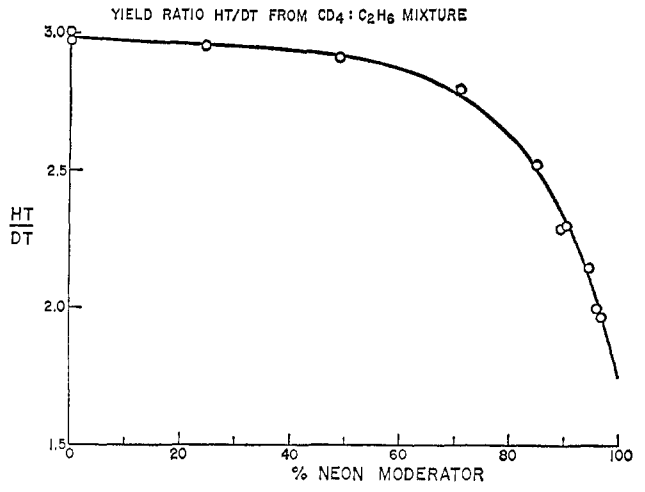

Figure 1. Variation of HT/DT ratio from recoil tritium reaction with equimolar CD_4–C_2H_6 mixture, as a function of moderation.

energies. In fact, by considering these magnitudes, it becomes evident that the mean energy of abstraction changes in the sequence CH_4, C_2H_6, C_3H_8 and also CD_4, C_2D_6, and C_3D_8. This is also the order of decreasing bond energies.

These findings are consistent with our result that abstraction by recoil tritium occurs, on the average, at higher energies than does displacement.[6,7]

$$T + RH \longrightarrow RT + H$$

They indicate further that the mean energy of abstraction increases with decreasing bond strengths. High-energy stripping reactions of neutral hot atoms were first postulated to account for the effect of bond strengths on hydrogen yields.[5] Confirmation of the consequent prediction[6] that abstraction can occur at high mean energies, and that it is this high-energy mode that seems to be enhanced when the hydrogen bond in the reactant is weakened,[8] provides strong support for the existence and importance of such a high-energy stripping mechanism.

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The Synthesis and Characterization of Poly d(I-C)·Poly d(I-C)[1]

Sir:

High molecular weight deoxyribopolynucleotides containing repeating base sequences are of interest as model compounds for a variety of chemical and biological studies. A series of such polymers has been recently synthesized by a combination of chemical and enzymatic techniques.[2] The copolymer which contains repeating adenylic and thymidylic acid units has been well characterized[3] and has been the subject of considerable investigation;[4] however, the polymer which contains repeating deoxyriboinosinic and deoxyribocytidylic acid units, poly d(I-C)·poly d(I-C),[5] has eluded preparation to date. Similarly, the synthesis of poly d(G-C)·poly d(G-C) has not been reported.

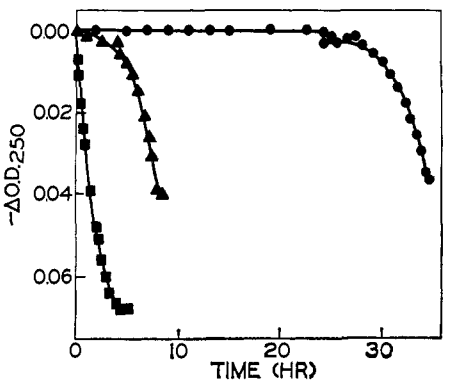

Figure 1. Polymer synthesis by the *Micrococcus lysodeikticus* DNA polymerase in the presence of dITP and dCTP. The reaction mixtures were prepared as described in the text. The development of hypochromicity at 250 mμ was used to follow polymer formation; ● indicates unprimed reaction in Tris-HCl buffer (pH 8.0), ▲ indicates poly dI·poly dC primed reaction in Tris-HCl buffer (pH 8.0), and ■ indicates poly dI·poly dC primed reaction in phosphate buffer (pH 7.3). Other details are described in the text.

We wish to report the synthesis of poly d(I-C)·poly d(I-C), a high molecular weight, helical deoxyribopolynucleotide containing strictly alternating deoxyribocytidylic acid and deoxyriboinosinic acid units. The polymer has been characterized by the following techniques: nearest neighbor frequency analyses, sedimentation velocity studies in both neutral and alkaline salt solutions, cesium chloride and cesium sulfate equilibrium buoyant density studies in both neutral and alkaline solutions, ultraviolet spectral data, and ultraviolet absorbance–temperature profiles. The physical properties of poly d(I-C)·poly d(I-C) are markedly different from the physical properties of poly dI·poly dC.[6]

Poly d(I-C)·poly d(I-C) was synthesized as a *de novo* product by the *Micrococcus lysodeikticus* DNA polymerase in the presence of dITP and dCTP (Figure 1). After a lag period of many hours the reaction exhibited exponential kinetics and was complete in 34.5 hr; the extent of hypochromicity developed at 250 mμ was 16%, which corresponded to the incorpora-

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(3) H. K. Schachman, J. Adler, C. M. Radding, I. R. Lehman, and A. Kornberg, *J. Biol. Chem.*, 235, 3242 (1960).

(4) H. Ch. Spatz and R. L. Baldwin, *J. Mol. Biol.*, 11, 213 (1965), and previous papers in this series.

(5) The abbreviations used herein have been explained in ref 2d.

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tion of approximately 50% of the radioactive triphosphates into acid-insoluble product. Poly d(I-C)·poly d(I-C) was synthesized as the major product in an anomalous reaction which was primed by poly dI·poly dC (Figure 1). This primed reaction was complete in 8.5 hr; 16% hypochromicity was developed, corresponding to the incorporation of 53% of the radioactive triphosphates. These syntheses of poly d(I-C)·poly d(I-C) were performed in Tris–HCl buffer at pH 8.0. However, when an identical poly dI·poly dC primed reaction was performed, except that phosphate buffer (pH 7.3) was substituted for the Tris–HCl buffer (pH 8.0), the product was pure poly dI·poly dC (see below for analyses of these reaction products). The faithful replication of poly dI·poly dC (Figure 1) in phosphate buffer (pH 7.3) proceeded quite rapidly under the standard reaction conditions; the reaction was complete in 5 hr, developing 27% hypochromicity which corresponded to 82% incorporation of the substrates.

The standard reaction mixture for the syntheses shown in Figure 1 was as follows: either Tris–HCl buffer (pH 8.0) or potassium phosphate buffer (pH 7.3) (0.05 *M*), magnesium chloride (0.005 *M*), mercaptoethanol (0.001 *M*), dITP[6a] and dCTP (each 0.1 m*M* including the appropriate radioactive deoxyribonucleoside triphosphate), poly dI·poly dC where indicated (buoyant density 1.598 g/cm^3 in Cs_2SO_4 (pH 7.3)) (0.025 m*M* total nucleotide), and *Micrococcus lysodeikticus* DNA polymerase[7] (specific activity 3370 units/mg; poly d(A-T)·poly d(A-T) assay) (50 units/ml). A second addition of enzyme (another 50 units/ml) was added to the unprimed reaction mixture after 24 hr of incubation. Reactions were performed at 37° in glass-stoppered quartz cuvettes (0.1-cm light path) in a thermostated Gilford spectrophotometer. For polymer isolation, the enzyme was heat denatured and the polymer was extensively dialyzed as previously described.[2d]

The reaction products were analyzed by nearest neighbor frequency analyses.[8] The product of an unprimed reaction which was prepared with α-^{32}P-labeled dCTP showed greater than 98% of the isotope was transferred to dIp. No radioactivity was observed in the region of dCp in two different chromatography systems. Nearest neighbor frequency analyses of polymers synthesized in the presence of poly dI·poly dC in Tris–HCl buffer (pH 8.0) showed, from preparation to preparation, variable amounts of contamination (from 1 to 25%) of poly d(I-C)·poly d(I-C) with poly dI·poly dC. As the primer contained no radioactivity these results indicate that both polymers were synthesized under these conditions. Hence, the product mixture was purified by preparative alkaline cesium chloride density gradient centrifugation.[9] A nearest neighbor frequency analysis on the purified product, which was prepared with α-^{32}P-labeled dCTP, showed greater than 99% of the isotope was transferred to dIp. No radioactivity was observed in the region of dCp; the dIp region (in two different chromatography systems) contained approximately 6000 counts/min. Hence poly d(I-C)·poly d(I-C) was not covalently joined by a phosphodiester linkage to the newly synthesized poly dI·poly dC since the two polymers were quantitatively separated by density gradient centrifugation.

This novel synthesis of poly d(I-C)·poly d(I-C) by the *Micrococcus lysodeikticus* DNA polymerase is not a general feature of the enzyme since it readily replicates poly dI·poly dC to provide more poly dI·poly dC in a phosphate-buffered (pH 7.3) reaction mixture (Figure 1). A nearest neighbor frequency analysis on this product, prepared with α-^{32}P-labeled dCTP in the reaction mixture, showed that greater than 99.5% of the isotope was transferred to dCp. Likewise, this enzyme has been used for the faithful replication of other DNA's by Zimmerman[7] (as well as unpublished work from our laboratory). Hence the role of poly dI·poly dC in the synthesis of poly d(I-C)·poly d(I-C) in Tris–HCl buffer (pH 8.0) may only be to provide a free hydroxyl growing point. Therefore, this enzyme, like the *Escherichia coli* DNA polymerase, seems very sluggishly (if at all) to initiate a new polynucleotide chain. Further studies of this anomalous behavior of the enzyme will be the subject of a later communication.

Pure poly d(I-C)·poly d(I-C) was efficiently and faithfully replicated under the reaction conditions detailed above (except that the triphosphate concentration was increased 5-fold and that poly d(I-C)·poly d(I-C) replaced poly dI·poly dC). An average of 13-fold synthesis was observed for two experiments. Nearest neighbor frequency analyses on preparations using either [α-^{32}P]dCTP or [α-^{32}P]dITP as the labeled triphosphate showed the quantitative transfer of the isotope to dIp and dCp, respectively. Thus, this reaction product can only be a polymer containing deoxyriboinosinic acid and deoxyribocytidylic acid units in strictly alternating sequence.

Poly d(I-C)·poly d(I-C) gave a single symmetrical band in analytical buoyant density gradient centrifugation experiments;[9] the density in cesium chloride solution (pH 7.3) was 1.735 g/cm^3 (marker DNA was poly d(A-T)·poly d(A-T), density 1.672 g/cm^3) and the density in cesium sulfate solution (pH 7.3) was 1.453 g/cm^3 (marker DNA was either T_4 bacteriophage DNA, density 1.444 g/cm^3, or poly d(A-T)·poly d(A-T), densty 1.424 g/cm^3). On raising the pH of the density gradient experiments to approximately 13.0 still only one symmetrical band was observed, a condition demanded for the structure poly d(I-C)·poly d(I-C) because of the identical base sequence of the two strands of the native polymer. The apparent buoyant densities of poly d(I-C)·poly d(I-C) in alkaline solutions were as follows: cesium chloride solution, 1.766 g/cm^3, and cesium sulfate solution, 1.436 g/cm^3. Poly d(A-T)·poly d(A-T) was the marker DNA in both salt solutions and had the following densities: 1.722 g/cm^3 [9] and 1.416 g/cm^3, respectively. The $s^0_{20,w}$ values of two different preparations of the polymer in 1 *M* NaCl solution were 19.9 and 15.8 S, indicating molecular weights of 6.4×10^6 and 3.3×10^6 daltons, respectively. The same two preparations had $s^0_{20,w}$ values in 0.1 *M* NaOH–0.9 *M* NaCl solution of 19.5 (2.7×10^6 daltons) and 17.3 S (1.9×10^6 daltons), respectively.[10] These data suggest that poly d(I-C)·poly d(I-C) was double helical in 1 *M* NaCl solution since the alkaline molecular

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weight value was approximately half (within ±8%) the value found in neutral salt solution.

That the polymer possesses an ordered helical structure was indicated by ultraviolet absorbance–temperature transition studies; poly d(I-C)·poly d(I-C) had a melting value (T_m) of 43° in 0.02 *M* sodium chloride solution (pH 7.2) and 54° in 0.1 *M* sodium chloride solution (pH 7.2). Ninety-five per cent of the absorbance transition occurred over a 3–5° range at both salt concentrations. For these studies the polymer was extracted with phenol and exhaustively dialyzed as previously described.[2d] Hence poly d(I-C)·poly d(I-C) has a considerably more stable structure than does poly dI·poly dC.[6] The molar extinction coefficient of poly d(I-C)·poly d(I-C) was 6.9×10^3 at the wavelength maximum (251 mμ) in 0.01 *M* NaCl–1×10^{-4} *M* EDTA (pH 7.0).

It is significant to note that the poly*ribo*nucleotide with alternating inosinic acid and cytidylic acid units is synthesized *de novo* by the *Azotobacter vinelandii* RNA polymerase[11] whereas poly dI·poly dC is synthesized *de novo* by the *Escherichia coli* DNA polymerase.[6a] Further properties of poly d(I-C)·poly d(I-C) as well as the formation of poly d(G-C)·poly d(G-C) are currently being studied.

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Catalytic Decomposition of Hydrogen Peroxide by Copper Chelates and Mixed Ligand Complexes of Histamine in the Presence of Phosphate Buffer in the Neutral pH Region[1]

Sir:

The catalytic decomposition of H_2O_2 to oxygen and water, *i.e.*, *catalatic reactions*, by metal salts and complexes has been extensively studied.[2] Catalatic activity is usually associated with catalase,[3] a hemoprotein, and iron compounds and chelates.[2,4–6] The nonheme, oxygen-carrying copper protein, hemocyanin, also exhibits catalatic activity[7–9] as do copper salts and chelates.[10–13] Many copper and iron complexes are also *peroxidatic; i.e.*, they promote the oxidation of hydrogen donors such as alcohols, phenols, and amines.[3,7,13d,14] Thus, the complex compounds of copper and iron provide relatively simple model structures for investigating the mechanisms of action of vitally important types of enzymes. However, for several reasons, no significant quantitative studies on catalatic reactions of copper complexes exist from which reaction mechanisms can be deduced. For example, previous investigations included various simplifying assumptions regarding the species of copper complexes present such as in 1:1 mixtures of Cu^{2+} and ligand, L, where it was arbitrarily assumed that no CuL_2 existed. Nor were the known[15] formations of hydrolyzed species taken into account, *e.g.*, LCuOH and $L_2Cu_2(OH)_2$. Experimentally, little or no attempt was made to maintain constant pH. In view of these oversimplifications, we are investigating catalatic systems involving copper and amino acids and related compounds in the pH region of ~6–9 in sodium dihydrogen phosphate buffer. We have taken into account the formation of simple, mixed, and hydrolyzed complex species. In this report, employing histamine as the ligand, our data conform best to a combination of a free-radical and molecular mechanisms involving the 1:1 chelate of copper–histamine.

The rates of decomposition of H_2O_2 were determined from manometric measurements of O_2 by the use of a differential syringe manometer.[16] The reproducibility of our readings was ±5%, and the absolute accuracy as determined by measurement of known volumes of O_2 released from spectrophotometrically standardized solutions of H_2O_2 by crystalline catalase was ±4%. The rates of O_2 evolution were determined by presetting the micrometer syringe corresponding to a desired volume, generally from 3 to 20 μl, and the time required for the manometric fluid to reach the reference mark in a horizontal capillary was recorded. At this point the micrometer was reset for the next reading, etc., until the run was complete. The apparatus was maintained on a single station of a Warburg bath and the reaction reference flasks were completely immersed in the constant-temperature water bath. The rates of O_2 evolution were independent of shaking speeds above 80 cycles/min: a speed of 115 cycles/min was used throughout. Under the experimental conditions employed, only those solutions containing H_2O_2, copper, and histamine possessed significant catalatic activity.

In a series of experiments run at 25° and pH 7.0, we found that the over-all reaction rate, k_{obsd}, varied as the first power of the total molar concentration of copper ion, $[Cu^{2+}]_T$ and $[H_2O_2]_T$. In order to identify the cata-

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