Reduction of Endotoxin Levels in Influenza Virus Vaccines by Barium Sulfate Adsorption-Elution

PATRICIA S. REICHELDERFER,* JODY F. MANISCHEWITZ, MARTHA A. WELLS, HERBERT D. HOCHSTEIN, AND FRANCIS A. ENNIS

Division of Virology and Division of Control Activities, Bureau of Biologics, Food and Drug Administration, Bethesda, Maryland 20014

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The level of endotoxin in influenza virus vaccine lots was reduced 10- to 20-fold after barium sulfate adsorption-elution. The amount of viral antigen lost was negligible.

Influenza vaccines are produced by concentrating allantoic fluids and this cannot be performed in a manner that will assure sterility. Control screening of vaccines from various manufacturers has revealed variation in the levels of contaminating bacterial endotoxins. Therefore, we investigated the various means by which the level of endotoxin could be reduced during virus purification. One of the techniques used in the past to purify and concentrate influenza virus has been the adsorption of virus onto barium sulfate (1, 3). This method was examined for its efficacy in removing bacterial endotoxins.

Vaccine lots purified by either zonal centrifugation or by column chromatography, but not treated with barium sulfate, were tested for endotoxin content. We then subjected four of these lots representing both whole and split virus vaccines and three different manufacturing techniques to barium sulfate treatment according to the following procedure.

Vaccines were mixed with an equal volume of 12.5% barium sulfate made up in pyrogen-free water. This mixture was stirred for 30 min at room temperature and then centrifuged at 800 × g for 15 min. The supernatant (i) was removed and the pellet was washed once with 1 volume of 0.5% sodium citrate, pH 7.0. After centrifugation the supernatant (ii) was removed and the virus was eluted by resuspending the pellet in 1 volume of 10.0% sodium citrate, pH 8.0, and agitating the suspension for 20 to 30 min at room temperature. After sedimentation, the eluate (iii) was removed for testing. In the initial experiments this last step was repeated using 10.0% citrate at 50% of the initial virus volume. However, since most of the virus was removed after the first elution, this second step was not employed with subsequent samples.

Each of the various supernatants was assayed for virus content and endotoxin concentration. Assays for viral hemagglutinin were performed in microtiter plates using a standard assay procedure (4, 5). The limulus amebocyte lysate test for endotoxin levels was performed by methods described earlier (2).

Assays of the various supernatants revealed that most of the virus became attached to the

\[ \text{FIG. 1. Reduction in the level of endotoxin after barium sulfate adsorption-elution. The numbers represent four sample vaccine lots from different manufacturers. Symbols: stipling, initial concentration of endotoxin (log}_{10} \text{ng/ml}; blank bar, final concentration of endotoxin (log}_{10} \text{ng/ml}; cross hatching, initial hemagglutinin titer (log}_{2}; diagonal hatching, final hemagglutinin titer (log}_{2}. \]
barium sulfate during the 30-min adsorption period. The endotoxin was lost primarily during the initial adsorption. The results of adsorption-elution with the four vaccine lots are shown in Fig. 1. The amount of virus lost in all cases was no greater than a twofold dilution of the initial concentration as measured by hemagglutinating activity, while the level of endotoxin was reduced 10- to 20-fold.

A second adsorption and elution step was tried to further reduce the endotoxin levels. The results obtained (Fig. 2) show that, while endotoxin levels were reduced to negligible amounts, virus yield was also greatly reduced. Assay of the initial supernatant, (i), showed that most of the virus failed to adsorb. Apparently, previous adsorption-elution reduces the possibility of the virus readsorbing, thus making a two-step process commercially undesirable.

Although a direct link between increased reactivity and levels of endotoxin in influenza virus vaccine has not been observed (D. Barry and H. Hochstein, unpublished data), it is generally felt that the lowest possible level of vaccine contamination with extraneous substances is desirable. During the purification of virus for vaccine manufacture by either ultracentrifugation or column chromatography it becomes necessary at some point to employ a concentration step. The barium sulfate adsorption-elution technique not only provides a means by which virus can be concentrated (1, 3), but also significantly reduces the amount of bacterial product contamination.

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