

will be given at a later date. Equation 1 (ref. 3) was used to calculate the charge on iron:

$$q_j = \sum_r \sum_{i \neq j} n_r C_{rj}^2 + \sum_r \sum_{i \neq j} n_r C_{rj} C_{ri} S_{ji}$$

$$q_{\text{atom}} = Q - \sum_j q_j \quad (1)$$

where q_{atom} = the signed charge of the atom in question; Q = number of electrons on the unperturbed atom; q_j = electron density in the j^{th} atomic orbital; n_r = number of electrons in the r^{th} filled energy-level; C_{rj} = wave function coefficient of the j^{th} atomic orbital in the r^{th} energy-level; S_{ji} = overlap integral value between the i^{th} and j^{th} orbitals of the system. The index j is over the orbitals of the atom in question, whereas index i is over all the orbitals of the system. Index r denotes only the filled energy-levels.

The result of our calculation is $q_{\text{iron}} = +2.02$ based on the Pauling structure⁴ for oxygenated haemoglobin. The Pauling theory of the oxygen-iron complex is that an electron pair (probably sp^2) is donated from oxygen to an empty d^2sp^2 orbital of iron as a normal octahedral chelate is formed. Then there is back donation from a $d\pi$ orbital of iron into a higher π_g orbital of oxygen. Essentially Weiss predicts that this back donation is quite large. If the first electron pair is shared equally between ferrous iron and oxygen, and if there is no back donation, the calculated charge would be $q_{\text{iron}} = +1$. However, if back donation is so large that the iron is essentially ferric, and if the first electron pair is again equally shared, q_{iron} would be $\sim +2$. Thus the calculated value of $q = +2.02$ shows that back donation is very largely in agreement with the theory proposed by Weiss.

This work was supported by the U.S. Public Health Service grants *FR-00009* and *RG 08285*.

One of us (R. O. V.) received financial support through a National Science Foundation graduate fellowship.

RICHARD O. VIALE
GERALD M. MAGGIORA
LLOYD L. INGRAHAM

Department of Biochemistry and Biophysics,
University of California,
Davis.

¹ Weiss, J. J., *Nature*, **202**, 83 (1964).

² Ballhausen, C. J., *Introduction to Ligand Field Theory*, 152 (McGraw-Hill Book Company, Inc., New York, 1962).

³ Muller, Norbert, Pickett, Lucy W., and Mulliken, Robert S., *J. Amer. Chem. Soc.*, **76**, 4770 (1954).

⁴ Pauling, L., in Roughton, F. J. W., and Kendrew, J. C., *Joseph Baneroff Memorial Symposium on Hemoglobin*, 57 (Interscience Pub. Duc., New York, 1949).

Sequence Specificity in Synthetic Polydeoxyribonucleotides

KORNBERG has shown that enzymatic synthesis of polydeoxyribonucleic acids from mononucleotides is possible even in the absence of a primer DNA molecule^{1,2}.

When mixtures of the two deoxyribonucleoside-5'-triphosphates, the heterocyclic bases of which were thymine and adenine, were used, Kornberg found that the polynucleotide formed after an initial time-lag was copolymer (Poly dAT) in which the bases alternated¹. When the same procedure was carried out with the corresponding nucleotides containing the bases, guanine and cytosine, the product was a mixture of the two homopolymers, Poly dG and Poly dC, not always in equal amounts.

In both cases, in which exactly equimolar amount of the two mononucleoside triphosphates were used, there was evidence for double helix formation with base-pairing of the Crick-Watson type².

In order to determine how far this sequence specificity is a result of enzyme stereochemistry or simply a result of

the molecular configurations of the nucleotides themselves, we have repeated the polymerization using equimolar amounts of the thymine and adenine deoxyribonucleoside-5'-phosphates, the thymine derivative being labelled with phosphorus-32 and a chemical condensing agent (dicyclohexylcarbodi-imide)³.

In syntheses leading to deoxyadenosine polynucleotides, Khorana found it necessary to acylate the 6-amino group of the purine⁴. In our work, however, we have avoided the use of this blocking group in order that the molecular configurations should be as close as possible to those in the unprimed enzymatic synthesis. Parallel experiments are planned with systems incorporating the acylated nucleotide to determine the influence of these protecting groups on the sequence specificity.

The nearest neighbour analysis method was used on the polymeric product which had been freed from small molecules by dialysis and afterwards degraded by the action of spleen phosphodiesterase and micrococcal DNase producing nucleoside-3'-phosphates. Thus the labelled phosphate which enters the polymer as thymidine-5'-phosphate appears in the hydrolysate as the 3'-phosphate of the nucleoside which was next to the thymidine in the polymeric chain.

The nucleotides were separated by paper chromatography; the spots were cut out and their activity measured.

If homopolymers are formed (for example, of the Poly dG-Poly dC type), all the activity should be found in the originally labelled nucleoside, thymidine, in the form of the 3'-phosphate. If, however, there is co-polymerization with a perfect alternation of bases, all the activity should be transferred to the initially unlabelled nucleoside, deoxyadenosine. Co-polymerization with a random sequence of bases should lead to both nucleoside-3'-phosphates having equal labelling.

In our experiments, we have found that the polymeric material, on degradation, yields a mixture of nucleoside-3'-phosphates in which approximately 20 per cent of the labelled phosphate has been transferred to the 3'-position of the deoxyadenosine.

Thus it is clear that there is some co-polymerization, but that there is neither a perfectly alternating sequence nor a completely random sequence.

Further experiments, in progress, using labelled deoxyadenylic acid should indicate how far the result may be explained by loss of deoxyadenylic acid from the polymerizing system as a result of the unprotected amino group.

We thank the Department of Scientific and Industrial Research for a grant for equipment and Unilever, Ltd., for a research studentship (G. R. B.). We also thank the Radiochemical Centre, Amersham, for a gift of ³²P-labelled thymidine-5'-phosphate.

D. COHEN
G. R. BANKS

Department of Chemistry,
University of Keele,
Staffordshire.

¹ Schachman, H. K., Adler, J., Radding, C. M., Lehman, I. R., and Kornberg, A., *J. Biol. Chem.*, **235**, 3242 (1960).

² Radding, C. M., Josse, J., and Kornberg, A., *J. Biol. Chem.*, **237** (1962).

³ Gilham, P. T., and Khorana, H. G., *J. Amer. Chem. Soc.*, **80**, 6212 (1958), and subsequent papers in this series.

⁴ Ralph, R. K., and Khorana, H. G., *J. Amer. Chem. Soc.*, **83**, 2926 (1961).

A Flow Method for Determining the Thermal Conductivity of Gas Mixtures

THE thermal conductivity of a mixture of two gases does not, in general, vary linearly with the composition of the mixture. For two gases of similar molecular weight the degree of non-linearity is small, while for systems of widely different molecular weight, linearity rarely extends beyond 0.05 mole fraction from either pure component.