ELECTRON MICROSCOPY OF SALIVARY GLAND VIRUSES*

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Plates 49 to 56

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Inclusions occurring in the salivary glands of rodents (1–4) and of monkeys (5) are morphologically similar to those sometimes present in the salivary glands of infants. In rodents their presence has been associated with a transmissible filtrable virus which has been in all cases species-specific. The characteristic nuclear inclusions have been identified in salivary glands of infants dying from various causes. Rarely a generalized infection, referred to as "cytomegalia" by Goodpasture (6), occurs in infants. In these cases enlarged cells with the distinctive inclusions are present in many organs (7–10). The agent causing the human inclusion disease has not been transmitted to experimental animals, nor has it been grown in embryonated eggs. It has, however, been serially transmitted in tissue cultures of fibroblasts derived from human myometrium (11).

Materials and Methods

The human strain of salivary gland virus used in this study was obtained from the submaxillary gland of a 7 month old infant who had died of an adrenal cortical carcinoma. The contralateral salivary gland contained the characteristic large nuclear inclusions of the salivary gland virus (11). The virus was cultured in roller tubes on fibroblasts derived from human myometrium and maintained in a medium comprised of Hanks's balanced salt solution and ox serum ultrafiltrate (2:1) to which was added embryonic chick extract (10 per cent) and horse serum (10 per cent).

Control uninfected cultures of cells from human myometrium were prepared for electron microscopy by fixing them *in situ*, in the roller tube, for 20 minutes. The fixative used was 1 per cent osmic acid in bichromate as recommended by Dalton (12). The colonies of fibroblasts (which were not removed from the tube) were rinsed in buffered normal saline and rapidly dehydrated in 70 per cent, 95 per cent, and absolute ethanol. The cells were then infiltrated with methacrylate monomer for 2 hours (3 parts of butyl to 1 of methyl; or 8 parts butyl to 1 of methyl, depending upon the type of microtome used). Finally they were transferred to gelatin capsules filled with the methacrylate mixture, to which benzoyl peroxide had been added as a catalyst. Polymerization was carried out at 60° C.

Cultures infected with the human strain of virus were similarly prepared 10 days following inoculation. No examination of the cultures at shorter intervals was made in the present investigation.

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A murine strain of the salivary gland virus was inoculated intraperitoneally into 3-week-old mice of a stock known to be free of the disease. The mice were killed 20, 30, and 48 hours following inoculation and portions of the spleens were fixed for 2 hours in a 1 per cent solution of osmic acid in bichromate. The tissue was rapidly dehydrated in ethanol solutions, transferred through 3 changes of a mixture of butyl and methyl methacrylate, and embedded in benzoyl peroxide catalyzed methacrylate as described for the tissue cultures. Spleens from control uninfected mice were similarly prepared. Salivary glands from older animals were examined 2 weeks after intraperitoneal injection of a suspension of the mouse salivary gland apparatus.

Thin sections for electron microscopy were cut with glass knives (15) in either a modified rotary microtome as described by Dempsey and Lansing (13) or a Porter-Blum microtome (14). Sections were floated onto a 20 per cent acetone solution, mounted on collodion covered copper grids, and examined without removing the plastic, in an EMU-2E RCA electron microscope. Micrographs were taken at original magnifications of 1,000 to 8,000 diameters and the negatives enlarged later as desired.

Spleens and salivary glands of mice, and fibroblasts from tissue cultures, were also examined by light and by phase microscopy. Adjacent thicker sections were taken at the time that the thin sections were cut, so that the same cells might be observed by electron, light, or phase microscopy. For the light microscope the methacrylate was removed in benzene, and the osmic acid in 1 per cent aqueous periodic acid, and the sections then stained with hematoxylin and eosin.

RESULTS

The fine structure of cells infected by (*a*) a human and (*b*) a mouse strain of the salivary gland virus will be presented in this paper.

Human Strain of Salivary Gland Virus

Normal uninoculated cultures are composed of elongated spindle-shaped fibroblasts, which frequently grow out in a layer one cell thick along the surface of the plasma clot. The cellular plasma membrane, as observed in the electron microscope, is intact with irregular small protrusions suggesting microvilli. The cytoplasm of the elongated cells contains prominent ergastoplasmic profiles with both granular and membranous components. Mitochondria are relatively small. Golgi material, although present, is not a conspicuous feature among the cytoplasmic constituents. Occasional osmiophilic lipide aggregates are noted in the cytoplasm. The cellular protoplasm is attenuated at both ends of the ovoid nucleus (Fig. 1). The nuclear membrane is uniform and the nuclear chromatin is evenly and finely dispersed. One or more dense nucleoli are usually present.

Infected cultures were examined 10 days after inoculation. By phase microscopy the diseased cell was easily identified by its increased size and large nucleus containing a central dense nuclear inclusion surrounded by a clear halo. The nuclear membrane was prominent with small clumps of chromatin adjacent to its inner margin. When stained with hematoxylin and eosin the distinctive nuclear inclusions of the salivary gland virus were present in these large cells.

In electron micrographs some cells thought to be in an early stage of infection were altered from the normal only by an increase in amount of cytoplasm and a globular cytoplasmic outline, while in others there was a decrease in nuclear chromatin which was irregularly clumped.

Definitely infected cells contained particles which were not seen in the uninfected control cells.

The infected cells were somewhat to markedly enlarged. In both the nucleus and cytoplasm of enlarged infected fibroblasts particles foreign to normal fibroblasts were evident in electron micrographs. The nucleoplasm in these cells was almost replaced by a central nuclear inclusion and its peripheral halo. In the thicker sections of osmic-fixed material, examined by phase microscopy and in low power electron micrographs, the nuclear inclusion resembled that seen in hematoxylin and eosin–stained sections: a large central dense mass with a surrounding clear zone. This appearance was altered in the thinner sections ordinarily examined with the higher powers of the electron microscope. In such specimens the inclusion was no longer a solid ovoid structure, but rather appeared to be a twisted skein of dense granular chromatin, with small particles interspersed (Figs. 2 to 4). A distinctly reduced amount of osmiophilic material was noted in the region surrounding the inclusion, although some of the particles could be seen there. When in the plane of section, the nucleolus was apparently little involved and usually was adjacent to the inner margin of the nuclear membrane, as is seen in Fig. 2. In the nucleus the particles thought to represent viral forms were most numerous in the inclusion where they were interspersed among its condensed chromatin and extended out into the surrounding pale zone. The most numerous nuclear particles varied from 65 to 110 mμ in diameter, and comprised a central dense dot of osmiophilic material surrounded by a zone of lighter substance, which, in turn, was enclosed by an outer osmiophilic shell of varying thickness. Transitions were seen from these structures to ones in which the central body was so enlarged as to fuse with the outer shell. A less prominent component of the nuclear inclusion was comprised of elongated forms as seen in Fig. 7. These comma-shaped particles have not been observed in the cytoplasm. Various types of nuclear particles are evident in Figs. 7 and 8.

The membranes surrounding the nucleus are double, with occasional minute openings or pores. The outer nuclear membrane was separated from the inner at some points (Fig. 7) and extended into the cytoplasm as a series of membrane-covered spaces indistinguishable from the ergastoplasmic sacs of the cytoplasm. This type of communication between the nuclear membranes and the ergastoplasm has been described by Watson (16). Occasionally membrane-surrounded clumps of the previously described particles were present within the nucleus (Figs. 5 and 6). Usually, these were so situated that one margin was adjacent to the nuclear membrane, making it difficult to distinguish the saccular membrane from that of the nucleus. In other cells invaginations of the nuclear membrane were incomplete. The sacs were then seen as isolated incorporations of the cytoplasm within the nucleus, surrounded by the nuclear membranes.

Particulate forms in the cytoplasm were numerous and pleomorphic. The most conspicuous cytoplasmic particle was a spherical body of dense homogene-

ous composition, 350 to 500 mμ in diameter (Fig. 9). Smaller target-like particles composed of a central dense structure surrounded first by a clear zone and peripherally by an osmiophilic shell of about 20 to 30 mμ in thickness were present either as isolated forms or as nests of many particles (Fig. 10). Both types of cytoplasmic particles were enclosed within vacuolar structures set off from the surrounding cytoplasm by a dense membrane. These vacuoles contain sometimes one particle and sometimes many. The vacuolar wall is similar in appearance to the membrane bounding the Golgi tubules which were prominent in the infected cells (Fig. 9). In some cells there was a finely granular material present on the outer margin of the vacuolar wall, which then resembled the ergastoplasmic sacs of the cytoplasm. At the margins of some of the particles the surrounding membrane extended into an adjacent ergastoplasmic sac without interruption.

Not only were groups of 2 to 4 particles surrounded by a membrane as is shown in Fig. 9, but other larger groups of as many as 15 to 30 spheres of various sizes were surrounded by a membranous sac within the cytoplasm as in Fig. 10. The particles within such larger sacs varied in size, perhaps in part related to the plane of section—but not entirely, since the same sac contained small target-like forms as well as the larger spheres. The exact relation between the different forms was not clear. Frequently the large saccular accumulations of particles were adjacent to the plasma membrane as in Fig. 10. It was considered that they might be accumulations of particulate forms of viral origin similar in type to that called a "plaque" by Bedson and Bland (17), and more recently by Gaylord (18), in cells infected with the meningopneumonitis virus.

Large homogeneous dense bodies, 2,000 to 2,800 mμ in diameter were, present in the cytoplasm of many cells. Small membrane-covered blebs 25, to 1,500 mμ in diameter, were present at the circumference of these bodies. Many of the blebs contained particles, some of which were the dense homogeneous spheres previously described, while others were the target-like forms. Either or both forms may occur within a single vesicle, as is shown in Fig. 11. Separation of these blebs from the large bodies may be the source of some of the membrane-surrounded isolated sacs of virus-like forms within the cytoplasm of cells (Figs. 11 and 12).

A conspicuous cytoplasmic alteration besides the presence of particles was an increase in the number of Golgi membranes, which were present as small vesicles associated with closely arranged flattened tubules surrounded by a smooth membrane (Fig. 9). In some cells particles were intimately associated with and partially surrounded by the Golgi material (Fig. 16).

Ergastoplasmic membranes were indistinct and poorly developed in many of the infected cells, and the granular component, in particular, was scant in comparison with that of the normal fibroblast. It is possible that the increased cytoplasmic volume had occurred without increase in the ergastoplasmic material,

or that some alteration had occurred in the ergastoplasmic membranes to explain their apparent decrease.

In infected cells prior to the formation of prominent nuclear inclusions and prior to the presence of virus in the cytoplasm, there seemed to be a stimulation of formation of mitochondria. The mitochondria were increased in number and in size, although their cristae may have been fewer or less complicated than usual. Occasionally there were mitochondrial forms which appeared to be open or incomplete. In contrast to these cells were those in which the nuclear inclusion was fully developed and in which cytoplasmic particles were present. In these cells there often were fewer mitochondria than usual, and as may be observed in Fig. 9, recognizable mitochondria were sometimes absent from large areas of the cytoplasm. Occasional vacuolated structures resembling mitochondria contained dense foci as in Fig. 14. The possibility of involvement of mitochondria in viral diseases has previously been suggested by Bang (19).

Dense spherical forms and occasional target-like forms were in close apposition to the external surface of the plasma membranes of many cells. Usually extracellular particles were not surrounded by a membrane and probably represented particles liberated by the breakdown of diseased cells (Figs. 9 and 13).

Mouse Strain of Salivary Gland Virus

It was considered of interest to compare the appearance of particles present in a human strain of virus with those of a mouse strain, since the morphologic alterations recognizable with the light microscope are similar and yet the viruses are species-specific. The pathologic changes in the spleens of the infected mice, as observed in electron micrographs, will not be described, but rather the morphology of the abnormal particles.

The infected cells were mainly of the reticuloendothelial group. The nuclear particles were similar in size and configuration to those previously described in fibroblasts infected with the human strain of the virus. The nuclear inclusion was formed by particles interspersed with condensed chromatin (Figs. 18 and 20). Particles were again prominent in the cytoplasm and, as observed in the infected fibroblasts, were of two sizes. Single dense spheres, 250 to 400 mμ in diameter, occurred in the cytoplasm and contained one or more small round clear foci about 60 mμ in diameter. These structures differed from the spheres described in the fibroblasts infected with the human strain of the virus in that they contained central clear zones (Fig. 20). A membranous sac containing multiple large forms similar to those occurring singly can be seen in Fig. 19. This sac measured 1.95μ in diameter; the dense spherical forms within it measured 150 to 430 mμ. Each of the spherical forms appears to be enclosed by a membrane, while within them one observes small pale zones, some of which contain a single dense dot and thus resemble the target-like forms.

Again, as in the cells infected with a human strain, the mitochondria were variable in number and had no constant pattern of arrangement of their cristae.

In minimally infected cells they appeared to be slightly increased in number as well as in variability of pattern, whereas in more definitely diseased cells there was an apparent reduction in number of mitochondria. Some of the mitochondria were vacuolated, while adjacent ones showed no such change.

Sections of salivary glands from older mice inoculated intraperitoneally 2 weeks previously were examined. Diseased cells were readily identified by phase microscopy, but were far less numerous than in the spleens of younger animals. The nuclear inclusion particles were identical with those seen in the spleen. However, within the cytoplasm of the infected cells there were numerous nests of target-like particles. The sacs of particles measured from 2.0 to 3.2μ in their greatest diameters and were surrounded by a thin membrane (Fig. 21). The enclosed particles measured 120 to 180 mμ and were identical with the target-like forms described in the cytoplasm of fibroblasts infected with the human strain of salivary gland virus.

It should be emphasized that the infected reticuloendothelial cells of the spleen were examined at the end of 3 days, and that in them spherical forms were present in which a suggestion of formation of target-like particles could be discerned. However, the salivary glands were examined at the end of 14 days, and in these no large spherical bodies were identified, but rather numerous membrane-surrounded aggregates of target forms (Fig. 21). The spleens of infected mice, in spite of their numerous nuclear inclusions, were a poor source of viable transmissible virus, whereas the salivary glands with fewer infected cells were an excellent source. This may indicate that the target forms seen in the cytoplasm of the salivary gland cells of mice, at 14 days, and in the cytoplasm of fibroblasts infected with the human strain 10 days previously, were the infective form of the virus.

DISCUSSION

Characteristic particles of 2 general sizes were readily identified in cells infected with either a human or a mouse strain of the salivary gland virus.

The *nuclear* forms of both strains of the virus were similar in size and appearance, and closely resembled the nuclear form of the herpes simplex virus as demonstrated by Morgan (20) and of the particles associated with the Lucké tumor reported by Fawcett (21). The distinctive nuclear inclusion was formed by granular chromatin and contained 3 types of particles: (*a*) numerous small particles surrounded by a membrane, (*b*) occasional membranes without a central particle, and (*c*) interspersed comma-like forms similar to those described by Fawcett (21), but more prominent. The nuclear inclusions formed by both strains of salivary gland virus were similar.

The *cytoplasmic* forms were of 2 types. One type, the smaller, was a target-like form comprised of a central dense dot surrounded by a pale zone and peripherally by a shell of dense material—the entire configuration often being

enclosed in a thin membrane. These small forms were morphologically similar in cells infected with either strain of the virus, and were reminiscent of the cytoplasmic and extracellular forms described in both the herpes simplex virus by Morgan *et al.* (20) and in the herpes B virus by Reissig and Melnick (22); they were almost identical with those of the Lucké carcinoma described by Fawcett (21). However, in neither of the herpetic virus–infected cells and rarely in the carcinoma were the particles present within membranous sacs as they were in both the cytoplasm of the epithelial cells of salivary gland in the mouse strain and in cytoplasm of fibroblasts infected by the human strain. Furthermore, in none of these infections were the other large abnormal cytoplasmic structures observed, as they were in cells infected with salivary gland viruses.

The other cytoplasmic particle is the large homogeneous dense sphere which occurs either as single or multiple particles within a thin membrane. These spheres, unlike the nuclear particles and the target-like forms, are dissimilar in cells infected with the two strains of salivary gland virus so far studied. In fibroblasts 10 days after inoculation with the human strain they occurred as solid spheres. Sometimes solid spheres and target forms were enclosed within the same vesicle (Fig. 10). In the virus of the mouse strain (not examined in tissue culture, but rather in the spleens of young mice 3 days after intraperitoneal inoculation) large forms occurred as spheres of the same size but with small target-like particles present within them (Fig. 19).

Although no interval studies were made, findings in both the tissue cultures infected with the human strain and in the splenic and salivary gland cells infected with the mouse strain lend support to Gaylord's thesis (18) that both binary fission and endosporulation may be possible methods of viral multiplication. In Fig. 9 there is suggestive evidence of budding, while in Fig. 10, also in an infected fibroblast, a sac containing forms of varying types is seen. Evidence possibly supporting endosporulation is demonstrated in Fig. 19, a splenic reticular cell in which a sac contains numerous dense spherical forms some of which contain smaller target-like structures.

The cytoplasmic particles associated with the salivary gland viruses in some ways are similar to those of the meningopneumonitis virus described by Gaylord (18). The fibroblasts infected by the meningopneumonitis virus show vesicles or sacs in the cytoplasm, containing large virus-like forms and associated smaller structures reminiscent of the target forms. However, the large particles are more numerous in the meningopneumonitis infection, practically filling the cytoplasm of the fibroblasts.

The relationship of the development of the nuclear forms to the origin of the cytoplasmic virus particles is not known. The presence of nuclear pores and the demonstration of the continuity of the canalicular systems of the cytoplasm with the outer nuclear membrane by Watson (16), and with the outside of the cell by Palade (23), suggest a mechanism whereby the nucleoplasm might be in open commu-

nication with the cytoplasm and the extracellular space. The presence of membranes surrounding particles in the cytoplasm may indicate that these particles are within either the ergastoplasmic canalicular system or segregated within the Golgi membranes of the cell. Their presence within membranes is distinct; but the definite identity of these membranes is less certain (Figs. 15 to 17). The possibility that the infective virus particle is ingested by the cell through some form of pinocytosis and transmitted through the membranous system of the cell thus reaching the nucleus might be considered.

The possibility of multiplication of the virus particles or of their transport within the membranous systems of the cell has been suggested by other investigators. Schwerdt and Pardee (24) studying the Lansing strain of poliomyelitis in the central nervous system of cotton rats found most of the virus to be free or associated with the microsomal fraction, in so far as they were able to determine by differential centrifugation. Friedlaender *et al.* (25) in a recent study with serial sections of the anopheles A virus growing in Ehrlich ascites tumor cells have found the virus to be within a tubular membranous system. They believe the external membrane about the particles to be the granule-covered membrane of the endoplasmic reticulum within which the virus is propagated. They suggest that paired particles within such a system need not prove binary fission, but rather may merely represent section through multiple particles in a folded tubular system.

Thus it seems likely that the virus may pass from either the extracellular space to the nucleus, or from the nucleus to the cytoplasm and outside of the cell by the canalicular system of the cytoplasm, and its connections with the plasma membrane, nuclear membrane, and nucleoplasm.

Bang (19) suggested in 1950 that vaccinia virus was present either within or closely associated with mitochondria. Others have mentioned a possible relation and in particular, Ackermann and Kurtz (26) working with herpes simplex–infected livers of 12-day chick embryos have found an association of virus with the mitochondrial fraction as determined by differential centrifugation. They postulate that the mitochondria are a possible intracellular site of viral synthesis with later deterioration of the mitochondria and thus free virus in the cytoplasm. Leyon (27) has also suggested that one of the plant viruses is intimately associated with chloroplasts; however, his work is on electron microscopy of fractionated and shadowed material and no sections were examined.

These investigations have pointed to some relationship between virus particles and mitochondria or endoplasmic reticulum. In the present work, cytoplasmic particles thought to be of viral origin have been identified (*a*) in double membrane–surrounded structures suggestive of altered mitochondria, (*b*) in vacuolated structures adjacent to vacuolated mitochondria and similar in appearance to the adjacent mitochondria except for the incorporated particle, (*c*) partially surrounded by or in close apposition to membranes identified as part of the Golgi complex, and (*d*) as single, double, or multiple forms within

membranes which often have a closely attached fine granular component and which at their margins extend into characteristic ergastoplasmic sacs.

The finding of abnormal particles in association with all three of these cytoplasmic constituents suggests the possibility of their interrelation and that the virus may be intimately connected with not one but all of them during its development.

SUMMARY

A human and a mouse strain of the salivary gland virus have been examined by electron microscopy. The human strain was transmitted, prior to examination, to tissue cultures derived from human myometrial cells, while the mouse strain was examined in mice inoculated intraperitoneally.

The nuclear forms associated with both strains of virus were morphologically similar. Nuclear inclusions, composed of particles interspersed with dense clumped chromatin, were a striking feature of infected cells. The cytoplasmic forms were of 2 types—one a 300 to 500 mμ homogeneous dense spherical form, and the other a target-like form composed of a central dense dot in a pale zone surrounded by a dense shell—the entire configuration measuring 100 to 180 mμ. The target-like particle appeared to be identical in both strains. The spherical cytoplasmic forms in cells infected with the human strain appeared to be solid, while in cells infected with the mouse strain there was evidence of formation of target-like forms within the spheres.

Possible mechanisms by which infection of the cell may occur, as well as possible mechanisms and sites of multiplication of virus, are discussed.

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BIBLIOGRAPHY

1. Jackson, L., *J. Infect. Dis.*, 1920, **26,** 347.
2. Thompson, M. J., *J. Infect. Dis.*, 1932, **50,** 162.
3. Kuttner, A. G., and Wang, S. H., *J. Exp. Med.*, 1934, **60,** 773.
4. Rector, E. J., and Rector, L. E., *Am. J. Path.*, 1934, **10,** 629.
5. Cowdry, E. V., and Scott, G. H., *Am. J. Path.*, 1935, **11,** 647.
6. Goodpasture, E. W., and Talbot, F. B., *Am. J. Dis. Child.*, 1921, **21,** 415.
7. Wyatt, J. P., Saxton, J., Lee, R. S., and Pinkerton, H., *J. Pediat.*, 1950, **36, 271.**
8. Smith, M. G. and Vellios, F., *Arch. Path.*, 1950, **50,** 862.
9. Mercer, R. D., Luse, S., and Guyton, D. H., *Pediatrics*, 1953, **11,** 502.
10. Haymaker, W., Stephens, J., Lillie, R. D., and Fetterman, G. H., *J. Neuropath. and Exp. Neurol.*, 1954, **13,** 562.
11. Smith, M. G., *Proc. Soc. Exp. Biol. and Med.*, 1956, **92,** 424.
12. Dalton, A. J., *Anat. Rec.*, 1955, **121,** 281.
13. Dempsey, E. W., and Lansing, A. I., *Proc. Soc. Exp. Biol. and Med.*, 1953, **82,** 253.

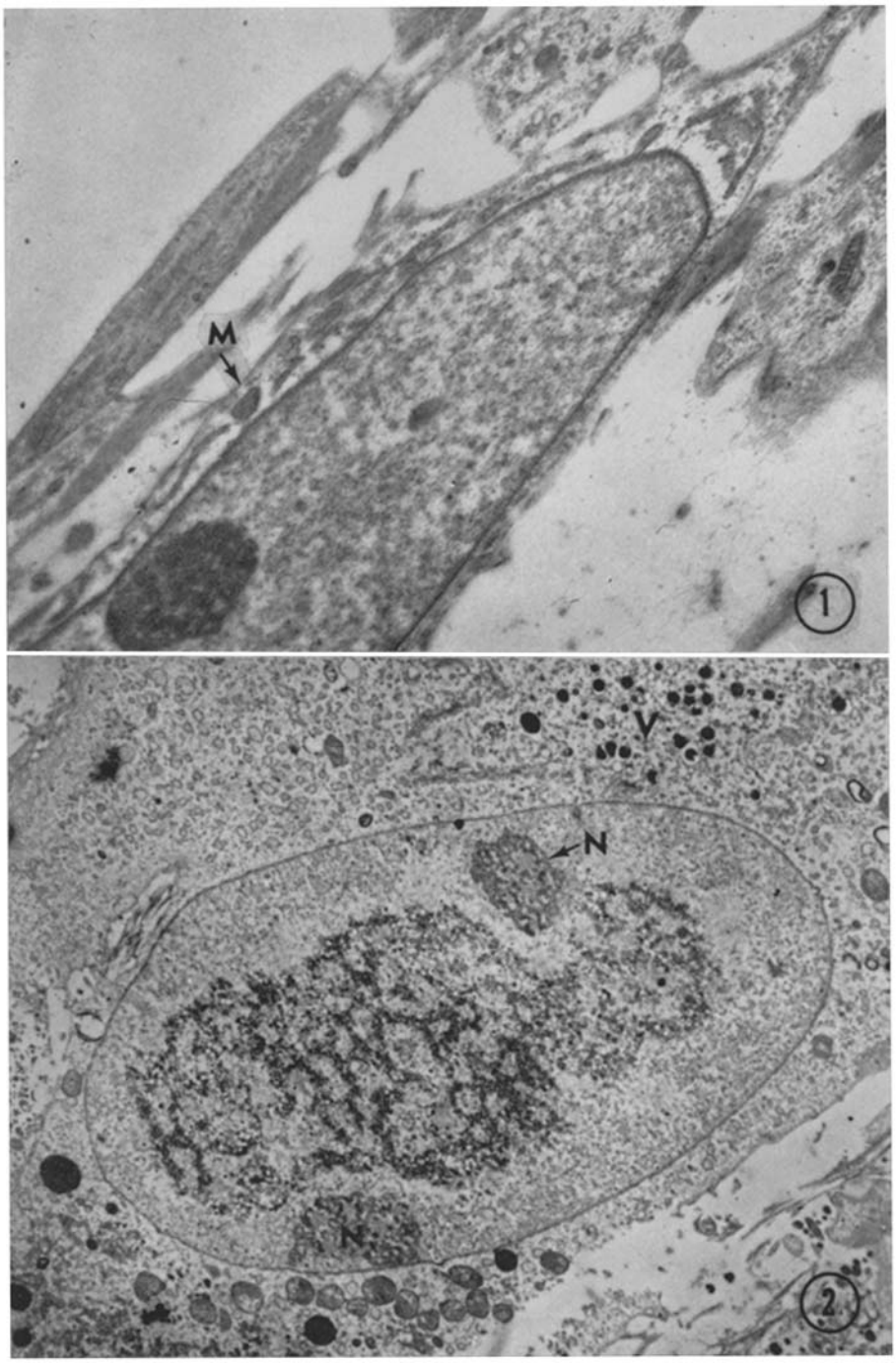
14. Porter, K. R., and Blum, J., *Anat. Rec.*, 1953, **117,** 685.
15. Latta, H., and Hartmann, J. F., *Proc. Soc. Exp. Biol. and Med.*, 1950, **74,** 436.
16. Watson, M. L., *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 257.
17. Bedson, S. F., and Bland, J. O. W., *Brit. J. Exp. Path.*, 1933, **13,** 461.
18. Gaylord, W. H., Jr., *J. Exp. Med.*, 1954, **100,** 575.
19. Bang, F. B., *Bull. Johns Hopkins Hosp.*, 1950, **87,** 511.
20. Morgan, C., Ellison, S. A., Rose, H. M., and Moore, D. H., *J. Exp. Med.*, 1954, **100,** 195.
21. Fawcett, D. W., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 725.
22. Reissig, M., and Melnick, J. L., *J. Exp. Med.*, 1955, **101,** 341.
23. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 567.
24. Schwerdt, C. E., and Pardee, A. B., *J. Exp. Med.*, 1952, **96,** 121.
25. Friedlaender, M., Moore, D. H., Love, R., Brown, R. A., and Koprowski, H., *J. Exp. Med.*, 1955, **102,** 361.
26. Ackermann, W. W., and Kurtz, H., *J. Exp. Med.*, 1952, **96,** 151.
27. Leyon, H., *Exp. Cell Research*, 1953, **4,** 362.

EXPLANATION OF PLATES

PLATE 49

FIG. 1. Electron micrograph of a portion of a normal fibroblast from an uninoculated tissue culture. To the left of the figure is a part of the nucleus with its dense nucleolus. The nuclear chromatin is finely and evenly dispersed. The cytoplasmic outline is attenuated, so that even at this relatively low magnification only a portion of the cell is present in the field. Mitochondria (*M*) are scattered through the cytoplasm and are rather small. The ergastoplasm is rather evenly dispersed. At the upper right of the figure are portions of the elongated cytoplasmic extensions of other fibroblasts. × 6,000.

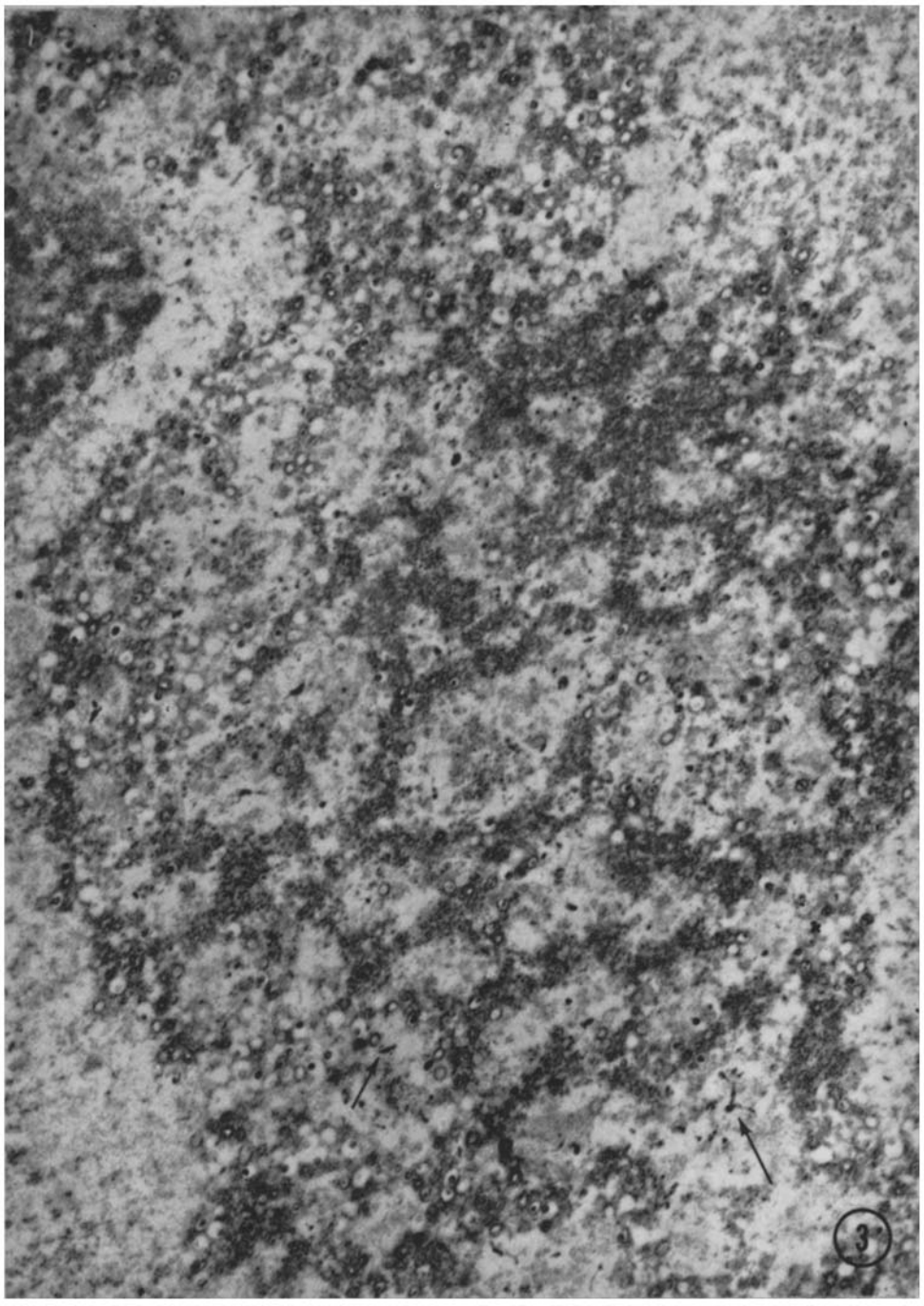
FIG. 2. Electron micrograph of a portion of a fibroblast infected by the salivary gland virus (human strain). Centrally in the nucleus there is a nuclear inclusion composed of what would appear to be virus particles and strands of dense finely granular chromatin material. Surrounding the centrally located inclusion is a pale zone. Two nucleoli (*N*) are present and uninvolved in the inclusion. There is a slight amount of finely granular chromatin present adjacent to the inner margin of the nuclear membrane. The mitochondria are apparently unaltered and are concentrated in the lower portion of the field. Densely osmiophilic inclusions are also present in the same region. Their exact origin is not definite. At the upper right (*V*) are spherical dense cytoplasmic forms which are surrounded by membranes. × 8,000.



(Luse and Smith: Electron microscopy of salivary gland viruses)

PLATE 50

FIG. 3. Higher magnification of nuclear inclusion seen in Fig. 2. Target-like forms are interspersed within the clumped chromatin. Also evident are the small comma-shaped forms (arrows). × 27,000.



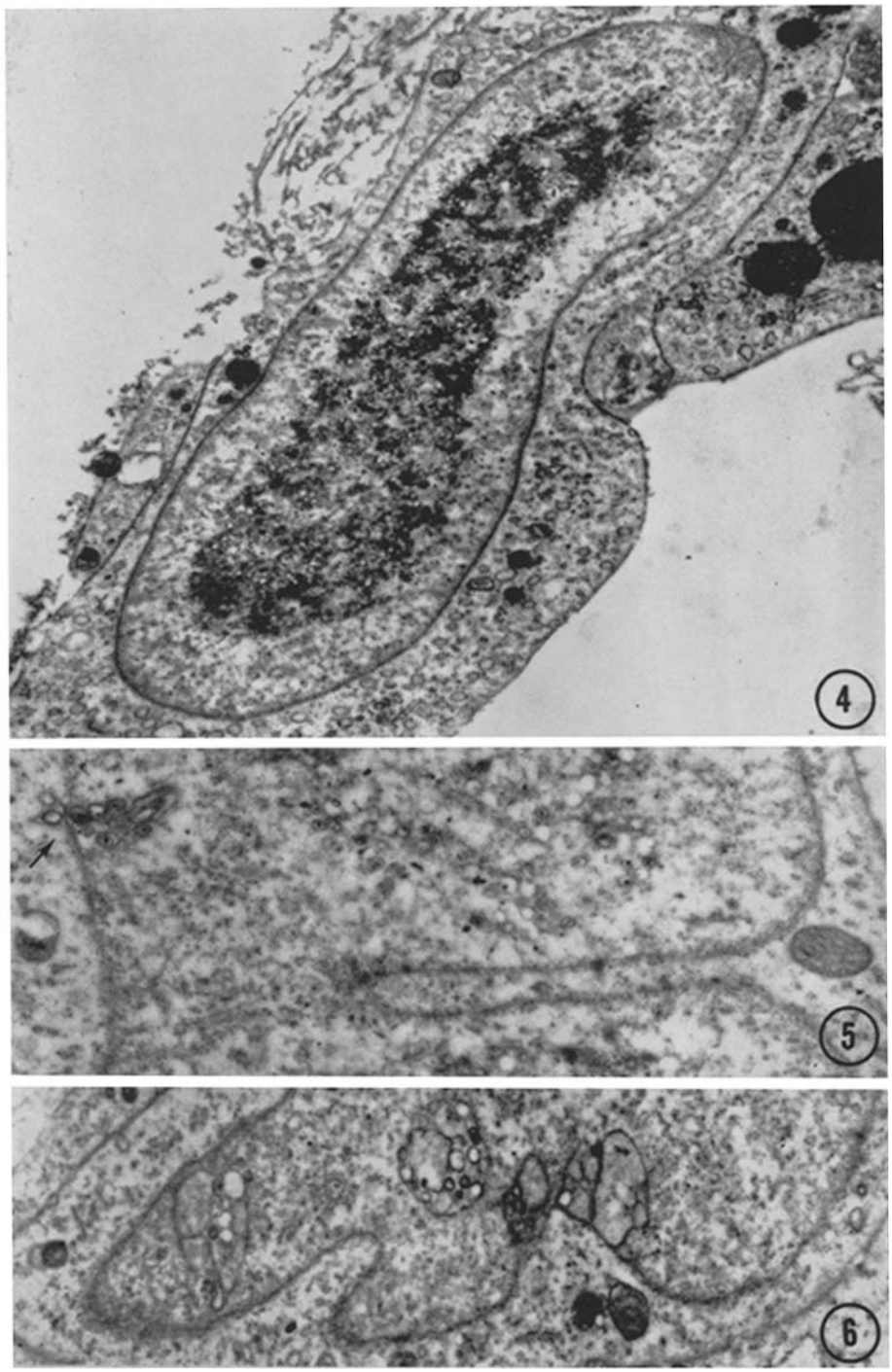
(Luse and Smith: Electron microscopy of salivary gland viruses)

PLATE 51

FIG. 4. Section of a fibroblast from a culture infected with a human strain of the salivary gland virus 10 days previously. A dense inclusion is present in the center of the nucleus. Surrounding the densely packed, dark particles and chromatin material forming the inclusion is a pale zone, the halo of light microscopy. Occasional particles are present in the cytoplasm at the lower right of the nucleus. This nucleus, when examined in adjacent sections by phase and light microscopy contains the type of inclusion distinctive for the salivary gland inclusion virus. × 8,500.

FIG. 5. Electron micrograph of portion of a fibroblast infected by the human strain of inclusion virus. A portion of the nucleus is present centrally. At the right margin there is a deep indentation of the nucleus by a narrow neck of cytoplasm. At the left, indicated by an arrow, is a membrane-surrounded collection of cytoplasmic particles within the nucleus. A similar particle between the outer and inner nuclear membranes is present at the left margin of this group. Within the nucleus there is what seems to be the beginning of an early nuclear inclusion formed by condensed chromatin and virus particles. × 18,000.

FIG. 6. Electron micrograph of a portion of a fibroblast infected with the human strain of salivary gland virus. The nucleus is similar to that of the cell shown in Fig. 5. Nuclear forms suggesting virus are scant. At the lower margin of the nucleus there are two sac-like forms present within the nucleoplasm. These membrane-surrounded spaces are in contact with the nuclear membrane. Centrally and at the left of the field are 2 other similar regions, set off from the nucleoplasm by membranes. Particles are evident in the 2 centrally placed foci. It is probable that at another level these also are in contact with the nuclear membrane and that their contents are of cytoplasmic origin. × 8,500.

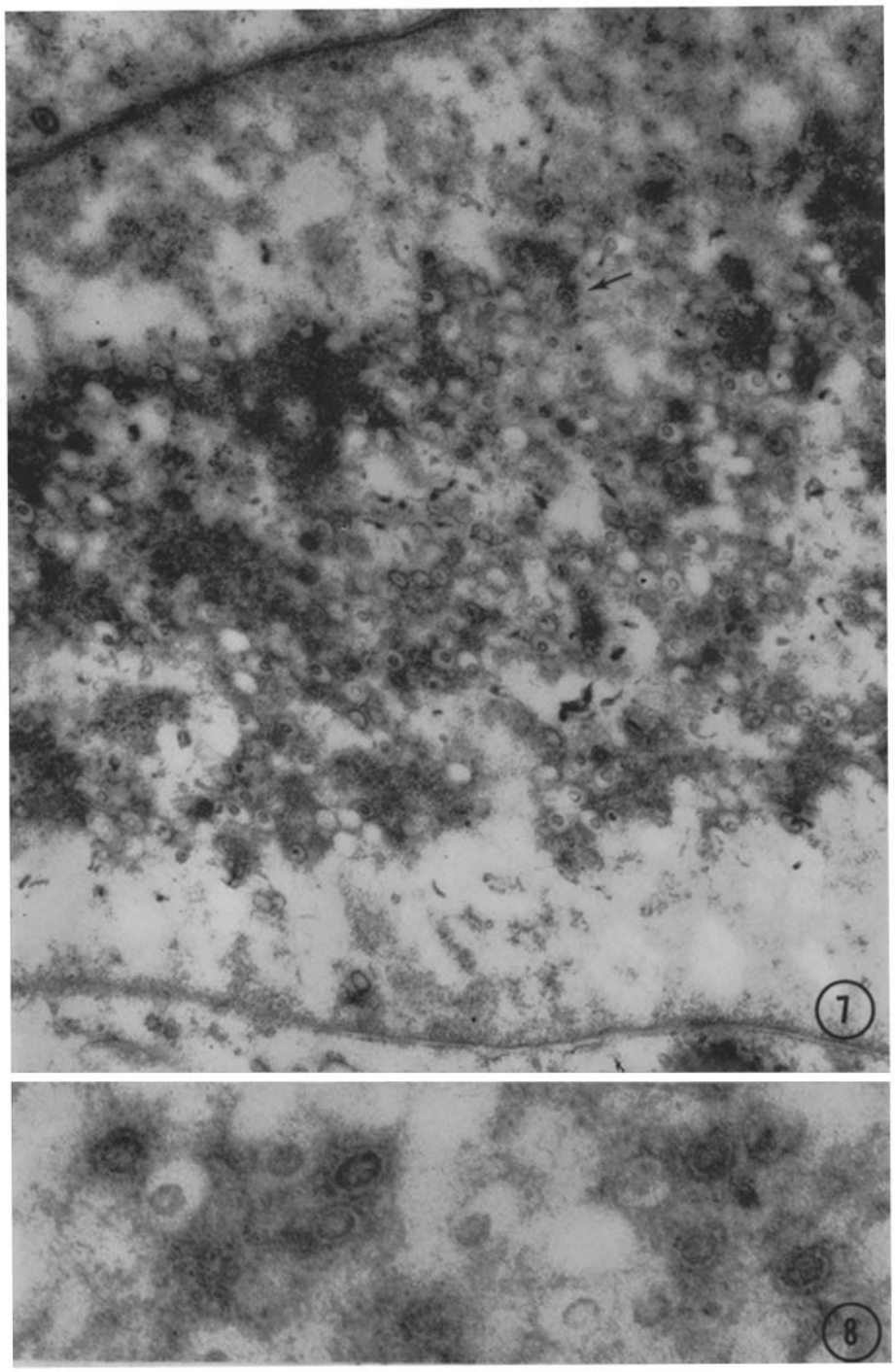


(Luse and Smith: Electron microscopy of salivary gland viruses)

PLATE 52

FIG. 7. Electron micrograph of a portion of nuclear inclusion in another infected fibroblast. The double nuclear membrane is visible at both the upper and lower margins of the field. Within the nuclear membranes there is a pale zone which represents the halo of light microscopy. Centrally the inclusion is made up of particles, one of which is indicated by an arrow, and of condensed chromatin. The particles are composed of a central dark spot about 40 mμ in diameter. This central dark body is surrounded by a pale zone and externally by a thin dark membranous shell. × 30,000.

FIG. 8. Higher magnification of particles in the nuclear inclusion of cell illustrated in Fig. 7. × 60,000.

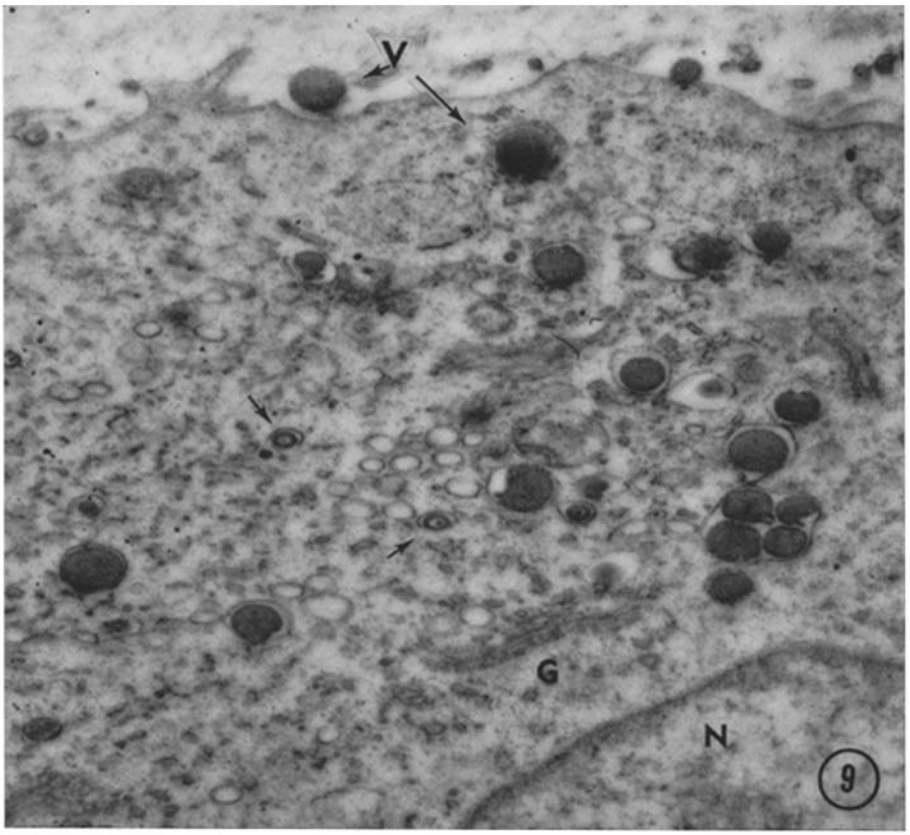


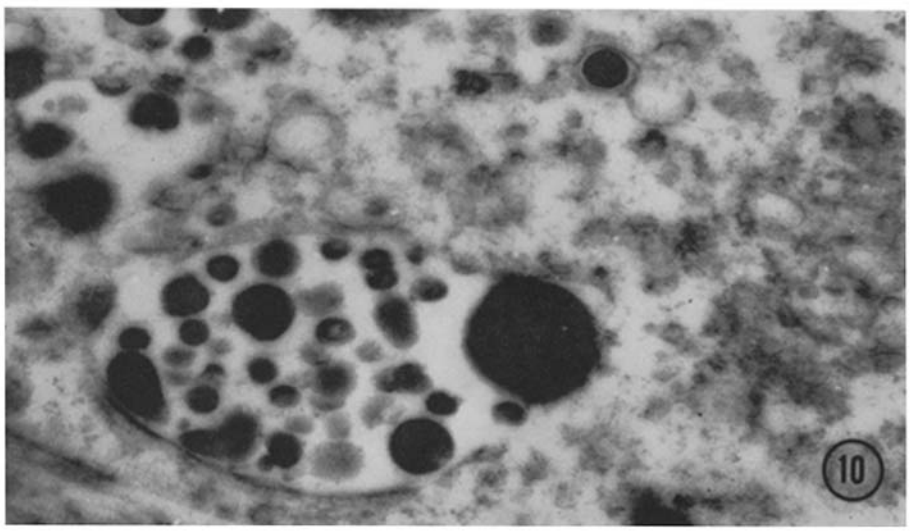
(Luse and Smith: Electron microscopy of salivary gland viruses)

PLATE 53

FIG. 9. Electron micrograph of cytoplasm of infected fibroblast. A portion of nucleus (*N*) is visible at the lower right. The plasma membrane of the cell surrounds several irregular cytoplasmic projections at the upper portion of the figure. An occasional particle (*V*) is present extracellularly. Within the cytoplasm are numerous smooth flattened membrane-surrounded spaces, the Golgi complex (*G*). Also present is a finely granular component associated with some small membrane-surrounded spaces. Mitochondria are absent except for a single questionable form. Multiple large (350 to 500 mμ) spherical abnormal particles (*V*) are present in the cytoplasm. They are all membrane-surrounded. More than one particle is noted within some of the membranes. At the arrows are abnormal forms of another type: target-like forms with a central dense dot surrounded by a pale zone, with a dense shell beyond it, the whole formation being within a membrane. × 24,000.

FIG. 10. Electron micrograph of part of the cytoplasm of an infected fibroblast. The cytoplasmic membrane is at the lower left. A large vesicular structure is present and contains numerous particles varying from a sphere of about 500 mμ to smaller target-like forms. Several single membrane-surrounded particles are present in the cytoplasm. × 25,000.





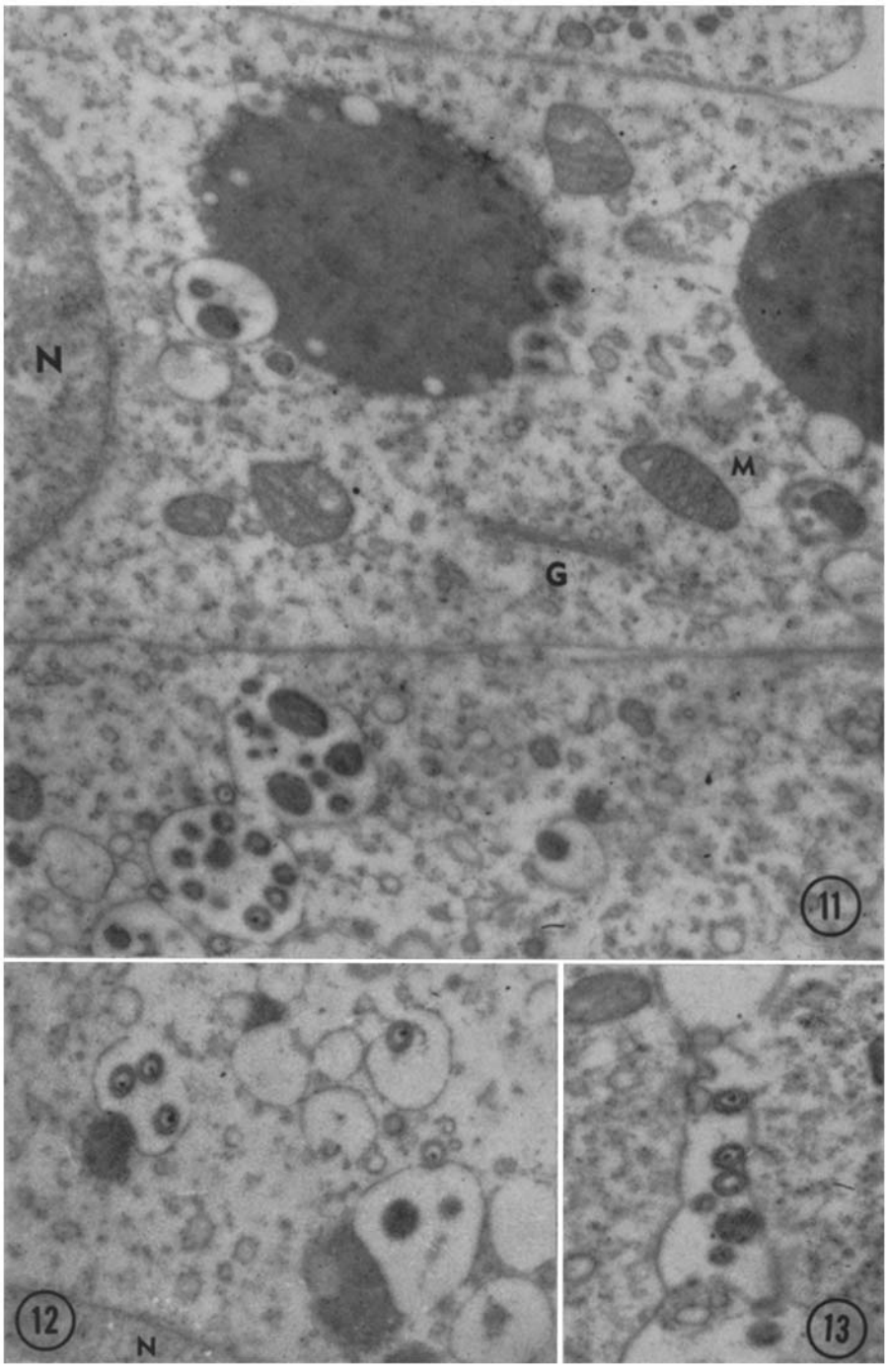
(Luse and Smith: Electron microscopy of salivary gland viruses)

PLATE 54

FIG. 11. Micrograph showing a portion of two fibroblasts infected with the human strain of virus. Their limiting plasma membranes extend across the field horizontally. The nucleus of one cell is at the left (*N*). A large solid body, over 2 μ in greatest diameter, is present centrally. Small and large blebs occur at its margin and particles are present within the blebs. Compare with Fig. 12. Several mitochondria (*M*) and Golgi membranes (*G*) are scattered in the cytoplasm. In the lower cell there are several membrane-surrounded vesicles containing particles. These may have originated as similar vesicles to those present on the surface of the large body in the upper cell. × 28,000.

FIG. 12. Part of another infected fibroblast in which the nucleus (*N*) is at the lower left. Several vesicles containing target-like forms are present within the cytoplasm. They are associated with similar but smaller masses of dense material as compared with that present in the large dense body in Fig. 11. × 30,000.

FIG. 13. Part of two infected fibroblasts and their intermediate extracellular space. Several target-like particles are present in the extracellular space. These extracellularly located particles are not surrounded by an external membrane. × 25,000.



(Luse and Smith: Electron microscopy of salivary gland viruses)

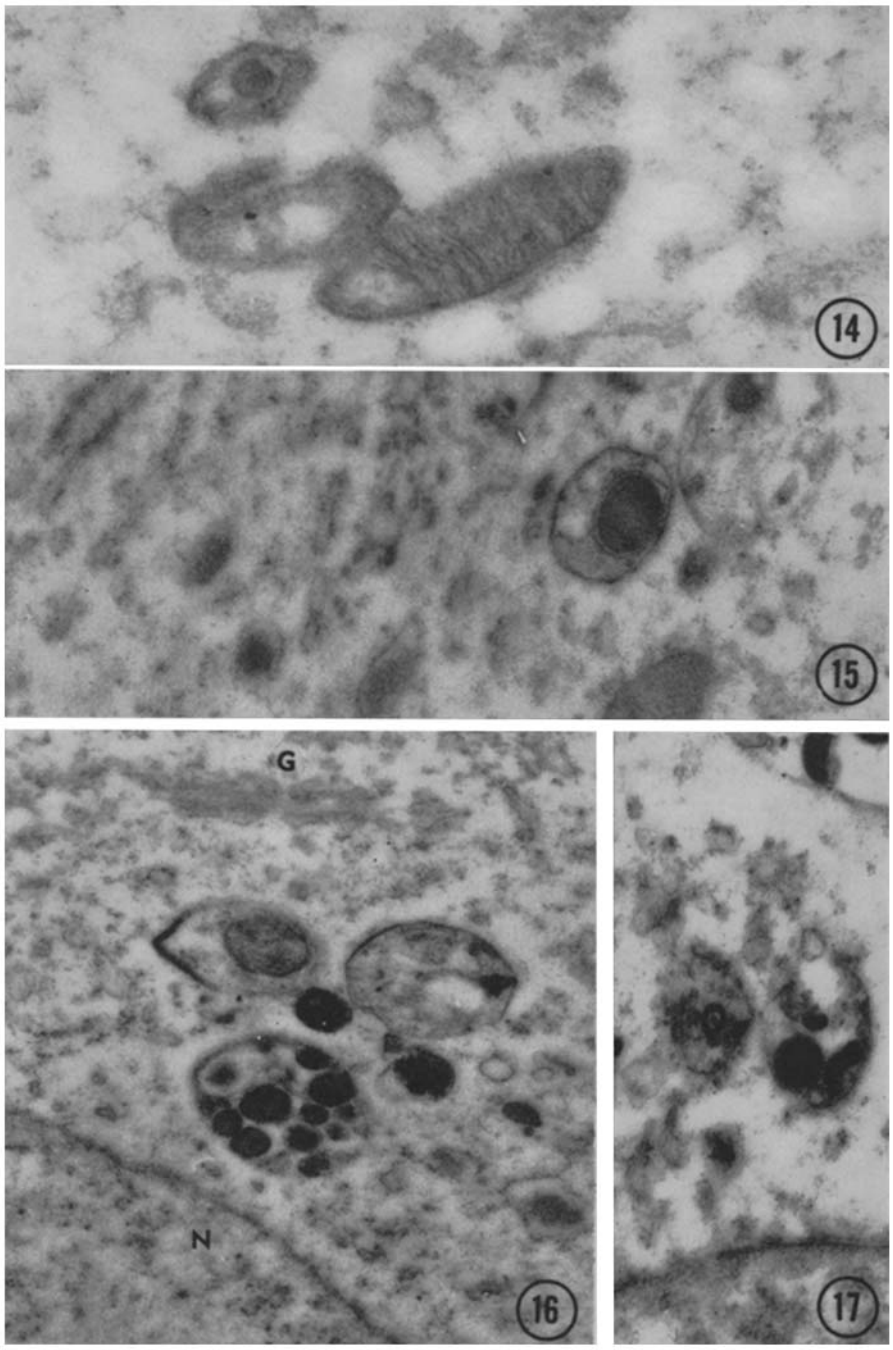
PLATE 55

FIG. 14. Portion of cytoplasm of infected fibroblast. At the lower center of the field is a mitochondrion with distinct inner cristae. At its lower left margin is a vacuole. The central form is similar to the mitochondrion. It has an external double membrane and possible internal cristae deformed by a large vacuole. To the upper left is another similar but smaller structure composed of an outer double membrane, and containing a central vacuole within which is a dense homogeneous round particle similar to the particles seen elsewhere. × 40,000.

FIG. 15. Portion of the cytoplasm of infected fibroblast. Ergastoplasmic membranes with a fine external granular component are at the left of the field. To the right is a spherical structure surrounded by a dense membrane. Within it there are vacuoles and a homogeneous dense material, and centrally another membrane within which is a sphere identical with the large spherical cytoplasmic particles seen in other cells. At the upper right is another faintly outlined structure, the left margin of which is composed of a double membrane. × 25,000.

FIG. 16. Part of an infected fibroblast. The nucleus of the cell (*N*) is present at the lower left. At the upper margin of the figure are flattened membranes of the Golgi complex (*G*). Centrally there are 3 structures, the lower central one of which contains definite virus-like particles. The upper right structure is partially surrounded by a double membrane. The significance and origin of these forms are not known. × 25,000.

FIG. 17. Section of an infected fibroblast. The nucleus is at the lower margin of the field. Two structures are present in the mid-portion of the field, the one to the left is made up of numerous small vesicles covered by a smooth membrane and reminiscent of Golgi membranes. However, another membrane seems to partially surround these small vesicles and centrally there is a possible target-like particle. The structure on the right contains similar vesicles, definite particles, and a tenuous surrounding membrane. × 25,000.



(Luse and Smith: Electron microscopy of salivary gland viruses)

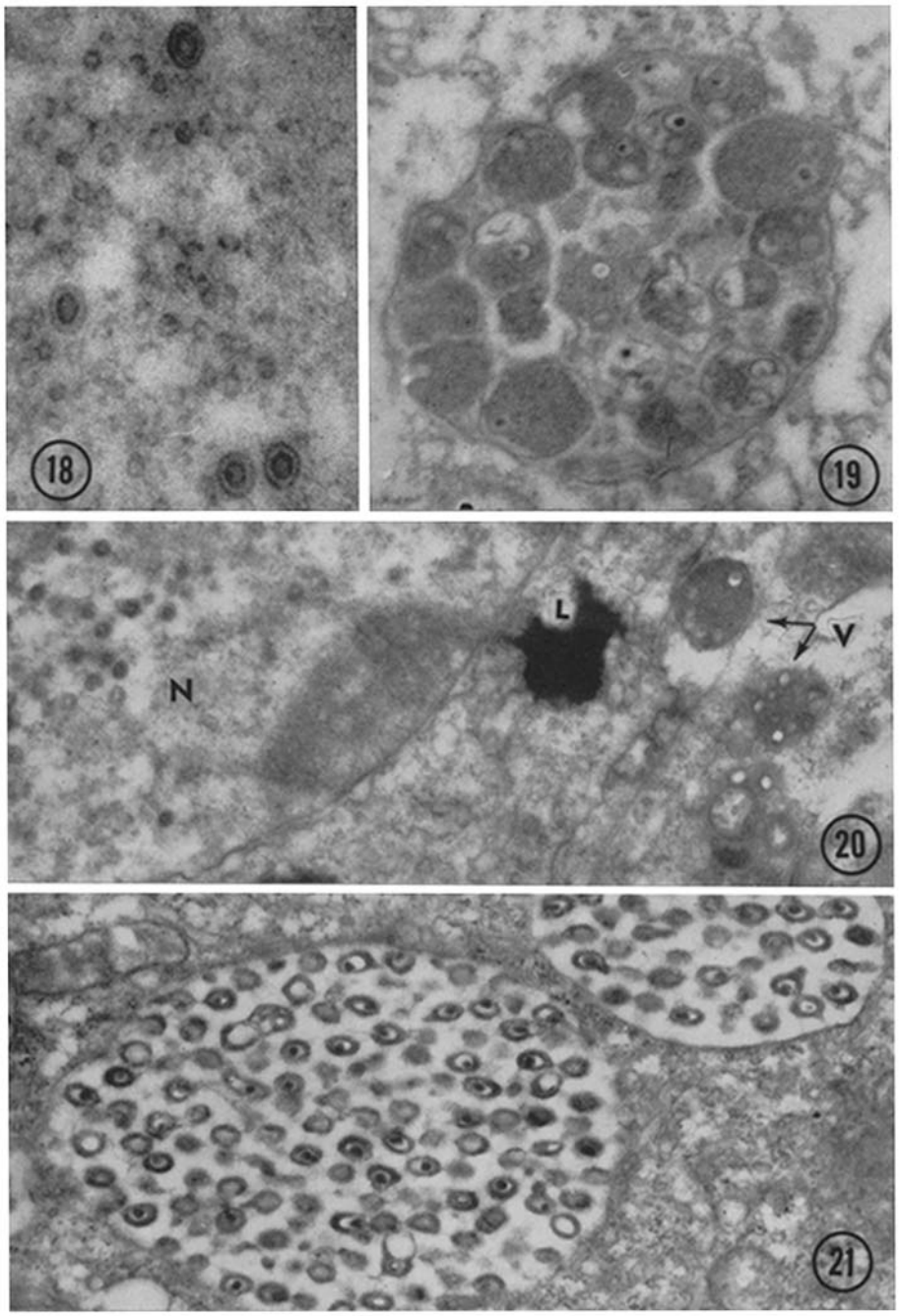
PLATE 56

FIG. 18. An electron micrograph of part of a nuclear inclusion in a splenic reticular cell infected by the mouse strain of the salivary gland virus. The nuclear virus-like particles are clearly demonstrated and are similar to those seen in the human strain, as illustrated in Figs. 6 and 7. × 55,000.

FIG. 19. Electron micrograph of a membrane-surrounded sac of spherical forms of the mouse salivary gland virus. This sac is present in the cytoplasm of a splenic reticular cell. The individual spheres measure 150 to 430 mμ in diameter and are membrane-surrounded. Within some of the spheres are smaller forms with a dense central dot, similar to the target-like forms of the cytoplasm. × 25,000.

FIG. 20. Electron micrograph of a part of an infected splenic reticular cell from a mouse injected three days previously with a murine strain of the salivary gland virus. The nucleus (*N*) is at the left of the field and contains nuclear abnormal particles at its left margin. A large lipid inclusion (*L*) is present centrally. At the right are several single spherical forms (*V*) similar to those seen in Fig. 19 and which also contain pale central zones. × 25,000.

FIG. 21. Electron micrograph of a portion of the cytoplasm of a salivary gland cell infected with the mouse strain of salivary gland virus. This mouse was injected intraperitoneally 2 weeks prior to examination. In the cytoplasm there are many nests of target-like particles thought to represent the mature form of the virus. × 25,000.



(Luse and Smith: Electron microscopy of salivary gland viruses)