THE RESPONSE OF DERMAL COLLAGEN TO CROTON OIL INJURY*

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An extensive and prolonged decrease in the dermal hydroxyproline concentration (and consequently collagen content) of skin of the rat distant to the site of local injury resulting from intradermal injection of croton oil has been reported (1). This reported response of "uninjured skin" distant to "local inflammation" has important physiological considerations relative to body response to various injuries of the skin. The present paper contains data obtained in an effort to confirm the above and associated observations.

PROCEDURE

Twelve, 1 year old, Holtzman strain male rats (average initial weight, 502 gms.) each received 0.4 ml. of 75% (V/V) croton oil (Magnus, Mabee and Reynard, Inc., New York, N. Y.) in peanut oil by intradermal injection into previously shaved abdominal skin. Groups of 4 rats were sacrificed 2, 4 and 6 days subsequent to croton oil injections. An additional group of 6 rats similar to those which received croton oil (average initial weight, 505 gms.) were utilized as a control group for determination of normal values (sacrificed in 2 groups of 3 each on the same days as those during which the 2 and 6 day croton oil-treated rats were sacrificed). All rats were housed individually and were fed a standard laboratory rat chow and tap water *ad libitum*.

Blood was removed from each control and from each croton oil-treated animal by direct cardiac puncture. The sera were collected and stored frozen until analyzed. The necrotic area in the skin resulting from the croton oil injection and skin from an equivalent area on the opposite side of the abdomen (henceforth referred to as uninjured skin) and both femurs were removed from each croton oil-treated animal. Skin samples equal in area to the necrotic areas in the skin of the croton oil-treated rats (henceforth referred to as normal skin) were removed from each side of the abdomen of the control rats. Both femurs were collected from the control animals. Samples of the lesion and of uninjured and normal skin from each experimental group were preserved for histological examination by fixation in 10% formalin immediately upon animal death.

The three types of skin samples were all dried to constant weight in an oven at 110°C., defatted with 1:1 (V/V) ether-alcohol in a Soxhlet extractor for 48 hours and dried again to constant weight. Aliquots of each dried fat-free skin sample were analyzed for hydroxyproline by the Prockop-Udenfriend method (2), hexosamine by the Boas modification (3) of the Elson-Morgan reaction and nitrogen by a micro-Kjeldahl procedure (4) as measures of collagen, hexosamine containing mucopolysaccharides of the ground substance and protein, respectively. Collagen content was calculated by multiplying hydroxyproline content by 7.46 (5). The collagen, hexosamine and nitrogen contents of each of the three types of skin samples are expressed as a percentage of dry fat-free tissue.

The serum samples were analyzed for total hydroxyproline, hexosamine and nitrogen by the same technics as applied to the dry fat-free skin samples. Analytical results for serum are calculated to represent quantities/ml. serum.

The collagen content of the femurs was evaluated by determination of the hydroxyproline content of sulfuric acid hydrolyzates of dry fat-free femurs by the Leach modification (6) of the Neuman-Logan procedure. Collagen content of the femurs was calculated by multiplying hydroxyproline content by 7.46.

Sections of each formalin fixed tissue sample were prepared for histological examination by staining with hematoxylin and eosin and Verhoeff's connective tissue stain according to conventional technics.

RESULTS AND DISCUSSION

The rats which received the croton oil appeared lethargic for 3–4 days following oil injection, but they did not lose significant weight. The lesions resulting from the croton oil injections had a hemorrhagic pattern of ulceration and necrosis typical of this type of injury such as has been previously described (1, 7). The uninjured skin from the croton oil-treated rats was histologically indistinguishable from the abdominal skin of control rats.

The results of all chemical analyses of the three types of skin samples are summarized in Table 1. The percent of solid matter in the croton oil lesions represented by collagen decreased consistently throughout the experiment while the concentration of hexosamine increased at each experimental period, thus resulting in a greatly elevated hexosamine to collagen (H/C) ratio in the croton oil lesion, as compared to normal skin, 6 days after injection of croton oil (normal skin H/C, 0.50, croton oil lesion H/C 6 days, 4.59). The percent nitrogen content of the dry fat-free tissue of the croton oil lesion was less than that of dry fat-free normal skin at each experimental period.

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These observations on changes in the chemical content of croton oil lesions in comparison to normal skin are in agreement with published reports (1).

The water and lipid contents of uninjured skin were not significantly different from those of normal skin at any experimental period (Table 1). The hexosamine and nitrogen contents of dry fat-free uninjured skin were similar to those of dry fat-free normal skin at each observation (Table 1). Two days after intradermal injection of croton oil, the collagen content of the dry fat-free uninjured skin was 7.5% less than that of normal skin (difference significant at $p < 0.02$). Four and six days following croton oil injection, the collagen content of the dry fat-free uninjured skin of the croton oil-treated rats was similar to that of normal skin. The 7.5% decrease in the collagen content of the dry fat-free uninjured skin of the croton oil-treated rats observed two days after oil injection is considerably less and much more transitory than the previously reported (1) 25% decrease in hydroxyproline content (or collagen content) which occurred within two days after croton oil injection and which remained significantly decreased for at least 14 days. No changes from control values in the collagen concentration of a second tissue (femurs) were observed in any of the groups of croton oil-treated rats (Table 2).

Serum hydroxyproline contents were unchanged from control values in rats which received croton oil (Table 2). If any extensive resorption of collagen from the connective tissues of the croton oil-treated rats had occurred, it would be reasonable to have expected to have observed an increase in serum hydroxyproline simultaneously with the collagen resorption. Serum hexosamine content (Table 2) was significantly elevated ($p < 0.01$) from that of control animals in the croton oil-treated rats sacrificed two days after oil injection, but the serum hexosamine contents of the croton oil-treated rats determined 4 and 6 days after oil injection were not significantly different from those of control animals. The observed elevation in serum hexosamine content in the croton oil-treated rats at the 2 day experimental period may

TABLE 1

*Analytical results of various components of normal skin, croton oil lesions and uninjured skin of adult male rats**

Experimental Group	Water	Lipid Wet Weight	Nitrogen Fat-Free Dry Weight	Hexosamine, Fat-Free Dry Weight	Collagen, Fat-Free Dry Weight	H/C†
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	
Normal skin	63 ± 0.3	9 ± 0.5	15.5 ± 0.3	0.28 ± 0.01	56.2 ± 0.4	0.50 ± 0.02
2 day lesion	67 ± 0.9‡	11 ± 0.8	13.5 ± 0.2‡	0.41 ± 0.03‡	43.5 ± 4.6‡	0.94 ± 0.17‡
2 day uninjured	62 ± 0.8	9 ± 0.5	15.1 ± 0.4	0.29 ± 0.02	52.0 ± 0.8‡	0.56 ± 0.04
4 day lesion	62 ± 0.5	7 ± 0.8	12.7 ± 0.3‡	0.70 ± 0.05‡	29.5 ± 2.2‡	2.37 ± 0.22‡
4 day uninjured	64 ± 0.6	8 ± 0.7	15.0 ± 0.1	0.30 ± 0.02	57.3 ± 2.3	0.52 ± 0.05
6 day lesion	62 ± 1.0	7 ± 0.9	13.0 ± 0.1‡	0.97 ± 0.05‡	21.1 ± 2.4‡	4.59 ± 0.62‡
6 day uninjured	61 ± 1.1	9 ± 1.2	15.9 ± 0.2	0.31 ± 0.02	56.8 ± 2.3	0.54 ± 0.04

* All results are expressed as the mean ± standard error of the mean.

† Per cent hexosamine × 100 ÷ % collagen.

‡ Means so marked are significantly different ($p < 0.02$) from values of control animals.

TABLE 2

*Analytical results of various blood components and femur collagen in control and croton oil-treated adult male rats**

Experimental Group	Blood			Femur Collagen
	Hydroxyproline	Hexosamine	Nitrogen	
	μg/ml.	*μg/ml.*	*per cent*	*per cent*
Control	25.2 ± 2.2	1290 ± 15	1.06 ± 0.02	13.2 ± 0.1
2 day croton oil	24.7 ± 2.0	1425 ± 43†	0.96 ± 0.01†	12.8 ± 0.2
4 day croton oil	26.8 ± 2.6	1275 ± 46	0.96 ± 0.02†	13.2 ± 0.4
6 day croton oil	27.3 ± 2.1	1366 ± 56	1.08 ± 0.02	13.6 ± 0.4

* All results are expressed as the mean ± standard error of the mean.

† Means so marked are significantly different ($p < 0.02$) from values of control animals.

have been a response to connective tissue damage in the croton oil lesion such as has been observed in various types of diseases in which urinary excretions or blood levels of hexosamine are increased (8, 9). The reason for the decrease in serum nitrogen in the croton oil-treated animals at 2 and 4 days is unknown (difference from control animals significant at $p < 0.01$), although the effect may have been a reaction to the toxic properties of croton oil.

In the present experiment only a slight and transitory decrease in the collagen content of uninjured skin and no change in the collagen content of a second collagen rich tissue (femurs) were observed in croton oil-treated rats as compared to normal animals. No changes from control values were observed in the serum hydroxyproline content of the croton oil-treated rats at any experimental period examined in the present study. It is thus concluded that the data obtained in the present study do not demonstrate an extensive and prolonged decrease in dermal hydroxyproline (or collagen) in the skin of the rat distant to the site of local injury resulting from intradermal injection of croton oil. The single observation in the present experiment which is in agreement with an effect of intradermal croton oil injection on collagen metabolism other than in the immediate area of the wound may represent a sampling error. Although the values obtained from the animals in which the difference in dermal collagen content of uninjured skin from normal skin was observed had a low variability (Table 1), the preparation of homogenous skin samples is difficult. It is therefore proposed that an extensive and prolonged decrease of dermal collagen in uninjured skin of croton oil-treated rats does not occur in all investigational conditions.

An attempt was made to duplicate the method of production of croton oil lesions and the preparation of samples utilized in the investigation (1) with which the results of the present experiment have been compared. In the previously reported study the samples were stored at −15°C. A constant percentage weight loss resulted after 5 days storage and subsequently the tissues did not lose weight upon further storage at −15°C. Analytical results were based upon the weight of the stored tissue. In the present experiment storage of the tissues at −15°C. resulted in inconsistent and continuing loss of weight and the samples were therefore heat dried to constant weight. Some of the difference between the results of the previous and present investigations may have resulted from the method of sample drying.

SUMMARY

Lesions in abdominal skin due to intradermal croton oil injections, abdominal skin on the side of the body opposite to croton oil injuries, abdominal skin of normal animals and serum from croton oil-treated and normal adult male Holtzman strain rats have been examined by chemical and histological technics. Chemical and histological changes previously reported as occurring in croton oil lesions were confirmed. An extensive and prolonged decrease in dermal collagen of abdominal skin on the side of the body opposite to the croton oil injury was not observed under the conditions of the present investigation.

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