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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TABLE I.
Serologic Response of Japanese Natives in Japan to Japanese B Encephalitis Mouse Brain Vaccine.

A. Influence of Potency of Vaccine as Determined by Mouse Assay.
B. Influence of Age of Vaccinated People.
C. Influence of Previous Inapparent Infection on Appearance of Complement-Fixing Antibodies.

| Lot of vaccine and potency $ID_{50}$ | Individuals vaccinated | | | Neutralization index* | | | Complement-fixing titer† | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. | Name | Age yr | Before vaccine | 10 days after 2nd dose | 10 days after 3rd dose | Before vaccine | 10 days after 2nd dose | 10 days after 3rd dose |
| A | 1 | Sak. | 5 | 3 | 320 | 3200+ | 0; 0 | 4(2) | 4(?) |
| 0.013 cc | 2 | Shi. | 3 | 3 | 160 | 3200+ | 0 | 0; 2(2) | 8(4); 16(4) |
| | 3 | Yam. | 5 | 4 | 63 | 3200+ | 0 | 0 | 2(2) |
| | 4 | Yag. | 4 | 6 | 25 | 500 | 0(2) | 2(2) | 4(2) |
| | 5 | Mor. | 3 | 3 | 3 | 63 | 0 | 0 | 8(4) |
| | 6 | Han. | 5 | —2 | 2 | 160 | 0 | 2(?) | 4(4) |
| | 7 | Kuy. | 4 | 3 | 2 | 400 | 0 | 0 | 4(?) |
| | 8 | Nis. | 4 | 2 | 2 | 2500 | 0 | 0 | 8(2) |
| | 9 | Hir. | 3 | 4 | 2 | 2000 | 0; 0 | 0 | 8(4) |
| | 10 | Has. | 5 | — | —2 | 3200+ | — | 0 | 8(2); 8(0) |
| B | 1 | Ino. | 5 | 1 | 3 | 500 | 0 | 0 | 0 |
| 0.077 " | 2 | Kaw., M. | 5 | 3 | 6 | 20—? | 0 | 0 | AC, <4(4) |
| | 3 | Nak., T. | 5 | 3 | 6 | 16—? | 0 | 0 | 2(?) |
| | 4 | Kaw., T. | 5 | 2 | —2 | 16—? | 0 | 0 | 0 |
| | 5 | Kat. | 5 | 2 | 3 | 5 | 0 | 0 | 0 |
| | 6 | Ish. | 5 | 1 | 2 | 6 | 0 | 2(2) | 4(2) |
| | 7 | Kai. | 4 | —2 | —2 | 6 | 0 | 0 | 8(4); 2(2) |
| | 8 | Kis. | 4 | 2 | 3 | 4 | 0 | 0 | 0 |
| | 9 | Nak., R. | 4 | 3 | —3 | 3 | 0 | 0; 2(2) | 0; 2(2) |
| | 10 | Yok. | 3 | 3 | —2 | 3 | 0; 0 | 0; 0(2) | 0 |
| | 1 | Kaw., Ts. | 65 | 2 | 2 | 4 | 0 | 0 | 0 |
| | 2 | ‡Aos., C. | 70 | 1 | 6 | 4 | 0 | 0 | 0 |
| A | 3 | Ish., J. | 70 | 3 | 3 | 25; 5 | 0 | 0 | 0 |
| | 4 | Sug. | 61 | 4 | 1 | 10; —2 | 0 | 0 | 0 |
| | 5 | Kob. | 76 | 1 | —3 | 3 | 0 | 0 | 0 |
| | 6 | Yas. | 71 | —2 | —2 | 3 | — | 0 | 0 |
| | 7 | Iba. | 63 | —2 | 2 | 4 | 0 | 0 | 0 |
| | 8 | Non. | 63 | —3 | 4 | 3 | 0 | 0(2) | 2(2) |
| | 9 | Tsu. | 63 | 3 | 2 | 4 | 0 | 0 | 0(2) |
| | 1 | Yok. | 73 | 320+ | — | — | 2(2) | 16(2) | 16(2) |
| | 2 | Sui. | 62 | 2500 | — | — | 2(4); 2(2) | 8(4); 8(2) | 8(2) |
| | 3 | Kan. | 80 | 3200+ | — | — | 0 | 16 | — |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| B | 4 | Tam. | 68 | 250 | — | — | 0 | 8 | 4 |
| | 5 | Kaw. | 78 | 320+ | — | — | 0 | 8 | 8 |
| | 6 | Kur. | 67 | 320++ | — | — | 0 | 16 | 4 |
| | 7 | Kaw., J. | 64 | 320+ | 320+ | 2500 | 0 | 32 | 8 |
| | 8 | Kom. | 73 | 250 | — | — | 2 | 32 | 8 |
| | 9 | Kaw., S. | 68 | 320++ | — | — | 2 | 64 | 32 |
| | 10 | ‡Aos., K. | 64 | 320+ | — | — | 4 | 16 | 8 |

* *The neutralization indexes not preceded by a minus sign represent the ratio of the combined, 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.*

† The complement-fixing titer represents the original dilution of serum yielding 2 plus (approximately 50%) fixation; the values given in parentheses represent the titers with normal mouse brain or Western Equine encephalitis antigen. When a titer is not followed by another in parentheses, it means that complement-fixation occurred only with the Japanese B encephalitis antigen. 0 = no fixation in the lowest dilution which was 1:2. Where 2 sets of values are given separated by a semicolon, they represent the results obtained on repetition of the test.

‡ Missed 2nd dose of vaccine; booster dose given 1 month after 1st.

region of Okayama, Japan, was carried out during the summer of 1946 in association with Professors K. Kitayama and E. Hamamoto, Doctors K. Hiraki and S. Shimomura and their associates on the medical faculty of the University of Okayama, and Professor M. Kitaoka of the Government Institute for Infectious Diseases, Tokyo Imperial University. In view of the limited amount of vaccine which was available for this investigation, our Japanese associates believed that the study should be limited to children 3 to 5 years of age and to old people over 60, because the case fatality rate among the latter can be 70 to 90%. Accordingly, approximately 19,000 children and 2,000 old people were inoculated with the same commercial vaccine, which was prepared in the U.S.A. and used for all the occupation forces, and by the same triple-dose method in advance of the season when epidemics of encephalitis usually appear. The children and the old people received the same amounts of vaccine, the first 2 doses of 1 cc each being given 6 days apart and the 3rd dose of 1 cc, one month after the first dose. The present study was undertaken not only to determine the antibody response of a small group of these people to vaccination but also to provide a base-line for interpreting the serologic pattern which might be encountered subsequently should any of these vaccinated people develop illnesses requiring laboratory investigation of the etiological relationship of the virus of Japanese B encephalitis.

Ten children and 10 old people living in Tsuyama City and a similar number living in a rural area (Kume-son and Miho-cho) were selected for these studies. Blood was obtained immediately before the first dose of

‖ We wish to express our indebtedness to Brig. Gen. S. Bayne-Jones, at the time deputy chief of the Preventive Medicine Service, Office of the Surgeon General, U. S. Army, Brig. Gen. J. I. Martin, U. S. A., Chief Surgeon of the U. S. Army Forces in the Pacific, Col. Crawford F. Sams, M.C., chief of the Public Health and Welfare Section of the Supreme Command for the Allied Powers, and Lt. Col. W. D. Tigertt, M.C., commanding officer of the 406th Medical General Laboratory, for making possible the work of this commission.

vaccine, 10 days after the 2nd dose, and 10 days after the 3rd dose. We are indebted to Capt. J. S. McKinney, M.C. and Lt. W. A. Scantland, M.C., who along with other medical officers worked with the Commission on this project, for obtaining the blood specimens on these people and shipping them in refrigerated containers to the Tokyo laboratory. The sera were then stored in the frozen state in an insulated box containing solid $CO_2$. First, the prevaccination specimens were all tested for neutralizing antibodies. All of the children and 9 of the 20 old people had no demonstrable antibodies. The 2 postvaccination specimens on each individual who had no antibodies were then tested together. The prevaccination and postvaccination specimens were tested simultaneously for complement-fixing antibodies. The neutralization tests were carried out as before with the undiluted, unheated sera against at least 3 or 4 dilutions of the Nakayama strain of Japanese B encephalitis virus; frozen portions of the same lot of virus were used in all the tests and the neutralization indexes, reported in Table I, were calculated from the combined, control $LD_{50}$ titer of $10^{-8.0}$, the individual, control, intracerebral titers having varied from $10^{-7.4}$ to $10^{-8.6}$. The complement-fixation tests were carried out as described before[1] with undiluted antigens and various dilutions (1:2 or higher) of the sera which were routinely heated at 60°C for 20 minutes; a few of the sera, treated in this manner, remained anticomplementary and had to be heated at 65°C for 20 minutes. Four mouse brain antigens were used, prepared from brains infected with the Japanese B, St. Louis or Western equine encephalitis viruses, and from normal mouse brain. One lot of vaccine (A) was used for all 3 doses in the urban group of this series, and another lot (B), for all 3 doses in the rural group. Aliquot portions of both lots were sent refrigerated to the Division of Virus and Rickettsial Diseases of the Army Medical School at Washington, D.C., and we are indebted to Doctors Joel Warren and Joseph E. Smadel for the results of the mouse assays. Lot A had a 50% immunogenic dose ($ID_{50}$) of 0.013 cc which is close to the minimal requirement of 0.01 cc. while Lot B (prepared at about the same time by another commercial company) had an $ID_{50}$ of 0.077 cc or 1/6 of the potency of Lot A.

The results, which are all summarized in Table I, clearly show the influence of the potency of the vaccine, as determined by mouse assay, the age of the vaccinated people, and of previous inapparent infection on the antibody response. In comparing the 2 groups of children, one finds that all 10 of those receiving the better vaccine had developed good titers of neutralizing antibodies, while only 1 of 10 receiving the poorer vaccine, with 1/6 the antigenic potency, developed a distinctly positive neutralization index; furthermore, after the 2nd dose none of the children inoculated with vaccine B had any antibodies while 3 of the 10 receiving vaccine A had distinctly positive indexes. The influence of age is clearly evident when one compares the 10 children and 7 old people, without antibodies prior to inoculation, from the same community who received the same vaccine A at the same time; not one of the old people developed antibodies while all the children did. It was pointed out in the preceding communication[1] that complement-fixing antibodies for the mouse brain component of the vaccine (as is evident from the reactions with the normal mouse brain and Western equine encephalitis virus antigens) as well as for the Japanese B encephalitis virus developed following inoculation. It is noteworthy, however, that all 10 children, inoculated with vaccine A had complement-fixing antibodies, and that the titers were higher with the Japanese B virus antigen in 8 of them with a 4-fold difference in titer in 5. This is to be contrasted with the absence of any evidence of specific response among the children inoculated with vaccine B, and of any significant response of any kind in the old people who were without neutralizing antibodies prior to vaccination. Quite a different response was found in the old people

1 Ginder, D. R., Matumoto, M., Schlesinger, R. W., and Sabin, A. B., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 130.

who had neutralizing antibodies prior to vaccination apparently due to previous inapparent infection with the virus of Japanese B encephalitis. All of these people showed a prompt appearance or very significant rise in complement-fixing antibodies only for the Japanese B virus antigen; this was most marked after the 2nd dose of vaccine and although it was still present about a month later (10 days after the 3rd dose) the titers were 2- to 4-fold less in 6 of the individuals. That this booster effect probably may result from a single dose of vaccine is suggested by the data on the last individual listed in Table I who did not receive the 2nd dose of vaccine. It is, furthermore, apparent that this booster effect may be produced by a vaccine of very poor antigenic potency, since 7 of the 10 old people exhibiting this effect were inoculated with vaccine B. It is also noteworthy, that none of these sera exhibited any specific group reaction with the St. Louis virus, even when the titers with the Japanese B virus were as high as 1:32 to 1:64.

*Discussion.* In view of the fact that this study was carried out in a region of Japan in which Japanese B encephalitis is known to be endemic, the question naturally arises to what extent the antibody response which was observed might have been the result of spontaneous infection during the period of investigation (14 June to 24 July) rather than due to vaccination. The following facts are against the possibility of spontaneous infection:

(a) Careful field, clinical, and laboratory investigations carried out as part of the vaccination program revealed no cases of Japanese B encephalitis in these areas during the summer and autumn of 1946.

(b) Concurrent studies revealed that during nonepidemic years, while the virus continues to be disseminated among certain domestic animals, the great majority, if not all, of the children born after the last epidemic are without antibodies.[2]

(c) Following inoculation with the vaccine of adequate potency, the neutralizing antibodies appeared in all the children but in none of the old people living in the same community.

(d) The final serum specimens for this study were obtained several weeks before the date when outbreaks usually reach their peak in this part of Japan.

The influence of the potency of the vaccine, as determined by mouse assay, on the antibody response in human beings, which was only suggested in the previous studies appears to be established by the results of the present investigation. That older people may not respond as well to the same dosage of vaccine was first suspected in a study carried out in the U.S.A.,[3] but the results of the present investigation, which are more striking because of the extremes of age and the seemingly all or none character of the response, add greater weight to this observation. The extent to which the larger amount of vaccine per unit of body weight might have been responsible for the better response of the children is not clear. It may be worth noting, however, that in the concurrent study on the response of U.S. personnel, aged 19 to 35 years, vaccinated by the same triple-dose method with preparations of equivalent or poorer potency, 45% had antibodies after the 3rd dose, in contrast to the completely negative results in the small group of old people; here the difference in the amount of vaccine per unit of body weight, if anything, favored the Japanese old people, who on the average probably weighed only about half as much as the group of American adults. It is interesting to speculate whether the poorer immunogenic response of the old people may be related to the high case fatality rate of the disease among them. The rapid and regular appearance of complement-fixing antibodies for the Japanese B virus following vaccination of the old people who exhibited evidence of previous inapparent infection, is in agreement with an observation made by Hammon[4] earlier in 1946 on a small group of Japanese adults. Further investigations are

[2] Sabin, A. B., Ginder, D. R., and Matumoto, M., in press.

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

[4] Hammon, W. McD., personal communication.

indicated to determine whether this may provide a method for distinguishing between neutralizing antibodies in people and animals which are the result of exposure to the virus, and "antibodies" which are occasionally found in animals and human beings under conditions where exposure to the specific virus is most unlikely. As an example, may be cited the American, reported in the preceding communication,[1] whose serum, prior to vaccination, neutralized the Japanese B encephalitis virus, but who did not develop complement-fixing antibodies following vaccination.

The results of the present investigation emphasize the importance of controlling the antigenic potency of Japanese B encephalitis vaccine by proper assay in mice, and indicate that the minimal requirement of 0.01 cc for the $ID_{50}$ should be retained until vaccines of greater potency can be prepared. It would also appear that some vaccines lose their potency more quickly than others (this seemed to be especially true for the vaccines prepared by one commercial company), and checks on the residual amounts of formalin and study of certain other factors which may be involved are indicated to insure minimal acceptable potency for the preparations finally used in the field.

*Summary.* Japanese children, 3 to 5 years old, and people, over 60 years of age, living in the endemic region of Okayama, Japan, were given commercial Japanese B encephalitis mouse brain vaccine—2 doses of 1 cc each, 6 days apart, and a 3rd dose of 1 cc one month after the first dose in advance of the season when outbreaks of the disease may be expected. None of the children had antibodies before vaccination, and of the 10, who were inoculated with a vaccine having a 50% immunogenic dose ($ID_{50}$) of 0.013 cc by mouse assay, all had neutralizing antibodies 10 days after the 3rd dose, while only 1 of the 10, who received a vaccine with an $ID_{50}$ of 0.077 cc, developed a significant neutralization index. However, in a group of 7 old people, without antibodies before inoculation, who received the same dosage of the better vaccine ($ID_{50}$ of 0.013 cc) none developed neutralizing or complement-fixing antibodies. Complement-fixing antibodies for the mouse brain component appeared as well as for the Japanese B virus contained in the vaccine, but in 5 of the 10 children, inoculated with the better vaccine, the titers were significantly higher with the Japanese B antigen. Complement-fixing antibodies, specific for the Japanese B virus, appeared rapidly in all of the 10 old people who had neutralizing antibodies (evidence of previous inapparent infection) prior to inoculation, but in none of the 9 old people who were without such antibodies.

## 15891 P

## Ragweed Reagins in Nasal Secretion.*

MAX SAMTER AND ELMER L. BECKER. (Introduced by W. H. Welker.)

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Although nasal secretion has been suspected by numerous observers to be an important factor in the mechanism of respiratory allergies, its mode of action is as yet unknown. The present study attempts to determine whether nasal secretion contains reagins in ragweed sensitive patients.

*Experimental Procedure.* Four normal subjects and 10 ragweed sensitive patients with marked skin reactivity and demonstrable circulating reagins were selected for the experi-

* This study was supported by a grant from the Asthmatic Children's Aid Society, Chicago, Ill.