PRNG 62 PUERTO RICO NUCLEAR CENTER Progress Report RADIATION CHEMISTRY AND PHOTOCHEMISTRY OF AQUEOUS SOLUTIONS OF OXYANIONS LER ORIGINATED BY UNIVERSITY OF PUERTO RICO UNDER CONTRACT NO. AT (40-1)-1899 FOR U.S. ATOMIC ENERGY COMMISSION ---Page Break--- PUERTO RICO NUCLEAR CENTER Progress Report of the Project RADIATION CHEMISTRY AND PHOTOCHEMISTRY OF AQUEOUS SOLUTIONS OF OXYANIONS by Malcolm Daniels April, 1965 ---Page Break--- RADIATION CHEMISTRY AND PHOTOCHEMISTRY OF AQUEOUS SOLUTIONS OF OXYANIONS Progress Report April, 1965 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 analyzed. 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 the Fourth International Congress of Photobiology, and the Ninth Annual Meeting of Biophysical Society. A. Mitrave 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 nitrate formation eventually decreases to a steady value, as shown in fig. 3 where a limiting rate R = 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. ---Page Break--- 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. Tate analysis of data meets 4 staple models 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-scavengable final rate is interpreted as the probability of decomposition or retention within the "solvent cage." Consequently, the overall course of photolysis in neutral and acid solutions is represented by: a) (003) = g(t) + $2), where f(t) = time-dependent. The quantum yield may also be written in the form: $v) = _ Tew Effect of Nitrate When this analysis is carried out on the previously reported ventilation results for the effect of nitrate concentration on the 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: Plo) = blo) + 45, [ow] ---Page Break--- 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: as a Bifest of Nitrite Concentration: Inhibition The slope of the concentration-time curve suggested that nitrite, a product of photolysis, was an inhibitor. To test this, the previously reported photolysis of a solution with nitrite added prior to irradiation has been extended and quantitatively analyzed. Some experimental results are shown in fig. 5 and the overall picture is summarized in fig. 6. It can be seen that the initial rate is rapidly decreased by all concentrations of nitrate without appreciably changing the final steady rate (or the rate constant), and follows until the concentration reaches a certain level.

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concentration ino a form tye af competition ---Page Break--- Kinetics, fig. 7, a = oe fs ta) Adastion of Hydrogen Peroxsce Awaentte being knowa to be a ccod soavenger for OM radieal, st bas been decided to try othe inom scavenvere for OH. Of these, tvirogen peroxide 4s of interest in that its behavior might cive some Might on the non-fomation of H.0, im nitrate photolyste. Addition of H,0, leads to en apparent increase in the rate of Photolynie, but tvo feature are note worthy. First, when the non-linear concentration curves are analysed st 1 found that the initial rate 4s oaly elightly higher than in the absence of Up, » and the major effect is on the save constant which decreases by « factor ©f 10, Second, in constrast to arsenite, fur which scavenging io complete at Limit, Hp, begins to have an effect at this concentration and 4e really only effective at concentrations 10-20 ai. Tt to inferred that 4,0, does not effectively compete in the primry Fadical reactions, ani tus ite main civoct te competing vith 70 tn the secondary reactions. Bifect of Bumaner The addition of ethanol Leads to an inerease in inttial rate at low concentrations. Acetaldajhyde ic formed at the same rate as nitrite together with very low yields of Hp, « Initial rates ao a function of etmancl concentration are shomn 4a fig. 8. (the curve is qaloulated from the relation: ---Page Break--- a eH, its Geom “on * Poo Xe ts tnterred chat ethanol, in conte 20 HLP, , te about as etteective & scavenger as aztontte aut Gi . However tare ween to be seus quuntitative differences re?! tea tn the Limiting quontum yields. ---Page Break--- | i | | | oF oe oz 1 To NI Saw vstyaeyd fo veymmp fo uoyoun$ © so aishtaoyd fo apes quareddy “Ton, oz ay ---Page Break--- —— Oe ---Page Break--- sysXjezes fo 2he4 yes vo valouuaoues” Kent ” fo ‘ese “6-9/4 ---Page Break--- Sp npn

os of ot W [ONJoz ot 9 fo “9 Wd 40, veyayeeoves Bnyw wo ywesucr aye pryrwiedke” fo suepuedeg “O14 ---Page Break--- oor o oot os ‘ - Ol Spon] OE 9 yd yo sphyazoyd we voyripest oy sold poppe gyi4yn fo g7effg 19 Old ---Page Break--- yayle yor sisKjeyoyd fo sazos wonpesyusoue> fo vei, +9 “91g ---Page Break--- ° he OF ywery fo pelle ‘L-org -o|°? a ---Page Break--- eo WY {|HO33] zo ro worpoyesruey jouoys7 wm sskppoyg fo yey peywy fo vorpersny ‘891d oT oz of ---Page Break--- B. Game Raltotysis Work caries oF A study of chs > gana: vactsu' > induce* reactions occurring tn aqueous sodium nitrats colwiicos a camerdy Bets cursed cat. To date ‘the investigution has Mattes vo tor dvvemstnaticn of G valueu of 10, and H03 fn neutral cerated celutione waove nitrate censoatrations mange ey 2, ‘free 1074¢ to At. A done rate of evans 2 x 20” ev. Liter”. min” has been caployed for al of the vork 9 date. Yeluee for G(H0%) lave ulso been determined for oxygen-caturated solutions. A typical yield-dose eurve for WO], formtion is show in Fig, 1 The shape of the curve at Lov dot not been explained, hovever, it has been observed that the adastion of 5 mtoro moles yer liter of Nel, results in a linear rate of formation of nitrite dn the sane dose range. The straight line whose slope gives watt 4s considered to be the initial rate of formtion of MOD is tMustrated by the broken Line. Fig. 2 shove the initia G(#,0,) values obtained by trradtating various concentrations of neutral aerated sodium nitrate solutions. A similar Plot of G(007) in neutral seroted ona cxygenstad solutions is given tn Fig. 3+ Although the vork is stilt tn tts initial stages, some interestiue points can be mentioned. ‘the ity, yield drops very rapidly vith increasing ---Page Break--- ba rttrate concentration to the aolccular yield vaiue of 0.7 where St resins essentially constant until rolatively high nttrate concentrations are reached. The decrease in O(#.0,) in this region is probably due to the decrease 4n the water electron fraction. Tho effect of caygen ts to

lower the nitrite yield. Mis can be attributed to the competition between oxygen and nitrate for the solvated electron. Values for k, and k, have been determined by other workers and are: 2 20 = 1s x 2 a? see’ i 9 x 10 20 el geet kein x wl ag ring ratio yy, % 2 Mike predicts that at nitrate concentrations above 0.1M O(W) should be partially independent of ©, concentration. The fact that oxygen lowers the nitrite yield even at 5M Mal indicates that this explanation is not the complete

one. The final plateau in the "Yoorsted" curve in Fig. 5 and the deflection of the “oxygenated” curve occur at about the same G(%) indicating that this O represents total electron scavenged by 20S. The increase in 60% at higher nitrate concentrations can be considered in part to be due to a direct action effect. Work is now in progress to determine (1) and G(H2O) for deoxygenated solutions and to investigate the effects of intensity, pH, and radical scavengers. ---Page Break--- I (2-01 ys %) 3 a soa oN] z L) (nw? ---Page Break--- a af oa %) 38 EFT AG i ---Page Break--- (e171 /ern) [foNnen] 01 yt Ol 5 , or Con) 9 ---Page Break--- Se FT lll x (Gavi fern) Eoney] ---Page Break--- ©, Luminescence Studies on 8) Cytosine ‘the luminescence behavior of the purines which occur naturally in constituent Pallas 48 well recognized (Duggan et al., 1957) and has been the subject of considerable research (Walass 1963, Drobntk 1964). The phosphorescence of purines (Bertchn and Ioesberg 1963) has been attributed solely to the adenine so asanine restover. Pyrimidines and their derivatives (with the exception of thymine) have been reported not to fluoresce at all (Udenfriend 1962). However, following our earlier work on the photochemistry of cytosine (Daniels and Grimston 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 Aninso.

Syactrophotofluorimeter coupled to a Duet type eM Cectioscope and recorded on an Hlectroinstrument Ie recorder. Apart from the first and second order Rayleigh and Raman scattering, a broad emission with maximum at 380 nm is seen; excitation maximum at 235 nm (wavelength uncorrected). The dependence of the intensity of the emission at 560 nm has been determined for 50° years (see vary in an ongoing TOI environment, according to need), using the microphotometer. Results are shown 16.2 (for comparison of instrument sensitivities, we get for 20 y/ad quinine in OL RHO, at Mom, a relative fluorescence intensity of 75). ---Page Break--- Luminescence Studies on Cytosine The luminescence behavior of the purines which occur naturally in JA. Constituent DaleAs 49 well recognized (Duggan et al. 1957) and has been the subject of considerable research (Valaas 1963, Drobatk 1964). The phosphorescence of DNA (Bersohn and Isenberg 1963) has been attributed solely to the adenine and guanine reaction, uracil and thymine derivatives (with the exception of thymine) have been reported not to fluoresce at all (Udenfriend 1962). However, following our earlier work on the photochemistry of cytosine (Dantels and Grimston 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 Anno Spectrophotometer coupled to a fluorescent type 5A microscope and presented on an Electro-instrument Xe recorder. Apart from the first and second order Rayleigh and Raman scattering, a broad emission with maximum at 360 nm is seen; excitation maximum is at 295 nm (wavelength uncorrected). The dependence of the intensity of the emission at 560 nm has been determined for 5 years (and varies by the minimum pH NaOH only, according to need), using the microphotometer. Results are shown in Fig. 2 (For comparison of instrument sensitivities, we get for 10^7/mL quinine 40.1 1H, at Mom, ...

selene tivzeecene intensity of 7), ---Page Break--- It can be seen that the fluorescence intensity shows a marked dependence on pH that can clearly be correlated with the concentration of the cytosine (yeh.45 pip-22.2) (Changatt and Davidson 1950). This 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 pHs. When corrected for vial light absorption, all curves show strong concentration quenching. Typical curves

at pH 1 and pH 2.5 are shown in Figs. 3 and 4. Analysis of this data shows that the quenching does not follow a simple Stern-Volmer relation in which cytosine participates. However, the curves for pH 3 fit the non-ionic form and can be analyzed into two components, one of which is quenched by a second-order mechanism 2 = H2(C)2 = 2-35008 a 206.8 wonder process 1 = i (G) where Xp = 9.tni0® (Fig. 5) and Fp is quenched by 8 0 *. at pH 8.5 (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 AYOSi20 and by A.E.C. Div. of Biology and Medicine. We thank Mrs. Wilde Reed de Sandoval for technical assistance. References Berechn R. and Taenvers I, Biophysical Research Communications 25,205 (1963) Bere A and Davidson Juli, The Nucleic Acids Vol. 5 Academic Press, N.Y. (1960) Saarete I and Grinison A. Dicenen. Biophysics, Res. Commun, 1964 (in press) Drobaik J, personal communication 1954 Creme S., Rowan Kei. Brodie 32 and Usenfriend, S. Arch. Biochem. Biophys., 68.1 (1957) Walass, £. Acta Chem. Scand, 17.462 (1963) ---Page Break--- Captions for Figures 1-6 Fig. 1 - Fluorescence emission spectrum of cytosine Fig. 2 - pH dependence of the fluorescence excitation at 38°C Fig. 3 - Corrected relative intensity of cytosine concentration at pH 6.8 as a function of Fig. 4 - Corrected relative intensity of

emtssicn as function of cytosine concentration et pil 1.8 Fig. 5 - Second-order contribution to fluorescence quenching at pit 6.8 Fig. 6 ~ Functional dependence of fluorescence quenching et pif 6.8 ---Page Break--- 100 2 2 2 ° + a AJISNBLNI SAlLVI3a 400 500 600 WAVELENGTH MILLIMICRONS 300 ‘200 ---Page Break--- ' [i 1 1 i ' ' 1 pK. z 3 = a a 5 a o < ABSORPTION 8 g 8 3° ° ° ALISNILNI JINIISIVONTA JAILV IIE 0.08 2 ---Page Break--- AAISNZLNI JONZDS3NONIs BAILV132N CYTOSINE mM ---Page Break--- AAISN3LNI 3IN3ISINONTS FALLVISY [cytosine] mm ---Page Break--- (wu [2a9]) ov ---Page Break--- wu [aNISOLAD] oz o1 ° ---Page Break--- -10- ints obi Ustnercerer aiesertse we nave previously resorted(}) the shotedeanination by 2537°A radiation of eytentne sn equecus solution and have dram atesticn to ite possible elgnttt- cance in photonsotegy. Investigation of Kinetics cf this desnination nas Led to the folleving results which, taken in conjunction with tae previously deseribea(®) Luntnescence venevior, allow clear conelustons to be dvaim ccneerning the nature and behsbior of the excited state of cytosine in aquecus sclution. ‘The deanination is Linear with abecrbed intensity up to considerable percentage change, (Fig. 1) and hence represents a major, primary reaction, As can be seen from Fig. 1, the reaction Le strongly dependent on concentration of cytosine, and quantum ylelde ever a twentyfold range of concentration are shown im Fig. 2. Grephtca2 analysis shove the reciprocal of the quantum yield to be & Linesr function of the reeiprecel of the cytosine concentration (Fig. 3) and gives ‘the result, = 6.2220 % (2) 1 +2 Fim) % ¢ his relationship is very inportant, because the form of it Indicates @ sinple ‘competition between deactivation of the chealcally active excited state and reaction of it with a further molecule of cytosine. Burthermore, if it 1s supposed ‘that deactivation is accompanied by enlasion of luminescence, then we can guanti- ively acecunt for both the Luminescence ani photochemical phennena by the folloving

mechanism. It was presented at the Fourth International Photobiological Congress, Oxford, England, July, 1951. ---Page Break--- We propose that an excited state of cytosine can luminesce in reverting to ground state by a first order process, such as cytosine can react with another cytosine molecule to give a transient excited dimer and that this can be deactivated by further collision with cytosine to give stable products, among them ammonia and other products. Treatment of this mechanism by stationary state kinetics, admitting 153P gives the following expression for the relative luminescence intensity A = K * [P]^n For the excited state (F = relative luminescence intensity, K = constant). and for the quantum yield of ammonia, = 1b K Mo a 4 (a =

rate constant for this reaction). These equations are to be compared with experimental equation (1) of the preceding communication and equation (1) of this case. 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 k_y from the experimental slopes k_y and k_g. We find k_y = 3.5 x 10^3, in good agreement with the assumption of the mechanism. ---Page Break--- 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 activation energy derived from the deamination kinetics k/x = 6.10^8, the maximum possible value which I can have due to the statistical considerations rate at 0.7 1 ut s" (calculated by method of G. v. seomate'), using a calibration substitute of 20° and so on. This gives an upper limit of 6.2 x 10^8 for ‘An alternative and complementary approach is to discuss the quenching constant in terms of k = 1.7 x 10^8, one of the intrinsic lifetime of the emission. If emission is from a singlet state, for which k is commonly 10^7, then k = 2.7 x 10^8, an impossibly high value. On the other hand, k_f

Emission ie trea a triplet level with T;= 10" sec, then k, = 1.7 x 20°, somewhat less than the activation-controlled rate. Accordingly, we conclude that the excited state 49 is probably a triplet level. To manifest, we find that the coordination of cytosine in aqueous solution leads to a relatively long-lived excited state, probably triplet, emitting weak luminescence at 580 nm, accompanied by self-quenching reactions leading eventually to deamination. A complete presentation of this work is in preparation. Puerto Rico was learned. Center: Malcolm Daniels, Department of Chemistry, University of Puerto Rico, Rio Piedras, Puerto Rico. Also Grimison (1) Hy Dandete and A. Gristcca, Biochemistry, Biophysics. Res. Commun. 16, 428 (1954) (2) Be RH 6, 2 (964) (5) 9. Ye sommes, a Pays. Shen. (Pexxurt) 8, 284 (1956) ---Page Break--- 200° ---Page Break--- W[aniso1ad] 01 40! 5 ° 3 = oo 01 x GHN) & ---Page Break--- [21180143] “orx” of o-ol o-3 ov oe Q ---Page Break--- Similar studies have been carried out on thymine. In aqueous solution at room temperature a clean extension X= 38cm has been found, confirming the report of Udenfriend. The pH variation of this extension 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 extension 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 dilute solutions at room temperature. The conditions for observing this extension, and its characteristics, are being determined. ---Page Break---