ELECTRON MICROSCOPY OF A BENIGN EPIDERMAL POX DISEASE OF RHESUS MONKEYS

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Electron microscopic studies of pox virus infection in monkeys are limited to those causing subcutaneous histiocytomas, the so-called Yaba lesions.[1-3] The present report describes the fine structure of a pox virus that caused epidermal papules in monkeys and that was morphologically indistinguishable from the mature Yaba virus. Other forms of cutaneous pox virus infections previously reported in monkeys include smallpox[4] and pox-like lesions in captive monkeys.[5-8]

The pox infection appeared at the USAF School of Aerospace Medicine in recently purchased monkeys (*Macaca mulatta*). Seven days following routine tattooing for purposes of permanent identification, 15 of 150 primates developed pox-like marking at the tattoo sites. The animals were nonfebrile and the lesions regressed 4–6 weeks after formation. The disease was transmitted experimentally to additional susceptible monkeys and was not associated with neoplastic tissue formation. A similar outbreak appeared at the Oregon Regional Primate Center in animals obtained from the same distributor.[9]

MATERIALS AND METHODS

Six days after appearance of the pox-like markings at tattoo sites, one of the 15 involved monkeys was sacrificed by means of intravenous sodium pentobarbital. Tissue from the lesion was placed in a test tube and quick-frozen in an acetone, dry ice bath. The tissue was stored in dry ice and later the same day minced, then mixed with Hanks's balanced salt solution (containing 500 U. of penicillin and 0.5 mg. streptomycin per milliliter) to form a 10% homogenized tissue suspension. An apparently normal primate, Monkey A, was inoculated with the suspension at multiple sites in the skin of the back, producing papules within 5 days (Fig. 1). A suspension of lesional tissue from this primate, prepared in an identical manner, was inoculated by needle prick into the back of a second normal animal, Monkey B. Specimens for study included 2-, 6-, and 13-day-old lesions from Monkey A, a 5-day-old lesion from Monkey B and a naturally occurring lesion of unknown age from Monkey C. Biopsies

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were performed under either general (sodium pentothal) or local (xylocaine) anesthesia.

Each biopsy specimen was halved and one portion was fixed in either 5% glutaraldehyde for 1 hr. (buffered with cacodylate at pH 7.2) or in 2% osmium tetroxide for 90 min. (buffered with phosphate at pH 7.2). Glutaraldehyde specimens were postfixed for 90 min. in 2% osmium tetroxide. Specimens were dehydrated with graded dilutions of ethyl alcohol and embedded in Epon 812.[10] Thin sections were cut with a diamond knife on a Porter Blum MT2 ultramicrotome, mounted on copper grids, stained with lead citrate,[11] and examined in a RCA EMU-3G electron microscope. Sections, 1 μ thick, were cut with glass knives, stained with Paragon 1301,[12] and examined under the light microscope.

The remaining half of each specimen was fixed in formalin, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined with an optical microscope.

Results

Initial observations were made by optical microscopy of paraffin and 1-μ thick Epon-embedded sections. In the 2-day-old papule, ballooned cells were visible in the slightly thickened epidermis. In older papules, the epidermis and hair follicle shafts were markedly increased in thickness, owing to cellular hyperplasia and increased cell size. Moderate polymorphonuclear infiltration was noted in the 5-day-old papule, and by the thirteenth day a suppurative inflammation was present with surface exudation and abscess formation. At low magnification the 13-day-old papule was composed of columns of swollen and granular epidermal cells with prominent cell membranes. High-magnification light microscopy (Fig. 2) revealed marked ballooning, cellular degeneration, and irregularly shaped, finely granular, cytoplasmic inclusions. The nuclei contained accumulations of amorphous pink-staining material that frequently appeared as vacuolated areas surrounded by distorted nuclear membranes. Oil immersion examination of thick (1 μ) sections (Fig. 3) provided additional cytological detail. The cytoplasm contained irregular clusters of dense particulate matter, and the intranuclear vacuoles were bordered by a marginated rim of chromatin material. Intercellular bridging was prominent.

Electron microscopy revealed the intracytoplasmic clusters seen in thick sections to be viral particles of the pox virus group (Fig. 4). An assortment of particulate viral forms was present within the clusters. Although it was not possible to formulate a developmental sequence from the static morphology available, arrangement of the viral forms according to their structural complexity was feasible. The least complex particulate forms consisted of semicircular shells located within or adjacent to a moderately dense granular-fibrillar cytoplasmic matrix (Fig. 4). A number of these semicircular shells appeared to have enclosed a portion of matrix (Fig. 4 and 5A). This semicircular form apparently preceded

the formation of a more complex circular form, *the formative shell.* The circular form (320–340 mμ in diameter) was comprised of a spiny double membrane (Fig. 4 and 5B) that enclosed granular-fibrillar material, *viroplasm,* which was indistinguishable from that of the adjacent cytoplasmic matrix. A portion of the enclosed viroplasm, in a later form, was condensed into a roughly circular, dense nucleoid (Fig. 5C). In a more complex stage, the nucleoid had elongated to form a central core made up of a membrane surrounding a collection of fibrillar material (Fig. 5D).

In succeeding stages (Figs. 5E, 5F, and 6) the core changed in configuration to assume a biconcave or dumbbell shape. It was uniformly covered by a core envelope of moderate electron-dense material which was occasionally separated from bordering side-bodies by an intervening membrane. The side-bodies, in turn, were covered by a series of layers comprised of alternating membranes and electron-translucent zones. Viral particles sequestered within the formative shell had a maximum of 3 external layers consisting of 2 dense membranes separated by an intervening translucent zone (Fig. 5E). Conversely, all viral particles that were free of formative shells had a minimum of 3 and a maximum of 7 distinct external layers (Fig. 6). In this regard, it appeared that development of additional external layers was contingent on the release of the immature particles from the formative shell. Evidence that the structurally immature particles were released from the formative shell was provided by the presence of numerous forms apparently in a stage of release (Fig. 5F).

The most complex particles were frequently found in larger numbers in sections prepared from the older papules. They ranged in length from 360 to 380 mμ, in width from 140 to 170 mμ, and were coated by 7 distinct layers comprised of 4 membranes and 3 translucent zones (Fig. 6).

In addition to the viral particles, the cytoplasm of infected cells contained numerous crystalloid structures (Fig. 7). Crystalloids were observed in sections prepared from all papules. These structures were frequently located adjacent to sites of viral multiplication but were not confined to these areas. They were usually rectangular in the plane of section and measured up to 0.6 × 3.6 μ in size. The crystalloids were composed of tubular-like structures giving a honeycombed appearance. The translucent centers of the tubules measured 280 Å across and the electron-dense walls, 80 Å wide. A vesicular network was visible around the periphery of each crystalloid, and virus particles possessing 3–7 external layers were often enclosed within the vesicular network (Fig. 7).

Electron microscopy revealed the nucleoli in infected cells from 2-, 5-, and 6-day-old lesions to be enlarged and densely granular. In sections

from the oldest lesion (13-day), membranous structures were observed within nuclei (Fig. 8). Electron microscopic examination demonstrated that the prominent intranuclear vacuoles seen on optical microscopy were actually composed of a sparse granular material (Fig. 9). No crystalloid structure or evidence of viral particle formation was noted within the nuclei.

Cytoplasmic forms of degeneration were observed in cells from all of the lesions. They included myelin figures (Fig. 8), swelling and distortion of mitochondria (Fig. 9), accumulation of randomly distributed glycogen granules, and membrane-bound lipid (Fig. 8).

Discussion

Somewhat similar morphologic features have been described for Yaba pox-virus,[2,3] vaccinia,[13,14] smallpox,[15] mousepox,[16] fowlpox,[17-19] swinepox,[20] and junco pox.[21] Most of the investigators postulate that the earliest detectable stage of pox virus formation is the development of membrane-bound spheres from cytoplasmic areas of granular-fibrillar material followed by the condensation of nucleoid material. Alternative ways by which the nucleoid is transformed into a viral core have been suggested. Avakyan and Byckovsky[15] infer that the nucleoid elongates into a membrane which encloses a portion of viroplasm forming a non-indented, uniform core. DeHarven and Yohn[2] have illustrated a viral particle containing a long membranous structure. The structure appeared to form in the nucleoid and was likened by them to a flat sac. They suggested that this sac subsequently opened up into a dumbbell-shaped viral core. In our material the early developmental forms resembled those described by Avakyan and Byckovsky,[15] but we also noted a single flat-sac form.

It is doubtful that the spherical shell provides the final coat for the mature virus. In our studies, the presence within shells of viral forms possessing a maximum of 3 external layers and the observation of unique "release" forms covered by equally thick coats suggest that the viral elements mature by a different method. The possibility that the release forms represent degenerative particles must be considered, but since their presence was limited to areas of active viral multiplication, they probably represent a stage in the development of the virus.

The manner in which the immature particle matures by the acquisition of 4 additional external layers was not clearly evident in our material. Yet, the close proximity of mature forms and immature forms unassociated with formative shells to the large cytoplasmic crystalloids suggests that the latter structures may take part in such a process. Such a function was proposed by Cheville[20] for similar lamellar bodies seen in swinepox. Polygonal inclusions superficially resembling the crystalloids

seen in the present material have been described in Yaba disease[2] and were considered by DeHarven and Yohn to represent, perhaps, accumulated viral nucleoprotein.

The electron-dense granules observed within viral particles in the present study are similar in size, shape, and location to granules found on molluscum contagiosum,[22] Yaba,[2] and swinepox.[20] In our studies, their location varied from within the core to the area of the side-body. Although the origin of the granules is unknown, they might represent remnants of nucleoid material left over following transformation of the nucleoids into viral cores.

Though the virus discussed in the present study shares a number of morphologic features with other pox viruses, its combined fine structure and pathologic effect on the target organ make it distinctive. Viral particles described by Prier and Sauer[8] in their cases of monkey pox disease ranged in length from 200 to 250 mμ and the infected cells contained rare nuclear inclusions. The pox virus under study most closely resembles the Yaba virus. The mature forms of both have the same number of layers in their external coat and are similar in size. Most recent calculations made by DeHarven and Yohn[2] define a particle 350–390 mμ in length and 150–200 mμ in thickness. The mature virus observed in our tissue had approximately the same dimensions, ranging in length from 360 to 380 mμ and in width from 140 to 170 mμ. Although morphologically similar, Yaba, in contrast, produces histiocytomas[23,24] in the dermis and subcutaneous tissues, and the cells infected by it are free of nuclear vacuoles.[1,2] Preliminary serologic studies with this newly recognized virus also have shown it to be readily distinguishable from the Yaba and vaccinia pox viruses.[25]

As in the present disease, fine structural study reveals that the nuclear vacuoles in junco pox[21] contain sparse granular material. Stained with hematoxylin and eosin, they appear bright red and have been likened to the intranuclear inclusions in smallpox lesions.[26] When stained in a like manner, eosinophilic "inclusions" were occasionally noted in nuclei from our material. In view of the absence of demonstrable viral particles within these vacuoles, it is suggested that the inclusions seen in paraffin-embedded tissue are actually artifacts of preparation resulting perhaps from aggregation of the sparse granular material within the vacuoles. The swinepox virus also produces intranuclear vacuoles, but ultrastructural studies show they are composed of fine fibrils.[20]

Summary

The fine structure of papular lesions produced in monkeys by a pox virus that morphologically closely resembles Yaba pox virus is described. Unlike Yaba, the lesions were confined to the epidermis and the infected

cells contained prominent intranuclear vacuoles similar to those seen in junco pox. The developmental cycle of the monkey pox virus is discussed, and a "release" form, previously undescribed for the pox virus group, is reported. The infected cells were shown to contain crystalloids, and evidence is provided that they may be associated with further maturation of viral particles following their release from "formative shells."

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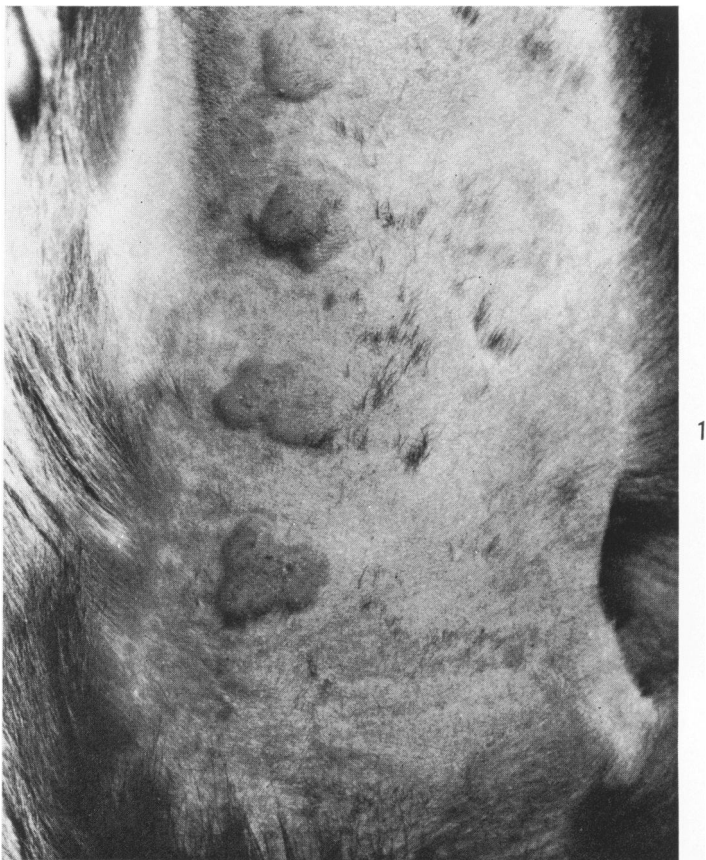
[*Illustrations follow*]

Legends for Figures

Fig. 1. Close-up of experimentally produced lesions on back of Monkey A, 6 days following appearance.

Fig. 2. Portion of papule showing ballooned epidermal cells and distorted nuclei. From 13-day-old lesion. Hematoxylin and eosin stain. × 400.



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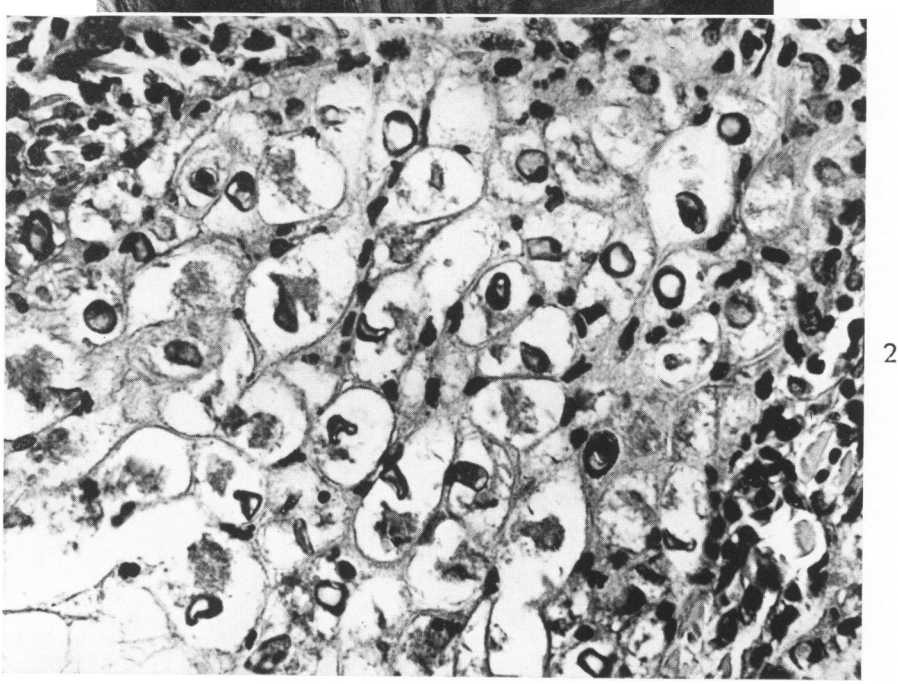

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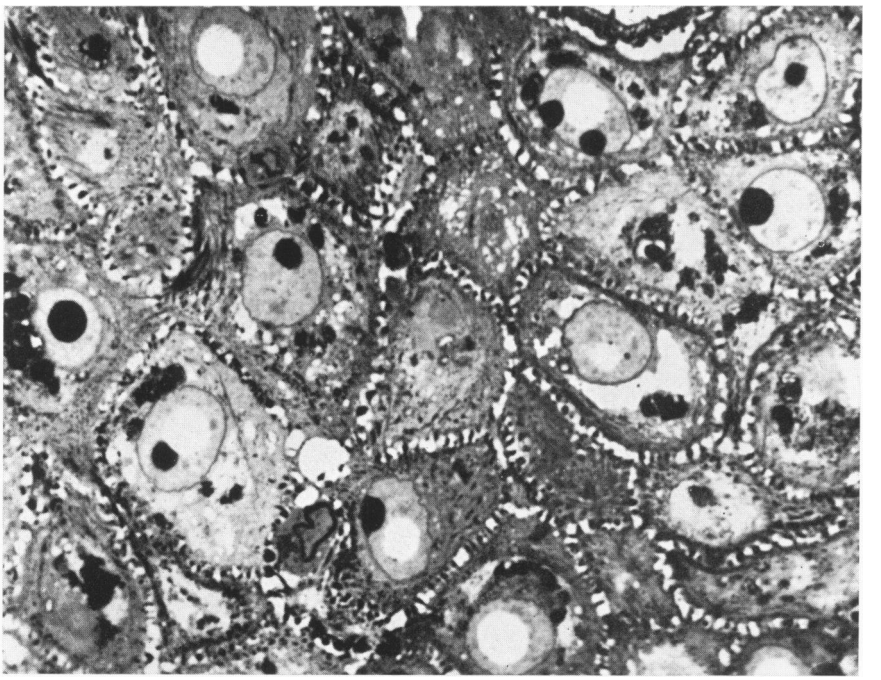
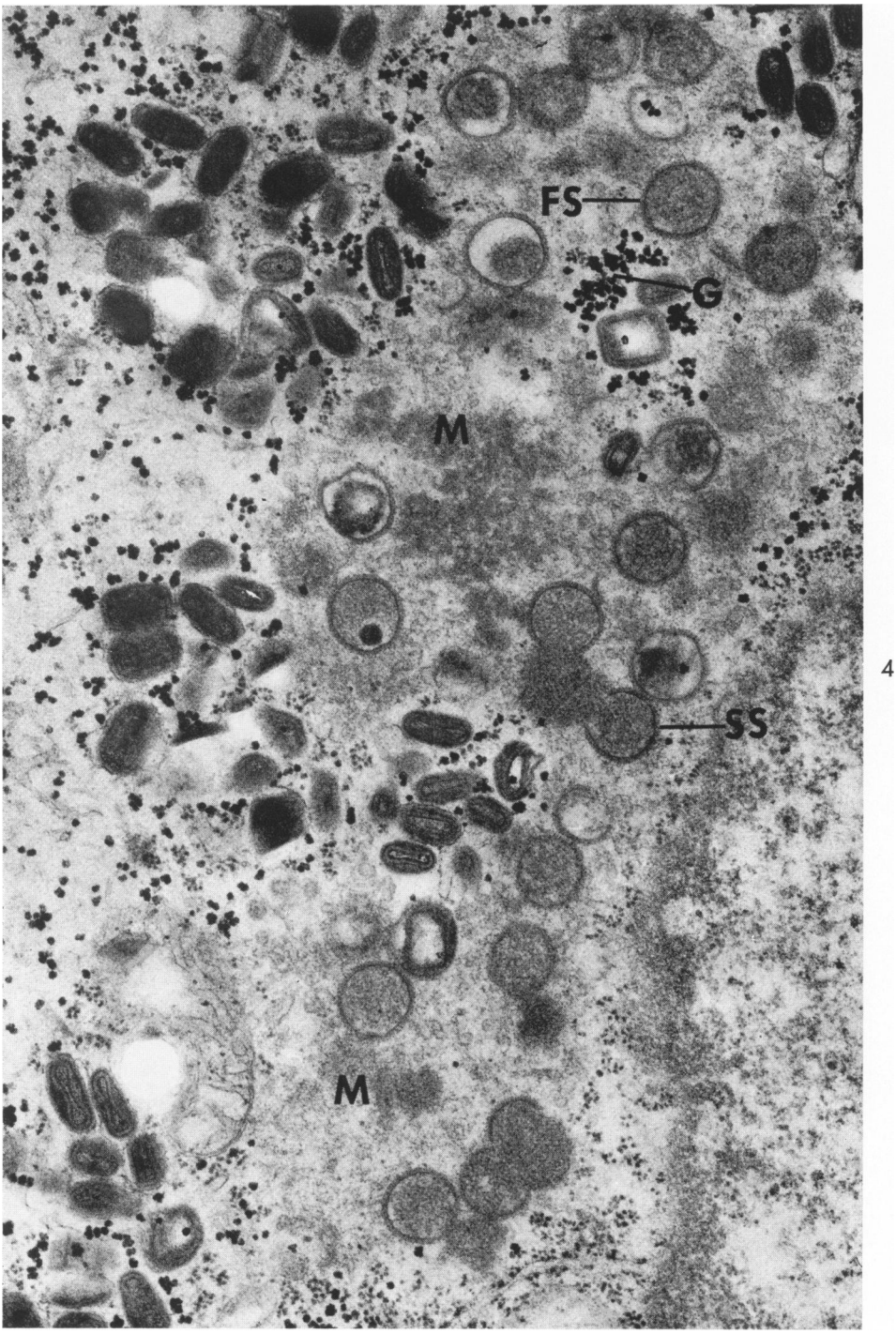


FIG. 3. Cells from 13-day-old lesion demonstrating particulate material in cytoplasm, nuclear vacuoles, and enlarged, often multiple nucleoli. Epon-embedded 1-μ section, Paragon stain. × 1000.

FIG. 4. Cytoplasmic focus of viral multiplication showing viral matrix (M) and assortment of particles including semicircular (SS) and formative shells (FS), as well as more mature forms. Randomly distributed glycogen granules (G) are evident. From 2-day-old papule. × 15,080.



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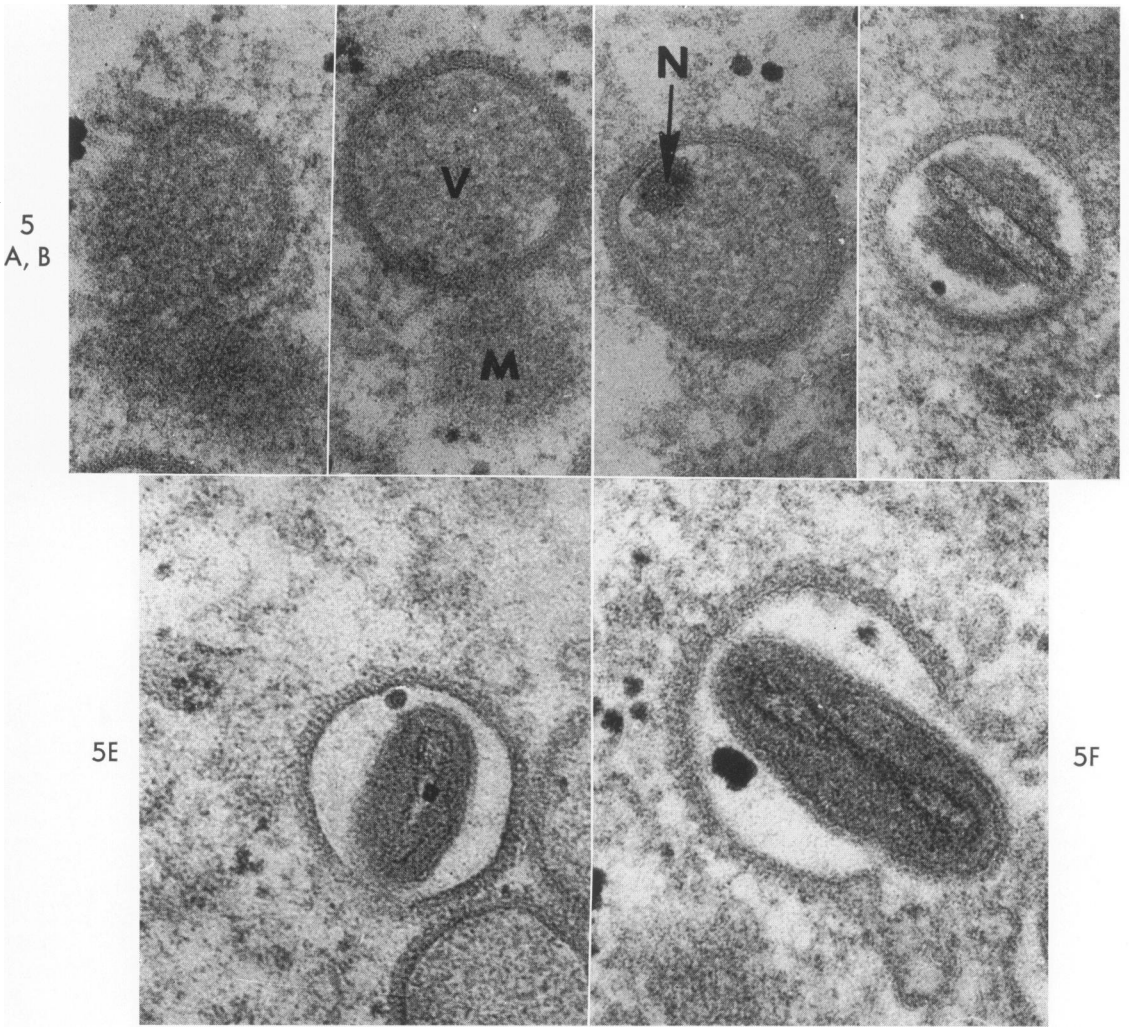

FIG. 5. Basic morphologic types of immature viral forms found in cytoplasmic areas of viral multiplication. A. Semicircular shell enclosing viral matrix. × 87,984. B. Circular "formative shells" enclosing viroplasm (V); and adjacent matrix, M. × 87,984. C. Formative shell containing dense nucleoid (arrow). × 87,984. D. Formative shell with early core development. × 83,200. E. Semimature form within formative shell. × 135,360. F. Semimature form apparently undergoing release from formative shell. × 128,000.

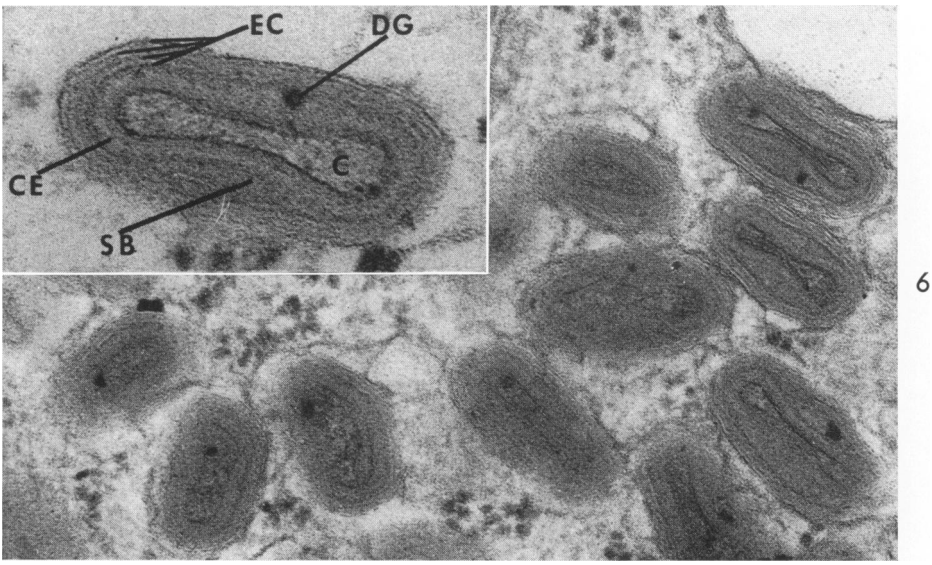

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FIG. 6. Mature viral particles free of formative shells, characterized by 7-layered external coats. C indicates core; CE, envelope enclosing core; DG, dense granule frequently seen in either core or area of side-body (SB); EC, external coat. From 2-day-old lesion. × 87,984. Inset: × 135,360.

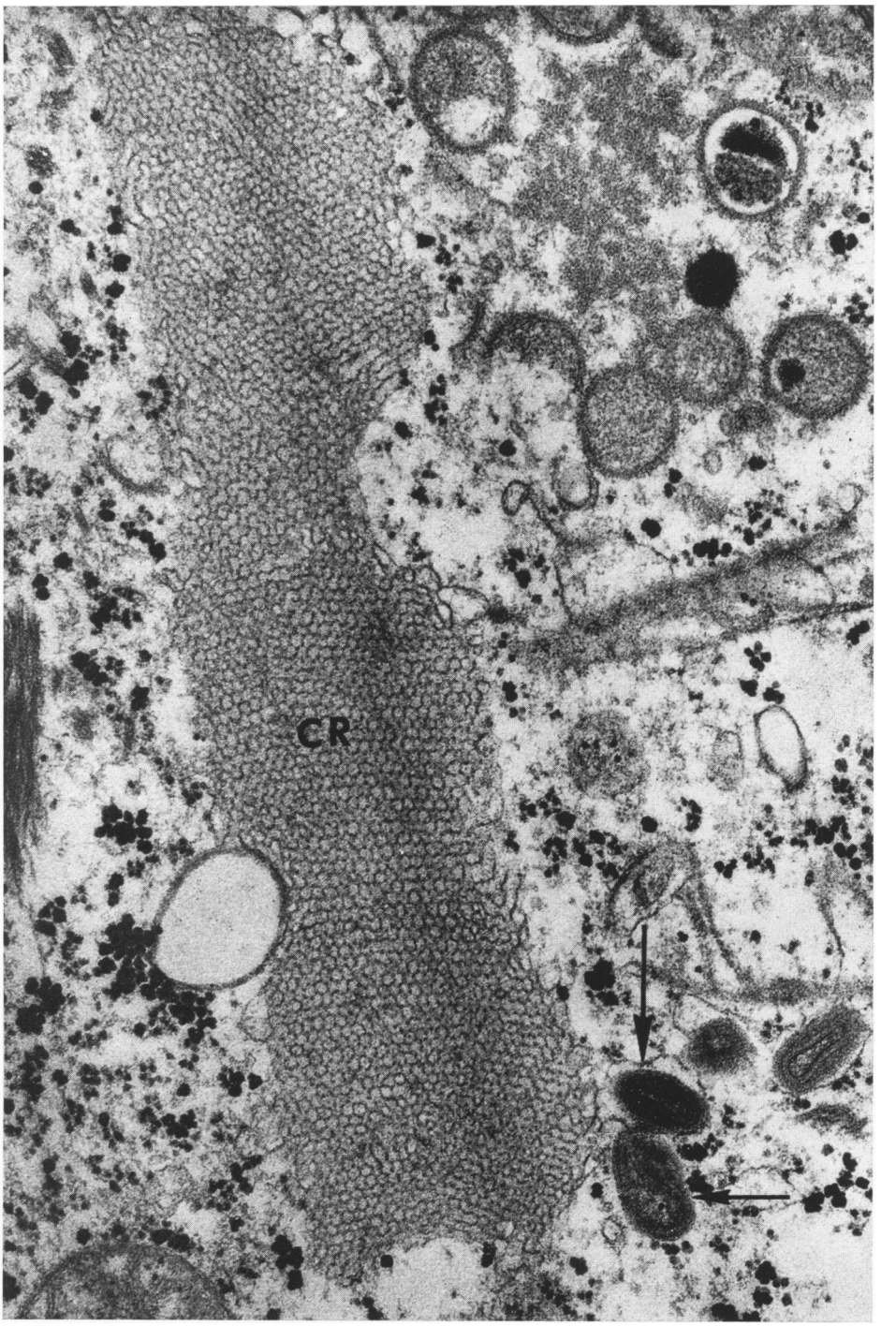

FIG. 7. Elongated crystalloid (CR) adjacent to area of viral multiplication. Two viral particles (arrows) are enclosed within vesicular network which is continuous with honeycombed structure. From naturally occurring lesion. × 49,600.

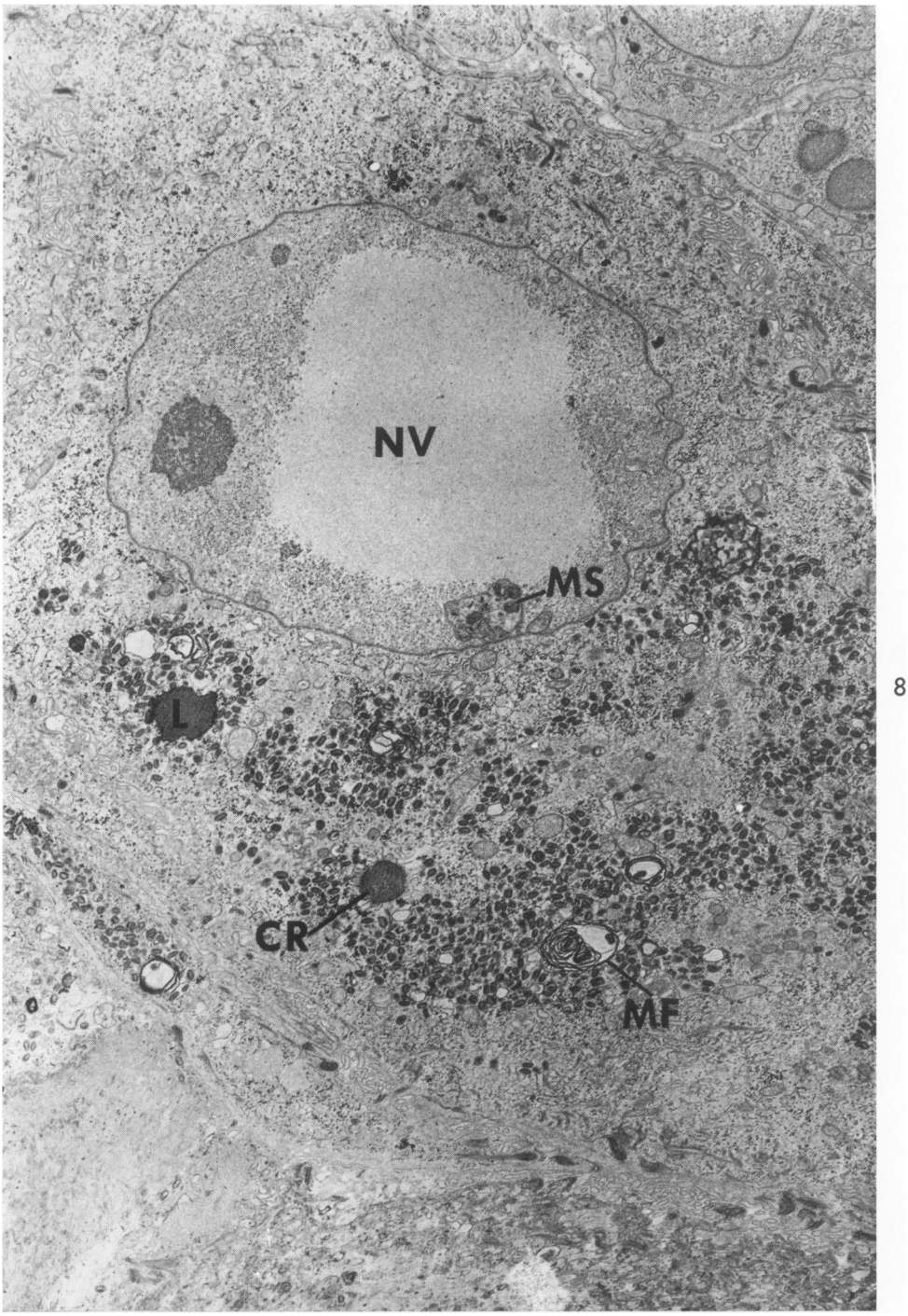

FIG. 8. Cell from 13-day-old lesion showing intranuclear membranous structures (MS) and densely granular nucleolus. Cell cytoplasm contains numerous viral particles. NV indicates intranuclear vacuole; L, membrane-bound lipid inclusion; MF, myelin figure; CR, crystalloid. × 10,790.

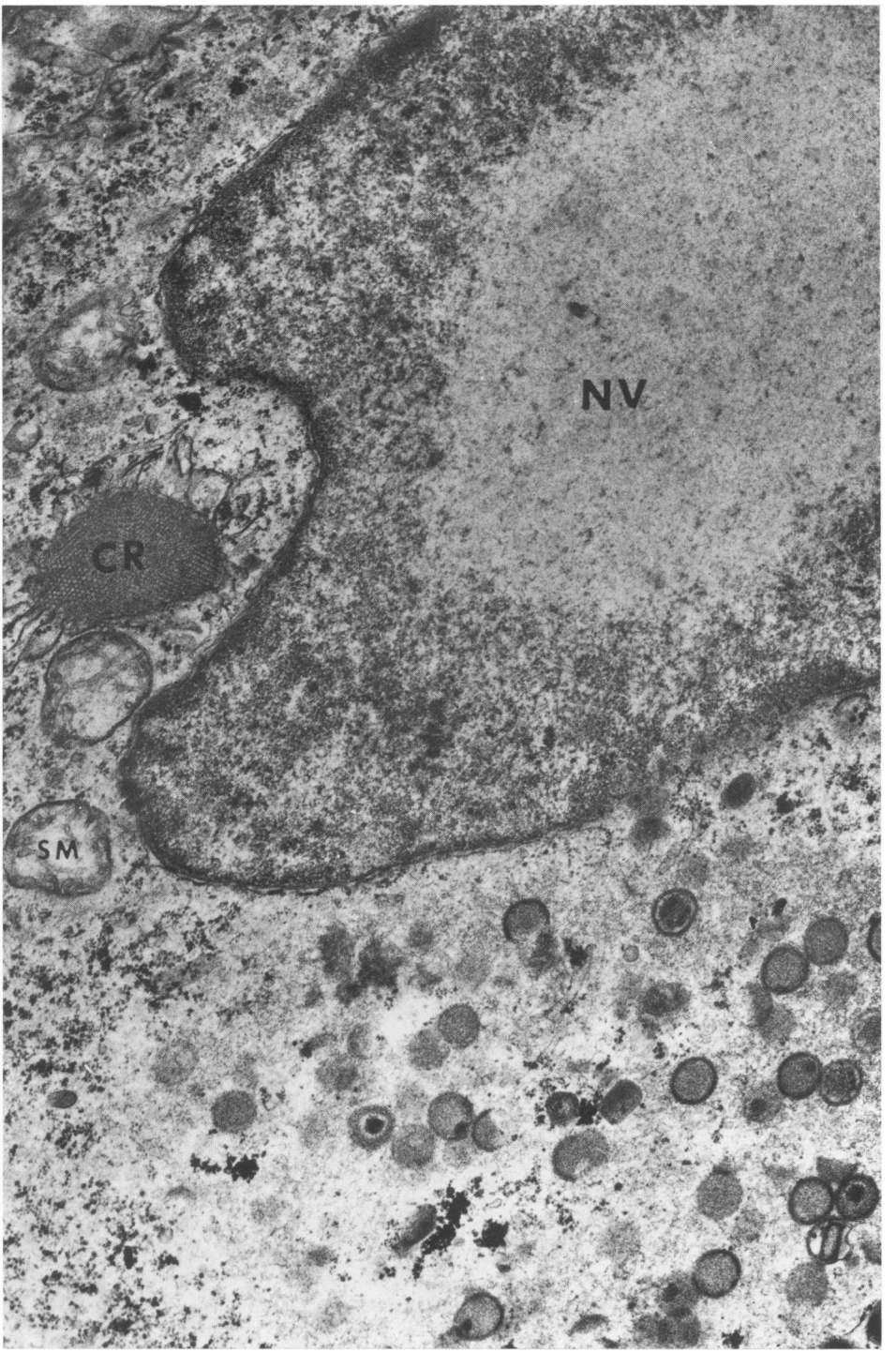

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FIG. 9. Large intranuclear vacuole (NV) surrounded by marginated chromatin. Vacuole contains sparse granular material. From 6-day-old papule. SM indicates swollen mitochondrion; CR, crystalloid. × 22,464.