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ELECTRON MICROSCOPY OF FOWL-POX VIRUS

By :

Kh. ZARRIN, D.V.M.,* A. AFSHAR, D.V. Sc., Ph. D.** A. MODJTABAI, M.D.* and A. RIAZI **
With technical assistance of G. RAHMANI*

Introduction :

Since 1947, when the technique of electron-microscopy was first applied to study the ultrastructure of fowl-pox virus, elementary bodies or Borrel bodies- (Boswell, 1947), a number of accounts have revealed the ultrastructure and development of viral particles and nature of the cytoplasmic inclusions - Bollinger bodies-associated with this virus (Morgan and Wyckoff, 1950; Morgan, Ellison, Rose and Moore, 1954; Eaves and Flewett, 1955; Kato, Hagiwara, Baba, Sato and Kamahora, 1955; Peters, 1959; Randall, Gafford and Arhelger, 1961; Arhelger, Darlington, Gafford and Randall, 1962; Beaver, Cheathem and Moses, 1963; Arhelger and Randall. 1964; Tajima and Ushijima, 1966).

These studies provide considerable information concerning the ultrastructure, the mode of development of viral particles and the nature of inclusions associated with fowl-pox virus. It has generally been considered that the Bollinger body is the site of gradual transition between undifferentiated and mature viral particles which have a dumb-bell like appearance with an outer membrane (Andrewes, 1964).

The present paper records the electron microscopic study on the chick chorioallantoic membrane (CAM) infected with a local strain of fowl-pox virus.

Materials and Methods :

A strain of fowl-pox virus, AP8, was isolated by chorioallantoic membrane inoculation using the technique described by Beveridge and Burnet (1946). For this study an inoculum was prepared in phosphate buffer saline (P.B.S.) solution from the infected chorioallantoic membrane of 2nd passage of AP8 strain of fowl-pox virus. The chorioallantoic membrane of 11-day-old embryonated eggs were inoculated with 0.1 ml. of the inoculum.

Sections of 216-hour infected chorioallantoic membrane were obtained from the site of inoculation where discrete pocks had been developed. The sections were

*From: Cancer Institute, Cancer Research Centre, Electron Microscopy Section Tehran, Iran.

**From: Dept. of Microbiology, College of Veterinary Medicine, Tehran University Tehran, Iran.

immediately immersd in a drop of 1% buffered osmium tetroxide (Dalton, 1955), and cut into 1 mm. cubes which were further fixed for one hour at 4°C. The tissue was rapidly dehydrated in graded alcohols, embedded in Epon 812, and sectioned on an automatic L.K.B. microtome with a glass knife. Suitable thin sections (300-500 A°) were mounted on specimen grids, stained with uranyl acetate for 10 minutes (Watson, 1958), and further 10 minutes with lead acetate (Reynolds, 1963). The specimen were examined in Siemens Elmescope A1 Electron Microscope.

Results :

Mature viral particles were observed in all the sections examined. Figure 1 and 2 show parts of cytoplasmic inclusions which contain several virus particles. Note that some of the virus particles sectioned transversally have a characteristic pox virus structure, consisting of a dumb-bell shaped or biconcave nucleoid (Fig. 1) which at times gives the impression of a figure eight (Fig. 2), and viroplasms surrounded by a clear zone which in turn is limited by a double layer envelope (Fig. 3, 4). The viral particles measured from 400 × 270 mμ, to 220 × 100 mμ. giving an average particle dimension of 288 × 159 mμ. They varied considerably in structure and frequently few of them clustered together (Fig. 3, 4, 5, 6). In most of the viral particles the inner core of the nucleoid appeared to be composed of a reticular network of coiled or interweaving filaments (Fig. 1, 5).

Cellular alterations included marked vesiculation of cytoplasm (Fig. 3).

Discussion :

Arhelger and Randall (1964) and Jajima and Ushijima, (1966) who studied the development of fowl-pox virus in chorioallantoic membrane reported that following the initial absorption and phagocytosis of fowl-pox virus at 96 hours, the first mature viral particles appeared within the cytoplasmic inclusions. They also found that at 120 and 144 hours numerous viral particles were emerging from the infected cells of the corioallantoic membrane. In the present study the infected chorioallantoic membrane was examined at 216 hours, beyond the maximum time set for by other investigators (Arhelger and Randall, 1964). At 216 hours, we were able to find only the mature forms of virus particles located within the inclusions (Fig. 1, 2) or scattered in the disrupted cytoplasm, frequently few particles clustered together (Fig. 3, 4, 5, 6). The morphological characteristics of fowl-pox viral particles reported here were similar to those described by previous workers (Morgan and Wyckoff, 1950; Beaver, Cheathem and Moses, 1963, Arhelger and Randall, 1964; Tajima and Ushijima, 1966).

Summary :

Chorioallantoic membrane were examined electron microscopically at 216 hours after inoculation with a local strain, AP8 of fowl-pox virus. The mature forms of viral particles were observed within the cutoplasmic inclusions and in the disrupted parts of the cytoplasm of infected cells. The morphological characteristics of fowl-pox viral particles were similar to those described by the previous investigators.

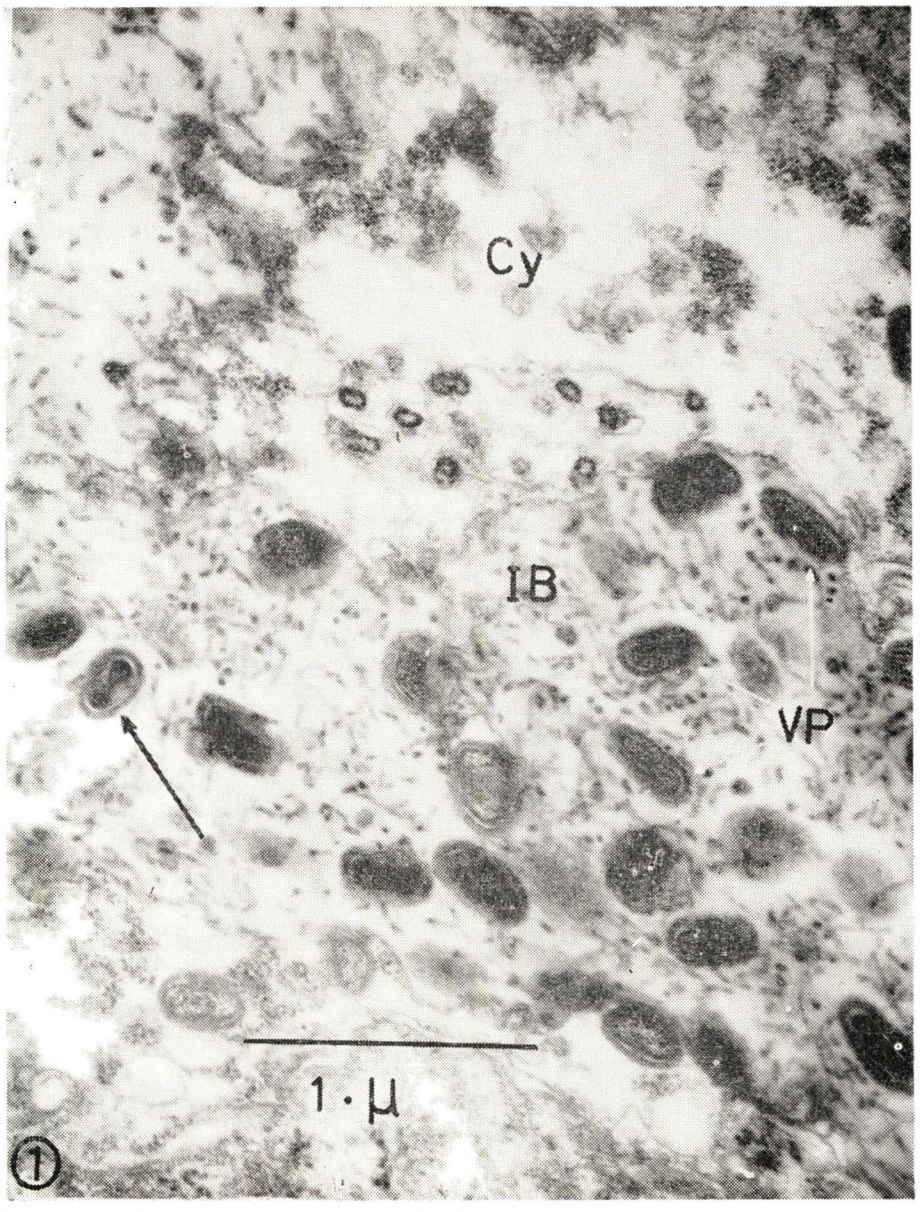

Fig. 1— Portion of the cytoplasm (CY) of a cell from the chorioallantoic membrane showing a part of an inclusion body (IB) containing numerous virus particles (VP). The arrow points to a typical virus particle with a dumb-bell shape osmophilic nucleoid. Magnification: X 48,000.

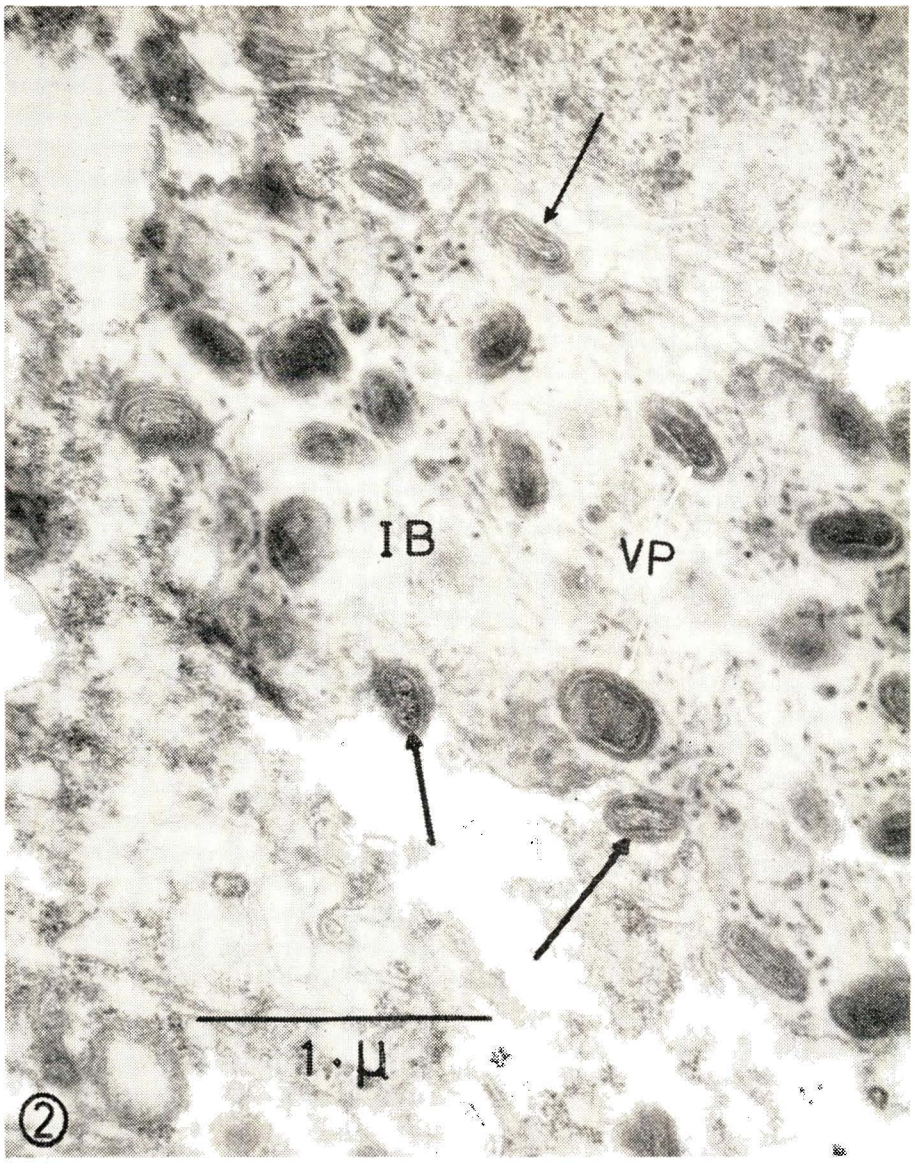

Fig. 2— Portion of the cytoplasm (CY) of a cell showing a part of an inclusion body (IB) containing numerous virus particles (VP). The arrow points to the reticular network of coiled or interweaving filaments of the nucleoid of the virus particles. Magnification: X 48,000.

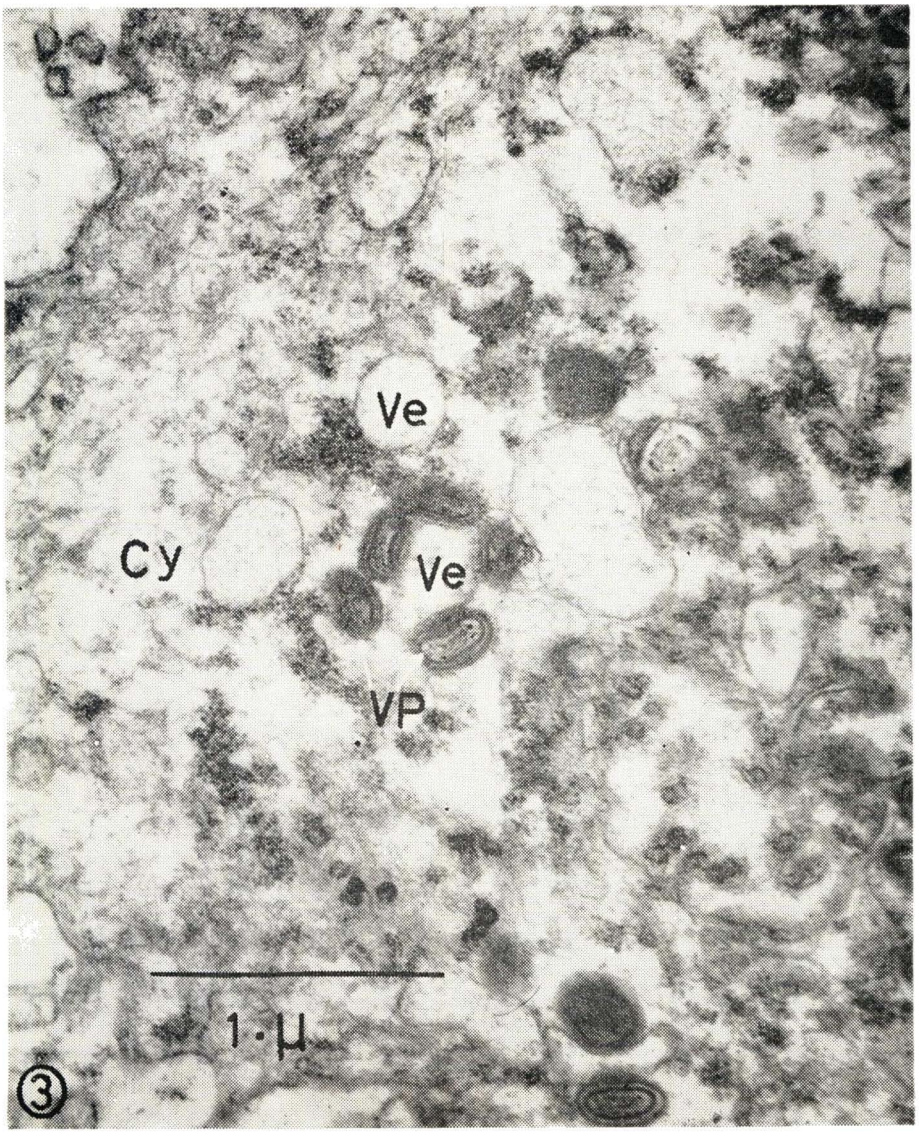

Fig. 3— A part of the disrupted cytoplasm (Cy) of an infected cell showing a cluster of viruses (VP) and numerous vesicules (Ve). Magnification: X 48,000.

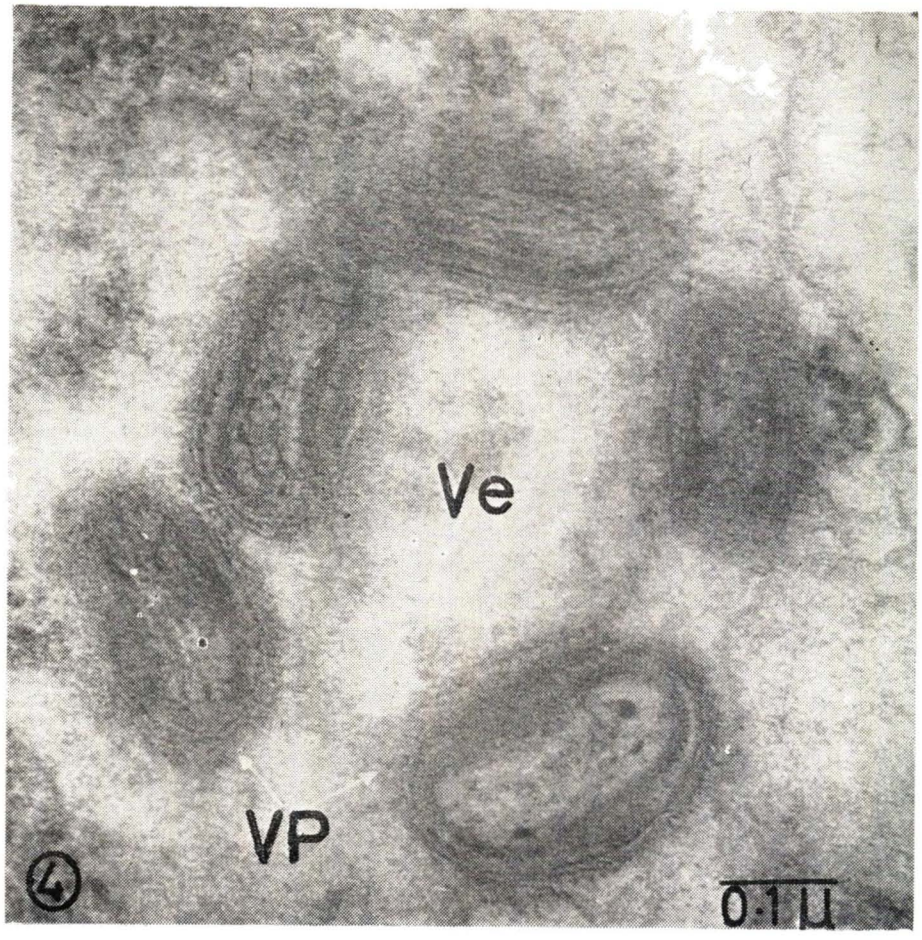

Fig. 4— High magnification electron micrograph showing five fowl-pox virus particles (VP) around a vesicule (Ve) in the cytoplasm of an infected cell. Note the double membranel outer coat (arrow) of a virus particle. Magnification: X 176,000.

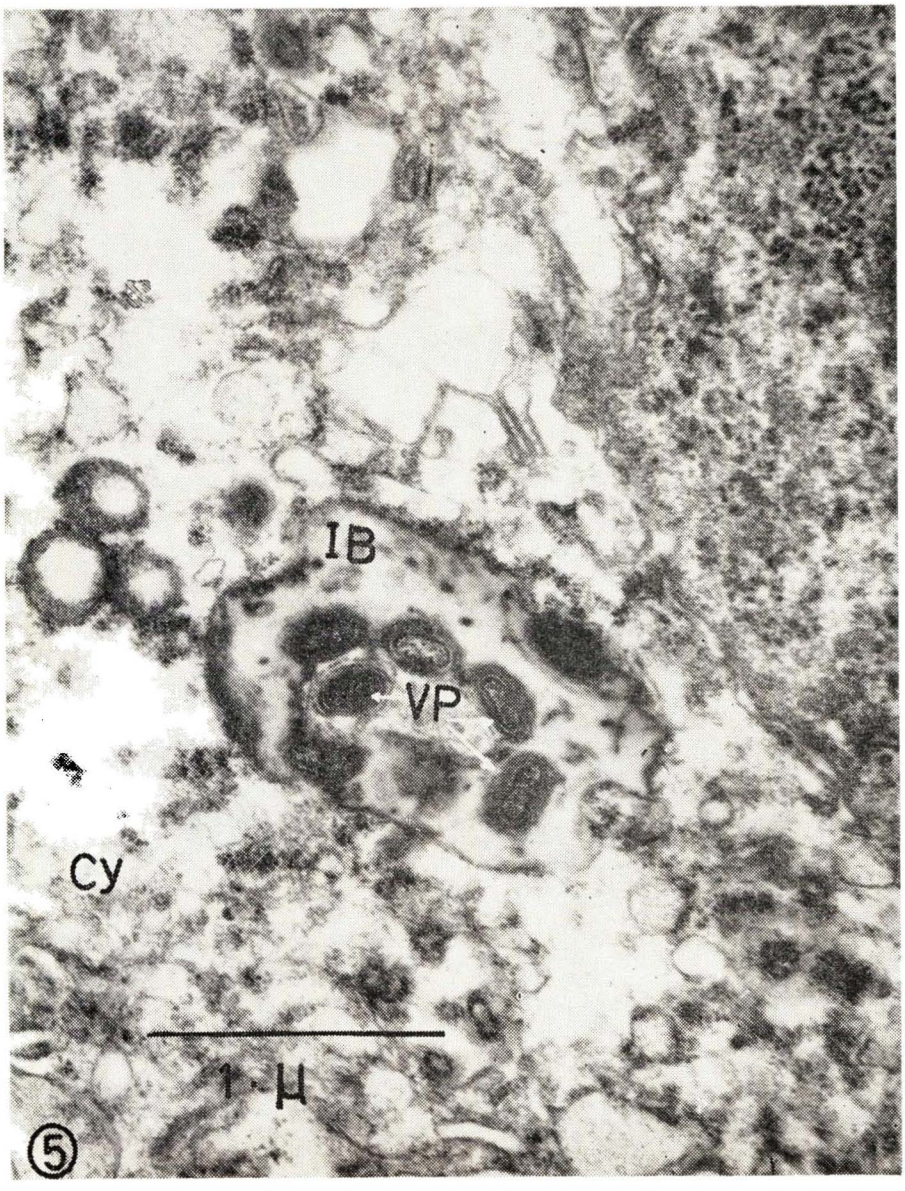

Fig. 5— A part of the disrupted cytoplasm (CY) of an infected cell showing an inclusion body (IB) containing some viral particles (VP).
Magnification: **X** 48,000.

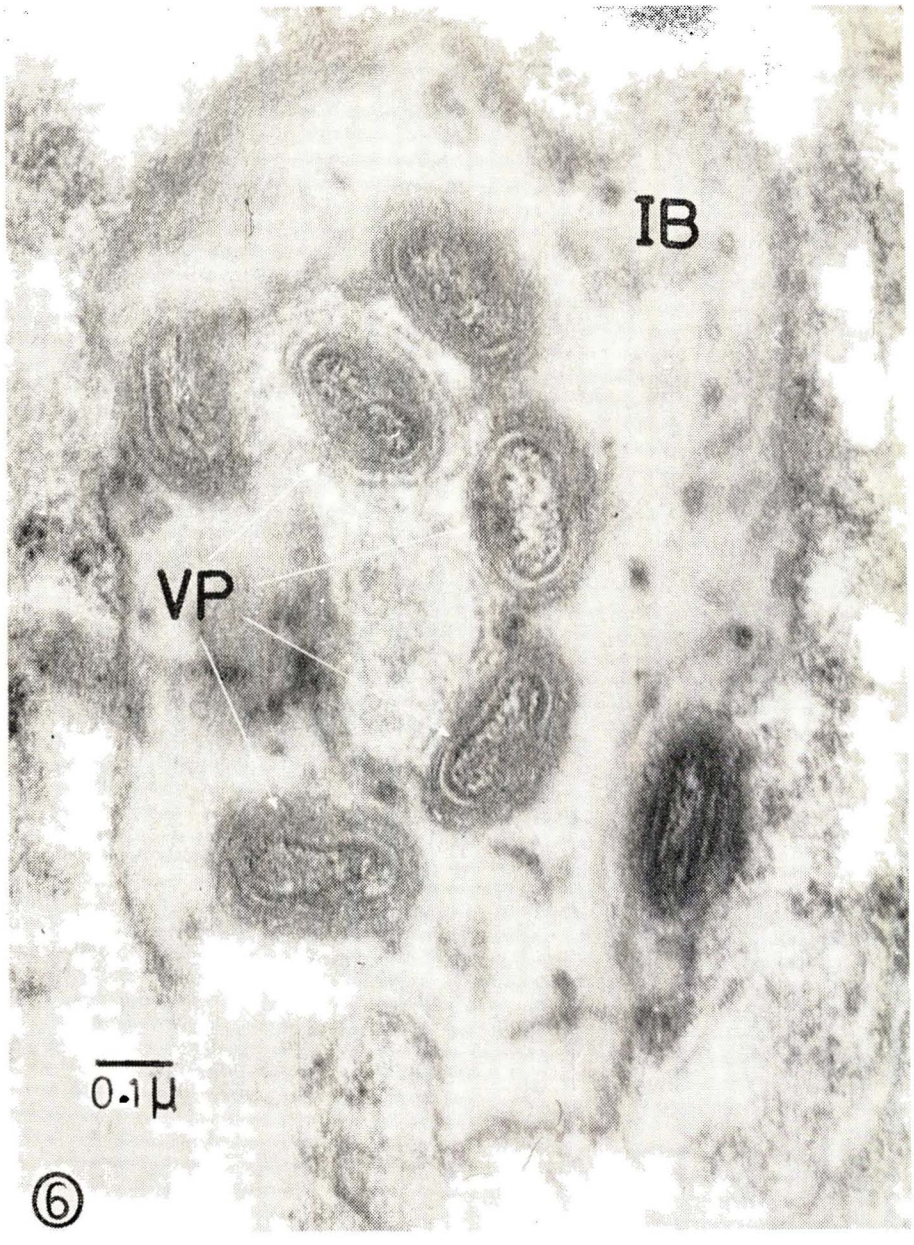

Fig. 6— High magnification electron micrograph showing an inclusion body (IB) and some viral particles (VP). Magnification: X 176,000.

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