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# Studies on Fibronectins in the Skin

## VIII. *Influence of Corticosteroids on Cell Cultures from Normal Human Skin*

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Fyrand O. Studies on fibronectins in the skin. VIII. Influence of corticosteroids on cell cultures from normal human skin. Acta Derm Venereol (Stockh) 1985; 65: 379–384.

Fibronectins are important glucoproteins of mesenchymal tissue. Fibronectins are also found in the human skin, and tissue cultures demonstrate the production of soluble dimers and insoluble fibrous polymers from dermal fibroblasts. Under the influence of different glucocorticoids, inhibited production of these fibronectins from cultured human skin cells is demonstrated. (Received September 4, 1984.)

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The therapeutic benefit of corticosteroids with systemic and local application in the treatment of inflammatory dermatoses is well known. Negative side effects of this treatment have been alarming, especially when local halogenated steroids are used. The lack of in vitro test systems for the ability of a substance to cause negative side effects has been a serious drawback.

Steroids show an antimitotic activity combined with a general reduction in the synthesis of proteins. This has been demonstrated in skin fibroblasts for the synthesis of fibrous proteins such as collagen (1, 2). Fibronectins are glucoproteins found in a soluble and a fibrous state in the human body. Fibronectins are produced in different mesenchymal cells. This has been extensively studied in fibroblast cultures (3) and from normal human skin (4). In the human skin, fibrous fibronectin is demonstraed with immunofluorescence technique at the dermoepidermal junction and in the papillary dermis (5).

Fibronectins mediate adhesion in cell/cell and cell/fibre interactions, important for the stabilisation of the tissue (6). The influence of steroids on the production of fibronectins in the skin could therefore be of importance.

In the present study, the effect of hydrocortisone and glucorticosteroids on the production of fibrous and soluble fibronectins from cultured cells from normal human skin is reported.

## MATERIALS AND METHODS

### *Cell cultures*

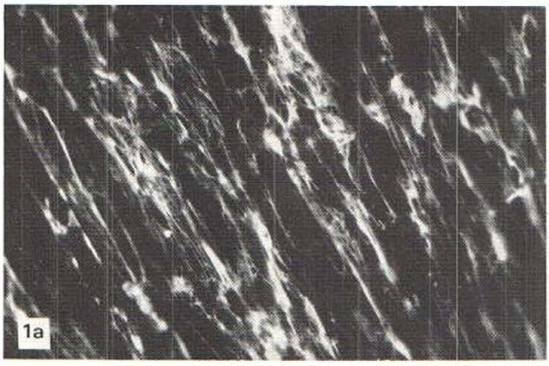
In vitro cultures of fibroblasts from normal human skin were taken from the same pool, cultivated and investigated for the presence of fibronectins as previously reported (4).

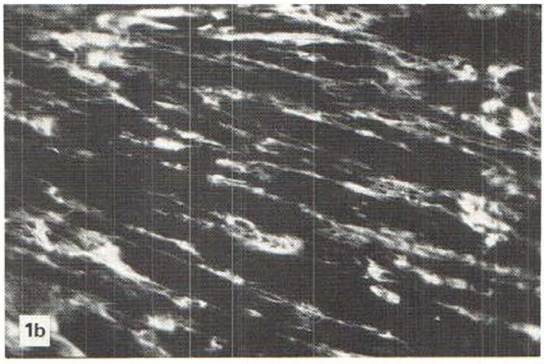
### *Steroids*

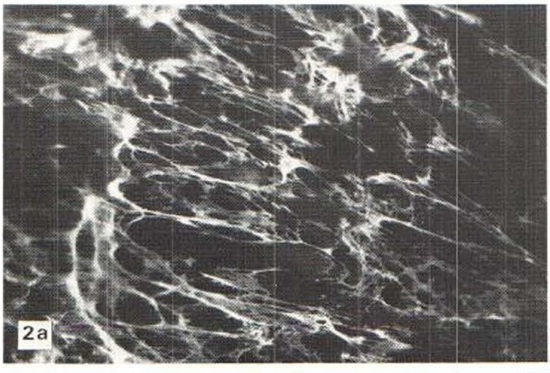
The following steroids were added to the culture medium: hydrocortisone, prednisone, dexamethasone, betamethasone valerate and clobetasol propionate. *Stock solutions:* hydrocortisone and prednisone: 5 mg/ml dissolved in 40% ethanol/0.15 M NaCl. Other steroids: 7.5 mg/ml dissolved in equal parts of DMSO and propylene glycol. These stock solutions were further diluted with the culture medium to a steroid concentration of 25 and 0.5 μg/ml.

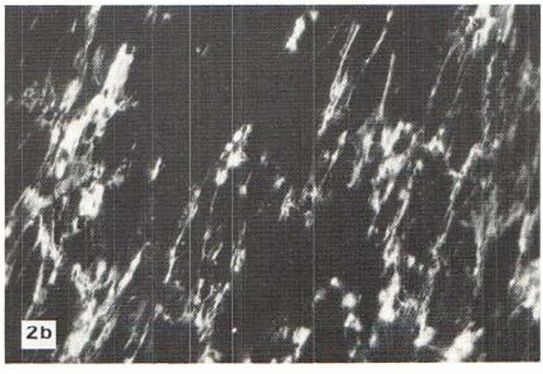
### *Controls*

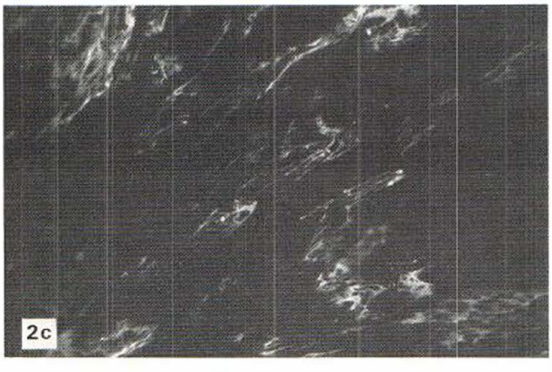
For all experiments the following controls were run in double samples: 1) plain culture medium and 2) culture medium with the addition of ethanol/0.15 NaCl or DMSO/propylene glycol corresponding to the highest concentration of the steroids investigated (25 μg/ml).




*Figs. 1–4.* Immunofluorescence micrographs of fibrous fibronectin in cell cultures from normal human skin, with and without the addition of steroids.

*Fig. 1.* 4 days culture. (*a*) Control. (*b*) Prednisone 25 μg/ml.

*Fig. 2.* 7 days culture. (*a*) Control. (*b*) Clobetasol propionate 0.5 μl/ml. (*c*) Clobetasol propionate 25 μl/ml.

*Production of fibronectins*

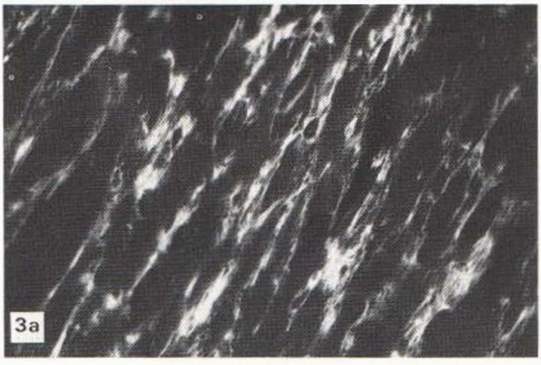
*Indirect immunofluorescence technique (IF)* was used for the demonstration of fibrous fibronectin on glass slides as previously reported (4). Medium containing steroids was used from the beginning of the culture, and in each run eight coverslips were investigated: two different controls and double samples of each steroid concentration. Hydrocortisone was also run at concentrations of 5 and 100 μg/ml.

*Quantitative electroimmunoassay* was used for the quantitation of soluble fibronectin in the culture medium as previously reported (4).

*Cell growth* was studied with samples running parallel to the IF studies. Double countings of cells were performed from separate wells, substituting the culture medium with 0.25% trypsin in Gibco Hanks balanced salt solution added antibiotics, at 37°C for 20 min and centrifuged at 1500 rpm for 10 min. The cell pellet was resuspended in culture medium and counted in a Bürcker cell chamber.

*Reagents*

Trypsin: Difco Lab, Detroit, USA. DMSO: Merck, W-Germany. Propylene glycol: ICI, Manchester, England. Hanks balanced salt solution: Gibco Biocult, Glasgow, Scotland. Hydrocortisone, predni-



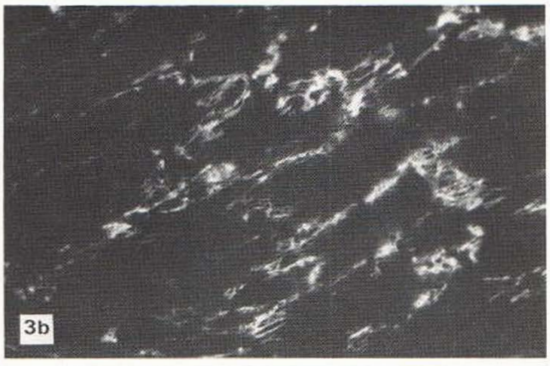




*Fig. 3.* 8 days culture. (*a*) Control. (*b*) Beta-metasone valerate 25 µl/ml.

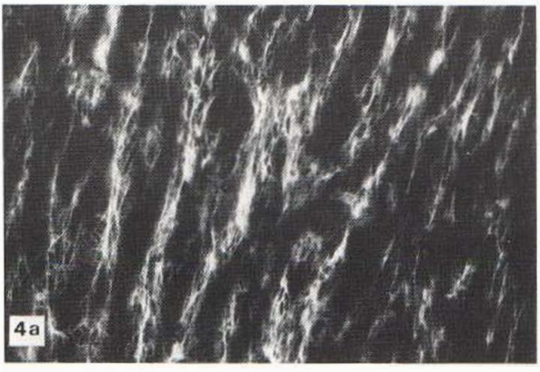




*Fig. 4.* 9 days culture. (*a*) Control. (*b*) Dexametasone 25 µl/ml.

sone, dexamethasone, betamethasone: Sigma, St. Louis, USA. Clobetasol propionate: Gift from Glaxo, Middelsex, England.

## RESULTS

### *Fibrous fibronectin*

The influence of steroids is judged by the IF intensity and the fading of the samples, together with changes in the distribution of the fibronectin pattern.

### *IF intensity and fading*

These phenomena are semiquantitative parameters of the amount of fibronectin present in the samples when the same standard routine is used. As fading of the conjugate occurs by exposure to the ultraviolet light in the microscope, fading is increased in the presence of less antigen.

Compared with the controls, the cultures with steroids show a small increase of the IF intensity at days 1–2. This is followed by a reduction and increased fading of the IF of the samples, correlating with the concentration and the potency of the investigated steroids (Fig. 1*a*, *b*). The lowest IF intensity and the most rapid fading was found at the highest concentrations (25 µg/ml) of clobetasol propionate (Fig. 2*a–c*). With hydrocortisone, only a small inhibition of the steroid cultures was found.

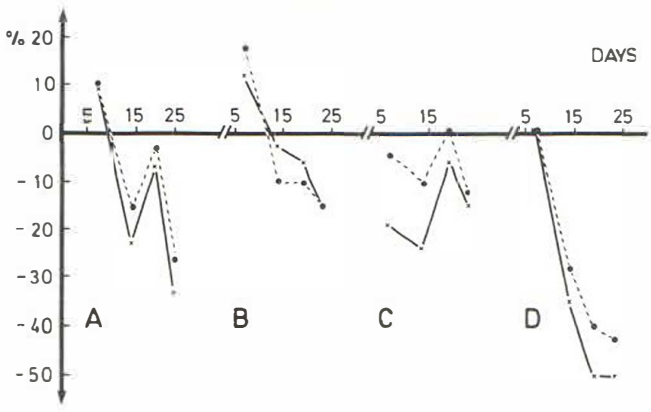



*Fig. 5.* Influence of steroids on the production of soluble fibronectin. The values are given in % compared with controls representing the baseline. A = hydrocortisone, B = prednisone, C = betametasone valerate, D = clobetasol propionate. ×—×, 25 μg/ml; O–O, 0.5 μg/ml.

*IF pattern*

Controls: the distribution of fibronectin develops into a uniform pattern on the coverslip (Figs. 1*a*, 2*a*). In the beginning fibronectin is located as small spots on the cell surface at days 1–2, mainly against the coverslips. In the following days, fibrous structures are formed, developing rapidly into a meshwork of long interconnecting fibres. These fibres are soon reoriented into parallel arrays (Figs. 1–4*a*). In this way, the cells are gradually built into a dense meshwork with a strong IF of the fibronectin fibres (Fig. 4*a*).

*Steroid cultures.* The development of the fibronectin meshwork is different under the influence of steroids. The production of fibronectin fibres is retarded and short and few interconnecting fibres are developed (Figs. 1–4*b*, 2*c*). Only a few areas are developing a meshwork pattern, which also appear smaller and isolated. A retarded development of the parallel orientation of the fibronectin fibres is also found (Fig. 1*b*). In some areas fibronectin fibres are unevenly distributed over the coverslips (Figs. 2–4*b*, 2*c*). These changes are most prominent in cultures with the highest concentrations (Fig. 2*c*) and with the most potent steroids (Figs. 2–4*b*).

*Soluble fibronectin*

The production of soluble fibronectin in cultures with and without steroids has been compared. In Fig. 5, the control values represent the baseline. With low-potent steroids (hydrocortisone and prednisone), a small increase is followed by reduction of the amount of soluble fibronectin. When higher concentrations and more potent steroids (betametasone valerate and clobetasol propionate) are investigated, a more pronounced reduction is found.

*Cell growth*

The study of the influence of steroids upon the cell growth showed that prednisone does not reduce the number of cells in lower concentrations (0.5 μg/ml). In contrast to this a reduction of the cell number is found with betametasone valerate, dexamethasone and clobetasol propionate, especially with the highest concentrations of the steroids.

## DISCUSSION

After the production of fibronectins in the cytoplasma of mesenchymal cells, the glucoproteins are rapidly transported to the cell surface where they are located as multimeric fibrous structures. By enzymatic cleavage, it has been shown that fibronectins have

specific binding sites for various proteins. Receptors have been demonstrated in the fibronectin molecule for fibrin, bacterias, cell surface structures and collagen (7). At the surface of mesenchymal cells, fibronectins are codistributed with collagen (8). In IF studies from normal human skin, fibronectins are mainly located in areas rich in collagen (5). Fibronectins are also produced in a dimeric soluble form into the culture medium of skin fibroblasts (4).

With their binding sites, fibronectins possess adhesive stabilizing properties of the intercellular system of the skin (6). This reflects the important mechanism of the dermoepidermal junction where two different tissue structures meet and are kept together. In this area collagen, laminin and fibronectins are concentrated. The inhibitory action of steroids upon the production and the presence of such proteins in tissue structures such as the skin is therefore of importance. This has been extensively studied in different systems. Generally an inhibitory effect of steroids upon the synthesis of proteins is found. An inhibition can be explained by the presence of specific steroid receptors and the action of steroids upon the RNA messenger system (9, 10). This has also been studied in human skin fibroblasts (2) where inhibited production of fibrous elements like collagen has been reported.

In the present study, an inhibition of the production and a change in the distribution of soluble and fibrous fibronectins have been demonstrated. The inhibition is dependent upon the concentration and the clinical efficacy of the steroids. This could be the consequence of a reduced cell number in the steroid cultures. However, in lower concentrations of prednisone (0.5 μg/ml), the cell number is not reduced although an inhibition of the production of fibronectin is found. Fibronectins act as opsonizing agents, attracting monocytes from peripheral blood (11), and stimulating phagocytosis (12). Fibronectins are also major components active in wound repair mechanisms (13). Together with the functions of fibronectins in cell/cell and cell/fibre interactions and their abundant presence in connective tissue structures such as the skin, an inhibitory influence of steroids on fibronectins may account for some of the observed side effects of local and systemic steroids in the human skin.

## ACKNOWLEDGEMENTS

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