COMPARATIVE TRIAL OF INFLUENZA VACCINES

1. IMMUNOGENICITY OF WHOLE VIRUS AND SPLIT PRODUCT VACCINES IN MAN

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Groups of about 100 persons aged 6 to 88 years were given 1 of 6 commercially prepared whole virus or split-product bivalent (A/England-B/Mass) influenza vaccines and 6 weeks later were given 1 of 5 monovalent (B/Hong Kong) vaccines. Hemagglutination-inhibiting (HI) antibody titers in serum specimens taken 6 and 12 weeks after inoculation were compared to those obtained before immunization. Overall antibody responses in all groups were adequate, yielding HI titers that are associated with relatively good levels of protection from infection. No differences were noted among the vaccines in their ability to boost pre-existing antibody. The tributyl phosphate (TBP) split-product vaccine, however, induced significantly lower homologous seroconversion and geometric mean antibody titers (GMT) to A/England and heterologous antibody titers to A/Aichi in persons without pre-existing antibody than did equivalent whole virus vaccines. Both the TBP and the ether-treated monovalent B/Hong Kong vaccines also induced lower heterologous GMT's to B/Mass in initially seronegative individuals. These data agree with previous observations that the primary response to influenza and other viral vaccines prepared from disrupted virions results in lower levels of antibody than does that to equivalent whole virus preparations. Studies are underway to determine whether the lesser immune response induced by these vaccines in seronegative persons is the result of smaller amounts of antigen in such preparations or because the antigen may be processed less efficiently by humoral or cellular immune mechanisms.

INTRODUCTION

Disruption of influenza viral particles by a variety of detergents and solvents (1, 2) has been employed in the preparation of certain inactivated influenza vaccines for over 15 years in an effort to separate the immunogenic glycoprotein surface antigens from the possibly reactogenic or pyro-

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Abbreviations: CCA, chick cell agglutination; EM, electron microscopy; ET, ether-treated; GMT, geometric mean titer; HI, hemagglutination inhibition; MID50, 50% minimum immunizing dose; TBP, tributyl phosphate; VP, viral particles.

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genic protein, lipid, or nucleic acid constituents of the virus (3). Previous clinical studies in humans have shown that commercially available split-product influenza vaccines induce approximately the same overall antibody response as whole virus vaccines (4–19). The interpretation of many of the studies, however, has been difficult because they were noncomparative (15–18) or because of differing routes of inoculation (11, 14, 19), the use of adjuvants (4, 5, 9, 13, 14, 17), the use of potency tests other than the chick cell agglutination (CCA) test (5, 6, 10, 13, 18) or the failure to segregate the immune response of individuals with and without pre-existing antibody (9, 10, 13–15, 19). Virtually all vaccines, however, made from a variety of plant (20–22) or animal (23–32) viruses are poor primary immunogens if the entire virus is not included in the vaccine. A notable example has been the observation that while ether disruption increases the hemagglutination titer of inactivated measles preparations (27), 3–4 times more disrupted antigen than whole virus antigen must be used to induce an equivalent serologic response (28).

Influenza vaccines disrupted by Tween 80 and either tri-n-butyl phosphate (TBP) or ether have also been found to be poor primary immunogens in mice (29–32) and ferrets (33), when compared to equivalent whole virus vaccines. In addition, a recent study indicated that a TBP split-product vaccine provided less protection from homologous infection than an equivalent whole virus vaccine (19) and that such a vaccine provided minimal protection from heterologous influenza infection and its sequelae in an elderly population (34). Because of the paucity of comparative data available concerning the primary and anamnestic immune response to modern, highly purified influenza vaccines, and since the level of antibody to the hemagglutinin component of influenza virus is closely correlated to the degree of protection from infection in man (35), the present study was designed to evaluate the hemagglutination inhibiting (HI) antibody response to split-product and whole virus inactivated influenza vaccines in seronegative and seropositive children and adults.

**Materials and methods**

**Vaccines.** Bivalent vaccines containing the A/England/42/72 (H3N2) and B/Mass/1/71 antigens produced by the six US manufacturers of influenza vaccine, and monovalent vaccines containing the B/Hong Kong/5/73 antigen produced by five US manufacturers, were purchased from commercial distributors. All vaccines had passed minimum requirements for safety and potency and were in general clinical use at the time of the study. The method of manufacture and CCA content of the vaccines are included in tables 1 and 2.

**CCA Testing.** CCA determinations were performed as previously described (36) and modified (37) to include a standard reference. Vaccine-reference (V/R) ratios thus obtained were multiplied by the assigned value of the 1972 reference (1886 CCA units/ml) and the results expressed as CCA units/human dose (0.5 ml). The coefficient of error of the CCA test has approximated 14 per cent in our laboratory over a two-year period. As the A and B components of the bivalent vaccines were formulated at a ratio of 7 to 3, 70 per cent of the CCA value for an individual bivalent vaccine was assigned to the A/England component and 30 per cent to the B/Mass component in calculating antigen doses in these studies.

**EM particle counts.** Enumeration of viral particles in the whole virus vaccines was performed by a modified sedimentation technique (38) in McIlvain's buffer to increase dispersion of aggregates. Forty randomly chosen electron microscopic fields of each preparation were examined for viral particle content, and an estimate was made of the amount of non-viral particulate material.
TABLE 1

Antigenic content of influenza vaccines (bivalent)

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Type*</th>
<th>A/England strain</th>
<th>B/Mass strain</th>
<th>Total CCA content†</th>
<th>Electron microscopic particle count x 10^4†</th>
<th>Mouse immunizing dose, potency§</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZP-1</td>
<td>ZPWV</td>
<td>A/England/42/72</td>
<td>B/Mass/1/71</td>
<td>990</td>
<td>15.15</td>
<td>0.85 0.31</td>
</tr>
<tr>
<td>ZP-2</td>
<td>ZPWV</td>
<td>A/Victoria/4/72</td>
<td>B/Mass/1/71</td>
<td>1150</td>
<td>9.00</td>
<td>1.68 1.50</td>
</tr>
<tr>
<td>ZP-3</td>
<td>ZPWV</td>
<td>X-37‡</td>
<td>B/Mass/1/71</td>
<td>1440</td>
<td>25.65</td>
<td>&lt;1.0 0.67</td>
</tr>
<tr>
<td>CP</td>
<td>CPWV</td>
<td>X-37A§</td>
<td>B/Mass/1/71</td>
<td>1050</td>
<td>22.70</td>
<td>&lt;0.73 0.39</td>
</tr>
<tr>
<td>TBP</td>
<td>TBP</td>
<td>X-37</td>
<td>B/Mass/1/71</td>
<td>1120</td>
<td>&gt;78</td>
<td>&gt;34</td>
</tr>
<tr>
<td>ET</td>
<td>ET</td>
<td>X-37</td>
<td>B/Mass/1/71</td>
<td>1240</td>
<td>&gt;87</td>
<td>&gt;37</td>
</tr>
</tbody>
</table>

* ZPWV, zonally purified whole virus; CPWV, chromatographically purified whole virus; TBP, tributylphosphate-treated split product; ET, ether-treated split product.
† Per 0.5 ml adult dose.
‡ High growth recombinant (H3N2) produced from A/England/42/72 x A/PR/8.
§ High growth recombinant (H3N2) produced from MRC-7 (A/England/42/72 isolated in eggs x A/PR/8) x A/PR/8.
¶ Number of CCA units required to seroconvert (<1:8→1:8 HI titer) 50% of mice tested (31).

TABLE 2

Antigenic content of influenza vaccines (monovalent)

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Type*</th>
<th>B/Hong Kong strain</th>
<th>Total CCA content†</th>
<th>Electron microscopic particle count x 10^4†</th>
<th>Mouse immunizing dose, potency§</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZP-1</td>
<td>ZPWV</td>
<td>B/HK/5/72</td>
<td>610</td>
<td>7.55</td>
<td>0.86</td>
</tr>
<tr>
<td>ZP-2</td>
<td>ZPWV</td>
<td>B/HK/5/72</td>
<td>620</td>
<td>3.70</td>
<td>1.87</td>
</tr>
<tr>
<td>ZP-3</td>
<td>ZPWV</td>
<td>BX-1‡</td>
<td>575</td>
<td>13.45</td>
<td>0.58</td>
</tr>
<tr>
<td>TBP</td>
<td>TBP</td>
<td>B/HK/5/72</td>
<td>785</td>
<td></td>
<td>24.5</td>
</tr>
<tr>
<td>ET</td>
<td>ET</td>
<td>B/HK/5/72</td>
<td>576</td>
<td></td>
<td>18</td>
</tr>
</tbody>
</table>

* Abbreviations as in table 1.
† Per 0.5 ml adult dose.
‡ High growth recombinant produced from B/Hong Kong/5/72 x B/Lee/40.
§ Number of CCA units required to seroconvert (<1:8→1:8 HI titer) 50% of mice tested (31).

Mouse immunogenicity tests. Fifty percent minimum immunizing dose (MID₅₀) calculations were obtained by immunizing 20 mice intraperitoneally with 0.5 ml of 3 Log₁₀ dilutions of the 11 vaccines and determining their HI antibody titers 14 days later as described previously (31). The results are given in terms of the number of CCA units required to induce an HI antibody level of ≥1:8 in 50 per cent of the mice.

Subjects. Individuals receiving the vaccine were students and staff members at the Arkansas Children’s Colony, a residential school for the mentally retarded located in a rural setting near Conway, Arkansas. Experimental protocols were formulated in accordance with the Declaration of Helsinki (39) and were approved by institution review committees of the Bureau of Biologics, the Food and Drug Administration and the Arkansas Children’s Colony. The Children’s Colony had been the site of previous trials of rubella (40) and mumps (41) vaccines, and the staff members, parents, and guardians were familiar with the procedures and conduct of viral vaccine trials.
It is the practice of the Arkansas Children's Colony to vaccinate all children against influenza on an annual basis, and the majority of the subjects had received an ether-treated vaccine containing A/Aichi and B/Mass antigens the year prior to the study. After appropriate informed consent had been obtained from the individual, parent, or guardian, serum samples were obtained from the 438 students and 149 staff members participating in the study. The subjects then received a single 0.5 ml subcutaneous inoculation in the right deltoid area of one of the six bivalent vaccines. Children aged 6-10 received only 0.25 ml of the vaccine in accordance with recommendations of the Committee on Infectious Diseases of the American Academy of Pediatrics (42). Six weeks later the identical procedure was repeated except that the monovalent B/Hong Kong vaccine was given rather than the bivalent A/England-B/Mass preparation. A final serum sample was taken six weeks after the monovalent B/Hong Kong inoculation. The students ranged in age from six to 33 years and the staff members from 18 to 88 years. Groups receiving each vaccine were matched for sex, age, and degree of mental development.

Serologic testing. Sera were treated with Vibrio cholerae neuraminidase, heated at 56 C for 30 minutes, and incubated with chick erythrocytes to remove nonspecific inhibitors (43) before being used in the HI tests. The treated samples were then tested in duplicate by the HI procedure as modified for microtitration plates (44) on an automatic diluting and dispensing apparatus (Dynatiter, Cooke Engineering Co., Alexandria, Va.). The serum samples were tested in twofold dilutions starting at 1:8, and 4 to 8 units of homotypic whole virus antigen was added to each well. For the B/Hong Kong antibody titrations, the BX-1 recombinant (B/Hong Kong/5/72 × B/Lee/40) strain which had been passed 22 times in embryonated eggs by Dr. Walter Dowdle of the Center for Disease Control was used, as lower passage B/Hong Kong virus was found to be relatively insensitive to homotypic antibody. The preinoculation and two postinoculation serum samples from any individual were tested at the same time and an equal number of sera from each vaccine group were titrated during any individual test day, as well as representative sample sera which had been tested repetitively and served as inter-test references. The day's test was repeated if the reference titers varied fourfold or greater, and individual sets of sera were retested if duplicate samples of any one of the set varied twofold or greater.

Statistics. Seroconversion (≤1:8 → ≥1:8) rates were compared by the Z-test of relative proportions, and postinoculation geometric mean antibody titers (GMT) of individuals who were initially seronegative were compared by the student t-test. In the calculation of mean antibody titers, sera which demonstrated no antibody at the lowest dilution (1:8) were considered as positive at 1:4.

RESULTS

Antigenic content. The total CCA content of the bivalent vaccines varied from 990 CCA units/dose for ZP-1 to 1440 CCA units/dose of ZP-3 (table 1). The split-product vaccines (TBP and ET) had a CCA content intermediate in this range and were similar to the value obtained for ZP-2. The monovalent vaccines varied to a lesser extent in CCA content, with vaccine TBP having 785 CCA units/dose and vaccines ZP-3 and ET the lowest at 575 and 576, respectively; vaccines ZP-1 and ZP-2 were intermediate with 610 and 620 CCA units/dose (table 2). A much greater variation among vaccines was noted when the viral particles (VP) were enumerated by electron microscopy. Bivalent vaccines ZP-2 and CP, for example, while having a similar CCA content, varied 25% per cent in their total viral particle content. Similarly,
monovalent vaccines ZP-2 and ZP-3 varied only 8 per cent in their CCA content but had a 265 per cent difference in their viral particle content. No one-to-one relationship, therefore, could be found between the number of CCA units in a given vaccine and its viral particle content. This observation is consistent with our previous findings that the CCA/VP ratio of vaccines prepared by different techniques may vary up to 400 per cent (38). Accurate EM quantitation of the viral particle content of split-product vaccines could not be obtained because many of the particles in the ET vaccines were disrupted or, in the TBP vaccines, were degraded and could not be distinguished from non-viral debris sedimented on the grid.

Great differences were noted, as previously described (31), between the whole virus and split product vaccines when the mouse immunogenicity test was used as a measure of antigenicity. For the A/England component, between less than 0.73 to 1.68 CCA units of whole virus vaccines were required to seroconvert 50 per cent of the mice tested, whereas greater than 78 to 87 CCA units of the split-product vaccines were required to produce the same seroconversion rate in mice (table 1). Likewise, for the B/Mass component, between 22 and 119 times as much split product vaccine as whole virus vaccine was required to produce 50 per cent seroconversion in mice. Differences in the MID50 titer of the monovalent Hong Kong vaccines were less striking (table 2) but nearly 10 times as much of the split-product vaccine as whole virus vaccine was required to produce 50 per cent seroconversion in mice. Differences in the MID50 titer of the monovalent Hong Kong vaccines were less striking (table 2) but nearly 10 times as much of the split-product vaccine giving the best MID50 titer (vaccine ET) as the whole virus vaccine having the worst MID50 (vaccine ZP-2) was needed to obtain an equivalent response.

**Overall serologic responses.** The geometric mean serum HI antibody titer (GMT) of the vaccinees (figure 1) to the homologous A/England antigen rose from 1:13.6 before inoculation to 1:31.8 six weeks after inoculation and dropped slightly to a level of 1:27.1 by 12 weeks after inoculation. The 2.34-fold mean increase in HI antibodies at the first postinoculation sampling was more modest than had been noted in previous studies of inactivated influenza vaccine immunogenicity in humans (5, 45), and may in part be related to the fact that the sample was taken several weeks after peak antibody levels had been reached and were in fact already falling. In the present study, intervals of 1½ to 3 months between inoculation and serum sampling were chosen, rather than the usual 2–4 week intervals when antibody response is maximal (46) because it was thought to be more relevant to the normal clinical situation where immunization is completed by mid-November and epidemic exposure occurs in December, January and February.

Antibody responses to the A/Aichi (H3N2) virus which caused a major pandemic in 1968–1969, rose and fell in a manner parallel to that of the A/England virus which caused moderate epidemic ill-

The rises in HI antibody titers to B/Mass six weeks after bivalent vaccine inoculation were less (1:22.6 to 1:35.8) than those to the A antigens. The 1.18-fold (1:35.8 to 1:42.2) increase in B/Mass antibody seen between six and 12 weeks was the result of a heterologous response to B/Hong Kong vaccine given at six weeks. This observation, together with the 1.18-fold (1:8.9 to 1:12.4) increase in HI antibodies to B/Hong Kong six weeks after inoculation with the bivalent vaccine containing B/Mass antigen, would indicate a greater degree of serologic cross-reactivity in humans between B/Mass and B/Hong Kong than had been noted in animals (48).

This study was conducted before introduction of the B/Hong Kong virus into the United States had been documented. The preinoculation GMT of 1:8.9 to this virus is in part a reflection of the fact that titers of <1:8 were calculated as positive at 1:4. Forty-three per cent of all sera had no detectable preinoculation antibodies to B/Hong Kong, and the majority of the remaining 57 per cent had low titers of 1:8 and 1:16. The possibility that a limited and undetected introduction of B/Hong Kong virus into the Arkansas population may have occurred before the initiation of the study (September 1973) cannot be discounted, because HI testing of 50 representative preinoculation sera from young subjects in the study were tested in parallel by the Center for Disease Control with sera obtained from students in Milwaukee in June of 1973; 38 per cent of the Arkansas sera had a titer of 1:10 to 1:80 whereas no detectable antibodies were found in the sera from Wisconsin (G. Noble, personal communication). The homologous rise of 1.89-fold (1:12.4 at six weeks to 1:23.4 at 12 weeks) to the B/Hong Kong antigen was slightly more than the homologous rise to B/Mass and may have been the result of either prior “priming” (49) by the B/Mass antigen or the fact that the recipients received on the average 66 per cent more B/Hong Kong than B/Mass antigen.

Response of individuals without antibody before vaccination. In comparing the antibody increases to split-product and whole virus vaccines, the responses of those who were initially seronegative (HI <1:8) were first examined in order to avoid artificial differences that might be caused by slightly differing levels of pre-existing antibodies in the various groups. Because the antibody responses of the individuals who received the whole virus vaccines were homogeneous, and significant differences were not observed in the seroconversion rates and GMT of these vaccinees, they are treated as a single group to simplify comparison with split-product vaccine recipients. The seroconversion (<1:8 —> ≥1:8) rates and GMT of initially seronegative individuals in all three groups to the four antigens tested are shown in table 3. For the A/England antigen, the TBP-treated vaccine produced a seroconversion rate of only 65 per cent by six weeks after inoculation, a rate that was significantly (p < .05) lower than that produced by the whole virus vaccines (86 per cent). The ether-treated vaccine induced an intermediate seroconversion rate of 78 per cent, and although this rate had fallen to 70 per cent by 12 weeks postinoculation, it was still not significantly different at the p < .05 level from the seroconversion rates produced by the whole virus vaccines. By 12 weeks, only 61 per cent of the TBP vaccinees who were initially seronegative had detectable A/England HI antibodies and the GMT of that group (1:12.6) was significantly lower than that of the whole virus vaccine recipients (1:20.4).

Statistically significant differences in the heterologous response to the A/Aichi antigen were more difficult to demonstrate because so few individuals had no pre-existing HI antibodies to this virus, with only eight of the ET vaccinees and five of
### Table 3

**Response of seronegatives to whole virus, ET and TBP vaccines**

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>A/England</th>
<th>A/Aichi</th>
<th>B/Mass</th>
<th>B/Hong Kong</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All vaccines combined</td>
<td>Whole virus</td>
<td>ET</td>
<td>TBP</td>
</tr>
<tr>
<td></td>
<td>6 weeks†</td>
<td>12 weeks</td>
<td>6 weeks‡</td>
<td>12 weeks‡</td>
</tr>
<tr>
<td>All vaccines combined</td>
<td>119/146 (82)*</td>
<td>35/48 (73)</td>
<td>92/121 (76)</td>
<td>104/121 (86)</td>
</tr>
<tr>
<td>Whole virus</td>
<td>86/100 (86)</td>
<td>29/35 (83)</td>
<td>66/84 (79)</td>
<td>69/169 (41)</td>
</tr>
<tr>
<td>ET</td>
<td>18/23 (78)</td>
<td>6/8 (75)</td>
<td>11/16 (69)</td>
<td>12/48 (25)%</td>
</tr>
<tr>
<td>TBP</td>
<td>15/23 (65)%</td>
<td>2/5 (40)</td>
<td>14/21 (67)</td>
<td>19/52 (37)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antigen</th>
<th>A/England</th>
<th>A/Aichi</th>
<th>B/Mass</th>
<th>B/Hong Kong</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 weeks†</td>
<td>12 weeks</td>
<td>6 weeks‡</td>
<td>12 weeks‡</td>
</tr>
<tr>
<td>All vaccines combined</td>
<td>1:20.8</td>
<td>1:24.8</td>
<td>1:20.3</td>
<td>1:16.7</td>
</tr>
<tr>
<td>Whole virus</td>
<td>1:22.8</td>
<td>1:31.3</td>
<td>1:24.9</td>
<td>1:17.4</td>
</tr>
<tr>
<td>ET</td>
<td>1:17.5</td>
<td>1:15.3</td>
<td>1:16.6</td>
<td>1:13.9%</td>
</tr>
<tr>
<td>TBP</td>
<td>1:17.0</td>
<td>1:12.6%</td>
<td>1:8.0%</td>
<td>1:14.0</td>
</tr>
</tbody>
</table>

* Number in parentheses indicates per cent.
† Homologous response.
‡ Heterologous response.
§ p < .05 when compared to whole virus.
‖ p < .01 when compared to whole virus.
†† 0.05 < p < 0.1 when compared to whole virus.
The TBP vaccinees having initial antibody titers less than 1:8. The GMT at both six and 12 weeks of those who had received the TBP vaccine, however, was less than one-third of that of the whole virus recipients and was significantly lower than the whole virus GMT at the \( p < .05 \) level.

The antibody responses to the B/Mass antigen six weeks after inoculation with the bivalent vaccine containing the B/Mass antigen, either in terms of seroconversion rates or GMT, did not differ appreciably among the recipients of the six vaccines. When the antibody response to the heterologous B antigens (six weeks after B/Hong Kong immunization and 12 weeks after the B/Mass inoculation) was examined, however, seroconversion rates in the ET and TBP vaccinees (69 per cent and 76 per cent, respectively) were lower than the 92 per cent seroconversion rate found in the whole virus vaccinees. GMT antibody levels likewise were lower (1:13.9 and 1:13.5 vs. 1:22.3) and significantly different at the \( p < .01 \) level. The “narrowing” of the antibody response to split-product vaccines is further suggested by the lower heterologous seroconversion rate of 25 per cent (vs. 41 per cent for the whole virus vaccines) to the B/Hong Kong antigen six weeks after inoculation with ET vaccine containing B/Mass antigen even though homologous seroconversion and GMT levels at 12 weeks for the three groups were indistinguishable.

Response of individuals with antibody before vaccination. The response of vaccinees who had initial HI antibody titers of \( \geq 1:8 \) is shown in figure 2. Differences in the degree of antibody rise to the different antigens among vaccinees in the three groups were small and in no instance were statistically significant. A general trend can be observed, nevertheless, that the TBP vaccine induced a lower increment of increase to the A/England, A/Aichi, and B/Mass antigens, and that both ET and TBP vaccinees showed a somewhat accelerated decrease in heterologous antibodies to the A/Aichi antigen between six and 12 weeks after inoculation with the A/England antigen. The degree of anti-B/Mass antibody increase among the ET vaccine recipients was the same following either B/Mass or B/Hong Kong vaccination and may have been the result of the relatively high preinoculation antibodies to B/Mass among vaccinees in that group. The homologous response of seropositives the recipients of TBP-treated vaccine to B/Mass was relatively poor, whereas the heterologous response (between six and 12 weeks) paralleled those in the ET and WV groups.

Discussion

The demonstration in the present study that split-product inactivated influenza vaccines, particularly those disrupted with Tween-80 and TBP, induce lower titers of antibody to homotypic and heterotypic antigens in seronegative children and adults is consistent with earlier experimental observations in animals following immunization with influenza and other viral vaccines. Antigens prepared by disrupting turnip yellow mosaic virus (20,21), tobacco mosaic virus (22), foot-and-mouth disease virus (23), rabies virus (24), poliovirus (25) and adenovirus (26) all induced lower levels of antibodies in animals than did equivalent amounts of whole virus antigen. Norrby found that 3–4 times as much ether-split than whole virus inactivated measles vaccine needed to be given to produce the same serologic response in children (28). This observation may be explained in part by the fact that while the hemagglutinin titer of paramyxovirus preparations often increases markedly after detergent disruption (27) presumably secondary to the increased availability and detection by in vitro assays of more hemagglutinin antigens, the total amount of viral antigen obviously does not change with such treatment. It would be likely that such a phenomenon may also pertain to
orthomyxoviruses such as influenza. Quantitation of myxovirus antigen by tests that depend on hemagglutination would therefore tend to give artifactually high readings for split-product antigens.

While one report has indicated that an experimentally-produced ET influenza vaccine induced somewhat better protection from infection in mice after repeated inoculations than did a whole virus vaccine (50), significant differences were not noted and the remainder of experimental studies of split-product influenza vaccines in animals would tend to indicate that the opposite is true. Davenport (29) has shown that ET vaccines made from PR/8, A/FM1/47, A/A/2/60, B/Lee/40 and B/A/3/62 all induced lower antibody levels and poorer protection from infection in mice than did equivalent whole virus preparations, but

![Graphs showing antibody titers over weeks after inoculation for different antigens and vaccine treatments.](https://academic.oup.com/aje/article-abstract/104/1/34/66270)
that this deficient response could be ameliorated by the addition of AlPO₄ adjuvant to the ET "subunit" vaccine. Neurath et al. (30) showed that although TBP split product A/Aichi vaccine in high doses (10,000 hemagglutinating units) induced a better mouse neutralizing antibody titer at four weeks after inoculation than did whole virus vaccine, the mouse neutralizing antibody titer at two weeks for this antigen, and at two and four weeks for A2/Japan and A2/Taiwan antigens was markedly lower than those induced by whole virus influenza antigens. Hinz et al. (32) also demonstrated that split-product A/Aichi vaccines induced lower antibody and protection rates in mice than did whole virus vaccines. We have shown in a previous study (31) employing the same lots of vaccine used in the present study, that the GMT and seroconversion rates in mice receiving dilutions of the ET or TBP vaccines were significantly (p < .05 to p < .001) lower than those induced by the same dilutions of whole virus vaccines. In addition, McLaren et al. (33) have shown that TBP-treated influenza vaccines induced poorer antibody and protective responses in ferrets than did smaller amounts of β-propiolactone-inactivated whole virus preparations.

Existing clinical data on the comparative immunogenicity and protectiveness of split-product influenza vaccines are somewhat less clear and are difficult to interpret. Previous published reports have indicated that ether-treated or TBP-treated vaccines induced equivalent or higher levels of antibody in humans than whole virus vaccines. Some of these reports have failed to segregate the response of seronegatives from those initially seropositive (9, 10, 13-15, 19). Others have used adjuvants that are no longer in commercially available ET influenza vaccines (4, 5, 9, 13, 14, 17). Furthermore, some studies have used measures other than the CCA test (a hemagglutination test that is currently the only official measure of influenza vaccine potency in the US) to quantitate the amount of antigen in the ET vaccine (5, 6, 10, 13, 18).

In two previous studies (14, 19) the ET vaccines were given by the intramuscular route, which is thought to produce a better immune response (11, 51) while the whole virus vaccine was given subcutaneously. In the present study, all vaccines were given by the subcutaneous route even though the package insert of the split-product vaccines recommended intramuscular inoculation, because many of the whole virus vaccines were not approved at that time for intramuscular injection. Since there was both published and unpublished information that differing routes of inoculation could affect subsequent antibody titer, it was thought that, in order to obtain valid data on the comparative immunogenicities of the vaccines, they should all be given by the same route.

Although studies on the comparative efficacy of ET vaccines in preventing influenza infection have not been published, such studies have been carried out with TBP vaccine and the results have been disappointing. Ruben et al. (19) found that whole virus vaccine given before an epidemic induced an 80 per cent reduction in clinical illness while TBP split-product vaccine induced only a 54 per cent reduction, a rate that was barely (p = .05) significantly different from those receiving no vaccine. In a more recent study conducted in a nursing home (34), no difference was noted in the A/England influenza attack rate and death from influenza rate between comparable patients who had received A/Aichi TBP vaccine and those receiving no vaccine. Such a difference could be noted, however, if the most debilitated inhabitants of the nursing home, housed on a single floor where virtually no vaccine was given, were included in the control group. In two other studies, where whole virus A/Aichi vaccine was given, good protection was noted during an A/England epidemic (52, 53).
The fact that only in individuals initially seronegative to an antigen did split-virus vaccines sometimes induce a lesser antibody response to that antigen than the antibody response following whole virus vaccines is consistent with our present knowledge of the immune response to inactivated influenza vaccines. It has been estimated that when overall antibody responses (seronegatives and seropositives combined) to influenza immunization are examined, a 10-fold difference in the amount of antigen in the vaccine will produce only a twofold difference in mean HI antibody titer (45, 54). Since primary antibody responses to influenza are more difficult to induce than anamnestic ones (55), it might be expected that the response of seronegatives would be a more sensitive indicator of the antigenicity of a vaccine than the response of those who have already had experience with the antigen. Such has been the case in experimental animals where the majority of the data was obtained from the response of mice that had had no prior antigenic experience with influenza (29–32). Comparison of the observations in tables 1 and 2 with those in table 3 indicates that the difference in immunogenicity between whole virion and split virus vaccines is far greater for mice than for seronegative humans. A possible explanation is that, in children and adults who are seronegative to a given antigen, the antibody response observed following influenza immunization may not be purely primary in character either because prior experience with the homologous antigen may have induced only small amounts of antibody, below the level detectable by the HI test (1:8), or because of prior experience with a closely or even distantly related antigen where the principles of "original antigenic sin" (56) would be operative and an accelerated immune response would be expected. Thus, only individuals (chiefly very young children) with no prior experience with an influenza antigen, either through infection or vaccination, might be expected to manifest a truly "primary" response comparable to that seen in experimental animals. Previous studies of the response of very young children to influenza immunization have found that significant and protective levels of antibody are very difficult to induce with either split-product (6, 18) or non-zonally purified whole-virus antigens (6). Recent reports, however, have shown that zonally purified whole virus vaccines induce adequate levels of HI antibodies in the majority of infants and young children who received them (57, 58).

The occasional observation of differences in heterologous but not in homologous response between whole virus and split-product antigens may be explained by the fact that decreasing amounts of viral antigen in influenza vaccine preparations induce antibodies to fewer heterologous antigens (59). Certain of the split product vaccines may therefore have retained sufficient antigenicity to produce a homologous antibody response that was not significantly different from the whole virus preparation but insufficient to induce an equivalent heterologous response.

The immune mechanisms underlying the lesser primary immunogenicity of split-product influenza vaccines can only be speculated upon. Whether it is merely the result of less antigen that might be present because of the "Norrby effect" or because the split product preparations, whose antigens are considerably smaller in size than those of whole virus vaccines, may not be handled optimally by the effectors of the immune response are the subject of current investigations. The use of such vaccines in young children with little or no prior influenza antigenic experience will provide additional information on the antigenicity of such vaccines in human immunologic "virgins" before the next major antigenic shift.
of the influenza antigen renders the majority of the population seronegative to the new strain.

REFERENCES


