Immunogenicity of Egg-Grown Influenza Vaccine: Inhibition by Immunization of Recipients with Host Antigen

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Rabbit antibody directed against normal allantoic fluid reduced the protective power of influenza virus prepared from egg-grown PR8 virus. A similar vaccine prepared from mouse lung virus was not inhibited. Preimmunization of mice with normal allantoic fluid or with egg-grown influenza B/Lee vaccine (crude or purified) inhibited the protective power of PR8 egg vaccine but did not affect a similar lung vaccine.

Virions of influenza virus contain protective antigen(s) as well as host-derived antigen(s) (6). We have shown previously that, for virus grown in Ehrlich ascites tumor cells, viral antigens exert a carrier effect with respect to host antigens (16). It seemed interesting to study, in the reverse situation, the effect which the host antigens exert on the immunogenicity of viral antigens. The rationale behind these experiments has been more fully developed elsewhere (15). It appears that immunity to host determinants reduces the response to later vaccination with egg-grown virus.

MATERIALS AND METHODS

Virus strains. The B/Lee and A5/PR8 strains of influenza virus were old laboratory strains with a long passage history in ferrets, mice, and eggs.

B/Lee egg vaccine. After a few passages in the allantoic cavity of hens’ eggs, virus pools were produced by injection of 10⁶ egg infective doses into the allantoic cavity of 10-day-old embryonated eggs. The allantoic fluids were harvested after 3 days of incubation at 35 C and pooled. The virus was purified by two cycles of absorption and elution with guinea pig red blood cells (10). The eluate was adjusted to a hemagglutinin titer of 1,600 to 3,200 and inactivated by the addition of Formalin at a final concentration of 1:1,000 (final concentration of formaldehyde, 0.04%). After incubation with Formalin at 37 C for 24 hr, the pH of the inactivated vaccine was adjusted to 7.2-7.4 with sodium bicarbonate before storage at +4 C. Such preparations were devoid of infectious virus, retained their hemagglutinin titer and immunizing power during 3 months of storage, and contained less than 0.5 mg of protein per ml.

PR8 mouse line. PR8 virus was passed three times serially at 10⁻⁴ dilutions in mice by the intranasal route, the lungs being harvested after 3 days. From the third passage, a pool of infected lungs was prepared by infecting 20 mice at a 10⁻⁴ dilution intranasally. The lungs were harvested after 3 days, ground in a mortar with sterile sand and 0.5 ml of saline per lung, and centrifuged at 3,000 x g for 20 min. The supernatant fluid was sealed in ampoules and kept at −70 C.

PR8 mouse lung vaccine. Mice were inoculated intranasally with a 10⁻³ dilution of the above PR8 mouse line stock. The lungs were harvested after 3 days, ground and centrifuged as above, purified by two cycles of absorption-elution with guinea pig red cells, and Formalin-inactivated exactly as described for B/Lee. Hemagglutination by such mouse lung vaccines was not inhibited by anti-allantoic sera. Storage properties and protein content were similar to those of B/Lee vaccine.

PR8 egg line. From the PR8 mouse line stock, an egg line was derived by two successive allantoic passages at 10⁻⁴ dilutions. Allantoic fluid from the second passage was sealed in ampoules and kept at −70 C.

PR8 egg vaccine. Ten-day-old chick embryos were inoculated by the allantoic route with a 10⁻⁴ dilution of the above PR8 egg line stock. The virus was harvested after 48 hr at 35 C. Purification by guinea pig red cell absorption and elution and inactivation with Formalin were carried out exactly as described for B/Lee. Hemagglutination by such egg vaccine was inhibited by anti-allantoic sera (see below). Storage properties and protein content were similar to those of PR8 mouse lung vaccine and B/Lee vaccine.

Rabbit serum against normal, allantoic fluid. Normal allantoic fluid was harvested from noninoculated 12-day-old chick embryos. Equal volumes of allantoic fluid and Freund’s complete adjuvant (Difco) were emulsified, and 2 ml of the emulsion was injected intramuscularly into the hind legs of rabbits. The procedure was repeated after 2 weeks. After another 2 weeks the rabbits were boosted three times at

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weekly intervals by intravenous injections of 4 ml of allantoic fluid and were bled 9 days after the last injection. The sera gave multiple precipitation lines which tested against normal or infected allantoic fluid in Ouchterlony tests. When tested against egg-grown, absorption-elution-purified PR8 and B/Lee virus, they had a hemagglutination-inhibition titer of 1:640 to 1:2,560. This titer was not affected by absorption with chick red cells or treatment with trypsin and periodate. When tested against crude infected allantoic fluid (PR8 or Lee) or mouse lung PR8 (crude or purified), only a non-specific inhibitory titer of 1:40 to 1:80, which could be reduced to less than 1:10 by treatment with chick red cell absorption, trypsin, and periodate (see below), was observed.

To remove chick cell agglutinins, one part of washed, sedimented (1,000 × g, 10 min) chick red cells was added to five parts of rabbit serum at 0 C. After 20 min the cells were removed by centrifugation. Nonspecific inhibitors were removed by treatment with trypsin at 56 C (17) and periodate as indicated by Laver and Webster (13).

Hemagglutination (HA) titrations. Serial twofold dilutions of virus, starting from an accurately prepared dilution of 1:10 or 1:100 (depending on final titer) were prepared in 0.25 ml volumes of saline in WHO (World Health Organization) standard plastic trays. Equal volumes of a suspension of washed fowl red cells containing 4 × 10^6 cells/ml (as estimated with a Coulter counter) were added. Sedimentation patterns were read when cells in control wells had settled to a button. Titers are expressed as the reciprocal of the highest dilution giving partial agglutination.

Hemagglutination-inhibition (HAI) titrations. Serial twofold dilutions of heat-inactivated (56 C, 30 min) or trypsin-periodate-treated (see above) serum (0.25 ml) were mixed with equal volumes of virus dilutions adjusted to an HA titer of 4. After 30 min at room temperature, 0.25 ml of fowl red cell suspension was added. Titers are expressed as the reciprocal of the highest dilution giving complete or partial inhibition of agglutination.

To determine antiviral antibody, virus in the form of crude allantoic fluid was used. To determine antibody to the chicken host component, egg-grown virus purified by two cycles of absorption-elution with guinea pig red cells served as antigen (6).

Mice. Female, noninbred, general-purpose white NMRI mice weighing between 18 and 22 g at the beginning of experiments were purchased from Tierfarm-AG, Sisseln, Switzerland.

Immunization procedure. Dilutions of vaccine were prepared in saline in fivefold dilution steps. Immunization consisted of a single 0.5-ml intraperitoneal injection. Control mice received 0.5 ml of saline intraperitoneally. The day of vaccine application will be referred to as day 0. Trial bleedings were done on day 10 (pooled orbital bleedings), the challenge virus was inoculated on day 15, and surviving animals were sacrificed on day 25. Pretreatment with normal allantoic fluid or heterologous vaccine was performed between days -17 and 0 as described in the text.

Virus challenge and scoring system. On day 15, PR8 mouse line stock stored at -70 C was diluted 1:100 in saline and inoculated intranasally into mice lightly anesthetized with ether. This represented 1,000 to 4,000 LD_{50} of virus as judged from parallel titration on mice of the same lot and scoring only for death or survival.

Deaths in the experimental mice were recorded twice daily; survivors were sacrificed on day 25, and the lungs were examined macroscopically. The following scoring system, modified from that of Burnet (2) and Horsfall (9) was adopted. Referring again to the day of vaccination as day 0 and the day of challenge as day 15, deaths occurring on days 18, 19, or 20 scored 7, deaths on days 21 or 22 scored 6, deaths on days 23 or 24 scored 5, and mice dying spontaneously on day 25 (a rare occurrence) or sacrificed on that day scored 4 to 0, depending on the degree of lung consolidation (no lesions = 0, lesions involving up to one-fourth of the lung surface = 1, lesions involving one-fourth to one-half of the lung surface = 2, lesions involving one-half to three-fourths of the lung surface = 3, lesions involving more than three-fourths of the lung surface = 4).

Groups of at least 7 mice were used. The average score for control (unvaccinated) groups of mice was usually between 6 and 7, but in an exceptional trial an average score as low as 5.6 was observed.

Statistical analysis. Although the control experiments suggested a linear relationship between average score values and log vaccine dose (Fig. 1), it was felt that the arbitrary and semiquantum character of the disease scores as defined above did not permit the application of a simple regression analysis. Rather, it seemed sufficient for the present purpose to test the null hypothesis that pretreatment had no effect on the level of protection induced by either vaccine. This could be done by simply comparing the numbers of mice dead by day 9 (scores 5, 6, and 7) and of mice surviving to day 10 (scores 0-4) at each vaccine dosage level for the different groups. The resulting fourfold tables were combined by adding the individual chi values and dividing by the square root of the number of tables to yield a probability estimate under the null hypothesis (20).

Comparisons of mouse lung vaccines in pretreated and control groups showed no significant deviations from each other or from the egg vaccine group in any of the experiments performed. The results of these three groups were therefore pooled and compared to the pretreatment egg vaccine group as indicated in Tables 2 and 3.

RESULTS

Dose-response relationships. Using the immunization and assay procedures outlined above, the relationship between dose of vaccine administered and average disease score was rather flat but approximately linear within a 25-fold range when average score values were plotted against log vaccine dose. Figure 1 is compounded of control values obtained in five representative experiments with five different vaccine lots, three
prepared from eggs and two from mouse lungs.

With vaccines adjusted to a hemagglutinin titer of 1,600 to 3,200, dilutions of vaccines between 1:20 and 1:500 gave intermediate levels of protection useful for the present purpose. Vaccines prepared from eggs and vaccines prepared from mouse lungs had comparable potencies.

**Effect of passive administration of rabbit antisera against normal allantoic fluid on immunogenicity of PR8 vaccines.** Preliminary experiments had shown that specific antiviral antibody (rabbit anti-PR8) was a powerful inhibitor of the immunogenicity of PR8 vaccines; normal rabbit serum, when employed undiluted, also exerted an inhibitory effect (*unpublished observations*). However, no inhibition occurred when normal rabbit serum had been treated with trypsin and periodate in an effort to eliminate nonspecific hemagglutinin inhibitors, or when the gamma globulin fraction of normal rabbit serum was used.

To see whether blocking of the host antigen present in influenza virions (6) by antibodies would inhibit the immunogenicity of virus-specific determinants located on the same viral particles, serum from rabbits immunized against normal allantoic fluid and treated with trypsin-periodate (see Materials and Methods) was used as a diluent for PR8 vaccines prepared in eggs or in mouse lungs. Similarly treated serum from the same rabbits taken before immunization served as controls. Table 1 shows a representative experiment.

The vaccine prepared from eggs was inhibited in its immunogenicity by antiserum against normal allantoic fluid. The effect was not spectacular, a final serum dilution of 1:20 being effective only in exceptional cases. This experiment was varied by using the gamma globulin fraction of the same sera. Preparations containing 2.3 mg of gamma globulin per ml, prepared by ion exchange chromatography (14) and pure as judged from immunoelectrophoresis, gave results similar to those presented in Table 1. Varying the time of contact between PR8 vaccine and antibodies to normal allantoic fluid before injection from 0.5 to 16 hr did not affect the result appreciably.

**Effect of active immunization with normal allantoic fluid on immunogenicity of PR8 vaccines.** Because passive immunization against normal allantoic fluid inhibited the immunogenicity of PR8 eggs vaccine, it seemed interesting to study the effect of active immunization.

Several experiments were designed as follows. Groups of mice received either 0.5 ml of normal allantoic fluid or 0.5 ml of saline intraperitoneally on day —15 (or on other days depending on the experiment). On day 0 they were vaccinated with three graded doses of either PR8 egg vaccine or PR8 lung vaccine. All mice were challenged on day 15 and scored on day 25 as indicated in Materials and Methods. Two additional groups of unvaccinated mice (pretreated and control) were always included, so that a total of 14 groups of mice were employed. The result of an experiment in which pretreatment was 5 days before vaccination is shown in Table 2.

The results of Table 2 are typical of several experiments done in the same manner or with minor variations. Mice pretreated with allantoic fluid invariably were less well protected by egg-grown vaccine than were corresponding controls. In marked contrast to this, the protective power of PR8 mouse vaccine was not reduced. When average scores for pretreated and control groups were plotted on semilogarithmic paper, it appeared that pretreatment resulted in a parallel shift of the dose-response curve. If this impression, which was not subjected to a critical statistical analysis, were true, it should be possible to express the effect of pretreatment by a "depression factor" indicating how many times more of a given egg vaccine had to be employed to reach the same protection in pretreated as compared to control mice. From crude graphical estimations, this factor appeared to vary from one experiment to another between 5 and 20. No attempt was made to calculate confidence limits.

The time at which pretreatment with normal allantoic fluid was performed influenced the degree of depression. The longest interval tested

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**FIG. 1.** Dose-response relationship for various PR8 vaccine preparations (controls without pretreatment). Open symbols: three different egg vaccine batches. Closed symbols: two different mouse lung vaccine batches.
Table 1. Effect of diluting PR8 vaccines in anti-allantoic serum on immunity to challenge

<table>
<thead>
<tr>
<th>Group</th>
<th>Vaccination</th>
<th>Individual disease scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 None (saline)</td>
<td></td>
<td>7 7 7 7 6 6 5 4</td>
</tr>
<tr>
<td>2 PR8 egg vaccine 1:100 in saline</td>
<td></td>
<td>7 6 4 3 2 1 0 0 0</td>
</tr>
<tr>
<td>3 PR8 mouse lung vaccine 1:100 in saline</td>
<td></td>
<td>6 4 3 1 1 1 0 0</td>
</tr>
<tr>
<td>4 None (anti-allantoic serum 1:10)</td>
<td></td>
<td>7 7 7 7 6 6 6 6</td>
</tr>
<tr>
<td>5 PR8 egg vaccine 1:100 in anti-allantoic serum 1:10</td>
<td></td>
<td>7 7 7 6 5 5 4 3 3</td>
</tr>
<tr>
<td>6 PR8 mouse lung vaccine 1:100 in anti-allantoic serum 1:10</td>
<td></td>
<td>5 3 3 1 1 0 0 0</td>
</tr>
<tr>
<td>7 None (normal rabbit serum 1:10)</td>
<td></td>
<td>7 7 7 7 6 5 5 5</td>
</tr>
<tr>
<td>8 PR8 egg vaccine 1:100 in normal rabbit serum 1:10</td>
<td></td>
<td>6 6 3 2 1 0 0 0</td>
</tr>
<tr>
<td>9 PR8 mouse lung vaccine 1:100 in normal rabbit serum 1:10</td>
<td></td>
<td>6 6 4 2 1 0 0 0</td>
</tr>
</tbody>
</table>

* The difference between group 5 and the pooled groups 2, 3, 6, 8, and 9 is statistically significant \( P < 0.01 \).

b For scoring system and statistical treatment, see Materials and Methods.

c Serum from rabbit 3596 immunized with normal allantoic fluid and treated with trypsin-periodate.

d Serum from rabbit 3596 taken before immunization and treated with trypsin-periodate.

Table 2. Effect of preimmunization with normal allantoic fluid on immunogenicity of PR8 vaccines

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Group</th>
<th>Vaccination</th>
<th>HAI titers</th>
<th>Individual disease scores</th>
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</thead>
<tbody>
<tr>
<td>None (saline)</td>
<td>1 None (saline)</td>
<td></td>
<td>&lt;10</td>
<td>7 7 7 7 6 6 6</td>
</tr>
<tr>
<td></td>
<td>2 PR8 egg vaccine 1:20</td>
<td></td>
<td>160</td>
<td>7 2 1 0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>3 PR8 egg vaccine 1:100</td>
<td></td>
<td>80</td>
<td>7 7 3 1 1 1 0</td>
</tr>
<tr>
<td></td>
<td>4 PR8 egg vaccine 1:500</td>
<td></td>
<td>80</td>
<td>7 7 7 6 5 5 4</td>
</tr>
<tr>
<td></td>
<td>5 PR8 mouse lung vaccine 1:20</td>
<td></td>
<td>80</td>
<td>2 1 1 1 0 0 0</td>
</tr>
<tr>
<td></td>
<td>6 PR8 mouse lung vaccine 1:100</td>
<td></td>
<td>80</td>
<td>7 7 6 6 2 0 0</td>
</tr>
<tr>
<td></td>
<td>7 PR8 mouse lung vaccine 1:500</td>
<td></td>
<td>80</td>
<td>7 7 7 7 5 1 0</td>
</tr>
<tr>
<td>0.5 ml of normal allantoic fluid on day -5</td>
<td>8 None (saline)</td>
<td></td>
<td>&lt;10</td>
<td>7 7 7 7 6 6 6</td>
</tr>
<tr>
<td></td>
<td>9 PR8 egg vaccine 1:20</td>
<td></td>
<td>40</td>
<td>7 6 6 5 5 2 1</td>
</tr>
<tr>
<td></td>
<td>10 PR8 egg vaccine 1:100</td>
<td></td>
<td>20</td>
<td>7 7 7 6 6 6 4</td>
</tr>
<tr>
<td></td>
<td>11 PR8 egg vaccine 1:500</td>
<td></td>
<td>&lt;10</td>
<td>7 7 7 7 6 6 4</td>
</tr>
<tr>
<td></td>
<td>12 PR8 mouse lung vaccine 1:20</td>
<td></td>
<td>80</td>
<td>2 1 0 0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>13 PR8 mouse lung vaccine 1:100</td>
<td></td>
<td>80</td>
<td>7 6 5 2 1 0 0</td>
</tr>
<tr>
<td></td>
<td>14 PR8 mouse lung vaccine 1:500</td>
<td></td>
<td>80</td>
<td>7 7 7 6 5 3 0</td>
</tr>
</tbody>
</table>

* The results of groups 9, 10, and 11 taken together differ significantly from those of the pooled groups 2 + 5 + 12, 3 + 6 + 13 and 4 + 7 + 14 \( P < 0.01 \).

b Hemagglutination-inhibition as measured against crude PR8 allantoic fluid.

c For scoring system and statistical treatment, see Materials and Methods.

Effect of preimmunization with B/Lee on immunogenicity of PR8 vaccines. It seemed likely that the effect produced by normal allantoic fluid would also be observed after pretreatment with crude infected allantoic fluid. To avoid complications from cross-reacting viral antigens, allantoic fluids containing influenza virus B/Lee, which shares no antigens with PR8, were used.

was 17 days, when a distinct depression regularly occurred. When the time interval between pretreatment and vaccine was shortened, depression increased, reaching a maximum around day -5.
Such pretreatment indeed caused inhibition of the immunogenicity of PR8 egg vaccine. The following application forms of B/Lee were tried with much the same results: crude undiluted infected allantoic fluid; B/Lee concentrated 10× by centrifugation from infected allantoic fluid; B/Lee concentrated by centrifugation and inactivated with Formalin; B/Lee purified from allantoic fluid by two cycles of absorption-elution with guinea pig red cells, inactivated with Formalin, and adjusted to a HA titer of 1,600. B/Lee was only applied on days -17 or -5 and was always given by the intraperitoneal route. Purification of the virus did not seem to impair its capacity to interfere with subsequent PR8 vaccination. As with allantoic fluid, pretreatment on day -5 was more effective than on day -17, particularly with respect to antihemagglutinin titers. Table 3 shows an experiment in which mice were pretreated with purified, inactivated B/Lee 17 days before vaccination with PR8.

**DISCUSSION**

It is not surprising that the passively administered antibody exerted an immunosuppressive effect. Exactly the same had been observed earlier, only in the reverse situation. In that case passive antiviral antibody inhibited the response to host determinants of the virus (16). Conceivably, antibody directed against the host component of egg-grown virus somehow covers viral antigens, just as it is able to inhibit hemagglutination by the virus (6). An analogy is presented by suppression, through anticarrier antibody, of the immune response to a hapten (7).

In contrast to this, the findings with active preimmunization were quite unexpected. In the reverse system already mentioned, the opposite had been found. Here active immunization against virus-specific determinants enhanced the response to subsequent injection of host determinants (16). It was therefore anticipated that
an animal actively immunized against allantoic fluid would be "primed" to an injection of egg-grown virus; in fact, preimmunization with allantoic fluid or heterologous egg-grown vaccine proved immunosuppressive to later vaccination with egg-grown PR8. It is unlikely that this effect was due to antigenic competition, since the immunogenicity of PR8 prepared in mouse lungs was not affected. For the same reason it is also unlikely that differences in distribution of antibody (secreted versus blood-borne) could account for the findings.

The outcome of experiments in which compound antigens of the type A-B and A-C are sequentially applied is unpredictable (15). In certain cases preimmunization with A-B followed by immunization with A-C enhances the response to C. This may be because A functions as a carrier, and the increase in numbers of A-reactive cells more than outweighs the immunosuppressive effect of the concomitantly induced anti-A antibody (11). In other instances the reverse may happen. Thus, in the experiments presented above, it is possible that preimmunization resulted in only a negligible increase in cells reactive with the chicken host antigen, but in sufficient circulating antibody to exert an immunosuppressive effect.

Any interpretation of my findings must be largely speculative since the situation is far more complicated than appears at first sight. The effect of preimmunization was more pronounced on the level of protection achieved than on the level of circulating antibody. Protection against challenge bears no simple relation to antibody titers as measured in vitro (5). Moreover, it is probably not only due to circulating antibody, but also to cellular immunity. It would be interesting to measure, in similar experiments, not the direct response to challenge, but the neutralizing capacity of serum; this would have the advantage of yielding inherently steeper dose-response curves (1, 3, 12) but would, of course, ignore an important aspect of vaccine efficacy.

Another difficulty is presented by the observation that a short interval between first and second treatment (5 days) was somewhat more suppressive than a longer one (15 to 17 days). If the idea is correct that circulating antibody acts by attaching to the host antigen in the virion, thereby covering or sterically hindering access to viral antigens, then it could be easily imagined that the bulkier 19S antibody arising early in immunization might be a more effective immunosuppressant than the smaller 7S antibody appearing later. Henry and Jerne (8), however, have shown that 7S antibody is effective as an immunosuppressant, whereas 19S antibody enhances immunogenicity. In contrast to this work which used a homologous antigen-antibody system, my experiments involved antigens which were not even cross-reactive, but simply happened to be located on the same viral particle. It is therefore difficult to apply the findings of Henry and Jerne to the present situation.

The statistical analysis was kept at a minimum, its only purpose being to test whether chance variations were likely to account for the reduced protection afforded by egg-grown vaccine in pretreated mice. Because the scoring system employed is not universally accepted and the distribution of its semiquantal data is unknown, it seemed safer to discriminate simply between mice dead by day 9 and those surviving to day 10. Although this procedure obviously wasted some rather elaborately won information, it was chosen because it was simple and unequivocal. It showed that the odds against running into results such as those shown in Tables 2 and 3 by mere chance were formidable. Considering that several experiments of the types exemplified by Tables 2 and 3 were done, each yielding similar degrees of significance, the compound probability of a spurious effect would seem remote indeed.

My experiments have certain intrinsic shortcomings which should not go unmentioned. It was felt that inclusion of a vaccine prepared in mouse lungs was important as an internal control to exclude any wholesale immunosuppressive or adjuvant effect. Furthermore, since it was unlikely that a moderate effect would show at either end of the dose-response curve (for instance, the contribution of the 1:500 vaccine dosage in Tables 2 and 3 to statistical significance is immaterial), three graded doses of each vaccine had to be employed in order to have one or two doses within the useful range. The usual experiment therefore comprised 14 groups of mice. With 7 animals per group this required approximately 100 mice, a number easily coped with by an experimenter assisted by a technician. However, in order to be able to repeat the same experiment a reasonable number of times, the only parameter which was varied (within rather narrow limits) was the time interval between pretreatment and vaccination, all other parameters being kept constant. Thus, pretreatment was always by a single intraperitoneal injection, as was vaccination, for which a single type of vaccine was employed; furthermore, the interval between vaccination and challenge was always the same, and the challenge dose was kept constant. It may be that simply postponing challenge by a few days or weeks would eliminate the difference observed, which would then be confined to the realm of mere epiphenomena of little practical
impact. To obtain a deeper insight into these complex interrelationships, multifactorial experiments should be designed. The logistic problems involved rapidly reach dimensions beyond the capacity of a small laboratory.

In spite of the doubts surrounding the interpretation of my data, they might have disturbing practical implications, for it is possible that repeated vaccination with any type of egg-grown influenza virus could lead to similar effects. Obviously it would be more difficult to reveal the phenomenon if the virus used for what I have called “preimmunization” and that used for immunization illustrated cross-reacting antigens. An experiment illustrating this point has been reported by Slnotnick (19). Mice received repeated injections of large doses of inactivated influenza vaccine shortly after birth; their response to a later (very large) “immunizing” injection of the same vaccine was measured and found to be considerably depressed. Although this outcome was interpreted in terms of immunological tolerance, alternative explanations were left open.

If effects similar to those reported here occur in man, one might expect that a high level of immunity against the chicken host antigen, built up in the course of repeated vaccination, could decrease the effectiveness of a further dose of influenza vaccine. Hence the usually recommended schedule of yearly vaccination against influenza with vaccines brought up to date with respect to the last epidemic strain, but always prepared in eggs, would be self-defeating, and particularly in those individuals one wishes to protect most (4). It should be noted in passing that highly purified vaccines or subunit vaccines would not solve the problem, since they still contain the host antigen (6, 13). Rather, one would have to consider the use of influenza vaccines prepared in human tissues.

The thought is worth pondering that the phenomenon, should it occur in man with influenza vaccines as currently employed, was almost certain to be missed, since vaccine potency is usually assessed by laboratory tests and by seroconversion rates in previously unvaccinated individuals. The efficacy of a vaccine in age groups having already been subjected to numerous vaccinations has never been adequately evaluated (4). Nevertheless, this is the main factor on which success or failure of a large-scale vaccination campaign against a new influenza pandemic would rest. Herein perhaps lies a key to the observation that influenza vaccines which seem promising from laboratory data yield indifferent prophylactic agents in the field (18). The questions at stake seem important enough to warrant a large-scale inquiry.

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LITERATURE CITED