

THE ACTIVITY OF HUMAN REAGINS EXPOSED TO THE DISINFECTANT, BETA-PROPIOLACTONE*

JOHN A. FLICK, M.D., AND RICHARD J. FEINBERG, SC.M., PHILADELPHIA, PA.

WHILE examining human serum for the presence of reagins by the Prausnitz-Küstner technique, one may transmit homologous serum jaundice to the recipient. Although the transmission of hepatitis virus type B is usually associated with the intramuscular or intravenous injection of human blood or some of its products, there is little reason to doubt that the intradermal injection of such materials, even in minute amounts, may result in hepatitis in the recipient. Loveless¹ observed a subject who developed viral hepatitis subsequent to repeated passive transfer skin testing with human sera. Also, the tattooing needle has been incriminated in the occasional transmission of viral hepatitis.^{2, 3}

Ultraviolet irradiation of infected plasma under suitable conditions apparently inactivates the virus.^{4, 5} Exposure of serum or plasma to such irradiation does not appreciably alter the electrophoretic patterns or solubility of the protein components,^{5, 6} nor does it inactivate serum reagins.¹ However, recent studies indicate that ultraviolet irradiation is not always a reliable means of rendering plasma noninfectious with respect to hepatitis.⁷⁻¹⁰

Storage of plasma for prolonged periods at room temperature may prove to be a thoroughly effective means of inactivating serum hepatitis virus¹¹⁻¹³ but, due to the known heat lability of many reagin specimens and to the desire to study reagins within a reasonably short period after obtaining the blood sample, it was considered unprofitable to explore this method of hepatitis prevention.

Hartman and his associates¹⁴ reported that the compound, beta-propiolactone† (betaprone), when added to plasma samples in a concentration of 0.15 per cent, inactivated seven unrelated viruses present therein. Hepatitis viruses were not included among those tested. At a concentration of 0.2 per cent the compound also killed six diverse species of bacteria in serum samples. Murray,¹⁵ studying the action of beta-propiolactone on hepatitis B virus in plasma, has indicated that the compound appears to reduce the infectivity of a given dose of the virus for human volunteers. Betaprone hydrolyzes rapidly in serum and its degradation products are relatively nontoxic for animals.^{14, 16, 17}

Hartman's¹⁴ report on the favorable disinfectant action of betaprone prompted a study of the possible inactivating effect of this compound upon serum reagins with the aim of using the compound to reduce the risk of viral hepatitis attending Prausnitz-Küstner skin testing. Evidence is presented here which indicates that betaprone does not produce appreciable loss of reagins in treated serum.

From the Department of Microbiology, School of Medicine, University of Pennsylvania.

Received for publication Aug. 18, 1954.

*This research has been aided by Grant G 3804 (c) from the United States Public Health Service.

†Manufactured by the B. F. Goodrich Chemical Company, Cleveland 15, Ohio.

METHODS

Beta-propiolactone,* a colorless liquid, was stored in a refrigerator. Just prior to use it was mixed with sterile saline to yield a solution of known and convenient concentration. This stock solution was then added aseptically to a quantity of serum so that the volume increment was small and the final concentration of betaprone was 0.2 per cent by volume. Precautions were taken to insure thorough mixing of the betaprone and the serum, even with the minute aspiration droplets of serum produced by expelling the serum from the delivery pipette on the upper wall of the tube. No pH adjustment of the mixture was made, but with some serum samples the pH was measured with a glass electrode before and after treatment. The betaprone-treated serum was then incubated at 37° C. for two to three hours in a water bath. An aliquot of the serum so treated was cultured in Bacto brain-heart infusion medium (Difco Laboratories, Inc.) containing 0.1 per cent agar. The cultures were examined for growth after incubation at 37° C. and at room temperature for three days. Serum dilutions were made after treating the serum with the disinfectant.

For comparative purposes in one case, serum was mixed with cold liquid ethylene oxide, a potent germicidal agent, at a final concentration of 6 to 7 per cent. The mixture was incubated at 37° C. for twenty-four hours, treated with sterile sodium thiosulfate solution to reduce the residual ethylene oxide, and stored until tested.

The sera under test were derived from hay fever patients. Nonatopic persons served as recipients in the Prausnitz-Küstner tests. Approximately twenty-four hours intervened between serum injection and the subsequent antigen injection into the same site. In all cases an excess of antigen was injected in order to bring out the maximal reaction. The wheal and the erythema were measured separately along two perpendicular diameters and these were then averaged to give a single dimension for each.

RESULTS

Passive transfer skin reactions obtained with treated sera were compared with reactions produced by controls in which saline was substituted for the disinfectant in the serum. Table I presents the data derived from a study of three human sera. There appears to be little or no inactivation of the reagents present in the betaprone-treated sera, judging from the size of the reactions developed by corresponding test and control sites. In Experiment IV, the reactions with betaprone serum are somewhat smaller than with the saline serum. This difference, of course, could be due to some inactivation of the reagents or to technical and chance error; experience suggests the latter. Experiment V indicates that ethylene oxide, at the concentration employed, inactivated most, if not all, of the reagent in the serum.

Since these initial observations were made, betaprone has been used routinely with all sera in human passive transfer tests as a safety precaution. Reagent titers of these sera from hay fever patients have varied from zero in a few instances

*One sample of this compound was generously supplied to us by Dr. J. Spizizen of Sharp and Dohme, Inc., West Point, Pennsylvania. Dr. Spizizen originally drew our attention to the activity of beta-propiolactone. A second sample was very kindly furnished by the B. F. Goodrich Chemical Company.

TABLE I. COMPARISON OF PRAUSNITZ-KÜSTNER TEST REACTIONS GIVEN BY DISINFECTANT- AND SALINE-TREATED SERA

EXPERIMENT NO.	TEST SERUM	SERUM DILUTION	ANTIGEN	MEAN DIAMETERS OF WHEEL AND ERYTHEMA (MM.)				
				SERUM + DISINFECTANT			SERUM + SALINE	
				DISINFECTANT (PER CENT)	WHEEL	ERYTHEMA	WHEEL	ERYTHEMA
I	A	1:1	Timothy	BP-0.1	14	37	13	36
			Timothy	BP-0.2	14	37		
II	B	1:1	Timothy	BP-0.2	12	39	11	38
		1:10	Timothy	BP-0.2	10	36	10	32
		1:50	Timothy	BP-0.2	0	0	0	0
III	C	1:1	Ragweed	BP-0.2	12	40	12	39
		1:10	Ragweed	BP-0.2	10	31	10	30
		1:50	Ragweed	BP-0.2	8	20	—	—
IV	C	1:1	Ragweed	BP-0.2	10	29	15	38
		1:5	Ragweed	BP-0.2	8	18	10	27
V	C	1:1	Ragweed	EO-6	0	22	14	45

BP, beta-propiolactone; EO, ethylene oxide. 1:1 dilution signifies no dilution (except for that produced by the additive, amounting to a serum dilution of 9:10). Each reaction figure represents the mean of two perpendicularly measured diameters.

to greater than 1:500. In most of these cases where the serum had a zero titer of reagin to a particular antigen it also had a relatively high titer of reagins to another antigen under test. Antigens employed were those indicated by the person's clinical history and skin test results.

Ethylene oxide at 6 per cent concentration caused a precipitate to form in the serum. This has not been observed so far with betaprone. However, 0.2 per cent betaprone did cause a precipitate to form in clear extracts prepared from human aorta.

Recently, a serum specimen was encountered which had its reagin activity markedly reduced by incubating the serum at 37° C. for two hours with or without added betaprone. A sample of serum obtained previously from the same atopic patient did not show this marked temperature lability of its reagins. Further studies would be necessary to determine whether or not treatment of the serum with betaprone at 6 to 10° C. for twelve to twenty-four hours (conditions which Hartman and his co-workers¹⁴ found satisfactory for destroying their viruses) could be substituted in this case.

TABLE II. PH VALUES OF SERUM SAMPLES BEFORE AND AFTER TREATMENT WITH BETA-PROPIOLACTONE

SERUM	BEFORE TREATMENT	AFTER BETAPRONE TREATMENT		SERUM	BEFORE TREATMENT	AFTER BETAPRONE TREATMENT	
		INITIALLY	AFTER TWO HOURS' INCUBATION			INITIALLY	AFTER TWO HOURS' INCUBATION
A	8.45	7.05	6.25	H	7.9	6.9	6.35
B	8.1	6.85	6.0	I	8.15	7.05	5.55
C	8.3	6.8	6.3	J	8.05	6.85	5.75
D	7.3	6.7	6.55	K	8.3	6.75	5.80
E	8.3	6.85	6.35	L	8.35	6.75	5.60
F	7.85	6.85	6.3	M	8.35	6.85	5.75
G	8.05	6.9	6.2	Average	8.11	6.86	6.06

Hartman and his associates¹⁴ apparently adjusted their serum and plasma samples to pH 6.5 before adding betaprone because of the more rapid hydrolysis of betaprone on the alkaline side of neutrality. The effect upon pH of adding betaprone to a final concentration of 0.2 per cent in serum was studied and the results were recorded in Table II. Measurements of the pH were made before and immediately after adding 2.0 per cent betaprone solution (dissolved in physiologic saline) and again after a two-hour incubation period at 37° C. in cotton-plugged tubes. Prior to testing, sera had been stored at -20° C. in rubber-stoppered vials for varying periods. Before treatment, the sera had a pH range of 7.3 to 8.4. Immediately after betaprone addition, the extreme values ranged from 6.7 to 7.05, with an average value of 6.86. Following the two-hour incubation period, the pH was found to be still lower, ranging from 5.55 to 6.55, with a mean value of 6.06.

An early sample of betaprone was kept at room temperature. After some months of intermittent use, it lost its ability to dissolve completely in saline. Gresham and his associates¹⁸ have studied some of the chemical properties of this compound and found that it polymerizes, slowly under some conditions and readily under others, to form water-insoluble complexes. Another sample stored in the dark at 4 to 10° C. for over one and one-half years, so far, has retained complete solubility in saline solution in the region of 2.0 per cent concentration.

Betaprone was reported to be not only a viricide, but also a bactericide.¹⁴ Although the sera listed in Table I were Seitz filtered before use, following this early experience the routine processing of sera for Prausnitz-Küstner testing has not included such filtration to insure sterility. Reliance has been placed on the betaprone treatment with subsequent culturing of the treated serum. So far all sera studied have been culturally negative. Most of these sera have been collected under reasonably aseptic conditions, but some were processed under conditions adverse for maintaining sterility and yet, following betaprone treatment, they have shown no evidence of living bacteria.

DISCUSSION

On the basis of the limited experience reported, beta-propiolactone does not appear to appreciably inactivate human serum reagins. Also, since its toxicity is low,^{14, 16, 17} there seems to be no obvious contraindication for its employment in sera under scrutiny for reagin activity. Whether or not betaprone will fulfill its primary duty of inactivating hepatitis viruses in serum has not been completely established as yet. Also, it is not possible to state definitely at this time that the concentration of this agent used here, 0.2 per cent, is optimal for hepatitis B virus inactivation. Judging from Hartman's report¹⁴ dealing with viruses other than those causing hepatitis, the minimal concentration of beta-propiolactone causing inactivation of a virus in serum depends upon the virus concentration and probably the pH. Thus, the recommended concentration of beta-propiolactone might be satisfactory for most hepatitis virus-infected serum samples but not for occasional ones possessing very high concentrations of virus.

Measurements of pH made on serum samples treated with betaprone to a concentration of 0.2 per cent indicate that betaprone will markedly reduce the

pH of serum, usually to values below pH 7.0. This reduction of pH probably is due to hydrolytic products of beta-propiolactone occurring as impurities or to rapid hydrolysis to some degree in the fresh 2.0 per cent solution in saline. Upon incubating the serum-betaprone mixture, there is a further drop in pH, probably due to continued hydrolysis of the compound. Within one minute of preparation the pH of a 2.0 per cent stock solution of betaprone is 3.3 in either water or 0.85 per cent sodium chloride solution. If this effect is due to hydrolytic impurities in the reagent before adding water, it may vary in degree with the age of the reagent. This hydrolytic process introduces a degree of uncertainty into any statement of concentration of beta-propiolactone in serum. In making up the stock solution of betaprone and also in adding the stock solution to the serum, an attempt was made to err slightly on the side of adding excess betaprone rather than to have the calculated concentration less than 0.2 per cent in the serum. These experimental conditions may or may not match those which ultimately are found best suited for inactivating hepatitis viruses in serum or plasma.

As a secondary consideration beta-propiolactone shows promise as a substitute for Seitz filtration of serum to render it free of living bacteria prior to skin testing. It would seem, however, that its use here would be limited to sera contaminated with nonsporulating bacteria, since spore formers are notorious for their resistance to many of the chemical disinfectant agents.

SUMMARY

The viricidal and bactericidal agent, beta-propiolactone, when admixed with serum in a concentration of 0.2 per cent, does not destroy reagins appreciably. The treatment of serum for Prausnitz-Küstner testing with this agent is recommended as a possible safety measure directed especially toward transmission of viral hepatitis. At this time, although there is some evidence from other sources that beta-propiolactone does inactivate hepatitis B virus in serum, the viricidal action of this compound on this particular virus has not been completely elucidated.

REFERENCES

1. Loveless, M. H.: Influence of Ultraviolet Irradiation on Antibodies of Pollen-Allergic Man, *Federation Proc.* **9**: 386, 1950.
2. Roberts, R. H., and Still, H.: Homologous Serum Jaundice Transmitted by a Tattooing Needle, *Canad. M. A. J.* **62**: 75, 1950.
3. Smith, B. F.: Occurrence of Hepatitis in Recently Tattooed Service Personnel, *J. A. M. A.* **144**: 1074, 1950.
4. Oliphant, J. W., and Hollaender, A.: Homologous Serum Jaundice. Experimental Inactivation of Etiologic Agent in Serum by Ultraviolet Irradiation, *Pub. Health Rep.* **61**: 598, 1946.
5. Blanchard, M. C., Stokes, J., Hampil, B., Wade, G. R., and Spizizen, J.: Methods of Protection Against Homologous Serum Hepatitis. II. The Inactivation of Hepatitis Virus SH With Ultraviolet Rays, *J. A. M. A.* **138**: 341, 1948.
6. Wolf, A. M., Mason, J., Fitzpatrick, W. J., Schwartz, S. O., and Levinson, S. O.: Ultraviolet Irradiation of Human Plasma to Control Homologous Serum Jaundice, *J. A. M. A.* **135**: 476, 1947.
7. Rosenthal, N., Bassen, F. A., and Michael, S. R.: Probable Transmission of Viral Hepatitis by Ultraviolet-Irradiated Plasma, *J. A. M. A.* **144**: 224, 1950.
8. Barnett, R. N., Fox, R. A., and Snavey, J. G.: Hepatitis Following the Use of Irradiated Human Plasma, *J. A. M. A.* **144**: 226, 1950.
9. James, G., Korns, R. F., and Wright, A. W.: Homologous Serum Jaundice Associated With Use of Irradiated Plasma: a Preliminary Report, *J. A. M. A.* **144**: 228, 1950.

10. Albrecht, R. M., Korns, R. F., Beadenkopf, W. G., Goodman, M. B., Locke, F. B., and Marks, V.: Serum Hepatitis Apparently Acquired From Irradiated Plasma, *J. A. M. A.* **152**: 1423, 1953.
11. Allen, J. G., Inouye, H. S., and Sykes, C.: Homologous Serum Jaundice and Pooled Plasma—Attenuating Effect of Room Temperature Storage on Its Virus Agent, *Ann. Surg.* **138**: 476, 1953.
12. Allen, J. G., Enerson, D. M., Barron, E. S. G., and Sykes, C.: Pooled Plasma With Little or No Risk of Homologous Serum Jaundice, *J. A. M. A.* **154**: 103, 1954.
13. Murray, R., Ratner, F., Diefenbach, W. C. L., and Geller, H.: Effect of Storage at Room Temperature on Infectivity of Ictero-genic Plasma, *J. A. M. A.* **155**: 13, 1954.
14. Hartman, F. W., Piepes, S. L., and Wallbank, A. M.: Virucidal and Bactericidal Properties of β -propiolactone, *Federation Proc.* **10**: 358, 1951.
15. Murray, R.: Personal communication, 1954.
16. Kelly, A. R., and Hartman, F. W.: Beta-Propiolactone: Its Toxicity, Degradation Products and Comparison With Nitrogen Mustard, *Federation Proc.* **10**: 361, 1951.
17. Kelly, A. R., and Hartman, F. W.: Biological Effects of Beta-propiolactone, *Federation Proc.* **11**: 419, 1952.
18. Gresham, T. L., Jansen, J. E., and Shaver, F. W.: Beta-Propiolactone: I. Polymerization Reactions, *J. Am. Chem. Soc.* **70**: 998, 1948.