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Toxic Effect of Calcium Alginate Swabs on *Neisseria gonorrhoeae*

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Calcium alginate (CA)-tipped swabs have been reported to interfere with the recovery of herpes simplex virus, *Chlamydia trachomatis*, and *Ureaplasma urealyticum* and may cause cytotoxicity in cell culture. To determine whether CA swabs also inhibit the growth of *Neisseria gonorrhoeae*, we carried out a series of experiments using either CA swabs that were toxic or nontoxic in a cell culture cytotoxicity assay or nontoxic rayon or cotton swabs. Leaving a toxic CA swab in 3 ml of Mueller-Hinton broth inoculated with 10^4 CFU/ml caused rapid killing within 6 h at 37°C; colony counts of five strains were <1% of those of Mueller-Hinton broth controls. When the tips of toxic CA swabs were inoculated directly and kept at 37°C without holding medium, the swabs were sterile at 6 h. If the same swabs were placed in Amies medium with charcoal, organisms could still be recovered at 6 h. Toxicity was less at room temperature than at 37°C. Inhibition of growth of *N. gonorrhoeae* was not seen with rayon or cotton swabs. The toxic component was neither the CA fiber nor the aluminum wire but probably the glue used to attach the fibers. We concluded that some lots of CA swabs kill *N. gonorrhoeae* in vitro. Survival of *N. gonorrhoeae* is improved with nontoxic swabs, particularly cotton swabs, and Amies medium with charcoal regardless of swab type.

Calcium alginate-tipped swabs have been reported to interfere with the recovery of herpes simplex virus (HSV), *Chlamydia trachomatis*, and *Ureaplasma urealyticum* from clinical specimens (1, 2, 5–7), and we and others have observed that calcium alginate swabs often cause cytotoxicity in cell cultures (5). Because it has been suspected that use of inhibitory swabs is also a problem in the isolation of *Neisseria gonorrhoeae* (4), we conducted a series of experiments to determine whether or not calcium alginate swabs are toxic for *N. gonorrhoeae*.

MATERIALS AND METHODS

Bacteria. Ten strains of blood culture isolates of *N. gonorrhoeae* from the culture collection of the Clinical Microbiology Laboratory, University of Colorado Hospital, Denver, were studied. All but one of the gonococci were of auxotype Arg^- Hyx^- Ura^- and serogroup I; the exception was Pro^- and serogroup II. Control organisms were *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and three clinical isolates of *E. coli*.

The inocula were prepared from pure cultures grown overnight (18 to 24 h) at 37°C on chocolate agar or 5% sheep blood agar (Pasco Laboratories, Wheatridge, Colo.). The growth was suspended and diluted in Mueller-Hinton broth (MHB) to a concentration of approximately 10^5 CFU/ml with a 0.5 McFarland $BaSO_4$ turbidity standard.

Cytotoxicity assay. Swabs were screened for cytotoxicity in human embryonic lung fibroblast cell cultures by using an assay developed in our laboratory. In the assay, a single swab was held in 3 ml of veal infusion broth overnight at 4°C; 0.5 ml of broth was then added to duplicate tubes of human embryonic lung fibroblast cells, incubated at 37°C, and observed daily for 3 days. A positive test was defined as generalized rounding of the cells without discrete foci, leading to detachment of the monolayer. Untreated veal infusion broth served as a negative control.

Swabs. Thirteen lots of calcium alginate-tipped aluminum wire shaft swabs (Calgiswab type 1; Inolex, Glenwood, Ill. [Spectrum Diagnostics]) were screened for cytotoxicity. Two lots of swabs that were highly cytotoxic (cytotoxicity assay positive at 24 h) and two lots that were nontoxic were selected for study. For purposes of comparison, we also studied a similar small, rayon-tipped swab (Marion Scientific, Div. Marion Laboratories, Inc., Kansas City, Mo.) and a cotton-tipped swab (Medical Wire ENT Swab; Medical Wire and Equipment Co., Cleveland, Ohio) that produced no cytotoxicity in human embryonic lung fibroblast cells.

Procedure. In experiment 1, we dissected the toxic calcium alginate swabs to determine the part of the swab responsible for cytotoxicity. We tested separately in the human embryonic lung fibroblast cell cytotoxicity assay a 2-cm segment cut from the middle of the wire shaft, the intact tip, fiber pulled from the swab tip, and pure calcium alginate fiber (Hardwood Products Co., Guilford, Maine). We were unable to obtain a sample of the glue used to attach the fibers to the shaft, so we were unable to test it in the cytoxicity assay. Whole swabs were used for all subsequent experiments.

In experiment 2, we placed a single calcium alginate swab in screw-cap tubes containing 1.0 ml of MHB inoculated with approximately 5×10^4 CFU of each strain per ml. The tubes were incubated at 37°C or room temperature (25°C) and sampled after 15 and 60 min with a calibrated platinum loop. Colony counts were done on sheep blood or chocolate agar plates after 24 to 48 h of incubation at 37°C in 5% CO_2. Percent survival was calculated by dividing the colony count after incubation with a swab by the colony count in a control tube of MHB without a swab.

Experiment 3 was modeled after the cytotoxicity assay and sought to confirm that the toxic component for *N. gonorrhoeae* was a soluble substance substance that could be eluted from the swab tip. A calcium alginate swab was placed into 3 ml of uninoculated MHB and kept at 4°C for 72 h. The swab was removed, the MHB was filtered through a 0.45-μm (pore size) filter to remove swab fibers, and then bacteria were added to give a final concentration of approximately 5×10^4 CFU/ml. The tubes were incubated and sampled, and the percent survival was calculated as previously described.

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In experiment 4, mock clinical specimens were prepared with five strains by inoculating the swab tips directly with 10 μl of MHB containing 10^4 to 10^5 CFU/10 μl by using a calibrated pipette. The swabs then were placed in sterile, dry tubes or tubes containing 3 ml of freshly prepared Amies transport medium with charcoal and incubated at 37°C or room temperature. Semiquantitative cultures were done after 1, 6, and 24 h by rolling each swab against the side of the tube and then inoculating a sheep blood or chocolate agar plate. Colonies were counted after incubation of the plates at 37°C in 5% CO_2 for 24 to 48 h. All of the experiments were done in duplicate, and the colony counts were averaged for analysis.

RESULTS

In experiment 1, we found that neither the wire shaft nor pure calcium alginate fibers were toxic in the cytotoxicity assay. Only the intact swab tip and fibers pulled from the swab tip of toxic lots were capable of causing cytotoxicity. Cytotoxicity was almost always apparent within 24 h but was enhanced by longer incubation. Negative controls and nontoxic swabs showed no evidence of cytotoxicity whatsoever. The only nontoxic lots of calcium alginate swabs were four lots several years old; all recent lots that we screened were cytotoxic.

In experiment 2, *N. gonorrhoeae* was rapidly killed in MHB at 37°C in the presence of toxic calcium alginate swabs (Table 1). The percent survival of two strains was greatly reduced within 15 min, and 8 of 10 strains showed <7% survival at 60 min compared with controls. In contrast, survival with nontoxic calcium alginate swabs was 100% for seven strains and >50% for the remaining three strains at 1 h. The toxic effect was also seen at room temperature but was less striking. Survival of *S. aureus* and *E. coli* was >100% at 60 min with toxic and nontoxic swabs at both temperatures.

In experiment 3, with MHB exposed to the swabs for 72 h and then filtered, toxic calcium alginate swabs led to decreased survival of *N. gonorrhoeae* (Table 2) within 60 min at 37°C compared with MHB controls or filtrate from nontoxic calcium alginate swabs. Colony counts of *S. aureus* and *E. coli* were undiminished in MHB exposed to toxic calcium alginate swabs.

In experiment 4, when bacteria were inoculated directly onto swab tips and placed in sterile tubes without holding medium, all swabs except cotton swabs were sterile at 6 h at 37°C (Table 3). Colony counts generally were higher with cotton swabs at both temperatures, and two strains survived for 24 h at room temperature only on cotton swabs (room temperature data not presented). In this experiment, nontoxic calcium alginate swabs were sterile at 60 min. When inoculated toxic and nontoxic swabs were kept in Amies transport medium with charcoal, four of five gonococcal strains survived for 6 h at 37°C on each of the swab types.

TABLE 1. Effect of calcium alginate swabs on survival of *N. gonorrhoeae* in MHB

N. gonorrhoeae strain	% Survival of inoculum at 60 min compared with controls[a]			
	25°C		37°C	
	Toxic swab	Nontoxic swab	Toxic swab	Nontoxic swab
1	79	100	5	100
2	82	100	5	100
3	57	52	3	100
4	100	100	4	54
5	14	91	1	50
6	100	100	6	100
7	48	100	<1	100
8	45	100	2	70
9	83	100	23	100
10	86	100	25	100

[a] The inoculum size was approximately 5×10^4 CFU/ml. The control was MHB without a swab.

TABLE 2. Effect of filtrate from calcium alginate swabs in MHB on *N. gonorrhoeae*

N. gonorrhoeae strain	% Survival of inoculum at 37°C compared with controls[a]			
	Toxic fluid		Nontoxic fluid	
	15 min	60 min	15 min	60 min
1	60	16	100	100
2	10	<1	100	100
3	23	<1	100	100
4	81	15	100	51
5	35	8	100	59
6	33	11	100	100
7	35	8	100	100
8	43	5	100	100
9	9	<1	100	100
10	29	4	100	100

[a] The inoculum size was approximately 5×10^4 CFU/ml. The control was MHB.

DISCUSSION

Recent studies of the effects of calcium alginate swabs have focused on interference with the recovery of agents that cause sexually transmitted diseases, particularly HSV (1, 2), *C. trachomatis* (5, 7), and *U. urealyticum* (6). In this study, we found that most lots of calcium alginate-tipped swabs were also highly inhibitory to the strains of *N. gonorrhoeae* that we tested.

Investigations of the effect of calcium alginate swabs on HSV have suggested that physical binding of viral particles to the calcium alginate fiber, rather than direct toxicity, is responsible for the observed inhibition (1, 2). Similar studies of an animal virus, bovine rhinotracheitis virus, however, seemed to indicate that calcium alginate swabs were directly virucidal (3).

Mardh and Zeeberg examined the effects of various types of swabs on chlamydial inclusion counts in cultures of experimentally infected transport medium and in clinical specimens (5). They found that a larger number of inclusions was obtained with cotton- and rayon-tipped swabs than with calcium alginate swabs. They also noted that calcium alginate-tipped swabs caused a cytopathic effect in McCoy cells exposed to the swabs for 48 h.

Smith and Weed reported that genital specimens transported on calcium alginate swabs grew *C. trachomatis* less often than specimens transported in 2-sucrose phosphate broth with the swab removed (7). In addition, they observed a trend toward higher inclusion counts with rayon-tipped swabs than with calcium alginate swabs. They speculated that the cement used to bind the fibers to the shaft may have been responsible.

Studies of the survival of *U. urealyticum* on calcium alginate, rayon, and cotton swabs showed that recovery of *U. urealyticum* from mock-infected urine was poorest with

TABLE 3. Effect of swab type and transport medium on survival of *N. gonorrhoeae*

N. gonorrhoeae strain	% Survival of inoculum at 37°C by swab type compared with controls[a]															
	No transport medium								Amies medium with charcoal							
	Toxic CA		Nontoxic CA		Rayon		Cotton		Toxic CA		Nontoxic CA		Rayon		Cotton	
	1 h	6 h	1 h	6 h	1 h	6 h	1 h	6 h	1 h	6 h	1 h	6 h	1 h	6 h	1 h	6 h
1	<1	0	0	0	0	0	10	0	20	<1	100	<1	100	4	100	7
2	0	0	0	0	100	0	100	0	100	9	100	100	100	20	100	30
4	0	0	0	0	0	0	100	0	4	0	16	0	100	13	100	7
7	100	0	0	0	100	0	100	2	100	2	100	7	100	100	100	<1
9	<1	0	0	0	8	0	100	4	100	1	100	30	100	100	100	100

[a] CA, Calcium alginate. The inoculum size was approximately 5×10^4 CFU/10 μl. The control was a matched swab plated immediately after inoculation.

calcium alginate swabs, but the researchers attributed their results to the decreased absorbancy of calcium alginate swabs rather than to a direct toxic effect (6).

We suspect that the same toxic factor contributes to the inhibitory effect of some calcium alginate swabs on *N. gonorrhoeae*, HSV, *C. trachomatis*, and *U. urealyticum*. On the basis of our own observations, the apparent inhibition of HSV by calcium alginate swabs is at least partly due to the cytotoxic effect on the target cell culture (unpublished data). Because the toxicity is not due to the fiber itself but to the glue or another unidentified substance, it should not be assumed that other brands of calcium alginate swabs are toxic nor should it be assumed that rayon or cotton swabs are always nontoxic. In related studies, we have found calcium alginate swabs that were consistently nontoxic and rayon swabs that exhibited cytotoxicity.

We concluded that some calcium alginate swabs are toxic for some strains of *N. gonorrhoeae* in vitro and that the toxic effect is predicted by cytotoxicity of swabs in cell culture. Whether the observed in vitro effects have practical significance in the recovery of *N. gonorrhoeae* when urethral specimens are plated directly on agar plates within a few minutes of collection is unknown and should be studied in clinical trials. Because other types of specimens frequently are collected with calcium alginate swabs, it will be important to learn whether the growth of other organisms, such as *Bordetella pertussis* in nasopharyngeal secretions, is adversely affected by toxic swabs. Screening of swabs by the cytotoxicity assay is a simple way to determine whether any particular brand or lot of swab is potentially toxic to microorganisms.

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