Soluble Collagen in Acute Inflammation.* (27722)

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The changes in total tissue collagen content during a cycle of acute inflammation in young(1) and mature rats(2) have been described. In view of the recent interest in the soluble fractions of collagen(3) and the significance of the neutral solvent-soluble fractions in the biosynthesis of the collagen fibers (4), a reinvestigation of acute inflammation was carried out. In the present study, attention was centered on the relative proportions of the various collagen fractions in the tissue at different stages of a standard experimental acute inflammation.

Methods. Acute inflammation was induced in the antemolar region of the palates of Sprague-Dawley rats‡ by the method of Forscher and Stanley(5). The young group averaged 150 g body weight. The mature group were retired breeders (300 g average). Animals were sacrificed in groups of 10 by chloroform inhalation at selected time intervals after induction of the lesion. The palates were frozen in dry ice immediately after excision, crushed in the frozen state and taken through the extraction sequence as a pool of 10 samples. The soluble fractions were extracted by (1) distilled water (10 ml, 10 minutes), (2) 0.2N NaCl (10 ml, overnight at 4°C) and (3) 0.15 M citrate buffer pH 3.5 (10 ml, overnight at 4°C). After each extraction step, the mixture was centrifuged at 27,000 × *g* and the supernatant fluid was filtered through a Millipore filter, size GS, to remove fines. The entire soluble fraction at each step was freeze-dried and these residues as well as the final residue from the citrate extraction were hydrolyzed in 3N HCl at 105°C in sealed tubes overnight. Quadruplicate aliquots of the hydrolysates were analyzed for hydroxyproline by the method of Neuman and Logan(6).

The value for total hydroxyproline for a group was obtained by adding the values for the soluble fractions to the value for the insoluble residue.

Results. The total hydroxyproline content of the inflamed palates followed the pattern reported by Forscher and Cecil(2). In the soluble fractions, there was a marked difference between the changes in neutral solvent-soluble fractions and citrate-soluble fraction, and a difference in response between young and mature animals.

a) Young animals. At 14 days in this experimental method, the tissue shows a normal appearance histologically(5) but total content of hydroxyproline is only 70% of normal. The total soluble fraction, when considered as percent of total hydroxyproline, decreased to a minimum at 8 days and returned to normal by 14 days. When individual soluble fractions are considered, it appears that the 8-day minimum is due to the citrate fraction. Both water-soluble and salt-soluble fractions increased after 4 days, corresponding to the healing and regeneration period. The changes in the citrate-soluble fraction correspond to the changes in total hydroxyproline content (Table I).

b) Mature animals. In older rats, total hydroxyproline did not decrease as much as in younger animals and returned to a more normal level by 14 days. The proportion of total soluble fraction was much lower (1/3 that of the younger rats) and did not change during the response. This was due to a decrease

TABLE I. Summary of Hydroxyproline Values for Young Animals.

Fraction	Normal as % of total	Days after injury, as % of normal value 2	4	8	14
Total hypro		91	88	73	71
Total soluble	6.54	81	78	62	99
Water soluble	1.01	82	77	95	172
Salt soluble	1.66	66	62	76	97
Citrate soluble	3.87	88	85	47	81

* Supported in part by grants from U.S.P.H.S.
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TABLE II. Summary of Hydroxyproline Values for Mature Animals.

Fraction	Normal as % of total	Days after injury, as % of normal value 2	4	8	14
Total hypro		85	88	92	94
Total soluble	2.20	112	96	95	93
Water soluble	.00	.00*	.54*	.45*	.44*
Salt soluble	1.10	117	71	80	65
Citrate soluble	1.10	105	71	68	80

* Since normal value = 0, these data reported as % of total hydroxyproline.

in both salt-soluble and citrate-soluble fractions with a corresponding increase in the water-soluble fraction (Table II).

Discussion. These results are in agreement with the concept that the neutral solvent-soluble fractions of collagen represent the early products of synthesis. It was found that in mature animals, in which connective tissue collagen turnover is extremely slow, the water-soluble fraction was absent in the uninflamed tissue. In both age groups, regeneration was accompanied by increases in neutral solvent-soluble fractions. It also appears that the citrate-soluble fraction is not associated with the synthesis sequence but is more likely a derivative of the mature collagen fiber.

The primary objective of this study was to determine the chemical nature of the collagen fiber disappearance seen histologically in acute inflammation. This phenomenon could be due to disaggregation of the gross fibers to units too small to be seen, to reversal of the synthesis sequence to convert the fibers to soluble forms or to digestion of the fibers to amino acids or peptides with removal of these end-products from the area. The work reported here indicates that the histologic observation of disappearance and reappearance of collagen fibers in acute inflammation in connecive tissue is due to proteolysis and *de novo* resynthesis.

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Received May 14, 1962. P.S.E.B.M., 1962, v111.

A Simple Rapid Method for Production of Viral Antibody in Mice.* (27723)

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A technic for production of antibody in mouse peritoneal fluid was described by Munoz(1) utilizing intraperitoneal inoculations of egg albumin or bovine serum albumin mixed in Freund's adjuvant. Ascites production was induced, however, in only ½ the animals tested. Both Herrmann and Kasel and their associates(2,3) used ascites fluid from immunized mice as a source of viral antibodies. Considerable quantities were induced by intraperitoneal injection of Sarcoma 180 cells by the first group and bacterial-adjuvant mixture(4) by the latter. A modification of these methods utilizing Ehrlich tumor cells has proved consistently reliable, required less time or fewer injections and is here described in detail.

*Supported in part by research grant from U. S. Public Health Service, Dept. of H.E.W.

†Postdoctoral Research Fellow of The National Foundation.