

Effects of beta-propiolactone (Betaprone) on reaginic (skin-sensitizing) antibodies

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The measurement of reaginic antibodies by transfer to nonallergic human beings has the potential risk of transferring the hepatitis virus. Since beta-propiolactone (BPL) is an effective virucidal agent in 0.40 per cent concentration and is rapidly denatured to harmless end products, its value in treating such reaginic serum was studied particularly as it may affect these labile antibodies. Despite the fact that this agent acts on various functional groups associated with proteins, the reduction in reagin titers of various sera directed against a number of allergens was slight. Only the reagin directed against penicillin was apparently completely destroyed. Experiments used nonallergic human beings and subhuman primates with the use of varying dilutions of BPL (from 0.2 per cent to 1.0 per cent) and serial dilutions of sera. The virucidal agent was added in some instances to each serum dilution and in other experiments only to the concentrated sera. The slight drop in the reagin titer was comparable in the various experiments. In vitro experiments with rabbit precipitating antibodies against several antigens showed no significant change in precipitating antibody titer after exposure to BPL. BPL (0.4 per cent), after incubation with serum, produced a minimal irritative effect in several subjects chiefly in the form of a small papule which did not interfere with the passive-transfer reaction.

The use of reaginic sera for the study of immediate-type hypersensitivity has for many years necessitated use of nonallergic human recipients. Although new techniques with the use of subhuman primates and in vitro methods are being developed, the classical passive-transfer technique of Prausnitz and Küstner (P-K) is still most acceptable for qualitative as well as quantitative studies of reaginic (skin-sensitizing) and blocking antibodies. This is especially true among investigators who do not have available nonhuman primates or special equipment which requires skilled technical assistance. However, there is much reluctance and in some areas rigid restriction on the experimental in-

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tion of human serum into volunteers. The primary concern is the hazard of transmitting microorganisms, particularly the virus of hepatitis. Authenticated occurrences of such unfortunate experiences are extremely rare, yet the specter of a single case has been the strongest deterrent to the use of this passive-transfer procedure.

An effective virucidal agent which would not denature this labile reaginic antibody would therefore be valuable in restoring and broadening the use of the passive-transfer procedure. Such an agent is being considered in beta-propiolactone (BPL). This chemical, in concentration that is sufficiently virucidal, is rapidly hydrolyzed to become free of local or systemic toxic effects and is found to have minimal denaturing effect on the reaginic antibodies directed to a number of allergens. Flick and Feinberg,¹ with the use of a single concentration of 0.2 per cent BPL with reaginic sera of timothy- and ragweed-sensitive patients, found that this agent did not affect reagin titers appreciably as measured by the passive-transfer method.

Our findings presented here will include studies on both human beings and subhuman primates with (1) various dilutions of BPL, (2) varying dilutions of sera, and (3) a variety of reaginic sera directed toward differing allergens.

BETA-PROPIOLACTONE (BPL)

This virucidal agent has been studied for a number of years and has been widely used for the sterilization of biological material.²⁻⁴ However, since its properties may not be familiar to many readers, a summary of those properties pertinent to our experiments is offered.

Physical and chemical properties of BPL

1. Stored in sealed polyethylene ampuls at room temperature, it will keep for from one to 2 weeks. At temperatures of -15°C ., it will keep for several months to a year.

2. The flash point is 70° to 74°C . (contact with heat must be avoided).

3. Contact with metal salts, especially ferrous salts, may result in catalyzed polymerization and may produce a violent explosion.

4. It reacts readily with hydroxyl, amino, carboxyl, sulfhydryl, and phenolic groups (these are associated with proteins).

5. It hydrolyzes rapidly in aqueous solution and disappears much more rapidly in plasma than in water. By the time a biological substance has been adequately treated with BPL, none of the original drug is present.

6. The degradation products (hydrolysates) are hydraacrylic acid, hydraacrylic polymer, and sodium beta chloropropionate. The toxicity (LD_{50}) of these end products is comparable to that of lactic acid.⁵

Biological properties

1. A vesicant effect occurs if the concentrated material is applied to the skin.

2. This drug itself has been found experimentally to be slightly carcinogenic, but this is not true of its end products.⁶

3. Its effect on the electrophoretic pattern of plasma was not appreciable with the use of 2,000 mg. of BPL (0.2 per cent) per liter and after 77 days of exposure. With 5,000 mg. of BPL (0.5 per cent) per liter of plasma, no effect was noted after storage for 7 days at 5° C.

4. Nontoxic, nonallergenic, noncarcinogenic results were reported in 4,000 transfusions of BPL-treated plasma to 1,391 patients.⁷⁻⁹

5. Sterilization effects of BPL in tissue and sera were superior to other chemical agents against viruses, bacteria, and fungi, including spores.^{2, 4}

Virucidal effect of BPL—general consideration

1. Concentrations of 0.4 per cent inactivated 22 different laboratory viruses seeded in plasma.⁴

2. The kinetic rate of BPL hydrolysis in plasma in relation to the rate of virucidal inactivity showed that at either 37° or 4° C, and at temperatures between, the virus of hepatitis is inactivated before the half-life of the drug. Half-life of BPL at 37° C. varies from 24 to 32 minutes and at 4° C. from 16 to 20 hours. Inactivation of various viruses to nondetectable quantity is attained in 15 to 30 minutes at 37° C. and in 8 to 10 hours at 4° C. However, it is recommended for conducting sterilization that, at 4° C., at least 4 days be allowed for complete hydrolysis before use of the product, while at 37° C. it is necessary to allow only 2 to 3 hours.

3. Products of BPL hydrolysis lack virucidal activity.

4. Virus inactivation is unaffected by pH changes due to the amphoteric nature of the hydrolyzing drug.

5. A disproportionate amount of drug is required to inactivate ultimate trace quantities of active viruses (tailing phenomena).⁴

6. The degree of inactivation of viruses cannot be reversed by dilution, prolonged storage, or immediate neutralization with sodium thiosulfate.

7. The concentration of BPL required for inactivation of a constant titer of virus in serum varies directly with the amount of protein present. In plasma concentrations of 30, 60, and 90 per cent, the amount of BPL required increased from 5,000 mg. per liter, to 15,000 mg. per liter, to 20,000 mg. per liter, respectively.

Virucidal activity of BPL—hepatitis virus

Results are of human trials with known hepatitis-infected plasma.^{8, 9}

1. Concentrations of 0.3 per cent to 0.4 per cent BPL were adequate for "apparent" sterilization of icterogenic plasma when 1 ml. of treated plasma was injected intramuscularly into human volunteers. However, when 600 ml. of plasma was administered intravenously, several of the volunteers developed hepatitis, indicating trace quantities of virus persisting with these drug concentrations.⁹

2. Combination of BPL with standard ultraviolet irradiation caused complete sterilization for hepatitis virus even after injections with large volumes of plasma. This latter has been evaluated by clinical pools of plasma and not with known icterogenic plasma in human volunteers.⁷⁻⁹

Table I. *Summary list of experiments, sera donors, recipients challenged, and allergens used*

<i>Exp. No.</i>	<i>Serum donor</i>	<i>Recipient</i>	<i>Allergens</i>
1	J. H.	Monkey No. 1346	Penicillin G.K., dog hair
	R. McQ.	Monkey No. 1346	Castor bean
2	J. H.	Monkey No. 1384	Penicillin G.K., dog hair
	J. H.	Monkey No. 1402	Penicillin G.K., dog hair
	J. H.	Monkey No. 1398	Penicillin G.K., dog hair, house dust
	W. O.	Monkey No. 1398	Dog hair, timothy pollen
3	J. H.	Human (H. W.)	Penicillin G.K., dog hair (1st and 2nd challenges)
	W. O.	Human (F. P.)	Dog hair (1st and 2nd challenges)
4	W. O.	Human (F. P.)	Timothy pollen
	W. O.	Human (H. W.)	Timothy pollen
	S. S.	{ Monkey No. 1322 }	Timothy pollen
	W. O.	{ and }	Dog hair
	J. H.	{ Monkey No. 1328 }	Dog hair
	L. M.	{ Monkey No. 1185 }	Timothy pollen
	L. R.	{ and }	Green pea
	R. W.	{ Monkey No. 1384 }	Timothy pollen
5	V. McG.	Human (D. M. and J. A.)	Lactalbumin
	L. M.	Human (J. N. and H. M.)	Green coffee, timothy pollen

EXPERIMENTS—GENERAL CONSIDERATION

The following experiments were carried out to determine the degree, if any, of denaturation of reaginic antibodies as measured biologically by transfer to human beings and subhuman primates (Table I): (1) effect of varying concentrations of BPL with constant dilution of reaginic serum, providing not only an indication of the effect of BPL on antibodies but also its possible irritative effect on skin after incubation at 4° C. for 24 hours; (2) varying dilutions of serum with constant concentration of BPL added to each serum dilution; (3) varying dilutions of serum with single concentration of BPL added to the original concentrated serum and further dilutions made with buffered saline; (4) varying dilutions of serum with varying dilutions of BPL added to the concentrated sera only and with further dilution of the sera with buffered saline.

Some observations were also made on the effect of BPL on rabbit precipitating antibodies directed against human serum albumin (HSA), timothy pollen (WST), and trinitrophenol (TNP) hapten.

MATERIALS AND METHODS

Beta-propiolactone or Betaprone, the designated name for the material recommended for biological sterilization, is 99 per cent pure lactone form. It was purchased from the manufacturer, Fellows-Testagar and Company, Inc., Detroit, Michigan, and was stored in sealed ampuls at -20° C. until used. When used to treat the sera, the dilutions were made with distilled water at 0° C., keeping the vials and other glassware immersed in ice baths. After mixing equal

volumes of sera and BPL, the dilution mixtures were stored at 4° C. for 24 hours until used for implantation in man and monkeys. Usually 4 to 6 hours would transpire between removal of these mixtures from refrigeration and transport to the subjects for implantation.

Sera which had been gathered over months were stored at -20° C. and thawed only when ready for use. These sera were collected under sterile conditions and were not filtered or treated with any chemical.

Allergens used in challenging the passively sensitized subjects were those currently used in our allergy clinic. The concentration was calculated on the basis of weight per volume. All had been bioassayed and found highly reactive on the basis of producing strong wheal and flare reactions on known allergic individuals. Fresh allergen dilutions were prepared for each experiment, and in the case of penicillin the powdered material was constituted in a designated dilution at the time of use.

Classical passive-transfer technique on human beings was used. Sites received their first challenge in most experiments 48 hours after implantation. The allergic-serum transfer (AST) to monkeys was done according to the technique of Layton.¹⁰ Intravenous challenge was carried out approximately 24 hours after implanting the sera.

Although the degree of passive reaction on the monkeys is recorded as negative (-) to greater than four plus (> ++++), the diameter of each reaction was measured and also recorded. Skin reactions (bluing or wheal size) were graded as follows: 5 to 10 mm. diameter, +; 10 to 15 mm. diameter, ++; 15 to 20 mm. diameter, +++; and greater than 20 mm. diameter, ++++. However, the relative degree of reaction is more quickly evident to the reader when designated by symbols. Before a second intravenous challenge with another allergen was carried out within the hour (e.g., dog hair after house dust), it would first be noted after 30 to 45 minutes that no measurable bluing change had occurred. The next challenging allergen produced a dramatic increase in net diameter and bluing intensity of the sensitized sites within 5 to 10 minutes.

Quantitative precipitin analysis of rabbit antisera (containing 1.8, 1.5, and 2.1 mg. of antibody protein per milliliter, respectively) directed against HSA, WST, and TNP antigenic determinants was performed as described by Campbell and associates.¹¹ Protein concentrations were determined by the Nessler method.¹² Rabbit antisera used in these experiments were diluted 1:4 with either buffered saline or 1 per cent BPL and stored at 4° C. for 24 hours prior to the addition of the appropriate antigen concentration.

RESULTS AND DISCUSSION

The effect of varying BPL concentrations on reagin directed against several allergens is demonstrated in Table II. Although studies on a single monkey are here reported, three additional animals were used and almost identical results were obtained. The serum of Patient J. H. contained reaginic antibodies directed against penicillin, dog hair, and house dust. Reagin directed against penicillin was evidently entirely destroyed in this experiment as well as in subsequent studies. After challenging within 45 minutes with dog hair, the

Table II. *Effect of BPL in varying concentrations on reaginic sera in 1:2 dilution only, Monkey No. 1346*

Serum donor	Allergen	Serum + BPL			Serum + saline
		0.2%	0.4%	0.75%	
J. H.	Penicillin G.K.	-	-	-	<++++
	Dog hair	+++	+++	+++	++++
		+++	+++	+++	++++
R. McQ.	Castor bean	+++	+++	+++	++++

Table III. *Effect of BPL, 0.4 per cent concentration, on serial dilutions of sera (BPL added to each dilution), monkey recipients*

A. Monkey No. 1384

Allergen	Serum diluent	Sera dilutions (Donor J. H.)							
		1:2	1:4	1:8	1:16	1:32	1:128	1:512	Control
Penicillin	BPL	-	-	-	-	-	-	-	-
	Saline	+++	++	-	-	-	-	-	-
Dog hair	BPL	++++	++++	+++	+++	+++	-	-	-
	Saline	>++++	++++	+++	+++	+++	+++	-	-

B. Monkey No. 1402

Allergen	Serum diluent	Sera dilutions (Donor J. H.)							
		1:2	1:4	1:8	1:16	1:32	1:128	1:512	Control
Penicillin	BPL	-	-	-	-	-	-	-	-
	Saline	+++	++	-	-	-	-	-	-
Dog hair	BPL	++++	+++	+++	+++	+++	-	-	-
	Saline	>++++	++++	++++	<++++	+++	+++	+	-

C. Monkey No. 1395

Allergen	Serum diluent	Sera dilutions									
		Donor J. H.						Donor W. C.			
		1:2	1:4	1:16	1:64	1:256	1:1024	1:5	1:25	1:125	1:625
Penicillin G.K.	BPL	-	-	-	-	-	-	-	-	-	-
	Saline	++	-	-	-	-	-	-	-	-	-
House dust	BPL	+++	++	++	-	-	-	-	-	-	-
	Saline	+++	+++	+++	++	-	-	-	-	-	-
Dog hair	BPL	++++	++++	++++	+++	-	-	+++	++	-	-
	Saline	++++	++++	++++	+++	-	-	+++	++	-	-
Timothy pollen	BPL	-	-	-	-	-	-	++++	+++	-	-
	Saline	-	-	-	-	-	-	++++	+++	-	-

BPL-treated serum showed a significant reaction which was slightly less pronounced than that produced with the untreated serum. This slight reduction in reactivity was also evident with reaginic serum of Patient R. McQ. and the allergen in castor bean.

The second important purpose of this experiment was to determine the necrotizing effect of BPL in varying dilutions. It was apparent that at 0.75

per cent final concentration, this agent still produced some tissue damage after incubation at 4° C. for 24 hours. This was evident not only from the small, circumscribed inflammatory reactions but also from some extravasation of Evans blue dye at the site of previous serum transplants before the intravenous challenge with the allergens was done. At a final strength of 0.4 per cent BPL, minimal, if any, local irritation was noted, and no dye extravasation occurred. Since at this concentration the BPL has been proved highly virucidal, subsequent experiments used this as a constant strength.

The effect of 0.4 per cent BPL on reaginic sera from patients sensitive to penicillin, house dust, dog hair, and timothy pollen was demonstrated by passive transfer to rhesus monkeys. A constant dilution of BPL at 0.4 per cent

Table IV. *Effect of BPL, 0.4 per cent concentration, on serial dilutions of sera (BPL added to each dilution), human recipients*

A. H. W.—recipient, implanted 7/14/66

<i>Allergen</i>	<i>Serum diluent</i>	<i>Serum of J. H.</i>					
		<i>First challenge 7/16/66</i>				<i>Second challenge 7/20/66</i>	
		<i>1:4</i>	<i>1:16</i>	<i>1:256</i>	<i>1:1024</i>	<i>1:4</i>	<i>1:256</i>
Penicillin	BPL	—	—	—	—		
	Saline	++++	—	—	—		
Dog hair	BPL	<++++	+++	++	+	+++	++
	Saline	++++	++++	++	+	++	+

B. F. P.—recipient, implanted 7/14/66

<i>Allergen</i>	<i>Serum diluent</i>	<i>Serum of W. O.</i>					
		<i>First challenge 7/16/66</i>				<i>Second challenge 7/20/66</i>	
		<i>1:5</i>	<i>1:125</i>	<i>1:625</i>	<i>1:3125</i>	<i>1:5</i>	<i>1:125</i>
Dog hair	BPL	<++++	++	±	—	<+++	±
	Saline	++++	+++	±	—	+++	±

Table V. *Effect of BPL, 0.4 per cent concentration, on serial dilutions of sera (BPL added to original sera and remaining dilutions made with buffered saline); human recipients**

A. Human recipient F. P.

<i>Allergen</i>	<i>Serum diluent</i>	<i>Dilutions of serum of W. O.</i>				
		<i>1:4</i>	<i>1:12</i>	<i>1:36</i>	<i>1:108</i>	<i>1:324</i>
Dog hair	BPL	++++	<++++	+++	++	++
	Saline	++++	<++++	+++	++	+

B. Human recipient H. W.

<i>Allergen</i>	<i>Serum diluent</i>	<i>Dilutions of serum of R. W.</i>				
		<i>1:4</i>	<i>1:12</i>	<i>1:36</i>	<i>1:108</i>	<i>1:324</i>
Timothy pollen	BPL	++++	<++++	+++	<++	±
	Saline	++++	+++	+++	++	+

*Implant 8/1/67; challenge 8/2/67.

was made by adding 0.8 per cent concentration to an equal volume of each of the serum dilutions. The results (Table III) on three monkeys indicate that the low titer antibodies directed against penicillin were destroyed. The reaginic antibodies directed against house dust and dog hair (Monkey No. 1395) were challenged first by house dust and then by dog hair 45 minutes later. The sites gave increasing reactions as indicated by the increasing diameter and intensity of bluing. BPL-treated sera showed slightly smaller diameters of the blue sites and a loss of reaction at high dilutions in several instances.

The BPL-treated and the untreated sera used on the two human volunteers (Author F. P. and a technician) shown in Table IV were also implanted into Monkey No. 1393 and Monkey No. 1402 on the same day. Results with first challenge were comparable for both monkey and human recipients. Again, this serum-containing reagin directed against penicillin was evidently inactivated.

Table VI. *Effect of BPL, 0.4 per cent concentration, on serial dilutions of sera (BPL added to original sera and remaining dilutions made with buffered saline): monkey recipients**

A. Monkey No. 1322

Allergen	Serum diluent	S. S.			W. O.			J. H.		
		1:8	1:16	1:32	1:20	1:80	1:160	1:20	1:80	1:160
Dog hair	BPL				+++	+	-	+++	<+++	-
	Saline				<++++	+++	+++	<++++	+++	-
Timothy pollen	BPL	+++	±	-						
	Saline	+++	++	±						

B. Monkey No. 1325

Allergen	Serum diluent	S. S.			W. O.			J. H.		
		1:8	1:16	1:32	1:20	1:80	1:160	1:20	1:80	1:160
Dog hair	BPL				+++	-	-	+++	++	-
	Saline				<++++	+	-	<++++	+++	±
Timothy pollen	BPL	+++	++	±						
	Saline	+++	++	+						

C. Monkey No. 1185

Allergen	Serum diluent	L. M.			L. R.			R. W.		
		1:8	1:16	1:32	1:20	1:80	1:160	1:20	1:80	1:160
Timothy pollen	BPL	+++	++	+				++		
	Saline	+++	++	+				<++++	+++	++
Green pea	BPL				<+++	-	-			
	Saline				+++	++	-			

D. Monkey No. 1384

Allergen	Serum diluent	L. M.			L. R.			R. W.		
		1:8	1:16	1:32	1:20	1:80	1:160	1:20	1:80	1:160
Timothy pollen	BPL	+++	++	+				+++	-	-
	Saline	+++	++	+				+++	++	+
Green pea	BPL				-	-	-			
	Saline				++	-	-			

*Implant 8/1/67; challenge 8/2/67.

while the reagin against dog hair (in the sera of "donors" J. H. and W. O.) showed a slight reduction in reactivity after treating with BPL. A second challenge on the human recipients at several of the same sensitized sites 96 hours later suggested comparable reduction of reactivity for both the treated and untreated sera. In fact, the untreated sera give slightly smaller reactions on second challenge, but no significance was attached to this.

One volunteer (H. W.) had little or no local reaction to serum dilutions to which BPL was added to a concentration of 0.4 per cent, but the other (F. P.) displayed vesicles after 48 hours which later umbilicated and dried, leaving some discoloration for several weeks. The degree of irritative reaction was greater at the sites of more dilute sera, suggesting some buffering effect of the serum present at greater concentration. These irritative reactions did not interfere with a clear wheal and flare reaction of direct challenge with the allergens. Subsequent passive-transfer experiments with 6 other human recipients with the use of 0.4 per cent BPL resulted in only an occasional local irritation with slight papule formation which disappeared in several days.

It was thought that at higher dilutions of serum the BPL might have both more denaturing and skin-irritating effect. The concentrated sera from patients sensitive to several allergens were treated with equal volumes of BPL to make a 0.4 per cent concentration of BPL. Further dilution of these BPL-sera mixtures were made with buffered saline. This procedure eliminated local reactions, even in the most reactive individual. It also permitted the use of a one per cent concentration of BPL in the original serum.

Table VII. *Effect of BPL in varying concentrations on serial dilutions of sera (BPL added to original sera and remaining dilutions made with buffered saline)*

A. *Serum of F. McG.; allergen, lactalbumin; recipients, J. A. and D. M.*

Serum dilutions	Untreated serum		With BPL 0.2%		With BPL 0.4%		With BPL 1.0%	
	D. M.	J. A.	D. M.	J. A.	D. M.	J. A.	D. M.	J. A.
1:4	+++	++++	+++	+++	<++++	<++++	Not applied	
1:16	++	+++	++	++	<+++	++	++	+
1:32	+	++	+	+	+	±	±	-
1:64	±	±	+	±	±	-	-	-
1:128	-	-	-	-	-	-	-	-

B. *Serum of L. M.; allergens, green coffee and timothy pollen;* recipients, J. N. and H. M.*

Serum dilutions	Untreated serum		With BPL 0.2%		With BPL 0.4%		With BPL 1.0%	
	J. N.	H. M.	J. N.	H. M.	J. N.	H. M.	J. N.	H. M.
1:4	+++	+++	+++	+++	+++	+++	Not applied	
1:16	<+++	++	<+++	++	<+++	++	++	++
1:32	++	++	++	+	+	+	+	+
1:64	++	++	+	+	+	±	±	-
1:128	±	±	-	±	±	-	-	-
1:128†	++++	+++	++++	+++	<++++	+++	++	+++

*Green coffee control, negative; timothy pollen control, negative.

†Timothy pollen challenge was made at the single 1:128 sites which were negative for green coffee and after a 15 minute wait.

In further experiments both human beings and monkeys were used on the same day as recipients of aliquot parts of the same sera to which BPL had been added to a final concentration of 0.4 per cent and serial dilutions made with buffered saline. In addition to the results shown in Tables V and VI, other allergens were used, including lactalbumin and green coffee (Table VII). The results (Tables V and VI) again indicate a slight degree of reduction in wheal and flare reactions with the BPL-treated sera in higher dilutions. This was not significantly different from the changes observed in Tables III and IV in which all the dilutions were treated individually with BPL.

Table VII shows the effect of treating allergic sera with BPL to make a concentration of 1.0 per cent, 0.4 per cent and 0.2 per cent. BPL was added to equal quantities of the undiluted sera, and subsequent dilutions were made with buffered saline. This permitted observing the relative effect of high and low concentrations of BPL on reaginic antibodies without the hazard of necrotizing effects of the higher concentration of BPL on the human recipients (the 1:4 dilution was not applied). It is again evident that the lower concentrations of BPL do not significantly denature reaginic antibodies. However, with a 1.0 per cent concentration of BPL, the passive-transfer reaction is slightly reduced.

In vitro experiments designed to evaluate the effect of 1 per cent BPL treatment on rabbit precipitating antibody directed against HSA, WST, and TNP showed less than a 3 per cent difference in the quantitative precipitin titers from the same antisera treated with saline. These studies suggest that treatment with one per cent BPL had no denaturing effect on rabbit antisera.

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