Onderstepoort J. vet. Res. 40 (2), 71–72 (1973)

RESEARCH NOTE

MECHANISM OF ENVELOPMENT OF HERPESVIRUS BY THE NUCLEAR ENVELOPE*

G. LECATSAS[1] and G. POSTE[2]

Published electron micrographs of the morphological development of herpesvirus have shown that the particle obtains an envelope derived from the inner component of the nuclear envelope (Siegert & Falke, 1966; Shipkey, Erlandson, Bailey, Babcock & Southam, 1967; Darlington & Moss, 1968; Nii, Morgan & Rose, 1968). However, details of this morphogenetic process have been limited by suboptimal staining and consequent poor resolution. In addition, the plane of section will influence the definition of the structures, particularly the membranes. Ben-Porat & Kaplan (1971) have shown that the viral envelope glycoproteins differ from those normally present in the nuclear envelope and have suggested that the proteins in the normal nuclear envelope do not become part of the viral envelope. It contains, instead, virus specific proteins. The phospholipids in the viral envelope, however, are derived from pre-existing cell membranes other than those of the nuclear envelope (Ben-Porat & Kaplan, 1972). There is a doubling of the phospholipid content of the nuclear membranes and consequently a doubling of these membranes. This communication provides structural evidence for doubling of the inner nuclear membrane and examines certain implications of this observation.

HEp-2 cells grown in Eagle's basal medium containing 3% calf serum were infected with a canine herpesvirus whose isolation has been previously described (Poste & King, 1971). After harvesting, cells were fixed in 3% gluteraldehyde for 1 hour, washed in phosphate buffer and post-fixed in 1% OsO_4 for 1 hour. The cells were dehydrated in graded ethanol dilutions and embedded in Epon. Thin sections were doubled stained in uranyl acetate and lead citrate and viewed in a Philips EM 300 electron microscope at 80 kV.

Fig. 1 depicts various stages in the budding of herpesvirus particles from the inner nuclear membrane. It is clear that granular, electron dense material collects on the nucleo-plasmic side of the inner nuclear membrane, so that a doubling of the inner nuclear membrane occurs with a space separating the two leaflets. Each leaflet is 10 nm thick and the width of the enclosed space is approximately 6,5 nm. At no stage is the continuity of the inner nuclear membrane interrupted in its continuity and it appears to act as a base for the assembly of new membrane material. At a later stage of budding the electronlucent space is reduced in width and the leaflets of the inner nuclear membrane become thinner. When budding is complete this space is no longer visible, suggesting that membrane material becomes tightly packed. No changes are evident on the cytoplasmic side of the inner nuclear membrane during budding, which indicates either that phospholipids may be added to this side of the membrane at earlier stages or that they pass through the nuclear envelope and then become deposited on the nucleoplasmic side of the inner nuclear membrane, possibly under the influence of nuclear-based viral or cellular enzymes. The sites of budding of virus particles from the nuclear membrane suggest that structural changes occur as a result of the presence of the viral capsid rather than virus particles finding portions of the nuclear envelope which have undergone structural changes. Ben-Porat & Kaplan (1972) have suggested that the latter sequence of events occurs. However, unless budding is complete certain portions of the nuclear envelope would show structural changes since the responsible virus particles may be detached during isolation procedures.

ACKNOWLEDGEMENTS

The senior author thanks Professor A. P. Waterson for facilities provided and the Meat Board of the Republic of South Africa for financial support.

REFERENCES

BEN-PORAT, T. & KAPLAN, A. S., 1971. Phospholipid metabolism of herpesvirus-infected and uninfected rabbit kidney cells. *Virology*, 45, 252-264.

BEN-PORAT, T. & KAPLAN, A. S., 1972. Studies on the biogenesis of herpesvirus envelope. *Nature, Lond.*, 235, 165-166.

DARLINGTON, R. W. & MOSS, L. H., 1968. Herpesvirus envelopment. *J. Virol.*, 2, 48-55.

NII, S., MORGAN, C. & ROSE, H. M., 1968. Electron microscopy of herpes simplex virus. *J. Virol.*, 2, 517-536.

POSTE, G. & KING, N., 1971. Isolation of a herpesvirus from the canine genital tract: Association with infertility, abortion and still-births. *Vet. Rec.*, 88, 229-233.

SHIPKEY, F. H., ERLANDSON, R. A., BAILEY, R. B., BABCOCK, V. I. & SOUTHAM, C. M., 1967. Virus biographies. II. Growth of herpes simplex virus in tissue culture. *Exp. mol. Path.* 6, 39-67.

SIEGERT, R. & FALKE, D., 1966. Elektronenmikroskopische Untersuchungen über die Entwicklung des Herpesvirus hominis in Kulturzellen. *Arch. ges. Virusforsch.*, 19, 230-249.

*Work carried out at the Royal Postgraduate Medical School, London, during the tenure of a bursary awarded to the senior author by the Meat Industries Control Board

[1]Veterinary Research Institute, Onderstepoort

[2]Present address: Department of Experimental Pathology, Roswell Park Memorial Institute, Buffalo, New York

Received 28 March 1973. Editor

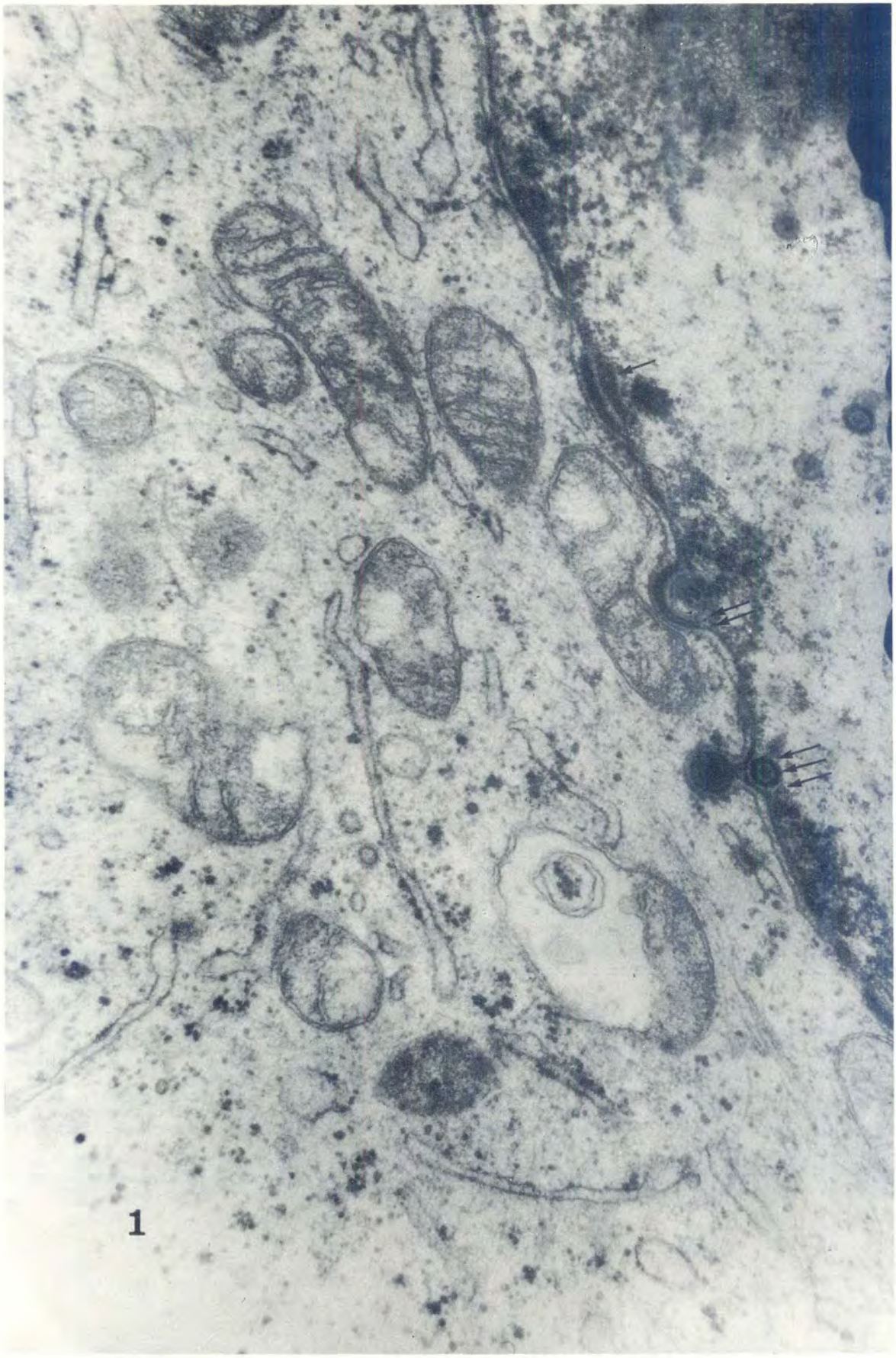

FIG. 1 Magnification: ×75 000. Stages in the process of budding of canine herpesvirus from HEp–2 cell nucleus. Single arrow indicates doubling of the inner component of the double-membraned nuclear envelope. This consists of thickening of the outer component and *de novo* development from granular, electron-dense material of a second layer. The two layers are separated by a space. Note thickness of the new layers compared to the rest of the inner component. Double arrow shows a later stage of virus budding from the nucleus. The two portions of the inner component of the envelope and the intervening space are still clearly visible. Triple arrow shows budding almost complete with the two layers derived from the nuclear envelope now appearing as one with no intervening space, suggesting that a packing of membrane molecules has occurred.