TABLE I.
Rate of Decline of Blood Alcohol Concentration in mg per 100 cc per Hour, With and Without Convulsion.

Subj.	First hr			First 2 hr		
	Control	Shock	Change	Control	Shock	Change
Sch.	27	12	—15	23	18	—5
Rag.	12	22	+10	16	22	+6
Doa.	20	27	+ 7	21	23	+2
Mc.	15	19	+ 4	13	16	+3
Cro.	18	23	+ 5	23	24	+1
Mos.	28	22	— 6	20	18	—2
Mean change			+ 0.9			+0.9
Stand. dev.			8.8			3.6

appear in Table I. Fig. 1 is a graphic representation of the rate of fall of blood alcohol concentration in one patient with and without convulsion.

Examination of Table I shows that the average rate of decline of blood alcohol concentration was slightly greater after electric shock than when no convulsion was induced. However, it also shows that these differences are very small in comparison with the standard deviation, and thus are certainly not of statistical significance. Ziskind,[6] working with rabbits, has found a similar lack of effect of shock on rate of fall of blood alcohol concentration.

For fear that we might be missing some precipitate drop of short duration occurring immediately after the convulsion, in several of the cases a blood sample was secured 15 minutes and 30 minutes after shock. These revealed no more evidence of acceleration than did the samples taken at longer intervals.

We conclude that electrically-induced convulsions are not effective in significantly influencing the rate of alcohol metabolism in man.

[6] Ziskind, E., personal communication, 1947.

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Antibody Response of Human Beings to Centrifuged, Lyophilized Japanese B Encephalitis Vaccine.*

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Since 1945, more than 350,000 people[1] have been inoculated with Japanese B encephalitis vaccine prepared in the U.S.A.[2] Since 1942, a number of studies have been carried out on the antibody response of human beings to various dosages and preparations, in an attempt to determine the proper dose in human beings particularly in relation to the potency of the vaccine as determined by assay in mice. Studies carried out without such reference to the quantitative determination in mice of the antigenic potency of the vaccine used for the human beings provide no useful information for comparative purposes, since vaccines prepared and stored in

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[1] Sabin, A. B., *J. A. M. A.*, 1947, **133**, 281.

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

TABLE I.
Data on Japanese B Encephalitis Vaccine Used in Tests Recorded in Table II.

Preparation: 7/24/42—10% mouse brain suspension in physiological salt solution, centrifuged on Swedish angle centrifuge at about 3,000 r.p.m. for 30 minutes. Titer of virus in centrifuged material before addition of formalin to a concentration of 0.2% was $10^{-8.4}$ (per 0.03 cc).
7/26/42—Vaccine lyophilized; tests for active virus negative; preliminary assay for immunogenic potency.
7/27/42—Lyophilized vaccine stored in refrigerator at 2°-3°C.
8/25/42—Vaccine assayed in mice and human volunteers inoculated.

"Long" Assay in 3- to 4-week-old Mice—Single Dose on 8/25/42—Challenge Test, 9/1/42.

Amount of vaccine cc	0.3 cc of indicated dilution of virus injected intra-abdominally									LD_{50} titer reciprocal log of dilution	Immunity index
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}		
None	9/10*	7/10	6/10	5/10	7/10	3/10	3/10	0/10	0/10	5.6	—
0.3	4/8	0/6	0/5	0/5	1/6	0/6	0/6			2.1—?	3,200+?
0.03	2/8	0/3	2/4	1/5	1/5	0/6	0/6			2.0—?	4,000+?
0.003	5/9	2/6	2/4	1/5	2/5	2/5	1/6			3.5	125

* Numerator = No. died of encephalitis; denominator = No. inoculated.

"Short" Assay in 10-week-old Mice.

10/16/42—first dose of vaccine; 10/19/42—second dose. 10/23/42—challenge with 0.3 cc of 10^{-4} virus suspension intraäbdominally. Intracerebral titer of challenge virus = $10^{-8.6}$ per 0.03 cc.

Amt of vaccine	Result of challenge inoculation
None	8/12
(0.15 × 2) — 0.3 cc	0/12
(0.015 × 2) — 0.03 cc	3/10
(0.005 × 2) — 0.01 cc	2/10

50% immunogenic dose = <0.01 cc

different ways can vary markedly in their immunogenic properties. In mice the development of significant, although occasionally small, amounts of neutralizing antibodies, which generally follows vaccination, has been found by us invariably to be associated with demonstrable resistance to infection by the intra-abdominal route. Although vaccination may give rise to a certain amount of resistance to infection without demonstrable antibodies, it appeared desirable that the dosage of vaccine selected for human beings should be capable of producing neutralizing antibodies at least in an appreciable proportion, even if not in all, of the inoculated people.

The first series of tests carried out in June, 1942 on a group of 6 people associated with the laboratory gave confusing results, first, because each one of the sera taken before vaccination had varying, but definite, amounts of neutralizing antibody, and secondly, because the vaccine used was prepared by a method which was subsequently found to be unsatisfactory. The formalin in the centrifuged vaccine was neutralized with NH_4OH one week after preparation, and it was stored in the frozen state in an insulated box with solid CO_2 for 24 days prior to its use in human beings and rhesus monkeys. An assay carried out simultaneously in mice showed a distinct loss in the antigenic potency of the stored vaccine. The second series of tests was carried out in August, 1942, with a lyophilized vaccine[2] which was stored in an ordinary refrigerator at 2 to 3°C. The data on this vaccine are given in Table I. Although the method of assay subsequently developed by one of us (A.B.S.) as the standard for the National Institute of Health was not available in 1942, it may be seen from the data shown in Table I that the vaccine inoculated into the second series of human volunteers had a 50% immunogenic dose (ID_{50}) within the range of the 0.01 cc which was subsequently adopted as the minimal requirement for an acceptable vaccine. The 12 human volunteers for this test were selected from among a group of 29 medical students because their sera were found to contain no antibodies for the Japanese B virus. These students were bled a second time just before injection of the vaccine as an additional check on the absence of antiviral substance in their blood prior to vaccination. The schedule of injections and the dosage are indicated in Table II. Further blood specimens were obtained 1 week, 2 weeks, and 4 weeks after injection of the first dose of vaccine. The sera were tested either in the fresh state, or when that was not practical, they were stored in the frozen state in an insulated box containing solid CO_2.

The data presented in Table II indicate that the angle-centrifuged, lyophilized vaccine, in single or multiple doses of 4 to 6 cc, was capable of giving rise to antibodies for the Japanese B encephalitis virus, but not in all inoculated individuals. The small numbers of volunteers in each group do not permit any conclusions regarding the relative merits of the same amount of vaccine administered in single or multiple doses. There was considerable question as to how to interpret the neutralization indexes in the range of 16 to 40 which appeared in individuals whose sera obtained prior to vaccination had indexes not greater than 3, and the tendency was to regard them as indicative of definite, though small, amounts of antibody. Two sera, obtained 4 weeks after vaccination, from students "Prit." and "Ulev." in the 3-dose group, which on repeated tests gave these equivocal intracerebral neutralization indexes, were tested in an intra-abdominal neutralization test in 2-week-old mice with completely negative results. In work done by one of us (A.B.S.) 3 years later it was found that in sera of patients with Japanese B encephalitis, an intracerebral neutralization index of 20 to 30 may be equivalent to an intra-abdominal neutralization index of 10,000 to 500,000.[1] It is apparent, therefore, that it may be best to continue to classify intracerebral neutralization indexes over 10 and under 50 as equivocal, and to disregard them in the final evaluation of the serological response to vaccination. On the basis of this criterion, only one of the 12 volunteers developed antibodies within one week, and 4 within 2 weeks; the

TABLE II.
Neutralizing Antibodies for Japanese B Encephalitis Virus in Medical Students Inoculated with Angle Centrifuged, Lyophilized Vaccine.

		Intracerebral neutralization index				
		Before vaccine		After vaccine		
Dosage and dates	Name	Bleeding 1 8/13/42	Bleeding 2 8/25/42	1 week 9/1/42	2 weeks 9/8/42	4 weeks 9/22/42
One dose	Bail.	3	3	10	**400**	**80**
4 cc	Cowg.	—2	3	*32*	*25*	5
8/25/1942	Bast.	1	1	4	1	3
	Earl.	—2	4	5	8	4
Two doses	Scha.	—1	3	**100**	**50**	**80**
2 cc each	Gill.	—2	1	5	8	3
8/25/1942	Schi.	1	3	1	2	2
28	Frie.	—2	1	1	—2	4
Three doses	Down	2	3	8	**2,500**	**800**
2 cc each	Jaco.	4	3	3	**2,500**	**3,200**
8/25/1942	Prit.	—1	3	*16*	*25*	10; *25*
28	Ulev.	1	—2	*25*	*40*	*40*; *25*
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Aliquot portions of the same preparation of virus frozen in separate ampoules were used in all the tests. The neutralization indexes were calculated from the combined LD_{50} titer of this virus preparation in control rabbit serum mixtures of many different tests, which was 10-8.2. All the sera shown in this table were included in 5 tests in which the LD_{50} titers of the controls were 10-8.3, 10-7.8, 10-8.5, 10-8.0 and 10-8.0. The prevaccination sera of 8/25/42, and the 1-week and 2-week postvaccination sera were tested simultaneously. The neutralization indexes not preceded by a minus sign represent the ratio of the control LD_{50} titer to that of the serum; when the titer of the serum was greater than that of the control, the ratio was reversed and the result recorded as a negative quantity preceded by a minus sign. The values which are not in boldface or italics are regarded as negative because similar variations which are inherent in the test can be obtained with the same serum and virus preparation; the values in italics are regarded as equivocal, and those in boldface as positive.

4 individuals with antibodies at 2 weeks, were still positive 4 weeks after vaccination.

Summary. Neutralizing antibodies for Japanese B encephalitis virus appeared in significant titers in 4 of 12 students inoculated with 4 to 6 cc of formalin-inactivated, angle-centrifuged, lyophilized mouse brain vaccine. Equivocal titers developed after vaccination in 3 additional members of this group, but intra-abdominal neutralization tests in very young mice gave negative results with these sera. The antibodies which appeared in significant titers persisted for at least 4 weeks. These data suggested that 4 cc of a vaccine, possessing a 50% immunogenic dose in mice of about 0.01 cc, was probably in the range of minimal dosage that would be required for human beings.