INVESTIGATIONS ON PARAINFLUENZA-3 VIRUS ISOLATED FROM CALVES IN POLAND. STRUCTURE OF VIRION AND INCLUSION BODIES

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The results of numerous investigations on structure and replication of the parainfluenza-3 virus (Pl-3) are not always in accordance. It is true, among other things, for appearance of inclusion bodies in infected cells, what is of importance for diagnostical examinations. These bodies were not observed by Cochen et al. (4) in the amnion cells of hen or man infected with the strain of this virus isolated from men, whereas by Bögel and Klinger (11, 12) the occurrence of eosinophilic inclusions in nuclei and cytoplasm of calf kidney cells infected with the above virus have been described, while Karten and Churchill (7), Kuhn and Kita (6) and Ané (1) have found these bodies only in cytoplasm of the same cells. It follows from the investigations of Kita (8) that the parainfluenza-3 virus strains isolated from cattle in Poland are capable to induce acidophilic inclusions in cytoplasm of the cells of calf kidney tissue cultures. The reason of such divergent observations can be perceived in different properties of various cells of hosts and various Pl-3 virus strains. In this connection it has been decided to investigate in the present work the structure of virions as well as the structure and location of inclusion bodies in the calf kidney cells infected with the virus.

Material and Methods

In the investigations two inland strains were used, isolated from calves, denoted as G-4/68 and G-82/68, kept in passages through calf kidney cell cultures. The cultures of the first implantation of cells designated for investigations on location and structure of inclusion bodies were infected on the 6th day after their establishment. Upon occurrence of a distinct cytopathic effect, usually on the 3rd day after infection, the cells were scraped off the glass surface, sedimented by centrifugation (1000 rpm), fixed with osmium tetraoxide and 1% potassium hypermanganate, washed up in the Ringer's fluid with 5% uranyl acetate, dehydrated in acetone and embedded in Westopal. The sections were cut up on the Porter-Blum ultramicrotome, stained with 8% solution of uranyl and 5% acetic acid as well as with lead citrate after Raynolds.

Initial material for the investigations on virion ultrastructure of the virus constituted cell cultures, in which almost all the cells showed cytopathic effect. Such cultures were scraped off the glass surface, freezed together with nutrient liquid in dry ice and alcohol and defreezed again. This material was centrifuged at about 4000 rpm for 10 minutes. Obtained supernatant was centrifuged at 50,000g for 2 hours. The scarce sediment formed in the test tube bottom together with a small liquid amount contained considerable amounts of material for investigations of virion ultrastructure. This material was contrasted negatively by means of 4% KPT with addition of 0.4% saccharose at pH 7. The preparations were made on the girds coated with formwar and carbon and reviewed under electron microscope Philips EM-100 at 80 kW.

Results and Discussion

Location and structure of inclusion bodies. In the respective investigations tissue cultures were used with non-synchronized growth and infected by introduction of viral inoculum into nutrient liquid, owing to which it was possible to observe particular stages of the infection process in the same culture. In Fig. 1 a fragment of single-layered culture consisting of three adjacent cells is presented, in which the replication process is at an initial stage. It can be seen that the process of release free viruses is low and the changes in ultrastructure of plasma, organellae and nucleus are insignificant. On the other hand, a striking sign is presence of inclusion bodies in cells being at an early infection stage (Fig. 1). They are of different size and shape, occur singly or by several in one cell section and are always located in plasm, often in the vicinity of cell nucleus. The inclusions are of irregular shape and, in view of electronic density approximating the nucleus density, are distictly marked against the background of protoplasma, although they have not any membraneous structure delineating them.

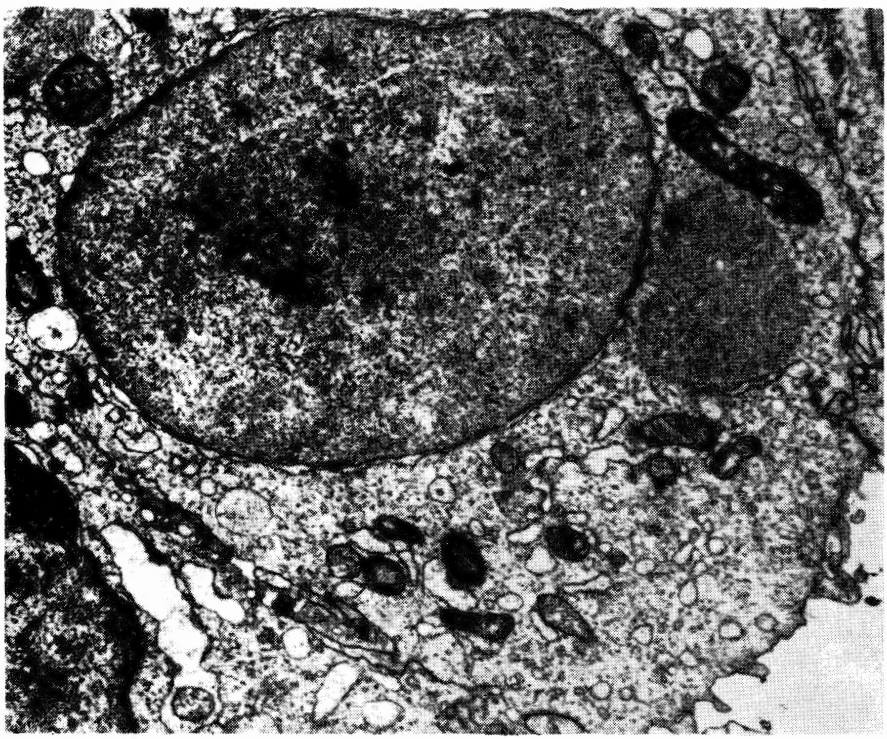

Fig. 1 — A fragment of calf kidney cell culture at early stage of infection with parainfluenza-3 virus. Cytoplasmic inclusions in the upper left corner and on the right side of nucleus. (× 14,200).

In the inclusions, irrespective of their size, a fine-fibrous internal structure can be perceived (Fig. 2). The fibres constituting a single ele-

ment of internal structure of the inclusions are arranged parallelly at the distance of about 300 Å, their thickness amounts to 90—110 Å. The structure of particular fibres is unequal, since two elements with different electronic density can be distinguished in them at greater enlargements (Fig. 3). The electronic-dense element consitute the granularities arranged along the fibres, with the diameter approximating the fibre thickness. A thinner, amorphous element is, as it seems, the substance binding the electronic-dense granularities. These fibres are of similar structure as the ribonucleo-proteinic structures obtained by Waterson (14) after disintegration of virions.

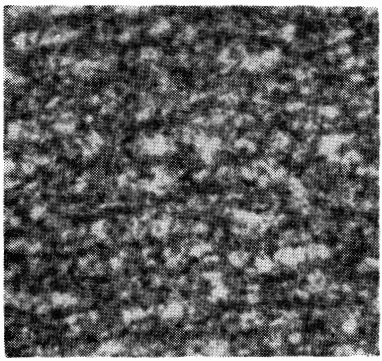

Fig. 2

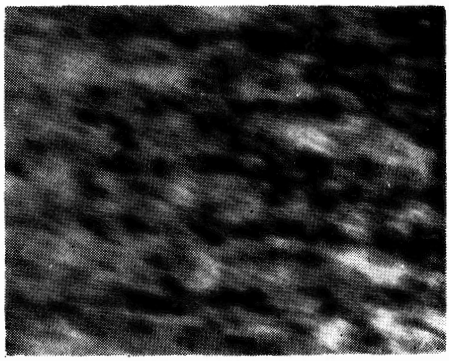

Fig. 3

Fig. 2 — Ultrastructure of cytoplasmic inclusion in a cell of calf kidney culture at production stage of infection with parainfluenza-3 virus. (105,000 ×, Fig. 3 — 247,000 ×).

An attempt of analysis of location, structure and inclusion number depending on infection stage of the cells has proved that during the whole production stage only their number increases, the structure and location remain unchanged. Distinct differences occur only in cells. which at the last non-productive stage of infection underwent a far advanced structural degradation (Fig. 4). The inclusions in such cells most often lose their fibrous internal structure and then a marked reduction of electronic density difference between both structural elements of particular fibres takes place (Fig. 5). The totality of structure of inclusions make the impression that they would be subject to morphological degradation progressing parallelly with degradation of cell and vanishing of virion replication process.

Structure of virion. The virions observed in ultrathin sections (Fig. 6) exhibit themselves as structures highly differentiated morphologically, usually of round shape, setting free from the cell surface and accumulating in intracellular space. The virion diameter varies within the limits of 120—200 mμ. In the structure of virions electronic-dense membrane with nucleocapside in its interior can be distinguished.

The examination of purified virus suspensions contrasted negatively, revealed also considerable differences in virion shape and size of this virus. On the whole, most virions are of irregulars spherical shape, their

diameter varying within 110—250 mμ, although larger virions can be encountered as well. In the structure of these virions (Fig. 7) a distinct electronic-dense about 80 Å thick membrane, covered from outside with dense, regularly arranged 100 Å long projections, could be distinguished. Inside the virion membrane, into which in consequence of injury contrasting dyestuff penetrated, a nucleocapside with helical symmetry can be observed. Fragments of the nucleocapside 150—170 Å in diameter, set free from membrane, are presented in Fig. 8. They consist of helically arranged elements.

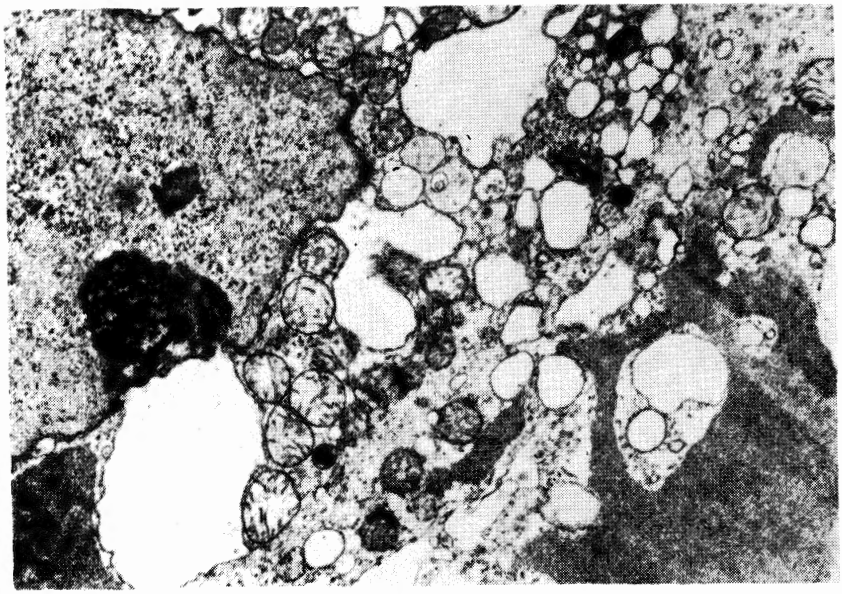

Fig. 4 — A fragment of calf kidney cell from tissue culture infected with parainfluenza-3 virus. Non-production, degradation stage of infection. In left and right corner — cytoplasmic inclusions. (27,000 ×).

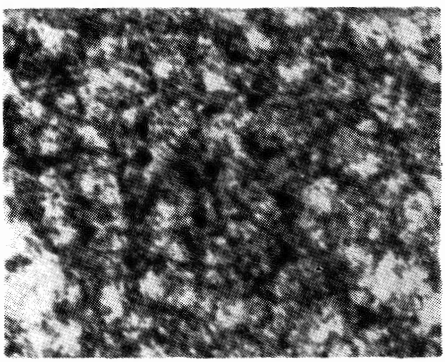

Fig. 5 — Cytoplasmic inclusion structure at structural degradation stage of cell infected with parafinfluenza-3 virus. (105,000 ×).

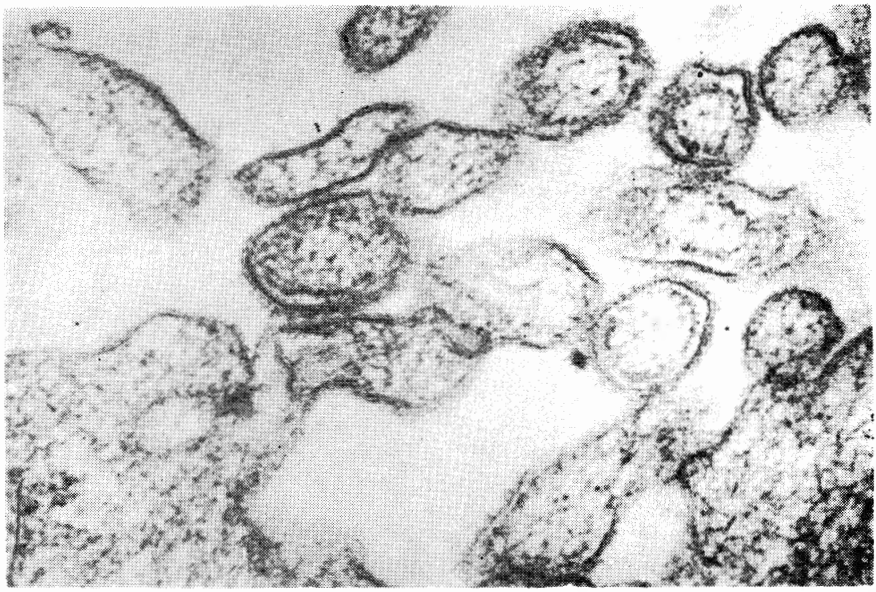

Fig. 6. — Virions of parainfluenza-3 virus in ultrathin sections of infected cells. Production stage. (69,000 ×).

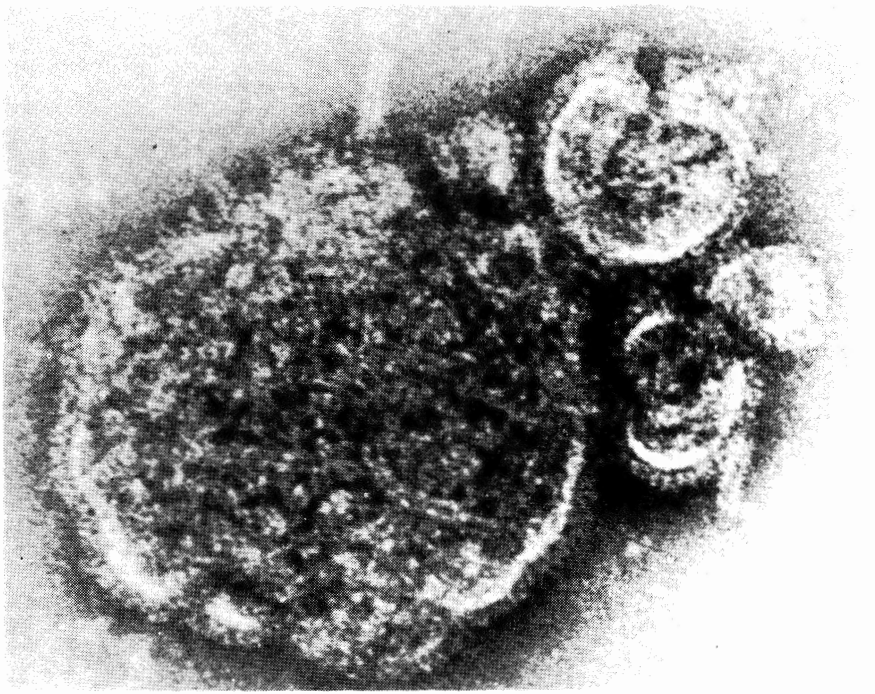

Fig. 7 — Structure of virions of parainfluenza-3 virus in negative staining. (180,000 ×).

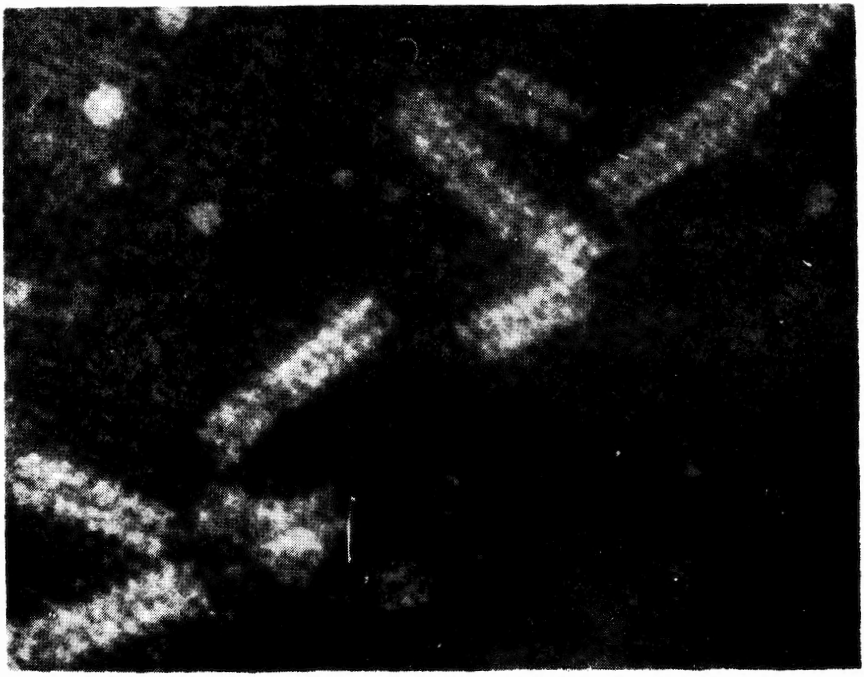

Fig. 8 — Structure of virion nucleocapside of parainfluenza-3 virus in negative staining. (440,000 ×).

The presented morphological features and sizes of virions of the parainfluenza-3 virus isolated in Poland do not differ significantly from sizes and structure of virions of other strains of this virus described by other authors, like Compans et al. (3). Horn et al. (5), Stevenson and Biddle (13). The observations concerning cytoplasmatic inclusions prove their regular occurrence in all the cells infected, their size and number in particular cells being different. The first inclusions appear at early infection stages and increase in number in the period of intensive replication of virions. At the non-productive stage of infection the inclusions are subject to morphological degradation parallelly with morphological degradation of cells. This phenomenon can prove a causal connection of replication process of the ribonucleo-proteinic material with the process of setting free complete viruses from the cells.

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