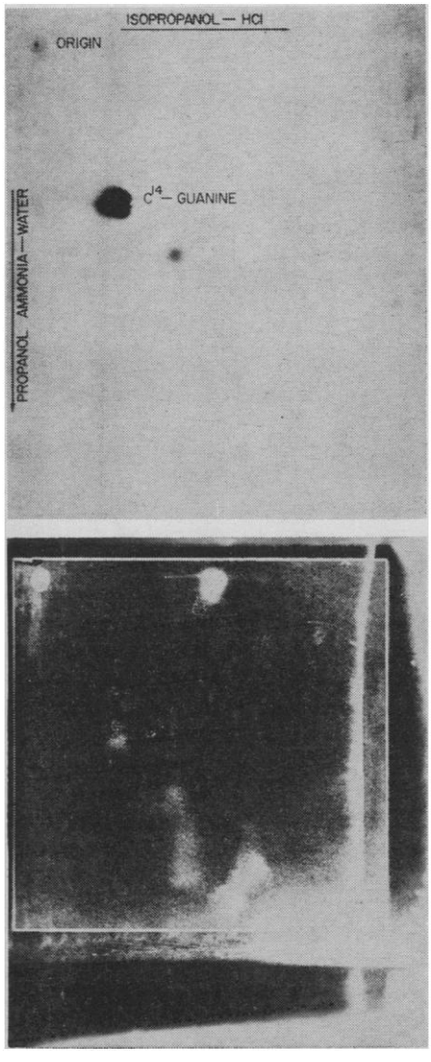


Fig. 2. The coincidence of the $C^{14}$-guanine spot on the autoradiograph (top) and the ultraviolet-absorbing spot on the shadowgram (bottom) illustrates the formation of guanine during the thermal polymerization of amino acids. The large ultraviolet absorbing spot to the right of the origin is probably due to a free amino acid.

absorbing areas on the chromatogram appeared as white spots on a dark background. By this method the smallest amount of purine or pyrimidine that could be detected was 0.03 $\mu$g. The paper chromatogram of the material eluted from the area corresponding to the guanine standard on the thin-layer chromatogram showed an ultraviolet absorbing spot having the $R_F$ values of guanine in the two solvents used.

In a second experiment the eluted material was chromatographed again with a trace of $C^{14}$-labeled guanine. The amount of guanine-$C^{14}$ used was large enough to darken an x-ray film after an exposure of 10 days, but too small to show any ultraviolet absorption on a shadowgram. The darkening of the x-ray film corresponded in shape and position to the ultraviolet-absorbing area of the shadowgram (Fig. 2). This coincidence was obtained in four different solvent systems: propanol, ammonia, and water (60:30:10 by volume); isopropanol and 2*N* HCl (65:35 by volume); butanol, anhydrous formic acid, and water (77:10:13 by volume); and butanol and water (86:14 by volume). Guanine was the only base detected by this technique. The ultroviolet spectrum at *p*H 1 of the material eluted showed maxima at 2750 Å and 2480 Å confirming the presence of guanine.

In a control experiment the identical mixture of amino acids was subjected to the entire experimental procedure except the polymerization by heat. No guanine was detected. In the experiment in which guanine was detected the conditions—heating to 200°C, hydrolysis with 6*N* HCl, extraction with 2*N* $NH_4OH$—were sufficiently rigorous to preclude any significant microbial contamination during the process itself.

The amount of material formed is extremely small. In six different runs 5 g of proteinoid gave an average yield of 0.6 $\mu$mole of guanine. During the hydrolysis of the proteinoid by 6*N* HCl some of the guanine must have been lost by conversion into xanthine. None of the other bases—adenine, cytosine, uracil, or thymine—appear to be formed.

In order to investigate the possibility of these bases being formed in quantities less than 0.03 $\mu$g, which was the smallest amount detectable by the technique used, further experiments were performed incorporating chromatographically pure aspartic and glutamic acids uniformly labeled with $C^{14}$ into the mixture of amino acids. Radioactive guanine was detected in this experiment and confirmed our previous finding. The experiment did not, however, reveal the presence of any of the other bases.

The mechanism of the synthesis is obscure. Hydrogen cyanide, which is known to be an intermediate in purine synthesis (*7*), may be produced by the pyrolysis of amino acids. The results of the experiments with labeled material show that both aspartic acid and glutamic acid may contribute to the synthesis of guanine. It is not clear to what extent the other amino acids which were used in the proteinoid synthesis took part in the formation of guanine. It is suggested that the formation of guanine during the thermal polymerization is relevant to a discussion on chemical evolution. The yield of guanine in this process, although small, may be considered significant in the context of a time scale of the order of several hundred million years.

CYRIL PONNAMPERUMA
RICHARD S. YOUNG
ELAINE F. MUNOZ
BARBARA K. MCCAW

*Exobiology Division,*
*National Aeronautics and*
*Space Administration, Ames Research Center, Moffett Field, California*

**References**

1. S. W. Fox, *Science* **132**, 200 (1960); ——— and K. Harada, *J. Am. Chem. Soc.* **82**, 3745 (1960).
2. E. Katchalski, *Advan. Protein Chem.* **6**, 123 (1951).
3. S. W. Fox and K. Harada, *Science* **128**, 1214 (1960); ——— and A. Vegeotsky, *Experimentia* **15**, 2 (1959).
4. The silica gel G for thin-layer chromatography was supplied by Research Specialties Company, Richmond, Calif.
5. T. Wieland and L. Bauer, *Angew Chem.* **63**, 511 (1951).
6. C. Ponnamperuma, R. M. Lemon, M. Calvin, *Science* **137**, 605 (1962).
7. J. Oro and A. R. Kimball, *Arch. Biochem. Biophys.* **93**, 166 (1961); C. Ponnamperuma and R. Mariner, 19th International Congress of Pure and Applied Chemistry, London (July 1963).

3 February 1964

## X-ray Diffraction Study of a DNA Which Contains Uracil

Abstract. *Three-dimensional structure of DNA from PBS2 bacteriophage, which contains uracil in place of the more usual thymine, is the same as that of "normal" DNA. The difference in secondary structure between DNA and RNA thus appears to be due to the 2'-hydroxyl group alone.*

The DNA of the *Bacillus subtilis* transducing bacteriophage PBS2 has the unusual property of containing uracil in place of thymine (*1*). Since all DNA samples studied so far appear to have essentially effectively identical three-dimensional structures (*2*) which differ from RNA (*3*), we were interested in finding the effect on the DNA structure of a base

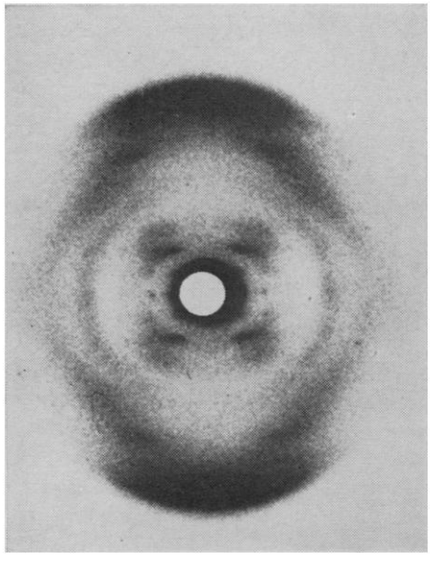

Fig. 1. X-ray diffraction photograph from a PBS2 DNA fiber 75 $\mu$ in diameter at 75 percent relative humidity. Fiber axis vertical.

which has usually been regarded as a component of RNA only.

It seems unlikely a priori that the absence of a methyl group in the 5 position, which constitutes the only difference between uracil and thymine, could have a marked effect on the three-dimensional structure of the nucleic acid. However, since this difference in base composition and the absence of the 2'-hydroxyl in DNA are the only major chemical differences between the two nucleic acids, it is a point which needs experimental clarification.

Bacteriophage PBS2 was grown on *B. subtilis* strain SB19. The bacteriophage lysate, concentrated by centrifugation, was shaken gently with buffered phenol to extract the DNA. After several extractions the DNA in the aqueous layer was dialyzed free of phenol. The DNA was then precipitated with alcohol and dried with acetone. Fibers about 0.05 mm to 0.1 mm in diameter were drawn from a rewetted gel of the DNA.

X-ray diffraction photographs of the fibers were taken in microcameras, the instances between specimen and film being 1.6 cm and 2.7 cm and exposure time about 24 hours. The water content of the fibers was controlled by filling the cameras with helium which had been bubbled through appropriate saturated salt solutions.

The x-ray diffraction photographs are not of high quality but show a pattern typical of that given by DNA in the B configuration, at both 75 and 92 percent relative humidity (Fig. 1). A simple comparison with other DNA's is made difficult by the extensive glucosylation of PBS2 DNA. Twenty percent of the guanine and 60 percent of the cytosine residues are glucosylated (*1*). The DNA from *Escherichia coli* bacteriophage T2 gives similar diffraction diagrams (*4*) and is also extensively glucosylated. This glucosylation has been proposed as the reason for the lack of crystallinity and for the poor diffraction patterns given by this DNA as compared to other DNA's—the glucosylation of the bases causes steric hindrance in the packing of the molecules (*4*). A similar explanation seems reasonable for PBS2 DNA. The diffraction pattern is quite characteristic of DNA, so that the structure is clearly a two-chain helical polynucleotide with chains running in opposite directions and with Watson-Crick base pairs between the chains. The base pairs are perpendicular to the helix axis.

While the conclusion is not completely clear-cut—a uracil-containing DNA which is not glucosylated would be a more suitable model—the similarity to DNA of bacteriophage T2, and the clear difference from RNA, makes it likely that the substitution of uracil for thymine has no major effect on the overall three-dimensional configuration of a two-chain base-paired helical nucleic acid. The presence or absence of the 2'-hydroxyl group appears to be the deciding factor.

ROBERT LANGRIDGE
*Children's Cancer Research Foundation, Children's Medical Center,* and *Harvard Medical School, Boston, Massachusetts*

JULIUS MARMUR
*Department of Biochemistry, Albert Einstein College of Medicine, New York*

**References and Notes**

1. I. Takahashi, *J. Gen. Microbiol.* **31**, 211 (1963); ——— and J. Marmur, *Biochem. Biophys. Res. Commun.* **10**, 289 (1963).
2. R. Langridge, W. E. Seeds, H. R. Wilson, C. W. Hooper, M. H. F. Wilkins, L. D. Hamilton, *J. Biophys. Biochem. Cytol.* **3**, 767 (1957); R. Langridge, H. R. Wilson, C. W. Hooper, M. H. F. Wilkins, L. D. Hamilton, *J. Mol. Biol.* **2**, 19 (1960).
3. R. Langridge and P. J. Gomatos, *Science* **141**, 694 (1963).
4. L. D. Hamilton, R. K. Barclay, M. H. F. Wilkins, G. L. Brown, H. R. Wilson, D. A. Marvin, H. Ephrussi-Taylor, N. S. Simmons, *J. Biophys. Biochem. Cytol.* **5**, 347 (1959).
5. Supported by NIH research grant CA-6570 to R. Langridge and a grant from the AEC to J. Marmur, who is a career scientist of the Health Research Council of the City of New York.

14 February 1964

## Unusual Aggregation of a Nonfunctional Tobacco Mosaic Virus Protein

Abstract. *The nonfunctional virus protein isolated from plants infected with the PM2 strain of tobacco mosaic virus aggregates to form elongated, two-stranded, open helical structures, in marked contrast with functional tobacco mosaic virus protein which aggregates into rods. This unique type of aggregation may explain why the PM2 protein is unable to combine with viral nucleic acid to form stable infectious virus particles.*

The isolation of two defective tobacco mosaic virus strains was recently reported by Siegel *et al.* (*1*). Both strains exhibited biological properties which indicated that in the host plant the virus nucleic acids were unprotected by protein. One of these strains (PM1) engendered no protein serologically related to tobacco mosaic virus. The other strain (PM2), however, was shown to have a protein of low molecular weight, which is serologically cross-reactive with tobacco mosaic virus protein but which apparently is unable to combine with the virus nucleic acid in vivo. This nonfunctional virus protein aggregates in vitro into elongated particles when the *p*H of the solution is lowered. Elongated striate loops have been seen in infected cells (*2*) suggesting that PM2 protein also aggregates in the host.

Low molecular weight tobacco mosaic virus protein can be made to aggregate into rods that are morphologically very similar to intact virus particles (Fig. 1). If this aggregation occurs in the presence of tobacco mosaic virus RNA, infectious virus particles are reconstituted (*3*). On the other hand, if PM2 protein is made to aggregate under the same conditions in the presence of tobacco mosaic virus RNA, no in-