Biochemical Pharmacology, Vol. 17, pp. 2081–2090. Pergamon Press. 1968. Printed in Great Britain

INDUCTION OF COLLAGENOLYTIC AND PROTEOLYTIC ACTIVITIES BY ANTI-INFLAMMATORY DRUGS IN THE SKIN AND FIBROBLAST*

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(*Received* 16 *March* 1968; *accepted* 3 *May* 1968)

Abstract—The administration of cortisol, oxyphenylbutazone and indomethacin to rats results in a marked and abrupt loss of cutaneous collagen from these animals. This collagen loss was associated with the appearance of both collagenolytic and proteolytic activities in the extracellular, extrafibrillar compartment of the skin. Cycloheximide and 5,5-diphenylhydantoin pretreatment inhibited both the cutaneous collagen losses and the appearance of these enzyme activities in the skin.

Kinetic studies have shown that within 4 hr after anti-inflammatory drug administration, peak concentrations of both collagenolytic and proteolytic activities were reached in the skin. These enzymatic activities were profoundly depressed by simultaneous administration of puromycin, cycloheximide and actinomycin-D.

Monolayers of cultured strain-L fibroblasts neither contained nor released either proteolytic or collagenolytic activities. Within 4 hr after administration of cortisol, idomethacin or oxyphenylbutazone, both types of enzymatic activities appeared within these cell cultures.

THE INSOLUBLE collagen components of rat skin have been shown to be relatively inert metabolically, with the biological half-life of this fibrous protein being as long as 1 yr in duration.[1] We have found that within 26 hr of the administration of cortisol[2] or of such nonsteroidal anti-inflammatory drugs as oxyphenylbutazone or indomethacin[3] to rats, large amounts of normally insoluble collagen disappear from the skin of these animals. This loss of cutaneous collagen is at least 10 times greater than the normal loss of *all* tissue collagen from the whole rat appropriate to this time period.[1, 3] Thus, inhibition of collagen anabolism alone cannot explain the observed decrease of collagen concentration in the skin; rather it would appear that the activation of a catabolic process for collagen must result from the administration of these drugs to the rat. In view of the extraordinary importance of collagen fibers to the biophysical properties of the connective tissue and wound repair[4] and in light of the fact that there is more collagen in the mammalian corpus than any other single protein,[5] the mechanism of this drug-produced catabolism of collagen would appear to be of biological importance.

* Supported in part by grants from N.I.H. (AM 08168), O.N.R. (NR 105-325) and the Life Sciences Division of the U.S. Army (6121).

Since collagen is essentially extracellular in location and since administration of the drugs mentioned above does not significantly alter either the number or the kinds of cells found in the skin,[1–3] catabolism of cutaneous collagen most probably proceeds via the release of collagenolytic enzymes from the cell into the extracellular compartment of the skin where the collagen fibers are located. We have presented evidence that this compartment of the skin contains both collagenolytic and proteolytic activity only during the time when losses of cutaneous collagen can be demonstrated subsequent to drug administration.[3]

This paper reports the results of experiments designed to show that: (1) the appearance of collagenolytic activity in the extracellular compartment of skin is probably causally rather than casually related to the concomitant catabolism of cutaneous collagen (2) this collagenolytic activity is most probably a result of enzyme induction by the appropriate drug; (3) this enzyme induction is likely to be due to a depression of an operon; and (4) it would appear that the most probable cell type in the skin in which most of this enzyme induction is occurring is the fibroblast.

MATERIALS AND METHODS

All animal experiments involved groups of 12 male Sprague–Dawley rats weighing 190–200 g each. After drug treatment, the animals were sacrificed by either anesthesia or exsanguination. The skins were shaven and as much skin as possible was removed from the trunk, neck and limbs. The weight of skin, after shaving and dissecting the tissue free of the underlying fat, fascia and *panniculus carnosum* varied by less than 5 per cent within or between the various groups of animals.

These excised skins were minced with scissors and extracted separately in the cold with 0·15 M NaCl as described previously to effect the quantitative extraction of the extrafibrillar, extracellular compartment of the skin.[3, 6]

After standing overnight at 4°, the supernatant was collected by centrifugation and the residue re-extracted sequentially with 0·5 M NaCl and 0·1 M citrate buffer. The final residue representing the insoluble collagen content of the tissue was autoclaved and all the collagen converted thereby into gelatin.[3, 6]

Aliquots of these extracts were hydrolyzed in 4 N HCl and the hydroxyproline concentration was determined.[3, 7] The hydroxyproline concentration was converted into mg of collagen per rat from the fact that 1 μmole hydroxyproline is equivalent to 1 mg collagen.[3, 8]

The results were expressed as the mean of 12 animals. The standard deviations of all these means were never more than 7 per cent and means which differed by more than 15 per cent were always significantly different ($P < 0{\cdot}05$). After dialysis and centrifugation of the 0·15 M NaCl extract to remove the euglobulins (including collagen), these extracts were lyophilized. The enzyme activities of 5 mg/ml of these extracts were determined at pH 7·5 on denatured hemoglobin[9] and at pH 5·5 on insoluble native cutaneous collagen[10] as described previously.[3] The results were expressed in terms of the number of μg of product solubilized after incubation for 16 hr at 32° by 5 mg/ml of this lyophilized skin extract (S_1). This S_1 preparation was also analyzed by two methods for RNA[11, 12] and for β-glucuronidase activity.[13] The latter enzyme represents a primary enzyme marker for lysosomes.[14]

Cortisol (17-OH corticosterone) and cortisol succinate, indomethacin, oxyphenylbutazone and 5′,5′-diphenylhydantoin (Dilantin) were gifts of the Upjohn Company.

Actinomycin-D was a gift of the Squibb Institute for Medical Research and cycloheximide and puromycin were purchased from Calbiochem.

RESULTS

Seven groups of 12 rats each received via the subcutaneous route 3 mg/kg of cortisol, cortisone acetate, prednisone, prednisolone, indomethacin or oxyphenylbutazone or 100 mg/kg of salicylate. One group of rats received carrier alone to serve as control. Twenty-four hr after this injection, another injection of the appropriate drug was administered i.p. Four hr after this second injection all animals were sacrificed and their skins removed, extracted and analyzed for collagen as described above.

Five similar-sized groups of rats were pretreated daily for 7 days with 100 mg/kg i.p. of 5′,5′-diphenylhydantoin (DPH), while 5 other groups of rats were pretreated for 24 hr with two separate s.c. injections of 0·5 mg/kg of cycloheximide. Four of these pretreated groups were then injected as described above with cortisol, prednisolone, indomethacin or oxyphenylbutazone separately. The skins of these animals were also shaven, excised, extracted sequentially as described above and analyzed for their respective collagen contents. The concentrations of the extractable collagens found in these three extracts (0·15 M NaCl, 0·5 M NaCl and 0·1 M citrate buffer) were added together to represent the total amount of extractable collagen found per rat skin. The effects of drug treatment upon the skin content of extractable and insoluble collagen in these animals were averaged per group and the results are presented in Table 1.

Control studies indicated that pretreatment of these rats with either DPH or cycloheximide alone did not alter significantly the analysis of skin collagen content from that of the control. The weight of skin collected from each rat was $11 \pm 0{\cdot}3$ g for the entire population of animals used in this study.

The data of Table 1 indicate first, that with the exception of salicylate, the other three anti-inflammatory drugs significantly reduced the amount of cutaneous insoluble collagen by about 30 per cent without altering the sum of the various extractable collagens in the tissue. Second, when rats were pretreated with either DPH or cycloheximide, the loss of insoluble cutaneous collagen usually associated with the administration of cortisol, indomethacin and oxyphenylbutazone was completely eliminated.

Separate studies were also performed by using steroids such as prednisone and cortisone, which did not have the β-11-OH group essential for glucocorticoid activity in the rat. Neither of these two compounds deficient in 11-OH groups had any effect upon the concentration of insoluble cutaneous collagen in these animals.

The results of analyzing the lyophilized, dialyzed 0·15 M NaCl extracts (S_1) of rat skins for RNA, proteolytic and collagenolytic activities and β-glucuronidase activity are presented for these animals in Table 2. These results demonstrate first, that the administration of cortisol, oxyphenylbutazone and indomethacin, but not salicylate, was associated with the appearance extracellularly of increased amounts of RNA and both proteolytic and collagenolytic activities. No increase in the extracellular activity of β-glucuronidase was observed, despite the appearance of proteolytic and collagenolytic activity after drug administration. The activities of these latter enzymes were also not increased after the administration of anti-inflammatory drugs. Second, both

Table 1. Inhibition of the cutaneous collagen loss associated with the administration of anti-inflammatory drugs to rats by pretreatment with either 5,5-diphenylhydantoin (DPH) or cycloheximide

Pretreatment	Drugs	Collagen per rat skin (mg)	
		Extractable	Insoluble
None	None	350	1480
None	Prednisone	365	1600
None	Prednisolone	350	1010*
None	Cortisol	367	1060*
None	Cortisone	355	1550
None	Indomethacin	360	1118*
None	Oxyphenylbutazone	390	1080*
None	Salicylate	330	1590*
DPH	None	380	1630
DPH	Cortisol	356	1495
DPH	Prednisolone	370	1375
DPH	Indomethacin	360	1350
DPH	Oxyphenylbutazone	390	1410
Cycloheximide	None	320	1450
Cycloheximide	Cortisol	330	1510
Cycloheximide	Prednisolone	350	1450
Cycloheximide	Indomethacin	318	1380
Cycloheximide	Oxyphenylbutazone	330	1555

* Significantly different from the untreated control ($P < 0.05$).

Table 2. Effects of pretreating rats with either 5,5-diphenylhydantoin (DPH) or cycloheximide upon the RNA concentration and enzyme activities of the nondialyzable, lyophilized 0·15 M NaCl extract of the skin of rats 28 hr after administration of anti-inflammatory drugs

Pretreatment	Drug	RNA*	Protease†	Coll'ase	β-Glucuronidase§
None	None	6·2	0	0	0·008
None	Prednisone	6·0	0	0	0·008
None	Prednisolone	9·0‖	270‖	70‖	0·007
None	Cortisol	8·9‖	280‖	60‖	0·006
None	Cortisone	6·5	0	0	0·008
None	Indomethacin	7·4‖	250‖	38‖	0·007
None	Oxyphenylbutazone	7·1‖	230‖	35‖	0·008
None	Salicylate	6·0	0	0	0·007
DPH	None	5·5	0	0	0·006
DPH	Cortisol	6·6	0	0	0·007
DPH	Prednisolone	6·8	0	0	0·008
DPH	Indomethacin	6·0	0	0	0·008
DPH	Oxyphenylbutazone	6·1	0	0	0·007
Cycloheximide	None	6·0	0	0	0·007
Cycloheximide	Cortisol	8·8‖	0	0	0·008
Cycloheximide	Prednisolone	7·5‖	0	0	0·008
Cycloheximide	Indomethacin	7·5‖	0	0	0·008
Cycloheximide	Oxyphenylbutazone	7·5‖	0	0	0·007

* RNA in μg/10 mg of skin extract.

† μg/ml of tyrosine equivalents released into TCA from denatured hemoglobin at pH 7·5 by 5 mg/ml of skin extract.

‡ μg/ml of peptide bound hydroxyproline released per 10 mg of purified insoluble collagen at pH 5·5 by 5 mg/ml of skin extract.

§ μmoles substrate hydrolyzed/min/mg saline extract.

‖ Significantly different from the untreated control ($P < 0.05$).

DPH and cycloheximide pretreatment completely eliminated the appearance of the proteolytic and collagenolytic activities extracellularly after drug administration. Although DPH could inhibit the action of either of these enzymatic activities *in vivo*,[3] cycloheximide was without effect *in vitro* in this regard.

Finally, the increased concentration of extracellular RNA with drug administration was not altered by cycloheximide pretreatment, whereas DPH pretreatment completely eliminated this increment of extracellular RNA.

The kinetics of the appearance of extracellular pH 5·5 optimal collagenolytic activity in the skin of rats 0, 3, 4, 5 and 6 hr after receiving a single i.p. dose of cortisol or indomethacin was studied. Groups of 30 rats each received either cortisol or indomethacin alone, while two similar-sized control groups received either desoxycorticosterone (DOCA) or the solvent alone. After the appropriate period of time, these animals were sacrificed in groups of 6 rats each, and the S_1 was prepared from each rat skin separately. The collagenolytic activity of each animal's saline extract was found not to vary by more than 14 per cent, with the S.D. within each group of 6 rats being ± 7 per cent of the mean. These results are presented in Table 3 and are

TABLE 3. COLLAGENOLYTIC ACTIVITY OF 5 mg/ml OF THE DIALYZED, CENTRIFUGED AND LYOPHILIZED 0·15 M NaCl EXTRACTS OF THE SKIN OF DRUG-TREATED RATS IN μg/ml OF PEPTIDE-BOUND HYDROXYPROLINE RELEASED FROM 10 mg/ml OF PURIFIED, NATIVE AND INSOLUBLE COLLAGEN AT pH 5·5 AFTER 16 hr OF INCUBATION AT 32°

Drugs	Hours after drug administration				
	0	3	4	5	6
Normal control (solvent)	0	0	0	0	0
Cortisol (3 mg/kg)	0	34	57	55	48
Cortisol + puromycin	0	20*	24*	12*	10*
Cortisol + cycloheximide	0	16*	16*	8*	8*
Cortisol + actinomycin-D	0	16*	20*	16*	8
Indomethacin (3 mg/kg)	0	20	32	23	19
Indomethacin + puromycin	0	12*	20*	16*	8*
Indomethacin + cycloheximide	0	8*	16*	8*	4*
Indomethacin + actinomycin-D	0	9*	11*	10*	10*
DOCA	0	0	0	0	0

* Significantly different from the appropriate drug alone ($P < 0.05$).

compared with those obtained from animals which had been treated simultaneously and subsequently with either puromycin, cycloheximide or actinomycin-D, in accordance with the dosage regimen of Thompson *et al.*[15] or Garren *et al.*[16] These results indicate that the collagenolytic activity found extracellularly in the skin of rats treated with either a steroidal or nonsteroidal anti-inflammatory drug was maximal about 4 hr after drug administration. All three inhibitors of protein biosynthesis, i.e. puromycin, cycloheximide and actinomycin-D,[17–19] reduced the amount of collagenolytic activity found extracellularly by as much as 80 per cent for cycloheximide and 80 per cent for actinomycin-D. Neither of the control S_1 preparations demonstrated any free collagenolytic activity at all during these time periods.

Separate experiments in which rats were pretreated for 4 hr with these inhibitors of protein biosynthesis prior to and during the administration of either cortisol or

indomethacin, as described above, indicated that most of the extracellular collagenolytic activity resulting from these anti-inflammatory drugs could be eliminated.

Similar data are recorded in Table 4 for the neutral pH optimal proteolytic activity found in the S_1 preparations of these rats. In this case the protein biosynthesis inhibitors were somewhat more effective in eliminating the extracellular appearance of this enzymatic activity.

TABLE 4. PROTEOLYTIC ACTIVITY OF 5 mg/ml OF DIALYZED, CENTRIFUGED AND LYOPHILIZED 0·15 M NaCl EXTRACTS OF THE SKIN OF DRUG-TREATED RATS IN μg/ml OF TYROSINE EQUIVALENTS RELEASED INTO TCA FROM DENATURED HEMOGLOBIN AT pH 7·5 AFTER 16 hr OF INCUBATION AT 37°

Drugs	Hours after drug administration				
	0	3	4	5	6
Normal control (solvent)	0	0	0	0	0
Cortisol (3 mg/kg)	0	225	300	280	225
Cortisol + puromycin	0	75*	112*	75*	38*
Cortisol + cycloheximide	0	45*	38*	45*	38*
Cortisol + actinomycin-D	0	75*	75*	93*	75*
Indomethacin (3 mg/kg)	0	150	262	244	225
Indomethacin + puromycin	0	60*	55*	60*	19*
Indomethacin + cycloheximide	0	38*	38*	38*	0*
Indomethacin + actinomycin-D	0	45*	50*	43*	50*
DOCA	0	0	0	0	0

* Significantly different from the appropriate drug alone ($P < 0.05$).

By using standard cell culture techniques, monolayers of strain-L fibroblasts (derived from the mouse) were grown up in 32-oz pharmacy bottles on Eagle's medium for 2 weeks from an inoculum of 1×10^6 cells to a monolayer of about 16–20 million cells per bottle in the presence of 10 μg/ml of vitamin C and 10% fetal calf serum. After remaining in monolayer for a few days, the cells were harvested and washed free of serum in medium alone. These cells were then suspended either in medium alone (1 ml/4 $\times$ 10^6 cells) or in medium containing 20 μg/ml of either cortisol succinate, indomethacin or oxyphenylbutazone for 4 hr at 37°. After this incubation, the entire cell suspension was extracted by crushing in a Potter–Elvehjem glass homogenizer. The resulting suspension was then incubated with either insoluble collagen or denatured hemoglobin as described above, with zero time incubation controls. The resulting collagenolytic or proteolytic activity of these cells was thus determined and calculated per 4 $\times$ 10^6 cells. Similar experiments were also performed in which the cell suspensions were pretreated with cycloheximide or actinomycin-D. The results are presented in Table 5. These results indicate that: first, strain-L fibroblasts normally do not contain any collagenolytic or neutral pH optimal proteolytic activities that can be measured by our methods; second, both proteolytic and collagenolytic activities could be demonstrated after exposing these cells to cortisol succinate, indomethacin or oxyphenylbutazone; and third, inhibitors of protein biosynthesis profoundly decreased the amounts of these enzymatic activities demonstrable after exposure of the cells to these anti-inflammatory drugs.

Separate experiments indicated that these cells accumulated about 0·45 mg collagen per 4 × 10^6 cells during the 3-day monolayer prior to harvesting for the drug experiments described above.

TABLE 5. PROTEOLYTIC AND COLLAGENOLYTIC ACTIVITY OF L-STRAIN FIBROBLAST CULTURES 4 hr AFTER THE ADDITION OF VARIOUS ANTI-INFLAMMATORY DRUGS WITH AND WITHOUT PRETREATMENT WITH INHIBITORS OF PROTEIN BIOSYNTHESIS

Pretreatment	Drug	Total enzyme activity	
		Proteolytic*	Collagenoyltic†
None	None	0	0
None	Cortisol succinate	85‡	65‡
None	Indomethacin	130‡	85‡
None	Oxyphenylbutazone	130‡	90‡
None	Salicylate	0	0
Cycloheximide	None	0	0
Cycloheximide	Cortisol succinate	10‡§	4‡§
Cycloheximide	Indomethacin	10‡§	8‡§
Cycloheximide	Oxyphenylbutazone	18‡§	8‡§
Actinomycin-D	None	0	0
Actinomycin-D	Cortisol succinate	12‡§	0
Actinomycin-D	Indomethacin	8‡§	4‡§
Actinomycin-D	Oxyphenylbutazone	15‡§	5‡§

* μgm/ml of TCA-soluble tyrosine equivalents released by the 0·15 M NaCl extract of 4 × 10^6 cells from denatured hemoglobin at pH 7·5.

† μgm/ml of peptide-bound hydroxyproline released by the 0·15 M NaCl extract of 4 × 10^6 cells from insoluble collagen at pH 5·5.

‡ Significantly different from untreated controls ($P < 0·05$).

§ Significantly different from cells which had *not* been pretreated ($P < 0·05$).

TABLE 6. DISTRIBUTION OF PROTEOLYTIC AND COLLAGENOLYTIC ACTIVITIES BETWEEN FIBROBLAST CELLS IN MONOLAYER, MEDIUM AND 0·15 M NaCl WASH OF THE CELLS ALONE AFTER EXPOSURE FOR 4 hr TO 20 μg/ml OF CORTISOL SUCCINATE (CORT), INDOMETHACIN (INDO) AND OXYPHENYLBUTAZONE (OXY)

Enzymatic activity	Control	Drugs		
		Cort	Indo	Oxy
Proteolytic (μg tyrosine/4 × 10^6 cells)				
medium alone	0	30	75	100
0·15 M NaCl wash	0	35	40	40
calc. total	0	65	115	140
observed total	0	78	134	134
% of total recovered	100	83	86	102
Collagenolytic (μg hydroxyproline/4 × 10 cells)				
medium alone	0	24	47	63
0·15 M NaCl wash	0	39	24	24
calc. total	0	63	71	87
observed total	0	63	79	86
% of total recovered	100	100	90	101

Similarly, monolayers of fibroblasts were grown, harvested, suspended in medium and treated with the three anti-inflammatory drugs described previously. After 4 hr of drug treatment, these cell suspensions were separated into two aliquots and the cell number counted in a homocytometer in the usual fashion. One aliquot of cells was centrifuged, the medium was removed and the cells were washed once with 0·15 M NaCl. This extract was also removed. The other aliquot was ground up, cells and medium together, in a Potter–Elvehjem glass homogenizer. The medium alone, the 0·15 M NaCl extract alone, and the homogenized cells and media together were then assayed for proteolytic and collagenolytic activity. The sum of the medium and saline-extracted enzymatic activities was then calculated and compared with the total activities determined by homogenizing both cells and medium together. These results are presented in Table 6 and demonstrate that at least 90 per cent of both enzyme activities can be removed from the fibroblasts by simple washing with isotonic saline.

DISCUSSION

Within 28 hr after the administration of cortisol, indomethacin or oxyphenylbutazone to rats, about 30 per cent of the insoluble cutaneous collagen concentration in these animals disappeared without demonstrating any significant changes in the total amounts of extractable collagens in these tissues. As has been shown previously by us, DPH at 100 mg/kg completely inhibited this cutaneous collagen response to these drugs.[20] Cycloheximide, a potent inhibitor of peptide chain lengthening,[19] also inhibited the drug-induced loss of cutaneous collagen.

Associated with this disappearance of cutaneous collagen was the appearance of collagenolytic and proteolytic activities in the 0·15 M NaCl extracts of these skins. The assay and general properties of these enzymes have been described previously.[3] The RNA contents of these skin extracts, representing largely the extracellular, extrafibrillar compartment of the skin,[2, 6] were also increased with the administration of drugs which resulted in collagen catabolism.

The two agents, DPH and cycloheximide, which inhibited the loss of cutaneous collagen, also inhibited the appearance of collagenolytic and proteolytic activities in the 0·15 M NaCl extract. DPH has been shown to inhibit partially these enzymatic activities *in vitro*.[3] Cycloheximide did not inhibit either enzymatic activity *in vitro*. DPH also inhibited the extracellular release of RNA; cycloheximide did not. Thus, two agents working in obviously very different fashions inhibit in a parallel manner both the appearance of collagenolytic activity in the skin and the loss of collagen from the skin. These results suggest that the appearance of collagenolytic enzyme activity in the skin is causally related to the demonstrable catabolism of cutaneous collagen produced by the administration of anti-inflammatory drugs.

The lack of a lysosomally derived enzyme marker increasing in extracellular concentration along with the collagenolytic and proteolytic activities described above suggests that these latter enzyme activities were not derived from the lysosomes, but from other regions in the cell.

The kinetics of the appearance of these proteolytic and collagenolytic activities in the skin of drug-treated rats followed closely the kinetics of the induction in the liver of the intracellular enzyme, tryptophane pyrolase.[16] Similar also, was the inhibition of the appearance of these enzymes in rat skin when the animals were pretreated with inhibitors of protein biosynthesis[17–19] to the pattern of enzyme induction described

by Thompson *et al.*[15] for the liver enzyme as a result of cortisol administration. The fact that an inhibitor of the synthesis of messenger RNA (actinomycin-D) inhibited the appearance of proteolytic and collagenolytic activities in rat skin suggests further that these enzymatic activities were induced by the various drugs via the derepression of an operon, which then made messenger RNA capable of directing the synthesis of these enzyme proteins.[15–19]

The most obvious cell type to be the target of these enzyme-inducing drugs is the fibroblast. The finding that monolayers of strain-L fibroblasts, which normally contained no commensurable amounts of these enzymatic activities, contained large amounts of these enzymes within 3 hr after exposing these cells to anti-inflammatory drugs suggests that this cell is the primary source of the enzymes involved in collagen catabolism in the rodent. The ability of cycloheximide and actinomycin-D to inhibit the appearance of proteolytic and collagenolytic enzymatic activities in these cultured fibroblasts suggests the interpretation that these enzymes were induced via a derepression of an operon within these cells.

Preliminary experiments with the skin from anti-inflammatory drug-treated rats indicated that removal of the upper 1/3 of the skin, including epidermis and other nonfibroblast-containing structures, did not reduce the total amounts of collagenolytic and proteolytic activities found within the whole skin on a per rat basis. This further confirms the probability that the bulk of these enzymes was associated with the fibroblastic cells within the tissue, since most of these kinds of cells are found in the lower 2/3 of the skin.

It is of interest to note that almost all of these induced enzymatic activities were released easily from these cells. In fact, 1/2 to 2/3 of the induced collagenolytic activity was released from the fibroblast into the culture medium. Thus not only was the collagenolytic enzyme(s) producing cutaneous collagen catabolism induced in the fibroblast by cortisol, indomethacin or oxyphenylbutazone, but these activities were also released easily from the cell into the extracellular space of the skin where the collagen substrate is located.

These inductive effects of both steroidal and nonsteroidal anti-inflammatory drugs upon the fibroblast recall the observations of Berliner *et al.*[21, 22] These workers found that the administration of anti-inflammatory steroids resulted in a loss of skin collagen, the morphologic "rounding-up" of rodent fibroblasts and an inhibition of their growth. Steroids which did not contain a β-11-OH group were essentially without effect upon the rodent fibroblast.[22] We have found that the administration of cortisone, desoxycorticosterone and prednisone, all steroids without an 11-OH group, was also without effect in the rat with regard to cutaneous collagen catabolism. Two groups of workers reported that they were unable to duplicate the steroid-induced catabolism of rat skin which we had described earlier.[2] One of these groups used prednisone;[23] the other group studied effects of cortisone[24] upon the collagen content of rat skin. When the varieties of these steroids containing β-11-OH groups (prednisolone and cortisol respectively) are used in the rat, changes in fibroblast morphology and growth[22] or catabolism of cutaneous collagen[2, 3] can be demonstrated.

Finally, phenylbutazone has been shown to be capable of inducing aminopyrine *N*-dimethylase activity in the liver, while salicylates apparently had no inductive effects upon drug-destroying enzyme.[25] These results are similar to our findings of Tables 1 and 2.

CONCLUSIONS

Cutaneous collagen catabolism in the rat appears to be a result of the induction, probably via the derepression of an operon in the skin fibroblast, of collagenolytic enzyme activity, which can readily diffuse out from the cell into the extracellular space of the skin where the collagen substrate is located. This induction can be demonstrated *in vivo* in skin or *in vitro* in fibroblast monolayers by cortisol and other anti-inflammatory steroids which contain a β-11-OH group, such as prednisolone, or by such nonsteroidal anti-inflammatory drugs as indomethacin or oxyphenylbutazone.

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