havior of the human Fraction I.

Summary. (1) The solubility of bovine fibrinogen in ethanol-water mixtures has been studied. The behavior was qualitatively similar to that of the human protein, but the influence of pH was much smaller. (2) A procedure for refractionating bovine Fraction I to give a 90% clottable product is described.

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Quantitative Studies on Prophylactic Effectiveness of Western Equine Encephalitis Antiserum in Mice.* (19172)

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The irregular, infrequent and unpredictable occurrence in the U. S. of sizeable epidemics of encephalitis due to the arthropod-borne viruses poses a difficult problem with regard to the various control measures which might be employed in the face of an epidemic. It has been demonstrated that the administration of suitable amounts of antiviral serum prior to infection, and under special conditions also at certain intervals after infection, can prevent development of the experimental disease (1-7). Previous studies were either of a qualitative nature in which large amounts of serum of unassayed potency were shown to have a protective effect, or, when sera of known potency were used in varying amounts, they were tested in animals infected by the intracerebral route. The purpose of the present communication is to present the results of certain pertinent quantitative studies which were carried out in 1942 and 1943 in relation to vaccination of human beings with western equine encephalitis chick embryo vaccine. The study was originally designed to indicate the significance of various levels of neutralizing antibody against western equine encephalitis virus as regards resistance to infection by a peripheral, extraneural route. The data, however, also throw light on the minimal amounts of passively introduced antibody which are required to protect against varying amounts of virus introduced by a peripheral, extraneural route, and thus provide a basis for estimating the amounts of antibody which may be required in humans.

Experimental procedure, methods and materials. The strain of WEE virus used was originally derived from California and since 1934 had been propagated in mice by Dr. P. K. Olitsky and his associates at the Rockefeller Institute and subsequently in this laboratory. The virus consisted of infected mouse brains, stored in dry ice as 10% centri-

TABLE I. Antibody Content of WEE Rabbit Antiserum Used in Prophylactic Tests.

Dilution of serum	Neutralization index* for .015 ml — Intra-abdominal test in 2-wk-old mice	Intracerebral test in 4-wk-old mice
Undiluted	5000000 +?	1600
1:10	2000000	200
1:100	1000000 +?	80
1:1000	6000	50
1:10000	13	6
1:100000	3	—
1:1000000	1	—

* Neutralization index = $\frac{\text{Titer of virus in mixtures with normal serum}}{\text{Titer of virus in mixtures with antiserum}}$

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TABLE II. Intracerebral Neutralization Tests on Sera of Mice Inoculated with WEE Rabbit Antiserum.

Route of inoculation	Amt of antiserum inj.	Avg wt of mice inoc., g	Time between inj. of antiserum and withdrawal of mouse serum, hr	Original dilution of mouse serum tested	Neutralization index (.015 ml in test)*
Fresh normal mouse serum control			—	U†	1
Intra-abdominal	.5 ml undilut.	18	4	U	320
			24	U	1300
			48	U	5000
	.15 ml of 1:10	11	4	U	1000
			24	U	500
			48	U	630
	.03 " " 1:10	10	4	U	500
			24	U	200
			48	U	320
Intravenous	.15 " " 1:10	7.2	4	U	500
				1:10	400
			72	U	800
	.15 " " 1:100	7.2	4	U	500
				1:10	10
	.15 " " 1:1000	7.3	4	U	63
				1:10	1
	.15 " " 1:33	19.5	4	U	160
				1:10	16

* This means that the .03 ml of serum-virus mixture inoculated contained .015 ml of serum.
† U = undiluted.

fuged suspensions in heated, undiluted rabbit serum. The intracerebral titer in mice of the freshly prepared suspension, diluted in 10% heated rabbit serum saline, was as a rule 10^{-9} per 0.03 ml. In intracerebral neutralization tests, following incubation for 2 hours at 37°C in the presence of equal parts of heated undiluted rabbit serum, the titers varied from $10^{-8.2}$ to $10^{-9.0}$. The WEE antiserum was prepared in 3 rabbits, each weighing 2.4 to 3.0 kilos, by a series of 5 inoculations of frozen or fresh, 10% mouse brain suspension according to the following schedule: 0.3 ml subcutaneously on 8/29/42; 0.3 ml intravenously on 9/8/42 and 9/10/42; 3 ml subcutaneously on 9/12/42 and 6 ml subcutaneously on 9/14/42; the rabbits were bled on 9/24/42 and the serum was pooled. Both the intracerebral and intra-abdominal neutralization tests were used to assay the potency of this antiserum by testing mixtures consisting of 10-fold dilutions of the serum and various 10-fold dilutions of the virus incubated for 2 hours at 37°C prior to inoculation (Table I). The dilutions of antiserum for these tests were made in heated undiluted rabbit serum to provide optimum protection for the diluted virus during the period of incubation at 37°C. Varying amounts of this antiserum were also injected intra-abdominally or intravenously in mice of known weight, which were subsequently bled at intervals, indicated in Table II, to determine the minimal amount of serum which had to be injected to yield an intracerebral neutralization index of approximately 50 in the undiluted mouse serum (Table II).† The final evaluation of the limits of the prophylactic effectiveness of the antiserum was carried out in various groups of approximately 2-week-old mice (weighing about 7 g) which were first inoculated intravenously with varying amounts of normal or WEE rabbit serum diluted in saline, and 4 hours later received an intra-abdominal injection of 0.3 ml of varying dilutions of WEE virus (Table III).

Results. The assay of the antiserum, shown in Table I, indicates that 0.015 ml of the

† The rabbit antiserum was itself lethal for mice within 10 to 20 minutes when given intravenously in doses of 0.15 ml of undiluted or 1:2 diluted serum, while 0.15 ml of the 1:10 or greater dilutions was harmless. By the intra-abdominal route 0.5 ml of the undiluted antiserum was harmless. The mouse-tissue antibodies contained in the antiserum may have been responsible for the toxic effect of the larger doses.

TABLE III. Limits of Prophylactic Effectiveness of WEE Rabbit Antiserum in Mice. Serum Intravenously 4 Hr Before Virus. Virus Intra-abdominally—.3 ml.

Date of test	Type of serum	Serum administered: Dilution of serum inj. in vol. of .15 ml	Serum administered: Avg wt of mice, g	Serum administered: Dose per kilo, ml	Intra-abdominal titer of virus in indicated groups of mice*	Protective index of antiserum log§
10-17-42	None—control	—	7.3	—	7.5†	—
10-23-42	Normal rabbit	1:10	7.2	2	6.4†	—
	WEE	1:10	7.2		1.4	5
1-21-43	Normal	1:100	7.2	.2	6.4‡	—
	WEE	1:100	7.2		2.2	4.2
2-8-43	Normal	1:1000	7.3	.02	5.8‡	—
	WEE	1:1000	7.3		3.2	2.6
	Normal	1:33	19.5	.23	1.4	—
	WEE	1:33	19.5		.8	.6

* Groups of 5 mice were tested at each serial 10-fold dilution of virus in the range of 10^{-1} to 10^{-9}, and the titer represents the reciprocal of the log of 50% endpoint for the .3 ml dose.

† Same lot of virus used on 10-17-42 and 10-23-42 with intracerebral titer of $10^{-9}/.03$ ml.

‡ Same lot of virus used on 1-21-43 and 2-8-43 with intracerebral titer of $10^{-8.5}/.03$ ml.

§ Protective index $= \frac{\text{titer of virus in mice inoculated with normal serum}}{\text{titer of virus in mice inoculated with antiserum}}$.

1:1,000 dilution was the limiting effective amount in both the intra-abdominal and intracerebral neutralization tests. It should be noted that this antiserum was relatively ineffective against larger amounts of virus by the intracerebral route. Thus, when the intracerebral 50% serum dilution endpoint for this antiserum was calculated from the same data by the method of Reed and Muench, it was 1:1,800 against 40 LD_{50} of virus and only 1:3 against 400 LD_{50} of virus. It is evident, however, that, when small doses of virus are used, the less cumbersome intracerebral test can be used for comparative assay of WEE antisera. While the intra-abdominal test yielded higher neutralization indexes at any given dilution of serum, it did not yield a higher serum-dilution protective endpoint.

The data presented in Table II indicate a good correlation between the neutralization index obtained in the regular intracerebral neutralization test and that obtained after the antiserum was diluted in the bodies of mice of known weight. Thus, 0.15 ml of the 1:1,000 dilution of antiserum given intravenously to mice weighing 7.3 g (0.02 ml/kg), after an interval of 4 hours, yielded a mouse serum with an intracerebral neutralization index of 63 in the undiluted state and no neutralization in the 1:10 dilution. Since 0.015 ml of the 1:1,000 dilution of the antiserum yielded a similar result in the original neutralization test (Table I), it would appear that the 0.15 ml inoculum underwent a dilution not greater than 10-fold in the bodies of 7.3 g mice. It is thus evident that the antibody neither gains nor loses any significant activity within a few hours after intravenous injection.

The data summarized in Table III show that: (a) the smallest amount of antiserum used (0.02 ml/kg) yielded a protective index of 400, *i.e.*, protected the mice against 400 times more virus than did a similar injection of normal rabbit serum; the actual amount of virus resisted by 50% of the mice was approximately 2 million (mouse) intracerebral lethal doses; (b) roughly proportionately larger amounts of antiserum were required to protect against larger amounts of virus; thus, 0.02 ml, 0.2 ml, and 2 ml of antiserum per kilo yielded protective indexes of 400, 16,000 and 100,000 respectively; (c) when older mice were used which, because of their natural maturation resistance, required very large amounts of virus for the production of encephalitis by the intra-abdominal route, much larger amounts of antiserum were needed to protect against the minimal intra-abdominal encephalitogenic dose.

Discussion. The data, here presented, emphasize some of the factors that need to

be taken into consideration in any attempt to determine a suitable dose of antibody for the protection of human beings during an epidemic of arthropod-borne virus encephalitis. Thus, the dose of virus is important, but there is, as yet, no precise information on the number of mouse intracerebral infectious doses of virus that mosquitoes transmit, nor on the number of such doses that are required to produce encephalitis in the most susceptible human beings. If the minimal human encephalitogenic dose corresponds to that obtaining for 2-week-old mice, 0.02 ml per kilo of an antibody solution, possessing the potency of the serum used in these tests, might be protective at the moment of inoculation, while 100 times as much or more may be required if the human encephalitogenic dose of virus should be much larger. The ideal dose of antiserum would, of course, be one which would supply enough antibody to provide protection for the whole period of potential exposure against the largest amounts of virus that mosquitoes might be able to transmit. The minimum dose of antiserum that might be considered for practical trial in human beings should provide enough antibody to be just detectable in the individual's undiluted serum at the end of the period of potential exposure.

The concentration of antibody achieved in the rabbit serum used in the present tests is not necessarily maximal, although Olitsky, Schlesinger and Morgan(6) have pointed out that merely increasing the number of inoculations beyond a certain minimum does not yield a WEE antiviral serum of greater potency. Nevertheless, it is possible that by the use of adjuvants and properly spaced booster inoculations in horses, cattle, pigs and sheep antiviral sera of greater potency than that used in the present tests might be prepared. Furthermore, concentration of approximately 15 to 30 times might be achieved by precipitation of the antibody with the gamma globulin fraction of these sera. Further studies are, therefore, indicated to determine whether or not antibody solutions can be prepared which are at least 100 times more potent than the serum used in the present tests. Such concentrated preparations, in doses of 0.02 to 0.1 ml per kilo, should provide sufficient antibody to warrant more detailed studies in human beings.

Summary. (1) Hyperimmune, WEE antiserum, prepared in rabbits by a series of 5 fairly closely spaced inoculations, yielded a 50% serum dilution titer of 1:1,800 against 40 LD_{50} of virus in the routine intracerebral neutralization test. When the intra-abdominal neutralization test was used, the amount of virus neutralized by various dilutions of the serum was always greater, but the limiting effective dilution of the serum was about the same as in the intracerebral assay against 40 LD_{50} of virus. (2) After intravenous injection of this antiserum in mice, the mouse serum acquired a neutralizing activity which indicated that the antibody underwent a dilution roughly in proportion to the size of the animal. (3) Prophylactic effectiveness was tested by injecting intravenously various dilutions of antiserum into 2-week-old mice, and 4 hours later inoculating these mice intra-abdominally with varying dilutions of the virus. Passive transfer of just enough antibody to impart to the undiluted serum an intracerebral neutralization index of approximately 50, protected against 400-4,000 intra-abdominal encephalitogenic doses of virus; the actual amount of virus contained in this dose was 2 million (mouse) intracerebral LD_{50}. The amount of antibody required for passive protection varied with the size of the infecting dose, so that 0.02 ml, 0.2 ml and 2 ml of the antiserum per kilo of body weight yielded protective indexes of 400, 16,000 and 100,000 respectively. The significance of these data for the problem of the prophylactic use in human beings of concentrated antisera against the arthropod-borne encephalitis viruses is discussed.

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