SYNERGISTIC INACTIVATION OF VIRUSES BY HEAT AND IONIZING RADIATION

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ABSTRACT Viral inactivation by heat and/or ionizing radiation is analyzed in terms of a kinetic model. The phenomenon of synergistic viral inactivation observed when viruses are exposed to the simultaneous application of heat and ionizing radiation is interpreted within the framework provided by this three-term model. Data on the inactivation of T4 bacteriophage by heat and/or ionizing radiation is presented, and the kinetic model is used to provide a description of observed dose rate and temperature dependences. Extension of the model to other viral systems inactivated by heat and ionizing radiation is considered, and the general applicability of these analyses suggests that the kinetic model may well serve as an extension of target theory in describing the radiobiological effects of ionizing radiation.

INTRODUCTION

The effect of ionizing radiation on bacterial, plant, and animal viruses has been extensively investigated (1, 2). The data from such studies has provided insights into the radiobiological effects of ionizing radiation on living systems; however, no theory is available which successfully describes and predicts the response of these living systems to ionizing radiation. Target theory (3, 4) provided an early and partially successful framework within which to interpret radiobiological phenomena, but simple target theory has lacked the flexibility to account for the ways in which radiation effects can be modified in living systems. For example, the inactivation of a number of viruses (5–7) by ionizing radiation has been shown to be strongly dependent on temperature, and target theory would not predict that the radiosensitivity of viruses could be modified by temperature. That thermal effects can result in viral inactivation is well-known (8), yet the increased inactivation observed when viruses are exposed to ionizing radiation in a thermal environment is not due to thermal inactivation per se since the temperatures at which the increased radiosensitivity of the viruses is observed are below those temperatures at which the thermal inactivation of viruses commences.

The realization that thermal effects can also affect the course of viral inactivation by ionizing radiation suggested that any attempt to describe the response of viruses

towards ionizing radiation should provide for the effects of temperature. This communication presents data on the temperature-dependent radiosensitivity of still another virus, T4 bacteriophage, and offers a three-term kinetic expression (model) which can be utilized to describe the inactivation of T4 bacteriophage as well as other viruses by heat, ionizing radiation, or the combined application of heat and ionizing radiation.

THEORY

Several potential mechanisms may be operative when viruses are inactivated in a composite heat and ionizing radiation environment. This section will present a simplified, yet very functional, first-order kinetic model of viral inactivation based on consideration of three such inactivating mechanisms. A basic assumption in the development of the kinetic analysis is that each virus has one critical substrate (e.g., the DNA or RNA) which must not be inactivated if the virus is to remain viable.

The first inactivating mechanism to be considered is that due to the thermal denaturation of the critical substrate. If the critical substrate essential for virus viability is denoted as having a population of A substrates within a population of P viruses, the heat inactivation of A to some inactivated state X may be represented by

$$A \xrightarrow{k_T} X , \tag{1}$$

where k_T is the rate parameter associated with the reaction. The parameter k_T may be described by Eyring's formulation,

$$k_T = \frac{kT}{h} e^{-\Delta F^{\neq}/RT} , \tag{2}$$

where $\Delta F^{\neq}$ is the change in free energy of the critical substrate as it goes from some original to some chemically activated complex in a first-order reaction. The terms K, h, and R refer to Boltzmann's constant, Planck's constant, and the gas constant respectively, while T is the Kelvin temperature. $\Delta F^{\neq}$ may be further described as

$$\Delta F^{\neq} = \Delta H^{\neq} - T\Delta S^{\neq} \tag{3}$$

where $\Delta H^{\neq}$ is the activation enthalpy in calories per mole and $\Delta S^{\neq}$ is activation entropy in calories per degree · mole.

A second inactivation mechanism considered present in the composite environment is that due to the direct effect of ionizing radiation (9) on the critical substrate, e.g., bond breakage. This inactivation may be represented by

$$A \xrightarrow{k_R} Y \tag{4}$$

where Y represents an inactivated complex. The reaction rate parameter k_R associated with this direct effect is characterized as a temperature-independent term which is related to the radiation dose rate in a linear manner. This relationship may be expressed as

$$k_R = Cr_d \tag{5}$$

where C is simply a constant of proportionality and r_d is the radiation dose rate in kilorads per hour. The preceding equation is identical to the doctrine of classical target theory since the rate of bond breakage or chain scission within the critical substrate is directly related to the rate of incidence of the photons of ionizing radiation.

A third basic inactivating mechanism considered operative in the composite environment is the reaction or intervention of free radicals generated by the radiation with the critical substrate (10–12). This may be represented by

$$A + R_a \xrightarrow{k_1} Z, \tag{6}$$

where k_1 is a temperature-dependent reaction rate parameter, R_a represents the population of radicals present, and Z is a third inactivated complex. For simplicity, assume that k_1 is represented by the Arrhenius rate parameter:

$$k_1 = ce^{-\gamma/RT}, \tag{7}$$

where c and γ are constants. Notice that the rate at which the free radicals inactivate the substrate A increases as temperature increases (13, 14).

The differential equation describing the rate of decrease in the critical substrate population A as a result of the three mechanisms described is

$$dA/dt = -(k_T + k_R + k_1R_a)A. \tag{8}$$

The solution of this nonlinear equation is dependent upon there being another equation or set of equations which describe the formation and decay of the free radical population R_a. In the most strict sense, this involves the solution of several simultaneous, nonlinear differential equations. For the purpose of these explanations, the assumptions are made that (*a*) the population of radicals develops very rapidly relative to the rate of decrease in A at the temperatures and dose rates of interest, and (*b*) the population R_a is much greater than that of A for all times of interest. These two assumptions allow R_a to be approximated as a constant value over the period of interest and the solution of equation 8 to be expressed as

$$A(t) = A(0)e^{-(k_T+k_R+k_1R_a)t}, \tag{9}$$

where $A(0)$ is the initial population of critical substrate. Recalling the assumed one-

to-one correspondence between A and P allows us to write

$$P(t) = P(0)e^{-(k_T+k_R+k_1R_a)t} = P(0)e^{-kt}, \tag{10}$$

where $P(t)$ is the virus population as a function of time t, and $P(0)$ is the initial virus population.

A point which remains to be explained concerns the magnitude of the free radical population R_a as expressed in equation 10. It has been demonstrated by Zimmer et al. (15) and Conger and Randolph (16) that stable free radical populations do reach a saturated steady-state value when a material is given a sufficient dose of radiation at a constant dose rate and temperature; however, the actual value of this steady-state population is dependent on the radiation dose rate and the temperature of the radiation environment since these conditions affect the generation and recombination rates of the free radicals. Moreover, it has been shown that the free radical–initiated rate of formation of polymers at ambient temperature is proportional to a fractional power of the radiation dose rate; hence, the population of free radicals present would also be proportional to a fractional power of the radiation dose rate (17). This power of dose rate must be inversely related to temperature since higher temperatures promote faster radical recombinations and reactions and result in a lower steady-state or quasi-steady-state level. On the basis of this logic, a simple expression selected to represent the free radical population R_a in equation 10 is

$$R_a = c_1 r_d^{\beta/T}, \tag{11}$$

where c_1 and β are constants. With this definition, the term k_1R_a of equation 10 is defined as

$$\begin{aligned} k_{TR} &= c_1 r_d^{\beta/T} c e^{-\gamma/RT} \\ &= r_d^{\beta/T} e^{\alpha} e^{-\gamma/RT}, \end{aligned} \tag{12}$$

where $\alpha = \log cc_1$. The rate parameter k_{TR} now provides a functional description of radiation dose rate and temperature-dependent free radical inactivation which is amenable to the analysis of actual data. Another description of k_{TR} is

$$\begin{aligned} k_{TR} &= e^{\alpha} e^{-(\gamma-\beta R\cdot\log r_d)/RT} \\ &= e^{\alpha} e^{-\Delta\mathfrak{F}/RT}, \end{aligned} \tag{13}$$

where $\Delta\mathfrak{F}$ is defined by

$$\Delta\mathfrak{F} = \gamma - \beta R\cdot\log r_d .$$

The final expression which describes the inactivation of viruses in a composite

heat and radiation environment is

$$P(t) = P(0)e^{-kt}, \tag{14}$$

where

$$k = k_T + k_R + k_{TR}. \tag{15}$$

The inactivation rate parameter k provides a means of describing viral inactivations due to heat and/or ionizing radiation. If only thermal effects are operating to inactivate viruses, k equals k_T. If inactivation is caused by irradiation at ambient temperature or lower, k is approximated by k_R (target theory); however, if heat and radiation are simultaneously applied, then k is described by equation 15.

As was pointed out earlier, the discussion here revolves around simple first-order kinetics and a decoupling of a very complicated set of nonlinear differential equations. The results presented are of sufficient accuracy to allow the correct modeling and analysis of synergistic inactivation data. For more detailed work numerical solutions could be obtained using higher order kinetic models of the heat and free radical inactivation. Brannen (18) has provided a framework for the analysis of higher order thermal inactivation kinetics.

The existence of a synergistic inactivation response to a composite heat and radiation environment is directly attributable to the properties of the k_{TR} inactivation parameter. This term defines a temperature-dependent inactivation rate for a chemical process sponsored by the action of ionizing radiation. It is the temperature dependence of this parameter which results in the experimental manifestation of synergism. Consider an initial viable population $P(0)$ which is sequentially treated with heat followed by radiation at low ambient temperature. Assuming first-order kinetics, the surviving population after the treatment with heat for time t_1 is

$$P(t) = P(0)e^{-k_T t_1}. \tag{16}$$

The surviving population expected after the subsequent treatment with radiation for the same time period t_1 is

$$\begin{aligned} P(t_1 + t_1) &= P(0)e^{-k_T t_1} \cdot e^{-k_R t_1} \\ &= P(0)e^{-(k_T + k_R)t_1}. \end{aligned} \tag{17}$$

Therefore, the combined rate parameter for additive heat and radiation inactivation is defined by $(k_T + k_R)$.

If an initial population is heated and irradiated simultaneously, however, the expected surviving population after a time t_1 is

$$P(t_1) = P(0)e^{-(k_T + k_R + k_{TR})t_1}, \tag{18}$$

where the composite inactivation rate parameter is given by $(k_T + k_R + k_{TR})$. This composite rate parameter is always larger than the combined rate parameter for the sequential heat and radiation treatment, because of the existence of the k_{TR} term, and therefore, more inactivation will always be observed in the composite environment after t_1 units of time than will be found after exposing a sample to heat alone for t_1 units and then to radiation at a low ambient temperature for t_1 units. This is the analytical basis for the synergistic response of biological systems to heat and radiation.

EXPERIMENTAL AND ANALYTICAL PROCEDURES

Materials and Methods

Dr. B. Barnhart, Los Alamos Scientific Laboratory, provided the *Escherichia coli* B and T4 bacteriophage used in this investigation. The freshly isolated phage was assayed by the agar layer method (19). A complete description of the growth, isolation, and assay methods used in this study has been given elsewhere (20).

Experimental Procedure

The experimental protocol employed in this study consisted of exposing a T4 phage suspension (approximately 5×10^6 phage/ml, 1% trypticase soy broth, 2 ml sample volume/glass test tube) to heat alone, radiation at ambient temperature, or a composite environment of heat and gamma radiation. Each data point was determined from duplicate samples assayed in replicate. A Blue M "Magniwhirl" constant temperature bath (Blue M Electric Co., Blue Island, Ill.) provided a temperature control of ±0.1°C at the desired temperature. Simultaneous heat and radiation exposure was achieved by placing the water bath, containing the samples, inside the Sandia Laboratory Gamma Irradiation Facility. The desired dose rate (30.6 krads/hr or 7.9 krads/hr) was attained by locating the water bath an appropriate distance from the 16-kCi cobalt-60 source. Silver phosphate or cobalt glass dosimeters were used to determine the dose delivered to each sample. Two dose rates were investigated in order to determine the dose rate dependency of the rate of phage inactivation in the composite environment.

Treatment of Survival Data

Since the semilog plots of the T4 phage inactivation data in this set of experimentation are linear, the population of phage $P(t)$ as a function of time t may be represented by the expression

$$P(t) = P(0)e^{-k_T t}, \tag{19}$$

for the thermal inactivation, and

$$P(t) = P(0)e^{-kt}, \tag{20}$$

for viral inactivation in a composite heat and ionizing radiation environment. $P(0)$ represents the initial viable phage population, and k_T and k are the inactivation rate parameters in hours^{-1} for thermal inactivation and composite heat and ionizing radiation inactivation

respectively. Values for k_T and k are determined for each experimental procedure by fitting the natural logarithm of the survival curve data with a straight line using a least squares norm.

As described in the section entitled Theory, the composite heat and ionizing radiation inactivation parameter k may be expressed as

$$k = k_T + k_R + k_{TR}.$$

The experimental procedures yield values for k and k_T. Since k is essentially constant in value up to temperatures above 40°C for T4 phage, the values of k at 23°C may be assumed to be very nearly equal to k_R for any given radiation dose rate. Subsequently, an experimental value for k_{TR} at any temperature and radiation dose rate may be determined by subtracting the sum of k_T and k_R from k.

Computational Procedures

The parameter values for k_T in equation 2 and k_{TR} in equation 12 were optimally determined for T4 bacteriophage by minimizing the sum of the absolute values of the differences between the mean of the data at each temperature and dose rate and the value provided by the rate parameter expression. This was done using a numerical search routine on a CDC 6600 computer (Control Data Corp., Minneapolis, Minn.).

The form for k_R in equation 5 was determined and the parameter value C has been obtained by fitting the means of the inactivation rate data at 23°C and the dose rates of 0, 7.9, and 30.6 krads/hr with a straight line using a least squares norm.

The parameter values for other viral systems considered were determined from inactivation data available in the literature. In most cases the parameters were optimized to best fit all of the available data as was done for the T4 phage data. For viral studies in which only one dose rate was examined, only the parameters α and $\Delta\mathfrak{C}$ were determined for the rate parameter k_{TR} as shown in equation 13.

RESULTS

The basic observation made during the course of this experimentation was that T4 bacteriophage is inactivated in a synergistic manner by the simultaneous application of heat and ionizing radiation. This phenomena is illustrated in Fig. 1 where typical survival curves for T4 bacteriophage are presented. The term synergistic inactivation may be applied to the survival data obtained on simultaneous exposure of T4 phage to heat (66°C) and ionizing radiation (30.6 krads/hr), since the rate of inactivation under these conditions is greater than that obtained by summing the inactivation rates due to heat alone at 66°C and irradiation at ambient temperature (23°C). For this particular case the values of k_T, k_R, and k are 6.6 hr^{-1}, 0.71 hr^{-1}, and 11.44 hr^{-1} respectively. The rate parameter k_{TR}, which may be determined from equation 15 and which provides a measure of the degree of synergism, has a value of 4.13 hr^{-1}.

The three types of survival data illustrated in Fig. 1, i.e. heat alone, radiation at ambient temperature, and composite heat and radiation, were obtained for every temperature reported in this investigation. As previously described, the negative

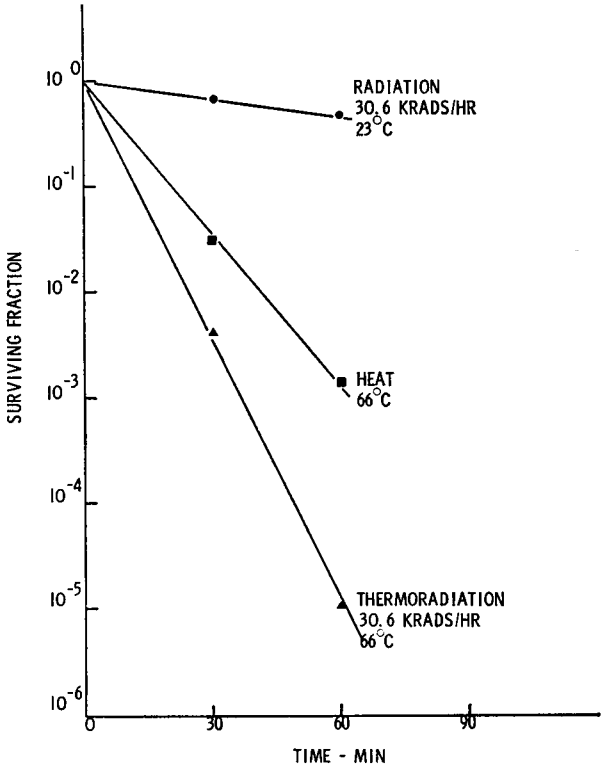

FIGURE 1 Synergistic inactivation of T4 bacteriophage at 66°C and 30.6 krads/hr.

slope of the heat-alone survival curve provided the inactivation rate parameter k_T, while the negative slopes of the irradiation at ambient temperature and the composite heat and radiation survival data yielded, respectively, the k_R and k inactivation rate parameters. These rate parameters, k_T, k_R, and k, were then analyzed in terms of the three-term model described under Theory.

Fig. 2 summarizes the survival data obtained for the inactivation of T4 bacteriophage on exposure to a composite heat and radiation environment. An important feature of the inactivation plots shown in Figs. 1 and 2 is that they are linear. All of the data for which results are reported in this investigation were of this general nature, and this fact allowed the inactivation data to be analyzed kinetically as described in Materials and Methods. Biphasic or two-component inactivation response curves have recently been reported for T4 phage (21). Studies in our laboratory on T4 phage stocks which had been held at 4°C for 3 wk or more also showed a biphasic response on exposure to heat or combined heat and radiation. Therefore, all experimentation reported in this communication was accomplished using a single T4 bacteriophage stock which was prepared, isolated, and studied within a 10 day period. Under these conditions, all T4 phage inactivation data yielded survival curves which were linear and amenable to first-order kinetic analysis.

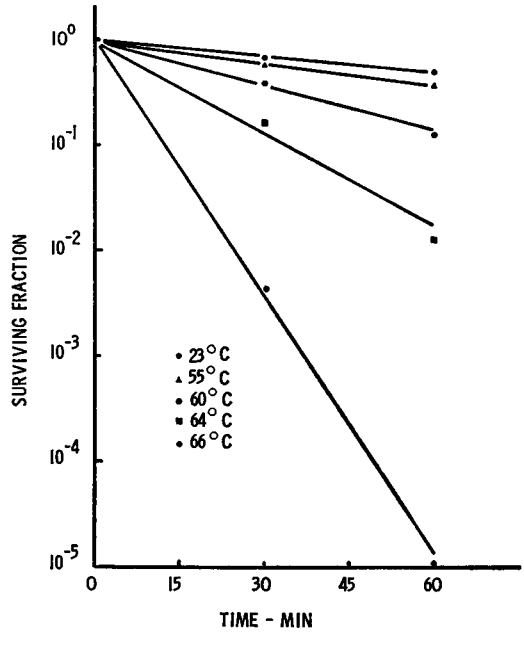

FIGURE 2 Composite heat and radiation inactivation of T4 bacteriophage for a set of five temperatures and a dose rate of 30.6 krads/hr.

Table I presents a list of the experimentally determined reaction rate parameters for both thermal (k_T) and combined thermal and radiation (k) inactivation of T4 bacteriophage. As previously indicated, k is equivalent to k_R at ambient temperature or lower. These rate parameters k_T and k are the negatives of the slopes of the straight lines which are fitted to the logarithm of the mean of the appropriate set of data at each point in time using a least squares norm. The standard deviations of the difference (d) between the survival curve data points (P_E) and the computed line (P_L), and the coefficient of correlation between the computed line and the data are also listed in Table I. Each survival curve data point (e.g., Figs. 1 and 2) was obtained by averaging four experimentally determined values. The usual variation between the extremes of these experimental points was approximately 10%. The values listed in Table I are to four significant figures to lessen the accumulation of computational errors.

The investigation of composite environment inactivation at a lower dose rate of 7.9 krads/hr yielded values of k for 0.17 hr^{-1} at 23°C, 1.23 hr^{-1} at 62°C, and 2.682 hr^{-1} at 64°C.

The general form of the three parameters which comprise the composite environment rate parameter k are described in equations 2, 5, 12, and 15. Using the optimization method discussed in the section entitled Computational Procedures, the

TABLE I

THERMAL (k_T) AND COMPOSITE HEAT AND IONIZING RADIATION (k) INACTIVATION RATES OF T4 PHAGE

Temperature	k_T	S.D.*	C.C.‡	k	S.D.*	C.C.‡
°C	$hours^{-1}$			$hours^{-1}$		
23	0			0.641	0.0204	0.997
23	0			0.666	0.0589	0.977
23	0			0.649	0.1079	0.926
23	0			0.732	0.0867	0.961
23	0			0.751	0.0484	0.988
23	0			0.708	0.0062	0.999
55	0.235	0.0289	0.957	1.019	0.0123	0.999
55	0.150	0.0083	0.991	0.973	0.0660	0.986
60	0.543	0.0227	0.995	2.118	0.0608	0.998
64	2.116	0.0633	0.997	4.554	0.0899	0.999
64	2.381	0.0500	0.998	4.438	0.1518	0.997
66	6.014	0.1564	0.998	8.887	0.0820	0.999
66	6.595	0.0797	0.999	11.439	0.1219	0.999

* Standard deviation of error = $\sqrt{\sum (P_E - P_L)^2/N}$.

‡ Coefficient of correlation = $\sqrt{1 - (\text{S.D.}/\sigma_y)^2}$ where $\sigma_y = \sqrt{[\sum (P_E)^2/N] - (\sum P_E/N)^2}$.

operational forms of the three rate parameters for T4 phage are:

$$k_T = \frac{KT}{h} e^{245.45/R} e^{-107416.0/RT}, \tag{21}$$

$$k_R = 0.0227 r_d \tag{22}$$

and

$$k_{TR} = r_d^{481.65/T} e^{95.09} e^{-66564.3/RT}. \tag{23}$$

The constants K, h, and R are respectively Boltzmann's constant, Planck's constant, and the gas constant. T is the temperature in degrees Kelvin and r_d is dose rate in kilorads per hour. At a dose rate of 30.6 krads/hr the parameter k_{TR} may be written.

$$k_{TR} = e^{95.09} e^{-63289.9/RT}. \tag{24}$$

Fig. 3 provides a comparison of the model predictions of $k = k_T + k_R + k_{TR}$ at dose rates of 30.6 krads/hr and 7.9 krads/hr with the means of the experimentally determined values of k. The use of the kinetic parameters presented in equations 18–20 provides a complete description of the inactivating process occurring in the composite thermoradiation environment for T4 phage. Fig. 3 again emphasizes the strong temperature dependence of the rate of the T4 phage inactivation. As ex-

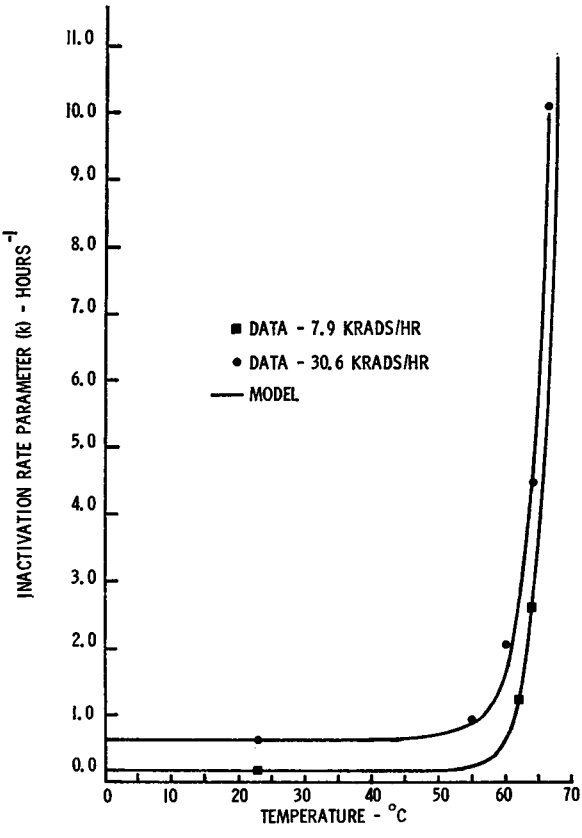

FIGURE 3 A comparison of data and model predictions for the inactivation parameter k which shows the temperature dependence of T4 radiosensitivity at 30.6 krads/hr and 7.9 krads/hr.

plained by the model of equations 21–23, this temperature dependence is not entirely a function of thermal denaturation (equation 21). The synergistic component of the inactivation is that temperature-dependent quantity represented by equation 23.

The general inactivation model which has been presented for T4 phage may also be applied to other viral systems. Reports on the inactivation of three other viruses by exposure to composite heat and radiation environments have been described in the literature (5–7), and the data from these studies were analyzed to determine the parameters for the kinetic inactivation model. A tabulation of the inactivation rate parameters for ϕX174, Newcastle disease virus (N.D.V.), and T1 and T4 bacteriophage is summarized in Table II, and this information may be used to draw several conclusions relative to the synergistic inactivation of viruses in a combined environment. The rate parameter k_T which describes pure thermal inactivation is defined in terms of entropy ($\Delta S^{\neq}$) and enthalpy ($\Delta H^{\neq}$). A comparison of these thermodynamic

TABLE II

FACTORS AND PARAMETERS DEFINING VIRAL INACTIVATION BY THERMORADIATION

Virus	ϕX174*	T1‡		N.D.V.§	T4
Radiation conditions, krads/hr	1500, ^{60}Co	25, X-ray		291, ^{60}Co	30.6, ^{60}Co
Physical conditions	Dry, vacuum	Dry	Wet	Wet	Wet
k_T					
$\Delta S\ddagger$, *cal/deg·mole*	−6.332	0	208	22.4	245.45
$\Delta H\ddagger$, *cal/mole*	26,763	28,000	95,000	30,900	107,416
k_{TR}					
α	9.13	22.50	91.4	83.15	95.10
$\Delta\mathfrak{C}$	6400.7	17,463.3	60,238.2	53,114.3	63,289.9
k_r					
C	0.00125	0.0043	0.0039	0.00618	0.0227
Nucleic acid mol wt, *daltons*‖	1.69×10^6	4.2×10^7		3.2×10^7	1.5×10^8

* Reference 6.
‡ Reference 5.
§ Reference 7.
‖ References 32 and 33.

parameters shows that the dry heat inactivation of ϕX174 and T1 phage is associated with very low entropy values while the wet heat inactivation of N.D.V. and T4 phage has much larger values. In addition, the values for enthalpies of activation for wet heat inactivation are characteristically higher than for dry heat inactivation. These same observations have been made in other inactivation studies (8, 22). The $\Delta S^{\neq}$ and $\Delta H^{\neq}$ values presented for the N.D.V. and T1 bacteriophage were obtained from the references cited in Table II. The values for the ϕX174 and T4 viruses were determined as described in the section entitled Experimental and Analytical Procedures using the appropriate data obtained either experimentally (T4) or from the cited reference (ϕX174).

Table II also presents a comparison of the viral nucleic acid molecular weight to the constant C, equation 5, for each virus studied. The constant C for each virus is intimately associated with k_R, i.e.. the temperature-independent radiation inactivation term. Notice that the value of C increases as the molecular weight of the virus increases. This important point is considered in detail in the Discussion.

A listing of the parameters which define the temperature-dependent radiosensitivity inactivation rate parameter k_{TR} for each virus is also presented in Table II.

These parameters are of basic importance since the term k_{TR} describes the degree of synergism present in the inactivation of a particular virus for a defined set of heat and radiation conditions. The two parameters α and $\Delta\mathfrak{C}$ (equation 13) are characteristically larger for the viruses inactivated in a wet environment than for those inactivated in a dry environment. This observation is similar to that made for the values $\Delta S^{\neq}$ and $\Delta H^{\neq}$ of the pure heat inactivation rate parameter k_T. A graphic illustration of the dependency of k_{TR} upon the temperature during irradiation is presented in Fig. 4. Again notice that viruses irradiated in a wet state have not only a more temperature-dependent radiosensitivity but also a much lower threshold for temperature dependency than do the viruses inactivated in the dry state. This implies that synergistic inactivation results would be confined to a smaller temperature band generally centered at a lower temperature for the wet system than for the dry system. This point is also further developed in the Discussion.

An explanation of the effect of the radiation dose rate on the curves of k_{TR} shown in Fig. 4 may be found by examination of equation 13,

$$k_{TR} = e^{\alpha} e^{-(\gamma - \beta R \cdot \log r_d)/RT}.$$

Notice that as the dose rate increases, the quantity

$$\Delta\mathfrak{C} = \gamma - \beta R \cdot \log r_d$$

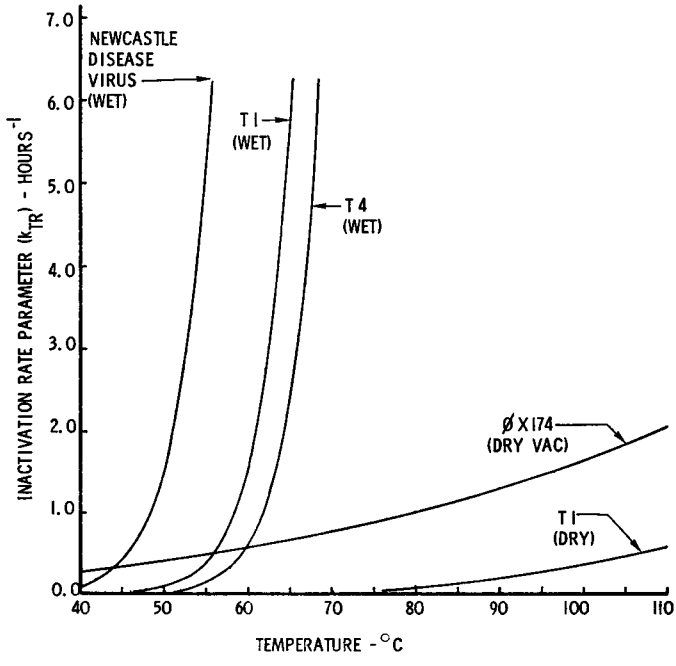

FIGURE 4 The temperature dependence of the rate parameter for four viruses. k_{TR} provides an absolute measure of synergism.

decreases. This is consistent with the data listed in Table II where the value of $\Delta\mathfrak{IC}$ for the N.D.V. is significantly smaller than that of either the T4 or T1 phage in the wet state, while the radiation dose rate of the Newcastle study was significantly larger than that of the T4 or T1 studies. A similar comparison of values for the dry ϕX174 and T1 studies yields this same dependency. If all the studies had been carried out at approximately the same radiation dose rate, the values of $\Delta\mathfrak{IC}$ would have been much closer for both the wet and dry systems, and the k_{TR} curves of Fig. 4 would be in a much more compact grouping. For example, lowering the dose rate of the Newcastle study should shift its k_{TR} plot closer to that of the T1 and T4 plots. Other implications of the fact that k_{TR} is also a nonlinear function of radiation dose rate have been described by Dugan (23) for a similar analysis of dry *Bacillus subtilis* var. *niger* spores.

DISCUSSION

The rate of inactivation of viruses by heat and/or ionizing radiation may be described by the three-term model which has been defined, equation 15, as $k = k_T + k_R + k_{TR}$. In this analysis, k_T is the inactivation rate due to heat alone, k_R is the temperature-independent radiation inactivation rate parameter associated with the direct effect (9) of ionizing radiation on a critical substrate, and k_{TR} is the temperature-dependent inactivation rate parameter of a chemical process sponsored by the action of the radiation.

As pointed out earlier, the thermal inactivation rate parameter is thought to represent the denaturation of some critical substrate within the viral system. The analysis of composite thermal and ionizing radiation inactivation presented here assumes that k_T is a function only of temperature and that it has the same value with or without any incident radiation.

The second inactivation rate component present in the composite environment is considered to be due to the direct effect of ionizing radiation on a critical substrate(s) of the viral system, e.g., bond breakage. The reaction rate parameter k_R associated with these direct effects is characterized as a temperature-independent term which is related to the radiation dose rate r_d in a linear manner. This relationship has been expressed as

$$k_R = Cr_d ,$$

where C is a constant of proportionality and is equivalent to $1/D_{37}$. D_{37} is the dose required to reduce a population to a 37% survival level. This analytical definition of the rate parameter k_R is equivalent to the basic postulate of target theory (3, 4, 24) in that each holds that the release of sufficient energy within a critical substrate by a single particle or photon can result in the loss of virus function. The formal resemblance between the k_R parameter and target theory can be verified experimentally since both would predict a direct relationship between the critical substrate

TABLE III

VIRUS NUCLEIC ACID CONTENT AND RADIOSENSITIVITY

Entity	Code No.	Nucleic acid content	Reference	C	Reference
		daltons		*krads*$^{-1}$	
Phage R17	1	9×10^5	34	1.28×10^{-3}	30
Tobacco ringspot virus	2	1.5×10^6	35	2.34×10^{-3}	36
Tobacco necrosis	3	1.5×10^6	35	1.61×10^{-2}	36
Tomato bushy stunt virus	4	1.6×10^6	37	2.34×10^{-3}	36
Phage ϕX174	5	1.7×10^7	38	2.56×10^{-3}	30
Phage ϕX174	6	1.7×10^7	38	3.03×10^{-3}	39
Phage ϕX174	7	1.7×10^6	38	4.3×10^{-3}	40
Phage ϕX174	8	1.7×10^6	38	1.25×10^{-3}	6
Sl3	9	1.7×10^7	40	2.56×10^{-3}	41
Tobacco mosaic virus	10	$1.95\text{–}2.16 \times 10^6$	35, 42	5.95×10^{-3}	43
Tobacco mosaic virus	11	$1.95\text{–}2.16 \times 10^6$	35, 42	3.33×10^{-3}	44
Tobacco mosaic virus	12	$1.95\text{–}2.16 \times 10^6$	35, 42	3.45×10^{-3}	45
Polyoma	13	3×10^6	46	2.0×10^{-3}	47
Fowl plague	14	$2.2\text{–}6.0 \times 10^6$	48	1.08×10^{-2}	36
Shope papilloma virus	15	5×10^6	49	2.45×10^{-3}	50
Rous sarcoma virus	16	$0.96\text{–}1.2 \times 10^7$	51	5.95×10^{-3}	52
Phage BM	17	2.49×10^7	53	5.59×10^{-3}	54
Newcastle disease virus	18	3.2×10^7	35	6.18×10^{-3}	7
Newcastle disease virus	19	3.2×10^7	35	2.34×10^{-2}	55
Phage alpha	20	3.1×10^7	56	4.9×10^{-2}	56
Phage T1	21	3.9×10^7	57	1.23×10^{-2}	58
Phage T1	22	4.2×10^7	59	4.1×10^{-3}	5
Phage T3 (T7)	23	4.2×10^7	59	1.26×10^{-2}	59
Phage T7	24	4.2×10^7	60	6.66×10^{-3}	58
Phage 22	25	5×10^7	57	9.8×10^{-3}	61
Phage λ	26	7×10^7	59	9.8×10^{-3}	62
Adenovirus, type V	27	6.6×10^7	63	1.3×10^{-2}	64
Phage P8	28	1.0×10^8	59	1.3×10^{-2}	59
Phage T2	29	1.29×10^8	65	1.0×10^{-2}	66
Phage T2	30	1.29×10^8	65	1.82×10^{-2}	58
Phage T2	31	1.29×10^8	65	2.1×10^{-2}	67
Phage T5	32	1.3×10^8	59	3.07×10^{-2}	59
Phage T4	33	1.51×10^8	59	1.0×10^{-2}	66
Phage T4	34	1.51×10^8	59	2.27×10^{-2}	This paper
Vaccinia virus	35	1.56×10^8	63	1.35×10^{-2}	68, 69

content, e.g. nucleic acid content, and the radiation sensitivity of biological systems. A pattern of quantitative relationships has been obtained over the years between nucleic acid content and radiation sensitivity of viruses, bacteria, and cells of mammalian and plant systems (25–27). These relationships have been used in support and justification of target theory. Similarly, Table III shows that the magnitude of the factor C contained in k_R, equation 5, is directly related to the molecular weight of the nucleic acid present in 35 viral systems. The formal and experimental re-

semblance between target theory and the k_R parameter suggests that the k_R parameter can be identified as a term which exhibits those radiobiological attributes associated with target theory.

A major limitation of classical target theory has been its inability to account for the temperature-dependent radiosensitivity exhibited by biosystems (2, 28). An advantage of identifying k_R as a target theory term lines in the fact that k_R is but one of the parameters which define the response of biosystems to heat and/or ionizing radiation (equation 15), and therefore, the inactivation of a biological system by heat, radiation, or composite heat and radiation can be successfully described by the three-term model. The effect of temperature on the radiosensitivity of viral systems can be accommodated within this new framework, and target theory can then be considered to be a limiting case of the more general three-term model in which only direct radiation effects (described by k_R) influence virus survival. Hence, the separation and identification of the parameters which influence radiation-induced inactivation produces an extension of target theory and an improvement in inactivation predictions for viruses.

A functional expression which relates C, equation 5, to the nucleic acid content of 35 viruses ranging in size from that of R17 phage (9×10^5 daltons) to vaccinia virus (1.56×10^8 daltons) is

$$C = 5.91 \times 10^{-6}\, \omega^{0.425}, \tag{25}$$

where ω is the nucleic acid molecular weight in daltons. The constants in this expression for C were obtained by fitting the expressed form to the data in Table III using a least squares norm. The coefficient of correlation of this fit is 0.824. This degree of correlation suggests that the approximate radiation sensitivities of practically any size virus may be predicted on the basis of equations 5 and 25 from their nucleic acid molecular weight. Conversely, for any given experimental value of C the approximate molecular weight of the nucleic acid present in the virus under investigation may be determined.

Fig. 5 illustrates the relationship between the experimentally determined values of C and the molecular weights of the corresponding nucleic acids for the 35 viruses. The scatter in these points about the fitted line of equation 25 can be attributed to a variation in the experimental conditions and procedures used in determining both the values of C and the nucleic acid molecular weights. Specifically, the variation in the energy of the radiation used in these experiments, the accuracy of dosimetry in defining the D_{37} dose, variations in test temperatures, and the difficulties in establishing a definitive molecular weight for the easily sheared nucleic acid polymers would all contribute to a randomization of data about this predicted norm. An indication of the experimental variability which can be expected may be obtained by comparing the data obtained for ϕX174 virus (Fig. 5 data, 5–8), tobacco mosaic virus (Fig. 5 data, 10–12), Newcastle disease virus (Fig. 5 data, 18, 19), T1 phage

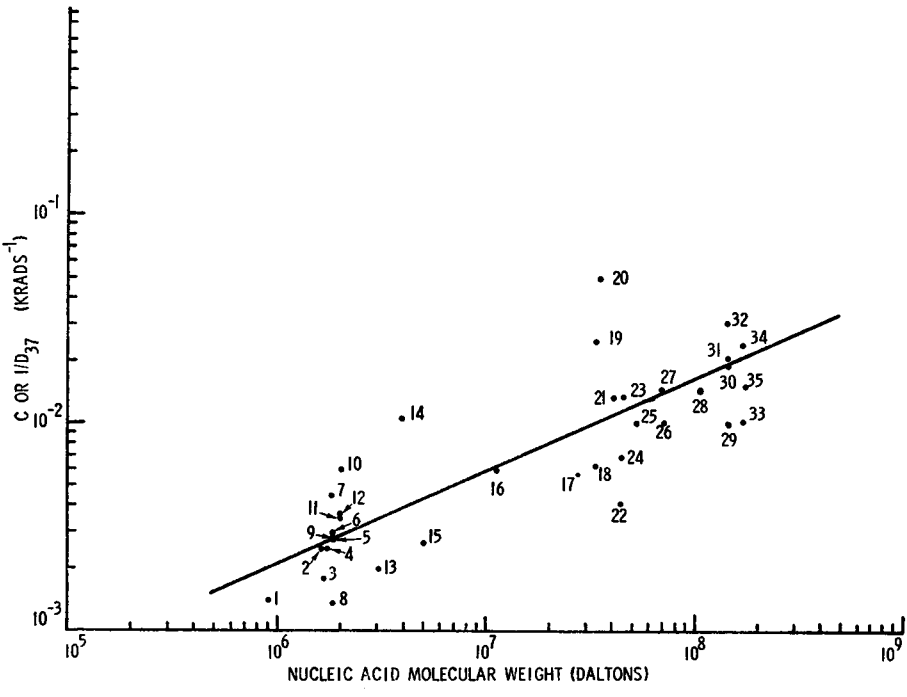

FIGURE 5 Relationship between C, $1/D_{37}$, in the rate parameter expression $k_R = Cr_d$ and viral nucleic acid molecular weight ω. The equation for the fitted line is $C = 5.91 \times 10^{-6}\ \omega^{0.425}$.

(Fig. 5 data, 21, 22), T2 phage (Fig. 5 data, 29–31), and T4 phage (Fig. 5 data, 33, 34) by different laboratories. Notice that data for each of these viruses are scattered about the fitted line.

The direct relationship between radiosensitivity C and nucleic acid molecular weight provides strong evidence for the designation of nucleic acid as the critical substrate or target being affected during radiation-induced inactivation (k_R), especially since the relationship is evidenced over such a large and diverse range of viruses (single and double strand, DNA and RNA viruses) irradiated under different environmental conditions (wet vs. dry, vacuum vs. oxygen).

Thus far the discussion of inactivation by radiation has been restricted to direct effects such as chain scission or bond-breaking phenomena caused by sufficiently energetic radiation. Radiation survival data for 35 different viruses has been analyzed (Fig. 5) in terms of the k_R inactivation rate parameter of the kinetic model and, allowing for experimental variability, this analysis has been successful and consistent with expectations concerning the nature of the critical viral substrate and the response of such a critical target to ionizing radiation.

The impetus for this study, however, was to obtain a kinetic model which could account for the temperature-dependent radiosensitivity of T4 bacteriophage. The

kinetic model must, therefore, not only describe radiation-induced inactivation (k_R) but also describe both the temperature and dose rate dependence of viral inactivation in a composite heat and ionizing radiation environment. The incorporation of a parameter (k_{TR}) into the kinetic model, which considers an inactivation mechanism based on radiation-induced free radical formation, has been described (section on Theory), and its success in predicting and describing the response of T4 bacteriophage to inactivation by composite heat and ionizing radiation is illustrated in Fig. 3. The hypothesized intervention of free radicals accounts for the temperature and radiation dose rate dependence of viral inactivation in a composite heat and radiation environment by assuming that the dose rate dependence stems from the dependence of the concentration of free radicals on the dose rate, and the temperature dependence is present because of the effect of temperature on the rate at which the dominant radicals react with critical substrate. Extension of this model to other viral systems is of necessity limited because of the fact that the radiosensitivity of only a few viruses has been studied as a function of temperature (5–7). The successful application of the kinetic model to those viruses for which such data was available is illustrated in Fig. 4, while a listing of the parameters which define the temperature radiosensitivity of these viruses is presented in Table II. The fact that other viral systems can be analyzed by the same kinetic model developed for T4 bacteriophage (the constants for any particular virus are, of course, uniquely defined for that virus) suggests that the three-term kinetic model has a general validity for the description of radiobiological inactivation phenomena.

This three-term model provides a means of interpreting the phenomena of increased viral radiosensitivity with increasing temperature; however, this phenomena can also be interpreted in terms of a model described by Freifelder and Trumbo (29) in which radiation-induced single-strand breaks (ssb) in opposite strands of DNA can result in a double-strand break (dsb). Temperature is one parameter which defines the maximum number of nucleotide pairs (h) which, when between two ssb in opposite strands, cannot maintain the double-strand structure, i.e., a dsb result. The strength of the three-term model lies in its capacity to *analytically describe* the phenomena of increased radiosensitivity with increasing temperature, while the ssb-dsb model provides a *graphic representation* of how free radical processes (ssb) can interact with temperature to produce a lethal biological event. It is not clear at this time that either model more accurately describes this phenomenon or that these models are in fact in basic conflict.

The interplay of inactivation rate parameters is illustrated in Fig. 6. The relative magnitude of the values for the k_T and k_{TR} terms at any given temperature is extremely important in defining the synergistic effect observed for a system. Consider the case of T4 phage; studies on the effect of temperature changes on the inactivating radiation sensitivity would report no effect in the temperature range from ambient to 50°C. This is true since the k_{TR} term representing the inactivating action of the radiation-induced free radicals is essentially zero for temperatures

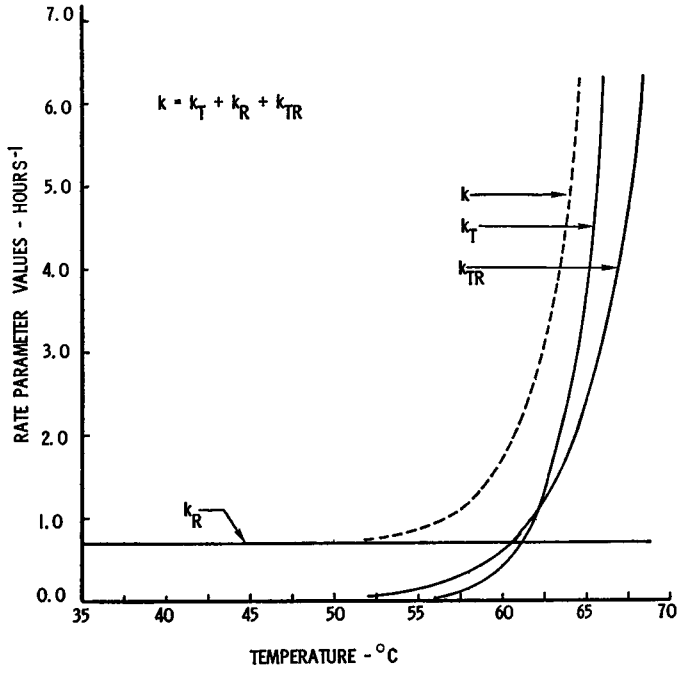

FIGURE 6 The temperature dependence of the components of the rate parameter $k = k_T + k_R + k_{TR}$ at 30.6 krads/hr for T4 bacteriophage. The comparison of the parameters describes the temperature range over which synergistic inactivation may be observed.

below 50°C, and the k_R term is constant (Fig. 5). On the other hand, for temperatures above approximately 66°C the k_T term in the expression for k would completely dominate the k_{TR} with increases in temperature. Investigating composite heat and radiation inactivation of T4 phage at temperatures above 66°C would result in a report that no synergistic inactivation existed. The k_{TR} term would simply be lost in the experimental noise when trying to determine it from the expression

$$k_{TR} = k - k_T - k_R . \tag{26}$$

Therefore, synergism would only be observed experimentally for T4 phage in the temperature range from ~55°C to ~70°C. Such considerations mean that an accurate determination of the effect of thermoradiation on a viral system depends on understanding the role which the individual inactivation rate parameters play in determining the over-all inactivation response. The report (30) that the target sensitivity of ϕX174 is independent of temperature over the range −180–+30°C is not in conflict with the finding (6) that ϕX174 shows a temperature-dependent radiosensitivity (temperature range −196–+160°C) when one recalls the fact that k_{TR} is a function of both temperature and radiation dose rate. This temperature dependence means that a radiosensitivity to temperature may or may not be observed

depending on the temperature range investigated. The effects of temperature and radiation dose rate on the inactivation process may help explain the inconsistencies observed when simple target theory is used to determine critical target volumes. For example, Brustad (31) observed that the apparent target volumes for trypsin determined from target theory differed by a factor of 12 depending on the sample temperature during irradiation.

The rational basis for the kinetic model lies in considering the inactivating phyisco-chemical transformations to be subject to the laws of chemical kinetics. The successful application of the model to such diverse types of viruses emphasizes the generality of the model in describing inactivation phenomena and suggests the extension of such a kinetic analysis to other biological systems. This possibility is currently under investigation.

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