Factors Influencing the Activity of Sterile Filtered and Heat-Sterilized Trypsin Solutions

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The mode of sterilization (filtration or heat) was found to significantly affect the activity of trypsin solutions. Trypsin activity was substantially reduced in the initial fractions of filtrate passed through asbestos filter pads; heat-sterilized trypsin was satisfactory for transfer of cell cultures grown on glass. Heat-sterilized trypsin may be useful when elimination of filterable organisms is required.

**Sterile filtered trypsin.** Asbestos contamination of parenteral drugs was reported recently by Nicholson et al. (1), House (2) and Brown et al. (1) had previously shown that cell plating efficiencies and virus titers were reduced by materials that are eluted from asbestos pads during filtration of culture media. In this report, we give results that indicate that asbestos pads are not suitable for sterile filtration of trypsin solutions.

In our earlier practice we prepared trypsin solutions in lot sizes of up to 100 liters. These lots were filtered through two Seitz presses, each containing 15 asbestos pads (Type ST-3, Columbia Filter Co., Hawthorne, N.J.), into five 5-gal (ca. 19-liter) bottles. The initial few liters of filtrate showing a tinge of yellowish-brown color were discarded. The solutions in each of the 5-gal bottles were then further transferred to 1-liter or 500-ml bottles. Labels on these bottles were coded with the letters "A" through "E," in addition to the lot number, to designate the 5-gal bottle from which they were filled. Technicians working with these solutions reported wide variations in time required for trypsin action.

Prefiltration samples were obtained from a 100-liter lot of 0.25% trypsin in tris(hydroxymethyl)aminomethane-saline. Postfiltration samples were taken from each of the five 5-gal bottles (A-E) used in the filling operation. One set of samples was immediately frozen at -20°C; other sets were subsequently frozen after being stored at 4°C for periods of up to 77 days. Trypsin activity was determined using the National Formulary assay (American Pharmaceutical Association) for trypsin in pancreatin. Results of this assay are shown in Fig. 1. Samples from bottles A and B, representing the first 40 liters of filtrate, had substantially less activity than samples from bottles C, D, and E.

An additional 100-liter lot of 0.25% trypsin in tris(hydroxymethyl)aminomethane-saline was prepared, A Millipore 293-mm press, containing a fiber glass prefilter and cellulose acetate RA (1.2 micron) and GS (0.22 micron) pads, was used for the initial portion of this lot and clogged after 53.5 liters had been filtered; 40 liters of the remaining solution were filtered through Seitz presses equipped with asbestos pads as above. Activity of pre- and postfiltration samples is shown in Table 1. Again, trypsin activity was reduced in the initial portion of filtrate passed through the asbestos pads.

Lot size of trypsin solutions used in our areas has been reduced to 50 liters, and all lots are sterilized by membrane filtration (Millipore Corp.). These changes have considerably reduced bottle-to-bottle differences in trypsin activity. Occasional lot-to-lot differences in trypsin activity have been caused by variations in potency of trypsin obtained from commercial sources.

**Heat-sterilized trypsin.** Crystalline trypsin in acid solution can withstand boiling, being denatured at temperatures above 50°C but reactivated on cooling (4). To determine whether partially purified trypsin can be sterilized in this manner, 8 × 10^6 Bacillus subtilis spores were added to 1 liter of a solution containing 0.05% (1:250, wt/vol) trypsin powder (Difco) and 0.02% ethylenediaminetetraacetic acid in Puck Saline A. The solution was adjusted to pH 2.3, and 40-ml samples were placed in a boiling-water bath. The temperature of the samples reached 97.5°C in 10 min; they were heated for an additional 10 min, but did not boil and were not otherwise visually altered. The solutions were kept at 4°C overnight, and samples were then inoculated onto plates of Trypticase (BBL) soy agar and incubated at 36°C. After 18 h, control plates inoculated with the acidified but
FIG. 1. Trypsin activity determined by using the National Formulary assay for trypsin in pancreatin. Symbols: A, 1st 20 liters of filtrate; B, 2nd 20 liters; C, 3rd 20 liters; D, 4th 20 liters; E, 5th 20 liters.

TABLE 1. Trypsin activity of filtered solutions

| Sample                        | Trypsin activity (mg/ml) |
|-------------------------------|--------------------------|
| Prefiltration                 | 1.37                     |
| Membrane-filtered, 1st 20 liters | 1.19                   |
| Membrane-filtered, 2nd 20 liters | 1.29                   |
| Membrane-filtered, last 13.5 liters | 1.30                   |
| Seitz-filtered, 1st 20 liters   | 0.80                     |
| Seitz-filtered, 2nd 20 liters   | 1.16                     |

unheated trypsin solution had a confluent lawn of bacterial growth; no growth was observed on plates inoculated with the heated trypsin solutions.

BS-C-1 cells, a green monkey kidney cell line that adheres tenaciously to glass, were harvested with the heated trypsin for five consecutive passages. (Trypsin was adjusted to pH 8.0 prior to use.) No cell toxicity was observed.

In a preliminary experiment, rabbit kidneys extracted with heated trypsin yielded 1.35 ml of packed cells; 3.10 ml of packed cells were obtained with membrane-filtered trypsin. Crystalline trypsin is known to be less effective for the digestion of intact tissues than is crude trypsin, which contains a variety of other enzymes (amylases, lipases, mucases, etc.) (4). The disparity in cell yields noted above may be due to loss of some or all of these other enzymes during the heat sterilization procedure.

Trypsin assays were performed by Paul Hartsaw and William Koch of our Analytical Chemical Development Department.

LITERATURE CITED

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