PRNC 62

PUERTO RICO NUCLEAR CENTER

Progress Report

RADIATION CHEMISTRY AND PHOTOCHEMISTRY OF AQUEOUS SOLUTIONS OF OXYANIONS

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PUERTO RICO NUCLEAR CENTER

Progress Report of the Project

RADIATION CHEMISTRY AND PHOTOCHEMISTRY OF AQUEOUS SOLUTIONS OF OXYANIONS

by

Malcolm Daniels

April, 1965

PUERTO RICO NUCLEAR CENTER
RADIOISOTOPE APPLICATIONS DIVISION

RADIATION CHEMISTRY AND PHOTOCHEMISTRY OF AQUEOUS SOLUTIONS OF OXYANIONS

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The work supported by A.E.C. Division of Biology and Medicine falls into three sections.

A) Photolysis of Nitrate Ion - previously reported work has been considerably extended and quantitatively analysed.

B) Radiolysis of Nitrate solutions. This work has been commenced this year.

C) Luminescence studies on D.N.A. constituents. Though not included in the proposal, this work has been carried out on equipment purchased from A.E.C. funds. Preliminary reports are appended. This work was presented at Fourth International Congress of Photobiology, and the Ninth Annual Meeting of Biophysical Society.

A. Nitrate Photolysis

The previously reported non-linear rates of photolysis found in the range pH 2-6 have now been analyzed quantitatively. Careful experiments at long irradiation times have shown that the rate of nitrite formation eventually decreases to a steady value. This is shown in fig. 1 where a limiting rate R_f = 0.60 μM/min. is indicated: Using this fact it is then found that there is a linear relationship between the reciprocal of the rate of photolysis and the duration of photolysis (fig. 2). Extrapolation to zero time then allows determination of the true initial rate.

In this way all previous non-linear data obtained in studying the effect of a) nitrate concentration; b) nitrite; c) arsenite scavenging can be decomposed into two linear terms; 1) an initial rate (usually high) which depends on the concentration of nitrate, or scavengers; 2) a 'final' low rate which is independent of these factors. This analysis of data suggests a simple model on which all subsequent discussion is based. It is envisaged that the initial rate is due to the escape of reactive species from the 'solvent cage' followed by their random diffusion and reaction with homogeneously distributed scavengers and inhibitors. The non-scavengeable final rate is interpreted as the probability of decomposition or reaction within the 'solvent cage'.

Consequently the overall course of photolysis in neutral and acid solutions is represented by:

a) $\phi(NO_2^-) = \phi(t) + \phi(f)$,

b) $\frac{1}{\phi(t)} = \frac{1}{\phi(o)} + k_{exp.}It.$

where $\phi(t)$ = time - dependent quantum yield
and $\phi(f)$ = time - independent quantum yield

which also may be written in the form:

$$\phi(t) = \frac{\phi(o)}{1 + k_2 t}$$

Effect of Nitrate Concentration

When this analysis is carried out on the previously reported results for the effect of nitrate concentration on rate of photolysis, it is found that the initial rate is only slightly dependent on nitrate concentration (fig. 3) and that the major effect is on the magnitude of the constant describing the non-linearity (fig. 4). Clearly, nitrate has a strong inhibiting effect on the secondary reactions during photolysis.

Although the experimental results may be written:

$$\phi(o) = \phi_1(o) + k_3 [NO_3^-]$$

mechanistic considerations suggest that the relationship may have a different form which the magnitude of the variation is inadequate to demonstrate.

The effect on secondary reactions may be described by the equation:

$$\frac{1}{k_{exp.}I} = k_4 \left[NO_3^-\right]$$

Effect of Nitrite Concentration: Inhibition

The slope of the concentration-time curves suggested that nitrite, a product of photolysis, was acting as an inhibitor. To test this the previously reported photolyses of nitrate with nitrite added prior to irradiation have been extended and quantitatively analyzed. Some experiments results are shown in fig. 5

and the overall picture is summarised in fig. 6. It can be seen that the initial rate is rapidly decreased by small concentrations of nitrate without appreciably changing the final steady rate (or the rate constant), and follows the rate law

$$\frac{1}{\phi} = \frac{1}{\phi(o)} + k_5 \left[NO_2^-\right]$$

Accordingly we describe the effect of nitrite as an inhibition of the overall process of photolysis and ascribe the non-linearity of the time curves to the accumulation of nitrite.

Addition of Arsenite

Following on previously reported work, it has been found that the secondary photolysis rate is unchanged by addition of up to 300 μM As. III and accurate values of the initial rate have been determined. The variation of $\phi(NO_2^-)$ with arsenite concentration has a form typical of competition

kinetics, fig. 7,

namely; $$\frac{1}{\Phi - 0.045} = \frac{1}{0.165} + \frac{1.35 \times 10^{-4}}{[\text{As III}]}$$

Addition of Hydrogen Peroxide

Arsenite being known to be a good scavenger for OH radical, it has been decided to try other known scavengers for OH. Of these, hydrogen peroxide is of interest in that its behavior might give some light on the non-formation of H_2O_2 in nitrate photolysis.

Addition of H_2O_2 leads to an apparent increase in the rate of photolysis, but two feature are note worthy.

First, when the non-linear concentration curves are analysed it is found that the initial rate is only slightly higher than in the absence of H_2O_2, and the major effect is on the rate constant which decreases by a factor of 10. Second, in constrast to arsenite, for which scavenging is complete at 1 mM, H_2O_2 begins to have an effect at this concentration and is really only effective at concentrations 10-20 mM.

It is inferred that H_2O_2 does not effectively compete in the primary radical reactions, and that its main effect is competing with NO_2^- in the secondary reactions.

Effect of Ethanol

The addition of ethanol leads to an increase in initial rate at low concentrations. Acetaldehyde is formed at the same rate as nitrite together with very low yields of H_2O_2.

Initial rates as a function of ethanol concentration are shown in fig. 8. (the curve is calculated from the relation:

$$\frac{1}{\phi - 0.061} = \frac{1}{0.095} + \frac{3.7 \times 10^{-4}}{[EtOH]}$$

It is inferred that ethanol, in contrast to H_2O_2, is about as effective a scavenger as arsenite and OH^-. However there seem to be some quantitative differences reflected in the limiting quantum yields.

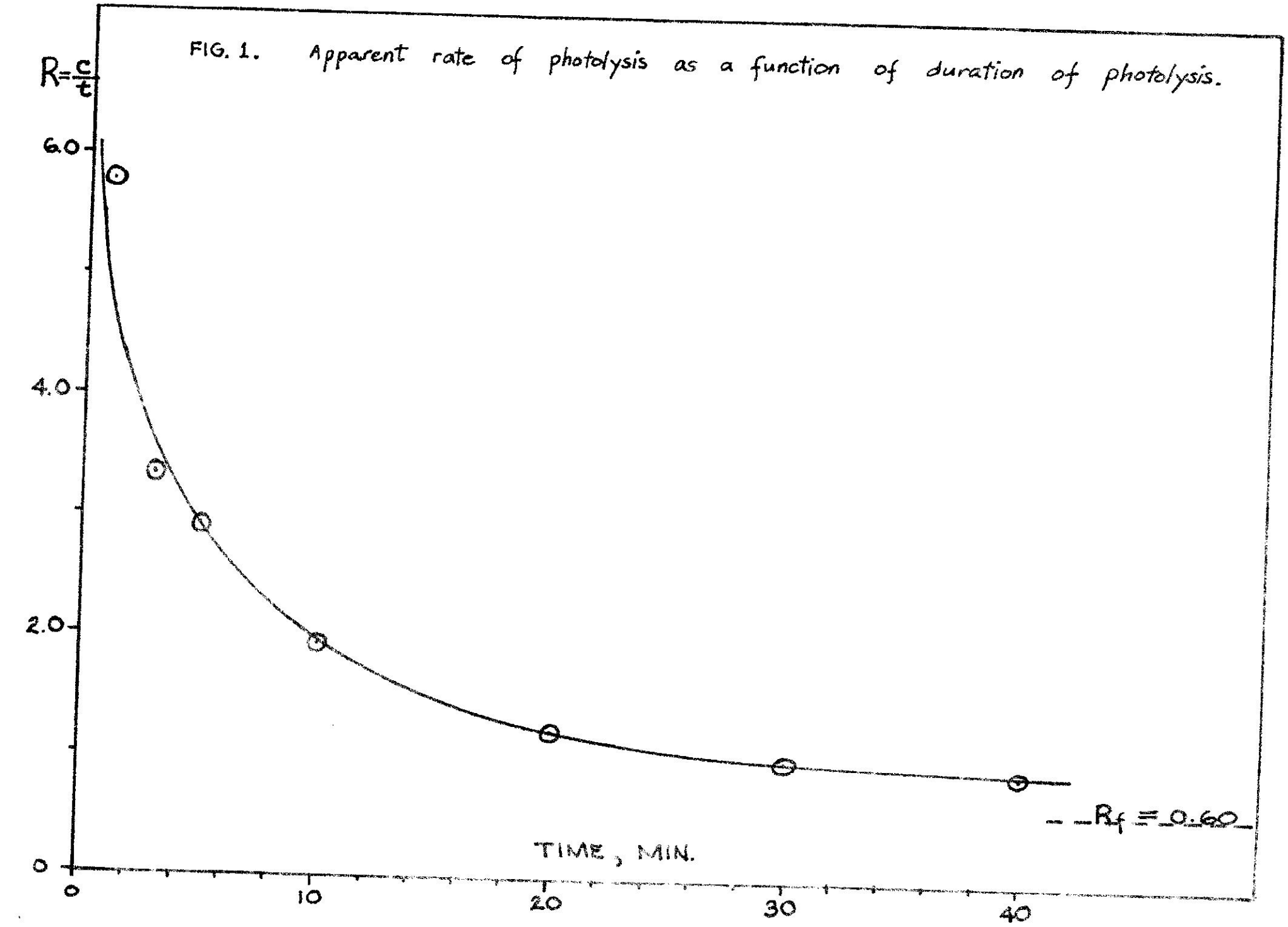


FIG. 1. Apparent rate of photolysis as a function of duration of photolysis.

FIG. 2. Reciprocal of rate of photolysis as a function of time.

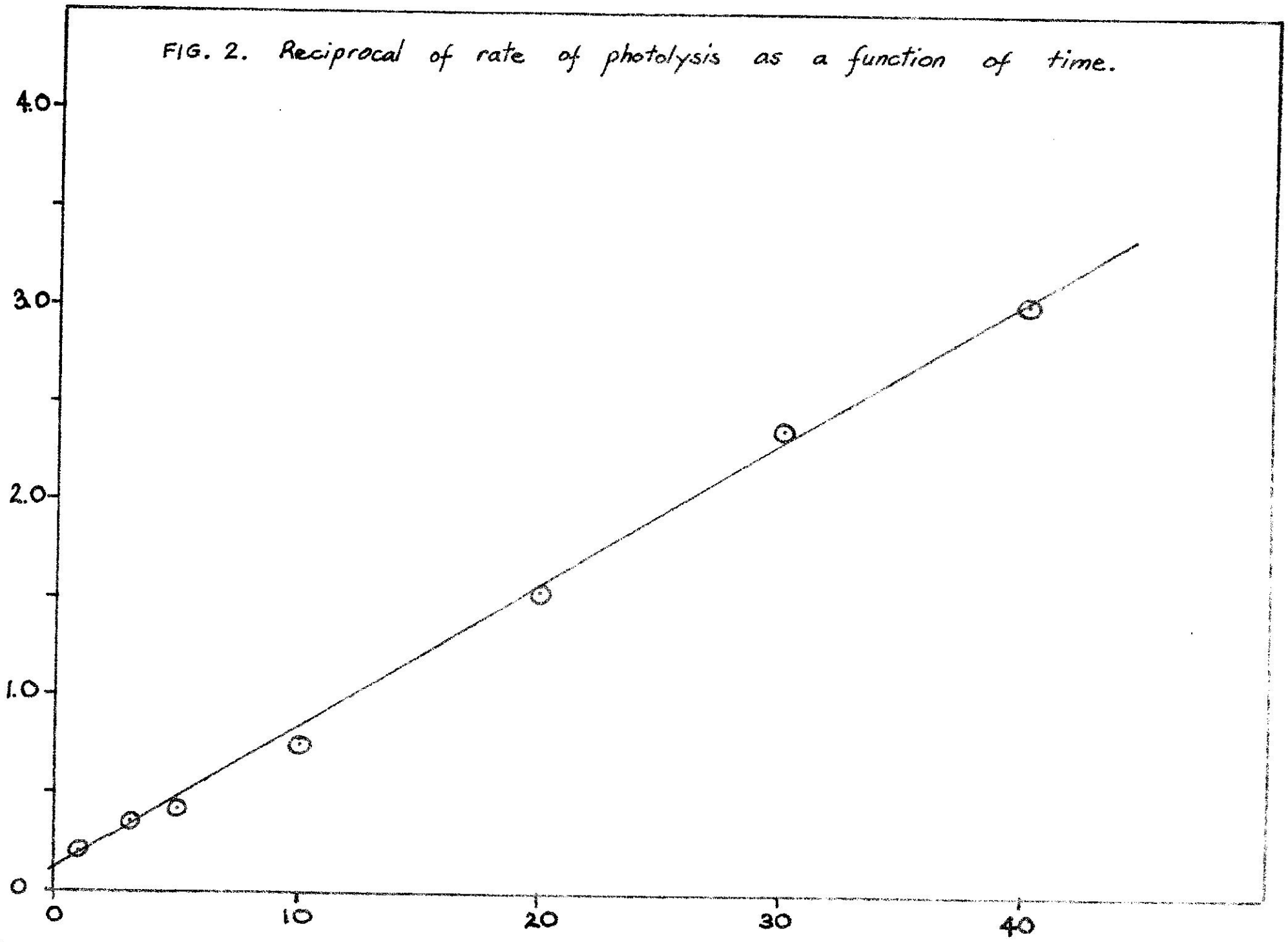


FIG. 3. Effect of nitrate concentration on initial rate of photolysis at pH 6.

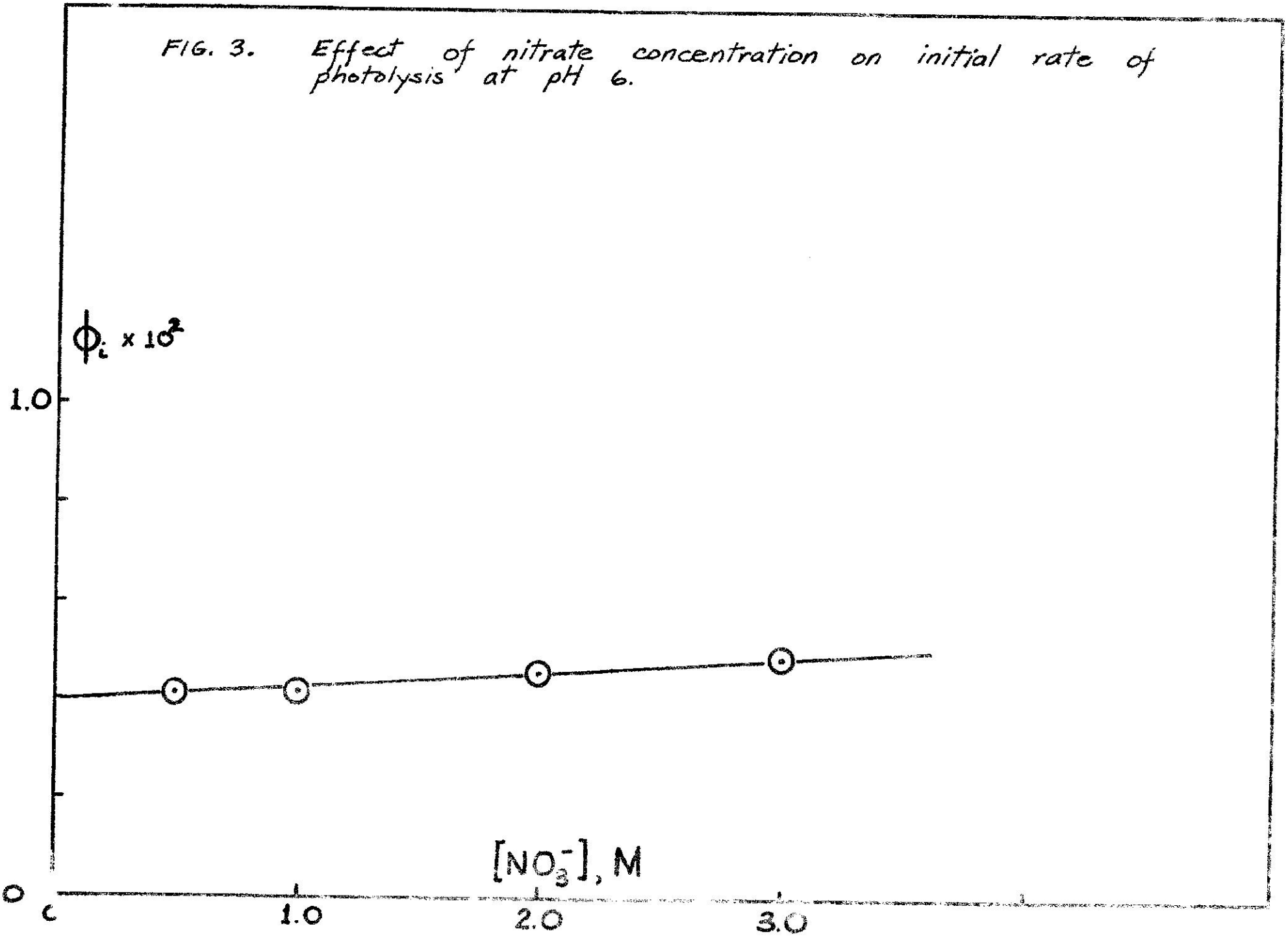


FIG. 4. Dependence of experimental rate constant on nitrate concentration at pH 6.

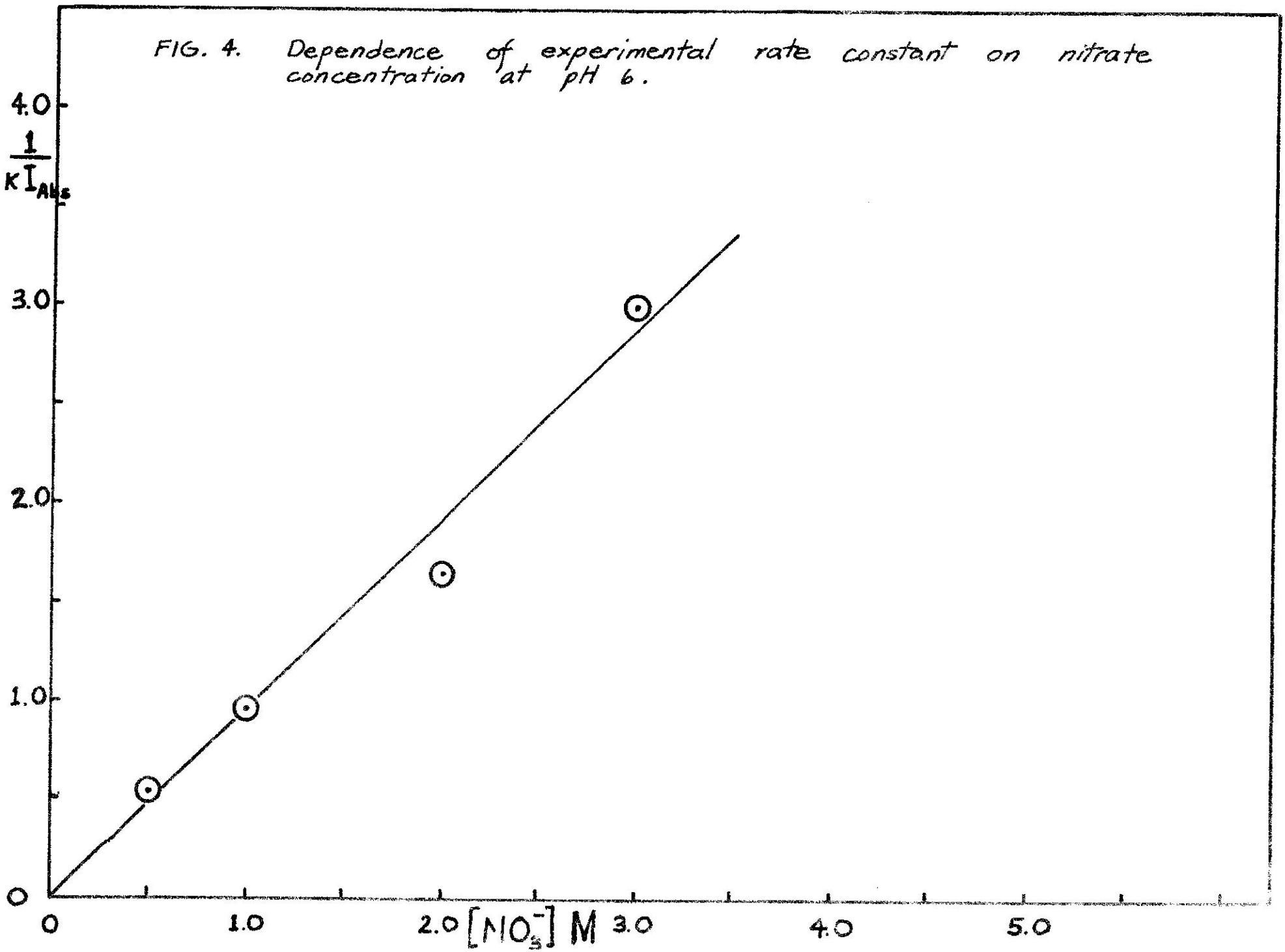


FIG. 5. Effect of Nitrite added prior to irradiation on photolysis at pH 6.

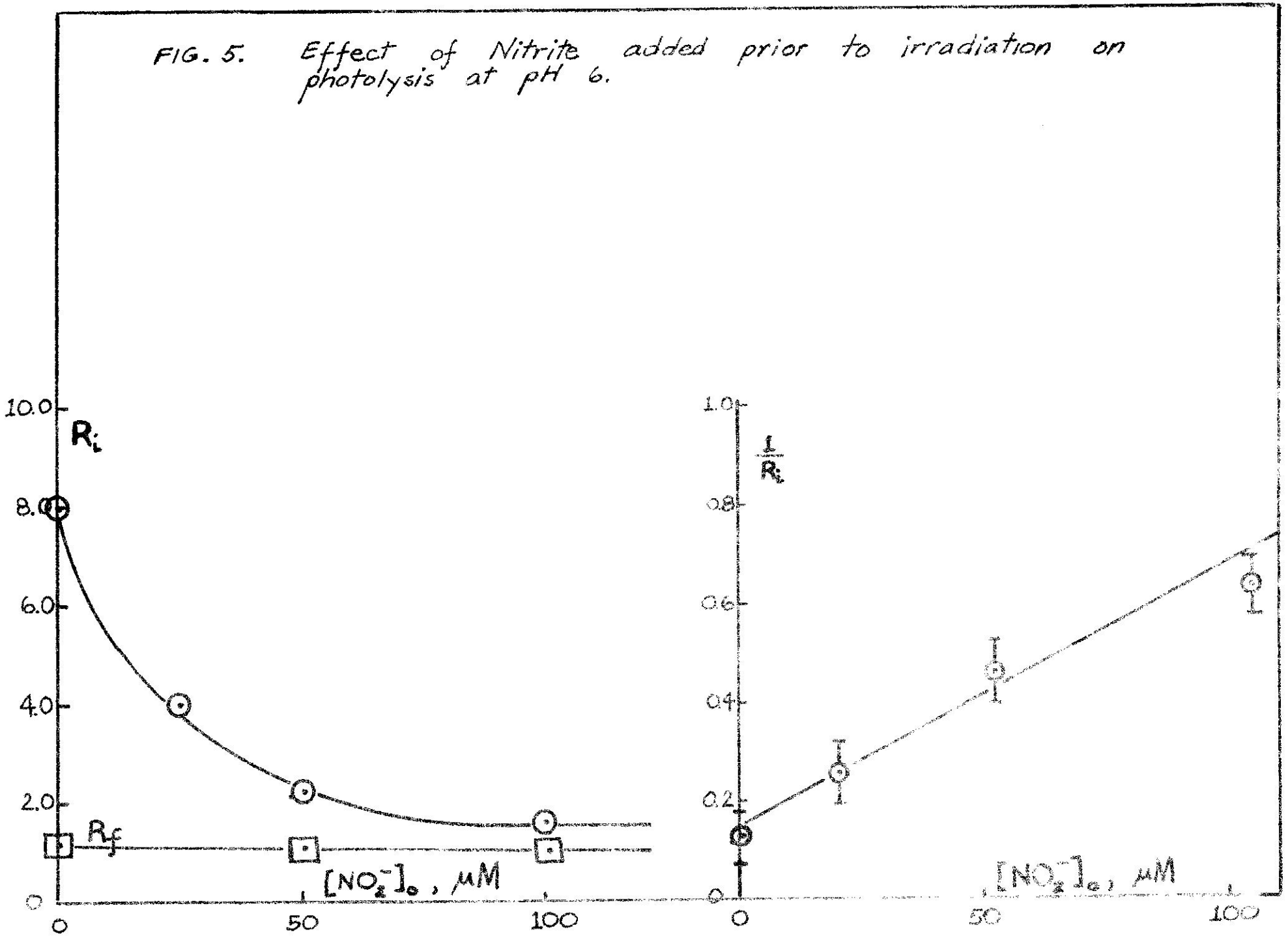
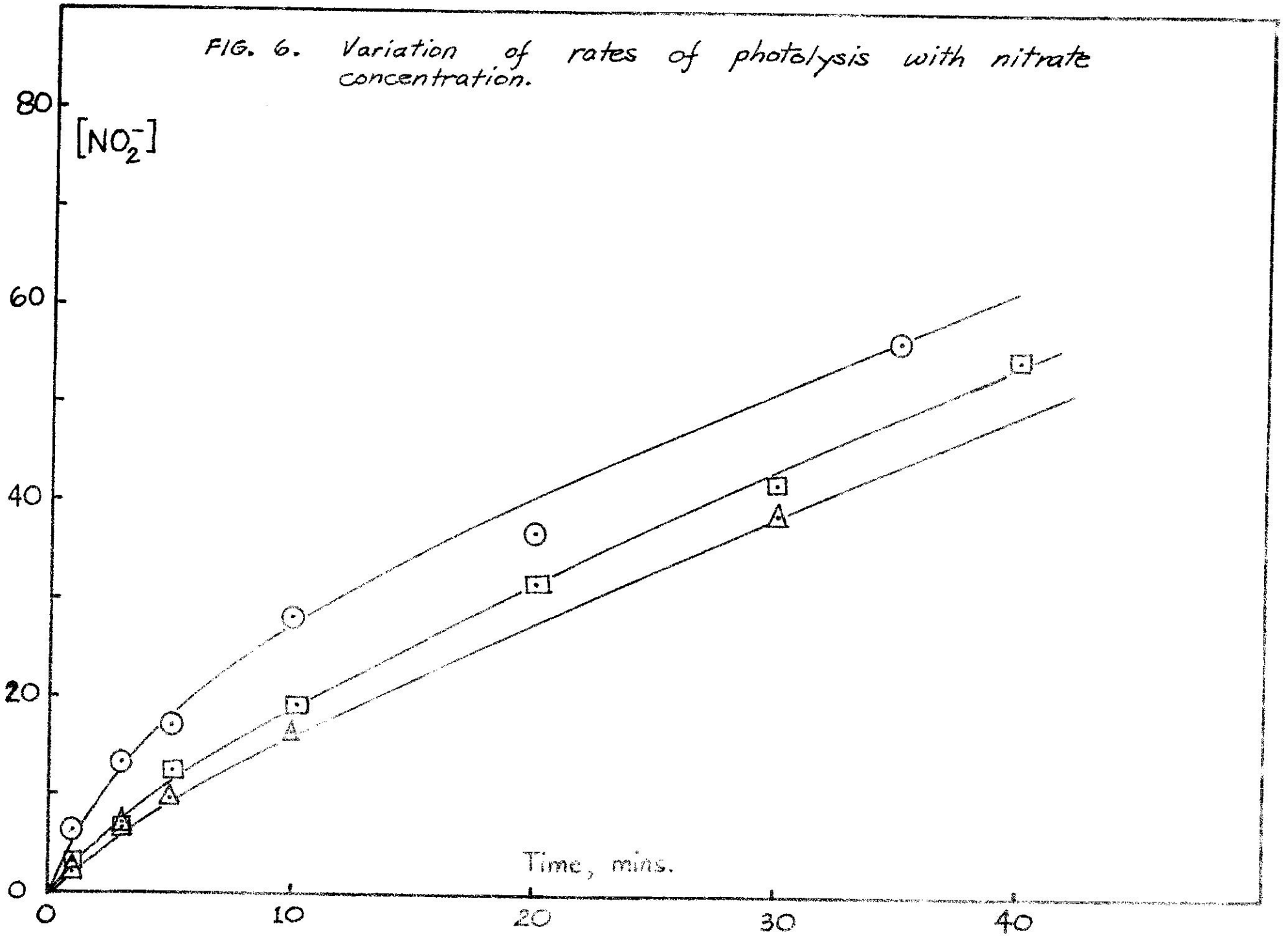


FIG. 6. Variation of rates of photolysis with nitrate concentration.



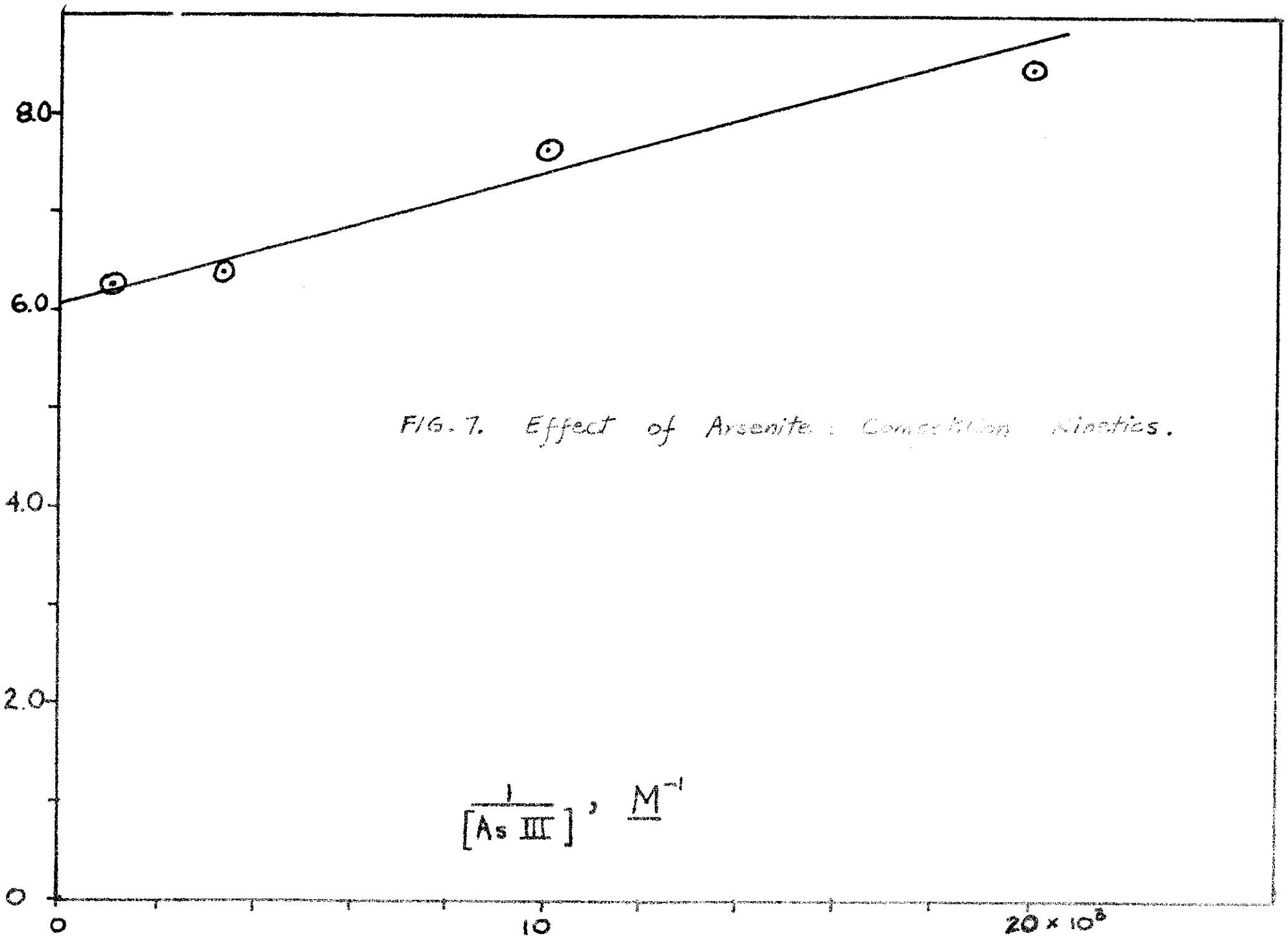
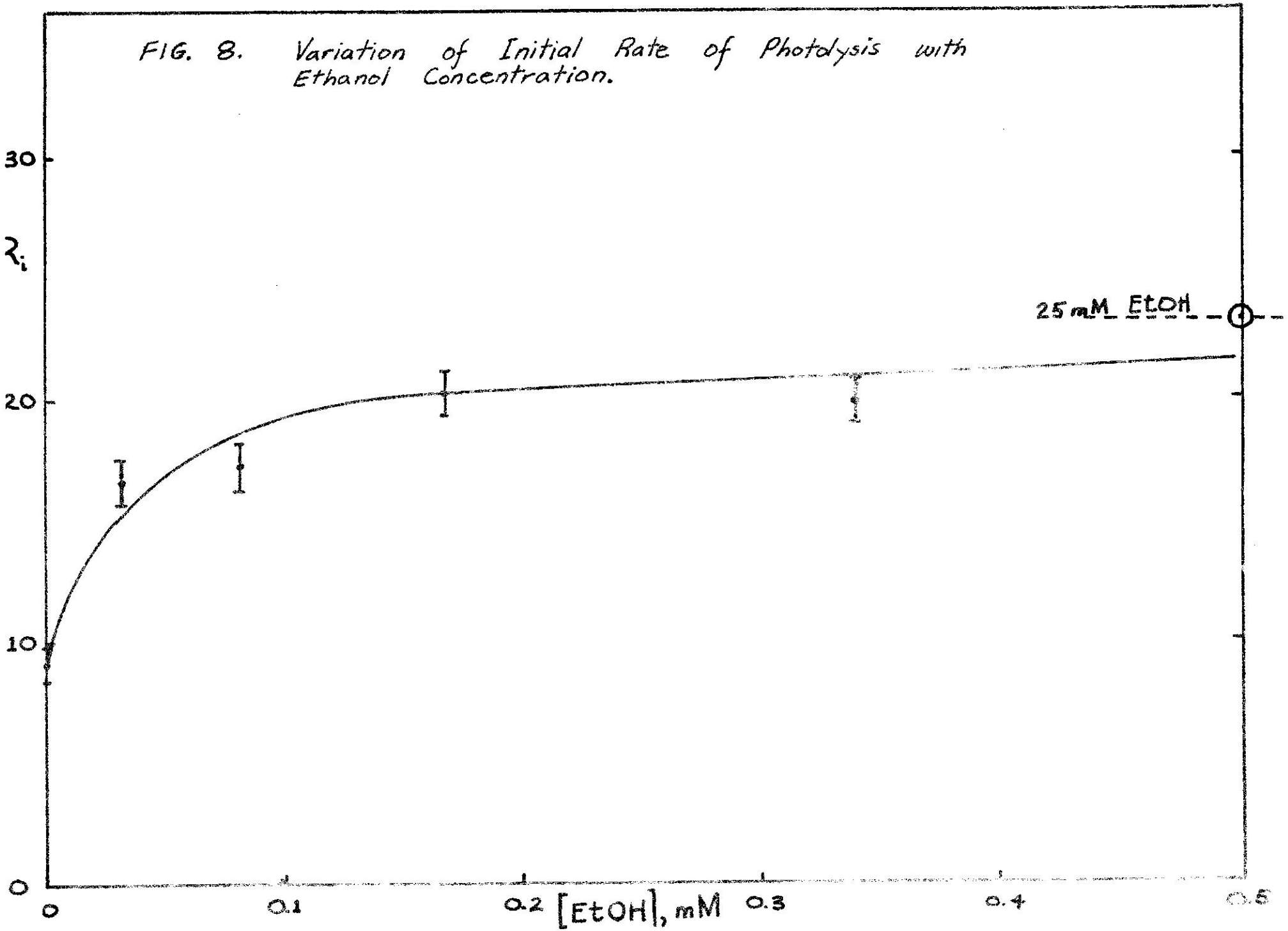


FIG. 7. Effect of Arsenite : Concentration Kinetics.

FIG. 8. Variation of Initial Rate of Photolysis with Ethanol Concentration.



B. Gamma Radiolysis of Aqueous Nitrate Solution
Work carried out by Dr. E. Wigg

A study of the γ gamma radiation induced reactions occurring in aqueous sodium nitrate solutions is currently being carried out. To date the investigation has been limited to the determination of G values of H_2O_2 and NO_2^- in neutral aerated solutions whose nitrate concentrations range from 10^{-4}M to 6M. A dose rate of about 1×10^{20} ev. liter^{-1}. min^{-1} has been employed for all of the work to date. Values for $G(NO_2^-)$ have also been determined for oxygen-saturated solutions. A typical yield-dose curve for NO_2^- formation is shown in Fig. 1. The shape of the curve at low dose has not been explained, however, it has been observed that the addition of 5 micro moles per liter of $NaNO_2$ results in a linear rate of formation of nitrite in the same dose range. The straight line whose slope gives what is considered to be the initial rate of formation of NO_2^- is illustrated by the broken line.

Fig. 2 shows the initial $G(H_2O_2)$ values obtained by irradiating various concentrations of neutral aerated sodium nitrate solutions. A similar plot of $G(NO_2^-)$ in neutral aerated and oxygenated solutions is given in Fig. 3.

Although the work is still in its initial stages, some interesting points can be mentioned. The H_2O_2 yield drops very rapidly with increasing

nitrate concentration to the molecular yield value of 0.7 where it remains essentially constant until relatively high nitrate concentrations are reached. The decrease in $G(H_2O_2)$ in this region is probably due to the decrease in the water electron fraction. The effect of oxygen is to lower the nitrite yield. This can be attributed to the competition between oxygen and nitrate for the solvated electron.

$$O_2 + e^-_{aq} \xrightarrow{k_1} O_2^-$$

$$NO_3^- + e^-_{aq} \xrightarrow{k_2} NO_2 + 2OH^-$$

Values for k_1 and k_2 have been determined by other workers and are:

$$k_1 = 1.9 \times 10^{10} \text{ l m}^{-1} \text{ sec}^{-1}$$

$$k_2 = 1.1 \times 10^{10} \text{ l m}^{-1} \text{ sec}^{-1}$$

giving a ratio $k_1/k_2 \cong 2$

This predicts that at nitrate concentrations above 0.1M $G(NO_2^-)$ should be essentially independent of O_2 concentration. The fact that oxygen lowers the nitrite yield even at 5M $NaNO_3$ indicates that this explanation is not the complete one.

The first plateau in the "aerated" curve in Fig. 3 and the inflection of the "oxygenated" curve occur at about the same $G(NO_2^-)$ indicating that this G represents total electron scavenge by NO_3^-. The increase in $G(NO_2^-)$ at higher nitrate concentrations can be considered in part to be due to a direct action effect.

Work is now in progress to determine $G(NO_2^-)$ and $G(H_2O_2)$ for deaerated solutions and to investigate the effects of intensity, pH and radical scavengers.

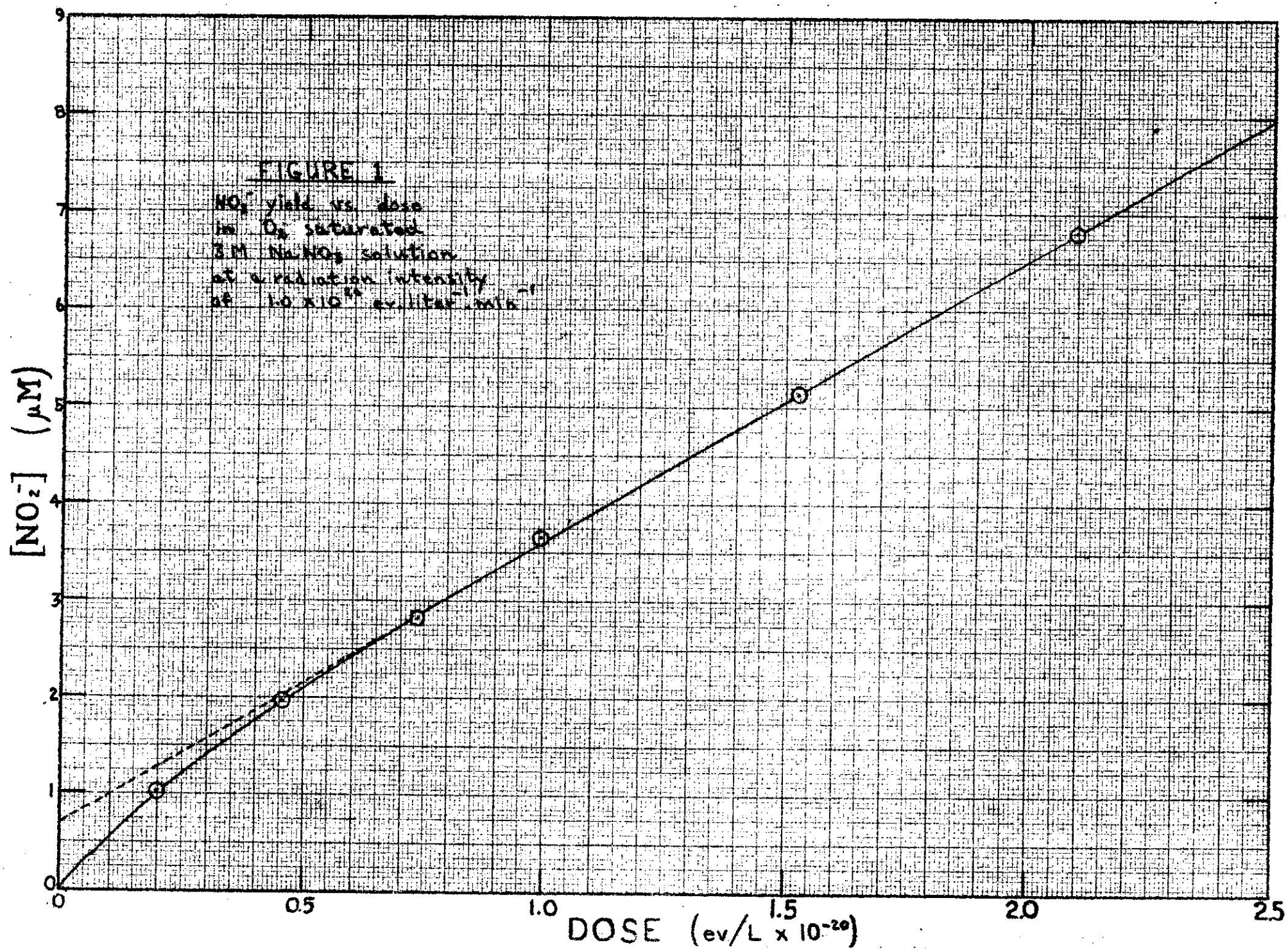


FIGURE 1
NO_2^- yield vs. dose
in O_2 saturated
3 M $NaNO_3$ solution
at a radiation intensity
of 1.0×10^{18} ev./liter.min.$^{-1}$
$[NO_2^-]$ (μM)
DOSE (ev/L x 10^{-20})
0
0.5
1.0
1.5
2.0
2.5
1
2
3
4
5
6
7
8
9

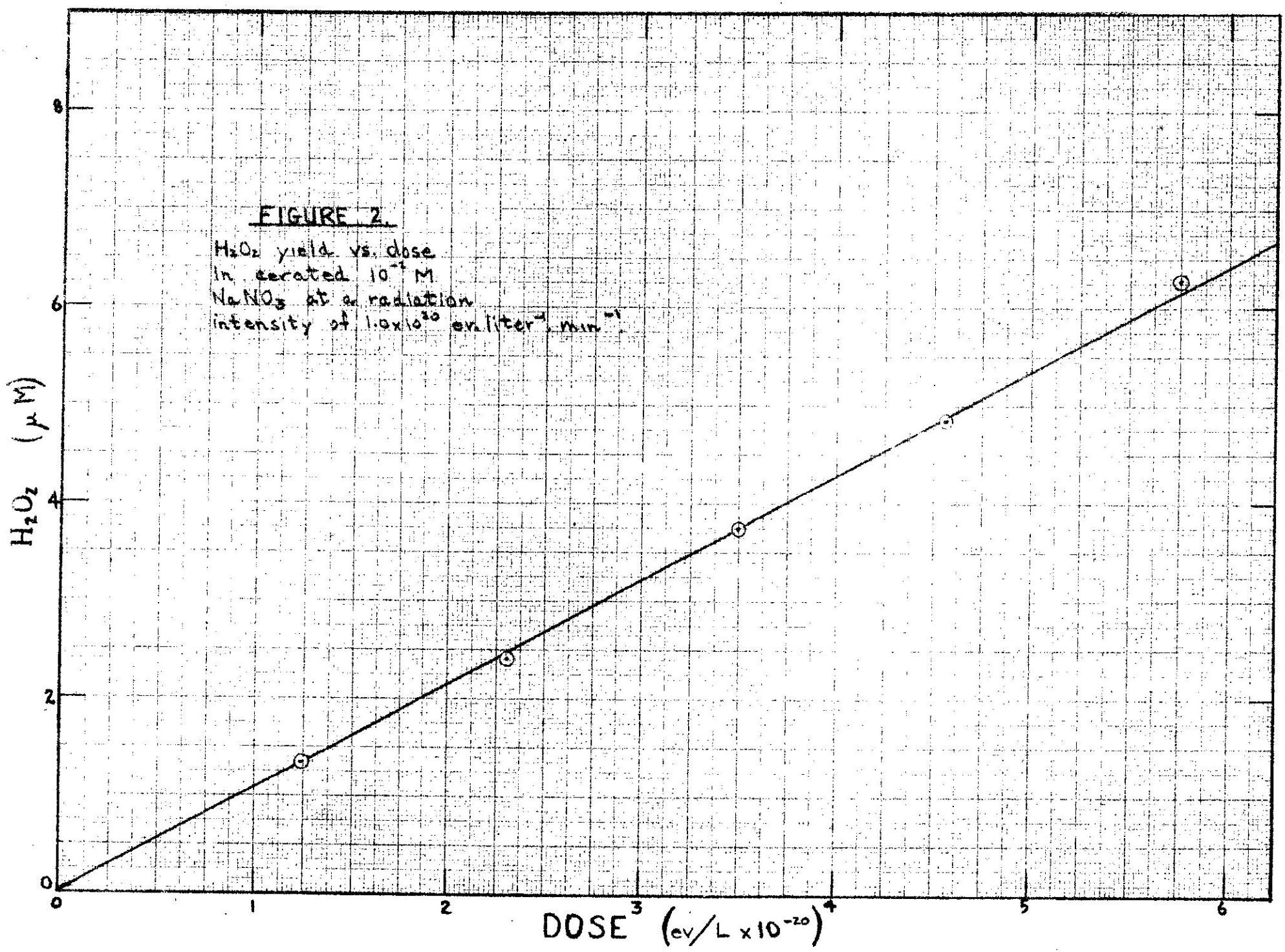


FIGURE 2.
H_2O_2 yield vs. dose in aerated 10^{-2} M $NaNO_3$ at α radiation intensity of 1.0×10^{20} ev liter^{-1} min^{-1}.
H_2O_2 (μM)
DOSE^3 (ev/L x 10^{-20})
0
2
4
6
8
0
1
2
3
4
5
6

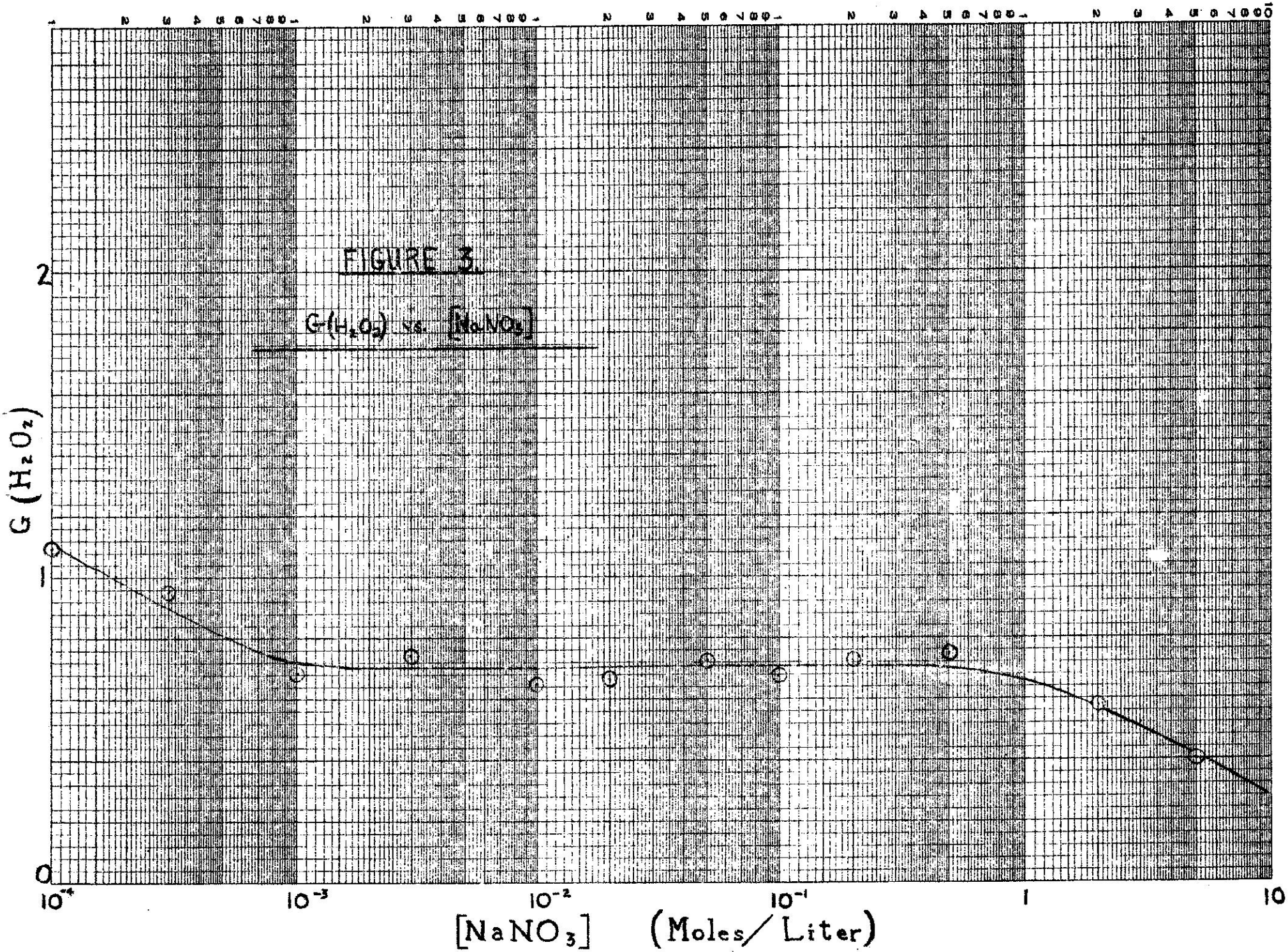


FIGURE 3.
G-(H2O2) vs. [NaNO3]
G (H2O2)
2
1
0
10⁻⁴
10⁻³
10⁻²
10⁻¹
1
10
[NaNO3] (Moles/Liter)

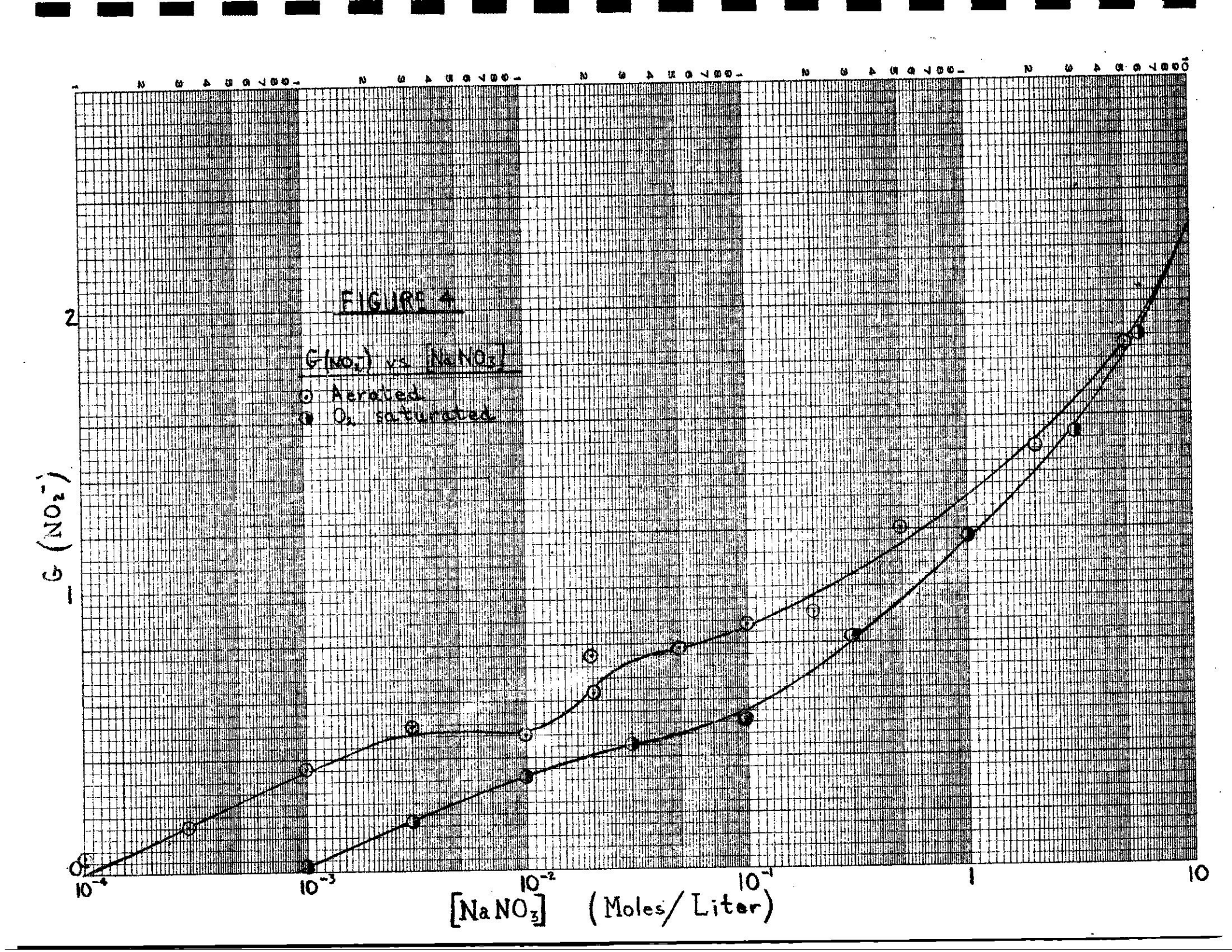


FIGURE 4
G(NO2-) vs. [NaNO3]
Aerated
O2 saturated
G (NO2-)
2
0
10-4
10-3
10-2
10-1
1
10
[NaNO3] (Moles/Liter)

C. Luminescence Studies on D.N.A. Constituent

a) Cytosine

The luminescence behavior of the purines which occur naturally in D.N.A. is well recognized (Duggan et. al. 1957) and has been the subject of considerable research (Walaas 1963, Drobnik 1964). The phosphorescence of D.N.A. (Bersohn and Isenberg 1963) has been attributed solely to the adenine and guanine residues. Pyrimidines and their derivatives (with the exception of thymine) have been reported not to fluorescence at all (Udenfriend 1962).

However, following our earlier work on the photochemistry of cytosine (Daniels and Grimison 1964) we now wish to report the fluorescence of aqueous solutions of cytosine at room temperature.

Cytosine (Calbiochem A Grade) in triple distilled water gave fluorescence spectra such as that shown in Figure 1. The spectra (emission and excitation) were obtained with an Aminco Spectrophotofluorimeter coupled to a DuMont type 304A Oscilloscope and recorded on an Electro-Instrument X-Y recorder. Apart from the first and second order Rayleigh and Raman scattering a broad emission with maximum at 380mμ is seen; excitation maximum is at 295mμ (wavelength uncorrected).

The pH dependence of the intensity of the emission at 380mμ has been investigated for 5×10^{-4}M cytosine (pH was varied by the addition of H_2SO_4 or NaOH only, according to need), using the microphotometer. Results are shown in Fig. 2. (For comparison of instrument sensitivities, we get for 10γ/ml quinine in 0.1 N H_2SO_4 at 480mμ, a relative fluorescence intensity of 75).

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It can be seen that the fluorescence intensity shows a marked dependence on pH which can clearly be correlated with ionization of the cytosine (pK_1=4.45 pK_2-12.2) (Chargaff and Davidson 1960). This pH variation confirms us in our belief that the fluorescence is truly that of the cytosine and not due to some adventitious impurity.

Further, we have investigated the concentration dependence of the emission at various pH's. When corrected for fractional light absorption, all curves show strong concentration quenching. Typical curves at pH6.8 and pH11.8 are shown in Figs. 3 and 4.

Analysis of this data shows that the quenching does not follow a simple Stern-Volmer relationship. However the curves for solutions in which cytosine is in the non-ionic form can be analyzed into two components F_1 and F_2 of which F_1 is quenched by a second-order mechanism $\frac{1}{F_1} = k_1(C)^2 k_1 = 2.35 \times 10^6$ at pH6.8 (Fig. 5) and F_2 is quenched by a first-order process $\frac{1}{F_2} = k_2 (C)$ where $k_2 = 9.4 \times 10^2$ at pH6.8 (Fig.6).

The nature of the fluorescing species and the mechanisms involved in quenching are the subject of continuing work.

This work is part of a program supported in part by National Institutes of Health Grant AM06420 and by A.E.C. Div. of Biology and Medicine.

We thank Mrs. Awilda Román de Sandoval for technical assistance.

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Chargaff E. and Davidson J.N. - The Nucleic Acids Vol. 3 Academic Press, N.Y.(1960)
Daniels M. and Grimison A. - Biochem. Biophys. Res. Commun. 1964 (in press)
Drobnik J. - personal communication 1964
Duggan D.E., Bowman R.L., Brodie BB and Udenfriend, S. - Arch. Biochem. Biophys. 68.1 (1957)
Walaas, E. - Acta Chem. Scand. 17.461 (1963)

Captions for Figures 1-6

Fig. 1 - Fluorescence emission spectrum of cytosine

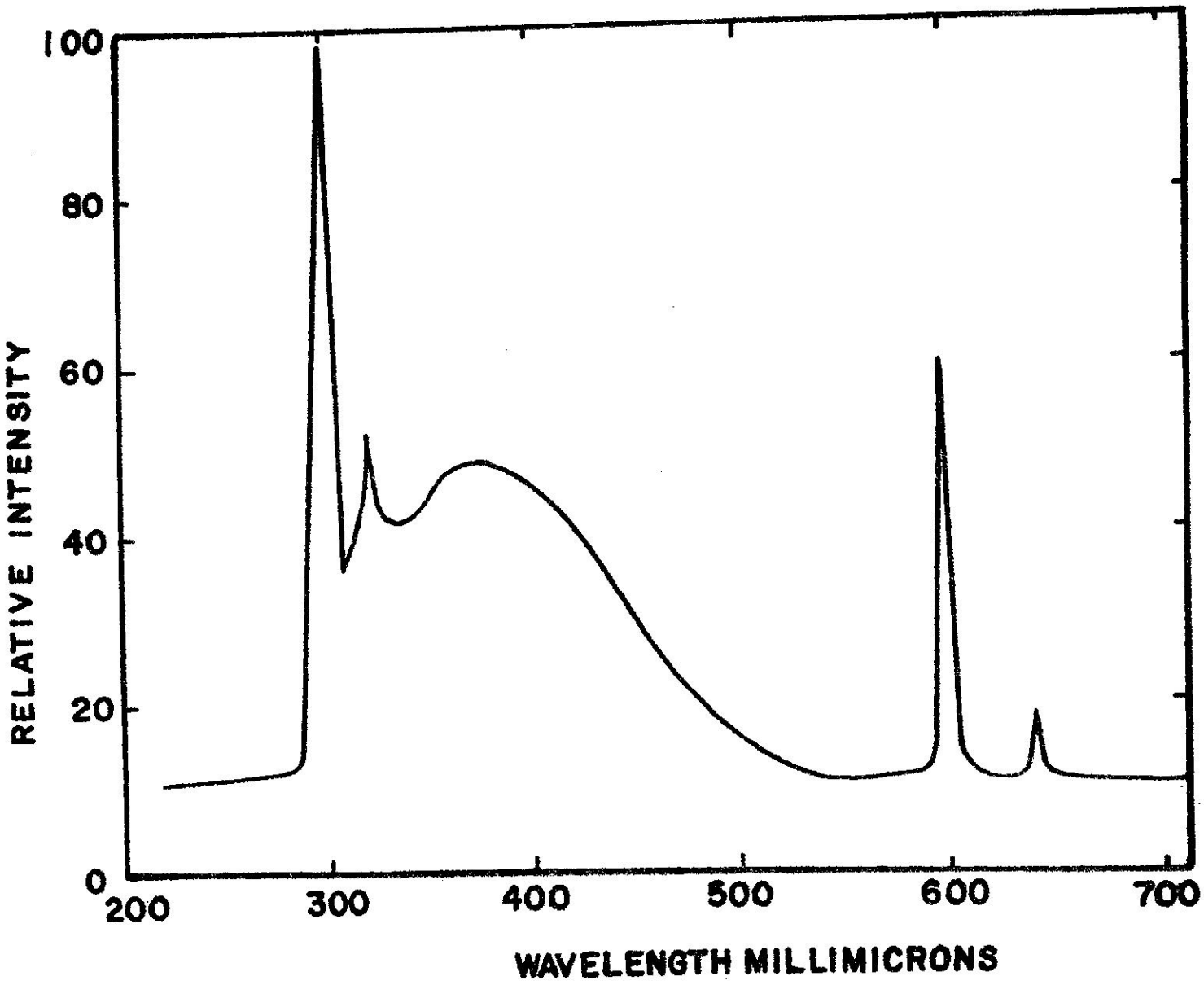
Fig. 2 - pH dependence of the fluorescence emission at 380mμ

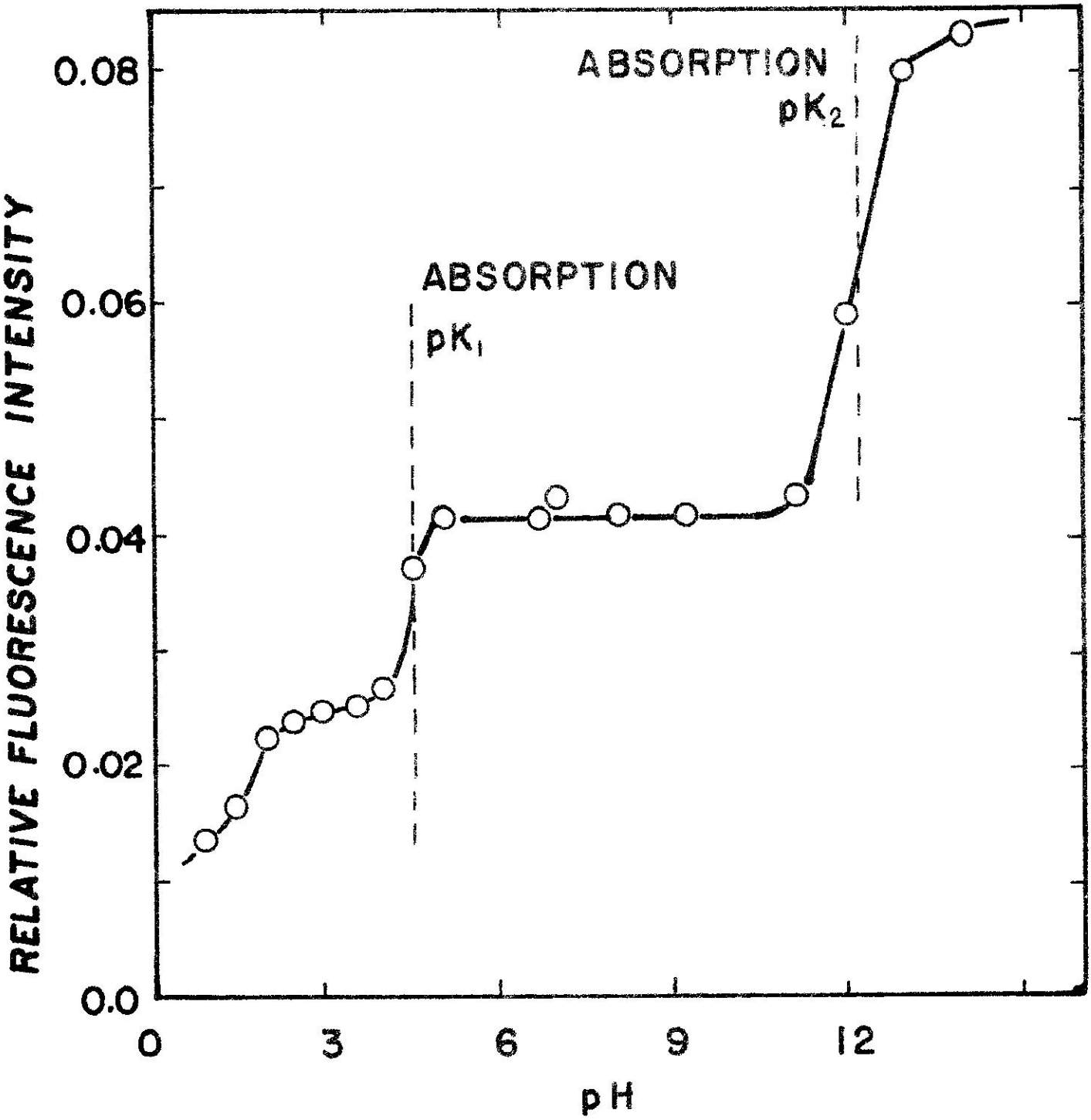
Fig. 3 - Corrected relative intensity of emission as function of cytosine concentration at pH 6.8

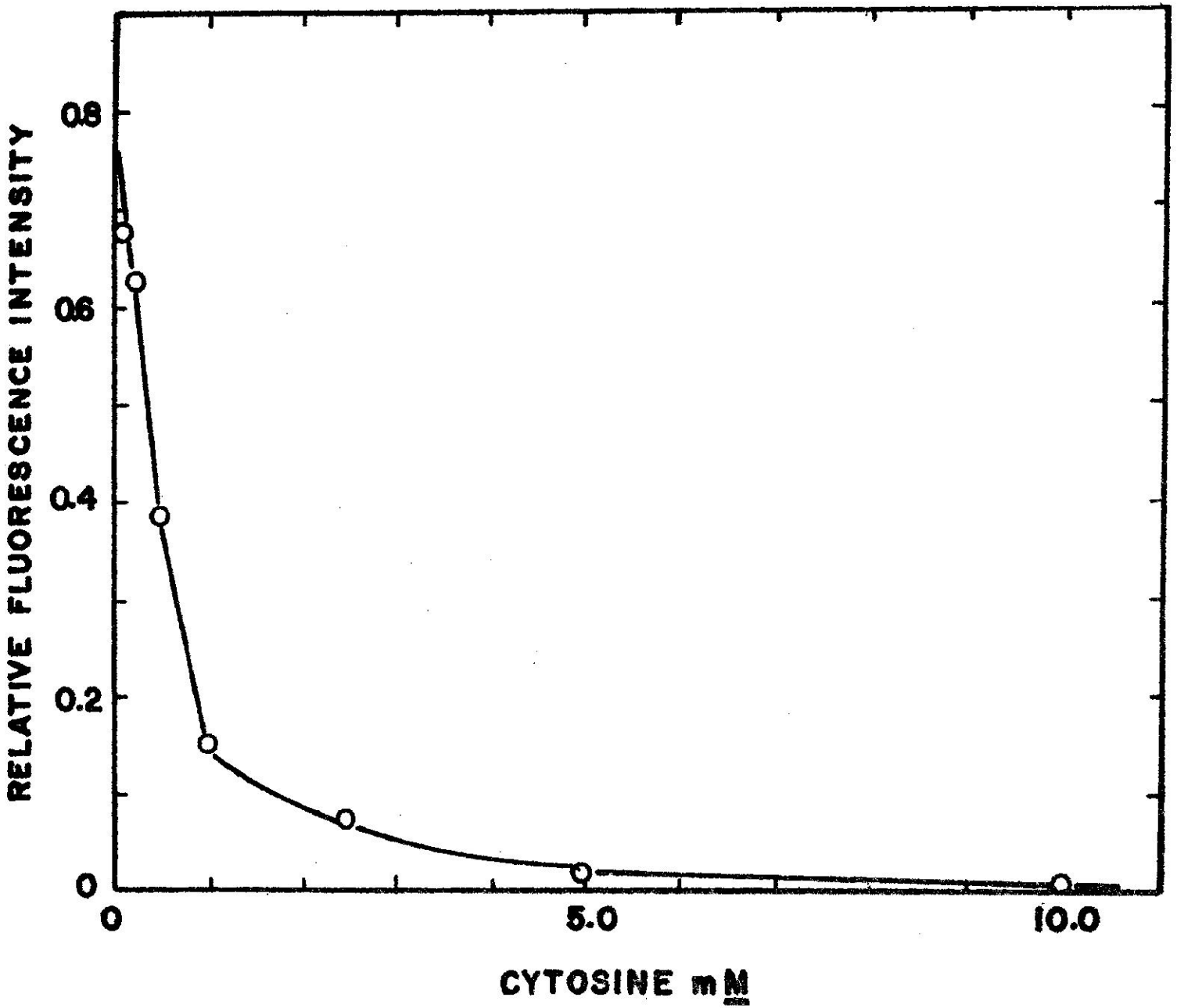
Fig. 4 - Corrected relative intensity of emission as function of cytosine concentration at pH 11.8

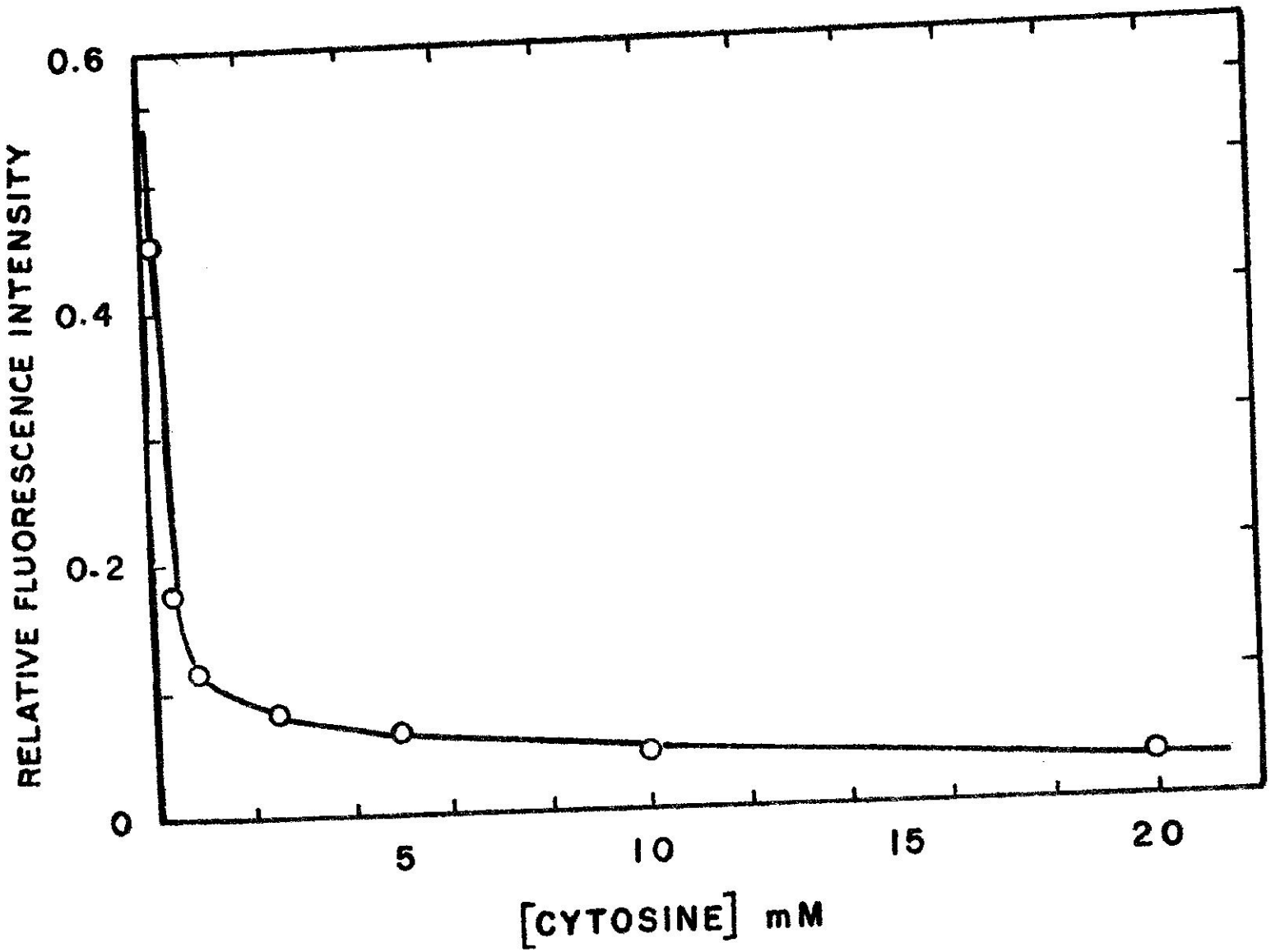
Fig. 5 - Second-order contribution to fluorescence quenching at pH 6.8

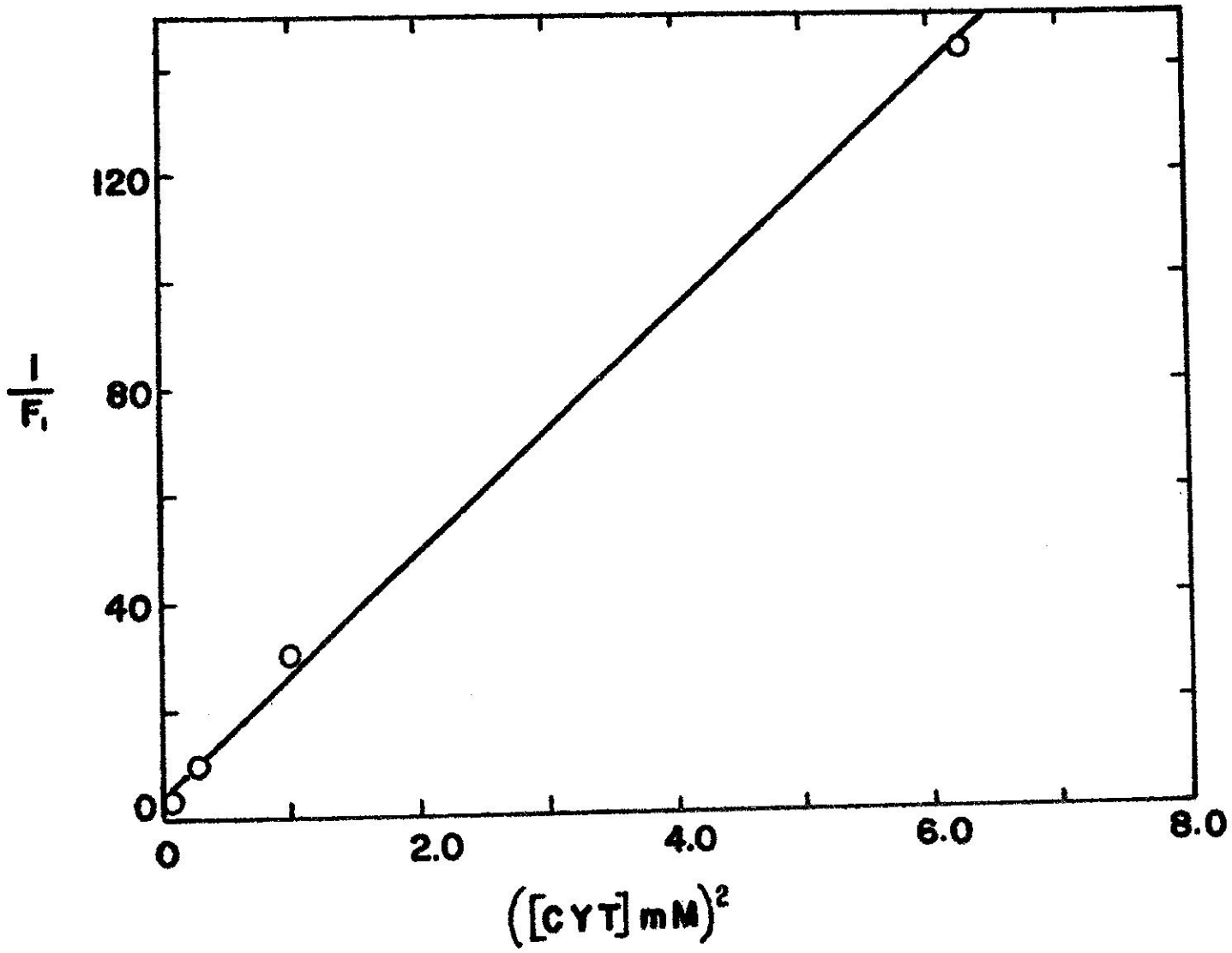
Fig. 6 - Functional dependence of fluorescence quenching at pH 6.8

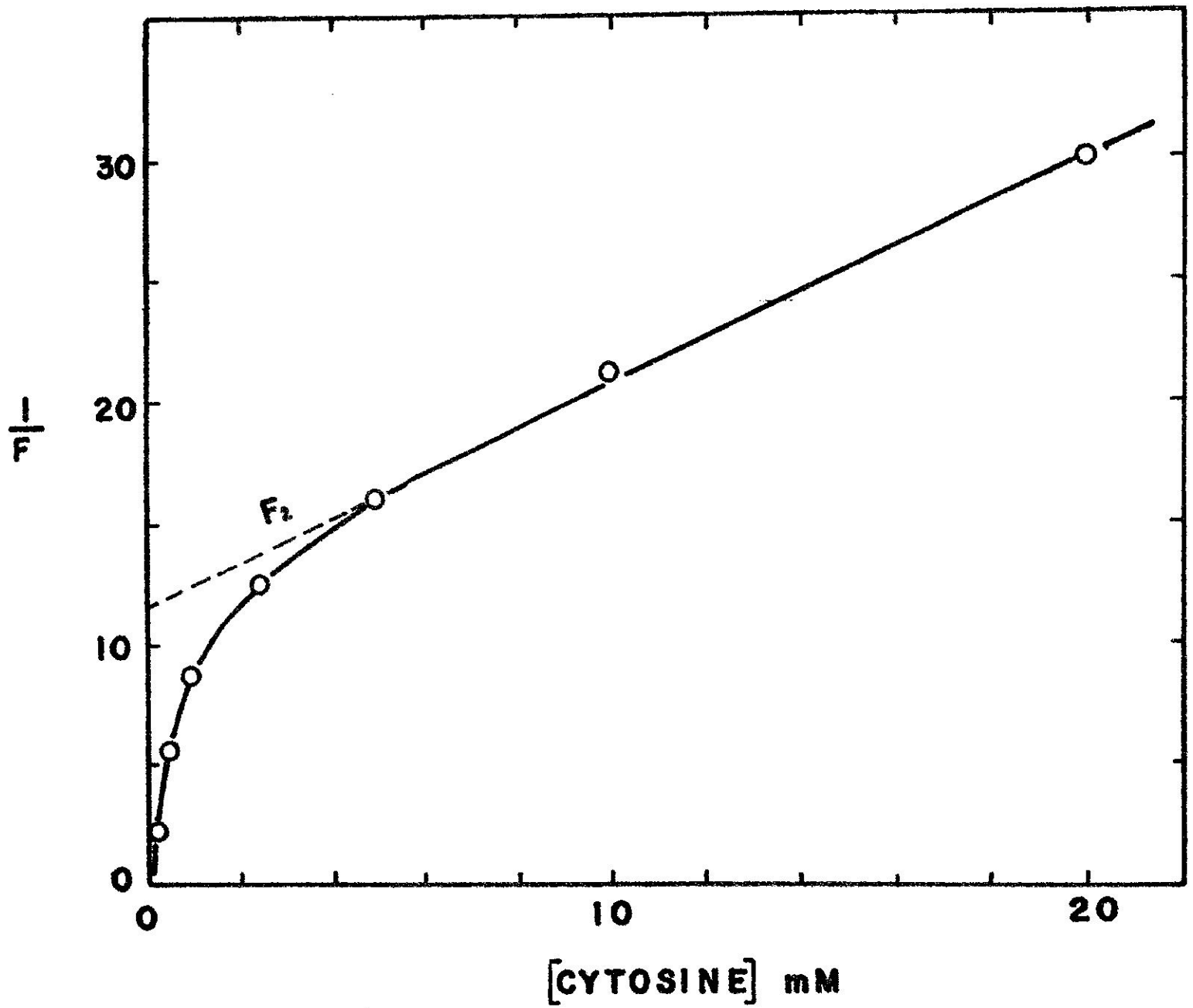

100
80
60
40
20
0
RELATIVE INTENSITY
200
300
400
500
600
700
WAVELENGTH MILLIMICRONS


ABSORPTION
pK2
ABSORPTION
pK1
0.08
0.06
0.04
0.02
0.0
0
3
6
9
12
pH
RELATIVE FLUORESCENCE INTENSITY


RELATIVE FLUORESCENCE INTENSITY
0.8
0.6
0.4
0.2
0
0
5.0
10.0
CYTOSINE mM


0.6
0.4
0.2
0
RELATIVE FLUORESCENCE INTENSITY
5
10
15
20
[CYTOSINE] mM


120
80
40
0
$\frac{1}{F_1}$
0
2.0
4.0
6.0
8.0
$([CYT]\ mM)^2$


30
20
10
0
1/F
F2
0
10
20
[CYTOSINE] mM

b) Nature of the Excited State of Cytosine: Evidence from Photochemical Kinetics and Luminescence Quenching*

We have previously reported[1] the photodeamination by 2537°A 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 behabior of the excited state of cytosine in aqueous solution.

The deamination is linear with absorbed intensity up 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

$$\frac{1}{\Phi(NH_3)} = \frac{1}{\Phi_0} + \frac{1}{\Phi_0} k_c \cdot \frac{1}{C} \qquad k_c = 6.2 \times 10^{-4}\ M \quad (1)$$

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.

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*Presented at the Fourth International Photobiological Congress, Oxford, England, July, 1964.

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

$$C + C \underset{k\text{-}2}{\overset{k_2}{\rightleftharpoons}} C_2^*$$

and that this can be deactivated by further collision with cytosine to give stable products, among them ammonia

$$C_2^* + C \xrightarrow{k_3} \text{Products } (NH_3)$$

Treatment of this mechanism by stationary state kinetics, assuming $k_3 \gg k_2$, gives the following expression for the relative luminescence intensity

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

(F = relative luminescence intensity, K = constant).

and for the quantum yield of ammonia.

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

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 $\frac{k_3}{k\text{-}2}$ from the experimental slopes k_a and k_c. We find $\frac{k_3}{k\text{-}2} = 3.5 \times 10^3$, 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 $k_2\tau_o = 1.7 \times 10^4$ where τ_o is the intrinsic lifetime of the emission. If emission is from a singlet state, for which τ_o 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 $\tau_o = 10^{-4}$ 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
Department of Chemistry
University of Puerto Rico
Río Piedras, Puerto Rico

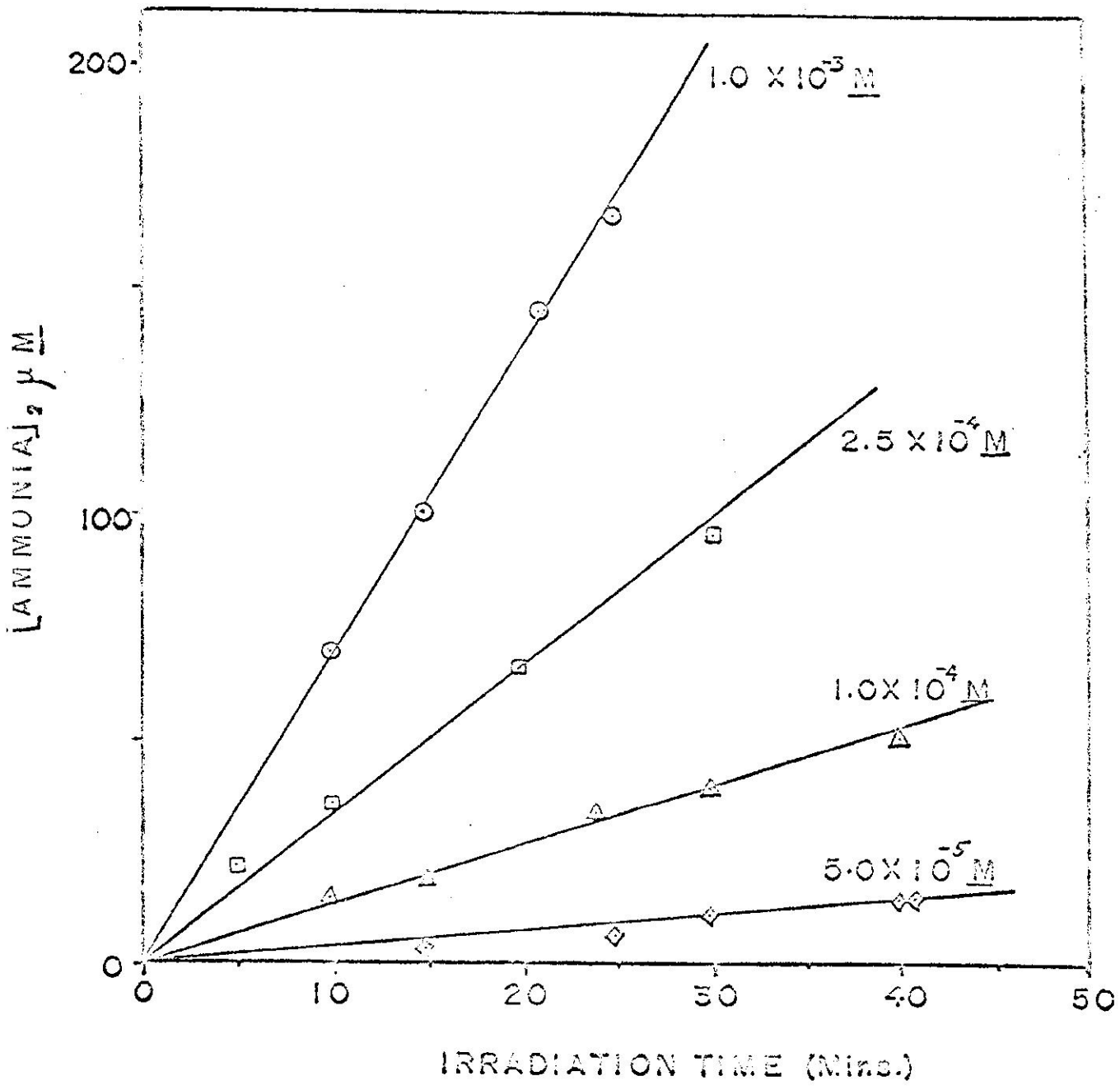
Malcolm Daniels

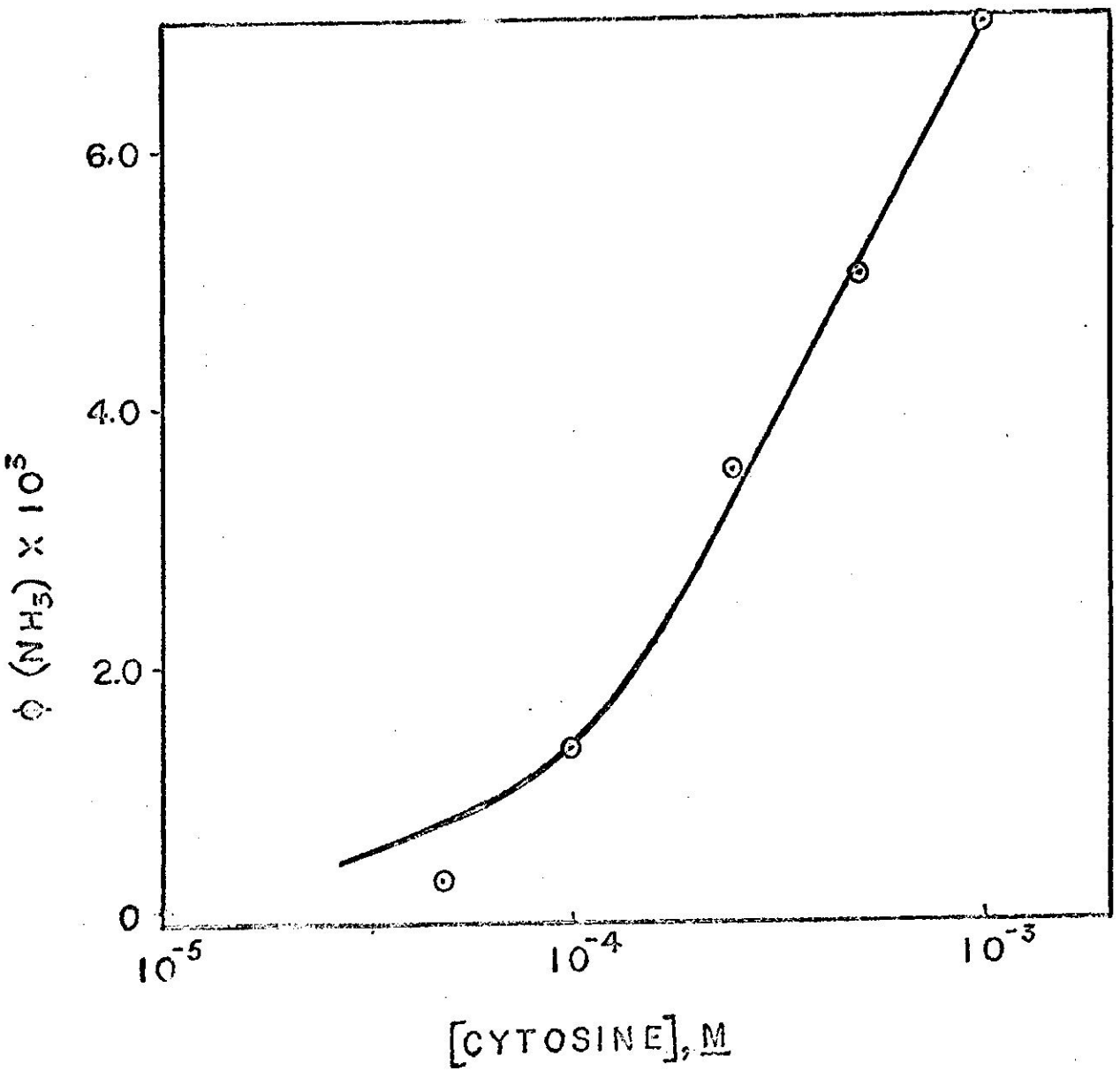
Alec Grimison

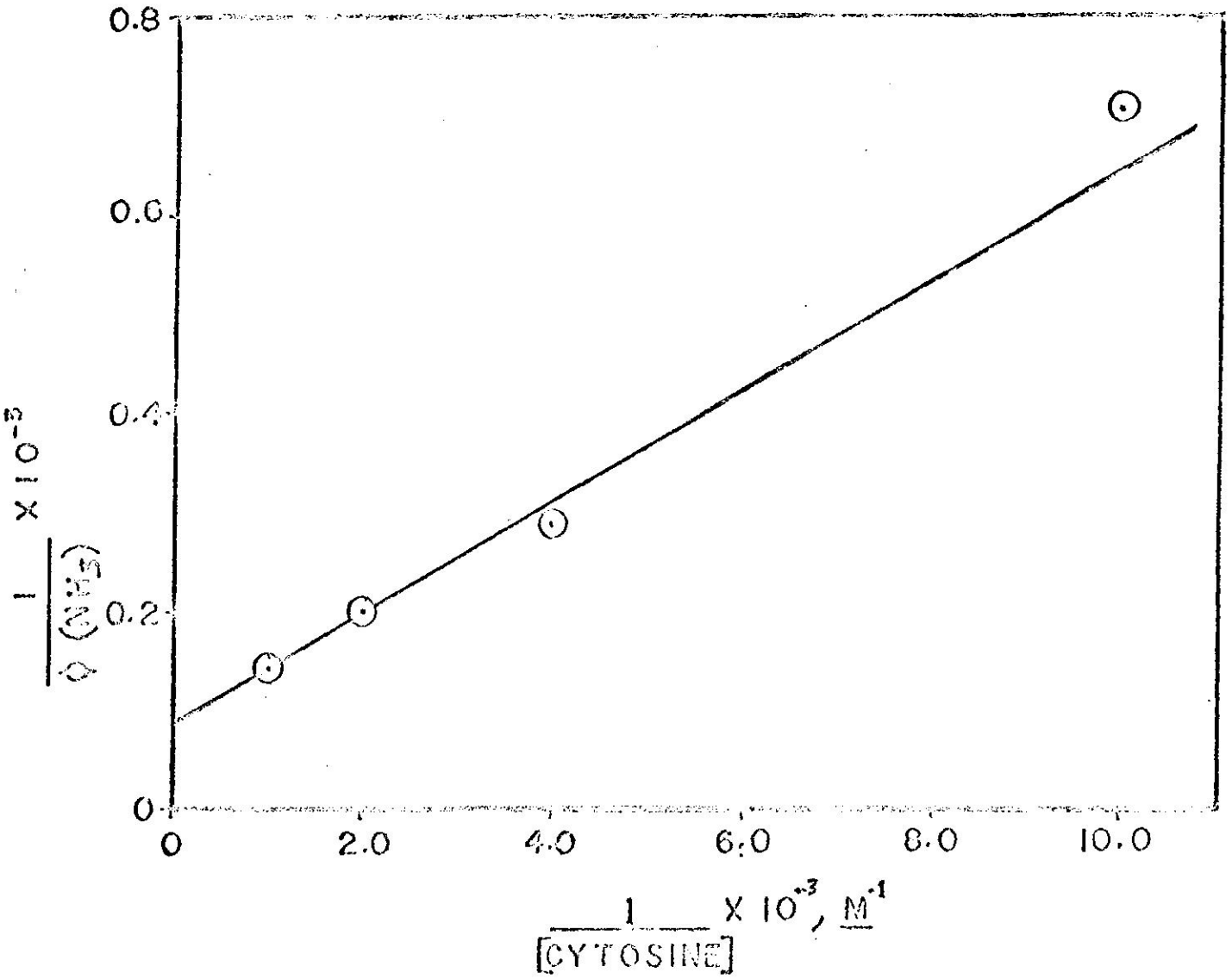
(1) M. Daniels and A. Grimison, Biochem. Biophys. Res. Commun. 16, 428 (1964)

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

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


200
100
0
0
10
20
30
40
50
1.0 X 10⁻³ M
2.5 X 10⁻⁴ M
1.0X 10⁻⁴ M
5.0X 10⁻⁵ M
[AMMONIA]₂ μ M
IRRADIATION TIME (Mins.)


6.0
4.0
2.0
0
Φ (NH_3) $\times 10^3$
10^{-5}
10^{-4}
10^{-3}
[CYTOSINE], M


0.8
0.6
0.4
0.2
0
0
2.0
4.0
6.0
8.0
10.0
$\frac{1}{\phi\,(NH_2)} \times 10^{-3}$
$\frac{1}{[CYTOSINE]} \times 10^{-3}$, M^{-1}

c) Thymine and D.N.A.

Similar studies have been carried out on thymine. In aqueous solution at room temperature a clean emission λ_{max} = 380mu has been found, confirming the report of Udenfriend. The pH variation of this emission parallels the pK of thymine, increasing strongly in alkaline pH.

In view of the evidence for the cytosine emission being a phosphorescence from a long-lived state, and the similarity between the behavior of cytosine and thymine, the possibility that the thymine emission is phosphorescence must also be investigated. Apparatus is being set up for lifetime studies.

It should be noted that a very weak emission has been observed from D.N.A. solutions at room temperature. The conditions for observing this emission, and its characteristics, are being determined.