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Connective Tissue III. Dermal Chemical Response to Age.* (26602

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Several investigators have shown that the collagen concentration of connective tissue increases with age(1-5) while changes in the hexosamine content of skin have been reported to decrease(2), remain constant(3) and even increase(1) with age. Kao and McGavack(6) have indicated that soluble dermal protein concentration decreased with age while there was a significant increase in the scleroprotein content of the tissue.

We have studied the effect of age upon the chemical anatomy of rat skin as revealed using a sequential extraction technic for dissection of skin into ground substance, neutral and citrate soluble collagens and insoluble collagen and scleroprotein(7). These results are described below.

Material and methods. Six groups of 12 male Sprague-Dawley rats weighing an average of 100, 150, 200, 270, 350 and 500 g each were sacrificed by etherization and exsanguination. About 2 g of abdominal skin were collected from each animal, shaven and dissected clean of adhering fat, fascia and muscle. Tissue from each animal was minced, and aliquots removed. The remaining minced skins were pooled into 2 collections from 6 animals each. Five g aliquots from each tissue pool were extracted sequentially in the cold with 0.15M NaCl, 0.50M NaCl, and 0.50M citrate buffer, pH 3.6 as has been described previously(7).

These extracts, as well as samples of skin from each animal separately (0.5 g) were hydrolysed in 4N HCl for 8 hours, and analyzed in triplicate for their hexosamine(8), hydroxyproline(9) and nitrogen(10) content. The results were expressed in μmoles per mmoles of dermal nitrogen to minimize variations in skin water and fat content. Subtraction of the sum of the soluble components from total dermal concentration permits the calculation of the insoluble dermal content of these tissues. These results were expressed as per cent of total hexosamine, collagen and noncollagenous protein which was insoluble.

Insoluble non-collagenous protein was calculated from the fact that one mg of collagen contains about one μmole of hydroxyproline and 13.3 μmoles of nitrogen(11). Therefore, the contribution of collagen nitrogen to total dermal nitrogen may be determined. The amount of non-collagenous nitrogen solubilized, when subtracted from total non-collagenous nitrogen, permits the amount of insoluble non-collagenous protein to be calcu-

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TABLE I. Effect of Age (in Terms of Body Weight) upon Chemical Construction of Rat Skin.

Analysis	Animal wt (g) 100	150	200	270	350	500
Total:						
Nitrogen*	3.75 ± .2	4.35 ± .3	4.70 ± .3	4.90 ± .3	4.15 ± .3	3.85 ± .2
Hexosamine†	2.50 ± .12	2.55 ± .12	2.17 ± .10	1.98 ± .10	1.45 ± .08	1.32 ± .07
Hydroxyproline†	28.8 ±1.4	35.4 ±1.8	38.5 ±1.9	49.0 ±2.4	53.0 ±2.7	47.5 ±2.4
.15M NaCl extract:						
Hexosamine†	1.69 ± .08	1.84 ± .09	1.51 ± .07	.93 ± .05	.43 ± .02	.36 ± .02
Hydroxyproline†	1.18 ± .06	1.91 ± .09	1.43 ± .07	1.04 ± .05	.70 ± .03	.57 ± .03
.50M NaCl extract:						
Hexosamine†	.83 ± .04	.73 ± .03	.66 ± .03	.39 ± .01	.39 ± .01	.34 ± .01
Hydroxyproline†	2.94 ± .15	3.45 ± .18	2.55 ± .13	4.00 ± .2	2.53 ± .13	1.17 ± .06
.50M citrate buffer:						
Hexosamine†	0	0	0	0	0	0
Hydroxyproline†	2.19 ± .10	2.60 ± .13	1.62 ± .08	1.63 ± .08	3.38 ± .16	3.38 ± .17
Insoluble hexosamine‡	0	0	0	35	44	50
Insoluble collagen§	65	71	82	85	87	95
Insoluble non-collagenous protein‖	50	35	22	2	21	46

* mmoles/g fresh skin. † μmoles/mmole whole skin nitrogen. ‡ % of total hexosamine. § % of total collagen. ‖ % of total non-collagenous protein.

lated from these results. The analytical results in terms of the mean of triplicate analyses of duplicate samples and their standard deviations are shown below. Duplicate extracts did not differ by more than 5%, and any means which differed by *more* than 10% were statistically significant ($p < .05$) for all the data presented below.

The 0.15M saline extract removed quantitatively the ground substance, while the 0.50M saline removed neutral soluble collagen(7). Citrate buffer effected the solubilization of the procollagen components of the skin(7).

Experimental and results: The analyses of whole skin and the various extracts are presented in Table I along with the calculations of percent of total hexosamine, collagen and non-collagenous protein which was insoluble. These results indicate that dermal nitrogen concentration increased with age until 270 g body weight was reached. Further increases in age (or weight) resulted in significant decreases in dermal nitrogen. Dermal hexosamine declined significantly with age, while hydroxyproline (or collagen) increased until an age equivalent to 350 g body weight was reached. Further aging then did not increase dermal collagen content.

Ground substance (0.15M saline soluble hexosamine) increased with age until 150 g body weight had been reached; further aging resulted in a significant decrease in ground substance. Neutral collagen bound hexosamine decreased with age, while no significant concentrations of hexosamine were found in the citrate buffer extracts.

Isotonic saline soluble hydroxyproline increased initially with age up to 150 g in body weight, then declined with further increases in body weight. Both soluble collagens changed in an apparently unmeaningful fashion with increasing animal weight and age.

No insoluble hexosamine was found until the animals reached a weight of 270 g. The percent of insoluble collagen increased continuously with age, while percent of non-collagenous scleroprotein decreased with age until 270 g body weight was reached; further aging results in increasing concentrations of dermal, insoluble, non-collagenous protein.

Summary and conclusions. Although the ground substance of rat skin appeared to decrease with age, dermal insoluble collagen content increased with age. In the face of decreasing concentrations of both total dermal hexosamine and ground substance, there was an abrupt appearance of insoluble hexosamine with age in excess of 200 g body

weight. Insoluble non-collagenous protein decreased with increasing body weight until 270 g. With further increases in age, dermal scleroprotein increased until the percent of total non-collagenous protein which was insoluble was essentially the same as that found in 100g rats. The maximum concentration of ground substance, total collagen and scleroprotein were found in the skin of rats weighing 150, 350 and 100g respectively. Maximum concentrations of insoluble hexosamine and insoluble collagen were found in the skin of rats weighing 500 and 270g respectively. The differing effects of aging upon ground substance, insoluble collagen and scleroproteins indicate that different mechanisms, possibly hormonal, were involved in controlling the concentration of each component, and suggest that these materials may be metabolically independent of each other.

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Melanophore-Dispersing Activity of Reserpine in Rana Frogs.* (26603)

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The effect of reserpine on the endocrine system in mammals and man has been extensively studied(1-10). We wish to report here on the stimulating effect of reserpine on melanophore dispersion in *Rana pipiens* frogs.

Materials and methods. Fifty adult male and female green-adapted Rana frogs, weighing 30-60 g each, were used. Reserpine stock solution was prepared according to the method described in Martindale's Extra Pharmacopoea, 1959, and diluted to the required concentration. *Exp. 1*: Pituitary homogenates of Rana frogs were injected into 5 Rana frogs to establish the course and extent of the melanophore-dispersing activity of normal frog pituitaries. *Exp. 2*: Reserpine was added *in vitro* to Rana frog skins preserved in Ringer solution to determine whether reserpine has a direct effect on frog-skin melanophores. *Exp. 3*: Single doses of 0.05-0.25 mg reserpine were subcutaneously injected into the dorsal lymph sac of 10 frogs, while 10 other frogs were injected with the solvent alone. Each frog was kept in a separate vessel and watched for 4 weeks.

The degree of darkening was evaluated quantitatively by determining the melanophore index (M.I.) according to Hogben's melanophore scale method(11-14). The M.I. of the middle hind webs of the frogs was determined microscopically.

Results. Exp. 1: Injection of Rana frog pituitary homogenates into normal Rana frogs resulted in darkening of the skin within 6-12 hours. This effect passed within 48 hours. *Exp. 2*: No effect was observed when reserpine was added to Ringer solution-pre-

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