

15888

Antibody Response of People of Different Ages to Two Doses of Uncentrifuged, Japanese B Encephalitis Vaccine.

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The development of neutralizing antibody in at least 4 of 12 students inoculated with 4 to 6 cc of an angle-centrifuged, lyophilized Japanese B encephalitis mouse brain vaccine was reported in the preceding communication.¹ Further experimental work on both Japanese B and St. Louis encephalitis mouse brain vaccines, indicated, however, that approximately 75% of the antigenic material may be lost by centrifugation.^{2,3} While it was possible to obtain satisfactory lyophilization of these uncentrifuged vaccines on a laboratory scale, pilot tests by the methods employed commercially in 1943 yielded lyophilized products which were largely denatured and unsatisfactory.† For these reasons it was decided in 1944, that the Japanese B encephalitis vaccine that was to be prepared on a large scale for the armed forces was to consist of 10%, uncentrifuged mouse brain suspension in physiological salt solution in which the virus is inactivated by

0.2% formalin at 2 to 5°C, the final product to be stored and shipped in the fluid state in the cold. On the basis of both experimental studies in mice² and preliminary observations on the development of neutralizing antibodies^{1,2} it was believed that for use in the face of an epidemic, the uncentrifuged, fluid vaccine should be administered in 2 doses, 2 cc each, 3 days apart. It was desirable, therefore, to know what the antibody response might be under these conditions, and the personnel of the commercial, biological houses, who were to produce this vaccine on a large scale, helped by giving their blood before and after vaccination in the manner described above.

The data on the preparation and assays of the vaccine for immunogenic potency in mice in this group of people are shown in Table I. The minimal immunogenic dose for mice determined, as indicated in Table I, was 0.0054 cc and 0.0056 cc in 2 separate tests, and almost identical values were obtained months later in several other laboratories, to which this vaccine was sent for use as a standard. Based on repeated assays on a number of vaccines prepared in this manner,³ the minimal requirement for the vaccines prepared on a large scale was set at 0.01 cc for the 50% immunogenic dose. The serum specimens available for this study were obtained and prepared through the courtesy and cooperation of Dr. Betty Lee Hampil of Sharp and Dohme, Inc. and of Dr. Clara Nigg of the E. R. Squibb Co. The vaccine was given subcutaneously in the arm, 2 cc for the first dose and the same amount 3 days later for the second dose. The group at the Squibb Laboratories received the vaccine as is, without neutralization of the formalin, which was the procedure to be followed in the field. In order to

* This work was done while the author was on active duty in the Army and on leave from the Children's Hospital Research Foundation and Department of Pediatrics, University of Cincinnati College of Medicine.

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¹ Sabin, A. B., and Duffy, C. E., *Proc. Soc. Exp. Biol. and Med.*, 1947, **65**, 123.

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

³ Sabin, A. B., unpublished tests.

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TABLE I.
Data on Japanese B Encephalitis Mouse Brain Vaccine (Lot AB) Used on Human Volunteers.

Preparation—12/22/44—10% mouse brain suspension in physiological salt solution; uncentrifuged but filtered through absorbent cotton and gauze.
 Lot A—370 cc—intracerebral titer = $10^{-9.3}$ per 0.03 cc.
 Lot B—344 cc—intracerebral titer = $10^{-8.8}$ per 0.03 cc.
 Formalin added to concentration of 0.2%.
 Stored in ordinary refrigerator at about 4°C.
 1/ 3/45—Lot A and Lot B pooled and phenylmercuric borate added to concentration of 1:50,000.
 1/ 5/45—Tests for residual, infective Japanese B virus, safety tests in mice and guinea pigs, and assay for immunogenic potency carried out.
 1/26/45 to 2/2/45—Used on human volunteers.

Assays for Immunogenic Potency in Mature Mice.

Mice— 18 to 20 g females.

Vaccination—Different dilutions of vaccine in volume of 1 cc injected intra-abdominally; 2 doses given 3 days apart.

Challenge— 1 week after first dose of vaccine; 0.3 cc of 10% virus suspension intra-abdominally.

Test No. and date	Intracerebral LD ₅₀ of virus in challenge dose given intra-abdominally	Mortality of unvaccinated controls	Mortality of mice vaccinated with indicated total dose					50% immunogenic dose ID ₅₀ cc
			0.1	0.03	0.01	0.003	0.001	
1. 1-12-45	108.8	20/20	1/20	1/10	3/9	5/10	10/10	.0054
2. 1-23-45	108.6	21/21	0/10	1/10	1/9	8/10	10/10	.0056

TABLE II.
Japanese B Encephalitis Virus Intracerebral Neutralization Test on Human Sera Before and Two Weeks After Vaccination with Lot AB.

Summary of test done on 27 February 1945.

Name and age	Serum specimen	Mortality at indicated final dilutions of virus					LD ₅₀ titer	Neutral- ization index
		10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸	10 ⁻⁹		
Normal rabbit serum heated at 56°C for 30 min	Control	—	5/5	5/5	5/5	0/5	8.5	—
Sus.—35	Prevaccination	5/5	5/5	5/5	5/5	—	8.5	1
	Postvaccination	5/5	4/5	2/5	0/5	—	6.7	63
Pan.—27	Prevac.	5/5	5/5	5/5	5/5	—	8.5	1
	Postvac.	4/5	1/5	0/5	0/5	—	5.5	1000
Fla.—20	Prevac.	5/5	5/5	5/5	5/5	—	8.5	1
	Postvac.	5/5	2/5	1/5	0/5	—	6.0	320
Dea.—18	Prevac.	5/5	5/5	5/5	4/5	—	8.4+?	1
	Postvac.	5/5	4/5	1/5	0/5	—	6.5	100
Hum.—19	Prevac.	5/5	5/5	5/5	3/5	—	8.2+?	2—?
	Postvac.	5/5	3/5	0/5	0/5	—	6.2	200

eliminate the pain resulting from the formalin in the vaccine the people at the Sharp and Dohme laboratories neutralized the formalin by the addition of NaHSO₃ just before inoculation. The sera, obtained before inoculation and 2 weeks after the first dose, were stored in the frozen state in an insulated

box containing solid CO₂. The prevaccination and postvaccination specimens were always included in the same test, a typical example of which is shown in Table II. Frozen portions of the same lot of virus, and the same lot of heated, undiluted rabbit serum for the control mixtures, were used in

TABLE III.
Appearance of Neutralizing Antibodies for Japanese B Encephalitis Virus in Human Volunteers of Different Ages. Inoculated with Uncentrifuged, Fluid Mouse Brain Vaccine.

Two doses—2 cc each given 3 days apart				
Intracerebral neutralization index*				
No.†	Name	Age yr	Before vaccine	2 wk after 1st dose of vaccine
1	Dea.	18	1	80
2	Hum.	19	2—?	160
3	Mas.	19	1	1000
4	Wil.	19	—1	100
5	Wal.	19	—1	1
6	Fla.	20	—1	320
7—	Har.	21	2—?	250
8	Bur.	22	—1	1—?
9—	Lon.	22	2—?	50
10—	Hil.	26	2—?	400
11	Dev.	26	—1	10
12—	New.	26	1	2—?
13	Pan.	27	—1	800
14	Mat.	27	1	250
15	McCon.	27	—1	8
16	Cra.	27	—1	1
17—	Ree.	28	—1	10
18	Pie.	30	2—?	13
19	Cla.	30	1	13
20—	Haw.	30	2—?	2—?
21—	Cat.	31	2—?	63
22—	Bis.	31	—1	6
23—	Sou.	31	1	1
24—	Hei.	34	—1	800
25	Sus.	35	—1	50
26	Fel.	36	—1	—1
27	Sho.	36	—1	1—?
28	Str.	39	—1	4—?
29	Glo.	40	4—?	800
30	Woo.	41	—1	1
31	Fit.	42	—1	2—?
32—	Lip.	47	—1	2—?
33—	Nig.	47	4—?	630
34	Ham.	48	1	2—?
35—	Jac.	56	1	1

* These neutralization indexes were calculated from the combined control LD₅₀ titer of 8.4, determined from the results obtained with the control mixtures in all the tests.

The distinctly positive indexes are in boldface.

The equivocal indexes are in italic.

† The numbers followed by a dash indicate the people who received the vaccination without neutralization of the formalin; all the others received vaccine to which NaHSO₃ was added prior to inoculation.

all the tests. The serum-virus mixtures were incubated in a water bath at 37°C for 2 hours prior to intracerebral inoculation in the mice. The LD₅₀ titers (the reciprocals of the log of the dilution) of the rabbit serum, control mixtures in the different tests were

8.8, 8.3, 8.0, 8.5, 7.7, and 8.7; that these variations are inherent in the test is evident from the fact that in a test in which the control mixture yields a titer of 7.7, the titer of the virus in a mixture with prevaccination serum may be 8.5, and that material from the same ampule of virus mixed with the same lot of rabbit serum which yielded the titer of 7.7 gave a titer of 8.7 in a test carried out 2 weeks later. For these reasons, the neutralization indexes shown in Table III were all calculated from a combined, control LD₅₀ titer (8.4) which was determined by the method of Reed and Muench from the sum of the results obtained with the control mixtures in the 6 separate tests.

The results of all the tests are shown in Table III, arranged according to the age of the vaccinated people. The complete absence of the slightest antiviral effect in any of the freshly frozen sera obtained before vaccination from this group of 35 people residing in the northeastern part of the U.S.A. is especially noteworthy, in view of the very high incidence of antibodies encountered among adults where Japanese B encephalitis is endemic⁴ as well as occasionally among residents in the U.S.A.² Those who developed antibodies following vaccination exhibited neutralization indexes of 50 to 1000, and only 4 had indexes of 10 to 13, the equivocal nature of which was discussed in the preceding communication.¹ Inspection of the results in Table III suggested the possibility that the younger adults may perhaps develop antibody more regularly than the older people. From the summary presented in Table IV, it appears that 52% of those 18 to 35 years of age developed antibodies (68%, if the equivocal indexes are included) as compared with 20% of those who were 36 to 56 years old. Although this data is not conclusive, it warrants further observations on the influence of the age of human beings on the development of antibodies following vaccination. The results summarized in Table IV also indicate that neutralization of the formalin with NaHSO₃, immediately before

⁴ Sabin, A. B., *J. A. M. A.*, 1947, **138**, 281.

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 NaHSO₃ immediately before injection of the vaccine had no effect on the development of antibodies.

15889

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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