TABLE IV.
Influence of Age of Vaccines and Neutralization of Formalin Prior to Inoculation, on Incidence of Neutralizing Antibodies.

Group	Total No.	Positive No.	Equivocal No.	Negative No.	Positive %	Positive + equivocal %
All ages	35	15	4	16	43	54
18-35 yr	25	13	4	8	52	68
36-56 "	10	2	0	8	20	20
Formalin not neutralized	13	6	1	6	46	54
Formalin neutralized with $NaHSO_3$ prior to inoculation	22	9	3	10	41	55

inoculation, has no effect on the development of antibodies.

The results obtained in this study indicated that approximately 50% of the people may develop neutralizing antibodies following injection of a total dose of 4 cc (2 doses of 2 cc, 3 days apart) of a Japanese B encephalitis vaccine possessing a 50% immunogenic dose of about 0.005 cc as determined by mouse assay. Thus far, it has not been possible to prepare vaccines of significantly greater antigenic potency, and the administration of larger amounts of mouse brain vaccine has been regarded as undesirable. Although resistance to infection may also be present among the 50% of vaccinated people who fail to develop antibodies, just as it can be demonstrated in mice inoculated with small amounts of vaccine,[2] this dosage of vaccine was selected for human beings because it was expedient rather than optimum.

Summary. Thirty-five people, aged 18 to 56, whose prevaccination sera were without any antiviral effect on Japanese B encephalitis virus, received 2 doses, 2 cc each, 3 days apart, of a fluid, uncentrifuged, formalinized vaccine with a 50% immunogenic dose for mice of about 0.005 cc. Two weeks after the first dose of vaccine, 43% had antibodies with intracerebral neutralization indexes of 50 to 1,000, and 54% were positive if the equivocal indexes of 10 or more are included. The incidence of antibody development appeared to be higher among the younger adults (18 to 35 years) than among the older ones (36 to 56 years) but this requires confirmation. Neutralization of the formalin with $NaHSO_3$ immediately before injection of the vaccine had no effect on the development of antibodies.

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Neutralizing and Complement-Fixing Antibodies for Japanese B Encephalitis Virus in Vaccinated U. S. Personnel in Japan.

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The explosive character and unpredictability of epidemics of Japanese B encephalitis, the lack of immunity of the occupation forces

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TABLE I.
Serological Response of U. S. Adult Personnel in Japan to 3 Doses of 1 cc Each of Japanese B Encephalitis Mouse Brain Vaccine

No.	Subject and age	Date of arrival in Japan	Neutralizing antibodies: Before vaccine	Neutralizing antibodies: 10 days after 2nd dose	Neutralizing antibodies: 10 days after 3rd dose	Complement-fixing antibodies: Before vaccine	Complement-fixing antibodies: 10 days after 2nd dose	Complement-fixing antibodies: 10 days after 3rd dose
1	B. (19)	10-45	13—?; 40?*	**4000+**	**4000+**	0	32(2)	16(2)†
2	McN. (30)	10-45	20—?; 1	**320+?; 8000**	**4000+**	0	32(2)	16(2)
3	T. (31)	9-45	2	*32*	**4000+**	0	2(2)	4(2)
4	S. (33)	6-46	3	**320+**	**400**	0	0	0
5	Br., J. (20)	10-45	5—?; 1	*20; 16*	**320**	0	4(4)	4(4)
6	K. (25)	10-45	4	*32*	*40*	0	0	0
7	P. (26)	5-46	3	*20*	**50**	0	0(2)	2(2)
8	C. (23)	11-45	4	4	**200**	0	2	4(2)
9	Po. (28)	4-46	5	6	**4000+**	0	0	2(2)
10	L. (20)	9-45	25—?; 8	20—?; 8	**800**	0	0(2)	0(2)
11	H. (20)	11-45	5—?	5—?	*32*—?; *25*	0	0	4(?)
12	Br., C.	?-?	20—?; —2	4—?	5—?	0	0(4)	4(4)
13	Ho. (22)	10-45	20—?; 6	8—?	8—?	0	0	0
14	Ca. (20)	11-45	4—?	8—?	5—?	0	0	0
15	E. (20)	3-46	5—?	5—?	8—?	0	0	0
16	Hol. (19)	4-46	8—?	5—?	8—?	0	0	0
17	McG. (19)	4-46	4	4	4—?	0	0	0
18	En. (30)	9-45	13—?; 4	4	5—?	0	0	0
19	Ke. (35)	4-46	6	8	8—?	0	0	0
20	Pow. (28)	5-46	1	3	8—?	0	0	0
21	M. (40)	4-46	—3	13—?	6	0	0	0
22	G. (61)	6-46	8—?	5—?	8—?	0(2)	2(2)	0(2)
23	A. (previous vaccine)	10-45	—1	**80**	*32*	0	0	0
24	Le. (23)	5-46	**320+; 500**	**320+**	**4000+**	0	2(2)	0

The neutralization indexes in boldface are regarded as positive, and those in italic as equivocal.

* Where 2 neutralization indexes are given, the second one represents the result obtained on repetition of the test.

† 16(2) = complement fixation with the Japanese B virus antigen in an original serum dilution of 1:16 and with the mouse brain component (*i.e.*, the Western Equine or normal mouse brain antigens) in a dilution of 1:2; the reactions with antigens other than Japanese B are given in parenthesis. These titers should be multiplied by 4 to make them comparable to the final serum dilution titers reported by others.

in Japan, and the administrative difficulties experienced in vaccinating large numbers of people during the outbreak on Okinawa in 1945, were factors in the decision to vaccinate the occupation forces in advance of the season when epidemics might occur. In view of the fact that the antigenic potency of the best Japanese B encephalitis vaccines that can be made at this time is limited,[1] and that it was considered desirable to keep the amount of mouse brain to be inoculated down to a minimum, it was decided to alter the dosage, from 2 doses of 2 cc each, 3 to 5 days apart, which was selected for use during an epidemic, to 2 doses of 1 cc each 4 to 7 days apart, to be followed by a booster dose of 1 cc one month later, or prior to 15 June on Okinawa and 15 July in Japan, or earlier if an epidemic appeared. The purpose of the present study was to determine the serological pattern, both as regards neutralizing and complement-fixing antibodies, in U.S. personnel in Japan receiving, accord-

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[1] Sabin, A. B., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 127.

ing to the schedule just outlined, the commercially prepared Japanese B encephalitis mouse brain vaccines produced in the U.S.A. The results were especially needed to provide a base-line for interpreting the serologic picture that might be found in vaccinated individuals with illnesses suggesting nonbacterial infections of the nervous system. It was furthermore desirable to correlate the data to be obtained with the antigenic potency of the commercial vaccines as assayed in mice during the period of their use, and with the results of previous studies in which vaccines of known potency were used in different dosage.

The studies were carried out on 24 Americans in Tokyo, whose arrival in Japan varied from late September, 1945 to early June, 1946. All but 2 were under 40 years of age. All but 2 had not previously been in any country where Japanese B encephalitis is known to occur; the 2 possible exceptions (No. 3 and 18 in Table I) had been in the Philippines from March to September, 1945. All but one (No. 23) had not previously received Japanese B encephalitis vaccine. The first dose of vaccine (1 cc) was given between 11 and 14, June, 1946; the second dose (1 cc), 4 to 5 days later; and the third dose (1 cc), 30 to 31 days after the first. The inoculations were given at an army dispensary where many others received the same lots of vaccine. Two lots of vaccine were used, samples of which were sent, refrigerated, to the Division of Virus and Rickettsial Diseases of the Army Medical School in Washington, D.C. for assay, and we are indebted to Doctors Joel Warren and Joseph E. Smadel for the results. The lot used for the first dose was prepared 30 November, 1945, (the one-year expiration date was 11-30-46) and upon assay in mice on 30 August, 1946, it yielded a 50% immunogenic dose (ID_{50}) of 0.028 cc. Since before being released for use it must have passed the minimal potency requirement of an ID_{50} of 0.01 cc in tests by the National Institute of Health, this lot of vaccine lost at least about ⅔ of its original potency. Whether this loss is the result of improper refrigeration, too small or too large an amount of residual formalin, or of other factors is not known. The lot of vaccine used for the 2nd and 3rd doses was prepared by another commercial company on 8 August, 1945, and upon assay in mice on 4 September, 1946, it yielded an ID_{50} of 0.0094 cc, and thus still fulfilled the minimal requirements.

Blood was obtained just before vaccination, 10 days after the 2nd dose and 10 days after the 3rd dose. The sera were all stored in the frozen state in an insulated box containing solid CO_2, and the pre- and postvaccination specimens were always tested simultaneously. The intracerebral neutralization tests were carried out and the indexes calculated from the combined, control LD_{50} titer in the manner described in the preceding communication.[1] The control LD_{50} titers of different portions of the same lot of virus used in the 6 separate tests were 8.5, 7.5, 8.5, 7.8, 8.0 and 8.2 (the reciprocals of the log of the dilution) and the combined titer of 8.1 was used for calculating all the neutralization indexes shown in Table I, with the exception of those obtained on repetition when different lots of virus were used. The complement fixation tests were carried out essentially according to the method of Casals and Palacios.[2] The sera were mixed with equal parts of physiological salt solution and heated at 60°C for 20 minutes just before the test; the undiluted sera were not tested because the results obtained with them cannot be regarded as significant. The antigens were all prepared from mouse brains infected with the Japanese B, St. Louis, or Western equine encephalitis viruses, or normal mouse brains for control, without freezing and thawing, by centrifugation at 18,000 r.p.m. on the International Centrifuge angle-head attachment (refrigerated by dry ice) for 60 minutes or longer, if necessary for the removal of anticomplementary material demonstrable by incubation overnight in the refrigerator. The preparations were used undiluted and had at least 4 to 8 units of antigen in the 0.25 cc amounts used in the test. Tests in which the amount of complement used turned out

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

TABLE II.
Types of Complement-Fixing Reactions with Various Antigens Exhibited by Sera of Americans in Japan Inoculated with Japanese B Encephalitis Mouse Brain Vaccine.

		Complement-fixation in mixtures with indicated antigens					C-F titer for Jap B	C-F titer for WEE or NMB
		Japanese B Serum 1:	St. Louis Serum 1:	WEE Serum 1:	NMB Serum 1:	Saline Serum 1:		
Name	Specimen*	2 4 8 16 32 64 128	2 4 8 16	2 4 8	2 4 8	2		
McN.	I	0 0 0 0	0 0	0 0	0	0	0	0
	II	4 4 4 4 3 ±	4 ± 0 0	2 0 0	2 0 0	0	1:32	1:2
	III	4 4 4 2 0 0 0	3 ± 0 0	1 0 0	4 0 0	0	1:16	1:2
Bag.	I	0 0 0 0	0 0	0 0	0	0	0	0
	II	4 4 4 4 3 1 0	4 2 1 ±	2 0 0	1 0 0	0	1:32	1:2
	III	4 4 4 3 ± 0 0	4 2 ± 0	2 0 0	2 0 0	0	1:16	1:2
Har.	I	0 0 0 0	0 0	0 0	0	0	0	0
	II	1 0 0 0	1 0	0 0	±	0	0	0
	III	4 3 ± 0	0 0	0 0	1	0	1:4	0?
Bro., J.C.	I	0 0 0 0	0 0	0 0	0	0	0	0
	II	4 3 1 ±	4 3	4 3	4	0	1:4	1:4
	III	4 3 1 0	4 3	4 3	4	0	1:4	1:4
Bro., C.A.	I	0 0 0 0	0 0	0 0		0	0	0
	II	1 ± 0 0	3 3	2 2		0	0	1:4
	III	3 2 0 0	3 2	2 1		0	1:4	1:2
Sch.	I	0 0 0 0 0	0 0	0 0	0	0	0	0
	II	0 0 0 0 0	0 0	0 0	0	0	0	0
	III	0 0 0 0 0	0 0	0 0	0	0	0	0

* I = serum before vaccine; II = 10 days after 2nd dose; III = 10 days after 3rd dose.
C-F = complement-fixing antibody; WEE = Western Equine encephalitis virus; NMB = normal mouse brain antigen; Saline = mixture with physiological salt solution instead of antigen to check on anticomplimentary properties of the serum.
Complete fixation is recorded as 4; different degrees of partial fixation as 3, 2, or 1; questionable trace as ±; no fixation, or complete hemolysis, as 0. The original dilution of serum giving 2 plus (approximately 50%) fixation represents the titer.

to be less than 1.7 or more than 2.5 units, as determined by simultaneous titration in the presence of the antigens were regarded as unreliable, and were repeated. The various types of reaction with the different antigens, exhibited by the sera obtained after vaccination, are illustrated by the results of a few tests in Table II. The results of all the complement fixation and neutralization tests are shown in Table I.

Only one of the 24 people had neutralizing antibodies for the Japanese B encephalitis virus before vaccination. This man was born in New York City where he lived all his life with the exception of approximately one year each in Arizona, California and Kansas. Prior to coming to Tokyo in May, 1946 he spent 2 months each in Saipan and Hawaii. His serum failed to neutralize the virus of St. Louis encephalitis. The occurrence of neutralizing antibodies for the Japanese B virus in one of 30 sera received from New Haven was reported in 1938 by Japanese investigators,[3] and also in 1943 among a varying number of medical students living in Cincinnati, Ohio.[4] Since these antibodies have been found in sera which do not neutralize the St. Louis encephalitis virus, they cannot be attributed to exposure to that virus and the slight antigenic relationship between it and the Japanese B virus. It is noteworthy that the one man with neutralizing antibodies in the present group had no complement-fixing antibodies for the Japanese B virus and that none appeared after vaccination. This is in contrast to an observation made by Dr. Hammon as well as ourselves, that complement-fixing antibodies appear rapidly and regularly after vaccination of Japanese natives possessing neutralizing antibodies as a result of inapparent infection with the virus.

[3] Takaki, I., Kudo, M., Kawakita, Y., and Tanaka, J., *Tokyo Izi Sinsi*, 1938, **62**, 716. (In Japanese; reference and translation provided by Dr. Y. Kawakita).

[4] Sabin, A. B., Duffy, C. E., Warren, J., Ward, R., Peck, J. L., and Ruchman, I., *J. A. M. A.*, 1943, **122**, 477.

TABLE III.
Neutralizing Antibodies for Japanese B Encephalitis Virus in Two Groups of People of Similar Age Following Vaccination with Different Amounts and Preparations of Different Potency.

Group	ID_{50} of vaccine by mouse assay cc	No. of persons in group	Dosage cc	Incidence of neutralizing antibodies at indicated times: Time after vaccine	Positive %	Positive + equivocal %
American adults 18-35 years in U.S.A. (reported by Sabin[1])	0.0055	25	1st dose—2 2nd dose—2 3 days later	14 days after 1st dose	52	68
American adults 19-35 years in Japan	0.028 (1st dose)	20	1st dose—1 2nd dose—1 4-5 days later	14-15 days after 1st dose	15	35
Present study	0.0094 (2nd & 3rd doses)		3rd dose—1 1 month after 1st	10 days after 3rd dose	45	55

In order to permit comparison of the results obtained in the present study (Table I) with those reported in the preceding communication,[1] the analysis of the data will be limited to the first 20 men, aged 19 to 35, who had no antibody or previous history of receiving Japanese B encephalitis vaccine prior to the present series of inoculations. Ten days after the second dose of 1 cc (*i.e.* 14 to 15 days after the first), 3 of the 20 (15%) developed significant titers of neutralizing antibody (indexes of 320, 4000+, and 8000) and 4 others (20%) exhibited equivocal titers of 20 to 32. Ten days after the 3rd dose (booster of 1 cc given one month after first dose), 3 of the 4 with equivocal titers after the 2nd dose became positive with indexes of 50, 320, and 4000+, and 3 additional individuals, who were previously negative, became positive with indexes of 200, 800, and 4000+. Thus, following the 3rd dose of vaccine, 9 of the 20, or 45%, had demonstrable neutralizing antibodies of significant titer and 2 additional ones (10%) exhibited equivocal titers. When these results are compared (Table III) with those obtained in 25 adults of similar age in the U.S.A. who received only 2 doses of 2 cc of a vaccine of greater potency, as determined by mouse assay, it is evident that:

(a) The 2 doses of 2 cc of the better vaccine produced better results (52% positive) than the 2 doses of 1 cc (15% positive) of the poorer vaccines.

(b) With the poorer commercial vaccines available in the field, a total dose of 3 cc (given in 2 doses of 1 cc, 4 to 5 days apart, followed by a booster of 1 cc one month later) gave practically the same results (45% positive) as a vaccine of 2 to 5 times greater potency in a total dose of 4 cc (given in 2 doses of 2 cc, 3 days apart).

The complement-fixing antibodies which were found following vaccination were of 2 kinds: one, reacting with the various virus antigens as well as with normal mouse brain in serum dilutions of 1:2 to 1:4, appeared in 7 of the 20 men (35%) after the 2nd dose and in 9 (45%) after the 3rd dose; the other, apparently specific for the Japanese B encephalitis virus and demonstrable in serum dilutions of 1:16 to 1:32, appeared in only 2 individuals (10%), and in both instances the titer was higher after the 2nd dose (1:32) than after the 3rd (1:16). In a third man ("Har." in Table II, No. 11 in Table I), with the serum obtained after the 3rd dose of vaccine, there was definite fixation in a dilution of 1:4 only with the Japanese B antigen, and, although entirely absent with the St. Louis and Western equine antigens, there was very slight (one plus) fixation with the normal mouse brain antigen. However, since the nonspecific reaction (*i.e.* against the mouse brain component) may yield, within the range of a

4-fold dilution, different titers with the different antigens (see No. 10 and 12 in Table I and Bro., C. A. in Table II), it seemed most probable that this seemingly specific fixation with the Japanese B virus antigen was actually nonspecific. For if this were interpreted as development of specific complement-fixing antibody, one would have to conclude in another case (Bro., C. A. in Table II) that the same vaccine gave rise to complement-fixing antibodies for the Western equine and St. Louis viruses, but not for the Japanese B virus. We concluded from these data that in people who had been inoculated with mouse brain vaccine, complement-fixation titers not greater than 1:4 of original serum dilution obtained with any mouse brain antigen, could not be regarded as specific (except for the mouse brain component) even when the reaction was positive with only one of a series of several antigens.

Summary. Commercial Japanese B encephalitis mouse brain vaccine was administered to the occupation forces in Japan in 1946 by the triple-dose method, and the antibody response was studied in a group of men who received the first 2 doses of 1 cc each 4 to 5 days apart and the 3rd dose of 1 cc one month after the first dose. The vaccine used for the first dose had a 50% immunogenic dose (ID_{50}) of 0.028 cc by mouse assay, which is considerably less than the minimal required potency of 0.01 cc, and the lot used for the other 2 doses just fulfilled the minimal requirements with an ID_{50} of 0.0094 cc. The results in 20 Americans, aged 19 to 35, who were stationed in Tokyo and were without antibody or previous history of having received this vaccine, were as follows:

(a) 15% had neutralizing antibodies 10 days after the 2nd dose, and 45% 10 days after the 3rd dose.

(b) Only 2 men (10%) developed specific complement-fixing antibodies for the Japanese B virus, which appeared after the 2nd dose of vaccine.

(c) Complement-fixing antibodies for the mouse brain component of the vaccine appeared in original serum dilutions of 1:2 to 1:4 in 35% after the 2nd dose and in 45% after the 3rd dose of vaccine.

The antibody response in this group of Americans in Japan, vaccinated by the triple-dose method with commercial preparations, which either just fulfilled or were below the ID_{50} of 0.01 cc which is the minimal requirement of potency, was about the same after the 3rd dose as that previously obtained after the 2nd dose in a group of 25 Americans, of similar age in the U.S.A., who received 2 doses of 2 cc each, 3 days apart, of a freshly prepared vaccine with an ID_{50} of 0.0055 cc.

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Serological Response of Japanese Children and Old People to Japanese B Encephalitis Mouse Brain Vaccine.

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A field trial to test the value of Japanese B encephalitis vaccine for the protection of children and old people living in the endemic

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