Short Communications

SC 2303

The electron microscopy of cesium chloride-treated DNA

During a study of Shope rabbit papilloma virus it was found that the virus-DNA molecules had an unusual appearance in the electron microscope, showing a fine electron dense "backbone" superimposed on the normal shadowed appearance. The virus had been purified by density-gradient centrifugation using CsCl, following the method described by CRAWFORD, CRAWFORD AND WATSON[1] for polyoma virus. It seemed possible that the appearance of the DNA was due to the uptake of cesium ions by the DNA during preparation. To investigate this further other DNA preparations were examined before and after exposure to CsCl.

The DNA samples were prepared as follows. DNA was extracted from purified Shope rabbit papilloma virus with sodium dodecyl sulphate as described by WATSON AND LITTLEFIELD[2]. *Micrococcus lysodeikticus* DNA was extracted from the bacteria using lysozyme and sodium dodecyl sulphate by the method of MARMUR[3]. The DNA solutions were diluted to 2–10 μg/ml in ammonium acetate (0.1 M, pH 6.8) and dried on to mica by a modification of the method of HALL[4] in the following way. Sheets of freshly cleaved mica were washed with a dilute solution of detergent (Photoflo, Kodak Ltd., diluted 1:1000 in water) to ensure that the surface was easily wetted.

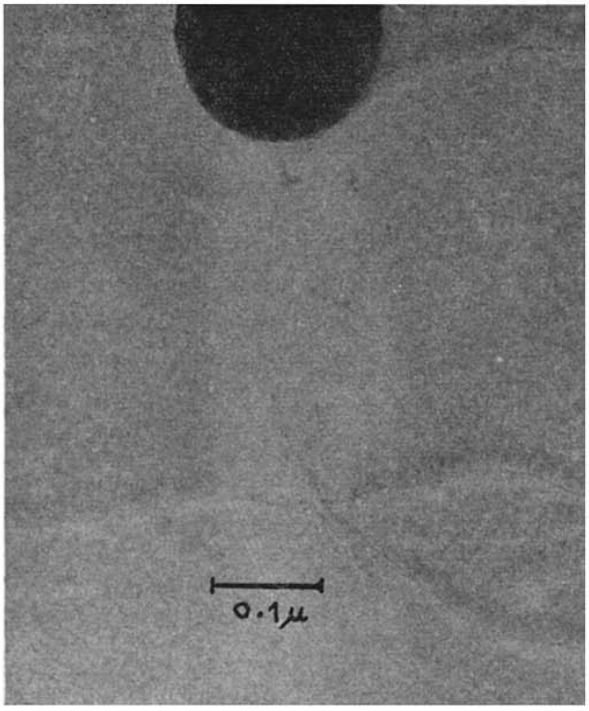

Fig. 1. Shope rabbit papilloma virus DNA passing through the shadow cast by a polystyrene latex particle. Instrumental magnification × 20000.

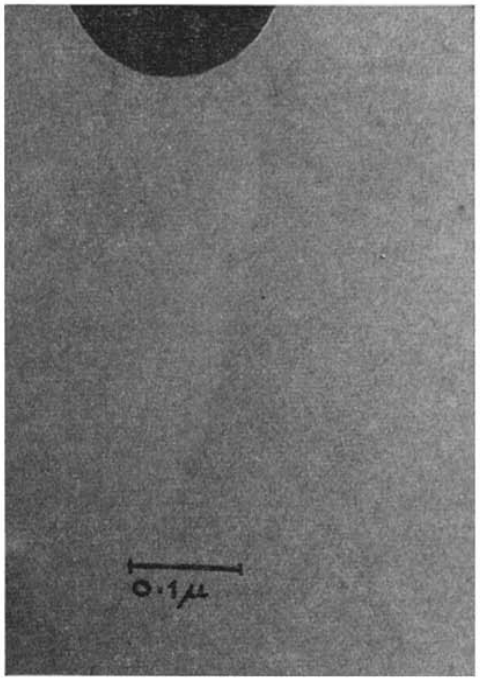

Fig. 2. *M. lysodeikticus* DNA passing through the shadow cast by a polystyrene latex particle. Untreated. Instrumental magnification × 40000.

The solution of Shope rabbit papilloma DNA was then pipetted on to the mica while the sheet was held at an angle of 45°, the excess liquid removed with filter paper and the residual solution allowed to dry. After shadowing with platinum–carbon, the mica was overlaid with 0.25% collodion in amyl acetate. The film was stripped off on to distilled water, picked up on copper grids and examined in a Siemens Elmiskop I.

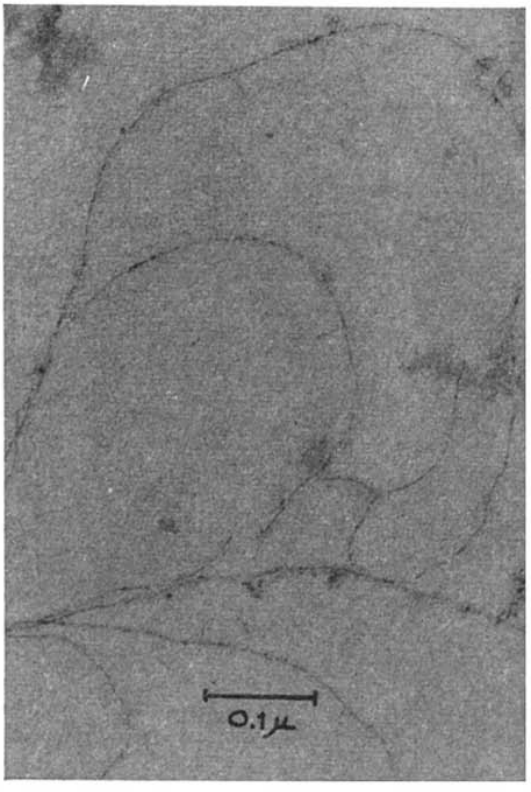

Fig. 3. *M. lysodeikticus* DNA treated with CsCl (10% solution for 30 min). Unshadowed. Instrumental magnification × 40000.

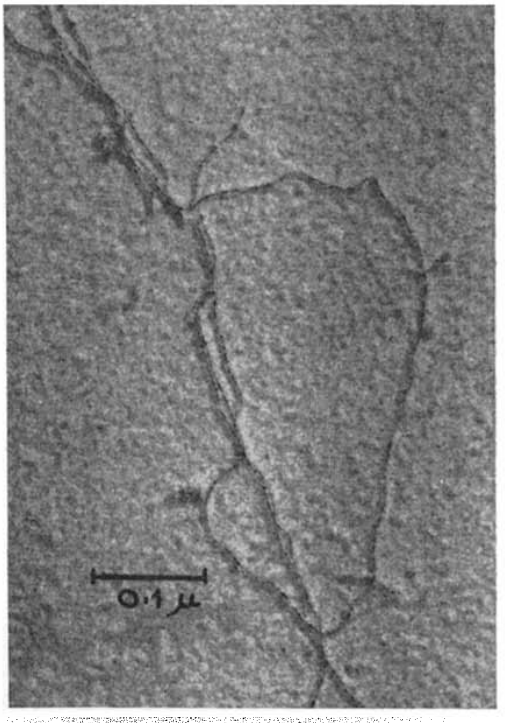

Fig. 4. *M. lysodeikticus* DNA treated with CsCl (10% solution for 30 min) and shadowed. Instrumental magnification × 40000.

The solution of *M. lysodeikticus* DNA was applied directly to collodion-covered grids held at an angle of 45°, drained on to filter paper and allowed to dry. These grids were treated in one of three ways: (a) Shadowed with platinum–carbon as above; (b) Treated with 10% CsCl for 30 min and then washed several times by inverting the grid on to distilled water and draining on to filter paper; (c) Treated with 10% CsCl for 30 min and then shadowed.

Fig. 1 shows part of a molecule of Shope rabbit papilloma virus DNA passing through the shadow of a polystyrene latex particle (188 mμ diameter). Outside the shadow of a polystyrene latex particle the molecule has an appearance typical of shadowed DNA, but with a dense "backbone". The "backbone" is seen to continue through the shadow as a dense line approx. 20 Å in width.

In Fig. 2 DNA from *M. lysodeikticus* (not exposed to CsCl) is shown passing through the shadow of a polystyrene latex particle; no "backbone" is visible. Fig. 3 shows the same DNA exposed to CsCl (10% solution for 30 min) but not shadowed. The molecules are seen as fine lines similar to the "backbone" of the DNA shown in Fig. 4 where the DNA has been exposed to CsCl and shadowed.

It was shown by BEER AND ZOBEL[5] that DNA fibrils were not visible in the electron microscope unless either stained with uranyl acetate or shadowed. The unstained fibrils were not visible within the shadow of a polystyrene latex particle although easily seen on either side of it. In general this was found to be the case in the present study. In some unstained preparations DNA molecules could be seen with difficulty, contrast being extremely low (Fig. 2).

Direct evidence for the uptake of cesium ions by DNA was obtained by the use of radioactive CsCl. Collodion films were prepared on pieces of glass 1 × 1 cm, washed with Photoflo and distilled water, then exposed either to *M. lysodeikticus* DNA (1 μg in 0.05 ml ammonium acetate) or to the solvent alone.

The excess liquid was pipetted off after 10 min and replaced with a 1% CsCl solution containing ^{137}Cs (specific activity 0.1 μC/μmole). After a further 30 min the CsCl was removed and the samples washed thoroughly with distilled water and allowed to dry.

The samples exposed to DNA took up radioactivity equivalent to 0.2 μg of Cs. Control samples took up less than 1/20 of this amount. The amount of radioactivity taken up by the DNA, 0.2 μg Cs/μg DNA is a minimum estimate since much of the DNA may not have adhered to the film.

Exposure to CsCl does not appear to destroy the biological activity of DNA, at least in the case of pneumococcal transforming principle[6]. The molecules seen after exposure to CsCl are therefore probably undamaged DNA molecules.

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SC 2315

On the mechanism of ultraviolet-induced mutations

It is generally acknowledged that ultraviolet and ionizing radiations may be mutagenic either by direct action on DNA or through different indirect effects, for instance by producing reactive substances able to interact with DNA or base analogs susceptible to be incorporated into DNA. However, while the ionizing radiations are on the whole non-selective, the irradiation with ultraviolet light is more specific. Thus, ultraviolet radiation is not ionizing, induces relatively few chromosome breaks, and its absorption is limited essentially to conjugated molecules. In fact, the similarity between the