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Glutaraldehyde Inactivation of Virus in Tissue

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High concentrations of influenza virus and T3 coliphage were inoculated into mouse tissue blocks. Exposure of the inoculated tissue blocks to 5% alkaline glutaraldehyde resulted in rapid inactivation of both viral agents.

Alkaline solutions of glutaraldehyde have been found to be effective in vitro against a variety of vegetative bacteria, tubercle bacilli, bacterial spores, a number of viruses, and several fungi (1, 3, 4, 6). Solutions of glutaraldehyde have also been used as fixatives prior to electron microscopy and cytochemical studies (5). More recently, Graham and Jaeger (2) concluded that a 5% solution of glutaraldehyde inactivated yellow fever virus contained in 1- to 2-mm blocks of mouse brain or monkey liver tissue within 2 hr. As a part of the research activity conducted within the Special Virus-Cancer Program of the National Cancer Institute, tissue specimens containing viral agents are fixed with 5% alkaline glutaraldehyde prior to electron microscopy studies. This study was initiated to determine the effectiveness of glutaraldehyde in inactivating viruses present in tissue blocks. An A_1 strain of myxovirus (influenza PR8) having properties thought to be similar to many murine leukemia viruses being studied in the Special Virus-Cancer Program was the test agent. A strain of T3 coliphage (ATCC $11303B_3$) was included as a second test agent.

An assay system using the T3 coliphage and the PR8 virus was developed. Laboratory mice were killed and their hind legs were skinned and removed at the pelvis. An 0.1-ml amount of a phage suspension (10^{11} plaque-forming units per ml) was inoculated intramuscularly into the posterior region of each thigh. Then a block of tissue ca. 0.5 by 0.5 by 0.25 cm was excised from the inoculated area of each leg. Tissue blocks were placed individually into test vials containing 5.0-ml amounts of 5.0% glutaraldehyde (*p*H 7.5) and into control vials containing 5.0-ml amounts of sterile 0.85% saline (*p*H 7.5). After an exposure period of 2, 4, 12, or 24 hr, each tissue block to be assayed was removed aseptically and placed into a glass tissue homogenizer containing 2.0 ml of an 0.25 M solution of sodium bisulfite. The tissue block was homogenized and samples were plaque-assayed against *Escherichia coli* (ATCC 11303B) directly or diluted (serial 10-fold dilutions) in tryptose broth and assayed. Portions of the saline solution were also diluted and plated after removal of each control tissue block. The results of this test are presented in Table 1. A bacteriophage titer of some 10^9 plaque-forming units per tissue block was readily inactivated during the 4-hr period of exposure to glutaraldehyde (Table 1). However, phage titer of controls did not change significantly throughout the experiment. Preliminary results indicated that 0.25 M sodium bisulfite effectively neutralized the test solution of glutaraldehyde, but did not inactivate the T3 coliphage or the PR8 virus.

TABLE 1. *Summary of results obtained with T-3 coliphage*

Exposure time	No. of plaque-forming centers recovered[a]		
	Glutaraldehyde test (tissue)	Controls	
		Tissue	Saline
hr			
Zero	Not done	3.8×10^9	Not done
2	1.3×10^4	4.6×10^9	1.3×10^9
4	0	6.5×10^9	2.9×10^9
12	0	1.4×10^9	1.3×10^9
24	0	6.2×10^8	5.5×10^8

[a] Each value is based on an average of three samples; each was plated in triplicate.

In another series of experiments, 0.1-ml amounts of influenza virus (7.1 EID_{50} per 0.1 ml) were used to inoculate mouse tissue, and virus survival was assayed in embryonated eggs. Toxicity studies had demonstrated that the mouse

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TABLE 2. *Summary of results obtained with PR-8 virus*

Exposure time	Infectivity titer of test inoculum[a]		
	Glutaraldehyde test tissue[b]	Controls	
		Tissue[b]	Saline[b]
hr			
Zero	Not done	6.3×10^4	Not done
2	0	1.8×10^5	1.4×10^5
12	0	2.0×10^5	3.7×10^5
24	0	2.8×10^5	1.7×10^5

[a] Infectivity titer based on a 4+ hemagglutination with two drops of allantoic fluid in 1.0 ml of a 0.25% suspension of chicken erythrocytes.

[b] Each value based on an average of 3 samples, each inoculated in 5 eggs.

tissue homogenates and an 0.25 M sodium bisulfite solution were not toxic at use concentrations to 10-day embryonated chicken eggs. Exposure time and conditions were identical to those used for the T3 coliphage (Table 2). PR8 virus in tissue blocks was completely inactivated during the 2-hr period of exposure to glutaraldehyde, but the controls remained unchanged throughout the test (Table 2).

Both of the agents studied were inactivated during the first 4 hr of exposure of the tissue to glutaraldehyde. It is recognized that the inactivation of the test agents was not accomplished during the intracellular phase of viral replication. Yet, it is reasonable to conclude that high concentrations of these viruses can be readily inactivated by 5% alkaline glutaraldehyde.

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