A detailed study of the kinetics and mechanism of each of these reactions is now underway in this laboratory and will be reported later.

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its structure [G. Oster, J. S. Bellin and B. Holmstrom, *Experientia*, **18**, 249 (1962)]. Polarographic studies in this laboratory indicate both FMN and lumichrome (LC) are formed upon reoxidation of photoreduced FMN.

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THE SEPARATION OF GUANOSINE OLIGONUCLEOTIDES: USE OF UREA TO AVOID AGGREGATE FORMATION

Sir:

Separation of guanine-rich oligonucleotides by usual methods is difficult and unreproducible, presumably because of aggregation of such compounds. We wish to report that a satisfactory separation can be carried

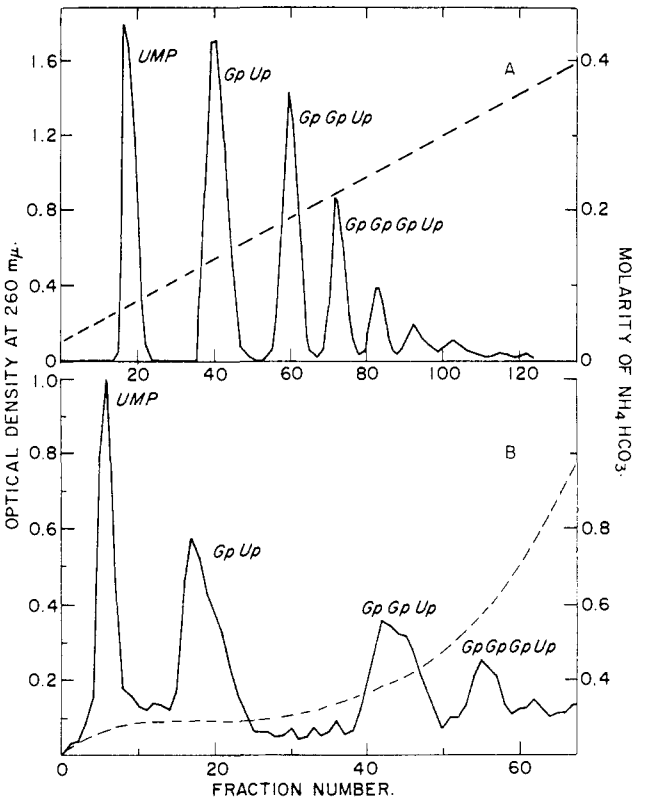

Fig. 1.—A. Separation of a pancreatic ribonuclease digest of poly GU (570 O.D. units at 260 mμ) adsorbed on a 12.8 × 2 DEAE-cellulose–bicarbonate column, eluted with a linear gradient. The mixing chamber contained 750 ml. of 0.03 *M* NH_4HCO_3 in 7 *M* urea, the reservoir 750 ml. 0.5 *M* NH_4HCO_3 in 7 *M* urea, both at pH 8.6. Samples of 8 ml. were collected at a flow rate of 2 ml./min. B. Similar to (A), 184 O.D. units at 260 mμ adsorbed on a 10 × 1 cm. column, eluted with a 600 ml. non-linear gradient[7] of NH_4HCO_3, pH 8.6, as shown, collecting 10 ml. samples at a flow rate of 0.75 ml./min.

out by DEAE-cellulose chromatography with the addition of 7 *M* urea, as described by Tomlinson and Tener[1] for the separation of ribonuclease digests of nucleic acids.

It has been shown[2] recently that deoxyguanosine oligonucleotides possess a secondary structure and are capable of forming aggregates. We have independent evidence that points to secondary structure in guanosine oligonucleotides. (1) A mixture of guanine-containing oligonucleotides of average chain length equal to 6 was unusually resistant to Takadiastase ribonuclease T_1[3] and pork liver nuclease.[4] A preliminary heating of the substrate resulted in somewhat more rapid hydrolysis, but the rates still were very slow. (2) Removal of terminal phosphate by *E. coli* alkaline phosphatase[5] was extremely slow and incomplete for GpGpGpGp, GpGpGpUp and homologs of higher molecular weight. (3) Paper chromatograms of pancreatic ribonuclease digests of poly GU[6] showed the presence of 3′-UMP, GpUp, GpGpUp and GpGpGpUp as discrete bands, but nothing else migrated from the origin even after several days development in the tank. Material at the origin was hydrolyzed and found to have a ratio guanine/UMP of 4.2/1. This indicates that oligonucleotides containing uracil did not display the expected mobility in the solvent system. Further, when GpGpUp, GpGp, GpG or larger homologs of these were re-chromatographed, over a third of the material remained at the origin.

The use of 7 *M* urea with the DEAE-cellulose column appears to prevent aggregation. With urea (Fig. 1), a simple linear gradient gave sharper peaks, better resolution and better return of optical density to the baseline between peaks than was obtained without urea and using a gradient deliberately flattened[7] to allow maximum resolution of the first four peaks.

Striking differences also were noted when single compounds were chromatographed on DEAE. Thus, GpGpUp (40.2 O.D. units at 260 mμ) was applied in 7 *M* urea to a DEAE-bicarbonate column (2.8 cm.^{3}) and eluted, with a linear gradient of NH_4HCO_3 in 7 *M* urea, as a single peak. Recovery was 40.8 O.D. units (101%). By contrast, chromatography of GpGpUp in the absence of urea led to recovery of only 65% of the material in the expected elution position, and even eluting the column with high salt did not completely remove the remainder.

Similarly, a digest containing a mixture of guanine oligonucleotides with 2′ (or 3′) phosphate end groups, eluted in the presence of urea, yielded a series of sharp peaks, of which the first five accounted for over 90% of the starting material. Chromatography as usually carried out with DEAE[7] or Dowex-1-Cl^-, 2× cross-linked,[8] was quite unsatisfactory, since even a compound as simple as GpG could not be recovered quantitatively.

(1) R. V. Tomlinson and G. M. Tener, *J. Am. Chem. Soc.*, **84**, 2644 (1962).
(2) R. K. Ralph, W. J. Connors and H. G. Khorana, *ibid.*, **84**, 2265 (1962).
(3) K. Sato-Asano and F. Egami, *J. Biochem.* (Japan), **44**, 753 (1957).
(4) M. N. Lipsett, L. A. Heppel and W. E. Razzell, unpublished data.
(5) A. Torriani, *Biochim. et Biophys. Acta*, **38**, 460 (1960).
(6) Poly GU is a random copolymer of uridylic and guanylic acids.
(7) Using the varigrad of E. A. Peterson and H. A. Sober, *J. Am. Chem. Soc.*, **78**, 751 (1956); *Anal. Chem.*, **31**, 857 (1959).
(8) E. Volkin and W. E. Cohn, *J. Biol. Chem.*, **205**, 767 (1953).

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CONCERNING THE STRUCTURE OF THE GRIGNARD REAGENT

Sir:

Although the Grignard reagent has been in use for over sixty years, considerable confusion exists as to the precise nature of this reagent in ether solution. The composition of the Grignard reagent has been represented most often by the equilibria[1]

$$2RMgX \leftrightharpoons R_2Mg + MgX_2 \leftrightharpoons R_2Mg \cdot MgX_2$$

(1) M. S. Kharasch and O. Reinmuth, "Grignard Reactions of Nonmetallic Substances," Prentice-Hall, Inc., New York, N. Y., 1954.