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Glucocorticoids Decrease Prolyl Hydroxylase Activity without the Cellular Accumulation of Undehydroxylated Collagen

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SUMMARY

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Cells were isolated from the dermis of control and triamcinolone-treated rats. The triamcinolone-treated cells were 86% viable as determined by trypan blue exclusion. Radioactive proline was added to the cell cultures and total proline incorporation and proteinaceous hydroxyproline synthesis were quantified. The cells isolated from triamcinolone-treated rats incorporated less proline into cellular protein than did control cultures. The amount of proteinaceous hydroxyproline synthesized by cells isolated from steroid-treated rats was decreased to a greater extent than total proline incorporation. The percent of cellular collagen was decreased in steroid-treated cells. Although prolyl hydroxylase activity (EC 1.14.11.2) in the cells isolated from glucocorticoid-treated rats was decreased, the extent of prolyl hydroxylation of total cellular collagen was not decreased. The data indicate that glucocorticoid treatment does not result in the synthesis and subsequent cellular accumulation of underhydroxylated collagen.

INTRODUCTION

The administration of glucocorticoids has potent antianabolic effects on protein and nucleic acid metabolism. These steroids decrease the synthesis of deoxyribonucleic acid and protein in liver as well as in peripheral tissues (1-10). Glucocorticoids inhibit collagen synthesis. Urinary hydroxyproline excretion is decreased following glucocorticoid administration (3, 11-14). The incorporation of radioactive proline into hydroxyproline, an amino acid found mainly in collagen, is markedly decreased in connective tissues following glucocorticoid administration (2, 5, 7, 15, 16). After multiple daily doses of glucocorticoid, the synthesis of collagen is selectively decreased since hydroxyproline formation is decreased to a greater extent than total proline incorporation (7).

Repeated daily injections of glucocorticoids in rats results in a decrease of prolyl hydroxylase in various tissues (7, 10, 16-18). Glucocorticoid administration also re-

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sults in a decrease of lysyl hydroxylase activity (10, 18) as well as collagen glucosyl and galactosyl transferases (18). Although prolyl hydroxylase activity is decreased in a dose- and time-dependent manner following glucocorticoid administration, there is no concomitant decrease in the extent of hydroxylation of nascent collagen peptides (10).

Prolyl hydroxylase is located within the cisternae of the rough endoplasmic reticulum (19–21). Hydroxylation of proline residues in collagen takes place while the nascent collagen peptide is being synthesized, as well as after the release of the polypeptide (22–24). Although Lazarides *et al.* (22) demonstrated in 3T6 cells that prolyl hydroxylase acts mainly on the nascent collagen peptides, Miller and Udenfriend (23) indicated that in guinea pig granuloma only 40% of the prolyl residues capable of being hydroxylated in nascent collagen peptides were hydroxylated. Recently Uitto and Prockop (24) demonstrated in tendon cells that the synthesis of hydroxyproline and hydroxylysine continues until some time after the assembly of collagen polypeptide chains is completed. Since some hydroxylation of proline residues in collagen occurs after collagen polypeptide synthesis on ribosomes, glucocorticoid treatment could have resulted in the synthesis of underhydroxylated collagen after the release of collagen peptides from ribosomes. In the present study, cells isolated from the dermis of glucocorticoid-treated and control rats were incubated *in vitro* with radioactive proline and the hydroxyproline to proline ratio of cellular collagen was determined. The data indicate that collagen synthesis is selectively decreased in glucocorticoid-treated rat dermal cells. The data, furthermore, indicate that although prolyl hydroxylase activity is decreased, underhydroxylated collagen is not synthesized intracellularly.

MATERIALS AND METHODS

Materials. Eagle's Minimum Essential Medium (F12) penicillin, streptomycin, trypsin (2.5 g/100 ml), and the trypan blue solution (0.4 g/100 ml) were obtained from Grand Island Biochemical Co. *Clostridium Histolyticum* collangenase Type I and trypsin inhibitor Type II-S were obtained from Sigma Chemicals. Protease-free collagenase, ABC form III (2500 units/ml) was obtained from Advanced Biofactures. [2,3-^{3}H]Proline (20 Ci/mmole) and [4-^{3}H]proline (15 Ci/mmole) were obtained from New England Nuclear. Powered triamcinolone diacetate was generously supplied by Dr. Edward Cantrall of Lederle Laboratories, Pearl River, N.Y.

Preparation of dermal cell suspension cultures. Sprague-Dawley rats (1–3 days old) were administered three daily injections of either 0.9% (w/v) NaCl or triamcinolone diacetate (15 mg/kg, i.p.). The skins from 15 control or triamcinolone-treated rats were rinsed in 0.9% NaCl (w/v) at 4°C and incubated for 30 min at 37°C in 90 ml of EMEM[4] containing 100,000 units of penicillin, 0.1 g of streptomycin and 5 g trypsin per liter. All incubations were done in EMEM. The dermis was then separated from the subcutaneous and epidermis layers at 4°C. The dermis tissue was minced in 65 ml of EMEM containing 0.2 g of trypsin and 77 mg of collagenase from *Clostridium Histolyticum.* The sample was incubated at 37°C for 40 min under 95% O_2 5% CO_2. Following incubation the cells were filtered through two layers of cheesecloth. The cell suspension was centrifuged at 900 × *g* for 10 min at 4°C. The cell pellet was resuspended in 20 ml of EMEM containing 6.6 mg of trypsin inhibitor and centrifuged at 900 × *g* for 10 min. The cells were washed twice with trypsin inhibitor solution and recentrifuged. To equilibrate the cells at 37°C, 3–4 × 10^8 cells were suspended in 120 ml EMEM and incubated for 2 hr. The number of cells in the suspension was counted after making a 1:10 dilution with EMEM in a hemocytometer with a phase contrast microscope. An equal number of cells isolated from control and steroid-treated skins were used in each experiment. The total cellular protein content per 10^8 was 3.77 ± 0.20 mg (mean ± SE) and 3.96 ± 0.14 mg for control and treated samples, respectively. Cellular protein was deter-

[4] The abbreviations used are: EMEM, Eagle's Minimum Essential Medium; TCA, trichloroacetic acid.

mined by the method of Lowry *et al.* (25) in 25 separate experiments.

Determination of cell viability. Five parts of the cell suspension were mixed with one part of 0.4% (w/v) trypan blue solution. The cells were allowed to stand at room temperature for 3 min and then examined by phase contrast microscopy. The cells which did not take up the trypan blue stain were counted as viable cells. At least 100 cells were counted in triplicate.

Prolyl hydroxylase activity. Dermal cells (1.5×10^6) were suspended in 3 ml of a buffer containing 0.25 M sucrose, 5×10^{-2} M Tris HCl (pH 7.5), 10^{-5} M dithiothreitol and 10^{-5} M ethylenediamine tetracetic acid. The sample was homogenized for 30 sec at full speed with the Polytron ST system, made 0.1% (w/v) Triton X-100 and homogenized for an additional 15 sec. The homogenate was centrifuged at 20,000 × *g* for 20 min and the resulting supernatant was used for enzyme assay. Enzyme activity was assayed by a modification of the method of Hutton *et al.* (26). Chick embryo substrate was prepared with [4-^{3}H]proline as described (26). The amount of tritiated water formed was linearly related to the time of incubation and the amount of supernatant protein assayed. Final radioactivity in the experimental samples was at least six times the amount of radioactivity of the blank (40 CPM). An aliquot of the 20,000 × *g* supernatant fraction was incubated at 30°C for 30 min in a mixture containing 220 μM Tris HCl (pH 7.5), 4.9 μM ascorbic acid, 0.30 μM ferrous ammonium sulfate, 0.6 μM α-ketoglutarate, 0.2 mg of catalase, 10 mg bovine serum albumin and [4-^{3}H]prolyl substrate (130,000 CPM) made up to a volume 1 ml with deionized H_2O. After incubation at 30°C for 30 min the reaction was terminated by adding one-tenth the volume of 50% TCA. The reaction mixture was vacuum distilled and the resulting tritiated water was counted in a 30% Triton X-100 cocktail. Prolyl hydroxylase is expressed as dpm of tritiated water formed per 30 min per mg supernatant protein.

Total proline incorporation into cellular protein. In order to determine linearity of proline incorporation, $1.4–1.8 \times 10^8$ dermal cells were suspended in 60 ml of EMEM and incubated for 2 hr. [2,3-^{3}H]Proline (120 μCi) was added to each incubation flask and the flasks were incubated for 0.5, 2 or 3 hr. At the end of the incubation period the cells were collected by centrifugation at 900 × *g* for 10 min. The cell pellet was suspended in 20 ml of EMEM and the cells were pelleted by centrifugation at 900 × *g* for 10 min. This wash procedure was repeated. The cells were then suspended in 3 ml of 0.1 M NaCl, 0.05 M Tris HCl (pH 7.4). The sample was homogenized for 45 sec with the Polytron ST system. The sample was placed in a boiling water bath for 10 min, and was then homogenized for an additional 30 sec. An aliquot of the homogenate was assayed for protein content. Two milliliters of 10% (w/v) TCA were added to a 20 μl aliquot of the homogenate. The sample was then filtered through a Millipore filter (type HA). The filter was washed twice with 5% (w/v) TCA, dried under a heat lamp for 10 min and counted in toluene, 2,5-diphenyloxazole, 1,4-bis-2-(4-methyl-5-phenyloxazolyl) benzene. Cells were also incubated with radioactive proline and 10^{-3} and 10^{-4} M cycloheximide. Protein synthesis was inhibited by 93%. This procedure of determining total proline incorporation was used since a large number of samples were assayed in certain experiments.

Total proline incorporation and hydroxyproline formed. Cells (3×10^8) isolated from control and triamcinolone-treated rats were incubated in 120 ml of EMEM for 2 hr. [2,3-^{3}H]Proline (240 μCi) was added and the cells were incubated for 30 min. After the cells were collected and washed as described above, the cell pellet was homogenized in 5 ml of H_2O with the Polytron ST homogenizer for 30 sec. An aliquot was used to determine protein content. An equal volume of 10% (w/v) TCA was added to homogenate at 4°. The sample was centrifuged at 10,000 × *g* for 10 min. The resulting pellet was resuspended in 10 ml of 5% (w/v) TCA and centrifuged. This washing procedure of the cell pellet was repeated. The cellular pellet was suspended in 10 ml of 6 N HCl and the sample was hydrolyzed for 18 hr. The hydrolyzed sample was evaporated, redissolved in 2 ml of water, neu-

tralized and chromatographed on Dowex 50W - X8 (200–400) mesh as previously described (7). The amount of radioactive proline incorporated into cellular protein and the amount of hydroxyproline formed were based on the amount of cellular protein. The proline incorporation and hydroxyproline formation data were calculated based on the specific activity of the TCA soluble proline pool. The TCA soluble material of the cellular homogenate and the two subsequent washes were filtered through Whatman #1 filter paper. The acid soluble fraction was then evaporated, redissolved in 10 ml of 6 N HCl and hydrolyzed for 18 hr in an autoclave. After the addition of 100 mg Norit A and filtration through Whatman #1 filter paper, the sample was evaporated, redissolved in 4 ml of water, neutralized and the proline was isolated by Dowex chromatography as previously described (7). The amount of proline was determined colorimetrically by the method of Troll and Lindsley (27).

Collagenase assay. The amount of labeled proline incorporated into collagen was determined by collagenase digestion of cellular protein. Dermal cells ($1.5–2.0 \times 10^8$) were incubated for 2 hr in 60 ml of EMEM. [2,3-^{3}H]Proline (120 μCi) was added to each flask and the cultures were allowed to incubate for the times indicated. The cells were harvested by centrifugation and washed twice as described above. The cells were suspended in 3 ml of 0.05 M Tris-HCl (pH 7.0), 0.1 M NaCl followed by homogenization with the Polytron ST homogenizer for 45 sec. The sample was then placed into a boiling H_2O bath for 10 min and then homogenized for 30 sec. A 50 μl aliquot was digested with 105 units of bacterial collagenase form III. An aliquot of the sample was added to an incubation mixture containing 80 mM sodium chloride, 0.7 mM magnesium chloride, 10 mM Tris-HCl (pH 7.5), 7.5 mM calcium chloride, 2.5 mM N-ethylmaleimide and bacterial collagenase form III. The total volume of the assay was 0.5 ml. The reaction mixture was incubated for 2 hr at 37°. The reaction was stopped by adding 2 ml of 10% (w/v) TCA on ice. The sample was filtered through a Millipore filter and the filter was counted as previously described. The amount of collagenase-digestible material was quantified by substracting the amount of radioactivity retained on the filter when the sample was digested with collagenase from the amount of radioactivity retained on a filter after incubation of the sample without collagenase. Maximum collagenase digestion was obtained at 1 hr. The amount of collagenase digestible product was not increased by doubling the amount of collagenase. Ninety per cent digestion was obtained as estimated by the amount of labelled hydroxyproline remaining in the tissue pellet after digestion.

Determination of the hydroxyproline to proline ratio of intracellular collagen peptides. Dermal cells ($3–3.5 \times 10^8$) were incubated for 2 hr in 60 ml of EMEM prior to the addition of [2,3-^{3}H]proline (360 μCi). The samples were incubated for an additional 5 hr. The cells were collected and washed twice with EMEM as described above; they were then suspended in 10 ml of H_2O containing 10^{-3} M phenylmethanesulfonyl fluoride and homogenized with a Polytron ST homogenizer for 45 sec at 4°C. TCA (10% w/v) was immediately added to the homogenate. The homogenate was centrifuged at 20,000 × *g* for 15 min. The resulting pellet was resuspended in 2 ml of 0.05 M Tris HCl (pH 7.5), 0.1 M NaCl, homogenized for 15 sec and dialyzed against three changes of this buffer. The cellular protein was digested with bacterial collagenase form III. Aliquots of the sample were added to the collagenase incubation mixture described above. The total volume of the collagenase assay was 1.2 ml. Two hundred units of collagenase (one unit of collagenase equals the amount of enzyme which is required to solubilize 1 μmole of leucine equivalents) were used to digest 1 mg of cellular protein. Following incubation for 2 hr at 37°C, twice the reaction volume of 10% TCA was added to the sample at 4°C for 15 min. The sample was then centrifuged at 20,000 × *g* for 15 min and the resulting supernatant was collected. The pellet was resuspended in 5 ml of 5% (w/v) TCA and centrifuged at 20,000 × *g* for 15 min. This wash procedure was repeated. The TCA soluble fractions were combined

and extracted with 15 ml of *n*-butylacetate. The acid soluble fraction was evaporated. The sample was then suspended in 10 ml of 6 N HCl and hydrolyzed *in vacuuo* for 18 hr at 110°. The sample was evaporated and chromatographed by Dowex cationic exchange chromatography as previously described (7). The hydroxyproline and proline peaks were collected, evaporated and counted to determine the hydroxyproline to proline ratio. The amount of proline and hydroxyproline were corrected for recoveries of [^{3}H]proline and [^{3}H]hydroxyproline added after collagenase digestion of nonradioactive intracellular protein.

RESULTS

Cells were isolated from dermis of control and glucocorticoid-treated rats. The morphology of dermal cells isolated from control animals is presented in Fig. 1. The cells were equilibrated in acetic acid to prevent clumping. The morphology of the cells was the same in the absence of acetic acid. In order to determine whether the cells were viable during the time in which these experiments were done, the viability was determined by trypan blue exclusion. The cells were allowed to incubate for up to 6½ hr at 37°. During this time, aliquots of the cell suspension were taken and assayed for trypan blue exclusion. Eighty-five per cent of the cells did not take up trypan blue during the course of 6½ hr of incubation at 37°C (Table 1).

Prolyl hydroxylase activity was determined in preincubated cells isolated from control and steroid-treated animals at three and six hours of incubation (Table 2). Enzyme activity in the steroid-treated cells was markedly decreased at both times of incubation. This experiment was duplicated and agrees with *in vivo* data (7, 10, 17).

In order to quantify the amount of hydroxyproline formation and the percent of cellular collagen synthesis, the time course of proline incorporation into total intracellular protein was determined (Fig. 2). The amount of proline incorporation in glucocorticoid-treated cells was decreased at all time points.

Previous *in vivo* studies demonstrated that collagen synthesis was selectively decreased after multiple injections of triam-

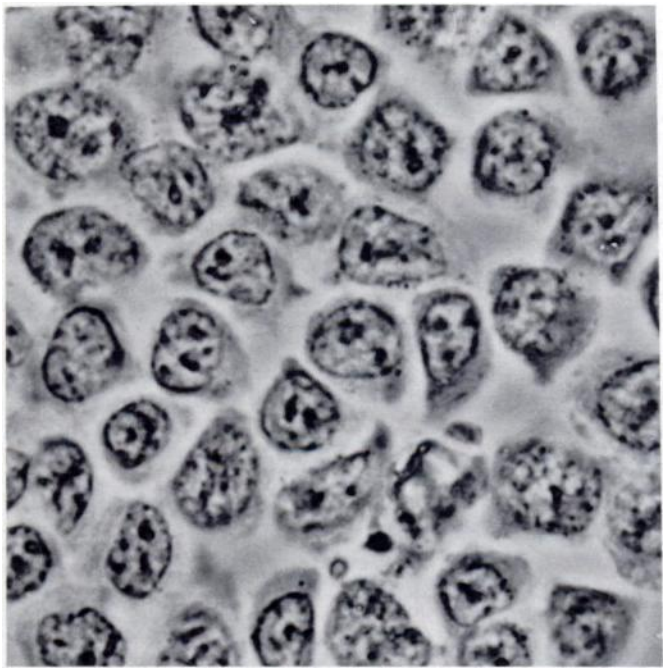

FIG. 1. *Phase contrast micrograph of cells isolated from control animals*
Cells were allowed to equilibrate with 50% acetic acid for 30 min before being photographed at 400 ×.

cinolone diacetate (7). In these studies hydroxyproline formation was decreased to a greater extent than total proline incorporation. This selective decrease of collagen synthesis was also demonstrated in dermal cells incubated with radioactive proline *in vitro* (Table 3). Hydroxyproline formation was decreased by 90% while total proline incorporation was decreased by 60%.

Cellular protein was isolated from control and glucocorticoid-treated cells after labelling with radioactive proline for either thirty minutes or one hour. The percent of collagenase-digestible radioactive proline was quantified in control and glucocorticoid-treated cell cultures (Table 4). The percent of the total cellular protein which was collagen was significantly less in glucocorticoid-treated cells. These data indicate that glucocorticoid treatment results in a selective decrease in collagen polypeptide synthesis.

Since hydroxyproline formation was selectively decreased in glucocorticoid-treated cell cultures at a time when prolyl hydroxylase was decreased, the hydroxyproline to proline ratio of total cellular collagen was determined (Table 5). Although prolyl hydroxylase activity was markedly decreased in glucocorticoid-treated cells, only a slight 5% decrease in the hydroxyproline to proline ratio was observed. When cells were incubated with 2,2′ dipyridyl, a 70% decrease of the hydroxyproline to proline ratio of intracellular collagen was observed. These data indicate that underhydroxylated collagen does not accumulate in dermal cells isolated from glucocorticoid-treated rats.

TABLE 1

Viability and cell number of dermal cells isolated from control and triamcinolone-treated rats as a function of time of incubation

Dermal cells were isolated as described in the text, preincubated for 2 hr and divided into four aliquots which were incubated for the times indicated. Five parts of the cell suspension were mixed with one part 0.4% trypan blue solution and allowed to stand at room temperature for 3 min. The percent viable cells was calculated by dividing the cells which excluded trypan blue by the total number of cells counted.

Time of incubation	Control		Triamcinolone	
	Cell number	Percent viability	Cell number	Percent viability
(hr)	*(× 10^{-6})*		*(× 10^{-6})*	
0	3.12	85	2.84	86
1.0	3.40	85	2.80	86
3.0	3.36	85	2.80	86
6.5	3.40	85	2.84	85

TABLE 2

Prolyl hydroxylase activity in dermal cells isolated from control and triamcinolone-treated rats

Dermal cells were isolated as described in the text from control and triamcinolone-treated rats. The cells (1.5×10^6) were preincubated for 2 hr and incubated for an additional 3 or 6 hr. Preparation of the supernatant fraction of cellular homogenates for enzyme assay was done as described in the text. Each sample was assayed in duplicate. All incubations were done in EMEM. Values in parentheses represent the percent decrease of enzyme activity from control values.

Time of incubation	Prolyl hydroxylase activity	
	Control	Triamcinolone
hr	*DPM of THO × 10^{-4}/mg supernatant protein*	
3	15.0	5.8 (61%)
6	13.4	6.4 (52%)

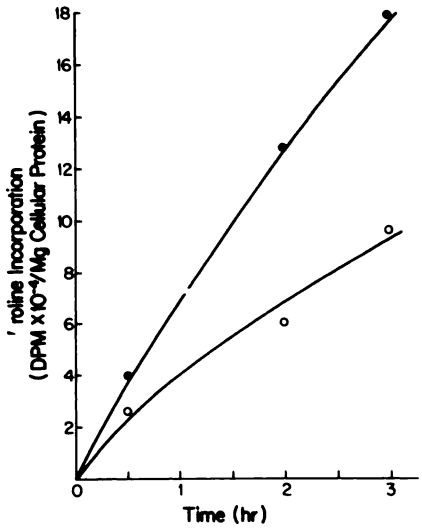

FIG. 2. *Time course of proline incorporation into protein of rat dermal cells*

Preincubated cells ($1.4–1.8 \times 10^6$) were incubated for the indicated times with radioactive proline. The cells were harvested and proline incorporation per milligram of cellular protein was determined as described in the text. Control —●—; steroid treated —○—.

DISCUSSION

Administration of glucocorticoid results in a decrease of collagen synthesis in connective tissues. Proteinaceous hydroxyproline synthesis is decreased in skin (2, 7), bone (5) and granuloma tissue (15, 16, 28) following glucocorticoid administration.

The decrease of collagen synthesis in connective tissues after a single injection of glucocorticoid was initially thought to result from a non-selective inhibition of protein synthesis since hydroxyproline formation was decreased to approximately the same extent as total proteinaceous proline incorporation (5). However, following multiple injections of triamcinolone diacetate to newborn rats, hydroxyproline formation was decreased in whole skin to a much greater extent than total proline incorporation (7). Since prolyl hydroxylase activity was concomitantly reduced in various tissues (7, 10, 16–18) this finding suggested that glucocorticoids selectively decreased the rate of collagen polypeptide synthesis and/or the rate of prolyl hydroxylation.

Recent studies quantified the rate of collagen synthesis relative to non-collagen

TABLE 3

Incorporation of [^{3}H]proline into total intracellular protein and hydroxyproline formation in dermal cells isolated from control and triamcinolone-treated rats

Dermal cells (3–4 × 10^6) from control and triamcinolone-treated animals were incubated for 2 hr and incubated an additional 30 min after the addition of [2,3-^{3}H] proline. The cellular protein was hydrolyzed and [^{3}H]hydroxyproline was separated from [^{3}H]proline by Dowex cationic exchange chromatography as described in the text. All values are calculated based on the specific activity of radioactive proline in the acid soluble fraction of the cellular homogenates which was determined as described in the text. The values represent the mean ± SE of four cultures. The values in parentheses represent the percent decrease of incorporation from control values.

Measurement	Control	Triamcinolone
Intracellular proline incorporated		
DPM/mg cellular protein / DPM/nmol proline	90.8 ± 9.2	28.6 ± 2.7[a] (68%)
Intracellular hydroxyproline formed		
DPM/mg cellular protein / DPM/nmol proline	9.2 ± 0.8	0.9 ± 0.2[a] (90%)

[a] Significantly different from control at $p < 0.05$.

TABLE 4

Percent of intracellular collagen synthesized by dermal cells isolated from control and triamcinolone-treated rats

Dermal cells were isolated from control and steroid-treated animals as described in the text. Preincubated cells (1.5–2.0 × 10^6) were incubated with [2,3-^{3}H]proline for the times indicated. The data from each time period represent a different experiment. Intracellular protein was prepared and aliquots of the cellular homogenates were digested with bacterial collagenase as described in the text. The per cent proline-labelled protein which was digested by collagenase was calculated by dividing the sample incubated with collagenase by the sample incubated in the absence of collagenase and multiplying by 100. The triamcinolone-treated samples were derived from more cells than the control samples.

Pulsing time	Treatment	−Collagenase	+Collagenase	Protein digestion
hr		*DPM* × 10^{-3}		
0.5	Control	1.6	1.1	28%
	Triamcinolone	1.8	1.5	17%
1.0	Control	2.7	1.9	27%
	Triamcinolone	3.8	3.1	19%

TABLE 5

Hydroxyproline to proline ratio of cellular collagenase-digestible protein of dermal cells isolated from control and triamcinolone-treated rats

Dermal cells were isolated from the skin of control and triamcinolone-treated rats as described in the text. Preincubated cells (3 × 10^6) were divided in half and incubated with or without 2,2'dipyridyl plus [2,3-^{3}H]proline for 5 hr and harvested. Cells (3.5 × 10^6) isolated from triamcinolone-treated tissue were incubated with radioactive proline for 5 hr and harvested. The amounts of radioactive hydroxyproline and proline released by collagenase digestion of cellular protein were determined as described in the text.

	[^{3}H]Hydroxyproline	[^{3}H]Proline	Hyp./Pro
	DPM × 10^{-4}		
Control	4.2	6.9	0.61
Triamcinolone	7.1	12.2	0.58
2,2' Dipyridyl treated cells	1.0	5.6	0.18

protein synthesis in dermis *in vivo* at both the tissue and ribosomal nascent chain levels (10). The activity of prolyl hydroxylase as well as the degree of prolyl hydroxylation of collagen nascent chains were determined. The results indicated that glucocorticoids selectively decreased collagen polypeptide synthesis in rat dermis sufficiently to account for the selective decrease in the newly synthesized acid-soluble collagen previously reported in skin (2, 3). Although prolyl hydroxylase activity was decreased in a dose- and time-dependent manner after administration of triamcinolone diacetate, there was no concomitant decrease in the extent of hydroxylation of nascent collagen polypeptides.

During the biosynthesis of collagen, hydroxylation of prolyl residues occurs before helix formation (29–33). The hydroxylation of prolyl residues is required for helix formation and resistance of the collagen molecule to proteolytic digestion. Underhydroxylated collagen exists in a random coil which is susceptible to proteolytic digestion (29). Prolyl hydroxylation is, therefore, a necessary step for the synthesis of collagen which is not susceptible to proteolytic digestion. Since some hydroxylation of prolyl residues occurs after release of collagen polypeptides from ribosomes (23, 24), we determined if underhydroxylated collagen was synthesized intracellularly after steroid treatment. Cells were isolated from rat dermis of animals treated with multiple injections of triamcinolone diacetate, and incubated with radioactive proline. The hydroxyproline to proline ratio of total cellular collagen was determined. Although prolyl hydroxylase activity is decreased in dermal cells isolated from glucocorticoid-treated rats, the extent of hydroxylation of total cellular collagen is not decreased. Therefore, underhydroxylated collagen is not synthesized and accumulated intracellularly for 5 hr of incubation following glucocorticoid treatment. The accumulation of underhydroxylated collagen was also not observed at 30 min of incubation.

As observed in *in vivo* studies, collagen polypeptide synthesis is also selectively decreased in the cells isolated from glucocorticoid-treated rats. The amount of proteinaceous hydroxyproline synthesis is decreased to a greater extent than total proline incorporation into cellular protein. A selective decrease of collagen synthesis is also indicated by the lower percent of collagen synthesized by glucocorticoid-treated cells.

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