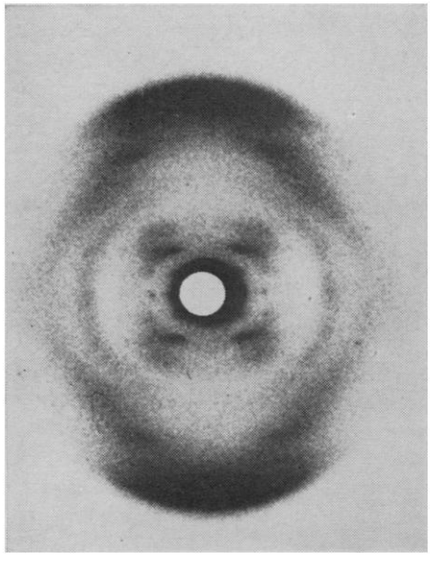

Fig. 1. X-ray diffraction photograph from a PBS2 DNA fiber 75 μ in diameter at 75 percent relative humidity. Fiber axis vertical.

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-

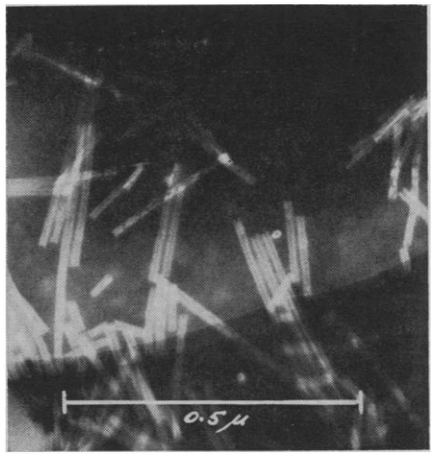

Fig. 1. Tobacco mosaic virus protein negatively stained at *p*H 5.3. The region at the bottom is supported by a carbon film, while the rest of the preparation has dried over a hole in the supporting membrane.

fectious virus particles are formed (*1*). The electron micrograph (Fig. 2) of the negatively stained aggregated PM2 protein may help to explain this behavior. The aggregated PM2 protein forms open, flexuous, helical structures rather than compact rods. The integrity and biological activity of tobacco mosaic virus RNA are normally stabilized when the RNA is sheathed in viral protein which thus shields it from the deleterious action of nucleases. Presumably, the open structure of aggregated PM2 protein could provide no such shield. Alternatively, the PM2 protein may have no affinity for virus RNA.

The electron micrographs of aggregated PM2 protein also show some single strands. We believe this may be an artifact of negative staining because micrographs of shadowed preparations show rope-like flexuous particles of a diameter characteristic only of double strands. Moreover, the particles are considerably longer in the shadowed preparations, indicating some breakage from negative staining with phosphotungstic acid (*4*).

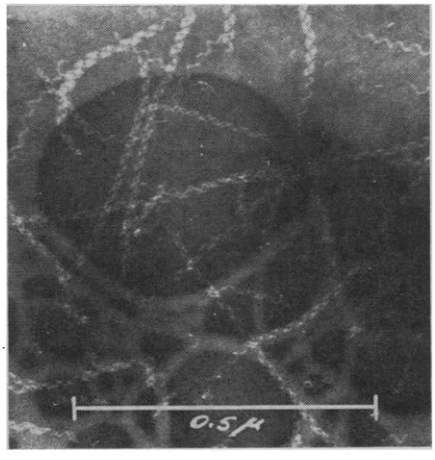

Fig. 2. An electron micrograph of PM2 protein negatively stained at *p*H 5.3. The protein particles extend over a network of holes in the membrane.

The increased resolution within the "holey" regions on both micrographs is of interest. Huxley and Zubay (*5*) have reported a reduction of flattening of *Escherichia coli* ribosomes and of turnip yellow mosaic virus particles accompanied by an increased visualization of structural detail where the droplet of phosphotungstic acid dries unsupported over the holes in the carbon film. The double strands of PM2 protein are most obvious in this region and appear to be narrower than where supported by the film. Outside of the hole the PM2 strands may be flattened during drying onto the membrane. Conversely, tobacco mosaic virus protein, does not show different dimensions in regions outside of the hole (Fig. 1).

Some of the PM2 helixes appear as right-handed coils whereas others seem left-handed. This is apparently an optical artifact, because in shadowed preparations all the particles are oriented in one way (*6*).

For negative staining with phosphotungstic acid we followed the procedure of Brenner and Horne (*7*). A 0.1-percent solution of either PM2 or tobacco mosaic virus "A" protein (*8*) was dialyzed in water at *p*H 8 for 4 hours at 2°C against 0.1*M* ammonium acetate adjusted to *p*H 5.3 with acetic acid. The aggregated protein was mixed with an equal volume of 2 percent phosphotungstic acid adjusted to *p*H 7, and sprayed onto carbon-coated screens. The screens contained holes and "pseudo holes" in the formvar membrane when prepared according to Harris (*9*). Before application of the specimens, the formvar was removed with chloroform.

MILTON ZAITLIN
WAYNE R. FERRIS
Departments of Agricultural Biochemistry and *Zoology, University of Arizona, Tucson*

References and Notes

1. A. Siegel, M. Zaitlin, O. P. Sehgal, *Proc. Natl. Acad. Sci. U.S.* **48**, 1845 (1962).
2. J. G. Bald, *Virology*, in press.
3. H. Fraenkel-Conrat and B. Singer, *Biochim. Biophys. Acta* **33**, 359 (1959).
4. Most particles in shadowed preparations are more than 5 μ in length, which explains why these aggregated preparations exhibit a very marked viscoelastic effect, characteristic of high molecular weight polymers.
5. H. E. Huxley and G. Zubay, *Proc. European Regional Conf. Electron Microscopy, Delft 1960* **2**, 703 (1961).
6. If you consider that you are viewing the particle end on, then the strands would be coiling clockwise as they approach you.
7. S. Brenner and R. W. Horne, *Biochim. Biophys. Acta* **34**, 103 (1959).
8. H. Fraenkel-Conrat, *Virology* **4**, 1 (1957).
9. W. J. Harris, *Nature* **196**, 499 (1962).
10. We thank Albert Siegel and Morris Cohen for their advice and interest. This work was supported in part by a grant from the National Science Foundation, by Atomic Energy Commission contract AT (11-1)-873, and grant award GM06327 of the National Institutes of Health. University of Arizona Experiment Station Technical Paper No. 865.

20 January 1964

Sensitivity of Female Inbreds of Cucumis sativus to Sex Reversion by Gibberellin

Abstract. *Two female inbred cucumbers were developed by substituting gene* Acr *for* acr *in the genetic backgrounds of the monoecious races Marketer and Tokyo, which exhibit weak and strong male tendency respectively. Marketer females are resistant and Tokyo females are sensitive to sex reversion in response to treatments with gibberellin* A_3. *Resistance and sensitivity of this type appear to depend upon the genetic system which controls sex tendency.*

The monoecious races of cucumber, *Cucumis sativus* L., are male early in development and female later. They vary greatly in sex tendency, depending on rate of sex reversion during plant development. Gene *Acr* accelerates the rate of sex reversion (*1*) and is often associated with complete femaleness (*2*).

Certain treatments with gibberellic acid or gibberellins increase male tendency in monoecious races (*3*) and induce staminate flowers in female inbreds (*1, 4*). However, most female inbreds, *Acr Acr*, are relatively resistant to sex reversion by gibberellic acid.

Previous results (*1*) suggested that some females might differ from each other in degree of resistance, depending on the potential sex tendencies of their genetic backgrounds. Hence, we attempted to synthesize resistant and sensitive female inbreds.

Two monoecious inbreds of the varieties Marketer and Tokyo were selected for synthesis. Marketer *acr acr* exhibits relatively weak male tendency, and Tokyo *acr acr* is strongly male