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Structure of Meningopneumonitis Virus Particles

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SUMMARY

The structure of purified extracellular meningopneumonitis virus particles liberated in a one step growth cycle was studied in electron microscopy, by shadowcasting, negative staining technique and ultrathin sections. The virus particle preparation has two distinct morphological forms, one a small dense-centered particle and the other a large, reticulated particle. The former looked like a "wrinkled pea with one flattened side" by shadowing, and had an unusual appearance by the negative staining technique.

Ultrathin sections or negative staining technique showed the fine inner structure of both forms of the meningopneumonitis virus. The dense-centered particle has a nucleoidal apparatus, which, in some particles, contains a number of nucleolus-like bodies, surrounded by a dense reticular region and often also by a paranucleoidal region inside the reticular region. Among these particles, there are observed morphological variations of MPV particles to be considered to represent differences in the stage of development.

The large, reticulated particle consists of a reticular system, a strand of which is approximately 30 - 40 A thickness, surrounding a nucleoid. In a ruptured particle helical structures 80-110 A side, can be seen, with a periodicity of 40-60 A. Both particles have an outer double 140-300 A width, each layer being 30-60 A thick.

INTRODUCTION

Meningopneumonitis virus (MPV) is a typical member of the psittacosis-lymphogranuloma group ranking between virus and rickettsia. Mary workers have found from electron micrographs that this group of viruses develop from the initial body to the elementary body in the intracellular growth cycle through intermediate stages (Gaylord, 1954; Tajima *et al.*, 1957; Mitsui *et al.*, 1958; Higashi, 1959; Higashi *et al.*, 1959; Letwin, 1959; Letwin *et al.*, 1961) and exist in two different forms, possibly representing mature and immature, even in the preparations of the elementary body (Rake *et al.*, 1946; Rake, 1947; Kurotchkin *et al.*, 1947; Heinmets and Golub, 1948; Moulder, 1954; Crocker, 1954; Sharp, 1960). However these elementary bodies were not obtained under one step growth cycle in a tissue culture.

We examined the structure of virus particles in an elementary body preparation of MPV, obtained under one step growth in L cells and confirmed the existence of different forms of MPV particles and studied their fine structure.

This paper describes these observations.

MATERIALS AND METHODS

1. *Virus*

Meningopneumonitis Virus (MPV), California 10 strain, adapted to L cells, kindly given by Drs. Higashi and Tamura (Inst. for Virus Research, Kyoto Univ.) was used throughout.

The virus titer was measured as the $TCID_{50}$ in L cells.

2. *Cells*

Strain L cells were used. 70 ml of suspension containing 5×10^6 cells was inoculated into a 1.5 l bottles and cultivated for 4 days as a monolayer (1.5×10^7 cells). The growth medium was Y.L.H. (0.1% yeast extract, 0.5% lactoalbumin hydrolysate in Hanks' solution), containing 5% bovine serum, free from penicillin.

3. *Purification of MPV*

L cell monolayers were inoculated with MPV by a multiplicity of approximately 10, adsorbed for 2 hours, then washed 3 times with PBS and added by 70 ml of maintenance medium. The maintenance medium was YLH containing 2% bovine serum, free from penicillin.

After 60 hours incubation at 37°C, the infected fluids were harvested (10^9 $TCID_{50}$ /bottle), clarified by centrifugation at 2,500 rpm for 10 minutes and then recentrifuged at 10,000 rpm for 20 minutes. Differential centrifugation was repeated and the resulting pellets were resuspended before electron microscopy.

4. *Electron microscopy*

A Hitachi HU 9D type electron microscope was used.

1) *Shadowing*

The purified MPV suspension in 0.85% NaCl was placed on a carbon-collodion grid and observed after shadowing with uranium.

2) *Negative staining*

The purified MPV suspension in distilled water was mixed with an equal volume of 2% phosphotungstic acid (PTA) adjusted to pH 7.0 with N-KOH, and placed on a carbon-collodion grid (Hosaka *et al.*, 1961) for observation.

3) *Ultrathin sections*

A part of the final suspension was centrifuged at 10,000 rpm for 30 minutes. The pellets were fixed for 60 minutes in a 1% osmium tetroxide (veronal-acetate buffer pH 7.4), dehydrated in graded ethanol and embedded in Epon 812 resin (Gibbons, 1958). Sections were cut with Porter-Blum ultramicrotome, stained for 30 minutes with 1% uranium acetate in 75% ethanol and examined in the electron microscope.

RESULTS

1. *MPV observed by shadowing*

Fig. 1 shows particles of MPV seen by shadowing. Two distinct morphological forms can be seen: one a particle of 400 - 550 mμ with a central mass surrounded by a peripheral rim and another a larger flat particle of 500 - 900 mμ of relatively homogeneous appearance.

The former has a height of about 200 mμ and the latter less than 100 mμ as judged from the shadow length. The former looks like a "wrinkled pea with one flattened side" as described by Rake *et al.* (1946).

Both types of particle have an uneven surface, respectively.

2. *MPV particles observed by negative staining technique*

Fig. 2 shows particles seen by negative staining. In this case also, two forms of particles can be seen, one small type of particle of 300 - 450 mμ having an irregular shaped dark PTA area and showing no visible inner structure and another larger type of particle of 500 - 800 mμ often with a crevice-like PTA area and an inner reticular structure. Some of the former particles showed a peripheral rim, in the photograph (Fig. 3-1). Possibly PTA penetrated easily over the peripheral rim, which was observed clearly under shadowing. This idea is supported by the fact that the size of this type of particle under PTA approximately corresponded to that the central mass of the first type of particle under shadowing.

It is improbable that the irregular shapes of PTA dark areas represent spaces corresponding shapes within the particles, because ultrathin sections of the MPV particles (Fig. 4) did not show such spaces. Possibly the irregular shapes of the PTA areas within the particles indicated PTA in uneven depressions formed on the surface of the MPV particles, while they were drying on the grid.

The appearance of the particles under PTA was compared with that seen by shadowing, and pairs of similar appearance could be selected, as shown in Fig. 3 (left: negative staining; right: shadowing). Therefore it seems certain that the irregular shape of the PTA areas within the MPV particles reflects unevenesses in the surface of the particles. Thus the two types of particle seen under PTA could correspond to the two types of the particle seen under shadowing.

Further, as shown in Fig. 3-8, from the PTA penetration over the peripheral rims of the particles, MPV particles usually exist separately, although the particles often appear to be connected when seen under shadowing.

3. *Ultrathin sections of MPV*

Figs. 4 and 5 show ultrathin sections of MPV. Two forms of particle can be seen; one is a dense-centered form of 350 - 480 mμ diameter and the other a large, reticulated form of 400 - 800 mμ diameter with or without a mucleoid of varying size (the peripherally sectioned particles were excluded). The dense-centered particle has a limiting membrane in a low contrast which is possibly a sac or envelope and contains a diffuse material. The duffuse material surrounds an irregular shaped inner membrane, 140 - 200 A thick, which is shown in Fig. 7 to consist of two layers, 30 - 50 A thick. The area, enclosed by the inner membrane, is 280 - 420 A across. The large, reticulated particle also has a membrane, 150 - 300 A thick composed of two layers.

As described above, two types of particles differing in size and structure can be seen by all 3 techniques. The two types of particles seen by the 3 techniques seems to correspond one another, one is a small particle with a central mass seen by shadowing, a small particle showing no visible inner structure under PTA and

a small dense-centered particle in section. The other type of particle is a large flat particle when seen by shadowing and a large reticulated particle when studied by negative staining or in section. From electronmicroscopical studies on the intracellular growth of the psittacosis group viruses, the large reticulated particles seem to develop into the dense-centered particles (Gaylord, 1954; Tajima *et al.*, 1957; Mitsui *et al.*, 1958; Higashi, 1959; Higashi *et al.*, Litwin *et al.*, 1961). There fore it seems no doubt that the former particles observed in the present experiments are a mature form and the latter particles, an immature from. In the purified MPV preparations used, the ratio of the large reticulated to the dense-centered particles was 4 - 8 to 50.

4. *Fine inner structure of the dense-centered particles*

Since PTA did not penetrate to the inside of the smaller compact particles their inner strudture could only be seen in ultrathin sections.

At a high magnification a nucleoidal apparatus could be seen in sections of the dense-centered particles (particles 1 and 2 in Figs. 6 and 7). The nucleoidal apparatus, approximately 1,000 A wide, contains a number of nucleolus-like bodies of 200 - 300 A diameter (particle 1), and is surrounded with a dense reticular substance (viroplasm). In other particles (particle 2), the nucleoidal apparatus has not so fine a structure but instead is surrounded by a paranuclear region of a low density. In the larger particles (particle 3), the paranuclear region is the clearer and the nucleoidal apparatus and the viroplasm the more diffuse. These morphological variations in the MPV particles (3-1) seem to indicate the development of the particles, judging from the size of the particles and the compactness and systematicity of their structure. The reticular strand in the viroplasm is observed of thickness of 80-100 A.

5. *Fine inner structure of the large reticulated particles*

Fig. 8 shows the fine structure of a large, reticulated particle seen by negative staining. A transverse crevis in the middle of the particle can be seen which is considered by comparison with the shadowed particles shown in Fig. 3 to indicate a depression on the surface. The double membrane 150 - 200 A thick, composed of two layers 40 -70 A thick, surrounding the crevis is possibly the membrane which covers the surface of thc particles. Within the particles is seen a reticular system of strands, 30 - 40 A thick. A nucleoid of 1,000 - 1,500 A width is present near the surface.

In a ruptured particle, strands, approximately 15 A thick, are coiled reticularly (Fig. 9). Often the strands appear a helical array 80 - 110 A side, with a periodicity of 40 - 60 A. In the middle of the particle, a nucleoid appears to be ruptured. It also consists of reticularly coiled strands. In some places reguelarly segmented strands of approximately 50 A width can be seen, which may be of helical array, in the nucleoid.

DISCUSSION

Purified MPV liberated extracellularly under one step growth condition was shown to consist of two distinct morphological forms of particles. This is consistent with the results of Higashi and Tamura (1962) and other data not obtained under one step growth conditions (Rake *et al.*, 1946; Kurotchkin *et al.*, 1947; Heinmets and Goloub, 1948; Moulder, 1954; Crocker, 1954; Sharp, 1960).

Our opinion of how the "wrinkled pea with one flattened side" or "Derby hat" appearance (Rake *et al.*, 1946) of the dense-centered particle seen by shadowing could be produced, is illustrated in Fig. 10.

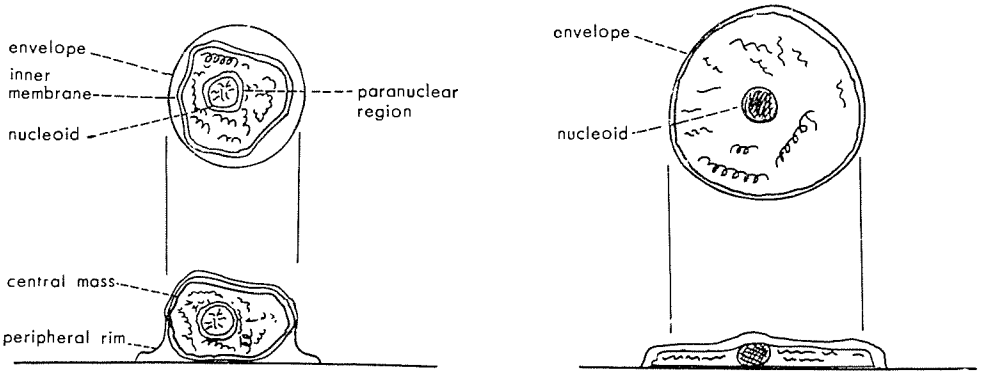

Fig. 10. The Alteration of the Two Forms of MPV during Drying on the Grid
left: the dense-centered particle
right: the large, reticulated particle

Fig. 10 shows how a dense-centered particle can produce a peripheral rim and a central mass on drying on a grid and thus a little increase the total width of the particle when seen by shadowing. Similarly a large, reticulated particle could become slightly larger when dried on a grid. This explanation is in principle similar to that of Rake *et al.* (1946). Thus it seems reasonable to consider that there is a jelly-like substance between the envelope and the inner membrane

Table 1. Comparison of the Size of MPV Particles Observed by 3 Techniques

Form of particle	Shadow	Negative staining	Section
dense-centered particle	400 - 550 mμ central mass 300 - 450	300 - 450 mμ	350 - 480 mμ inner membrane-area 280 - 420
large, reticulated particle	500 - 900	450 - 800	400 - 700

and the wrinkling of the surface reflects irregularities in the inner membrane, rather than only an artifact caused by drying. This is consistent with an earlier inferential interpretation of Heinmets and Goloub (1948).

The apparent size of the MPV particles varied a little with the technique employed, as shown in Table 1. This size variation can be explained by the norphological alterations, as illustrated in Fig. 10 occurring while the particles are drying on the grids. The size of the particles in the sections which were not dried on a grid is probable the nearest to the size of the particle in nature, because it corresponds approximately to the true size expected from the illustration in Fig. 10. Distortion of the structure of viral material (Epstein, 1962) and of cellular material is much less when the material is embedded in epon 812 than in methacrylate. In the present work, material was embedded in epon. An inner membrane within the envelope and a series of MPV particles differing in appearance of the nucleoidal apparatus could clearly be seen. The latter seem to represent the development of the nucleoidal apparatus.

The size (80-100 A) of the reticular fiber in the dense-centered particle in section (Fig. 6, 7) was different from that (30-40 A) in the large, reticulated particle under PTA (Fig. 8). At present the significance of this difference is not certain.

It is interesting that a large MPV virus contains a regular helical structure within the particle, as shown in Fig. 9. It is not yet known whether this structure is of DNA or RNA-line. Possibly the helical structure constitutes the viroplasm of MPV.

The nucleoidal apparatus seen in the present experiments corresponds to the "central star" of Trachoma virus described by Mitsui *et al.* (1958).

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EXPLANATION OF PHOTOGRAPHS

Fig. 1. MPV particles under shadow
A. small particle with a central mass surrounded by a peripheral rim
B. large flat particles of relatively homogeneous appearance

Fig. 2. MPV particles by negative staining
A. small compact particle showing no visible inner structure
B. large, reticulated particle

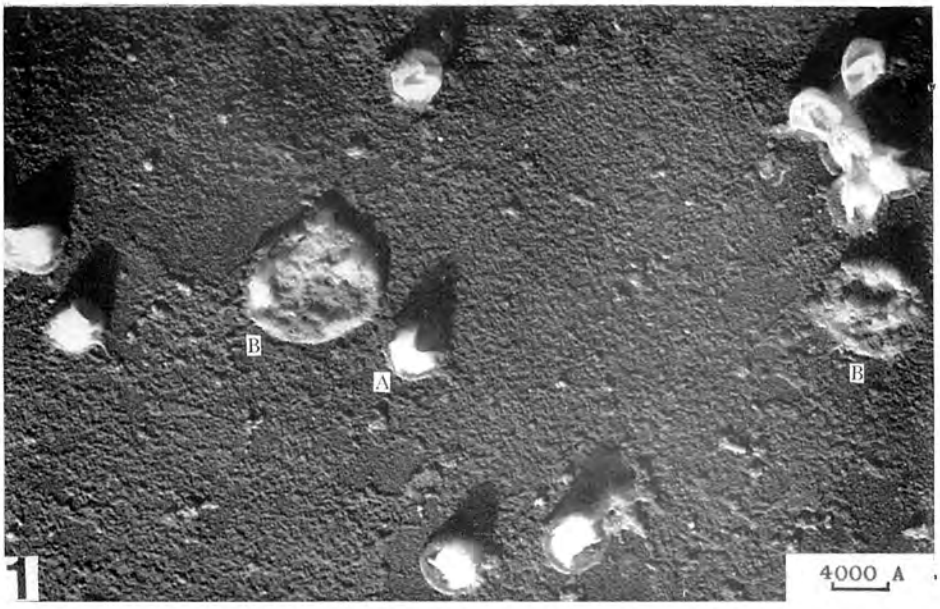
Fig. 3. Comparison of MPV particles seen by negative staining with those seen by shadowing (left: negative staining, right: shadowing)
Shadowed particles were photographed directly from the negative plates, to show more clearly a fine uneveness on the surface of the particles
1 - 6, dense-centered particles
7, large, reticulated particle
8, by negative staining MPV particles are seen to be relatively separate (left) but are fused with each other when seen by shadowing (right)

Fig. 4,5. MPV particles in ultrathin section
A. dense-centered particle
B. large, reticulated particle with or without a nucleoid
In upper left in Fig. 4, a double membrane (M) of a large, reticulated particle can be seen. Long arrows indicate the inner membrane and short arrows, the envelope.

Fig. 6,7. MPV particles in section at higher magnification
Particles 3 to 1, show the change in the nucleoidal apparatus (N) from a diffuse form to a compact form.
The compact nucleoidal apparatus (particle 1) contains a number of nucleolus-like bodies. Within the envelope, a double membrane can be seen.

Fig. 8. A large, reticulated particle of MPV seen by negative staining
The reticular system consists of strands, approximately 30 A thick.
The middle transverse crevis seems due to a depression on the surface of the particle. The double surface membrane are clearly shown.

Fig. 9. A ruptured MPV particle
Within the particle, helical structures (H) can be seen. The helix has a width of 80 - 110 A and a periodicity of 40 - 60 A. Further in the ruptured nucleoid (N), a reticular system can be seen and in some places, segmented strands (S).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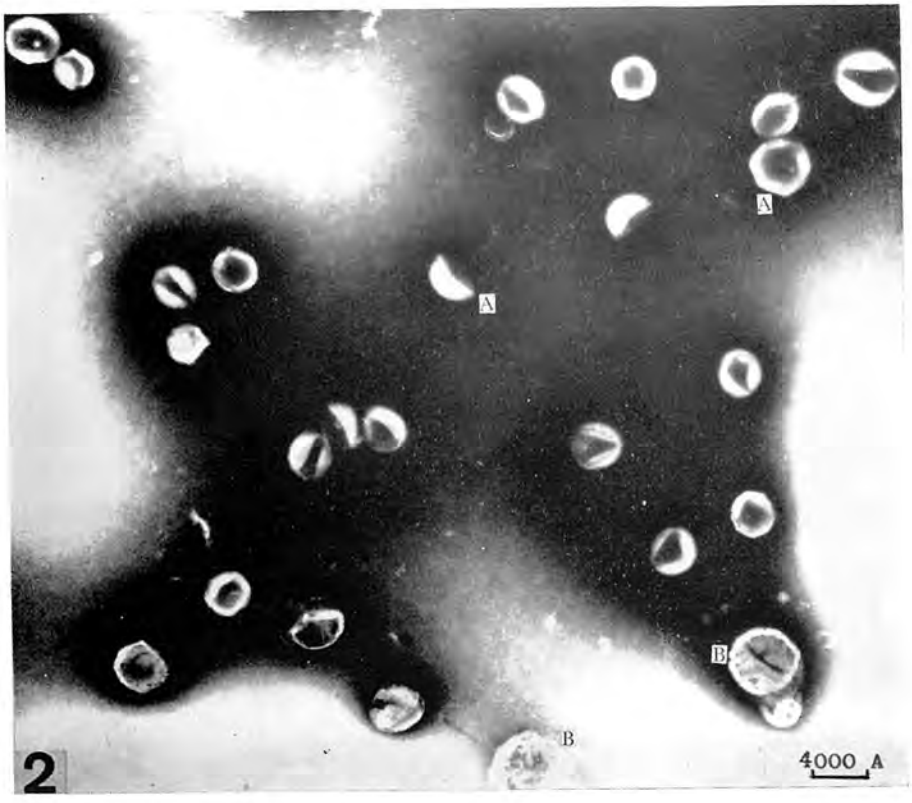
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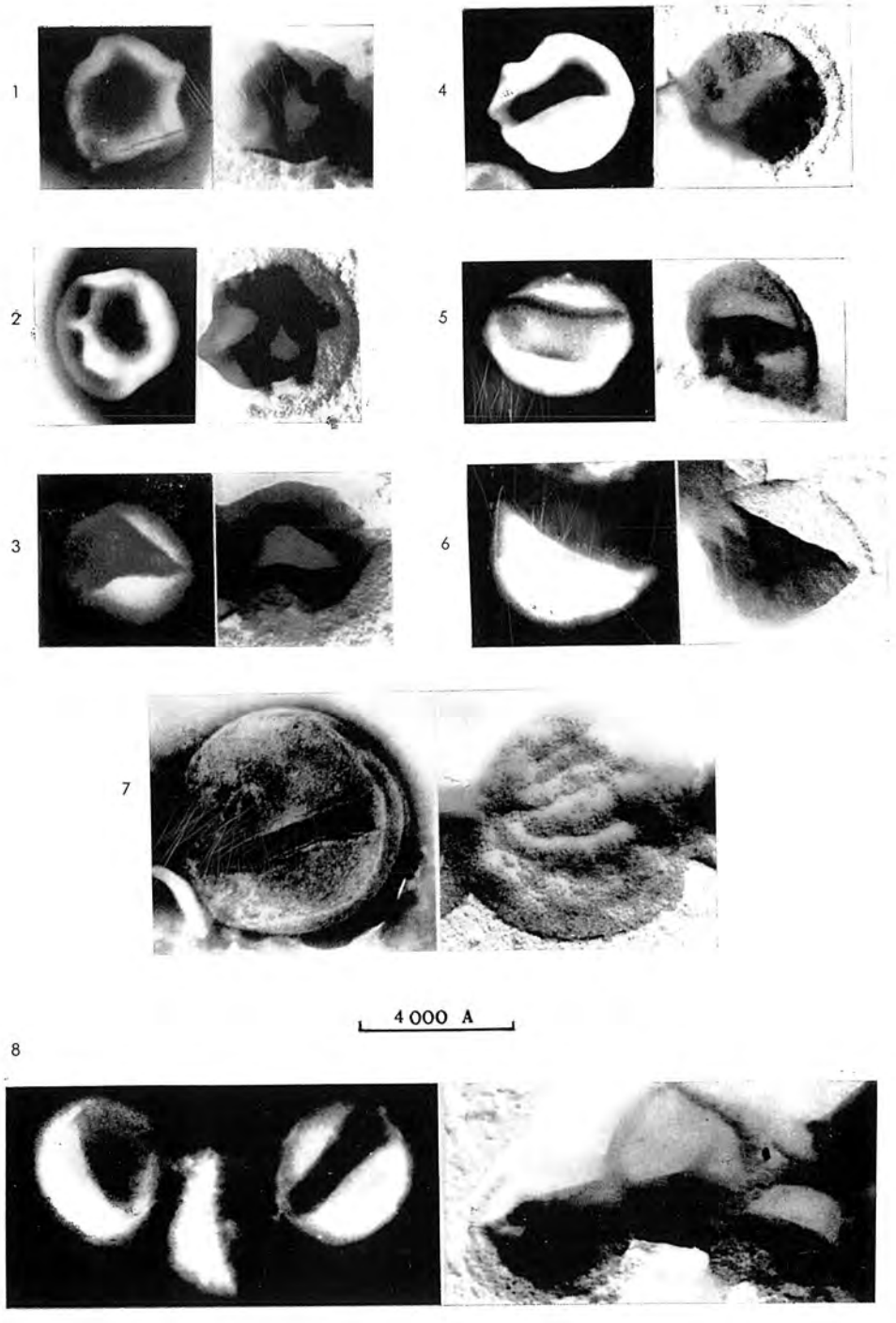
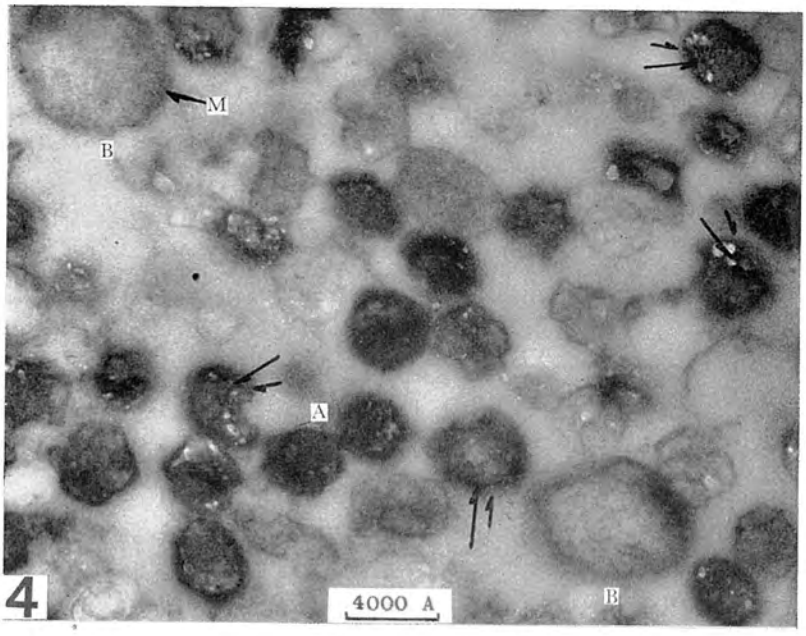


Fig. 3.



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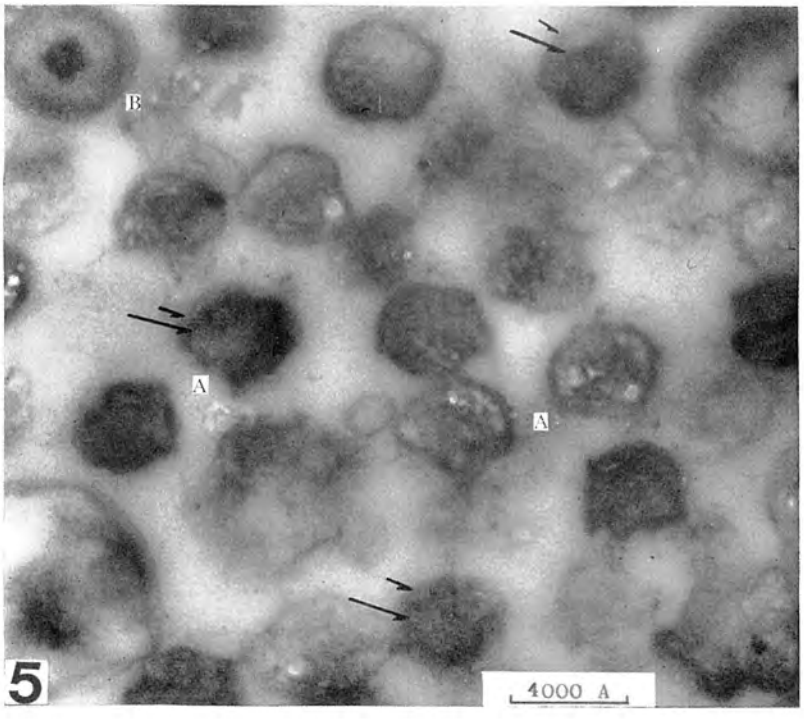

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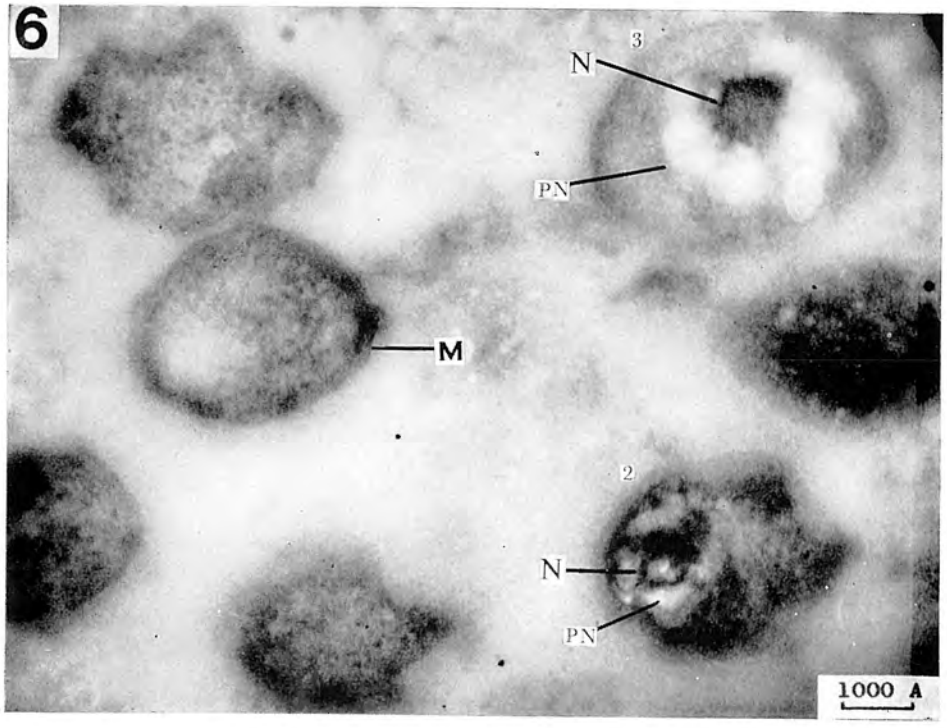
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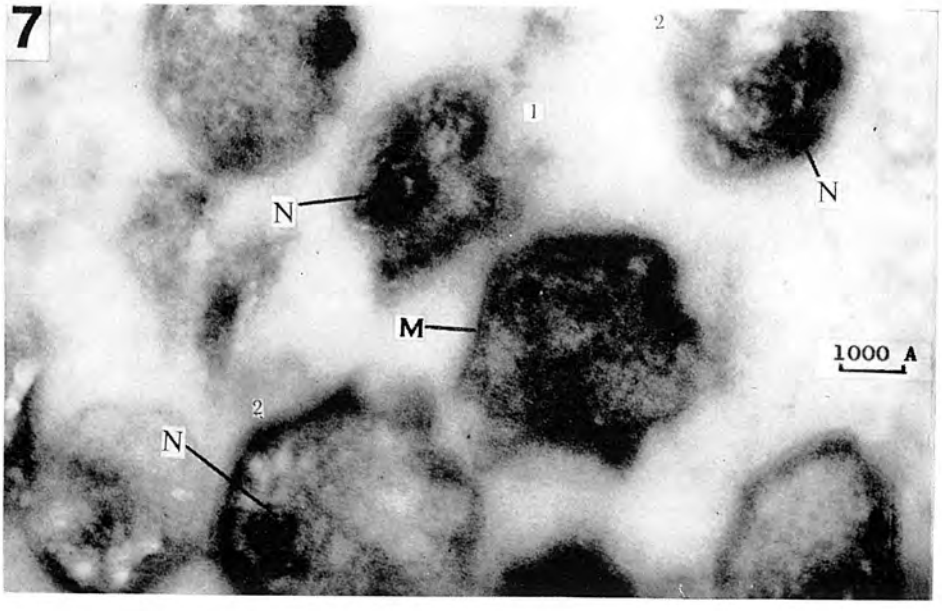
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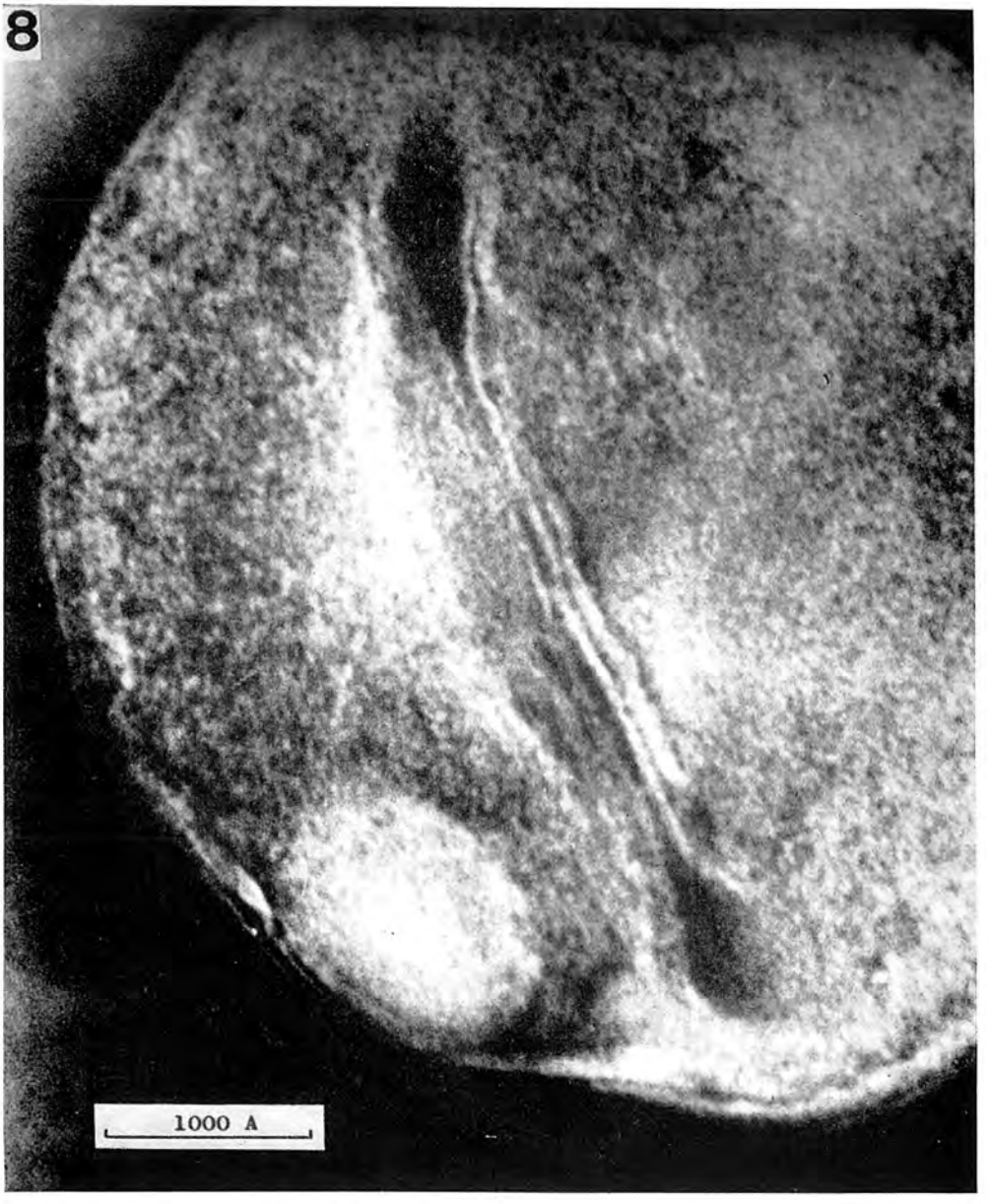
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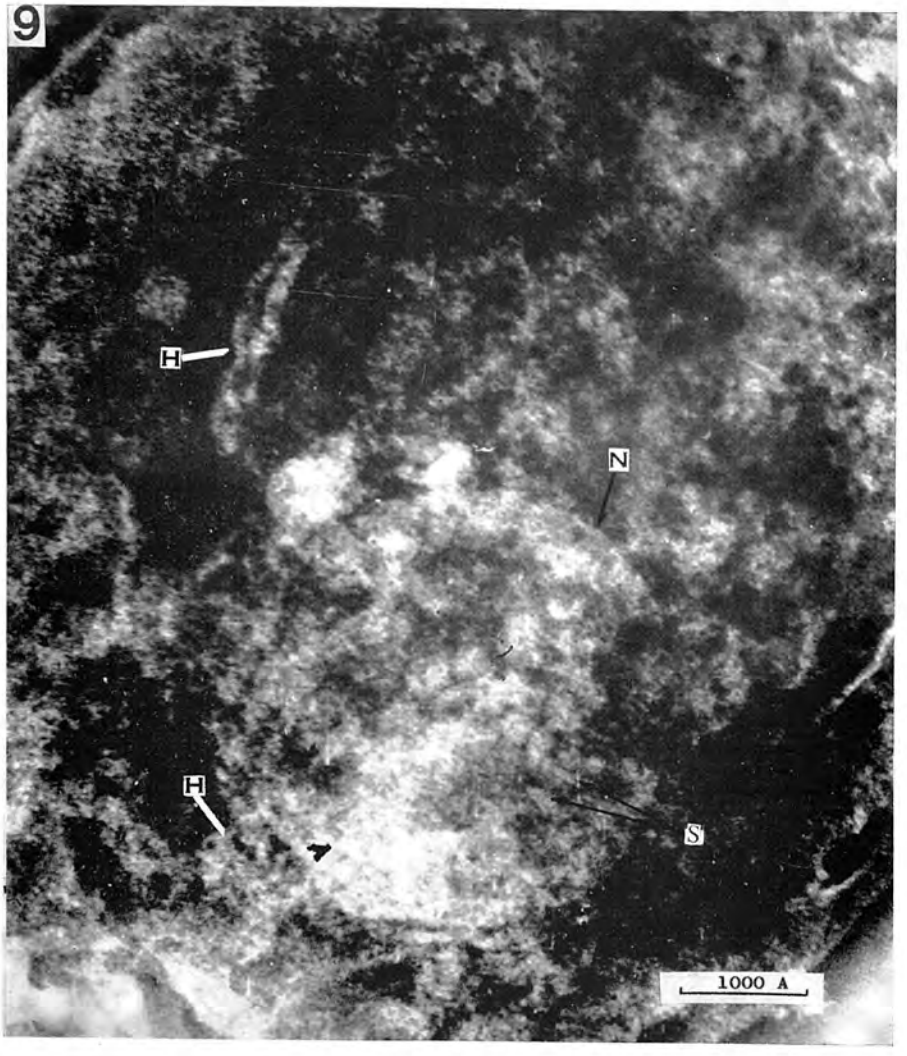
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