compound V. The results strongly suggest that the photoisomers are more toxic than their parent compounds because of rapid metabolism to compound V, itself a more toxic material than the photoisomers. Aldrin and dieldrin are not metabolized to compound V in insects, although this transformation has been observed in male mice (*7*).

No photodieldrin was detected in mosquito larvae (in contrast to flies) which were treated with photoaldrin. This suggests that photodieldrin is either metabolized to compound V much more rapidly in this species than in flies, or that, in mosquitoes, conversion to the ketone VI (not found) precedes epoxidation. Possibly ketone formation and epoxidation occur at the same enzymatic site.

Only negligible amounts of photodieldrin have been recovered in certain foods, and it has been concluded that photolysis of cyclodienes does not represent an increase in the toxicological significance of cyclodiene residues (*4*). However, the rapid biological activation of the photoisomers to the more toxic compound V in insects and possibly similar metabolism in other organisms suggests that residues of compound V might be important.

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Multiplicity Reactivation as a Test for Recombination Function

Abstract. *Multiplicity reactivation of bacteriophage inactivated by ultraviolet light is dependent on the recombination function of either the host bacterial cell or the infecting bacteriophage. Absence of both recombination systems leads to a loss of multiplicity reactivation.*

The inactivation of bacteriophage by ultraviolet light is essentially a first-order reaction (*1*) and can be expressed as:

$$P/P_0 = e^{-kT} \quad (1)$$

where P_0 is the initial bacteriophage titer, P is the bacteriophage titer after the ultraviolet dose T (measured in seconds), and k is a constant dependent on the sensitivity of the bacteriophage and the ability of the host bacterium to repair ultraviolet damage. If bacteria are infected with irradiated bacteriophage at a multiplicity greater than one, the survival of infective centers as a function of dose is expected to be (*2*)

$$Y/Y_0 = 1 - (1 - e^{-kT})^m \quad (2)$$

where Y_0 is the initial infective center titer, Y is the infective center titer after ultraviolet dose T, and m is the multiplicity of infection. As the dose increases, Eq. 2 reduces to

$$Y/Y_0 = me^{-kT} \quad (3)$$

Thus the ratio of survivors of multiple infection to those of single infection at any dose T will be the multiplicity of infection, m.

This is not the case for a number of bacteriophages. The ratio of survivors of multiple infection to those of single infection increases with dose to values much greater than the multiplicity used (*3*). This phenomenon is termed muliplicity reactivation, and its explanation is based on the observation that ultraviolet irradiation stimulates recombination in bacteriophage; and recombination acts between two damaged genomes may lead to an undamaged genome and, eventually, to an infective center (*4*). With the isolation of both bacterial and bacteriophage mutants deficient in recombination, it is possible to test the recombination theory for multiple reactivation.

Bacteriophage lambda undergoes multiplicity reactivation (*5*) especially in strains of *Escherichia coli* which do not efficiently repair radiation damage (*uvr*−) (*6*). These bacterial strains are more sensitive to killing by ultraviolet light because of the low efficiency for repair of radiation damage. Similarly, bacteriophage lambda is more sensitive to radiation killing on *uvr*− bacteria than on *uvr*+. Moreover, the fact that the recombination system of the host cell is not required for reactivation is in agreement with the observation that lambda contains information for its own recombination system (*7*). Bacteri-

Table 1. Effect of recombination (*rec*) functions on multiplicity reactivation of bacteriophages irradiated with ultraviolet. Bacteriophage lambda, *rec*+, and φtde120R, *rec*−, were irradiated with ultraviolet by a germicidal lamp (General Electric). Samples removed at stated times were adsorbed to either *Escherichia coli* AB1886 *rec*+ or AB2480 *rec*− at a multiplicity much less than one (monocomplexes) or greater than one (multicomplexes). The infected bacteria were assayed for infective centers on AB2480. The multiplicity of infection (MOI) for multicomplexes was calculated from a microscopic count of bacterial cells and the initial titer of bacteriophage. The entire experiment was conducted under yellow light to avoid photoreactivation.

Dose (sec)	P/P_0		B/A	MOI
	A monocomplex	B multicomplex		
	Phage, rec+; *host*, rec+			
15	1.8×10^{-1}	1.7×10^{-1}	0.9	3
30	1.4×10^{-2}	1.0×10^{-1}	7.1	
45	6.3×10^{-4}	6.6×10^{-2}	100	
60	5.5×10^{-5}	3.8×10^{-2}	690	
90	2.2×10^{-5}	1.7×10^{-2}	770	
120	1.1×10^{-5}	5.9×10^{-3}	540	
	Phage, rec+; *host*, rec−			
15	4.8×10^{-2}	3.5×10^{-1}	7.3	5
30	9.3×10^{-4}	1.0×10^{-1}	110	
45	3.1×10^{-5}	1.7×10^{-2}	550	
	Phage, rec+; *host*, rec−			
15	4.8×10^{-2}	1.8×10^{-1}	3.8	2.5
30	9.3×10^{-4}	3.7×10^{-2}	40	
45	3.1×10^{-5}	3.5×10^{-3}	110	
60	3.9×10^{-6}	5.9×10^{-4}	150	
	Phage, rec−; *host*, rec+			
15	2.3×10^{-1}	2.7×10^{-1}	1.2	3
30	3.4×10^{-2}	1.8×10^{-1}	5.3	
45	4.9×10^{-3}	1.3×10^{-1}	27	
60	5.1×10^{-4}	1.0×10^{-1}	200	
90	1.0×10^{-4}	3.8×10^{-2}	380	
120	5.5×10^{-5}	1.5×10^{-2}	270	
	Phage, rec−; *host*, rec−			
15	3.8×10^{-2}	1.7×10^{-1}	4.5	4
30	8.4×10^{-4}	3.2×10^{-3}	3.8	
45	2.2×10^{-5}	1.2×10^{-4}	5.5	
60	2.0×10^{-6}	7.0×10^{-6}	3.5	
90	1.5×10^{-7}	5.6×10^{-7}	3.7	
	Phage, rec−; *host*, rec−			
15	3.8×10^{-2}	1.1×10^{-1}	2.9	2
30	8.4×10^{-4}	2.1×10^{-3}	2.5	
45	2.0×10^{-5}	7.6×10^{-5}	3.5	
60	2.0×10^{-6}	5.2×10^{-6}	3.1	

ophage lambda (*8*) and a recombinationless mutant of a lambda-ϕ80 hybrid, ϕtde120R (*9*), were tested for multiplicity reactivation on *E. coli* strains AB1886 *uvr*−, *rec*+, and AB-2480 *uvr*−, *rec*− (*10*).

Bacteriophage suspended in dilution buffer ($10^{-2}M$ tris (hydroxymethyl) aminomethane, *p*H 7.4; $10^{-2}M$ $MgSO_4$; 0.01 percent gelatin) were irradiated with ultraviolet by a germicidal lamp (General Electric). Samples removed at various times were assayed for survivors by adsorbing to the appropriate bacterial strains at multiplicities of much less than one (monocomplexes) or greater than one (multicomplexes). The infected bacteria were then assayed by the soft-agar technique (*11*) for infective centers on *uvr*−, *rec*− bacteria to avoid multiplicity reactivation on the plate.

The ratio of multicomplex survival to monocomplex survival (B/A) increases with ultraviolet dose when either the bacteriophage or the host or both are *rec*+ (Table 1). But when both host and bacteriophage are *rec*−, the ratio of multicomplex to monocomplex survival remains constant and essentially the same as the multiplicity of infection.

This indicates that the bacterial and bacteriophage recombination systems are involved in multiplicity reactivation. Thus multiplicity reactivation becomes another method for detecting the presence of recombinational systems in bacteria and bacteriophage and does not require the use of genetic markers.

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Metal Ion Activation of Phosphate Transfer by Bidentate Coordination

Abstract. *The hydrolysis of methyl phosphate bound to the triethylenetetraminecobalt(III) ion is much faster than the hydrolysis of either dimethyl phosphate bound to the same cation or methyl phosphate bound to the pentamminecobalt-(III) ion. The rate enhancement is attributed to bidenate coordination of the methyl phosphate. This feature suggests a pseudorotation mechanism analogous to that proposed by Westheimer for the hydrolysis of ethylene methyl phosphate. Stabilization of bidentate coordination might play a role in metal ion activation of phosphate-transfer enzymes.*

Divalent metal ions are required by most enzymes which catalyze phosphate-transfer reactions (*1*). Using fluorine nuclear magnetic resonance, Mildvan *et al.* (*2*) have demonstrated direct interaction between Mn^{2+} bound to pyruvate kinase and fluorophosphate, which is the product of the fluorokinase reaction. In none of the enzymes which take part in phosphate transfer has the mode of coordination of the metal ion activator been elucidated. We report here on a model system which may be of relevance.

For studies of mechanisms, cobalt(III) complexes offer the advantage of a well-defined coordination sphere, in which ammine ligands are quite inert to substitution. We have studied the hydrolysis of methyl phosphate esters bound to the complex ions $Co(trien)^{3+}$ ("trien" is an abbreviation for triethylenetetramine) and $Co(NH_3)_5^{3+}$. In the former case, two coordination sites are available to the phosphate; in the latter case, only one site is available.

A $0.5M$ solution of $[Co(trien)(PO_4CH_3)]NO_3$ in D_2O was prepared by treating $\beta[Co(trien)CO_3]NO_3$ (*3*) with $H_2PO_4CH_3$ (*4*). Its nuclear magnetic resonance (NMR) spectrum showed a doublet for the methyl protons, whose resonance is split by the phosphorus nuclear spin, at 3.47 and 3.65 parts per million relative to tetramethylsilane. The intensity of the doublet diminished when the solution was heated, and a new peak, attributable to methanol, arose at 3.27 ppm. On the basis of the NMR peak areas, the kinetics were determined to be first order, with a rate constant of 2.7×10^{-4} sec^{-1} at 78°C. The rate was reduced by half for a temperature decrease of approximately 10°C. For $HPO_4CH_3^-$ (*5*), the species with the highest rate of hydrolysis in the aqueous methyl phosphate system, the rate constant at 100°C is 8.23×10^{-6} sec^{-1}, about 130 times slower than the value for $Co(trien)PO_4CH_3^+$ obtained by extrapolation to the same temperature.

A $0.6M$ solution of $[Co(NH_3)_5(PO_4CH_3)]NO_3$ in D_2O, prepared by treating $[Co(NH_3)_5CO_3]NO_3$ (*6*) with $H_2PO_4CH_3$, was heated in a sealed NMR tube at 68°C. From the equilibrium constant for dissociation of $Co(NH_3)_5(PO_4H)^+$, calculated from the results of Schmidt and Taube (*7*), we estimate that the extent of dissociation of $Co(NH_3)_5(PO_4CH_3)^+$ was less than 1 percent. After 48 hours, no trace of a methanol NMR peak could be observed and the methyl phosphate doublet maintained its original intensity. The rate of hydrolysis is at least two orders of magnitude less than that for $Co(trien)(PO_4CH_3)^+$.

Dimethyl phosphate was also investigated. A $0.5M$ solution of $[Co(trien)(PO_4(CH_3)_2)](NO_3)_2$ was prepared from $\beta[Co(trien)CO_3]NO_3$, $HPO_4(CH_3)_2$ (*8*), and HNO_3 (1:1:1 mole ratio). As with methyl phosphate bound to pentamminecobalt(III), no methanol production could be observed by NMR after extended heating at 68°C. Schmidt and Taube (*7*) have failed to detect methanol in solutions of $\{Co(NH_3)_5[PO_4(CH_3)_2]\}^{2+}$.

Solutions of $[Co(trien)PO_4CH_3]NO_3$ have a *p*H of ~ 3.7. Titration with acid or base produced well-defined, reversible buffer regions at about *p*H 2.4 and 6.7, respectively. The titration curve (Fig. 1) reflects protonation and deprotonation of a monodentate aquo complex, $[Co(trien)(PO_4CH_3)(H_2O)]^+$. The pK_a of this species, ~ 6.7, may