THE APPLICATION OF FERRITIN-CONJUGATED ANTIBODY TO ELECTRON MICROSCOPIC STUDIES OF INFLUENZA VIRUS IN INFECTED CELLS

II. The Interior of the Cell*

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In a previous publication (1) it was shown that freezing and sectioning cells enabled ferritin-conjugated antibody globulin to penetrate the cytoplasm and localize on the surface of intracellular vaccinia virus. Subsequently it has been found that freezing alone renders the cell sufficiently permeable for ferritin to gain entrance. The purpose of this paper is to illustrate and describe the location and appearance of viral antigen in cells infected with the PR8 strain of influenza virus.

Materials and Methods

The manner of inoculating the chicken embryos and the technique of conjugating ferritin with antibody globulin were described in the preceding paper (2).

Introduction of Ferritin-Conjugated Globulin into Cells.—Infected chorioallantoic membranes attached to the shell were washed in Tyrode's solution, fixed for 5 minutes in 5 per cent phosphate buffered formalin at pH 7.5, rinsed in Tyrode's solution, and placed in a test tube containing 0.88 M sucrose. The membranes were frozen by immersing the tube in a CO_2-alcohol bath, thawed at room temperature, covered with ferritin-conjugated specific antibody globulin for 30 minutes, removed from the shell, and washed in Tyrode's solution. They were then fixed in osmium tetroxide, dehydrated, embedded, and cross-sectioned for the electron microscope.

RESULTS

Fig. 1 illustrates the nucleus of an infected cell. The discontinuity of the nuclear membrane and the extracted appearance of the cytoplasm are probably due in part to the freezing procedure and in part to the infection. Within the

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nucleus are irregular strands and clumps of dense material, which, at higher magnification (Fig. 2),[1] is seen to contain numerous ferritin granules. The intervening nuclear matrix is relatively free of ferritin. Fig. 3 shows part of a nucleus in a cell presumed to be at an early stage of infection. The ferritin has tagged small aggregates of dense material resembling that illustrated in Fig. 2. Only a few scattered ferritin granules are present in, or at the surface of, the nucleolus, which occupies the upper portion of the field.

In Fig. 4 the cytoplasm of an infected cell occupies the upper two-thirds and is separated by an irregular intercellular cleft (indicated by arrows at the margins of the picture) from an adjacent cell at the bottom. The surface of the upper cell, although it is not shown in this field, exhibited viral particles tagged with ferritin. No virus was found to be associated with the lower cell, and it is presumed that this cell was not infected. In the cytoplasm of the upper cell are numerous dispersed ferritin granules, which are not localized on the walls of vacuoles, the peripheral double membranes of the mitochondrion at the top, or the collection of ribonucleoprotein particles at the upper right. Only a few ferritin granules are evident in the lower cell.

In order to ascertain whether the localization of ferritin was the result of a specific antigen-antibody reaction, preparations of infected chorioallantoic membranes were treated with ferritin alone and with ferritin-conjugated nonspecific antibody globulins. As was noted in studies of vaccinia virus (1), washing failed to remove all the unreacted ferritin granules, and therefore the cells contained some ferritin, but much less than did cells treated with specific ferritin-conjugated globulin. Moreover, there was no localization of ferritin on the dense intranuclear material. Finally, in preparations of infected membranes exposed to specific ferritin-conjugated antibody the amount of ferritin in uninfected cells (see Fig. 4) did not exceed that observed in the controls.

DISCUSSION

Nuclear changes of the type illustrated in Figs. 1 and 2 were first noted in electron microscopic studies of an Asian strain of influenza virus, and it was suggested that this alteration was related to the elaboration of soluble "S" antigen (3), which Liu (4) had demonstrated within nuclei of infected cells by the method of immunofluorescence.[2] Subsequently, similar nuclear changes were observed in cells infected with other strains of influenza virus. In view of their specific tagging with ferritin-conjugated antibody there is little doubt that the aggregates of dense material are composed of, or contain, viral antigen. It should be emphasized that the small, scattered aggregates shown in Fig. 3

[1] An arrow has been drawn in the same region of Figs. 1 and 2 to assist in orientation.

[2] Liu commented on the fact that the antigen sometimes formed a "network pattern," which is precisely the appearance that the dense material would give if the nucleus shown in Fig. 1 were seen in a thick section.

were most often encountered, the large accumulations seen in Fig. 1 being unusual.

Watson and Coons (5) originally observed that influenza viral antigen first was detectable in the nuclei of infected cells. Breitenfeld and Schäfer (6) in a study of cells infected with fowl plague virus noted that the soluble antigen first appeared in the nucleus and later in the cytoplasm, whereas the hemagglutinin was confined to the cytoplasm. They suggested that the soluble antigen forms in the nucleus, diffuses into the cytoplasm, and is assembled with hemagglutinin into infectious virus at the cellular surface. Recently, Holtermann *et al.* (7), employing beef embryo kidney tissue cultures, and Traver *et al.* (8), using chorioallantoic membranes, described a similar sequence of events for influenza virus. Our findings are in accord with these observations. The nuclei of cells which show little alteration of architecture and therefore are believed to be at an early stage of infection contain small scattered aggregates of antigen (Fig. 3). The remarkable paucity of ferritin granules either within, or at the surface of, nucleoli indicates that antigen is not formed within this structure. Entry into the cytoplasm presumably results from the diffusion of soluble antigen through the nuclear membranes, since cells producing virus generally exhibit intact nuclei. Once outside the nucleus the antigen becomes so dispersed, however, that its presence can only be detected by electron microscopy after ferritin tagging (Fig. 4). The failure of conjugated ferritin to localize on mitochondrial membranes or in collections of ribonucleoprotein particles (Fig. 4) suggests that neither of these cytoplasmic components synthesizes antigen. These studies have not provided information regarding the site of hemagglutinin production.

SUMMARY

Freezing of the chorioallantoic membrane after brief fixation in formalin preserves antigenicity of cellular components and allows penetration of ferritin-conjugated antibody. Dense aggregates of viral antigen, presumed to be of the soluble type, were found in the nuclei of cells infected with influenza virus. Intracytoplasmic antigen, on the other hand, was widely dispersed. The experimental observations are consistent with the hypothesis that soluble antigen diffuses into the cytoplasm through intact nuclear membranes. Nucleoli do not appear to be the sites of antigen synthesis.

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EXPLANATION OF PLATES

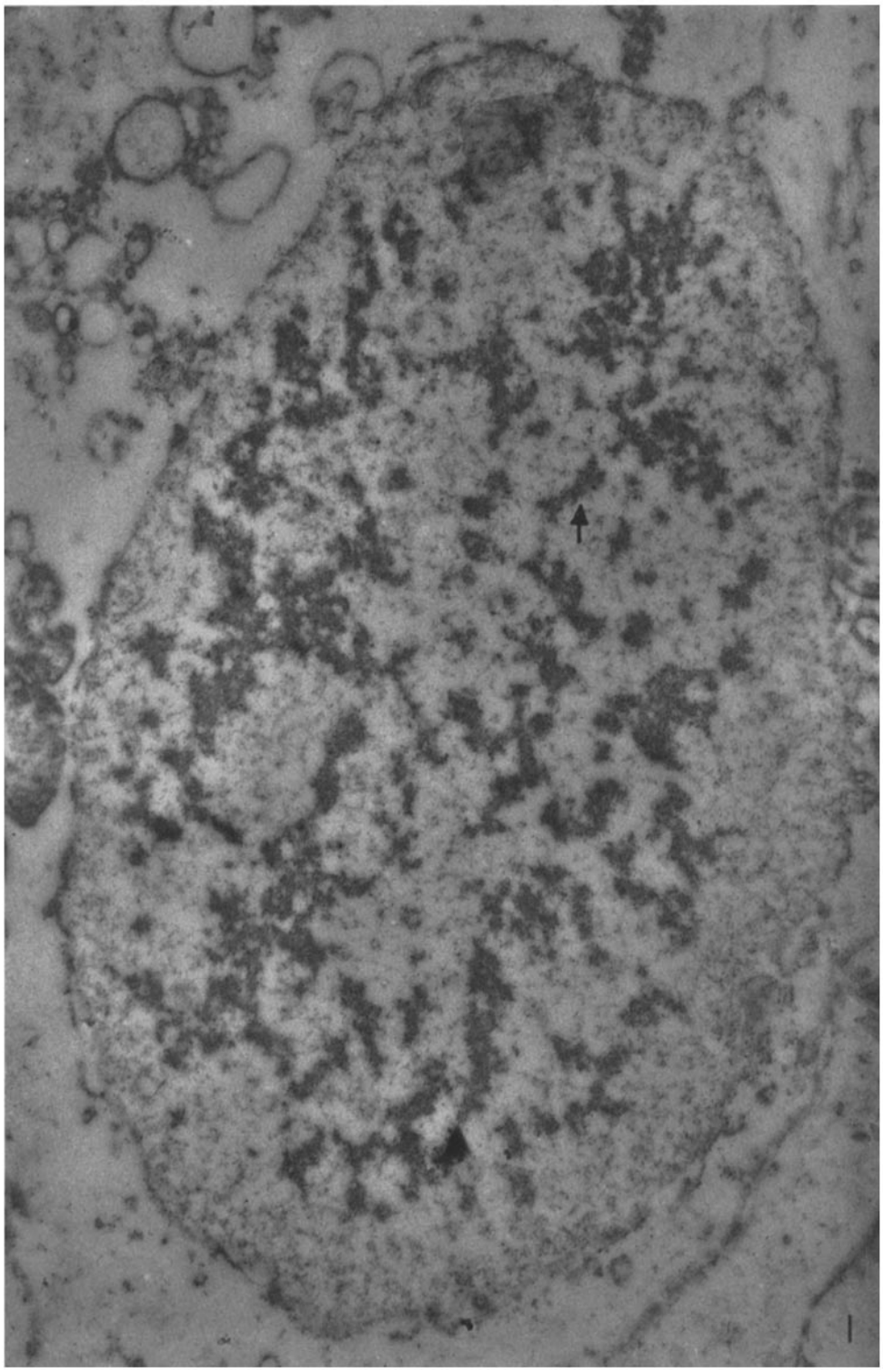
PLATE 85

FIG. 1. A nucleus containing aggregates of dense material. The chromatin is sparse and the nuclear membranes have disrupted. × 26,000.

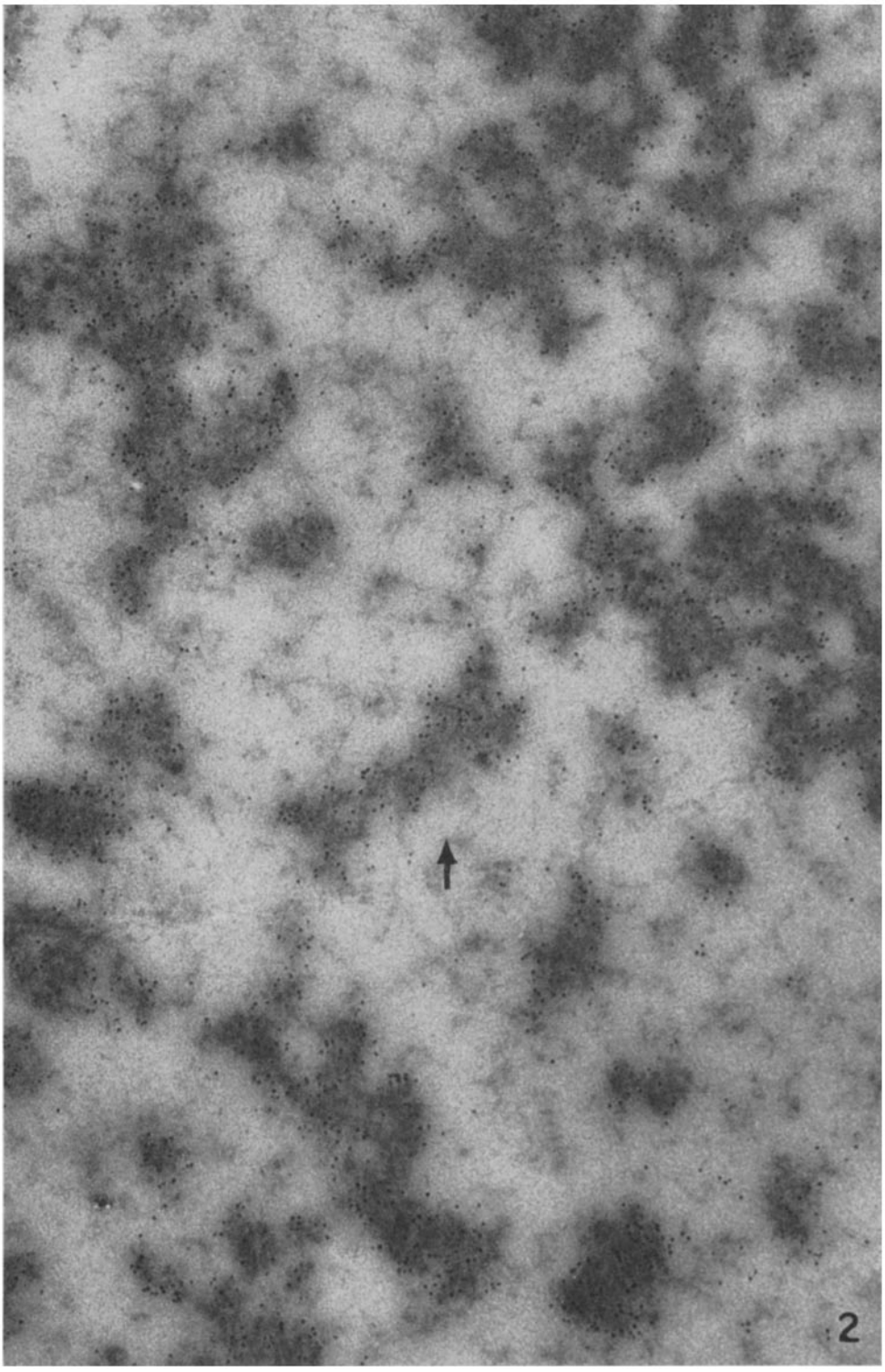
PLATE 86

FIG. 2. Part of the preceding nucleus (see arrow) viewed at higher magnification. Ferritin-conjugated antibody is present within the aggregates of dense material. The intervening nuclear matrix is nearly devoid of ferritin. × 97,000.

PLATES



(Morgan *et al.*: Ferritin-conjugated antibody and interior of cell)

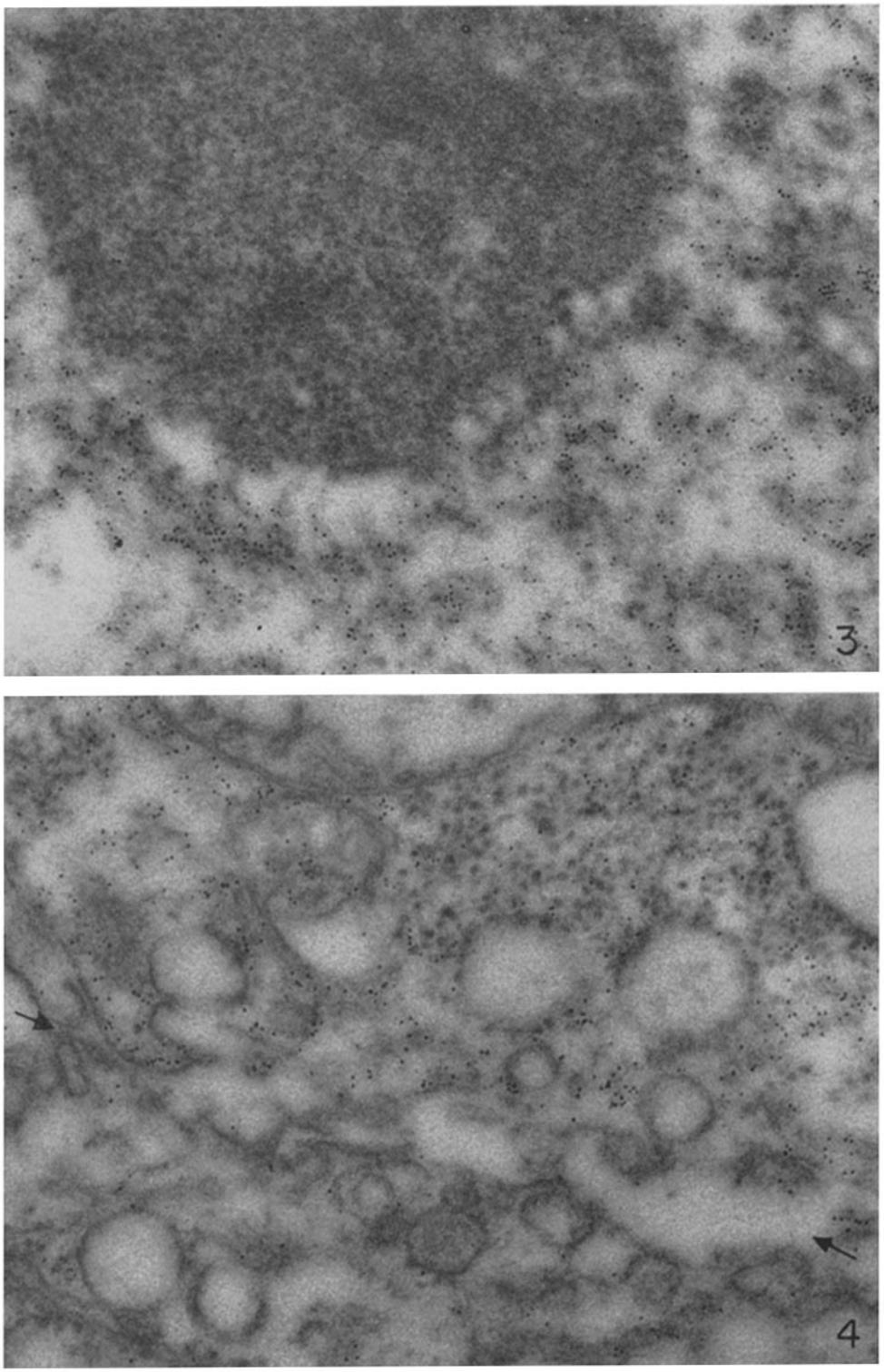


(Morgan *et al.*: Ferritin-conjugated antibody and interior of cell)

PLATE 87

FIG. 3. The nucleus of a cell believed to be at a relatively early stage of infection. The ferritin-conjugated antibody has tagged small aggregates of dense material. Only a few ferritin granules are visible within, or at the surface of, the nucleolus. × 97,000.

FIG. 4. Part of the cytoplasm of two cells. The intercellular space is marked by arrows at the margins of the field. The lower cell, whose surface was devoid of virus, contains few ferritin granules. The upper cell, whose surface was lined with ferritin-tagged viral particles, contains numerous scattered ferritin granules presumably attached to dispersed viral antigen. There is no concentration of ferritin on the double membranes bordering the mitochondrion at the top nor on the ribonucleoprotein particles at the upper right. × 97,000.



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