

Composition of the Rous Virus Nucleoid

A TECHNIQUE, modified from that of Gessler, Bender and Parkinson¹, was recently devised to investigate the purifying effect of a fluorocarbon on vaccinia virus².

The technique has now been applied to nodules of Rous tumour grown on the chick chorio-allantois in order to prepare suspensions of the Rous virus. Such suspensions have been subjected to high-speed centrifugation and the pellets which resulted have been fixed, embedded, sectioned and examined in the electron microscope. It has been found that the only recognizable formed structures present in the pellets were uniform spherical particles about 75 m μ in diameter (Fig. 1), and biological tests integrated with the morphological work have established that these particles were in fact the Rous virus³. Fragments of the pellets containing the virus are ideal both for examination by electron and fluorescence microscopy after enzyme digestion, and for cytochemical study.

The nucleoid of the Rous virus is known to lie in a central zone separated from the viroplasm by a fine membrane⁴. In order to investigate the nature of the nucleoid, samples of the pellets containing the virus have been treated with ribonuclease. The samples were first fixed with potassium permanganate⁵ which, unlike the more commonly used osmium-sucrose fixative for electron microscopy⁶, allows subsequent digestion by both deoxyribonuclease⁷ and, under certain conditions, ribonuclease. After fixation, the samples were placed in iced 30 per cent alcohol and allowed to warm to room temperature⁸; they were then incubated for 2 hr. at 37° C. in 30 per cent alcohol containing 0.1 per cent by weight of ribonuclease, protease-free, and recrystallized three times (Worthington Biochemical Corporation, Freehold, New Jersey). Control samples were incubated simultaneously in 30 per cent alcohol without the enzyme. After incubation, all the samples were dehydrated, embedded in *n*-butyl methacrylate and sectioned for examination in the electron microscope.

The incubation procedure made the particles appear ragged and extracted (Fig. 2). It is nevertheless clear that in the control preparations, which were not exposed to the ribonuclease, the nucleoid and outer limiting membrane remained intact (Fig. 2a). On the other hand, the nucleoids were removed from

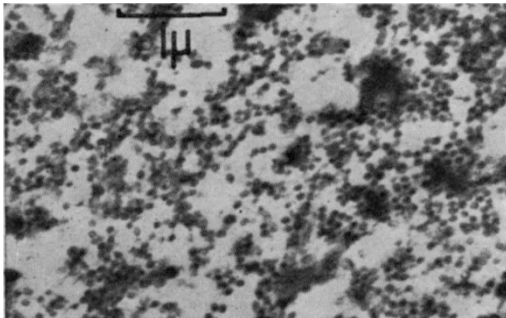


Fig. 1. Electron micrograph of a section through a pellet prepared from a suspension of the Rous virus which had been treated seven times with a fluorocarbon. Uniform virus particles about 70-75 m μ in diameter are almost the only formed structures present. Permanganate fixation. $\times 14,000$

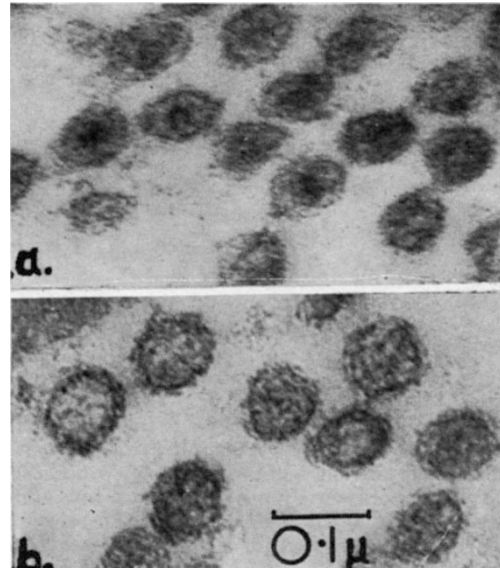


Fig. 2. Electron micrographs of thin sections of Rous virus particles. (a) Control particles which had been incubated in 30 per cent alcohol at 37° C. for 2 hr.; the nucleoid and outer limiting membrane can be distinguished. (b) Particles which had been incubated in the same way as the controls except that the 30 per cent alcohol contained 0.1 per cent by weight of ribonuclease; the nucleoids have been removed and the particles appear slightly swollen. Permanganate fixation. $\times 130,000$

particles which had been subjected to ribonuclease digestion (Fig. 2b); such particles also appeared slightly swollen as if some central supporting structure had been removed. The specificity of the ribonuclease digestions in conjunction with the findings of the control experiments demonstrate that the nucleoid of the Rous virus contains a substantial amount of ribonucleic acid. This work will be reported in detail elsewhere⁸ together with a discussion of its significance.

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¹ Gessler, A. E., Bender, C. E., and Parkinson, M. C., *Trans. N.Y. Acad. Sci.*, **18**, 701 (1956).

² Epstein, M. A., *Brit. J. Exp. Path.* (in the press). Holt, S. J., and Epstein, M. A., *ibid.* (in the press).

³ Epstein, M. A., *Brit. J. Cancer* (in the press).

⁴ Epstein, M. A., *Brit. J. Cancer*, **11**, 268 (1957).

⁵ Luft, J. H., *J. Biophys. Biochem. Cytol.*, **2**, 779 (1956).

⁶ Palade, G. E., *J. Exp. Med.*, **95**, 285 (1952). Caulfield, J. B., *J. Biophys. Biochem. Cytol.*, **3**, 327 (1957).

⁷ Holt, S. J., and Epstein, M. A., *Brit. J. Exp. Path.* (in the press).

⁸ Epstein, M. A., and Holt, S. J., *Brit. J. Cancer* (in the press).

A Cobalt-Accumulator Plant, *Clethra barbinervis* Sieb. et Zucc.

DURING an investigation to determine if variations in the heavy-metal content of vegetation and soil could be used in prospecting for chromite ore bodies in the Hinokami district, Tottori Prefecture, a tree, *Clethra barbinervis* Sieb. et Zucc., was unexpectedly found to be an accumulator of cobalt.

Leaves of seventeen different kinds of trees collected from 199 locations in the neighbourhood of the mine were analysed for iron, nickel, cobalt, copper, zinc, manganese and chromium. The cobalt and nickel