Effect of Glass on pH-Dependent Stability of Typhoid Vaccine

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Vaccines made from the Ty-2 strain of *Salmonella typhosa* were tested periodically for stability of pH and of potency. The acetone-treated cultures prepared in buffered saline solutions retained potency beyond 30 months of storage at 0 to 5 C. Similar vaccines in unbuffered saline solutions lost potency coincident with increase of alkalinity. Vaccines packaged in United States Pharmacopeia borosilicate glass vials retained potency and pH stability, whereas those in Type III United States Pharmacopeia soda-lime glass vials were less stable.

Strain Ty-2 of *Salmonella typhosa* was designated in 1969, for commercial production of typhoid vaccines in the United States (2). A new and more potent vaccine, lot 6A, became the reference vaccine for potency testing.

Our unpublished preliminary studies indicated that several acetone-treated vaccines were of more consistently higher potency than were portions of the same cultures inactivated with formol and/or heat and phenol.

According to Landy (6), the Vi antigen is retained by the acetone process.

In early studies, clinicians studied commercial heat-phenolized vaccine and acetone-treated experimental vaccine in human volunteers. Essentially the same levels of agglutinating antibodies to H, O, and Vi antigens were observed after immunization with either type of vaccine. Clinicians were unable to distinguish (blind study) between the effects of either kind of vaccine at the sites of injection or relate them to general reactions. However, in potency tests in mice, the phenolized vaccine failed to meet the new potency standard. The acetone vaccine was equal to the new official reference vaccine.

Experimental lots (acetone processed) tested for stability at 0 to 5 C retained adequate potency demonstrated in mice 18 months after which it was observed that vaccine in 10-ml vials appeared to retain potency better than those in 1.2-ml vials. A check of the pH of these (unbuffered) vaccines packaged in flint glass vials (type III) showed a range of pH values from 7.92 to 9.22 in the small vials and pH 7.40 to 7.62 in the others. This problem was eventually corrected by the use of buffered saline and borosilicate glass packages. This report describes repeated observations of pH and potency tests of four commercial lots of vaccine beyond 30 months of storage at 0 to 5 C.

MATERIALS AND METHODS

Vaccines. The Ty-2 strain of *S. typhosa* reference vaccine, lot 6A, was obtained from the Division of

Biologics Standards, National Institutes of Health, Bethesda, Md., now named the Bureau of Biologics, Food and Drug Administration. The reference vaccine is an acetone-precipitated and washed suspension of the Ty-2 strain, vacuum-dried.

Experimental and commercial vaccines were prepared from broth cultures grown in fermentors. Acetone-precipitated cultures were washed in acetone and air-dried. Dried cultures were resuspended in physiological salt solution, 0.85% NaCl, (PS) as concentrated vaccine. Final dilutions were made in PS or buffered PS with Merthiolate, (thimerosal, Lilly) at a final concentration of 1:10,000. Experimental vaccines were made in PS and in PS buffered with mono- and disodium phosphates, 0.013 M, pH 7.0 to 7.2 (PBS). Commercial lots of vaccine were made in PBS.

All vaccines conformed to specifications for purity, potency, etc., in *Additional standards, typhoid vaccine*, Public Health Service Regulations, title 42, part 43 (2). The vaccines contained 0.20 ± 0.002 mg of nitrogen (Kjeldahl) and less than 10^9 organisms per ml.

Potency tests. The procedures and statistical treatment of the mouse potency tests were conducted as prescribed (2). Tests in triplicate were scheduled after 9, 18, 24, 30, and 36 months of storage at 0 to 5 C. Additional tests were made whenever statistically "invalid" (2) data indicated a need for more information.

The contents of three vials were pooled when 1.5-ml vials were tested; i.e., nine vials were used in each triplicate test series. Single vials were tested when 15-ml vials were examined. Vials were randomly selected.

The reference vaccine is rated at 8 units. The effective dose, 50% (ml) is determined for each vaccine (2). The lowest acceptable limit is 5 units or a ratio of 0.625 compared to the reference vaccine.

Packaging. Borosilicate glass vials were type I, United States Pharmacopeia (U.S.P.) (11) and flint glass vials were type III, soda-lime glass (U.S.P.). Both are permitted for parenteral products. Vials of 4-ml capacity were filled to provide three 0.5-ml doses. Ten-dose vials of 25-ml capacity were filled to ensure availability of 15.0 ml of vaccine.

Mice. The CFW strain of mice (Carworth Farms) was used when available. When locally supplied

white Swiss mice were substituted, many "invalid" (2) tests resulted. Thereafter, Harlan (Harlan Industries, Cumberland, Ind.) SPF (and a few Harlan ICR) mice were specified, effecting greater consistency and fewer "invalid" (2) tests.

pH Tests. A Corning model 7 pH meter was used with a model 4094-60 A.H. Thomas dual electrode. The repeatability of the meter is specified at ± 0.02 pH. Small vials were vigorously shaken before readings were made with the electrode immersed in the vial. The 15-ml vials were sampled by aseptic (syringe) removal of 0.5 ml to a plastic test tube (12 by 75 mm) into which the electrode was immersed.

Preliminary experiments. Before producing commercial lots, we observed the stability of vaccines prepared in unbuffered PS, packaged in type III glass vials. Two lots (with 10 ml per vial) were of adequate potency after 36 months of storage at 0 to 5 C. Three similar vaccines in 1.2 ml (note) portions were potent through 18 months, after which they frequently failed to pass tests. In vaccines stored 36 months at 36 to 37 C, pH readings of the larger vials were 7.41 to 7.62 and those of the small vials ranged from pH 7.92 to 9.22.

Two more vaccines were packaged in type III glass. Within 3 weeks at 37 C, the pH of the unbuffered vaccines showed a rise to about pH 9.0. Only slightly lower values were obtained in type I vials. Clearly, a buffer was needed.

For an estimate of the buffering efficacy of 0.013 M PBS, lot 79 was prepared at pH 7.1 and filled into type I and type III vials. After 4, 11, and 18 days of storage at 36 to 37 C, the pH values rose about 0.2 pH in either type of vial. The pH did not change in vials stored at 0 to 5 C.

Commercial vaccines. Four sublots of 1.5-ml vials were prepared from one lot of vaccine concentrate. Lots 25 and 26 were not buffered. Lots 27 and 28 were diluted in PBS, pH 7.2. Lots 25 and 27 were in type III glass and lots 26 and 28 in type I vials. The vials stored at 36 to 37 C were to be checked for pH weekly, those at 0 to 5 C checked monthly. However, pH readings of the unbuffered sections were above pH 9.2 in 1 week in type III glass stored at both 0 to 5 C and 36 to 37 C, whereas in type I vials, the pH ranged from 8.7 to 9.0. Buffered vaccine maintained stable pH through 18 months at 0 to 5 C. A rise of 0.3 pH was noted after 16 weeks at 36 to 37 C (type I glass).

After the 16-week period, all vaccines were discarded except lot 28 (buffered, type I glass). It was then decided to prepare all commercial vaccines in PBS and type I glass vials.

Commercial vaccine lots 12, 39, and 43 were subjected to the same 9- to 36-months schedule of surveillance of pH and potency as applied to lot 28. All lots except lot 43 were of 1.5-ml vials. The latter was in 15-ml packages.

RESULTS

Individual pH readings and vaccine potency (in units) are shown in Fig. 1, 2, 3, and 4. Four commercial vaccines remained essentially neutral in pH and potent after 30 months of storage at 0 to 5 C. Two lots are yet to be tested at 36

FIG. 1. Stability of lot 28 typhoid vaccine.

FIG. 2. Stability of lot 39 typhoid vaccine.

months. Lot 39 was potent after 41 months at 0 to 5 C, and lot 28 was acceptable after 36 months at 0 to 5 C.

To verify our belief that it was the Vi antigen which deteriorated in the experimental or pre-

FIG. 3. Stability of lot 12 typhoid vaccine.

liminary preparations, we injected mice (intraperitoneally, 0.001 ml) with a lot (N) held 29 months in flint glass at 0 to 5 C (pH 7.6) and 36 to 37 C (pH 8.7). After 10 days the mouse sera were tested with sheep erythrocytes sensitized with Vi antigen (*Citrobacter freundii*, purified antigen). The hemagglutination test showed no presence of Vi antibody in the sera of mice injected with the heated-stored alkaline vaccine. Anti-Vi titers up to 1:64 were detected in sera of mice injected with cold-stored vaccine. Tests for O antibody with Difco O antigen (group D) and boiled Ty-2 cells revealed no O antibody in any of these serum samples.

Slide agglutination tests of one vial of each of the two experimental vaccines mentioned above (lot N) were made as a check on the antigens. The contents were washed in PBS (pH 7.2) and reconstituted to 1:10 volume in PBS. The two suspensions were tested on slides with appropriate O and Vi antisera (Difco). The cold-stored vaccine reacted with Vi antiserum, but did not react with the O antiserum. The vaccine stored at 37 C (alkaline) did not agglutinate in the Vi antiserum, but did react with the O antiserum.

DISCUSSION

Typhoid vaccines are assigned an expiration date of 18 months from date of issue from the manufacturer's cold storage. When appropriately packaged and stored, fluid typhoid vac-

cine may be expected to retain potency well beyond this limit. One vaccine was found potent after 15 years (3, 4). No mention of pH control has been observed in reports of potency loss related to age and/or storage conditions (3, 4, 6, 9, 10).

Leaching of alkali from vials, especially type III glass, is, as expected, accelerated at 36 to 37 C. Rise in pH coincided with diminution of potency.

The lability of Vi antigen to mild alkali has been reported (5, 7, 8). Landy noted that alkali-hydrolyzed Vi antigen was not immunogenic for rabbits or mice. Earlier studies (13) showed alkaline extracts of *S. typhosa* to be ineffective. Landy (8) regarded the mouse protection test "for all practical purposes" to be an assay for Vi antigen.

The importance of selecting packaging materials can easily be overlooked. Both types I and III glass are permitted for packaging parenterals (11). *National Formulary* 1975 (1) specifies maximum limits of 0.02 N sulfuric acid required to neutralize extract of 10.0 g of powdered glass: 1.0 ml for type I and 8.5 ml for type III glass.

Whether or not the mouse protection test accurately reflects the effectiveness of the vaccines in humans is a current controversy beyond the scope of this report. It must be noted, however, that the mouse protection test is the

FIG. 4. Stability of lot 43 typhoid vaccine.

only official criterion for estimating vaccine potency in the United States. The preservation of Vi antigen is thus essential for successful passing of the required tests (14, 15).

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