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NOTES

Arrangement of Herpesvirus Deoxyribonucleic Acid in the Core

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The core of the herpes simplex virion consists of an electron-dense toroidal structure 50 nm high, with an inside diameter of 18 nm and an outside diameter of 70 nm, penetrated by a less dense cylindrical mass. The toroid contains deoxyribonucleic acid (DNA), as evident from the observation that uranyl ion staining is removed by ethylenediaminetetraacetic acid under conditions which result in the extraction of uranyl ions bound to nuclear DNA. Studies on negatively stained preparations of purified capsids suggest that the toroid consists of DNA arranged as if it were spooled around the cylindrical mass.

Herpes virions consist of three major architectural elements, i.e., a core, a capsid, and an envelope (9). Epstein (3) and Zambernard and Vatter (10) demonstrated that viral deoxyribonucleic acid (DNA) is contained in the core of the virion, but the precise structure of the core has been largely unknown. In this paper we are reporting on the arrangement of the DNA in the core.

The electron microscope studies were done on three kinds of preparations derived from infected cells. Two of the preparations involved thin sections of infected cells. Briefly, human epidermoid carcinoma (HEp-2) cells were infected with subtype 1 of herpes simplex virus at a multiplicity of 10 plaque-forming units/cell in mixture 199 supplemented with 1% calf serum. After 11 hr of incubation, the cells were scraped off the glass with a rubber policeman and collected by centrifugation at 2,000 rev/min for 5 min in a refrigerated International centrifuge (model PR-2). The cells were fixed in 2.5% gluteraldehyde in phosphate buffer (*p*H 7.2), dehydrated in ethanol, stained with 0.1% fast green FCF in 100% ethanol for 10 min to reveal their location at the time of sectioning, and then embedded in Epon. Sections were cut on a Porter-Blum MT2 microtome by using a diamond knife. The first preparation examined with the electron microscope consisted of thin sections stained with saturated uranyl acetate in 50% ethanol for 1 hr and counterstained with lead citrate (7) for 90 sec just prior to viewing in a Siemens Elmiskop 1a electron microscope operated at an accelerating voltage of 80 kv. In these preparations, the core appeared as an electron-opaque toroid partially surrounding a less dense cylindrical mass. Figure 1 shows three views of the core together with three dimensional drawings of the core in these preparations. The volume of the torus appears to be between 1.44×10^5 and 1.77×10^5 nm^3. The torus itself occupies only about 50 to 60% of the volume enclosed by the outer capsid.

The second preparation consisted of thin sections cut from the same batch of infected cells but stained for 1.5 min with 5% uranyl acetate in distilled water. Some of the sections were then soaked in 0.2 M ethylenediaminetetraacetic acid (EDTA), *p*H 7.0, for 30 min followed by three rinses of 30 sec each in distilled water. They were then counterstained with Reynold lead citrate solution for 40 sec followed by a vigorous rinse with 0.02 M NaOH (2). The purpose of the EDTA treatment was to selectively solubilize the stain bound to the DNA; in preparations treated in this fashion, the DNA was unstained in both virus particles and nuclear chromatin, but was observed as unstained areas outlined by the background of stained macromolecules. Figure 2 shows an electron micrograph of a thin section stained in this fashion. Based on the fact that the torus remained unstained, we conclude that it contains the DNA. It is noteworthy that the material immediately surrounding the DNA stains very intensely. The nature of this material is now known. The cylindrical mass passing through the center of the torus stains in a less intense fashion, suggesting that it contains protein.

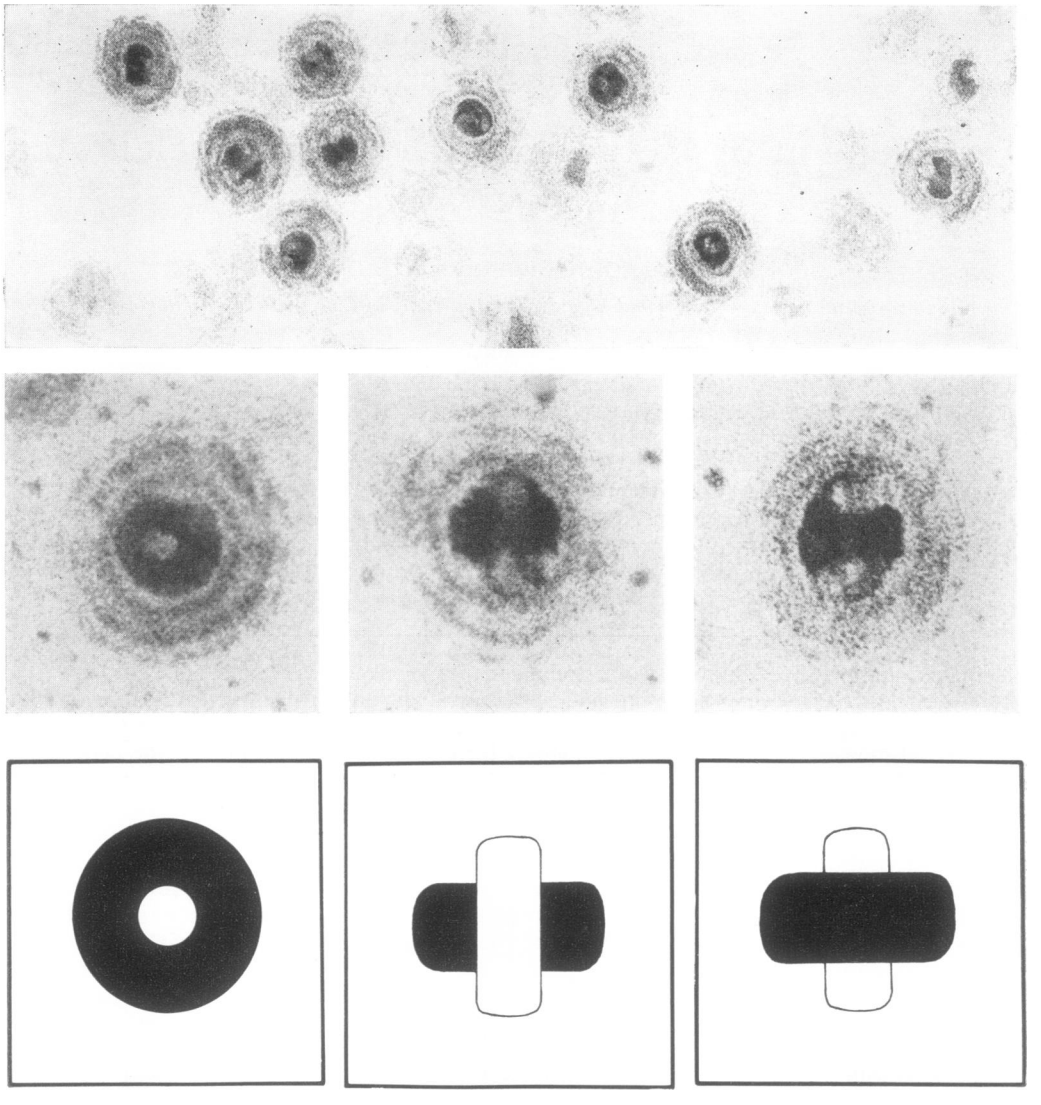

FIG. 1. *Electron micrographs of thin sections of herpes virions showing the core cut at various angles and drawings of a model cut in the same plane (left and center) or viewed from the same angle (right). The cylindrical structure passing through the toroid varies somewhat from virion to virion. ×200,000; figures are drawn on larger scale.*

The third preparation consisted of purified capsids containing DNA (B capsids). The method of preparation of B capsids is as described by Gibson and Roizman (Virology, *in press*). Cells infected for 24 hr were lysed with Nonidet P-40. The nuclei were then collected and lysed with 0.5% sodium deoxycholate. The lysate was digested with deoxyribonuclease (50 μg/ml) to reduce the viscosity, made 0.5% and 0.5 M with respect to BRIJ-58 (polyoxyethylene [19] cetyl ether, Atlas Chemical Industries, Inc., Wilmington, Del.) and urea, respectively, and centrifuged on a 10 to 40% (w/w) sucrose density gradient prepared in 0.15 M NaCl and 0.01 M sodium phosphate (*p*H 7.2) for 60 min at 23,000 rev/min and 4 C in a Spinco SW27 rotor. This procedure separated two populations of capsids differing in DNA and two proteins; both populations formed bands near the middle of the tube. The bottom band, consisting of B capsids which contain the DNA, was collected mechanically. The material was stained with saturated uranyl acetate in 1% sodium borate. The negative stain permeating the capsids made apparent threadlike strucures 4.0 to 5.0 nm wide on the surface of the core (Fig. 3).

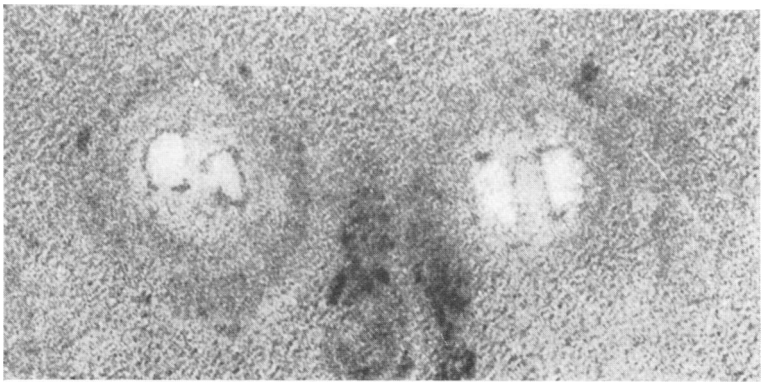

FIG. 2. *Electron micrograph of a thin section of virions in the cytoplasm of infected cells. The sections were treated with EDTA to selectively remove uranyl binding to DNA. Both virions were sectioned in the same plane as that shown in the center electron micrograph of Fig. 1.* ×200,000.

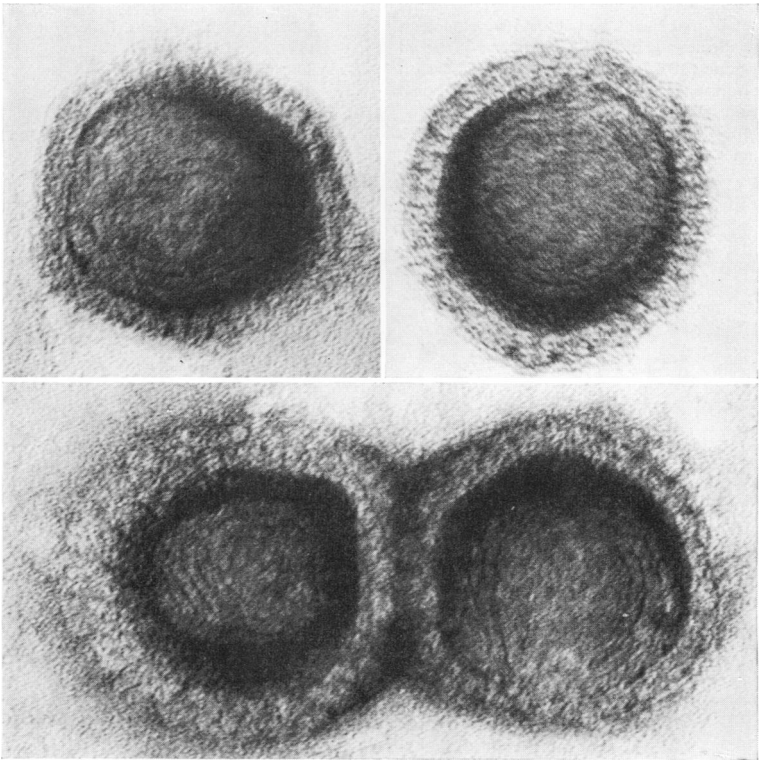

FIG. 3. *Capsids containing DNA (B capsids) prepared from nuclei of infected cells and permeated with uranyl acetate. Electron micrographs show details of the surface of the core.* ×380,000.

We conclude from these studies the following. (i) The core of the DNA is a toroid which normally stains intensely with heavy-metal stain. The toroid may explain the variation in the shape of the core reported in numerous studies (6, 8). (ii) Passing through the center of the torus is a cylindrical structure which binds heavy-metal ions less intensely. (iii) The torus contains DNA which is wound around the cylindrical structure in strands with a spacing of 4.0 to 5.0 nm. (iv) The volume occupied by the DNA may be calculated to be 1.57×10^5 nm^3, based on the previously determined molecular weight of the DNA ($99 \pm 5 \times 10^6$; reference 5) and length (50 μm; reference 1) and the assumption that it is a cylinder with a radius of 1.0 nm. Although the calculated volume of the DNA is close enough to the volume of the toroid to argue against the possibility that the

DNA is bound to large amounts of protein, there is indeed space in the grooves of the DNA which could accommodate polyamines and some proteins. In fact the 4.0-nm to 5.0-nm spacing of the strands suggests that the DNA either possesses tertiary structure or is complexed with other molecules. Polyamines sufficient to neutralize 40 to 60% of the phosphate in the DNA were previously reported in the capsid (4). It is noteworthy that the capsids containing the DNA differ from those lacking cores with respect to two proteins, i.e., no. 21 and 22a, and of these two proteins 22a appears to be on the surface of the capsid, leaving only protein 21 as a possible structural protein of the core (W. Gibson and B. Roizman, Virology, *in press*).

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