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Connective Tissue. II. Distant Dermal Collagen Response to Local Inflammation.* (26265)

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It has been demonstrated that there is a significant decrease in total dermal concentration of hydroxyproline or collagen in skin distant from the site of local croton oil induced inflammation(1,2,3). This loss of dermal collagen from uninjured tissue is not associated with a change in either total dermal nitrogen or hexosamine(3).

Recently, we have developed a technic for extractive disection of rat skin into ground substance, neutral soluble collagen, procollagen and insoluble collagen(4,5). Employing this procedure, we have investigated the nature of the "distant dermal collagen response to local inflammation."

Material and methods. Twenty-four Sprague-Dawley rats, averaging 270 g in weight were divided into 3 equal groups of 8 each, 2 of which were injected intradermally with 0.4 ml of a 75% solution of croton oil in peanut oil, the third group with a similar volume of the carrier oil alone. This latter group was sacrificed by etherization within a few minutes after injection, and 5 g of shaved abdominal skin distant from site of injection (left abdominal area) were removed. The chemical analysis of skin from animals injected with carrier alone did not differ significantly from normal 0, 4 or 14 days after injection(3). The eschar formed 4 days after injection of the irritant, and one group of animals was sacrificed at this time. Similarly, the third group was killed after 14 days when the eschar had sloughed, and the apparently uninjured skin collected. All tissues were shaven, dissected clean of adhering fat, fascia and muscle, and finely minced. Triplicate samples of pooled tissue from 8 animals were extracted sequentially in 0.15 and 0.5M NaCl and citrate buffer as described previously(5). The hexosamine(6), hydroxyproline(7) and nitrogen content of the extracts, as well as that of the whole skin of each animal separately, was determined in triplicate after acid hydrolysis(5). These extracts have been shown to remove quantitatively the ground substance, neutral soluble collagen and "procollagen" respectively(5). The difference between total tissue content of each component and the sum of the soluble material in μmoles/g of starting tissue defined the composition of the insoluble residue.

All results were expressed in μmoles/g of skin as the mean of triplicate analyses performed upon triplicate tissue extracts obtained from the pooled tissue of each group of 8 animals. The analytical variation between the triplicate extracts obtained from each group of animals was less than 10%. Mean values of 9 determinations for each fraction are presented along with the appropriate standard deviation below. The tissue samples were stored for a few weeks in the frozen state after cleaning but prior to either hydrolysis or extraction. Water loss *via* sublimation was essentially constant for all tissues at 30% w/w as has been described previously(4).

Results and discussion. The pathology of this type of injury, a non-suppurative ulcerous defect lightly outlined by erythema, has been described(2). No significant changes in dermal hexosamine or nitrogen were found in the skin distant from site of local croton oil induced inflammation. Changes in hy-

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TABLE I. Distribution of Dermal Hydroxyproline (μmoles/g) in Rat Skin Distant from Site of Local Croton Oil Induced Injury.

Extract	Days after injury 0	4	14
.15M NaCl	7 ± .4	7 ± .6	8 ± 1
.50M "	29 ± 2	* 15 ± .8	* 12 ± .6
.50M citrate buffer	12 ± .6	* 43 ± 3	* 52 ± 3
Insoluble	312 ±20	*185 ±14	*224 ±13
Total	360 ±15	*253 ±15	*296 ±16

* Significantly different ($p < .05$) from control.

droxyproline were significant, however (Table I). These data demonstrate that while there was no change in isotonic saline soluble hydroxyproline, there was a profound drop in dermal concentration of neutral soluble collagen and insoluble collagen. These decreases occurred in the face of a marked increase in procollagen. These dermal collagen changes were not significantly affected by either formation or sloughing of the eschar.

Dermal extracts were mixed 5:1 with 40% trichloracetic acid. Over 95% of the soluble nitrogen was precipitated by this procedure. Therefore dermal protein content of skin is accurately measured by total nitrogen, the constancy of which indicates that insoluble collagen is specifically decreased in skin distant from the site of local injury, and that there is no general protein catabolic response to inflammation.

The specificity of this dermal collagen response to local inflammation suggests the possibility that a dermal collagenolytic enzyme is hydrolysing the insoluble collagen. Recently a mammalian collagenase has been described(8). Quantitatively, the increased concentration of procollagen cannot account for the loss of insoluble protein. More probably, collagenolysis would result in peptide sized products, which would be soluble in either saline fraction. Since no increase in saline soluble hydroxyproline was found, these materials may drain away from the skin *via* dermal lymphatics.

Rat skin from animals subjected to massive daily doses of sodium dialantin also contain less neutral soluble collagen and more procollagen than normal skin(4). These dermal collagen changes with dilantin result in an increased synthesis of insoluble collagen, however. If such changes in soluble dermal collagen are characteristic of the synthesis of insoluble collagen, why with inflammation are similar changes in soluble collagens found associated with a profound decrease in insoluble and total dermal collagen? Perhaps the distant dermal collagen response to local inflammation involves firstly the enzymatic hydrolysis of insoluble collagen and secondly in response to this loss, an attempted synthesis of replacement collagen. Rate of insoluble collagen synthesis being slower than rate of hydrolysis(4,9), a steady state would be established in which dermal concentration of 0.5M saline soluble collagen was low, since it rapidly aggregates to form procollagen(4), and this latter component would increase in concentration because of the slower rate required for collagen fiber formation(9).

Collagen concentration in mg/g of skin is equal to the concentration of hydroxyproline in μmole/g and total non-collagenous protein may be calculated from the difference between total and collagenous nitrogen as previously described(5). After correction for dermal water loss (30%) *via* sublimation with storage of tissue samples in the frozen state(5), the chemical anatomy of the skin before injury, after eschar formation and after eschar sloughing is shown in Table II. Water content was determined by lyophilization and fat by difference. This table clearly demonstrates the specific decrease in dermal collagen with local inflammation, and accumulation of non-collagenous protein.

Summary and conclusions. Skin distant from local croton oil induced inflammation demonstrates no significant change in total hexosamine or nitrogen, despite a marked de-

TABLE II. Chemical Anatomy of Rat Skin Distant from Site of Local Injury.

Component (mg/g)	Days after injury 0	4	14
Collagen	240 ± 10	*169 ± 10	*200 ± 10
Other protein	150 ± 8	*230 ± 12	*200 ± 10
Water	550	550	550
Fat	60	51	50

* Significantly different from control.

crease in 0.5 M saline soluble, insoluble and total collagen. Since less than 5% of total soluble nitrogen is not protein, this constancy of dermal nitrogen means that non-collagenous dermal proteins or glycoporteins are being synthesized. Therefore insoluble collagen is specifically decreased in response to local inflammation. It is suggested that this distant dermal collagen response to local inflammation results from enzymatic collagenolysis which stimulates the attempted synthesis of replacement collagen. A steady state with respect to collagen anabolism and catabolism could then be established in which, by reason of the relative rates of synthesis and degradation, the skin would contain subnormal amounts of 0.5M saline soluble and insoluble collagen and supernormal concentrations of pro-collagen.

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Placental Transfer of Fluorine in the Human Fetus.* (26266)

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Recent studies have established certain relationships between daily fluorine intake by pregnant women and fluorine content of the maternal blood, placental tissue and cord blood(1,2,3,4). Gardner *et al.*(1) found significantly higher fluorine values in maternal blood and placental tissues of pregnant women living in Newburgh, N. Y. where fluorine content of the water is 1 ppm, than in those living in Rochester, N. Y., where the water is fluorine-free. Held(2) reported a marked increase in maternal and cord blood fluorine levels after supplemental fluorine had been given. Feltman and Kosel(3) compared fluorine concentration in cord blood and placental tissue in women drinking fluorine free water with that in women given fluoridated water or fluorine tablets. In both groups placental tissue contained much more fluorine than cord blood. Ziegler(4), using pooled materials, compared fluorine values in placental tissue, maternal blood and cord blood of a small number of women drinking almost fluorine-free water, with those in women given additional fluorine in milk. In the latter group there was a very significant increase of fluorine concentration in maternal blood and placental tissue; in cord blood the value increased only slightly.

The present study shows a similar relationship in a larger number of subjects, each individually investigated.

Materials and methods. Fluorine determinations were carried out on blood samples taken from 25 healthy, pregnant women shortly before their delivery in the obstetrical department of Hadassah-University Hospital, and from 32 healthy, non-pregnant women. All the subjects lived in Jerusalem, which is supplied by drinking water containing 0.55 ppm fluorine(5). Placentas were obtained at normal deliveries occurring at full term. Net weights of the placentas ranged

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