Fine Structure of Cellular Inclusions in Measles Virus Infections*

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PLATES 177 TO 179

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ABSTRACT

Cells which are infected with measles virus have been known for some time to contain inclusion material that is distinguishable from normal cellular components by application of traditional staining methods and observation in the light microscope. The fine structure of the inclusion material contained in HeLa cells infected with Edmonston strain of measles virus has been examined in the electron microscope. Two steps have been found necessary in this study: (1) the recognition by phase-contrast microscopy of the living cell of bodies that are defined as inclusion material when the cells are classically stained; and (2) the recognition in the electron microscope of inclusion-body material that had previously been identified in the living cell.

The fine structure of the nuclear and cytoplasmic inclusion material in osmium-treated cells was found to consist mainly of randomly arrayed filaments of low electron density. Dense, highly ordered arrays of filaments were found near the center of the nuclear inclusions, sometimes as a two-dimensional, nearly orthogonal arrangement. If the size of the measles virus is taken to be around 100 mμ in diameter, the strands seen in the inclusions cannot be fully formed virus.

INTRODUCTION

Nuclear inclusion bodies within the epithelial cells lining the lungs of infants who had died of sporadic cases of virus pneumonia secondary to measles were first reported in 1939 (9). In 1941 (1) an examination of cases from an epidemic of primary infant virus pneumonitis showed the existence of cytoplasmic inclusion bodies within epithelial cells of the lungs. Later it was shown that morphologically similar inclusion bodies (both nuclear and cytoplasmic) can be found in distemper infections of dogs and ferrets (2, 5, 14), and in tissue culture cells infected with measles virus (5, 8). A few instances of inclusion material appearing like that found in measles and distemper infections have come from a variety of pathological sources (14, 4). This similarity in appearance derives from light microscope observation of fixed cells stained with hematoxylin and eosin in which are seen clear, pink, cytoplasmic bodies, constituting as much as an estimated one-quarter of the volume of the cell. In both diseases eosinophilic inclusions are also seen in the nucleus, particularly prominently in cells infected with measles virus. Inasmuch as an immunologic (3, 7) as well as a morphologic relationship has been demonstrated between distemper and measles, the examination of the fine structure of the cellular inclusions is of comparative interest. The present paper describes only the inclusion material found in cells subjected to one pathogenic agent, the Edmonston strain of measles virus.

When cultured cells, strain HeLa, are infected with measles virus, vast inclusion areas develop in the cytoplasm and smaller ones in the nucleus. Large groups of cells, grown as tube cultures, are particularly convenient to use for the study of the fine structure of the inclusion bodies because a large fraction of the cells of such cultures is found to contain them upon examination in the light microscope. Preliminary examination in the elec-

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tron microscope shows that some cells have undergone changes in their fine structure and that within them are regions that might be considered inclusion bodies. But the statistical shortcomings of the sampling of masses of cells by electron microscopy allow only an *inference* to be made that these regions are, in fact, the inclusion bodies. A *positive* morphological identification can be made, however, by first finding an inclusion in a selected living cell and subsequently examining the same inclusion in the electron microscope.

Inclusion bodies, such as those resulting from measles infections, have traditionally been identified by their appearance following the application of classical fixation and staining procedures. These methods, however, are not compatible with good electron microscopy. But if large, distinctive inclusion areas can first be unequivocally identified in the living cell through a correlation of their appearance in the phase contrast microscope with their appearance when subsequently stained by classical procedures, their recognition in the electron microscope can be secured following procedures of fixation and staining appropriate to that instrument. The method employed in accomplishing such recognition is outlined in this paper.

Materials and Methods

Stock cells, strain HeLa, were carried by serial passage as bottle cultures in a growth medium consisting of 20 per cent human serum and 80 per cent solution 199 (13). For present purposes, the cells were removed from the bottle by adding 5 ml. of 0.0037 per cent versene in a modified Hanks' solution[1] to a bottle of cells from which the growth medium had previously been drained. The cells, which could be seen to slough off in 1 or 2 minutes, were then set down by adding 1 ml. of the cell suspension (approximately 500,000 cells/ml.) to the surface of a 43 x 50 mm. coverslip trimmed to fit in the bottom of a Petri dish, 50 mm. in diameter. Five ml. of growth medium were then added to the dish and the preparation was incubated overnight at 37°C. under 5 per cent CO_2 in air. The following day, when the cells had formed a well attached sheet, they were infected by adding 0.2 ml. of measles inoculum to the dish. The inoculum of Edmonston strain measles virus consisted of cleared fluid from tissue culture tubes, having a titer of approximately 10,000 TCD_{50} per 0.1 ml.

After approximately 5 days holes appeared in the cell sheet, representing places where groups of cells had sloughed off the glass, and numerous giant cells had formed and were frequently found in these areas. The large coverslips were then removed from the Petri dish and were inverted over a monel metal slide (0.025 inch thick) containing a hole 38 mm. in diameter, the under side of which had been previously covered with a sealed-on 43 × 50 mm. coverslip. The top coverslip was sealed, and the preparation examined in the phase contrast microscope. Large, clear areas which sometimes appeared brilliantly opalescent were seen in irregular shaped patches in the nucleus and cytoplasm of some cells, particularly the giant ones. A sketch was made of a few of these areas of characteristic morphology, and the areas marked by scoring the top of the coverslip with a diamond pencil. When the coverslip was subsequently fixed in Bouin's fixative, washed, and stained with hemotoxylin and eosin, the previously marked and sketched nuclear and cytoplasmic areas were observed to have changed from clear or opalescent to a pink color. They appeared in photomicrographs as lakes of dark material in the cytoplasm (*CI* in Fig. 1), and as areas of medium density in the nuclear area (*NI* in Fig. 1), sometimes surrounded by a clear, non-staining region. Since this is the classical appearance of measles inclusion bodies, it was felt that their recognition under phase contrast was safely established.

A region of infected cells was selected which, by phase contrast microscopy, exhibited both cytoplasmic and nuclear inclusions. The region was sketched, photomicrographed, and marked, and the preparation fixed, dehydrated, and embedded by a method quite similar to that of Howatson and Almeida (10). Orientation provided by the previous marking and sketching of the cells allowed the flat surface of the block to be trimmed to include only the cell area desired for sectioning. Sectioning was performed using a diamond knife and a Porter-Blum microtome. The first sections secured were mounted (15) on formvar films over single-slot grids and examined in an RCA electron microscope, EMU-3C.

CYTOLOGICAL OBSERVATIONS

Low magnification electron micrographs showed that the sections passed through the same cells as those previously seen in their entirety in the living state. In areas of distinctive morphology, the limits of the nuclear and cytoplasmic inclusion areas could be seen in the same cell in both the light and electron micrographs. For example, Fig. 2 shows a cell area photographed under phase contrast in which the inclusion areas are seen as the refractile or clear areas in the cytoplasm and nucleus. Fig. 3 is an electron micrograph of a section passing through a portion of this cell area, shown in the marked rectangle in Fig. 2. Some

[1] The Hanks' solution used was deficient in Ca^{++}, Mg^{++}, and $PO_4^{=}$. It contained 8 gm. NaCl, 0.4 gm. KCl, 1 gm. glucose, and 0.35 gm. $NaHCO_3$ per liter.

degree of distortion resulting from sectioning is apparent.

Since the electron micrograph is of a section no thicker than about one one-hundredth of the cell thickness represented in the light micrograph, a complete and detailed correspondence of appearance is not to be expected. Matching of these two pictures is made easier by the presence of a morphologically distinct and bizarre inclusion of unknown significance and seen at *A* in both Figs. 2 and 3.

As seen in Fig. 3, the nuclear and cytoplasmic inclusion material appears to consist of fairly homogeneously stranded material of low density. Examination of electron micrographs secured from preparations like that providing Figs. 2 and 3, compared with ones obtained from random sections of infected, tube-cultured cells, showed that inclusion bodies could be unequivocally identified in the latter preparations by their fine structure alone. Since the specialized type of preparation designed to provide positive recognition of the inclusion areas is time-consuming and unnecessary after recognition has been established, the rest of the electron micrographs described here are of sections of cells grown in tube cultures.

Figs. 4 to 7 are electron micrographs of sectioned inclusions seen in cells sampled from tube cultures infected 5 to 12 days. These cells were fixed while attached to the side of the tube by adding veronal-buffered 1 per cent OsO_4 (pH 7.4) directly to the test tube after pouring off the culture fluid. They were then scraped from the side of the tube and processed through washing, dehydration, and embedding with methacrylate as a sedimentable pellet by a method previously used for suspended cells (11). In Fig. 4, a nuclear inclusion *NI* may be seen as a rather sharply delineated area of low density in the nucleoplasm. The bulk of the material consists of randomly arrayed strand-like structures. Within this inclusion, there may be seen two areas in which there appears to be a two-dimensional array of linear structures. (These arrays, distinctive in electron micrographs, cannot be distinguished from the remainder of the inclusion body by light microscopy.) A cytoplasmic inclusion area is shown in Fig. 5 and is seen to consist of randomly arrayed, strand-like material morphologically similar to the bulk of the nuclear inclusion. These cytoplasmic inclusions exist in tremendous volume in some cells which otherwise do not show severe morphological disturbances. For example, the cell, which in Fig. 5 shows the mitochondria *M*, and fat *F*, to be normal, appeared to display other normal features over much of its cytoplasm.

Some cells of the culture showed pathologic changes other than the nuclear and cytoplasmic inclusions; presumably some of these were due to the measles infection, but no investigation has been made to establish this presumption.

Fig. 6 represents an example of a nuclear inclusion area in which oriented filaments are found in patches and are seen to extend continuously into strands that are randomly arrayed. Fig. 7 is a higher magnification electron micrograph of another nuclear inclusion area showing both a one-dimensional and two-dimensional ordered array. No oriented structures have been observed in the inclusion material in the cytoplasm.

It is most unlikely that the strand-like structures found in the measles inclusion bodies represent mature measles virus. If one assumes the evidence from ionizing radiation and gradocol membrane filtration to be correct, the size of measles virus is 100 to 200 mμ (6). The strands shown here have a length in the range of 0.2 to 1.0 μ and a width too small to be accurately measured, but estimated at 20 mμ. The constant association of the inclusion bodies with both natural and experimental measles infections, however, seems to relate them specifically with the disease.

The arrays seen in the present study appear in electron micrographs to be somewhat similar to the protein crystals found in cells infected with type 5 adenovirus (12). In the latter instance, however, there is no report of the crystals being surrounded by larger areas of randomly arrayed material.

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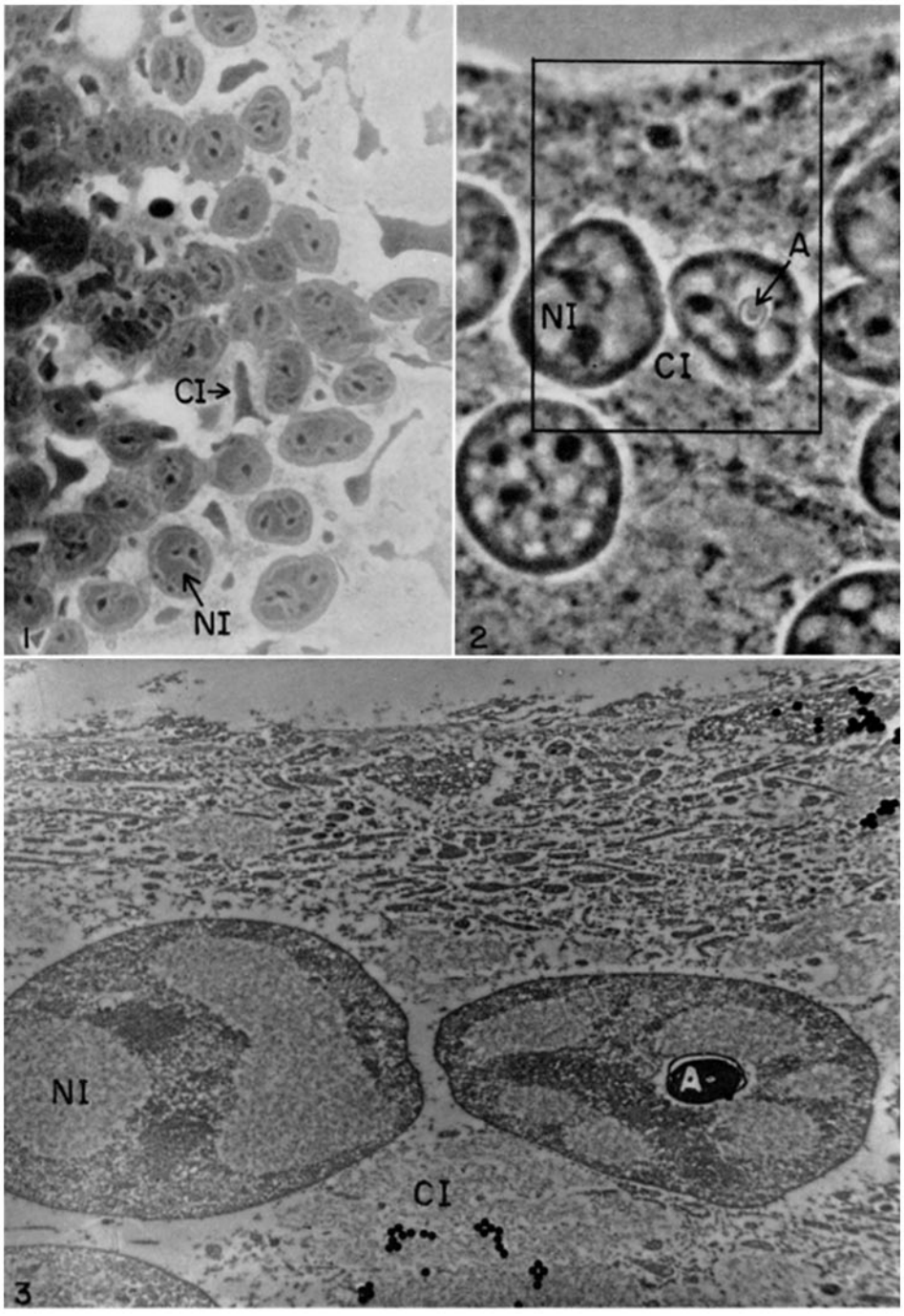
Explanation of Plates

Plate 177

FIG. 1. A light micrograph of a group of HeLa cells grown as a sheet on glass and infected with Edmonston strain of measles virus. The cells were fixed in Bouin's fluid and stained with hematoxylin and eosin. Large eosinophilic cytoplasmic inclusions are seen at *CI* and at many places throughout the culture. Eosinophilic nuclear inclusions are seen at *NI* and in many other nuclei. × 750.

FIG. 2. A phase-contrast micrograph of a portion of a giant, multinuclear tissue culture HeLa cell grown on a glass coverslip and infected with Edmonston strain of measles virus for approximately 5 days. Clear or refractile nuclear inclusions, *NI*, and cytoplasmic inclusions, *CI*, develop as a result of the infection and are seen in this cell in the living state, but can be seen as well after fixation in 1 per cent veronal buffered OsO_4 (pH 7.4). This cell was subsequently sectioned and examined in the electron microscope. The boxed area represents the area to match with the electron micrograph of Fig. 3. (Recognition of the identity of the configuration in the two pictures is made easier by the object of unknown significance, seen at *A*.) × 1300.

FIG. 3. A low-power electron micrograph of a section of the same osmium-treated cell area shown in the rectangle marked in Fig. 2. Nuclear inclusion areas, *NI*, and cytoplasmic inclusions, *CI*, consist of fairly homogeneously stranded material of low density and are seen to match in general profile in the two pictures. Recognition of the similarity of the configuration in the two pictures is clear, although matching is not perfect owing to (1) distortion of the section during microtomy, and (2) a disparity of at least one-hundredfold between the thickness of the section and the thickness of the whole cell photographed by phase-contrast microscopy. (The black spheres seen in this and subsequent micrographs are images of polystyrene latex reference particles). × 4000.



(Kallman *et al.*: Fine structure of measles inclusion bodies)

PLATE 178

FIG. 4. An electron micrograph of a HeLa cell taken from a culture infected 7 days with Edmonston strain of measles virus. A nuclear inclusion, *NI*, is seen to consist of randomly arrayed, strand-like structures within which lie two distinctive arrays, *C*, appearing to consist of doubly arrayed, close-packed fibrillar material. × 35,000. Figs. 4 to 7 represent methacrylate-embedded sections of cells grown as tube cultures, fixed in veronal-buffered 1 per cent OsO_4 (pH 7.4), and scraped from the side of the tube.

FIG. 5. A cytoplasmic inclusion, *CI*, is shown in an electron micrograph of a HeLa cell taken from the same measles-infected tissue culture as that shown in Fig. 4. The inclusion is seen to consist of randomly arrayed strand-like material identical to the bulk of that found in the nuclear inclusions. Normal cytoplasmic constituents seem not to have been radically altered as partially evidenced by the appearance of the mitochondria, *M*, and of the fat bodies, *F*. × 54,000.

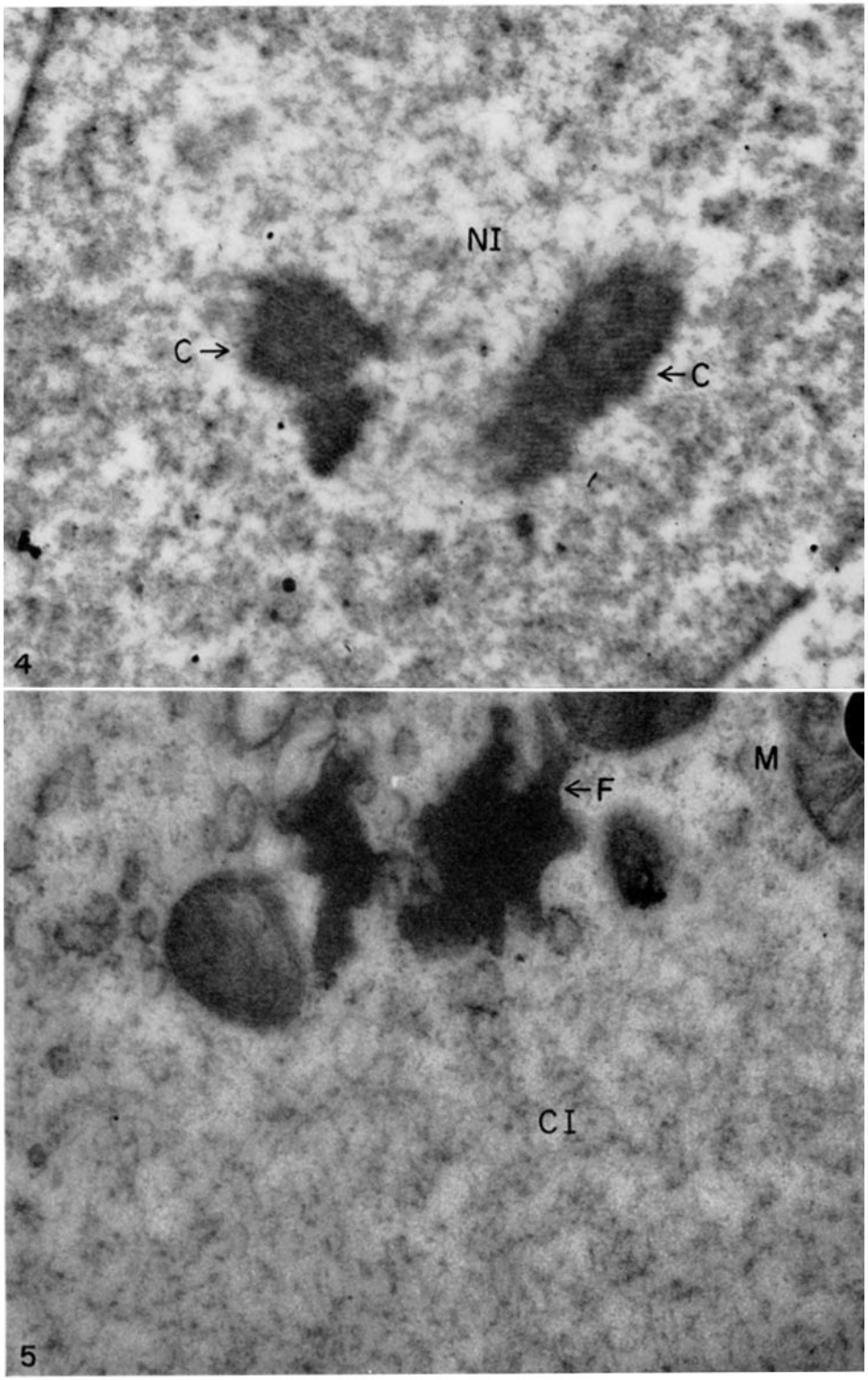


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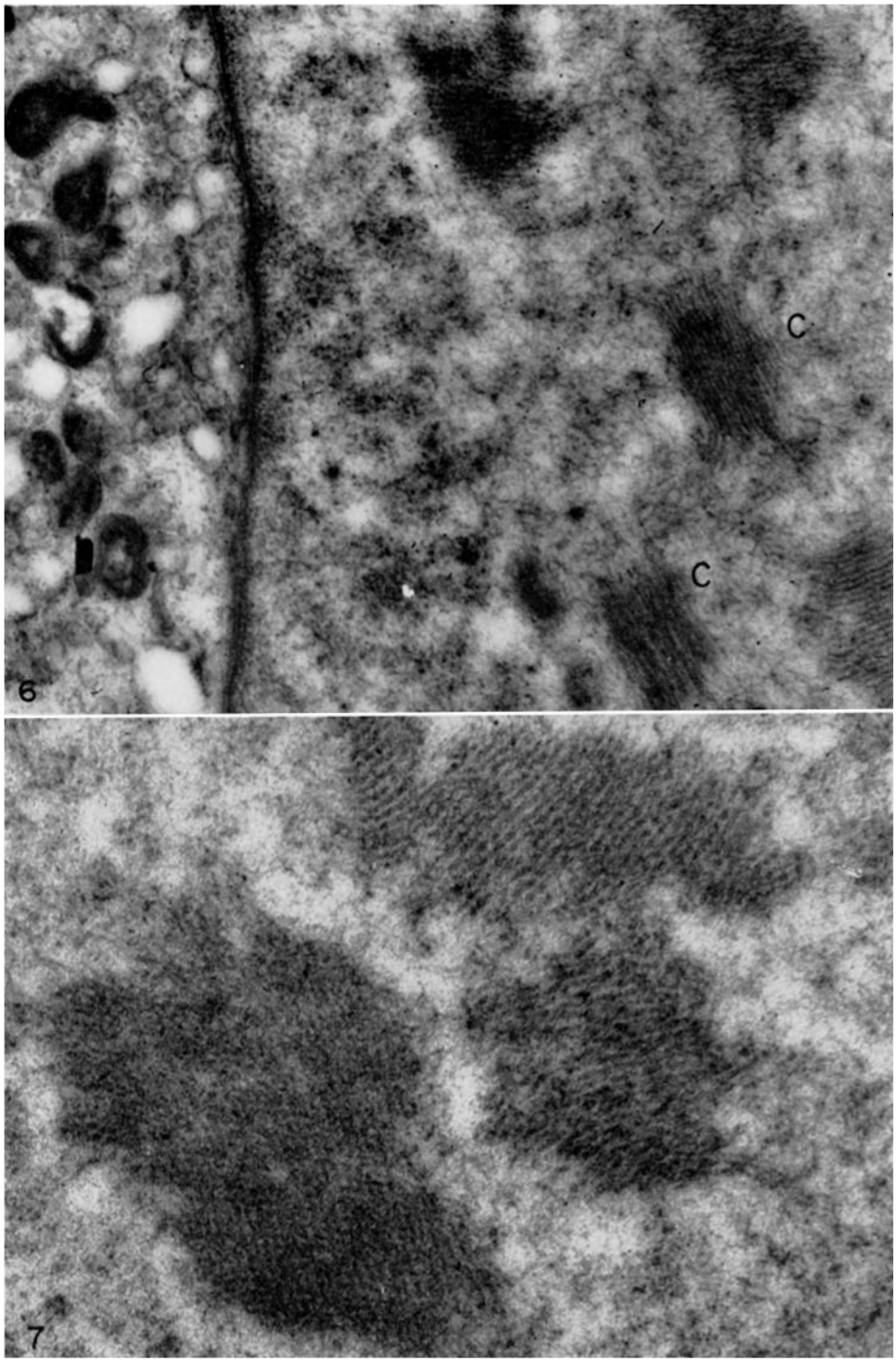
PLATE 179

FIG. 6. An electron micrograph of a nuclear inclusion area from a HeLa tissue culture cell infected with Edmonston strain of measles virus. Several strands of the ordered arrays in the nucleus, *C*, are seen to extend continuously into the region of randomly arrayed filaments. × 32,000.

FIG. 7. A higher magnification electron micrograph of a nuclear inclusion area taken from a measles-infected cell similar to those shown above. Two kinds of orientation (linear and cross-hatched) may be seen in the two different ordered areas. × 65,000.



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