Susceptibility to influenza is greatest in childhood, and tends to decline progressively with age (1-3). Recent studies have shown a striking correlation between an increase in resistance to influenza and the acquisition with age of broadly reacting antibody due to repeated exposures to many antigenic variants of influenza virus (4-7). Viruses of influenza A may be divided serologically into three antigenic families: swine, A, and A-prime. In each family a characteristic set of components is dominant. Minor antigenic components are widely shared between them (8). Viruses of each family have been prevalent during successive periods of time, and as a result, different age segments of the population have had different amounts of experience with each family of viruses; they have different antibody patterns, and different states of immunity (5, 6, 8). For example, the antibody pattern of children consists principally of A-prime antibodies. The attack rate is at all times highest during this period of life. From age 12 to 29, the antibody pattern is broader, comprising antibodies to A as well as the A-prime strains. The incidence of influenza is considerably lower at these ages. The antibody pattern of persons over 30 is the broadest, and is composed of swine, A, and A-prime antibodies. The incidence of influenza is lowest in this age category.

To induce at all ages a broad composite of antibody which resembles that of the older segments of the population, whose antigenic experience is greatest and whose susceptibility is least, has become a goal for artificial immunization. Previous studies with monovalent aqueous influenza virus vaccines have emphasized the dominant effects of the major antigens of strains of past infection upon the range of antibody response to vaccination (9). Thus, while antibody to strains previously encountered was reinforced in all age groups regardless of the antigenic composition of the vaccine administered, children generally did not develop antibodies to swine or A strains unless given virus of that character. Persons of military recruit age generally did not develop
swine antibodies unless given swine vaccine. Persons over 30 developed only very low levels of A-prime antibodies unless A-prime vaccines were used. Consequently, composite or broadly reacting antibody could not be induced at all ages by any of the monovalent aqueous vaccines tested.

The purpose of the present study was to determine how to induce by vaccination high levels of composite antibody in persons of all ages, despite differences in their prior antigenic experiences. The range of antibody response in children, recruits, and persons over 30, following a single inoculation of monovalent adjuvant, polyvalent adjuvant, or polyvalent aqueous influenza virus was studied. These results prompted investigations carried out in children and in persons over 30 using multiple doses of vaccine. It was demonstrated that vaccination, like infection, can predetermine the range of antibody response to subsequent antigenic stimulation. In consequence, it becomes feasible to obtain high levels of composite antibody, despite differences in age and past experiences, if suitable preparations and schedules are employed.

Materials and Methods

Vaccines.—All vaccines used were prepared on request by commercial pharmaceutical firms. Centrifugation was used for concentration and purification of virus intended for adjuvant vaccines, adsorption and elution from chicken red blood cells for virus intended for aqueous vaccines. In either case, virus suspensions were then inactivated with formalin (1:4,000), and merthiolate (1:10,000) was added as a preservative. Adjuvant vaccines were made, by emulsifying a volume of virus concentrate containing 4 mg per ml of aluminum phosphate with an equal volume of a mixture containing 9 parts of light medicinal mineral oil (drakol 6-VR) and one part emulsifier (purified arlacel A). A volume of 0.25 ml of adjuvant vaccine was given intramuscularly in the posterior belly of the triceps muscle. Aqueous vaccines were administered subcutaneously in 1.0 ml doses.

Subjects.—Antibody response to these experimental vaccines was studied in children aged 4 to 10 (median = 7), military recruits aged 17 to 28 (median = 18), and in adults 30 or more years of age (median = 47). The children and persons over 30 years of age were inmates of state mental institutions. Military recruits were airmen stationed at Sampson Air Force Base, Geneva, New York, for basic training.

Immunization and Bleeding Schedules.—In groups of 25, children, recruits, and persons over 30 were bled and vaccinated. A second bleeding was obtained 2 weeks after aqueous vaccine and 6 weeks after adjuvant vaccine.

Treatment of Sera.—Serum was promptly separated from each blood sample, and merthiolate (1:1,000) was added to yield a final concentration of 1:10,000. For certain experiments, pools of sera derived from samples obtained before and after each vaccination were made by combining appropriate aliquots. Sera were stored at 4°C, and heated at 56°C. for 30 minutes prior to use.

Hemagglutination-Inhibition Titration.—The hemagglutination-inhibition titers of sera were determined by a pattern method with 4 units of virus and 0.5 per cent chicken erythrocytes suspended in saline (10). Antibody titers are expressed as the reciprocal of final dilutions of sera.

It is a pleasure to acknowledge the assistance of Dr. Harold B. Houser, Director, and of the officers and enlisted men of the Laboratory on Housing and Illness, Sampson Air Force Base.
Virus.—Strains of influenza virus used for vaccination and for antibody determinations were chosen from the collection of the Strain Study Center, Commission on Influenza, School of Public Health, Ann Arbor. They were: swine No. 1976 (1931), PR8 (A-1934), FM1 (A'-1947), Cuppett (A'-1950), PR301 (A'-1954), and Malaya 302 (A'-1954).

Solutions.—Saline refers to 0.15 M NaCl buffered at pH 7.2 with 0.01 M phosphate.

EXPERIMENTAL

Antibody Response in Three Age Groups to Monovalent Adjuvant Influenza Virus Vaccines.—It has been demonstrated that antibody response to adjuvant influenza virus vaccines tends to be broader as well as higher than to aqueous vaccines (11). However, the influence of differences in the prior antigenic experiences of persons of different ages upon antibody response to adjuvant vaccines has not been systematically investigated. It seemed important, therefore, to determine whether the superior immunizing potency of adjuvant vaccines would invoke a composite of antibodies at all ages, even though a single strain was used in the vaccine. For this purpose, groups of 25 children, military recruits, or persons over 30 years of age were given monovalent adjuvant vaccine containing 250 CCA (chicken cell agglutinating) units of either swine, PR8, FM1, or Malaya virus. The results of antibody determinations measured by hemagglutination inhibition in pools of sera obtained from each age group before and after vaccination are reproduced in Fig. 1 as paired bar graphs. The first member of each pair represents pre-vaccination, and the second, post-vaccination levels of antibody. Antibody levels measured with the strain used for vaccination (homologous) are shown as open bars, while hatched bars represent amounts of antibody measured with heterologous viruses.
Swine Vaccine.—Swine influenza virus vaccine induced high post-vaccination antibody levels to swine virus in each of the three age groups. The highest was found in persons over 30 who alone showed swine antibody before vaccination, and who are believed to have been exposed to swine-like strains during their childhood (4, 5).

Swine virus vaccine caused a remarkable increase in antibody to PR8 in military recruits. The same vaccine induced little antibody to PR8 in children, and only a moderate increase in persons over 30. When antibody determinations were carried out with the pairs of sera that had been pooled from each of the three age groups, it was found that only 16 per cent of the children showed an antibody increase to PR8 after vaccination with adjuvant swine vaccine. In contrast, all the recruits and 80 per cent of the persons over 30 showed a rise in PR8 antibodies after adjuvant swine vaccine. These serologic findings correlate nicely with what is known of the period of prevalence of influenza A and of the age-specific attack rate of that illness. Thus, most children aged 4 to 10 who were born after the last wide-spread prevalence of influenza A in 1943, show no antibody increase to PR8 after vaccination with swine virus. The few children in whom antibody increase was observed are believed to have been infected previously with A-like strains. A limited distribution since 1943 of the major antigens of the viruses of influenza A has recently been postulated and discussed (7). Persons now of military recruit age were children during the period of prevalence of influenza A, and the attack rate of influenza A has been shown to be highest in the first decade. Persons now over 30 were children during the period of prevalence of influenza caused presumably by swine-like strains, and these persons were exposed secondarily to influenza A probably while in their “teens” or “twenties.” At these periods of life, the age-specific attack rate of influenza A ordinarily has declined from its maximum. Hence, the results obtained indicate that swine virus adjuvant vaccine does not induce antibody to PR8 unless previous infection with influenza A has oriented antibody-forming mechanisms to respond in that manner.

Adjuvant swine vaccine reinforced antibody to FM1 in children and in recruits to approximately the same extent. The post-vaccination titer to FM1 in persons over 30 was lower, even though the pre-vaccination level approximated that of the younger age groups. Antibody to Malaya strain after adjuvant swine virus vaccine decreased progressively with the age of the group vaccinated.

These serologic findings can be explained by correlating information on the time of appearance of influenza A-prime, the age-specific attack rate of that illness, and the occurrence of antigenic variation among strains of the A-prime family of influenza virus. For example, the data available indicate that the attack rate of influenza A-prime is highest in childhood and declines sharply thereafter (3, 7). The children studied in this report were all born during the
period of prevalence of influenza A-prime, and many of the recruits (medium age: 18) were in the first decade of life when influenza A-prime was first encountered in 1947. On the other hand, persons now aged 30 or more were at least 20 when influenza A-prime appeared. It seems likely, therefore, that many of the older children and the younger recruits had comparable exposures with the early strains of influenza A-prime, of which FM1 is a prototype, while persons over 30 were less intensely affected by virus of that antigenic character. Such inferences are in accord with the observation that antibody response to FM1 was the same in children and recruits but less in persons over 30. Since the appearance of influenza A-prime, persons in each of the groups studied have become older, and concurrently, shifting in antigenic composition of A-prime strains of influenza virus has occurred. Therefore, because the attack rate of influenza A-prime declines after childhood, it follows that children have had the greatest experience with recent strains of influenza A-prime, recruits less, and persons over 30 least. The post-vaccination levels of antibody measured in each age group with the Malaya strain of 1954 are in agreement with these considerations. It seems quite clear, especially in view of the consistently low levels of post-vaccination A-prime antibody found in persons over 30 that adjuvant swine virus vaccine did not broaden antibody response to A-prime strains except as can be explained by previous infection.

**PR8 Vaccine.**—PR8 adjuvant vaccine induced high post-vaccination antibody titers to swine influenza virus only in persons over 30. The low post-vaccination antibody level to swine virus present in pools of sera obtained from children and recruits was shown to be due to antibody increase in but a small proportion of the individuals of each group, and this proportion was similar to that found after aqueous PR8 vaccine (9). Evidence suggesting a limited distribution of the major antigens of swine-like strains in recent years has been presented previously (7). Clearly children and military recruits have had relatively minimal prior antigenic experience with the major antigens of swine influenza virus and vaccination with adjuvant PR8 vaccine did not broaden antibody response as measured with swine virus beyond limits determined by the effects of previous exposures.

Antibody response to the homologous strain (PR8) was greatest in military recruits, less in persons over 30, and least in children. The explanation for these findings is again offered that persons of military recruit age have had the greatest, persons over 30 less, and children the least previous antigenic experience with the major antigens of influenza A strains.

The pattern of antibody increase to FM1 and Malaya strains, after PR8 adjuvant vaccine, was like that observed after swine virus adjuvant vaccine, although post-vaccination levels were somewhat lower. The interpretation of the antibody responses is essentially the same offered to explain the findings with the swine vaccine.
**FM1 Vaccine.**—FM1 adjuvant vaccine induced antibody responses to swine influenza virus in the three age groups much like that seen after vaccination with PR8. Antibody to PR8 in response to FM1 adjuvant vaccine was absent in children and moderate, though equal, in recruits and in persons over 30. Again the failure of monovalent adjuvant vaccines to broaden antibody response in children to swine or PR8, and in recruits to swine virus is noted. FM1 vaccine induced high levels of homologous antibody in each of the three age groups studied. Nevertheless, the final titer achieved by the oldest age group was the lowest. Antibody response as measured with Malaya virus was of the same pattern.

The results of these experiments are in remarkable agreement with those observed previously with aqueous monovalent influenza virus vaccines (9). They demonstrate that heterologous antibody responses to monovalent adjuvant vaccines do not broaden beyond the confines predetermined by the major antigens of past infections. Hence, composite antibody could not be induced at all ages by vaccines containing a single strain of virus of the nature here employed.

**Antibody Response to Polyvalent Aqueous and Adjuvant Vaccines in the Same Three Age Groups.**—Recognizing the limitations in antibody response to monovalent vaccines, it seemed logical to formulate and to test polyvalent vaccines, to ascertain whether preparations containing representative strains of swine, A, and A-prime virus were capable of inducing a composite of antibodies irrespective of age and differences in prior exposures. To measure the breadth and height of antibody response to a polyvalent aqueous and a polyvalent adjuvant vaccine the following experiment was carried out.

Groups comprising 25 children, recruits, or persons over 30, respectively, were bled, vaccinated, and bled again. The aqueous vaccine given to children and persons over 30 contained equal parts of swine, PR8, FM1, and Cuppett strains in a total concentration of 750 CCA units per ml; that given to recruits contained one part each of swine, PR8, and PR301 in a final concentration of 800 CCA units per ml. The adjuvant vaccine given to all three age groups contained equal parts of swine, PR8, and PR301 strains in a final concentration of 200 CCA units per 0.25 ml. Table I presents the geometric mean HI antibody titers determined with swine, PR8, FM1, and Malaya 302 strains in pairs of sera obtained before and after vaccination.

**Children.**—In children, while antibodies to all three families of virus were evoked by both types of vaccine, post-vaccination antibody levels to swine and PR8 were relatively low. The antibody response to the two vaccines appeared equal except that the level of FM1 antibody was higher after aqueous vaccine. This difference may be related to the fact that FM1 was contained in the aqueous vaccine, and that the antibody response of children tends to be strain-specific. The pattern of antibody response in these children demonstrates again that their exposures were largely limited to influenza A-prime strains.
Recruits.—In recruits, post-vaccination antibody levels to the four test strains were higher after adjuvant than after aqueous vaccine. In either case, the highest titer was found with PR8. With adjuvant vaccine, the response to A-prime strains was also broader. As a result, the height of the titers of the composite of antibodies against influenza was more uniform. Aqueous vaccine induced in recruits low levels of swine antibody similar to those found in children after either vaccine. Post-vaccination A-prime antibody titers in recruits given aqueous vaccine were slightly lower than those of children receiving aqueous vaccine, but the titers after adjuvant vaccine were considerably higher. The antibody response to swine, A, and A-prime strains seen in recruits is ordered in magnitude in accordance with the amount of exposure persons of this age have had with each of the families of virus.

Persons over 30.—In persons over 30, post-vaccination antibody levels after either vaccine were much higher against swine virus than in the younger age groups. PR8 antibody levels were intermediate between those found after vaccination in recruits and in children. Post-vaccination A-prime antibody levels were the lowest of the three age groups studied, and it is noted that before vaccination this older group had little or no antibody to the recent Malaya strain. They behave with respect to Malaya antibody as children do when given the swine and PR8 strains. There was no real difference in response to aqueous and adjuvant preparations. Clearly, the pattern of antibody response of persons over 30 reflects the gradation in amounts of experience this age group has had with swine, A, and A-prime viruses.

These findings indicate that although a composite of antibody to swine, A, and A-prime viruses can be stimulated by polyvalent aqueous and polyvalent adjuvant vaccines, the magnitude of the antibody increases observed in each age group was proportionate to the prior antigenic exposure of each cohort as reflected in initial antibody titers. Hence, the most noticeable effect of the polyvalent vaccines used was to provide a “booster” stimulus. Nevertheless, the primary vaccination may establish a foundation of antibody against strains

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### TABLE I

**Comparison of Antibody Response in Three Age Groups to Polyvalent Aqueous and Adjuvant Influenza Virus Vaccine**

<table>
<thead>
<tr>
<th>Age</th>
<th>Swine</th>
<th>PR8</th>
<th>FM1</th>
<th>Malaya</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous vaccine</td>
<td>Adjuvant vaccine</td>
<td>Aqueous vaccine</td>
<td>Adjuvant vaccine</td>
</tr>
<tr>
<td>Children</td>
<td>&lt;32/64</td>
<td>&lt;32/64</td>
<td>&lt;32/77</td>
<td>32/61</td>
</tr>
<tr>
<td>Recruits</td>
<td>&lt;32/77</td>
<td>&lt;32/77</td>
<td>128/1,434</td>
<td>83/4,996</td>
</tr>
<tr>
<td>&gt;30 yrs.</td>
<td>282/1,638</td>
<td>179/1,843</td>
<td>92/922</td>
<td>48/717</td>
</tr>
</tbody>
</table>

* Geometric mean pre- and post-vaccination HI antibody titers.
not previously encountered, as witnessed by a comparison of pre- and post-vaccination antibody levels.

**Predetermination of Antibody Response by Vaccination.**—Since single doses of aqueous or adjuvant monovalent vaccine, and single doses of aqueous or adjuvant polyvalent vaccine had failed to induce high levels of composite antibody at all ages, the next experimental approach was to learn whether vaccination could meet the deficit in natural antigenic experience that characterizes each of the age groups studied. As a beginning, it was decided to ascertain whether vaccination with strains prevalent before their birth could persistently orient the antibody response of children to revaccination with strains antigenically like those of recent circulation. By such an experiment, it was also recognized that a direct test could be derived of the thesis that antibody stimulated by exposure to heterologous strains of influenza virus is persistently oriented by the major antigens formerly encountered.

**Antibody Orientation to Primary Vaccine.—**

For this purpose, a group of 17 children was available who had been vaccinated 1 year previously with aqueous suspensions of swine and PR8 viruses. Because of the design of experiments reported previously, these children had received in sequence at 2 week intervals 1.0 ml. subcutaneously of monovalent aqueous influenza virus vaccines containing swine, Cuppett, PR8, and FM1 strains, respectively. The concentration of virus in each vaccine was 750 CCA units per ml. (9).

To determine the effects of the previous vaccinations upon antibody response, 0.25 ml. of adjuvant vaccine, containing 250 CCA units of Malaya 302, was given intramuscularly. As a control, Malaya adjuvant vaccine was given to 24 children of the same age who had not been vaccinated previously. HI antibody levels determined with swine, PR8, FM1, and Malaya strains of virus in sera obtained before and 6 weeks after vaccination with the adjuvant preparation are shown in Table II.

The sera of the children vaccinated one year previously with swine, Cuppett, PR8, and FM1 aqueous vaccine still contained low levels of antibody to swine,

---

**TABLE II**

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Antibody titer to strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Swine</td>
</tr>
<tr>
<td>Malaya adjuvant</td>
<td></td>
</tr>
<tr>
<td>Previously vaccinated children (17)</td>
<td>77<em>461</em></td>
</tr>
<tr>
<td>Unvaccinated children (24)</td>
<td>&lt;32/&lt;32</td>
</tr>
</tbody>
</table>

* Geometric mean HI antibody titer before and after vaccination.
and PR8, but high levels to FM1 and Malaya strains. Vaccination with adjuvant vaccine containing Malaya virus caused a marked reinforcement of antibody to swine virus, a significant but less dramatic reinforcement of antibody to PR8 and, despite high pre-vaccination levels, an extensive increase in antibody to FM1 and Malaya. Vaccination with adjuvant vaccine containing Malaya virus did not increase antibody to swine or PR8 strain in the control group of children. Pronounced reinforcement of A-prime antibody levels, however, did occur. These results clearly demonstrate that prior contact with the major antigens of swine and PR8 given by vaccination has oriented antibody response so that subsequent vaccination with Malaya virus, which apparently contains swine and PR8-like antigens as minor components, results in reinforcement of antibody to swine and PR8.

TABLE III
Additive Effects of Revaccination upon Antibody Foundation Constructed by Primary Vaccine

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Date</th>
<th>Antibody Titer to strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Swine</td>
</tr>
<tr>
<td>Polyvalent aqueous</td>
<td>Dec. 1954</td>
<td>&lt;32*/64*</td>
</tr>
<tr>
<td>Malaya adjuvant</td>
<td>Feb. 1956</td>
<td>&lt;32/243</td>
</tr>
<tr>
<td>FM1 adjuvant</td>
<td>Aug. 1956</td>
<td>192/922</td>
</tr>
<tr>
<td>PR8 adjuvant</td>
<td>Jan. 1957</td>
<td>614/1,951</td>
</tr>
<tr>
<td>PR8 adjuvant</td>
<td>Previously unvaccinated children</td>
<td>&lt;32/32</td>
</tr>
</tbody>
</table>

* Geometric mean HI antibody titer before and after vaccination.

Persistence of Antibody Foundation Laid Down by the Primary Vaccine.—The orienting effect produced by vaccination was found to be remarkably persistent.

A second group of 15 children was studied who had received in December, 1954, a polyvalent aqueous vaccine containing swine, PR8, FM1, and Cuppett strains in a concentration of 750 CCA units per ml. In February, 1956, these children were revaccinated intramuscularly with an adjuvant vaccine containing 250 CCA units of Malaya virus; in August, 1956, they were given an adjuvant preparation containing 250 CCA units of FM1 virus, and in January, 1957, they received an adjuvant PR8 vaccine containing 250 CCA units per dose. Bloods were obtained before each vaccination and 2 weeks after aqueous or 6 weeks after adjuvant vaccines were given. The results of antibody determinations with swine, PR8, FM1, and Malaya virus are shown in Table III.

The antibody response to polyvalent aqueous vaccine recorded in these tests is similar to that shown in Table I since these 15 children were a part of that
original group of 25. Antibody titers measured in sera obtained before vaccination with Malaya adjuvant vaccine demonstrate the decline in the ensuing 14 months of antibody levels from those observed 2 weeks after vaccination with polyvalent aqueous vaccine. The results shown 6 weeks after vaccination with Malaya adjuvant vaccine may not be attributable solely to the effects of stimulation by revaccination since simultaneously an epidemic of influenza A-prime occurred in the institution where the children were cared for. However, pools of serum from an unvaccinated control group of children bled at the same intervals showed antibody increase only to A-prime strains and it has been demonstrated (cf. Table II) that Malaya adjuvant vaccine does not ordinarily cause antibody increase in children to swine or PR8 strains. Therefore, for convenience in presentation, the antibody response of these children in this interval will be spoken of as antibody response to revaccination, even though it is realized that an indeterminate, though probably minor amount of the antibody increases observed, may have resulted from infection. With these reservations, vaccination with Malaya adjuvant vaccine was followed by a sharp increase in antibody to swine virus, and a lesser response to PR8. As would be expected in children, a marked increase in A-prime antibodies occurred.

Six months later, at a time when influenza was not occurring at the institution (August, 1956), these children were revaccinated with FM1 adjuvant. An antibody increase to swine virus was again observed and the level attained was greater than that found after the previous vaccinations; it was about 8-fold higher than those observed after either initial polyvalent vaccine (cf. Table I). This result indicates a persistence of antibody orientation to swine virus for 20 months after the initial vaccination.

It is noteworthy that antibody increase to A-prime strains was observed after each vaccination and that the amounts of antibody produced were large. Once again these findings illustrate the phenomenon of reinforcement by vaccination of antibody to strains of primary infection.

There was a less efficient orientation of antibody production to PR8 by the original vaccination of the children. While both A-prime adjuvant vaccines did cause some enhancement of PR8 antibodies, the amounts obtained after revaccination were relatively low. Previous studies have indicated a greater antigenic crossing between swine and recent A-prime strains than with PR8 (9). The comparatively poor PR8 response may then reflect the relative paucity of PR8 antigen in the FM1 and Malaya A-prime strains. It is also possible that when given together swine virus may preempt the antibody-forming sites capable of being sensitized through vaccination.

To determine whether the initial polyvalent vaccine containing PR8 had exerted any significant orientation upon antibody formation to that strain, these 15 children were then vaccinated in January, 1957, with an adjuvant monovalent PR8 vaccine. The PR8 antibody had not changed significantly during the 5 month interval since vaccination in August, 1956. After revaccination
F. M. Davenport and A. V. Hennessy

with PR8 strain, antibody measured with PR8 virus rose to high levels. The geometric mean titer of 9011 achieved in these children is in marked contrast to that of 384 observed in a group of 25 previously unvaccinated children following an initial vaccination with the same PR8 adjuvant vaccine. The marked difference in the titers achieved in these two groups of children indicates that the PR8 given these 15 children 25 months before in the polyvalent aqueous vaccine, had in fact effectively sensitized the PR8 antibody-forming mechanism although it had been less responsive to heterologous stimuli than was the case with swine antigen.

Antibody to swine strain had persisted at a high level since the last vaccination 6 months before, and after revaccination with PR8 was reinforced to a still higher level. This result indicates that once the antibody-forming mechanism is properly oriented successive vaccinations with related but heterologous strains result in progressively higher titers.

Antibody reacting with A-prime strains was very high before revaccination and increased to higher levels following vaccination although not to the heights noted after vaccination in August with FM1 strain. The observations suggest that the antibody production to those dominant antigens had become so complete as to be less reactive to an additional stimulus.

The findings demonstrate that high levels of composite antibody can be achieved and maintained in children by an initial sensitization with a polyvalent aqueous vaccine, followed by successive revaccination with selected monovalent adjuvant vaccines. In addition, the results afford a striking illustration of the principle that antibody response to vaccination or infection with various but related strains is oriented by the major antigens of the viruses previously encountered. Similar experiments have been carried out with vaccines made from strains of influenza B and the results are similar (12).

Influence of Repeated Vaccination with Polyvalent Vaccine upon Antibody Response.—In the light of the objective for artificial immunization previously stated, that is, to induce at all ages high levels of a broad composite of antibody, the data obtained made it of special interest to ascertain whether high levels of composite antibody could be induced in children by giving polyvalent influenza virus vaccines in sequence.

To test this possibility, there was available a group of 25 children who had received a polyvalent adjuvant vaccine in July, 1956. These children were revaccinated with polyvalent aqueous vaccine in January, 1957. The polyvalent adjuvant vaccine contained per dose 50 CCA units each of swine, PR8, and PR301 strains. The polyvalent aqueous vaccine contained per ml. 200 CCA units each of the same viruses. The results of hemagglutination inhibition antibody determinations made with swine, PR8, FM1, and Malaya strains are represented in Table IV as geometric mean titers.

Children.—The antibody response of these children to polyvalent adjuvant vaccine was somewhat greater to each virus than that of another group given, in October, 1955, a different preparation of adjuvant vaccine of the same com-
Nevertheless, the pattern of antibody response is very similar in both groups with the dominant increases to A-prime strains. The adjuvant vaccine given in the summer of 1956 appears to be a better antigenic agent than that given in the fall of 1955. Antibody levels against swine, PR8, FM1, and Malaya strains induced by the polyvalent adjuvant vaccine were maintained until January, 1957. Antibody increase to aqueous vaccine given 7 months later as measured with swine virus, was more than 5-fold, with PR8 9-fold; antibody increases measured with FM1 and Malaya strains were less than 2-fold. However, levels of antibody against these strains were already high before revaccination. As a result of revaccination, good titers were now evident against all test strains; the lowest titer, that of swine virus, was over 800. Comparison of antibody levels attained to the same test strains after a single vaccination with either polyvalent adjuvant or aqueous vaccine (cf. Table I) demonstrates the serologic advantage of using these two vaccines in sequence. Clearly the revaccinated children achieved higher levels of a composite of antibodies. The suggestion is obvious, that after a primary vaccination with a good adjuvant vaccine, the aqueous vaccine may then be used after an appropriate interval as a booster to induce high levels of antibody to multiple strains, including those to which original antigenic experience is gained by vaccination.

Persons over 30.—A similar experiment was carried out in a group of 20 persons over 30 years of age who had received the less potent polyvalent adjuvant vaccine in September of 1955 and were revaccinated 17 months later in February, 1957, with polyvalent aqueous vaccine. The results are shown in the lower part of Table IV. Since the 20 persons used in this group were part of the 25 individuals whose response to adjuvant vaccine is shown in Table I, the initial

<table>
<thead>
<tr>
<th>Group</th>
<th>Vaccine</th>
<th>Date</th>
<th>Antibody titer to Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Swine</td>
</tr>
<tr>
<td>Children</td>
<td>Polyvalent adjuvant</td>
<td>July 1956</td>
<td>&lt;32*/141*</td>
</tr>
<tr>
<td></td>
<td>Polyvalent aqueous</td>
<td>Jan. 1957</td>
<td>154/819</td>
</tr>
<tr>
<td></td>
<td>Polyvalent adjuvant</td>
<td>Sept. 1955</td>
<td>154/1,843</td>
</tr>
<tr>
<td></td>
<td>Polyvalent aqueous</td>
<td>Jan. 1957</td>
<td>1,434/3,072</td>
</tr>
</tbody>
</table>

* Geometric mean HI antibody titer before and after vaccination.
response to vaccination will not be discussed. It is notable that the antibody levels to all strains declined but slightly in the 17 months after adjuvant vaccination. Revaccination with polyvalent aqueous vaccine caused a prompt and substantial increase in antibody to all test strains. The increase in antibody was about 2-fold to swine, FM1, and Malaya strains, and 4-fold to PR8. The results are the reverse of those in children; here the swine antibody is the dominant one, but firm and good levels of antibody to the strains of secondary exposure are well established. Again, comparison of antibody levels achieved after a single dose of adjuvant or aqueous polyvalent vaccines (cf. Table I) with those observed after use of polyvalent vaccines in sequence after a substantial interval, demonstrates the superiority of the antibody response following the latter procedure and the resultant higher levels of a composite of antibodies against antiogenically representative strains of group A influenza viruses. It is noteworthy that the antibody-orienting effect of the first vaccination persisted in this experiment for at least 18 months. The results of all these studies exemplify again the characteristic antibody pattern and responses in different segments of the population—differences based upon first experiences and conditioned responses.

The data presented demonstrate that it is possible to obtain high levels of composite antibody, despite differences in age and past experience, if suitable preparations and schedules are employed. It should be pointed out that much of the considerations of vaccination against influenza has been dominated by the results of studies of repeated inoculation of adults at short intervals, i.e. weeks, with limited strain distribution and with the military emphasis on a single dose. The accumulating information demonstrates that this view must be thoroughly reconsidered. The probability is that repeated injections at more widely spaced intervals of a selected group of antigens will give a broader, firmer, and more composite coverage.

DISCUSSION

The results of the present investigation extend the demonstration that antibody responses to influenza viruses are persistently oriented by the major antigens of strains previously encountered by infection. The antibody orientation of persons of different ages varies owing to recurrent epidemics caused by strains of changing antigenic composition. It is now recognized that children aged 10 or less have been principally exposed to A-prime strains; that persons of military recruit age have been exposed in succession largely to A and A-prime viruses, and that persons over 30 have been exposed seriatim to the swine, A, and A-prime families of virus. Differences in the exposures of each age group are attributable to coincidence in the time of their childhood and in the periods of prevalence of these major antigenic variants of influenza A (4, 5, 7).

It has been shown in this and in a preceding study that antibody response
to vaccination, like infection, is dominated by the persistent effects of "original antigenic sin" (9). Vaccination greatly reinforces antibody to strains previously encountered but antibody response to strains encountered for the first time is relatively poor. Thus regardless of whether aqueous monovalent vaccines were given repeatedly at short intervals (9), whether monovalent adjuvant vaccines were given as a single inoculation, or whether polyvalent vaccines of either kind were administered once, high levels of A-prime antibodies were induced in children, high levels of A and A-prime antibodies in recruits, and high levels of swine and A antibodies in persons over 30. In contrast, children remained relatively deficient in swine and A antibodies, recruits in swine antibodies and persons over 30 in A-prime antibodies unless the corresponding strain was given in high dosage as a monovalent preparation. The concordance of these findings with the thesis that prior experience predetermines antibody response to subsequent exposures, given either by infection or by vaccination, emphasizes the validity of that concept.

The epidemiologic and immunologic implications of this "doctrine of original antigenic sin" are extensive. At present, the phenomenon of recurrent epidemics of influenza caused by viruses of varying virulence and of varying antigenic composition poses a unique problem in the control of communicable disease. Several solutions have been proposed. One presupposes that immunity results from highly strain-specific antibody, and that the proper course to take is to rely, for protection against next year's antigenic variant, solely upon antibody that is produced by vaccines manufactured this year. Such a proposal ignores the implications of the accumulating data on the relation between antibody and protection in man. A series of studies has shown that resistance to influenza correlates with the existence of broadly reacting antibody which affords protection regardless of minor antigenic caprice in the prevalent strain. To recapitulate, despite highest levels of antibody against the prevailing types of virus, the incidence of influenza is greatest in childhood; conversely, the incidences are lower in the older segments of the population who possess high levels of antibody against formerly prevalent strains of virus, but relatively low levels of antibody against the prevailing strain (4, 5, 6). Moreover, experience has shown that it is logistically unrealistic to expect that sufficient quantities of vaccine could be regularly produced from strains isolated during one respiratory disease season in time for effective use before the next.

An alternate proposal has been that of using, as a single inoculation, polyvalent vaccines formulated by inclusion of prototypic strains, to span the major antigens that comprise influenza viruses. It was hoped that such a vaccine would induce high levels of composite antibody at all ages which would simulate that characteristic of older persons whose experience with antigenic variants of influenza virus is greatest and whose susceptibility is least. Part of the data reported in this communication tested this possibility and found it wanting when the results of a single vaccination were considered. It seems clear, then,
that composite antibody cannot be produced at all ages by current monovalent
or polyvalent vaccines used as a single dose, owing to the predeterminate effects
of prior infections.

However, the demonstration that vaccination, like infection, can lay at will
the foundations for future antibody responses, opens a new and promising vista
not circumscribed by age or by commitment to artificial though convenient
schedules of vaccination. Vaccination has been shown to be capable of initiating
the processes of accumulating additional antibody dividends upon restimulation
with the same or even with antigenically remotely related strains. Repeated
vaccinations at properly spaced intervals with appropriate viruses would be
expected to yield a more durable immunity not jeopardized by minor, though
at times seemingly dramatic, antigenic changes in the "virus of the year." Since
major antigenic rearrangements yielding new families of A strains occur
at widely spaced intervals, the antigenic coverage of a polyvalent vaccine can
be further broadened by the addition of virus antigenically representative of
each new family, once its status is recognized. The goal for attaining high com-
posite antibody levels and a higher degree of immunity at all ages, despite
differences in prior antigenic experiences in the population, seems attainable
if a selected group of antigens is given at widely spaced intervals. Progress
towards this goal encourages future studies designed to determine the mechanics
of producing a broader, firmer, more composite coverage of antibody prote-
ction against epidemic influenza.

The literature contains but one study which examines the hypothesis that
repeated vaccinations yield a superior immunity to epidemic influenza. The
data accumulated in this investigation of an outbreak which occurred in a
boys' school suggest that greater resistance was conferred upon those individu-
als who had received two A-prime vaccines at about yearly intervals,
than on those who had been vaccinated only once with an A-prime vaccine
(13). Obviously, this important lead should be investigated further and trials
on the efficacy of vaccines of broadest coverage, used in the most efficient man-
ner, in populations of different age categories must be continued if the optimal
solution to the problem of vaccination against influenza is to be found.

Finally, it should be pointed out that the epidemiologic and immunologic
findings described do not represent phenomena peculiar to influenza. Rather
they illustrate a general principle applicable to other infections of man. Data
which support this conclusion have been observed in rickettsial infections (14),
in antibody response to adenovirus vaccines (15), and in infections caused by
the arthropod-borne group of animal viruses (16).

SUMMARY

The antibody orienting effects of prior infections with antigenic variants of
influenza viruses were confirmed by studies with monovalent adjuvant vaccines
and with polyvalent aqueous and adjuvant preparations. In either case, the
predominant antibody response was of a “booster” type, directed against the major antigens of strains of original infection. It was shown that vaccination with appropriate strains, selected as antigenic prototypes, could orient or predetermine subsequent antibody response upon revaccination. Moreover, the effects of exposure by vaccination were found to be durable and to constitute a foundation upon which future antibody dividends could be accumulated. As a result, it seems feasible to induce by vaccination a more lasting broad composite antibody protection against influenza if appropriate preparations and schedules are used.

BIBLIOGRAPHY