REGULATION OF MORPHOLOGY AND PHYSIOLOGY OF EPITHELIAL CELLS BY COLLAGEN FIBRILS

KATSUTOSHI YOSHIKATO¹, AYAKO MAKINO^{1,2} and KIMIKO NAGAYOSHI³

¹Developmental Biology Laboratory, Department of Biology, Faculty of Science, Tokyo Metropolitan University, Fukazawa, Setagayaku, Tokyo 158, and ³Department of Ultrastructural Research, Kitasato University School of Nursing, Kitasato, Sagami-hara 228, Japan

ABSTRACT

Interactions between epithelial cells and collagen fibrils were investigated using primary cultures of newborn rat epidermal cells and adult rat hepatic parenchymal cells. Morphology and the rate of DNA synthesis of these cells were regulated by collagen fibrils in a dose-dependent manner. Collagen fibrils at more than 50 $\mu\text{g}/\text{cm}^2$ of growth area greatly reduced spreading of the two types of cells. The rate of DNA synthesis of these cells was inversely related to the quantity of the collagen fibrils available to the cell: when cultured on the collagen fibrils at 500 $\mu\text{g}/\text{cm}^2$, the rates of synthesis of epidermal and hepatic cells decreased to 42% and 14% of those on the plastic culture dish, respectively. Collagen in a heat-denatured or a native, but non-polymerized form showed no such actions or less effective on the cells, indicating the importance of a fibril formation in the cell to collagen interactions. The transglutaminase activity of epidermal cells and the secretion of albumin by hepatic parenchymal cells increased proportionately to the amounts of collagen fibrils, suggesting that collagen fibrils support to induce and stabilize the expressions of differentiated functions of the epithelial cells.

Cells *in vivo* have close relationships with collagen fibers (5), which are a major component of extracellular matrices (ECM), though the extent of the interactions varies from one type of cells to another. Cells in connective tissues are surrounded with ECM and therefore receive intense biochemical and morphologic regulations by collagen fibers (13, 15). On the other hand epithelial cells interact with ECM through a half of their surface (ventral surface); they attach to and spread on basement membranes. Therefore, it is inferred that the epithelial cells receive regulations by collagen

fibers in a way different from the connective tissue cells.

Generally, collagen has been used as a natural substrate for cells and attracts many cell biologists to investigate cell to collagen interactions (6). We previously reported the interactions of fibroblasts of dermal connective tissues with collagen (21, 30-33), and showed that reconstituted collagen fibrils markedly influence the morphology and the rate of DNA synthesis of the cell.

We were interested to know if such interactions observed in fibroblasts are also present in epithelial cells. In the present study, effects of collagen on two types of epithelial cells have been investigated for their spreading, the rate of DNA synthesis and differentiated functions. We report herein that the morphology

²Present address: Preclinical Evaluation Section, Technical R & D Division, Terumo Corporation, Shibuyaku, Tokyo 151, Japan

and physiology of the epithelial cells are regulated by collagen fibrils in a manner almost identical to that observed in fibroblasts.

MATERIALS AND METHODS

Chemicals and Culture Materials

Transferrin, aprotinin, prolactin, linoleic acid, insulin, and trypsin were from Sigma Chemical; glucagon from Calbiochem-Boehringer; epidermal growth factor (EGF) from Collaborative Research; ethylenediaminetetraacetate (EDTA) and *N*-2-hydroxyethylpiperadine-*N'*-2-ethansulfonic acid (HEPES) from Dojin Chemical Institute; fetal calf serum (FCS) from Commonwealth Serum Laboratory; culture media, RPMI 1640 and medium 199 (M199) from GIBCO; antibiotics (penicillin and streptomycin) from Meiji Seika Kaisha; bacterial collagenase for preparation of hepatocytes from Wako Pure Chemical Industries; dispase for preparation of epidermal cells from Godo Shusei Co.; collagenase (form III, *Clostridium histolyticum*, nonspecific protease-free) from Advance Biofactures; methyl- ^3H thymidine (specific radioactivity, 20 Ci/mmol) from New England Nuclear; and Falcon culture dishes from Becton Dickinson Labware. All other chemicals were of reagent grade and purchased from Wako Chemical Industries or Nakarai Chemicals.

Animals

Sprague-Dawley rats (male, 6-week-old, weighed 150–200 g) and newborn rats of inbred Fisher (male, specific pathogen-free, within 48 h after birth) were purchased from Shizuoka Laboratory Animal Center.

Cells

Epidermal cells were obtained from the newborn rat skin as described previously (16, 28). The whole skin of newborn rats was dissected into small pieces (5 mm $\times$ 5 mm) and digested with dispase (1,000 units/ml) at 4°C for 24 h. The epidermis was separated from dermis with forceps, treated with 0.25% trypsin for 20 min at 25°C, transferred into medium of M199 with 20% FCS, and was stirred gently for 30 min at room temperature. The cell suspension thus obtained was passed through

a nylon mesh (250 mesh) and epidermal basal cells were separated on a density gradient medium of Percoll as described previously (21, 22). The basal cells were inoculated at 10^5 cells/cm² in 35-mm dishes and were cultured in 2 ml of M199 containing 10% FCS, 10 mM NaHCO₃, 20 mM HEPES, 100 $\mu\text{g}/\text{ml}$ streptomycin and 100 units/ml penicillin in a moist atmosphere of 5% CO₂ and 95% air. The first medium change was performed after 24 h and then media were changed every other day.

Hepatic parenchymal cells were obtained from Sprague-Dawley rats by the method of collagenase perfusion according to Seglen (27). The cells were suspended in culture medium and inoculated in 35-mm diameter dishes at 2×10^4 cells/cm² of growth area. The culture medium was a defined serum-free medium prepared mainly according to Hasegawa *et al.* (14) and Enat *et al.* (7). This medium contained following ingredients in RPMI 1640: 20 mU/ml prolactin, 10 ng/ml human growth hormone, 0.5 $\mu\text{g}/\text{ml}$ insulin, 0.5 $\mu\text{g}/\text{ml}$ glucagon, 50 $\mu\text{g}/\text{ml}$ EGF, 2.5 $\mu\text{g}/\text{ml}$ linoleic acid, 0.5 $\mu\text{g}/\text{ml}$ transferrin, 0.3 $\mu\text{g}/\text{ml}$ aprotinin, 0.1 μM CuSO₄·5H₂O, 3 nM H₂SeO₃, 50 μM ZnSO₄·7H₂O and 2.1 $\mu\text{g}/\text{ml}$ phosphoenolpyruvate.

Preparation of Collagen Coated Dishes

Collagen was extracted and purified as described previously (21, 32); following procedures were carried out aseptically. Pieces of the calf skin dermis were digested by pepsin in a dilute HCl solution (pH 3) for 3 days. The digest was passed through Millipore filters. After inactivating pepsin, collagen was precipitated by adjusting pH of the filtrate to 7. The precipitate was resolubilized in the dilute HCl. Collagen was further purified by repeating this cycle of precipitation and solubilization three times. The final precipitate was dissolved in the dilute HCl and used for the present study. The collagen thus obtained was electrophoretically pure and was completely digestible by nonspecific protease-free bacterial collagenase. This preparation of collagen contained type I and type III collagens at a ratio of 49:1. Plastic dishes were coated with collagen as detailed previously (32): One milliliter of collagen solution was placed in 35-

mm dishes and air-dried aseptically. Culture medium (2 ml) was added to the coated dishes 1 h prior to use and the dishes were kept at 37°C to polymerize collagen molecules into fibrils. When it is necessary to avoid a formation of collagen fibrils, coated dishes were irradiated by a 6W-UV lamp (maximum absorbance at 254 nm and the distance between the dishes and the UV lamp was 20 cm) for 30 min at 4°C in an atmosphere of N₂ and were processed as described above. The UV irradiation did not denature collagen molecules as evidenced by measuring helical contents of collagen with optical rotary dispersion.

Cell Morphometry

The extent of cellular spreading was quantitated by measuring area of a cell as follows. Cells were photographed through an inverted phase contrast microscopy (Nikon, TMD-2). The cells on photomicrographs were analyzed by the digitizing interactive image analysis system (Model IBAS-1, Carl Zeiss Co., Oberkochen).

Measurement of the Rate of DNA Synthesis

Cells (hepatic parenchymal cells or epidermal cells) on collagen coated dishes were labelled with 1 μ Ci/ml [³H]thymidine for 4 h in the culture condition described above for each type of the cells. The isotope incorporation was terminated by adding 1,000 times excess amounts of non-labelled thymidine to the media. Cells were dissociated from dishes by digesting with 0.2% collagenase and were pelleted into conical test tubes. The pellets were processed as described previously (32) and were dissolved in a solution of 0.2% Triton-0.1 N NaOH. Aliquots of the dissolved materials were used for fluorometric measurement of DNA concentrations (10), and the remainder was measured for radioactivity.

Assay for Transglutaminase of Epidermal Cells

Epidermal cells cultured for 6 days were harvested from dishes by treatment with 0.2% collagenase, suspended in 50 mM Tris-HCl buffer, pH 8.6, containing 1 mM EDTA and

1 mM dithiothreitol and were homogenized by a Kontes sonicator (Vineland, NJ). The homogenate was centrifuged at 15,000 g for 60 min and transglutaminase activity in the supernatant was determined by the method of Buxman *et al.* (3). Casein solution (0.2%) 0.5 ml was combined with 0.25 ml of monodansyl cadaverine solution (2 mM) and 0.25 ml of the supernatant and incubated at 37°C for 60 min. Reaction products were precipitated with an equal volume of 10% trichloroacetic acid. The extent of dansyl cadaverine incorporation into casein was determined on a Hitachi F-3000 spectrophotofluorometer with excitation and emission wavelengths at 355 nm and 525 nm, respectively. One unit of the enzyme activity was defined as the amounts which produce 1 μ M monodansyl cadaverine-casein complex from casein and monodansyl cadaverine.

Determination of Albumin Produced by Hepatic Parenchymal Cells

The parenchymal cells were cultured in the serum-free defined medium as described above. Aliquots of the medium were subjected to polyacrylamide gel (10%)-electrophoresis in the presence of 0.1% sodium dodecyl sulfate according to Laemmli (18). The gels were stained with Coomassie Brilliant Blue G-250 and were dried. The amounts of albumin separated on the gels were determined by analyzing the gels with a slab gel scanner (Shimazu, SD-910).

Electron Microscopic Observations

One milliliter of collagen solution (0.5%) was placed on a cover glass slip (30 mm) and air-dried. When necessary, dry slips were subjected to a UV-irradiation as described above. The slips were placed in a dish (35 mm) containing 2 ml of phosphate-buffered saline (PBS) and incubated at 37°C for 2 days. The slips were then dipped in 1% glutaraldehyde, 0.5% tannic acid in 0.1 M PBS (pH 6.8), post-fixed with 1% OsO₄ in 0.1 M PBS (pH 7.4) for 1 h at 4°C, dehydrated in a graded series of ethanol, dried by the critical point method, and coated with gold. The slips were viewed through a Hitachi S-700 scanning electron microscope (SEM).

Fig. 1 Epidermal cells on collagen fibrils. One million epidermal cells were cultured for 2 days on 35-mm plastics which had been coated with varied amounts of collagen. On a plain plastic (A), coated with 0.5 μg (B), 5 μg (C), 50 μg (D) and 500 μg (E) of collagen per cm^2 of growth area. $\times 180$

RESULTS

Cellular Spreading on Collagen Fibrils

Epidermal cells Plastic dishes were coated with various amounts of collagen and epidermal cells were cultured upon them for 2 days (Fig. 1). The cells on the dishes coated with

lower amounts (less than 5 $\mu\text{g}/\text{cm}^2$) of collagen spread well and made well-developed epidermal sheets as on plain dishes. However, when the amounts of collagen increased more than 50 $\mu\text{g}/\text{cm}^2$, the extent of spreading and the size of epidermal sheets decreased in a dose-dependent manner.

Fig. 2 SEM observations of UV-irradiated collagen. Collagen was viewed as detailed in the text. A: Collagen ($714 \mu\text{g}/\text{cm}^2$) without a UV-irradiation, B: Collagen ($714 \mu\text{g}/\text{cm}^2$) subjected to a UV-irradiation. The scale bars represent $1 \mu\text{m}$.

The UV-irradiation of collagen which had been coated to plastics and air-dried completely inhibited the formation of fibrils on plastics (Fig. 2). The epidermal cells cultured on the UV-irradiated collagen did not show any collagen-dependent change in cell morphology observed in collagen fibrils described above

(Fig. 3). The collagen which had been heat-denatured also did not show such a morphologic effect on epidermal cells as native collagen even at $500 \mu\text{g}/\text{cm}^2$ (Fig. 3F). These two results suggest the importance of a fibril formation in the cell to collagen interactions.

The collagen-induced change in the cellular

Fig. 3 Epidermal cells on collagen treated with UV or heat. One million epidermal cells were cultured for 2 days on 35-mm plastics coated with collagen which had been treated with UV or heat. A: Cells on a plain plastic. B through E: Cells on UV-irradiated collagen (B: $0.5 \mu\text{g}/\text{cm}^2$, C: $5 \mu\text{g}/\text{cm}^2$, D: $50 \mu\text{g}/\text{cm}^2$, and E: $500 \mu\text{g}/\text{cm}^2$). F: Cells on collagen at $500 \mu\text{g}/\text{cm}^2$, which had been boiled for 10 min. $\times 180$

morphology was quantitated by measuring the area of a cell (Fig. 4). Up to $5 \mu\text{g}/\text{cm}^2$, collagen manifested rather some improvement in the spreading. The ratio (%) of the area at $50 \mu\text{g}/\text{cm}^2$ and $500 \mu\text{g}/\text{cm}^2$ to that at $5 \mu\text{g}/\text{cm}^2$

was 62% and 37%, respectively. In contrast, UV-irradiated collagen showed a significant improvement of the cellular spreading up to $500 \mu\text{g}/\text{cm}^2$, where 2-fold more spreading occurred as compared to plain dishes.

Fig. 4 Spreading of epidermal cells on collagen. One million epidermal cells were plated on 35-mm collagen-coated dishes and were cultured for 2 days. ●—●: UV-Irradiated collagen, ○—○: Native collagen. Δ: Plain plastic. Each point includes 50 determinations; bars represent S.E.M.

Hepatic parenchymal cells Collagen induced the same morphologic changes in the hepatic parenchymal cells as in the epidermal cells and the UV-irradiation abolished these effects of collagen (Fig. 5). A formation of fibrils could not be observed with naked eyes or photomicroscopically when collagen had been coated at concentrations less than 5 $\mu\text{g}/\text{cm}^2$. At a concentration of 50 $\mu\text{g}/\text{cm}^2$, the fibril formation could not be noticed with naked eyes, but could be observed photomicroscopically. Thin fibrils at this concentration were scattered on the plastic surface and looked unstable and sticky. At 500 $\mu\text{g}/\text{cm}^2$, the fibril formation was easily noticed even with naked eyes, because the surface of plastic turned opaque. The fibrils at this concentration were firmly interconnected each other and looked very stable.

The changes in cell morphology are clearly demonstrated quantitatively in Fig. 6, by measuring the area of a cell. Collagen improved the cellular spreading up to 5 $\mu\text{g}/\text{cm}^2$, where 50% increase in the cell area was seen as compared to plain dishes. Beyond this concentration, collagen manifested opposite effects on the cells. The parenchymal cells drastically diminished the size of both the cell and the colony. At 50 $\mu\text{g}/\text{cm}^2$ collagen, the cellular size was about 40% of that at 5 $\mu\text{g}/\text{cm}^2$. The area of a cell at 50 $\mu\text{g}/\text{cm}^2$ seemed to be in-

fluenced not only by interactions of the cells with collagen fibrils, but also by a fragility of the fibrils. As described above, collagen fibrils at this concentration looked too unstable to bear with a contractile force generated by hepatocytes. As a result, the area of a cell at 50 $\mu\text{g}/\text{cm}^2$, was less than that at 500 $\mu\text{g}/\text{cm}^2$, where the area seemed to be determined only by interactions between cells and collagen fibrils. The area of a cell on the UV-irradiated collagen was 2,500 μm^2 which was independent on the quantity of collagen.

DNA Synthesis on Collagen Fibrils

Epidermal cells The effect of collagen on the rate of DNA synthesis of epidermal cells was quantified by labelling with [^3H]thymidine for 16 h at day 3 of culture (Fig. 7). The rate of DNA synthesis decreased with the increase of the amount of collagen up to 50 $\mu\text{g}/\text{cm}^2$, where the maximum effect was observed; the rate on 50 $\mu\text{g}/\text{cm}^2$ was about one third of that on plain dishes (Fig. 7A). The UV-irradiation of collagen partially recovered the rate of DNA synthesis, but not completely (Fig. 7B).

Hepatic parenchymal cells Hepatocytes on plastic dishes started to slough off from the dishes at day 4 of culture. The same cells could survive up to 7 days when they were cultured

Fig. 5 Hepatocytes on collagen. Two hundred thousand hepatocytes were plated in 35-mm collagen-coated dishes with 2 ml of medium and cultured for 2 days. A: Without collagen, B: $0.5 \mu\text{g}/\text{cm}^2$ collagen, C: $5 \mu\text{g}/\text{cm}^2$ collagen, D: $50 \mu\text{g}/\text{cm}^2$ collagen, E: $500 \mu\text{g}/\text{cm}^2$ collagen, and F: $500 \mu\text{g}/\text{cm}^2$ collagen irradiated with UV. $\times 180$

on the dishes coated with collagen at the concentrations more than $0.5 \mu\text{g}/\text{cm}^2$. [^3H]Thymidine-incorporation study showed that the rate of DNA synthesis reached the highest level around day 3 and collagen regulated the rate of synthesis in a dose-specific manner; higher amounts of collagen suppressed it and lower

amounts enhanced it as compared to that on plastics (Fig. 8A). Hepatocytes on $500 \mu\text{g}/\text{cm}^2$ collagen synthesized DNA at the rate of one tenth of that on $5 \mu\text{g}/\text{cm}^2$ collagen. The UV-irradiation almost abolished the suppression observed at high doses of collagen (Fig. 8B), suggesting again the importance of a forma-

Fig. 6 Spreading of hepatocytes on collagen. Two hundred thousand hepatocytes were plated in 35-mm collagen-coated dishes with 2 ml of medium and cultured for 2 days. ●—●: UV-Irradiated collagen, ○—○: Native collagen. △: Plain plastics. Each point includes 50 determinations and the error bars represent S.E.M.

Fig. 7 DNA synthesis of epidermal cells on collagen. Epidermal cells were plated at a density of 10^5 cells/cm² in 35-mm dishes which had been coated with native or UV-irradiated collagen and were cultured in 2 ml of medium for 3 days. The rate of DNA synthesis was determined by labelling the cells with [³H]thymidine at the concentration of 1 μCi/ml for 16 h. Each column shows the average of two determinations and error bars indicate their ranges. A: Native collagen, B: UV-Irradiated collagen. The control value (the rate of DNA synthesis on a plain dish) of the experiment B was the same as in the experiment A, because both experiments were done simultaneously.

Fig. 8 DNA synthesis of hepatocytes on collagen. Hepatocytes were plated at a density of 2×10^4 cells/cm² in 35-mm dishes which had been coated with native collagen (A) or UV-irradiated collagen (B) and cultured in 2 ml of medium for 3 days. The cells were labelled with 1 $\mu\text{Ci/ml}$ of [^3H]thymidine for 4 h. Each column shows the average of two determinations and error bars indicate their ranges. The control value in the experiment B was the same as in the experiment A, because both experiments were done at the same time.

tion of fibrils in the cell-collagen interactions.

Stabilization of Differentiated Functions of Epithelial Cells on Collagen Fibrils

The following study showed that collagen fibrils improve the expression of differentiated functions of epithelial cells.

Epidermal cells The activity of transglutaminase was determined for epidermal cells as a marker enzyme of the differentiation of the cells (Fig. 9). Epidermal cells were cultured on collagen at a lower ($0.5 \mu\text{g/cm}^2$) or a higher ($500 \mu\text{g/cm}^2$) concentration. This activity was significantly elevated in the cells on collagen as compared with those on plastic. The lower amount of collagen was also effective, though the extent of the elevation was less than that of the higher amount of collagen. The UV-irradiation of collagen reduced this effect of collagen, especially at the concentration of $0.5 \mu\text{g/cm}^2$.

Hepatocytes The secretion of albumin was quantitated as a marker of the functions of hepatocytes (Fig. 10). Collagen at less than

$5 \mu\text{g/cm}^2$ did not show any effect on this function. Hepatocytes could not survive beyond the 5th day on plain dishes. Albumin secretion was greatly improved when the cells were cultured more than 3 days on collagen at the concentrations more than $50 \mu\text{g/cm}^2$. The UV-irradiation of collagen completely abolished this improvement (data not shown).

DISCUSSION

The present study clearly demonstrated that collagen modulates the shape, proliferation and expression of differentiated functions of epithelial cells. In this regulation, collagen exhibits quite different actions on the cells, depending upon its form of existence in cell to collagen interactions (a monomeric or a poorly polymerized form and a fibrillar form); the first form of collagen promotes spreading or flattening of the cells and the other form induces less flat shape of the cells.

In the present study, the monomeric or poorly polymerized collagen was produced by heat-denaturing native collagen or by irradiat-

Fig. 9 Transglutaminase activity of epidermal cells cultured on collagen. Epidermal cells were plated at a density of 10^5 cells/ cm^2 on 35-mm dishes coated with varied amounts of collagen and were cultured for 6 days with medium change at every other day. Each column represents the average of 3 determinations $\pm$ S.E.M. A: Native collagen, B: UV-Irradiated collagen

Fig. 10 Secretion of albumin by hepatocytes. Hepatocytes were plated at a density of 2×10^4 cells/ cm^2 in 35-mm dishes in 2 ml of medium and cultured for 6 days. The media were changed with fresh ones at days 1, 3 and 5. The amount of albumin released into the medium was determined every day. The 5 and 6 day time-points for cells on plain plastics are not shown because the hepatocytes could not survive beyond the 5th day. Each point represents the average of 3 determinations and their range was within 10% of the average. $\square$ — $\square$: Without collagen coating. Coated with 0.5 $\mu\text{g}/\text{cm}^2$ ($\circ$ — $\circ$), 5 $\mu\text{g}/\text{cm}^2$ ($\bullet$ — $\bullet$), 50 $\mu\text{g}/\text{cm}^2$ ($\triangle$ — $\triangle$), and 500 $\mu\text{g}/\text{cm}^2$ collagen ($\blacktriangle$ — $\blacktriangle$)

ing air-dried collagen with UV. Electron microscopical observations presented the evidence that UV-irradiated collagen is not capable of aggregating into a fibrillar form (Fig. 2). The mechanism of action of UV upon collagen has not been well understood. However, it is most probable that UV is absorbed by residues of aromatic amino acids and modifies their chemical structures. The irradiated collagen molecules cross-link each other through these modified side chains of amino acids, which inhibits a formation of fibrils when the collagen monomers are incubated at 37°C in a physiological salt concentration (12).

We previously reported that there is a correlation between cell shape and the rate of DNA synthesis of fibroblasts (31, 32). Fibroblasts take on a more flat appearance and show a higher rate of DNA synthesis on a monomeric or poorly polymerized form of collagen, whereas fibroblasts on fibrillar collagen take on a slim and more elongated appearance and show a lower rate of DNA synthesis. The same conclusions can be drawn for cells of epithelial origin, hepatic parenchymal cells and epidermal cells. Therefore, the mode of interactions of fibroblasts with collagen,

which was investigated extensively in the previous work, appears to be applicable to various types of animal cells. However there seems to be a discrepancy for this generalization when the results in Fig. 4 are compared with those in Fig. 7B, because high concentrations of UV-treated collagen do not reduce keratinocyte spreading (Fig. 4), but do cause a decrease in [^3H]thymidine incorporation (Fig. 7B). These results were reproducible. At present, we can not give a satisfactory explanation for this discrepancy. However, when we compare the results of shape *versus* DNA synthesis at the same concentrations, it is seen that native collagen fibrils cause a decrease in both spreading and the rate of DNA synthesis as compared with UV-treated collagen. Furthermore, it is plausible that UV-treated collagen itself has some suppressive effect on the rate of DNA synthesis in keratinocytes through mechanism other than cellular shape.

Folkman and Moscona presented evidence that cell shape determines the rate of DNA synthesis and cell proliferation (11); as cells become more flattened, the rate of their proliferation increases. Our results on the cell shape *versus* DNA synthesis support their study in that low concentrations of fibrillar collagen and all concentrations of UV-treated collagen stimulate the rate of DNA synthesis in hepatocytes (Fig. 8) and also cause increased cell spreading relative to control cultures on plastic (Fig. 6). A similar relationship between cell shape and DNA synthesis, which is induced by collagen, is observed in keratinocytes, although some apparent discrepancy is noticed as discussed above. These results may also be relevant to the observation that stretching stimulates proliferation of epithelial cells (2).

The present study demonstrated the other interesting aspect of cell to collagen interactions. Both epithelial cells, hepatocytes and epidermal cells, expressed higher levels of their differentiated functions on collagen as compared to those on bare plastic. This effect of collagen was also dose-dependent and had some connection to the form of collagen. This was especially the case for hepatocytes. Monomeric or a poorly polymerized form of collagen had no effect on the enhancement of albumin release by the cells.

The difference in the mechanism of inter-

actions of cells with monomeric collagen and polymeric collagen remains to be solved. The first step of cell to collagen interactions is a binding of collagen to the cell surface. Fibronectin has been shown to bind to collagen (8, 9) and to mediate the attachment of cells to collagen (17, 23, 29). However, various types of cells are also capable of binding directly to collagen (1, 19, 20, 24-26). In this regard the result reported by Dedhar *et al.* (4) appears to be suggestive for the interpretation of our finding that cells show quite different behaviors toward collagen fibrils and monomeric collagen. They showed the presence of a cell surface receptor complex which binds to fibrillar Type I collagen, but not to denatured collagen molecules. Therefore, there is a possibility that cells bind to collagen fibrils and denature collagen molecules via different types of cell surface receptors. These initial different binding could induce different responses of the cells to both types of collagen.

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