TABLE IV.
Effect of decreasing percentage of ovalbumin in 2% protein solution during sensitization of collodion particles. One-half hour exposure.

% of protein as ovalbumin	Agglutination with anti-ovalbumin serum dilutions									
	1:2	1:4	1:8	1:16	1:20	1:40	1:80	1:160	1:320	Saline
100	....	....	....	....	+	+	+	+	—	—
75	....	....	+	+	±	±	—	—	—	—
50	....	±	—	—	—	—	—	—	—	—
25	—	—	—	—	—	—	—	—	—	—

\+ complete agglutination
± partial agglutination
— no agglutination
.... not examined

Effect of the presence of other proteins in agglutination by adsorption. The effect of the large amount of non-specific tissue protein in suspensions of virus-containing tissue was approached artificially. In 2 experiments, when collodion particles were sensitized in mixtures of ovalbumin and sheep serum, the agglutination titer of an anti-ovalbumin rabbit serum decreased with the amount of ovalbumin in the protein mixture. In the first experiment the collodion particles remained in the protein solution overnight, while in the second the exposure was for half an hour. Results of these experiments are given in Tables III and IV.

There is 0.5% protein by micro-Kjeldahl analysis in the supernate from a 10% W.E.E. mouse brain suspension prepared according to Casals and Palacios[16] method for complement-fixing antigen. 0.02 ml of a 10^{-6} dilution of this suspension was infectious for young mice. Assuming 50 virus particles per mouse infectious dose—special methods are necessary to show one virus-particle infectious[19, 20]—and a mass of 3.7×10^{-15} g per particle, then each ml of suspension would contain about 10^{-6} g of virus. The virus content would then be 0.0001% and the portion of protein as virus would be 0.02%. The decrease in agglutinating titer of an antiserum with the percentage decrease of specific protein in a mixture of proteins indicates that the low percentage of virus in respect to tissue might be responsible for negative results.

Conclusions. Only a small amount of the virus of western equine encephalomyelitis is adsorbed upon collodion particles from the clear supernates of virus-containing tissue suspensions during an overnight period in the icebox. This amount is not sufficient to give a positive agglutination reaction with specific antiserum. Experiments with collodion particles sensitized with protein mixtures indicate that it will probably be necessary to concentrate and partially purify the W.E.E. virus in order to obtain collodion particles adequately sensitized for agglutination with virus antisera.

19 Parker, R. F., and Rivers, T. M., *J. Exp. Med.*, 1936, **64**, 439.

20 Parker, R. F., *J. Exp. Med.*, 1938, **67**, 725.

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Vaccination of Mice Against Typhus.

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Ever since the discovery of its susceptibility by Nicolle *et al.*[1] the guinea pig has been the animal most widely used in typhus studies. Its reaction to established murine strains,

TABLE I.
Vaccination of Mice with Epidemic Typhus Vaccine.

Exp. No.	No. of mice used	Vaccine	Vaccine dose	Yolk sac infecting dose	Strain	Route*	Results: After 24 hrs. Living	Results: After 24 hrs. Dead	Results: After 7 days Living	Results: After 7 days Dead
1	10 test	30% yolk sac	0.5 cc	1.5 MLD	Epidemic	i.v.	10	0	....	....
	10 controls		0				0	10	....	....
2	10 test	20% " "	0.5 "				10	0	....	....
	10 "	10% " "	0.5 "	<1 "	"	"	10	0	....	....
	20 controls		0				5†	15	....	....
3	8 test	10% " "	0.5 "				8	0	....	....
	8 "	10% " "	0.25 "	3 "	"	"	8	0	....	....
	16 controls		0				0	16	....	....
4	8 test	10% " "	0.1 "	3 "	"	"	7	1‡	....	....
	8 controls		0				0	8	....	....
5	16 test	10% " "	0.5 "	<1 "	Murine	i.p.	16	0	8	8
	16 controls		0				13	3	4	12

* i.v.—intravenous; i.p.—intraperitoneal.
† Control mice in this experiment slightly heavier than test mice.
‡ Mouse in poor condition at time of challenge inoculation.

when a scrotal reaction occurs regularly, is quite satisfactory. With the epidemic disease, however, where a definite pattern of febrile reaction is the only evidence to be relied on for interpretation, the guinea pig leaves much to be desired. Wohlrab[2] reported that white mice could be infected with murine typhus by various routes, though he did not obtain one hundred percent morbidity. Various workers have shown that murine typhus could be carried in passage in the mouse, but that the epidemic strain was lost after a few transfers.[3,4] By means of intraperitoneal inoculation with murine infected yolk sacs of embryonated eggs, Gildermeister & Haagen[5] were able to kill mice after 24 or 48 hours. This finding was extended to the epidemic strain by Topping and Bengtson[6] who used the intravenous route of inoculation.

In the course of studies on the development of agglutinins in various species of animals[7] it was observed that mice acquired good titers of this antibody rapidly as the result of inoculation, both with vaccine and with infectious material. It was felt, therefore, that they would be useful as test animals for typhus vaccines, the early formation of agglutinins suggesting that they could be tested for immunity much sooner than has been possible in the case of guinea pigs. This proved to be the case as will be shown below.

Materials and Methods. 30% ether-extracted formalin inactivated yolk sac vaccines were prepared from Breinl epidemic and Wilmington murine strains of typhus; 10% material was made from this. All vaccinations were performed by the intraperitoneal route, employing a single dose of vaccine. Test mice and controls (C.F.W. strain of Swiss mice from Carworth Farms) about 4 weeks of age weighing 11 to 14 g were selected at the same time. All mice were kept under identical conditions at a temperature of 68 to 70° F. The challenge dose was given 7 days after vaccination, and consisted of intravenous or intraperitoneal administration of 0.5 cc of infectious yolk sac suspension. (This material was kept frozen at —70°C in small samples after titration. The highest dilution required to kill 8 out of 8 mice was called 1 mld.) Mice receiving epidemic material were observed for 24 hours; those infected with murine typhus for one week.

1 Nicolle, C., Consiel, E., and Coner, A., *Compt. rend. Acad. d. sc.*, 1911, **152**, 1632.

2 Wohlrab, R., *Zentr. f Bakt.*, 1937, **140**, 193.

3 Laigret, J., and Jadin, J., *Arch. Inst. Past. Tunis*, 1933, **21**, 381.

4 Kligler, I., *B. J. Exp. Path.*, 1936, **17**, 53.

5 Gildermeister, E., and Haagen, E., *Deut. Med. Woch.*, 1940, **66**, 878.

6 Topping, N., Bengston, I., Henderson, R., Withheld from publication.

7 To be published.

TABLE II.
Vaccination of Mice with Murine Typhus Vaccine.

Exp. No.	No. of mice used	Vaccine	Vaccine dose	Yolk sac infecting dose	Strain	Route*	Results: After 24 hrs. Living	Results: After 24 hrs. Dead	Results: After 7 days Living	Results: After 7 days Dead
1	8 test	10% yolk sac	0.5 cc	3 MLD	Murine	i.v.	8	0	....	....
	8 controls		0				0	8	....	....
2	8 test	10% " "	0.5 "	>2 "	"	i.p.	8	0	8	0
	8 controls		0				3	5	0	8
3	8 test	10% " "	0.5 "	3 "	Epidemic	i.v.	3	5	....	....
	8 controls		0				0	8	....	....

* i.v.—intravenous; i.p.—intraperitoneal.

Results. Table I. summarizes the results obtained in mice vaccinated with epidemic vaccine, and tested with epidemic and murine typhus yolk sac suspensions.

The results obtained in the first 4 experiments show that it is possible to protect mice with as little as 0.1 cc of 10% yolk sac vaccine against a very large dose of epidemic typhus given intravenously. In order to show that the animals acquire not merely an ability to neutralize the toxic factor in the challenge material but a complete immunity to the whole antigen, further experiments with murine typhus were performed. Mice vaccinated with epidemic vaccine were challenged by intraperitoneal inoculation with infectious murine yolk sac (Table I., Experiment No. 5) and the results compared with those obtained when mice vaccinated with murine vaccine were tested with the homologous strain by the same route (Table II., Experiment No. 2). The amount of epidemic strain vaccine used was insufficient to protect all mice against death from the murine infection, but no early deaths occurred as in the controls, indicating that all the vaccinated animals were protected from the toxic factor in the inoculum and suggesting that this agent is probably identical in both types of typhus.

From Table II it may be seen that the administration of murine vaccine to mice will protect them not only against a very heavy intravenous injection with the homologous strain but against intraperitoneal inoculation as well. Cross-protection against the epidemic strain is only partial however, as shown previously in mice vaccinated with the epidemic vaccine and challenged with the murine strain. Presumably a greater quantity of vaccine in either case would afford complete cross-protection. Further experiments along these lines are in progress.

Summary. A simple and rapid method for the testing of murine and epidemic typhus vaccines in mice is described. By this means, the use of guinea pigs is eliminated and the period of testing shortened by about two weeks as compared with methods currently in use for testing typhus vaccines. The results suggest that the toxic factor in the two strains is identical.

The inoculations were performed by Miss Jane Burrows, to whom we express our thanks.