cephalitis infections by attempted direct isolations of the virus. Secondly, it should be possible to gain fundamental knowledge about the mode of spread of the encephalitis viruses within a monolayer of cells or through the fluid medium such as we now have for poliovirus and others of the more stable viruses. Thirdly, such a system may well provide a source of relatively pure virus particles for preparation of diagnostic antigens or inactivated vaccines. Lastly, continued passage of virus in tissue culture may lead to selection of attenuated strains of virus suitable for production of live virus vaccines such as are now available for prophylaxis against yellow fever.

Summary. (1) Monolayer HKC tissue cultures can be prepared on a routine basis by methods essentially identical with those currently in use for preparation of monkey kidney tissue cultures. In screen tests with 13 arthropod-borne agents and 3 types of poliovirus, HKC proved markedly susceptible to the CPE of Japanese B encephalitis, West Nile, Uganda S, Ilheus, Bunyamwera, and Semliki Forest viruses, only moderately susceptible to effects of the St. Louis encephalitis agent and the New Guinea C, Hawaiian, and Trinidad strains of dengue virus. In contrast, the Ntaya, Zika and Bwamba agents and the 3 types of poliovirus failed to show CPE. (2) Evidence has been presented for serial propagation of JBE virus through 8 serial transfers in HKC tissue cultures. CPE was eventually complete. After 6 passages the virus yield was greater than the starting inoculum, although the dilution factors had carried beyond the extinction point of infectivity of the original inoculum. (3) Identity of the Japanese B encephalitis virus after 6 serial passages in tissue culture was established by neutralization tests performed both in tissue cultures and in mice and thus indicates that the "test tube" procedure may prove useful as a serological tool in the laboratory.

1. Sanders, M., Kiem, I., Lagunoff, D., *Arch. Path.*, 1953, v56, 148.

2. Scherer, W. F., Syverton, J. T., *Am. J. Path.*, 1954, v30, 1075.

3. McCollum, R. W., Foley, J. F., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 556.

4. Banta, J. E., Report to 8th Ann. Meet. of Tissue Culture Assn., Baltimore, Md., April, 1957.

5. Bhatt, P. N., Work, T. H., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 213.

6. Kissling, R. E., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 290.

7. Melnick, J. L., Chapter in *Diagnostic Procedures for Virus and Rickettsial Diseases*, 2nd ed., 1956, p97, Am. Pub. Health Assn., N. Y.

8. Hammon, W. McD., *ibid.*, p169.

9. Casals, J., Brown, L. V., *J. Exp. Med.*, 1954, v99, 429.

Received January 2, 1958. P.S.E.B.M., 1958, v97.

Reactivity of Globulins from Rheumatoid Sera in the Latex-Fixation Test.* (23828)

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While discussing the various parameters of the latex-fixation test for rheumatoid arthritis, Singer and Plotz(1) reported that 11% of 150 rheumatoid sera agglutinated a standardized suspension of latex particles from which globulin had been deliberately omitted. These sera were those which were strongly positive with the standard "antigen", *i.e.* with a latex particle suspension containing 25 γ of human γ-globulin/ml. The tentative explanation presented was that, in such instances, the latex particles became coated with γ-globulin, or a fraction thereof, from the pa-

* This investigation was supported by research grant from Nat. Inst. of Arthritis and Metabolic Diseases, Bethesda, Md., and by research funds from Franklin County Chapter of Arthritis and Rheumatism Fn.

tient's own serum and subsequently were agglutinated with another serum fraction. In the companion paper, these authors(2) found, while comparing the reactivity of rheumatoid sera by the sheep cell agglutination technic and by latex-fixation, that 5 of 107 sera positive by the latter test were reactive only when undiluted. The total number of reactive undiluted sera was not determined.

We reported(3) that the disfunction of normal undiluted sera (human and animal) as the source of "antigen" globulin when mixed with a suspension of latex particles is restored by dilution or by testing the globulins recovered after Na_2SO_4 precipitation. In view of these findings and those of the above authors, it seemed advisable to investigate the possible role of albumin in the demonstration of the agglutinating (or flocculating) factor present in undiluted rheumatoid sera and to relate these results, if possible, to some distortion of serum components.

Materials and methods. Sera were obtained from blood samples of patients attending Rheumatology Clinic of the University Hospital. These sera were maintained in the frozen state and thawed only when aliquots were needed for serologic testing. *Latex-fixation test.* Details of the technic have been described(4). The test is similar to that suggested by Singer and Plotz(1), and differs principally in the quantities of reagents required and length of incubation. *Serum fractionation.* The Na_2SO_4 precipitation procedure(5) is a modification of more standard methods and designed for fractionating small quantities of serum (1 ml). Simplicity of fractionation permits simultaneous treatment of multiple samples. The reconstituted globulins are free of albumin and contamination of the albumin supernate with globulins is minimized. *Paper electrophoresis.* Stained patterns of serum samples subjected to electrophoresis in a Durrum-type apparatus were quantitated by photoelectric scanning with simultaneous area integration (Spinco Analytrol).

Results. Frequency with which undiluted sera react positively in the latex-fixation test. One hundred and seven sera, collected from patients, were diluted 1:20 and tested by the latex-fixation test. Of these, 94 reacted positively and were from cases of typical peripheral rheumatoid arthritis. The remaining 13 sera were from patients ill with other collagen disorders or from patients tentatively suspected of having rheumatoid arthritis. All sera then were tested in the undiluted state, but with the same latex-globulin "antigen". The only undiluted sera found to react positively were among the group which also gave positive tests when diluted 1:20, as in the standard screening procedure(4). Thirty-four % (32 sera) reacted in this manner.

TABLE I. Effect of Precipitating Globulin on Reactivity of 12 Rheumatoid Sera.

Serum No.	Reactivity by latex-fixation test when: Titrated as serum (initial dilution 1:20)	Titrated as globulin (initial dilution 1:20)
1	5120*(—)†	5120 (3+)
2	" (—)	" (4+)
3	2560 (3+)	" (3+)
4	" (—)	2560 (2+)
5	1280 (—)	" (2+)
6	" (—)	1280 (3+)
7	" (—)	640 (2+)
8	640 (—)	1280 (2+)
9	320 (2+)	320 (2+)
10	" (—)	160 (1+)
11	160 (—)	320 (1+)
12	80 (—)	80 (1+)
13–22	— (—)	— (—)

* Titers expressed as reciprocals of dilutions.
† Results in parentheses indicate intensity of reactivity recorded as —, ±, 1+, 2+, 3+, 4+. In first column, results are from tests performed with undiluted sera; in second column, from tests conducted with reconstituted, but undiluted globulins.

Reactivity of globulins recovered after precipitation. A large group of rheumatoid and other sera were diluted 1:20 and screened by the latex-fixation test. Subsequently, the positive sera were titrated and 12 were selected for experimentation on the basis of their varied titers. Ten other sera, negative by the screening test, were included for purposes of control. All sera then were tested in the undiluted state for reactivity (Table I). Only 2 of the undiluted sera were positive, and these, as before, were from rheumatoid patients. Peculiarly, this reactivity was not confined to those sera exhibiting higher titers (to be discussed). One ml of each serum was treated with Na_2SO_4 and the precipitated globulins, reconstituted to original volumes,

TABLE II. Relationship between Latex-Fixation Titer, Activity as Undiluted Sera, and Albumin Content.

Serum No.	Reactivity by latex-fixation of diluted sera (titer)	% albumin
1	5120*(2+)†	56
2	" (—)	78
3	" (2+)	
4	1280 (±)	
5	320 (—)	
6	160 (—)	
7	" (±)	70
8	" (—)	90
9	" (—)	
10	— (—)	87
Normal	— (—)	100

* Titers expressed as reciprocals of serum dilutions.

† Intensity of reactivity recorded as —, ±, 1+, 2+, 3+, 4+, and represents activity of undiluted sera.

were re-examined for reactivity with latex-globulin "antigen". The globulins from all rheumatoid sera (those reactive at dilutions of 1:20 or above) reacted positively, despite the fact that only 2 such sera were reactive when undiluted (Table I). The globulins from sera which were negative when diluted to 1:20 uniformly failed to demonstrate reactivity. Titrations of the flocculating component of the globulins were performed in the usual manner with latex-"antigen". As seen from the Table, virtually no alteration in reactivity resulted from the precipitation procedure.

Since, seemingly, removal of albumin (Table I) conferred reactivity to reconstituted, but undiluted, globulins, it seemed possible that reactivity of undiluted sera might be a reflection of the distribution of serum components. Accordingly, 9 rheumatoid sera, varying in latex-fixation titers and in reactivity when undiluted, were subjected to paper electrophoretic analysis. A 10th serum from another collagen disease, and non-reactive by the latex-fixation test, was included as was a normal serum. The activities of these sera are recorded in Table II.

For purpose of illustration, the albumin content of sera 1, 2, 7, 8 and 10 are included in the Table. The normal serum was subjected to electrophoresis simultaneously with the test sera to facilitate accurate comparisons. The albumin content of normal serum was represented as 100%. Concentrations of albumin in the test sera were calculated to this reference. It should be recalled that the normal serum was from a single blood specimen and while concentrations of the component protein fractions were within the normal range, they do not correspond necessarily to the averaged concentrations of normal serum proteins.

A comparison of the albumin content of test serum 1 and of normal serum readily reveals the decreased albumin content of test serum 1 (56% of normal). In view of the high concentration of latex-flocculating factor in this serum, it is not surprising that this serum reacted positively when undiluted. Test serum 2, although demonstrating the same latex titer as serum 1 when diluted, contained significantly more albumin (78%). This serum was non-reactive in the undiluted state. Sera 7 and 8 exhibited the same modest latex-fixation titer of 1:160. However, serum 7 reacted as undiluted serum with the latex-globulin "antigen". As before, the albumin content of this serum is decreased (70%) while the concentration of albumin in serum 8 more nearly approaches that of the control serum (90%). The albumin content of the non-reactive serum 10 (87%) also is similar to that of normal serum.

The concentration of albumin in the remaining 5 sera bore the same relationship to reactivity in the undiluted state as did the sera just described.

Discussion. In experiments not reported here, attempts to definitely assign the inhibitory property of whole serum to the albumin fraction met with failure. Reconstituted sero-reactive globulins were mixed with appropriate quantities of human albumin and subjected to the latex-fixation test. In no instance was inhibition observed. These findings, however, do not eliminate albumin from consideration, inasmuch as it is well established that mere admixture of serum components does not endow the mixture with the properties of whole serum.

It should be understood clearly that the inhibition described here and correlated with serum albumin is distinct from the inhibitor reported by Heller *et al.*(6) who were re-

ferring to the inhibitor of agglutination of sensitized sheep cells by rheumatoid sera and assigned this activity to some component of the FII fraction of serum. Based on this observation, tanned sheep erythrocytes were coated with γ-globulin and employed for detection of the agglutinating factor in rheumatoid sera. The test for agglutination-inhibitor as devised by Ziff *et al.*(7) is associated with the euglobulin fraction of serum. It was postulated that all sera probably contain inhibitor, but that it cannot be readily demonstrated in the euglobulin fraction of rheumatoid sera because of neutralization by the rheumatoid factor. Albumin was found to be free of inhibitive properties by both groups of workers.

In the present report the influence of serum albumin in the latex-fixation technic most probably is a function of its protective colloid properties and may reflect certain, as yet unexplained, aspects of the test.

Summary. 1. Thirty-two of 94 rheumatoid sera, positive by the latex-fixation test when diluted 1:20, also reacted in the undiluted state. 2. Sodium sulfate precipitation of the remaining 62 sera permitted positive demonstrations with the reconstituted globulins. 3. No alteration in titer was associated with the precipitation procedure. 4 Electrophoretic patterns revealed that the reactive undiluted sera manifested a reduced albumin content.

1. Singer, J. M., Plotz, C. M., *Am. J. Med.*, 1956, v21, 888.

2. Plotz, C. M., Singer, J. M., *ibid.*, 1956, v21, 893.

3. Rheins, M. S., McCoy, F. W., Buehler, E. V., Burrell, R. G., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 67.

4. Rheins, M. S., McCoy, F. W., Burrell, R. G., Buehler, E. V., *J. Lab. Clin. Med.*, 1957, v50, 113.

5. Thurston, J. R., Rheins, M. S., Buehler, E. V., *ibid.*, 1957, v49, 647.

6. Heller, G., Jacobson, A. S., Kolodny, M. H., Kammerer, W. H., *J. Immunol.*, 1954, v72, 66.

7. Ziff, M., Brown, P., Lospalluto, J., Badin, J., McEwin, C., *Am. J. Med.*, 1956, v20, 500.

Received January 3, 1958. P.S.E.B.M., 1958, v97.

Biological Activity of Soluble Antigen-Antibody Complexes I. Skin Reactive Properties.* (23829)

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As a result of some preliminary studies made in conjunction with an investigation on the effect of soluble complexes on smooth muscle(1), it became obvious that soluble antigen-antibody complexes formed in excess antigen also produced a marked increase in permeability of capillaries in guinea pig skin. This was shown by the fact that when a small amount of soluble antigen-antibody complex was injected into the skin of normal guinea pigs which had previously received an intravenous injection of Evans Blue, the area of injection became blue. The following paper presents a more detailed investigation of this phenomenon. The first work involved fractionation of antiserums by starch electrophoresis to determine if the antibody involved was a precipitating gamma globulin. The second study was made on normal and immune serums without the addition of any antigen when it became apparent that immune as well as normal serums contained a skin-irritating protein component.

Materials and methods. Antiserums: In the present investigation, only Armour's bovine serum albumin (BSA) and rabbit antiserum were studied. Three different pools (1, 2 and 3) of antiserum were used but immunization was the same in all rabbits. The schedule consisted of 10 mg of BSA intravenously 3 times a week for 4 weeks, then bleeding 7 days after the last injection. Serum from each rabbit was titrated by qualitative

* The authors are indebted to Mr. Bror Clark for his valuable technical assistance. This work was supported by grant from Nat. Inst. of Allergy and Infectious Diseases.

Contribution No. 2302.