Connective Tissue VIII: Effect of Rickets upon Chemistry of Inflammation.* (28072)

J. C. Houck and R. A. Jacob
Biochemical Research Laboratory, Children's Hospital and Department of Biochemistry, Georgetown University Medical School, Washington, D.C.

The connective tissue of rats rendered rachitic *via* the Templin and Steenbock diet (1) have been shown to be refractory to exogenous cortisol(2), presumably because of the chronic stress of partial starvation occurring with this diet(2). In view of the obvious influences of the adrenal corticoids upon the character of the inflammatory response, the chemistry of croton oil induced dermal lesions in the normal rat was compared with the results obtained in the rachitic rat. These results would be of particular interest with regard to the losses of insoluble collagen from uninjured skin distal to the site of local injury(3,4). This "distant dermal collagen response" has been duplicated by cortisol injection, sham adrenalectomy and intraperitoneal injections of large volumes of saline, and is presumed therefore to be a result of the change in the adrenal corticosteroid pattern in the rat due to the stress of local trauma(5). No such response should occur with rickets.

Materials and methods. Two groups of 42 male rats, formerly Osborn-Mandell strain animals, but now considered to be the Nutrition Laboratory strain of the Food and Drug Agency, Department of Health Education and Welfare, were used in this study. One group of 42 normal animals weighing 240-270 g were injected intradermally with 0.4 ml of a 5% solution of croton oil in a bland carrier (peanut oil) in the left abdominal area as has been described previously(6,7,8). At 0, 2, 4, 6, 10, 15 and 23 days after injury, groups of 6 rats each were sacrificed *via* ether and exsanguination, and samples of both the wounded tissue and uninjured skin from the uninjured side (right) of the same animal were removed. Cleaned aliquots of these tissue samples were subjected to acid hydrolysis and the concentration of hydroxyproline, hexosamine and nitrogen determined as has been described previously(9). The remaining portions of uninjured tissue were pooled into 2 equal groups each representing 3 of the 6 rats sacrificed, and extracted sequentially with 0.15 M NaCl, 0.5 M NaCl and citrate buffer (pH 3.6) as had been previously described(9). The wounds from animals sacrificed 10 days after injury were sufficiently large to permit the pooling of aliquots of necrotic tissue from all 6 rats so that a similar sequential extraction of the injured skin could be performed. These extracts were also hydrolyzed in acid, and their hydroxyproline, hexosamine and nitrogen contents determined.

A similar experiment was performed using 42 rachitic rats which since weaning had been maintained on the Templin and Steenbock diet(1) in which the calcium to phosphate ratio was 5:1 and which weighed about 100-110 g as compared to the 220-250 g appropriate for their age. These animals, which were generously supplied from the Nutrition Laboratory of the F.D.A. by Mr. Walter Hooper, were also injected intradermally with croton oil, and the experimental protocol described above was followed for 23 days after injury, during which time these animals were maintained *ad lib* on the rachitogenic diet.

All results were expressed in terms of μmoles of hexosamine, hydroxyproline and mmoles of nitrogen per g of whole skin. During the entire course of these experiments no significant alterations in dermal nitrogen were observed in either the normal or the rachitic group of rats. Standard deviations of each mean value never exceeded 7% of the mean and values which differed by more than 15% (2 standard deviations) were found to be significantly different ($p<.05$).

Results and discussion. The effects of croton oil induced lesions upon the chemistry of the skin is shown in Table I, and the results

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TABLE I. Kinetics of Dermal Chemical Response to Croton Oil Induced Lesions in Normal and Rachitic Rats.

Days after injury	Normal			Rachitic		
	Hex	Hypro	N	Hex	Hypro	N
Control	10.0	240	5.2	9.5	198	5.4
2	19.0*	86.8*	4.8	*14.4**	*216*	4.8
4	25.9*	68.6*	5.1	*20.0**	*150**	4.9
6	39.8*	59.5*	5.7	*21.0**	*148**	4.6
10	32.2*	71.7*	5.7	*16.0**	*140**	5.7
15	36.6*	72.2*	5.7	*11.0*	*190*	5.5
23	12.8*	139 *	5.0	*9.5*	*180*	5.2

* Significantly different from control ($p<.05$).
Italicized means are significantly different from injured normal animals.
Hex = μmoles of hexosamine per g fresh skins; Hypro = μmoles of hydroxyproline per g fresh skin; N = mmoles of nitrogen per g fresh skin.

from normal and rachitic rats are compared with the control (day 0) values as well as with each other. These data indicate that the rachitic rats demonstrated a dermal chemical response to local inflammation which was qualitatively similar to that produced in the non-rachitic animal. Quantitatively, however, the wound in the rachitic rats did not lose as much hydroxyproline (collagen) or gain as much hexosamine or non-collagenous nitrogen as did the non-rachitic animals. Interestingly enough, the wounds in the rachitic rats were largely healed chemically by the 23rd day after injury. Such was not the case with the non-rachitic animals.

TABLE II. Chemistry of Croton Oil Induced Lesions in Normal and Rachitic Rats 10 Days After Injury.

Analyses†	Normal		Rachitic	
	Control	Injured	Control	Injured
Total				
Hex	8.7	33.0*	9.0	16.0*
Hypro	240	72.0*	210	140 *
N	4.9	5.3	5.2	5.7
.15M NaCl				
Hex	3.8	25.3*	4.1	*12.6**
Hypro	4.7	3.5*	8.0	*6.9*
N	0.8	3.5*	0.5	*2.6**
.5M NaCl				
Hex	2.0	4.0*	1.5	*1.2*
Hypro	20.4	7.3*	20.0	*11.4**
.5M Citrate				
Hex	0	2.3*	0	0
Hypro	8.0	7.3	25.0	*29.0*
Insoluble				
Hex	3.2	1.4*	3.4	*2.2**
Hypro	207	54 *	157	*93 **
N	2.9	.75*	2.4	*1.7**

* Significantly different from control ($p<.05$).
† Hex = μmoles of hexosamine per g fresh skin; Hypro = μmoles of hydroxyproline per g fresh skin; N = mmoles of nitrogen per g fresh skin.
Italicized means are significantly different from injured normal animals ($p<.05$).

Analyses of the extracts of the wounds 10 days after injury are shown in Table II. Subtraction of the sum of the various soluble components from the respective totals permit calculation of the concentration of the insoluble dermal materials to be made. The data of this table indicate, first, that most of the increased concentration of hexosamine in the wounds from both rachitic and non-rachitic rats was soluble in isotonic saline, and that this increase in isotonic saline soluble hexosamine was associated with a similar increase of nitrogen soluble in this fraction. Quantitatively, this concentration of soluble nitrogen was about equal to the increase in non-collagenous dermal nitrogen required to maintain a constant total dermal nitrogen in the face of marked losses of hydroxyproline. Second, these data indicate that most of the loss of hydroxyproline from the wound involved insoluble collagen, and finally the data of Table II confirm the observation of Table I that quantitatively, the rachitic rats responded less to inflammation than did the non-rachitic animals.

TABLE III. Dermal Concentration of Saline Soluble Hydroxyproline (μmoles/g) in Injured Skin Distal to Site of Local Injury in Normal and Rachitic Rats.

Days after injury	Normal		Rachitic	
	.15M NaCl	.50M NaCl	.15M NaCl	.50M NaCl
Control	4.7	20.4	*8.0*	20.0
2	4.2	11.7*	*8.8*	*18.3*
4	7.4*	12.2*	*9.2*	*21.8*
6	6.5*	16.0*	*7.1*	*22.0*
10	17.0*	15.0*	*7.0*	*19.3*
15	5.0	14.0*	*7.3*	*21.2*
23	4.2	19.2	*8.4*	*18.8*

* Significantly different from control ($p<.05$).
Italicized means are significantly different from injured normal animals ($p<.05$).

Table III shows the effects of inflammation upon concentration of saline soluble hydroxyproline in the uninjured skin distal to the site of local injury. These data indicate that the decreased amount of 0.5 M NaCl soluble

TABLE IV. Dermal Concentration of Citrate Soluble and Insoluble Hydroxyproline (μmoles/g) in Injured Skin Distal to Site of Local Injury in Normal and Rachitic Rats.

	Normal		Rachitic	
Days after injury	.50M citrate	Insoluble	.50M citrate	Insoluble
Control	8.0	207	*25.0*	*157*
2	18.0*	148*	*22.3*	*178*
4	12.6*	90*	*28.0*	*160*
6	12.5*	120*	*23.3*	*180*
10	12.6*	149*	*28.1*	143
15	13.0*	156*	*26.8*	150
23	8.4	200	*22.0*	*142*

* Significantly different from control ($p < .05$). Italicized means are significantly different from injured normal animals ($p < .05$).

hydroxyproline (salt-soluble collagen) found in the uninjured skin of non-rachitic rats with local injury was not found in the uninjured skin from rachitic animals. Similarly no changes in concentration of isotonic saline soluble hydroxyproline were found in the skin from these animals, whereas the skin from normal animals demonstrated some significant increases in the amount of hydroxyproline soluble in this solvent.

The changes in the citrate soluble and insoluble concentrations of hydroxyproline from the uninjured skin of non-rachitic rats are contrasted in Table IV with the lack of effect of local inflammation upon these components of the skin from rachitic animals. The increased concentration of citrate soluble collagen and the loss of insoluble collagen from the uninjured skin of non-rachitic rats is typical of the previously described "distant dermal collagen response"(3,4). The rachitic rat does not demonstrate, therefore, such a response.

Summary and conclusion. Rachitic rats, which have been shown to be refractory to cortisol insofar as their connective tissue chemistry is concerned, demonstrated a chemical response to local injury which was qualitatively similar to that evidenced in non-rachitic animals. Quantitatively, the chemical alteration in inflammation in the rachitic animals was less profound than that found in the non-rachitic rats. Indeed, the wounds in the rachitic animals were chemically healed within 23 days after injury whereas the non-rachitic rats were not. Finally, the cortisol refractory rachitic animals did not demonstrate a "distant dermal collagen response" to local inflammation, confirming previous reports suggesting that this response was a hormonally potentiated response to the stress produced by local trauma.

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Production of Octadecenoic Acid in Plasma by *Staphylococcus aureus*. (28073)

JULIA T. WELD, B. H. KEAN AND WILLIAM M. O'LEARY
Departments of Public Health and Microbiology, Cornell University Medical College, New York City

The development of unique red bands around the growth of certain strains of *S. aureus* on blood agar plates has been reported (1). During these studies, gray plaques were observed on the surface of the blood agar. The purpose of this paper is to describe methods of producing these plaques and to report on their chemical identification.