toxicity which, it has been hypothesized, may be mediated through the formation of hydroperoxides(4).

Summary. Intraperitoneal injection of concentrates of methyl linoleate hydroperoxide into adult rats was lethal at a level of about 150 μM per 100 g. Previous injection of equal amounts of tocopherol or ethoxyquin did not change the LD_{50} level. Sixteen hundred but not 800 μM per 100 g were lethal when administered by stomach tube.

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Received October 7, 1963. P.S.E.B.M., 1963, v114.

Inactivation of Streptococcal Bacteriophage by Sulfhydryl Reagents.* (28810)

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Work reported previously indicated that the host range of beta-hemolytic streptococcal bacteriophage is a group-specific phenomenon. Thus, the Group A phage lyses Group A streptococci but not Group C and similarly, Group C phage lyses Group C streptococci but not Group A. Such group specificity of these phages suggests that the cell wall carbohydrate, a significant constituent shared by all streptococcal strains of the same serologic group, may serve as a receptor substance for attachment of the phage to the organism(1). Additional support for this view has been obtained by experiments which demonstrated the inactivation of Group C phage but not Group A phage with the purified cell wall carbohydrate of Group C streptococci. It is clear, however, that factors other than the nature of the specific attachment substance of the bacterial cell wall control the adsorption and penetration of the virus into the host cell. For example, recent studies by Kosloff have emphasized the role of sulfhydryl groups in T2 bacteriophage invasion of *E. coli*(2).

In related experiments with animal viruses, Choppin and Philipson reported inactivation of enteroviruses by treatment with mercaptide forming reagents such as p-chloromercuribenzoate; mercuric ion and Thimerosal; an inactivation which was reversed with sub-

* This investigation was supported in part by research grant from Nat. Heart Inst., Division of Research Grants, U.S.P.H.S.

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sequent treatment by the thiol compound, reduced glutathione(3). In the experiments reported here, inactivation of streptococcal phage with the mercaptide forming reagents was also partially reversed by reduced glutathione.

Materials and methods. Group A phage lysates were made by lysis of streptococcal Group A strain T25/41 with A25 phage stock and similarly Group C phage lysates were prepared by the lysis of Group C strain 26RP66 with C1 phage stock. The soft agar layer technique was employed to determine the number of plaque forming phage particles (4). The special media employed as the nutrient source for the agar plates has been described(1). Streptococcal strains T25/41 and 26RP66 were used as indicator strains in the soft agar layer for phages A25 and C1 respectively.

The various phage inactivation experiments were carried out as follows. Two-tenths ml of an appropriate concentration of sulfhydryl reagent was added to 1.8 ml of phage lysate which had been diluted in dialysate broth to a concentration of approximately 10^7 plaque forming units per ml. The mixture was incubated at 24°C and 0.1 ml samples, taken at intervals were diluted immediately into 9.9 ml of Todd-Hewitt broth to halt inactivation. Additional dilutions made from this diluted sample were plated to determine the titer of plaque forming units. Controls were performed in a manner similar to the experimental system and final results were calculated as percent of control values. The dialysate broth employed in the inactivation step has been described(1).

Stock solutions of p-hydroxymercuribenzoate (PHMB)§ were prepared in glycylglycine buffer at pH 8.6. The reagent remained in solution upon subsequent dilution into dialysate broth at pH 7.5. Aqueous solutions of Thimerosal, $HgCl_2$, $ZnCl_2$ and $CdCl_2$, were diluted to appropriate concentration with the dialysate broth.

Results. The inactivation of Groups A and C phage with p-hydroxymercuribenzoate, (which will hereafter be referred to as PHMB), is shown in Fig. 1. Rapid inactivation of the Group C phage Cl occurred with a concentration of 1.0×10^{-3} M PHMB; the Group A phage A25, on the other hand, was consistently less sensitive to the action of this reagent. At a concentration of 1×10^{-3} M PHMB 99% of the Cl phage was inactivated in 10 minutes whereas only 50% of the A25 phage was inactivated.

Fig. 2 illustrates the inactivation of phage by Hg^{++} and demonstrates the fact that phage is less sensitive to PHMB than to this ion. For instance, at a concentration of 3.6×10^{-4} M Hg^{++}, Cl phage inactivation is comparable to that achieved with 1×10^{-3} M PHMB as depicted in Fig. 1. This difference in the effectiveness of the inactivation by these 2 substances was a consistent finding in several experiments over a range of inactivator concentrations. In general a concentration of PHMB 3 to 5 times that of $HgCl_2$ gave comparable inactivation. 1×10^{-3} M Thimerosal, another organic mercurial, also inactivates the phage, but is somewhat less effective than PHMB at the same concentration.

Because mercuric ion is bivalent and in Group IIb of the periodic table the inactivation of phage by the other elements of this group is of interest. Fig. 3 illustrates a comparison of the inactivation of Group C phage by zinc ion, cadmium ion, and mercuric ion. At a concentration of 1×10^{-3} M zinc or cadmium ion, phage is inactivated more slowly than by 3.6×10^{-4} M mercuric ion. Thus the ions of the elements of Group IIb inactivate the Cl phage, but Hg^{++} is more effective in this respect.

In view of the recent finding of Choppin and Philipson that the inactivation of enterovirus infectivity with mercaptide forming reagents is reversed with subsequent treatment by reduced glutathione(3), it was of interest to determine the effect of similar treatment on bacteriophage previously inactivated with PHMB or mercuric ion. In the following experiments Cl bacteriophage inactivated by PHMB or mercuric ion was subsequently treated with reduced glutathione. Cl phage was treated with either 5×10^{-4} M PHMB or 3.3×10^{-5} M mercuric ion. After 5 min-

§ Nutritional Biochemicals Co.

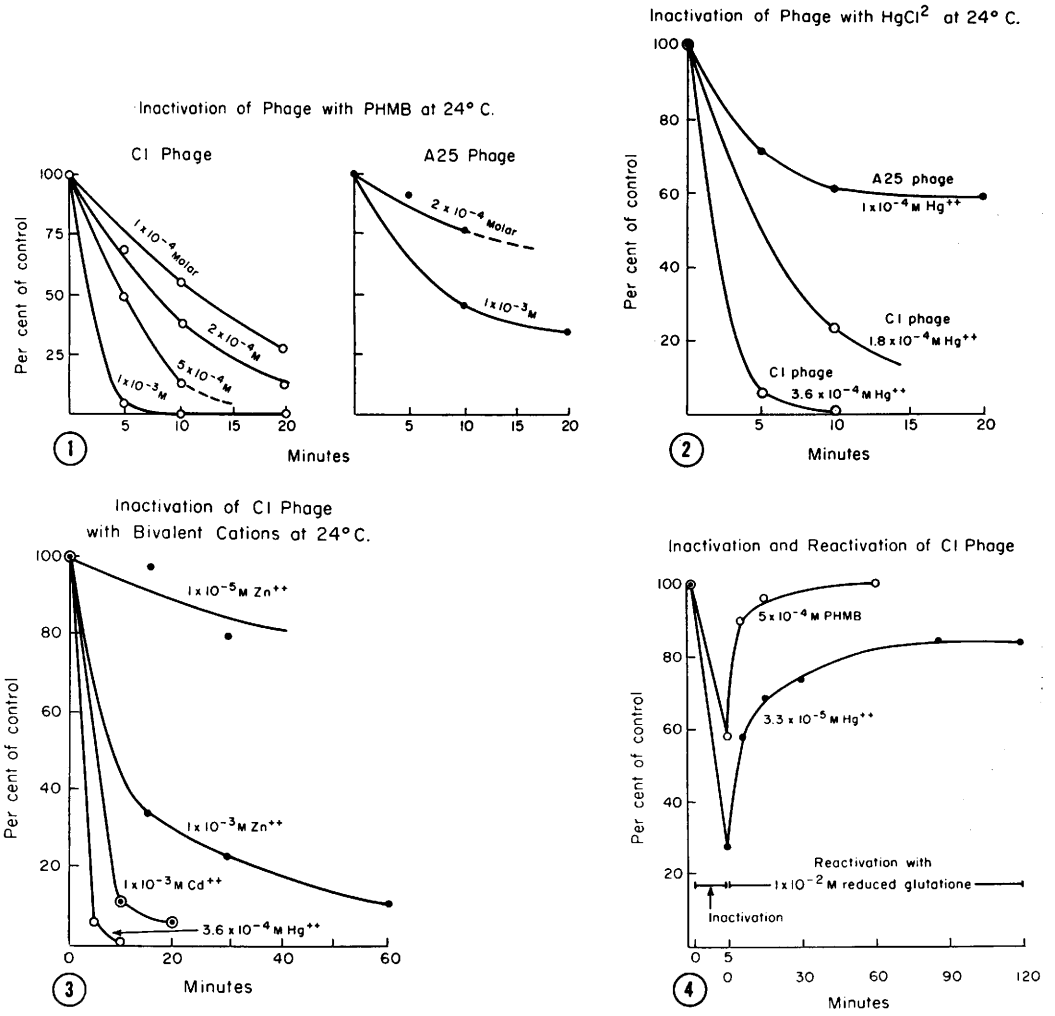

FIG. 1. Inactivation of C1 phage and A25 phage infectivity by mercuric ion at 24°C. Infective phage count expressed as percent of control.

FIG. 2. Inactivation of C1 phage and A25 phage infectivity by mercuric ion at 24°C. Infective phage count expressed as percent of control.

FIG. 3. Inactivation of C1 phage infectivity by bivalent cations at 24°C. Infective phage count expressed as percent of control.

FIG. 4. Reactivation by reduced glutathione of C1 phage infectivity inactivated by PHMB or mercuric ion. Phage was treated for 5 min at 24°C, with either PHMB or mercuric ion in dialysate medium. After dilution 100-fold to terminate the inactivation, the virus was treated at 24°C with reduced glutathione at a final concentration of 10^{-2} M. Infective phage count expressed as percent of control.

utes of inactivation phage counts were determined on aliquots of the mixtures diluted 100-fold. Other aliquots diluted 100-fold were held at 24°C in broth containing 10^{-2} M reduced glutathione, and samples were taken for phage counts at intervals for the next 2 hours. Phage suspensions which were not subjected to the chemical reagents served as controls. Experimental results were expressed as percent of these controls. The results are depicted in Fig. 4. In 5 minutes 75% of the C1 phage were inactivated by 3.3×10^{-5} M mercuric ion. Inactivated phage suspension diluted 100-fold into reduced glutathione and held at 24°C was rapidly reactivated. After treatment for one hour with reduced gluthione reactivation was essentially complete as demonstrated by the fact that the phage

TABLE I. Inactivation of Phage with Mercuric Ion and PHMB, and Subsequent Reactivation with Reduced Glutathione.

Phage	Inactivation			Reactivation for 60 min with reduced glutathione
	Inactivator	Time, min	Phage count, % of control	Phage count, % of control
C1	1.8×10^{-4} M Hg^{++}	5	29	76
	5.0×10^{-4} M PHMB	5	47	106
	5.0×10^{-4} M PHMB	10	12	75
	1.0×10^{-3} M PHMB	5	7	68
A25	3.6×10^{-4} M Hg^{++}	5	34	110
	1.0×10^{-3} M PHMB	10	38	94

count was 80% of control value. Fig. 4 also depicts a similar experiment with Cl phage except that inactivation was carried out with PHMB, but again the inactivation was reversed by addition of reduced glutathione.

The conditions of inactivation require comment. If inactivation proceeded too long with too high a concentration of sulfhydryl reagent the process was not reversible with reduced glutathione. Thus inactivation with 10^{-3} M Hg^{++} resulted in a 3 log fall in phage count, a much more marked effect than the inactivation at 10^{-4} M Hg^{++}; however, with subsequent addition of reduced glutathione there was no detectable reactivation of the phage treated with 10^{-3} M Hg^{++}. Temperature and pH, as well as the buffer system in which inactivation and reactivation proceed, are important factors in the reaction. These variables have not been studied in detail. Several examples of inactivation and reactivation of Cl and A25 phages are tabulated in Table I. The concentration of inactivating reagent was somewhat greater for the A25 than the Cl phage. Inactivation varied from 7 to 47% of control values. Reactivation, determined at 60 minutes, the time at which maximum reactivation occurred, ranged from 68% to 110% of control values.

When Cl phage inactivated with zinc ion and cadmium ion were treated with reduced glutathione, instead of the expected phage reactivation a marked additional inactivation occurred. For example, inactivation with 5×10^{-4}M zinc ion effected a 24% drop in phage titer after 30 minutes, but upon subsequent treatment with reduced glutathione the phage count was further diminished to less than 1% of control value.

A critical question at this point is the cause of the inactivation of the phage treated with mercaptide forming compounds. While it is conceivable that phage adsorption has been inhibited, there is some evidence to suggest that PHMB treated phage is in fact adsorbed to the bacteria. This would indicate interference with the infectious process after the adsorption step. The results of the following experiment support this view. Cl phage was inactivated by PHMB for 7 minutes with a consequent fall in titer from 1.1×10^9 to 0.28×10^9 plaque forming particles per ml. The phage, diluted 100-fold to terminate inactivation by PHMB, was added to streptococci held at 24°C. After 15 minutes the mixture was cooled rapidly and centrifuged at 4°C. Adsorption of the inactivated phage to the streptococci was demonstrated by the fact that the phage titer in the supernatant after treatment with 10^{-2} M reduced glutathione was only 1.4×10^8. The phage titer of an aliquot of the PHMB treated phage which had not been exposed to the streptococci prior to reactivation was 0.75×10^9 or 70% of the initial phage count.

The view that the PHMB treated phage although adsorbed to the streptococcus was not infective also receives support from the subsequent treatment of the streptococcal pellet collected by centrifugation in the above experiment. The organisms to which the inactive phage were adsorbed, were resuspended in broth and divided into 2 equal portions. Lysis occurred in one portion after incubation

for 2 hours in the presence of reduced glutathione at a concentration of 1×10^{-2} M while lysis did not occur in the other portion which had not been treated with reduced glutathione. Thus addition of the sulfhydryl compound to the inactive adsorbed phage resulted in infection, virus multiplication, and cell lysis.

Summary. Although there is evidence to suggest that cell wall components play a role as receptors for phage attachment to the cell, the details of virus adsorption and the prerequisite conditions for infection are not clearly elucidated. In the experiments reported here, it has been demonstrated that phages of Groups A and C streptococci are inactivated by certain sulfhydryl reagents such as PHMB and mercuric ion. The results suggest that these agents did not prevent phage adsorption but rather affected some later step in infection. The inactivation of phage infectivity by the sulfhydryl reagents was reversed by the thiol compound reduced glutathione, a finding consistent with the view that sulfhydryl groups of the phage tail may play a role in the early stages of phage infection.

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Received October 2, 1963. P.S.E.B.M., 1963, v114.

Effects of Insulin on Renal Hemodynamics.* (28811)

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Intravenous administration of glucose elevates blood glucose and causes a significant rise in renal blood flow(1,2). To investigate whether renal blood flow might be correlated with blood sugar levels during hypoglycemia, Bounous and Shumacker injected insulin intravenously into anesthetized dogs(2). One-half unit of regular insulin per kg of body weight decreased renal blood flow gradually during 20 minutes to only 60% of the control value. Blood sugar fell from a mean of 77 mg % to a mean of 50 mg %. On the average, total urine flow fell from 0.04 to 0.02 ml per min. However, in one of the 6 experiments there was no detectable urine flow either before or after the insulin. Bounous and Shumacker ascribed the decrease in renal blood flow to the lowering of blood sugar produced by insulin.

In the experiments reported below, intravenous administration of 1.0 unit of regular insulin per kg of body weight produced no significant effect on renal blood flow and a slight, but probably significant, increase in urine flow.

Methods. Adult mongrel dogs were anesthetized with a barbiturate or with chloralose. The ureters were exposed retroperitoneally and cannulated so that separate urine samples could be collected from each kidney. Blood was obtained from a femoral artery. For measurement of glomerular filtration rate, creatinine, and for measurement of effective renal plasma flow, para-aminohippurate (PAH) were given by continuous intravenous infusion after a priming dose, and were determined in plasma and urine by methods described by Smith(3). Renal blood flow was calculated from the PAH clearance and the hematocrit, assuming an extraction ratio of 0.85. In addition, renal blood flow through one kidney was directly measured with a Shipley-Wilson rotameter in 3 of the 6 ex-

* This investigation was supported in part by USPHS fellowship and in part by Training Grant from Div. of Gen. Med. Sciences, U.S.P.H.S.