MATERIAL AND METHODS

Appendices with pinworms were obtained from Šikl's Department of Pathology, Faculty of Medicine, Plzeň; fresh females and eggs from the Regional Department of Hygiene and Epidemiology, Prague and from a kindergarten from children with massive oxyuriasis.

The female worms isolated from the appendices were fixed in either 10% formol, or in Zenker's, Bouin's or Baker's fixatives, and embedded in paraffin or gelatine (for paraffin or frozen sections). Fresh eggs were obtained either directly with Schüffner's tube, or extracted from viable female worms stored in saline at 5 °C. Spontaneous oviposition was initiated by raising the temperature to 37 °C. Uterine secretions were removed from the eggs by centrifugation in a solution of 0.01% NaOH and water. Empty egg-shells only were used for the microanalysis. For hatching the eggs were placed in a veronal-acetate buffer with 0.5% pepsin, at 37 °C. The egg-shells were fixed in 1% OsO_4 or 6% glutaraldehyde for 3 hr and dehydrated in either the alcoholic series or in absolute acetone (Arenberg's method 1970). Completely dehydrated eggs were coated in a vacuum chamber with either Au or C (thickness 200 Å). The intensity of the spectral line Sk alpha and Pk alpha were recorded on the screen and their wave length measured with an X-ray spectrometer. Current intensity $1 . 10^{-9}$ A, accelerating tension 15 kV, or current intensity $3 . 10^{-9}$ A, accelerating tension 17.5 kV respectively. Photographs were taken with the Asahi-Petax camera from the screen using cine-film 20 DIN ORWO.

We used standard histological methods in this investigation, and histochemical methods given in Pearse (1960).

RESULTS

In young female worms measuring from 7—8 mm × 0.3 mm, the proximal end of the ovary is occupied by germinal, genital cells (5—5.5 μ in diameter) with basophilic nuclei measuring 3.4 μ. These cells divide by mitosis. Germinal cells with smaller nuclei (2.5 μ) are present in the distal terminal end of the ovary which, in transverse section, measure 18 μ. In another transverse section, an ovary measuring from 23—25 μ contained spindle-shaped oogonia arranged radially around the central rachis. In longitudinal section, the shape of the oogonium appeared to be conical attenuating towards the site of attachment to the rachis; its length was 7.5 μ, maximum width 4.5 μ. Young oocytes started to differentiate from oogonia measuring 15.5—18 × 8.5—14 μ. At first, the arrangement of the oocytes which measured 20.5 × 16.5 μ was similar to that of the oogonia except for their plasma which contained numerous phospholipid and glycogen granules; later, the growing oocytes separated from the rachis. Densely packed oocytes were observed in that part of the ovary, where the ovarial tube measured 45—50 μ in diameter. They were arranged into the so-called germinal columns (Muso 1930) composed of two to three rows of oocytes, in parallel direction to the course of the ovary. The plasma of the oval oocytes (28.5 × 15 μ) contained two types of granules; of these, the smaller granules stained intensely with specific histochemical methods, while the larger granules situated mainly around the nucleus, showed a minimum reaction to these methods. The plasma of the oocytes as well as the plasma of cells in the ovarial wall contained finely dispersed glycogen. Mature oocytes which had passed from the distal end of the ovary to the oviduct, measured 30.5 by 25 μ. In transverse section through the oviduct we found an occasional oocyte only. Its plasma showed dark granulation on the periphery, and a superficial membrane of 0.4 μ in thickness. Large granules were present in the middle plasma of the oocyte. In another series of sections we encountered fertilized oocytes in the anterior uterus portion. These measured 31.5 × 20 μ and were surrounded by a thickened membrane (vitelline membrane) which reacted to acid stains. Glycogen and darkly stained granules were found to concentrate in the peripheral plasma close below the vitelline membrane. We failed in observing egg-shell formation in these young females, because an occasional oocyte only matured and passed through the oviduct to the uterus which, at that time, was filled with secretion only. Neither glycogen nor phospholipid granules could be demonstrated with histochemical methods in

oogonia from the proximal end of the ovary. The plasma of larger oocytes contained PAS positive granules, which remained unaffected by the saliva test. They stained for glycogen with Best's carmine. In frozen sections, the plasma surrounding the nucleus of the oocyte contained phospholipid granules. In the distal portion of the ovary, the plasma of the oocytes arranged in germinal columns contained two types of granules (Plate I, Fig. 1). The smaller granules stained with methods for proteins (Hg-bromphenol blue), for tyrosine (Morel-Sisley and TC), for phospholipids (Luxol blue, Sudan IIIB), for neutral lipids (Fettrot), for acid mucosubstances (AB, pH = 2.6, and Hale). The larger granules did hardly react to these methods, but stained faintly with PAS and acid stains (Azan, Mallory); they were found to be eosinophilic and could be impregnated with Gomori's method. The plasma of the mature oocyte stained faintly with acid stains (Azan, Goldner) differing in that from the darkly stained larger granules in the middle plasma of the oocyte. The periphery of the plasma showed a homogeneous granulation and a thin refringent superficial membrane staining with Luxol blue.

The proximal end of the uterus contained fertilized oocytes with a thickened vitelline membrane staining at the surface blue with Hale's method. Lipoprotein granules were present in the peripheral part of the plasma. The uteri were filled with secretion composed of basic proteins, tyrosine and tryptophan, and lipid droplets.

The formation of the primary egg-shell was observed in the developing egg in a series of histological sections through females measuring 9.5 mm in length, 0.5 mm in width. In the distal end of the ovary, the maturing oocytes were closely pressed together, at a right angle to the course of the ovarial tube, and appeared to be disc-shaped. Large, oval granules were present near the centre of their plasma, while small granules were situated near the periphery. The oviduct contained oocytes with their original vitelline membrane, in which another component of the egg-shell is formed from reserve granules (Plate I, Fig. 2). Small granules were observed in the peripheral plasma of the oocytes. These adhered together with glycogen to the inner surface of the originating primary egg-shell. The thickness of the primary egg-shell was 0.8 μ. Large plasmic granules were present at the poles of the pyriform oocytes. In another section we observed eggs measuring 40 × 25 μ in diameter. The developing primary egg-shell did not abut the superficial plasma of the egg and it was possible to view the perivitelline space. The peripheral plasma of the egg containing large granules was bounded, however, by its own thin membrane (Plate I, Fig. 3).

Histochemical methods disclosed lipoproteins and acid mucosubstances in the small granules, in the large granules an elastin-like protein staining with orcein (Tänzer-Unna orcein method) and aldehyde fuchsine. The distal end of the oviduct contained oocytes with a thickened vitelline membrane which, after treatment with hot HCl stained black with Fontana's impregnation method. Lipoprotein granules were present in the surface of the plasma, large granules at the poles of the oocytes. The primary egg-shell originated on the surface of the vitelline membrane and stained with Luxol blue and Alcian blue at pH = 2.6. Large granules containing protein were found on the surface of the egg plasma and formed a thin plasmic membrane.

Transverse sections through eggs from the anterior portion of the uteri showed a characteristic trihedral shape due to the structure of the completely differentiated primary egg-shell consisting of an inner, plastic layer (0.5—0.8 μ) and an outer solid refringent layer (Plate I, Fig. 4). In longitudinal section, the outer layer of the primary egg-shell showed a marked thickening (up to 1 μ) at three sites responsible for the formation of three edges and for the trihedral shape seen in transverse section. The lumen of the middle uterus contained eggs with a newly formed layer on the surface of the primary egg-shell which originated apparently from adhering secretory granules observed earlier in the proximal portion of the uterus. The thickness of this layer was not uniform; at

the apical pole it was very thin and, with several methods, did not seem to be covering the apical pole. The outer layer of the shell was followed by a middle layer (the original primary egg-shell) and this was followed by an inner layer. The thin site at the apical pole measuring 1.3 × 0.9 μ resembled a pore; it was filled by the middle layer, the differentiation of which into an outer and inner layer has been described in the foregoing text. Special histochemical methods suggested that the filling of the pore was formed by the inner layer of the primary egg-shell only, which had thickened at this site. The entire surface of this portion showed transverse striation. The layer contained a neutral polysaccharide and a protein with SS groups. In the completely formed primary egg-shell, a refringent layer differentiated from its surface. It stained with Luxol blue (Plate II, Fig. 3) and with the method Azan, Ponceau S, pH = 2.6, and reacted to staining for tyrosine (Morel-Sisley and TC) (Plate II, Figs. 2,4). This layer forms the three edges seen in longitudinal section through the egg and the trihedral shape in transverse section (Fig. 1). The inner layer of the egg-shell contained mucosubstances with COOH groups, and lipids. In the middle portion of the uterus, a new outer lipoprotein layer of the egg-shell was formed, which was of ununiform thickness. The apical pole of the egg-shell proper reacted faintly to methods for tyrosine, tryptophan and lipids. In eggs at the gastrula stage and beyond it, the apical pole did not react to tyrosine and tryptophan. The filling of the pore, however, reacted intensely to methods for proteins with SS groups and neutral polysaccharides (Plate II, Fig. 1) similar to the complete inner portion of the middle layer, the thickened part of which formed the filling of the pore. It did not react to Luxol blue and Azan, which stained the outer portion of the middle layer.

The distal end of the uterus and the ovijector contained eggs at different stages of cleavage—at the morula and blastula stage, but not at the so-called "tadpole" stage. The latter stage was found in eggs of females obtained from the anal region.

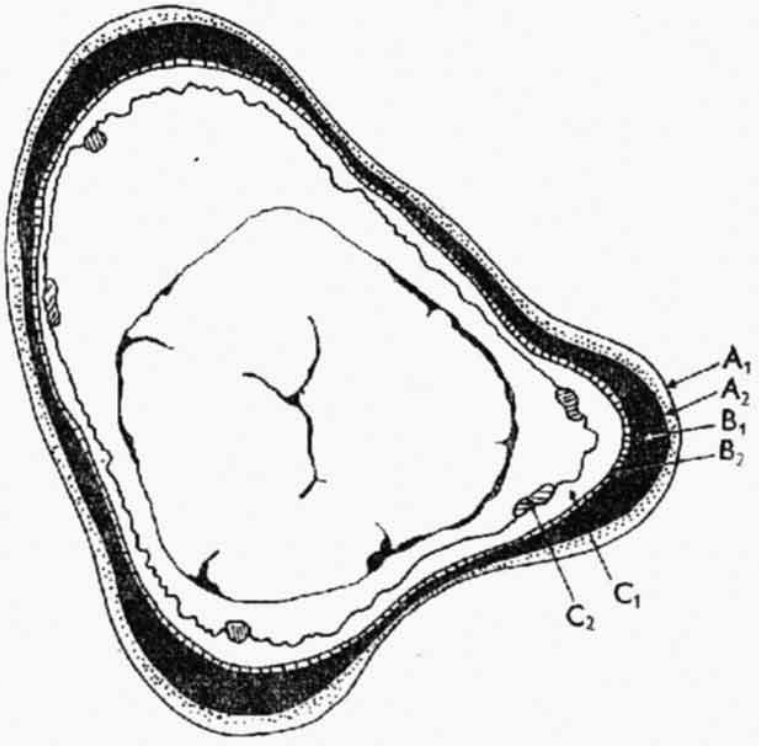

Fig. 1. Schematic representation of a transverse section through the egg of *Enterobius vermicularis*. Note the trihedral shape of the primary egg-shell with thickened edges at the three peaks of the trihedral formation.

Explanations: A_1 — outer superficial layer; A_2 — inner superficial layer; B_1 — outer solid portion of the middle layer; B_2 — inner part of the middle layer; B_3 — plug; C_1 — inner layer; C_1 — innermost layer with adhering granules

The uteri of these females were sac-shaped and packed with eggs, the proximal ends of the ovaries were empty. The structure of the egg-shell of these eggs did not differ substantially from that of eggs from mature females isolated from the appendices. Hence, the definitive egg-shell containing a "tadpole" stage embryo, consists of three principal layers. A subdivision of the three principal layers was observed with histochemical methods (Fig. 2). The first, outer, layer subdivided into two layers; the second, middle, layer (the original primary egg-shell) into two layers, i.e., the outer layer with the typical three edges at the sites of its thickening, and the inner layer which shows transverse striation and fills the pore at the apical pole of the egg. Also the third, principal, layer subdivides into two layers, an outer- and an innermost layer. The surface of the latter is most uneven, because granules of varying size adhere to it.

Histological methods disclosed in the distal portion of the uterus and the ovijector that the outer lipoprotein layer of the egg-shell reacted also with DDD and NBAF for proteins with SH groups. The transverse striation of the inner polysaccharide layer of the middle layer was observed with impregnation methods (Gomori, Rogers).

No changes were observed in the staining properties of the inner layer of the egg-shell containing acid mucosubstances with COOH groups and lipids except for several sites of its inner surface (innermost layer) thickened by adhering spherical bodies which stained intensely with PAA-aldehyde fuchsine for proteins with SS groups (Table 1). We failed, however, in observing either this layer or the granules in eggs collected from the anal region which contained a coiled motile larva already. Treatment of the eggs with various digesting solutions affected the outer layer only; the middle layer remained intact and curved only in accord with the movements of the larva inside it, without changing its histochemical properties. During hatching of the larva, the egg-shell ruptures at the thinned margin of the "operculum" (filling of the pore) and this part of the shell separates together with the remnants of the "operculum".

In eggs from anal scrapings, histochemical methods failed to disclose the presence of lipids in the innermost layer, and that of spherical, PAA-AF positive bodies on its inner surface.

No changes were observed in the histochemical properties of the middle layer after digestion of the outer lipoprotein layer. After hatching, the ruptured filling ("operculum") at the apical pole is turned away, but retains its intense PAA-AF positivity.

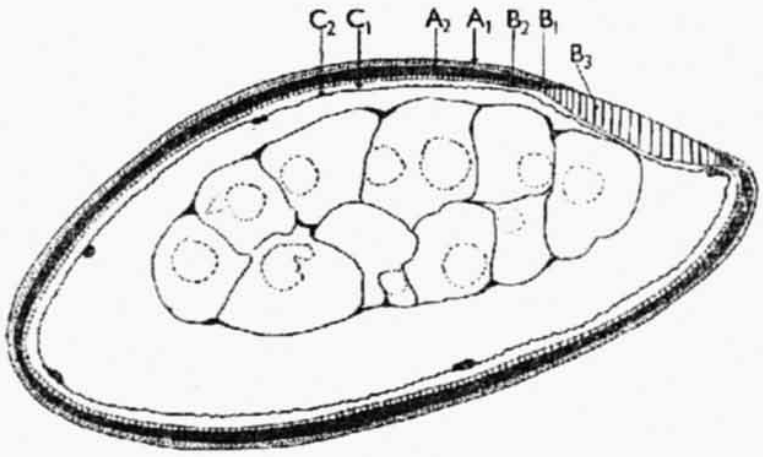

Fig. 2. Schematic representation of the three principal layers of the egg-shell (longitudinal section) and their histochemical subdivision. Note the differentiation of the outer layer into two layers, and the filling of the pore at the apical pole with the thickened inner part of the principal middle layer. The middle layer divides into a solid layer with three thickened edges (seen in longitudinal section), and an inner layer forming the filling of the pore (operculum). The inner layer differentiates into an outer and innermost layer with granules of different size sticking to its surface.

Table 1. Histochemistry of the completely formed egg-shell

Test	Outer layer		Middle layer		Inner layer		Plug
	superficial layer	inner layer	outer layer (forming 3 edges)	inner layer (forming the plugs)	outer layer	innermost layer	
Sudan black B	+++	–	–	–	+++	–	–
Sudan black B with chloroform extraction	–	–	–	–	–	–	–
Luxol blue for phospholipids	–	–	++++	–	++/+	–	–
Luxol blue with pyridine extraction	–	–	–	–	–	–	–
Peracetic acid aldehyde fuchsin reagent for -SS-.	–		–	++++	–	+++	++++
Performic acid alcian blue reagent for -SS-	–	–	–	++++	–	++/+	++++
AB pH 0.2, control	–	–	–	–	–	–	–
Dihydroxy-dinaphthyl-disulphide (DDD) reagent for -SH	–	+++	–	–	–	–	–
Thioglycollic acid + DDD reagent for -SS	–	–	–	++++	–	+++	+++++
Dinitrofluorobenzene (DNFB) method for SH and NH_2	–	+++	–	–	–	–	–
Nitrobromacetofenone (NBAF) method for -SH-	–	+++	–	–	–	–	–
Morel-Sisley method for tyrosine	–	+++	+++	–	–	–	–
DMAB-nitride method for tryptophan	–	++++	–	–	–	–	–
Coupled Tetrazonium (TC) reaction for tyrosine, tryptophan	–	++++	++	–	–	–	–
Mercuric bromophenol blue for proteine	–	++++	–	–	–	–	–
Sakaguchi oxine reaction for arginine	–	+++	–	–	–	–	–
Lugol-aldehyde fuchsin	–	–	–	++	–	–	–
AB pH 2.6	–	–	–	–	++++	+++	–
Demethylation + AB	–	–	–	–	++++	+++	–
Methylation + AB	–	–	–	–	–	–	–
PAS	–	–	–	+++	+	++	+++
Desacetylation + PAS	–	–	–	++++	+/++	+++	+++++
Acetylation 58 °C 48 h + PAS	–	–	–	–	–	–	–
Dialysed iron method (Hale) for acid mucopolysaccharides	–	–	–	–	++++	++	–

In order to obtain exact information on the surface and pattern of the egg-shell, and on the spatial distribution of trace elements in the chemical compounds, a microanalysis was made with the electron microanalyser JXA-5. For this purpose we selected eggs with completely differentiated shells after hatching of the tadpole-like embryo. The stereoscopic picture (taken with a microprobe) of a transverse section through two eggs, one cut longitudinally, the other with an embryo at the gastrula stage transversely, showed the lay-out of the egg-shell (Plate III, Fig. 1). The thickest was the inner layer displaying a fine fibrous structure with an irregular inner surface. This was overlaid by a compact, solid, inner, layer showing a considerable thickening at the site of the apical pole of the egg and partly covering this pole. A transverse slite (pore) was seen in the outer surface of this layer at the apical pole. Another transverse section through the egg taken at the level of its apical pole situated at the basal portion of the trihedral section, showed the massive thickening of the middle layer, and the transverse slit (Plate III, Fig. 2).

The microanalysis of trace elements was made in two egg-shell from which the embryos had been removed in order to obtain exact information on the quantity of these elements in the shell, which otherwise could be influenced by the presence of similar elements in the embryo. We traced the course of the beam (Plate IV, Fig. 1) and measured the intensity of its spectral line with an x-ray spectrometer. After disclosing that the spectral lines under consideration were those of sulphur (Sk alpha), a line scan (Plate IV, Fig. 3) was made from which the maximum and minimum values of density and spatial distribution of sulphur could be read. The viewing screen showed always two lower and one highest maximum at the moment when the line beam past through that layer of the shell which, in the previous picture, had been dark and compact. The line scan disclosed also a certain elevation at the site of location of the granules of uterine secretion. Density and distribution of sulphur in both shells was indicated by spots of light on the viewing screen (Plate IV, Fig. 2).

The microanalysis disclosed also traces of phosphorus in the egg-shell, and the line scan showed the maximum during the passage of the beam through the compact layer of the shell (Plate IV, Fig. 4).

DISCUSSION AND CONCLUSION

Examination of eggs of various parasitic nematodes disclosed three characteristic layers in the egg-shell, i.e., the outer lipoprotein layer (protein coat); the middle chitinous layer (egg-shell proper); the inner lipoid layer. This finding has been confirmed by numerous authors (Christenson 1950; Fairbairn 1955; Fairbairn and Passey 1955; Rogers 1956; Kochhar 1960; Lee 1961; Monné 1962; Anya 1964, and others).

Also we observed three principal layers in the egg-shell of mature *Enterobius vermicularis* eggs, i.e., the outer-, middle- and inner layer. Histochemical methods, however, disclosed that each of the three principal layers was subdivided into two layers. The outer into a superficial, lipoid, layer and an inner, protein, layer; the middle layer into a refringent layer reacting intensely to staining with Luxol blue, and with three thickened edges seen in longitudinal section and a trihedral shape seen in transverse section, and into a layer containing neutral polysaccharides and proteins with SS groups. The inner layer subdivided into a layer containing lipids and acid mucosubstances with COOH groups, and a layer forming the innermost surface of the shell; this reacted intensely to staining and bore PAA-aldehyde positive granules on its surface. Zawadowsky and Schalimov (1929) apparently differentiated two layers in the middle ("chitinous") layer which accounts for the four membranes of the egg-shell described by them. Ina-

tomi (1957) studying the ultrastructure of the egg-shell in the electron microscope disagreed with Leuckart (1876) and Yoshida (1923) in that an operculum or chitinous plug was present in the egg-shell, but supported Nishio's (1924) suggestion that the egg-shell consisted of two layers which were both of chitinous structure and lacked the presence of a distinct plug. The thinnest part of the outer layer was observed at the site which ruptured during the hatching of the larva. Our observations suggested that the "operculum" differentiated as a filling at the site of the pore on the apical pole of the egg, and originated on the surface of the primary egg-shell after the formation of the outer lipoprotein layer which failed to cover this pole. We agree with Wilson (1958) in that it is difficult to encounter the "operculum" region in histological sections; in a not fully developed egg this region looks like an asymmetry. The filling of the pore (operculum) is formed by a thickening of that portion of the middle layer which contains a neutral polysaccharide and a protein with SS groups. This inner part of the middle layer showing transverse striation with impregnation methods appears to be analogous to the submicroscopic tubules observed by Inatomi (1957). Yanagisawa and Ishii (1954) and Yasaki (1958) did not mention an operculum in *Ascaris* eggs, but suggested the presence of a chitinous plug in view of the chitinous structure of the middle layer. Several other authors (Fauré-Frémiet 1913; Chitwood 1938; Christenson 1950; Yanagisawa 1955; Yasaki 1958; Kochhar 1960; Lee 1961; and others) also considered this layer to be chitinous. Monné (1962) observed an intense reaction for SH groups in the outer lipoprotein layer of the *Ascaris* egg-shell, and for proteins with SS groups in the chitinous middle layer. We found, however, that the outer lipoprotein layer reacted for SH groups only with eggs located in the distal end of the uteri of mature females. We confirmed a positive reaction for proteins with SS groups in the inner portion of the middle layer in eggs from the mid-uterus, while eggs from the proximal end of the uterus reacted only faintly to staining for SS groups. Histochemical methods confirmed that this protein with a high cystine content was associated with a polysaccharide. Jaskoski (1962) disclosed with chromatography the presence of cysteine-cystine in the middle and inner layer of *Ascaris lumbricoides* egg-shells. Yasaki (1958) also found a protein with SS groups associated with a polysaccharide, in the middle layer of the egg of *Ascaris suum*. According to our observation the outer lipoprotein layer of the egg-shell reacted more intensely for tyrosine than the refringent part of the middle layer forming the three edges. Anya (1964) confirmed a more intense reaction for tyrosine in the middle chitinous layer of *Aspiculuris tretaptera* eggs. Zawadowsky (1929) found a higher percentage of amino acids in the outer layer of the egg-shell, Jaskoski (1962) reported also the presence of arginine and glycine-serine for eggs of *Ascaris suum*.

Kochhar (1960) studied the formation of the egg-shell from glycogen and reserve substances during the oogenesis of *Porrocaecum angusticolle*. He observed two types of granules in the oocytes which he called "lipoid" and "protein yolk" (also hyaline spheres), but failed to find an association between the protein yolk ("hyaline spheres") and the formation of the middle "chitinous" egg-shell layer, as did Rogers (1956), Mercer (1962), Monné (1962). On the other hand, Kochhar (1960) observed that these granules and the glycogen move towards the surface of the oocyte during fertilization, although glycogen only is important for the origin of chitin in the middle layer of the egg-shell. Also we found two types of granules in the oocyte, i.e., small darkly staining lipoprotein granules, and large protein granules. The first were present in the peripheral part of the plasma of oocytes with a thickened vitelline membrane, the second were first found in the centre of the oocyte, and moved from there to below the thin membrane bounding the plasma of eggs which, at this time, are forming the primary egg-shell. No large granules, however, were present in eggs with a completely differen-

tiated egg-shell. Anya (1964) demonstrated that granules of the "hyaline spheres" form the protein- or phospholipid fraction of the "chitinous" layer. Fauré-Frémiet (1913) and Fauré-Frémiet et al. (1954) suggested that the content of these granules consisting mainly of protein, is secreted through the membrane of the fertilized oocyte to the perivitelline space, while Yanagisawa (1955) maintained that the hyaline granules form the third layer of the "hard-shell" which Rogers (1956) failed to demonstrate.

In experimental digestion of the egg-shell, no changes were observed in the histochemistry of the thickened filling (operculum) at the apical pole of the egg. Changes, however, were observed in its morphology.

The method of a microanalysis of elements with the electron microanalyser JXA-5 has not been used previous to this study for biological material in Czechoslovakia. We are convinced that this method will provide a considerable amount of information on the surface of the tissue (Green 1967; Ishii and Myazaki 1970) and will make it possible to demonstrate with great exactness the content and spatial distribution of trace elements in chemical compounds forming the tissue. The spatial distribution of elements as observed by us shows clearly that the high content of sulphur in the egg-shell is not homogeneous in its distribution, but depends entirely on the morphological structure of the egg-shell. Phosphorus was demonstrated in the middle layer of the shell only. This is consistent with the histochemical evidence on the presence of phospholipids.

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ГИСТОЛОГИЧЕСКИЕ И ГИСТОХИМИЧЕСКИЕ ИССЛЕДОВАНИЯ ЯЙЦЕОБОЛОЧКИ У *ENTEROBIUS VERMICULARIS*

Д. Гулинска и В. Гулински

Резюме. В работе приведены результаты гистологического и гистохимического исследования оболочки яйца острицы. Оболочка яйца состоит из трех основных слоев. Наружный слой составляет липопротеин; средний слой составляет нейтральный полисахарид, связанный с протеином содержащим группы дисульфидов (SS) и перекрыт плотным липоидным слойком, содержащим тирозин. Внутренний слой составляют мукополисахариды с группами COOH и липиды. Главную яйцеоболочку ооцита образуют цитоплазмические гранулярные запасы яйцевода и после оплодотворения таковая является окончательной у яиц в верхней части матки. Внешний новый слой окончательной оболочки образован секрецией матки. Гистохимическим путем обнаружена покрышка (operculum), возникающая в точке поры на аникальном полюсе яйца; ее образуют нейтральные полисахариды и протеин с группами дисульфидов (SS). Эту пору наблюдали с помощью электронного микроанализатора JXA 5. Метод микроанализа элементов дал возможность уточнить содержимое и распределение серы и фосфора в яйцеоболочке.

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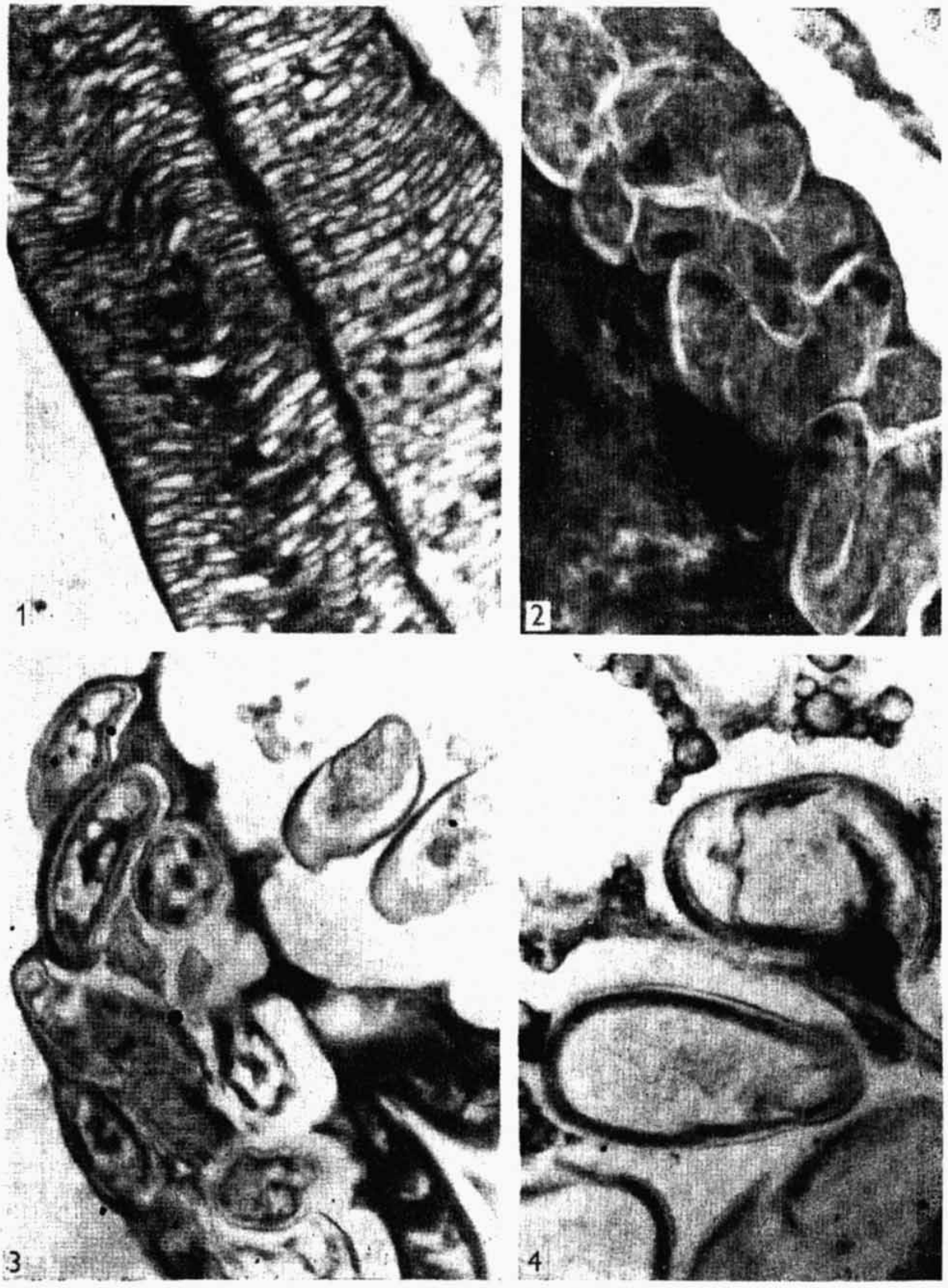

Fig. 1. Tangential section through the distal end of the ovary of mature females with oocytes pressed into the so-called germinal columns. Note the darkly stained oval granules and the faintly stained homogeneous granules in the plasma of the oocyte (Azan and Luxol blue; × 82).

Fig. 2. Longitudinal section through the distal portion of the oviduct containing mature oocytes. The primary egg-shell differentiates from the vitelline membrane on the surface of the oocytes. Note the small, darkly stained granules below and the large granules at the poles of the oocyte. (Goldner + + Luxol blue, × 53.7.)

Fig. 3. Longitudinal section through the upper end of the uterus with the eggs. The primary egg-shell consists of an outer lipoid layer (outer outline) and an unstained inner layer. The membrane thickened with coarse granular particles is seen on the plasma surface (Azan, × 37).

Fig. 4. Tangential section through the eggs in the mid-uterus. The middle layer of the primary egg-shell is darkly stained. Note the granules of uterine secretion among the eggs. (PAA-AF × 89.)

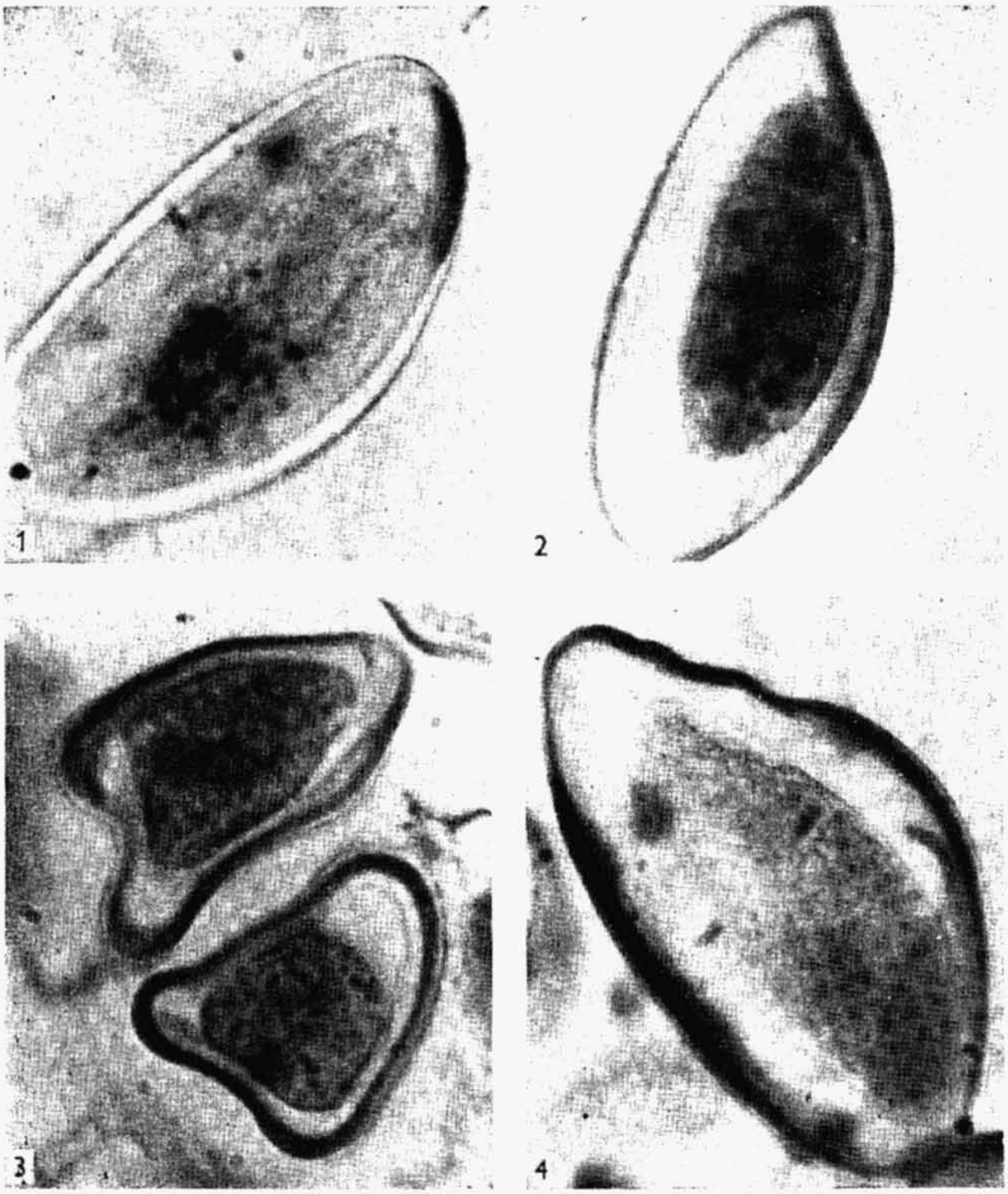

Fig. 1. Oblique, longitudinal section through the egg containing a coiled embryo. Intense staining for proteins with SS groups in the "operculum" at the site of the pore, and of the granules in the oesophageal region of the embryo. (PAA-AF, ×530.)

Fig. 2. Longitudinal section through an egg with a cleaving embryo. Intense staining by tetrazonium coupling of the outer lipoprotein layer and the outer part of the middle layer. (TC, ×530.)

Fig. 3. Transverse section through eggs in mid-uterus. Note the thickened supports on the three peaks of the trihedral formation formed by the outer part of the middle layer. (Luxol blue + PAA-AF, ×530.)

Fig. 4. Longitudinal section through an egg with a darkly stained outer portion of the middle layer forming the thickened edges. (DMAB — Morel-Sisley, ×530.)

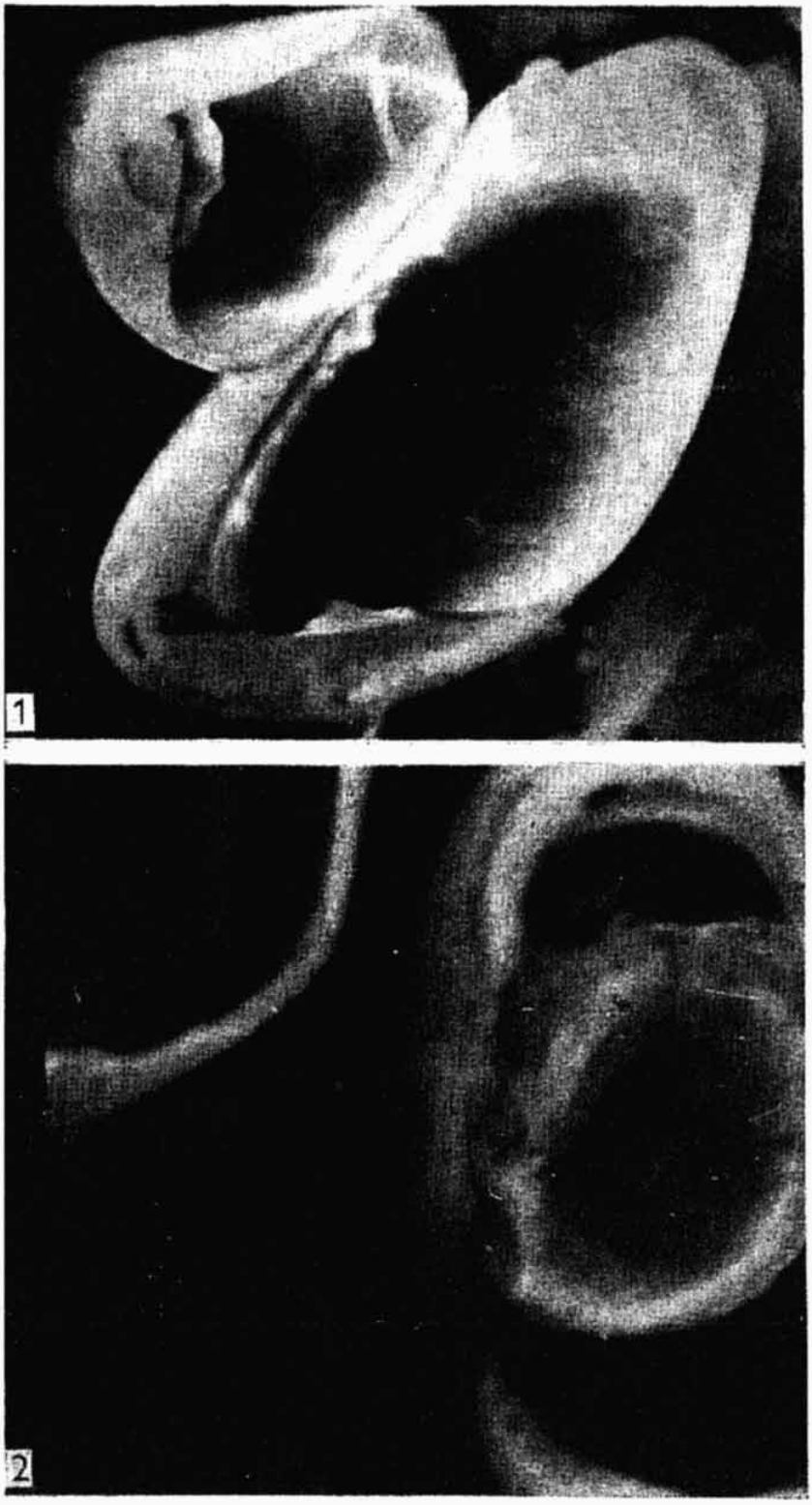

Fig. 1. Stereoscopic picture of the egg-shell (longitudinal and transverse section) taken with the microprobe JXA-5. Note the "operculum" region and the transverse slit (pore) at the apical pole in the longitudinal section. The inner layer appears as a fine fibrous structure (×980; Au-coating; accelerating voltage 15 kV; current intensity 1.10^{-9} A.

Fig. 2. Stereoscopic picture of an egg with the embryo at the gastrula stage (transverse section). Note the distinct "operculum" region and the pore in the outer layer at the apical pole of the egg-shell. (× 1057.)

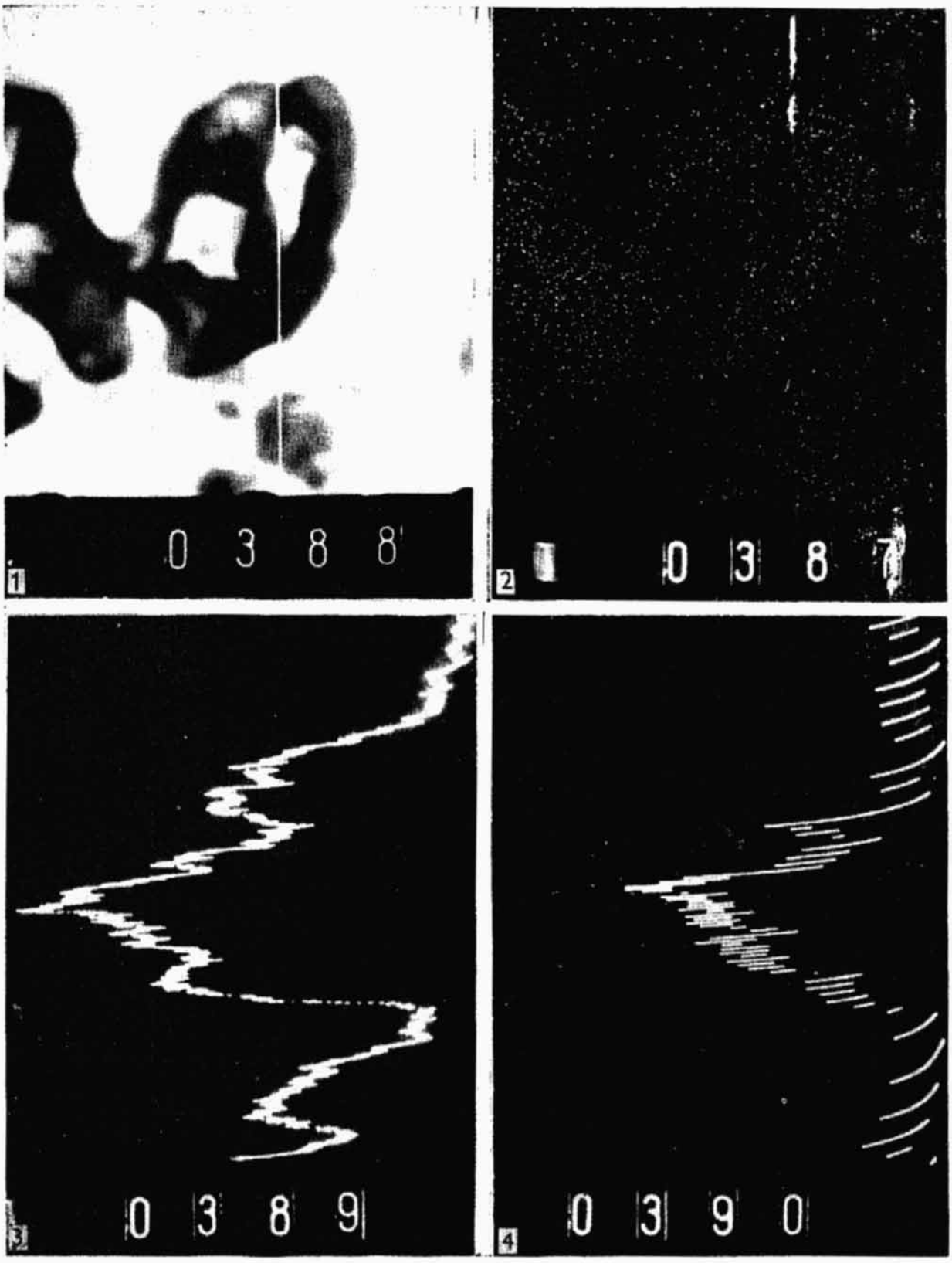

Fig. 1. Linear analysis with trace elements. Note the high concentration of sulphur in the egg-shell with a solid middle layer; egg-shell partly deformed by removal of "tadpole" embryo. (Current intensity 3.10^{-9} A; accelerating voltage 17 kV; × 642.)

Fig. 2. Spatial distribution of sulphur in two egg-shells, of which one was scanned. The spots indicating the sites of sulphur location are clearly visible on the screen (PET crystall, × 642).

Fig. 3. Line scan of sulphur content in the egg-shell (values the same as in Fig. 1). Sulphur was highest at the site of the solid layer (middle layer), but generally high throughout the egg-shell. A low percentage of sulphur is indicated by the scan in the secretory uterine granule.

Fig. 4. Line scan of the content of phosphorus in the egg-shell from Fig. 3. Although phosphorus content was considerably lower than sulphur content, the site of maximum incidence was identical (× 642; accelerating voltage 17 kV, current intensity $3 . 10^{-9}$ A).

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STUDIES ON THE MORPHOLOGY AND HISTOCHEMISTRY OF THE MALE GONAD AND SPERMATOGENESIS IN ENTEROBIUS VERMICULARIS (LEACH, 1853)

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Abstract. The male reproductive system consists of a testis, a seminal vesicle, a vas deferens and an ejaculatory duct, the latter forming the cloaca together with the rectum. In the vas deferens, we distinguished three sections differing in histological structure and histochemical reactions of the various granular substances. The granules of the proximal section were mostly proteinaceous, of the medial section lipoidal, of the distal section lipoproteinaceous; the latter were pyroninophilic and gave a positive reaction for alkaline phosphatase. The testis contained oval spermatogonia, spherical spermatocytes with concentrically arranged basophilic granules, and spermatids with a basophilic rodlike formation [the microtubule—chromatin complex (Lee and Anya, 1967)]. Scanning electron micrographs of the spicule surface disclosed verrucae and a groove in the ventral surface of the attenuated proximal spicule portion. The shape of the anal papillae was mammillate.

Information on the morphology of the male gonad of *E. vermicularis* is available only from the older literature. We have studied in detail the histology and histochemistry of the male gonad, and spermatogenesis of the human pinworm. The microscopical anatomy has been described for oxyurids parasitic in animals, i.e., for *Oxyuris curvula* by Martini (1926); for *Aspiculuris tetraptera* by Anya (1966). The sperms have been described by Meves (1920) for *Passalurus ambiguus*, and by Lee and Anya (1967) for *Aspiculuris tetraptera*. Numerous studies are available on the morphology of the male *Ascaris megalocephala* and *Ascaris lumbricoides* (Mayer 1908; Musso 1930; Sturdivant 1934; Pasteels 1948; Favard 1961, and others). Grassé (1965) reviewed the morphogenesis and spermatogenesis of various nematodes. Leuckart (1876) and Heller (1903) distinguished four sections in the gonad of the male *E. vermicularis*, i.e., testis, sperm duct, seminal vesicle and ejaculatory duct. Leuckart maintained that the seminal vesicle was below the sperm duct and observed several layers of large, spherical, lipoidlike cells in its wall; the wall of the seminal vesicle was formed by a cylindrical epithelium. Inside the vesicle he observed minute cells showing amoeba-like movement which he suggested to be spermatozoa. Various other authors, however, observed in the epithelium of the vas deferens (sperm duct) of various parasitic nematode species differently large granules arranged in several layers, and expressed various ideas on their origin. In histochemical studies on the male gonad of *Aspicularis tetraptera*, Anya (1966) disclosed granules of different histochemical composition and suggested that these may have been formed by the secretion of the epithelium of the vas deferens.