Connective Tissue VII. Factors Inhibiting the Dermal Chemical Response to Cortisol.* (28464)

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Administration of cortisol to rats has been shown to result in a loss of both hexosamine (1) and hydroxyproline from the skin(2). Castor(3) has shown that fibroblasts *in vitro* synthesize less hyaluronic acid in the presence of cortisol than in its absence. We have recently found that dermal concentrations of both the "ground substance" and hyaluronic acid as well as of insoluble collagen were reduced in animals which had been subjected to daily administration of physiological doses of cortisol(4).

Recent studies in this laboratory of factors altering the chemistry of the inflammatory response have suggested that both chronically stressed rachitic rats(5) and animals of advanced age(6) might be refractory to exogenous cortisol. Further, recent studies of the inhibition of the effects of Dilantin administration upon the chemistry of the connective tissue by cortisol(7) have suggested that this drug might also inhibit the dermal chemical response to steroid.

Results of a comparison of the dermal chemical response to one dose of cortisol in young, normal rats with that produced in rachitic, aged and Dilantin treated animals.

Materials and methods. Four groups of 30 male Osborne-Mendel strain rats each obtained from the Nutrition Laboratory, Food and Drug Agency, Department of HEW, through the courtesy of Mr. Walter Hooper, were used in this study. One group of animals, weighing 230-250 g each, received one subcutaneous dose of 3 mg/kg of cortisol (Cortril, C. Pfizer) suspended in 0.2 ml of isotonic saline. Another group of rats of similar weight received daily i.p. injections of 100 mg/kg of Dilantin (Parke-Davis) suspended in 1.0 ml of isotonic saline as well as one dose of cortisol on the first day of Dilantin administration. A third group of these animals had previously been allowed to grow for over 2 years to a weight of 470-550 g before receiving one dose of cortisol. The fourth group of animals had been rendered rachitic in the Nutrition Laboratory using their modification of the Templin and Steenbock diet(8), and after receiving one dose of cortisol, these animals were maintained on this diet. All 3 other groups of rats had been maintained on the usual rat chow diet *ad lib.*

At 0, 1, 3, 7 and 10 days after receiving one dose of cortisol subcutaneously, 6 rats from each of the 4 experimental groups of animals described above were sacrificed and 2.5 g samples of abdominal skin were removed from each animal. After shaving and removing the underlying fat, fascia and muscle, aliquots of each skin sample were hydrolyzed in 10 ml/g of 4 N HCl for 8½ hours at 100°C. The remaining portions of skin were pooled into 2 duplicate pools of 3 rats each, representing each day of sacrifice and extracted sequentially with 0.15 M NaCl, 0.5 M NaCl and 0.5 M NaCl and 0.5 M citrate buffer (pH 3.6) as has been described previously(9). This procedure has been shown to extract quantitatively the "ground substance" neutral and acid soluble collagens respectively from the tissue(9,10). These fractions were also hydrolyzed as described above and all hydrolysates were analyzed for hexosamine(11), hydroxyproline(12) and nitrogen(13) content. The sum of the various soluble components when subtracted from the total concentration of each material permits calculation of the chemistry of the insoluble portion of the tissue(9).

All results were expressed in terms of μmoles of hexosamine or hydroxyproline per mmole of *total* dermal nitrogen, to obviate local variations in tissue content of water and fat. These results are presented as the mean for each day of sacrifice with the standard deviation for each mean never exceeding 7% of the mean. Means which differed

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TABLE I. Dermal Chemistry of Whole Rat Skin at Various Days After a Single Injection of Cortisol.

Animals	Days after cortisol injection 0	1	3	7	10
Normal					
Hex†	2.10	1.76*	1.70*	2.00	2.00
Hypro†	45.5	35.0 *	32.0 *	38.3 *	47.5
Rachitic					
Hex	1.80	1.86	1.80	1.80	1.80
Hypro	38.6	38.1	38.6	40.0	40.0
Aged					
Hex	1.68	1.60	1.63	1.50	1.55
Hypro	56.0	52.5	51.0	54.0	51.3
Dilantin treated					
Hex	2.10	1.85	1.80	1.88	1.80
Hypro	45.5	44.0	43.0	45.0	47.0

* Significantly different from day 0 ($p < .05$).
† Hex = μmoles of hexosamine per mmole of total dermal nitrogen. Hypro = μmoles of hydroxyproline per mmole of total dermal nitrogen.

by 2 standard deviations were always found to be significantly different by the "t" test ($p < .05$).

Results. The dermal chemical response of all 4 groups of rats to a single injection of cortisol is shown in Table I. These results indicate that the decreased amounts of either total hexosamine or total hydroxyproline found in the skin of young untreated rats injected with cortisol were not found in the skin of rachitic, aged or Dilantin treated animals. The loss of dermal hydroxyproline produced by one injection of cortisol in the young, normal animal endured for about 7 days and only after 10 days did the concentration of this imino acid, considered a quantitative measure of collagen concentration(14), return to normal (day 0). The loss of total dermal hexosamine with cortisol administration was no longer statistically significant by the 7th day after injection, however.

The hexosamine and nitrogen content of the isotonic saline soluble portion of rat skin from each experimental group at various days after cortisol injection is shown in Table II. These data demonstrated that for one week after administration of a single dose of cortisol to young, untreated rats the amounts of both hexosamine and nitrogen soluble in this fraction were reduced significantly below normal (day 0). No similar decrease in amounts of isotonic saline soluble hexosamine and nitrogen were found with cortisol administration to rachitic, aged or Dilantin treated animals.

The amounts of neutral and acid soluble collagens in the skin of animals from all 4 experimental groups are shown in Table III.

TABLE II. Hexosamine and Nitrogen Content of Isotonic Saline Extracts of Skin from Rats at Various Days After a Single Injection of Cortisol.

Animals	Days after cortisol injection 0	1	3	7	10
Normal					
Hex†	.98	.60*	.57*	.71*	.85
N†	.20	.11*	.13*	.13*	.18
Rachitic					
Hex	.40	.36	.43	.37	.38
N	.16	.17	.14	.16	.17
Aged					
Hex	.51	.50	.46	.46	.46
N	.07	.06	.06	.07	.06
Dilantin treated					
Hex	1.00	.98	.91	.95	1.10
N	.20	.21	.18	.18	.22

* Significantly different from day 0 ($p < .05$).
† Hex = μmoles of soluble hexosamine per mmole of total dermal nitrogen. N = mmoles of soluble nitrogen per mmole of total dermal nitrogen.

These results indicate that skin from young, normal rats lost a significant proportion of its neutral soluble collagen content and accumulated a large amount of acid soluble col-

TABLE III. The 0.5M NaCl and 0.5M Citrate Buffer Soluble Hydroxyproline Concentration (in μmoles per mmole Total Dermal Nitrogen) of Rat Skin at Various Days After a Single Dose of Cortisol.

Animals	Days after cortisol injection 0	1	3	7	10
Normal					
Salt	4.00	3.00*	2.90*	3.50*	4.25
Citrate	1.60	9.00*	6.75*	3.48*	1.70
Rachitic					
Salt	4.60	4.48	4.40	4.75	4.61
Citrate	5.00	5.00	4.86	4.79	5.28
Aged					
Salt	2.27	2.20	2.31	2.00	2.15
Citrate	.65	.60	.68	.58	.70
Dilantin treated					
Salt	4.00	3.80	4.30	4.18	4.36
Citrate	1.60	1.78	1.53	1.60	1.70

* Significantly different from day 0 ($p < .05$).

TABLE IV. Concentration of Insoluble Hexosamine and Hydroxyproline in Rat Skin at Various Days After Cortisol Injection.

Animals	Days after cortisol injection 0	1	3	7	10
Normal					
Hex†	.7	.6	.7	.6	.8
Hypro†	39.0	22.2*	22.0*	29.5*	41.0
Rachitic					
Hex	.5	.4	.5	.4	.5
Hypro	28.6	27.7	29.4	29.4	29.0
Aged					
Hex	.1	.1	.1	.1	.1
Hypro	52.3	49.0	47.2	51.0	48.0
Dilantin treated					
Hex	.7	.8	.8	.6	.6
Hypro	39.0	37.4	36.0	38.2	40.0

* Significantly different from day 0 ($p < .05$).
† Hex = μmoles of hexosamine per mmole of total dermal nitrogen. Hypro = μmoles of hydroxyproline per mmole of total dermal nitrogen.

lagen with cortisol administration. These changes from normal in the dermal concentrations of both soluble collagens with cortisol had disappeared by the tenth day after steroid administration. The rachitic, aged and Dilantin treated animals did not demonstrate any changes in dermal concentrations of either soluble collagen with cortisol administration.

The effects of cortisol administration upon the insoluble dermal concentrations of hexosamine and hydroxyproline in all 4 experimental groups of rats are shown in Table IV. These results indicate that with steroid administration to the young, normal rat the amount of insoluble collagen in the dermis was markedly decreased below normal (day 0). This effect of cortisol lasted for at least one week. The rachitic, aged and Dilantin treated animals did not demonstrate any changes in dermal concentration of insoluble collagen with cortisol administration, however.

Summary and conclusions. A single injection of a physiologic dose of cortisol (3 mg/kg) into young, normal rats resulted in a marked decrease in dermal concentrations of 0.15 M saline soluble hexosamine and nitrogen and neutral soluble and insoluble collagens. Associated with these changes was a profound increase in dermal concentration of acid soluble collagen. These changes in dermal chemistry produced by one dose of cortisol were maintained for at least one week after steroid administration. This dermal chemical response to administration of one dose of cortisol was not found in rachitic, aged or Dilantin treated rats. These results suggest that with the chronic stress resulting from the starvation imposed by a rachitogenic diet, or with advanced age rats become refractory to cortisol. As little as one dose of Dilantin was sufficient apparently to inhibit or antagonize the usual dermal chemical response to cortisol.

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