Preliminary tests suggest that columns made up from bottom to top of layers of 8, 7, 6, 5, 4 and 3 per cent agar are superior to those containing only 4 or 8 per cent agar for the separation of particles of different sizes. Additional studies are under way which should give us more information regarding optimum conditions for purification and separation of viruses, cell particulates, nucleic acids, enzymes, and proteins.

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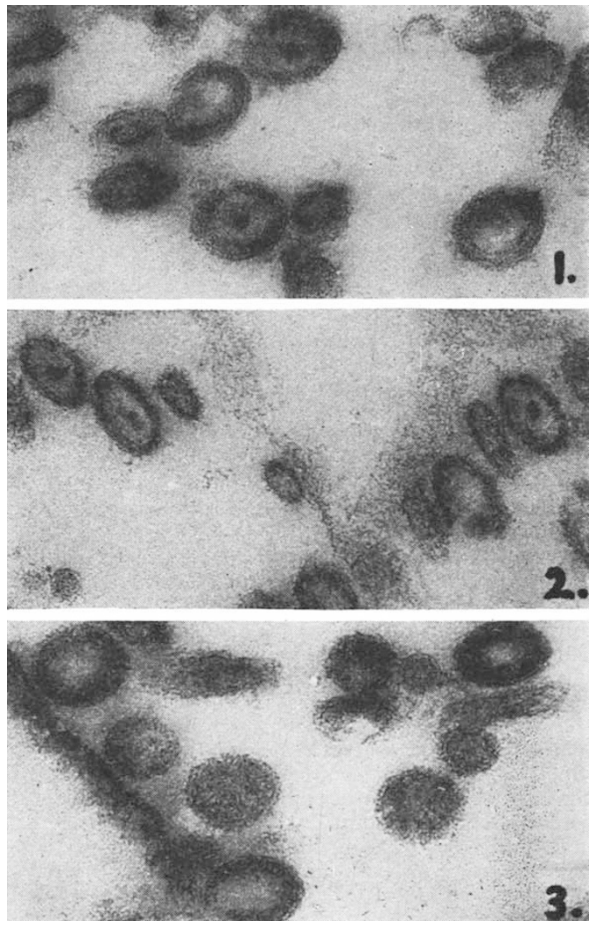
Nucleic Acid of Mature Herpes Simplex Virus: its Type and Location

THE nuclear inclusions of herpes infections have been shown, cytochemically, to contain deoxyribonucleic acid (DNA)[1], and it is further known that this material is synthesized in excess by cells soon after the herpes virus enters them[2]. However, no connexion has been established between the DNA and constituents of the agent, the nucleic acid type of which has not, as yet, been elucidated.

In order to investigate the nature of herpes virus nucleic acid, mature extracellular particles have been treated with specific nucleases, and the resulting changes caused within them have been observed in thin sections with the electron microscope, such methods having proved successful with the adeno and Rous viruses[3]. For the experiments, the *HFEM* strain of herpes was grown in HeLa cultures and the readily recognizable mature virus[4] was gathered along with the cells around which it accumulated. The cells were collected, fixed, dehydrated, embedded in *n*-butyl methacrylate, and sectioned for electron microscopy as already described[5], except that potassium permanganate was used as the fixative[6] since it permits subsequent digestion of nucleic acids by appropriate enzymes[7]. After fixation, samples of the material were placed in iced 30 per cent alcohol and brought to room temperature; the samples were then divided into three groups before continuing with dehydration and embedding. One group was incubated for 2 hr. at 37° C. in 30 per cent alcohol containing 1 mgm./ml. ribonuclease at *p*H 7, a second group was similarly incubated in the same medium containing 1 mgm./ml. deoxyribonuclease together with magnesium chloride (0·003 *M*), and a third group was incubated in the medium containing a similar concentration of this salt but no enzyme.

On examination, the control preparations which had been in the enzyme-free medium all contained numerous extra-cellular particles with well-marked nucleoids (Fig. 1), while the particles in material exposed to ribonuclease also retained these dense structures (Fig. 2). Particles which had been subjected to the action of deoxyribonuclease were quite unlike those of the other groups; their nucleoids were digested, leaving an empty space in the central area (Fig. 3).



Figs. 1–3. Electron micrographs of thin sections cut through permanganate-fixed mature herpes simplex virus particles lying between microvilli close to infected HeLa cells. The particles were treated in various ways before embedding in methacrylate. (× 80,000).

Fig. 1. Control preparation incubated in enzyme-free 30 per cent alcohol for 2 hr. at 37° C. The dense viral nucleoids are present.

Fig. 2. Preparation incubated as the foregoing but with ribonuclease (1 mgm./ml. at *p*H 7) in the alcohol; here, too, the nucleoids are intact, despite some compression of the particles as a result of sectioning

Fig. 3. Particles in a preparation which was incubated with deoxyribonuclease (1 mgm./ml.) and 0·003 *M* magnesium chloride in the alcoholic medium. The nucleoids have all been removed

This finding establishes that herpes virus contains nucleic acid of deoxyribose type and demonstrates that this material is localized, in mature particles, within the viral nucleoid. A full account of these experiments has been reported elsewhere[8].

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