

KINETICS OF THE RADIOLYSIS OF ENZYMES  
AND OTHER ORGANIC SOLUTES

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## LIST OF SYMBOLS

$k_n$	second order rate constant
$A_n$	solute or species derived from solute
B	intermediate species derived from solute
$C_m$	solute independent impurity or molecular species derived from solute
D	dose
G	yield of primary radicals
$G_A, G_{\text{solute}}$	yield of solute molecules
t	time

## CHAPTER I

### INTRODUCTION

#### History

Fricke (1) has shown that the effects of radiation on solutes in dilute aqueous solutions are "indirect", i.e. caused by reaction with radicals produced in water by the radiation. These radicals, which are produced directly from action of radiation on water, will be called primary radicals. The most common primary radicals are the hydrated electron, hydroxyl radical, and hydrogen atom. The rate at which they are produced from water is called the G value, and is commonly expressed in units of radicals/(100 eV).

Radiation-induced changes in enzymes and other organic solutes have often been described by an exponential function of dose (2). However, loss of enzymatic activity has, in many instances, been shown to conform to non-exponential inactivation curves (3,4,5,6,7). Generally the rate of inactivation decreases with increasing dose, though even this observation has met with some contrary evidence; under some conditions linearity has been reported for lysozyme (8).

The fact that the rate of inactivation<sup>1</sup> of many solutes does decrease with increasing dose is generally interpreted as a result of

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<sup>1</sup>In this work, a solute is said to be inactivated when that property which is assayed is destroyed.

competition between inactivated molecules and the active ones for the primary radicals (9,10).

Some models have been proposed for the kinetics of solute inactivation in aqueous solution (9,10,11,12,13,14,15,16). However, they have several shortcomings. It has often been assumed that the inactivation is exponential, and, on the basis of this assumption, a model is devised instead of devising a model which does, or may under certain conditions, show exponential behavior. Also, it has often been assumed implicitly that a single interaction with a primary radical suffices to inactivate the solute. It seems plausible that an enzyme or macromolecule might react with a primary radical without losing that property which is assayed to determine activity. It will be demonstrated that the aforementioned assumptions are not necessary in order to maintain a manageable model. Previous models have also neglected the possibility that a solute radical, formed from a primary radical and a solute molecule, might contribute to the inactivation of solute. Also, since the kinetics seem to be determined by competition between solute molecules, intermediates, and impurities for the primary radicals, disproportionation or dimerization reactions involving the solute radicals should be included.

This work will present mechanistic schemes which contain both of the preceding possibilities. The results predicted by these schemes can then be compared to experimental data. This comparison will provide evidence for or against the inclusion of specific steps in the mechanistic scheme.

It should be noted that the philosophy behind this work is not one of "curve fitting". It will be quite apparent that even a very simple and unrealistic model can fit a variety of curves according to the rate constants which may be assigned to particular reactions. At this time it



is impossible to obtain these rate constants from experimental procedures. Therefore, it will be the purpose of this work to devise methods which can be used to give evidence for generalized mechanisms from experimentally obtainable parameters.

#### Assumptions

A solute species reacting with primary radical  $\omega\cdot$  will be represented as follows,



where A is the solute,  $B\cdot$  is the product of the reaction and  $k_1$  is the rate constant. If A is a large organic solute,  $B\cdot$  may represent several different species, according to the number of sites in A which may undergo reaction with the primary radical. Furthermore, more than one primary radical may react with A. In that case,  $k_1$  represents an average rate constant which is weighted. Since  $B\cdot$  is probably still susceptible to further reaction with primary radicals, the following reaction may take place,



$k_2$  is a rate constant that is doubly weighted, once for the different primary radicals and once for the different species of  $B\cdot$ . It is an assumption that  $k_1$  and  $k_2$  exist such that they can describe quantitatively the preceding system. It is not an assumption that the actual rate constants for the individual reactions collectively represented by (1.1) and (1.2) will yield rate constants  $k_1$  and  $k_2$  when weighted according to the G yield of radicals and proportions of different species of  $B\cdot$ , but only

that constants  $k_1$  and  $k_2$  exist such that they can describe the preceding system (or an extension of it) in terms of ordinary chemical kinetics.

A second assumption is that the G yield for radicals remains constant. Most radiolysis work is done with a buffer present. At constant pH, Haissinsky (17) gives some evidence to support this assumption. This assumption, restated, simply means that the solute concentration is such that a) the solute does not react with primary radicals in the spur, the region of interaction of the radiation particle or wave and solvent, and b) the primary radicals do not recombine outside the spur (i.e., all radicals react with the solute or species derived from the solute). Kupperman (18) also provides some evidence to support this assumption by means of diffusion kinetic studies.

A third assumption is that a solute molecule is inactivated solely by indirect action. No direct inactivation takes place. This assumption is the logical conclusion of the work of Fricke (1).

Finally, it will be assumed that all components of the radiolyzed system other than those specified in the models undergo negligible change in concentration relative to the changes in concentration of the species which are specified by the models. Particularly, this applies to buffers and dissolved gases. This assumption does not mean that such components do not scavenge primary radicals, but that this scavenging effect is constant throughout the radiolysis.

A series of models will now be presented using the preceding assumptions. The first two models are unrealistic for all but the simplest solutes, but are helpful in developing the general mechanisms which follow.

## CHAPTER II

### CONSECUTIVE MECHANISMS

#### Mechanism I

The simplest model for the inactivation of a solute in the presence of an added impurity is



A is the monitored solute, C is an added impurity and I represents a radical insensitive product. The G yield is the yield of radical  $\omega^\bullet$  per unit dose and  $dD/dt$  is the dose rate. (The reader is reminded that it is an assumption that G is constant throughout the radiolysis.)

The rate of inactivation of solute is given by

$$\frac{dA}{dt} = -k_1 A \omega^\bullet \quad (2.4)$$

The change of concentration of impurity C with respect to time is given by

$$\frac{dC}{dt} = -k_2 C \omega^\bullet \quad (2.5)$$

Finally, the rate of change of concentration of primary radical  $\omega^\bullet$  is

given by

$$\frac{d\omega^*}{dt} = G\left(\frac{dD}{dt}\right) - k_1 A \omega^* - k_2 C \omega^* \quad (2.6)$$

From Equations (2.4) and (2.5),  $dA/dC$  can be obtained,

$$\frac{dA}{dC} = \frac{k_1 A}{k_2 C} \quad (2.7)$$

Integrating Equation (2.7) from  $A = A_0$ ,  $C = C_0$  to  $A = A$ ,  $C = C$ , one obtains an expression for the concentration of  $C$  in terms of  $A$  and the rate constants,  $k_1$  and  $k_2$ ,

$$C = \frac{C_0 A^{(k_2/k_1)}}{A_0^{(k_2/k_1)}} \quad (2.8)$$

Assuming a steady-state for  $\omega^*$ , (which seems very reasonable at most dose rates since  $k_n \sim 10^6 - 10^{10}$  l/mole-sec), Equation (2.6) can be written as follows:

$$\frac{d\omega^*}{dt} \approx 0 \approx G\left(\frac{dD}{dt}\right) - k_1 A \omega^* - k_2 C \omega^* \quad (2.9)$$

The steady-state expression for  $\omega^*$  is thus given by

$$\omega^* = \frac{G\left(\frac{dD}{dt}\right)}{(k_1 A + k_2 C)} \quad (2.10)$$

By substituting Equation (2.10) into Equation (2.4), Equation (2.11) is obtained.

$$\frac{dA}{dt} = \frac{-G\left(\frac{dD}{dt}\right)k_1 A}{k_1 A + k_2 C} \quad (2.11)$$

Thus, the change of solute concentration with respect to change in dose,  $D$ , can be written,

$$\frac{dA}{dD} = \frac{-Gk_1A}{k_1A + k_2C} \quad (2.12)$$

Substituting Equation (2.8) into (2.12) and rearranging, an integrable expression is obtained.

$$\int_{D=0}^{D=D} dD = -\frac{1}{G} \left[ \int_{A=A_0}^{A=A} dA + \int_{A=A_0}^{A=A} \frac{k_2 C_0 A^{(k_2/k_1)}}{k_1 A_0^{(k_2/k_1)} A} dA \right] \quad (2.13)$$

If  $D_x$  is defined as the dose required to reduce the original concentration to fraction  $x$ , ( $x \equiv A/A_0$ ), the following expression is obtained, using  $dA = A_0 dx$ ,

$$D_x = \frac{A_0}{G} \int_{x=1}^{x=x} dx - \frac{C_0}{G} \frac{k_2}{k_1} \int_{x=1}^{x=x} x^{(k_2/k_1-1)} dx \quad (2.14)$$

or,

$$D_x = \frac{A_0}{G} (1-x) + \frac{C_0}{G} (1-x^{k_2/k_1}) \quad (2.15)$$

$D_x$  is linear with initial concentration of solute  $A_0$ , if the original concentration of solute independent impurity is kept constant. A plot of  $D_x$  versus  $A_0$  will yield a straight line with an intercept proportional to the amount of added impurity if Mechanism I is the case.

#### Mechanism II

Since it seems reasonable that a primary radical will react with the product of  $A + \omega\cdot$ , this possibility should be included in a mechanism. Also, it seems reasonable that a molecule of solute  $A$  might react with primary radical  $\omega\cdot$  without destroying that property which is assayed. This can be represented by  $A_{(1)} + \omega\cdot \xrightarrow{k_0} A_{(2)}$ , where  $A = \sum A_{(n)}$ . All  $A$ 's retain the property which is monitored.



A is again the original solute, B is an intermediate formed from A, C is an added impurity, and I is an inert product. In this case the original solute may or may not be inactivated upon reaction with primary radical  $\omega^\bullet$ , and the intermediate B can still react with primary radicals. From Mechanism II the following equations are obtained.

$$\frac{dA}{dt} = -k_1 A \omega^\bullet \quad (2.21)$$

$$\frac{dB}{dt} = (k_1 A - k_2 B) \omega^\bullet \quad (2.22)$$

$$\frac{dC}{dt} = -k_3 C \omega^\bullet \quad (2.23)$$

By dividing (2.21) by (2.22), Equation (2.24) results,

$$\frac{dA}{dB} = \frac{-k_1 A}{k_1 A - k_2 B} \quad (2.24)$$

Rearranging,

$$k_1 A dB - k_2 B dA = -k_1 A dA \quad (2.25)$$

This differential equation may be solved by using  $A^{-(k_2/k_1+1)}$  as an integrating factor. Multiplying by  $A^{-(k_2/k_1+1)}$  gives

$$k_1 A^{-k_2/k_1} dB - k_2 B A^{(k_2/k_1 - 1)} dA = -k_1 A^{-k_2/k_1} dA \quad (2.26)$$

Equation (2.26) can now be integrated between the limits ( $A = A_0, B = 0$ ) and ( $A = A, B = B$ ), giving,

$$\left[ \frac{k_1 B}{A^{k_2/k_1}} \right]_{\substack{B=0 \\ A=A_0}}^{\substack{B=B \\ A=A}} = \frac{k_1 A^{(-k_2/k_1 + 1)}}{(k_2/k_1 - 1)} \left[ \right]_{A=A_0}^{A=A} \quad (2.27)$$

Evaluating (2.27) and solving for B gives

$$B = \frac{A - A_0 \left( \frac{k_2}{k_1} \right) \left( 1 - \frac{k_2}{k_1} \right)}{\left( \frac{k_2}{k_1} - 1 \right)} \quad (2.28)$$

B is now expressed in terms of A and rate constants  $k_1$  and  $k_2$ .

An expression for C is obtained by dividing (2.21) by (2.23), and integrating. It is identical to the expression for C derived in the previous section (Equation (2.8)), except the subscript on the rate constant for the reaction of the added impurity with  $\omega^\bullet$  is now 3 rather than 2.

$$C = \frac{C_0 A^{(k_3/k_1)}}{A_0^{(k_3/k_1)}} \quad (2.29)$$

From (2.16) - (2.20),  $d\omega^\bullet/dt$  is written as follows, assuming steady state,

$$\frac{d\omega^\bullet}{dt} \approx 0 = G \left( \frac{dD}{dt} \right) - (k_0 A + k_1 A + k_2 B + k_3 C) \omega^\bullet \quad (2.30)$$

By solving Equation (2.30) for  $\omega^\bullet$ , Equation (2.31) is obtained.

$$\omega^\bullet = \frac{G \left( \frac{dD}{dt} \right)}{k_0 A + k_1 A + k_2 B + k_3 C} \quad (2.31)$$

Substituting  $\omega$  into Equation (2.21), the following expression for  $dA/dD$  results.

$$\frac{dA}{dD} = \frac{-k_1 AG}{k_0 A + k_1 A + k_2 B + k_3 C} \quad (2.32)$$

By rearranging Equation (2.32), Equation (2.33) results.

$$dD = -\frac{1}{G} \left[ \frac{k_0}{k_1} + 1 + \frac{k_2 B}{k_1 A} + \frac{k_3 C}{k_1 A} \right] dA \quad (2.33)$$

Now, by substituting Equations (2.28) and (2.29) into Equation (2.33), an expression for  $dD$  is obtained.

$$dD = -\frac{1}{G} \left\{ \left( \frac{k_0}{k_1} + 1 \right) dA + \frac{k_2}{k_1 A} \left[ \frac{A - A_0 \frac{k_2/k_1 (1 - k_2/k_1)}{(k_2/k_1 - 1)}}{A_0} \right] dA + \frac{k_3}{k_1 A} \left[ \frac{C_0 A \frac{(k_3/k_1)}{A_0}}{(k_3/k_1)} \right] dA \right\} \quad (2.34)$$

Using  $x = A/A_0$  and  $A_0 dx = dA$ , we may integrate Equation (2.34).

$$\int_{d=0}^{D=D} dD = -\frac{1}{G} \left\{ A_0 \left( \frac{k_0}{k_1} + 1 \right) \int_{x=1}^{x=x} dx + A_0 \int_{x=1}^{x=x} \frac{k_2}{k_1} \left[ \frac{1 - (xA_0) \frac{(k_2/k_1 - 1) (1 - k_2/k_1)}{A_0}}{(k_2/k_1 - 1)} \right] dx + A_0 C_0 \frac{k_3}{k_1} \int_{x=1}^{x=x} x \frac{(k_3/k_1 - 1) - k_3/k_1 (k_3/k_1 - 1)}{A_0} dx \right\} \quad (2.35)$$

Simplification of Equation (2.35) yields



$$\int_{D=0}^{D=D} dD = -\frac{1}{G} \left\{ A_0 \left( \frac{k_0}{k_1} + 1 \right) \int_{x=1}^{x=x} dx + A_0 \int_{x=1}^{x=x} \frac{k_2}{k_1} \left[ \frac{1-x}{(k_2/k_1-1)} \right] dx \right. \\ \left. + C_0 \frac{k_3}{k_1} \int_{x=1}^{x=x} x^{k_3/k_1-1} dx \right\} \quad (2.36)$$

Integration of Equation (2.36) gives

$$D_x = -\frac{1}{G} \left\{ A_0 \left( \frac{k_0}{k_1} + 1 \right) (x-1) + A_0 \left[ \frac{k_2}{k_2-k_1} (x-1) + \frac{k_1}{k_2-k_1} (x^{k_2/k_1-1}) \right] \right. \\ \left. + C_0 (x^{k_3/k_1-1}) \right\} \quad (2.37)$$

Rearrangement yields the final result.

$$D_x = \frac{A_0}{G} \left[ \left( \frac{k_0}{k_1} + 1 \right) (1-x) + \left\{ \frac{k_2}{k_2-k_1} (1-x) + \frac{k_1}{k_2-k_1} (1-x^{k_2/k_1}) \right\} \right] \\ + \frac{C_0}{G} (1-x^{k_3/k_1}) \quad (2.38)$$

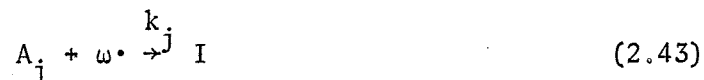
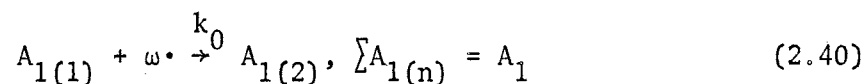
Again,  $D_x$  is linear with  $A_0$ , the original solute concentration, if the initial concentration of C,  $C_0$ , is kept constant as  $A_0$  varies. The intercept is again proportional to the amount of solute independent impurity.

The slope of Equation (2.38) is a function of  $k_1$  and  $k_2$ . Therefore, if  $k_1$  and  $k_2$  are temperature dependent, as one might expect, a series of plots of  $A_0$ 's versus  $D_x$ 's taken at different temperatures might yield lines with different slopes. This is not the case for Mechanism I (Equation (2.15) of the previous section). Since there is some doubt as to whether the rate constants are temperature dependent, what can be said is this: If the slope of  $A_0$  versus  $D_x$  is temperature dependent the first mechanism is ruled out and the second mechanism remains a possibility.

Conversely, if Mechanism I has been ruled out (perhaps for purely chemical reasons) the temperature dependence or independence of the slope might suggest whether, and possibly to what degree,  $k_1$  and  $k_2$  are temperature dependent. In the same manner, it might be noted that the intercept will be temperature dependent if  $k_3$  and  $k_1$  are temperature dependent.

### Mechanism III

It will now be shown that for any number of consecutive steps for both solute and impurity,  $D_x$  will be linear with  $A_0$ , the original concentration of solute, if the initial concentration of solute independent impurity is kept constant as  $A_0$  varies. The general consecutive mechanism will include the reaction of solute with  $\omega\cdot$ , without the destruction of the property which is assayed to determine activity. The general consecutive mechanism is represented by:



and





where  $A_{1(n)}$  is the original solute and  $A_n$ ,  $n \neq 1$  is the subsequent product of  $A_{n-1}$  and  $\omega \cdot$  that has lost the monitored property of  $A_1$ , whatever it may be.  $C_1$  is an impurity independent of solute and  $C_m$ ,  $m \neq 1$ , is the subsequent product of  $C_{m-1}$  and  $\omega \cdot$ .

The original solute,  $A_1$ , disappears according to the following expression,

$$\frac{dA_1}{dt} = -k_1 A_1 \omega \cdot \quad (2.48)$$

Assuming steady state conditions for radical species  $\omega \cdot$ ,

$$\frac{d\omega \cdot}{dt} \approx 0 = \frac{dD}{dt} (G) - [(k_0 A_1 + k_1 A_1 + k_2 A_2 + \dots + k_j A_j) + (K_1 C_1 + K_2 C_2 + \dots + K_i C_i)] \omega \cdot \quad (2.49)$$

or,

$$\omega \cdot = \frac{G \left( \frac{dD}{dt} \right)}{(k_0 A_1 + k_1 A_1 + k_2 A_2 + \dots + k_j A_j + C_1 K_1 + C_2 K_2 + \dots + K_i C_i)} \quad (2.50)$$

Then the expression for the disappearance of  $A_1$ , the original solute

becomes

$$\frac{dA_1}{dt} = \frac{-k_1 A_1 G \left( \frac{dD}{dt} \right)}{(k_0 A_1 + k_1 A_1 + k_2 A_2 + \dots + k_j A_j + C_1 K_1 + C_2 K_2 + \dots + K_i C_i)} \quad (2.51)$$

or,

$$\frac{dA_1}{dD} = \frac{-k_1 A_1 G}{(k_0 A_1 + k_1 A_1 + k_2 A_2 + \dots + k_j A_j + C_1 K_1 + C_2 K_2 + \dots + K_i C_i)} \quad (2.52)$$

Likewise, the expression for the change in concentration of  $A_n$ ,

where  $n = 2, \dots, j$ , (any subsequent molecule or radical formed from the reaction of  $A_{n-1}$  and  $\omega\cdot$ ) is

$$\frac{dA_n}{dt} = (k_{n-1}A_{n-1} - k_n A_n) \omega\cdot \quad (2.53)$$

Using the steady state expression for  $\omega\cdot$ , we obtain

$$\frac{dA_n}{dD} = \frac{(k_{n-1}A_{n-1} - k_n A_n)G}{(k_0 A_1 + k_1 A_1 + \dots + k_j A_j + K_1 C_1 + K_2 C_2 + \dots + K_i C_i)} \quad , n = 2, \dots, j \quad (2.54)$$

At this point it is important to note that the expressions for the change of  $A_n$  and  $A_1$  are written with respect to the change in dose, and are independent of dose rate  $dD/dt$ . A plot of  $A_1$  versus dose should not change with different or even varying dose rates if, in reality, a consecutive mechanism obtains in the radiolysis of a solute. This will be discussed further in Chapter III.

From (2.52)

$$\int_{D=0}^{D=D} dD = -\frac{1}{G} \int_{A_1=A_{10}}^{A_1=A_1} \left\{ 1 + \frac{k_0}{k_1} + \frac{k_2 A_2}{k_1 A_1} + \frac{k_3 A_3}{k_1 A_1} + \dots + \frac{k_j A_j}{k_1 A_1} + \frac{K_1 C_1}{k_1 A_1} \right. \\ \left. + \frac{K_2 C_2}{k_1 A_1} + \dots + \frac{K_i C_i}{k_1 A_1} \right\} dA_1 \quad (2.55)$$

where  $A_{10}$  is the original concentration of solute.

If  $x \equiv A_1/A_{10}$ ,  $dA_1 = A_{10} dx$  and

$$\int_{D=0}^{D=D} dD = D_x = -\frac{1}{G} \int_{x=1}^{x=x} \left\{ 1 + \frac{k_0}{k_1} + \sum_{n=2}^j \frac{k_n A_n}{k_1 x A_{10}} + \sum_{m=1}^i \frac{K_m C_m}{k_1 x A_{10}} \right\} A_{10} dx \quad (2.56)$$

or

$$D_x = -\frac{1}{G} \left[ A_{10} \left\{ 1 + \frac{k_0}{k_1} \right\} \left\{ x-1 \right\} + \sum_{n=2}^j \frac{k_n}{k_1} \int_{x=1}^{x=x} \frac{A_n dx}{x} + \sum_{m=1}^i \frac{K_m}{k_1} \int_{x=1}^{x=x} \frac{C_m dx}{x} \right]. \quad (2.57)$$

From Equation (2.57) it is apparent that  $D_x$  will be linear with  $A_{10}$  if and only if

(a) for any given  $x$  and all  $A_n$ ,  $n = 2, \dots, j$ , we have at that  $x$ ,

$$\sum_{n=2}^j \frac{k_n}{k_1} \int_{x=1}^{x=x} \frac{A_n dx}{x} \propto A_{10} \quad (2.58)$$

or

$$\sum_{n=2}^j \frac{k_n}{k_1} \int_{x=1}^{x=x} \frac{A_n dx}{x} \quad (2.59)$$

is constant (the latter seems unlikely), and

(b)

$$\sum_{m=1}^i \frac{K_m}{k_1} \int_{x=1}^{x=x} \frac{C_m dx}{x} \quad (2.60)$$

is constant for any given  $x$  and all  $A_n$ ,  $n = 2, \dots, j$ .

Requirements (a) and (b) will be proven by induction. First, requirement (a):

From (2.52) and (2.54)

$$\frac{dA_1}{dA_n} = \frac{-k_1 A_1}{(k_{n-1} A_{n-1} - k_n A_n)}, \text{ for all } n = 2, \dots, j \quad (2.61)$$

Rearranging, the following differential equation is obtained.

$$k_1 A_1 dA_n - k_n A_n dA_1 = -k_{n-1} A_{n-1} dA_1 \quad (2.62)$$

Using  $A_1^{-(k_n/k_1+1)}$  as an integrating factor the preceding equation may be

solved. Multiplying by  $A_1^{-(k_n/k_1+1)}$

$$k_1 A_1^{-(k_n/k_1)} dA_n - k_n A_n A_1^{-(k_n/k_1+1)} dA_1 = -k_{n-1} \frac{A_{n-1}}{A_1^{(k_n/k_1+1)}} dA_1 \quad (2.63)$$

for all  $n = 2, \dots, j$ .

The left side of the preceding equation is now an exact differential.

$$\begin{aligned} & \int_{A_n=0, A_1=A_{10}}^{A_n=A_n, A_1=xA_{10}} \left[ k_1 A_1^{-(k_n/k_1)} dA_n - k_n A_n A_1^{-(k_n/k_1+1)} dA_1 \right] \\ &= \int_{A_1=A_{10}}^{A_1=xA_{10}} -k_{n-1} \frac{A_{n-1}}{A_1^{(k_n/k_1+1)}} dA_1 \end{aligned} \quad (2.64)$$

Hence, by inspection

$$\frac{k_1 A_n}{A_1^{k_n/k_1}} \Big|_{A_n=0, A_1=A_{10}}^{A_n=A_n, A_1=xA_{10}} = -k_{n-1} \int_{A_1=A_{10}}^{A_1=xA_{10}} \frac{A_{n-1}}{A_1^{(k_n/k_1+1)}} dA_1 \quad (2.65)$$

When  $A_n = 0$ , the left side of the preceding equation is equal to zero.

Therefore the preceding equation can be written as

$$\frac{k_1 A_n}{A_1^{k_n/k_1}} = -k_{n-1} \int_{A_1=A_{10}}^{A_1=xA_{10}} \frac{A_{n-1}}{A_1^{(k_n/k_1+1)}} dA_1 \quad (2.66)$$

Since  $x \equiv A_1/A_{10}$ , the limits on the right hand side of the equation become  $x = 1$  to  $x = x$ . By this substitution, the following expression is obtained,

$$\frac{k_1 A_n}{x^{(k_n/k_1)} A_{10}^{(k_n/k_1)}} = -k_{n-1} \int_{x=1}^{x=x} \frac{A_{n-1}}{x^{(k_n/k_1+1)} A_{10}^{(k_n/k_1+1)}} dx \quad (2.67)$$

Solving for  $A_n$ ,

$$A_n = - \frac{k_{n-1}}{k_1} x^{k_n/k_1} \int_{x=1}^{x=x} \left[ \frac{A_{n-1}}{x^{(k_n/k_1+1)}} \right] dx$$

for all  $n = 2, \dots, j$ . (2.68)

For  $n = 2$ , the following expression is obtained, from (2.68)

$$A_2 = - \frac{k_1}{k_1} x^{k_2/k_1} \int_{x=1}^{x=x} \left[ \frac{A_1}{x^{(k_2/k_1+1)}} \right] dx \quad (2.69)$$

By definition  $A_1 \equiv xA_{10}$ ; hence

$$A_2 = - x^{k_2/k_1} A_{10} \int_{x=1}^{x=x} x^{-(k_2/k_1)} dx \quad (2.70)$$

At a given  $x$ ,

$$- x^{k_2/k_1} \int_{x=1}^{x=x} x^{k_2/k_1} dx \quad (2.71)$$

is a constant. Let

$$P_{A_2} = - x^{k_2/k_1} \int_{x=1}^{x=x} x^{k_2/k_1} dx = f_2(x) \quad (2.72)$$

Therefore  $A_2 = P_{A_2} A_{10}$ , or  $A_2$  is proportional to  $A_{10}$  at a given  $x$ . Also,

$A_2 = f_2(x)A_{10}$  at a given  $x$ .

For  $n = 3$ , Equation (2.68) gives

$$A_3 = - \frac{k_2}{k_1} x^{k_3/k_1} \int_{x=1}^{x=x} \left[ \frac{A_2}{x^{(k_3/k_1+1)}} \right] dx \quad (2.73)$$

Since  $A_2 = f_2(x)A_{10}$ ,

$$A_3 = - \frac{k_2}{k_1} x^{k_3/k_1} \int_{x=1}^{x=x} \left[ \frac{A_{10} f_2(x)}{x^{(k_3/k_1+1)}} \right] dx \quad (2.74)$$

$A_{10}$  can again be factored out of the integral, resulting in the following expression,

$$A_3 = -\frac{k_2}{k_1} x^{k_3/k_1} A_{10} \int_{x=1}^{x=x} \frac{f_2(x)}{(k_3/k_1+1)x} dx \quad (2.75)$$

Let

$$P_{A_3} = f_3(x) = -\frac{k_2}{k_1} x^{k_3/k_1} \int_{x=1}^{x=x} \left[ \frac{f_2(x)}{(k_3/k_1+1)x} \right] dx \quad (2.76)$$

$P_{A_3}$  is constant for a given  $x$ ; therefore  $A_3 = K_{A_3} A_{10}$  or  $A_3$  is proportional to  $A_{10}$  at a given  $x$ .

Similarly all subsequent  $A_n$ 's are proportional to  $A_{10}$  by some function of  $x$ ,  $A_n = P_{A_3} A_{10} = A_{10} f_n(x)$ . Furthermore, this proportionality factor is given by the expression

$$f_n(x) = \left(\frac{-k_{n-1}}{k_n}\right) x^{(k_n/k_1)} \int_{x=1}^{x=x} \left[ \frac{f_{n-1}(x)}{(k_n/k_1+1)x} \right] dx \quad (2.77)$$

$$n = 2, \dots, j.$$

Therefore, the second term inside the brackets of Equation (2.57) becomes, since  $A_n = A_{10} f_n(x)$

$$\sum_{n=2}^j \frac{k_n}{k_1} \int_{x=1}^{x=x} \left(\frac{A_n}{x}\right) dx = A_{10} \sum_{n=2}^j \frac{k_n}{k_1} \int_{x=1}^{x=x} \left[ \frac{f_n(x)}{x} \right] dx \quad (2.78)$$

Let

$$P'_A = \sum_{n=2}^j \frac{k_n}{k_1} \int_{x=1}^{x=x} \left[ \frac{f_n(x)}{x} \right] dx$$

$P'_A$  is constant at a given  $x$ . Hence at a given  $x$

$$\sum_{n=1}^j \frac{k_n}{k_1} \int_{x=1}^{x=x} \frac{A_n dx}{x} = K'_A A_{10} \quad (2.79)$$

Therefore requirement (a) has been met.

Now, for requirement (b), the following expressions are obtained for the change in concentrations of  $C_1$  and  $C_m$  with respect to time.



$$\frac{dC_1}{dt} = -K_1 C_1 \omega \quad (2.80)$$

$$\frac{dC_m}{dt} = (K_{m-1} C_{m-1} - K_m C_m) \omega \quad (2.81)$$

From (2.80) and (2.81) and the same treatment that led to Equation (2.68), we obtain

$$C_m = -\frac{K_{m-1}}{K_1} x^{K_m/K_1} \int_{x=1}^{x=x} \left[ \frac{C_{m-1}}{x^{(K_m/K_1+1)}} \right] dx \quad (2.82)$$

From Equations (2.80) and (2.48),

$$\frac{K_1 C_1}{k_1 A_1} = \frac{dC_1}{dA_1} ; \quad \frac{dA_1}{k_1 A_1} = \frac{dC_1}{K_1 C_1} \quad (2.83)$$

Integration of the expression on the right between the limits ( $A_1 = A_{1_0}$ ,  $C_1 = C_{1_0}$ ) and ( $A_1 = A_1$ ,  $C_1 = C_1$ ) is now possible.  $C_{1_0}$  is the original concentration of impurity.

$$\frac{K_1}{k_1} \int_{A_1=A_{1_0}}^{A_1=A_1} \frac{dA_1}{A_1} = \int_{C_1=C_{1_0}}^{C_1=C_1} \frac{dC_1}{C_1} \quad (2.84)$$

$$\frac{K_1}{k_1} \ln \frac{A_1}{A_{1_0}} = \ln \frac{C_1}{C_{1_0}} \quad (2.85)$$

Using  $x = A_1/A_{1_0}$ , exponentiating, and rearranging, an expression for  $C_1$  may be obtained.

$$C_1 = C_{1_0} x^{K_1/k_1} \quad (2.86)$$

Let  $P_{C_1} = f_1(x) = x^{K_1/k_1}$ . At a given  $x$ ,  $P_{C_1}$  is constant. From equation (2.82) the following expression is obtained for  $C_2$ ,

$$C_2 = -x^{K_2/K_1} \int_{x=1}^{x=x} \left[ \frac{C_1}{x^{(K_2/K_1+1)}} \right] dx \quad (2.87)$$

Since  $C_1 = f_1(x)C_{1_0}$ ,

$$C_2 = -x^{K_2/K_1} C_{1_0} \int_{x=1}^{x=x} \left[ \frac{f_1(x)}{x^{(K_2/K_1+1)}} \right] dx \quad (2.88)$$

Again, let

$$P_{C_2} = f_2(x) = x^{K_2/K_1} \int_{x=1}^{x=x} \left[ \frac{f_1(x)}{x^{(K_2/K_1+1)}} \right] dx \quad (2.89)$$

$P_{C_2}$  is constant at a given  $x$  and  $C_2 = P_{C_2} C_{1_0}$  or  $C_2 = f_2(x)C_{1_0}$ .

Likewise, from expression (2.82),  $C_3$  is given by,

$$C_3 = -\frac{K_2}{K_3} x^{K_3/K_1} \int_{x=1}^{x=x} \left[ \frac{C_2}{x^{(K_3/K_1+1)}} \right] dx \quad (2.90)$$

Since  $C_2 = f_2(x)C_{1_0}$

$$C_3 = -\frac{K_2}{K_3} x^{K_3/K_1} \int_{x=1}^{x=x} \left[ \frac{f_2(x)C_{1_0}}{x^{(K_3/K_1+1)}} \right] dx \quad (2.91)$$

Again, let

$$P_{C_3} = f_3(x) = -\frac{K_2}{K_3} x^{K_3/K_1} \int_{x=1}^{x=x} \left[ \frac{f_2(x)}{x^{(K_3/K_1+1)}} \right] dx \quad (2.92)$$

Hence,  $C_3 = f_3(x)C_{1_0} = P_{C_3} C_{1_0}$  or  $C_3$  is proportional to  $C_{1_0}$  at a given  $x$ .

Similarly, all subsequent  $C_m$ 's are proportional to  $C_{1_0}$  at a given  $x$  by the proportionality factor  $f_m(x) = P_{C_m}$ ,

$$f_m(x) = -\frac{K_{m-1}}{K_m} x^{K_m/K_1} \int_{x=1}^{x=x} \left[ \frac{f_{m-1}(x)}{x^{(K_m/K_1+1)}} \right] dx \quad (2.93)$$

$m = i, \dots, i$

or

$$C_m = f_m(x)C_{10} \quad (2.94)$$

The third term inside the brackets of Equation (2.57) can be rewritten,

$$\sum_{m=1}^i \frac{K_m}{k_1} \int_{x=1}^{x=x} \frac{C_m dx}{x} = C_{10} \sum_{m=1}^i \frac{K_m}{k_1} \int_{x=1}^{x=x} \left[ \frac{f_m(x)}{x} \right] dx \quad (2.95)$$

Let

$$P'_C = \sum_{m=1}^i \frac{K_m}{k_1} \int_{x=1}^{x=x} \left[ \frac{f_m(x)}{x} \right] dx \quad (2.96)$$

$P'_C$  is a constant at a given  $x$ . Therefore, if  $C_{10}$  is constant the entire expression is constant at a given  $x$ .

$$\sum_{m=1}^i \frac{K_m}{k_1} \int_{x=1}^{x=x} \left( \frac{C_m}{x} \right) dx = P'_C C_{10} = \text{constant} \quad (2.97)$$

The second requirement, (b) is met. A plot of  $D_x$  versus  $A_{10}$  (holding  $C_{10}$  constant) will give a straight line if consecutive reactions occur. The intercept of this line is proportional to the amount of solute independent impurity in the system.  $A$  is the solute, and

$$D_x = P'_A A_0 + P'_C C_0, \quad P'_C C_0 = \text{constant} \quad (2.98)$$

$C_0$  is the original solute independent impurity concentration.

Since  $A_0/D_x$  is proportional to the  $G$  yield of solute (molecules inactivated/radiation unit) at  $x$ , the slope of a  $D_x$  versus  $A_0$  plot is the reciprocal of the  $G$  yield for the solute at that  $x$  as the initial concentration of solute,  $A_0$ , is increased. It is evident, then, that the slope of a linear  $A_0$  versus  $D_x$  plot is proportional to the radical scavenging capacity of the solute and all subsequent species derived from the solute

and the intercept is proportional to radical scavenging capacity of the solute-independent impurity and all species derived from the solute independent impurity

### Logarithmic Behavior and Consecutive Mechanisms

As previously noted, solutes often show exponential behavior when they are radiolyzed. Some possibilities concerning logarithmic behavior will now be discussed.

If the solute decrease is exponential with dose, the following equation is necessarily valid.

$$\frac{dA_1}{dD} = -K_{ex} A_1 \quad (2.99)$$

where  $K_{ex}$  is a constant and  $A_1$  is the solute. By referring to Equation (2.57) of the general consecutive mechanism,

$$\frac{dA_1}{dD} = \frac{-k_1 A_1 G}{k_0 A_1 + \sum_{n=1}^j k_n A_n + \sum_{m=1}^i K_m C_m} \quad (2.100)$$

it is apparent that

$$K_{ex} = \frac{k_1 G}{k_0 A_1 + \sum_{n=1}^j k_n A_n + \sum_{m=1}^i K_m C_m} \quad (2.101)$$

From Equation (2.101), it is apparent that

$$k_0 A_1 + \sum_{n=1}^j k_n A_n + \sum_{m=1}^i K_m C_m = \text{constant} \quad (2.102)$$

All of the terms in the preceding equation are radical scavenging terms. The conclusion must be that if consecutive reactions occur and exponential behavior is observed, the total radical scavenging ability of the

system remains constant throughout the radiolysis. (The steady state concentration of primary radicals remains constant.)

Equation (2.102) can be shown to be valid if all species of A scavenge primary radicals at equal rates and all species of C scavenge primary radicals at equal rates, and, if enough intermediates are formed such that no inert product reaches appreciable concentration. That is,

$$k = (k_0 + k_1) = k_2 = k_3 = \dots = k_n \quad (2.103)$$

$$K = K_1 = K_2 = K_3 = \dots = K_n \quad (2.104)$$

and

$$\sum A_n \approx A_0 \quad (2.105)$$

$$\sum C_m \approx C_0 \quad (2.106)$$

By substituting Equations (2.103), (2.104), (2.105) and (2.106) into Equation (2.100),

$$\frac{dA_1}{dD} = \frac{-kA_1G}{kA_0 + KC_0} \quad (2.107)$$

or

$$\frac{dA_1}{dD} = \frac{-kA_1G}{C_s} \quad (2.108)$$

where  $C_s$  is a constant. This is the exponential behavior sought.  $C_s$  is a radical scavenging constant for the solution.

It is interesting to note that Hutchinson and Ross (10) and Augenstine (9) concluded that all rate constants for primary radical reactions are essentially equal, after they assumed exponential behavior and implicitly assumed that if a radical reacts with a solute molecule,

the solute is inactivated (i.e.,  $k_0 = 0$ ).

It is not the author's contention that the set of conditions proposed above is the only way in which Equation (2.102) can be valid. Indeed, in chemical terms, the proposed constancy of the radical scavenging ability of the system seems reasonable without the preceding conditions. A solution of an organic compound would eventually be broken down into elementary components:  $\text{CO}_2$ ,  $\text{NH}_3$ ,  $\text{H}_2\text{O}$ , etc. The solution resulting from long-term radiolysis would likely have a very small radical scavenging ability. However, if the radiolysis is considered complete when ninety per cent of the original solute remains, the resulting solution might contain products which are of comparable complexity to the original molecule. The number of "sites" which are susceptible to radical attack might be nearly the same or even slightly higher than the initial number of sites after radiolysis to  $D_{.90}$ . The averaging effect of several molecules of varying sensitivities to radical attack might account for the exponential behavior. It should be noted that often, with enzymes, the assays are subject to relatively large experimental error. Also, a buffer is often employed for radiolysis work. Its presence might have a leveling effect on the total radical scavenging ability of the solution (see page 4, Chapter I, Section II).

Equations (2.99), (2.101) and (2.102) give Equation (2.108) where  $C_s$  is the radical-scavenging constant of Equation (2.102).

$$\frac{dA_1}{dD} = \frac{-k_1GA_1}{C_s} \quad (2.108)$$

Equation (2.108) is necessarily true if consecutive reactions take place and exponential behavior is observed. If the radical scavenging ability, represented by  $C_s$ , decreases,  $dA_1/dD$  will have a value less than that

expected from exponential behavior. If the value  $C_s$  decreases as the radiolysis proceeds,  $dA_1/dD$  will have a larger value than that expected from logarithmic behavior. A careful, systematic study of the products of the radiolysis of a number of organic solutes that show non-exponential behavior might support or discredit the preceding hypothesis. Figure 1 illustrates the non-exponential behavior of papain as reported by Sanner and Pihl (6). In this case the activity decreased at a faster rate than that which would be expected from exponential behavior. This suggests, according to the hypothesis, that the total radical scavenging ability of the system decreases as the radiolysis proceeds. This is assuming, of course, that the mechanism is consecutive.

#### Impurity in the Solute

If an impure solute is present, the original concentration of pure solute is proportional to the fraction of purity  $f$ .

$$A_{p_0} = A_{t_0} f \quad (2.109)$$

$A_{t_0}$  is the total original concentration of pure and impure solute and  $A_{p_0}$  is the original concentration of pure solute. Therefore, the initial amount of impure solute is given by  $A_{I_0}$ ,

$$A_{I_0} = A_{t_0} - A_{t_0} f \quad (2.110)$$

or,

$$A_{I_0} = A_{t_0} (1-f) \quad (2.111)$$

By assuming consecutive mechanisms, Equation (2.98) may be used and an expression for  $D_x$  can be written,

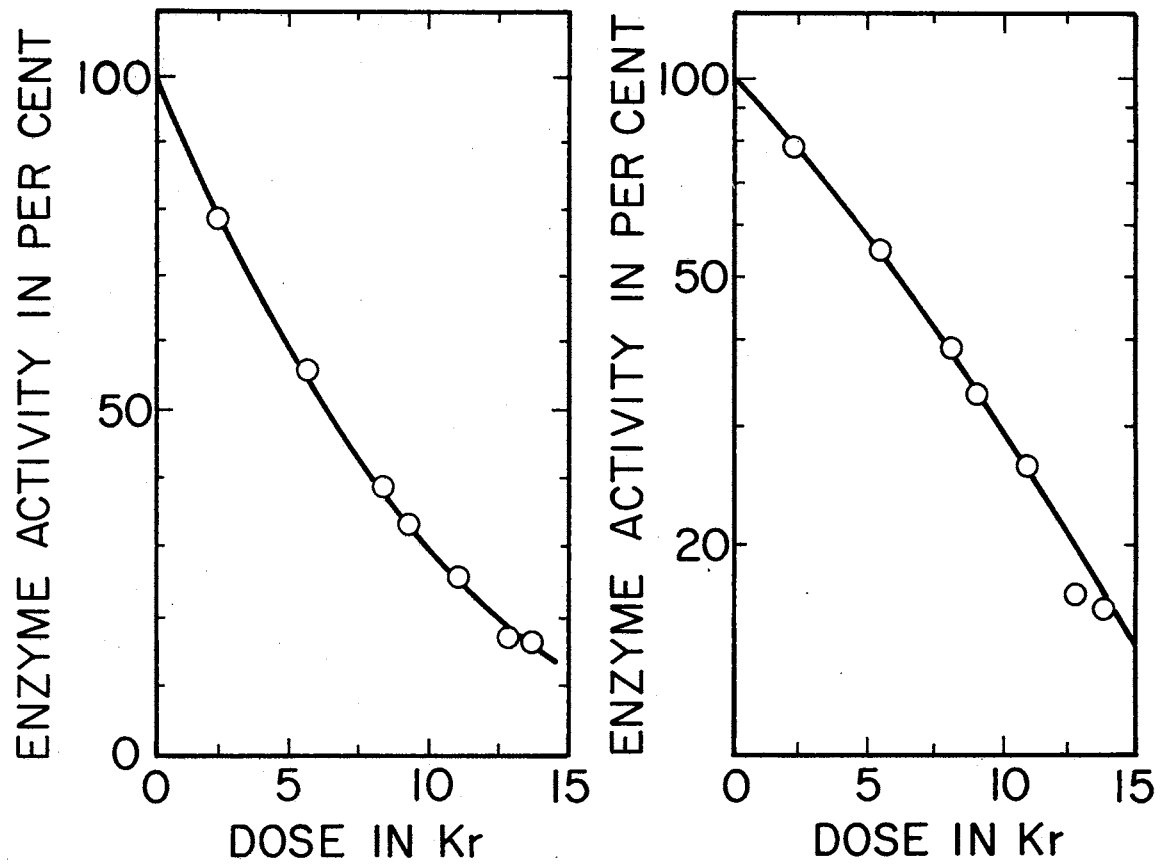


Figure 1. Non-Exponential Behavior of Papain



$$D_x = P'_{A_p} A_{t_0} f + P'_{A_I} A_{t_0} (1-f) \quad (2.112)$$

where  $P'_{A_p}$  is a constant corresponding to  $A_p$  and  $P'_{A_I}$  is a constant corresponding to  $A_I$ . No solute-independent impurity term is included in Equation (2.112). A solute-independent impurity could, of course, be included by the addition of a constant to Equation (2.112). By rearranging Equation (2.112),

$$D_x = \left[ P'_{A_p} f + P'_{A_I} (1-f) \right] A_{t_0} \quad (2.113)$$

If  $P'_{A_p} = P'_{A_I} = P'_A$ , that is, the radical scavenging ability of the pure and impure solutes are equal,

$$D_x = P'_A A_{t_0} \quad (2.114)$$

Therefore, an impure solute will have no effect on the slope of a  $D_x$  versus  $A_0$  plot if the pure and impure solute have identical radical scavenging capacity, which might be expected with an enzyme. However, if  $P'_{A_p}$  and  $P'_{A_I}$  are not equal, the slope may be greater or less than the slope that would be obtained from the pure solute,  $A_p$ , alone, depending on the radical scavenging capacity of the impure solute  $A_I$ .

#### Estimation of Rate Constants

Because the radiolysis of an organic solute is a complex affair and usually subject to considerable experimental error, the extraction of rate constants is practically impossible by the traditional method of taking the first derivative of solute versus dose plots. However, if consecutive reactions occur, Equation (2.86) of page 19 [the general consecutive mechanism] will always be valid. Pulse radiolysis studies have provided values for rate constants of certain simple organic

molecules. Using, Equation (2.86), the rate constant for the inactivation step can be obtained in terms of fraction of inactivation and the concentration of added impurity.

$$\frac{K_1 (\ln x)}{\ln\left(\frac{C_1}{C_{10}}\right)} = k_1 \quad (2.115)$$

where

$K_1$  = the known rate constant of the added substance;

$x$  = fraction of inactivation of solute;

$C_1$  = concentration of impurity at  $x$ ; and

$C_{10}$  = original concentration of impurity.

Hence if both the solute and impurity are simultaneously monitored and if the rate constant for reaction of radical  $\omega\cdot$  with the impurity is known, the rate constant for inactivation step is obtainable. If chemical evidence suggests that more than one primary radical participates in the inactivation, a weighted average of known rate constants should be used and the resulting rate constant would be a weighted average rate constant.

#### Plots Reciprocal G Yield and of $D_x$ Versus $A_0$

Huthinson and Ross (10) provide some  $D_{.37}$  versus  $A_0$  plots with and without the addition of impurity. This plot for methylene blue, coenzyme A and sulfanilide are given in Figures 2, 3, and 4, respectively.

Augenstine (9) uses a similar plot. He postulates that a plot of the reciprocal of the G yield for the solute versus the reciprocal of the original solute concentration is a linear relationship at  $D = D_{.37}$ . This may be written as,

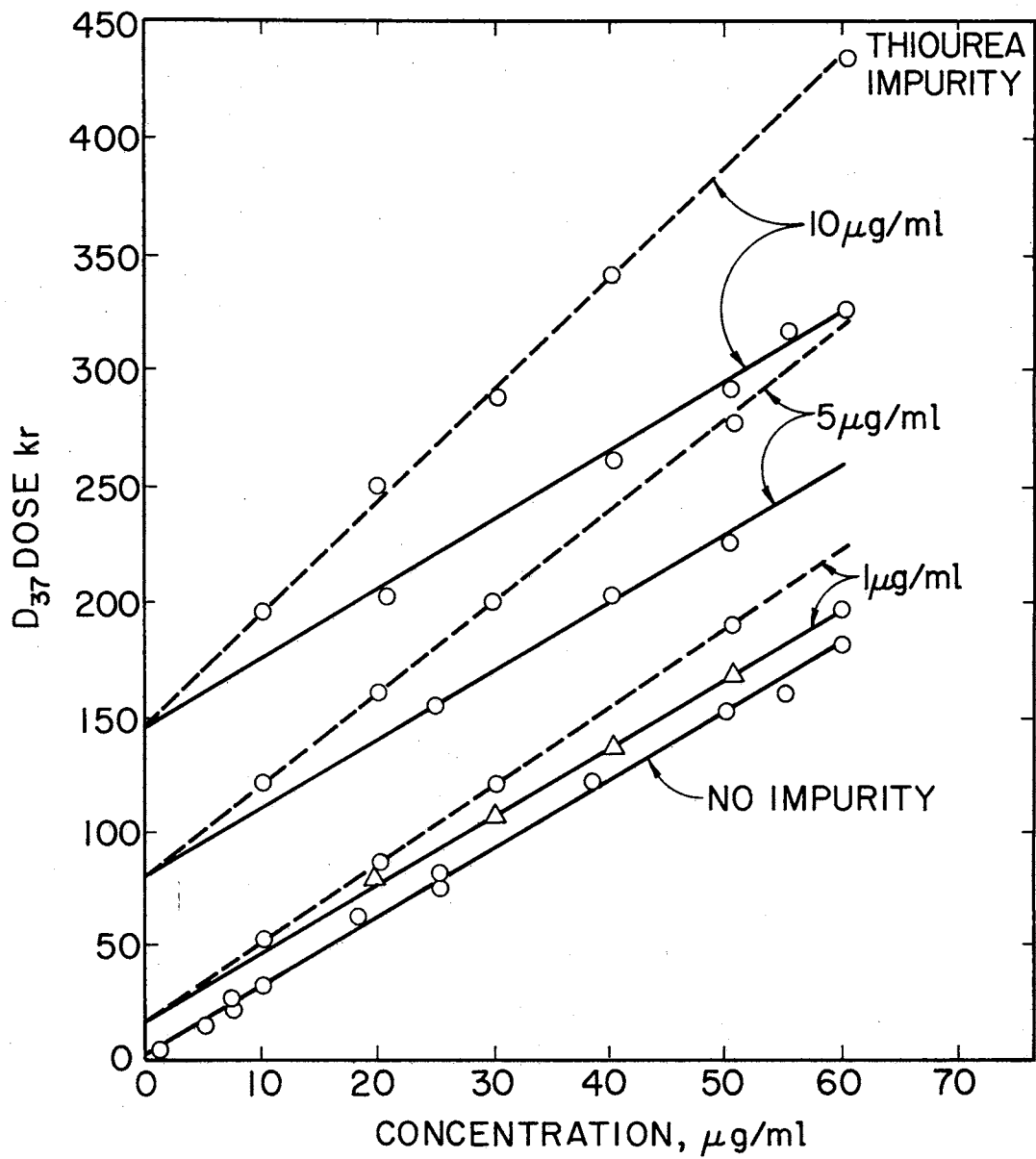


Figure 2. Methylene Blue,  $D_{.37}$  Versus  $A_0$ .

(— in  $\text{O}_2$ ; - - - in  $\text{N}_2$ )

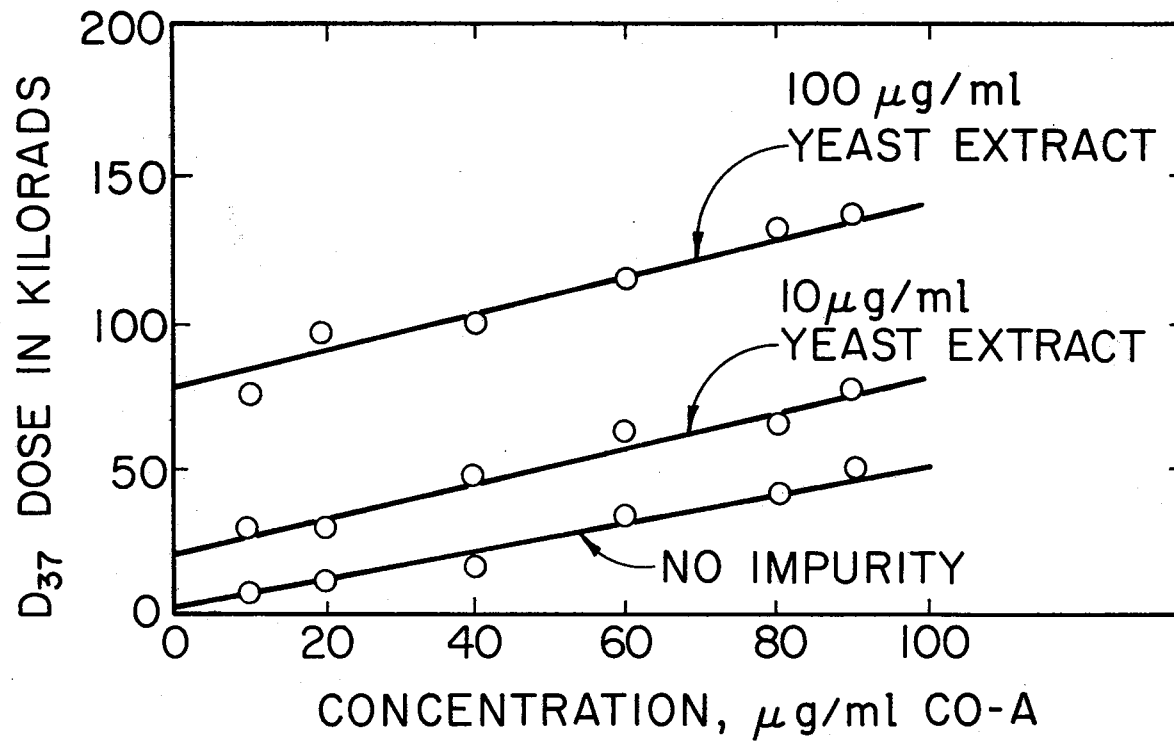


Figure 3. Sulfanilamide,  $D_{.37}$  Versus  $A_0$

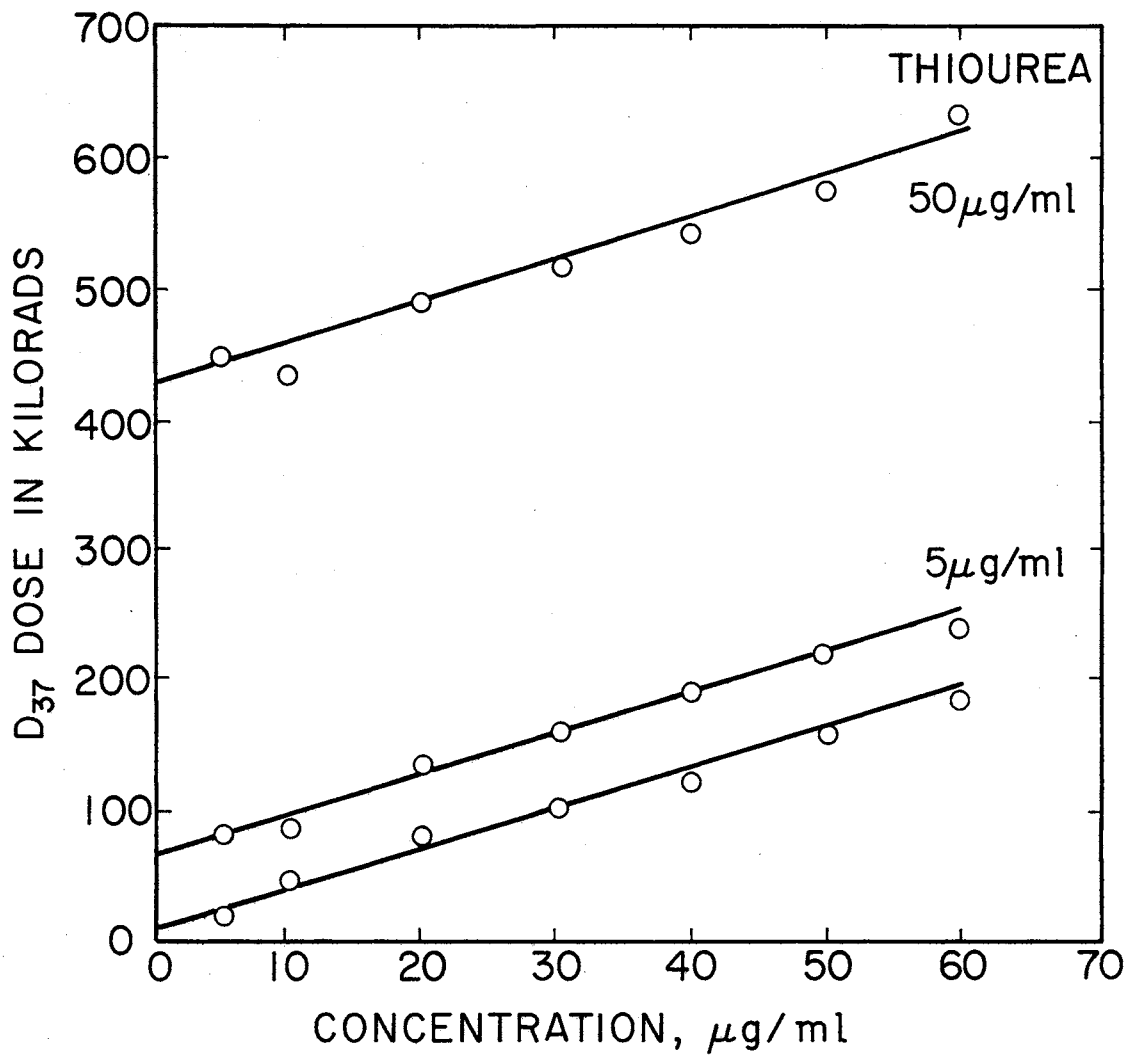


Figure 4. Coenzyme A,  $D_{.37}$  Versus  $A_0$

$$\frac{1}{G_{\text{solute}}} = \left(\frac{1}{A_0}\right) C_1 + C_2 \quad \text{at } D = D_{.37} \quad (2.116)$$

where  $C_1$  and  $C_2$  are constant. It is apparent that the  $G$  yield of solute (molecules/unit of radiation) is proportional to  $A_0/D_{.37}$  at  $D = D_{.37}$ ;

$$G_{\text{solute}} = (C) \frac{A_0}{D_{.37}} \quad (2.117)$$

where  $C$  is a constant.

Substituting (2.118) into (2.117)

$$\left(\frac{1}{C}\right) \frac{D_{.37}}{A_0} = \frac{1}{A_0} C_1 + C_2 \quad (2.118)$$

or

$$\frac{D_{.37}}{A_0} = \frac{1}{A_0} C_1' + C_2' \quad (2.119)$$

where  $C_1'$  and  $C_2'$  are constants. This equation can be written as

$$D_{.37} = C_1' + C_2' A_0 \quad (2.120)$$

which is identical to Equation (2.98), page 21.

Augenstine's plots, Equation (2.117), for trypsin, D-amino oxidase, ribonuclease, and chymotrypsin, are given in Figures 5, 6, 7, and 8, respectively [1 rad =  $1.76 \times 10^{12}$  ion pairs/cc/roentgen]. Trypsin and D-amino oxidase show linearity, supporting consecutive mechanisms. However, chymotrypsin and ribonuclease show a downward deviation from linearity much greater than experimental error should allow (see Figures 7 and 8). Accordingly, consecutive mechanisms should be ruled out for chymotrypsin and ribonuclease.

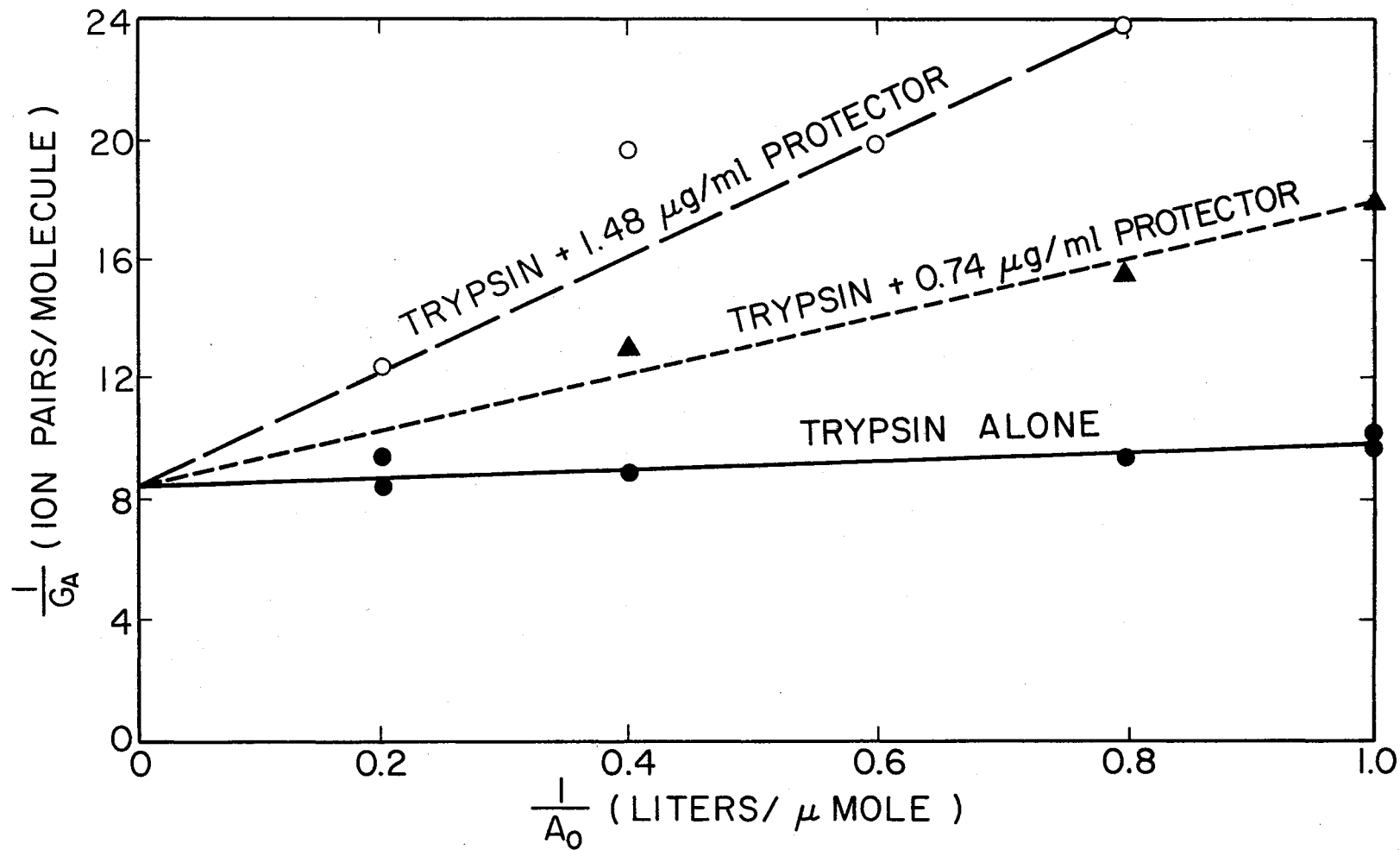


Figure 5. Trypsin,  $1/G_A$  Versus  $1/A_0$

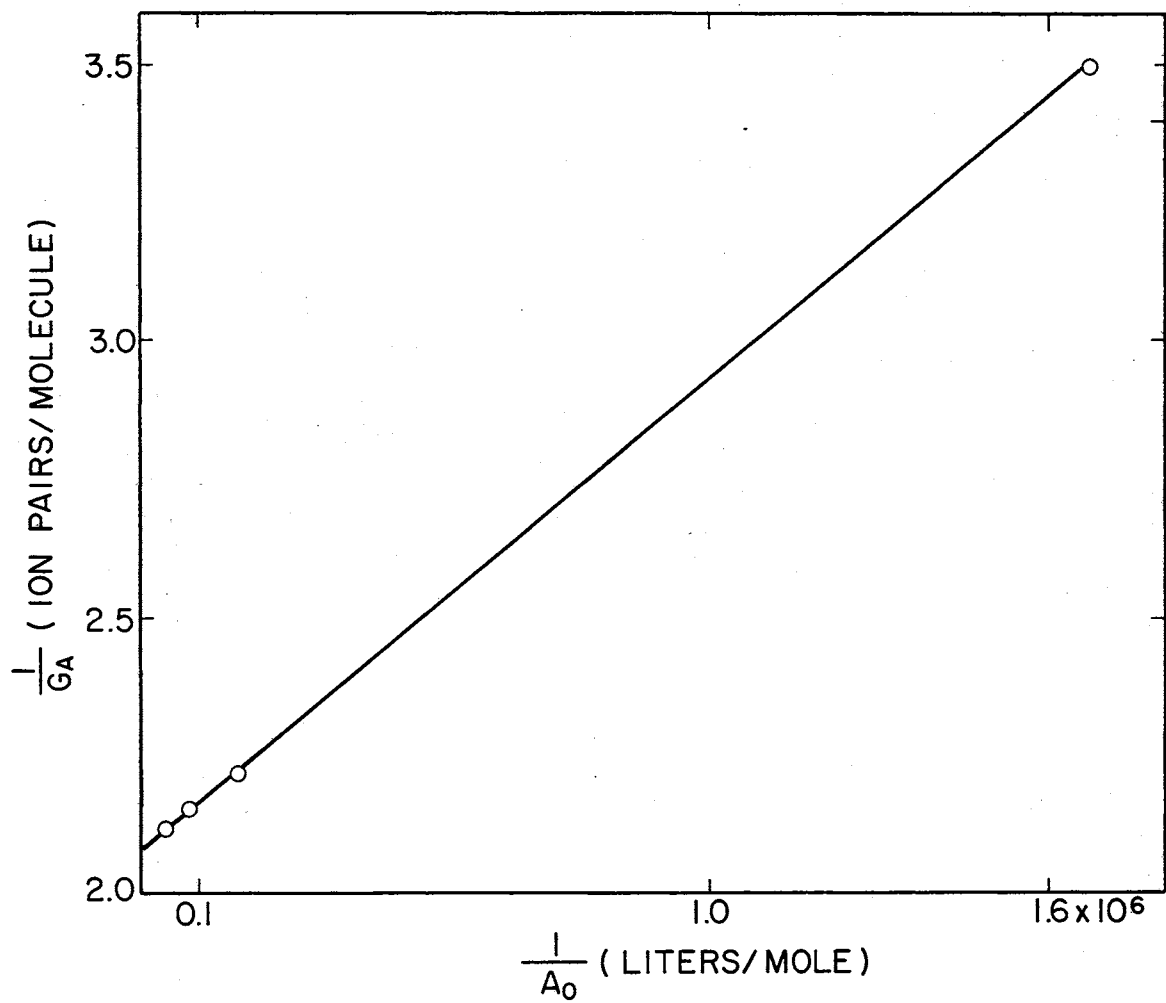


Figure 6. D-Amino Acid Oxidase,  $1/G_A$  Versus  $1/A_0$



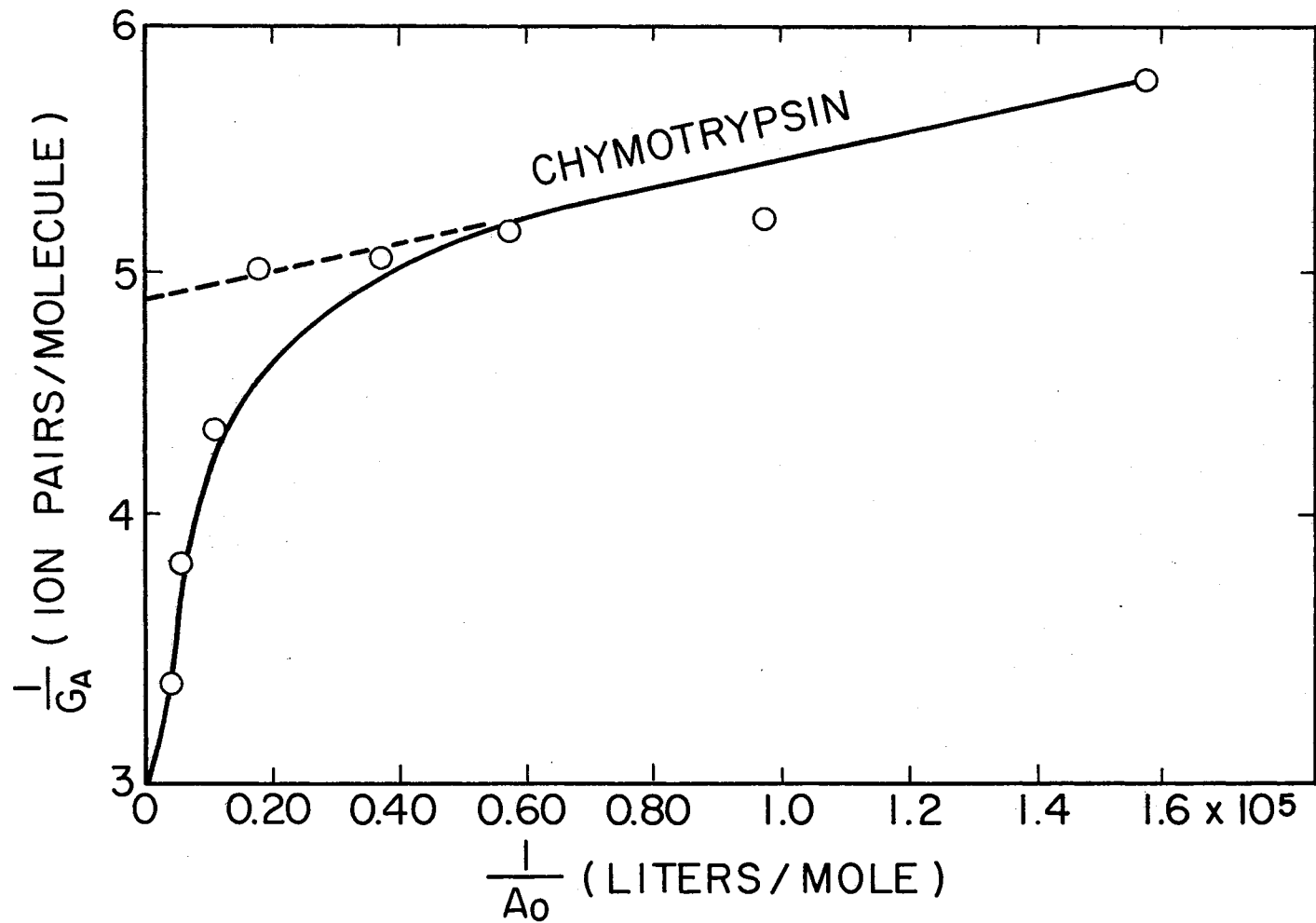


Figure 7. Chymotrypsin,  $1/G_A$  Versus  $1/A_0$

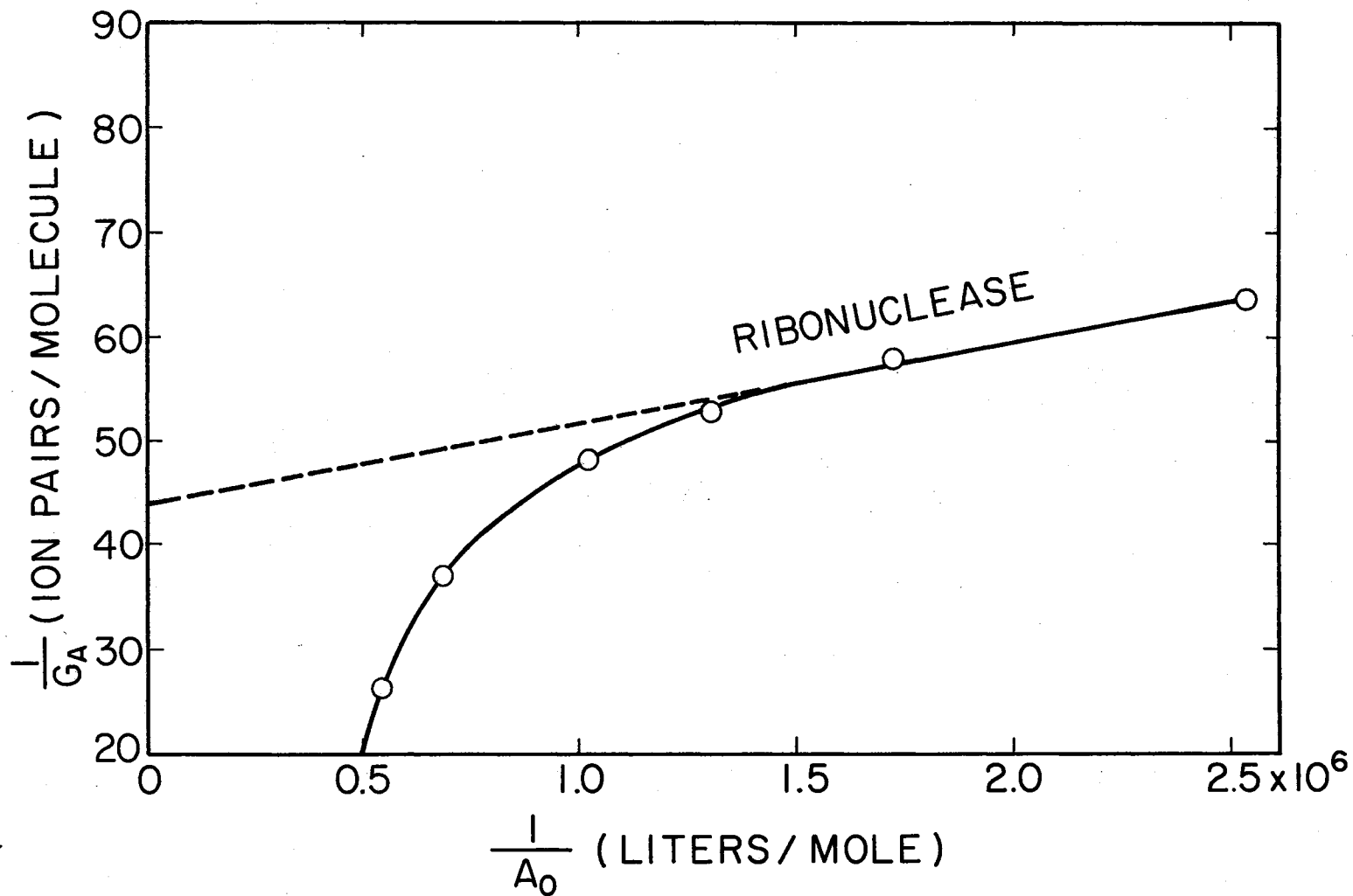


Figure 8. Ribonuclease,  $1/G_A$  Versus  $1/A_0$

## CHAPTER III

### DEVELOPMENT OF PARALLEL MECHANISMS

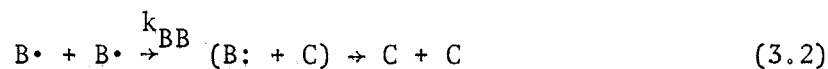
#### Mechanisms IV - V

The product of the highly reactive radical species  $\omega\cdot$  and the solute A is probably a radical  $B\cdot$ ,



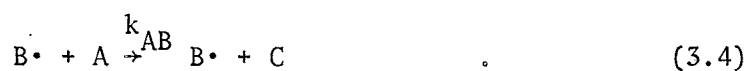
This radical, although not as reactive as  $\omega\cdot$ , should undergo some further reaction in addition to reaction with another primary radical. The secondary solute radical could react with another secondary solute radical, the original solute molecule, or another molecular species.

The product of the  $B\cdot + B\cdot$  reaction might be molecular species or a molecular species and a diradical, depending on a number of factors, particularly, the size of the original solute molecule.



C now represents a molecular species that is capable of scavenging primary radicals.

The product of the  $B\cdot + A$  step is probably a molecular species C and another secondary radical.

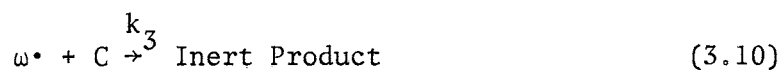
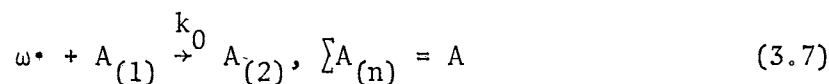


The product of  $B\cdot + C$  is probably another molecular species and another secondary radical.



This step will have no ultimate effect on the kinetics if the assumption concerning the weighted average rate constants is correct (Section II, Part I). Also, it will be assumed that the diradical of expression (3.3) will undergo the same reactions as the radical  $B\cdot$  (expressions (3.3), (3.4), and (3.5), and, again, a weighted average rate constant can describe the reactions of both  $B\cdot$  and  $B_2$ . The simplest mechanism using expressions (3.2), (3.4), and (3.5) is Mechanism IV. No impurity will be used in Mechanism IV; C now represents a molecular species formed from A.

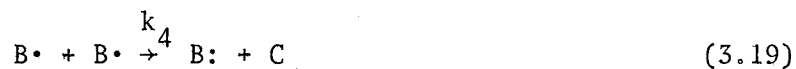
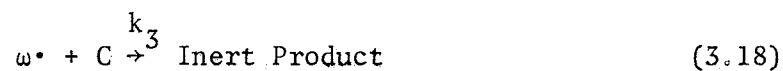
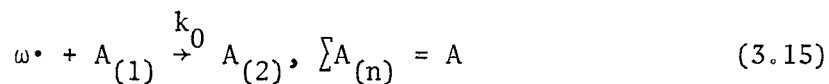
#### Mechanism IV



Similarly, using expressions (3.3), (3.4), and (3.5), the simplest

possible mechanism is Mechanism V.

Mechanism V



Again, no impurity is included in Mechanism V.

It seems to be a reasonable postulate that Mechanisms IV and V are related to some general mechanism containing non-consecutive steps in much the same way that Mechanism II, a specific consecutive mechanism, is related to Mechanism III, the general consecutive mechanism. However, no general proof of this postulate will be given. Restated, the postulate implies that a comparison of the kinetics resulting from Mechanism IV and V to the kinetics resulting from Mechanism III will show the same general patterns as a comparison of the kinetics resulting from a general non-consecutive mechanism which is analogous to Mechanisms IV and V and the general consecutive Mechanism III. Of course, since no impurity is present in Mechanisms IV and V, the analogy should hold when  $C_0$  (initial

impurity concentration) is zero for Mechanisms II and III. Similarly the analogy should hold if an impurity which does not form inactivating secondary radicals, but only undergoes consecutive reactions, is added to the general non-consecutive mechanism.

From Mechanism IV, the following differential equations are obtained.

$$\frac{dA}{dt} = -k_1 A \omega^\bullet - k_5 AB \quad (3.22)$$

$$\frac{dB}{dt} = (k_1 A - k_2 B) \omega^\bullet - k_4 B^2 \quad (3.23)$$

$$\frac{dC}{dt} = -k_3 C \omega^\bullet + 2k_4 B^2 + k_5 AB + k_2 B \omega^\bullet \quad (3.24)$$

The steady state expression for  $\omega^\bullet$  is

$$\omega^\bullet = \frac{G \left( \frac{dD}{dt} \right)}{k_1 A + k_2 B + k_3 C + k_0 A + k_6 B} \quad (3.25)$$

Substituting (3.25) into (3.22), (3.23) and (3.24), respectively, Equations (3.26), (3.27) and (3.28) are obtained.

$$\frac{dA}{dD} = \frac{-k_1 GA}{(k_1 A + k_2 B + k_3 C + k_0 A + k_6 B)} - \frac{k_5 (A)(B)}{\left( \frac{dD}{dt} \right)} \quad (3.26)$$

$$\frac{dB}{dD} = \frac{G(k_1 A - k_2 B)}{(k_1 A + k_2 B + k_3 C + k_0 A + k_6 B)} - \frac{k_4 B^2}{\left( \frac{dD}{dt} \right)} \quad (3.27)$$

$$\frac{dC}{dD} = \frac{(k_2 B - k_3 C)G}{(k_1 A + k_2 B + k_3 C + k_0 A + k_6 B)} + \frac{2k_4 B^2}{\left( \frac{dD}{dt} \right)} + \frac{k_5 AB}{\left( \frac{dD}{dt} \right)} \quad (3.28)$$

Similarly, Mechanism V yields the following equations.

$$\frac{dA}{dD} = \frac{-k_1 AG}{(k_1 A + k_2 B + k_3 C + k_0 A + k_6 B)} - \frac{k_5 AB}{\left( \frac{dD}{dt} \right)} \quad (3.29)$$

$$\frac{dB}{dD} = \frac{(k_1 A - k_2 B)G}{(k_1 A + k_2 B + k_3 C + k_0 A + k_6 B)} - \frac{k_4 B^2}{\left( \frac{dD}{dt} \right)} \quad (3.30)$$

$$\frac{dC}{dD} = \frac{(k_2B - k_3C)G}{(k_1A + k_2B + k_3C + k_0A + k_6B)} + \frac{k_4B^2}{\left(\frac{dD}{dt}\right)} + \frac{k_5AB}{\left(\frac{dD}{dt}\right)} \quad (3.31)$$

Solutions to Mechanisms IV and V,

$D_{1/3}$  Versus  $A_0$  Plots

Equations (3.26-3.28) and (3.29-3.31), corresponding to Mechanisms IV and V, respectively, were solved numerically using a computerized application of the Runge Kutta numerical method for simultaneous first order differential equations. An IBM 360 computer was used. The results that follow are examples of the patterns which were observed. All parameters not specified by the figures designated in the discussion are to be found in Table I.

Figure 9 illustrates the deviations from linearity of the  $D_{1/3}$  versus  $A_0$  plot for some selected variations in rate constants  $k_4$  (corresponding to the  $B \cdot + B \cdot$  reaction) and  $k_5$  (corresponding to the  $A + B \cdot$  reaction). Both Mechanisms (IV and V) are represented in Figure 9. The results illustrate that linearity increases as the  $k_4/k_5$  ratio increases. The limiting initial slope of all the curves is the slope of the  $D_{1/3}$  versus  $A_0$  plot when  $k_4$  and  $k_5$  are both zero (curve 1), corresponding to a consecutive mechanism. Mechanism IV tends to show greater deviation from linearity than Mechanism V when identical parameters are used (curve 3; curve 6; curve 4, curve 7; curve 5, curve 8). Mechanisms IV and V differ only in the fact that in one case the  $B \cdot + B \cdot$  reaction yields two molecules in one case (Mechanism V) and a diradical and a molecule in the other (Mechanism IV). Therefore, if a stoichiometric relationship that represents both the preceding possibilities is used, deviation from linearity less than Mechanism IV and greater than Mechanism V would be

TABLE I  
PARAMETERS FOR NUMERICAL INTEGRATION OF MECHANISMS IV AND V

Parameter	Value
$k_1$	$1.0 \times 10^9$ (liters/mole-second)
$k_2$	$1.5 \times 10^9$ (liters/mole-second)
$k_3$	$1.8 \times 10^9$ (liters/mole-second)
$k_4$	Varied (see Figures 9 and 10)
$k_5$	Varied (see Figures 9 and 10)
$k_6$	$1.0 \times 10^9$ (liters/mole-second)
$k_7$	$1.0 \times 10^9$ (liters/mole-second)
G	$3.0 \times 10^{-8}$ mole radicals/rad
$A_0$	$.5 \times 10^{-5}$ - $5.0 \times 10^{-4}$ in increments of $.5 \times 10^{-3}$
$C_0$	0.0
$B_0$	0.0



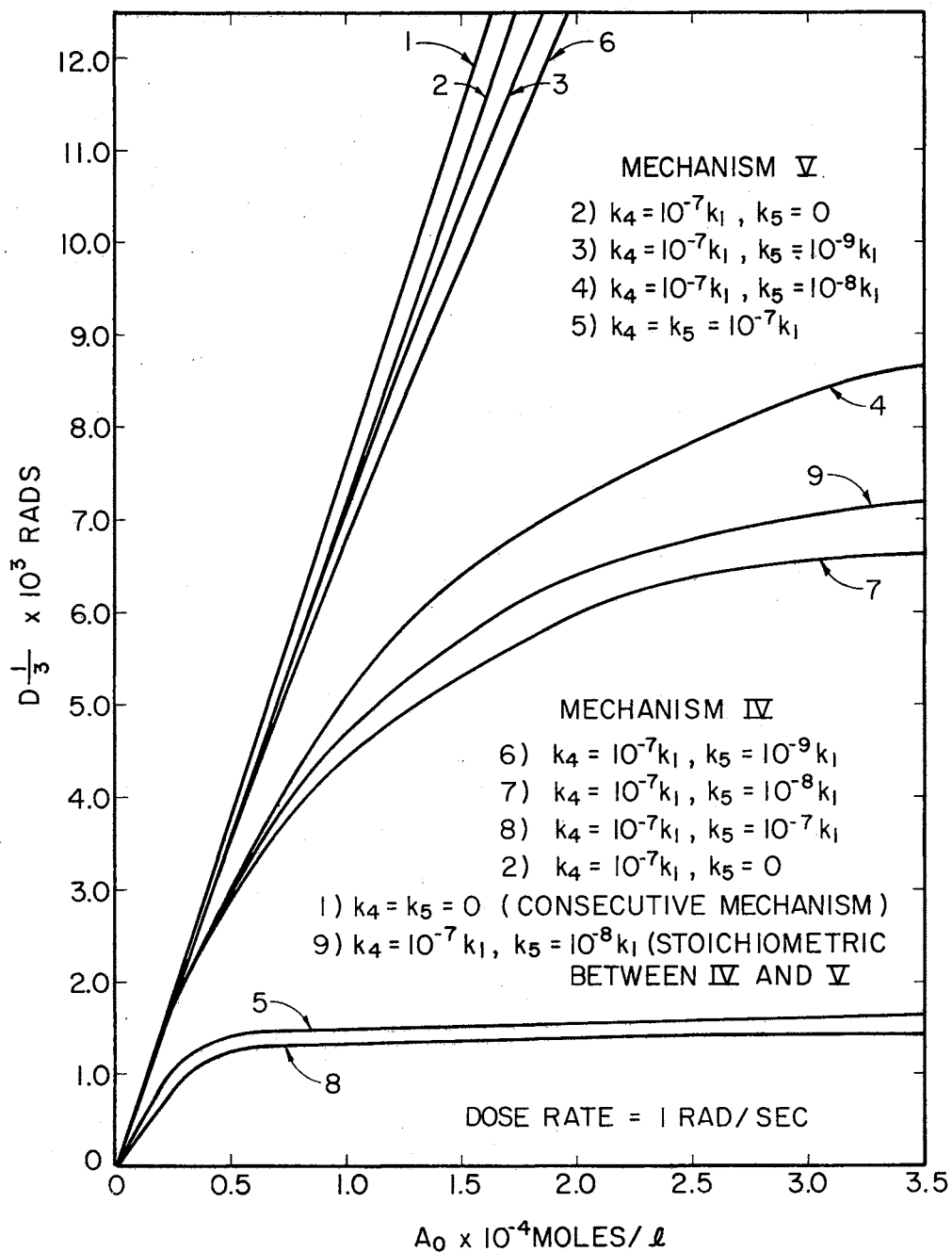
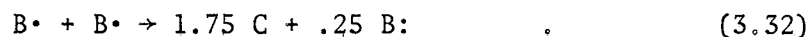


Figure 9. Results of Numerical Integration of Mechanisms IV and V

expected. This is observed in curve 9, where the following stoichiometry was assumed:

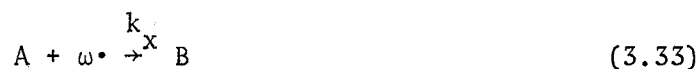


Generally, it seems that the deviations from linearity in Figure 9 increase as the proportion of solute molecules inactivated by secondary solute radicals increase.

The highest  $k_4/k_5$  ratio, when  $k_5 \neq 0$ ; that illustrated in Figure 9 is 100, curves 6 and 3. Generally, the  $B\cdot$  radical could not be considered in a steady state in the individual integrations that resulted in the lines of Figure 9, as it normally rose to from  $\sim 5$  to  $\sim 15$  percent of the concentration of  $A_0$  at some time during the integrations.

Figure 10 illustrates a  $k_4/k_5$  ratio of 1000 for Mechanism V. Generally, the  $B\cdot$  radical could be considered in the steady state, as its concentration never reached a level greater than  $10^{-3}$  times that of  $A_0$  in the individual integrations. (The author does not mean to imply that the  $B\cdot$  radical is in the steady state because of the higher  $k_4/k_5$  ratio; the steady state of  $B\cdot$  is undoubtedly a result of the fact that either  $k_4$  or  $k_5$ , or both, are larger in Figure 10 than they are in Figure 9.)

All of the curves represented in Figure 10, with the possible exception of curve 6, are good approximation of a straight line. It is apparent, however, that curves 5 and 6 represent cases in which there is considerable inactivation of solute molecules by secondary solute radicals. A possible explanation of linearity of the lines in Figure 10 is the steady state behavior of the  $B\cdot$  radical. That is, the following processes,



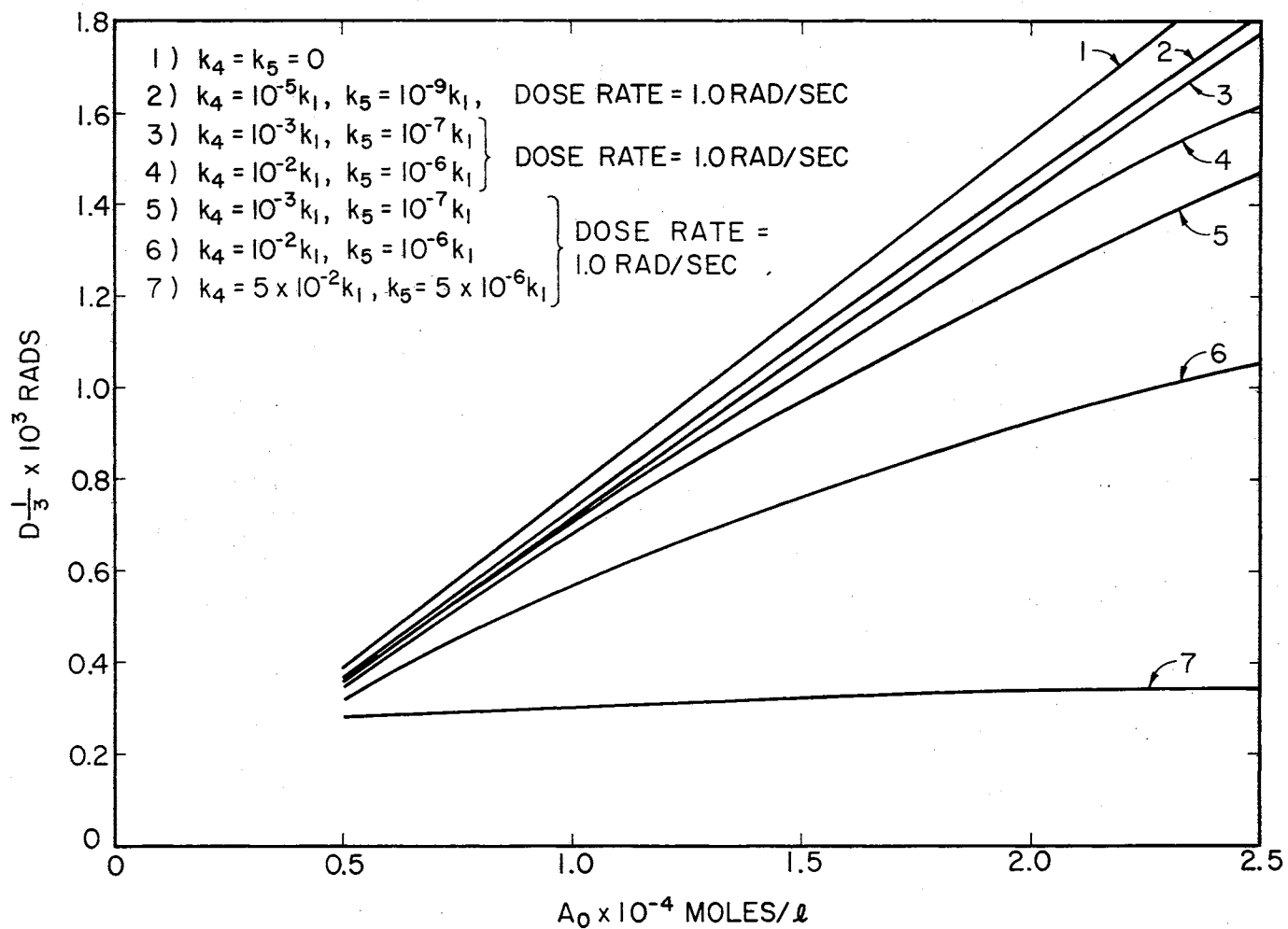


Figure 10. Results of Numerical Integration of Mechanism V,  $k_4/k_5 = 10^4$



and



might be represented by



and



if  $B\cdot$  is in the steady state.

When the dose rate is increased, the slope of the linear, or semi-linear,  $A_0$  versus  $D_x$  plots increases (curves 6 and 4; curves 5 and 3, Figure 10). This is to be expected from Equations (3.26) and (3.29). It is obvious from Equations (3.27) and (3.30) that an increase in dose rate decreases the number of  $B\cdot + B\cdot$  and  $A + B\cdot$  reactions. Therefore, an increase in dose rate should increase the slope of a linear or semilinear  $A_0$  versus  $D_x$  plot according to the proportion of solute molecules which are inactivated by secondary solute radicals. If no increase in slope is observed, the conclusion would be that very few solute molecules are inactivated by secondary solute radicals. However, curve 2 of Figure 9 and curve 2 of Figure 10 indicate that the  $B\cdot + B\cdot$  reaction might take place to a very appreciable extent without significant deviation from the behavior expected if only consecutive reactions occurred.

It seems probable that under certain conditions a semilinear or

linear  $D_x$  versus  $A_0$  plot might show some curvature when the dose rate is increased. However, the author did not observe such behavior with the mathematical models.

It has been shown that a plot of  $1/G$  for the solute versus the reciprocal of the original concentration should also give a straight line, if consecutive mechanisms occur. Augenstine's plots for chymotrypsin and ribonuclease (Figures 7 and 8) are not straight lines. When a constant term for added impurity undergoing consecutive mechanisms was added to the  $D_{1/3}$  resulting from the numerical solution from Mechanism V, Figure 11 results. (All parameters except  $k_4$  and  $k_5$  are given in Table I.) In Figure 11,  $D_{1/3}(1/3 A_0)$ , which is proportional to  $1/G_A$ , was plotted against  $1/A_0$ . Figure 11 is quite similar to Figures 7 and 8 in form, suggesting a parallel mechanism. However, this type of curvature was not always observed for the  $1/G$  solute versus  $1/A_0$  plot when the  $D_x$  versus  $A_0$  showed deviations from linearity.

#### Dose Rate Effects

Since dose rate changes can change the slope of the  $D_{1/3}$  versus  $A_0$  plots of Figure 10, an example of dose rate effects for one  $A_0$  seems to be in order. Figure 12 illustrates some dose rate effects for Mechanism V. The  $k_4$  and  $k_5$  rate constants are given on the graph and the dose rate was varied from 1.0 rad/sec to 3.75 rad/sec.  $A_0 = 5.0 \times 10^{-5}$  moles/l. The remainder of the parameters are given in Table I.

Generally, the dose rate effect is more pronounced as the number of secondary radicals inactivating the solute increases (see Figure 12). The limiting value of  $D_{1/3}$  is approximately  $3.8 \times 10^4$  rads, which is the same value at  $A_0 = 5.0 \times 10^{-5}$  when  $k_4$  and  $k_5$  are zero in Figure 9. It is

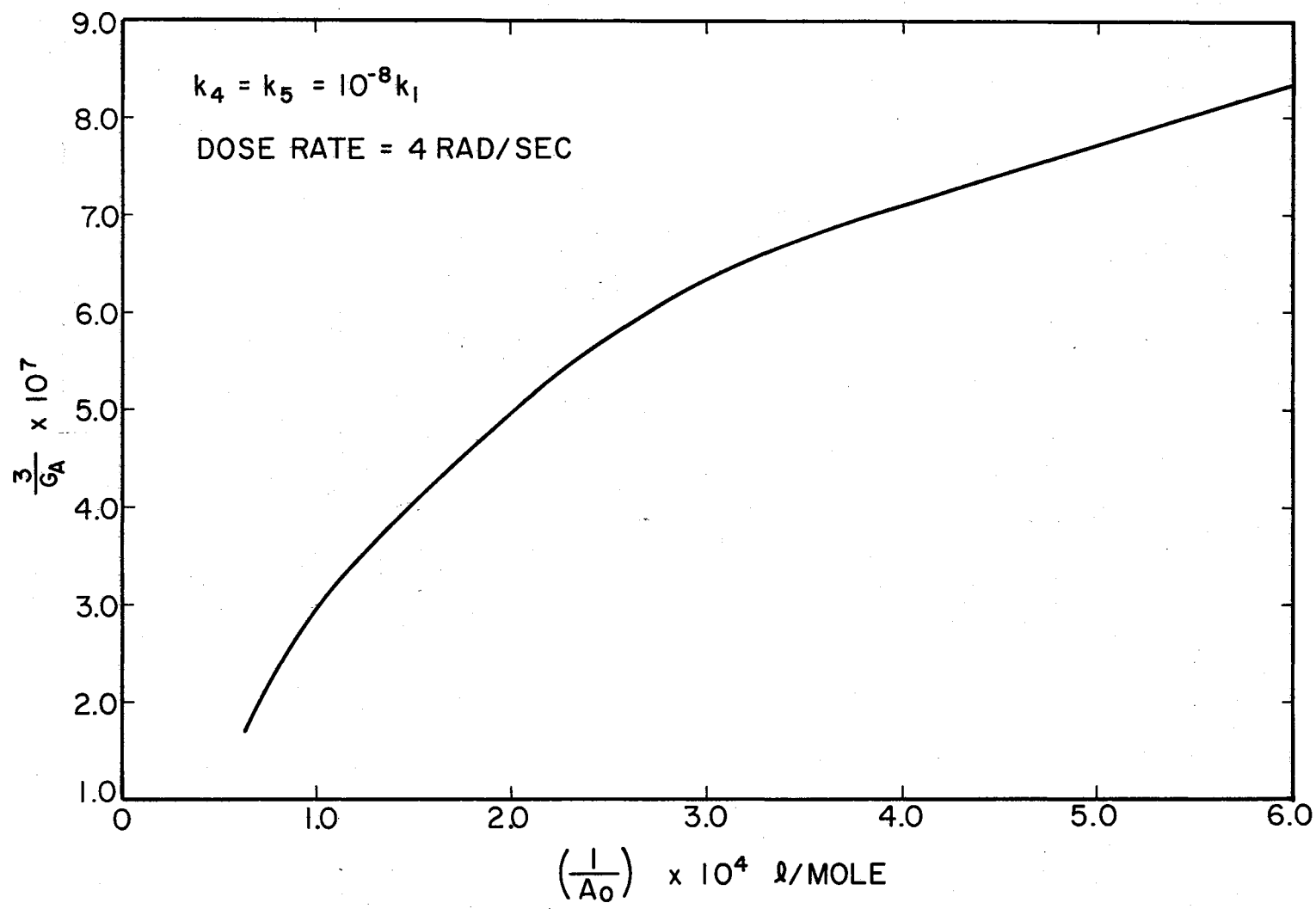


Figure 11. Simulated Non-Linear  $1/G_A$  Versus  $1/A_0$

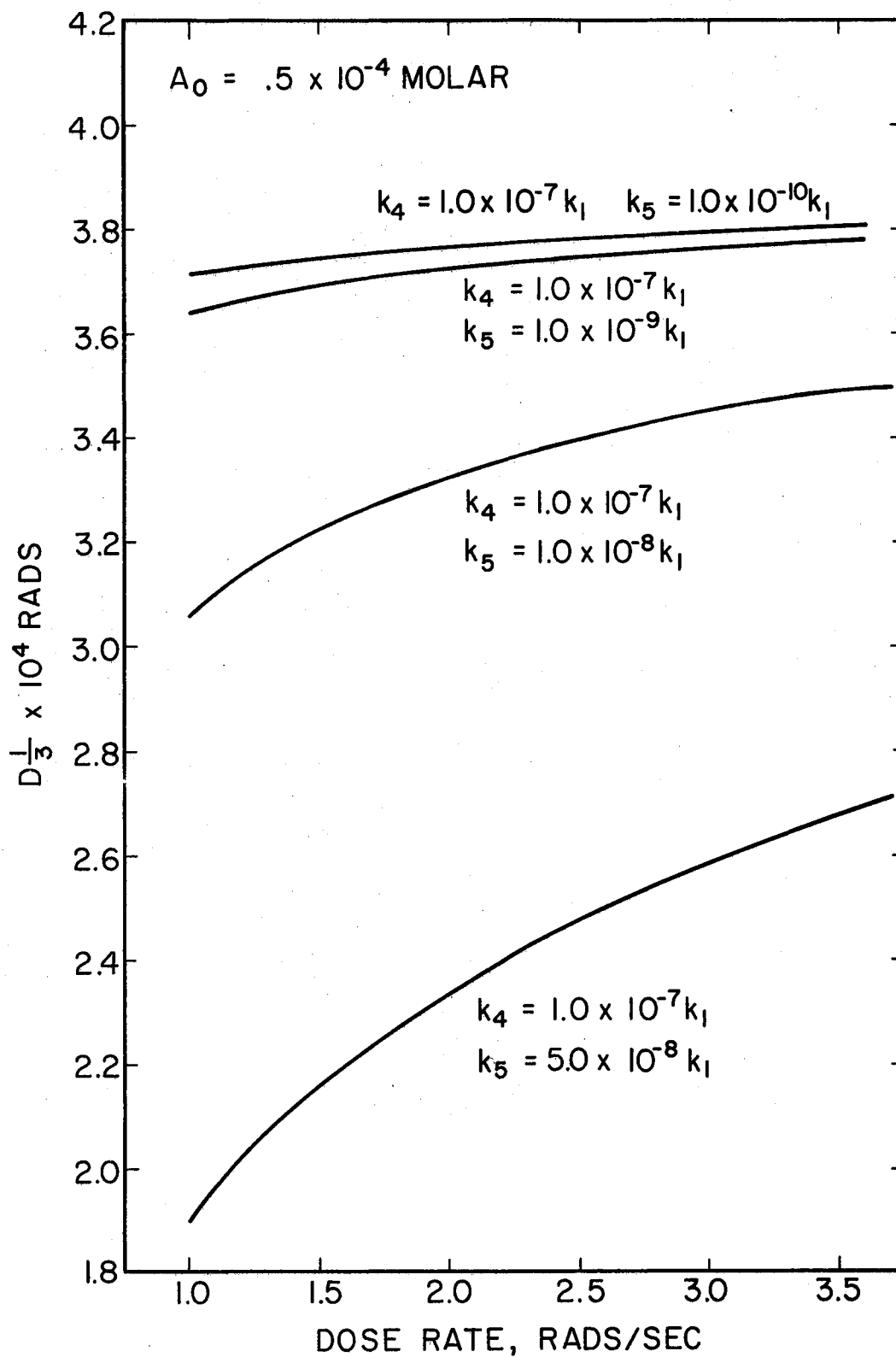


Figure 12. Dose Rate Effects of Mechanism V

obvious from Figure 11 that a dose rate effect would be observed when  $k_4 = 1.0 \times 10^{-7} k_1$  and  $k_5 = 10^{-10} k_1$  if the dose rate were decreased below 1.0 rad/sec. Similarly, the dose rate effect would disappear when  $k_4 = 10^{-7} k_1$  and  $k_5 = 5.0 \times 10^{-8}$  if the dose rate were increased several fold. Similar results were obtained for Mechanism IV, which would be expected from Equations (3.26-3.31).

Figure 13 illustrates the dose rate effect at  $A_0 = 5 \times 10^{-5}$  (moles/l) corresponding to lines 6 and 7 of Figure 10. It is not readily apparent why the dose rate effects are so small, even though a substantial number of solute molecules are inactivated by secondary solute radicals.



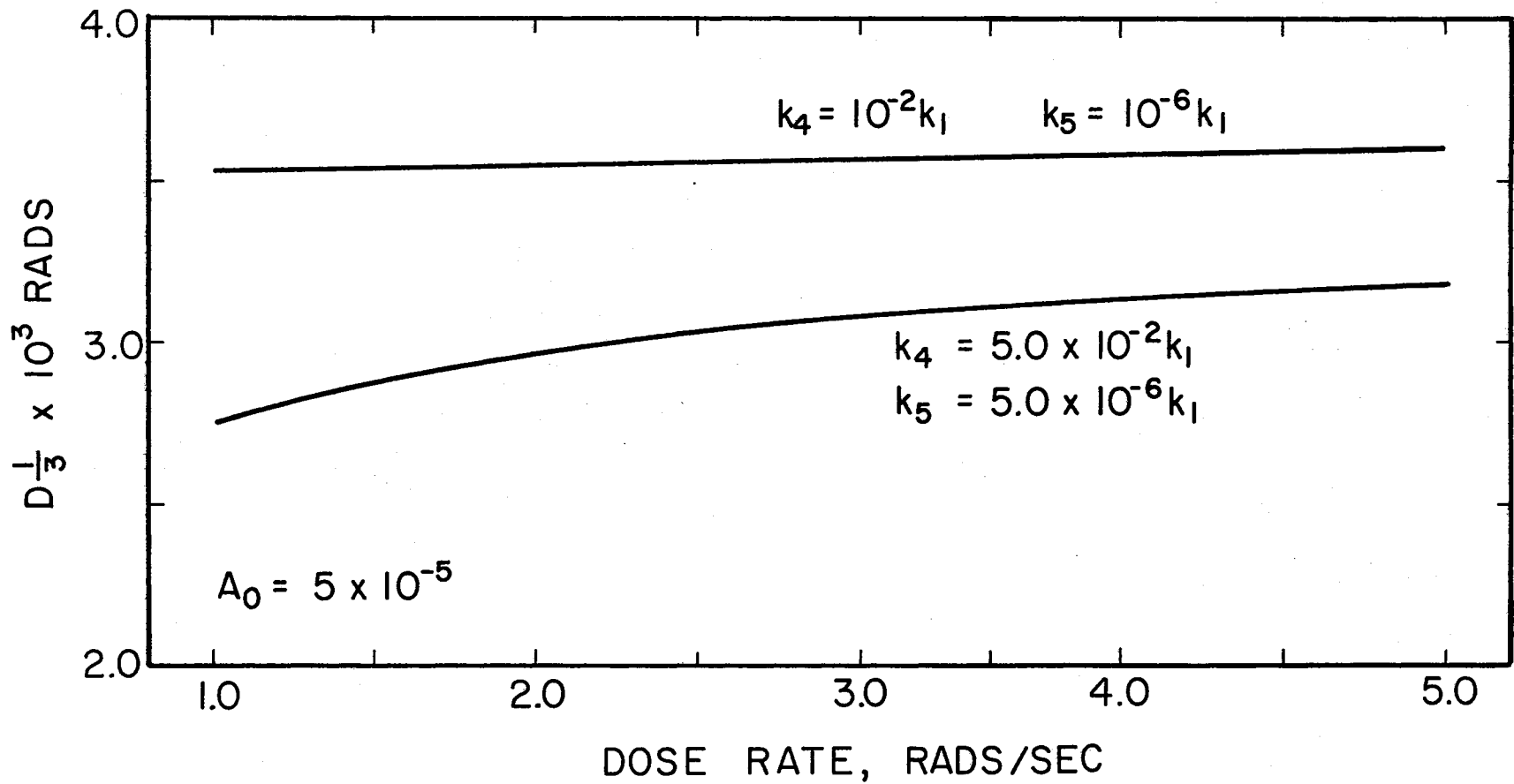


Figure 13. Dose Rate Effects for Semi-Linear  $D_x$  Versus  $A_0$

## CHAPTER IV

### CONCLUSIONS AND RECOMMENDATIONS

If consecutive reactions occur a plot of  $D_x$  versus  $A_0$  yields a straight line which has an intercept proportional to the amount of solute independent impurity and a slope which is a measure of the radical scavenging capacity of the solute and all subsequent species formed from the solute.

Furthermore, the rate of change of any species in the consecutive mechanism with respect to dose is independent of dose rate. Therefore no dose rate effect should be observed for consecutive mechanisms.

Finally, if consecutive mechanisms occur and Mechanism I is ruled out, a temperature dependence of the slope of the  $D_x$  versus  $A_0$  plot indicates a temperature dependence of the rate constants for the reactions of the solute and subsequent intermediates from the solute and primary radicals.

When solved numerically, the parallel mechanisms, Mechanisms IV and V, give various deviations from linearity with the  $D_x$  versus  $A_0$  plots, depending on the rate constants of the parallel steps. It appears that the deviation from linearity increases as secondary radicals contribute more to the inactivation of the original solute (see Figure 9). However, if the secondary radicals are in a steady state, a  $D_x$  versus  $A_0$  plot will sometimes approximate linear behavior, even though a substantial portion of the original solute is inactivated by secondary solute radicals (see

Figure 10). In that case an increase of the dose rate increases the slope of the  $D_x$  versus  $A_0$  plot (see Figure 11). Also, when the parallel mechanisms are solved numerically for one  $A_0$ , a dose rate effect of the type demonstrated in Figures 12 and 13 is observed. That is, the dose required to inactivate the solute to 1/3 of the original solute concentration increases as the dose rate increases. The dose rate effect also seems to increase as a greater proportion of the solute molecules are inactivated by secondary solute radicals. This is to be expected from Equations (3.26) and (3.29).

It appears that the  $B \cdot + B \cdot$  reaction may take place to a substantial extent without significantly altering the kinetics from those expected from consecutive reactions.

The preceding discussion allows the following statements to be made.

(1) If a  $D_x$  versus  $A_0$  plot is not linear, the mechanism is not consecutive.

(2) A dose rate effect will not be observed for consecutive mechanisms.

(3) A non-linear  $A_0$  versus  $D_x$  plot suggests inactivation by secondary solute radicals.

(4) A dose rate effect of the type described above suggests inactivation by secondary solute radicals.

(5) A linear  $A_0$  versus  $D_x$  plot that undergoes a change in slope with a change in dose rate suggests inactivation by secondary solute radicals.

The author does not wish to imply that an experimental  $D_x$  versus  $A_0$  plot giving similar curvature to one of the lines given in Figures 9 or 10 necessitates rate constants of the magnitudes used in the numerical

integration. The actual mechanisms are probably more complicated than either Mechanisms IV or V. However it is the author's assertion that a general parallel mechanism will show the same patterns as Mechanisms IV and V.

Experimental evidence of Huthinson and Ross (10) seems to support either consecutive reactions or predominately consecutive reactions with some secondary radical-secondary radical reactions (Figures 2-5). An electron spin resonance spectra of the solutions taken while (or shortly after) the solutions are being radiolyzed might indicate the relative abundance of radicals during the radiolysis. A rather constant signal would suggest little radical build-up and support the inclusion of the  $B\cdot + B\cdot$  reaction. A signal that jumps several orders of magnitude would suggest strictly consecutive reactions.

Augenstine (9) provides two plots which suggest inactivation of solute by secondary solute radicals (Figures 7 and 8).

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VITA<sup>8</sup>

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