Connective Tissue IV. Effect of Age upon Dermal Chemical Response to Adrenal Hormones.* (26849)

P. SETHI, E. R. RAMEY AND J. C. HOUCK
Biochemical Research Laboratory, Children's Hospital Research Foundation, and Georgetown University Medical Center, Washington, D. C.

The adrenal gland plays a major role in hormonal control of the connective tissue(1). Considerable information is available concerning the effect of cortisone upon the histochemistry of this tissue(2,3). Chemically, Layton(4) has shown that rate of synthesis of chondroitin sulfate was inhibited by massive doses of cortisone, while Sobel *et al.*(5,6) have reported that large doses of hormone resulted in a decrease in dermal hexosamine, but had no effect upon dermal collagen content.

Using a sequential extraction procedure for chemical dissection of connective tissue into ground substance, neutral and acid soluble collagen, and insoluble collagen(7), this paper describes the effect of both deoxycorticosteroid (Doca) and hydrocortisone (cortisol) administration upon the chemistry of rat skin. These results were obtained using rats of 3 different weights; 150, 270 and 370 g representing young, mature and aged animals respectively(8).

Materials and methods. Three groups of 18 male Sprague-Dawley rats each weighing an average of 150, 270 and 370 g were employed. Six animals from each group were injected daily for 7 days with 3 mg/kg body weight subcutaneously of cortisol (Cortril, C. Pfizer & Co.); another 6 animals from each weight group were injected similarly with 3 mg/kg of Doca (Organon); the remaining animals were used as normal controls. All animals were sacrificed on the seventh day and about 2 g of abdominal skin from each animal were shaven, removed, dissected free of adhering fat, muscle and fascia, and minced. Aliquots from each tissue sample (0.5 g) were hydrolysed in 4N HCl for 8 hours at 100°C. The cleaned skin remaining from each group of 6 rats was pooled and sequentially extracted in duplicate with 0.15M NaCl, 0.5M NaCl and 0.50M citrate buffer (pH 3.6) in the cold as described previously (7). These extracts were also hydrolysed in 4N HCl. The hydrolysates of both extracts and whole skin were analysed in triplicate for their hydroxyproline(9), hexosamine(10) and nitrogen(11) concentration as a measure of the collagen(12), ground substance (13) and protein content of the skin, respectively. These results were expressed as the ratio of the mean hexosamine of hydroxyproline content (in μmoles) per mmole of total dermal nitrogen of each group of animals, to minimize the changes in dermal water and fat produced by hormone administration.

Experimental and results. Analyses of the saline soluble components of rat skin 7 days after starting either daily cortisol or daily Doca administration are shown in Table I with the results obtained from normal animals of a similar weight. These results indicate that both hormones produce a decrease in ground substance (ratio of hexosamine to nitrogen in the 0.1M NaCl extract) except in the aged animals. Isotonic soluble hydroxyproline/nitrogen was increased with Doca administration only in the aged animal, while both hormones produced decreases of this material in younger animals. The increase in hydroxyproline/nitrogen ratio of the isotonic extracts of the skin of aged animals with Doca was the only difference in the dermal chemical effects of this hormone as compared with cortisol.

Both the hydroxyproline and hexosamine to nitrogen ratios in the 0.5M NaCl extracts were decreased below normal with both hormones in animals of all 3 weights studied.

Analyses of both the citrate buffer soluble and the insoluble components of the skin of rats after either cortisol or Doca administration are shown in Table II. These results show that only with the older animals did

* Supported in part by a grant-in-aid from N.I.H., P.H.S., Bethesda, Md.

TABLE I. Effect of Age upon Dermal Chemical Response to Adrenal Hormones (Saline Soluble Components).

Wt, g		0.15M NaCl Hex/N†	0.15M NaCl Hypro/N†	0.50M NaCl Hex/N†	0.50M NaCl Hypro/N†
150	Normal	1.90	1.90	.74	3.51
	Cortisol	.87*	1.10*	.61	2.90
	Doca	.50*	.85*	.25*	1.23*
270	Normal	.96	1.05	.37	4.00
	Cortisol	.47*	.20*	.27*	1.00*
	Doca	.48*	.34*	.33	2.60*
370	Normal	.43	.70	.38	2.51
	Cortisol	.50*	.38*	.26*	.38*
	Doca	.53*	1.00*	.29*	1.06*

* Significantly different from normal ($p < .05$).
† Hex = μmoles hexosamine; Hypro = μmoles hydroxyproline; N = mmoles nitrogen.

both hormones result in appearance of citrate soluble hexosamine. Animals of all 3 weights demonstrated an increase in citrate soluble collagen with cortisol. Doca was less effective in this regard than cortisol, and with increasing body weight, this hormone became even less active than cortisol until, in the aged animal, Doca resulted in a decrease in dermal concentration of citrate soluble collagen.

The insoluble hexosamine content of the skin from young animals was increased only with administration of Doca. With older animals, both hormones resulted in an increase in this material in the skin. Insoluble collagen was decreased by both hormones in all 3 weight-ranges of animals studied.

The percent changes from normal with respect to total dermal hexosamine and hydroxyproline to nitrogen ratios induced by cortisol or Doca in young (150 g), mature (270 g) and aged (370 g) animals are shown in Table III. These data indicate that although both hormones produced qualitatively similar changes in dermal chemistry, these changes were quantitatively different. Further, Doca and cortisol produced quantitatively different effects upon soluble collagen concentration only in the skin of aged rats.

With increasing age, the total hexosamine to nitrogen ratio in rat skin responded differently to administration of both cortisol and Doca. Total dermal collagen concentration was increasingly depressed from normal with increasing age in response to both cortisol and Doca administration. In either young or mature animals, both hormones decreased the dermal concentration of ground substance. In the aged animal, however, both cortisol and Doca resulted in an increase in ground substance.

Except for the obverse effect of Doca in the aged animal, both hormones decreased the neutral soluble collagen and increased the

TABLE II. Effect of Age upon the Dermal Chemical Response to Adrenal Hormones (Citrate Soluble and Insoluble Components).

Wt, g		0.50 citrate Hex/N†	0.50 citrate Hypro/N†	Insoluble Hex/N†	Insoluble Hypro/N†
150	Normal	0	2.7	0	28.5
	Cortisol	0	4.3*	0	21.0*
	Doca	0	4.0*	.83*	24.5*
270	Normal	0	1.6	.60	41.5
	Cortisol	0	5.1*	1.30*	28.0*
	Doca	.30*	4.2*	1.00*	28.1*
370	Normal	0	3.4	.65	47.0
	Cortisol	.33*	5.0*	1.00*	26.1*
	Doca	.27*	1.7*	1.08*	30.0*

* Significantly different from normal ($p < .05$).
† Hex = μmoles hexosamine; Hypro = μmoles hydroxyproline; N = mmoles nitrogen.

TABLE III. Percent Changes from Normal in Dermal Chemistry with Hormone Administration in Young, Mature and Aged Rats.

		Hormones					
		Cortisol			Doca		
		Young	Mature	Aged	Young	Mature	Aged
Total:	Hex/N	−42	0*	+80*	−39	0*	+40*†
	Hypro/N	−20	−29*	−41*	−16	−26*	−36*
	.15M NaCl Hex/N	−52	−51	+25*	−73†	−50*	+25*
Soluble Hypro/N:	.5M NaCl	−42	−80*	−46	−55†	−67*†	+43*†
	.5M citrate	+59	+210*	+47*	+48†	+163*†	−50*†
	Insoluble Hypro/N	−26	−33	−45*	−14†	−33*	−36

* Significantly different from effect in the previous age group ($p < .05$).
† Significantly different from effect produced by cortisol in a similar age group ($p < .05$).

citrate soluble collagen concentration of the skin from animals of all 3 ages. Finally, both hormones were increasingly effective with age, decreasing the dermal concentration of insoluble collagen.

Discussion. With the exception of the aged animal, both cortisol and Doca produced remarkably similar effects upon the chemistry of rat skin. In view of the primary mineralocorticoid effect of Doca, the similarity of effects produced by cortisol and Doca suggests either a hitherto unknown property of Doca or that changes in electrolytes and water eventually have profound consequences upon the metabolism of the connective tissue. Since the data reported above were based on nitrogen content of the skin, it would seem unlikely that these changes in dermal chemistry with cortisol or Doca would result directly from changes in tissue water content.

The primary effect of adrenal hormones upon the chemistry of the skin is to decrease total and insoluble dermal collagen concentration. Changes in total hexosamine and ground substance with hormone administration seem to depend largely upon the age of the animal. Presumably this represents either the synergistic or antagonistic activities of various sex and growth hormones with adrenal hormones acting upon the connective tissue or changes with ageing in responsiveness of the connective tissue itself to these hormones.

Sobel *et al.*(5,6) found that cortisone was without effect upon collagen content of rat skin. In young animals, such as this group employed in their studies, collagen concentration decreased 20%. Either these workers could not analytically determine a change in dermal collagen concentration of this magnitude, or the large doses of drug employed by them over a long period of time resulted in a different response to cortisone. Our data and those reported by them with respect to both dermal hexosamine loss and decrease in hexosamine to collagen ratios with cortisol administration are essentially in agreement.

Both Doca and cortisol resulted in a decrease in neutral soluble and insoluble collagen and an increase in citrate soluble collagen in young or mature animals only. Quantitatively these changes in dermal collagen are remarkably similar to those produced in uninjured skin distant from the site of local injury(11,14). Perhaps these changes with local inflammation proceeded *via* the trauma of injury and consequent release of minute amounts of adrenal hormones. This work on inflammation was performed only on mature animals, and no changes in total hexosamine with local injury were either found(14) or should be expected from the data on Table III. If adrenal hormonal mechanisms are involved in this "distant dermal collagen response to local inflammation"(14,15), then inflammation in young or aged rats should result in either a loss or an increase in hexosamine concentration of the uninjured skin (Table III) respectively.

Summary. Both cortisol and Doca similarly affect the chemistry of the connective tissue in that they result in a decrease in total and insoluble collagen concentration of rat skin. The effects of these hormones upon rat skin are profoundly altered by the age

of the animal. The aged rat demonstrates a considerably different dermal chemical response to cortisol and particularly Doca than does either the young or the mature animal.

The authors wish to acknowledge the assistance of Jean E. Johnston.

1. Iverson, K., *Connective Tissue in Health and Disease,* ed. Asboe-Hansen, Munksgaard, Copenhagen, 1954, pg. 130.

2. Castor, W. C., Baker, B. L., *Endocrinol.,* 1950, v47, 234.

3. Grossfeld, H., Ragen, C., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 63.

4. Layton, L. L., *Arch. Biochem. Biophys.,* 1951, v32, 224.

5. Sobel, H., Gabay, S., Johnson, C., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 296.

6. Sobel, H., Marmorsten, J., *Endocrinol.,* 1954, v55, 21.

7. Houck, J. C., Jacob, R. A., PROC. SOC. EXP. BIOL. AND MED., 1960, v105, 324.

8. Houck, J. C., De Angelo, L., Jacob, R. A., *ibid.,* 1961, v107, 280.

9. Martin, C. J., Axelrod, A. E., *ibid.,* 1953, v83, 461.

10. Blix, G., *Acta Chem. Scand.,* 1948, v2, 467.

11. Houck, J. C., Jacob, R. A., *J. Invest. Dermatol.,* 1961, v36, 451.

12. Neuman, R. E., Logan, M. A., *J. Biol. Chem.,* 1950, v186, 549.

13. Boas, N. F., *Arch. Biochem. Biophys.,* 1955, v57, 367.

14. Houck, J. C., Jacob, R. A., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 145.

15. Sethi, P., Houck, J. C., *J. Invest. Dermatol.,* 1961, v37, 85.

Received June 28, 1961. P.S.E.B.M., 1961, v108.

Relation of Selenium, Vitamin E and Other Factors to Muscular Dystrophy in the Rabbit. (26850)

J. F. PROCTOR,* D. C. MAPLESDEN,† D. E. HOGUE, AND J. K. LOOSLI
Dept. of Animal Husbandry, Cornell University, Ithaca, N. Y.

Among various species there are widely differing effects of a dietary lack of Vit. E, the most common manifestation of which is muscular degeneration. Morgulis and Spencer (10) found evidence for a factor in alfalfa or whole wheat germ which was necessary for preventing muscular dystrophy in the rabbit in addition to a fat-soluble factor (Vit. E). MacKenzie and McCollum(9) reported that Vit. E alone was capable of preventing this deficiency syndrome. A lack of many other nutritional factors has also been implicated in producing muscular dystrophy; among them are choline(6,8), potassium(7), phosphorus(13), Vit. C(4), Vit. B_6(3), and selenium(11,12). Other factors have been shown to increase the severity, such as iodide (1) and even exercise. Differences in these factors could explain the contradictory evidence presented by Morgulis and Spencer (10) and by MacKenzie and McCollum(9).

Methods. Two experiments were independently conducted at the Ontario Agricultural and Veterinary College and at Cornell University. The objectives of each were to determine the effect of alpha tocopherol and selenium upon incidence of muscular dystrophy in the rabbit. In addition, the Cornell experiment was designed to include diets to which natural feedstuffs had been added. These feeds were chosen because muscular dystrophy often occurs in lambs when these feeds are fed(5,12). Diets used in the experiments are shown in Table I.

Results and discussion. An alopecia was noticed in all of the rabbits fed diets containing no "natural feed." A loss of the long guard hairs began along the ventral part of the abdomen about one week after the rabbits were placed on the experimental diets. Since the rabbits were housed in groups of 5 it was not possible to determine the extent of hair chewing that might have taken place.

* National Dairy Products Corp., Research and Development Division, Glenview, Ill.

† Dept. of Medicine and Surgery, Ontario Veterinary College, Guelph, Canada.