ABNORMAL SEDIMENTATION SCHLIEREN DIAGRAMS OF BLOOD SERUM OF RHEUMATIC PATIENTS AS AN INDICATOR OF REVERSIBLY DISSOCIATING IMMUNE COMPLEXES

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Circulating immune complexes (CIC) play an important role in the phathogenesis of rheumatic diseases [1, 2]. Despite much research the problem of detection and classification of CIC is still far from being solved, mainly because methods of serum protein fractionation are insufficiently specific. This applies also to sedimentation analysis on analytical ultracentrifuges [3]. In particular, the long-established classification of CIC based on sedimentation coefficients [8] evidently does not reflect all their physicochemical diversity. However, not all the potential of analytical ultracentrifugation has yet been realized. Insufficient attention has so far been paid to the sawtooth fluctuations of the gradient curves frequently observed on sedimentation schlieren diagrams of the type shown in Fig. 1. Nevertheless, patterns of this kind indicate that conditions specifically disturbing normal sedimentation of proteins exist in a solution of blood serum in the cell of an analytical ultracentrifuge, and as a result, alternate negative and positive protein concentration gradients appear (saw teeth). It was shown in [5] that these disturbances may be caused by reversible reactions of the formation and dissociation of immune complexes.

This paper shows how sedimentation analysis of blood serum can be used to detect a particular protein population, namely reversibly dissociating CIC, in patients with various rheumatic diseases.

EXPERIMENTAL METHOD

Blood serum was diluted with 0.05 M phosphate buffer, pH 7.2, containing 0.1 M NaCl, to a total protein concentration of 30 mg/ml. Sheep antisera against human IgG, IgA, and IgM, produced by the Gor'kii Research Institute of Epidemiology and Microbiology, Ministry of Health of the RSFSR, were used. Sedimentation analysis was carried out on a "Beckman" (USA) Model E analytical ultracentrifuge, in the An-H rotor at 56,000 rpm and at 20°C, in one-sector aluminum cells 12 mm in diameter. In all the experiments the schlieren analyzer (phase plate) was at an angle of 65°C. The CIC concentration was determined by the method in [6]

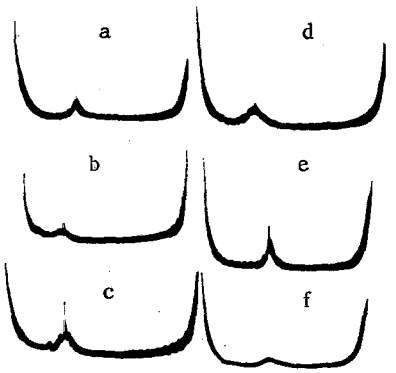

Fig. 1. Sedimentation schlieren diagrams of patients' (a-e) and donor's (f) blood sera. Sedimentation diagram (d) obtained in an experiment with a solution of serum from a patient with systemic scleroderma, at pH 4.0. Direction of sedimentation from left to right.

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TABLE 1. Brief Characteristics of Blood Sera of Patients Whose Schlieren Sedimentation Diagrams are Given in Fig. 1

Sedimentation diagram in Fig. 1	Patient No.	Diagnosis	Total protein, mg/ml	CIC, conventional units	RF	Intensity of "saw" on sedimentation diagram
a	1	Rheumatoid arthritis	69,3	0,093	1/40	++++
b	2	Sjögren's disease	82,1	0,030	Negative	++
c	3	Systemic scleroderma	76,8	0	1/40	++
d	3	The same	76,8	Not determined		+
e	4	Systemic lupus erythematosus	86,6	0,200	Negative	+++
f	Donor	None	82,0	None	None	—

(normal value 0.1 conventional unit). The rheumatoid factor (RF) was titrated by the latex test [9] (normal value not more than 1/20).

EXPERIMENTAL RESULTS

Typical schlieren diagrams of patients with rheumatoid arthritis (a), Sjögren's disease (b), systemic scleroderma (c), and systemic lupus erythematosus (e) and also of a healthy blood donor (f) are given in Fig. 1. Table 1 gives brief characteristics of the corresponding blood sera. The intensity of the "saw," which can be seen clearly on the sedimentation diagrams (Fig. 1: a-c, e), was assessed on a 5-point system (—, +, ++, +++, ++++). No "saw" was present on the donor's sedimentation diagram. Sedimentation diagram "d" was obtained in an experiment with the serum of a patient with systemic scleroderma, transferred by dialysis into acetate buffer, pH 4.0. Clearly the intensity of the "saw" was considerably reduced. Reduction or total disappearance of the "saw" also occurred on dilution of the sera to 5-7 mg/ml and on the addition of a small volume of a mixture of specific anti-IG-sera.

Qualitative analysis of the phenomena in the analytical ultracentrifuge cell shows that the alternate negative and positive concentration gradients (the saw "teeth" on the schlieren sedimentation diagrams) could arise in a protein solution if the following conditions were satisfied simultaneously: 1) two or more types of proteins participate in reversible association—dissociation reactions; 2) the sedimentation coefficients of the associates are significantly higher than those of their components; 3) the life span of the associates is such that before reverse dissociation they are able to anticipate their free components by a distance which can be noted by means of a schlieren optical system. If diffusion is unable to blur the sedimentation boundaries arising as a result of this anticipation, local falls of total protein concentration will take place along the cell, and "pits" will appear, corresponding on the Schlieren diagrams to "teeth" in alternate directions (Fig. 2). Disturbance of the normal monotonous distribution of concentration along the cell also takes place when the molar volume of the reaction differs from zero, as a result of which the hydrostatic pressure in the ultracentrifuge cell, which rises rapidly with increasing distance from the axis of the rotor, in accordance with the Le Chatelier principle, causes an even more rapid change in the value of the equilibrium constant of the reaction [4, 7].

These theoretical considerations indicate that the sawtooth schlieren diagrams of the blood serum of patients with rheumatic diseases, illustrated in Fig. 1, definitely show the presence of reversibly reacting proteins, with kinetic reaction parameters and sedimentation coefficients which satisfy the conditions for "saw" formation mentioned above. Sawtooth sedimentation diagrams are observed much less often and in a less marked form in solutions of isolated, noninteracting proteins and in healthy human blood serum and serum from patients not affected by autoimmune diseases. This suggests that abnormal sedimentation of blood proteins of rheumatic patients is generated by a special CIC population. The difference from CIC detected by precipitation methods or having the appearance of secondary fractions in the ultracentrifuge [3] is that this population is formed by proteins which associate—dissociate reversibly in situ and satisfy the conditions specified above required for the appearance of a "saw."

The existence of these reversible protein reactions in the patients' serum stems not only from theoretical considerations, but also has experimental confirmation: a "saw" is observed usually if the total serum protein concentration is relatively very high (in the present ex-

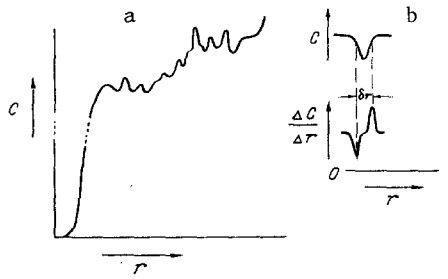

Fig. 2. Scheme of appearance of sawtooth pattern during ultracentrifugation. a) Scheme showing distribution of concentration in analytical ultracentrifuge cell in the case of a "saw" on the schlieren diagram; b) one of the "pits" and the corresponding saw "teeth"; C0 protein concentration (in mg/ml); r) distance from axis of rotor (in cm).

periments 30 mg/ml). Lowering of the concentration to 5-7 mg/ml leads, as a rule, to disappearence of the "saw," the possible explanation of which is a shift of equilibrium of the reversible reaction in accordance with the law of mass action toward dissociation. As a result the concentration of heavy associates falls below that required to initiate sawtooth disturbances of sedimentation.

The immune nature of the protein components of the reversible reaction is confirmed by the appreciable decrease in intensity of the "saw" at pH 4.0 (at pH 4.0 weak immune complexes dissociate, see Fig. 1d) and by disappearance of the "saw" after addition of mixed antiserum against human immunoglobulins. Meanwhile, the data in Table 1 show absence of correlation between the appearance of the "saw" and its intensity, on the one hand, and the content of "ordinary" CIC and of rheumatoid factor, on the other hand; this can be taken as evidence of the special character of those CTC which are responsible for the "saw." This special feature is the weak bond between antibodies and the antigens forming these CIC, as a result of which their reaction is reversible. At physiological pH and ionic strength values this can take place only when the concentration of antibodies is much greater than the amount of antigen, or if the autoantibodies have low affinity.

During this study we investigated more than 160 patients with rheumatic diseases and observed sawtooth schlieren diagrams with a varied intensity of the "saw" in roughly 70% of cases.

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