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RESEARCH NOTE

FORMATION OF WALLAL VIRUS IN CELL CULTURE

B. M. GORMAN[1] and G. LECATSAS[2]

Wallal virus, isolated from *Culicoides* spp. (Doherty, 1972) has been shown to replicate in the presence of fluorodeoxy-uridine, to be relatively resistant to lipid solvents and to lose infectivity when exposed to pH 6,0 (Gorman, Goss, Sayers & Symons, 1971). These properties suggest that the virus belongs to the group of bluetongue-type viruses (Verwoerd, 1970).

In order to substantiate its classification in this group we report here the characteristics of the fine cytopathology induced by the virus in both BHK 21 and horse kidney (EK) cells. Mouse brain-derived strain Ch 12048 of Wallal virus was inoculated on to the EK269A line of horse kidney cells (Gorman, Leer & Goss, 1962) and subsequently on BHK 21 and EK cells in Eagle's medium without serum.

Infected BHK 21 and EK cells were harvested at 40 hours after infection with virus, centrifuged at low speed and the resultant pellet prefixed in 3% glutaraldehyde for 1 hour, and postfixed in 1% OsO_4 in Millonig's buffer for 1 hour (Millonig, 1961). Subsequently the cells were dehydrated in graded ethanol dilutions, cleared in propylene oxide and embedded in Epon 812 according to the methods described by Lecatsas & Weiss (1969). Thin sections were cut on a Reichert OmU2 ultramicrotome, double stained in uranyl acetate and lead citrate and examined with a Siemens Elminskop 1A electron microscope operated with a double condenser system and at an accelerating voltage of 80 kV.

Partially degraded virus particles are found in myelin-type inclusion bodies as shown in Fig. 1 and 2. The presence of granular viral inclusion bodies which characterises the bluetongue-type viruses is demonstrated in Fig. 3. The swelling of vesicles of the rough endoplasmic reticulum (Fig. 3 and 4) characterises the infection process. A specific characteristic of the fine cytopathology is the presence of large granular inclusions in the cytoplasmic matrix of cells infected with this virus. The appearance of these structures is coupled with the disintegration of the nuclear membrane and the liberation of nuclear material into the cytoplasmic matrix (Fig. 5, 6 and 7). Prominent, rounded nucleoli characterise the intact nucleus in infected cultures and they apparently correspond to the granular cytoplasmic structures in cells where the nucleus has disintegrated. These observations have been verified in light microscopic investigations of Wallal-infected BHK cells stained with haematoxylin and phloxin (Fig. 7). The fact that these granular inclusions occur in both BHK 21 and EK cells infected with Wallal virus strongly suggests that the feature is virus specific and not a reaction to virus infection by the particular cells.

At this stage, the disintegration of the nucleus and the liberation of nucleolar-like granular bodies appear to be characteristic for this virus, at least in BHK 21 and EK cells. Sectioned particles have an approximate diameter of 57 nm. This compares well with our figure for negatively stained particles of Wallal virus (Lecatsas & Gorman, 1972).

Acknowledgements

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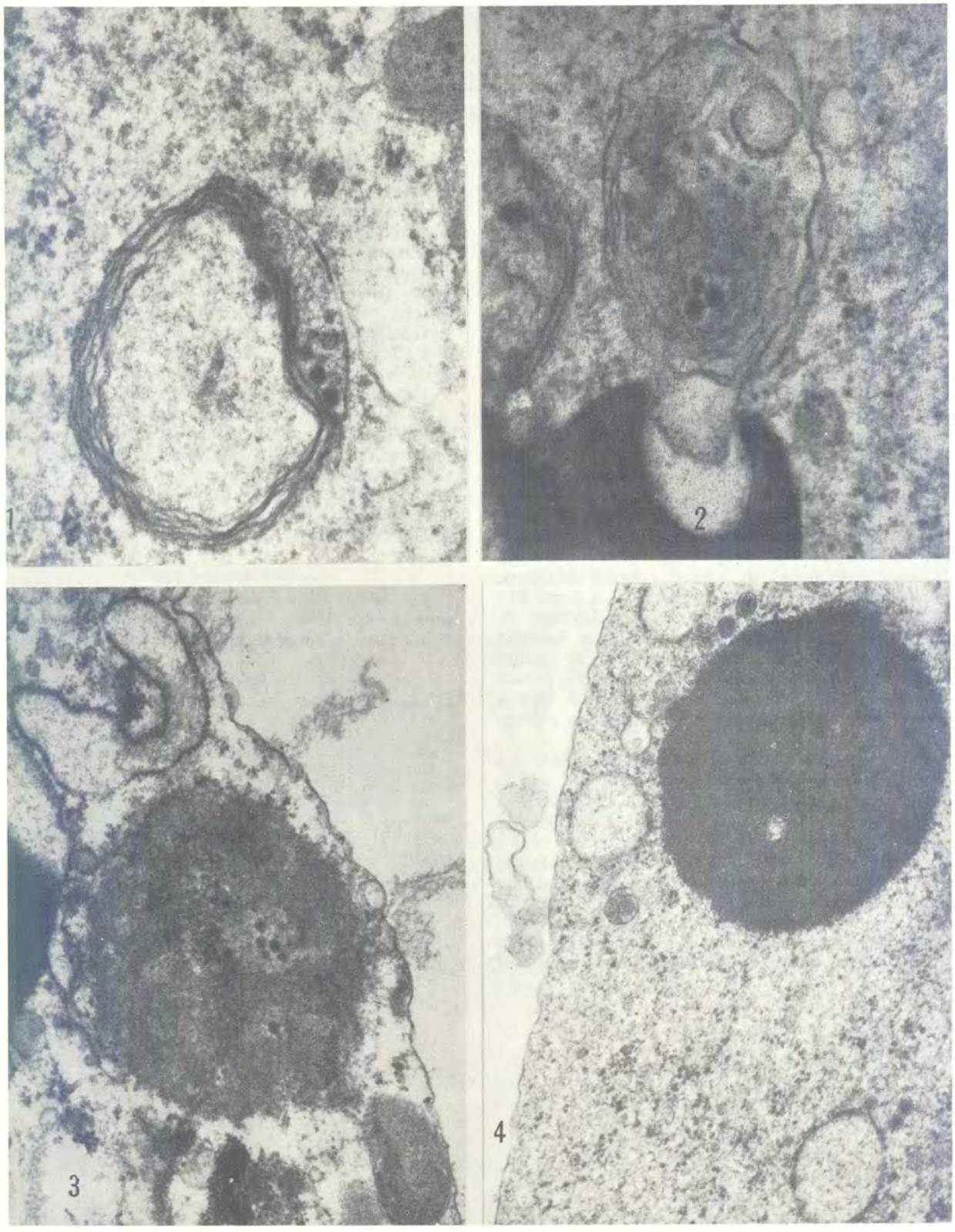

FIG. 1 & 2 Lysosome-type inclusions containing degraded virus particles in Wallal-infected EK cell. Magnification × 80 000
FIG. 3 Granular virus inclusion body in cytoplasmic matrix of EK cell. Note virus particles in centre of body. Magnification × 80 000
FIG. 4 Characteristic dense, spherical inclusion body in cytoplasmic matrix of BHK 21 cell. Magnification × 80 000

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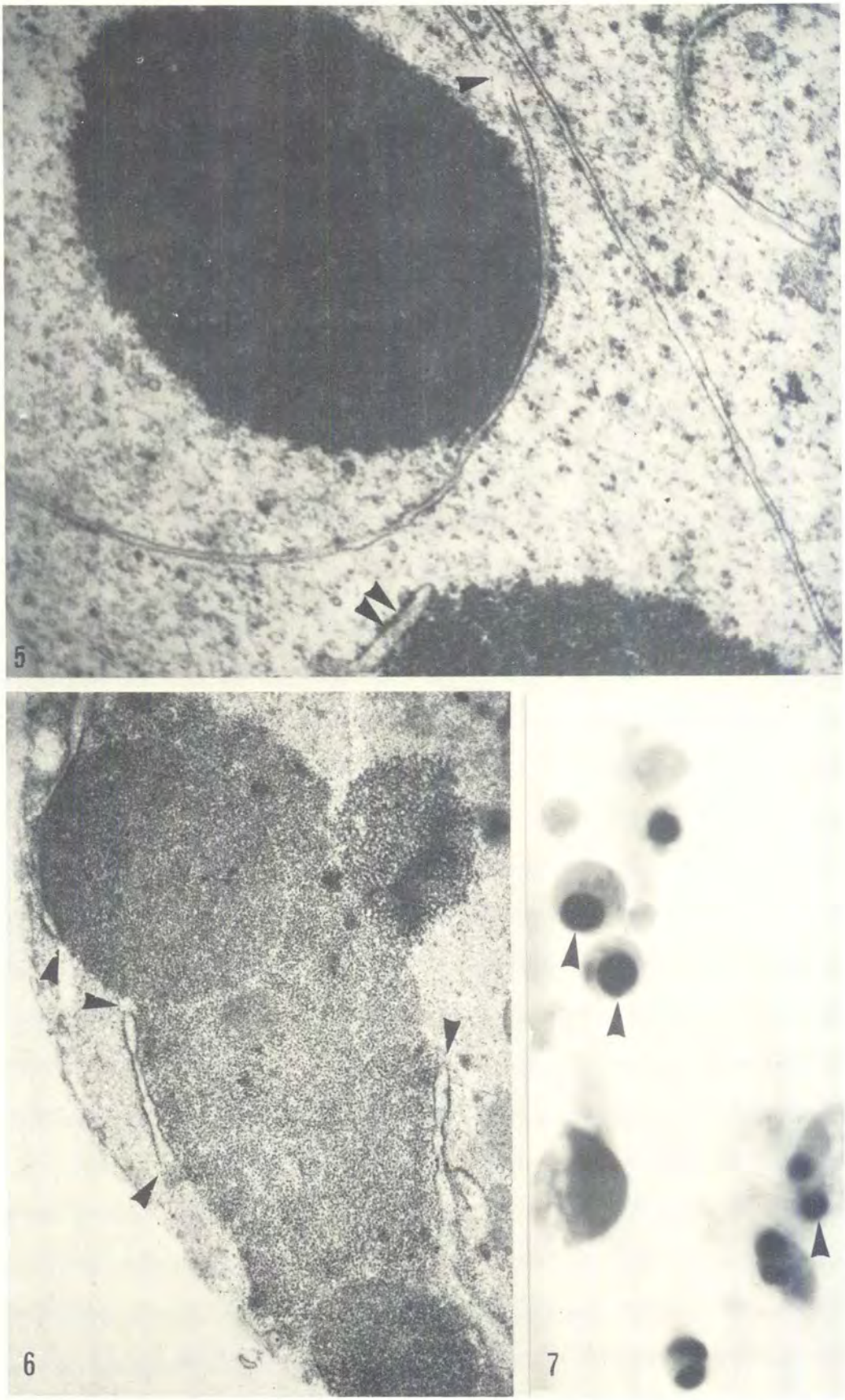
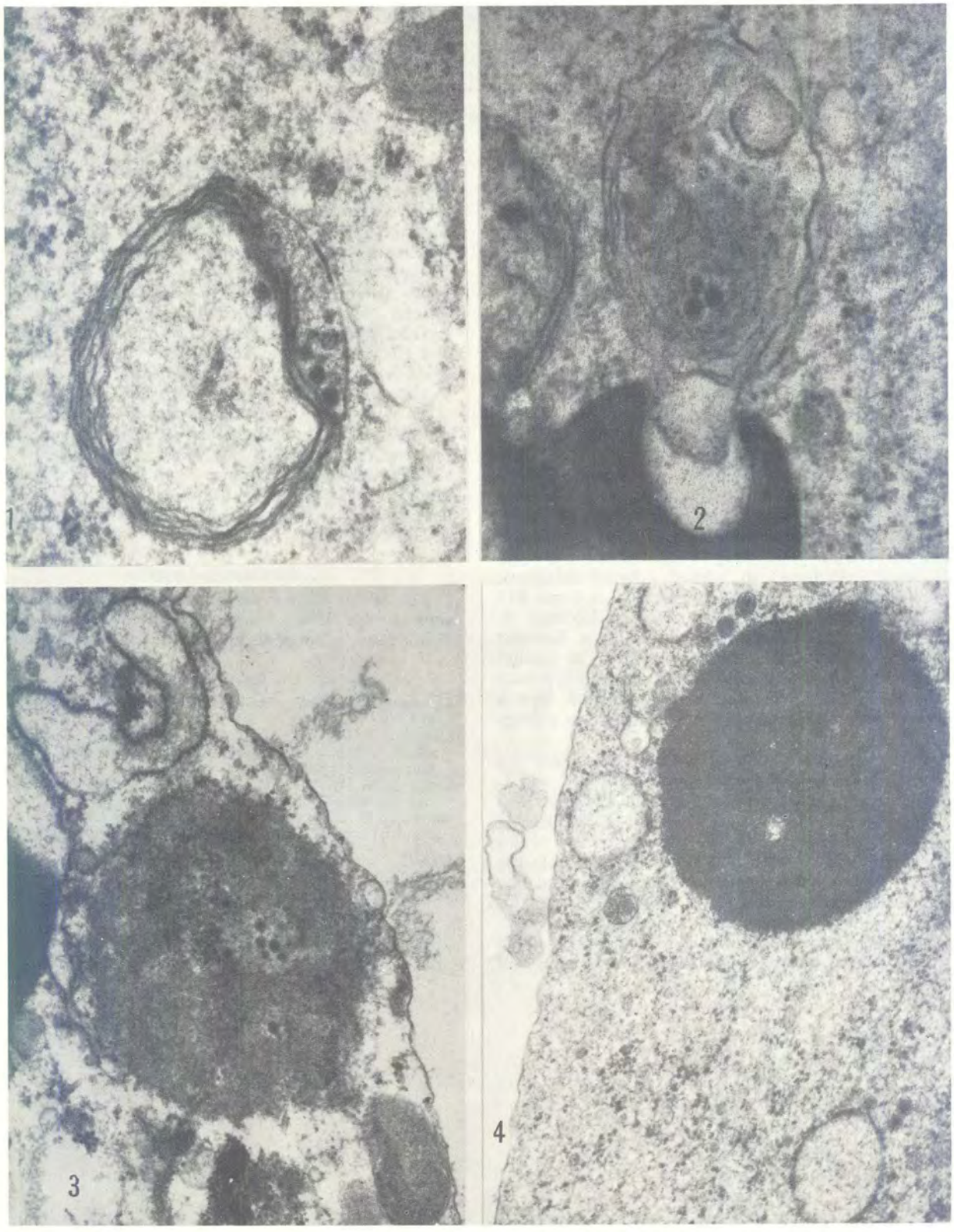


FIG. 5 Granular, dense, spherical inclusion bodies in Wallal-infected BHK 21 cell. Note disrupted membrane (arrow) surrounding inclusion body which appears to be an isolated nucleolus. Double arrow shows attachment of rough endoplasmic reticulum to the lower inclusion. Magnification × 40 000

FIG. 6 Disintegrating nucleus (note discontinuous nuclear envelope marked by arrows) with spherical, dense granular inclusions. Magnification × 20 000

FIG. 7 Spherical, dense nucleoli (arrows) in culture of BHK 21 cells infected with Wallal virus. Magnification × 1 200



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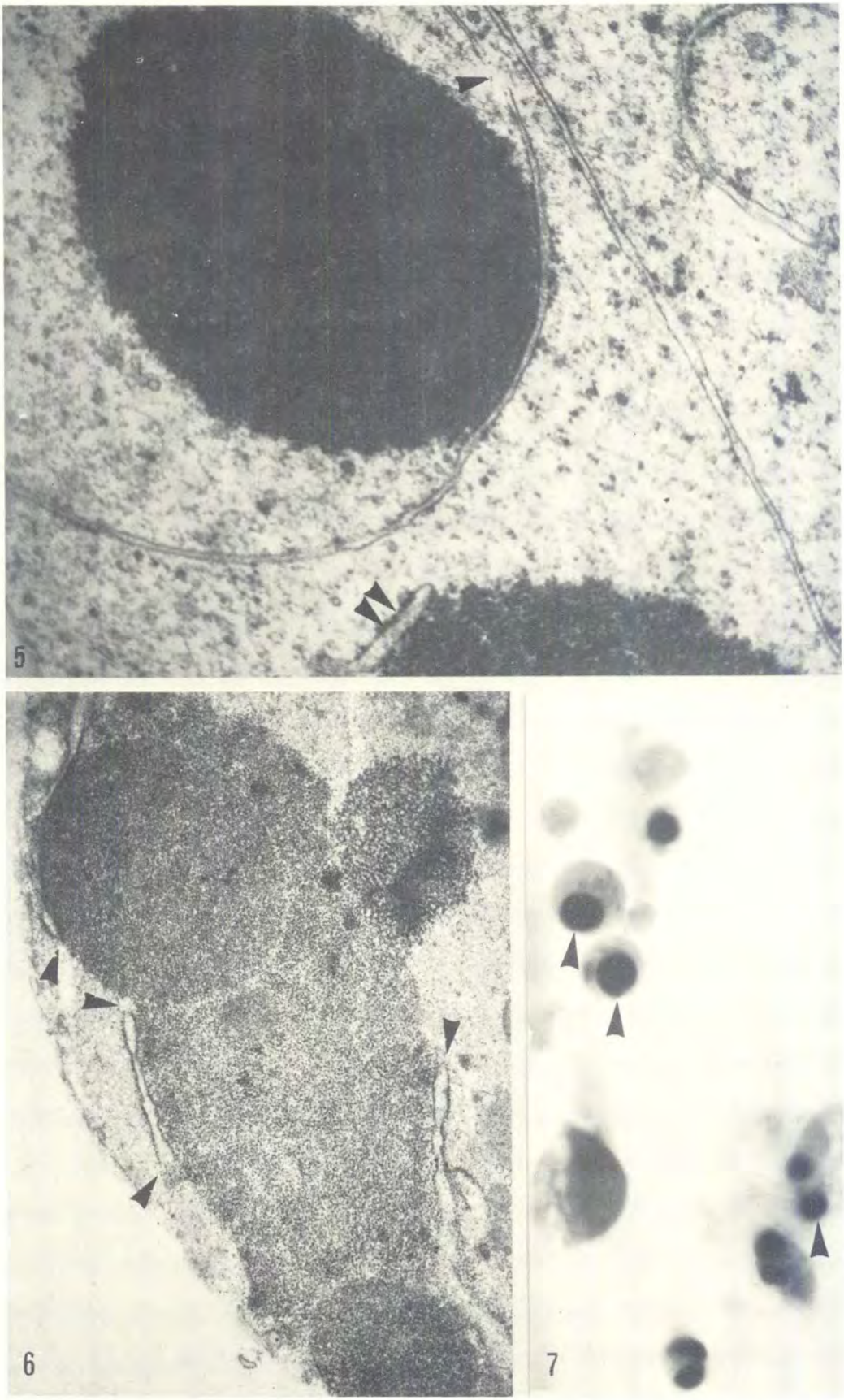

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