

The structure and formation of the *Syngamus trachea* egg-shell (Nematoda: Syngamidae)

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Abstract. The structure and formation of the egg-shell in *Syngamus trachea* has been examined for the first time in an electron microscopic investigation. The egg-shell of *Syngamus trachea* is composed of four layers: the uterine, the vitelline, the middle chitinous, and the inner lipid layer. The latter layer is up to 0.2 µm thick. It contains electron-transparent vesicles, dispersed in a light matrix. The middle chitinous layer is approximately 1 µm thick. The vitelline layer originates in the oolemma of the fertilized oocyte, its thickness varying between 0.05 and 0.1 µm. The surface of *Syngamus trachea* eggs is covered by a uterine fibrous layer.

Ultrastructural studies on nematode egg-shells have been carried out by several authors and their results were summarized by Wharton (1980). A few types of nematode egg-shells have been described to consist of three to five layers. However, no paper has yet been published stating which of the mentioned types the *Syngamus trachea* egg-shell can be classified with. Our paper will try to fill the gap in the knowledge on the ultrastructure of the fertilized oocyte and on the formation of the egg-shell in this helminth which inflicts grave parasitic diseases in free-living gallinaceous birds.

MATERIALS AND METHODS

Syngamus trachea (Montague, 1811) nematodes were obtained from the trachea of experimentally infected chickens. The helminths and their isolated reproductive tract were immersed in 3% glutaraldehyde in 100 mM cacodylic buffer (pH 7.3) and kept in the fixing solution for 2 h. The uteri were separated from the reproductive tract and processed individually by the procedure described earlier (Bruňanská 1991).

RESULTS

The lumen of the proximal portion of the uterus is filled with fertilized oocytes (Fig. 1). These are demarcated by an electron dense layer, very similar to the oolemma of mature oocytes. On close examination we can see that it is formed by an outer thin dense layer and an inner thin membrane (Fig. 2). The inner membrane is likely a newly-created "secondary oolemma", separating the cytoplasm of the egg from the developing shell. The outer layer (0.2 µm thick) is the first egg-shell layer, the vitelline layer, developed from the vitelline membrane, derived from the original oolemma.

As result of fertilization, the cytoplasm of the oocytes shrinks, thus giving rise to the chitinous layer between the

vitelline layer and the new oolemma. This layer first appears as an electron lucent zone (Fig. 3). At this time lipids as well as dense and shell granules can still be observed in the cytoplasm of the fertilized oocytes, just like in the oocytes of the ovary (Bruňanská 1992). Shell granules and mitochondria tend to concentrate all over the area of the fertilized oocyte, the inside of which is filled with numerous small dense granules and lipids (Fig. 4). Shell granules gradually disappear from the egg cytoplasm and at the same time an electron dense chitinous layer is formed (Fig. 5). The chitinous layer rapidly becomes thicker (Fig. 6). It is approximately 1 µm thick and it consists of chitin-protein microfibrils which form an irregular reticulum (Fig. 7). The outer surface of this layer is lined with a vitelline layer. In some eggs a light band of reticular character (0.1 - 0.2 µm wide) is formed in the chitinous layer, immediately subjacent to the vitelline layer (Figs. 6 and 7). In this band small dense granules also occur sporadically.

Under the chitinous layer, round to oval vesicles with light centre and clear-cut outlines are accumulating in the perivitelline space (Figs. 5, 6, 7). Some of the vesicles closely adjoin the inner side of the chitinous layer, thus forming the lipid layer of the egg-shell with a thickness varying between 0.05 - 0.1 µm.

The uterine layer of the egg-shell is deposited on the surface of the eggs (Figs. 5, 6, 7). It is of a fibrous appearance and it is not demarcated by any membrane.

The cytoplasm of the shell-forming eggs is filled with numerous small dense granules and small droplets of saturated lipids (Figs. 5, 6). Large nuclei contain condensed chromatin (Fig. 8).

The complete shell of *Syngamus trachea* eggs thus consists of four layers: the uterine, the vitelline, the middle chitinous and the inner lipid layer.

The uterine wall of *Syngamus trachea* is composed of epithelial cells, adjoining the basement membrane (Fig. 9).

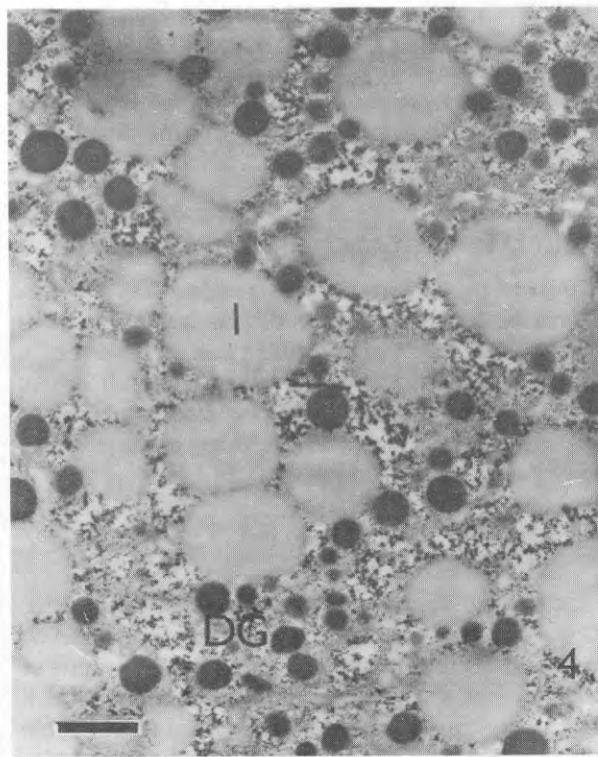
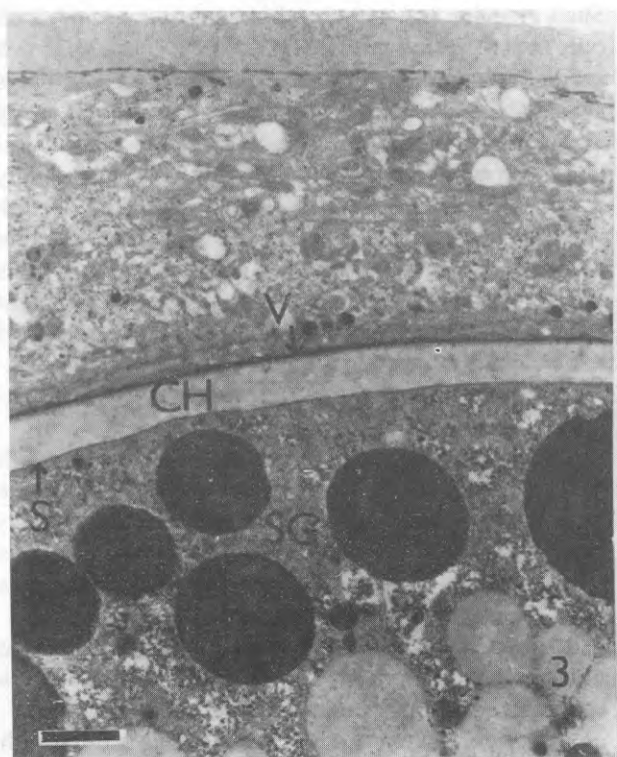
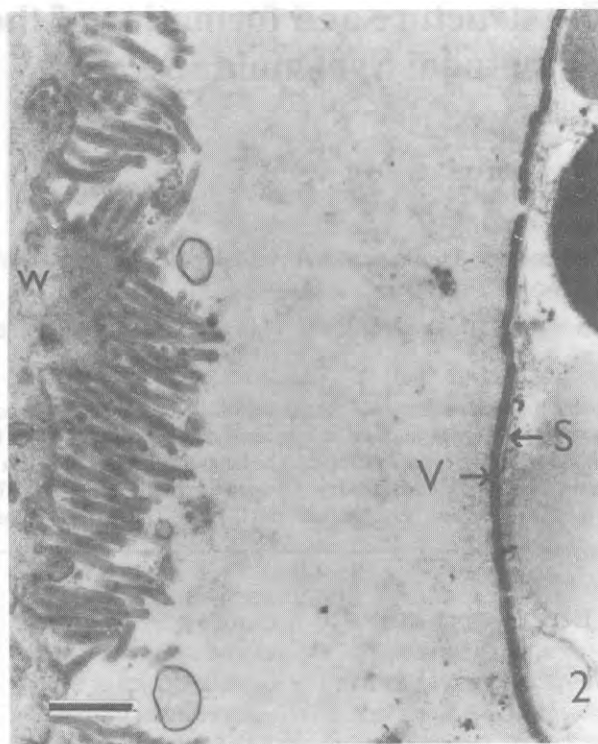
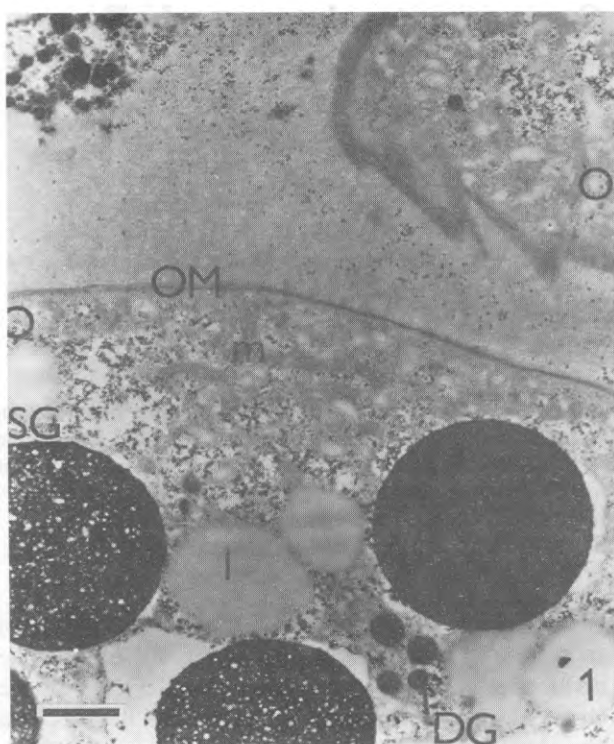


Fig. 1. Fertilized oocytes (O) in the proximal portion of the uterus. Their cytoplasm contains lipids (l), dense and shell granules (DG, SG) and mitochondria (m). Oolemma (OM) transforming into the vitelline layer. Bar = 1 μ m. **Fig. 2.** The surface of the fertilized oocyte is demarcated by an outer electron dense vitelline layer (V) and by a thin inner membrane (S). Bar = 0.5 μ m. **Fig. 3.** Between the vitelline layer (V) and the newly-formed secondary oolemma (S) an electron lucent zone is formed (CH). Large shell granules (SG) tend to be localized all over the area of the fertilized oocyte. Bar = 1 μ m. **Fig. 4.** The inside of the fertilized oocyte is filled with numerous small dense granules (DG) and saturated lipids (l). Bar = 1 μ m

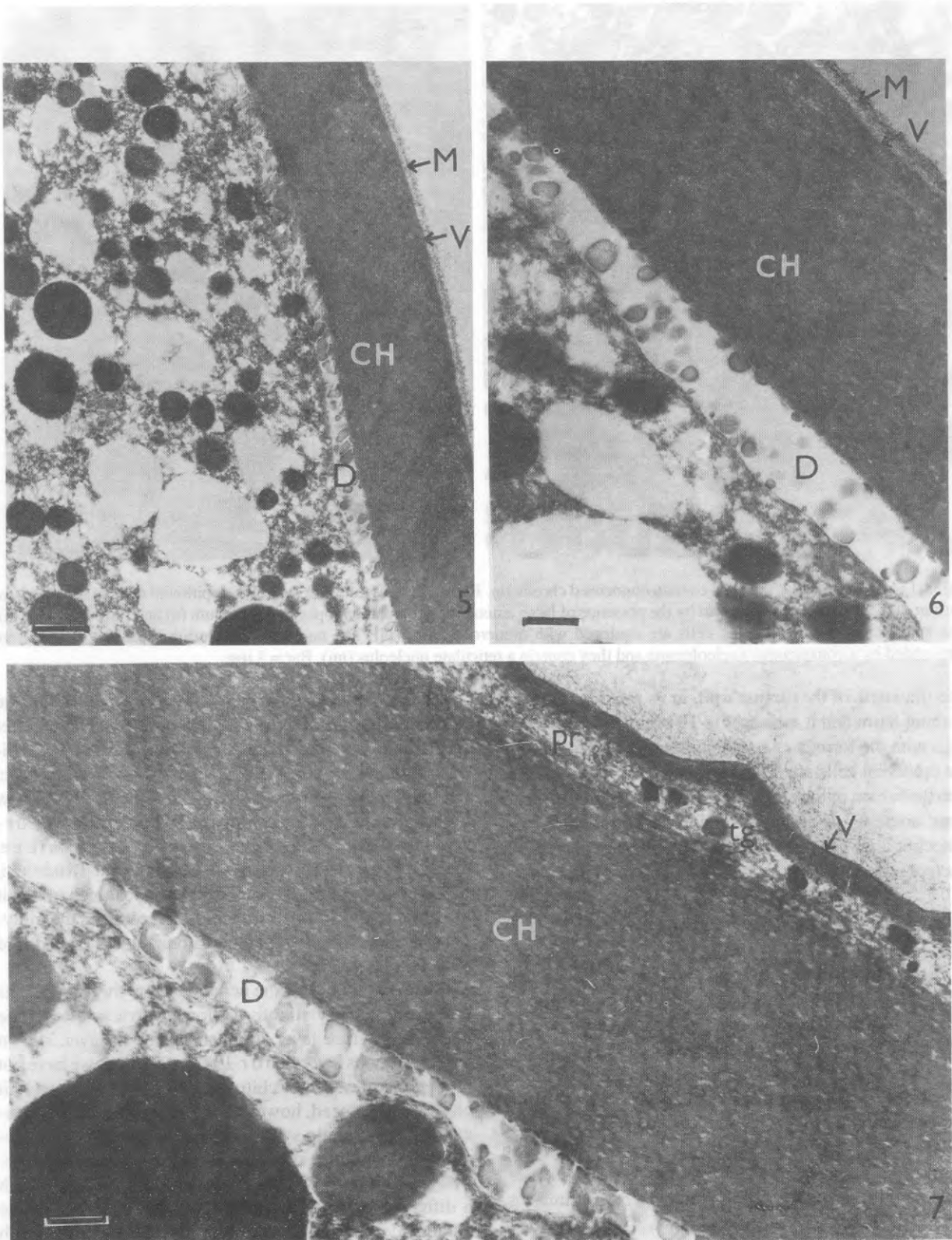


Fig. 5. Formation of the egg-shell lipid layer (D). CH - chitinous layer, V - vitelline layer, M - uterine fibrous layer. Bar = 0.9 μ m. **Fig. 6.** The complete shell of eggs consisting of four layers: uterine (M), vitelline (V), middle chitinous (CH) and lipid layer (D). Bar = 0.4 μ m. **Fig. 7.** In the chitinous layer of some eggs a wider light band of thin reticular character is formed (pr), containing also small dense granules (tg). V - vitelline layer, CH - chitinous layer, D - lipid layer. Bar = 0.25 μ m.

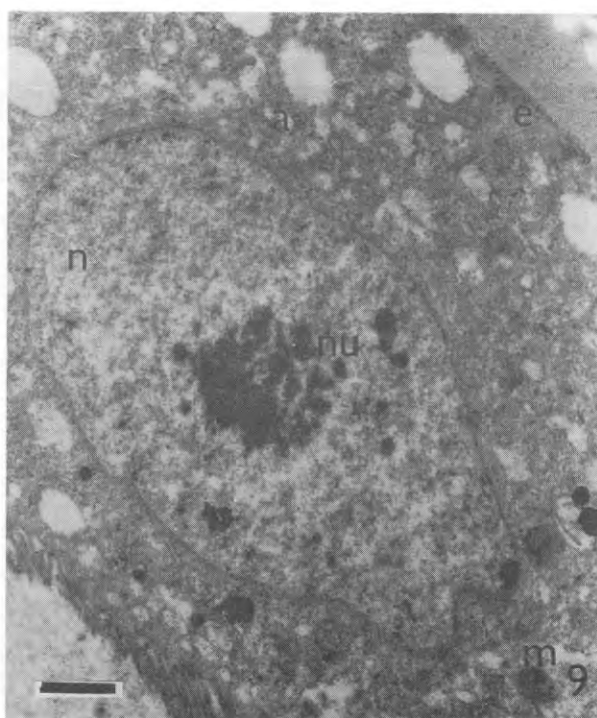
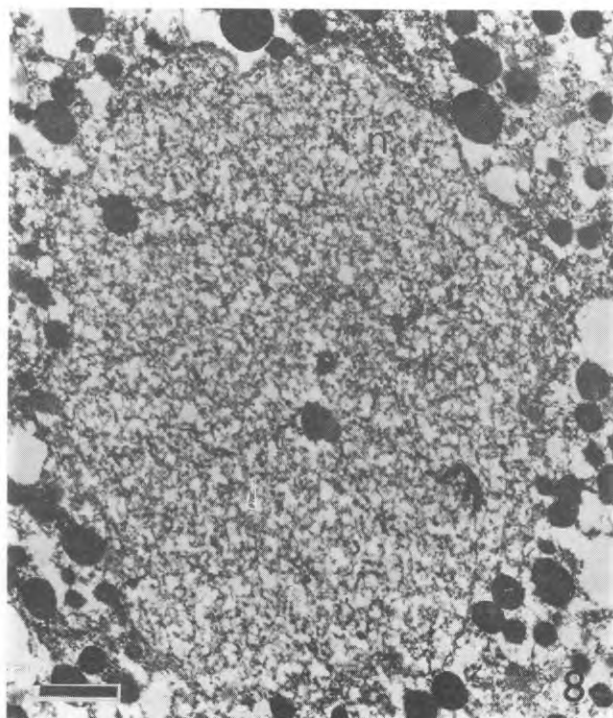


Fig. 8. Large nuclei (n) of the eggs contain condensed chromatin. Bar = 1 μ m. **Fig. 9.** The wall of the epithelial cells in the proximal portion of the uterus is characterized by the presence of large amount of agranular endoplasmic reticulum (a) and mitochondria (m). The apical ends of the epithelial cells are equipped with numerous microvilli. The nuclei of the uterine epithelial cells (n) are surrounded by a conspicuous nucleolemma and they contain a reticulate nucleolus (nu). Bar = 1 μ m.

The thickness of the uterine wall, in its proximal portion, is about 8 μ m and it increases to 19 μ m in the area where eggs with the formed egg-shell appear. The apical ends of the epithelial cells are equipped with microvilli, oriented into the lumen of the uterus. The inside of these cells have large amounts of agranular endoplasmic reticulum and mitochondria. The nuclei are surrounded by a conspicuous nucleolemma and they contain a reticulate nucleolus.

DISCUSSION

Electronmicroscopic investigations of nematode eggshells have been the subject of research conducted by several authors. They have been reviewed by Anya (1976), Wharton (1980), and Foor (1983).

The first conspicuous change observed in the fertilized oocytes of *Syngamus trachea* concerns the redistribution of shell granules in the cytoplasm. Migration of these granules to the periphery of eggs can also be observed in *Porrocaecum angusticolle* (Molin, 1860), *Aspicularis tetraptera* (Nitzsch, 1821), *Ascaris lumbricoides* Linnaeus, 1758, *Heterakis gallinarum* (Schränk, 1788), *Trichuris muris* (Schränk, 1788), (Kochhar 1960, Anya 1964, Foor 1967, Lee and Lešťan 1971, Preston and Jenkins 1984). The displacement of shell granules entails changes in their appearance. Such a change has been recorded in the eggs of *Trichuris muris* (Preston and Jenkins 1984) and *Dictyocaulus viviparus* (Bloch, 1782) (Gutteková and Bruňanská 1990).

Like in *Aspicularis tetraptera* (Wharton 1979a), the original oolemma of the oocyte in *S. trachea* changes after fertilization into the vitelline membrane. From the vitelline membrane having the character of a unit membrane, the vitelline layer of the egg-shell is derived. This is how the vitelline layer also develops in *Trichuris suis* (Wharton and Jenkins 1978). In *Heterakis gallinarum* (Lee and Lešťan 1971) and *Syphacia obvelata* (Rudolphi, 1802) (Wharton 1979b) it has the appearance of a unit membrane, and in some tylenchids, it resembles the trilayer surface membrane of the nematode cuticle (Bird and McClure 1976).

The second layer of the nematode egg-shell appears as a pellucid or very slightly yellowish structureless zone under the vitelline layer. It transforms into a layer, known as the chitinous layer (Foor 1983). Although we have not proved the presence of chitin in the eggs of *S. trachea*, it has been evidenced, however, by the results of biochemical studies on chitin synthesis intermediary products, suggesting that chitin synthesis takes the same course in *S. trachea* as it does in *Ascaris suum* Goeze, 1782 but with a different intensity (Turčková et al. 1991). Some authors identify the formation of the chitinous layer with the occurrence of shell granules also known as reticulate granules (Preston and Jenkins 1984) or as heterogeneous granules (Gutteková and Bruňanská 1990). In our opinion, shell granules are also connected with the formation of the chitinous layer in *S. trachea*. This is evident from the morphological similarity of the electron-density in shell granules and of that in a newly-synthesized

chitinous layer. We believe that shell granules can, by their content, participate in the formation of protein sheaths of chitinous microfibrils.

The ultrastructural studies on the eggs of *Ascaris suum* have shown that the chitinous layer is composed of chitinous microfibrils, 75-400 Å in diameter (Rogers 1956). According to Wharton (1980), chitin in the chitinous layer is often connected with protein. Chitin-protein complexes appear as 2.8 nm thick chitinous microfibrils, covered by a protein matrix (Neville 1975). Preston and Jenkins (1984) believe that the changed appearance of the developing shells, assuming the fibrillar character, may be reflecting the formation of chitin-protein complexes. Such an arrangement was observed in the egg-shell of *Capillaria hepatica* (Bancroft, 1893) (Grigoris and Solomon 1976) and *Trichuris suis* (Schrank, 1788) (Wharton and Jenkins 1978). The ultrastructure of the chitinous layer in *Syngamus trachea* bears a strong resemblance to the chitinous layer in *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, (Bird and McClure 1976). In both the cases a light band of thin reticulate character can be observed under the vitelline layer. The eggs of *Syngamus* in this band also show the presence of dense granules. The presence of granules and fibrils was also described in the chitinous layer of *Ascaris* eggs (Rogers 1956). The origin and function of these granules are obscure.

Between the inner surface of the chitinous layer and the oolemma in *S. trachea*, the inner shell layer is formed. This layer, in other nematodes known as the lipid layer, is of varied appearance in different species. It may be either thin and membranous, as is the case, e. g., in *Aspiculuris tetraptera*, *Syphacia obvelata*, *Hammerschmidtella die-singi* (Hammerschmidt, 1938) Chitwood, 1932 (Wharton 1979 a,b,c), and *Dictyocaulus viviparus* (Gutteková and Bruňanská 1990), or composed of several membranes, distinct on the specimens prepared by freeze etch, as seen in some tylenchids (Bird and McClure 1976). It may also be removed by processing in the laboratory (Rogers 1956) or poorly preserved (Wharton and Jenkins 1978). This layer may, however, not be only of membranous appearance, as mentio-

ned before, but can be formed by three bands of electron-transparent and electron-dense material as observed in *Meloidogyne javanica* (Bird and McClure 1976) or in *Trichuris muris* (Preston and Jenkins 1984). The inner layer of *Syngamus trachea* eggs is not membranous either. It contains round to oval vesicles with a lighter centre. Subpellicular vesicles of similar morphology were described by Bouchet et al. (1986) in the eggs of *Toxocara canis* (Werner, 1782). The presence of vesicles in the inner layer of *Syngamus trachea* is indicative of this layer being formed by a lipoprotein complex. The protein constituent of vesicles may originate from shell granules which by their content contribute to the formation of the inner layer of the nematode egg-shell (Foor 1967, Wharton 1986, Gutteková and Bruňanská 1990).

The layer of fibrous material, deposited on the surface of *Syngamus* eggs, is designated as the uterine layer. In its appearance it resembles a glycocalyx on the surface of the intestinal cell microvilli in some nematodes (Zmoray and Gutteková 1978, Gutteková and Zmoray 1982). The surface (uterine) layer of the *Syngamus* egg-shell, like that of other nematodes (Foor 1983), is of protein character (Foor 1967, Zulauf and Gut 1978, Poljakova et al. 1985).

The formation of *Syngamus* egg-shells entails changes in the micromorphology of the uterine epithelial cells. A gradual thickening of the uterine wall can be observed in the distal direction, as also described in the ovoviviparous species, *Dictyocaulus viviparus* (Gutteková and Bruňanská 1990). The ultrastructure of the uterine wall, namely the presence of endoplasmic reticulum and mitochondria, is suggestive of the high metabolic activity conducive to the creation of the optimum environment for the developing eggs.

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REFERENCES

- ANYA A. O. 1964: Studies on the structure of the female reproductive system and egg-shell formation in *Aspiculuris tetraptera* Schulz (Nematoda: Oxyuroidea). *Parasitology* 54: 699-719.
- ANYA A. D. 1976: Physiological aspects of reproduction in nematodes. *Adv. Parasitol.* 14: 267-351.
- BIRD A. F., McCLURE M. A. 1976: The tylenchid (Nematoda) egg shell: structure, composition and permeability. *Parasitology* 72: 19-28.
- BOUCHET F., BOULARD Y., BACCAM D., LEGER H. 1986: Ultrastructural studies of alterations induced by microwaves in *Toxocara canis* eggs: prophylactic interest. *Z. Parasitenkd.* 72: 755-764.
- BRUŇANSKÁ M. 1991: An ultrastructural study on the germinal zone and rachis of the ovaries in *Syngamus trachea*. *Helminthologia* 28: 165-171.
- BRUŇANSKÁ M. 1992: The ultrastructure of the growth zone of the ovaries in *Syngamus trachea*. *Helminthologia* 29: 7-12.
- FOOR W. E. 1967: Ultrastructural aspects of oocyte development and shell formation in *Ascaris lumbricoides*. *J. Parasitol.* 53: 1245 - 1261.
- FOOR W. E. 1983: Nematoda. In: K. G. Adiyodi and R. G. Adiyodi (Eds.), *Reproductive Biology of Invertebrates*. Vol. 1: Oogenesis, Oviposition and Oosorption. J. Wiley and Sons, Ltd., New York, pp. 223-256.

- GRIGONIS G. I., SOLOMON G. B. 1976: *Capillaria hepatica*: Fine structure of egg shell. Exp. Parasitol. 40: 286-297.
- GUTTEKOVÁ A., BRUŇANSKÁ M. 1990: The ultrastructure of *Dictyocaulus viviparus* uterus. Helminthologia 27: 153-162.
- GUTTEKOVÁ A., ZMORAY I. 1982: Ecomorphological aspects of the intestinal cells ultrastructure of *Thelazia gulosa* (Railliet et Henry, 1910). Biológia 36: 547-557.
- KOCHHARD D. M. 1960: Histochemical studies on the oogenesis and fertilization of the nematode *Porrocaecum angusticolle* (parasite in vulture). Res. Bull. Punjab Univ. 11: 207-219.
- LEE D. L., LEŠTAN P. 1971: Oogenesis and egg shell formation in *Heterakis gallinarum* (Nematoda). J. Zool. Lond. 164: 189 - 196.
- NEVILLE A. C. 1975: Biology of the Arthropod Cuticle. Springer-Verlag, Berlin, 448 pp.
- POLJAKOVA - KRUSTEVA O., BRUŇANSKÁ M., DUBINSKÝ P. 1985: The influence of fertilization on the ultrastructure of *Ascaris suum* eggs. Helminthologia 22: 233-244.
- PRESTON C. M., JENKINS T. 1984: *Trichuris muris*: Structure and formation of egg shell. Parasitology 89: 263-273.
- ROGERS R. A. 1956: A study of eggs of *Ascaris lumbricoides* var. *suum* with the electron microscope. J. Parasitol. 42: 97-108.
- TURČEKOVÁ L., DUBINSKÝ P., RYBOŠ M. 1991: Intermediate products of chitin synthesis in *Ascaris suum* and *Syngamus trachea*. Second International School "Parasite - Host - Environment", 28 May - 1 June, Sofia, Bulgaria.
- WHARTON D. A. 1979a: The structure of the egg-shell of *Aspiculuris tetraptera* Schulz (Nematoda: Oxyuroidea). Parasitology 78: 145-154.
- WHARTON D. A. 1979b: The structure and formation of egg-shell of *Syphacia obvelata* Rudolphi (Nematoda: Oxyurida). Parasitology 79: 13-28.
- WHARTON D. A. 1979c: The structure and formation of the egg-shell of *Hammerschmidtella diesingi* Hammerschmidt (Nematoda: Oxyuroidea). Parasitology 79: 1-12.
- WHARTON D. A. 1980: Nematode egg-shells. Parasitology 81: 447-463.
- WHARTON D. A. 1986: A functional biology of Nematodes. Croom Helm. London and Sydney: 60-87.
- WHARTON D. A., JENKINS T. 1978: Structure and chemistry of the egg-shell of nematode (*Trichuris suis*). Tissue Cell 10: 427-440.
- ZMORAY I., GUTTEKOVÁ A. 1978: Ultrastructure of intestinal cells of *Heterakis gallinarum*. Angew. Parasitol. 19: 106-111.
- ZULAUF E., GUT Ch. 1978: Isolation of messenger RNA coding for eggshell protein of the DNA - eliminating nematode *Ascaris lumbricoides*. Eur. J. Biochem. 82: 577-583.

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