# ELECTRON MICROSCOPE STUDY OF THE DEVELOPMENT OF THE PAPILLOMA VIRUS IN THE SKIN OF THE RABBIT*

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Plates 26 to 31

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A recent communication (1) reported electron microscopic observations of the rabbit papilloma virus in thin sections of tumors which had been stored in glycerine for periods of more than 1 year. The poor cytological preservation of such material did not permit study of viral development but did permit positive identification of profiles of the mature virus itself. At the time of that preliminary report it had not been possible to recognize the viral bodies in fresh optimally fixed papillomas.

The present paper describes a study of freshly fixed papillomas in which extensive visualization of viral particles has now been possible with retention of cytological detail, and a concept of the mode of maturation or development of these particles in a remarkable relationship to epidermal cell nuclei has been gained.

## *Materials and Methods*

Papillomas were taken from New Jersey cottontail rabbits which had been inoculated with virus originally from Kansas rabbits, by the skin abrasion method. The tissues were fixed in 1 per cent buffered osmium tetroxide, dehydrated in ethanol and embedded in methacrylate in the usual fashion. Thin sections have been studied in the electron microscope (RCA, EMU2A) and for orienting purposes thicker sections (*ca.* 1 $\mu$) have been stained with the Feulgen technique, azure B and toluidine blue, then studied in the light microscope.

## *Observations with the Electron Microscope*

In uninvolved rabbit skin, epidermal cell nuclei of the stratum germinativum and lower stratum spinosum have thin limiting membranes and contain rather evenly distributed chromatin in very short strands or small granules. The nucleoli tend to be well demarcated by reason of being composed of small and more closely packed granules and often present a retiform pattern in the

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two dimensions of the sections. The nucleoli are closely surrounded by chro matin without intervening electron-lucent regions (*v.i.*). In the uppermost portions, the chromatin is sometimes more coarsely aggregated and there is accumulation of that material along the inner aspect of the nuclear membrane (margination). In other respects rabbit skin is similar in ultrastructure to skin described by other investigators (2, 3).

In sections of papilloma from cottontail rabbits, virus of a size and configuration similar to that reported previously (1) is found in epidermal cells in various stages of development. The first morphological evidence of the presence of the virus appears in nucleoli of cells of the lower stratum spinosum (Figs. 1 and 2). There the nuclei are more compact. Single or occasionally multiple sharply demarcated aggregates of fine closely packed granules are often found in the nucleolar area. These aggregates are separated from the more coarsely granular material of the associated chromatin in some places by narrow regions of low density.

The involved nuclei tend to be somewhat enlarged with partial margination of chromatin, the chromatin itself appearing a little coarser than normal. However, the cytoplasm of these cells is in general similar to that of normal epidermal cells with tonofibrils, mitochondria, numerous RNA granules, and desmosomes at the borders.

In epidermal cells more peripherally situated, the finely granular regions of the nucleolus contain occasional clearly separated, circular dark areas approximately 33 m$\mu$ in diameter surrounded by concentric paler zones and then a partially developed very fine net. At this stage characteristic mature viral particles are not present elsewhere in the nucleus or in the cytoplasm of such cells.

Still closer to the surface of the tumor the virus particles not only occupy most of the nucleolar region but also extend centrifugally and are distributed widely throughout the nucleus although still contained within the limiting membrane of that structure (Fig. 3). In these nuclei chromatin margination is still more pronounced than in cells deeper in the Malpighian layer and the central portions of the nucleus contain only small amounts of electron-dense material other than fully developed viral particles. The network enmeshing the particles within the nucleolar area does not extend to include those having generalized intranuclear distribution as is illustrated in Fig. 3.

Cells near the surface contain numerous large and dense keratohyaline granules usually with little remaining "normal" cytoplasm. In such regions some nuclei are considerably enlarged and contain innumerable viral particles with random distribution. The nuclear membranes are irregularly thickened by dense remnants of chromatin.

At the papilloma surface some tumor cells retain nuclei which are densely

packed with virus. In some instances the viral bodies assume ordered arrays of widely varying size and complexity. Often the nuclear membranes are no longer recognizable and the cells consist of thick-walled, heavily keratinized shells containing pools of virus (Figs. 4 to 6). Some times the packed virus forms rows, squares, pentagons, hexagons, or larger orderly patterns.

## DISCUSSION

The observations suggest that the first morphological evidence of the presence of the virus is in the cells of the lower stratum spinosum containing no or infrequent keratohyaline granules. The first visible stage of virus multiplication is judged to be the finely granular material appearing in the nucleolar area. The particles then become clearly outlined within this matrix, later they are dispersed more widely through the nucleus and ultimately throughout the whole cell. If the earliest stage of viral development is indeed in the nucleolus it might be supposed that the virus would contain RNA. However, it is difficult to establish this on a histochemical basis. No evidence is available from this study with regard to the transmissibility of virus of the finely granular stage. The investigations of Noyes (4) who employed a microcautery technique to selectively destroy either keratinized or proliferating cells suggest that transmissibility is associated with the more keratinized cells. The present investigation has not been helpful in demonstrating the ability of the viral infected epidermal cells to proliferate. In fact the late morphological changes in the nucleus are sufficiently severe to suggest that mitosis is then impossible. It is possible that the virus acts indirectly to cause hyperplasia in cells not themselves infected. Alternatively the infecting virus may be directly stimulating cell proliferation at the germinal layer before viral multiplication becomes evident by electron microscopy; perhaps earlier even than the stage shown in Fig. 1.

## SUMMARY

Rabbit papilloma virus seems uniquely to begin its proliferation in the nucleolus of infected cells. In cells near the germinal layer of the stratum Malpighii spherical viral bodies seem to develop within a reticulum which forms out of the fine granular matrix of the nucleolus. The virus may later fill the nucleus and spread into the whole cell. The age of the cell, determined by its position in the Malpighian layer and by the extent of keratinization, can be correlated with the viral development.

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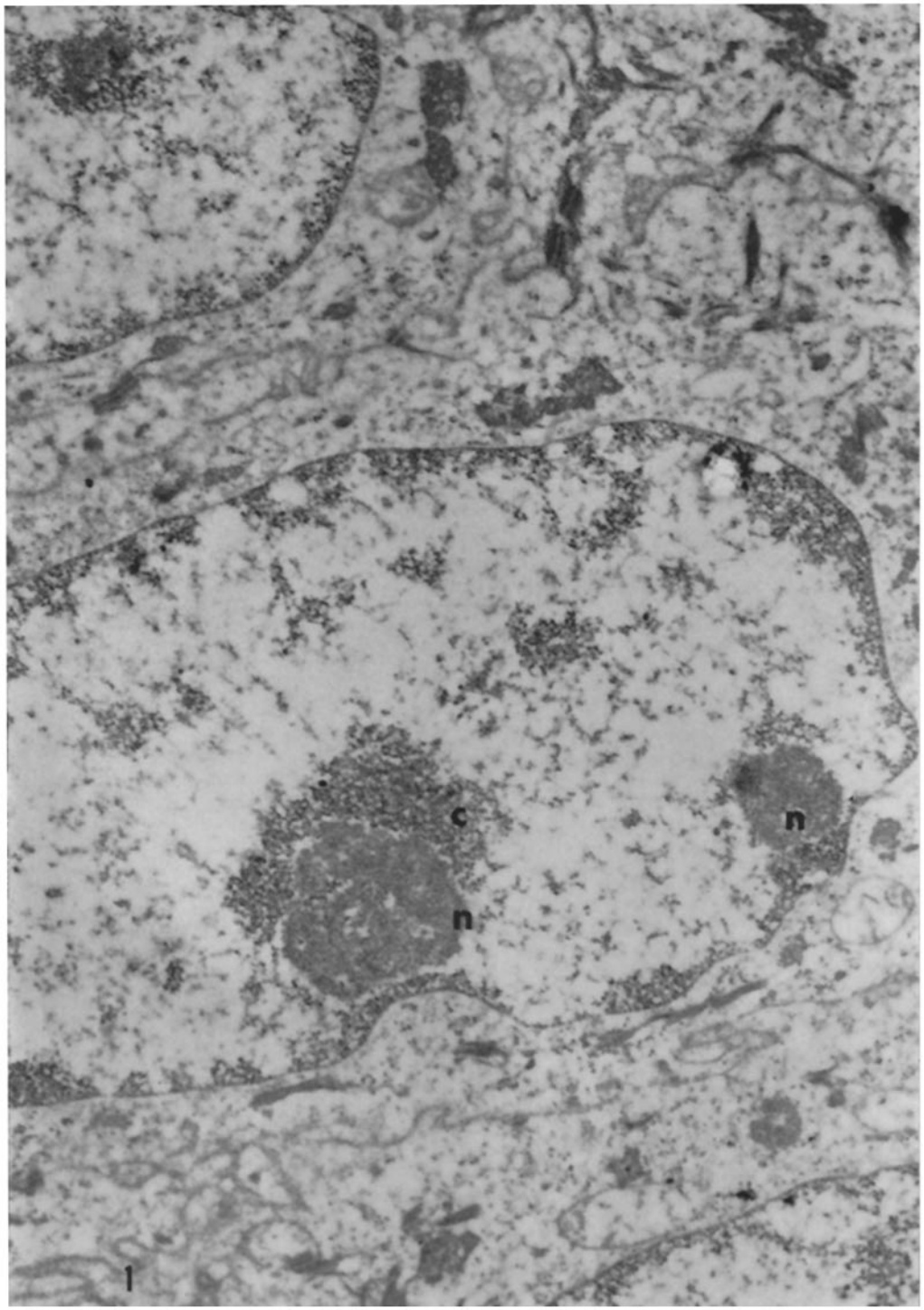
## EXPLANATION OF PLATES

All of the figures are electron micrographs of papillomas from cottontail rabbits.

### PLATE 26

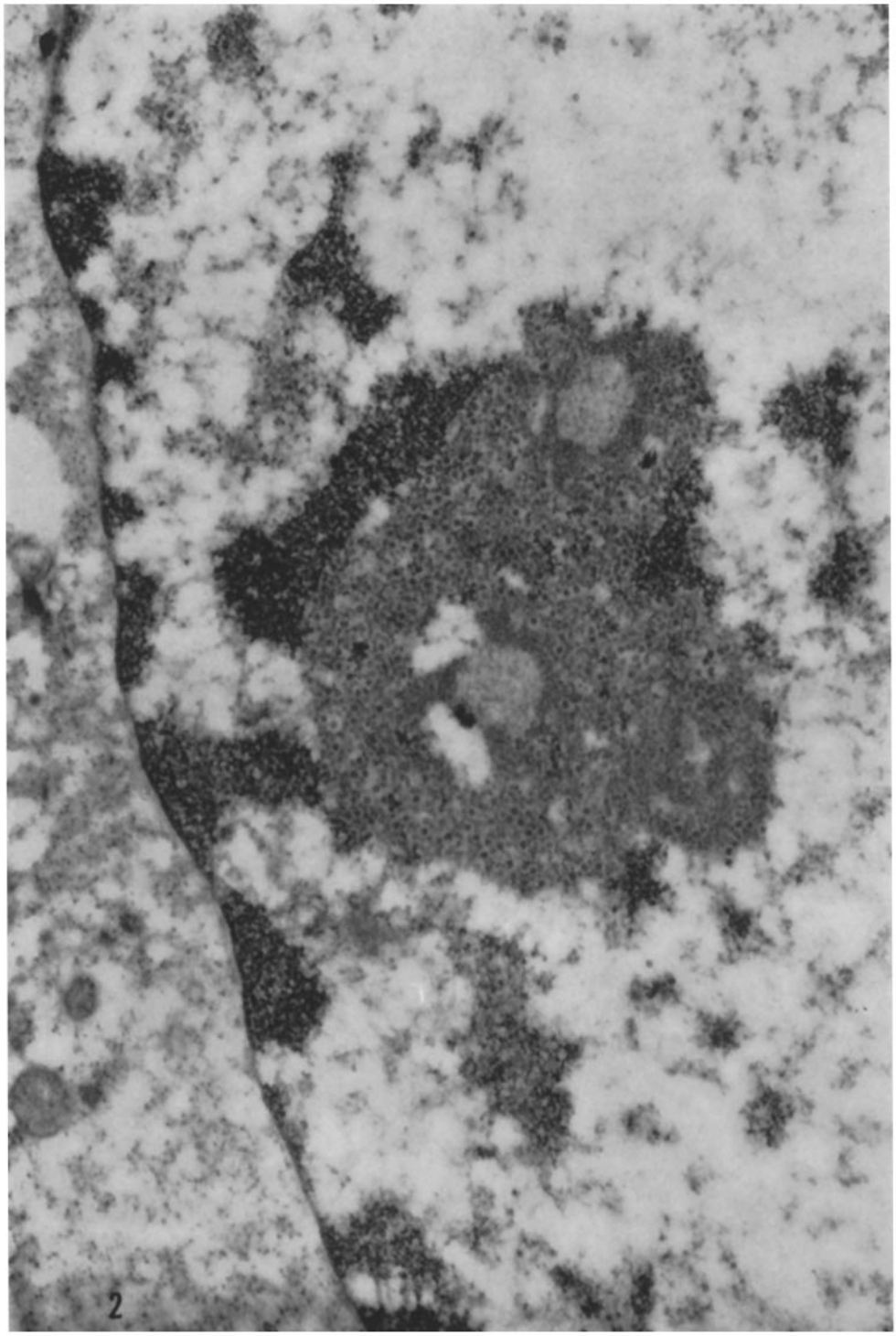
FIG. 1. An area in the lower stratum spinosum including portions of nuclei and cytoplasm of three cells. Two abnormal nucleoli, *n*, appear in the central nucleus. The nucleus is swollen and the chromatin is somewhat marginated and aggregated (*c*) above the larger nucleolus. Another nucleolus in the midst of some associated chromatin is visible in the cell at the upper left corner. × 25,000.




(Stone *et al.*: Electron microscope study of papilloma virus)

PLATE 27

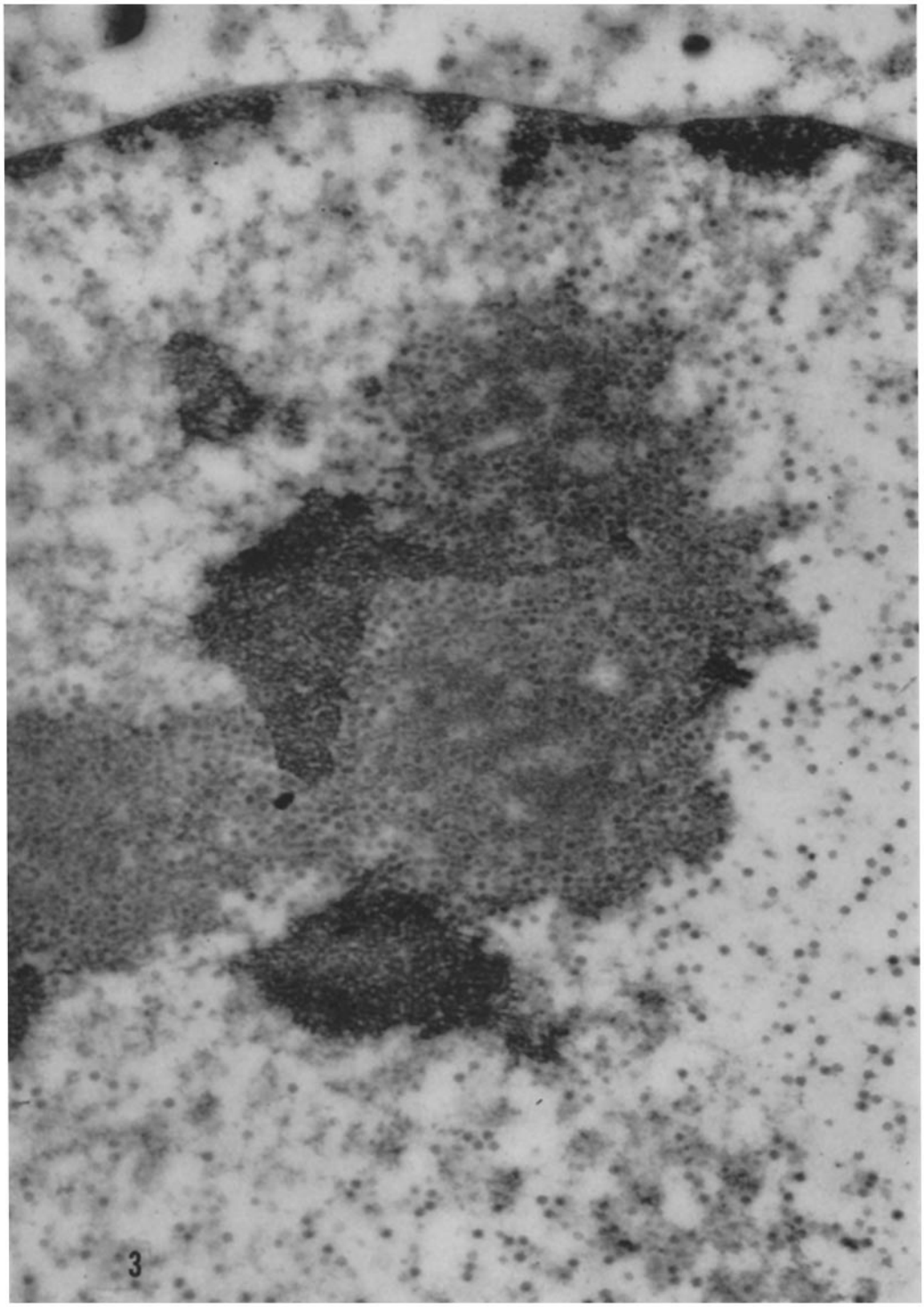
FIG. 2. Nucleolus in which viral bodies surrounded by zones of low density and strands forming a network are apparent. Patches of chromatin are scattered around the nucleolus, along the nuclear margin, and throughout the nucleus, but at this stage of development the viral bodies appear only in the nucleolus. × 37,000.




(Stone *et al.*: Electron microscope study of papilloma virus)

PLATE 28

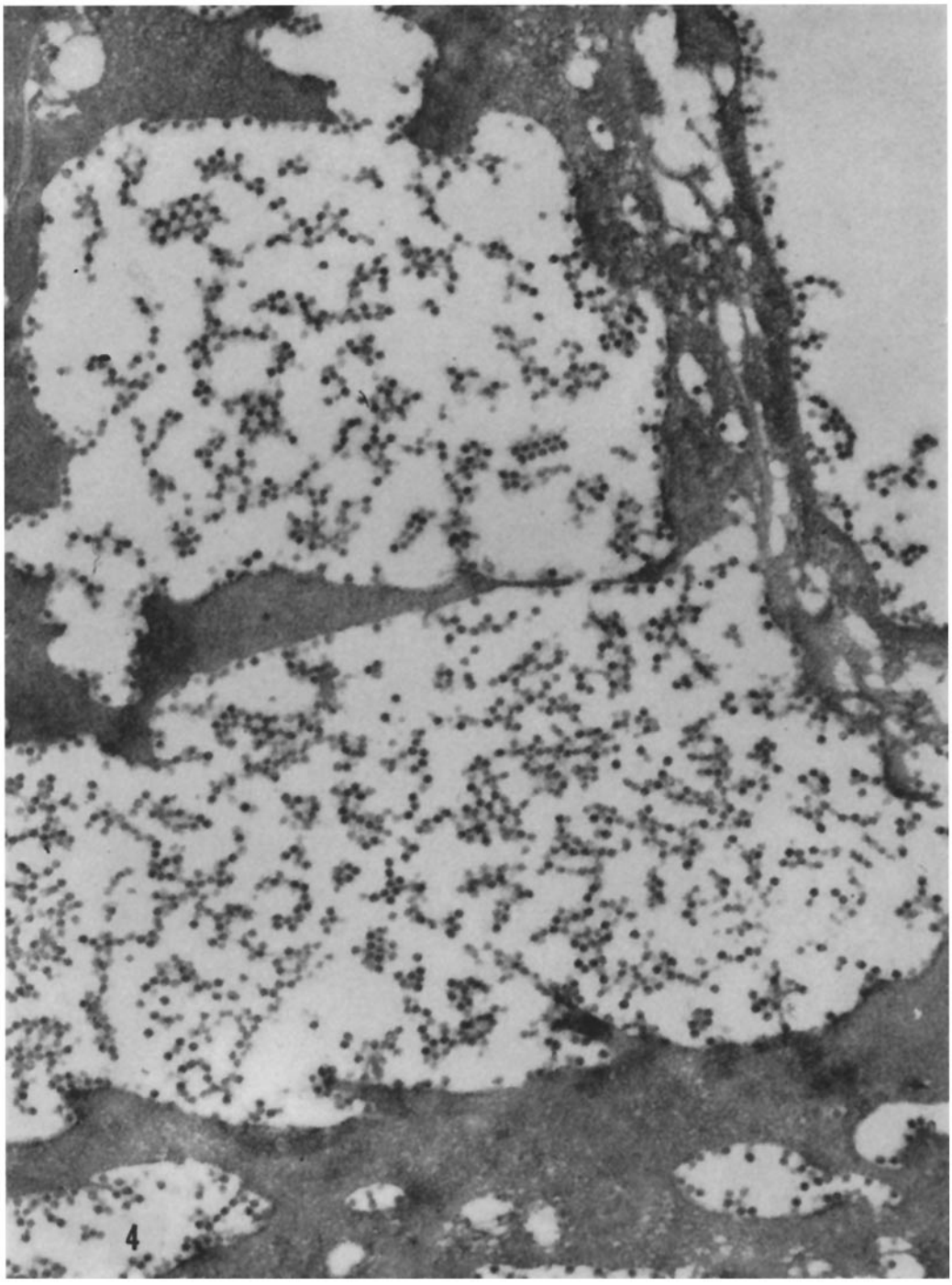
FIG. 3. Nucleus containing viral bodies not only in a network in the nucleolus, but also scattered throughout the whole intranuclear area. The nuclear membrane runs along the upper border of the micrograph and cytoplasm free of virus is above. × 49,000.




(Stone *et al.*: Electron microscope study of papilloma virus)

PLATE 29

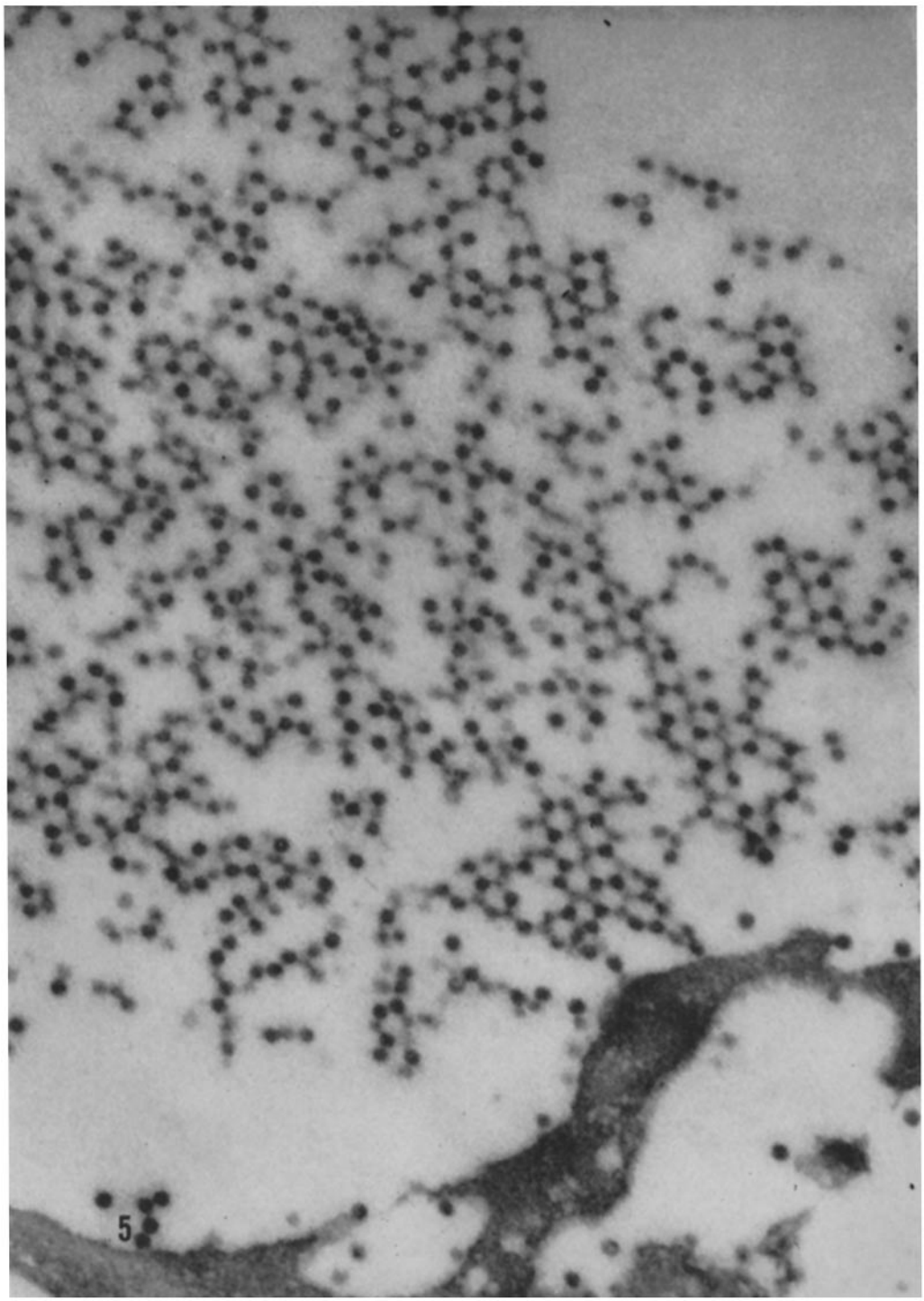
FIG. 4. Keratinized cell remnants of the cornified layer containing myriads of viral particles. × 42,000.




(Stone *et al.*: Electron microscope study of papilloma virus)

PLATE 30

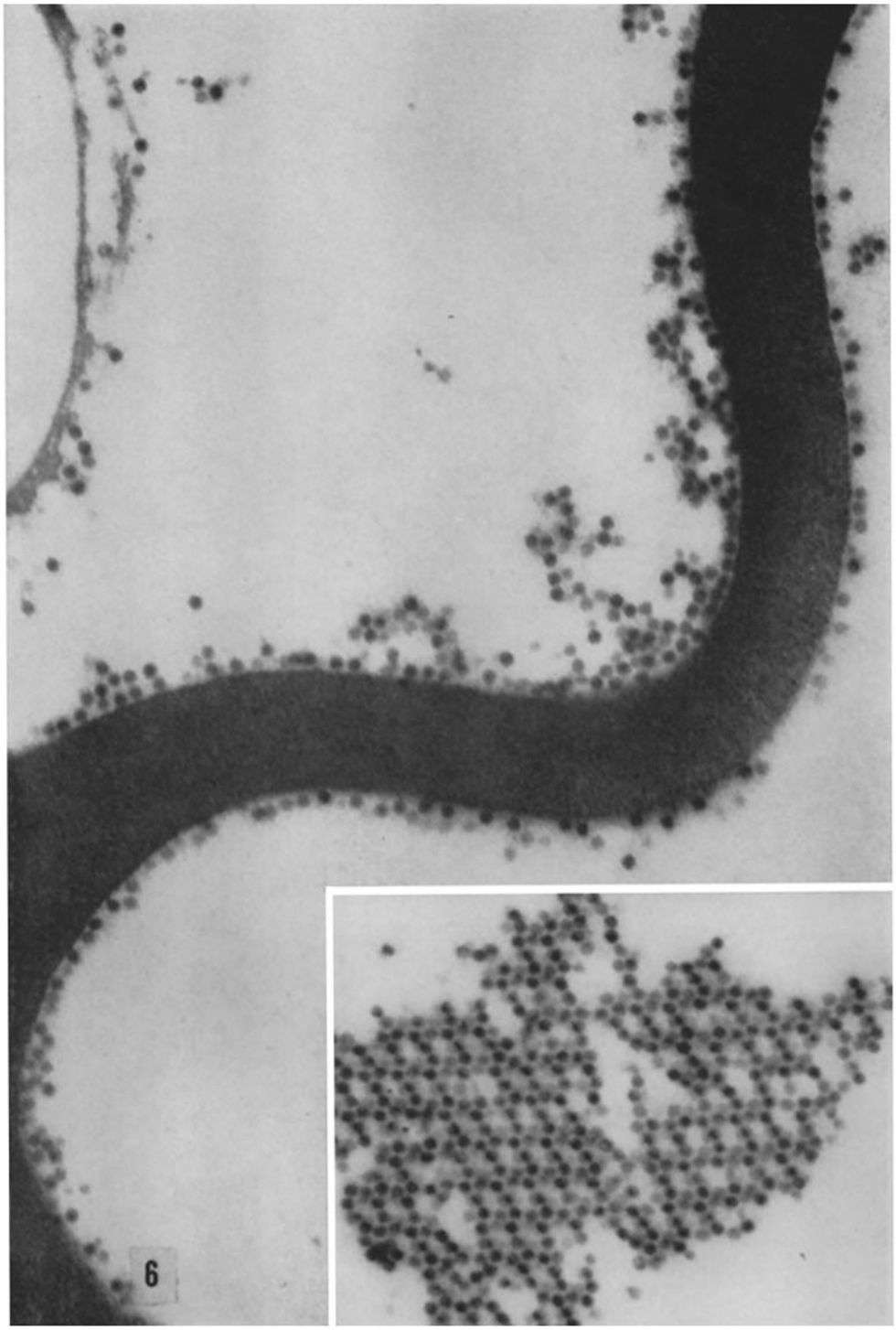
FIG. 5. Very thin section showing viral profiles. Some show eccentric densities whereas others show hollow circles. Variation in apparent size and density probably result from variation in proportion of individual bodies caught within this section. × 83,000.




(Stone *et al.*: Electron microscope study of papilloma virus)

PLATE 31

FIG. 6. A heavily keratinized cell border remaining intact in the upper corneum with numerous adherent viral bodies. Insert shows an array of viral particles from the same general area. × 62,000.




(Stone *et al.*: Electron microscope study of papilloma virus)