cells were detected in 100% of the hosts having donors of incompatible *A*, *C* or *D* blood type. Where incompatibility at the *B* locus was involved, only one host was found to be circulating donor-type cells during the post-irradiation period.

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Collagen Synthesis and C^{14}-Labeled Proline Uptake and Conversion to Hydroxyproline in Steroid-Treated Granulomas.* (29539)

M. R. NOCENTI, G. E. LEDERMAN, C. A. FUREY AND A. J. LOPANO
(Introduced by L. J. Cizek)
Departments of Physiology and Urology, College of Physicians and Surgeons, Columbia University, New York City

Although it has been shown by morphological and histochemical studies that certain steroids depress fibroblastic activity and collagen formation(1-5), there have been few chemical determinations of collagen metabolism following steroid treatment. Robertson and Sanborn obtained a reduction in total collagen concentration in subcutaneous granulomas with large dosages of an 11-oxy steroid(6), while Jorgensen(7) found that steroid treatment decreased the proliferation of dermal granulation tissue, but not its collagen concentration. Studying the specific collagen fractions, Houck showed that sham adrenalectomy and cortisol administration decreased the total neutral soluble and insoluble collagen concentration in rat skin but increased the acid-soluble content(8). The following studies were undertaken to determine the influence of certain steroids on: 1) granuloma collagen content, and 2) proline incorporation into the salt-soluble collagen fraction and its subsequent hydroxylation.

Methods. In the initial study, granulomas were induced by implanting weighed sterile gauze sponges (20 $\pm$ 1 mg) subcutaneously in the dorsum of 120-150 g male Sherman rats. Penicillin G (1500 units) was injected prophylactically. Five days after implantation, a group of animals was sacrificed; a second group was treated with the steroid to be tested (by direct injection into the sponge); and a third group remained untreated. On the ninth day the second and third groups of animals were sacrificed, and their sponges were removed. All of the sponges were weighed immediately after removal and again after drying to constant weight. The sponges were then macerated and extracted

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with distilled water to remove free hydroxyproline. The remaining material was subjected to hydrolysis with 6N HCl at 110°C for a period of 16 hours. The hydrolysate was analyzed colorimetrically for hydroxyproline(9). The steroids used were dexamethasone (Decadron, Merck) in dosages of 125, 250 and 500 μg, and cortisone acetate (Cortone, Merck) in dosages of 250, 500 and 1000 μg.†

In the second study, growth of granulomatous tissue was induced by injecting 0.8 ml of a 1.0% lambda-Carrageenan solution subcutaneously into the dorsum of 8 male Sherman rats weighing 130 to 150 g.‡ Penicillin G (1500 units) was given to all animals at a site distant from the Carrageenan injection. Two days later, 10 mg of hydrocortisone acetate (Hydrocortone, Merck) was injected directly into the center of the developing granulomas in one-half of the animals. Preliminary studies had shown that this dosage of hydrocortisone would cause a 50% reduction in weight of the connective tissue formed. Three days later, all animals were injected intraperitoneally with 0.9 ml of uniformly labeled C^{14}-L-proline (25 μc/ml), and food was withdrawn. Twenty hours after the labeled proline injection, the granulomas were carefully removed and immediately weighed after blotting on filter paper. The salt-soluble fraction of collagen was extracted from the entire granuloma according to the procedure of Gross(10) and hydrolyzed by the method of Hausmann and Neuman(11). Aliquots of the hydrolysate were taken for: (A) hydroxyproline and proline determinations by the method of Neuman and Logan (9), and (B) C^{14} counting for 5 minutes in a liquid scintillation counter by New England Nuclear Assay Corp. after chromatographic separation(11) of hydroxyproline and proline.

Results. Under the experimental conditions of the first study, the amount of connective tissue formed in the gauze sponges increased over the 9 days in the control animals. Both cortisone and dexamethasone administration reduced the wet and dry weights of the granulomas; a progressive reduction being noted with increasing dosage (Table I). Furthermore, the total bound hydroxyproline (collagen) was reduced by these agents below that of the 9-day control level. Steroid treatment did not alter the total hydroxyproline when calculated as a percent of dry weight from the 9-day control value (approximately 3.6%).

In the second study, the mean wet weight of the control granulomas was 6.04 ± 0.61 g while that of the experimental group was 3.04 ± 0.48 g, a highly significant reduction ($p<.01$). In addition, the specific activities of the hydroxyproline and proline in the treated granulomas were significantly reduced ($p<.02$, $p<.01$, respectively). However, the ratio of specific activities (hydroxyproline/proline) was not altered (Table II).

Discussion. The results of the first study confirm the well-known effect of 11-oxy steroids in depressing the growth of granulomatous tissue(1-3). Most investigators have observed a reduction in wet weight(7,12,13). Bush and Alexander(14) obtained a reduction in dry weight, while Jorgensen(7) did not, even though the wet weight was reduced. However, in the latter study, Prednisone, in dosages of 5 and 10 mg/kg did reduce both wet and dry defatted weights. In our series, both wet and dry weights were significantly reduced by the steroid dosages employed with the exception of the lowest cortisone dosage. Aside from the different methods of granuloma induction, these differences probably can be attributed to the different modes of hormone administration. Our injections were made directly into the developing granuloma, while Jorgensen administered the steroids subcutaneously. It is presumed that a considerable difference in effective dosage is involved.

Despite the above noted differences in regard to dry weight changes, in both studies steroid administration did not alter the total hydroxyproline when calculated as a fraction of the dry weight(7). This suggests that the

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‡ Carrageenan was kindly donated by Marine Colloids, Inc., N.Y.C.

TABLE I. Influence of Cortisone Acetate and Dexamethasone on Weight and Hydroxyproline Content of Experimental Granulomas.

Group	No. of animals	Mean wet wt (mg)	Mean dry wt mg	Mean dry wt Avg change from 5 day control (mg)	Bound hydroxyproline μg	Bound hydroxyproline Avg change from 5 day control (μg)
Cortisone:						
5 day control	4	96.5 ± 4.6*	12.5 ± .7†	—	257 ± 36*	—
9 day control	4	159.5 ±12.9	19.8 ± 2.2	+ 7.3	719 ± 72	+462
250 μg	4	127.8 ± 7.8	16.6 ± 1.0	+ 4.1	399 ± 40*	+142
500 μg	5	98.7 ± 1.9*	11.6 ± .2*	— .9	383 ± 48*	+126
1000 μg	5	90.8 ± 5.6*	9.5 ± 1.2*	— 3.0	351 ± 28*	+ 94
Dexamethasone:						
5 day control	5	130.7 ± 6.5*	12.4 ± 1.0*	—	437 ± 32*	—
9 day control	5	298.8 ±25.2	34.3 ± 2.6	+21.9	1256 ±111	+819
125 μg	5	139.7 ± 6.2*	16.4 ± 1.1*	+ 4.0	400 ± 24*	— 37
250 μg	5	106.0 ±12.5*	11.5 ± 1.3*	— .9	275 ± 44*	—162
500 μg	5	101.3 ± 6.0*	8.9 ± .5*	— 3.5	298 ± 27*	—139

All values = average ± standard error of mean.
* $P<.01$ compared to 9 day control.
† $P<.02$ compared to 9 day control.

TABLE II. Influence of Hydrocortisone Acetate on Uptake and Conversion of C^{14}-labeled Proline to Hydroxyproline in Experimental Granulomas.

	No. of rats	Wet wt, g	Proline (Pro) cpm/μmole	Hydroxyproline (OHPro) cpm/μmole	OHPro/Pro
Control	4	6.04 ± .61	749 ± 74	706 ± 66	.95 ± .04
Treated	4	3.04 ± .48*	455 ± 27*	471 ± 42†	1.01 ± .08

All values are the average ± standard error of mean. * = $P<.01$ † = $<.02$
cpm = counts per min. Treated = 10 mg hydrocortisone acetate.

steroid inhibitory effect is not specifically directed upon collagen synthesis alone, but may represent a generalized depression of the activity of those cells responsible for connective tissue formation. It should be noted, however, that although Houck obtained a decreased collagen *concentration,* his data were derived from studies using dermal tissue and calculated on a wet weight basis(8). Robertson and Sanborn report that cortisone in large dosages decreases collagen concentration in subcutaneous granulomas(6). It is possible that reduced total collagen and collagen concentration can occur independently as influenced by steroid dosages. Our results are also in keeping with those biochemical studies demonstrating a steroid-induced decrease in total collagen formation(6,7,8,15).

That the rate of collagen synthesis was reduced is indicated by the reduction in the uptake of C^{14}-labeled proline by the salt-soluble collagen fraction in steroid-treated Carrageenin-induced granulomas. Daughaday and Mariz found that hydrocortisone treatment in rats (2 mg/100 g body wt/day × 7) did not impair the ability of subsequently isolated cartilage to form labeled hydroxyproline *in vitro*(16). However, when hydrocortisone was added to the incubation medium (0.9 μg/ml), the formation of hydroxyproline from labeled proline was inhibited. In contrast, Gerarde and Jones obtained a prolonged *in vitro* inhibition of growth and collagen synthesis, but only when lung tissue from cortisone-treated chick embryos was used with cortisone present in the medium (17).

The ratio of OHPro/Pro specific activities remained at unity for both the control and treated granulomas. This ratio agrees with the ratio of the specific activities obtained by Hausmann and Neuman in the salt-soluble

collagen fraction of guinea pig skin(11). Since it has been shown that collagen hydroxyproline arises only from the hydroxylation of bound proline(11), it may be concluded that the steroid depression of collagen synthesis is not related to an interference with the subsequent hydroxylation of proline.

Since the reduction in collagen synthesis appears to coincide with a depressed formation of other constituents (no change in collagen concentration), the primary steroid effect may be to depress a more basic cellular metabolic process. Thus, the steroid effect probably is not directed specifically at collagen synthesis, and certainly not at the conversion of bound proline to hydroxyproline.

Summary. Cortisone acetate and dexamethasone administered directly into 5-day-old cotton gauze-induced granulomas significantly reduced the wet and dry weights and the total hydroxyproline (collagen) in the granulomas. The total hydroxyproline remained a constant fraction of the dry weight. The injection of hydrocortisone acetate directly into developing (2 days) Carrageenin-induced granulomas significantly reduced the granuloma wet weight and the C^{14}-proline uptake and conversion to hydroxyproline. The hydroxyproline:proline specific activities ratio was not altered from the control value of 1.0 by steroid treatment.

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Estimation of Thyroid-Stimulating Hormone (TSH) Secretion Rates of New Hampshire Fowls.* (29540)

C. E. HENDRICH AND C. W. TURNER†
Department of Dairy Husbandry, University of Missouri, Columbia

A method of estimating the thyroxine secretion rate (TSR) of fowls has been described(1). It has been shown that the mean TSR of mature fowls is significantly lower during summer months than during winter months(2,3). TSR's measured during the period of rapid growth in the chick(4) were significantly higher than were TSR's of mature fowls, determined at comparable seasons of the year(2).

Therefore it seemed of interest to develop a method of directly estimating the thyroid-stimulating hormone secretion rate (TSH-SR) of fowls and to compare it with their

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