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REFERENCES

1. E. F. Elslager and J. Davoll, *Lectures in Heterocyclic Chemistry* (Eds. R. N. Castle and L. B. Townsend), Vol. 2, pp. S97–S133. Hetero Corp., Orem, UT (1974).
2. W. E. Richter, Jr. and J. J. McCormack, *J. med. Chem.* **17**, 943 (1974).
3. J. J. McCormack, in *Chemistry and Biology of Pteridines*, (Ed. W. Pfleiderer), pp. 125–132. Walter de Gruyter, New York (1976).
4. J. R. Bertino and W. L. Sawicki, *Proc. Am. Ass. Cancer Res.* **18**, 168 (1977).
5. K. W. Ledig, *Ger Offen* 2,731,039; *Chem. Abstr.* **88**, 105410d (1978).
6. W. E. Gutteridge, J. J. Jaffe and J. J. McCormack, *Biochim. biophys. Acta* **191**, 753 (1969).
7. J. R. Bertino and G. A. Fischer, *Meth. med. Res.* **10**, 297 (1964).
8. Y-M. Wang, E. Lantin and W. W. Sutow, *Clin. Chem.* **22**, 1053 (1976).
9. A. R. Cashmore, R. T. Skeel, D. R. Makulu, E. J. Gralla and J. R. Bertino, *Cancer Res.* **35**, 17 (1975).
10. A. H. Calvert, J. S. Cridland and K. R. Harrap, *J. clin. Path* **30**, 1154 (1977).
11. P. R. Finley and R. J. Williams, *Clin. Chem.* **23**, 2139 (1977).
12. R. J. Kaufman, J. R. Bertino and R. T. Schimke, *J. biol. Chem.* **253**, 5852 (1978).

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Decreased protein degradation in the skin of glucocorticoid-treated newborn rats*

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The administration of pharmacological doses of glucocorticoids results in decreased growth of experimental animals [1,2] and humans [3,4]. Protein synthesis [2,5–8] and DNA synthesis [2] are decreased in skin following glucocorticoid treatment. Reports in the literature of anti-anabolic effects of glucocorticoids on protein synthesis in skin are numerous [2,5–8]. Although glucocorticoids markedly decrease protein synthesis in skin, we previously observed that the administration of three daily pharmacological doses of the synthetic glucocorticoid, triamcinolone, did not result in the loss of either total body weight or skin weight as compared to zero day controls [2].

Glucocorticoids have been shown to increase catabolic processes in skin. Reports in the literature demonstrate that glucocorticoids increase both collagenolytic and proteolytic activities [9–11] in skin as well as collagenolytic activity in corneal tissue [12] and corneal fibroblasts [13]. Protein degradation in skeletal muscle is also increased by cortisone treatment [14]. The marked decrease of protein synthesis and the increase of catabolic processes by glucocorticoids in the skin should have resulted in an appreciable decrease of protein content. However, the content of total protein did not change as compared to zero day controls [8]. The present study was undertaken to determine the half-lives of skin protein of control and glucocorticoid-treated neonatal rats *in vitro*. Skin proteins labeled with either radioactive proline or tryptophan from triamcinolone-treated newborn rats had longer half-lives *in vitro* than labeled proteins from control animals. During these protein degradation studies, cycloheximide was present at a level which indicated protein synthesis by 91 per cent, thus negating the problem of radioactive precursor reutilization. The present report indicates that, besides having an anti-anabolic effect, glucocorticoids also have an anti-catabolic effect on protein metabolism in skin.

Sprague–Dawley rats (1 to 2-days-old) were used throughout these studies. Powdered triamcinolone diacetate was kindly supplied by Dr. E. W. Cantrell of Lederle Laboratories (Pearl River, NY). Steroid was suspended in 0.9% NaCl. [^{3}H]Tryptophan (3 Ci/mmole) and [2-3-^{3}H]proline (20 Ci/mmole) were purchased from New England Nuclear, (Boston, MA). Eagle's minimal essential medium (F-12) with Hank's balanced salt solution (pH 7.4), 2 × glucose and 2 × $NaHCO_3$, and penicillin–streptomycin solution were obtained from Grand Island Biologicals (Grand Island, NY).

In order to determine the pulse time which gave maximum labeling of skin protein, newborn rats were given radioactive proline for various times before death (Table 1). Skin protein

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Table 1. Time course of [^{3}H]proline incorporation into rat skin protein*

Time (hr)	[^{3}H]Proline incorporation (dis./min./mg protein. × 10^{-3})
0.5	2.9 ± 0.1
1	5.1 ± 0.2
2	6.9 ± 0.4
4	5.9 ± 0.1
9	7.1 ± 0.1

* Fifteen newborn rats received 5 μCi of [2,3-^{3}H]proline per rat. The skin of one rat was homogenized in 15 ml of H_2O at 4°. An aliquot was assayed for protein content by the method of Lowry *et al.* [15]. The homogenate was made 5% (w/v) trichloroacetic acid. The sample was centrifuged at 20,000 *g* for 10 min. The resulting pellet was resuspended in 25 ml of 5% (w/v) trichloroacetic acid and recentrifuged. The wash procedure was repeated. Finally, the tissue pellet was solubilized in 15 ml of 1 N NaOH by incubating at 70° for 1 hr. An aliquot of the sample was neutralized with HCl and counted in a 30% Triton X-100 scintillation mixture. Three rats were killed at each time indicated. The values represent the means ± S.E. of three samples.

was maximally labeled with radioactive proline at 2 hr. Accordingly, the animals used in the decay studies were pulse labeled for 3 hr.

The amount of cycloheximide to be used in the decay studies was determined by incubating skin minces with various concentrations of cycloheximide and by determining the specific activity of radioactive protein (Table 2). The minces were preincubated with cylcoheximide for 30 min and radioactive proline was added for an additional 60 min. Maximum inhibition of protein synthesis was obtained with 10^{-3} M cycloheximide. Accordingly, this concentration of cycloheximide was used in the decay studies.

The half-lives of [^{3}H]proline-labeled proteins of control and glucocorticoid-treated skin are given in Fig. 1. The proline-labeled protein from triancinolone-treated rats had approximately a 4-fold greater half-life than that of control skin. The decay of radioactive protein labeled with tryptophan was also determined (Fig. 1). The half-life of tryptophan-labeled skin protein from steroid-treated rats was 1.6-fold greater than that for skin protein of control rats.

In the present study, the half-lives of skin protein prelabeled with either radioactive proline or tryptophan were higher in steroid-treated tissues than in control tissues. Since tryptophan is not present in mature collagen, the half-life of the total pool of non-collagen protein in the skin is increased by glucocorticoid treatment. A significant portion of the soluble and insoluble pools of collagen is labeled with radioactive proline as early as 30 min after radioisotope administration (data not shown). However, the degradation of proline-labeled protein in skin probably does not represent a significant amount of collagen since the half-lives of degradation in 5-week-old rats of soluble collagen and insoluble collagen in skin are 17 days and 28 days, respectively [16], although the degradation of newborn rat skin collagen may be much more rapid. Furthermore, the studies of degradation of rat skin collagen in 5-week-old rats [16] did not take into account reutilization of labeled precursor. In the *in vitro* mince system, cycloheximide is added to prevent reutilization.

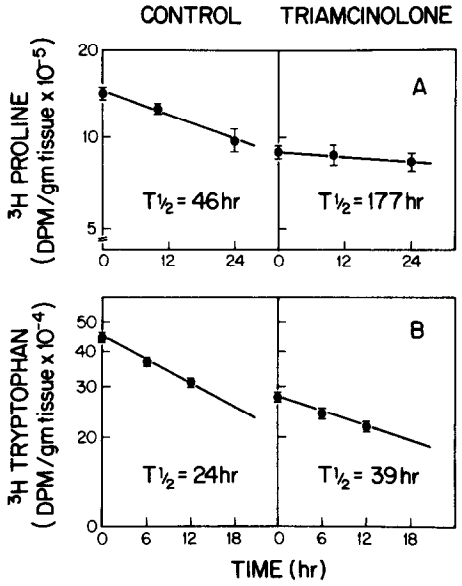

Fig. 1. Decay of proline (A) and tryptophan. (B) labeled skin protein from control and triamcinolone-treated newborn rats (1- to 2-days-old). Newborn rats were injected intraperitoneally with either 0.9% NaC or triamcinolone diacetate (15 mg/kg) for 3 days. Three hours prior to death on day 4, each rat was injected intraperitoneally with 5 μCi of either [^{3}H]proline or [^{3}H]tryptophan. The skins were rinsed in Eagle's minimal essential medium with Hank's balanced salt solution (pH 7.4), 2 ×glucose and 2 ×$NaHCO_3$, 0.05 M Tris (hydroxymethyl) methyl-glycine, penicillin (100 units/ml) and streptomycin (100 μg/ml). All subsequent operations were carried out under sterile conditions. The skins were minced in minimal essential media and drained on cheesecloth. One gram of tissue was placed into a sterile dialysis bag containing 10 ml of minimal essential medium with 10^{-3} M cycloheximide. Following incubation, the contents of the bag were placed in a centrifuge tube and homogenized with a Polytron ST homogenizer for 1 min. An equal volume of trichloroacetic acid (10%, w/v) was added to the homogenate. The sample was washed with 5% (w/v) trichloroacetic acid, solubilized in NaOH and total incorporation was determined as described in Table 1. Decay curves were fitted to the data by the least squares method and the half-lives of loss of radioactivity were calculated using the first order decay rate equation.

Table 2. Dose–response of cycloheximide inhibition of protein synthesis in rat skin*

Addition	Proline incorporation (dis./min./mg protein × 10^{-3})
None	3.88
Cycloheximide (M)	
10^{-7}	3.39 (13%)
10^{-6}	2.58 (34%)
10^{-5}	0.64 (84%)
10^{-4}	0.45 (88%)
10^{-3}	0.34 (91%)

* Newborn rat skin was minced in minimal essential medium. The tissue was drained through cheesecloth. Two g of minced tissue was suspended in 20 ml of Eagle's minimal essential media with cycloheximide. After 30 min, 50 μCi of [2,3-^{3}H]proline was added and the samples were incubated for an additional 60 min. The sample was next homogenized for 1 min with a Polytron ST homogenizer at 4°. Trichloroacetic acid (10%, w/v) was added and total incorporation was determined as described in Table 1. Protein content of the homogenate was determined by the method of Lowry *et al.* [15].

The mechanism of inhibition of protein degradation may be via glucocorticoid-mediated stabilization of lysosomal membranes. Pharmacological doses of glucocorticoids given *in vivo* result in hepatic lysosomes which *in vitro* release reduced amounts of their enzymes [17–20].

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REFERENCES

1. J. N. Loeb, *New Engl. J. Med.* **295**, 547 (1976).
2. R. A. Newman and K. R. Cutroneo, *Molec. Pharmac.* **14**, 185 (1978).
3. Z. Laron and A. Pertzelan, *J. Pediat.* **73**, 774 (1968).
4. J. M. Reinisch, N. G. Simon, W. G. Karow and R. Gandelman, *Science* **202**, 436 (1978).
5. K. I. Kivivikko, O. Laitenen, J. Aer and J. Halme, *Biochem. Pharmac.* **14**, 1445 (1967).
6. Q. T. Smith and D. J. Allison, *Endocrinology* **77**, 785 (1965).
7. J. Uitto, H. Tier and K. K. Mustakallio, *Biochem. Pharmac.* **21**, 2161 (1972).
8. K. R. Cutroneo and D. F. Counts, *Molec. Pharmac.* **11**, 632 (1975).
9. J. C. Houck and Y. M. Patel, *Nature, Lond.* **206**, 158 (1965).
10. J. C. Houck and V. K. Sharma, *Science* **161**, 1361 (1968).
11. J. C. Houck, V. K. Sharma, Y. M. Patel and J. A. Gladner, *Biochem. Pharmac.* **17**, 2081 (1968).
12. S. I. Brown, C. A. Weller and A. M. Vidrich, *Am. J. Ophthalmol.* **70**, 744 (1970).
13. R. M. Hook, C. W. Hook and S. I. Brown, *Invest. Ophthamol.* **12**, 771 (1973).
14. A. L. Goldberg, *J. biol. Chem.* **244**, 3223 (1969).
15. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 267 (1951).
16. K-Y. T. Kao, D. M. Hilker and T. H. McGarack, *Proc. Soc. exp. Biol. Med.* **106**, 121 (1961).
17. G. Weissmann and L. Thomas, *J. exp. Med.* **116**, 433 (1962).
18. S. H. Pollack and J. H. Brown, *J. Pharmac. exp. Ther.* **178**, 609 (1971).
19. L. J. Ignarro, *J. Pharmac. exp. Ther.* **182**, 179 (1972).
20. E. B. Thompson and M. E. Lippman, *Metabolism* **23**, 159 (1974).

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The effect of modification at the carbocyclic ring of nogalamycin on the interaction with DNA

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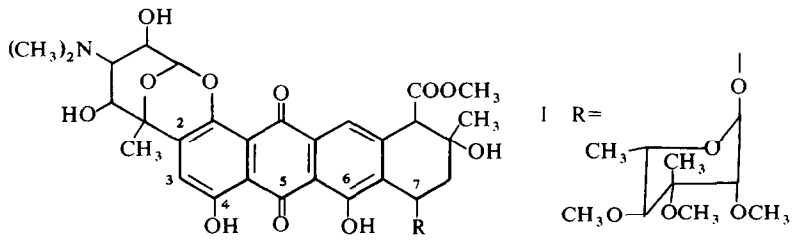
Nogalamycin (I) is an anthracycline antibiotic, but is atypical of this group in that it contains the sugar nogalose rather than an amino-sugar at position 7. Also, the aglycone is in glycosidic linkage at the 1 position with an amino-sugar residue which is additionally bound to the 2 position of the aglycone by a C–C bond [1].



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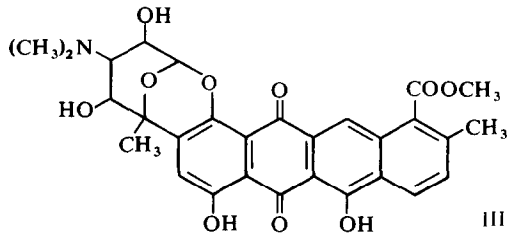

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