TABLE I.
Effect of Thiamine Compounds on Contraction of Muscle Induced by Acetylcholine and Potassium.

Conc. of the thiamine compounds in mols	Magnitude of contraction in % of control after immersion in the thiamine compounds for 5 min.*								
	Contraction induced by acetylcholine in muscles immersed in			Contraction induced by acetylcholine in sensitized muscles immersed in			Contraction induced by potassium in muscles immersed in		
	Thiamine chloride	Thiamine pyrophosphate	Acetylthiamine	Thiamine chloride	Thiamine pyrophosphate	Acetylthiamine	Thiamine chloride	Thiamine pyrophosphate	Acetylthiamine
0	100	100	100	100	100	100	100	100	100
3.10^{-7}	96	93	94	94	94	98	134	139	132
3.10^{-6}	93	93	92	96	93	93	149	166	162
3.10^{-5}	86	90	82	87	91	89	170	178	172
3.10^{-4}	78	89	68	76	86	64	180	232	207
3.10^{-3}	45	75	38	41	71	35	199	300	218

* Each value represents the average of 12 separate experiments. The S.E. of the mean for each value was less than $\pm 5\%$.

higher concentrations did not increase the effect of acetylcholine in inducing muscle contraction, while in high concentrations the effect of acetylcholine was depressed. The time necessary to exert this depression is very short. An adequate explanation cannot be offered at the present. It is possible that the presence of the thiamine compounds in relatively large concentrations prevents some of the receptor mechanisms from reacting to the presence of acetylcholine.

The above observations may throw some light on the mechanism of action of the thiamine compounds in regulating the contraction of muscle.

Summary. 1. The effect of thiamine chloride, thiamine pyrophosphate, and acetylthiamine in inducing muscle contraction and the effect of the thiamine compounds on the muscle contraction induced by acetylcholine and potassium were investigated. 2. Thiamine compounds did not induce a contraction of the muscle in concentrations lower than 1.10^{-2} M in a period of 3 minutes. 3. The thiamine compounds did not sensitize the muscle to acetylcholine. In higher concentrations the effect of acetylcholine in inducing muscle contraction was depressed. 4. The thiamine compounds significantly increased the effect of potassium in inducing muscle contraction.

Thiamine pyrophosphate and acetylthiamine was kindly supplied by the Merck Research Laboratories, Rahway, N.J.

14605 P

Embryonic Chick Antigens for Complement Fixation with Viruses of Eastern and Western Equine Encephalomyelitis.*

GORDON C. BROWN. (Introduced by Thomas Francis, Jr.)

From the Department of Epidemiology and the Virus Laboratory, School of Public Health, University of Michigan.

The complement-fixation test has been increasingly employed in the identification of virus infections of the central nervous system. With one exception all complement-fixing antigens thus far employed with equine encephalomyelitis have been prepared from animal brain tissue, usually that of the mouse.[1-7] Mohler,[8] using a formolized antigen

* Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

prepared from chick embryos inoculated with the virus of Western equine encephalomyelitis, reported complement fixation with serum from convalescent or immunized horses.

Because of its size a single infected chick embryo contains many times as much virus as an infected mouse brain and in view of the greatly decreased incubation period for maximal production of virus further investigation of this source of antigen was suggested.

Equine encephalomyelitis antigen. A strain of Eastern virus obtained from Dr. A. B. Sabin and the McMillan strain of Western virus received from Dr. J. Casals were employed. The viruses had been maintained at high titer by continued intracerebral passage through mice. They were then subjected to 13 rapid passages in hens' eggs containing 10-day-old embryos by inoculation of 0.1 cc of 1:100 suspension of infected embryonic tissue onto the chorioallantoic membrane. The embryos were removed after 18-24 hours and suspended in physiological salt solution to give a concentration of 20% by weight. The suspension was centrifuged at 2,500 r.p.m. for 15 minutes and the supernatant removed and stored at −70°C. Before use as antigen the virus suspension was thawed and centrifuged at 3,000 r.p.m. for 15 minutes. The clear supernatant was diluted with an equal volume of physiological salt solution. The preparations, still infectious for mice in titers of 10^{-8} with Eastern virus and 10^{-6} with Western virus, were then tested in order to determine their complement-fixing ability in the presence of immune serum.

Hyperimmune Serum. Guinea pigs were inoculated intraperitoneally with a 10% suspension of active virus derived from mouse brain. A total of 8 injections was given at intervals of 3 or 4 days over a period of 3 weeks and consisted of 3 inoculations of 0.05 cc, 4 of 0.1 cc, and 1 of 0.2 cc. Four days after the last injection they were bled by cardiac puncture and the sera pooled and frozen in small tubes at −70°C.

Complement Fixation. Serum of guinea pigs served as complement. It was titrated in the presence of the amount of antigen used in the test. The unit of complement was selected as the highest dilution which in the presence of antigen caused complete lysis of the hemolytic system. One and one-half such units of complement were chosen for most tests.

The sera from immune guinea pigs were inactivated at 56°C for 30 minutes. Two-fold dilutions were employed in 0.2 cc amounts. No great difference in titer obtained whether single or multiple pipettes were used in preparing the dilutions of serum. 0.2 cc of complement was then added followed by 0.2 cc of the clarified 10% suspension of virus. The tubes were incubated in the water bath at 37°C for one hour. 0.3 cc of a suspension of sensitized sheep cells representing a concentration of 2% by volume of packed cells and 2 units of anti-sheep cell hemolysin was then added and the tests read after exactly 15 minutes' incubation at 37°C.

By this method it was found that specific complement fixation uncomplicated by cross reactions could be obtained with antigens prepared from the viruses of Eastern and Western equine encephalomyelitis and homologous immune sera. Antigens prepared in the same manner from normal chick embryos failed to react with either serum.

In Table I are presented the results of a typical test. Complete fixation was usually obtained with dilutions of homologous serum as high as 1:64.

Effect of Inactivation of Virus upon the Antigenic Activity. The highly infectious antigens of virus titering 10^{-8} for the Eastern type and 10^{-6} for the Western type were exposed to ultraviolet irradiation at a distance of 4 cm from an 8-watt General Electric

1 Howitt, B. F., *J. Immunol.*, 1937, **33**, 235.

2 Howitt, B. F., abstracted in *J. Bact.*, 1938, **36**, 52.

3 Casals, J., and Palacios, R., *Science*, 1941, **94**, 330.

4 Casals, J., and Palacios, R., *J. Exp. Med.*, 1941, **74**, 409.

5 Casals, J., *Am. J. Pub. Health*, 1941, **31**, 1281.

6 Howitt, B. F., *J. Immunol.*, 1943, **47**, 293.

7 Havens, W. P., Watson, D. W., Green, R. H., Lavin, G. I., and Smadel, J. E., *J. Exp. Med.*, 1943, **77**, 139.

8 Mohler, W. M., *J. Am. Vet. Med. Assn.*, 1939, **94**, 39.

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.