NATIONAL ACADEMY OF SCIENCES

JEROME VINOGRAD
1913–1976

A Biographical Memoir by

ROBERT L. SINSHEIMER

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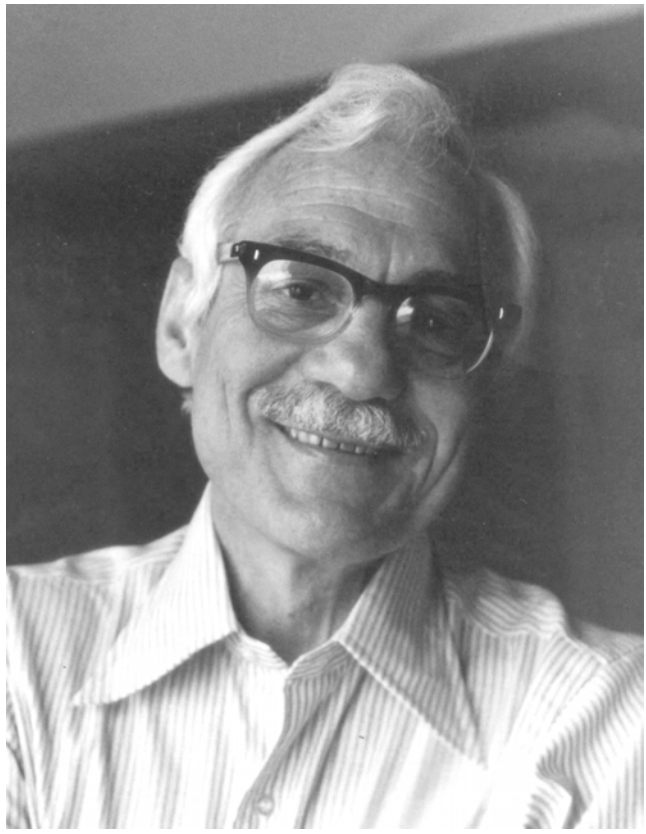
Biographical Memoir



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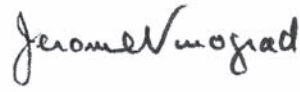

JEROME VINOGRAD

February 9, 1913–July 7, 1976

BY ROBERT L. SINSHEIMER

JEROME VINOGRAD WAS BORN ON February 9, 1913, in Milwaukee, Wisconsin. His academic career began with a series of two-year programs at the University of Minnesota (1929-1931), University of Berlin (1931-1933), and the University College, London (1933-1935). He then studied at the University of California, Los Angeles, receiving an M.S. in 1937 and then at Stanford, receiving a Ph.D. in 1940 in physical and colloid chemistry. His work at Stanford resulted in five publications concerning the use of detergents to solubilize otherwise insoluble dyes, among other topics.

In 1937 he married Sherna Shalett. They had two daughters, Julie and Deborah. He was subsequently divorced and in 1975 married Dorothy Colodny.

During 1941-1951, Vinograd worked for the Shell Development Company on the use of emulsion polymerization to produce synthetic rubber and on problems of catalysis to manufacture aviation gasoline. In 1951 he moved to the Department of Chemistry at the California Institute of Technology, first as a senior research fellow, then in 1956 as a research associate, and in 1966 as a professor of chemistry and biology.

Professor Vinograd was elected to the National Academy of Sciences in 1968. He received the Kendall Award

from the American Chemical Society in 1970 and the Helen Hay Whitney Foundation Duckett Jones Award in 1972.

Active with a variety of problems, Professor Vinograd was best known for two major areas of scientific accomplishment: the theory and application of density gradient ultracentrifugation and the study of the properties of closed circular DNA rings.

DENSITY GRADIENT ULTRACENTRIFUGATION

Vinograd's initial major contribution was the development of density gradient ultracentrifugation. This was stimulated by Matthew Meselson and Frank Stahl, who were seeking a means to implement their bold experiment to verify the hypothesis that DNA replication involved the separation of the two parental strands, one going into each of the two daughter DNA molecules.

Meselson and Stahl initially wanted to make the parental strands heavier than normal by incorporation of 5-bromouracil and sought Vinograd's advice as to whether 5-bromouracil-containing DNA could be separated from normal DNA by velocity ultracentrifugation. Vinograd indicated this seemed unlikely unless the velocity difference could be magnified by approximately matching the density of the DNA of the strands with a salt solution. From this germinated the concept of equilibrium sedimentation of macromolecules in density gradients and subsequently the famous experiment of Meselson and Stahl using the isotope of N^{15} instead of bromouracil.

In the equilibrium sedimentation method the macromolecule (DNA) is dissolved in a salt solution (CsCl) of the appropriate density and centrifuged to equilibrium (approximately 24 hours). At equilibrium, driven by sedimentation and diffusion, the CsCl will form a stable gradient of concentration, increasing in density with the radius. The larger

DNA molecules will form a narrow Gaussian band centered about the solution density equivalent to their own density. At equilibrium the DNA molecules are then distributed with respect to concentration in a band of width inversely related to their molecular weight. If the DNA molecules are of more than one discrete density (as in the Meselson and Stahl experiment) more than one discrete Gaussian band will appear. If the DNA varies broadly with respect to composition a skewed non-Gaussian distribution will appear. From the width of a Gaussian band and the gradient of CsCl density the molecular weight of the DNA can be calculated.

Several later papers refined the theory and extended the density gradient technique in its application to macromolecules. A variation that employed a lamella of the macromolecules layered on a self-generating density gradient permitted more rapid determination of their sedimentation and diffusion coefficients. This was applied to hemoglobin, MS2 RNA, T7 DNA, φX174 virus, and southern bean mosaic virus.

Further papers considered the solvation of DNA in CsCl as a function of the CsCl density and the solution temperature, the viscosity of CsCl as a function of its density, and the banding of RNA in a CsCl gradient. The addition of dimethylsulfoxide was shown to enhance the ability to band RNA in CsCl without aggregation.

STUDIES OF CIRCULAR DNA

Jerome Vinograd opened a new chapter in his research with his studies on closed circular DNA from several sources. It is interesting to follow the progression of this research project. The first paper of this series concerned the double-stranded DNA of polyoma virus, which was shown to sediment monomolecularly and not to lose infectivity after heating

to 100 degrees for 10 to 20 minutes. Sedimentation analysis under varied conditions and electron microscopy confirmed the presence of cyclic DNA (form I) and what were believed to be linear DNA molecules (form II).

Subsequent research demonstrated that form II was also circular but with one strand of the DNA helix cut. By investigating why the circular form I (an intact duplex) sedimented 20 percent faster than form II (also a duplex with one or more strand scissions) Vinograd demonstrated that form I was a twisted circular structure that could be converted to form II by enzymatic scission of one strand. This twisted circular form appeared twisted in electron micrographs. Its sedimentation behavior implied the presence of secondary left-handed turns. The binding of intercalative dyes, such as ethidium bromide, was shown in Vinograd's and other laboratories to cause a partial unwinding of the duplex DNA structure. In closed circular DNA such unwinding is accompanied by a change in the number of superhelical turns so that the total number of turns in the molecule remains constant. At a critical amount of dye binding the number of superhelical turns is zero. More dye binding results in superhelical turns of the opposite sign.

All of this can be followed in the ultracentrifuge. As the maximum amount of dye that can be bound by the closed circular molecule is less than can be bound by the linear or nicked circular molecule and as the buoyant density of the DNA-dye complex is inversely related to the amount of dye bound, the buoyant density of the closed circular DNA-dye complex is greater than that of the linear DNA or nicked circular DNA-dye complexes. At saturating amounts of ethidium bromide the buoyant density difference is approximately 0.04 gm/ml in CsCl. This difference provided a means to isolate closed circular DNA from the mitochondria of HeLa cells. Electron microscopy of this preparation

demonstrated not only the presence of closed circular DNA but also of small amounts of duplex or larger multiples of the mitochondrial DNA.

All of this work resulted in the formulation of the topological winding number,α, an invariant number, which characterizes the molecule. α is smaller than the secondary winding number, β, the expected number of turns for a DNA double helix of the size involved. γ is the number of superhelical secondary turns needed to achieve the maximum chemical stability, and $\gamma=\beta-\alpha$. Alkaline titration revealed an early titration of 3 percent to 4 percent of the base pairs of polyoma virus DNA, which suggested that the superhelix density is 0.03-0.04 yielding 15-20 superhelical turns in a DNA of 3 million molecular weight.

Further research demonstrated that intercalation of ethidium bromide causes an unwinding of the superhelical turns of 12 degrees per bound dye molecule. The binding of 30 dye molecules results in the removal of one superhelical turn. Thus, the native superhelical density can be determined by measurement through the region in which the superhelix changes sign by dye titration of the buoyant density.

Alternatively, at high dye concentration the buoyant density difference between open and closed forms of SV40 DNA was shown to be approximately constant because of the free energy required to form positive superhelixes. This buoyant energy difference is quantitatively related to the native superhelix density. Vinograd demonstrated that the initial superhelix density σ_c is related to v_c, the molar binding ratio at the dye concentration at which all superhelical turns are removed by $\sigma_c = 0.62\, v_c$.

The superhelix density of a closed circular DNA thus can be determined by measurement of its buoyant density in CsCl-ethidium bromide relative to that of a nicked ver-

sion of the same DNA or to that of a reference DNA (with appropriate correction for base composition).

The superhelix densities, determined by these methods, differ for DNA from various sources. The density for mitochondrial DNA from SV40-transformed mouse cells in culture is ~ 2×10^{-2}, while for DNA from the bacterial virus PM2 it is ~ 5×10^{-2}. Mitochondrial DNA from cells grown in media containing the intercalating dye ethidium bromide can have a superhelix density as high as 12×10^{-2}.

Subsequent studies were undertaken with "nicking and closing" enzymes, now called topoisomerases, which converted closed circular DNAs into a product set with a zero mean degree of supercoiling. The individual species of the sets differed by one, two, three, etc., of supercoils with the relative masses of each type fitting a Boltzmann distribution, with the energy of supercoiling proportional to the square of the degree of supercoiling. The enzymes can relax both positive and negative superhelical turns. The same set of products could be obtained by action of the enzymes upon separated rings of varied supercoiling.

MITOCHONDRIAL DNA

Further studies of mitochondrial DNA from varied sources found the common presence of circular oligomers and of catenated oligomers. In a CsCl-ethidium bromide density gradient the closed oligomer bands at the same density as the monomer, although its sedimentation velocity is significantly greater.

Catenated molecules can (for dimers) have both rings closed, one closed and one relaxed (nicked), or both relaxed. If both are closed, their sedimentation rate is the same as the circular dimer. If one ring is closed and one relaxed, the sedimentation rate is intermediate between a closed circular dimer and a relaxed circular dimer, demon-

strating that the two rings are connected. If, however, both rings are relaxed the sedimentation value is surprisingly less than that of a circular monomer (i.e., the two rings behave as though they are more or less independent).

Continuing studies of mitochondrial DNA molecules revealed the presence of replicating circles. These involved an initial D loop of some 450 nucleotides (the "heavy" strand), which is subsequently continued in the presence of a nicking process. Later replication of the "light" strand commences at about 60 percent from the "origin," proceeding counterclockwise.

The discovery that closed circular mitochondrial DNAs of about 10 million molecular weight invariably suffered chain scissions at high pH led to the suggestion of covalently incorporated ribonucleotides. This was confirmed by the quantitative conversion of closed mitochondrial DNA to nicked DNA by ribonuclease H. From the biphasic activity of the enzyme, two populations are present, one containing about 10 ribonucleotides, the other some 30 ribonucleotides.

SCIENTIFIC ACTIVITIES

The 1950s and 1960s were an era of great advances in molecular biology, and Caltech, with George Beadle and Max Delbruck and Linus Pauling, was a major center for this progress. Professor Vinograd thrived in this environment and, as described, made many contributions to this vibrant field. During this period of intensive research and discovery, Professor Vinograd was highly active in the scientific community. He gave nearly a dozen lectures each year at various colleges and universities, including the Burroughs Wellcome Lecture at Harvard in 1970, the Jesse W. Beams Lecture at the University of Virginia in 1972, and the Falk-Plaut Lecture at Columbia University in 1972, and participated in many scientific meetings. He mentored a continu-

ous stream of graduate students and postdoctoral fellows, including William Bauer, Robert Bruner, David Clayton, Lawrence Grossman, John Hearst, Bruce Hudson, James Ifft, Harumi Kasamatsu, Roger Radloff, Robert Watson, William Upholt, and Hans-Peter Vosburg.

Jerry, as he was known to his friends and colleagues, had an open personality, always ready to discuss scientific questions of common interest and quick to provide analytical insight.

Professor Vinograd died suddenly at the age of 63.

FOR THEIR ASSISTANCE, I am indebted to the staff of the archives of the California Institute of Technology.

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