min 20 sec; 1-3, 11 min 52 sec; 1-4, 18 min 10 sec.



Figs. 5-8.—Enlargements ( $\times 120$ ) from 16 mm cine-film. Longer axes of figures represent 470 nm. Elapsed times are as follows: 1-5, 1 hr 30 min 30 sec; 1-6, 1 hr 35 min 18 sec; 1-7, 3 hr 18 min 30 sec; 1-8, 4 hr 35 min 16 sec.

In major respects, the behaviour of synovial cells displayed by the time-lapse technique was similar to that of other types of cell, and the different appearances on direct microscopic examination and in "still" micrographs can simply be attributed to a wider range of changes in shape, which would also be made more obvious by the larger mean bulk of the cells as found by electronic cell-sizing (Fraser, unpublished data).

*(b) Synovial Cells with Erythrocytes*

The Brownian movements of erythrocytes, like the spontaneous movements of the synovial cells, were accelerated and more easily seen in time-lapse projection, and served well to outline the clear pericellular gels. In the cine-study, the most extensive clear areas were seen after the rapid retraction of part of a cell, or behind a migrating cell. On the other hand, practically no clear areas were seen ahead of quickly moving cells. This indicated that the gels did not move in unison with the cells, and the irregular and random movements of cells, in part or whole, were thus responsible for the discrepancies between outlines of the cells and the clear pericellular areas.

Figures 1-8 show frames selected from one record to demonstrate these points. Relatively stationary groups of cells at the upper right and lower left aspects serve as reference points. In zone A, expansion and contraction of a cell is shown in Figures 3-5. Movements of the cells within the clear area occur between Figures 4 and 5 and Figures 6 and 8; and between Figures 1 and 8 the whole zone of cells and its investment have moved anticlockwise, up and to the left. In zone B, part of a cell is seen without a clear pericellular area. In Figure 5, this cell has moved left into the erythrocyte mass, but in the next figure it has retreated to the right leaving a gap between the erythrocytes which took an hour to fill in. In zone C, Figure 1 shows that virtually the whole of the gap in the erythrocyte mass is occupied by thinly spread cytoplasm. In Figure 2, three aspects of the cytoplasm have retracted rendering the cytoplasm more opaque especially in the lower aspect. The extensive clear area which resulted remained for over 4 hr. The subsequent figures show that the cell moved through this area to join the cell at D, moved randomly, and then further to the right leaving a large cell-free void.

This series of figures also demonstrates the predominance of changes in shape mentioned earlier. In these rather sparse cultures, random short movements appeared to be the rule rather than extensive migration, which may reflect the paucity of contacts between cells and consequent lack of contact guidance.

#### IV. DISCUSSION

The development of clear areas shown by "still" photographs might simply be attributed to displacement of erythrocytes during movements of the tissue cells, and to a failure of the non-motile erythrocytes to move into the cleared areas. However, the intensity of Brownian movements seen in time-lapse projection leaves no doubt that the erythrocytes would quickly fill the clear areas if there were no physical impediment to their doing so. Evidence has previously been given to show that the clear pericellular areas are occupied by a gel containing hyaluronic acid,

a conclusion that rests particularly on the rapidity (1–3 min) with which surrounding particles fill the clear areas after introduction of hyaluronidases (Clarris and Fraser 1968).

The irregularities in these gels result primarily from their failure to follow cell movements closely. In some instances, no pericellular gel is apparent until the cell moves, which indicates that the added erythrocytes can overlap the cell boundaries by overriding the margins of the gel coatings in the vertical plane. It follows that demonstration of pericellular gels by the present techniques depends critically on the relationships between rates of cell movement, of hyaluronic acid secretion, and of dissolution of the gel into the surrounding medium. If a cell line were to secrete more rapidly dispersing colloids, any pericellular investments of such colloids would be correspondingly more difficult to demonstrate. In the case of encapsulated streptococci, the hyaluronic acid coatings are presumably more regular in outline because the bacteria are not attached to a culture surface and do not undergo comparable deformation, so that there is a uniform concentration gradient between cell surface and the medium.

The pericellular investments of hyaluronic acid do not provide individual coatings for each synovial cell, but are irregular aggregations which may enclose several cells. Unlike the glycoproteins (Curtis 1967) they cannot be construed as part of the plasma membrane. Finally, it should be emphasized that the gels do not appear to hinder movement or contact between synovial cells, although it has been observed that they do interfere with contact between synovial cells and motile lymphocytes (Fraser and Clarris 1970). This apparent anomaly can be attributed to the fact that lymphocytes *in vitro*, unlike synovial cells, do not develop any firm attachment to the culture vessel, and therefore cannot develop sufficient thrust to overcome the elastic properties (Ogston and Stanier 1953) of hyaluronic acid gels.

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