Combined Use of Ultraviolet Irradiation and Beta Propiolactone Sterilization of Sera Infected with a Virus.* (21157)

JOSEPH SMOLENS AND JOSEPH STOKES, JR. (Introduced by Werner Henle.)

From the Children's Hospital of Philadelphia, and the Department of Pediatrics, School of Medicine, University of Pennsylvania

The transmission of serum hepatitis by blood and blood products presents a vexing and serious problem. Physical (ultraviolet) and chemical methods (B-propiolactone, etc.) have been utilized in efforts to sterilize plasma and serum and it would appear that these technics have some value in reducing the incidence of hepatitis resulting from infusion of these products. However, they are frequently not completely sterilized by any method used currently as was shown in recent clinical tests(1-3).

The purpose of this communication is to describe a method of treatment which seems to offer a rationale for use. Orienting experiments have shown that either ultraviolet treatment or beta propiolactone resulted in the death of *almost* all of the micro-organisms. The problem then would involve the inactivation of the relatively few micro-organisms which survive the first type of treatment. An analogy may possibly be found in the field of chemotherapy where the combination of drugs has been used to combat diseases in which minute numbers of the etiologic agent are refractory to one drug but succumb to another drug which has a mechanism of action different from the first drug. It was decided, therefore, to utilize ultraviolet irradiation to destroy the bulk of virus particles (bacteriophage) added to serum, followed by beta propiolactone in order to kill the relatively few survivors. This sequence was chosen since it was not known whether ultraviolet irradiation would have any effects on the beta propiolactone although the reversal of the sequence might be perfectly satisfactory.

Materials and methods. The suspending fluid used in all of the experiments was normal human serum. The sera were kept frozen until the day they were to be used at which time they were thawed and filtered through a Seitz sterilizing pad and the bacteriophage was added directly before treatment in order to give the concentrations shown in Tables I and II. The virus used was the T4r coliphage furnished through the generosity of Dr. S. S. Cohen.

The ultraviolet source was the standard Dill apparatus† and the method recommended by the National Institutes of Health was employed in which the rate of flow is about 250 ml per minute. The B-propiolactone was supplied through the courtesy of Dr. George H. Mangun. The treatment was carried out at room temperature.

Results. Table I shows that a minute number of virus particles escaped the effects of ultraviolet irradiation or of beta propiolactone when used separately but that when the two methods were employed in sequence complete sterilization was effected.

One of the major criticisms concerning the use of chemical agents for sterilization of plasma or serum is the possibility of deleterious action on the proteins. With this in mind it was thought advisable to determine the minimal concentration of beta propiolactone that would be efficacious. The results presented in Table II show again that small numbers of virus particles remained viable following ultraviolet irradiation but that sterilization was obtained with 1.5 g beta propiolactone per liter of serum when it is added after the ultraviolet treatment.

We are indebted to Prof. Alfred Chanutin for the electrophoretic analyses of serum before and after treatment with the combined therapy of ultraviolet irradiation and 1.5 g of beta propiolactone per liter. The patterns were identical showing no changes in the serum proteins as determined by this technic.

* Presented in part on April 7, 1954, before the Panel on Sterilization of Blood and Plasma of the National Research Council.

† Manufactured by J. J. Dill Co., Kalamazoo, Mich.

TABLE I. Effect of Ultraviolet Irradiation, Beta Propiolactone and the Combination of These Two Agents in Serum Contaminated with Bacteriophage.

	Original No. phage particles per ml serum*	Treatment	Phage counts/ml serum after treatment 15 min.	1 day	2 days	6 days
1.	560×10^6	UV†	990		300	170
2.	560×10^6	UV + BPL‡		0	0	
3.	494×10^6	BPL‡	210×10^6		49×10^4	30
4.	56×10^6	Control			30×10^6	9×10^6
5.	100 ml of #1 after UV	BPL‡			0	

* 200 ml normal human serum (Seitz filtered) per bottle.
† UV apparatus used was standard Dill machine. Aerogenes control was completely killed.
‡ BPL—B propiolactone (3 g/liter). In #2 BPL added 15 min. after UV.

The results reported here based on a combined method of treatment of virus infected serum would appear to be promising and certainly worthy of further consideration, particularly since the type of ultraviolet apparatus used here is already extensively in use throughout the United States and since the subsequent step of adding beta propiolactone is relatively simple.

TABLE II. Effect of Varying Amounts of Beta Propiolactone following Ultraviolet Irradiation in Serum Contaminated with Bacteriophage.

Sample	Phage counts/ml serum Before UV	After UV
A	876×10^6	1070
B	944×10^6	340

A and B divided into 4 equal portions as follows:

	BPL* in g/liter	16 hr phage counts/ml of serum		BPL* in g/liter	16 hr phage counts/ml of serum
A 1	3	0	B 1	1.5	0
A 2	1.5	0	B 2	.19	90
A 3	.75	20	B 3	.05	160
A 4	.38	110	B 4	0	120

* Beta propiolactone added about 30 min. after UV.

Aerogenes control 3.5×10^6 was completely killed by UV.

Conclusions. Serum heavily infected with a virus (bacteriophage T4r) was sterilized by a combined method of treatment which consisted of ultraviolet irradiation followed by the addition of beta propiolactone. The beta propiolactone in concentrations as low as 1.5 g per liter could be used effectively. Under these conditions there is no change in the serum proteins as determined by electrophoretic analyses. Under the conditions in this study, either ultraviolet irradiation or beta propiolactone individually did not sterilize the serum.

1. Murphy, W. P., Jr., and Workman, W. G., *J.A.M.A.*, 1953, v152, 1421.
2. Murray, R., personal communication.
3. Murray, R., Oliphant, J. W., Tripp, J. T., Hampil, B., Ratner, F., William, C. L., Diefenbach, M. D., and Geller, H., to be published.

Received June 16, 1954. P.S.E.B.M., 1954, v86.