method was tested and confirmed in experimental melioidosis in mice and hamsters, and its advantages over the usual titration method were discussed. 2. The number of *M. pseudomallei* necessary to generate one lesion, called the "ratio," is obtained by dividing the inhaled dose by the total number of primary pulmonary foci. This "ratio" multiplied by 0.7 equals the respiratory LD_{50}. Data and graphs are presented to estimate the total number of primary pulmonary lesions when they are so numerous that only the lesions on the surface of the lung can be counted. Estimates of the 95% confidence interval of the respiratory LD_{50} obtained by this lesion-count method overlapped those obtained by the titration method.

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A Chick Embryo-Derived Complement-Fixing Antigen for Western Equine Encephalomyelitis. (22549)

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Complement-fixing antigens for the diagnosis of certain neurotropic viral infections, for example, Western equine encephalomyelitis, St. Louis encephalitis, Japanese B encephalitis, are in general derived from infected rodent (usually mouse) brain tissue. Howitt(1) used ether-extracted mouse brain as an antigen for the detection of antibodies to the Western equine and St. Louis encephalitis viruses but found that the margin between specific and nonspecific reactions was narrow. Casals and Palacios(2), also using mouse brain as an antigen source, were able to remove some of the nonspecifically reacting substance by means of repeated freezing and thawing. Havens *et al.*(3) used high-speed centrifugation to remove nonspecifically reacting substances from mouse and hamster brain antigens.

In the case of the Western equine encephalomyelitis virus, complement-fixing antigens have also been prepared from homogenized chick embryos. Mohler(4), who used formalinized preparations, found them to be highly anticomplementary and frequently nonspecific. Brown(5), however, found that homogenized embryo suspensions, either uninactivated or inactivated by ultraviolet irradiation, gave specific fixation with guinea pig hyperimmune sera. One of the difficulties with WEE (and EEE) mouse brain antigens has been the low antibody titers obtained with human sera (WEE(6,7)) and horse sera (EEE and WEE(8)), and also the occurrence of nonspecific results(6). DeBoer and Cox(6) found that antigens prepared from homogenized chick embryos or from mouse brains treated by repeated freezing and thawing reacted with syphilitic sera and that this non-specific reactivity could be removed by benzene extraction. España and Hammon(9) modified the method in order to obtain more sensitive antigens. Preparation of mouse brain antigen by the method of DeBoer and Cox requires approximately 4 days(9), and by the method of España and Hammon, approximately 36 hours(9). A simpler and less expensive means of preparing antigens is de-

sirable, and the present communication describes a WEE virus antigen prepared from the embryonated hens' egg.

Preparation of WEE egg antigen. Eleven-day-old embryonated hens' eggs are inoculated into the allantoic cavity with 0.1 ml of egg-passaged WEE virus in 10% normal rabbit serum broth. The virus suspension is appropriately diluted so that approximately 50% of the embryos will be moribund within 24 to 30 hours. The A-42 strain of WEE virus, which was isolated in this laboratory in 1946 from the brain of a horse and which has been passaged only in the chick embryo since its original isolation in the egg, is used. As soon as moribund embryos are detected, they are placed in the refrigerator, and when approximately 50% of the embryos are moribund, the remainder of the eggs is placed in the refrigerator and held overnight at 4°-6°C. The next day, the allantoic and amniotic fluids are harvested and pooled, and the allantoic and amniotic membranes are removed and also pooled. The membranes are made into a 50% suspension in the fluids either by grinding in a mortar or by a 2- to 3-minute cycle in a blendor. The suspension is spun at 10,000 rpm in the angle rotor of a Spinco centrifuge for one hour. The clear supernatant fluid is removed and merthiolate is added to give a final concentration of 1:10,000. This constitutes the final antigen, which is distributed into ampoules of convenient size and stored on dry ice. The antigen yield from 48 embryonated eggs approximates 75 ml.

Detection of WEE complement-fixing antibody in human sera. Egg membrane-fluid antigens, prepared essentially as described above, have been used in this laboratory continuously since 1950. Up to 1953, the antigen suspension was centrifuged at 4500 rpm for one hour in an angle head at 4°C in a refrigerated International centrifuge; preparations spun at 10,000 rpm for one hour in a Spinco machine (refrigerated) have been used since then, as a much clearer antigen is produced, and the incidence of nonspecific reactions is also reduced. Complement fixation tests for the detection of WEE virus antibodies were performed according to the technic described several years ago(10), and latterly according to a slightly modified method recently described(11).

TABLE I. Results of Complement Fixation Tests with WEE Egg-Derived Antigen and Sera of Patients with Encephalitis.

Patient No.	Name	Days after onset	WEE antibody — CF titer	WEE antibody — Neutralization indices
1	H. Ca	24	<1:8	1.8
		45	1:16	1.7
2	G. Da.	6	<1:8	1.9
		13	1:16	1.9
3	G. Ag.	5	<1:8	2.6
		23	1:32	1.7
4	F. Ar.	5	<1:8	2.5
		17	1:32	3.0
5	J. Ba.	1	<1:8	1.6
		18	1:32	2.9
6	L. Ga.	12	<1:8	1.9
		26	1:32	2.1
7	C. An.	6	<1:8	2.1
		16	1:64	2.1
8	D. Br.	8	<1:8	3.1
		22	1:64	2.9
9	G. Cl.	2	<1:8	1.0
		14	1:64	2.1
10	E. LaS.	2	<1:8	1.9
		18	1:64	2.7
11	I. Br.	9	<1:8	1.8
		22	1:128	2.7
12	R. Ac.	4	<1:8	1.9
		23	1:128	>3.2
13	E. Cl.	4	<1:8	3.4
		19	1:128	4.0
14	M. Et.	10	<1:8	1.3
		19	1:128	2.4
15	M. Fr.	4	<1:8	1.3
		14	1:128	2.4
16	L. Ki.	2	<1:8	1.9
		12	1:128	2.0
17	J. Bo.	5	<1:8	2.6
		19	1:256	2.1
18	F. Fa.	6	<1:8	1.6
		13	1:512	2.2
19	I. Cc.	9	1:32	1.6
		24	1:512	2.9
20	M. Ch.	7	1:64	3.1
		21	1:256	3.3

Table I illustrates rises in complement-fixing antibody in 20 patients with encephalitis due to WEE virus infection. By the time the first blood specimen was obtained after onset of illness, neutralizing antibody had already attained maximal levels, *i.e.*, it was not possible to show a diagnostically significant rise in titer between acute and convalescent phase serum specimens. The neutralization

test employed was the usual one in which undiluted serum is tested against serial 10-fold dilutions of the virus by intracerebral inoculation of the mixtures into adult Swiss mice. Since a change in virus titer of one dilution step (10-fold or 1.0 log) is within the experimental error of this method, and if a rise of 1.5 logs in the neutralization indices is considered as the minimal significant change(12), then only in Patient 12 could a diagnosis be made by a significant rise in neutralizing antibody level. On the other hand, it was possible to show a rise in complement-fixing antibody in all 20 individuals. The patients listed in Table I represent only 20 of the more than 350 cases of Western equine encephalitis diagnosed in the 1952 outbreak in California(12) by means of complement fixation tests in which an egg membrane-fluid antigen was used.* In 18 of the 20 patients listed, the complement-fixing antibody titer of the acute phase specimen was less than 1:8. This was typical of the preponderance of the entire series of patients mentioned; of this group, only a very small number had antibody levels of 1:8 or greater in the acute-phase serum specimen.

The egg antigen described here is easily prepared and, equally important, preparations of good antigenic potency (one unit = 1:16 or 1:32 dilution) can be consistently prepared. In our hands, antigen prepared from infected mouse brain by Casals' modification(13) of the original freeze-thaw method of Casals and Palacios(2) while satisfactory, varies as to antigenic potency; occasional lots approaching the potency of the egg antigen are obtained, but in general the antigenic potency is lower. An occasional human serum will give some degree of nonspecific fixation with a control egg membrane antigen prepared in the same fashion as the specific antigen. In such cases, the sera are retested with mouse brain antigen prepared by the Casals method(13).

Summary. A complement-fixing antigen of the WEE virus is described. The antigen is prepared from the fluids and membranes of the infected chick embryo and has a high degree of sensitivity. Since complement-fixing antibody in human WEE virus infections is slower in appearing than the neutralizing antibody, diagnosis can usually be made by the complement fixation method whereas diagnostically significant rises in antibody frequently are not demonstrable by the neutralization test.

* The patients in this series were tested with a "4500 rpm" antigen. The "10,000 rpm" antigen subsequently adopted gives essentially similar results with respect to specific fixation, but is freer of nonspecifically reactive substances due to the higher gravitational field of force applied during centrifugation.

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