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Protein concentration of synovial fluid in chronic rheumatoid arthritis. Estimation of protein in the synovial fluid of chronic rheumatoid arthritis by gel filtration and paper electrophoresis

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Protein concentration of synovial fluid in chronic rheumatoid arthritis. Estimation of protein in the synovial fluid of chronic rheumatoid arthritis by gel filtration and paper electrophoresis*

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Abstract

For the purpose to reveal the characteristics of the synovial fluid of the chronic rheumatoid arthritis the proteins of the synovial fluid and blood serum have been analysed by employing the methods of electrophoresis, gel filtration on Sephadex G-200 column and ultracentrifugation. Waaler-Rose test and latex fixation test have also been made on each protein fraction, and the following results were obtained. 1) The total protein level of synovial fluid, which is 3/5 of that of the serum, is slightly higher than that of control. 2) Fractionation of the synovial proteins by electrophoresis revealed nearly the same protein contents in each fraction in percentage as that of comparable fraction of the serum protein, with a slight increase in γ -globulin fraction. 3) The fractionation by Sephadex column G-200 give three peaks both in serum and synovial fluid, 19 S, 7S and 4S. 4) 19S fraction of the synovial fluid, which is mainly of γ -globulin, showed a higher level than that of the synovial fluid from the controls. 5) Rheumatoid tests gave positive reaction in the 1st peak containing 19S γ -globulin from the synovial fluid and blood serum.

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**PROTEIN CONCENTRATION OF SYNOVIAL FLUID IN
CHRONIC RHEUMATOID ARTHRITIS**

**ESTIMATION OF PROTEIN IN THE SYNOVIAL FLUID
OF CHRONIC RHEUMATOID ARTHRITIS BY GEL
FILTRATION AND PAPER ELECTROPHORESIS**

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There are many reports dealing with the assay of serum proteins in chronic rheumatoid arthritis by means of electrophoresis (1, 2), column chromatography (2) and ultracentrifugation (1, 2, 3) revealing the specific change to the disease but only a few reports on the analysis of the proteins of synovial fluid by the assays of paperchromatography and diselectrophoresis, that is to show most clearly the changes specific to chronic rheumatoid arthritis (4).

Since the introduction of the gel filtration method for protein assay by PORATH (1959) the analysis and the purification (5) of the serum proteins in chronic rheumatoid arthritis have made a great advance. Therefore, it would be worthwhile to analyze the synovial fluid proteins by applying the same method, though some papers have already appeared. In view of this, the author attempted to study change of proteins in the synovial fluid of chronic rheumatoid arthritic joints by the gel filtration on Sephadex G-200 column, and found that the synovial proteins can be separated into three fractions just as in the case of serum proteins and the precipitin constant of the fractions are identical with that of the serum protein. It was also found that the percentage of the second peak increases along with the increase of total protein in synovial fluid. The present communication describes briefly the results obtained by using gel filtration technique and paper electrophoresis.

MATERIALS AND METHODS

Blood serum and synovial fluid from the patients of chronic rheumatoid

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rthritis served as materials.

The serum was obtained from the blood drawn from the cubital vein of six patients whose RA test of serum proved positive. Synovial fluid was obtained from 16 patients with chronic rheumatoid arthritis, and four controls, two patients with osteoarthritis and two of traumatic arthritis. Of all these samples total protein contents were measured by Hitachi proteinometer.

Paper-electrophoresis (6, 7) was carried out with 0.01 ml each of serum and synovial fluid employing Toyo filter paper No. 50 soaked with buffer, and 0.5 mA of the current per 1 cm width of the filter paper at 5°C with Veronal buffer, pH 8.5 (1M=0.11). After running the paper was dried, stained with BPB stain for 30 minutes, treated with acetic acid, dried, and each fraction was eluted in 10 ml of 2M NaHCO₃ solution. The optical density of each eluate was measured by the Hitachi electrospectrometer, GPO type, at the optical density of 570 m μ , and the protein contents of each eluate was computed.

Gel filtration on Sephadex column, 3.0 \times 43.0cm: The elution was conducted with 3 ml of synovial fluid and 0.9% NaCl solution. The synovial fluid was pretreated with hyaluronidase in order to lower the viscosity (8) of the synovial fluid to make its separation easier, i. e. 3 ml of synovial fluid were added with 200 units of Harotase (hyaluronidase, a product of Takeda Pharmaceutical Co.). After mixing well, the mixture was centrifuged at 2,500 r. p. m. for 10 minutes, and 2 ml of the supernatant were used for the gel filtration, one drop per ten seconds.

The protein contents of the collected fractions were measured with the spectrophotometer (Hitachi type) at the optical density of 280 m μ . The protein ratio of eluted fractions was calculated from the ratio of the areas giving by the protein concentration curves of individual fractions. Waaler-Rose test (9) (S. S. C. A. -test) and latex fixation test (10) (LFT) were made in each protein fraction to determine rheumatoid factor.

Sedimentation constants were estimated in the eluates obtained from two fractions giving the individual peaks which were put into vials in tube and condensed to 1% in Sephadex G-25 and subjected to the ultracentrifugation (Hitachi UCA-I type analytical ultracentrifugation apparatus) as 52,300 r. p. m. for 10 minutes.

Double diffusion test: The precipitin reaction in agar was conducted with anti-human 19S and anti-human 7S of globulin goat serum (the products of Mann Research Lab.) against the eluate from each peak as antigen.

RESULTS

Total protein contents of the synovial fluid of the rheumatoid arthritis were 4.50 g/dl in mean and somewhat higher than that from the synovial fluid of arthritis other than rheumatoid arthritis 4.45 in mean. Total serum protein of the patients was 7.8 g/dl in mean and relatively low comparing to the value of normal human serum, 8.13 g/dl (Tables

1, 2).

The analysis of the synovial fluid protein by electropaperchromatography gave the similar levels in every constitution, albumin and globulins, as those in serum, with a relatively large value in γ -globulin (Table 1).

Table 1 Proteinometric and Electropaperchromatographic Analyses of Chronic Rheumatoid Arthritis

	Total Protein (g/dl)	Albumin (%)	Globulin			
			α_1 (%)	α_2 (%)	β (%)	γ (%)
Synovial Fluid*	4.34	36.66 ± 0.67	7.95 ± 0.08	9.01 ± 0.08	13.57 ± 0.08	32.28 ± 0.75
Serum**	8.05	37.96 ± 0.87	7.39 ± 0.87	10.31 ± 0.15	14.16 ± 0.23	30.04 ± 0.63

* mean value of 11 cases ** mean value of 12 cases

Table 2 Proteinometric and Gel filtration analyses of Blood Serum and Synovial Fluid on Sephadex G-200 Column

Data on 16 patients of Rheumatoid Arthritis, 4 patients of Arthritis other than Rheumatic Disease and 3 Normal Individuals

Material	Diseases	Cases	Total protein (g/dl)	Protein Fraction by Sephadex G-200 column		
				Peak 1 (%) (19S)	Peak 2 (%) (7S)	Peak 3 (%) (4S or albumin)
Synovial Fluid	Rheumatoid arthritis	16	4.76 ± 1.17	13.03 ± 3.97	45.67 ± 10.20	41.17 ± 14.60
	Arthritis other than rheumatism	4	4.45 ± 0.97	8.4 ± 2.5	42.90 ± 12.00	48.5 ± 13.3
Serum	Rheumatoid arthritis	6	7.65 ± 0.70	21.24 ± 2.80	40.85 ± 6.20	37.85 ± 3.40
	Normal Individuals	3	8.13 ± 0.29	11.67 ± 11.30	40.22 ± 4.30	48.06 ± 4.80

The analysis by gel filtration with sephadex G-200 revealed that the proteins of the synovial fluid can be separated into three fractions as in the case of serum (Table 2, Figs. 1, 2). Concerning the protein contents of each fraction, percentage of protein contents in peak 1 showed a marked high level, with a slight decrease in peak 3, while the protein in peak 2 showed less significant change (Table 2). Ultracentrifugation test revealed that the proteins in peak 1 contains 19S γ -globulin, peak 2 7S γ -globulin, and peak 3, 4S or albumin (Fig. 3). Precipitin reaction tests on

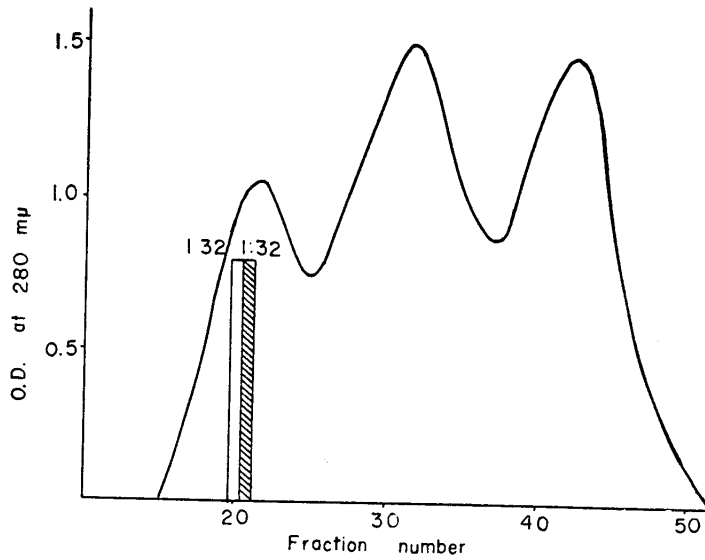


Fig. 1 Fractionation pattern of the serum protein in rheumatoid arthritis by Sephadex G-200 column and rheumatoid test of the protein at each peak
Hatched column: S. S. C. A. titer, Open column: L. F. T. titer,
Method: see text

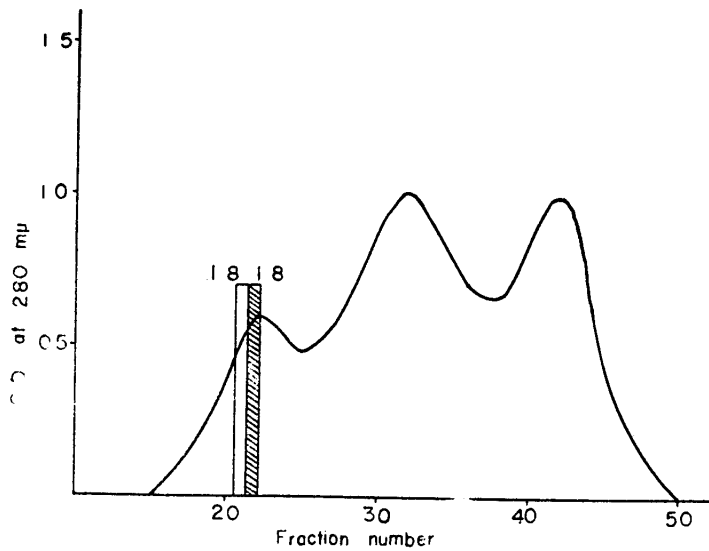


Fig. 2 Fractionation pattern of the synovial protein in rheumatoid arthritis by Sephadex G-200 column and rheumatoid test of the protein at each peak
Hatched column: S. S. C. A. titer, Open column: L. F. T. titer
Method: see text

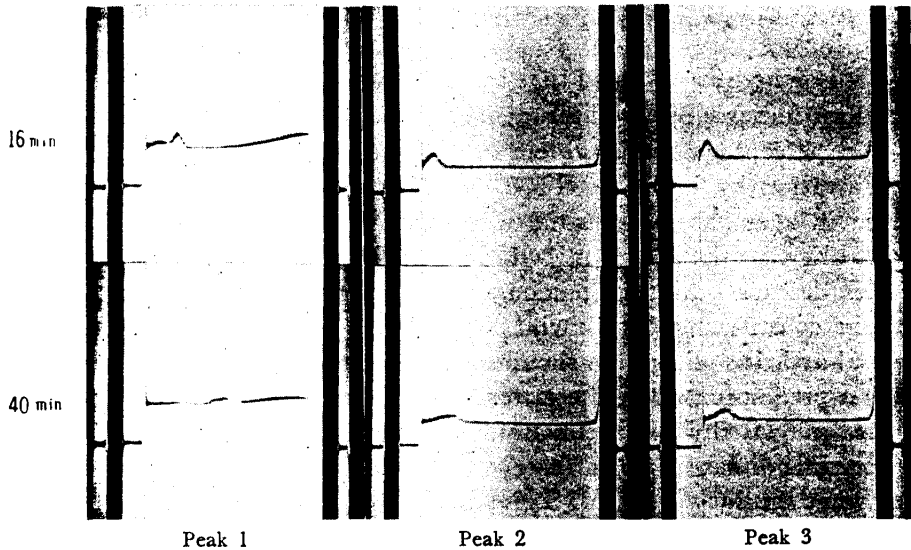


Fig. 3 Ultracentrifugation patterns of the protein from 1st, 2nd and 3rd peaks appearing in Fig. 3

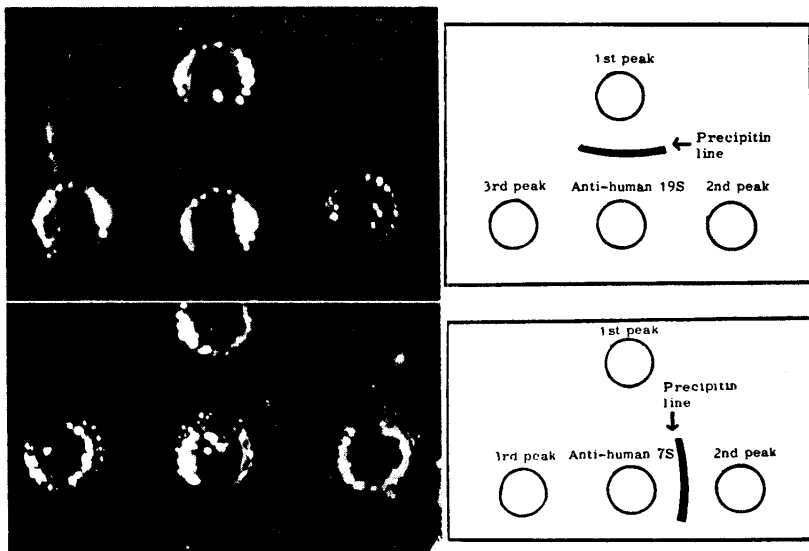


Fig. 4 Double diffusion test made on the proteins from 1st, 2nd and 3rd peaks appearing in Fig. 1

every protein by using the antibodies to 7S, 19S and globulins reconfirmed the finding obtained by analytical centrifugation (Fig. 4). Waler-Rose test and LFT on 3 peaks in synovial fluid and serum disclosed that

the factor giving a positive rheumatoid arthritic reaction is in peak 1 (Figs. 1, 2).

It is generally accepted that in allergic disease γ -globulins 7S and 19S globulins increase in blood serum. This is also true in rheumatoid arthritis, i. e. percentage of the protein in the fraction appearing as peak 1 in gel filtration by Sephadex G-200, or 19S globulin, showed a marked increase in rheumatoid arthritis, with a slight increase in 7S globulin, indicating that the tissue antibody is largely concerned to the development of this disease. Similar tendency has also been revealed between the protein constituents of synovial fluids from rheumatoid arthritis and general arthritis other than rheumatism, though the increase in 7S globulin was predominated in the latter. Comparing the protein constituent of the synovial fluid of rheumatoid arthritis to there of the blood serum of the same disease some difference in the percentage of 19S fraction but somewhat similar values in 7S globulin and 4S or albumin fractions.

DISCUSSION

The electropaperchromatographic analysis of the synovial fluid and blood serum of the chronic rheumatoid arthritis also indicates that the protein constituents of the synovial fluid of the chronic rheumatoid arthritis are very similar to those of the serum protein, though slightly high in γ -globulin in the synovial fluid. This fact suggests the possibility that most of the proteins of the synovial fluid are of serum proteins transudated. But it does not mean the direct communication as there is always some difference between protein constituents of synovial fluid and serum. The present study has demonstrated that in the case of chronic rheumatoid arthritis there occurs an increase of 7S component (mainly 7S γ -globulin by paper chromatography) in the synovial fluid, and this increase may be responsible for the increase in the protein concentration of the synovial fluid (Fig. 5). It still remains unclarified whether the 7S γ -globulin is derived from γ -globulin of serum proteins or derived from the tissue cells (mainly plasmacytes) of inflammatory site of the joint. As the protein contents of the serum are found always higher than those of the synovial fluid, protein in serum is transudated into synovial cavity. However, concerning the communication of synovial fluid with serum there is a paper¹² reporting the translocation of I^{131} γ -globulin from synovial fluid to serum. Therefore, it is probable that there is a mutual communication between synovial fluid and serum protein. Rheumatoid tests also showed a positive reaction in 19S fraction of synovial protein as in the case of

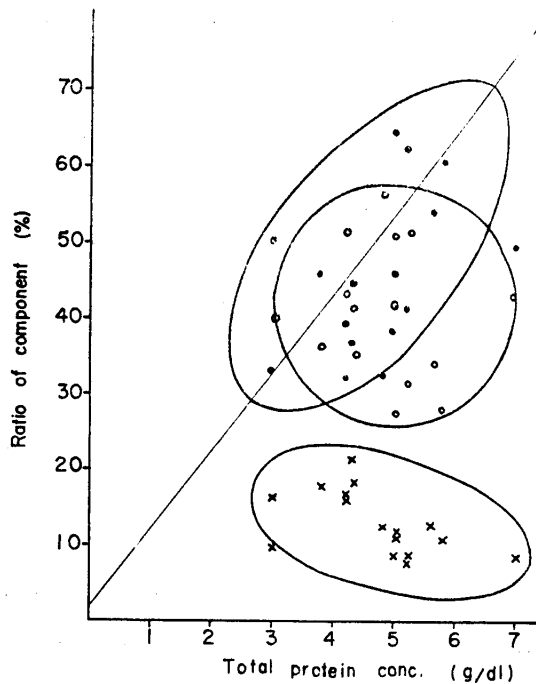


Fig. 5 Correlation between total protein concentration of synovial fluid in R. A. and relative protein percentage of Peaks 1, 2, 3, respectively by gel filtration
Crosses: Peak 1, Filled Circles: Peak 2, Open Circles: Peak 3

serum protein. Although we have no definitive answer to this problem at present, in view of the fact that the γ -globulin percentage of synovial fluid surpasses the γ -globulin of serum, it is probable that the γ -globulin from the local tissue cells of a portion of it might have seeped into serum. For the solution of this point further study is required.

CONCLUSION

For the purpose to reveal the characteristics of the synovial fluid of the chronic rheumatoid arthritis the proteins of the synovial fluid and blood serum have been analysed by employing the methods of electrophoresis, gel filtration on Sephadex G-200 column and ultracentrifugation. Waaler-Rose test and latex fixation test have also been made on each protein fraction, and the following results were obtained.

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nearly the same protein contents in each fraction in percentage as that of comparable fraction of the serum protein, with a slight increase in γ -globulin fraction.

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