1-Phenyl-2-benzoyl-8-azacycl(2.2.2)azine, VII.—Heating 450 mg of VI in a sublimation tube at 240° caused rapid evolution of gas. The residue was taken up in a 2:1 mixture of benzene-Skelly Solve B and chromatographed over alumina (Woelm, activity 3). The solid obtained from the first eluate fraction was recrystallized from an ether-Skelly Solve B mixture to give 312 mg of orange crystals, mp 112–114°.

Anal. Calc. for $C_{21}H_{14}N_2O$: C, 81.27; H, 4.55; N, 9.03. Found: C, 81.49; H, 4.66; N, 9.19.

1-Phenyl-8-azacycl(2.2.2)azine, VIII.—A solution of 812 mg of VII and 5.0 gm of potassium hydroxide in 25 ml of 80% aqueous ethanol was sealed in a Carius tube and heated at 130° for 20 hr. After the tube was cooled and opened, the ethanol was removed and water was added. Extraction of the mixture with ether followed by drying and concentration gave 596 mg of a brown solid. This was taken up in a 3:1 mixture of benzene-Skelly Solve B and chromatographed over alumina (Woelm, activity 3) to give 456 mg of a pale-orange solid. Recrystallization of this from ethanol gave almost-white crystals, mp 124–125°.

Anal. Calc. for $C_{14}H_{10}N_2$: C, 81.53; H, 4.89; N, 13.58. Found: C, 81.50; H, 4.99; N, 13.86.

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TRIPLET EXCITONS IN POLYADENYLIC ACID

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Bersohn and Isenberg¹ reported that the phosphorescence of polyadenylic acid (poly A), observed in glasses at 77°K, can be quenched by small quantities of Mn^{2+} ions and suggested that triplet diffusion could explain the observations. We have made quantitative measurements of this quenching, and our data provide strong support for the triplet exciton model in which the diffusion length is at least 100 adenine residues.

Poly A is known² to exist in two conformations: the acid form is more than half protonated and is a hydrogen-bonded double helix, while the nonprotonated neutral form is a single strand. One cannot study the excited states of the double-strand form because, as we have shown,^{3, 4} both fluorescence and phosphorescence are quenched. In the single-strand form, ORD and optical absorption measurements have indicated⁵ a large degree of base stacking in aqueous solution at room temperature, which also exists⁴ in ethylene glycol:water (EG:H₂O) glasses below 250°K. The luminescence at 77°K from the single-strand form resembles that of the constituent AMP except for a red shift of ~ 100 Å (see Fig. 1), and higher quantum yields of both fluorescence and phosphorescence. Divalent paramagnetic ions like Mn^{2+} and Co^{2+} have been shown^{6, 7} to bind strongly to polynucleotides such as poly A and for the particular case of RNA, it has been established⁸ that they bind

to the phosphates. Considering all the binding experiments, it seems quite certain that they also bind to the phosphates in poly A.

Different poly A samples and the oligonucleotide (Ap)₇ were obtained from the Miles Chemical Corp., Elkhart, Indiana, as the potassium salts, and used without further purification. The sedimentation velocities were determined by the supplier. Random, single-stranded poly A₂U with $s = 4.2$, synthesized with *M. lysodeikticus* polymerase, was a gift from Professor J. Fresco. Poly d(AT) was extracted from the crab *Cancer borealis* by Dr. T. Yamane using the method of Davidson *et al.*⁹

Metal ions were added as chlorides. The polynucleotide concentration was 2×10^{-3} M in phosphates, unless otherwise specified. The pH was controlled by phosphate buffers at $\sim 1 \times 10^{-3}$ M. The divalent metal ions were bound almost exclusively to the polymers because their binding constants are higher than those of the inorganic phosphate buffers.

Emission spectra were obtained by means of a single-beam luminescence spectrometer, which by means of 150-cps choppers at auxiliary foci in the excitation and emission beams, can be operated in fluorescence, phosphorescence, or luminescence modes. Bausch and Lomb 500-mm grating monochromators were used as dispersing elements for the exciting and emitted light, and the wavelength drum was driven by a motor. The emitted light was detected by an EMI 9558QC photomultiplier, and after amplification and phase-sensitive detection, was recorded on a Sanborn recorder. All optical measurements were made at about 85°K in ethylene glycol: water (1:1) glasses with the samples in quartz tubes of 3-mm diameter.

The luminescence spectra of poly A with varying amounts of Co²⁺ added are shown in Figure 2. Note that the phosphorescence, which shows well-resolved

FIG. 1.—A comparison of the luminescence from poly A and AMP in EG:H₂O at 85°K. The dotted line shows the division between fluorescent and phosphorescent emission.

vibrational structure, is severely quenched, while the fluorescence, centered at about 3300 Å, is hardly affected by the Co²⁺ ions. For example, when r , the fraction of bases which have an ion bound to their phosphates, is 0.015, i.e., $r = [\text{Co}^{2+}]/[\text{PO}_4] = 0.015$, the phosphorescence intensity is reduced by a factor of 2, while there is no decrease in fluorescence intensity even when r is 20 times larger.

For small values of r when the average distance between bound ions is large compared to β , the number of residues quenched by a single bound ion, we may write

$$[P(0) - P(r)]/P(0) = \beta r, \quad (1)$$

where $P(r)$ is the phosphorescence intensity observed at a given value of r . As shown in Table 1, the phosphorescence quenching by all four iron group ions, i.e. Mn²⁺, Co²⁺, Ni²⁺, and Cu²⁺, was the same, within experimental error, while Mg²⁺ and Zn²⁺ did not introduce any measurable quenching. From the data at small values of r one finds that $\beta \sim 100$ for the ions which do quench and $\beta \sim 0$ for Mg²⁺ and Zn²⁺. The similar quenching range of the different iron group ions is consistent with a model in which triplet diffusion limits the extent of the quenching. To test this hypothesis, triplet quenching was measured in samples of poly A of different

FIG. 2.—The effect of Co^{2+} ions upon the luminescence of poly A at 85°K in $\text{EG:H}_2\text{O}$ showing the phosphorescence quenching.

FIG. 3.—The measured phosphorescence of poly A of different chain lengths showing the limiting quenching behavior at $s = 11$. The data for this sample are fitted to equation (2) by setting $\tau_j = 4.1 \times 10^{-4}$ sec.

TABLE 1

MEASURED VALUES OF $P(r)/P(0)$ FOR DIFFERENT METAL IONS IN THE POLY A ($s = 11$) SAMPLE SHOWING THE SIMILAR QUENCHING EFFICIENCIES OF THE TRANSITION METAL IONS AND THE LACK OF QUENCHING BY Mg^{2+} AND Zn^{2+}

r	Mn^{2+}	Co^{2+}	Ni^{2+}	Cu^{2+}	Mg^{2+}	Zn^{2+}
0	1.00	1.00	1.00	1.00	1.00	1.00
0.0030	0.63	0.71	0.70	0.87	1.02	1.00
0.0075	0.37	0.35	0.54	0.46	0.97	1.00
0.015	0.15	0.14	0.21	0.28	0.99	0.96
0.030	0.072	0.068	0.064	0.64	1.00	0.98
0.075	0.010	0.014	0.014	0.013	0.98	1.03
0.150	0.004	0.004	0.004	0.004	1.02	0.97

lengths, where the lengths were assumed to increase with the sedimentation velocity s . Figure 3 shows how the quenching of poly A with different s values depends on r . Note that the quenching efficiency increases with increasing s values, but that there is relatively little change in going from $s = 4$ to $s = 11$. This suggests that for the latter the quenching is limited by the triplet diffusion length rather than by the length of the polymer. Therefore, the $s = 11$ results can be compared with the diffusion theory developed by Bersohn and Isenberg¹ for DNA, although they lacked sufficient accurate data to test the model. The solid line in Figure 3 was calculated from their expression for diffusion-controlled quenching,

$$Q \equiv P(r)/P(0) = 1 - \alpha^2 \left\{ \frac{1}{\alpha} - \frac{1}{2} \Psi \left[1 + \frac{\alpha}{4} \right] + \frac{1}{2} \Psi \left[\frac{1}{2} + \frac{\alpha}{4} \right] \right\}, \quad (2)$$

where $\alpha = 2r(\tau_d/\tau_j)^{1/2}$ in which τ_d is the lifetime of the triplet state and τ_j is the time between jumps of the triplet to a neighboring chromophore. Ψ is the digamma function whose numerical values have been tabulated.¹⁰

The agreement between the curve calculated on this model and the data is excellent. The only adjustable parameter is τ_j and the best fit with the data was obtained for $\tau_j = 4 \times 10^{-4}$ sec. This value is consistent with our previous con-

clusions⁴ that $\tau_j > 2 \times 10^{-10}$ sec which was based upon the failure to observed spin-spin motional averaging of the triplet ESR in poly A as compared to AMP. From the measured value of τ_j one can calculate a one-dimensional diffusion constant $D = l^2/\tau_j = 2.8 \times 10^{-12}$ cm²/sec, where l is the distance between stacked bases. The diffusion length $L = \sqrt{D\tau_d}$ equals 78 residues or 260 Å during the 2.4-sec lifetime¹ of the adenine triplet. This is consistent with the approximate value of $\beta \sim 100$ determined from the initial slope of the quenching. (Note that β should be twice L .)

Preliminary ESR and phosphorescence measurements of decay times of the poly A triplets show no change of the decay times with quenching. These results are not consistent with a diffusion-limited model which we have used above, but could be explained by a model in which kinks or barriers to diffusion limit the range. The theoretical curve for this case¹¹ fits the data about as well as equation (2), and τ_j would be shorter than 4×10^{-4} sec.

When the number of residues per polymer is much smaller than β or $2L$, the observed residual phosphorescence will be proportional to the probability that all of the binding sites on a polymer are unoccupied by quenchers. In that case,

$$P(r)/P(0) = (1 - r)^n, \quad (3)$$

where n is the number of residues per polymer.

Figure 4 shows a plot of $\log P(r)$ as a function of $\log (1 - r)$ for the oligonucleotide (Ap)₇ with Co²⁺ added. The straight-line dependence is the one expected from equation (3), and the slope of the straight line agrees with $n = 7$. For comparison, the straight lines for $n = 5$ and 9 are also presented. This means that essentially all of the metal ions act as quenchers and one can reject the possibility¹ that the quenching action is due to a minority of metal ions binding, not at the phosphates, but at some position on the bases.

FIG. 4.—Quenching of the phosphorescence in (Ap)₇ by Co²⁺ compared with the solid lines representing $(1 - r)^n$ where $n = 5, 7$, and 9.

in 2'-5' ApC disagrees with the conclusions of Hélène *et al.*^{13, 14} who interpreted their results on 3'-5' ApC in ethanol:H₂O at 77°K, and in 3'-5' GpA H₂O:propylene glycol as indicating transfer.

Considering the absence of triplet transfer⁴ from A to U in both UpA and poly A₂U in which the polynucleotide was a random polymer in a single strand, we decided to test the hypothesis of triplet excitons by studying the metal ion quenching of the adenine phosphorescence in poly A₂U. The sample was prepared at a concentration of 2 mg/ml and Co²⁺ added up to $r = 0.10$. Whereas in poly A at this value of r the quenching factor was ~ 100 , in poly A₂U it was about 1.1. Therefore, in this polymer $\beta \sim 1$ instead of ~ 100 as observed in poly A of approximately the

essentially all of the metal ions act as quenchers and one can reject the possibility¹ that the quenching action is due to a minority of metal ions binding, not at the phosphates, but at some position on the bases.

The question arises whether the phenomenon of triplet transfer and triplet diffusion occurs in more heterogeneous nucleotide systems than poly A.

We have shown¹² that $\tau_j > \tau_d = 2.4$ sec in the stacked dinucleotides 2'-5' ApC, and 3'-5' UpA and ApG in EG:H₂O glasses at liquid nitrogen temperatures. This absence of triplet transfer

same length. This result is consistent with a model in which a U residue in poly A acts as a trap or a barrier for triplet energy transfer.

However, from the lack of transfer from A to U in poly A₂U and UpA, even though both have stacked bases,¹⁵ we conclude that the U acts as a barrier rather than a trap.

The measured value of τ_j in poly A is at least 10^4 times shorter than in the above-mentioned dinucleotides where we observe no triplet transfer. On the other hand, it is considerably longer than the jump time observed in benzene¹⁶ and anthracene¹⁷ crystals. Although the calculated exchange integrals¹⁷ are extremely sensitive to distance, it does not seem that this sensitivity alone could explain these large differences in jump times.

Summary.—Bersohn and Isenberg¹ reported that the phosphorescence of polyadenylic acid (poly A), observed in glasses at 77°K, can be quenched by small quantities of Mn²⁺ ions and suggested that triplet diffusion could explain the observations. We have made quantitative measurements of this quenching and our data provide strong support for the triplet exciton model in which the diffusion length is at least 100 adenine residues.

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