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Intranuclear Virus-like Bodies in the Amoeboflagellate *Naegleria gruberi**

FREDERICK L. SCHUSTER

Dept. of Biology, Brooklyn College, Brooklyn, N. Y. 11210

SYNOPSIS. Intranuclear virus-like bodies were seen in cultures of the EG strain of *Naegleria gruberi* from soil. Introduction of these virus-like particles into cultures seemed to coincide with use of chicken embryo extract as a supplement in culture media used to maintain axenic amoebae in the laboratory; the appearance of the virus-like units is triggered by transfer of axenic lines of *N. gruberi* EG into monobacterial culture medium. The particles, ~ 100 nm in diameter, are mainly restricted to the nucleus of the cell. Passage of particles from the nucleoplasm into the cytoplasm is suggested by their association with tubular projections from the nuclear membrane, and particles have been seen in the cytoplasm of the amoebae. The virus-like bodies resemble reovirus.

NAEGLERIA GRUBERI is a soil protozoon with an amoeba-to-flagellate transformation during its life cycle (4-6, 10). While studying this transformation with the electron microscope, virus-like bodies were found in the nuclei of one of the strains. Designated EG (after its site of isolation, the Eucalyptus Grove of the University of California at Berkeley), this strain has been cultivated for ~ 8 years. It had been examined at times in the electron microscope without evidence of virus-like particles. As noted later, appearance of the particles coincided with the use of chicken embryo extract in the culture medium.

This preliminary report describes these virus-like units along with some attempts to trace their origin.

MATERIAL AND METHODS

Strain EG was maintained axenically in a yeast-peptone-liver medium supplemented with heat-killed *Aerobacter aerogenes* (10) or, more recently, chicken embryo extract (11). Monobacterial cultures were established by transferring amoebae from axenic lines onto peptone-yeast-glucose agar plates (1) in the presence of living *A. aerogenes*.

For electron microscopy, amoebae were flushed off the agar surface with dilute saline, fixed in veronal-acetate-buffered 2% (v/v) glutaraldehyde plus 2% (v/v) acrolein and 0.01% (w/v) $CaCl_2$, post-fixed in similarly buffered OsO_4 and, following dehydration, embedded in Maraglas epoxy resin. Sections were stained with Pb citrate (9) before being examined in an RCA EMU-3G electron microscope operating at 100 kv.

OBSERVATIONS AND DISCUSSION

The apparent viral-like material was first seen in sectioned amoebae sometime after chicken embryo extract was employed to supplement the yeast-peptone-liver medium; it seems possible that the source of the "infection" was the embryo extract. The virus-like elements, however, were not immediately evident in amoebae grown axenically but appeared only after transfer from an axenic to a bacterized culture medium. Under these conditions, the nuclei of up to 30% of the amoebae seen in section contained sparse to fairly heavy aggregations of particles (Fig. 1). The nucleoplasm of nuclei with the heavier concentrations of virus-like particles assumed an empty appearance, in contrast to the uniformly dense matrix in nuclei of cells lacking particles. The large nucleolus was not affected by the virus-like bodies. In nuclei with relatively few particles, there were apparent generative bodies—densely granular spheres—which might have been giving rise to the virus-like units (G in Figs. 2 & 3).

Another characteristic of nuclei with particles was the presence of crystalloids having faint periodicity within the nucleoplasm (C in Fig. 3). These were also seen in nuclei of living cells examined under phase-contrast as needle-like shafts, ~ 2 μ long, traversing the nucleus. Typical of the heavily "infected" nucleus were pegs on the nuclear membrane projecting into the cytoplasm (Figs. 4 & 5). These projections were formed by a double membrane continuous with the double membrane of the nucleus and therefore an extension of it. A nuclear pore appeared to be present at the distal end of the projection and, on the cytoplasmic side of the projection, a virus-like body (V in Figs. 4 & 5). Typically, too, cisternae of the endoplasmic reticulum were attached to the pegs at their distal ends. It would appear that the projection serves as a means by which the virus-like particle passes into the cytoplasm of the amoebae, and virus-like units have been seen in the cytoplasm.

The bodies themselves are ~ 100 nm in diameter and are composed of a central core surrounded by a wall which, in turn, is coated with a diffuse layer of material. The units strikingly resemble reovirus both in ultrastructure and location (2, 3). They also resemble the virus-like bodies reported by Perkins (7) in the nuclei of the oyster pathogen *Labyrinthomyxa marina*, as well as similar elements associated with kappa particles from *Paramecium aurelia* as reported by Preer and Jurand (8).

Since the trigger for appearance of the virus-like particles was transfer of amoebae from an axenic to a bacterized culture medium, perhaps growth conditions in the axenic culture medium are not entirely optimal either for the amoebae or for the virus-like units, and only when the amoebae are growing in the presence of living *A. aerogenes*—presumably a more complete nutrient medium—can the virus-like bodies reproduce. Seven other strains of *N. gruberi* from various sources maintained under condi-

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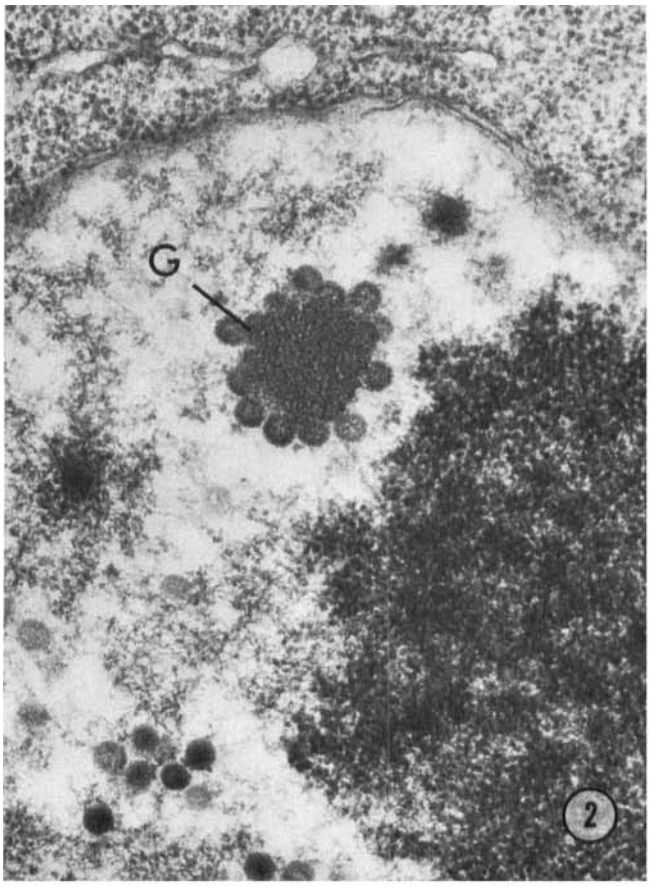
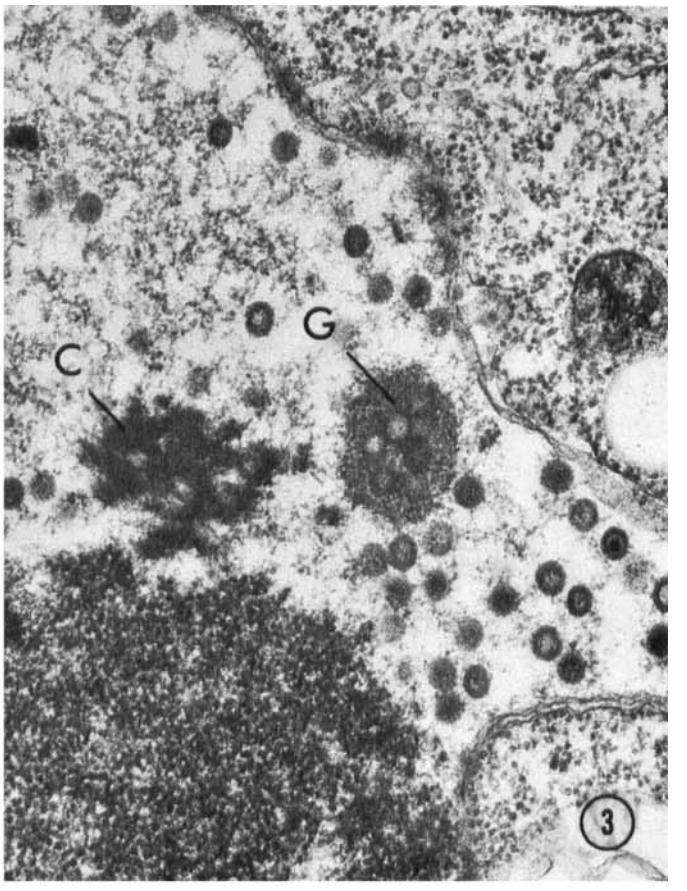
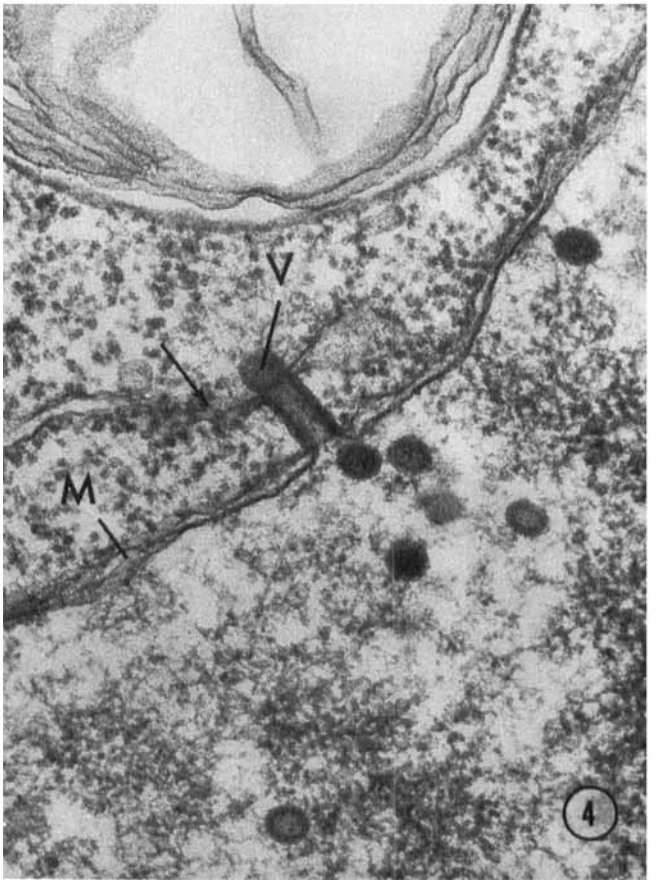
Fig.1. Section thru the nucleus and nucleolus of an EG amoeba. Virus-like particles (V) ~ 100 nm in diameter scattered thruout the nucleoplasmic matrix. Nucleoplasm somewhat "empty," a feature which accompanies the presence of large numbers of particles. × 25,000.


V
1


G
2


C
G
3


V
M
4

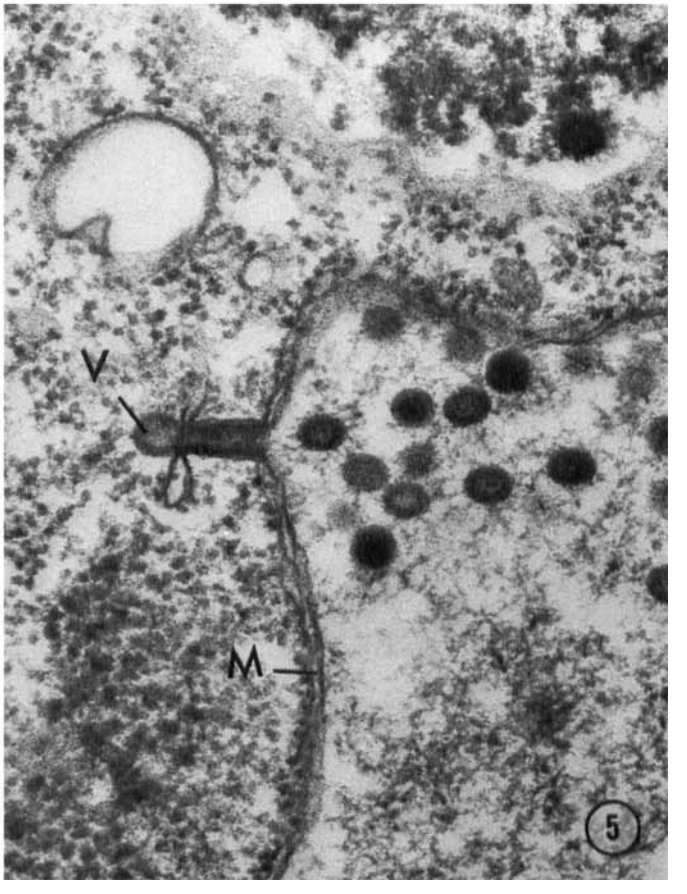

V
M
5

Fig. 2. Portion of a nucleus showing generative body (G) alongside the nucleolus. Virus-like particles appear to be arising from the periphery of the generative body. × 41,000.

Fig. 3. Section thru nucleus showing generative body and a portion of a crystalloid (C) in the nucleoplasm. × 41,000.

Fig. 4. Projection on the nuclear membrane (M) of an "infected" nucleus. Note virus-like particles in the nucleoplasm at the proximal end of the projection and a single virus-like body (V) at the distal end of the projection. Note also the cisterna of the endoplasmic reticulum (arrow) just below the particle at the distal end of the projection. × 58,500.

Fig. 5. Another nucleus with a projection on the nuclear membrane with a virus-like body at its distal end. × 58,500.

tions identical to those used for the EG strain have been examined for virus-like particles with the electron microscope. None has had such bodies. On the chance that the virus-like units were being brought into the cells by the *A. aerogenes* upon which the amoebae were feeding, a strain of *E. coli* was used to establish a monobacterial culture of the EG amoebae. Amoebae feeding on *E. coli* also had virus-like bodies, suggesting that the strain of *Aerobacter* being used as a food source was not the source of the "infection."

Immediately after transfer to a bacterized culture medium from the axenic state, very few amoebae had the virus-like units. By the time the culture was in its logarithmic phase—reached on about the 2nd day after transfer—the number of cells with particle-containing nuclei attained a maximum of ~ 30%. Whether this indicates activation of particles latent in the nuclei of the axenic amoebae or spread of "infection" in the culture is not yet clear. There was no evidence of plaque formation on the agar medium on which the cells were growing, but there are technical problems in proving the existence of plaques in these amoeboid populations. Much cellular debris was seen on the agar surface suggestive of lysis, but this requires confirmation.

We are trying to develop a less cumbersome and more reliable technic for assay of virus-like particles than examination of sectioned amoebae in the electron microscope. Along these lines we are attempting isolation of the virus-like bodies from the EG strain as well as trying to improve plating procedures for revealing plaque formation.

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