

Replication Cycle of an Insect Granulosis Virus

WHEN a lepidopterous larva such as a cutworm (Noctuidae) in a late stage of infection with a granulosis is shaken up in distilled water, as much as 10 ml. of a fluid of the consistency of thin cream is obtained. This consistency is due to the immense numbers of protein crystals, the granules, each containing a short virus rod, which are liberated from the insect's tissues. Centrifugation of this fluid for 30 min at 5,000 r.p.m. causes the granules to form a sediment and gives a clear supernatant with a greenish opalescence. When this opalescent fluid is examined with the electron microscope it is found to contain large numbers of long virus-like threads branched in an intricate manner.

This communication is concerned with the origin of these threads and the part they are thought to play in the replication cycle of the virus.

In a previous communication¹ the suggestion was made that the short thick virus rod emerged from its occluding crystal, lengthened in the process and commenced to branch. Since these observations were all made on purified preparations of the virus threads and their concomitant granules, the criticism could be made that some of these phenomena were artefacts arising from the negative staining with phosphotungstic acid. The peculiar

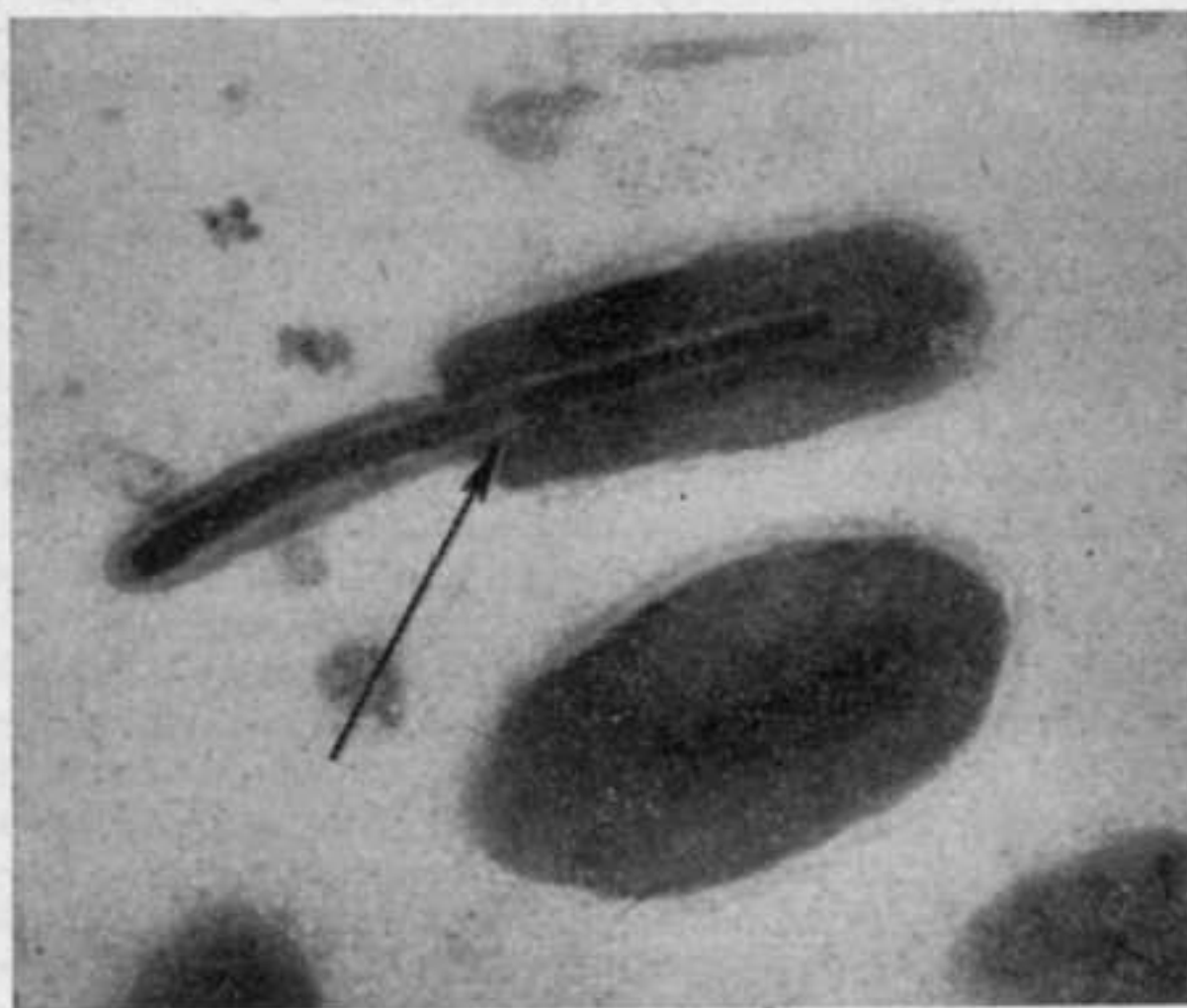


Fig. 1. Section through two granules, side by side in the same cells; note the apparent extrusion and elongation of the virus rod; the outer membrane now encloses only half the rod (arrows); note also an apparent membrane surrounding the granules. ($\times 82,500$)

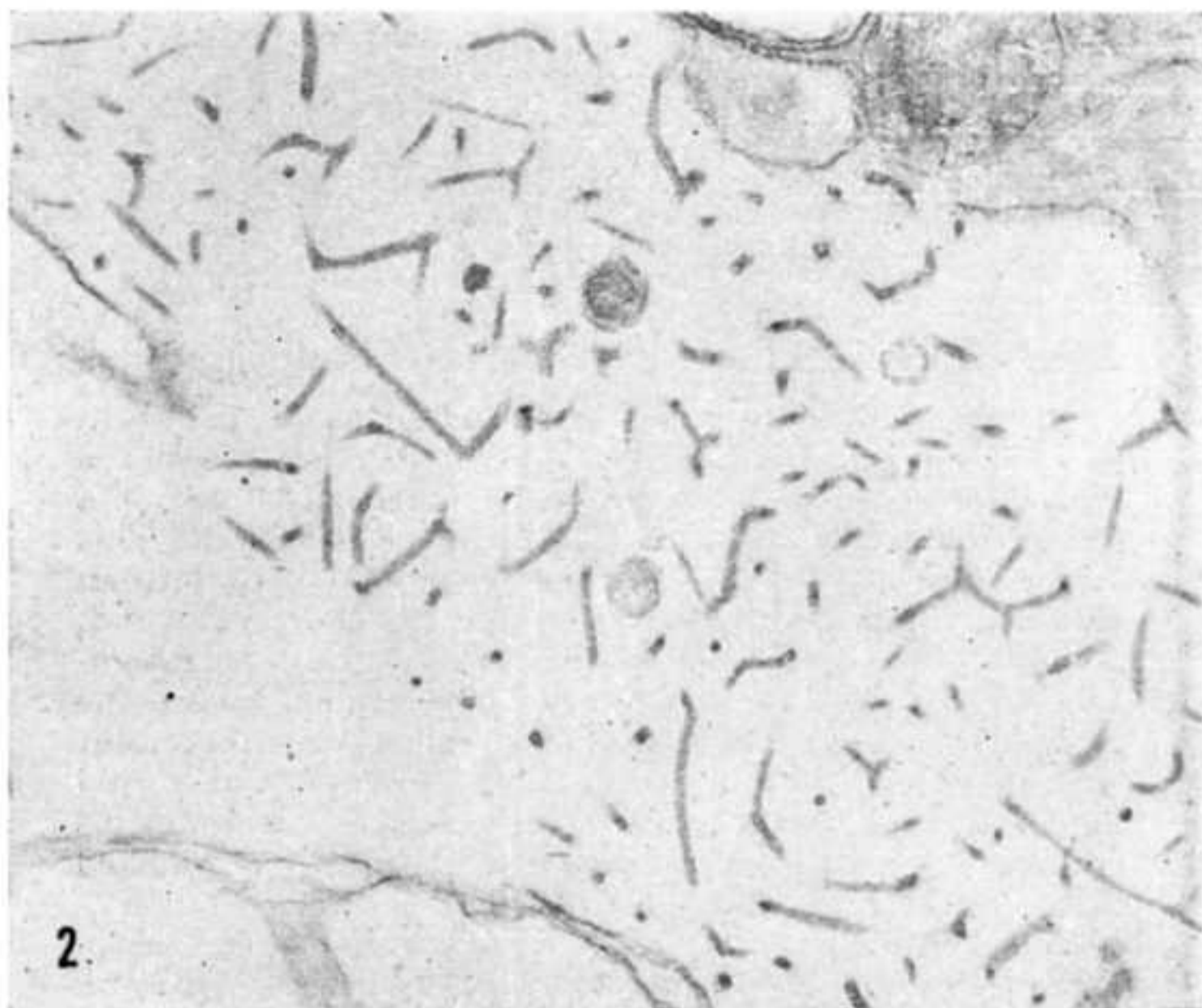


Fig. 2. Low power electron micrograph of a section through a mass of branching virus threads inside the cell. ($\times 40,000$)

branching of the virus might also be attributed to adsorption of the various lengths.

Further investigations have now been made, but this time on thin sections of infected tissue fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. The changes described here all occurred inside the cell itself. Based on these results an explanation is offered of the origin of the long virus threads and of their role in the replication of the virus. The cycle proposed is a tentative one, but it appears to fit most of the observed phenomena.

As previously suggested¹, the initial step in the replication cycle seems to be the extrusion of the short thick virus rod from its occluding protein crystal, the granule. To this end there appears to be a cap on one end of the crystal which lifts up to allow exit of the virus particle. In addition to being occluded in the crystal, the virus is also completely enclosed in an outer membrane.

Numerous examples have now been observed of this extrusion process occurring inside the cell. As the rod emerges from the crystal it increases in length and decreases in diameter; the outer membrane in which the particle was originally completely enveloped is sometimes pushed out with it. In Fig. 1 are sections of two crystals side by side in the same cell; in one is the short thick virus rod with its enveloping membrane. In the other the virus rod is in process of emergence, and it is noteworthy that the outer mem-

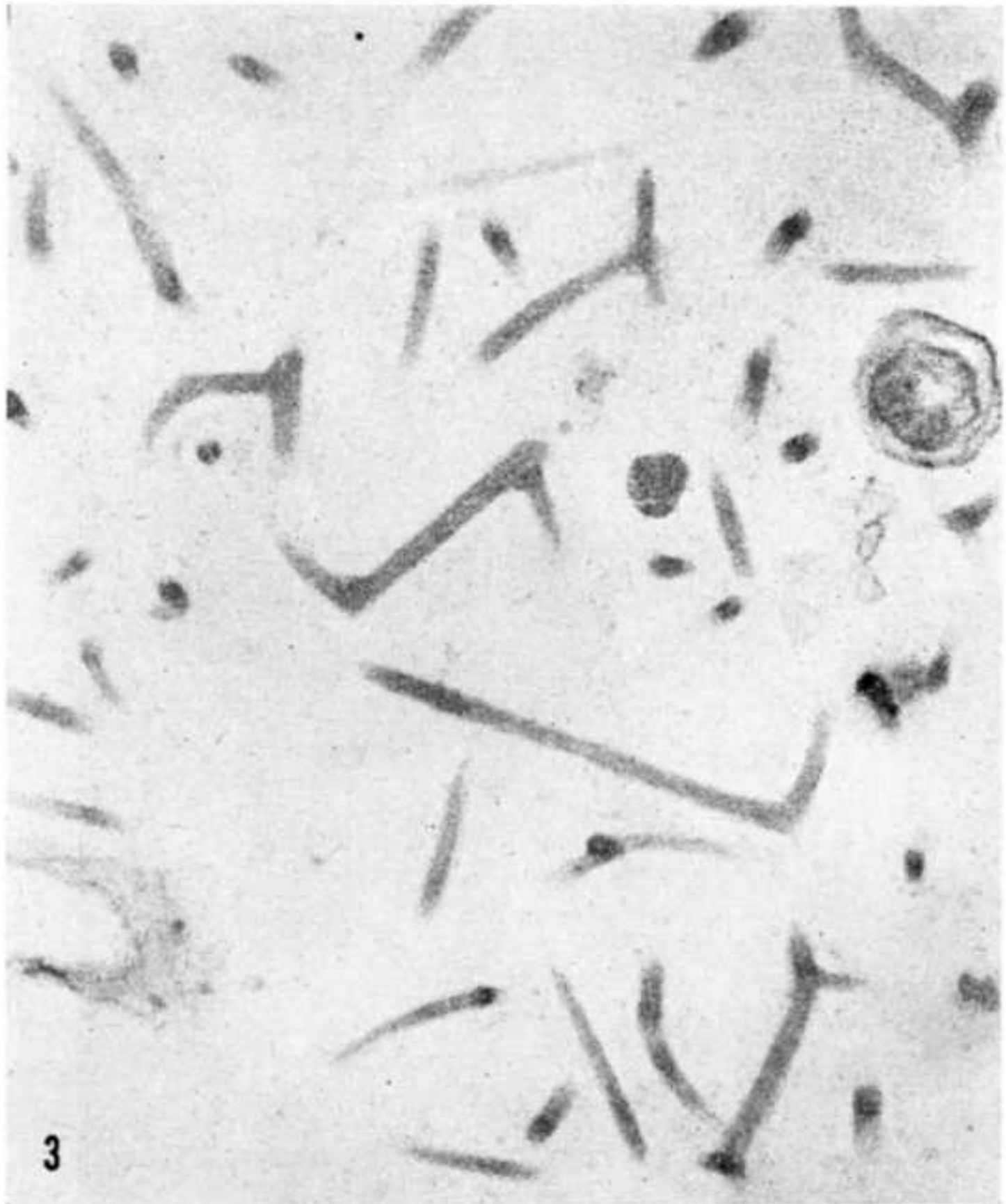


Fig. 3. Similar to Fig. 2 but at higher magnification; the branching of the threads is clearly visible. ($\times 100,000$)

brane, pushed out by the emerging rod, now envelops only half its length. Empty membranes and the U-shaped empty granules can be found loose in the cell.

It is of interest to speculate on the nature of the stimulus which causes the virus rod to emerge from the crystal.

Perhaps the most interesting phenomenon in this replication process is the apparently unlimited and uncontrolled branching of the long virus threads, a phenomenon that, so far as we know, is unique among viruses. That the branching is a genuine phenomenon and that it occurs inside the cell cytoplasm are shown in Figs. 2 and 3. The reason for the branching is at present obscure, unless it is a means of increasing the DNA which must be inside the long thread.

High-resolution electron micrographs of the virus threads suggest that they are composite with an inner helical core. Fig. 4 shows one of these threads negatively stained with phosphotungstic acid. The stain has apparently entered, possibly by a small break in the outer coat, and delineated the internal helix.

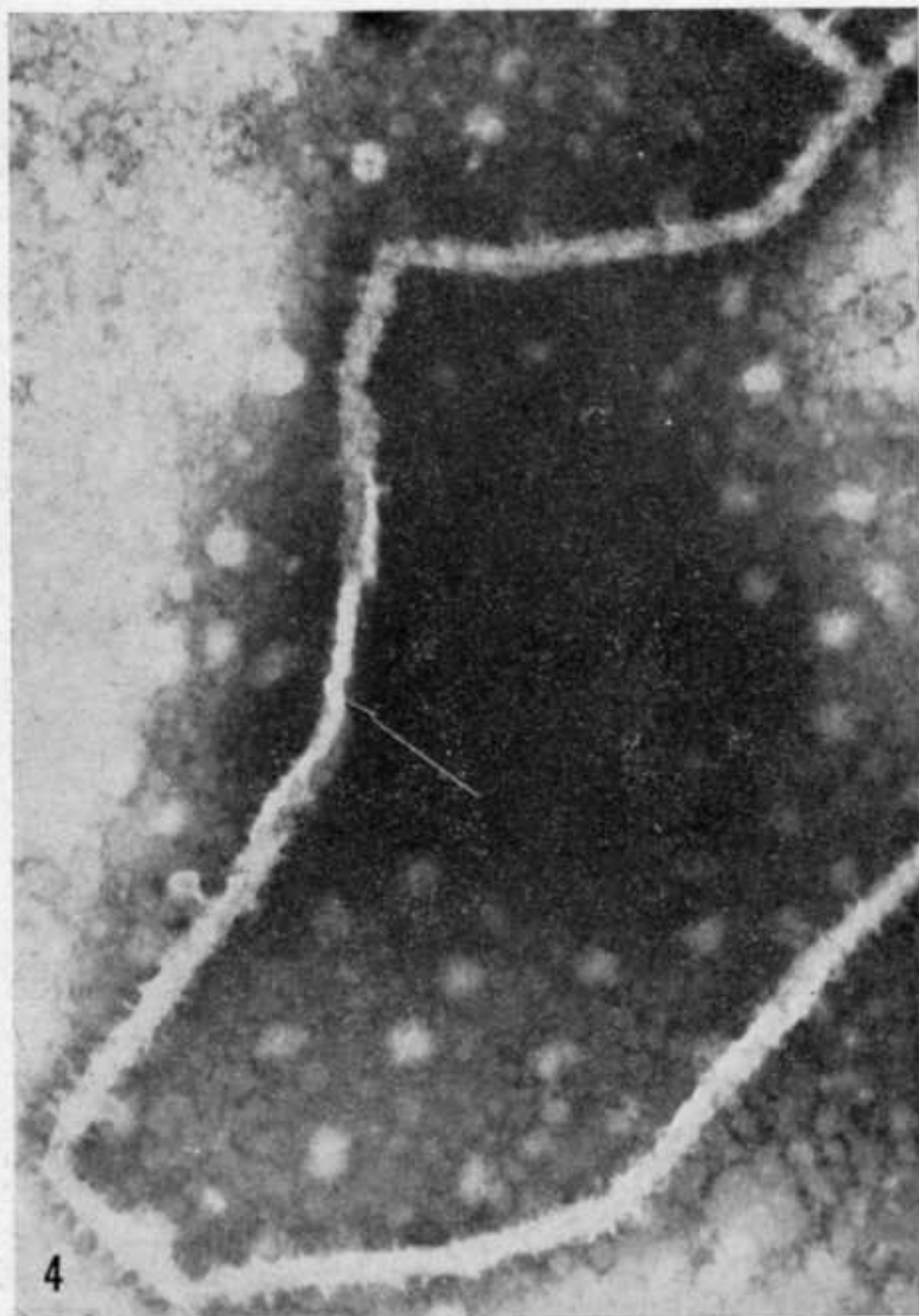


Fig. 4. A long virus thread negatively stained with phosphotungstic acid; note the internal helix (arrow). ($\times 100,000$)

Presumably the next step in the replication process is the break-up of the long thin virus threads into the short rods. Lying free in the cell cytoplasm there occur single short rods, some with, and some without, their outer membrane. The last stage in the replication process seems to be the deposition on the finished virus rod of the protein to form the occluding granule as originally suggested by Hughes².

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¹ Smith, Kenneth M., Trontl, Z. M., and Frist, R. H., *Virology*, **24**, 508 (1964).

² Hughes, K. M., *J. Bact.*, **64**, 375 (1952).