though engorgement of DDT-poisoned insects might occur shortly after an application, it is doubtful that it could recur often enough to damage a fish population. Even after deliberate addition of DDT to a stream or pond, its rate of disappearance is rapid (*8*). Thus, it is doubtful that the concentrations of DDT capable of damaging a fishery by chronic toxicity, as determined in this experiment, would be reached frequently enough to do so, barring repeated flagrant misuse of the insecticide.

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Electron Microscopy of the Replicative Form of the DNA of the Bacteriophage ϕX174

Abstract. *Electron micrographs of surface films containing the replicative form of the DNA of bacteriophage ϕX174 show ring structures, whose average contour length is 1.64 μ, which have the characteristic appearance of double-stranded DNA throughout most of their length.*

The DNA of bacteriophage ϕX174 has been shown to be single-stranded (*1*) and to have a ring structure (*2*).

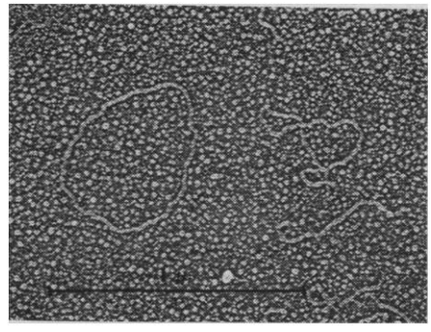

Fig. 1. Opened and twisted rings of ϕX-RF DNA with filamentous DNA of *E. coli*. Uranium contrast, negative. × 75,000.

Evidence has been presented (*3*) that during the intracellular reproduction of this virus, the viral DNA is converted to a double-stranded form, referred to as a "replicative" form, or RF, which is then multiplied. A purification of the replicative form has recently been described (*4*).

Our purification of the RF has included the use of fractional precipitation by cetyltrimethylammonium bromide (*5*) and of column chromatography, as described by Mandell and Hershey (*6*). The preparation obtained in this way is infective to protoplasts (*7*), and this infectivity has the resistance to inactivation by ultraviolet light and the buoyant density characteristic of the replicative form (*3*).

This material has been examined in the electron microscope by the monolayer technique of Kleinschmidt *et al.* (*8*). A solution of 2×10^{-6} g DNA per ml is mixed with 10^{-4} g/ml cytochrome *c* in 1*M* ammonium acetate, and 0.2 ml of the mixture is spread upon a clean surface of 0.1*M* ammonium acetate as hypophase in a Langmuir trough.

The DNA threads diffuse and part of them become adsorbed to the protein film. After full expansion to about 0.85 m² per mg of protein, the film is transferred to carbonized support films (*9*) and rotary shadowed with uranium (*10*). An appreciable fraction of the DNA threads are seen in the form of rings, either open or twisted to varying extent (Fig. 1). The appearance of these circular structures throughout most of their length is that characteristic of double-stranded DNA. Measurement of the length of the DNA in over 200 of these rings, both open and twisted, has given an average length of 1.64 ± 0.11 μ. Many of the

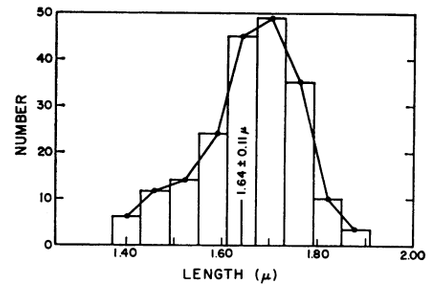

Fig. 2. Length distribution of ϕX-RF DNA of over 200 rings.

DNA threads in the preparation appear to have two ends, but only a small fraction of these are comparable in length with the rings.

If a Watson-Crick structure (*11*) is assumed for a double-stranded ϕX174 DNA, it should have a weight of 1.96×10^6 avograms (1 avogram = 1g/avogadro number) per micron (Na^+ salt). Thus the observed mean length corresponds to a molecular weight of 3.2×10^6, in remarkably good agreement with the calculated value of twice the weight of the viral DNA (1.7×10^6 avograms) (*1*).

It thus appears plausible to associate these structures with the replicative form of ϕX174 DNA and to conclude that the RF, like the viral form, occurs in a ring structure during the vegetative stage of the virus (*12*).

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