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NOTE

Formalinized Bacterial "Antigens" as a Potential Infection Hazard

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It is widely thought that after enteric bacteric have been "formalinized" (treated with an equal volume of 0.6% formalin) for 1 h, the bacteria become "antigens" and are no longer viable. None of the 27 cultures of *Salmonella* and other *Enterobacteriaceae* were entirely killed within 1 h after formalin was added, but all 27 were reduced from 10^9 viable cells per ml to less than 10^2 per ml within 7 h. Thus, mouth pipetting of cultures formalinized for only 1 h is a possible infection hazard.

H or flagella antigens of bacteria are heat labile, so a method other than boiling must be used to prepare them. Since the early serological studies of the *Salmonella* (2, 3), formalin treatment has been the method of choice for preparing "H antigens" of *Enterobacteriaceae* (2, 3). The bacterial culture is grown in broth, and an equal volume of "formalinized saline" (0.6% formalin) is added. It is generally thought (2) that after 1 h of exposure to formalin, the culture becomes an antigen with no viable cells. Many workers who would never dream of mouth-pipetting viable cultures do mouth-pipet antigens. Since there was anecdotal evidence that cultures formalinized for 1 h do contain viable cells, I thought it worthwhile to investigate this point further.

A fresh bottle of commercial formalin was opened and used to prepare formalinized saline (NaCl, 0.85%; formalin, 0.6%, [vol/vol]). On the same day, 1 ml of the formalinized saline was mixed with 1 ml of 24-h Trypticase soy broth cultures containing about 10^9 bacteria/ml of the 27 *Enterobacteriaceae* listed in Table 1. At the times listed, 0.01 ml of each tube was inoculated onto sheep blood agar plates. The plates were placed in a stream of air in a vertical laminar flow safety cabinet for 1 h to help remove formaldehyde and then incubated at 36 ± 1 C. Some killing may have occurred on the plate, but the blood plus the air drying essentially stopped the reaction.

Figure 1 shows the killing curve of formalin on *Salmonella typhimurium* 36; about 90% of the surviving bacteria are killed each hour. Table 1 shows the survival of other *Enterobacteriaceae* in formalinized saline. The data show that after 1 h the culture contains many viable

TABLE 1. *Survival of Enterobacteriaceae in formalinized saline*

Species	No. of isolates tested	Bacterial survival[a] in 0.01-ml sample at h:					
		1	2	3	5	7	30
Salmonella (different serotypes)	11	+	+	+	+	−	−
Salmonella typhi	2	+	+	+	−	−	−
Arizona hinshawii	2	+	+	+	+	−	−
Citrobacter freundii	2	+	+	+	+	−	−
Escherichia coli	4	+	+	+	+	−	−
Serratia marcescens	2	+	+	+	+	−	−
Klebsiella pneumoniae	2	+	+	+	+	−	−
Enterobacter cloacae	2	+	+	+	+	−	−

[a] Initially there were 10^9 viable cells per ml. If there were no viable cells in a 0.01-ml sample, then at least 99.99999% of the bacteria were killed.

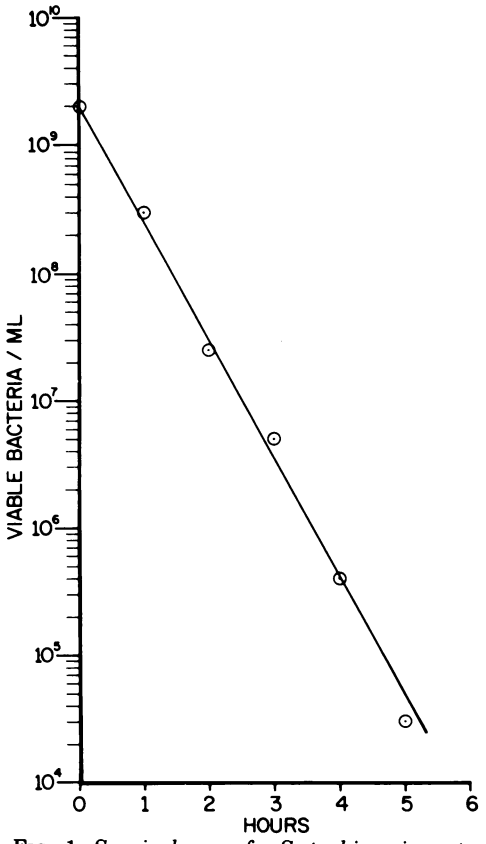

FIG. 1. *Survival curve for S. typhimurium strain 36 in formalinized saline (done by a standard plate count on blood agar).*

cells and is not a "killed antigen," since over 10^8 cells/ml of *S. typhimurium* 36 are still alive. Kauffman recommends that formalinized cultures be left overnight at 37 C to kill the cells (3). This approach appears feasible, but cultures formalinized overnight may still contain a few viable cells. This fact, plus the possible toxic and mutagenic effects of formaldehyde, indicate that a safety pipettor should be used to dispense formalinized bacterial cultures. Pipetting with a safety pipettor would comply with safety precedures at the Center for Disease Control, but mouth pipetting does not. A statement of the Center's policy on mouth pipetting follows (1).

"Mouth pipetting of material containing etiologic agents, or sera, of toxic or corrosive chemicals, and of other known hazardous materials is strictly forbidden. Effective hand-pipetting devices are available and must be used when any element of risk exists."

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