Bacteriophages in Live Virus Vaccines (37683)

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(Introduced by H. M. Meyer, Jr.)
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Fetal bovine serum (FBS) is used extensively as a component of nutrient medium to promote the growth of cells *in vitro*. FBS from different suppliers has recently been shown to have a high rate of contamination with bacteriophages (1, 2). Since some commercially prepared biological products are produced in cell culture systems it seemed possible that phages would persist through the various steps of manufacture and be included in the final products. This report presents evidence of the presence of coliphages in live virus vaccines currently licensed for human use in the United States.

Materials and Methods. Vaccines. Samples of vaccines for poliomyelitis, measles, mumps and rubella were selected from those submitted to the Bureau of Biologics (BB) for quality control testing.

Bacteriophage assays. The agar layer method (3) was used to detect the presence of *E. coli*-specific phages in FBS and vaccines. The vaccines could not be tested directly since neomycin sulfate was present at concentrations up to 50 μg/ml which interfered with the growth of *E. coli,* the host organism for phage. Vaccine was concentrated and the interfering antibiotic was eliminated by centrifuging 5 to 10 ml of the samples at 100,000*g* for 5 hr and resuspending the pellet in 1 ml of phosphate buffered saline (pH 6.8) containing 0.01 *M* $MgSO_4$. A volume of 0.1 ml of the concentrated vaccine or FBS was added to 0.5 ml of individual suspensions of 2 strains of *E. coli,* and the mixtures were incubated at 37° for 10 min. Strain C-3000 was obtained from the American Type Culture Collection, Rockville, MD, and strain K-12 was a gift of Dr. M. Gottesman, NIMH. Tryptone top agar (2.5 ml) was then added to the suspension and layered onto tryptone plates. Replicate plates were incubated at 37 and 40°, and plaques were counted after 18 to 48 hr. Vaccine diluent, cell culture media, and aseptically drawn human serum were carried through the same procedures as the vaccine samples.

Electron microscopy. The procedures described by Adams (3) were used to isolate phage and to produce concentrates. Single plaques were incubated with 0.25 ml suspensions of *E. coli* at 37° for 10 min, and then 10 ml of medium MAB 87/3 (4) was added. After overnight incubation at 37°, 1 ml of the supernatant solution was transferred to a fresh suspension of *E. coli,* and treated as just described. This procedure was repeated daily until a titer of at least 1×10^{10} PFU/ml was obtained. Electron microscopic studies of the virus isolates were made using the pseudoreplica technique (5).

Results and Discussion. A total of 60 lots of bulk or final product live virus vaccines were assayed for coliphages and 11 (18%) were positive.

The appearance of the most frequently found type of plaque produced by phages in vaccines is compared in Fig. 1 with the type found most frequently in FBS. In Fig. 2, the morphologic characteristics of the respective viruses isolated from single representative plaques shown in Fig. 1 are presented. Both plaque morphology and electron microscopic appearance of the phage isolated from one of the vaccines were very similar to those isolated from FBS. The virus particles were hexagonal, measured 50 nm, and showed no evidence of tails. These features suggest that they fit into Bradley's class E (6) except that their size (50 nm) is more consistent with the viruses of class C. Studies are in progress to determine the identity of

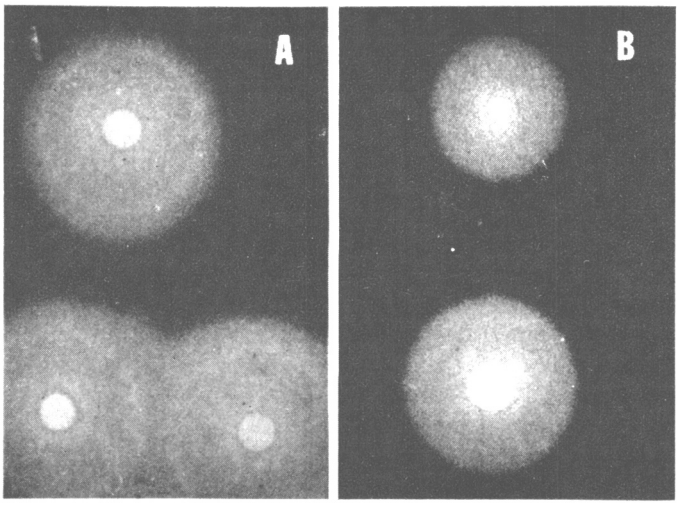

FIG. 1. Appearance of phage plaques from (a) FBS, and (b) measles vaccine (2.5×).

the isolates.

Table I summarizes the data on those vaccines which contained coliphages. Each of the four vaccines had phages in one or more of the lots tested, and phages could be found in final products as well as in the bulk samples.

Federal Regulations (7) require that sufficient rinsing of the cells be made prior to infection to reduce the concentration of the serum component in the growth medium to 1:10^6 or less. This procedure should theoretically eliminate phages from the system since the maximum concentration of coliphage we detected in FBS was 1.5×10^4 plaque forming units (PFU)/ml. However, phages may be adsorbed to the cells surface and/or gain entry into the cells, thus preventing at least a portion of them from being removed by the rinsing. This concept is supported by experiments in which we inoculated cultures of chick embryo fibroblasts and WI-38 cells

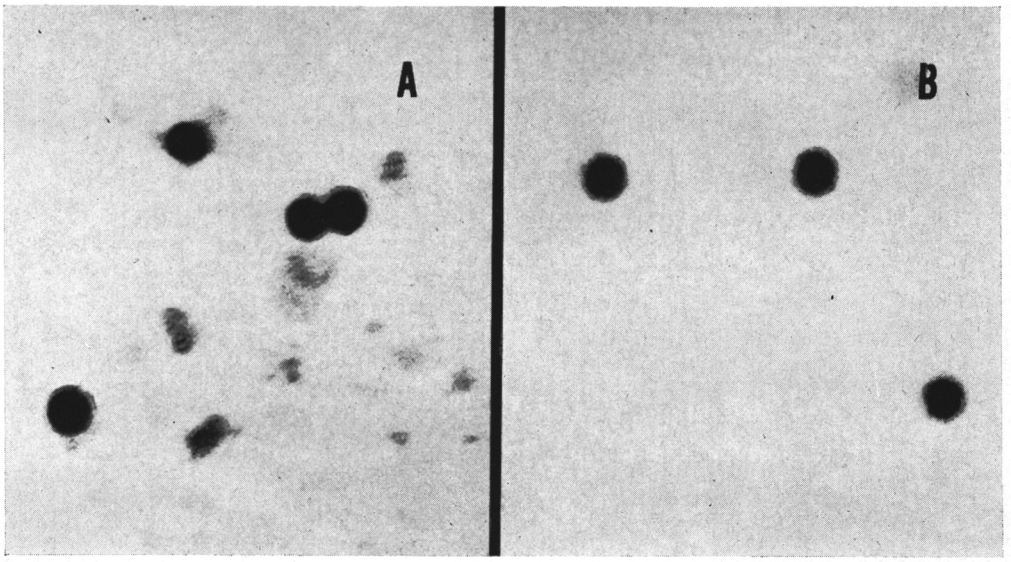

FIG. 2. Electron micrographs of phages isolated from the plaques shown in Fig. 1 (a) FBS isolate, and (b) measles vaccine isolate. Scale: 100 nm.

TABLE I. Vaccines Containing Coliphage.

Vaccine	Phage (PFU/ml)[a]	No. lots positive
Polio	1	1
Mumps	2–54	3
Measles	1–40	6
Rubella	2	1

[a] Values based on 1 ml of original bulk vaccine or final product.

with 10^4 PFU of the vaccine coliphage isolate and were able to recover 10 PFU/ml at 9 and 12 days after inoculation and after 3 changes of medium. The effect of the changes of fluid were estimated to be equivalent to a $1:10^5$ dilution of the original inoculum.

Two alternate explanations for the presence of phage in vaccines which cannot be excluded at this time are: (a) the inadvertent introduction of phages and/or bacteria-containing-phages during some other phase of the manufacturing process; and (b) replication of phages in mammalian cells.

In general, the number of PFU of phage per milliliter of vaccine ranged from 1 to 5. Since only *E. coli* was used for the assay, our findings must be considered a minimum estimate of the total phage content. Also, it is possible that unknown quantities of inactivated but still immunogenic phages are actually present in vaccines. Additional phages might be detected using various other bacterial strains as hosts. Studies to assess these possibilities are now in progress.

The highest concentration of phage found in a vaccine final product was 20 PFU/ml. Since this occurred in measles vaccine, and the individual dose is 0.5 ml, only 10 PFU of coliphage would have been administered to a vaccinee. This is 10,000-fold less than the minimum number of phage required to observe transcription or translation of phage DNA by a single human cell *in vitro*. (8).

There is no evidence that adverse reactions related to the presence of phages have occurred in any of the hundreds of millions of persons who have received live virus vaccines for polio, measles, mumps, and rubella for over a decade. Another factor to be considered in assessing the possible hazard associated with phages in vaccines is that man has an enormous environmental contact with them. The human intestinal tract consistently harbors phages and, under certain conditions, phages can be expected to be present transiently in human blood and tissues. In addition, massive doses of phages have, in the past, been administered to humans in an attempt to treat certain bacterial diseases (9), or for experimental purposes (10). In no instance was there evidence that administration of phages to man or to animals was harmful.

Even though the available information about phages in vaccines suggests that their presence has had no adverse effect on the health of the vaccinees, studies have been initiated to define the extent to which phages can interact biologically with mammalian cells *in vitro* and *in vivo*.

The finding of coliphages in vaccines points out the potential for persistence of an extraneous viral agent in cell cultures after introduction via FBS. Whether coliphages have been responsible in part for any of the unexplainable or variable results in past studies using cell cultures remains to be determined. However, it would seem to be desirable from an experimental cell research point of view, to eliminate phages from FBS, as it would be from a public health point of view to exclude them from vaccines.

Summary. Bacteriophages were isolated from 11 of 60 samples of live virus vaccines for human use. One of the phages isolated gave similar plaques and had similar morphologic characteristics to the phage found most frequently in fetal bovine serum.

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