J. Cell Sci. 79, 67–81 (1985)
Printed in Great Britain © The Company of Biologists Limited 1985

STUDIES ON THE MECHANISM OF HYDRATED COLLAGEN GEL REORGANIZATION BY HUMAN SKIN FIBROBLASTS

CLYDE GUIDRY AND FREDERICK GRINNELL

Department of Cell Biology, University of Texas Health Science Center, Dallas, Texas 75235, U.S.A.

SUMMARY

During reorganization of collagen gels by human skin fibroblasts the total protein content of the gels remained approximately constant. Only 5% of the collagen was degraded, although the volume of the gels decreased by 85% or more. It could be concluded, therefore, that gel reorganization required physical rearrangement of pre-existing collagen fibrils rather than degradation of the original collagen and resynthesis of a new matrix. Collagen molecules in the gels were not covalently crosslinked or otherwise modified enzymically during gel reorganization, as determined by sodium dodecyl sulphate/polyacrylamide gel electrophoresis and collagen repolymerization studies. Serum was required for gel reorganization and, in the absence of serum, cell spreading was predominantly filipodial, i.e. there was little cytoplasmic reorganization. At the electron-microscopic level it was found that many more collagen fibrils became associated with the cells in the presence of serum than in its absence. Serum was also found to promote the synthesis and secretion of proteins by the cells, and conditioned medium could take the place of serum in promoting gel reorganization. The involvement of cell-secreted factors was also demonstrated by the ability of cycloheximide to inhibit gel reorganization. Finally, when gel reorganization was stopped by adding cytochalasin D to the incubations or removing cells by detergent treatment, a small but significant re-expansion of the collagen fibrils was observed. Consequently, a portion of the collagen that had been physically reorganized by the gels was unstable and could not hold its position without continued force exerted by the cells.

INTRODUCTION

A few years ago it was shown that culturing fibroblasts in hydrated collagen gels led to gel reorganization (Bell, Ivarsson & Merrill, 1979), a finding that was subsequently confirmed in other laboratories (Bellows, Melcher & Aubin, 1981; Steinberg, Smith, Colozzo & Pollack, 1980). The contraction of collagen gels by fibroblasts has been suggested as a possible *in vitro* model for connective-tissue reorganization during development (Stopak & Harris, 1982) or granulation tissue contracture during wound healing (Coulomb, Dubertret, Bell & Touraine, 1984; Ehrlich & Wyler, 1983). In addition, the contracted gels have potential clinical importance in the development of 'artificial skin' that could be used in treating major wounds involving loss of substantial amounts of skin such as result from serious burns (Bell, Ehrlich, Buttle & Nakatsuji, 1981).

In our laboratory we have examined contraction of hydrated collagen gels, using a model somewhat different than that studied by others. Instead of placing fibroblasts

Key words: collagen gels, fibroblasts, skin.

within gels, we have placed them on top of gels attached to an underlying solid support (Grinnell & Lamke, 1984). Under the latter circumstances, the reorganization of collagen gels occurred in two dimensions rather than three. There was a decrease in gel thickness of around 90 % without any decrease in gel diameter. Using this model, we observed that the initial binding interaction between cell processes and collagen molecules led to reorganization of the collagen fibrils in the plane of cell spreading. While the initial reorganization of the gels occurred only in the region of cell–collagen interactions, after additional time the distal portions of the gels also became reorganized.

The mechanism by which fibroblasts cause the reorganization of hydrated collagen gels has yet to be determined. It has not been shown, for example, whether gel reorganization involves collagen degradation or covalent modifications of the collagen molecules in the gels. Experiments designed to test these and related points were carried out, and the results are reported in this paper.

MATERIALS AND METHODS

Cells

Human skin fibroblasts were established from foreskins obtained at circumcisions. Cells were grown in stationary culture in 75 cm^2 tissue culture flasks using Delbecco's Modified Eagle's Medium supplemented with 20 mM-Hepes buffer, 1 % penicillin, streptomycin, Fungisone (Gibco), gentamycin (50 $\mu g\ ml^{-1}$), and 20 % foetal bovine serum. The cultures were incubated in an humidified incubator containing 5 % CO_2 and 95 % air. Cultures were discarded before the 15th passage.

Hydrated collagen gels

Hydrated collagen gels were prepared as previously described (Grinnell & Lamke, 1984) using Vitrogen 100 collagen (Flow Laboratories) and 10× phosphate buffered saline, neutralized with 0·1 M-NaOH, and gelled at 37°C for 60 min in an humidified incubator. The final collagen concentration in the solutions was 1·5 $mg\ ml^{-1}$.

For analysis of contraction rates the lattices were prepared in 35 mm tissue culture dishes, which contained a 12 mm (diameter) circular score, within which the polymerizing collagen solution was added. The thickness of the collagen gels was measured on a Zeiss inverted phase-contrast microscope equipped with a Mitutoyo dial test indicator (0·01–100 mm). The plane of focus was adjusted from the top to the bottom of the collagen lattice and the distance of stage movement noted on the dial test indicator. This method of measuring lattice thickness was determined to be reproducible to 0·02 mm.

Preparation of radiolabelled collagen

Radiolabelled collagen was prepared by ^3H acetylation of lysine residues (Schor, Allen & Harrison, 1980). The commercial collagen solution (20 ml at 3·0 $mg\ ml^{-1}$) was brought to pH 8·9 by addition of 1 M-K_2HPO_4. Tritiated acetic anhydride (2·5 mCi) dissolved in 1 ml of toluene, was added dropwise, with continuous stirring, to the collagen solution at 4°C. The solution was stirred for another 60 min at 4°C and then dialysed exhaustively against 0·015 M-acetic acid to remove the unbound radioactivity. Hydrated collagen gels were prepared with the radiolabelled collagen in the same manner as with unlabelled collagen.

Incubation of cells with collagen gels

Logarithmically growing human fibroblasts were harvested from cultures using 0·05 % trypsin/0·06 M-EDTA (Gibco) for 15 min at 37°C. Cells were washed and resuspended in the

experimental media at the desired concentration; 100 μl of the cell suspension was placed on top of the collagen lattice. This volume remained within the previously enscribed circle. The cells were allowed to attach to the matrices for 30 min before the dish was overlaid with 3 ml of experimental media. Incubations were carried out at 37°C for the times indicated in the Fig. legends in an humidified incubator containing 5 % CO_2 and 95 % air.

Preparation of conditioned medium

Conditioned medium was prepared by incubating confluent layers of human foreskin fibroblasts in 75 cm^2 tissue culture flasks with serum-free DMEM at 37°C in an atmosphere of 5 % CO_2 and 95 % air. The medium was replaced with fresh serum-free medium each week. The first collection was discarded. All subsequent collections were dialysed, concentrated, and recombined with fresh serum-free media to give the final protein concentration desired.

Materials

Cycloheximide and cytochalasin D were purchased from Sigma Chemical Co.; tritiated amino acid mixture (196 Ci g^{-1}) and tritiated acetic anhydride (100 Ci mol^{-1}) were obtained from ICN Biomedicals Ltd.

RESULTS

Collagen reorganization involves a physical rearrangement of pre-existing collagen fibrils

As shown in Fig. 1, collagen gels decreased to less than 15 % of their starting thickness when cultured with cells for 72 h, while gels incubated in the absence of cells did not change in thickness. Since the diameters of the gels were constant, their volume had decreased by greater than 85 %. In trying to understand the mechanism of this decrease it was essential to determine whether the protein in the gels was destroyed during the reorganization process. At various times during reorganization, the cells were lysed in de-ionized water in the presence of proteinase inhibitor. Following extensive washing to remove unbound proteins (72 h), the gels were hydrolysed with NaOH and total protein content was measured. There was little change in protein content in the presence or absence of cells, although there was a slight increase in protein observed in parallel cultures of cells incubated on plastic substrata (Fig. 1).

The constant level of total protein in the gels during reorganization could have occurred as a result of extensive secretion of new protein into the matrix, accompanied by an equivalent amount of degradation of the pre-existing collagen. To test this possibility, cells were cultured on collagen lattices prepared with collagen that had been radioisotopically labelled. Control experiments revealed that the radiolabelled collagen formed a gel similarly to unlabelled collagen, and that exogenously added collagenase solubilized 100 % of the radioactivity in the gel (data not shown). Cells cultured on top of radiolabelled collagen gels were found to reorganize the gels as shown in Fig. 2. It was found that under these conditions, even though collagen reorganization resulted in an 85 % decrease in gel thickness, less than 5 % of the radioactivity was released from the gels. Moreover, this was only slightly greater than the amount of radioactivity released from control gels in the absence of cells.

Taken together, the results in Figs 1 and 2 suggested that reorganization of the collagen gels by human fibroblasts occurred by physical rearrangement of pre-existing collagen fibrils, rather than replacement of the pre-existing collagen fibrils with a newly synthesized matrix.

Collagen molecules are not covalently modified during collagen reorganization

It also was of interest to determine whether the collagen fibrils in the gels were modified during reorganization. It was possible, for instance, that the cells secreted factors that promoted covalent crosslinking of collagen or other enzymic modifications of the pre-existing fibrils. To study this possibility, collagen gels were allowed to reorganize for 24 h and then the cells were removed and the gels washed,

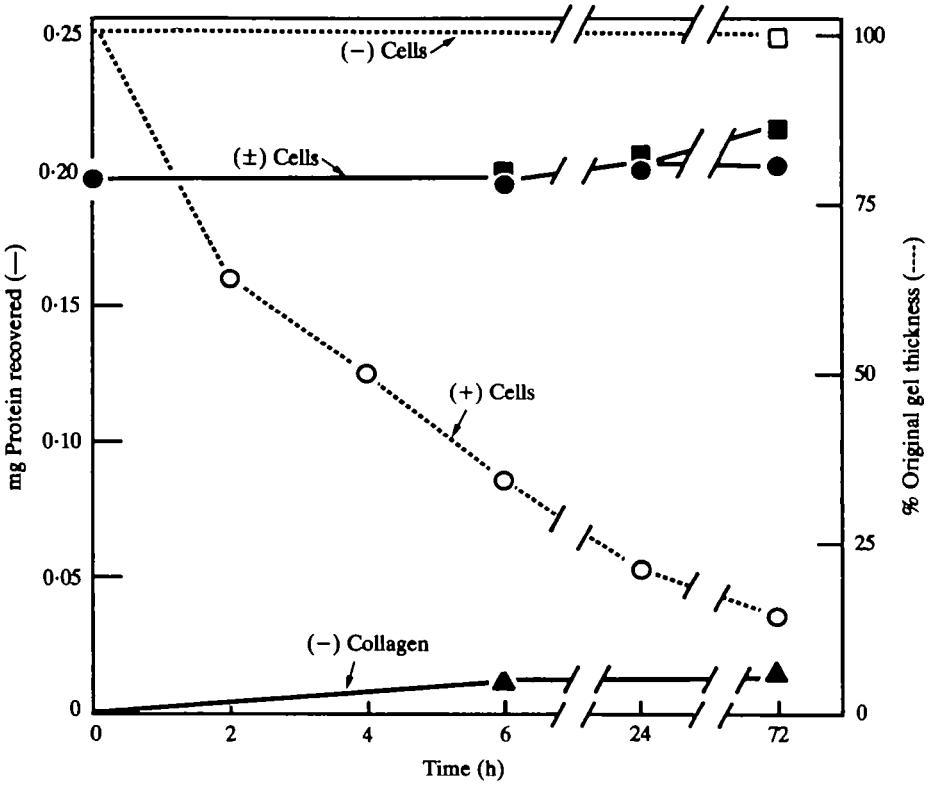

Fig. 1. Total protein content of collagen gels during fibroblast reorganization. Cells (1×10^5) were plated on either collagen gels or tissue culture dishes and incubated at 37 °C. At the times indicated, the matrices were washed with water containing 0·2 mM-phenylmethylsulphonyl fluoride (PMSF) for 60 min to lyse the cells, and then with phosphate buffered saline for 72 h to remove unbound proteins. Each gel was dissolved in 1 ml of 1 M-NaOH and assayed for protein content: (■——■) +cells; (●——●) −cells; (▲——▲) +cells, −collagen (Lowry, Rosebrough, Farr & Randall, 1951); or gel thickness (□····□) +cells. Data presented are the means of results for duplicate cultures. Other details are described in Materials and Methods.

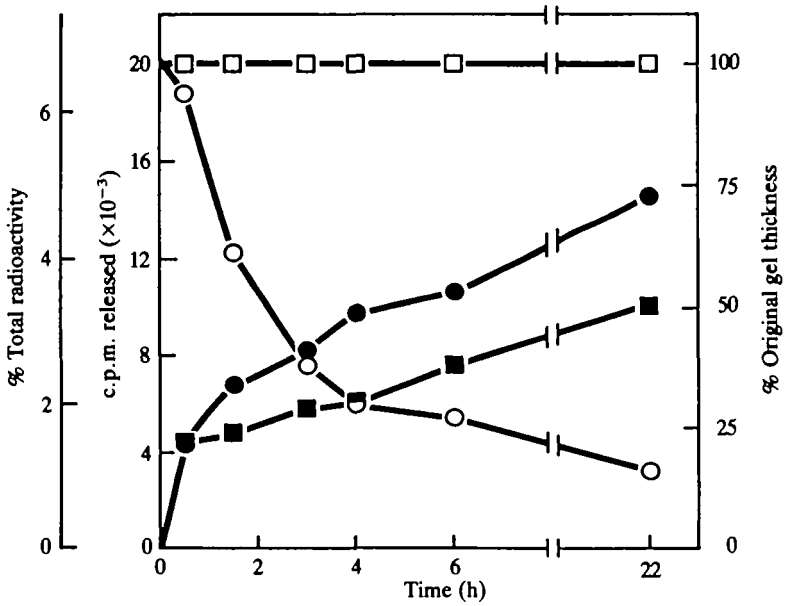

Fig. 2. Fibroblast degradation of pre-existing collagen during reorganization. Cells ($2{\cdot}5\times10^5$) were plated on collagen gels prepared with radiolabelled collagen and incubated at 37°C. At the times indicated, gel thickness was measured and 0·1-ml samples of the culture were removed from the cultures and analysed for radioactivity. Data presented are the mean results from quadruplicate cultures. Standard deviations were less than 5%. Other details are described in Materials and Methods. (□——□) Gel thickness (−) cells; (○——○) gel thickness (+) cells; (■——■) c.p.m. released (−) cells; (●——●) c.p.m. released (+) cells.

as described above. Subsequently, all of the collagen in the reorganized gels could be resolubilized in dilute acetic acid. Experiments were then carried out to determine the rate of polymerization of the resolubilized collagen compared with that of fresh collagen. The results shown in Fig. 3 indicate that the polymerization rate was the same for re-isolated collagen from the reorganized gels and control collagen. Samples of the reorganized collagen, collagen from non-reorganized gels, and fresh collagen, were compared by sodium dodecyl sulphate/polyacrylamide gel electrophoresis (SDS/PAGE), and no differences in the amounts or mobilities of the various polypeptide chains were observed (data not shown). From these observations it was concluded that covalent modification of the collagen molecules did not occur during gel reorganization.

Collagen gel reorganization requires serum and, or, factors secreted by the cells

In order to learn more about other factors that might play a role in reorganization of collagen fibrils, we studied the serum requirement. As shown in Fig. 4, there was little change in gel thickness in the absence of serum in the incubation medium, but

re-addition of serum led to rapid reorganization of the collagen in the gels. The serum requirement for gel reorganization might have resulted from an effect on cell growth. It has been reported from other laboratories that serum is required for reorganization of collagen gels containing fibroblasts (Steinberg *et al.* 1980; Buttle & Ehrlich, 1983), but in their experimental system reorganization was markedly dependent on cell number (Bell *et al.* 1979; Steinberg *et al.* 1980). In the model system we are using, with 50 000 or more cells per gel, the cell number is well above saturating conditions, as can be seen in Table 1. We could conclude, therefore, that serum was required for gel reorganization *per se*, not just for cell growth.

A major effect of serum appeared to be on the ability of cells to spread. In the presence of serum, many cells were observed to become completely spread after 2 h (Fig. 5A). In the same time period, however, cell spreading in the absence of serum was primarily filipodial (Fig. 5C). At the electron-microscopic level, it was found that there was a marked reduction in the binding of collagen fibrils to the cell surface in the absence of serum compared to the presence of serum (Fig. 5D and B, respectively), indicating that serum had an effect on the cells' ability to attach to the collagen fibrils. The possibility that plasma fibronectin might be required for this interaction was eliminated, since serum depleted of fibronectin had effects similar to

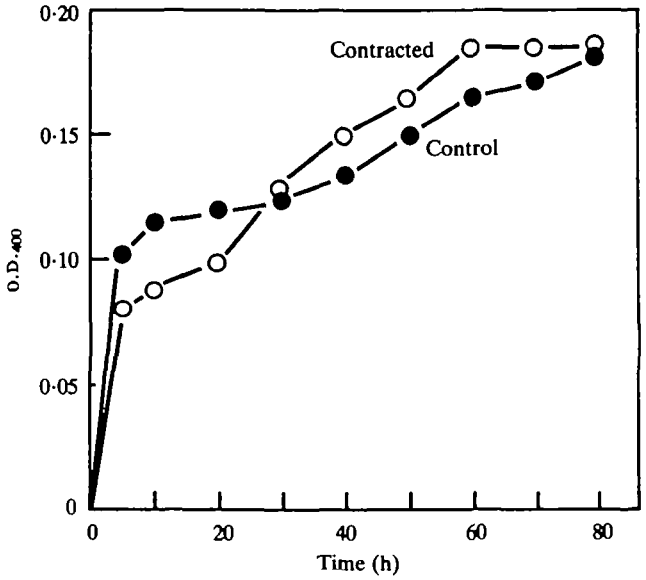

Fig. 3. Polymerization kinetics of collagen recovered from reorganized gels. Cells (4×10^5) were plated on 0·9 ml collagen gels and incubated at 37 °C for 24 h. The matrices were washed with a 10 mM-Tris-buffered solution of 0·5% sodium deoxycholate and 1 mM-PMSF (pH 8·0) to remove the cells. The reorganized lattices were solubilized in 0·1% acetic acid and concentrated to 3·0 mg ml^{-1}. Collagen solutions were prepared for polymerization and incubated at 37 °C. At the times indicated, polymerization was measured by increase in turbidity ($O.D._{400}$). Other details are described in Materials and Methods.

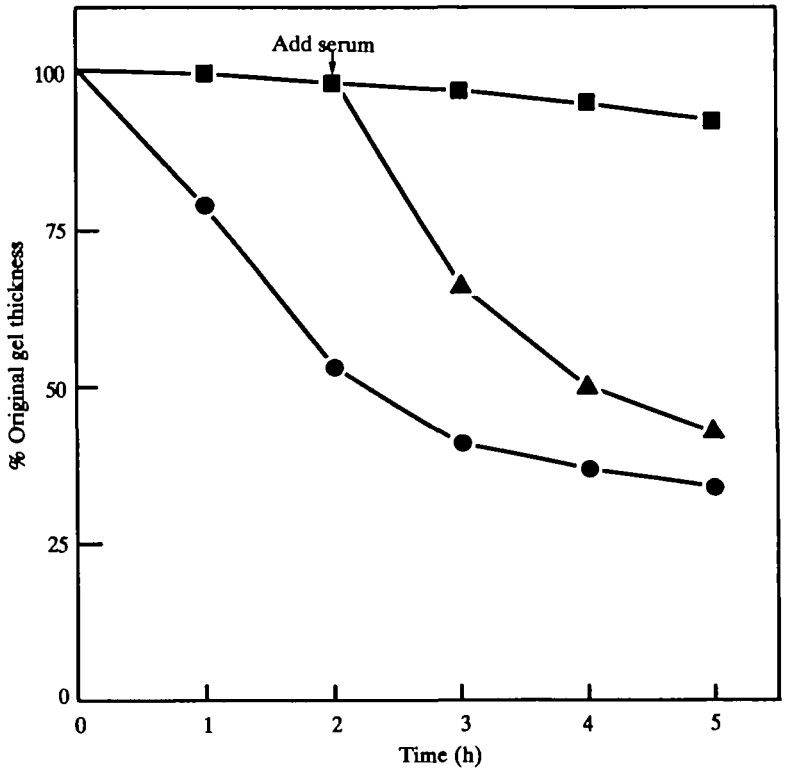

Fig. 4. Fibroblast reorganization of collagen gels in the presence and absence of serum. Cells (1×10^5) were plated on collagen gels in the presence (●) or absence (■) of 20% foetal bovine serum and incubated at 37 °C. After 2 h of incubation, the media in half of the serum-free cultures were exchanged for media containing serum (▲). At the times indicated, the gel thickness was measured. Data presented are the means of results from quadruplicate cultures. Other details are described in Materials and Methods.

undepleted serum, and addition of fibronectin to serum-free medium did not compensate for the absence of serum (data not shown).

Additional studies suggested that serum might be required for the cells to secrete factors that promoted their interaction with the collagen gels. Conditioned medium

Table 1. *Effect of cell number on fibroblast reorganization of collagen gels*

Number of cells added per gel	% Decrease in gel thickness
50 000	82
25 000	77
12 500	67
6250	53
3125	36

Cells (as indicated) were plated on collagen gels and incubated at 37 °C for 21 h, at which time gel thickness was measured. Other details are described in Materials and Methods.

produced by fibroblasts cultured on plastic surfaces was tested, and Table 2 summarizes results comparing different concentrations of serum and conditioned medium. It can be seen that replacement of serum by conditioned medium, as well as serum, allowed collagen gel reorganization to occur.

If serum influenced the secretion of cell factors, then it might have been possible to demonstrate directly a serum effect on secretion of new proteins by the fibroblasts. To test the effect of serum on biosynthesis of new proteins, cells were incubated on

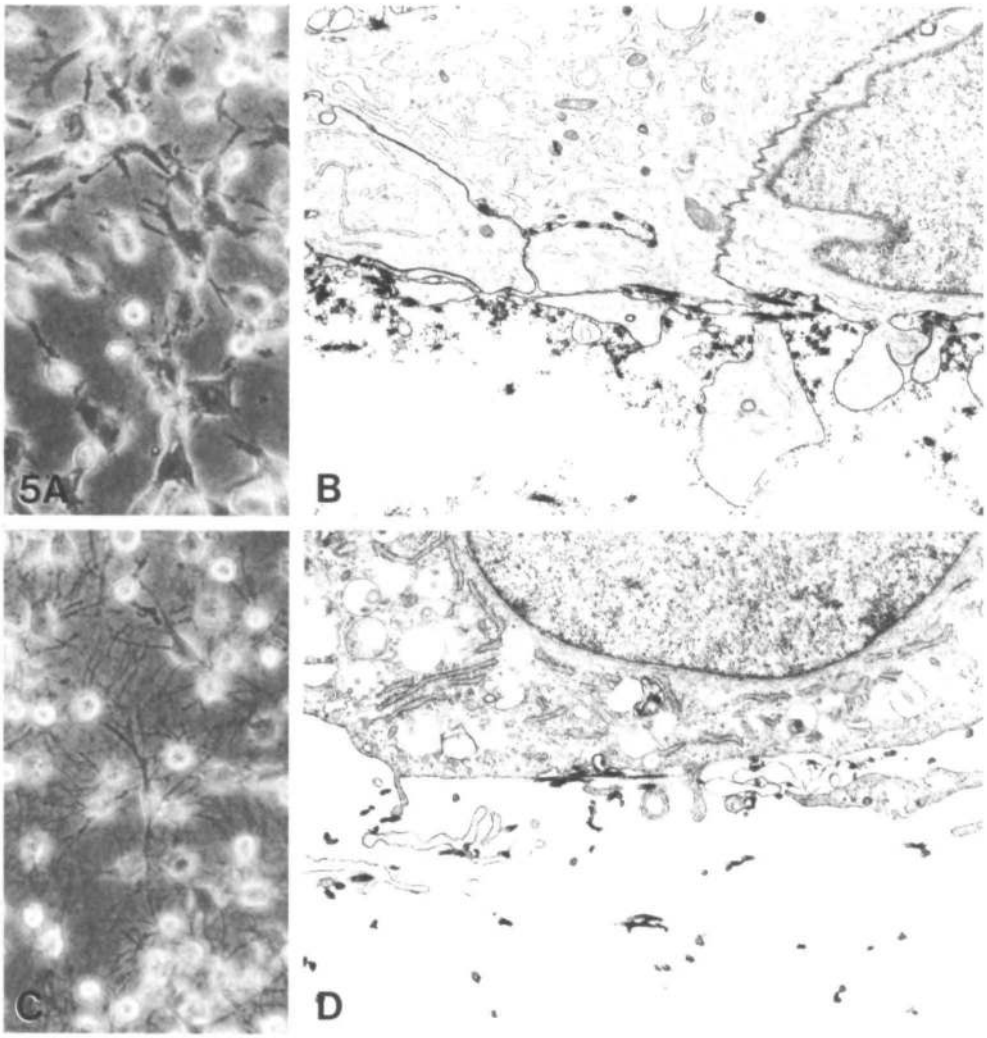

Fig. 5. Morphology of fibroblasts on collagen gels in the presence and absence of serum. Cells (1×10^5) were plated on collagen gels in the presence (A,B) or absence (C,D) of serum and incubated at 37 °C for 2 h. The samples were photographed under phase-contrast microscopy and then processed for transmission electron microscopy as described previously (Schor *et al.* 1980). Other details are described in Materials and Methods. A,C, ×175; B,C, ×7600.

the gels in the presence of tritiated amino acids. At various times the extent of gel reorganization was measured. After removing the incubation medium the gels were solubilized with SDS. The solubilized gels and incubation medium were each dialysed extensively to remove free amino acids. In control experiments in the absence of cells, essentially all of the radioactivity was dialysable. As shown in Fig. 6, there was a marked enhancement in the total amount of non-dialysable radioactivity in the presence of serum compared to the absence of serum. Of the radioactivity incorporated after 24 h, 48 % was in the medium and 52 % was matrix-associated in the presence of serum, while 21 % was in the medium and 78 % was matrix-associated in the absence of serum. In terms of absolute counts this means that the matrix-associated radioactivity was almost 50 % higher in the presence of serum than in its absence.

If the proteins produced by cells were important in gel reorganization, then inhibition of protein synthesis would have been expected to inhibit gel reorganization. To test this possibility, experiments were carried out in serum-containing medium in the presence and absence of cycloheximide. Addition of cycloheximide at the beginning of the incubation of cells on the collagen did not have any effect on the first 4 h of reorganization. Subsequently, however, further reorganization on the collagen gels did not occur (data not shown). This suggested that the cells might contain a pool of proteins necessary for gel reorganization, so studies were carried out in which cycloheximide was added to the cells 4 h before the cells were added to the gels. Under these conditions, little reorganization of the gels occurred compared to cycloheximide-free controls (Fig. 7) and cell spreading was inhibited (Fig. 8). The inhibition by cycloheximide was readily reversible, and when cycloheximide-containing medium was replaced with fresh medium, gel reorganization continued normally after a short lag period (Fig. 7).

Table 2. *Effect on conditioned medium on fibroblast reorganization of collagen gels*

Serum or conditioned medium protein added	% Original gel thickness
None	65
0·25 mg/ml:	
Serum	59
Conditioned medium	52
0·5 mg/ml:	
Serum	57
Conditioned medium	46
1·0 mg/ml:	
Serum	51
Conditioned medium	36
5·0 mg/ml:	
Serum	27

Cells (1×10^6) were plated on collagen gels in medium containing serum or conditioned medium as shown and incubated at 37 °C for 24 h after which the gel thickness was measured. Other details are given in Materials and Methods.

A lag chase occurs between physical rearrangement of collagen fibrils and stabilization of the reorganized state

Since it appeared that synthesis and secretion of cellular factors was important in collagen gel reorganization, it was important to determine whether these cellular factors played some role in stabilization of the reorganized collagen fibrils. That is, one possibility was that fibroblasts first rearranged the collagen fibrils and then secreted a factor that stabilized the rearranged state. To test this possibility, cells were allowed to reorganize collagen gels for various times, after which cytochalasin D was added to the incubations. Others had shown that cytochalasin could prevent collagen gel reorganization, but no previous attempt had been made to see whether the gels were able to re-expand after the cells' contractile activity was inhibited (Bell *et al.* 1979). Addition of cytochalasin with the cells almost completely inhibited subsequent reorganization of the gels (Fig. 9). When the cells were first allowed to contract the gels, and then the cytochalasin was added, it was found that the gels partially re-expanded (Fig. 9). Similarly, if the cells were removed from the gels by

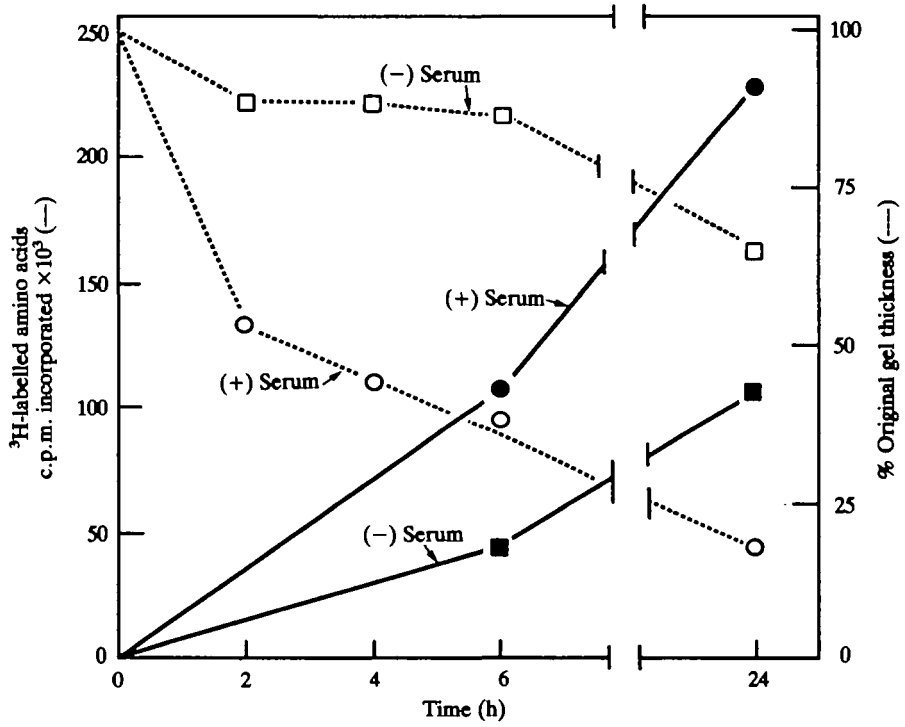

Fig. 6. Total incorporation of tritiated amino acids by fibroblasts during reorganization of collagen gels. Cells (1×10^5) were plated onto collagen gels with 25 μCi ml^{-1} tritiated amino acids and incubated at 37 °C in the presence or absence of 20% foetal bovine serum. At the times indicated, the gel thickness was measured and the gels were washed and solubilized with 1% SDS. Both the media and the solubilized gel samples were dialysed to remove free amino acids. Data presented are the mean of duplicate cultures. Other details are described in Materials and Methods.

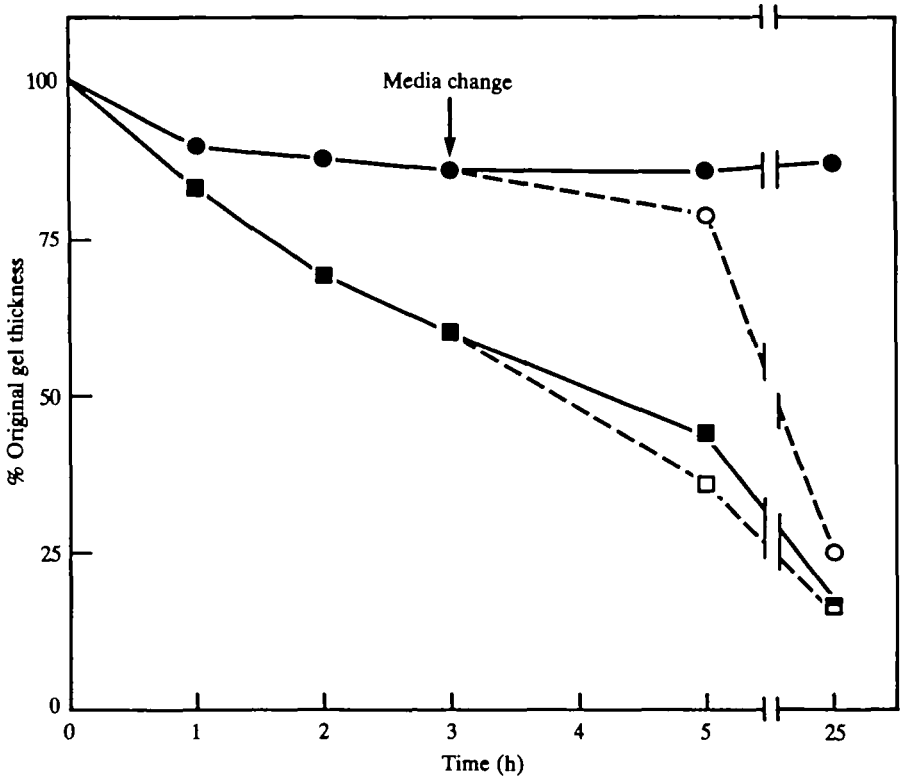

Fig. 7. Fibroblast reorganization of collagen gels in the presence of cycloheximide. Control cells (5×10^4) or cells pretreated for 4 h with DMEM containing 0·1 mM-cycloheximide and 20% foetal bovine serum were plated onto collagen gels and incubated at 37 °C in medium containing 0·1 mM-cycloheximide and 20% serum. At the times indicated, the gel thickness was measured. After 3 h of incubation, the medium in half of the treated (●, ○) and untreated (■, □) cultures was replaced with fresh medium containing no cycloheximide. Data presented are the mean results from quadruplicate cultures. Other details are described in Materials and Methods.

treatment with the detergent sodium deoxycholate, the results were almost identical to the findings observed using cytochalasin. That is, there was no further gel reorganization after solubilization, and a slight expansion of the previously reorganized gel occurred (data not shown).

DISCUSSION

The purpose of the studies described in this paper was to learn more about the mechanism by which human skin fibroblasts reorganize hydrated collagen gels. We have shown previously that the sparse, randomly packed collagen fibrils originally found in the gels become densely packed and aligned during gel reorganization (Grinnell & Lamke, 1984). The present results show that during this reorganization only 5% of the starting collagen was degraded under conditions where the volume of

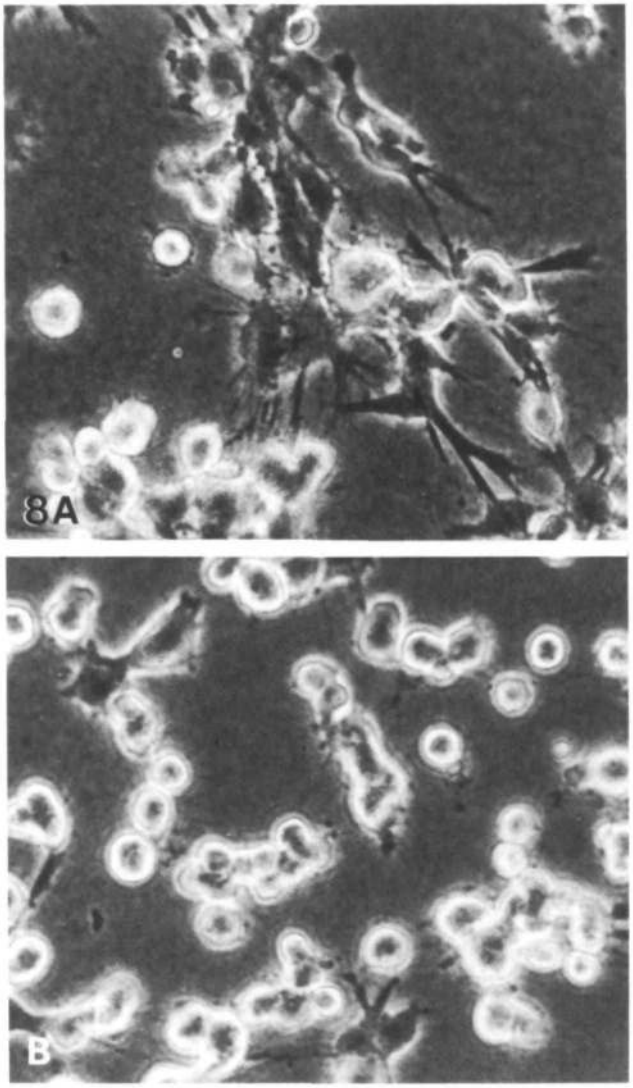

Fig. 8. Morphology of fibroblasts on collagen gels in the presence (A) or absence (B) of cycloheximide. Appearance of cells from the experiment described in the legend to Fig. 7 as observed by phase-contrast microscopy after 2 h of incubation in the absence (A) or presence (B) of cycloheximide. Other details are described in Materials and Methods. A,B, ×175.

the gels decreased by 85% or more. In addition, covalent modifications in the collagen molecules of the reorganized gels were not detected by comparing the polymerization rates of fresh collagen and collagen re-solubilized from reorganized gels or by SDS/PAGE analysis.

Examination of the serum requirement for collagen gel reorganization revealed that in the absence of serum, cell spreading was limited to the extension of prominent filipodia (cf. Grinnell & Minter, 1978). From electron-microscopic observations it

appeared that fewer collagen fibrils were bound at the cell surface if serum was absent from the incubations. In previous studies it has been found that the time of initial collagen gel reorganization correlates with initial cell spreading (Bellows *et al.* 1981; Grinnell & Lamke, 1984), and that cells that spread poorly compared to normal cells because of cytoskeletal or other defects, have a decreased ability to cause collagen gel reorganization (Steinberg *et al.* 1980; Delvoye, Nusgens & Lapiere, 1983). Taken together these findings indicate that collagen gel reorganization requires normal cell spreading.

The serum factor required for normal cell spreading and collagen gel reorganization has not been identified. The possibility that fibronectin was involved was ruled out by experiments with fibronectin-depleted serum and with exogenously added fibronectin. Significantly, serum was found to promote the synthesis and secretion of cellular proteins, and conditioned medium could substitute for serum in promoting cell spreading and collagen gel reorganization. It may be, therefore, that in this system the serum factor functions indirectly to promote the secretion of

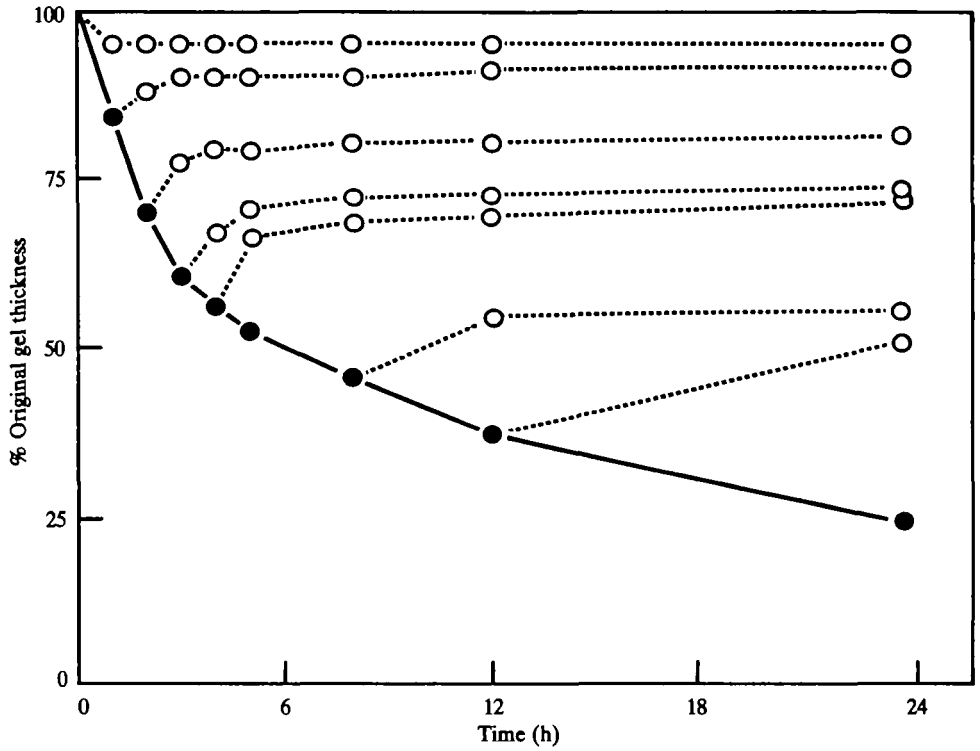

Fig. 9. Fibroblast reorganization of collagen gels in the presence of cytochalasin D. Cells (1×10^5) were plated onto collagen gels and incubated at 37 °C in DMEM containing 20% foetal bovine serum. Cytochalasin D (10 mM) was added as shown and gel thickness was measured at the times indicated. Data presented are the means of results of duplicate cultures. Other details are described in the Materials and Methods. (●——●) Control; (○——○) +10 mM-cytochalasin.

endogenous cell factors that are necessary for cell spreading and, or, gel reorganization. Consistent with this idea, cycloheximide treatment inhibited the ability of the cells to spread on the collagen gels and also inhibited gel reorganization. Whatever the factors are whose synthesis is inhibited by cycloheximide, they must be present in the cells in a significant pool, since cycloheximide had little effect on the cells unless it was added several hours before the cells were incubated with the collagen gels.

Studies in which collagen gel reorganization was inhibited by addition of cytochalasin D or removal of cells from the gels with detergent indicated that the reorganized collagen gels could re-expand partially following these treatments. This suggests that some collagen fibrils had been physically moved by the cells but were not yet fixed in place. The chemical basis of this instability is intriguing. One attractive hypothesis is that physical activity on the part of the cells – either spreading or tractional forces (Harris, Stopak & Wild, 1981) – brings the collagen fibrils close to each other, and the factors secreted by the cells non-covalently crosslink the fibrils together. Alternatively, there may be time-dependent changes in the intramolecular non-covalent bonding between adjacent collagen fibrils that are independent of cell secretions.

It is worth noting that the effects of serum, cycloheximide and cytochalasin on gel reorganization that have been described in this paper cannot be accounted for in terms of cell growth. The cell number that we are using is well above saturating level. This point is important because of potential misinterpretation regarding results from studies carried out under conditions of limiting cell numbers. For instance, while others have found that colchicine inhibits gel reorganization (Bell *et al.* 1979), colchicine had no effect in our model system (unpublished observations). Similarly, it has been suggested that cyclic AMP inhibits the ability of fibroblasts to reorganize collagen gels by interfering with their cytoskeletal function (Ehrlich & Griswold, 1984), but many previous reports have demonstrated that cyclic AMP promotes cytoskeletal interactions necessary for cell adhesion (reviewed by Grinnell, 1978). In the model system we are studying, cyclic AMP had no effect on gel reorganization (unpublished observation), and it is possible that the studies showing that cyclic AMP inhibits collagen gel reorganization were detecting an effect of cyclic AMP on cell growth.

The present studies suggest a number of interesting questions for future analysis. The identity of the serum factor(s) required for complete cell spreading need(s) to be determined. Also, the important factor(s) in conditioned medium require(s) identification. Finally, the question of what cell components are synthesized and incorporated in the matrix under these conditions may provide considerable insight into the mechanism by which the physically reorganized collagen fibrils are stabilized.

We are grateful to Dr William Snell for his helpful comments during this work and regarding the manuscript. This research was supported by a grant from the NIH (GM31321). Clyde Guidry was supported by a pre-doctoral fellowship from the Samuel Roberts Noble Foundation Inc.

REFERENCES

BELL, E., EHRLICH, H. P., BUTTLE, D. J. & NAKATSUJI, T. (1981). Living tissue formed *in vitro* and accepted as skin-equivalent tissue of full thickness. *Science* **211**, 1052–1054.

BELL, E., IVARSSON, B. & MERRILL, C. (1979). Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential *in vitro*. *Proc. natn. Acad. Sci. U.S.A.* **76**,1274–1278.

BELLOWS, C. G., MELCHER, A. H. & AUBIN, J. E. (1981). Contraction and organization of collagen gels by cells cultured from periodontal ligament, gingiva and bone suggest functional differences between cell types. *J. Cell Sci.* **50**, 299–314.

BUTTLE, D. J. & EHRLICH, H. P. (1983). Comparative studies of collagen lattice contraction utilizing a normal and transformed cell line. *J. Cell. Physiol.* **116**, 159–166.

COULOMB, B., DUBERTRET, L., BELL, E. & TOURAINE, R. (1984). The contractility of fibroblasts in a collagen lattice is reduced by corticosteroids. *J. invest. Derm.* **82**, 341–344.

DELVOYE, P., NUSGENS, B. & LAPIERE, C. M. (1983). The capacity of retracting a collagen matrix is lost by dermatosparactic skin fibroblasts. *J. invest. Derm.* **81**, 267-270.

EHRLICH, H. P. & GRISWOLD, B. S. (1984). Epidermolysis bullosa dystrophica recessive fibroblasts produce increased concentrations of cAMP within a collagen matrix. *J. invest. Derm.* **83**, 230–233.

EHRLICH, H. P. & WYLER, D. W. (1983). Fibroblast contraction of collagen lattices *in vitro*: Inhibition by chronic inflammatory cell mediators. *J. cell. Phys.* **116**, 345–351.

GRINNELL, F. (1978). Cellular adhesiveness and extracellular substrata. *Int. Rev. Cytol.* **53**, 65–144.

GRINNELL, F. & LAMKE, C. R. (1984). Reorganization of hydrated collagen lattices by human skin fibroblasts. *J. Cell Sci.* **66**, 51–63.

GRINNELL, F. & MINTER, D. (1978). Attachment and spreading of baby hamster kidney cells to collagen substrata: Effects of cold-insoluble globulin. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4408–4412.

HARRIS, A. K., STOPAK, D. & WILD, P. (1981). Fibroblast traction as a mechanism for collagen morphogenesis. *Nature, Lond.* **290**, 249–251.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. (1951). Protein measurements with the Folin phenol reagent. *J. biol. Chem.* **193**, 265–275.

SCHOR, S. L., ALLEN, T. D. & HARRISON, C. J. (1980). Cell migration through three-dimensional gels of native collagen fibres: Collagenolytic activity is not required for the migration of two permanent cell lines. *J. Cell Sci.* **46**, 171–186.

STEINBERG, B. M., SMITH, K., COLOZZO, M. & POLLACK, R. (1980). Establishment and transformation diminish the ability of fibroblasts to contract a native collagen gel. *J. Cell Biol.* **87**, 304–308.

STOPAK, D. & HARRIS, A. K. (1982). Connective tissue morphogenesis by fibroblast traction. *Devl Biol.* **90**, 383–398.

(Received 11 April 1985 – Accepted 8 May 1985)