clinical states and the amounts of 17-KGS they excreted.

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Connective Tissue X. Dermal Chemical Response to Atropine.* (29020)

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In studying the effects of atropine-induced vasodilatation upon the chemistry of inflammation(1) it was noticed that administration of daily doses of this drug apparently produced major alterations in the chemical composition of uninjured rat skin. This paper concerns the effects of atropine upon the chemistry of uninjured skin in the intact rat and indicates another effect of this drug upon tissue metabolism(2).

Materials and methods. Seventy-two male Sprague-Dawley rats weighing 250-275 g each were given daily dorsal subcutaneous injections of 0.1 ml of 0.15 M NaCl containing initially 5 mg of atropine sulfate/kg body weight. This dose was increased daily by 1 mg/kg, to overcome the well known ability of rats to become refractory to this drug. Groups of 12 animals each were sacrificed 24 hours after receiving 0, 2, 4, 6, 9 and 14 daily doses. The abdominal skin, not including skin from the area of injection, was removed from each animal, shaved and dissected free of fat, fascia and muscle. Aliquots of each skin were hydrolyzed in acid and the hydrolysate analyzed for its hydroxyproline, hexosamine and nitrogen content as described previously(3). The remaining skin was collected into 3 equal pools representing 4 rats each, and these pools were extracted sequentially with 0.15 M NaCl, 0.5 M NaCl and citrate buffer as has been described previously (3). The fucose(4) and protein bound sialic acid, or sialate(5), contents of these fractions, and concentrations of hydroxyproline, hexosamine and nitrogen were determined

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TABLE I. Effect of Daily Administration of Atropine upon Chemical Analysis of Whole Rat Skin.

Dosage	Hydroxyproline[1]	Hexosamine[1]
0	48.0 ± 3	1.83 ± .2
2	*35.7 ± 2	2.10 ± .2
4	*40.9 ± 2	1.91 ± .2
6	*34.0 ± 2	2.00 ± .2
9	*40.7 ± 3	1.98 ± .2
14	44.5 ± 3	1.93 ± .2

[1] μmoles/mmole total dermal nitrogen.
* Significantly different from day 0 ($p < .05$).

(3). Data were expressed as the mean hydroxyproline, hexosamine, fucose and sialate contents of both tissue and the various fractions in μmoles per mmole *total* dermal nitrogen, obviating variations in tissue water and fat(3). The standard deviations of these means, which represent triplicate samples per day of sacrifice, never exceeded 8% of the mean, and means differing by more than 2 standard errors were significantly different by the "t" test ($p < .05$).

Results. The effects of atropine administration upon the ratio of hydroxyproline and hexosamine to nitrogen in rat skin are shown in Table I. Atropine administration did not affect hexosamine concentration of the dermis, but did result in a rapid and significant loss of hydroxyproline, or collagen(6), from the tissue. Within 14 days of daily atropine injection, however, this effect of the drug upon dermal collagen was no longer apparent.

The effects of atropine administration upon the analysis of the 0.15 M NaCl soluble components ("ground substance") of rat dermis are shown in Table II. With the exception of bound sialic acid, only a very transient effect of drug administration upon the isotonic saline soluble components of rat dermis was observed. The concentration of bound sialate, presumably deriving from serum glycoproteins(7), was immediately and profoundly increased with atropine administration, however, and this effect continued throughout drug administration.

TABLE II. Effects of Daily Administration of Atropine upon Chemical Analysis of Isotonic Saline Soluble Components of Rat Skin.

Dosage	Hypro[1]	N[1]	Hex[1]	Fucose[1]	Sialate[1]
0	1.10	.15	1.10	.90	.04
2	1.15	.14	.96	*1.34	*.16
4	*7.41	*.29	1.00	*1.36	*.35
6	1.03	.13	.91	1.00	*.18
9	.93	.13	.98	1.00	*.20
14	.90	.11	.93	1.00	*.20

[1] μmoles of 0.15 M NaCl soluble hydroxyproline (Hypro), nitrogen (N), hexosamine (Hex), fucose and sialic acid per mmole of total dermal nitrogen.
* Significantly different from day 0 ($p < .05$).

The effects of atropine upon the chemistry of 0.50 M NaCl soluble materials from skin are shown in Table III.

The only response demonstrated in this extract to the drug was an eventual decrease in amount of collagen soluble in this solvent, and an initial and transient increase in concentration of bound sialic acid.

TABLE III. Effect of Daily Administration of Atropine upon Chemical Analysis of 0.50 M NaCl Soluble Components of Rat Skin.

Dosage	Hypro[1]	N[1]	Hex[1]	Fucose[1]	Sialate[1]
0	4.50	.13	.59	1.22	.00
2	5.00	.13	.60	1.40	*.05
4	*6.31	.15	.68	1.11	*.05
6	*3.40	.11	.49	1.18	.00
9	*3.60	.11	.50	1.10	.00
14	*3.60	.12	.53	1.28	.00

[1] μmoles of 0.5 M NaCl soluble hydroxyproline (Hypro), nitrogen (N), hexosamine (Hex), fucose and sialic acid per mmole of total dermal nitrogen.
* Significantly different from day 0 ($p < .05$).

TABLE IV. Effects of Daily Administration of Atropine upon Chemical Analysis of 0.5 M Citrate Buffer (pH 3.6) Soluble Components of Rat Skin.

Dosage	Hydroxy-proline[1]	Nitrogen[1]	Hexosamine[1]
0	1.60	.14	.00 ± .30
2	*14.0	*.28	.00 ± .30
4	*10.5	*.22	.00 ± .30
6	* 7.8	.17	.00 ± .30
9	* 7.8	.18	.00 ± .30
14	* 7.0	.18	.00 ± .30

[1] μmoles of citrate buffer soluble materials per mmole of total dermal nitrogen.
* Significantly different from day 0 ($p < .05$).

The analysis of the citrate buffer soluble components of rat skin after various doses of atropine is shown in Table IV. With atropine administration, there was a marked and prolonged increase in dermal concentration of acid soluble collagen. The increased nitrogen content of this fraction was presumably due

TABLE V. Effect of Daily Administration of Atropine upon Chemical Analysis of Insoluble Portion of Rat Skin.

Dosage	Hydroxyproline[1]	Nitrogen[1]	Hexosamine[1]
0	40.0	.55	.33
2	*15.5	.45	.30
4	*16.5	*.34	.38
6	*21.7	.60	.35
9	*28.4	.49	.38
14	33.4	.60	.39

[1] μmoles of insoluble materials per mmole of total dermal nitrogen.
* Significantly different from day 0 ($p < .05$).

to the increased amount of collagen found in the extract.

Subtraction of the sum of the various soluble hexosamine, hydroxyproline and nitrogen components of the tissue from total dermal concentration of these materials permits calculating the composition of the insoluble portion of the tissue as has been described previously(3). These results (Table V) indicate that the primary effect of daily atropine administration upon the connective tissue is to effect a decrease in dermal concentration of collagen. This effect was profound initially, and had disappeared by the 14th dose of drug.

Discussion. Atropine administration increased the amount of protein-bound sialic acid in the "ground substance," presumably reflecting the increased amount of plasma glycoproteins found in the tissue due to peripheral vasodilatation. This effect was pronounced throughout the drug administration.

Despite the general independence of the hexosamine content of the whole tissue and its various fractions with respect to atropine dosage, the collagen composition of the connective tissue was initially altered by the drug. That is, atropine administration resulted in marked decreases in the total, saline soluble and insoluble collagen concentrations of the skin, and in a profound increase in amount of acid soluble collagen found in this tissue. The decreased amounts of both total and insoluble dermal collagen found with daily atropine administration were not apparent by the 14th dose of the drug, perhaps indicating that the animals had become refractory to atropine, despite the increasing dose of atropine being used. Concentrations of the soluble collagens remained significantly different from normal throughout the drug administration. The difference in response of insoluble and soluble dermal collagens to atropine dosage might suggest that the altered concentrations of the soluble collagens did not derive from the degradation of insoluble collagen into the soluble forms, but rather represent the anabolic activity of the tissue to replace independently catabolized collagen.

It may be pertinent that although with stress(8) cortisol(9), atropine and Dilantin (10), similar changes in the dermal concentrations of the various soluble collagens were observed, in the former 3 situations these changes were associated with losses from the skin of insoluble collagen, while in the last situation there was a marked increase in dermal concentration of insoluble collagen. One possible explanation of these findings might be that if insoluble collagen were rapidly degraded, perhaps enzymatically, and removed *via* lymphatic drainage from the tissue, the fibroblasts might then synthesize more acid soluble collagen at the expense of the salt soluble variety in an automatic attempt to replace the amount of insoluble collagen lost from the dermis. If rate of loss of insoluble collagen was only slightly greater than rate of conversion of the acid soluble material into the insoluble form, then the latter would accumulate in the face of deficits in the tissue concentration of the former. That is, while Dilantin stimulated the fibroblast directly, as has been previously suggested(11), to make acid soluble collagen at the expense of the salt soluble variety, stress, cortisol and atropine indirectly stimulated fibroblast production of acid soluble collagen by effecting in some fashion the rapid removal of insoluble collagen from the tissue. Therefore it might be possible for the animal to become refractory to the "insoluble collagen removal effect" of atropine, while still permitting alterations in the dermal concentrations of the soluble collagens to take place in response to this now statistically insignificant loss of in-

soluble collagen from the tissue. Speculatively to separate the catabolic mechanism of insoluble collagen from the anabolic process does not appear to be inconsistent with what is currently known of collagen metabolism *in vivo*(12,13) and might be consistent with the cyclic rate of isotope incorporation into newly synthesized collagen recently described by Bentley and Jackson(14). That is, the independent disappearance of a mature form of collagen from a biological "compartment" results in a marked increase in rate of synthesis of the precursors of this component. Whether this disappearance of a more mature form of collagen from the biological "compartment" refers to its leaving the cells as tropocollagen or to its being precipitated as insoluble collagen or to its loss from the tissue entirely, the net result of its loss would be an increased rate of synthesis of its appropriate precursors.

These speculations are not inconsistent with what is currently known and they do to a degree partially explain the data described here as well as the effect of stress (8), cortisol(9) and Dilantin(10) upon the chemistry of the connective tissue described elsewhere.

Summary and conclusions. The dermal chemical response to atropine administration involved primarily losses of salt soluble, insoluble and total collagens and increased amounts of acid soluble collagen effecting the hexosamine content of the tissue. The increased dermal concentration of acid soluble collagen and the decreased dermal concentration of salt soluble collagen were maintained during the entire course of atropine administration, whereas the decreased concentration of both total and insoluble collagen was no longer significant by the last day of drug administration. To explain these changes in the dermal concentrations of the various collagens the possibility was suggested that atropine stimulated specifically the catabolism of insoluble collagen, and the changes in the dermal concentrations of the soluble collagens represented an anabolic response to this loss.

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Effect of Amphetamine on the Learning and Performance of Mice in a Swimming Maze.* (29021)

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The ability of the amphetamines to enhance performance has been studied extensively. In a recent review Weiss and Laties (1) concluded that these drugs improve performance on a wide variety of tasks. In some

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