TABLE I.
Complement Fixation Tests with Eastern and Western Equine Encephalomyelitis Viruses and Guinea Pig Hyperimmune Serum

Serum	Antigen	Original dilution of serum 1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	Controls: Antigen Anti-comp.	Antigen Hemol.	Serum Anti-comp.	Serum Hemol.
WEE	WEE	4*	4	4	4	4	4	2	0	0	4	0	4
	EEE	0	0	0	0	0	0	0	0	0	4	0	4
	Normal	0	0	0	0	0	0	0	0	0	4	0	4
EEE	WEE	0	0	0	0	0	0	0	0	0	4	0	4
	EEE	4	4	4	4	4	4	1	0	0	4	0	4
	Normal	0	0	0	0	0	0	0	0	0	4	0	4

* 4 = no hemolysis. 0 = complete hemolysis.

germicidal lamp.† Irradiation for more than 45 minutes destroyed completely the infectivity of the Eastern type of virus but the complement-fixing ability was unchanged even after 120 minutes' exposure. Furthermore, no obvious decrease in antigenic titer occurred after 10 weeks' storage at 4°C.

The infectivity of the Western antigen was destroyed after 20 minutes' irradiation while complement fixation was obtained with antigen irradiated as long as 30 minutes. Loss of potency occurred with prolonged irradiation as well as irregularity in the anti-complementary power of the antigen.

Improved methods of preparation, the effect of other inactivating procedures and the efficacy of these antigens with human sera are being studied.

† Further details will be described in a later communication.

14606 P

Slide Agglutination Test for Rapid Diagnosis of Pre-Eruptive Typhus Fever.

A. A. SMORODINTZEFF AND R. V. FRADKINA. (Introduced by J. E. Smadel.)

From the Virus Department of the All Union Institute of Experimental Medicine, Moscow, U. S. S. R.

A specific antigen occurs in the serum of patients during the first few febrile days of typhus fever.[1] Approximately 60% of 107 typhus patients studied during the pre-eruptive stage had sufficient amounts of this specific immune substance in their bloods to be detected by the complement fixation technic; furthermore, the antigen was absent from sera of 57 patients with typhoid, dysentery, and pneumonia. The circulating antigen was no longer demonstrable after specific antibodies made their appearance, *i.e.*, 6th to 9th day.[1] Although satisfactory results were obtained with the complement fixation technic, it seemed desirable to employ a simpler and more rapid method for demonstrating the antigen in the blood of patients with early typhus.

Various attempts were made to develop a slide agglutination test sufficiently sensitive for the detection of the comparatively small amounts of antigen present in blood of typhus patients. For this purpose a number of particulate substances were added to typhus antigens, usually Cox type vaccine, which were

1 Drobyshevskaya, A. I., and Smorodintzeff, A. A., *J. Epid. and Microbiol.* (Moscow), 1942, No. 1.

employed in preliminary studies. The addition of neither animal charcoal, erythrocytes nor *B. prodigiosum* had the desired effect. On the other hand, suspensions of carmine, of indigo, and of other water-insoluble aniline dyes when mixed with rickettsial material gave results which suggested their usefulness in testing for typhus antigen in patients' sera. Carmine has been used most extensively in our work.

Method. The technic of the slide agglutination test in current use is as follows: Chemically pure carmine is ground to a fine powder in a mortar and a 2% suspension is prepared by the gradual addition with constant trituration of freshly distilled water. The suspension is shaken for 10 minutes in a bottle with glass beads. Large particles are removed by short centrifugation at slow speed. The suspension is allowed to stand 24 hours and then centrifuged in order to sediment most of the carmine particles: the supernatant fluid is decanted and discarded. The sediment is resuspended to the original volume in a 0.25% solution of NaCl. The dye is now ready for use.

Serum to be tested for the presence of typhus antigen is diluted 1 to 5 with distilled water and ¼ volume of carmine suspension is added. The mixture is shaken for 5-10 minutes then lightly centrifuged (500 r.p.m. for 5 minutes) after which most of the supernatant fluid is decanted and the sediment resuspended in the residual fluid. While such antigen-laden particles of carmine are specifically agglutinated by the human serum rich in antibody, preliminary experiments have indicated that the intensity of reaction is increased several-fold if the antibody is used not as such but also adsorbed on particles of carmine. In the actual test, two drops of the suspension of carmine, laden with serum collected during the acute phase of the disease (antigen), are placed separately on a glass slide and to one of them is added a drop of a suspension of carmine particles laden with antibody-containing serum, and to the other, as control, is added a suspension of particles to which negative serum was adsorbed. Additional control tests of the obvious type are carried out. Agglutination becomes visible macroscopically within a few minutes.

It is important in the test to employ solutions with relatively low concentrations of electrolytes in order to avoid spontaneous agglutination of carmine particles; even so, certain lots of carmine are unusable because of nonspecific aggregation. Tests with acute phase sera stored for longer than 5 days are generally unsatisfactory.

Results. Over 100 sera from early cases of typhus fever and other infectious diseases were tested for typhus antigen by the complement fixation technic[1] and the carmine particle agglutination method. The immunological specificity of the results obtained by the two methods were comparable.

Preliminary studies suggest that instead of serum, whole blood obtained by finger prick can be used in the test for antigen. The blood is lysed by dilution with distilled water and the test carried out as above.

The presence of typhus antibodies in sera of convalescent patients and hyperimmune guinea pigs and rabbits is readily demonstrated by this same slide agglutination method. Antigen for use in this procedure is prepared by adding an equal volume of 2% carmine suspension to appropriately diluted (usually 1/20) typhus vaccine of the Cox type. Serum to be tested for antibody is prepared as described above.

Summary. A specific antigen found in the serum of typhus fever patients during the pre-eruptive stage of typhus fever can be demonstrated by a slide agglutination test which employs sensitized dye particles.