An Electron Microscope Study of Polyoma Virus in Hamster Kidney*

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ABSTRACT

Electron microscope studies were made of hamster kidneys taken at daily intervals after injection of a variant of polyoma virus into newborn animals. Particular attention was paid to the period 5 to 6 days after injection at which time the necrotizing response was at its peak and virus particles were seen in greatest numbers. The most numerous particles were about 28 mμ in diameter. They were observed mainly within nuclei of stromal cells and are similar to the particles seen in large numbers in polyoma-infected mouse cells growing *in vitro*. They were not observed in cells of fully developed tumors. Filamentous or tubular structures closely associated with the 28 mμ particles and probably concerned in their formation are described. Considerable quantities of viral material were contained within cytoplasmic inclusions. In some of the inclusions larger particles of diameter 60 mμ were observed. The origin of these particles and their relation to the 28 mμ particles is discussed.

The recovery of a cytopathogenic virus from a mammary tumor in a C3H mouse has been reported (1). Subsequent investigations (2) showed that this virus is a variant of polyoma (parotid tumor) virus (3, 4). Perhaps the most striking property of this variant of the polyoma virus is its ability to induce in less than 2 weeks large diffuse renal sarcomas in Syrian hamsters injected shortly after birth with the virus preparation (1, 5). The rapid induction of renal tumors in a high percentage of the animals allowed a day-to-day histological study to be made of the sequence of changes in the kidneys of the hamsters during viral carcinogenesis (6); this showed that within 3 or 4 days after infection, changes, both degenerative and proliferative, were visible in the stromal cells in the corticomedullary region. At first the degenerative changes were prominent but later the proliferative response became dominant and the lesions assumed the characteristics of typical sarcomas. The peak of the necrotizing response occurred 5 to 6 days after

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the animals were injected, at which time preliminary electron microscope studies showed that particles presumed to be polyoma virus were present in greatest numbers (2). The present report deals mainly with this stage in the infective sequence. In it are described in some detail the appearance and arrangement of the particles, and changes in the fine structure of the kidney cells associated with viral infection. Since cells at many different stages of infection were present in animals sacrificed at this time it was also possible to obtain information on the mode of development of the virus within the cells.

Materials and Methods

The origin of the virus preparation used in this investigation has been described (1).

For the electron microscope study two litters of Syrian hamsters were injected a few hours after birth with the virus preparation. Two hamsters, one from each litter, were sacrificed at daily intervals beginning at the 4th day after injection and continuing until the 10th day at which time the kidneys were heavily infiltrated with tumor and deaths began to occur. One kidney from each animal was processed for light micro-

scopy, the other was immersed immediately in a drop of osmium tetroxide and a thin slice passing through the corticomedullary region was cut with a razor blade. After further trimming the tissue was fixed for an hour in osmium tetroxide, dehydrated in alcohol, and embedded in methacrylate. Thin sections were cut with a Porter-Blum microtome and examined in an RCA EMU-3b electron microscope. Kidneys from normal hamsters of the same age range were processed in the same way and examined by Dr. T. S. Leeson, Department of Anatomy, University of Toronto (7). A second series of kidney specimens was obtained and processed in identical manner but in this instance the sequence started on the 1st day after injection and no control material was taken. Four additional specimens of kidneys from 6-day old infected hamsters were prepared and examined in a similar manner to supplement the information obtained from the sequence experiments.

Most of the sections were stained with lead hydroxide after the method described by Watson (8).

OBSERVATIONS

Kidneys of animals sacrificed 4 to 7 days after infection when examined at low magnification in the electron microscope had an appearance corresponding closely to that observed in the light microscope. Tubules and glomeruli presented for the most part a normal appearance, but compared with normal kidneys of the same age the mesenchymal intertubular tissue was greatly increased in amount. Proliferation of some cells of this tissue was accompanied by degeneration of others. Inclusions were present in the cytoplasm of many cells.

The appearance of a typical intertubular area of a 6-day infected hamster kidney viewed at low power in the electron microscope is shown in Fig. 1. Cells marked *a* appear fairly normal, though irregularities of nuclear outline and prominence of nucleoli may indicate abnormal proliferative activity. In the cell marked *b* alterations from the normal distribution of the chromatin in the nucleus are apparent but no specific particles can be detected within it. In the nucleus of the cell marked *c* the chromatin is abnormally dense and distributed mainly peripherally, close to the nuclear membrane. Throughout the remainder of the nucleus are scattered numerous small dense particles of uniform size; such particles have never been seen in normal hamster kidneys. Fig. 2 illustrates at higher magnification another cell of this type. Dense round particles occur singly or in small groups throughout the nucleus which otherwise appears relatively empty except near the

nuclear membrane to which a dense band of chromatin is closely applied. This appearance is typical of the majority of infected nuclei. In a smaller number of infected cells the nucleus appears very dense, containing closely packed particles and clumped chromatin. Some nuclei contain much chromatin and relatively few particles (Fig. 3); in others, presumably at a later stage of infection, the substance of the nucleus is almost entirely replaced by arrays of closely packed particles (Fig. 4). The particles are sometimes so regularly arranged that in places they have the appearance of crystalline formations. The diameter of individual particles was about 28 mμ.[1] Measurements made in crystalline arrays, however, gave minimum centre-to-centre separation of 38 mμ. The particles had no obvious limiting membrane and were usually of uniform density. Particles of identical morphology have been observed by us in large numbers in cultures of mouse kidney and mouse embryo cells on which polyoma virus was being propagated, and in small numbers in tumors induced by the virus in Swiss mice (2). Several authors have described independently similar particles in cells growing *in vitro* after infection with polyoma virus (9–11). Dmochowski *et al.* (11) have observed them also in cells of tumor and spleen of mice infected with the virus. Although complete proof is lacking it seems highly probable that these particles represent the infective polyoma agent and we shall refer to them henceforth as viral particles.

Many of the stromal cells contained inclusions within the cytoplasm. Some of these appeared to consist of material derived from the break down of virus-infected cells, and were recognizable as whole degenerate cells or large fragments thereof; others consisted of collections of virus particles, often associated with a granular or filamentous matrix and separated from the cytoplasm of the cell by a single limiting membrane (Fig. 5). Within many of the inclusions complex membranous formations were observed in close association with the virus particles; layers of particles separated by membranes were commonly seen in the form of spirals or long tortuous convolutions (Fig. 6). In some areas membranes alone were present, often in the form of multiple layers in spiral formation.

A second type of particle, considerably larger than that already described, was observed within

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[1] In sections stained with lead hydroxide isolated particles may appear somewhat larger.

some of the inclusions. The number of these varied from one kidney specimen to another. They were always less numerous than the small particles and in some specimens none of the larger particles have been detected. They had a diameter of about 60 mμ and consisted of a nucleoid of diameter about 30 mμ surrounded by a clear zone and a single limiting membrane. The large particles were observed most frequently scattered more or less at random among the 28 mμ particles which usually greatly outnumbered them (Fig. 7). Sometimes however, the 60 mμ particles were arranged in groups set apart from the other elements of the inclusion and occasionally they were the most numerous constituent of an inclusion (Fig. 8). They have not been identified within nuclei at any stage of infection.

Other structures observed in only a small percentage of infected cells but which appear to be of significance in the development of the intranuclear virus particles will now be described. Fig. 10 shows a cell thought to be in an early stage of infection. It contains no clearly defined virus particles but exhibits margination of the chromatin and invagination of the nuclear membrane, features commonly associated with infection. Filamentous structures of diameter about 28 mμ may be seen in two main locations close to the chromatin on the side removed from the nuclear membrane. Fig. 11 shows another example of intranuclear filaments but this time in a cell containing recognizable virus particles, with which the filaments are in close association. Cells in which the nuclear material has been largely replaced by closely packed virus aggregates may also show filamentous structures. Fig. 12 shows part of a nucleus in which many filaments are present in random orientation. In other nuclei the filaments are arranged more regularly in the form of bundles. In some sections the filaments appear as cylinders or tubules having a dense outer layer and less dense core. This is clearly seen where the tubules are cut in cross-section (Fig. 13).

Although most of the virus was present in the stromal cells, parenchymal cells both tubular and glomerular were occasionally found with infected nuclei, without, however, any indication of the accompanying proliferative changes found in the mesenchymal tissue. Fig. 14 shows an example of an infected tubular cell.

DISCUSSION

The light microscope study of polyoma-infected hamster kidney (6) showed that at one stage during a carcinogenic process, the final outcome of which was a typical sarcoma, many of the cells showed degenerative changes of the type commonly associated with infection by virus. In animals sacrificed 4 to 7 days after infection both light and electron microscopy revealed in the intertubular tissue in the corticomedullary region of the kidney, actively proliferating cells side by side with cells showing all degrees of degeneration. The ability to elicit, to certain circumstances, either a degenerative or a proliferative response in the cells that they infect is common to many viruses (12). Non-tumor viruses have a predominantly necrotizing effect, but many of these have been shown to evoke, at least temporarily, a proliferative response. The effect of tumor viruses, on the other hand, is usually seen as a proliferation, but degenerative effects can in some cases be demonstrated. The Rous virus, for example, when injected into chicks induces tumors, but in chick embryos degenerative hemorrhagic vascular lesions result (13). The system that we have been studying provides a particularly good example of a virus with a twofold effect for, as we have already mentioned, the polyoma virus evokes, apparently simultaneously and in the same region of the kidney, proliferation of some cells and degeneration of others.

The electron microscope study reveals little or nothing of the role of the virus in exciting proliferation. Actively proliferating cells are difficult to distinguish from normal stromal cells and as far as can be ascertained do not contain virus particles. Nor has virus been detected so far in the cells of fully developed tumor in the hamster. Thus, if viral material is present in cells that are neoplastic or about to become so, it must be in small amount or in a form that is not recognizable in the electron microscope.

Study of the virus-host interaction that results in degenerative changes has been more rewarding, for in this the virus plays a more obvious role. In cells in which the nucleus is heavily infected, normal structures in both nucleus and cytoplasm are almost completely destroyed and the cells clearly cannot survive; in these cells the necrotizing effect of the virus is directly attributable to the multiplication of the virus within the nucleus. Less heavily infected cells present more of a problem. In these, the cytoplasm may present a fairly normal appearance but more often shows some degenerative changes. It may be that some of these lightly infected cells can survive and multiply and thus should be classed with the proliferative

group. It seems more likely, however, that most of these cells are at an early stage of degeneration. Such cells are often seen in groups around others at an advanced stage of degeneration suggesting that they have been recently infected by virus released from the heavily infected cells. Finally, there are many cells showing degenerative changes but containing no detectable virus particles. It is possible that these cells have been infected and have released virus without any major disruption of cell architecture. Another possibility is that these cells have never been infected and that their degeneration is not due to virus multiplication within them but to release of some toxic substance from adjacent infected cells. It should be noted, however, that degenerate cells are quite common in the intertubular tissue of kidneys of normal hamsters especially in very young animals (7). The presence of apparently non-infected degenerate cells in the infected kidneys may, therefore, be a normal condition made more obvious by the increased amount of intertubular tissue.

We have described two main types of infected nuclei, the condensed type containing closely packed particles and dense chromatin masses, and the large, swollen type of low average density containing scattered particles and marginated chromatin. Leeson (7) has described in the normal hamster kidney two distinct stromal cell types; (a) cells with dense clumped chromatin and marked separation between the inner and outer layers of the nuclear membrane, and (b) cells with normal chromatin distribution and density, and no marked separation of the nuclear membrane layers. The similarities between the two types of infected cells and the types present in normal kidney mesenchyme make it seem likely that the differences observed in response to virus infection are due mainly to differences in the type of infected cells. However, the different responses of the cells to infection could depend to some extent on the degree of differentiation of the cells, the point in the cycle of multiplication at which infection occurred or the multiplicity of infection. The two different appearances do not seem to represent different stages in the infective process, for cells of either type may contain virus particles ranging in number from very few to several millions per nucleus. In both types of infected cell there is evident a close association of the virus particles with the chromatin, which is denser and composed of coarser filaments and granules than normal

chromatin. Virus particles are seen in close relation to and sometimes within the chromatin masses, but details of how they are formed are difficult to discern. The filamentous or tubular structures illustrated in Figs. 10 to 13 may play a part in their formation. Filaments have been seen in both the condensed and swollen type of nuclei but only in a small percentage of infected cells. Occasionally, large numbers of tubular forms are present in a single nucleus as shown in Figs. 12 and 13. Very similar appearances have been observed by us in infected cells grown *in vitro*. Bernhard *et al.* (9) in a recent article show a group of similar filamentous forms in an infected tissue culture cell and suggest that the spherical virus particles are formed from the tubules. We have observed in some of our preparations apparent thickening of the walls of the tubules at regular intervals and accumulations of dense material within them, suggesting that virus particles may be forming. The frequent occurrence of the particles in linear arrays even where the packing is not dense supports this theory of their mode of formation. It is difficult to be certain, however, that the densities seen within the tubules are not due to superposition effects. The thickness of the average section is about 70 mμ; *i.e.*, about twice the diameter of the tubules and virus particles. Thus, more than one layer of these will often be present in a section. Because of the large depth of field in the electron microscope all elements in this thickness will be in equally good focus and particles apparently lying within tubules may actually be above or below them. Examples of effects that are clearly due to superposition may be seen in Fig. 6, especially at the lower centre, where there occur virus particles in roughly parallel rows separated by membranes. In some places the particles instead of being round have the appearance of linear densities transverse to the rows, the distance between the densities being approximately half the centre-to-centre spacing of the round particles. The diagram in Text-fig. 1 shows how the appearance illustrated in Fig. 6 could be obtained by superposition of two rows of round virus particles.

To return to the discussion of the tubules, these are seen rather infrequently; so it would seem that if they play a part in the formation of virus, their presence is only a transitory one or else tubule formation is not an essential step in the process of virus formation. A possibility that cannot be excluded is that they represent another form of the
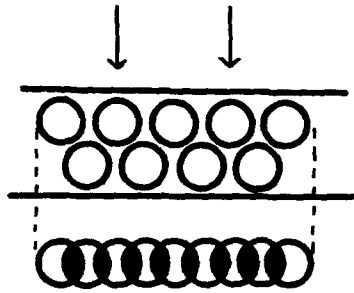
TEXT-FIG. 1. Effect of superimposition. The upper part of the diagram shows two rows of virus particles contained within the thickness of a section. In the lower part of the diagram, the appearance that such an array of particles would have in the electron microscope is shown. Compare with the area marked S in Fig. 6.

virus. There is a precedent for this in the instance of the myxoviruses which occur in two forms, one spherical and the other filamentous; but their mode of formation is quite different in that the filaments are formed at the cell surface. The tubules associated with polyoma virus have not been definitely identified outside of nuclei. The membranous structures that occur in association with viruses in the cytoplasm (Fig. 6) do not appear to be related to the intranuclear tubules.

The close association of the 60 mμ particles with the 28 mμ particles present in the cytoplasm and the fact that the central bodies or nucleoids within the larger particles appeared to be identical with these 28 mμ particles suggested that they might be a further development of the smaller particles. It appears that in the case of several tumor viruses including the mammary tumor virus (14, 15), and the Friend leukemia virus (16), the outer membrane of the mature particle is derived from the cell membrane of the cell in which the virus is formed. We found no evidence that a similar process occurred in the cells that we were studying. There were, however, regions within the cytoplasm of some cells which appeared to be sites of formation of the larger particles. One such region is illustrated in Fig. 9. It consists of a collection of 28 mμ particles without enveloping membranes, 60 mμ particles with fully formed membranes and what appear to be intermediate stages between the two. It is difficult to determine with certainty whether the particles round which the membranes are formed have been released from the nucleus of the cell in which they occur, have formed in the site or have been ingested by the cell. The nuclei

of most of the cells in which virus-membrane complexes of the type described were seen contained scattered virus particles, and some evidence of these particles emerging into the cytoplasm was occasionally encountered. One interpretation is that the formation of the membranes is a reaction of the cell to the presence of viral material within the cytoplasm—an attempt to isolate the particles by surrounding them with membranes. Alternatively the process may be regarded as a stage in the formation of mature virus particles. In either case, if our interpretation of the sequence of events is correct, the wrapping-up process does not often go to completion for the ratio of large particles to small particles is quite low and in fact in some infected kidneys no large particles were found. The process of membrane formation is probably halted when the nucleus becomes so heavily infected that cellular function is disturbed. The type of inclusion shown in Fig. 6 may represent material derived from a cell which disintegrated before the coating process was complete. The large membrane-bound particles are very rare in cultured cells presumably because degeneration occurs more rapidly in vitro than in vivo.[2]

The fact that the small 28 mμ particles occur in large numbers in tissue culture preparations that are known to be highly infective is good evidence that they are the infective agents. Additional evidence was provided by Kahler et al. (17) who established a correlation between the hemagglutination titres of aliquots of samples of a purified virus preparation obtained from infected tissue culture fluids by density gradient centrifugation, and the number of particles of recognizable form and size that were observed in the electron microscope in shadowed specimens prepared from these aliquots. The particle size (44 mμ) was larger than that of the particles observed in sectioned material (28 mμ) but the difference may be due to the different techniques employed in the preparation of the specimens. A similar difference between the size of particles in shadowed and sectioned specimens of rabbit papilloma virus, has been observed (18, 19). It is worthy of note, however, that although the diameter of individual particles of polyoma virus in sectioned material was about

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[2] In an article that came to hand after the completion of this report, Negroni et al. (Brit. Med. J., 1959, **1**, 359) describe the finding of particles of diameter 50 to 60 mμ in the cytoplasm of polyoma-infected cells growing in vitro.

28 mμ, the minimum centre-to-centre separation in crystalline arrays was about 38 mμ. This apparently larger diameter could be due to the presence round the particles of a layer of material that is not normally visible in osmium-fixed material. A similar explanation has been suggested in the instance of the adenoviruses to account for a similar difference which is observed between the size of particles measured as individual units or as members of crystalline arrays (20).

There is as yet no good correlation between infectivity of polyoma virus and hemagglutination titre and to this extent the identification by Kahler et al. of the infectious agent with the small particle is uncertain. Nevertheless, their evidence adds weight to the conclusion from the study of sectioned cells that the small particles are the infective agents. If this is so what is the role of the larger (60 mμ) particles? On this we can only speculate. They are, apparently, not necessary for infection because, in our experience, they occur very rarely in cells infected in vitro, from which lysates showing infectivity in hamsters at dilutions of 10^5 or more can be obtained. The large particles have the general appearance associated with many known viruses in mature or infective form, so it is possible that the large particles are an infective form of the polyoma virus. There is evidence that another tumor virus—the mammary tumor virus—may occur in two distinct infective forms. Moore et al. (21) concluded from their studies, which involved three different methods of particle size discrimination viz., filtration, irradiation, and diffusion, that the infective agent was present in their extracts in two forms—one a small particle of diameter 20 to 30 mμ, the other a much larger particle of diameter about 100 mμ. The nucleoids of the larger particles were considered to be identical with the small particles. Another possibility that cannot be excluded is that the 60 mμ particles and the associated 28 mμ particles found in the cytoplasm of polyoma-infected cells are not identical with the 28 mμ particles formed in the nucleus, but may represent a passenger virus originating either in the tissue culture cells or in the host animals.

We now consider briefly three other viruses that have some points in common with polyoma virus: herpes simplex virus, rabbit papilloma virus, and human skin papilloma virus, all of which originate in the nucleus. Morgan et al. (22) have recently described the development of herpes simplex virus in HeLa cells. Infected cells exhibit margina-

tion of the chromatin and extensive convolution of the nuclear membrane, and sometimes contain aggregates consisting of nearly uniform granules that occasionally form short rows. These are somewhat reminiscent of the intranuclear arrays of polyoma virus but are smaller in size and less regular in arrangement. Particles identified with herpes simplex virus and consisting of a central body or nucleoid and a dense limiting membrane are found scattered within the granular aggregates; these form within the nucleus, unlike the large particles associated with polyoma virus for which no evidence for intranuclear formation was found. Moreover, the central body of the herpes simplex particle does not seem to be related to the granules which are much smaller. In this they differ from polyoma particles in which the central bodies of the large particles appear to be identical with the more numerous small particles.

The virus of rabbit papilloma resembles polyoma virus more closely. Stone et al. (19) have recently described its appearance in cells of freshly fixed papillomas. The particle size is given as 33 mμ, slightly larger than the 28 mμ polyoma particles. The papilloma virus shows some tendency to form regular arrays but the regularity is not as striking as that seen in some polyoma-infected cells. No large particles corresponding to the 60 mμ particles observed in polyoma-infected tissue have been described. The association of the initial stages of proliferation with the nucleolus which is a feature of rabbit papilloma virus has not been observed with polyoma virus.

Finally, intranuclear crystalline arrays of particles presumed to be virus have been described by Bunting (23) in thin sections of a human skin papilloma. Their diameter is given as 20 to 38 mμ. The resemblance of these particles to polyoma and rabbit papilloma virus suggests that further studies of human papilloma virus, using improved techniques that have become available since Bunting's work was done, would lead to useful information about intranuclear tumor viruses in general.

For studying the sequence of changes in cells during viral carcinogenesis the polyoma-hamster kidney system that we employed possesses several advantages including a short tumor induction period and uniformity of response in the host. The present study dealt mainly with one stage in the carcinogenic process and further investigation will

be required to ascertain in detail the whole sequence of events in the kidney from inoculation of the virus until widespread tumor is established. Studies already made, however, have shown that from a morphological standpoint the fully developed tumor is not a good place to study virus-cell relationships. Indeed, if the present investigation had been confined to the later stages of the disease when tumor is well established, little or no information on the virus or its mode of development would have been obtained.

Many questions remain to be answered. For instance, what is the significance of the close association of the 28 mμ particle with tubular structures and the chromatin in the nucleus, and the association of a similar particle with a 60 mμ membrane-bound particle in the cytoplasm; or what is the action of the virus in producing two distinct responses, one degenerative and the other proliferative in cells of the same type? Morphological observations alone may not be able to answer such questions but at least they provide a framework within which biological findings must be fitted.

These studies form part of an investigation on viral carcinogenesis being carried out in the Biological Research Division, Ontario Cancer Institute. The authors gratefully acknowledge the valuable cooperation of other members of the Division.

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EXPLANATION OF PLATES

With the exception of Fig. 1 the micrographs were obtained from sections treated with lead hydroxide.

PLATE 389

FIG. 1. A general view of intertubular tissue in a hamster kidney 6 days after infection with polyoma virus. Cells marked a are stromal cells which show no obvious abnormalities but are present in considerably larger numbers than in normal 6-day hamster kidney.

The cell marked b shows nuclear degeneration with margination of the chromatin but no demonstrable virus particles.

In the cell marked c the chromatin is markedly marginated and unusually dense; the remainder of the nucleus is mainly occupied by small dense virus particles. X 18,000.
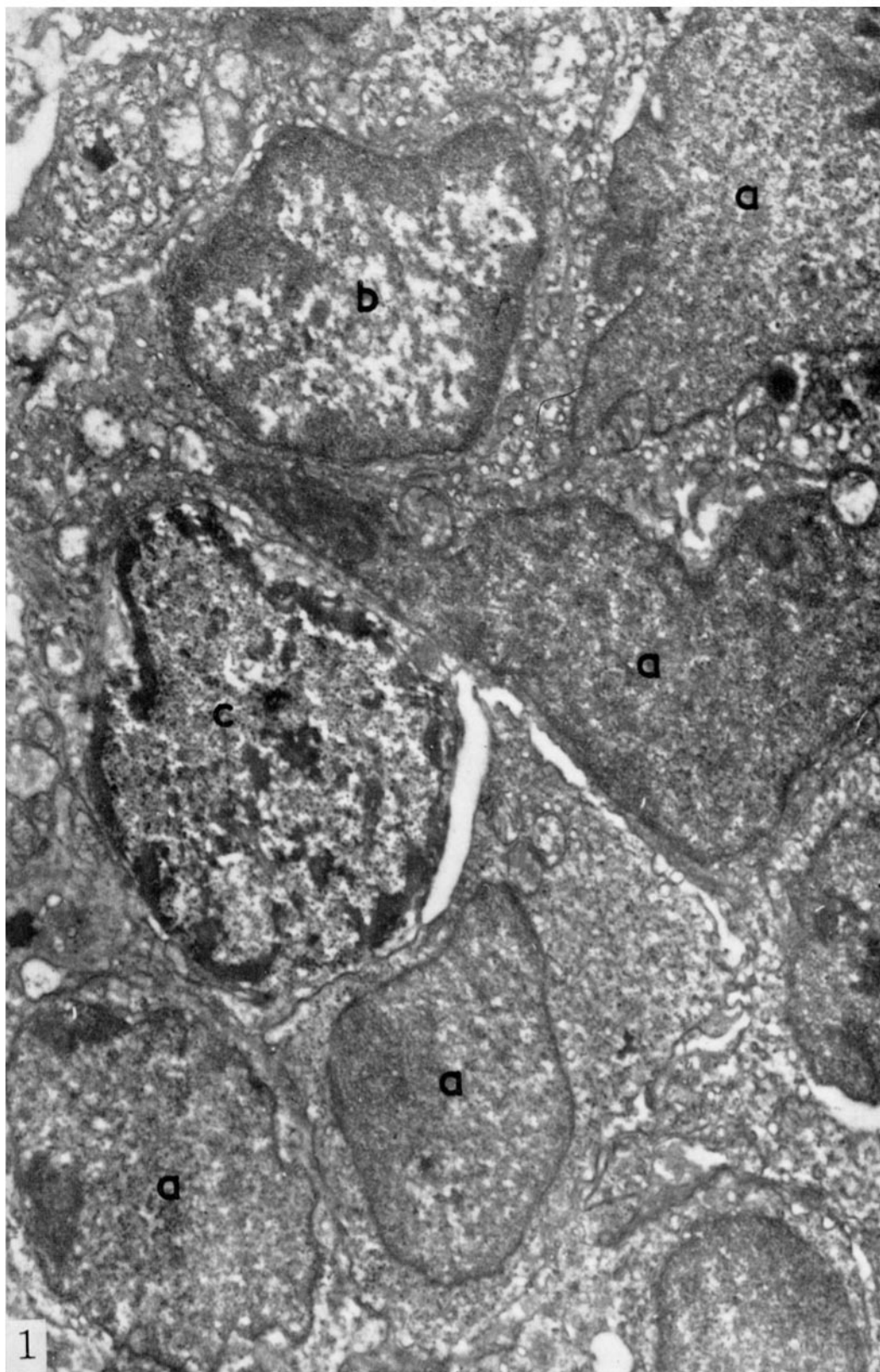
(Howatson and Almeida: Polyoma virus in hamster kidney)

PLATE 390

FIG. 2. The upper part of the figure shows the cytoplasm of two adjoining tubule cells. Separated from them by a basement membrane is a stromal cell, the nucleus of which contains scattered virus particles. A dense rim of chromatin is applied close to the nuclear membrane. This appearance is typical of the majority of infected cells. × 42,000.
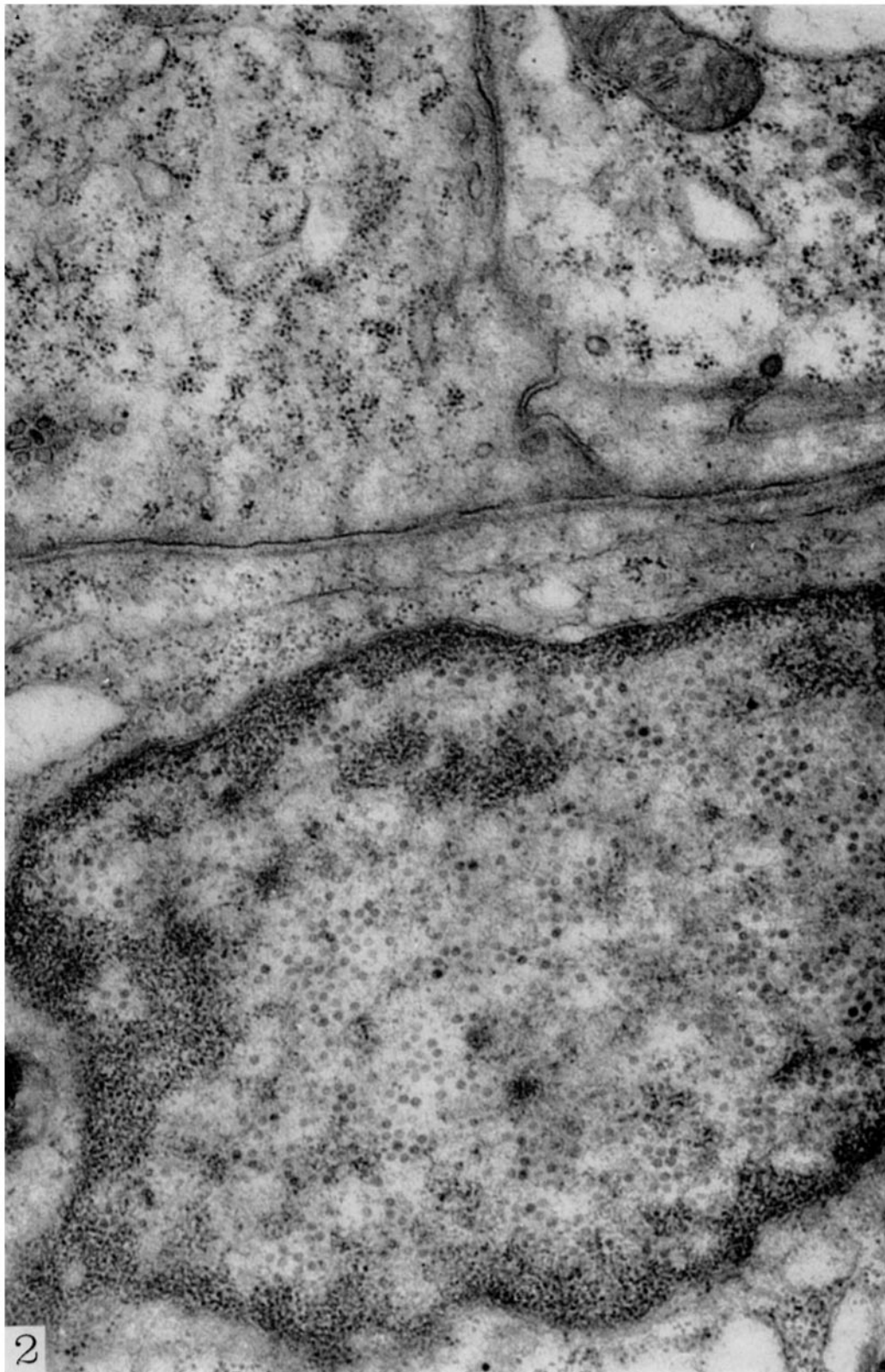
(Howatson and Almeida: Polyoma virus in hamster kidney)

PLATE 391

FIG. 3. Infected cell showing the dense type of nucleus. Much of the nucleus consists of masses of tightly packed virus particles. A large area of dense chromatin occupies the lower right portion of the nucleus. Several very dense aggregates of material of unknown nature are present in the lower part of the nucleus. × 40,000.
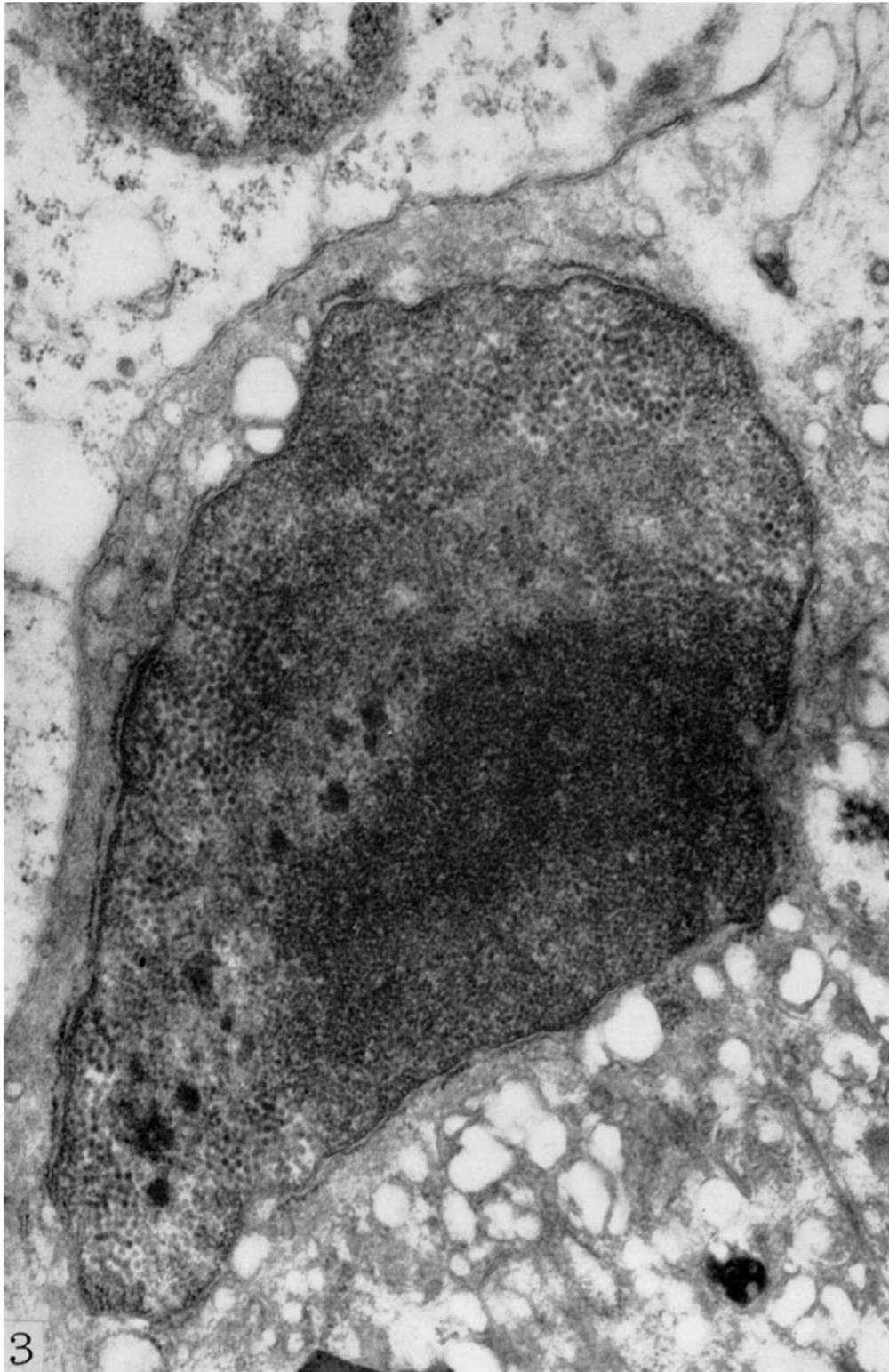
(Howatson and Almeida: Polyoma virus in hamster kidney)

PLATE 392

FIG. 4. Degenerate infected cell. The nucleus contains densely packed virus particles arranged in places in crystalline formation. × 30,000.

FIG. 5. Group of cytoplasmic inclusions containing virus particles associated with granular or filamentous matrix. × 34,000.
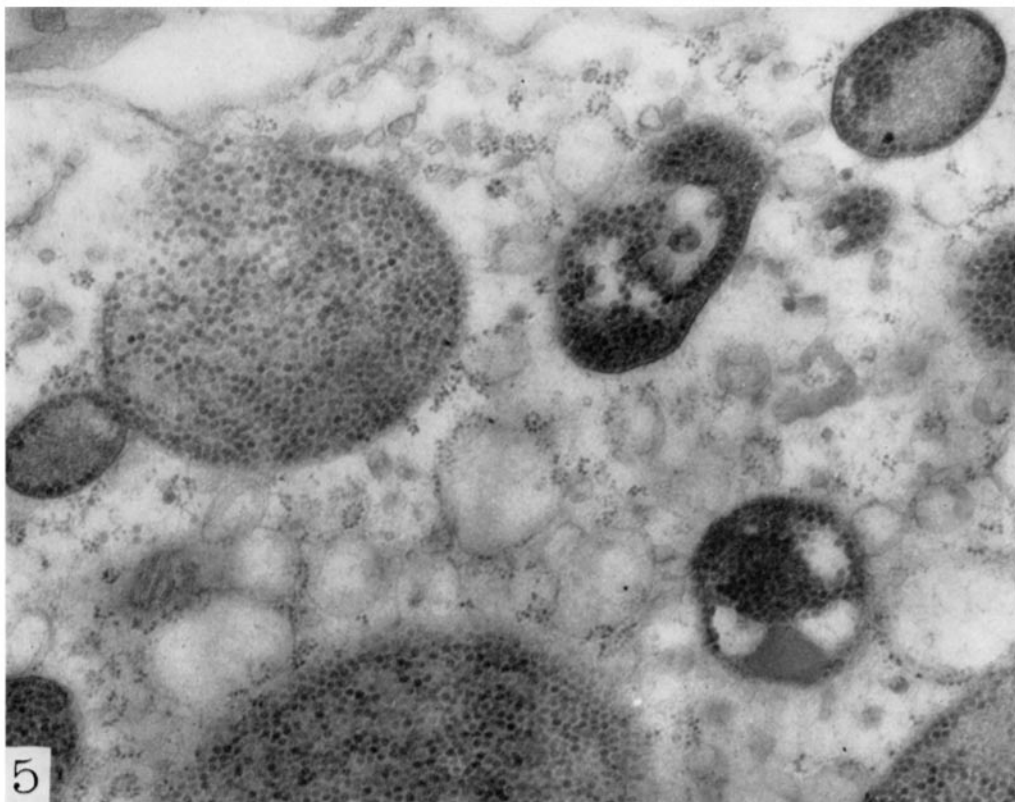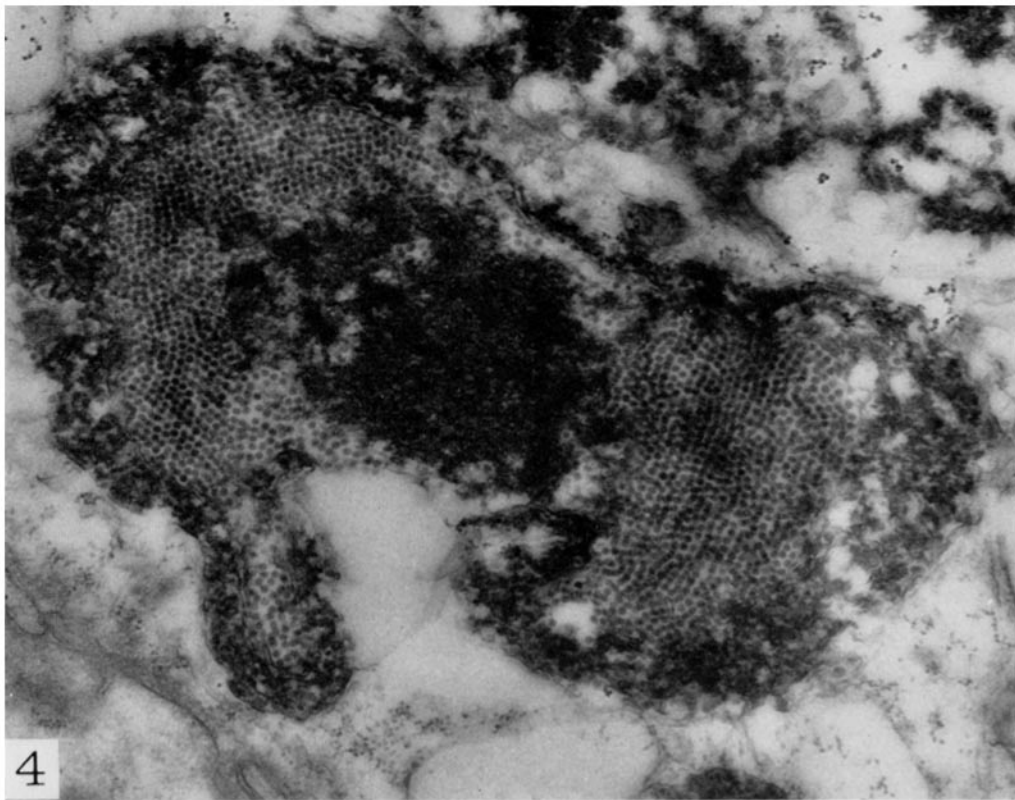
(Howatson and Almeida: Polyoma virus in hamster kidney)

PLATE 393

FIG. 6. Portion of a large inclusion showing free virus particles and at several places parallel rows of particles separated by membranes. At the lower centre the appearance marked S is due to the superimposition of more than one layer of particles. $\times$ 67,000.

FIG. 7. Inclusion containing both the large (60 mμ) and small (28 mμ) types of particles. The large particles are less numerous than the small and are scattered randomly among them. $\times$ 40,000.
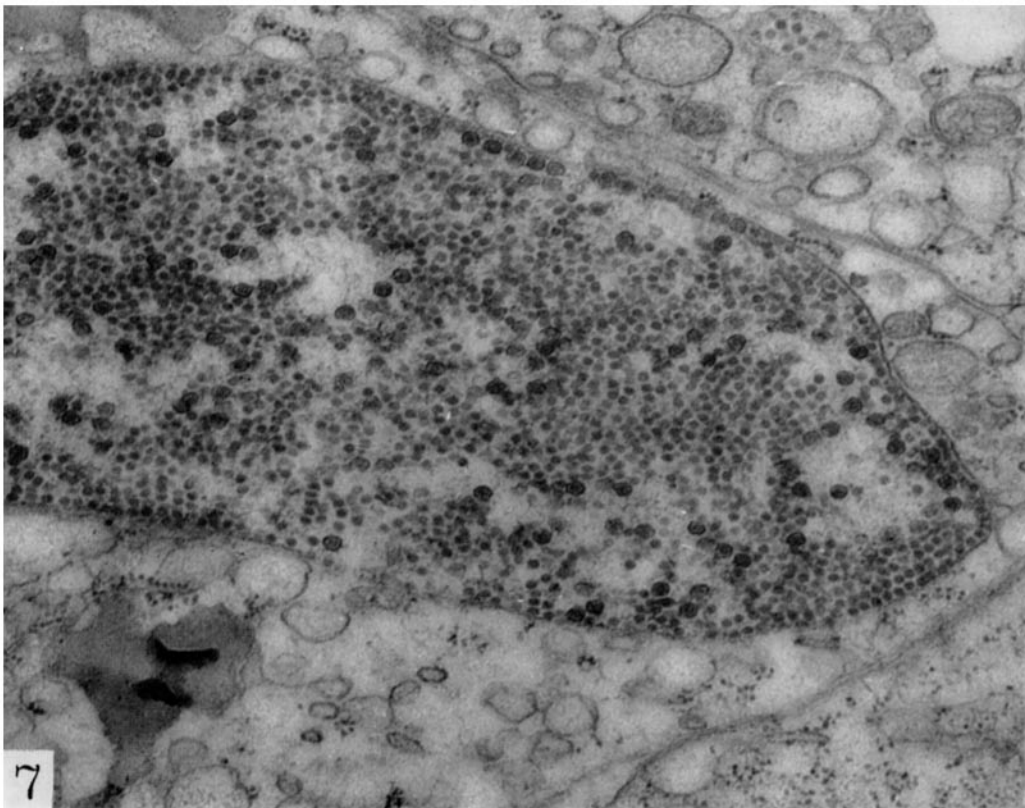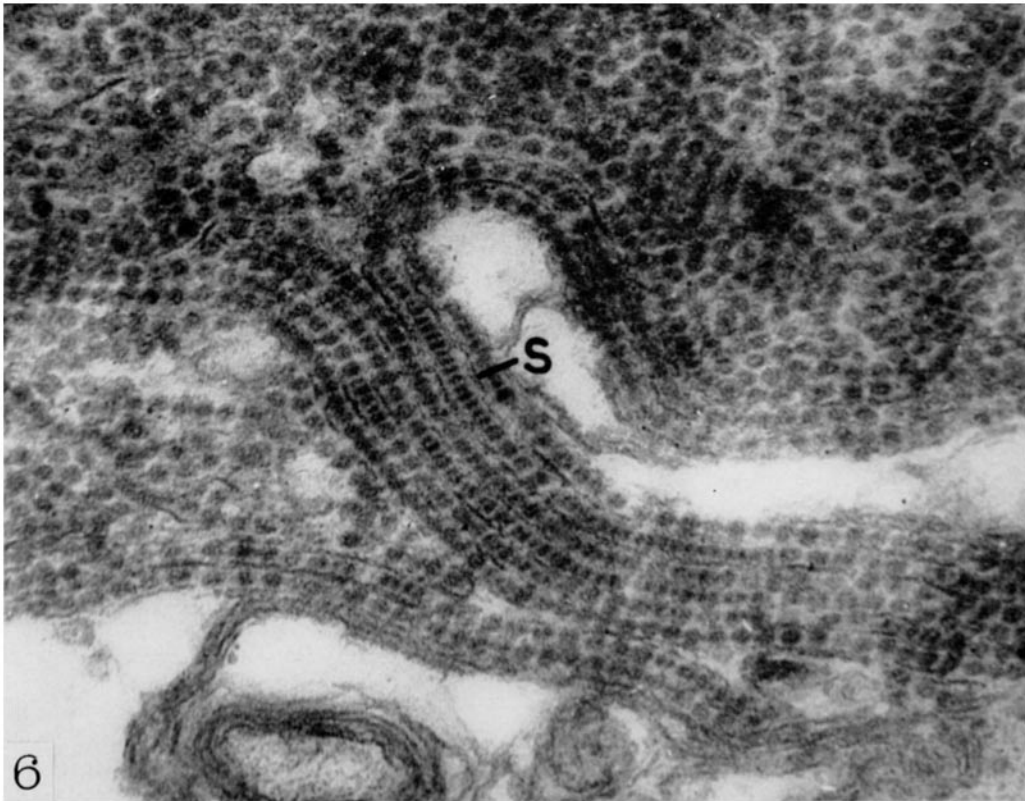
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PLATE 394

FIG. 8. Membrane-bound cytoplasmic inclusion lying adjacent to the nucleus. The inclusion contains 28 and 60 mμ particles, the latter being in the majority. $\times$ 52,000.

FIG. 9. Portions of two adjacent cytoplasmic inclusions. Both contain 28 mμ particles, 60 mμ particles, and what appear to be intermediate forms. $\times$ 120,000.
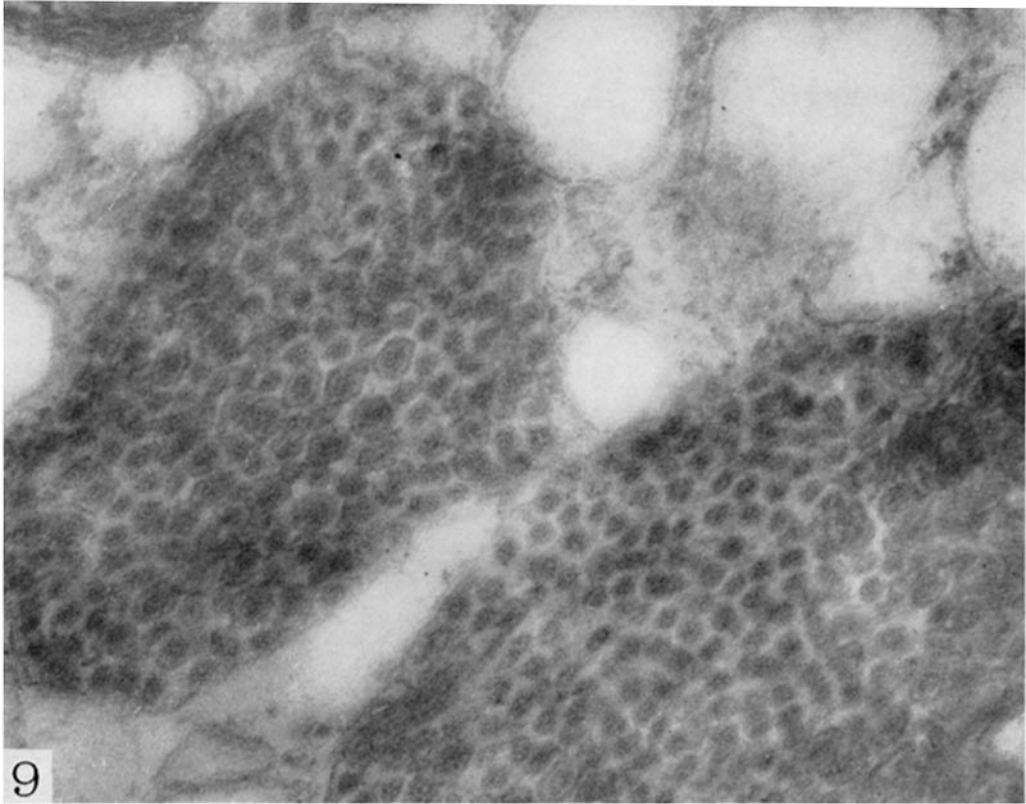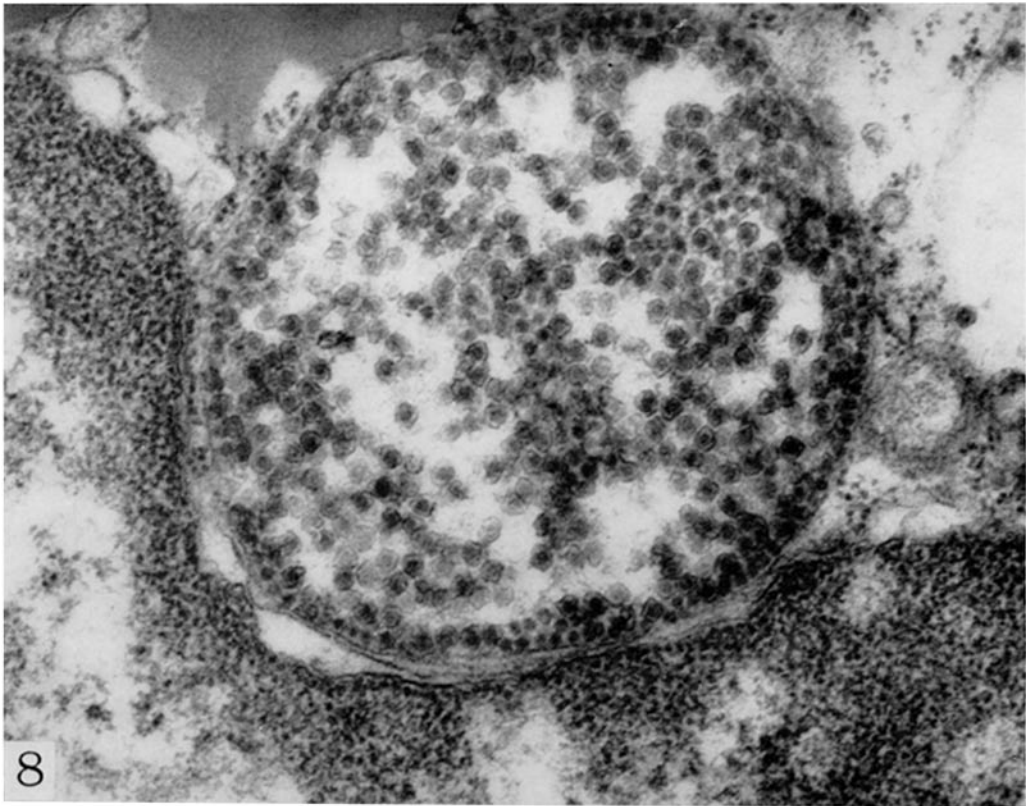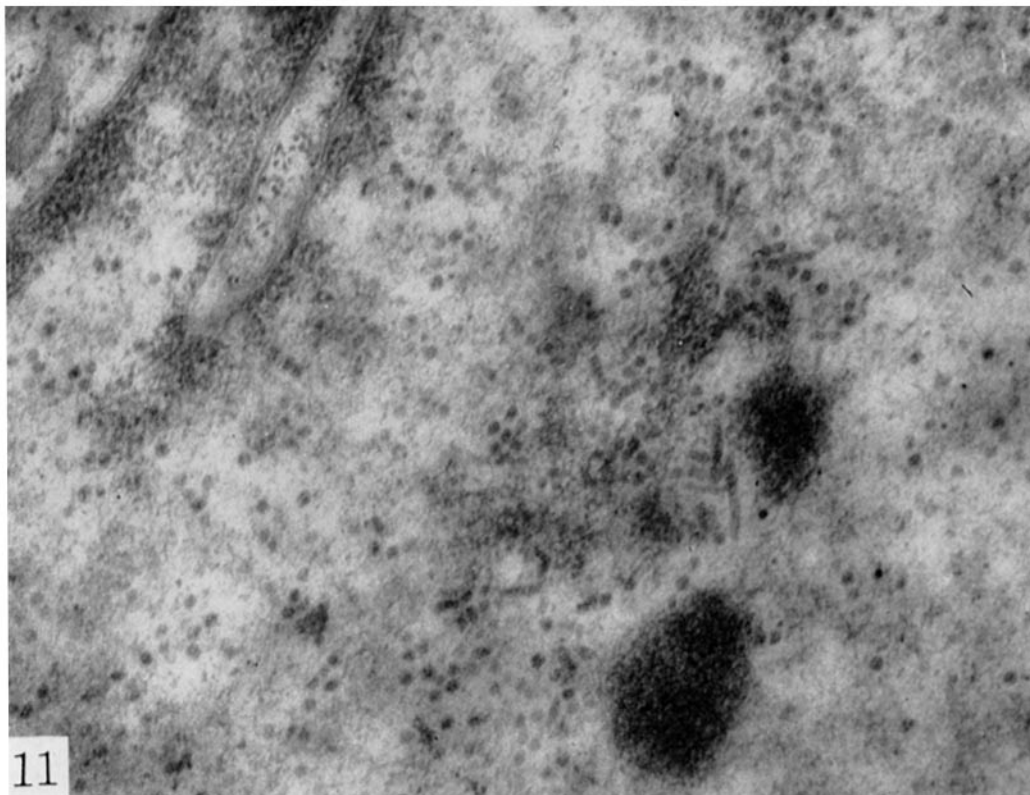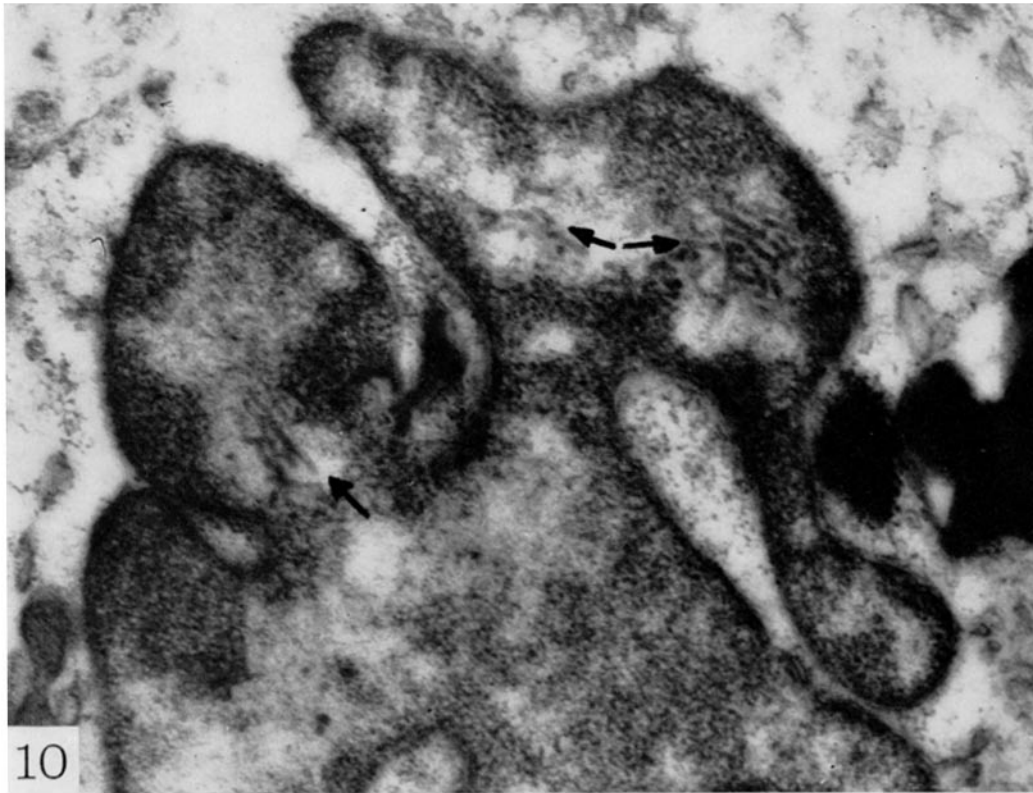
(Howatson and Almeida: Polyoma virus in hamster kidney)

PLATE 395

FIG. 10. Part of a nucleus showing marked invagination of the nuclear membrane and some margination of the chromatin. Within the nucleus in two main areas marked by arrows there are groups of filamentous structures measuring about 30 mμ in diameter. × 35,000.

FIG. 11. Portion of an infected nucleus containing filamentous structures similar to those in Fig. 10 in close association with groups of virus particles. × 38,000.
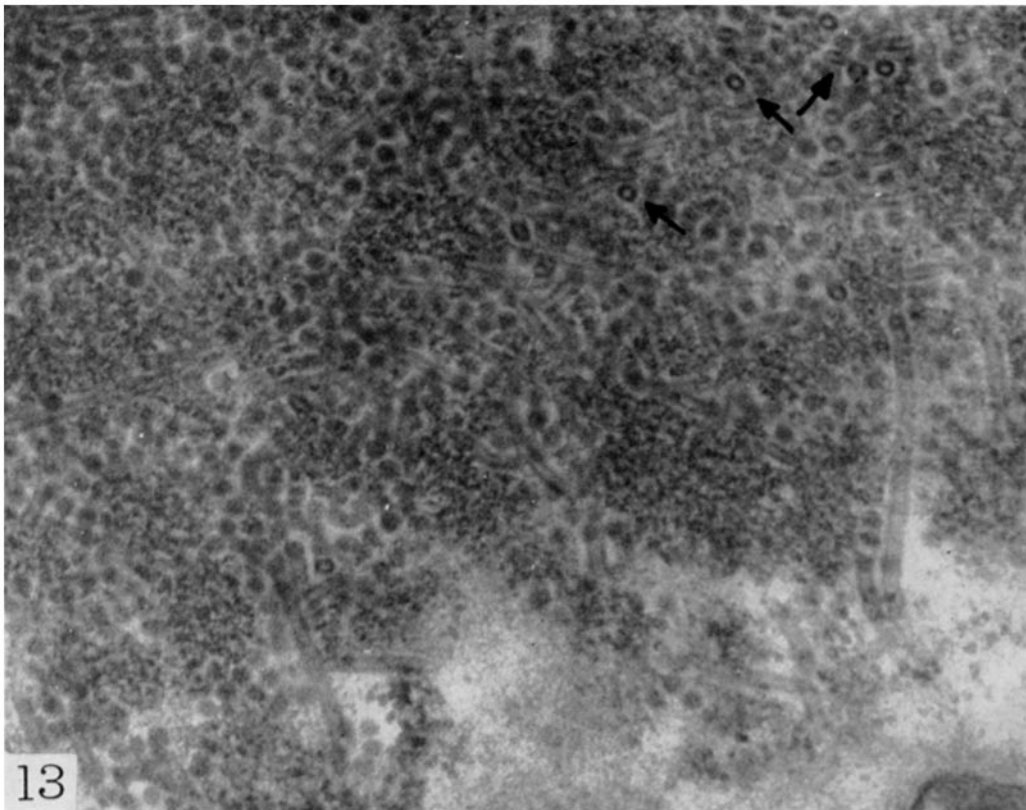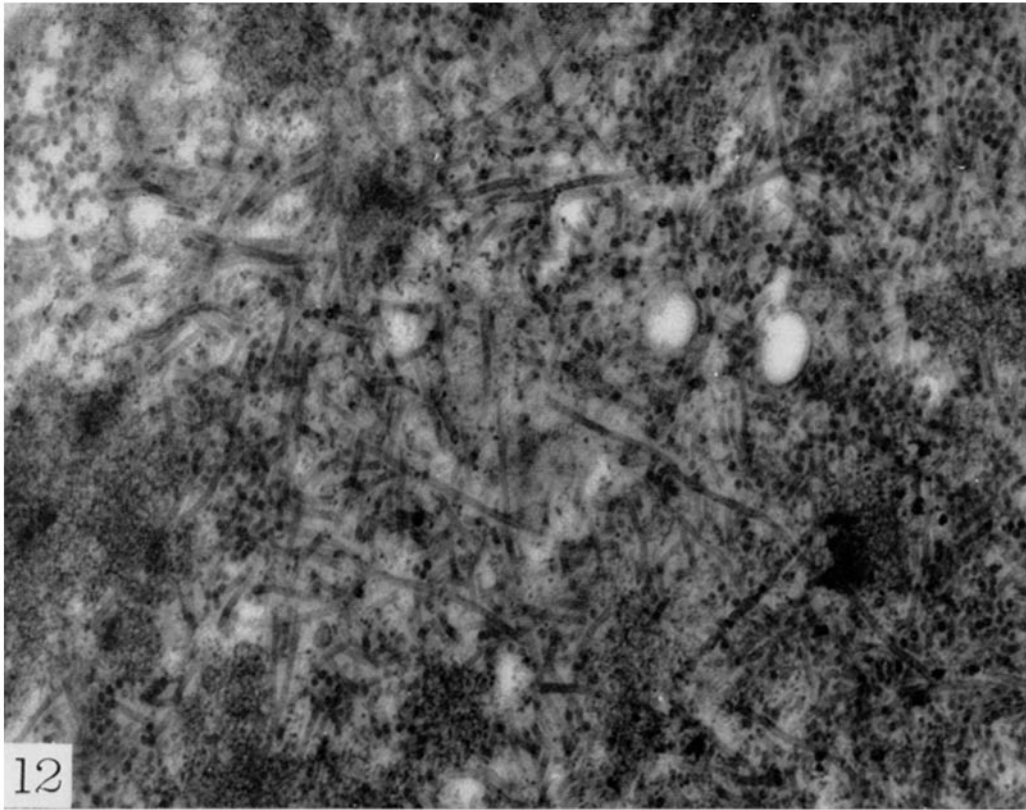
(Howatson and Almeida: Polyoma virus in hamster kidney)

PLATE 396

FIG. 12. Portion of a nucleus in which the viral material is mainly in the form of filaments distributed at random. × 36,000.

FIG. 13. Similar area from another kidney specimen showing numerous filaments cut in various planes. Where cut in cross-section (arrows) they appear as a dense ring with a clear central zone. × 67,000.
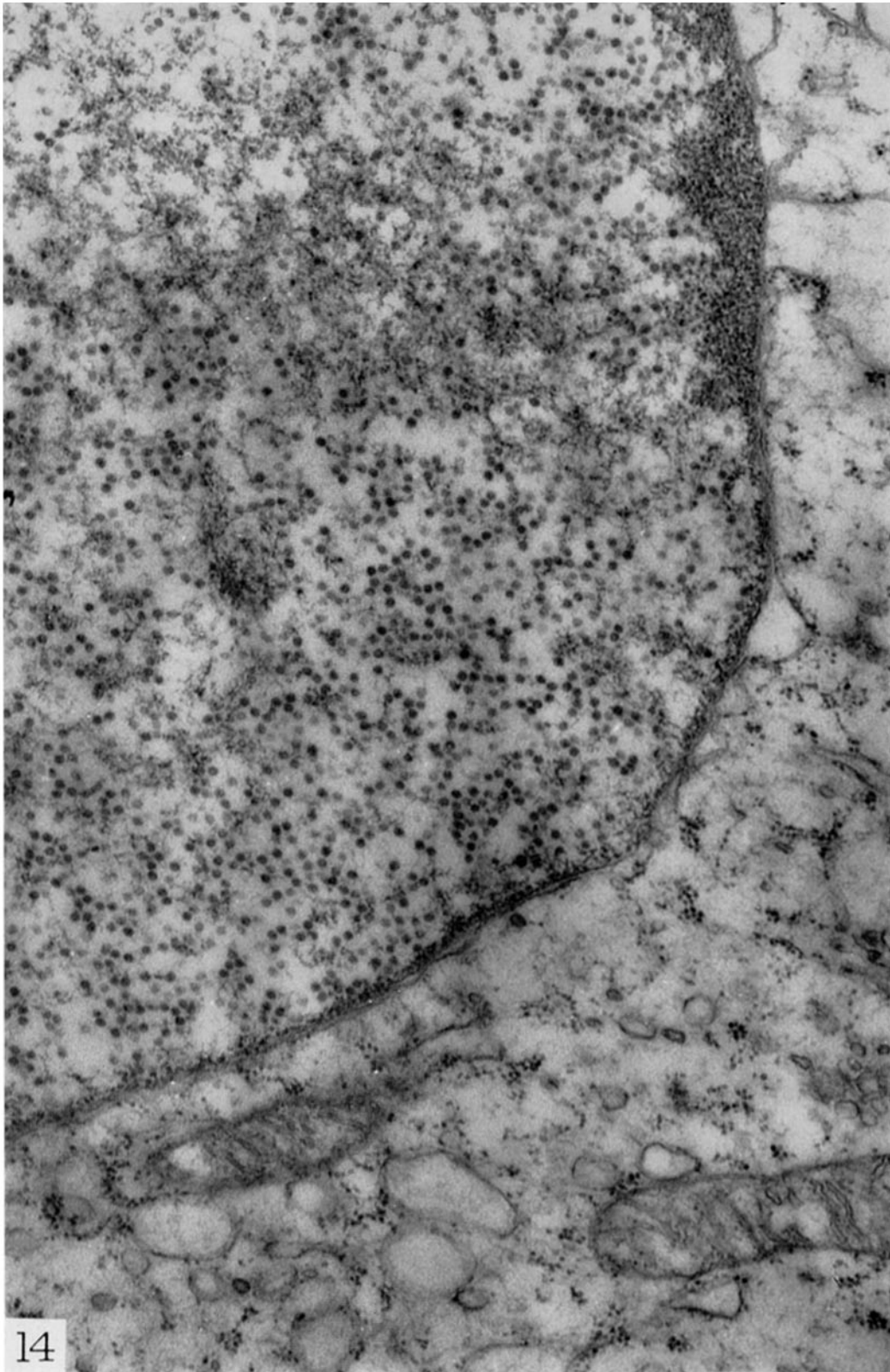
(Howatson and Almeida: Polyoma virus in hamster kidney)

PLATE 397

FIG. 14. Part of a parenchymal cell from a kidney tubule. The nucleus shows scattered virus particles similar to those seen in stromal cells. × 39,000.

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(Howatson and Almeida: Polyoma virus in hamster kidney)