Inhibition of the Ascorbic Acid-Induced Depolymerization of Hyaluronic Acid by Serum Fractions[1] (34156)

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A decrease in the viscosity of solutions of hyaluronate by L-ascorbic acid was reported by several authors (1–3). This decrease in viscosity which is observed already at a concentration of ascorbic acid equal to that found in human blood has been attributed to a rapid depolymerization of hyaluronic acid. However, it is not known whether ascorbic acid plays a role in the pathogenesis of certain connective tissue diseases, such as rheumatoid arthritis, in which degradation of hyaluronate *in vivo* is thought to occur. Serum proteins (4), as well as antioxidants and free radical scavengers (5) have been found to inhibit the action of ascorbic acid on purified hyaluronic acid. In earlier work, alpha-2 globulin fractions (4) and ceruloplasmin (6) were identified as the serum proteins that are most active in inhibiting this reaction. However, no fraction investigated showed inhibition of the depolymerization of hyaluronic acid greater than that exhibited by whole serum on a protein weight basis. The present study was undertaken to determine whether some fraction of human serum could exceed in potency this inhibition afforded by whole serum.

Materials and Methods. Purified hyaluronic acid ($[\eta] = 45$ dl/g) used in these experiments contained less than 4% protein. It was prepared from bovine synovial fluid by a method described previously (7). Purified Cohn serum fractions were supplied by the American National Red Cross, Washington, D. C.

Depolymerization of the hyaluronic acid by ascorbic acid alone (control) or in admixture with a serum fraction was measured at 30° by changes in specific viscosity using Cannon-Manning glass viscometers. The system was composed of hyaluronic acid (0.35 mg/ml) in 0.2 *M* phosphate buffer, pH 7.2, 0.33 m*M* L-ascorbic acid, and a serum fraction of variable concentration.

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TABLE I. Inhibition of Ascorbic Acid Induced Depolymerization of Hyaluronic Acid by Protein Fractions of Human Serum.[a]

Inhibitor	Specific fluidity after 120 min
None	>3
Fresh whole serum	0.70
Cohn fraction II	1.40
III-2	1.42
IV combined	0.69
IV-1	0.65
IV-4	0.56
IV-5, 6	0.99
IV-7	1.50
V combined	>2

[a] Final concentrations: hyaluronic acid 0.4 mg/ml; ascorbic acid 0.33 m*M*; serum fraction 518 mg /100 ml; phosphate buffer 0.2 *M*; pH 7.3, 30°. Buffered hyaluronic acid (0.4 mg/ml) had a specific fluidity value of 0.56.

The inhibitory effect of human serum fractions was compared by first making up solutions of equal weight of protein (518 mg/100 ml). Each fraction was mixed with a buffered solution of hyaluronic acid. Ascorbic acid dissolved in water was then added, and the change in fluidity ($1/\eta_{sp}$) with time of the polymer substrate was measured.

Results. The results, shown in Table I, indicate that combined Cohn fraction IV, and both basic fractions IV-1 and IV-4 showed inhibition greater than whole serum. Serial dilutions of these fractions and of whole serum were also examined for inhibitory effect on the viscosity reducing activity of

ascorbic acid on hyaluronic acid (Table II). A plot of serum protein concentration against the corresponding inhibition showed that on a basis of weight, basic fraction IV-4 was 10–20 times more effective in inhibiting the ascorbic acid induced depolymerization of hyaluronate than fresh whole serum; at higher concentration, fraction IV-1 exhibited an inhibition that was almost twice that shown by fresh serum (Table II). When compared with sodium diethyldithiocarbamate, one of the strongest inhibitors known for this system (5), fraction IV-4 had an inhibition comparable, if not greater than that obtained with the aforementioned chelating agent. Fraction IV-1 contains α-globulin mainly, fraction IV-4 albumin and α- and β-globulins (8).

Discussion. The possibility arose that the observed inhibition of the hyaluronic acid–L-ascorbic acid system in the presence of serum Cohn fraction IV-4 might be caused by destruction of the autoxidant by the serum protein. To test this hypothesis, we compared the effect of fractions IV-4 and V on the stability of an L-ascorbic acid solution in phosphate buffer at pH 7.3. The titration curve (9) shows that both of these fractions have a pronounced stabilizing effect on ascorbic acid under these conditions, although neither of the proteins prevents its slow oxidation (Fig. 1). These observations would suggest that serum fraction IV-4 inhibits the degradation of hyaluronic acid by competing

TABLE II. Inhibitory Effect of Cohn Fractions IV-4 and IV-1 at Various Concentrations.[a]

Conc of inhibitor (mg/100 ml)	Inhibition (%)		
	IV-4	IV-1	Serum
10	80	0	57
25	84	36	58
50	92.5	56	59.5
100	97.5	68	63.5
200	99	75.5	68.5
250	99	79.5	71.5
300	99.5	82	74
400	99.9	85	80
500	100	88.5	86

[a] Hyaluronic acid, 0.4 mg/ml; 0.2 *M* phosphate buffer, pH 7.3; serum fraction; 0.33 m*M* L-ascorbic acid; 30°.

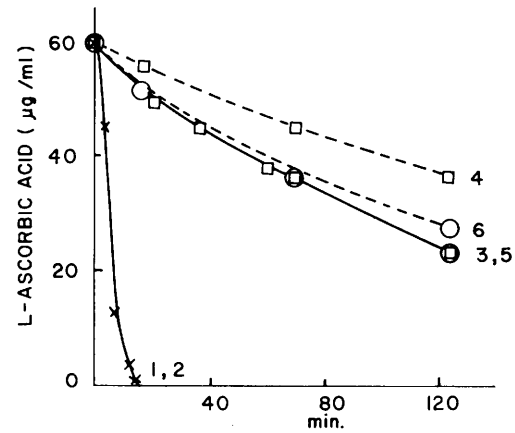

FIG. 1. Disappearance of L-ascorbic acid (0.33 m*M* in 0.2 *M* phosphate buffer, pH 7.3, 30°) in the presence of serum fractions IV-4 and V (5 mg/ml). AA = L-ascorbic acid; HA = hyaluronic acid, 0.4 mg/ml: 1, 2 = AA with and without HA; 3, 5 = AA + IV-4, AA + V; 4 = AA + IV-4 + HA; and 6 = AA + V + HA.

efficiently for the free radical species that are produced in the course of the autoxidation of ascorbic acid. Indeed, the formation of hydroxyl free radicals (10), and the ability of some proteins to act as scavengers have been reported. The results also suggest that combined fraction V has no scavenging ability, and therefore, does not prevent the depolymerization of hyaluronic acid by ascorbic acid. The possibility that hyaluronic acid forms a complex with a constituent of fraction IV and thereby is protected against attack by free radicals evolved in the system, cannot be ruled out either. However, since fractions IV and V show opposite behavior on the depolymerization of hyaluronic acid in the presence of L-ascorbic acid, it is conceivable that the two protein fractions act by different mechanisms in stabilizing the reducing agent.

It is not known whether ascorbic acid may be implicated in the pathogenesis of diseases wherein depolymerization of hyaluronate occurs. In such an event, however, the import of a protein constituent of serum that has the ability to control the breakdown of hyaluronic acid by ascorbic acid is obvious. Pathologic variations in the proportions of the serum fractions shown in this paper to be

the most effective inhibitors in the above system have yet to be investigated.

Summary. Cohn fraction IV-4 was 10–20 times more effective than fresh whole serum in inhibiting the ascorbic acid-induced depolymerization of hyaluronic acid from synovial fluid.

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