A SEROLOGIC RECAPITULATION OF PAST EXPERIENCES WITH INFLUENZA A; ANTIBODY RESPONSE TO MONOVALENT VACCINE*

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The results of previous studies on the distribution by age of antibodies to influenza viruses have shown a remarkable correlation between the type of antibody found now in the sera of persons at three stages of life, and the antigenic character of the strains prevalent during the childhood of each age group (1–4).

For example, the principle antibody of children born after the appearance of influenza A-prime in 1946 is antibody that reacts with A-prime strains. The principle antibody of young adults who were children during the period of prevalence of influenza A, which extended until 1943, is antibody that reacts with strains of Type A influenza virus. Likewise, in persons over 30 years of age, the principal antibody recognized is one that reacts with swine influenza virus. Many of the persons now over 30 years of age were children during a period when influenza viruses antigenically closely related to swine strains are presumed to have prevailed. This period included the pandemic of 1918 (5, 6, 1–4). Analogous results have been found with respect to antigenic variants of Type B influenza virus and influenza B (1, 2).

To explain these phenomena the thesis was presented that at the time of the initial infections with influenza viruses, which occur predominantly in childhood, the antibody-forming mechanisms are persistently oriented by the dominant antigens of the strains encountered. Upon subsequent exposure to influenza viruses of varied but related antigenic composition reinforcement of the level of antibody to the strains of primary infection occurs while the serologic response to prevailing viruses may be dampened. This thesis has been epitomized in a colloquial expression as "the doctrine of original antigenic sin." It was previously stated that the data which led to formulation of this "doctrine" were in part derived by measuring interepidemic levels of antibody

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with prototypic strains of virus. Under these circumstances, the time, rate, and character of prior antigenic exposures of the population studied are not precisely known. In order to define more accurately the dimensions of the persistent serologic influences of the dominant antigens of the strains of childhood infection, it was decided to control these variables by vaccination with monovalent vaccines containing representative strains of swine influenza, influenza A, and influenza A-prime. The vaccines were given to children born during the period of prevalence of influenza A-prime, to adults who were children during the epoch of influenza A, and to adults over 30 years of age. Antibody response in each age group was measured with each strain of virus. In addition, it was recognized that the data obtained might provide a serologic index of the relative extent of the antigenic experience of these segments of the population with influenza A, A-prime, and swine-like strains. It is believed that this use of vaccines to assess the relative frequency of infection in a population represents a new development in serologic epidemiology.

Materials and Methods

Vaccines.—Monovalent influenza virus vaccines, each containing 750 CCA units of virus per ml., were prepared by a commercial pharmaceutical firm with the following strains: Swine 1976 (1931), PR8 (1933), FM1 (1947), and Cuppett (1950). The viruses were inactivated with formalin (1:4000). Merthiolate (1:10,000) was added as a preservative. Inadvertently, the monovalent Cuppett vaccine contained a trace of Conley virus (1952 A'), but in an amount judged to be too small to influence the results obtained.

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 were inmates of State mental institutions. Military recruits were airmen stationed at Sampson Air Force Base, Geneva, New York, for basic training.[1]

Immunization and Bleeding Schedules.—In groups of 20 to 25, children, recruits, and persons over 30 were bled and vaccinated according to the following plan. Four groups in each age category received three doses of monovalent vaccines containing either swine, PR8, FM1, or Cuppett virus. Six groups received 4 different monovalent vaccines alternated so that the order in which swine, PR8, or Cuppett strains of virus was given, was varied to include all possible permutations, and each group received FM1 as the last vaccine. Vaccination was carried out at 2-week intervals. Bleedings were obtained before each vaccination and at 2 and 4 weeks after the last. The dose of all vaccines was 1 cc. subcutaneously.

Treatment of Sera.—Serum was promptly separated from each blood sample, and merthiolate was added to yield a final concentration of 1:10,000. 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 Titrations.—The hemagglutination-inhibition titer of serum pools was determined by a pattern method with 4 units of virus and 0.5 per cent chicken erythrocytes suspended in saline. (7).

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

[1] 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.

EXPERIMENTAL

Antibody Response in Children to Monovalent Vaccines.—Antibody responses to swine, PR8, FM1, and Cuppett strains were measured in pools of serum obtained before and 2 weeks after vaccination with monovalent vaccines containing these viruses. In Fig. 1, pre- and post-vaccination antibody titers are shown as paired bar graphs. Open bars represent antibody levels measured

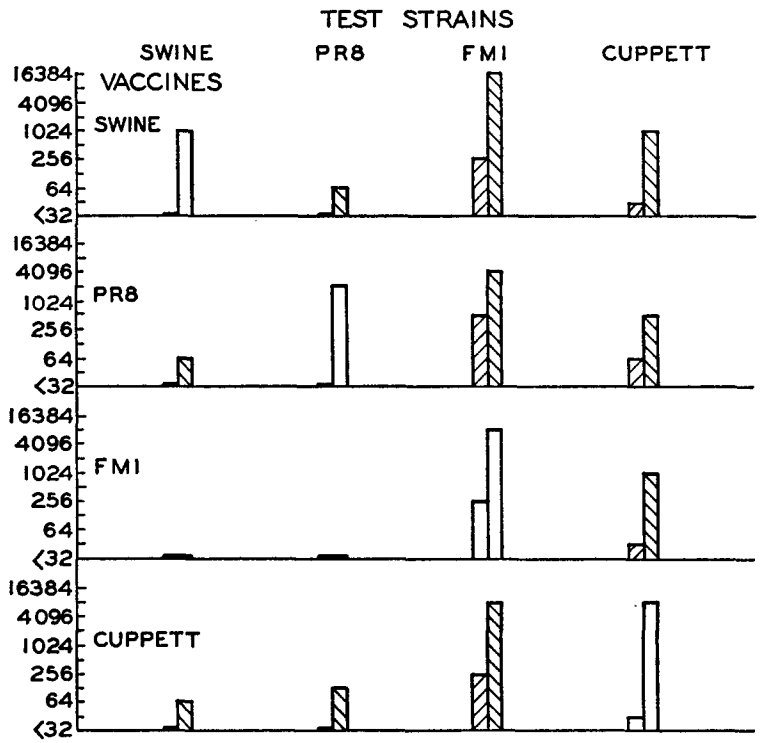

FIG. 1. Antibody response in children aged 4 to 10 years to monovalent influenza virus vaccines.

with the strain used for vaccination. Hatched bars represent antibody levels measured with heterologous strains. Antibody to swine virus was not detected in the lowest dilution of prevaccination sera tested. As would be expected, a high level of homologous antibody was found 2 weeks after vaccination with the swine strain. Of greater import is the antibody response after swine vaccine to heterologous viruses. Note that before vaccination, antibody to PR8 was inapparent and the level developed after vaccination was low. In contrast, postvaccination antibody levels to FM1 and Cuppett were high, and the levels reached represent a marked increase over the amounts found before vaccination. Analogous results were obtained by vaccination with PR8. The ho-

TABLE I

*H-I Antibody Titers in Pools of Sera Obtained before and after Administration of Monovalent Vaccines to Children Aged 4 to 10**

Vaccines given	Group	Bleeding	Titer with test strains			
			Swine	PR8	FM1	Cuppett
Swine	1	1	0	0	256	32
Swine		2	1,024	128	>16,384	1,024
Swine		3‡	1,024	64	>16,384	1,024
		–				
		4	2,048	32	>16,384	2,048
		5	512	32	8,192	512
PR8	2	1	0	0	1,024	64
PR8		2	64	2,048	2,048	128
PR8		3	64	2,048	2,048	128
		–				
		4	64	1,024	2,048	128
		5	32	1,024	2,048	128
FM1	3	1	0	0	256	32
FM1		2	0	0	4,096	512
FM1		3	0	32	4,096	512
		–				
		4	0	32	4,096	256
		5	0	32	2,048	256
Cuppett	4	1	0	0	256	32
Cuppett		2	32	128	4,096	1,024
Cuppett		3	0	64	8,192	512
		–				
		4	0	32	4,096	512
		5	0	32	4,096	512
Swine	5	1	0	0	256	32
PR8		2	1,024	32	>16,384	2,048
Cuppett		3	512	512	8,192	1,024
FM1		4	256	256	4,096	512
		–				
		5	256	128	8,192	512
		6	256	128	8,192	1,024
Swine	6	1	0	0	256	32
Cuppett		2	1,024	64	>16,384	1,024
PR8		3	1,024	64	>16,384	1,024
FM1		4	512	1,024	4,096	512
		–				
		5	512	512	4,096	512
		6	512	1,024	4,096	512

TABLE I—*Concluded*

Vaccines given	Group	Bleeding	Titer with test strains			
			Swine	PR8	FM1	Cuppett
PR8	7	1	0	0	256	64
Swine		2	128	2,048	4,096	1,024
Cuppett		3	256	1,024	4,096	1,024
FM1		4	128	1,024	4,096	512
		–				
		5	64	1,024	4,096	512
		6	64	512	2,048	256
PR8	8	1	0	0	128	32
Cuppett		2	64	1,024	2,048	256
Swine		3	64	1,024	4,096	512
FM1		4	256	512	4,096	512
		–				
		5	256	512	4,096	512
		6	256	512	4,096	256
Cuppett	9	1	0	0	256	32
PR8		2	64	256	8,192	1,024
Swine		3	64	1,024	8,192	512
FM1		4	256	512	4,096	512
		–				
		5	128	256	4,096	512
		6	128	256	4,096	512
Cuppett	10	1	0	0	128	0
Swine		2	64	128	4,096	512
PR8		3	512	256	4,096	512
FM1		4	256	512	4,096	256
		–				
		5	128	128	2,048	256
		6	128	128	2,048	128
Control	11	1	0	32	512	32
No Vaccine		2	0	32	512	32
		3	0	32	512	32

* The first bleeding was obtained at the time of the first vaccination. Successive bleedings were carried out at 2-week intervals.

‡ Last vaccine given.

mologous antibody response was excellent. Low levels of antibody to swine virus developed. PR8 vaccine effected a marked reinforcement of antibody to FM1 and Cuppett, although the final yield of A-prime antibody was less than that observed after swine vaccine. Vaccination with either FM1 or Cuppett yielded a good homologous antibody increase. The heterologous antibody response to

the corresponding A-prime strain was also of a high order. However, FM1 vaccine did not stimulate the production of measurable antibody to swine or PR8 virus, and the response after Cuppett vaccine was minimal.

The most striking characteristic of these results is that all the vaccines induced a marked heterologous antibody response to A-prime strains, while none produced more than minimal heterologous antibody levels to PR8 or swine. These findings are a clear demonstration of persistent orientation to antibody formation resulting from initial experience with influenza. The primary infections of this age group were influenza A-prime, and a marked heterologous antibody response to A-prime strains resulted even though the vaccines given were as remotely related antigenically to influenza A-prime as PR8 and swine.

Table I shows antibody levels determined before and at 2-week intervals after multiple vaccinations with swine, PR8, FM1, or Cuppett given alone or in the sequences indicated. Maximal antibody levels to homologous and heterologous viruses were generally reached at 2 weeks (Groups I to IV). It is noteworthy that repeated vaccination of children with the same strain did not lead to a broadening of heterologous antibody response to PR8 or swine. The dependence of the development of significant levels of antibody to PR8 or swine in children upon vaccination with these viruses is emphasized by the findings in Groups 5 through 10. In all instances the appearance of high levels of antibody to swine or PR8 was delayed until 2 weeks after the use of the homologous vaccine.

Close scrutiny of the data in Table I yields several provocative observations. In children, swine vaccine induced higher levels of antibody to FM1 and Cuppett than did vaccination with either of these A-prime strains. (Group I, *c.f.* Groups 3 and 4). Vaccination with PR8 was less effective in stimulating high levels of anti-influenza A-prime antibodies than swine, FM1, or Cuppett vaccines. (Group 2, *c.f.* Groups 1, 3, and 4). These findings suggest that swine virus contains a more representative selection of the antigenic components common to strains of influenza A-prime than are apparent in either PR8, FM1, or Cuppett viruses. However, prior vaccination with PR8 or Cuppett viruses appeared in most instances to suppress the capacity of swine vaccine to stimulate maximal A-prime antibody levels (Groups 7 to 10, *c.f.* Groups 5 and 6). Further examples of suppression of maximal antibody yield when different vaccines were given in sequence are the lower titers of swine antibody found when PR8 or Cuppett strains were administered before swine vaccine (Groups 7 to 10, *c.f.* Groups 5 and 6), and the lower titer of PR8 antibody resulting when swine vaccine (Group 5) or Cuppett and swine vaccines (Group 10) were used before PR8 vaccine (*c.f.* Groups 2, 7 to 9). The mechanism of these results cannot be explained at present. Suppression of maximal antibody yield when vaccines are given in sequence could result through neutralization of antigen by anti-

bodies already evoked by the preceding vaccines, or perhaps antigens given after the initial one may fail to provide a maximal challenge to an antibody-forming mechanism already stimulated to produce antibodies to closely related viruses. Whatever the mechanism may be, the results illustrate the suppressive effect of prior experience with antigenic variants of influenza viruses upon the antibody response to strains subsequently experienced. In this sense, the find-

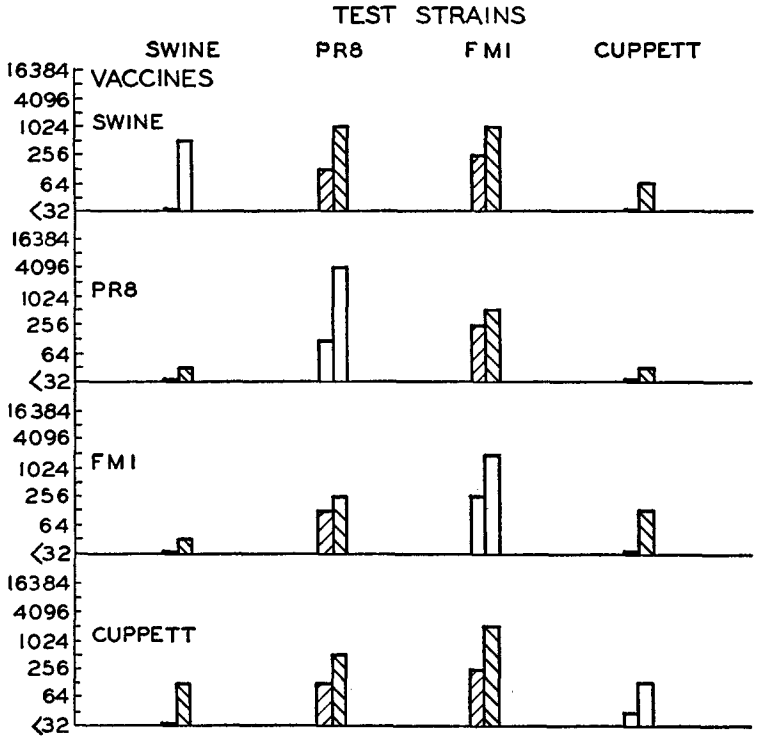

FIG. 2. Antibody response in recruits aged 17 to 26 years to monovalent influenza virus vaccines.

ings herein described after vaccination are similar to those reported previously after infection (2, 4).

Antibody Response in Military Recruits to Monovalent Vaccines.—The same vaccines were given to military recruits whose initial infections at childhood occurred during the period of prevalence of influenza A. Fig. 2 shows antibody levels found in pools of sera obtained before and 2 weeks after each vaccination. In contrast to the results in children, swine virus vaccine produced in recruits a marked reinforcement of antibody to PR8, and only slightly reinforced A-prime antibody levels. The final titer of homologous antibody to PR8 vaccine was greater than in children but the heterologous antibody in-

crease measured with A-prime strains was considerably less. Vaccination of recruits with A-prime strains stimulated levels of A-prime antibody, which were lower than those observed in children. The A-prime vaccines supported antibody levels to PR8, though to a lesser extent than did swine vaccine. Minimal heterologous antibody levels to swine virus followed the administration of PR8 and A-prime viruses. The dominant effects of the initial influenza A infections of this segment of the population upon their antibody response when subsequently exposed to influenza viruses are again demonstrated by reinforcement of PR8 antibody levels by all vaccines even though they were as distantly related antigenically to PR8 as swine and A-prime strains. It will be recalled that persons currently of military recruit age have repeatedly been exposed to influenza A-prime since 1946. The spectrum of antibodies at this age in interepidemic periods is broader than in childhood and comprises antibody to many A and A-prime strains (1, 2, 4). The suppressive effect of this broader composite of antibody upon the serologic response to influenza viruses is seen by the development of lower levels of antibody to swine and A-prime strains in recruits, than in children, when the homologous vaccines were given.

The results of other experiments in which the same vaccines were given repeatedly or alternately, as described in the preceding section on antibody response in children, were confirmatory of the observations illustrated and will not be presented in detail. Broadening of antibody response in recruits on repeated administration of the same vaccine was not observed.

Antibody Response in Persons over 30 Years of Age to Monovalent Influenza Virus Vaccines.—The results of previous studies led to the inference that people over 30 years of age had probably encountered strains of influenza virus antigenically closely related to those of swine influenza at the time of their childhood experience with influenza (1–4). To test this inference further and to evaluate the antibody-orienting capacity of such childhood experiences, the same monovalent vaccines were given to adults aged 30 or more. In Fig. 3 representative antibody levels found before and 2 weeks after vaccination are reproduced. In this age group antibody to swine virus is present before vaccination (1–4). The level of postvaccination antibody homologous for that strain was the highest observed in this study. Heterologous antibody response to Swine vaccine, as measured with PR8 or A-prime strains, was the least seen in the three age groups tested. PR8 vaccine increased heterologous antibody to swine virus, but did not consistently increase antibody levels to A-prime strains. The homologous antibody response to PR8 was less than that observed in children or recruits. A-prime vaccines given to persons over 30 years of age yielded an antibody response to the A-prime viruses used for testing similar to that seen in recruits, but considerably less than was found in children. Reinforcement of antibody levels to PR8 and to swine virus was affected by A-prime vaccines, and it is noteworthy that the postvaccination levels of antibody

measured with swine virus after vaccination with either PR8 or A-prime strains were almost equal to that observed after swine vaccine was given.

The results observed in persons over 30 years of age add further support to the inference that the initial experiences of this cohort of the population were with viruses antigenically closely related to swine virus since reinforcement of antibody levels to swine virus followed the use of all of the vaccines given.

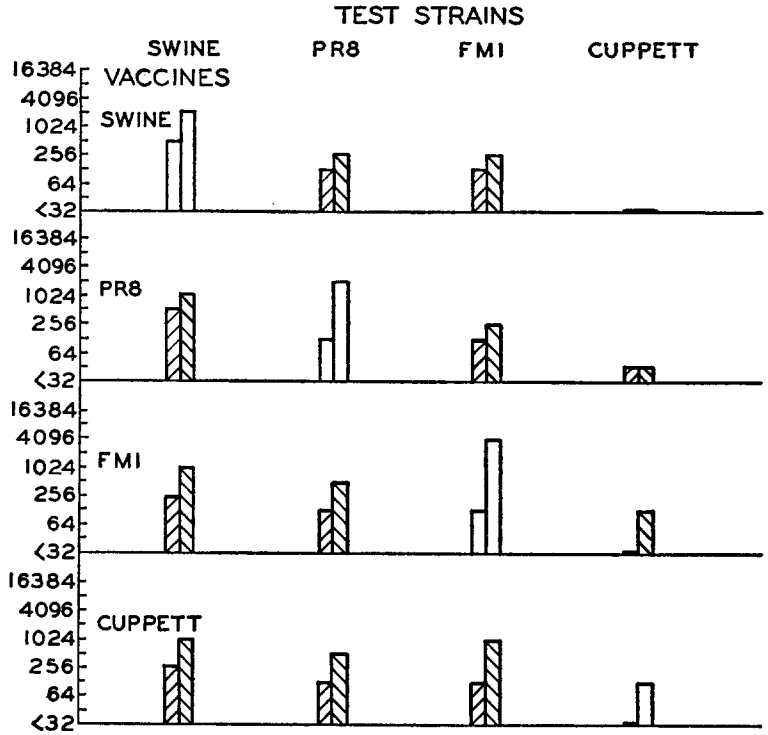

FIG. 3. Antibody response in persons over 30 years of age to monovalent influenza virus vaccines.

Suppression of antibody response owing to the broad composite of antibodies characteristic of this age group, which comprises antibodies to swine, A, and some A-prime strains, is seen in the diminished homologous antibody response to PR8 vaccines. The postvaccination level reached was the lowest observed in this study. As in recruits, vaccination with A-prime strains led to lower levels of homologous antibody than use of A-prime vaccines in children. Administration of the four monovalent vaccines repeatedly or alternately to persons over 30 years of age did not produce a broadening of antibody response.

Antibody Response to Monovalent Vaccines Measured with Many A and A-prime Strains Representative of the Known Period of Prevalence of These Types of

Influenza Virus.—Primary experience with influenza viruses by vaccination or infection leads to the production of antibody of limited scope, which in general reacts most readily with the strain responsible for that particular antigenic stimulus. Familiar examples are those seen in the antibody response

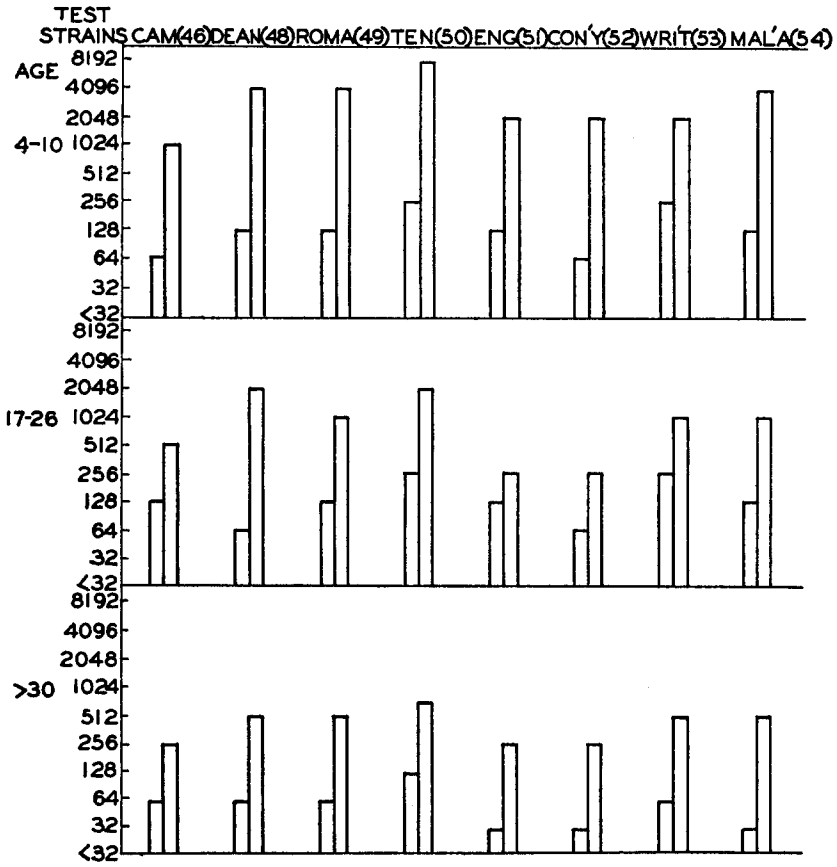

FIG. 4. Antibody response to Cuppett virus vaccine in children, recruits, and persons over 30 years of age as measured with Type A-prime strains.

of ferrets, mice, chickens, and occasionally of humans (8). However, it would seem reasonable to expect that the variety of antigenic experiences of the bulk of the human population would be much richer than that of the examples cited, owing to the frequent recurrence of influenza caused by the prevalence of viruses of different antigenic composition. In consequence, it seemed likely that the antibody response of humans might be broader than that seen in experimental animals. To ascertain the dimensions of the antibody response of humans to vaccines prepared from prototypic viruses, antibody levels in sera

obtained before and 2 weeks after vaccination with A and A-prime strains were measured with viruses isolated during the period of prevalence of these infections.

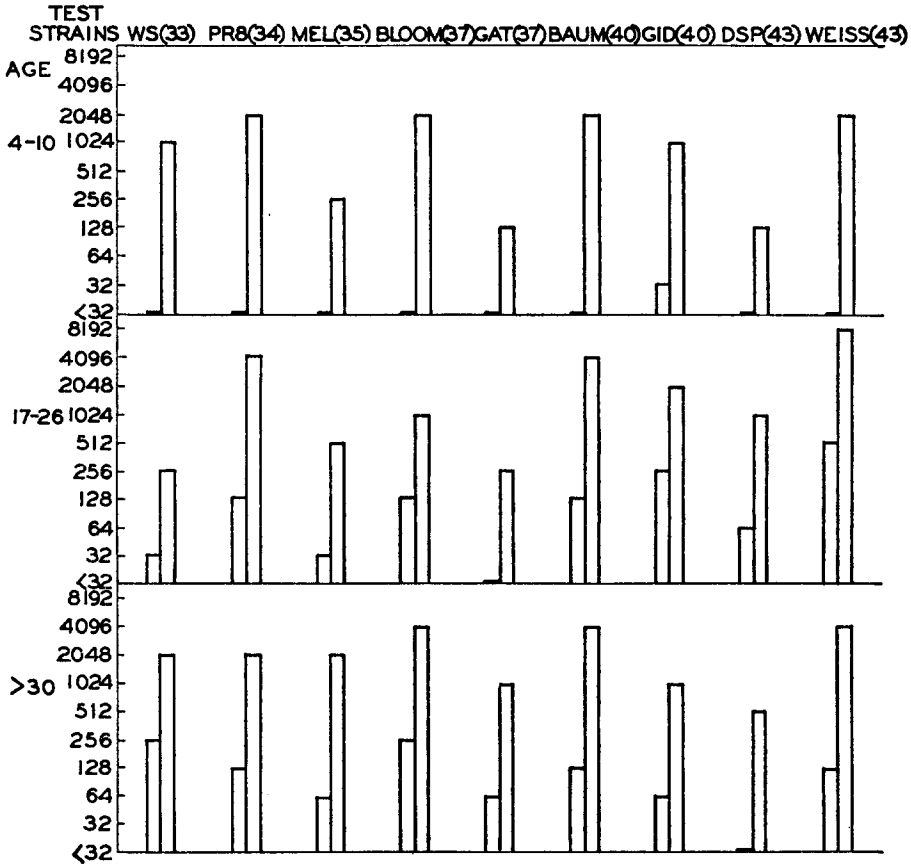

FIG. 5. Antibody response to PR8 virus vaccine in children, recruits, and persons over 30 years of age as measured with Type A strains.

In Fig. 4, the antibody response to Cuppett vaccine in children, recruits, and persons over 30 years of age, as determined with A-prime strains isolated between 1946 and 1954 is shown. It is evident that the antibody response to Cuppett vaccine as measured with antigenically and chronologically representative strains of influenza A-prime was greatest in children, less in recruits, and least in persons over 30. Moreover, within each age group, the antibody response to the viruses used for testing were remarkably uniform. Similar results were obtained with FM1 vaccine. In Fig. 5 antibody response to PR8 vaccine as measured with representative strains of influenza A, isolated between 1933

and 1943, is shown. High levels of antibody were induced to all of the test viruses, although the final yield to Melbourne (1935), Gatenby (1937), and DSP (1943) were low in children. These findings emphasize the richness of the human populations' past experience with the antigens that comprise influenza virus, and sharply differentiates the antibody response of humans from that seen in experimental animals not previously exposed to influenza viruses.

DISCUSSION

The experimental findings reported in this paper provide a remarkable illustration of the persistent antibody-orienting effects produced by the major antigens of the viruses encountered in the primary infections of childhood. Thus, regardless of the strain of virus given by vaccination, the children responded by producing antibodies to strains of influenza A-prime, the military recruits to strains of influenza A, and the persons over 30 years of age to a strain of swine influenza. The childhood infections of these three segments of the population were with influenza A-prime, with influenza A, and with influenza caused by swine-like strains respectively (1, 2). However, the results of this study disclose more than the antigenic characteristics of the strains of primary infection. They would appear to provide a serologic index of the amount of infection which each of these three age groups have experienced with influenza A-prime, influenza A, and influenza caused by swine-like strains. Such a serologic appraisal becomes possible because it was found that the antibody response of humans to monovalent vaccines was strikingly type-specific; *i.e.*, to swine, A, or A-prime strains, save for those instances wherein antibody response to heterotypic viruses could be explained by previous experience. For example, in children little or no antibody to PR8 or swine virus was produced except by vaccination with the homologous strain. Nevertheless, very high levels of A-prime antibodies developed after vaccination with swine, Type A, or A-prime viruses. Clearly this result indicates that anti A-prime antibodies produced in human sera by vaccination show little or no intrinsic serologic cross-reactivity with A or swine strains. In like vein, recruits did not develop more than minimal antibody levels to swine virus, despite the development of high levels of Type A antibody. Again the lack of intrinsic serologic cross-reactivity between anti-influenza A antibodies and swine virus is emphasized.

If it is granted that the evidence presented warrants the inference that heterotypic antibody response to vaccination largely reflects prior experience rather than serologic cross-reactivity, it becomes possible to reconstruct the previous antigenic experiences of each of the age groups studied. The antibody response of the children to monovalent vaccines indicates that as a group they have had little exposure to the dominant antigens of Type A or swine strains. Recruits have also had a limited experience with the major antigens of swine virus but their heterotypic antibody response to strains of influenza A-prime

indicates that this age group has had considerable experience with influenza A-prime, in addition to their primary infections with influenza A. Likewise persons over 30 years of age react as if after their initial infections with swine-like strains, they subsequently experienced an important amount of infection with influenza A, but a greatly diminished amount of infection with influenza A-prime. It would appear, therefore, that delineation of the characteristics of antibody response obtained in three segments of the population after vaccination with monovalent influenza virus vaccines, provides a serologic recapitulation of each cohort's past experiences with antigenic variants of influenza virus. The use of vaccines for this purpose is believed to be a new development in serologic epidemiology.

SUMMARY

The results of the present study provide a striking demonstration of antibody orientation produced by the dominant antigens of the strains of influenza virus encountered at childhood. The homologous and heterologous antibody response to monovalent vaccines containing swine, PR8, FM1, or Cuppett viruses show that the antibody-forming mechanisms of children born after 1943 are oriented to strains of influenza A-prime; of recruits to strains of influenza A; and of persons over 30 years of age to a strain of swine influenza. It was shown that antibody response to heterotypic viruses appears to provide a serologic index of the amount of experience each of the segments of the general population studied have had with antigenic variants of influenza virus. Finally it was demonstrated that the antibody response of humans to strains of influenza A or influenza A-prime is remarkably uniform for isolates of each antigenic type.

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