Purification and Isolation of Rheumatoid Factor. (24417)

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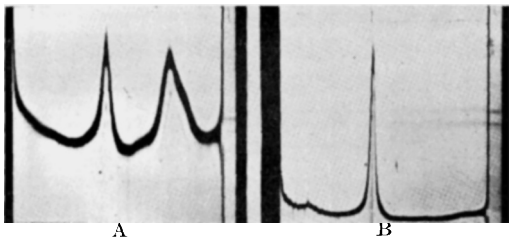
Serum of patients with rheumatoid arthritis has been shown to agglutinate bacteria (1) and particles or cells sensitized with human or rabbit gamma globulin. Sensitized sheep erythrocytes(2) have been commonly used to demonstrate activity of rheumatoid agglutination activating factor. Partial purification has been achieved by column chromatographic separations of serum proteins of rheumatoid serum on anionic and cationic cellulose ion exchangers(3). Also purification has been achieved by dissociation of complexes precipitated from rheumatoid serum by heated human Cohn Fraction II preparations followed by differential ultracentrifugation through a sucrose density gradient(4). Svartz *et al.*(5) used cold precipitable globulins of rheumatoid serum as starting material for fractionation on carboxymethyl cellulose to yield an apparently homogeneous protein with a sedimentation coefficient of 18.7. Concentration of one of the preparations(5) was too low to demonstrate homogeneity in the ultracentrifuge unequivocally, and no concentration dependence data were given for the sedimentation coefficient. The other preparation (4) was obtained after dissociation at pH 3.0. At this reaction the material eventually loses all activity(3). As a result, even brief exposures to pH 3.0 buffers might be expected to produce some denaturation of the rheumatoid factor. It, therefore, seemed useful to present some data on an active material prepared by a different method. Furthermore, we are able to present some precise quantitative data(6,7) on the relative specific activities of the material at successive stages of purification.

Methods and materials. Relative specific activities were determined by a method we developed, which measures inhibition of hemolytic activity of sensitized Newcastle Disease Virus particles by the rheumatoid factor(6,7). By using this method relative specific activities of the material at successive stages of purification with a precision of $\pm$ 5% for duplicate samples can be determined. The method of fractionation proceeded as follows: 500 ml of serum derived from 550 ml of rheumatoid plasma by thrombin treatment was dialyzed against 0.01 M phosphate pH 5.8. The resulting euglobulin was redissolved in phosphate buffered isotonic saline and dialyzed with 2 g of diethylamino-ethyl (DEAE) cellulose against 0.01 M phosphate pH 7.9. 93% of the activity was adsorbed. The DEAE and adsorbed euglobulin were transferred to the top of 18 g DEAE column for elution by stepwise increased salt and decreased pH method of Lospalluto and Ziff (3). An active peak emerged during elution with 0.15 M phosphate pH 5.0. An aliquot of this material was adjusted to 0.1 M phosphate pH 7.4 by dialysis after freeze drying. The remainder was dialyzed against 0.1 M phosphate pH 4.4. After concentration by freeze drying to 30 ml, 20 ml of this solution was chromatographed on 10 g of carboxymethylcellulose (CMC) equilibrated with pH 4.4 phosphate. A sharp peak containing the activity emerged at pH 4.4. This material was freeze dried and dialyzed against 0.1 M phosphate pH 7.4.

Results. Fig. 1 shows sedimentation patterns of DEAE activity peak and CMC activity peak. The DEAE material shows 4 components with sedimentation coefficients of approximately 7, 9, 19, and 30. The CMC eluate shows at least 90% of $S^{\circ}_{20,w}$ 18.8 com-



A B

FIG. 1. Sedimentation diagrams of rheumatoid factor preparations: (A) Activity peak eluted from DEAE. (B) 1A after elution from CMC at pH 4.4. Both pictures taken after 24 min. at 59,780 r.p.m.

TABLE I. Sensitized NDV Hemolysis Inhibition Activity.

Fraction	Units/ml	Nitrogen (mg/ml)	Specific activity (units/mg N)	Specific activity × serum
Serum	37	11.2	3.3	1
Euglobulin	118	.82	143	43
DEAE, pH 7.4	628	.98	641	194
" , pH 4.4	656	1.08	607	181
CMC	1751	.98	1786	543

ponent and a small component with $S^{\circ}_{20,w}$ greater than 30, possibly an aggregate of the 18.8 component. A trace of slow material is also seen. The peak is sharper and more homogeneous in appearance than the material prepared by Edelman *et al.*(4).

Relative specific activities at successive stages of purification are shown in Table I. Fourteen per cent of the nitrogen and 39% of the activity were recovered from the CMC column. The material shown in Fig. 1B had a sensitized sheep cell agglutination titre of 1:5120.

Fig. 2 shows the data on concentration dependence of sedimentation coefficient, and the extrapolated value of 18.8 S at zero concentration.

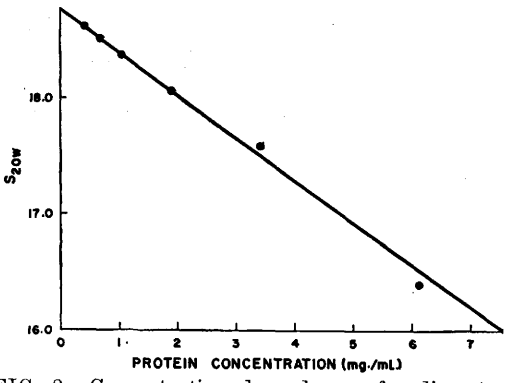


FIG. 2. Concentration dependence of sedimentation coefficient of major peak from Fig. 1B.

Summary. A method for purification of the rheumatoid factor has been presented. A euglobulin fraction of serum was chromatographed on DEAE cellulose. The resulting active fraction was further chromatographed on carboxymethyl cellulose to yield a preparation which contained more than 90% of a component with a sedimentation coefficient of 18.8. Relative specific activities at successive stages of purification were determined. Concentration dependence data for the sedimentation coefficient were obtained.

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