PUERTO RICO NUCLEAR CENTER

PHOTOCHEMISTRY AND RADIATION CHEMISTRY PROJECT

Nature of Excited State of Cytosine: Evidence from
luminescence quenching and photochemical kinetics

NATURE OF THE EXCITED STATE OF CYTOSINE: EVIDENCE FROM
PHOTOCHEMICAL KINETICS AND LUMINESCENCE QUENCHING

Malcolm Daniels
Puerto Rico Nuclear Center

Alec Grimison
Chemistry Dept., University of Puerto Rico

December, 1964

NATURE OF THE EXCITED STATE OF CYTOSINE: EVIDENCE FROM PHOTOCHEMICAL KINETICS AND LUMINESCENCE QUENCHING*

We have previously reported[1] the photodeamination by 2537 A$^\circ$ radiation of cytosine in aqueous solution and have drawn attention to its possible significance in photobiology.

Investigation of kinetics of this deamination has led to the following results which, taken in conjunction with the previously described[2] luminescence behavior, allow clear conclusions to be drawn concerning the nature and behavior of the excited state of cytosine in aqueous solution.

The deamination is linear with absorbed intensity uo to considerable percentage change, (Fig. 1) and hence represents a major, primary reaction. As can be seen from Fig. 1, the reaction is strongly dependent on concentration of cytosine, and quantum yields over a twentyfold range of concentration are shown in Fig. 2. Graphical analysis shows the reciprocal of the quantum yield to be a linear function of the reciprocal of the cytosine concentration (Fig. 3) and gives the result

$$\frac{1}{\phi^{(NH_3)}} = \frac{1}{\phi_o} + \frac{1}{\phi} k_c \cdot \frac{1}{(C)} \qquad k_c = 6.2 \times 10^{-4} \text{ M} \qquad (1)$$

This relationship is very important, because the form of it indicates a simple competition between deactivation of the chemically active excited state and reaction of it with a further molecule of cytosine. Furthermore, if it is supposed that deactivation is accompanied by emission of luminescence, then we can quantitatively account for both the luminescence and photochemical phenomena by the following mechanism.

We propose that an excited state of cytosine

$$C \xrightarrow{h\nu} C^*$$
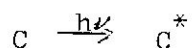
which can luminesce in reverting to ground state by a first order process,

$$C^* \xrightarrow{k_1} C + h\nu$$
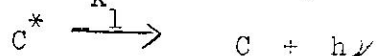
can react with another cytosine molecule to give a transient excited dimer

$$C + C \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C_2^*$$
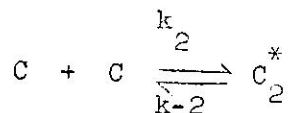
and that this can be deactivated by further collision with cytosine to give stable products, among them ammonia

$$C_2^* + C \xrightarrow{k_3} Products\ (NH_3)$$
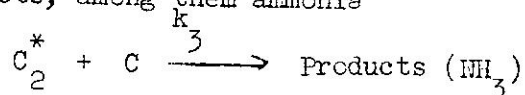
Treament of this mechanism by stationary state kinetics, assuming $k_3 \gg k_2$, gives the following expression for the relative luminescence intensity

$$\frac{1}{F} = \frac{1}{K} + \frac{1}{K} \frac{k_2}{k_1} \cdot \frac{k_3}{k_{-2}} (C)^2$$

(F = relative luminescence intensity, K = constant).
and for the quantum yield of ammonia.

$$\frac{1}{\phi(NH_3)} = \frac{1}{\phi_0} + \frac{1}{\phi_0} \frac{k_1}{k_2} \cdot \frac{1}{(C)}$$

These equations are to be compared with experimental equation (1) of the preceeding Communication and equation (1) of this one. The internal consistency of the mechanism, and hence the relationship between results obtained by two entirely different experimental techniques, is shown by the evaluation of the ratio $\frac{k_3}{k_{-2}}$ from the experimental slopes k_a and k_c. We find $\frac{k_3}{k_{-2}} = 3.5 \times 10^3$, in agreement with the assumption of the mechanism.

Hence we conclude that the excited state which luminesces is the same one which on quenching leads to the deamination reaction.

Further consideration of the experimental constants leads to the conclusion that the excited state C^* is long lived. Thus from the deamination kinetics $k_1/k_2 = 6.2 \times 10^{-4}$. The maximum possible value which k_2 can have is the diffusion controlled rate of 10^9 l m$^-$ sec^{-1} (Calculated by method of G. V. Schultz[3], using a diffusion coefficient of 10^{-5} cm^2 sec^{-1}). This gives an upper limit of 6.2×10^5 sec^{-1} for k_1 .

An alternative and complementary approach is to discuss the quenching constant in terms of $k_2 \Upsilon = 1.7 \times 10^4$ where Υ is the intrinsic lifetime of the emission. If emission is from a singlet state, for which Υ is commonly 10^{-8} secs, then $k_2 = 1.7 \times 10^{12}$, an impossibly high value. On the other hand, if emission is from a triplet level with $\Upsilon = 10^{-4}$ sec, then $k_2 = 1.7 \times 10^8$, somewhat less than the diffusion-controlled rate. Accordingly we conclude that the excited state is probably a triplet level.

To summarize, we find that irradiation of cytosine in aqueous solution leads to a relatively long-lived excited state, probably triplet, emitting weak luminescence at 380 mμ, and undergoing self-quenching reactions leading eventually to deamination.
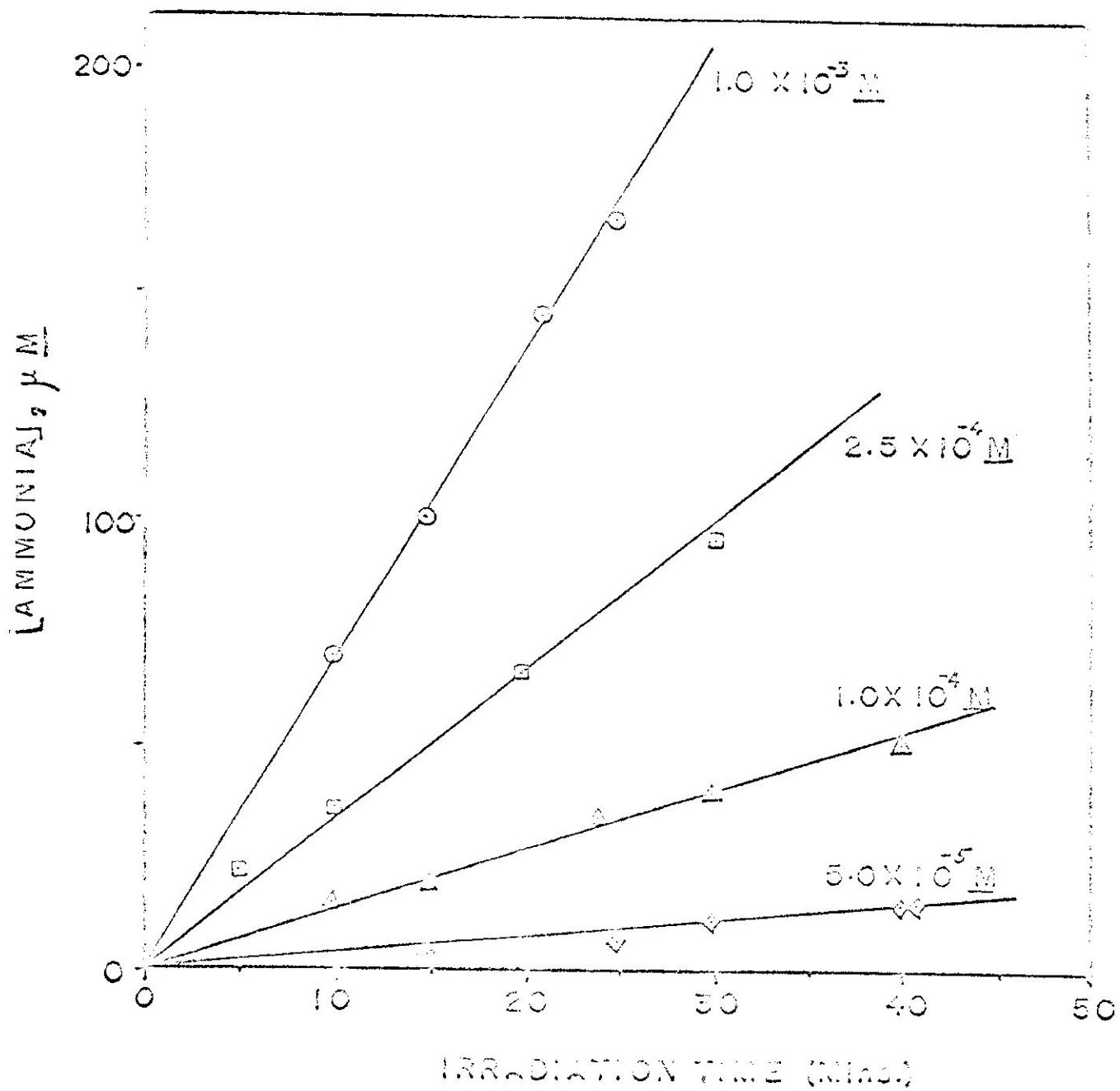
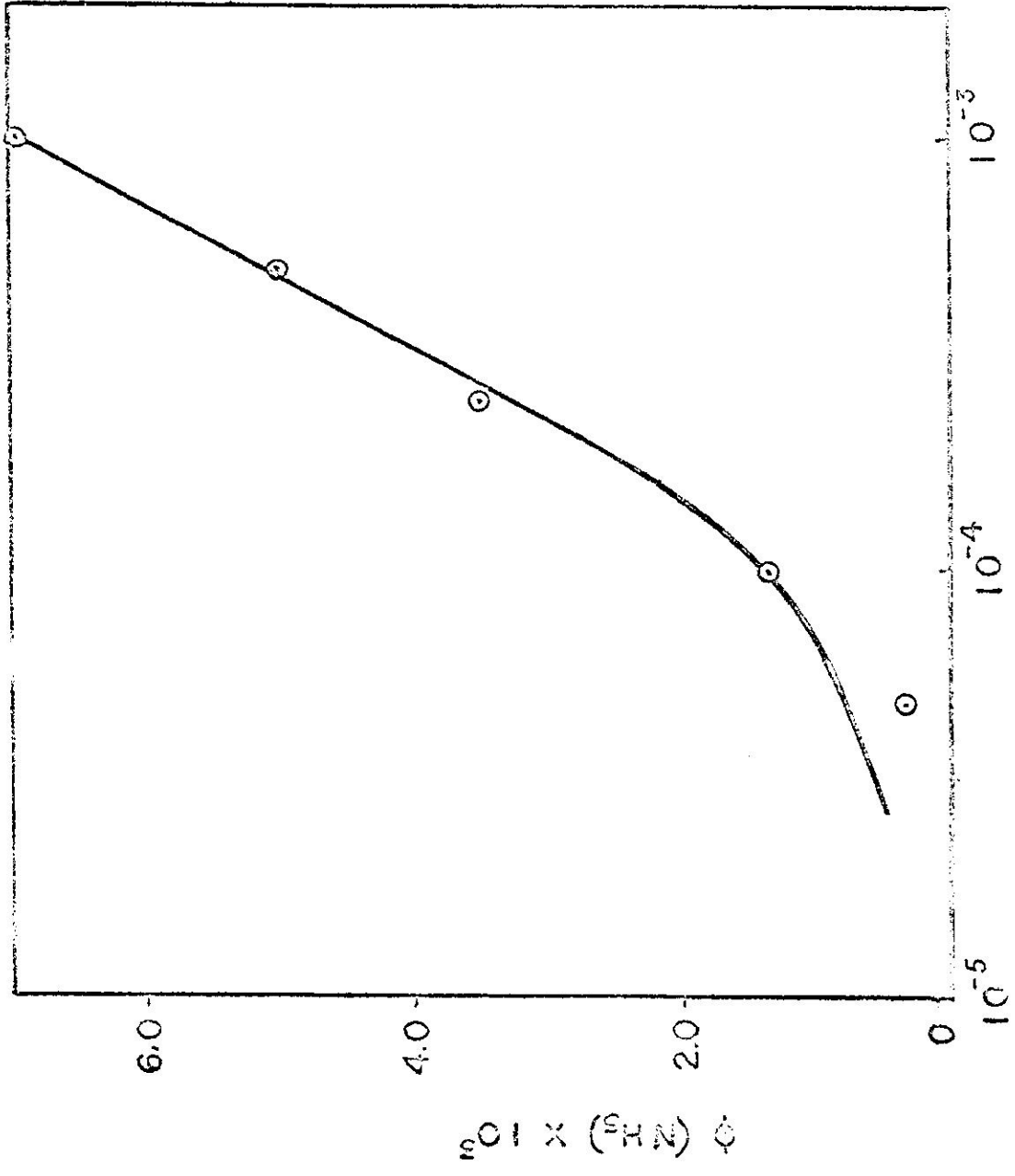A complete presentation of this work is in preparation.

Puerto Rico Nuclear Center   Malcolm Daniels
Department of Chemistry
University of Puerto Rico
Río Piedras, Puerto Rico
                                            Alec Grimison

(1) M. Daniels and A. Grimison, Biochem. Biophys. Res. Commun.
     16, 428 (1964)

(2) P. R. N. C. 42 (1964)

(3) G. V. Schultz, Z. Phys. Chem. (Frankfurt) 8, 284 (1956)

ϕ (NH$_3$) X 10^3

[CYTOSINE], M