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# A Selective Decrease of Collagen Peptide Synthesis by Dermal Polysomes Isolated from Glucocorticoid-Treated Newborn Rats

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SUMMARY

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Polysomes were isolated from the dermis of control newborn rats treated with various pharmacological doses of triamcinolone diacetate and allowed to incorporate radioactive proline into collagen and noncollagen protein in the wheat germ lysate system. Proline incorporation into collagen and noncollagen protein was linearly related to the $A_{260}$ unit of polysomes added to the lysate system. Proline incorporation into collagen and noncollagen was maximal at 4.6 mM magnesium acetate, 155 mM potassium chloride, 0.017 mM amino acids and 40 $\mu$g protein of the wheat germ extract. Proline incorporation into collagen and noncollagen protein was dependent on the presence of amino acids, polysomes, adenosine triphosphate, potassium chloride, magnesium and wheat germ extract. Polysomes isolated form glucocorticoid-treated rats incorporated less proline into collagen as compared to proline incorporation into noncollagen protein, indicating a selective decrease of collagen synthesis, although proline incorporation into noncollagen protein was also decreased. This selective decrease of collagen synthesis was dose dependent. Significant inhibition of collagen synthesis was observed at a dose of 2 mg/kg. Maximum inhibition of collagen synthesis was observed at a dose of 12 mg/kg. This selective decrease in collagen synthesis was also dependent on the number of daily injections of glucocorticoid. A selective decrease of collagen synthesis was observed six hours after a single intraperitoneal injection of 12 mg/kg triamcinolone diacetate. After three daily injections of drug at this dose (12 mg/kg), the inhibitory effect on noncollagen and collagen syntheses was doubled. Since amino acids are not rate limiting in this protein synthesizing system *in vitro*, the glucocorticoid-mediated selective decrease of collagen synthesis in skin does not result from an inhibition of amino acid uptake.

## INTRODUCTION

The administration of pharmacological doses of glucocorticoids to experimental animals and man results in diminished growth of somatic and connective tissues (1-3). Glucocorticoids have potent antianabolic effects on nucleic acid and protein metabolism. Pharmacological doses of these steroids decrease the synthesis of deoxyribonucleic acid and protein in liver (4-6). These antianabolic effects are also evident in skin (7-9) and other peripheral tissues (10).

Glucocorticoid-mediated antianabolic ef-

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fects on collagen metabolism have been reported in both normal and inflamed connective tissues. Hydroxyproline excretion is decreased in both young rats (7, 8, 11–13) and in children (14) following glucocorticoid therapy. Administration of glucocorticoids results in decreased incorporation of radioactive amino acids into collagen in skin (7–9) and granuloma tissue (15–17). The decrease of collagen synthesis, at least in skin, is not associated with an alteration in the specific activity of the acid soluble proline pool (9).

The decreased rate of collagen synthesis in connective tissues following a single injection of glucocorticoid was initially thought to result from a nonselective inhibition of protein synthesis, since hydroxyproline formation was decreased to approximately the same extent as total proline incorporation (18). However, following multiple injections of glucocorticoid, hydroxyproline formation was decreased to a much greater extent than proline incorporation into protein (9). A recent study (19) indicated that glucocorticoids selectively decrease collagen polypeptide synthesis in rat dermis sufficiently to account for the selective decrease in the newly synthesized acid-soluble collagen previously reported in skin (7, 8).

The inhibition of amino acid uptake in target tissues is a possible mechanism of the antianabolic effect of glucocorticoids on collagen and noncollagen protein syntheses. An inhibition of amino acid uptake would result in diminution of the intracellular pools of amino acids available for activation and subsequent incorporation into protein. Glucocorticoids inhibit both the uptake of $\alpha$-aminoisobutyric acid (20–22) and the uptake of utilizable amino acids in rat diaphragm (22). The uptake of $\alpha$-aminoisobutyric into mouse ear strips (23) and thymocytes (24) is also inhibited by glucocorticoids. Cortisol decreases the rate of collagen synthesis in cloned granuloma fibroblast cells concomitantly with decreases of both the intracellular concentration of free amino acids and $\alpha$-aminoisobutyric uptake (25). The data above indicate that the glucocorticoid-mediated antianabolic effect on protein synthesis in peripheral tissues could result from a decrease in activated amino acid precursor pools.

In the present study, polysomes were isolated from the dermis of glucocorticoid-treated newborn rats and translated in a wheat germ lysate system in which amino acids are not rate limiting. The data indicate that proline incorporation into protein directed by polysomal preparations isolated from glucocorticoid-treated rats is markedly decreased. The data also indicate that polysomes isolated from steroid treated rats incorporate less radioactive proline into collagen as compared to proline incorporation into noncollagen protein, indicating a selective decrease of collagen synthesis.

## MATERIALS AND METHODS

One-day-old Sprague-Dawley rats were used throughout these studies. Animals were given 1, 2, or 3 intraperitoneal injections of triamcinolone diacetate suspended in 0.9% (w/v) sodium chloride. Triamcinolone diacetate was kindly supplied by Dr. E. W. Cantrell of Lederle Labs. All chemicals used throughout these studies were analytical reagent grade. [2,3-$^3$H]Proline (20 Ci/mmole), [G-$^3$H]tryptophan (10 Ci/mmole) and Triton X-100 were purchased from New England Nuclear. [5-$^3$H]Proline was obtained from Amersham/Searle. Protease-free bacterial collagenase (Form III) was purchased from Advanced Biofactures Corp. The wheat germ was kindly supplied by Mr. Malhot of General Mills.

*Isolation of dermal polysomes.* Animals were killed by decapitation. The skins were removed and placed in the polysome isolation buffer containing 200 mM potassium chloride, 0.01 mM DTT[2], 0.3 mM EDTA, 250 mM RNAase free sucrose, 10 mM magnesium chloride, 40 $\mu$g/ml polyvinylsulfate and 50 mM Tris-HCl (pH 7.5). The facia and musculature of the subcutaneous layer were removed by dissection. The skins were then soaked in buffer for 20 min. The dermis was scraped free of the epidermis and weighed. The removal of the epidermis and

[2] The abbreviations used are: DTT, dithiothreitol; EDTA, ethylenediamine tetraacetate; HEPES, N-2-hydroxyethylpiperazine-N'-2 ethanesulfonic acid; ERW, *E. coli* K12 strain.

subcutaneous layers was periodically accessed by histological examination. The dermis was minced in three volumes of buffer and then homogenized for 45 sec with the Polytron PT system. The homogenate was made 0.5% (w/v) sodium deoxycholate and 0.25% (w/v) Triton X-100. The homogenate was centrifuged at 10,000 × *g* for 30 min. The resulting supernatant was strained through cheesecloth and layered over a 1.5 M sucrose cushion. The sample was centrifuged at 136,000 × *g* for 4.5 hr. The polysomal pellet was suspended in 250 mM sucrose, 1 mM DTT, 0.1 mM EDTA (pH 7.0) and the sample was centrifuged at 8,000 × *g* for 10 min. The resulting supernatant was stored in aliquots at −80° until assayed.

*Preparation of wheat germ S-30 extract.* The wheat germ S-30 fraction was prepared by a modification of the method of Roberts and Paterson (26). Six grams of wheat germ were suspended in 28 ml of a buffer containing 100 mM potassium chloride, 1 mM magnesium acetate, 2 mM calcium chloride, 1.2 mM DTT and 20 mM HEPES (pH 7.6) and homogenized with the Polytron PT system for 1 min. The wheat germ S-30 fraction was then prepared as previously described (19). The protein concentration of the wheat germ preparation was determined by the method of Lowry et al. (27).

*Protein synthesis* in vitro. The protein synthetic system consisted of 40 μg protein of wheat germ S-30 extract, 0.2 $A_{260}$ unit of polysome, (1 $A_{260}$ unit = 50 μg RNA per ml), 2 mM DTT, 0.8 mM adenosine triphosphate, 16.7 μM guanosine triphosphate, 6.7 mM creatine phosphate, 33.3 μg/ml creatine phosphokinase, 155 mM potassium chloride, 4.6 mM magnesium acetate, 0.033 mM of 19 amino acids minus proline, 10 μCi of [2,3-$^3$H]proline and 20 mM HEPES (pH 7.6) made up to a total volume of 60 μl with water. The reaction mixture was incubated for 1 hr at 25°.

*Collagenase digestion of product.* To the protein assay mixture was added 9.4 mM potassium chloride, 0.7 mM magnesium chloride, 7.1 mM calcium chloride, 2.4 mM N-ethylmaleimide, 1 mM cycloheximide, 9.4 mM Tris-HCl (pH 7.5), plus and minus 38 units of collagenase made up to total volume of 1.06 ml. The samples were incubated for 2 hr at 37°. Two hundred μl of 1.5 M Tris (pH 9.0) was added and the samples were incubated for an additional 20 min at 37°. The samples were then placed on ice, precipitated with 2 ml of 10% (w/v) trichloroacetic acid and filtered through a 0.45 μm Millipore filter. The dried filters were counted in 10 ml of toluene-2,5-diphenyloxazole-1,4-bis [2-(4-methyl-5-phenyloxazolyl)] benzene.

*Collagen synthesizing polysome recovery from control and glucocorticoid-treated dermal homogenates.* Non-steroid-treated rats were injected intraperitoneally with [5-$^3$H]proline (10 μCi/g rat) for 30 min. A homogenate of dermis was prepared as described above. Ten ml aliquots of this [5-$^3$H]proline-labeled homogenate were mixed with 10 ml aliquots of non-radioactive tissue homogenates prepared from control and triamcinolone-treated rat dermis. Polysomes were isolated as described above. The amount of radioactive collagen synthesizing polysomes recovered was determined by the collagenase digestion assay as described above.

*Preparation of [2,3$^3$-H]proline labeled skin collagen and [G-$^3$H]tryptophan-labeled* Escherichia coli *protein.* The efficiency of different preparations of collagenase to digest a [2,3-$^3$H]proline-labeled skin collagen substrate was monitored throughout these studies. Rats were treated with β-aminopropionitrile, labeled with [2,3$^3$-H]proline, and collagen was isolated as previously described (19). The collagenase preparations used in these studies solubilized 80% of the collagen preparation.

The collagenase preparations were also assayed for protease contamination by incubating with [G-$^3$H]tryptophan-labeled *E. coli* protein. To prepare radioactive-labeled *E. coli* protein, ERW cells were inoculated and incubated for 20 hr at 37°. Cultures containing $10^9$ cells/ml were diluted 1:10 with minimal media plus 25 μg/ml tryptophan. The cultures were incubated aerobically at 37° for 2 hr, at which time the cell concentration was 5 × $10^8$ cells/ml. One millicurie of [G-$^3$H]tryptophan was added to each of three 500 ml flasks. The cultures were then incubated for 7 hr until they had

attained a stationary phase. The cells were collected by centrifugation at 12,000 × *g* for 15 min. The cells were suspended in 120 ml of 100 mM sodium chloride and 50 mM Tris-HCl (pH 7.5). The sample was homogenized with the Polytron PT system for 1 min. The homogenate was centrifuged at 15,000 × *g* for 30 min. The resulting supernatant was dialyzed against four changes of 4 liters each of the NaCl-tris buffer. No collagenase preparation used in these studies digested *E. coli* protein.

## RESULTS

Polysomes were isolated from the rat dermis of control and glucocorticoid treated rats. The amount of proline incorporation into collagen and noncollagen peptides versus the $A_{260}$ unit of polysomes is presented in Fig. 1. Proline incorporation into collagen and noncollagen peptides was linearly related to the amount of $A_{260}$ unit of polysomes added to the protein synthesizing system *in vitro*. Proline incorporation into collagen and noncollagen protein for all polysome preparations was linear up to 0.2 $A_{260}$ unit of polysomes. Therefore, all polysome preparations were assayed for the synthesis of collagen and noncollagen protein at 0.2 $A_{260}$ unit of polysomes.

The effect of omission of various components of the protein synthesizing system on incorporation of radioactive proline into collagen and noncollagen protein is seen in Table 1. Optimum incorporation of radioactive proline into collagen and noncollagen protein required the presence of amino acids, adenosine triphosphate, potassium chloride, magnesium and the S-30 wheat germ extract. The omission of polysomes resulted in no synthesis of collagen and a background of noncollagen wheat germ protein synthesis, which was only 2% of the incorporation of the complete system. This low background of the wheat germ system resulted in a significant level of incorporation into collagen and noncollagen protein directed by rat dermal polysomes.

The amount of proline incorporation into collagen and noncollagen protein as a function of the time of incubation of the synthetic system is seen in Fig. 2. Maximum proline incorporation into collagen and noncollagen protein was observed within 60 min of incubation at 25°. Subsequent experiments were incubated for 60 min.

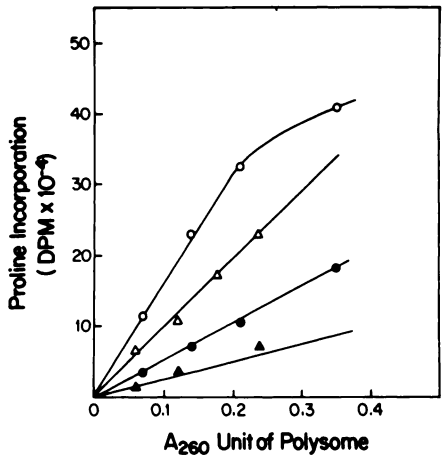


FIG. 1. *Proline incorporation into collagen and noncollagen protein per $A_{260}$ unit of polysome in the wheat germ lysate system*

Polysomes were isolated from nonsteroid-treated animals and rats receiving three daily intraperitoneal injections of triamcinolone diacetate at 12 mg/kg. Varying amounts of polysomes were incubated in the wheat germ lysate system and the amounts of radioactive proline incorporated into collagen and noncollagen protein were determined by collagenase digestion, as described in the text. Control noncollagen (-○-), control collagen (-●-) steroid-treated noncollagen (-△-) and steroid-treated collagen (-▲-).

TABLE 1

*The effect of omission of various components of the wheat germ system on collagen and noncollagen protein syntheses*

Polysomes (0.2 $A_{260}$ unit) were prepared from nonsteroid-treated rats. The polysomes were translated in the protein synthetic assay mixture minus various components as indicated. Proline incorporation into collagen and noncollagen protein was determined by collagenase digestion as described in the text.

| Percent incorporation of the complete system | | |
|---|---|---|
| | *Collagen* | *Noncollagen* |
| Complete | 100 | 100 |
| minus amino acids | 36 | 29 |
| minus polysomes | 0 | 2 |
| minus ATP | 6 | 15 |
| minus KCl | 16 | 28 |
| minus $Mg^{++}$ | 6 | 3 |
| minus S-30 | 3 | 5 |

Proline incorporation into collagen and noncollagen protein was brought to optimal levels for the amount of wheat germ extract

and the concentration of amino acids of the protein synthetic system. Maximum proline incorporation into collagen and noncollagen protein was observed at 40 μg protein of wheat germ extract (Fig. 3). Greater than 40 μg protein of wheat germ extract caused a slight decrease of proline incorporation into noncollagen protein. Maximum incorporation of proline into collagen and noncollagen protein was observed at 0.017 mM of amino acids minus proline. Higher concentrations of amino acids did not affect the amount of proline incorporation. A concentration of 0.034 mM of each amino acid was used in subsequent experiments.

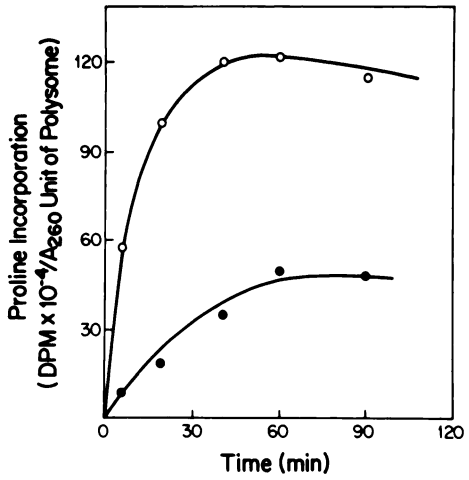


FIG. 2. *Proline incorporation into collagen and noncollagen protein versus the time of incubation of the wheat germ lysate system*

Polysomes were isolated from the dermis of 4-day-old rats, as described in the text. The polysomes (0.2 $A_{260}$ unit) were incubated in the wheat germ lysate system for the times indicated. The amounts of radioactive proline incorporated into collagen (-●-) and noncollagen (-○-) protein were determined by collagenase digestion, as described in the text.

Proline incorporation into protein was also optimized for the magnesium and potassium chloride concentrations (Fig. 4). Optimum proline incorporation into collagen and noncollagen protein was observed at 4.6 mM magnesium acetate. Addition of more magnesium resulted in a dramatic decrease of proline incorporation into both collagen and noncollagen protein. Maximum proline incorporation into collagen and noncollagen protein was observed at 155 mM potassium chloride (Fig. 4). Concentrations greater than 200 mM of this salt resulted in marked inhibition of proline incorporation into collagen and noncollagen protein.

Previous studies *in vivo* indicated that the selective decrease of collagen polypeptide synthesis was dependent on the dose and the number of daily injections of triamcinolone diacetate (19). Accordingly, polysomes were isolated from rats which were treated with three daily intraperitoneal injections of various doses of triamci-

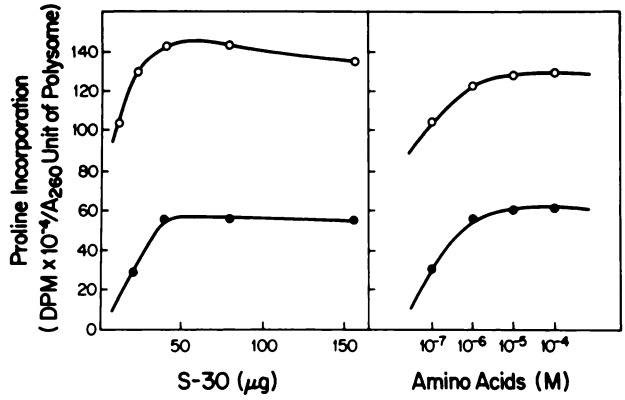


FIG. 3. *Proline incorporation into collagen and noncollagen protein versus the amounts of wheat germ extract and amino acids*

Polysomes were isolated from control 4-day-old rats, as described in the text. The polysomes (0.2 $A_{260}$ unit) were incubated with the components of the wheat germ system as described in the text and various amounts of either wheat germ extract or amino acids. The amount of proline incorporated into collagen (-●-) and noncollagen (-○-) protein was determined by the collagenase digestion assay.

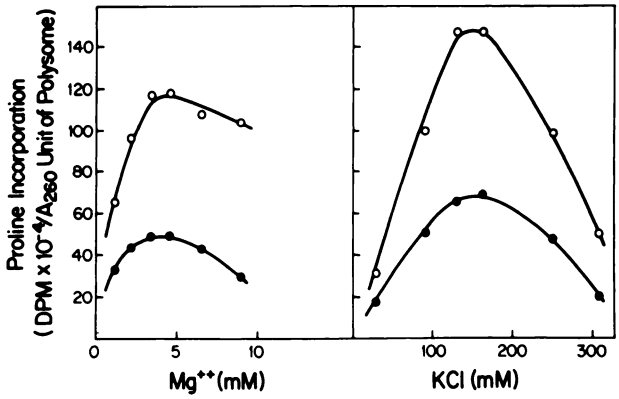


FIG. 4. *Proline incorporation into collagen and noncollagen protein versus the concentrations of magnesium acetate and potassium chloride*

Polysomes were isolated from control 4-day-old rats, as described in the text. The polysomes (0.2 $A_{260}$ unit) were incubated with the components of the wheat germ system, as described in the text with various concentrations of either magnesium acetate or potassium chloride. The amount of proline incorporated into collagen (-●-) and noncollagen (-○-) was determined by the collagenase digestion assay.

nolone diacetate. The amount of proline incorporated into collagen and noncollagen protein per $A_{260}$ unit of polysome was determined (Table 2). A dose as low as 2 mg/kg resulted in a selective decrease of collagen synthesis. Collagen synthesis was decreased by 31%, whereas noncollagen protein synthesis was decreased by only 13%. Maximum inhibition of collagen and noncollagen protein syntheses was observed after multiple injections of a dose of 12 mg/kg of steroid. Higher doses of triamcinolone were not used since a dose of 12 mg/kg of this steroid gave the maximal decrease of collagen synthesis in studies *in vivo* (19).

The temporal response of inhibition of collagen and noncollagen protein synthesis is seen in Fig. 5. Polysomes isolated from rats treated with a single injection of 12 mg/kg of triamcinolone diacetate for 6 hr incorporated less proline into collagen as compared to proline incorporation into noncollagen protein. This selective decrease of collagen synthesis was observed after either a single or multiple injection of steroid (12 mg/kg). After three intraperitoneal injections of drug (12 mg/kg), proline incorporation into collagen was decreased by 70%. Noncollagen protein synthesis was decreased by only 35%.

The selective decrease of collagen synthesis may have resulted from a decreased yield of collagen-synthesizing polysomes isolated from glucocorticoid-treated rat dermal homogenates. Therefore, recovery of collagen-synthesizing polysomes from control and steroid-treated homogenates was determined. Equal aliquots of a proline-labeled homogenate were mixed with control and steroid-treated nonradioactive homogenates, and polysomes were isolated. The yield of collagen-synthesizing poly-

TABLE 2

*Dose response of collagen and noncollagen protein syntheses by dermal polysomes isolated from triamcinolone treated rats*

The values represent the mean ± standard errors of determinations from 3 to 5 polysome preparations. Steroid-treated 1-day-old animals received three daily intraperitoneal injections of triamcinolone diacetate at the doses indicated and were killed 24 hr after the last injection. Polysomes were isolated and translated in the wheat germ lysate system, as described in the text. Proline incorporation into collagen and noncollagen proteins was determined by the collagenase digestion assay.

| Triamcinolone-dose | Collagen | Noncollagen |
|---|---|---|
| *mg/kg* | *dpm* × $10^{-4}/A_{260}$ | *unit of polysome* |
| 0 | 49.9 ± 2.2 | 136.5 ± 2.6 |
| 2 | 34.6 ± 1.7[a] (31%) | 118.3 ± 1.8[a] (13%) |
| 4 | 22.8 ± 1.1[a] (54%) | 109.2 ± 4.9[a] (20%) |
| 12 | 14.1 ± 1.1[a] (72%) | 87.9 ± 4.6[a] (36%) |

[a] Significantly different from nonsteroid-treated control at $p \leqq 0.05$.

somes was determined by the amount of collagenase-digestible nascent chains. The yield of proline-labeled collagen synthesizing polysomes from control and steroid-treated homogenates was the same (data not shown).

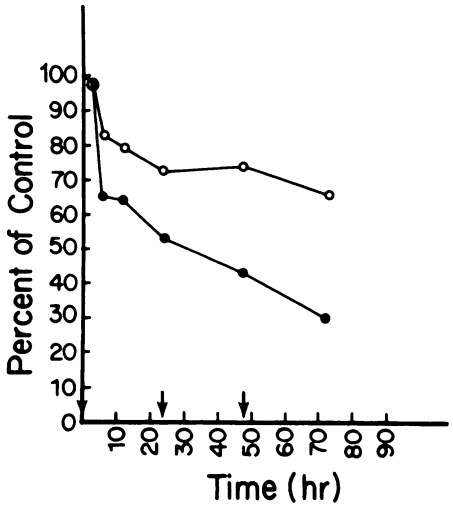


FIG. 5. *Temporal response of collagen and noncollagen protein synthesis by dermal polysomes isolated from triamcinolone-treated rats*

One-day-old rats were injected with either 12 mg/kg of triamcinolone diacetate or no drug at the times indicated by the arrows. Polysomes were isolated and translated in the wheat germ lysate system, as described in the text. The values represent the mean percentage of from 3 to 4 different polysome preparations. Proline incorporation into collagen (-●-) and noncollagen (-○-) protein was determined by the collagenase digestion assay.

In order to determine if the selective decrease of proline incorporation into collagen resulted from either greater collagenolytic or proteolytic activities associated with glucocorticoid-treated polysome preparations, these activities were measured. Negligible amounts of collagenolytic and proteolytic activities were associated with the polysome preparations (Table 3). Furthermore, the control preparations had more collagenolytic and proteolytic activity than the steroid-treated polysomes. Accordingly, differences in either collagenolytic or proteolytic activity between control and steroid-treated polysome preparation did not account for the observed selective decrease of proline incorporation into collagen.

## DISCUSSION

An antianabolic effect of glucocorticoids on collagen metabolism was originally suspected since glucocorticoid administration resulted in a decrease of urinary hydroxyproline excretion (7, 8, 11–14). Subsequent studies quantified the amount of proteinaceous hydroxyproline synthesis in connective tissues after administration of glucocorticoids (7, 9, 15–17, 28, 29). These studies demonstrated that collagen synthesis was markedly decreased in connective tissues following glucocorticoid administration. An

TABLE 3

*Collagenolytic and proteolytic activities of control and glucocorticoid-treated polysome preparations*

The [$^3$H]proline labeled collagen substrate was prepared as previously described (19). The [$^3$H]tryptophan labeled protein substrate was prepared as described in the text. The polysome preparations were incubated with the substrates in the protein assay system described in the text. After incubation at 25° for 60 min, the assay mixtures were deacylated, acidified, and filtered. The percent digestion was calculated by dividing the radioactivity that passed through the filter by the amount of radioactivity retained by the filter in the absence of polysomes.

| Substrate | Polysome preparation assayed | $A_{260}$ unit or polysome assayed | Radioactivity retained on the filter | Percent digestion |
|---|---|---|---|---|
| | | | *dpm* × $10^{-2}$ | |
| [$^3$H]Proline labeled collagen substrate | No addition | | 45.0 | |
| | Control | 0.19 | 41.0 | 9% |
| | Triamcinolone | 0.19 | 44.9 | 1% |
| [$^3$H]Tryptophan labeled *E. coli* substrate | No addition | | 35.9 | |
| | Control | 0.19 | 34.7 | 3% |
| | Triamcinolone | 0.19 | 35.3 | 2% |

antianabolic effect of these steroids on collagen metabolism was also indicated since prolyl hydroxylase (9, 17, 19, 30–32), lysyl hydroxylase (19, 32) collagen galactosyltransferase and glucosyltransferase (32) were decreased following glucocorticoid administration.

Although hydroxyproline formation was decreased to the same extent as total proline incorporation after a single injection of steroid (18), our study demonstrated that hydroxyproline formation was decreased to a greater extent than proteinaceous proline incorporation after multiple injections of steroid (9). Since prolyl hydroxylase was decreased at a time when hydroxyproline formation was selectively decreased (9), a possible decrease of the prolyl hydroxylation step in collagen synthesis was suspected. A subsequent study demonstrated that although prolyl hydroxylase was decreased in a time- and dose-dependent manner, the hydroxyproline-to-proline ratio of collagen ribosomal nascent chains was not decreased (19). This study also quantified the rate of synthesis of collagen and noncollagen peptides at the tissue and ribosomal levels by the collagenase digestion assay. The data indicated that glucocorticoids selectively decrease the rate of collagen polypeptide synthesis.

The present studies were undertaken to define this selective antianabolic effect of glucocorticoids on collagen synthesis. Amino acid uptake is inhibited by glucocorticoid administration (20–25). Furthermore, glucocorticoid administration to isolated cloned fibroblasts decreased the intracellular concentration of free amino acids concomitantly with a decrease of collagen synthesis (25). In the present studies, polysomes were isolated from glucocorticoid-treated rats and translated in an protein-synthesizing system *in vitro* where amino acids are not rate limiting. The data indicate that collagen and noncollagen protein syntheses are decreased. Furthermore, polysomes isolated from glucocorticoid-treated rats incorporate significantly less proline into collagen as compared with the amount of proline incorporation into noncollagen protein. Thus, the selective decrease of collagen synthesis is observed both *in vivo* and in this heterologous protein synthesizing system.

Since the proline content of the collagen core is much different than that of the procollagen extension peptides, the use of collagenase digestion to quantify collagen may have resulted in an underestimation of the collagen synthesized. Alternately since collagen is proline rich as compared to other proteins, the amount of collagen synthesized is probably greater than the actual percent of collagen synthesized. However, there is a selective decrease of collagen synthesis since the data may be expressed as the percent decrease of control (see Fig. 5).

The mechanism of this selective decrease in collagen synthesis is not completely defined by the present studies. However, an inhibitory effect of glucocorticoids on amino acid uptake is not responsible for the selective decrease of collagen polypeptide synthesis. The data indicate that the glucocorticoid-mediated decrease of collagen synthesis results from a regulation of collagen biosynthesis at either the translational or transcriptional level. One possible explanation is that collagen messenger ribonucleic acid is selectively decreased following glucocorticoid administration. This selective decrease of collagen messenger ribonucleic acid could result from either a selective decrease in the transcription of collagen messenger ribonucleic acid or a decrease in the processing of messenger to become functionally active messenger ribonucleic acid associated with ribosomes. The selective effect of glucocorticoids on collagen synthesis could also result from a selective decrease of the activity of ribosomal messenger ribonucleic acid. A factor(s) required for protein synthesis on polysomes may be decreased in the glucocorticoid-treated polysomal preparations. This latter possibility would also necessitate that a specific factor for collagen synthesis would have to be selectively decreased since collagen synthesis is decreased to a greater extent than noncollagen protein synthesis. Future studies will quantify the amount and activity of collagen messenger ribonucleic acid following glucocorticoid administration.

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