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# GLUCOCORTICOID RECEPTOR IN CULTURED HUMAN SKIN FIBROBLASTS*

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## SUMMARY

Human skin fibroblasts were grown in minimal essential medium with Earle's salts and 15% fetal calf serum. Confluent monolayer subcultures were incubated at 37°C, for 30 min with tritiated glucocorticoid dissolved in medium without fetal calf serum. Free and bound labelled steroids in cell sonicates were separated by chromatography using Sephadex G-25 columns. The specifically bound [$^3$H]-cortisol and [$^3$H]-trimacinolone acetonide represented 75% of the total bound radioactivity. There was no significant displacement of [$^3$H]-cortisol by progesterone, 17-hydroxyprogesterone, testosterone, dihydrotestosterone or estradiol-17$\beta$, but 5$\alpha$-dihydrocortisol, cortisone, corticosterone and asdosterone produced partial displacement. Scatchard analysis of data showed $K_D$ and $B_{max}$ values of 0.4–8.6 M $\times$ $10^{-9}$ and 1.5–11.4 fmol/$\mu$g DNA for triamcinolone acetonide. Using exclusion chromatography on Sephadex G-150 and sucrose gradient, the molecular weight of the glucocorticoid-receptor complex was estimated to be 40,000. In a cell-free system the glucocorticoid-receptor in cytosol was translocated to nuclei while the glucocorticoid alone was not. Thus subcultured human skin fibroblasts contain a cytosol protein which specifically binds glucocorticoid and which appears to be similar to glucocorticoid receptors described in other cell systems.

## INTRODUCTION

High-affinity low-capacity glucocorticoid binding proteins have been demonstrated in the cytosol and nuclear fractions of various types of cells. In a stable line of rat hepatoma tissue culture cells the binding characteristics for glucocorticoids correlated well with their degree of induction of tyrosine aminotransferase activity [1, 2]. The activity of alkaline phosphatase in the cells of subline HeLa 65 had a similar relationship [3]. It was also true for the suppression of glucose uptake by rat thymus lymphocytes [4] or by mouse lymphoma [5, 6] and for the growth inhibition of mouse fibroblasts [7]. Bovine mammary epithelial cells [8], bone cells [9], brain and pituitary of rats [10, 11], and fetal lung of rabbit and sheep [12], all appeared to possess similar glucocorticoid binding proteins. Data obtained in human material other than in HeLa cells [3] are limited to the work with leukemic cells [13], fetal lung [14], lymphocytes [15, 16] and fetal palate [17].

The purpose of this study is to obtain information in man using subcultured skin fibroblasts as fibroblasts can be easily grown from a small skin explant, thereby facilitating multiple experiments. A specific high-affinity, low-capacity binding protein with the characteristics of a glucocorticoid receptor has been demonstrated.

## MATERIALS AND METHODS

[$^3$H]-Cortisol (91.8 Ci/mmol) and [$^3$H]-dexamethasone (20.7 Ci/mmol), obtained from New England Nuclear and [$^3$H]-triamcinolone acetonide (16 Ci/mmol) from Schwartz Bioresearch were subjected to chromatography before use. Tritiated steroids were purified by chromatography every 3 months ([$^3$H]-cortisol on paper in benzene–methanol–water, (100:55:45, by vol.) [$^3$H]-triamcinolone acetonide on silica gel thin layer in $CHCl_3$–ethanol (9:1, V/V)). Non-radioactive dexamethasone was a generous gift from Merck, Sharp and Dohme's Research Division. Other steroids used were purchased from Sigma and Mann Research Laboratories. Protease (type IV), protease-free RNA-ase A (type XII-A) and DNA-ase I were obtained from Sigma.

Crystalline bovine serum albumin and egg albumin (Nutritional Biochemical Company) and purified soybean trypsin inhibitor (Worthington) served as standards in density gradient ultra-centrifugation. This was done in a Beckman type L ultracentrifuge, equipped with a swinging-bucket rotor (SW 50.1) holding 5 ml tubes.

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Processing of skin samples and serial subculturing of the fibroblasts in monolayer were described earlier [18–20]. From 2 to 6 × $10^6$ fibroblasts were grown to confluence in 15 × 100 mm petri dishes. After washing the cells with Hanks' solution (Grand Island Biological) the fibroblasts were incubated at 37°C for 30 min with various steroids in serum free minimal essential medium with Earle's salts (MEM). The MEM was supplemented with non-essential amino-acids obtained from Grand Island Biological, 27 mM sodium bicarbonate, penicillin (25 U/ml), streptomycin (25 μg/ml), amphotericin (2.5 μg/ml) and kanamycin (100 μg/ml) [19]. After trypsinization for 12 min at 37°C with 0.25% trypsin (Grand Island Biological) in Hanks' solution and neutralization of trypsin activity by rapid cooling to 0°C and addition of 2 ml of a mixture of 20 mM Tris, 0.32 M sucrose, 1 mM $MgSO_4$ and 0.1% bovine gammaglobulin (Calbiochem, fraction II) the loose cells were centrifuged for 10 min at 1,250 *g*. For detection of binding in whole cells the cell pellet was resuspended in 1 ml of 0.02 M Tris buffer pH 7.5, containing 0.5 M KCl and 1.5 mM EDTA. It was then sonicated in a Bransonic III ultrasonic cleaner (Branson Instruments Co.) to break up cell and nuclear membranes. A second centrifugation at 1,600 *g* for 20 min gave a clear solution. Steroid bound to the macromolecular fraction was then separated from non-bound by gel-exclusion chromatography using Sephadex G-25 coarse grade columns (15 × 70 mm) and the same "high-salt" buffer as indicated for resuspension [18].

Specific binding was defined as the amount of [$^3$H]-glucocorticoid binding which could be displaced by an excess of unlabelled glucocorticoid. The fraction which could not be displaced was considered to represent non-specific binding and amounted to about 25% of the total binding at the saturating concentration of [$^3$H]-steroid. Column chromatography of cell sonicate labelled with [$^3$H]-triamcinolone acetonide was done on Sephadex G-150 superfine grade, 450 × 9 mm, using as eluant 0.5 M KCl and 0.5 mM EDTA in 0.02 M Tris–buffer (pH 7.4) with a flow rate 0.11 ml/min. When measuring molecular weights, a Sephadex G-75 column (fine grade, 450 × 25 mm) was calibrated with ribonuclease A (M.W. 13,700), chymotrypsinogen A (M.W. 25,000), ovalbumin (M.W. 45,000) and aldolase (M.W. 158,000), obtained from Pharmacia kit. The eluant buffer was the same as mentioned for the Sephadex G-150 procedure (flow rate 8.5 ml/h).

Unless mentioned otherwise all procedures were carried out in an ice-bath or in a cold room at 4°C. Counting [$^3$H]-activity in effluent (1 ml) was done in New England Nuclear "Formula 947" liquid scintillation counting fluid (10 ml). Binding data were derived from Scatchard plots [21] constructed by linear regression analysis.

DNA was measured following Burton's method [22]. Protein assays were done according to Lowry[23].

For binding studies in a cell-free system trypsinized fibroblasts were lysed by hypotonic shock in 20 mM Tris-buffer (pH 7.5) with 0.5 mM $MgCl_2$ and 1.0 mM $CaCl_2$ and passage of the cells through a 25 G needle (10×). After restoration of isotonicity by adding 1/8 volume of the same Tris-buffer with 2.2 M sucrose, crude cytoplasmic and nuclear fractions were separated by centrifugation at 2,000 *g*. Cytosol was then obtained by centrifugation of the cytoplasmic fraction for 1 h at 149,000 *g* in a Beckman type L ultracentrifuge. Incubation with [$^3$H]-triamcinolone acetonide (1.5 nM) in the absence or presence of a 100-fold concentration of non-radioactive steroid followed for 2 h at 0°C. The crude nuclear pellet was resuspended in 20 mM Tris-buffer (pH 7.5) containing 0.5 mM $MgCl_2$, 1.0 mM $CaCl_2$ and 1.8 M sucrose. Aliquots of 3 ml of this nuclear suspension were layered over 2 ml portions of the same Tris-sucrose buffer in 5 ml polyallomer tubes. After ultracentrifugation for 1 h at 149,000 *g* in a swinging bucket rotor (SW 50.1) a pellet of well purified and intact nuclei was obtained, whereas a layer of other particulate material remained floating on top of the buffer solution. The nuclear pellets were washed with 5 ml aliquots of 20 mM Tris-buffer (pH 7.5), containing 0.5 mM $MgCl_2$, 1.0 mM $CaCl_2$ and 0.25 M sucrose and thereafter combined with the preincubated cytosol fractions for 1 h at 22°C.

After separation of cytosol and nuclei by centrifugation the nuclei were washed again, resuspended in 1.0 ml aliquots of 20 mM Tris-buffer (pH 7.5), containing 0.5 M KCl, 1.5 mM EDTA and 1 mM $MgSO_4$ and sonicated. 0.7 ml aliquots of cytosol or nuclear sonicate were chromatographed on Sephadex G-25 columns as for the separation of bound and unbound [$^3$H]-activity in the whole-cell sonicate. Also, 0.25 ml aliquots of sonicate were taken for DNA-estimation.

## RESULTS

Incubation of intact cells with increasing concentrations of tritiated cortisol, triamcinolone acetonide

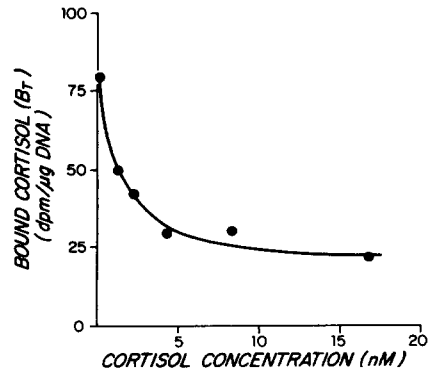


Fig. 1. Competition between cortisol and [$^3$H]-cortisol (3.0 nM) for total binding ($B_T$) in human skin fibroblasts. Whole cells were incubated with the steroid and thereafter treated as described in Methods.

Table 1. Specificity of the glucocorticoid receptor*

| Compound added | Displacement of 3.0 nM [$^3$H]-cortisol by a 100-fold non-radioactive steroid (%) | Displacement of 5.8 nM [$^3$H]-triamcinolone acetonide by a 50-fold amount of non-radioactive steroid (%) |
|---|---|---|
| Cortisol | 100 | 44 |
| Triamcinolone | 100 | 100 |
| Dexamethasone | 100 | 92 |
| Corticosterone | 71 | 60 |
| Cortisone | 61 | — |
| 5α-dihydrocortisol | 68 | — |
| 5β-dihydrocortisol | — | 23 |
| Aldosterone | 29 | 16 |
| Progesterone | 0 | — |
| 17-hydroxyprogesterone | 0 | — |
| 11-deoxycortisol | 13 | 35 |
| Testosterone | 14 | 2 |
| 5α-dihydrotestosterone | 0 | 20 |
| Estradiol-17β | 14 | — |

* The difference in the relative amounts of non-radioactive steroids used to displace [$^3$H]-cortisol and [$^3$H]-triamcinolone acetonide should be noted.

or dexamethasone resulted in the apparent saturation of a macromolecular binder present in whole cell sonicates. Saturation usually occurred at 6–10 nM concentration. Figure 1 shows the displacement of [$^3$H]-cortisol by increasing concentrations of non-radioactive cortisol. A 100-fold excess concentration of non-tritiated cortisol displaced 70–75% of the bound labelled cortisol. This displaceable fraction was considered as the specifically bound fraction $B_S$. The non-displaceable fraction was proportional to the [$^3$H]-glucocorticoid concentration used and was considered as non-specific ($B_N$).

Since binding equilibrium was reached in intact cells within the incubation period of 30 min at 37°C, $B_S$ could be calculated as the difference between the total amount of bound radioactivity, $B_T$ and $B_N$. Because of the linear proportionality of $B_N$ to the concentration up to 20 nM of $^3$H-steroid used, $B_N$ could be derived for any $^3$H-steroid concentration from the value for $B_N$ obtained at one particular concentration of [$^3$H]-steroid in the presence of an excess of nonradioactive steroid [18].

Binding was observed in intact fibroblasts of skin of both male and female subjects from the neck, wrist, buttock, inguinal area, mons and foreskin. The values for the apparent dissociation constants $K_D$ and the maximum binding $B_{max}$ at 37°C were derived from Scatchard-analysis of data from incubation experiments with triamcinolone acetonide. $K_D$ values ranged from 0.4 to 8.6 M $\times 10^{-9}$ and $B_{max}$ values from 1.5 to 11.3 fmol/μg DNA. Values for cortisol were in the same range. Using the average DNA-content of 12.5 μg per $10^6$ cells [19] it could be calculated that approx. 35,000 (range 3250–85,000) binding sites were present per cell. The variation in $K_D$ and $B_{max}$ could not be explained on the basis of source of skin, sex of patient, or subculture number of cells. Glucocorticoid binding was also observed in the skin fibroblasts from a subject with the androgen insensitivity syndrome, in which no dihydrotestosterone-receptor complex could be demonstrated [19].

The specificity of binding of intact cells was investigated by using various non-radioactive steroids in large concentrations to study displacement of [$^3$H]-cortisol or [$^3$H]-triamcinolone acetonide. The results as reported in Table 1 are the means of two series of experiments, each one carried out in duplicate. No significant displacement was seen with progesterone, 17-hydroxyprogesterone, 11-deoxycortisol, testosterone, dihydrotestosterone or estradiol-17β. Partial displacement occurred with aldosterone, corticosterone or 5α-dihydrocortisol. The same appeared to be true for cortisone. Displacement of [$^3$H]-dexamethasone by various concentrations of non-radioactive dexamethasone, triamcinolone acetonide and cortisol revealed that binding was strongest with triamcinolone acetonide and weakest with cortisol. [$^3$H]-triamcinolone acetonide in turn was most efficiently displaced by itself and dexamethasone; it was inefficiently displaced by prednisolone and cortisol (Fig. 2).

Aliquots of a whole cell sonicate of intact cells previously labelled with $^3$H-triamcinolone acetonide, in the presence or absence of a 100-fold excess of cold triamcinolone acetonide, were incubated with protease (type IV), RNA-ase A, DNA-ase I (200 μg per ml of sonicate for 30 min at 37°C). The results were compared with those obtained from incubations with buffer solution only. Specific binding $B_S$ decreased to 0.3% of the control in the sonicate treated with protease, to 91.2% when treated with RNA-ase and did not change at all when treated with DNA-ase, demonstrating the protein nature of the specific binder.

For the first 4 h, the relative stability of the steroid–protein complex in the whole cell sonicate at 0°C was lowest for cortisol, higher for dexamethasone and highest for triamcinolone acetonide (Fig. 3). After 24 h, the relative stability for triamcinolone acetonide

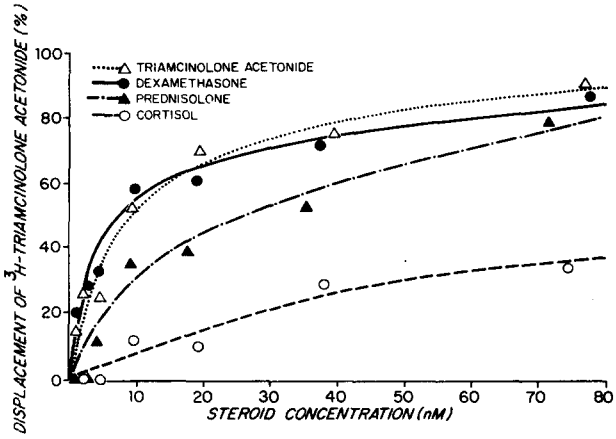


Fig. 2. Displacement of [$^3$H]-triamcinolone acetonide (3.3 nM) from its receptor by various amounts of non-radioactive triamcinolone acetonide, dexamethasone, prednisolone and cortisol. The data were obtained from whole cell sonicates. The 0% displacement represented the total binding of [$^3$H]-triamcinolone acetonide at a 3.3 nM concentration; the displacements obtained with various steroid concentrations were expressed as percentage of this original value.

remained the highest, that of dexamethasone being smaller than that of cortisol.

A whole cell sonicate prepared from approx. $15 \times 10^6$ intact cells previously incubated with 2.5 nM [$^3$H]-cortisol in the usual way was extracted with carbon tetrachloride (10 fold volume × 2). After addition of [$^{14}$C]-cortisol for recovery purposes the extract was chromatographed on silica gel thin layer (benzene–ethyl acetate, 110:90, V/V) as well as on paper (methanol–water–benzene, 55:45:100, by vol.). In both systems, 72% of the [$^3$H]-activity had the same mobility as cortisol.

For the purpose of a further characterization of the triamcinolone receptor complex gel exclusion chromatography on Sephadex columns was done. Three radioactive peaks were eluted from a G-150 column (Fig. 4). The first [$^3$H]-triamcinolone peak was very small and was associated with the main protein peak. It was non-displaceable by large amounts of non-radioactive steroid and was considered as the non-specifically bound activity. The second $^3$H-peak followed the main protein peak and was entirely displaceable, representing therefore the specifically bound activity. The third peak was the unbound steroid. For [$^3$H]-dexamethasone practically identical results were seen. No significant second peak could be demonstrated with [$^3$H]-cortisol. G-100 columns gave very similar patterns. Using a Sephadex G-75

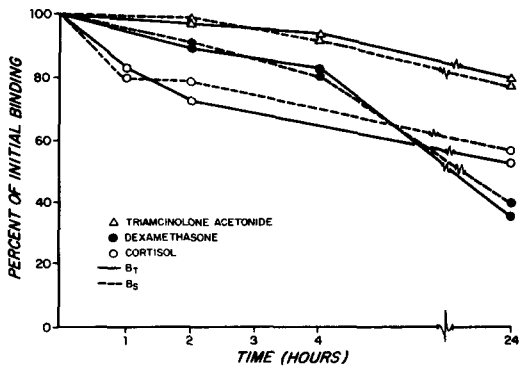


Fig. 3. Effect of time on the stability of the glucocorticoid-receptor complex at 0°C. The 100% mark represents binding values obtained at zero time with [$^3$H]-steroid concentrations of 3.3 nM and with or without a 100-fold excess of non-radioactive steroid. Stability of total binding ($B_T$) and specific binding ($B_S$) were similar.

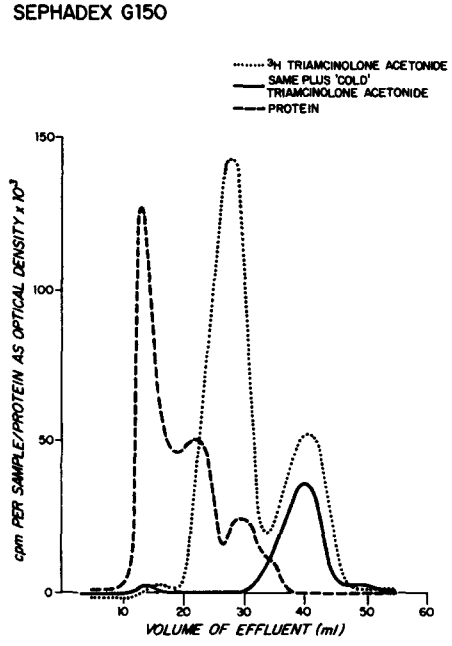


Fig. 4. Elution pattern of radioactivity from a fibroblast sonicate following a 30 min incubation at 37°C with [$^3$H]-triamcinolone acetonide (3.9 nM) in absence or presence of a 100-fold excess of non-radioactive steroid.

column calibrated with purified globular proteins of known molecular weight, an attempt was made to determine the molecular weight of the specific binder [24]. A molecular weight of approx. 42,000 resulted with this method. When adding 10% sucrose to the eluant similar results were obtained.

In a 5–20% sucrose gradient made in the 0.02 M Tris-buffer (pH 7.5) with 0.5 M KCl and 1.5 mM EDTA, a single displaceable radioactive peak of [$^3$H]-triamcinolone acetonide was consistently demonstrated after centrifugation at 149,000 *g* for 18 h to be located between the protein peaks of ovalbumin (3.65 S, molecular weight 45,000) and soybean trypsin inhibitor (molecular weight 21,500) (Fig. 5). The difference of the sites of the peak obtained for [$^3$H]-triamcinolone acetonide alone and for the same plus "cold" cortisol was minimal and could well be explained by the technique of fraction collection. Displacement of the [$^3$H]-triamcinolone acetonide peak by cortisol was less extensive than by equal concentrations of triamcinolone acetonide or dexamethasone.

In order to examine the significance of the cytoplasmic binder for the transfer of [$^3$H]-triamcinolone acetonide to the nucleus, purified nuclei were incubated with aliquots of cytosol preincubated with either [$^3$H]-triamcinolone acetonide, or [$^3$H]-triamcinolone acetonide in the presence of a 100-fold concentration of non-labelled triamcinolone acetonide. Nuclei were also incubated with [$^3$H]-triamcinolone acetonide in buffer solution but without cytosol. In the first experiment, 1355 d.p.m. were recovered in the nuclear preparation while 195 d.p.m. were found in the second experiment, suggesting that the specifically bound [$^3$H]-activity translocated from cytosol to nuclei represented 1160 d.p.m. or 85% of the total. No detectable [$^3$H]-activity could be recovered from the nuclei incubated with buffer.

## DISCUSSION

Extensive evidence exists supporting the concept that a specific glucocorticoid receptor-protein is essential for glucocorticoid action at a cellular level. Most studies were done with cell lines from experimental animals, the only exception being the investigation of cells from human fetal lung and leukemic patients [13, 14]. Furthermore, several cell lines arose from tumors, the genetic properties of which may have been different from those of the original cells. The study of subcultured human skin fibroblasts seems to offer a more direct insight into human physiology or pathology. Problems related to aging and eventual death of these cells can be avoided by the use of a cell bank, i.e., storage of young subcultures suspended in culture medium containing 8% dimethyl sulfoxide and frozen in liquid nitrogen.

In this study a binding capacity for glucocorticoids was demonstrated in whole cell sonicate as well as cytosol. The data showed a fairly large variation, probably due to methodology, but gave the order of magnitude of maximal binding capacity and apparent $K_D$. Because of the greater stability of the triamcinolone acetonide binding protein complex and its virtual absence of metabolism during incubation, most of our experiments were done with this steroid. This is in contrast with our cortisol studies where at least 30% of the steroid was metabolized, the nature of the metabolites remaining to be determined. Others have shown that human skin is able to convert to cortisol to cortisone, allodihydrocortisol, tetrahydrocortisol, Reichstein's substances E and epi-E, U and epi-U [25, 26]. Mouse fibroblasts, however, were not able to interconvert cortisol and cortisone [27]. The results obtained in the specificity studies (Table 1) were not unexpected, regarding the known importance of an 11-hydroxyl-group for glucocorticoid ac-

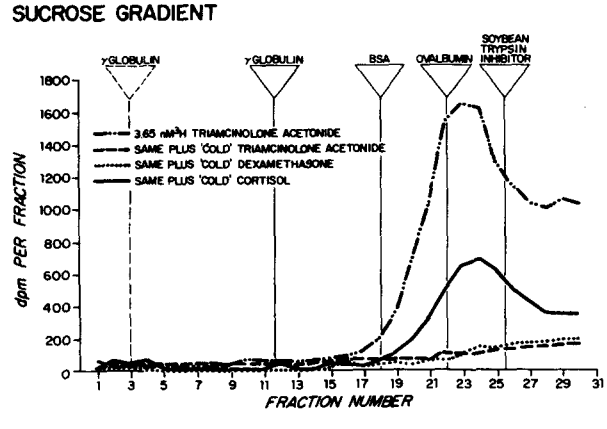


Fig. 5. Radioactivity distribution after sucrose gradient ultracentrifugation (149,000 *g* for 18 h) of a fibroblast sonicate following a 30 min incubation at 37°C with [$^3$H]-triamcinolone acetonide (3.65 nM) in absence or presence of a 100-fold excess of non-radioactive triamcinolone acetonide, or dexamethasone, or cortisol.

tivity [28]. Since human skin fibroblasts are known to possess ample 5α-reductase activity [18], part of the $^3$H-steroid assumed to be specifically bound may represent [$^3$H]-5α-dihydrocortisol. The displacement of [$^3$H]-cortisol by cortisone excess may very well result from conversion of cortisone to cortisol. Cortisone binding itself was found to be absent or minimal in other systems [4, 9, 10, 29]. It is possible that the displacement of dexamethasone by cortisone in hepatoma cells [12] may be due to a metabolite of cortisone rather than to this steroid itself. Although metabolism of cortisol during incubation may explain in part the difference in binding between cortisol and triamcinolone acetonide or dexamethasone, it would appear that lower affinity of the binder for cortisol as demonstrated by displacement studies (Fig. 2), must also play an important role. The affinity for either triamcinolone acetonide or dexamethasone did not differ greatly. Results shown in Fig. 2 suggest an intermediate binding affinity for prednisolone.

The molecular weight of the steroid–receptor complex was determined to be approx. 40,000 by gel-exclusion chromatography as well as by sucrose-gradient centrifugation. The difference in buffers used in the two techniques being sucrose, we repeated the Sephadex procedure with addition of 10% sucrose, this percentage being estimated to be the sucrose concentration on the site in the gradient where the [$^3$H]-triamcinolone acetonide peak was seen. Similar results were obtained with or without addition of sucrose. Since the very nature of the receptor protein is not yet known and Sephadex gel filtration methodology for molecular weight estimation has its pitfalls, the data given may be regarded with some caution.

When skin fibroblasts of a patient with androgen insensitivity were incubated with either [$^3$H]-cortisol or [$^3$H]-triamcinolone acetonide specific glucocorticoid binding was demonstrated, similar to that observed in normal subjects. These findings further emphasize the specificity of cytoplasmic receptors for various steroids in man, and in particular give further support for the specificity of the defect in androgen receptor as previously reported for the syndrome of androgen insensitivity [18–20].

Our experiments with a cell-free system confirmed the existence of a protein in cytosol which binds specifically glucocorticoids. Furthermore the steroid–receptor complex can be readily translocated into nuclei whereas the steroid itself is unable to do so.

This study demonstrates the presence of a glucocorticoid receptor in the human skin fibroblast. A protein present in cytosol specifically binds glucocorticoid with characteristics similar to those reported for glucocorticoid receptor proteins in other cell systems.

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## REFERENCES

1. Baxter J. D. and Tomkins G. M.: The relationship between glucocorticoid binding and tyrosine aminotransferase induction in hepatoma tissue culture cells. *Proc. natn. Acad. Sci. U.S.A.* **65** (1970) 709–715.
2. Baxter J. D. and Tomkins G. M.: Specific cytoplasmic glucocorticoid hormone receptors in hepatoma tissue culture cells. *Proc. natn. Acad. Sci. U.S.A.* **68** (1971) 932–937.
3. Melnykovych G. and Bishop C. E.: Specific binding of cortisol in subcellular fractions of HeLa cells: Temperature dependence and effects of inhibitors. *Endocrinology* **88** (1971) 450–455.
4. Munck A. and Wira C.: Glucocorticoid receptors in rat thymus cells. *Advances in Biosciences* 7 (Edited by G. Raspe). Pergamon, Vieweg, Oxford (1971) pp. 301–330.
5. Hollander N. and Chiu J. W.: *In vitro* binding of cortisol by a substance in the supernatant fraction of P 1798 mouse lymphosarcoma. *Biochem. biophys. Res. Commun.* **25** (1966) 291–297.
6. Baxter J. D., Harris A. W., Tomkins G. M. and Cohn M.: Glucocorticoid receptors in lymphoma cells in culture: Relationship to glucocorticoid killing activity. *Science* **171** (1971) 189–191.
7. Hackney J. F., Gross S. R., Aronow L. and Pratt W. B.: Specific glucocorticoid binding macromolecules from mouse fibroblasts growing *in vitro*. *Molec. Pharmacol.* **6** (1970) 500–512.
8. Tucker H. A., Larson B. L. and Gorski J.: Cortisol binding in cultured bovine mammary cells. *Endocrinology* **89** (1971) 152–160.
9. Feldman D., Dziak R., Koehler R. and Stern P.: Cytoplasmic glucocorticoid binding proteins in bone cells. *Endocrinology* **96** (1975) 29–36.
10. Watanabe H., Orth D. N. and Toft D. O.: Glucocorticoid receptors in pituitary tumor cells. *J. biol. Chem.* **248** (1973) 7625–7630.
11. DeKloet R., Wallach G. and McEwen B. S.: Differences in corticosterone and dexamethasone binding to rat brain and pituitary. *Endocrinology* **96** (1975) 598–609.
12. Giannopoulos G.: Variations in the levels of cytoplasmic glucocorticoid receptors in lungs of various species at different developmental stages. *Endocrinology* **94** (1974) 450–458.
13. Lippman M. E., Halterman R. H., Leventhal B. G., Perry S. and Thompson E. B.: Glucocorticoid-binding proteins in human acute lymphoblastic leukemic blast cells. *J. clin. Invest.* **52** (1973) 1715–1725.
14. Ballard P. L. and Ballard R. A.: Glucocorticoid receptors and the role of glucocorticoids in fetal lung development. *J. clin. Invest.* **53** (1974) 477–486.
15. Smith K. A., Crabtree G. R., Kennedy S. J. K. and Munck A. U.: Glucocorticoid receptors and glucocorticoid sensitivity of mitogen stimulated and unstimulated human lymphocytes. *Nature (Lond.)* **267** (1977) 523–525.
16. Neifeld J. P., Lippman M. E. and Tormey D. C.: Steroid hormone receptors in normal human lymphocytes; induction of glucocorticoid receptor activity by phytohemagglutinin stimulation. *J. biol. Chem.* **252** (1977) 2972–2977.
17. Goldman A. S., Shapiro B. H. and Katsumat M.: Human foetal palatal corticoid receptors and teratogens for cleft palate. *Nature (Lond.)* **272** (1978) 464–466.
18. Keenan B. S., Meyer W. J. III, Hadjian A. J. and Migeon C. J.: Androgen receptor in human skin fibroblasts. Characterization of a specific 17β-hydroxy-5α-androstan-3-one protein complex in cell sonicates and nuclei. *Steroids* **25** (1975) 535–552.

19. Keenan B. S., Meyer W. J. III, Hadjian A. J., Jones H. W. and Migeon C. J.: Syndrome of androgen insensitivity in man: Absence of 5α-dihydrotestosterone binding in skin fibroblasts. *J. clin. Endocr. Metab.* **38** (1974) 1143–1146.
20. Meyer W. J. III, Migeon B. R. and Migeon C. J.: Locus on human X chromosome for dihydrotestosterone receptor and androgen insensitivity. *Proc. natn. Acad. Sci. U.S.A.* **72** (1975) 1469–1472.
21. Scatchard G.: The attractions of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.* **51** (1949) 660–672
22. Burton K.: A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* (1956) 315–323.
23. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J.: Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193** (1951) 265–275.
24. Laurent T. C. and Killander J.: A theory of gel filtration and its experimental verification. *J. Chromatogr.* **14** (1964) 317–330.
25. Malkinson F. D., Lee M. E. and Cutukovic I.: *In vitro* studies of adrenal steroid metabolism in the skin. *J. Invest. Dermat.* **32** (1959) 101–107.
26. Hsia S. L. and Hao Y. L.: Metabolic transformations of cortisol-4-($^{14}$C) in human skin. *Biochemistry* **5** (1966) 1469–1474.
27. Berliner D. L. and Ruhmann A. G.: Comparison of the growth of fibroblasts under the influence of 11β-hydroxy and 11-keto corticosteroids. *Endocrinology* **78** (1966) 373–382.
28. Rousseau G. G., Baxter J. D. and Tomkins G. M.: Glucocorticoid receptors: relations between steroid binding and biological effects. *J. Molec. Biol.* **67** (1972) 99–115.
29. Litwack G., Morey K. S. and Ketterer B.: Corticosteroid binding proteins of liver cytosol and interactions with carcinogens. In *The Effects of Drugs on Cellular Control Mechanism* (Edited by B. R. Rabin and R. B. Freedman). University Park Press. Baltimore (1972) pp. 105–131.