Food Uptake by the Insect-parasitic Nematode, Sphaerularia bombi (Tylenchida)

GEORGE O. POINAR, JR. and ROBERTA HESS[1]

Abstract: The insect-parasitic female of *Sphaerularia bombi* everts its uterus and associated reproductive structures into the body cavity of its bumblebee host. This uterine sac then takes over the normal functions of the parasite and leads an independent existence. An examination of this sac shows that the surface of the uterine cells are differentiated into a network of saccular indentations separating off fine cytoplasmic extensions. The folding of adjacent cytoplasmic extensions around portions of the host's hemolymph results in the formation of pinocytotic vacuoles. Intracellular vacuoles are also formed at the base of the saccular indentations. It appears that the first stage of intracellular digestion in *S. bombi* initially occurs by pinocytosis in the outer surface of the uterine cells. The inner and outer surfaces of the ovary and oviduct are modified into lobelike projections to increase the absorptive surface area, and electron-dense droplets originating in this tissue were also observed in the developing eggs. The hypothesis is presented that soluble nutrients are passed into the ovary-oviduct tissue where, after being reconstituted into droplets, they enter the developing eggs. *Key Words:* nourishment, parasite, bumblebee.

Little information is available on the uptake of nutrients by nematodes. Absorption normally occurs through the intestinal cells, although simple diffusion of nutrients may also occur through the cuticle in animal-parasitic nematodes. However, there has been no evidence of phagocytosis, pinocytosis, or intracellular digestion in the roundworms (2). Special adaptations associated with the uptake of nutrients have been reported in some entomogenous nematodes. Riding (5) reported finding microvilli on the body wall of the parasitic tylenchid female of a species of *Bradynema* from the phorid fly, *Megaselia halterata* Wood, and the present report discusses still another adaptation for obtaining nourishment in an insect nematode.

A special case of parasitism is exhibited by *Sphaerularia bombi* (Dufour), a parasite of bumblebee queens, since in the host, the uterus and associated reproductive structures of the nematode become everted through the vulva, a condition referred to as "prolapsed uterus". The uterine cells greatly expand, forming a huge uterine sac containing the ovary, oviduct, sperm, and dwarfing the remainder of the female body (Fig. 1).

The eggs develop normally within the uterine sac and are passed out into the host's hemocoel where they develop to third-stage juveniles (3). Since there is no growth or development of the original female, it appeared that host nutrients must be absorbed by the uterus, which has assumed an independent role. The purpose of this study was to determine how nutrients were absorbed by the developing parasite and whether special morphological modifications were present.

MATERIALS AND METHODS

Mature uterine sacs of *Sphaerularia bombi* (Dufour) were removed from *Bombus lucorum* L. and *Bombus terrestris* L. collected during May and June, 1971, in Holland. They were prefixed for 2 hr in 3% gluteraldehyde, then washed in 0.05 M phosphate buffer and fixed in 1% osmium in phosphate buffer (pH 7) for 1 hr. After dehydration in a graded ethanol series, the specimens were embedded in Araldite 6005 and sectioned with glass knives mounted in a Porter-Blum microtome (MT-2). The sections were stained with a saturated aqueous solution of uranyl acetate followed by lead citrate (4) and examined with an RCA EMU-3F and Philips EM-300 electron microscope. For light microscopy, similar specimens were fixed in 3% Formalin for 1 week and processed to glycerin using alcohol dehydration.

RESULTS

During the development of *Sphaerularia bombi* in the hemocoel of *Bombus*, the internal surface of the prolapsed uterus makes contact with the hemolymph of the host. The uterus is one cell thick, and enlargement apparently occurs not by cell division, but by hypertrophy, causing cell nuclei and surrounding cytoplasm to stand out as projections over the surface of

Received for publication 6 March 1972.

[1] Division of Entomology, University of California, Berkeley 94720. The senior author thanks Dr. M. Oostenbrink and members of the Laboratorium voor Nematologie, Wageningen, The Netherlands for providing laboratory space and facilities during the course of these studies, and Dr. A. van Kammen, Dr. T. S. Ie and Miss Magda Usmany of the Laboratorium voor Virologie, Wageningen, The Netherlands for aid in the preparation of specimens.

the organ (Fig. 1). The cell borders remain distinct and become interdigitated with desmosomes during the enlargement of the uterus.

In the fully developed uterus, the nucleated portion of the cells with associated cytoplasm becomes enlarged and swollen. Each cell contains a pleiomorphic nucleus with sparse chromatin, extensive rough endoplasmic reticulum, elongate mitochondria, lipid droplets and numerous heterogenous cytosomes. The cytosomes varied from membrane-bound vacuoles containing flocculent material in the apical regions of the cell to basally located electron-dense vacuoles containing membranous inclusions.

The entire surface of the uterus exposed to the host's hemolymph was modified into a network of saccular indentations separating off fine cytoplasmic extensions (Fig. 2, 3, 4). The

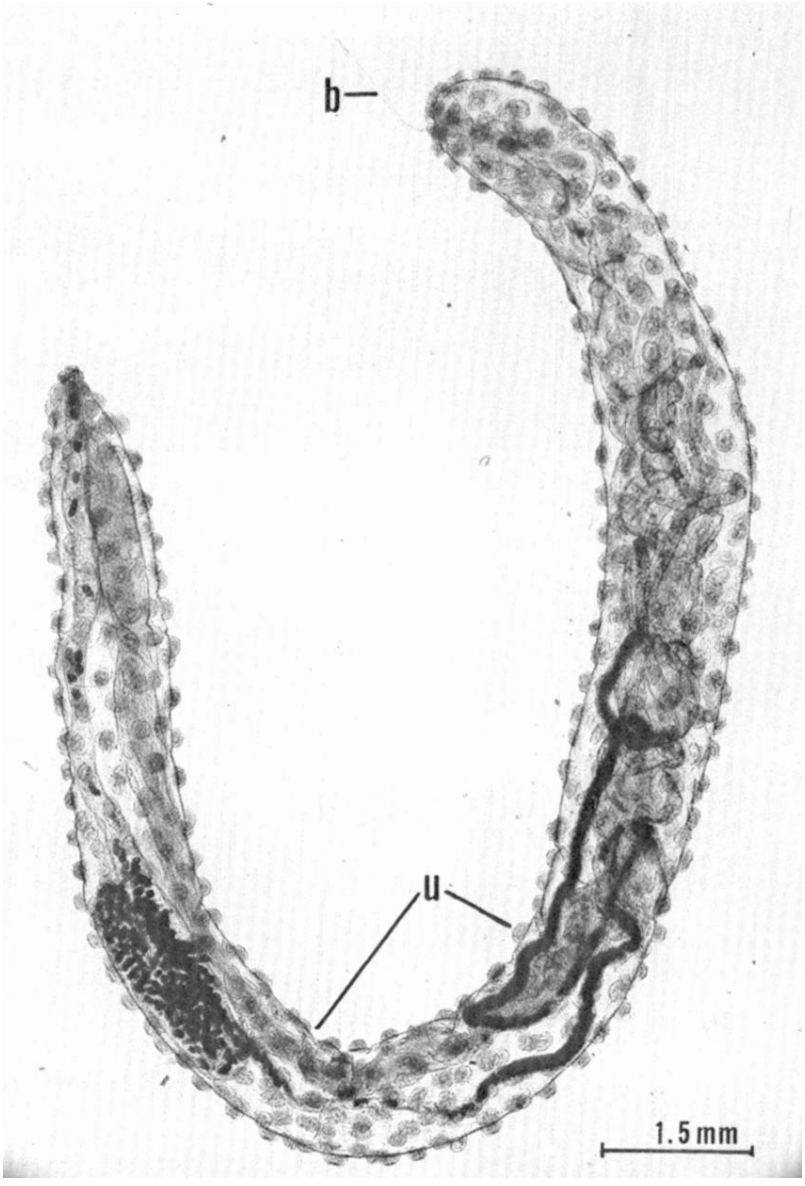

FIG. 1. Mature female of *Sphaerularia bombi* removed from the body cavity of a queen *Bombus* sp. (b = body of female, u = enlarged uterine sac).

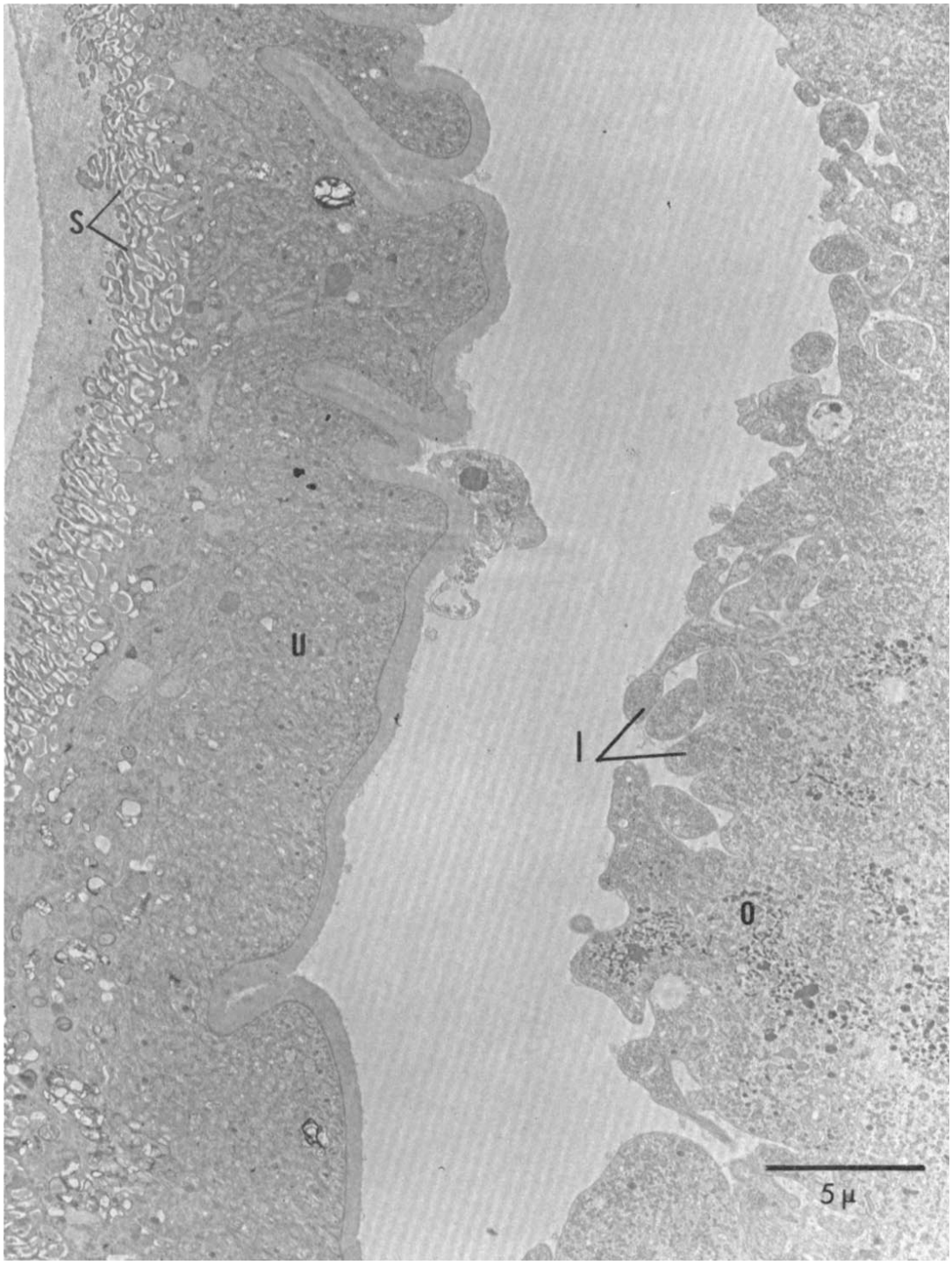

FIG. 2. Section of the wall of the uterus (u) and ovary-oviduct (o) of *S. bombi* (s = saccular indentations on the surface of the uterus; l = lobe-like extensions on the surface of ovary-oviduct).

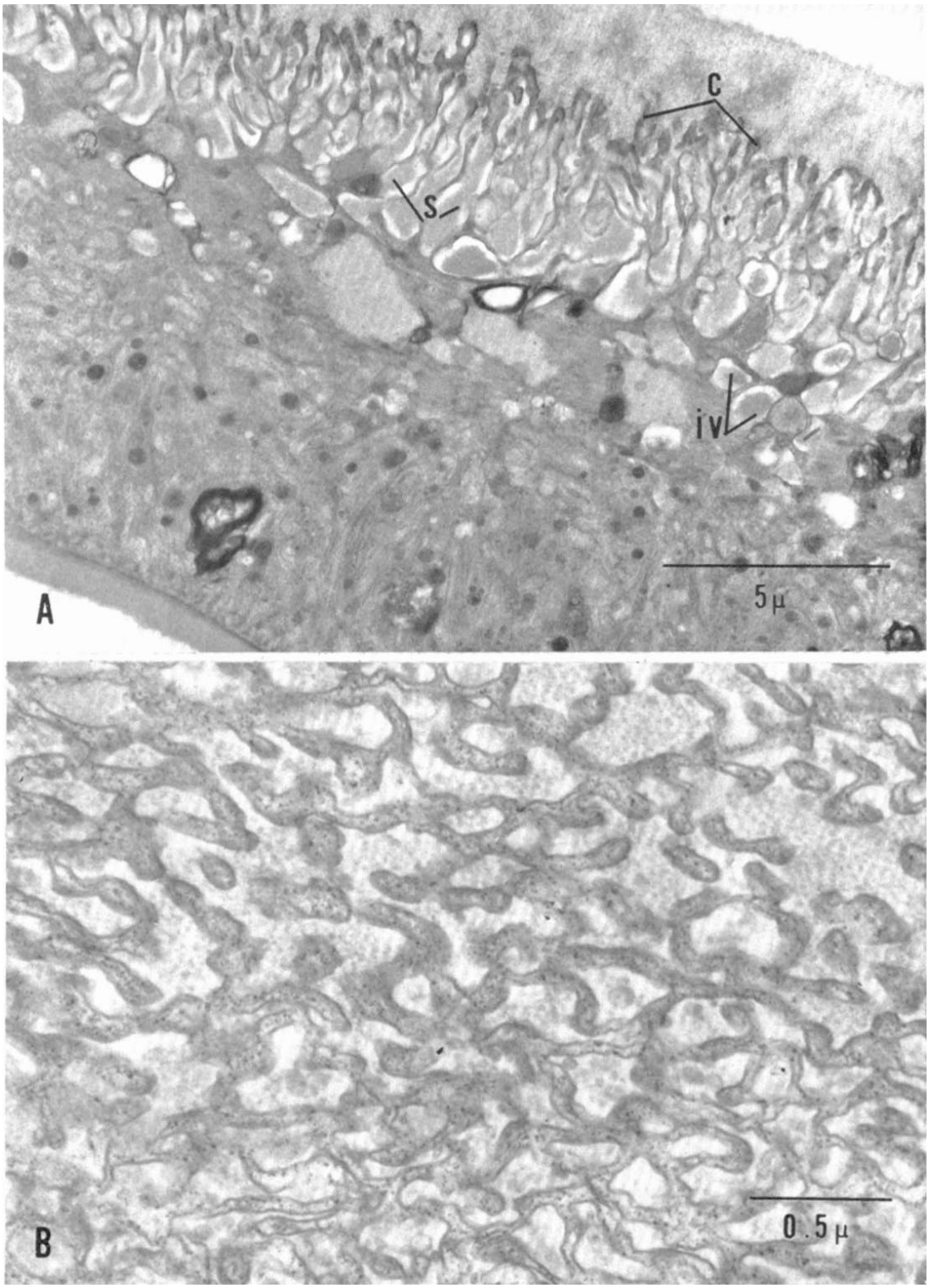

FIG. 3. **A.** Outer surface of the uterus showing the saccular indentations (s) surrounded by cytoplasmic extensions (c) and the formation of intracellular vacuoles (iv) containing flocculent deposit. **B.** Section cutting through the cytoplasmic extensions parallel to the outer surface of the uterus.

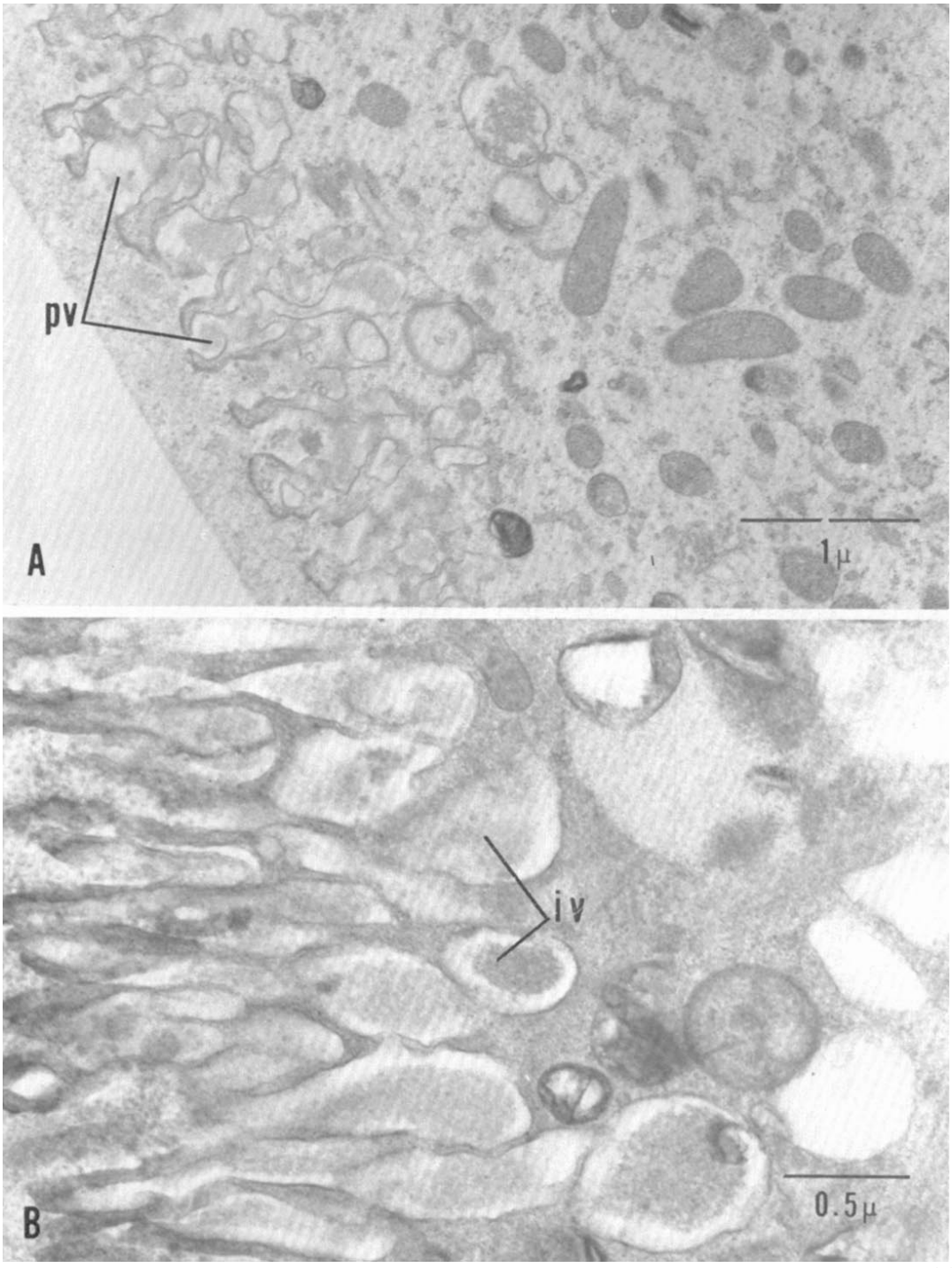

FIG. 4. **A.** Outer surface of uterus showing tips of cytoplasmic extensions enclosing portions of host material in "pinocytotic vacuoles" (p.v.). **B.** Close-up of 3A showing the presence of intracellular vacuoles (i.v.) forming at the base of the saccular indentations.

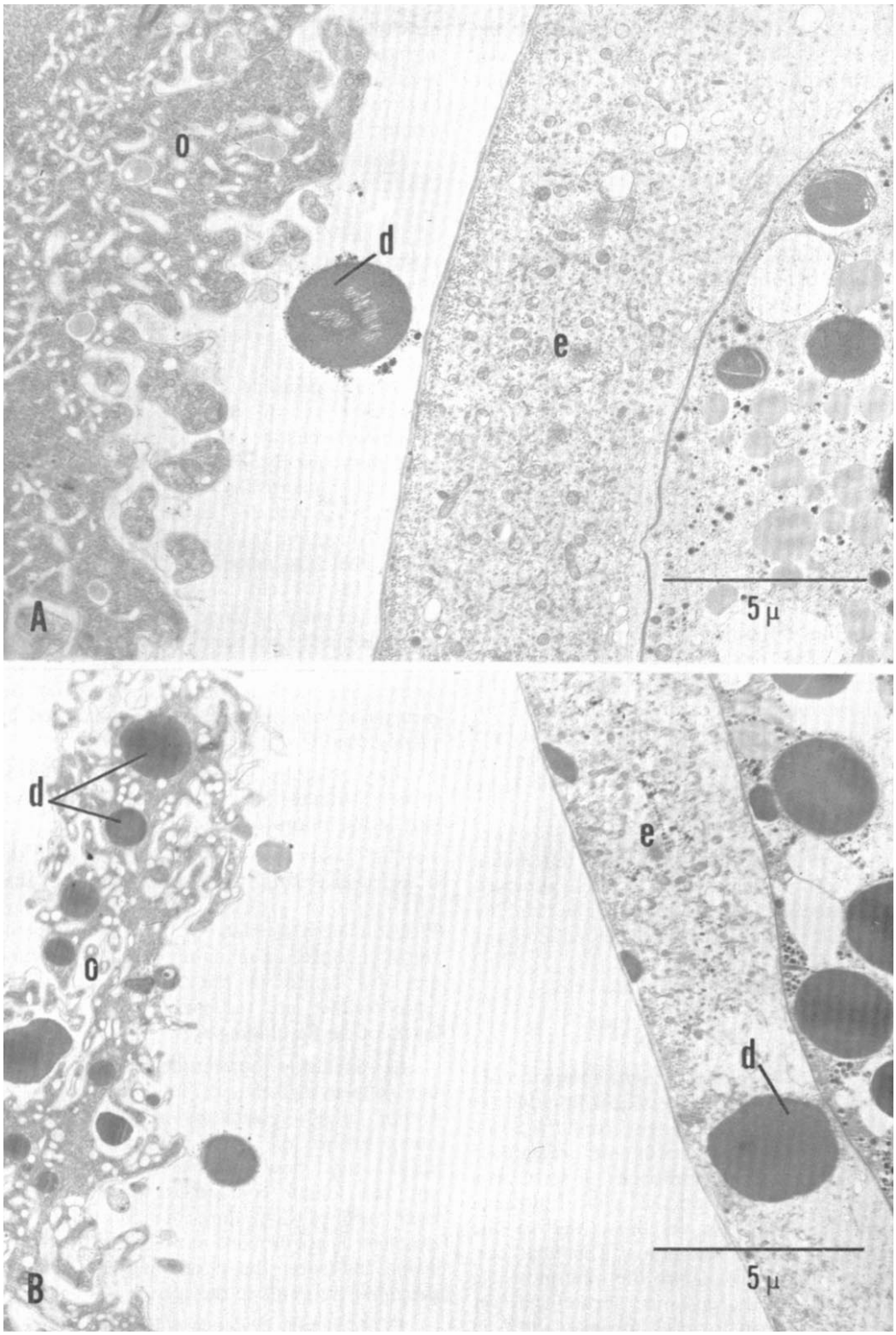

FIG. 5. **A.** Electron-dense droplet (d) between the ovary-oviduct (o) and developing egg (e). **B.** Electron-dense droplets (d) in the tubular indentations of the ovary-oviduct (o) and outer and inner portions of the developing egg (e).

outer surface of the uterus, as well as the contents of the saccular indentations, was covered with a fine flocculent deposit that ultrastructurally resembled a mucous or mucopolysaccaride (Fig. 2, 3, 4) (1). The tips of the cytoplasmic extensions were sometimes bent towards each other and appeared to be enclosing portions of the flocculent deposit into pinocytotic vacuoles (Fig. 4A).

Intracellular vacuoles or vesicles containing flocculent deposit also were formed at the base of the saccular indentations, and these structures were seen at various distances from the surface of the cells (Fig. 3A, 4B). The lower surface of the uterine cells was ramified by extensive infolding of the cell membrane and contained cytosomes (Fig. 2). A basal lamina (typically found in transporting cells) occurred along the outer edge of these cells, and a flocculent deposit, similar to the one covering the apical surface but denser, also lined the lower surface of the cells (1).

The inner and outer surfaces of the ovary-oviduct were modified by projections and indentations differing from those on the uterus surface. The ovary-oviduct cells contained branched tubular indentations surrounded by lobelike extensions of the cytoplasm (Fig. 2, 5). Both surfaces of the oviduct were coated by a fine deposit 30-45 nm thick. The cytoplasm of the ovary-oviduct cells contained electron-dense droplets concentrated near the egg side of the cell (Fig. 5). These droplets appeared to form within the rough endoplasmic reticulum. Similar electron-dense droplets were observed in the space between the ovary-oviduct and the developing eggs as well as in the outer and inner portions of the developing eggs (Fig. 5). They were never observed in the uterine cells.

DISCUSSION

The extruded uterus of *Sphaerularia bombi* in the hemocoel of its insect host functions as an organ for absorbing nutrients from the host's hemolymph. The uterus in nematodes commonly has several functions. It serves as a portion of the reproductive system through which the eggs pass on their way to the exterior; parts of it function as a spermatheca, other portions may secrete the outermost egg membrane, and the posterior portion may act as a storage organ for eggs. We now show an additional function—that of absorbing nutrients from the environment.

The uterus is everted during its extrusion into the host's hemocoel so that its absorptive surface is exposed to the exterior. This surface is increased by the bulging shape of the cells and by the differentiation of the cell membranes into saccular indentations.

The flocculent deposit covering the uterine cells may serve as a protective layer to keep the host's blood cells from adhering to the surface of the cells. It could be a residue originating from the host's hemolymph, or a secretion arising from the uterine cells. However, if the deposit were being produced by the uterine cells, one would expect to find well-developed Golgi apparti, since these are generally assumed to be responsible for the synthesis and concentration of substances like mucopolysaccharides. Yet, these cell organelles were very poorly developed. This, plus the presence of microvillar type projections which are generally accepted as transporting material in one direction (as in renal cells), makes us believe that host material is being incorporated into the uterine cells.

The presence of pinocytotic vacuoles in the terminal portions of the cytoplasmic extensions and intracellular vacuoles at the base of the saccular indentations strongly suggests that the incorporation of nutrients is accomplished by pinocytosis.

These vacuoles and vesicles (earlier referred to as cytosomes) become modified in the lower part of the uterine cell and disappear.

The "coated" tubular indentations of the outer surface of the ovary-oviduct suggest that absorption occurs in that locality. Electron-dense droplets are formed within the rough endoplasmic reticulum of these cells and are also found in the space between the ovary-oviduct and the eggs as well as in the layers of the developing eggs.

In conclusion, our results indicate that the intracellular digestion of food material by *S. bombi* initially occurs by pinocytosis in the outer surface of the uterine cells. We then suggest that these food vacuoles are digested and the soluble constituents passed into the ovary-oviduct where they are reconstituted into droplets. These droplets move out of the ovarial tissue and enter the developing eggs, thereby providing nutrients to the eggs.

Experimental evidence will be necessary to prove the above hypothesis concerning the movement of nutrients into the ovary-oviduct and developing egg since our evidence is based

solely on visual interpretations of the electron micrographs.

LITERATURE CITED

1. FAWCETT, D. W. 1966. An atlas of fine structure, the cell, its organelles and inclusions. W. B. Saunders Co., Philadelphia, Pa. 448 p.
2. LEE, D. L. 1965. The physiology of nematodes. Freeman and Co., San Francisco. 154 p.
3. POINAR, G. O., JR. and P. A. VAN DER LAAN. 1972. Morphology and life history of *Sphaerularia bombi* (Dufour) (Nematodea). Nematologica (in press).
4. REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17:208-212.
5. RIDING, IRENE L. 1970. Microvilli on the outside of a nematode. Nature 226:179-180.