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EFFECT OF HYDROCORTISONE ACETATE, FLUOCINOLONE ACETONIDE, FLUCLOROLONE ACETONIDE, BETAMETHASONE-17-VALERATE AND FLUPREDNYLIDEN-21-ACETATE ON COLLAGEN BIOSYNTHESIS

JOUNI UITTO and KIMMO K. MUSTAKALLIO

Departments of Medical Chemistry and Dermatology, University of Helsinki, Helsinki, Finland

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Abstract—The effect of hydrocortisone acetate, fluocinolone acetonide, fluclorolone acetonide, betamethasone-17-valerate and fluprednyliden-21-acetate on collagen biosynthesis was studied both *in vivo* and *in vitro*. In experiments *in vivo*, test substances and [^{14}C]proline were injected on the chorioallantoic membrane of 11-day-old chick embryos. In experiments *in vitro*, chick embryo tibiae were incubated in a medium containing the corticosteroids to be tested and [^{14}C]proline. In both types of experiments, the formation of [^{14}C]hydroxyproline was taken as a measure of the rate of collagen biosynthesis. In addition, the effect of these corticosteroids on the activity of protocollagen proline hydroxylase was tested.

[^{14}C]hydroxyproline formation *in vivo* was inhibited by all the corticosteroids tested, and no clear difference in the degree of inhibition could be observed between the various corticosteroids. *In vitro*, betamethasone-17-valerate inhibited hydroxyproline formation more than the other substances tested. The concentration of corticosteroids required to inhibit collagen formation was considerably higher *in vitro* than *in vivo*. The corticosteroids tested did not affect the activity of protocollagen proline hydroxylase in the bones. In all these experiments, total protein synthesis, measured as incorporation of total ^{14}C-radioactivity into the bones, was inhibited by corticosteroids to the same extent as hydroxyproline formation. The results seem to suggest that corticosteroids inhibit collagen biosynthesis by inhibiting the formation of polypeptide precursors of collagen.

CORTICOSTEROIDS are widely used as therapeutic agents in various clinical disorders, especially those involving connective tissue. Consequently, their effect on the metabolism of connective tissue has received special attention (for review, see Refs. 1 and 2). The mode of action of corticosteroids on the connective tissue components has been shown to be mainly anti-anabolic. Cortisone and hydrocortisone have been demonstrated to inhibit collagen biosynthesis in the skin of animals,[3–5] in developing chick embryos[2,6] and in fibroblast cultures.[7] However, the exact nature of the inhibition is still obscure.

Prolonged clinical use of cortisone and hydrocortisone has some adverse effects, and therefore, some newer corticosteroid preparations have been introduced. They have been reported to differ in their clinical efficacy and in the extent of side effects.[8,9] In the present study, the effect of hydrocortisone acetate, fluocinolone acetonide, fluclorolone acetonide, betamethasone-17-valerate and fluprednyliden-21-acetate on collagen biosynthesis was studied both *in vivo* and *in vitro*. Collagen biosynthesis was

measured *in vivo* by injecting radioactive proline into chorioallantoic membranes of chick embryos or *in vitro* by incubating chick embryo tibiae in a medium containing [^{14}C]proline. The amount of radioactive hydroxyproline in non-dialysable form was taken as an index of collagen formation. In addition, the activity of protocollagen proline hydroxylase, the enzyme that participates in the intracellular biosynthesis of collagen by hydroxylating peptide-bound proline to collagen hydroxyproline, was assayed in the bones.

MATERIAL AND METHODS

Test substances. The effect of the following corticosteroids on collagen biosynthesis was studied: hydrocortisone acetate, fluocinolone acetonide, fluclorolone acetonide, betamethasone-17-valerate and fluprednyliden-21-acetate. These substances were dissolved in physiological saline containing 80% (v/v) ethyl alcohol. In control incubations, physiological saline containing 80% ethyl alcohol was used.

In vivo *incubations.* Fertilized eggs (Saarioinen Oy, Sahalahti, Finland) were incubated in a moist atmosphere at 37°. On the 11th day of embryonic development, 0·1 ml of test substances were pipetted on the chorioallantoic membrane in the concentrations indicated in the legends to the tables. After the periods indicated, 2 μc of [^{14}C]proline, specific activity 180 mc/m-mole (New England Nuclear Corporation), was injected on the chorioallantoic membrane as well. After 3 hr incubation at 37°, the embryos were removed and the tibiae carefully dissected out. Tibiae from five to ten embryos were used for each determination; the bones were cross-paired and used in duplicate for further analyses.

In experiments designed to study the effect of corticosteroids on the activity of protocollagen proline hydroxylase, the tibiae were removed after incubation with the test substances. For each determination the enzyme activity was assayed from two groups of cross-paired tibiae taken from five to eight embryos.

In vitro *incubations.* In order to study the effect of corticosteroids on collagen biosynthesis *in vitro*, the tibiae of 11-day-old chick embryos were pooled and then randomly divided into portions containing seven to ten bones each. The tibiae were preincubated with test substances in 2 ml of Krebs–Ringer A solution,[10] pH 7·4 at 37°, and 2 μc of radioactive proline was added to the incubation mixture. Incubation in a metabolic shaker was stopped after 90 min by chilling the samples and the bones were immediately used for further analyses.

Preparation of the bones. After incubation, the bones were homogenized in 3 ml of distilled water, with a Teflon and glass homogenizer. The homogenates were dialyzed against running tap water for 24 hr, and then hydrolyzed in 6 M HCl for 6 hr at 136°. After hydrolysis, the samples were evaporated to dryness and then eluted with distilled water. Aliquots of the eluate were used for determination of total and radioactive hydroxyproline and total ^{14}C-radioactivity.

Assay of protocollagen proline hydroxylase. The tibiae were homogenized in 2 ml of 0·1 M KCl and 0·02 M tris–HCl (pH 7·8 at 24°) with a Teflon and glass homogenizer. The 15,000 *g* supernatant of the bone homogenate was used as a source of the enzyme. Aliquots of the supernatant were incubated with [^{14}C]proline-labelled protocollagen substrate,[11] 50 mM tris–HCl buffer (pH 7·8 at 24°), 2 mM ascorbic acid, 0·5 mM α-ketoglutarate, 0·08 mM $FeSO_4$, and 0·05 mg/ml catalase, all in a final volume of 4·0 ml.[11,12] After incubation, the samples were hydrolyzed in 6 M HCl for 6 hr at

136°. Thereafter, total ^{14}C-radioactivity and [^{14}C]hydroxyproline were determined in the hydrolysate. Another aliquot of the 15,000 *g* supernatant of the bone homogenate was hydrolyzed as above and then used for determination of α-amino nitrogen.

Determination of [^{14}C]hydroxyproline and total ^{14}C-radioactivity. Radioactive hydroxyproline was determined by the method of Juva and Prockop[13] and total ^{14}C-radioactivity by the method described by Prockop and Ebert.[14] The measurements of radioactivity were made with a three-channel liquid scintillator (DECEM-NTL 314, Wallac Ltd., Finland). The observed counts were corrected for quenching by comparison of the external standard, with a channel ratio technique, and the radioactivities were expressed as disintegrations per minute.

Determination of hydroxyproline and protein contents. The amount of hydroxyproline was assayed by the method of Kivirikko *et al.*,[15] and α-amino nitrogen according to Rubinstein and Pryce.[16] The protein content was calculated by multiplying the values of α-amino nitrogen by a factor of 7·2.

RESULTS

Effect of corticosteroids on [^{14}C]hydroxyproline formation and total ^{14}C-incorporation in vivo. When 200 μg of hydrocortisone acetate, fluocinolone acetonide, fluclorolone acetonide, betamethasone-17-valerate or fluprednyliden-21-acetate was injected on the chorioallantoic membrane, three times at 3-hr intervals, a marked decrease in formation of radioactive hydroxyproline could be observed (Table 1). The observed decrease was of the same order of magnitude, i.e. about 80 per cent, with all the corticosteroids tested. Further, the incorporation of total ^{14}C into the bones was inhibited to the same extent, and thus, no changes in the ratio of radioactive hydroxyproline to total radioactivity could be observed. In essentially similar experimental conditions, injection of 100 or 20 μg of test substances on the eggs inhibited collagen

TABLE 1. EFFECT OF 200 μg OF HYDROCORTISONE ACETATE, FLUOCINOLONE ACETONIDE, FLUCLOROLONE ACETONIDE, BETAMETHASONE-17-VALERATE OR FLUPREDNYLIDEN-21-ACETATE ON [^{14}C]HYDROXYPROLINE FORMATION AND TOTAL ^{14}C INCORPORATION IN CHICK EMBRYO TIBIAE *IN VIVO*

Test substance	Amount (μg)	[^{14}C]hydroxyproline (dis./min/μg hypro)	[^{14}C]hydroxyproline (dis./min/bone)	Total ^{14}C (dis./min/bone)
Control		40·5	4340	11800
Control		37·8	4480	15200
Hydrocortisone acetate	200	10·2	1130	3380
Hydrocortisone acetate	200	6·8	1040	3210
Fluocinolone acetonide	200	3·4	270	860
Fluocinolone acetonide	200	2·5	300	1000
Fluclorolone acetonide	200	6·5	580	2120
Fluclorolone acetonide	200	7·0	620	2035
Betamethasone-17-valerate	200	7·4	710	2320
Betamethasone-17-valerate	200	7·8	680	2340
Fluprednyliden-21-acetate	200	8·4	700	1950
Fluprednyliden-21-acetate	200	6·7	530	1520

200 μg of a test substance was injected, three times at 3-hr intervals, on the chorioallantoic membrane of 11-day-old fertilized eggs. Thereafter, 2 μc of [^{14}C]proline was injected, as well. After 3 hr incubation, the tibiae were dissected out, and [^{14}C]hydroxyproline, total ^{14}C and hydroxyproline content were assayed, as described in Material and Methods.

TABLE 2. EFFECT OF 5 μg OF HYDROCORTISONE ACETATE, FLUOCINOLONE ACETONIDE, FLUCLOROLONE ACETONIDE, BETAMETHASONE-17-VALERATE OR FLUPREDNYLIDEN-21-ACETATE ON [^{14}C]HYDROXYPROLINE FORMATION AND TOTAL ^{14}C INCORPORATION IN CHICK EMBRYO TIBIAE *IN VIVO*

Test substance	Amount (μg)	[^{14}C]hydroxyproline (dis./min/μg hypro)	[^{14}C]hydroxyproline (dis./min/bone)	Total ^{14}C (dis./min/bone)
Control		38·1	1090	3700
Control		36·1	1060	3480
Hydrocortisone acetate	5	32·1	915	3190
Hydrocortisone acetate	5	33·0	980	3030
Fluocinolone acetonide	5	14·2	390	1425
Fluocinolone acetonide	5	11·7	330	1225
Fluclorolone acetonide	5	14·1	550	1910
Fluclorolone acetonide	5	12·5	480	1670
Betamethasone-17-valerate	5	19·0	415	1470
Betamethasone-17-valerate	5	21·1	440	1400
Fluprednyliden-21-acetate	5	13·7	290	1200
Fluprednyliden-21-acetate	5	15·7	260	1010

For experimental conditions, see legend to Table 1.

formation by *ca.* 75 and 55 per cent, respectively. Injection of 5 μg of test substances on the chorioallantoic membrane of the chick embryos caused about 50 per cent inhibition of [^{14}C]hydroxyproline formation or of total ^{14}C incorporation (Table 2).

In further experiments, 200 μg of test substance was injected on the chorioallantoic membrane, and after 1 hr incubation 2 μc of [^{14}C]proline was added. A slight inhibition of [^{14}C]hydroxyproline formation could be observed with fluocinolone acetonide and fluclorolone acetonide, whereas the other corticosteroids tested did not exert any effect (Table 3).

TABLE 3. EFFECT OF 200 μg OF HYDROCORTISONE ACETATE, FLUOCINOLONE ACETONIDE, FLUCLOROLONE ACETONIDE, BETAMETHASONE-17-VALERATE OR FLUPREDNYLIDEN-21-ACETATE ON COLLAGEN FORMATION IN CHICK EMBRYO TIBIAE *IN VIVO*

Test substance	Amount (μg)	[^{14}C]hydroxyproline (dis./min/μg hypro)	[^{14}C]hydroxyproline (dis./min/bone)	Total ^{14}C (dis./min/bone)
Control		35·6	1510	4280
Control		33·4	1770	5210
Hydrocortisone acetate	200	36·6	2080	5800
Hydrocortisone acetate	200	34·4	1950	5700
Fluocinolone acetonide	200	25·2	1380	3690
Fluocinolone acetonide	200	24·7	1630	5390
Fluclorolone acetonide	200	30·8	1835	5100
Fluclorolone acetonide	200	30·0	1810	5850
Betamethasone-17-valerate	200	37·8	1910	5570
Betamethasone-17-valerate	200	39·5	1990	5450
Fluprednyliden-21-acetate	200	37·0	2040	6320
Fluprednyliden-21-acetate	200	35·9	1860	5290

200 μg of a test substance was injected on the chorioallantoic membrane of 11-day-old fertilized eggs. After 1 hr incubation, 2 μc of [^{14}C]proline was injected into the eggs. Three hr later the tibiae were dissected out, and [^{14}C]hydroxyproline, total ^{14}C and hydroxyproline content were assayed, as described in Material and Methods.

TABLE 4. EFFECT OF HYDROCORTISONE ACETATE, FLUOCINOLONE ACETONIDE, FLUCLOROLONE ACETONIDE, BETAMETHASONE-17-VALERATE OR FLUPREDNYLIDEN-21-ACETATE ON PROTOCOLLAGEN PROLINE HYDROXYLASE ACTIVITY IN CHICK EMBRYO TIBIAE *IN VIVO*

Test substance	Amount (μg)	Protocollagen (dis./min/mg supernatant protein)	Proline (dis./min/mg total protein)	ψydroxylase activity* (dis./min/bone)
Control		6400	1190	1700
Control		9590	1959	2547
Hydrocortisone acetate	200	9002	1660	1657
Hydrocortisone acetate	200	10,950	2300	2431
Fluocinolone acetonide	200	18,030	3749	3280
Fluocinolone acetonide	200	20,105	4220	2680
Fluclorolone acetonide	200	10,640	2880	2547
Fluclorolone acetonide	200	13,550	2710	2875
Betamethasone-17-valerate	200	7990	1450	2240
Betamethasone-17-valerate	200	8720	1619	2352
Fluprednyliden-21-acetate	200	10,810	2650	2919
Fluprednyliden-21-acetate	200	13,060	3060	3252

200 μg of a test substance was injected, three times at 3-hr intervals, on the chorioallantoic membrane of 11-day-old fertilized eggs. After 3 hr incubation, the tibiae were dissected out and the protocollagen proline hydroxylase activity in the bones was assayed, as described in Material and Methods.

* The values are expressed as dis./min [^{14}C]hydroxyproline formed in an aliquot of 50,000 dis./min [^{14}C] proline-labelled protocollagen substrate per hr.

Effect of corticosteroids on protocollagen proline hydroxylase activity in the bones. When 200 μg of the test substances were injected on the chorioallantoic membranes, three times at 3-hr intervals, no changes in the activity of protocollagen proline hydroxylase could be observed when the enzyme activity was expressed as dis./min [^{14}C]hydroxyproline synthesized in an aliquot of 50,000 dis./min [^{14}C]proline-labelled protocollagen substrate per bone. On the other hand, when the values were related to the protein content of the bones or to that in the 15,000 *g* supernatant of the tissue homogenate, fluocinolone acetonide, fluclorolone acetonide and fluprednyliden-21-acetate seemed to increase the enzyme activity as compared to the corresponding controls (Table 4).

Effect of corticosteroids on [^{14}C]hydroxyproline formation and total ^{14}C incorporation in vitro. The effect of corticosteroids on collagen formation was studied *in vitro* by incubating chick embryo tibiae in the presence of test substances, and the conversion of [^{14}C]proline into [^{14}C]hydroxyproline was taken as an index of collagen formation. When the bones were incubated in a medium containing test substances 100 μg/ml, and radioactive proline was added after 180 min preincubation, hydrocortisone acetate, fluocinolone acetonide, fluclorolone acetonide and fluprednyliden-21-acetate slightly inhibited [^{14}C]hydroxyproline formation, whereas in the bones incubated with betamethasone-17-valerate the values for radioactive hydroxyproline were less than one-tenth of those in the controls (Table 5). In addition, all the substances tested inhibited total ^{14}C incorporation and hydroxyproline formation to the same extent, and thus no changes in the ratio [^{14}C]hydroxyproline/total ^{14}C could be observed. When the time of preincubation with corticosteroids was shortened to 15 min, a reversal of the betamethasone-17-valerate-induced inhibition of collagen formation

TABLE 5. EFFECT OF HYDROCORTISONE ACETATE, FLUOCINOLONE ACETONIDE, FLUCLOROLONE ACETONIDE, BETAMETHASONE-17-VALERATE OR FLUPREDNYLIDEN-21-ACETATE ON [^{14}C]HYDROXYPROLINE FORMATION AND TOTAL ^{14}C INCORPORATION IN CHICK EMBRYO TIBIAE *IN VITRO* AFTER 180 min PREINCUBATION

Test substance	Concentration (μg/ml)	[^{14}C]hydroxyproline (dis./min/μg hypro)	(dis./min/bone $\times 10^{-4}$)	Total ^{14}C (dis./min/bone $\times 10^{-4}$)
Control		165·8	4·6	17·8
Control		198·0	4·4	12·8
Hydrocortisone acetate	100	105·9	3·4	10·6
Hydrocortisone acetate	100	86·0	3·1	10·3
Fluocinolone acetonide	100	137·0	3·9	13·4
Fluocinolone acetonide	100	86·5	2·4	8·6
Fluclorolone acetonide	100	143·0	3·8	12·5
Fluclorolone acetonide	100	119·0	3·1	10·4
Betamethasone-17-valerate	100	10·7	0·3	1·1
Betamethasone-17-valerate	100	10·1	0·3	1·2
Fluprednyliden-21-acetate	100	122·0	3·2	11·7
Fluprednyliden-21-acetate	100	115·8	3·0	10·0

Tibiae from 11-day-old chick embryos were incubated in 2 ml of Krebs–Ringer A solution containing 100 μg/ml test substance. Each culture contained eight bones. After pre-incubation, 2 μc of [^{14}C]proline was added. Incubation was continued for 90 min at 37°, and thereafter [^{14}C]hydroxyproline, total ^{14}C, and hydroxyproline content were assayed in the bones, as described in Materials and Methods.

TABLE 6. EFFECT OF HYDROCORTISONE ACETATE, FLUOCINOLONE ACETONIDE, FLUCLOROLONE ACETONIDE, BETAMETHASONE-17-VALERATE OR FLUPREDNYLIDEN-21-ACETATE ON [^{14}C]HYDROXYPROLINE FORMATION AND TOTAL ^{14}C INCORPORATION IN CHICK EMBRYO TIBIAE *IN VITRO* AFTER 15 min PRE-INCUBATION

Sample	Concentration (μg/ml)	[^{14}C]hypro (dis./min/bone $\times 10^{-4}$)	Total ^{14}C (dis./min/bone $\times 10^{-4}$)
Control		3·3	9·6
Control		3·2	10·7
Control		3·1	12·1
Control		2·6	8·3
Hydrocortisone acetate	100	2·7	8·3
Hydrocortisone acetate	100	2·5	8·5
Hydrocortisone acetate	100	2·1	8·2
Fluocinolone acetonide	100	2·7	8·2
Fluocinolone acetonide	100	2·2	8·3
Fluocinolone acetonide	100	2·0	8·1
Fluclorolone acetonide	100	2·3	8·1
Fluclorolone acetonide	100	2·3	7·9
Fluclorolone acetonide	100	2·2	7·7
Betamethasone-17-valerate	100	2·0	8·1
Betamethasone-17-valerate	100	1·8	7·8
Betamethasone-17-valerate	100	1·7	6·6
Fluprednyliden-21-acetate	100	2·4	9·1
Fluprednyliden-21-acetate	100	2·2	8·5
Fluprednyliden-21-acetate	100	1·8	6·6

For experimental conditions, see legend to Table 5.

TABLE 7. EFFECT OF FLUCLOROLONE ACETONIDE AND BETAMETHASONE-17-VALERATE ON [^{14}C]HYDROXYPROLINE FORMATION AND ^{14}C INCORPORATION IN CHICK EMBRYO TIBIAE *IN VITRO*

Test substance	Concentration (μg/ml)	[^{14}C]hypro (dis./min/bone $\times 10^{-4}$)	Total ^{14}C (dis./min/bone $\times 10^{-4}$)
Control		4·7	15·4
Control		5·2	19·3
Control		4·2	15·3
Fluclorolone acetonide	100	3·9	15·1
Fluclorolone acetonide	100	3·5	14·2
Fluclorolone acetonide	100	3·1	12·2
Fluclorolone acetonide	10	3·8	15·2
Fluclorolone acetonide	10	5·3	19·4
Fluclorolone acetonide	10	5·0	17·8
Fluclorolone acetonide	1	4·8	18·7
Fluclorolone acetonide	1	4·8	18·9
Fluclorolone acetonide	1	4·1	17·4
Betamethasone-17-valerate	100	0·5	3·1
Betamethasone-17-valerate	100	0·7	3·1
Betamethasone-17-valerate	100	0·6	2·2
Betamethasone-17-valerate	10	4·1	15·4
Betamethasone-17-valerate	10	2·8	11·5
Betamethasone-17-valerate	10	4·5	16·5
Betamethasone-17-valerate	1	4·2	15·8
Betamethasone-17-valerate	1	4·3	17·1
Betamethasone-17-valerate	1	5·3	18·6

For experimental conditions, see legend to Table 5.

could be observed (Table 6). In addition, the degree of [^{14}C]hydroxyproline formation was found to be dependent on the concentration of corticosteroids in the incubation medium: 100 μg/ml of fluclorolone acetonide or betamethasone-17-valerate inhibited hydroxyproline formation significantly, whereas no inhibition could be observed when 10 or 1 μg/ml was added to the incubation medium (Table 7).

DISCUSSION

The biosynthesis of collagen, the main fibrillar protein of the connective tissue, follows the same basic principles as that of other proteins. However, collagen is characterized by a high content of hydroxyproline, an imino acid which is not derived from the free amino acid pool, but from hydroxylation of proline residues already incorporated into polypeptide precursor of collagen, called protocollagen (for review, see Ref. 17). Consequently, the formation of radioactive hydroxyproline from radioactive proline can be taken as an index of the rate of collagen formation. On the other hand, the rate of total protein synthesis can be estimated in the same specimens by the over-all incorporation of ^{14}C-radioactivity into non-dialyzable proteins. The hydroxylation of proline residues is catalyzed by an enzyme, protocollagen proline hydroxylase. This enzyme has recently been purified to 95 per cent purity,[18] and its activity has been demonstrated in various animal[19–21] and human tissues.[20,22,23] The activity of protocollagen proline hydroxylase has been shown to be high when active synthesis of collagen occurs, e.g. in embryonic[24,25] and granulomatous[24,26]

tissues. In addition, a parallelism between protocollagen proline hydroxylase activity and the rate of hydroxyproline formation has been demonstrated in granuloma tissue.[26]

In the present study, collagen formation was studied in chick embryo tibiae. Chick embryos have been reported to synthesize collagen at a rapid rate,[2] and consequently, they offer a convenient system for studies on the metabolism of connective tissue. When administered *in vivo* all the corticosteroid preparations included in this study, i.e. hydrocortisone acetate, fluocinolone acetonide, fluclorolone acetonide, betamethasone-17-valerate and fluprednyliden-21-acetate, decreased [^{14}C]hydroxyproline formation. The inhibition was found to be dependent on the dose and the length of administration. No clear difference in the degree of inhibition could be demonstrated between the various corticosteroids *in vivo*. On the other hand, *in vitro* betamethasone-17-valerate exerted a considerably greater effect on collagen biosynthesis than did the other corticosteroids tested. The inhibition of [^{14}C]hydroxyproline formation *in vitro* was found to be dependent on the preincubation time and on the concentration of these substances. In general, inhibition of collagen biosynthesis required considerably higher concentrations of corticosteroids *in vitro* than *in vivo*. This observation is consistent with earlier observations indicating that hydrocortisone and its active analogues are less effective in inhibiting protein synthesis *in vitro* than *in vivo* (see Ref. 27). Possibly, active metabolites of corticosteroids contribute to the total inhibition of collagen biosynthesis in whole chick embryos.

In the present study, overall protein synthesis, measured as total ^{14}C incorporation, was found to be inhibited to the same extent as collagen formation. Therefore, the results seemed to suggest that the inhibition of collagen biosynthesis is a reflection of more general impairment of protein synthesis, and that the corticosteroids tested do not exert any action on the hydroxylation of proline residues. This suggestion gained support from the assays of protocollagen proline hydroxylase activity in the bones. The enzyme activity was not changed in bones treated with corticosteroids as compared to the controls. On the other hand, when the values for enzyme activity were related to the protein content of bones, a marked increase in the activity of protocollagen proline hydroxylase in corticosteroid-treated bones could be observed. This finding suggests that the synthesis of this enzyme protein is not inhibited by corticosteroids or else that the degradation of the enzyme protein is slower in corticosteroid-treated bones than in the controls. In addition, the activation of inactive forms of enzyme by some metabolites cannot be excluded.[28] Nevertheless, the dissociation of protocollagen proline hydroxylase activity and the rate of [^{14}C]hydroxyproline formation under the experimental conditions used in this study seems to cast some doubt on the use of the level of protocollagen proline hydroxylase activity in tissues as an indicator of the rate of collagen biosynthesis, as has been suggested earlier.[24]

In conclusion, the present results suggest that corticosteroids inhibit the biosynthesis of collagen by interfering with the formation of polypeptide precursors of collagen. This effect is probably part of a more general inhibition of protein synthesis and not limited to collagen. The mechanism of this inhibition is not clear. Corticosteroids may be involved in the regulation of protein synthesis by acting as effector molecules in repression and induction.[29] On the other hand, the possibility that corticosteroids affect the uptake of amino acids into the cells and, consequently, decrease

the amount of amino acids available for protein synthesis cannot be excluded for the accurate measurement of the intracellular amino acid pool in tibiae would be extremely difficult. The corticosteroids included in this study, i.e. hydrocorticone acetate, fluocinolone acetonide, fluclorolone acetonide, betamethasone-17-valerate and fluprednyliden-21-acetate, inhibited collagen formation *in vivo* to the same extent. Betamethasone-17-valerate, however, seemed to affect collagen formation *in vitro* to a greater degree than the other corticosteroids tested. The latter finding may have relevance to the clinical efficacy and to the extent of side-effects of various corticosteroids when applied locally, e.g. in dermatological disorders.

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