Fibrinoid and Hydroxyproline of the Rheumatoid Subcutaneous Nodule.* (19063)

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In a number of diseases which have been designated as "collagen diseases", degeneration of collagen with replacement by a fibrinoid material is described. Fibrinoid has been variously considered to be fibrin(1,2), degenerated collagen(3,4) and a complex between degenerated collagen and mucopolysaccharide(5).

The amino acid, hydroxyproline, constitutes approximately 14% of collagen as derived from a variety of sources(6), and except for the small amounts present in elastin, is found in no other protein of the body. In view of the fact that fibrinoid has been described as a degenerated form of collagen, it was of interest to determine whether the total hydroxyproline content of rheumatoid subcutaneous nodules, which contain relatively large amounts of fibrinoid material, could be accounted for entirely in the collagen and elastin fractions. Three subcutaneous nodules, containing considerable amounts of fibrinoid material as demonstrated by histological examination of tissue sections, were analyzed. It was found that the hydroxyproline content of the collagen fraction of each nodule corresponded closely to the recorded values of Neuman and Logan(6) for purified collagen from a number of sources, and that the total hydroxyproline content of the nodules was accounted for entirely by the collagen and elastin fractions.

Experimental. Nodules were excised under local procaine anesthesia. A portion of the nodule was reserved for histological examination.‡ The remaining tissue, varying in weight between 1 and 5 grams, was minced finely and divided into several portions. Extractable lipid was determined by a single extraction with a solution of 3 volumes of alcohol and 1 volume of ether, followed by three extractions with ether. Dry weight was determined following fat extraction by drying at 105°C for twenty-four hours. Repeated determinations showed that constant weight was achieved by the drying procedure. Collagen and elastin were analyzed on 0.5 to 1 gram samples of tissue by the method of Lowry, Gilligan and Katersky(7), the collagen being determined on the basis of the nitrogen content of the gelatin supernatant and calculated on the basis of 17.5% nitrogen in collagen. Hydroxyproline was determined by the method of Neuman and Logan(6). The hydroxyproline content of collagen was determined in the gelatin solution obtained in the procedure of Lowry *et al.*(7). Total hydroxyproline content was determined by hydrolyzing approximately 0.1 to 0.3 gram of minced tissue in 1 ml of 6 N hydrochloric acid for four hours in a sealed tube at fifty pounds pressure, then neutralizing and diluting to appropriate volume. There was no interfer-

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TABLE I. Analysis of Rheumatoid Nodules and Synovia.

Tissue	% water	% lipid	Collagen as % wet wt	Hydroxyproline as % collagen	Elastin as % wet wt	Hydroxyproline as % wet wt of tissue	% Hydroxyproline* from collagen and elastin
Nodule B	80.6	2.44	10.1	14.7	0.52	1.41	1.49
Nodule S	85.8	1.86	7.08	13.9	0.43	1.11	0.992
Nodule K	72.6	2.46	13.5	12.8	1.70	1.72	1.76
Synovial membrane	72.8	2.93	17.4	13.9	1.29	2.63	2.44

* Calculated on the basis of 1.8% of hydroxyproline in elastin(6).

ence with color development, and the absorption spectrum obtained (between 500 and 600 mμ) corresponded quantitatively with that of a standard solution of hydroxyproline. When 3.0 mg of hydroxyproline were added to duplicate samples of 0.2 gram of minced tissue which were subsequently hydrolyzed in 6N hydrochloric acid as described above, 3.0 and 3.1 mg, respectively, were recovered.

Results. Water, collagen and lipid comprised 90 to 95% of the net weight of the 3 nodules as well as of the synovial membrane (Table I). Whereas the nodules and the synovial tissue were relatively rich in collagen, the elastin content of these tissues was low. This fact coupled with the low hydroxyproline content of elastin indicates that elastin contributes negligibly to the total hydroxyproline content of the tissue. The values of 13.9, 14.7 and 12.8% respectively found for the hydroxyproline content of the collagen of the 3 rheumatoid nodules, and that of 13.9% in the synovial membrane are in fair agreement with recorded values observed in samples of normal collagen derived from a variety of sources(6,8), and suggests that there is no significant variation in the composition of the collagen of the subcutaneous nodule.

Camera lucida tracings of representative sections of nodules S and B indicated that the areas occupied by fibrinoid material were approximately 25 to 26% as large as the areas occupied by collagen. This was demonstrated by a group of staining procedures including the van Gieson and Trichrome stains. If fibrinoid were a protein of collagenous origin, one might reasonably expect that it would contribute significantly to the total hydroxyproline content of the nodule tissue, unless, of course, the change of collagen to fibrinoid involved the loss of hydroxyproline specifically from the protein chain. The latter, though it is unlikely, cannot be ruled out.

Fibrinoid is extracted from subcutaneous nodule tissue(9) during the preliminary treatment with 0.1 N sodium hydroxide in the procedure of Lowry *et al.* for the determination of collagen and elastin. If fibrinoid contained hydroxyproline in the amounts present in collagen, one would expect significant differences between the total hydroxyproline content of nodule tissue obtained by analysis of the wet tissue (expressed as % wet weight in Table I) and the value calculated from the collagen and elastin contents (Table I). With experimental errors due to small samples taken into account, there is no large discrepancy in the % hydroxyproline arrived at by the two procedures. This would preclude the loss of significant amounts of hydroxyproline on alkaline extraction and indicates that fibrinoid, since it is removed by such treatment, does not contain hydroxyproline, at least in amounts approaching that in collagen. The latter observation would suggest that the fibrinoid material of the rheumatoid nodule is not of collagenous origin. Further quantitative studies are in progress(9).

Summary. Three subcutaneous nodules and one specimen of synovial membrane from patients with rheumatoid arthritis have been subjected to analysis for water, lipid, collagen, elastin and hydroxyproline. The hydroxyproline content of the collagen in these tissues was similar to that found in collagen from

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other sources. The total hydroxyproline content of the nodules and the synovial tissue was accounted for completely by the collagen and elastin fractions. The suggestion is made on this basis that the fibrinoid material of the rheumatoid nodule is not of collagenous origin.

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The *In vitro* Interchange of Cholesterol Between Plasma and Red Cells.* (19064)

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In previous studies on the incorporation of C^{14}-acetate carbon into cholesterol in various tissues of intact animals(1), it was observed that newly synthesized cholesterol appeared in liver, plasma, and red blood cells with surprising rapidity and attained uniform distribution within a few hours. Further investigation in dogs led to the conclusion that a rapid interchange of cholesterol must be assumed to occur in the living animal to explain the rapidity of equilibration of isotopic cholesterol between liver, plasma, and red cells(2,3). It therefore seemed of interest to investigate cholesterol interchange *in vitro*. Hahn and Hevesy explored the *in vitro* interaction between P^{32}-labeled phospholipids of plasma and red cells and found a very limited interchange—5% in 4.5 hours in rabbit blood(4). Although only total phospholipid P was measured, these authors concluded that some phosphatides exchanged with great speed and others not at all.

It is now generally accepted that the phospholipids and cholesterol of plasma are present in lipoprotein form and this would appear to be true also for red cells. However, the unesterified cholesterol moiety of both plasma and red cell lipoproteins is chemically identical and is therefore particularly suitable for interchange studies.

Procedure and results. Whole blood containing C^{14}-labeled cholesterol of high specific activity was obtained from a dog previously maintained on a cholesterol-free diet by the oral administration of 100 to 400 μc of C^{14} as carboxyl-labeled acetate, followed by the exsanguination of the animal about 24 hours later. The blood was treated with an anticoagulant (A.C.D. solution or oxalate) and cells and plasma separated. The interfacial layer was rejected and the cells were washed 3 times with isotonic salt solution. Unlabeled dog blood was drawn and treated in the same way except that the cells were not washed. In most experiments labeled cells and labeled plasma from one dog were each incubated with their unlabeled counterparts from a normal dog. In one experiment, normal human red cells were incubated with radioactive dog plasma. Measured volumes of red cells and of plasma were mixed and incubated at 37°C with gentle agitation and a slow stream of 95% oxygen plus 5% carbon dioxide was passed through the flasks. Aliquots were removed at intervals up to 4 hours and centrifuged. The interfacial layer was rejected and total lipid extracts were prepared from aliquots of plasma and red cells by 3 extractions with hot alcohol-ether (3:1), to a total of 10 volumes of solvent. Aliquots of the clear lipid extract were analyzed for free and total cholesterol by the Sperry - Schoenheimer

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