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THE HISTOLOGY AND HISTOCHEMISTRY OF THE CUTICLE OF FEMALES OF ENTEROBIUS VERMICULARIS

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Dedicated to Dr. J. Šlais D. Sc. on the occasion of his 50th birthday

Abstract. The cuticle of the human pinworm was studied with histological and histochemical methods and with the method of electron microanalysis. The results indicate that the cuticle is approximately 4 μm thick and that it consists of 8 layers: the outer and inner cortical layer, the intermediary layer, the homogeneous layer, the outer, middle and inner fibrillar layer, the basal layer and the 0.1 μm thick refractive layer on the surface. All layers pass without interruption into the lateral cuticular ledge. Acid mucosubstances were demonstrated in the content of the anterior portion of the ledge. Histochemically, the ledge represents a differentiated cuticular formation. The presence of proteins with SS and SH groups and that of phospholipids was demonstrated in the cuticle with histochemical methods and studied in detail with the electron microanalyser JXA-5 employing the method of a microanalysis of the elements. This enabled the exact detection of substances in the content, and showed the spatial distribution of sulphur and phosphorus in the cuticle.

The cuticle of small parasitic nematodes consists of three basic layers: the cortex, the matrix (called also homogeneous layer) and the fibrillar layer (Inglis 1964). The three basic layers may be subdivided into several layers; this depends on the body measurements and the nematode species as pointed out by Mueller (1927), Watson (1965) and Lee (1960).

In spite of the interest in the histological and histochemical structure of the cuticle of various nematode species, only Bogoyavlenskiy and Drynochkina (1967) studied this subject and published a brief survey of the histological character of the cuticle of *Enterobius vermicularis*. In our opinion, some deeper knowledge of the histochemistry of the cuticle of this nematode may help to solve various questions of its biology and may also be useful in the search for effective anthelmintics. and in the identification of pinworm remnants in oxyuric granulomas.

MATERIAL AND METHODS

Our material was obtained from Šikl's Department of Pathology, Medical Faculty, Plzeň; from the Centre of Hygiene and Epidemiology, Prague, and from several persons with massive oxyuriasis. Fresh material was collected from the morning stool of three children before medication. Viable females were fixed either immediately or transported in test tubes cooled in thermos flasks with dry ice to the laboratory. For standard morphological and histological inspection female pinworms were fixed in 10% formol. For histochemical purposes, fresh material was fixed for 24 hrs in either formol (using Baker's method), or in neutral 5% formol; unfixed tissue was used for the preparation of frozen sections. For electron microscopy, fresh material was fixed with a special fixative consisting

of 6% glutaraldehyde and a 0.1% solution (after Millonig) for 90 min. Standard histological methods were used for embedding paraffin sections in paraffin and frozen sections in gelatine. Very good results were obtained in some histological reactions by using the method of faster embedding of the tissue which, fixed with Baker, was cleared with benzene, dehydrated with absolute alcohol and embedded in paraffin.

In addition to standard histological staining methods we used the following histochemical reactions: PAS; PAS with acetylation; PAS with deacetylation; Best's carmine; Alcian blue pH = 2.5; Alcian blue with methylation and demethylation; Hale's method in Müller's modification using colloidal iron for the control preparations; Hale-PAS; 0.1 % toluidine blue for metachromic substances; bromphenol blue; aqueous bromphenol blue for basic proteins; Morel—Sisley's reaction for tyrosine; Adams' method with p-dimethyl-aminobenzaldehyde-nitrite (DMAB) for tryptophan; dihydroxy-dinaphthyl-disulphide (DDD) for sulphydryl (SH) groups of proteins; control sections were blocked with N-ethylmaleinimide; dihydroxy-dinaphthyl-disulphide (DDD) with thioglycolic acid for disulphide (SS) groups; Alcian blue with performic acid (PFA AB) for disulphide groups; coupled tetrazonium for tyrosine; dinitrofluorobenzene (DNFB) for SS groups; Sudan black B with hot chloroform extraction; oil red 0; luxol blue with pyridine extraction.

For the special method of the microanalysis of elements in the cuticle with the microanalyser JXA-5 we used only fresh material fixed in either 10% neutral formol or 6% glutaraldehyde, or unfixed material frozen at —25 °C [Noi (1969)]. The material employed consisted partly of whole females, partly of extracted cuticle. For embedding we used paraffin, gelatine. For cutting longitudinal and transverse sections (thickness 3 to 30 μm) we used the frigistor microtome and the microtome MSE. The sections were glued to special short quartz slides covered with a thin layer of glue Durofix, or with a fine film of gelatine, or they were placed onto a thin stripe of Scotch tape. Deparaffined and dehydrated paraffin sections and dehydrated gelatine sections were covered with a 200 Å thick layer of Au and C in a vacuum to be used for spectral micro analysis. The intensity of the spectral lines Sk alpha and Pk alpha were recorded on the viewing screen of the cathode-ray tube, their wave length was determined with a crystal x-ray spectrometer. The best image of the surface of the cuticle was obtained from tissue fixed with formol, or from unfixed tissue frozen at -25 °C, stretched either on Scotch tape or sectioned on the frigistor microtome. Metal-coating of the tissue had also to be perfomed at -25 °C (Nei 1969). Photographs from the cathoderay tube of the electron microanalyser were taken with an ASAH-PETAX camera on 20 Din ORWO cine-film and treated with standard techniques.

RESULTS

a) THE MORPHOLOGY OF THE CUTICLE OF *ENTEROBIUS VERMICULARIS*

The cuticle of the pinworm consists of several layers, as does also that of other nematode species, and its surface is divided by grooves into rings. These are visible in longitudinal sections and are mostly narrower in the middle part of the ventral side of the body than in the dorsal side; this may be in accord with the physiological bending of the worm to the ventral side after isolation from the intestine. The width of the rings on the cephalic and tail end of the body, however, is the same both dorsally and ventrally. In most of the histological staining methods, the proper surface of the cuticle differs more in its refractive properties than in specific staining (Fig. 1). The thickness of this superficial layer (A) is always below 1 μm. The cortical layer (B) following the superficial layer is generally subdivided into an outer layer (B_1) and an inner layer (B_2). On a stretched dorsal surface of the body, it is sometimes difficult to distinguish the annulation in longitudinal sections, because the grooves are very shallow and seem to be extended only as far as the outer cortical layer, while the inner cortical layer lies uninterrupted under the shallow grooves. In longitudinal sections the cortical layer is thickest in the centre of the ring and attenuates towards both ends. The maximum width of the outer cortical layer is 0.6 μm in the centre of the ring, while the corresponding part of the underlying inner cortical layer attains almost 1 μm. The intermediary fibrillar layer (C) is situated between the cortical and the homogeneous layer. The homogeneous layer (D) (width 1.6 μm) proceeds as an almost equally wide stripe

similar as the fibrillar layer (E, F, G) (width 1.5 μm). The ventral side of the cuticle is bordered by a distinct limiting membrane (H) of approximately 0.1 μm in thickness. Under the cuticle is also a hypodermis and a muscle layer of an ununiform thickness similar as that in the layer of parenchymatous tissue, which forms an irregular layer under the muscle cushion of the wall and establishes the communication between the body wall and the internal organs. The grooves in the cortical layer of a densely folded wall (Fig. 2) are relatively deep in the ventral side close to the opening of the genital organ extending almost to the homogeneous layer. The refractive properties of the superficial layer covering the surface of the rings and grooves remain. however, unchanged and the layer is of an almost uniform thickness (0.1 μm). At this functional state of the cuticle, the outer cortical layer is thinnest in the centre of the ring and thickens towards both ends. The same situation occurs in the inner cortical layer which is elevated at both ends. The outer cortical layer is slightly thinner in the centre of the ring (approximately 0.5 μm) similar as in the centre of the ring of the inner cortical layer (0.5 μm), because the main bulk of the cortical layer is forced towards the groove. An undulation of the course of both the intermediary and the homogeneous layer is the natural consequence of these conditions; the thickness of these layers, however, is 1.6 μm and that both under the centre of the ring and under the groove. Also the fibrillar layer being compressed in the part facing the centre of the ring showed an undulated course

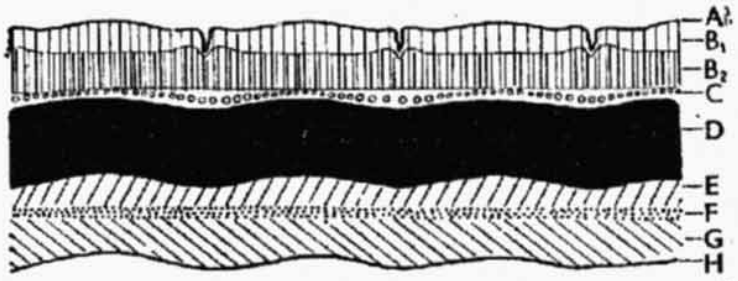

Fig. 1. Reconstruction of the cuticle of *Enterobius vermicularis;* the extension of the wall is normal in the longitudinal section. The cortical layer covered by a superficial layer is divided into rings by grooves. These penetrate the complete outer cortical layer, but affect only moderately the inner cortical layer. An intermediary layer visible only with certain staining methods, lies between the slightly undulated homogeneous layer and the cortex. The three fibrillar layer—the outer, middle and inner layer—are bordered by a basal membrane.
Key to lettering of figures: A — superficial layer; B — cortex; B_1 — outer cortical layer; B_2 — inner cortical layer; C — intermediary layer; D — homogeneous layer; E — outer fibrillar layer; F — middle fibrillar layer; G — inner fibrillar layer; H — basal membrane; Ch — branching of the outer fibrillar layer into the lateral ledge; I — matrix of the ledge; J — homogeneous layer in the distal portion of the ledge; K — ribbonlike fibrillar layer in the basal part of the ledge; L — homogeneous fibrillar layer in the basal portion of the ledge; M — inner fibrillar layer in the basal portion of the ledge

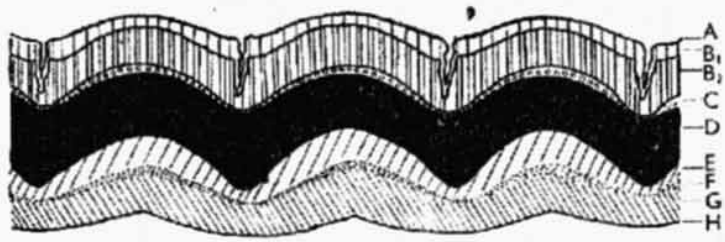

Fig. 2. Reconstruction of a densely folded cuticle on the ventral side of the body close to the vulval opening. The grooves in the cortical layer are deep touching almost the homogeneous layer. The outer cortical layer is narrowest in the centre of the ring and thickens towards the margins as does also the inner cortical layer. The course of both the homogeneous and the fibrillar layer is undulated.

and an ununiform appearance; it attained a thickness of over 4 μm, being thinner under the groove (approximately more than 2 μm). The limiting basal membrane adapts its course to that of the fibrillar layer. Extremely undulated is also the course of the hypodermis, but the musculature and the parenchyma of the body wall are not affected by this functional state.

In a transverse section throught the worm (Fig. 3) the superficial refractive membrane showed up very clearly. The thickness of the cortical layer varied in relation to the shape of the body and it was difficult to dinstinguish the inner and outer cortical layer. The measurements of the homogeneous and fibrillar layers were similar to those observed in a longitudinal section through the stretched cuticle. The character of the basal membrane remained unchanged. The cuticle changed at the site of entrance into the lateral cuticular ledge (Fig. 3) which, in the adult female, attained a general height of 30 μm. Its surface is formed by a layer of medium thickness (17 μm) and into it enter both the cortical layer (B) and the homogeneous layer (D). Considerable changes were observed in the fibrillar layer which enters the ledge and differentiates in its inner content. In one portion of the content of the lateral ledge, leading from the margin toward the centre, we distinguished an area of about 17 μm with a different matrix (I),

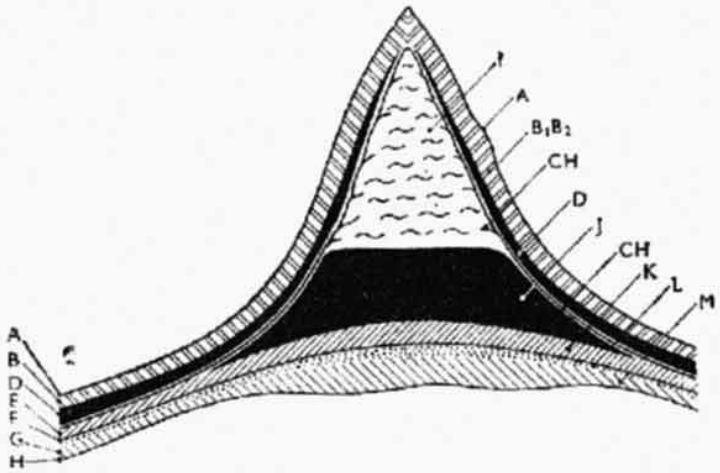

Fig. 3. Reconstruction of the lateral cuticular ledge and the cuticle in transverse sections. The ledge is covered by a superficial layer and by an undifferentiated cortex. The inner content of the ledge consists of an upper portion formed by outer fibrillar branches and a matrix, and of a distal portion formed by the homogeneous layer. The basal part of the ledge consists of three fibrillar layer: the middle ribbonlike layer, the homogeneous layer and the inner layer.

into which entered and branched refractive fibrils (Ch), apparently from the distal border of the homogeneous layer (Fig. 3). It is possible that these fibrils belong to the superficial (outer) fibrillar layer, which is only little differentiated outside the area of the lateral ledge. The content of the distal portion of the lateral edge is formed by the homogeneous layer (J) which is 5 μm thick and has no distinct structure. The homogeneous layer is followed by a ribbonlike undulated layer (K) attaining up to 2 μm in thickness; the structure of this layer is different and it may be considered to be the swollen and undulated middle fibrillar layer, because the upper half of the fibrillar layer situated at the side of the ledge passes smoothly into it. In the middle of the ledge, an almost 1 μm thick homogeneous layer (L) of the content separates the extended, 3 μm thick, basal layer, which receives the distal half of the fibrillar layer around the ledge. This layer appears to be the swollen inner fibrillar layer (M). The cuticle is bordered by a limiting basal membrane (H) which is uniform in its thickness even in the area of the ledge and, hence, confirms that the lateral ledge is a differentiated cuticular

formation. In younger females with a not fully differentiated genital organ, the lateral ledge is smaller. Its height attains about 20 μm, its surface is formed by a layer of 10—12 μm consisting of a cortical and homogeneous cuticular layer.

b) THE HISTOCHEMISTRY OF THE CUTICLE OF *ENTEROBIUS VERMICULARIS*

As regards its histochemistry, the cuticle is a highly differentiated organ as indicated by the results of histochemical reactions (Tables 1, 2). The superficial layer differs histochemically from the cortical layer more in its refractive than staining properties. In almost all reactions in which the inner cortical layer stains positively, the staining of the superficial layer is more intensive with a specific refraction of light. This layer as demonstrated in the tables (Tables 1, 2) consists of a scleroproteid with a high content of cystine and has a high refraction index. This was confirmed by reactions which demonstrated an accumulation of SS and SH groups of proteins (Plate I, Figs. 1, 2) bonded by the present neutral lipids. With methods reacting to the presence of acid mucosubstances, the refractive index of the feebly stained superficial layer was increased. It was not always possible to demonstrate with histochemical methods the division of the cortical layer into an outer and an inner layer. In extremely extended tissue, these layers could not be distinguished particularly with methods for proteins with SS and SH groups, in which the staining of the superficial layer was identical with the staining of this layer in a cuticle in which the cortical layer was clearly differentiated into an outer and an inner layer. In a densely folded wall, the outer cortical layer did not stain with methods for proteins with SS and SH groups, while the inner cortical layer reacted positively to these methods. Also a certain amount of lipids (Plate I, Fig. 3) and especially phospholipids, was found in the inner cortical layer. Neither acid mucosubstances nor several amino acids (arginine, tyrosine, tryptophan) could be demonstrated in the cortical layers. A thin differentiated intermediary layer, sometimes appearing as if consisting of fibrils, was demonstrated with several methods between the cortical and the underlying homogeneous layer. The staining properties of this layer were similar to those of the middle fibrillar layer in some methods only (Table 1). The presence of neutral polysaccharides and tryptophan was indicated by the results of histochemical reactions. The fibrillar structure in the intermediary layer did not stain with Gomori's method and, therefore, its fibrils seemed to be of the collageneous type as suggested also by the PAS positive reaction. The homogeneous layer was demonstrated with several histochemical reactions independent on the functional state of the cuticle. The appearance of this layer was that of a moderately undulated stripe characterized by the presence of a high amount of tyrosine and arginine and a smaller amount of acid mucosubstances. There was also present a minute amount of lipids, but no proteins with SS and SH groups. This layer was followed by three fibrillar layers, the outer, middle and inner fibrillar layer. The outer and inner layer contained a high amount of reducing substances which, with impregnation methods, are responsible for the black staining. Apart from the middle layer, the presence of proteins with an accumulation of SS groups was demonstrated in these layers; their intensity however, was less high than that in the surface and the cortex of the cuticle. Of the amino acids we found a small amount of tyrosine and arginine. By contrast to the middle layer, which was found to be highly PAS positive and to contain tryptophan, neither tryptophan nor polysaccharides were found in the remaining two layers. All other histochemical methods referred to in the foregoing text gave a negative reaction in the middle fibrillar layer. Silver-reducing substances and lipids were found in the basal membrane.

The results of the histochemical reactions of the cuticular ledge are shown in Table 2. The character of the superficial layer remained unchanged and histochemical reactions are the same as those in the remaining parts of the cuticle. The staining properties of the cortical layer are similar to these of the cortex of a cuticle in which some methods are unable to disclose the differentiation of this layer into an outer and an inner cortical layer. The outer superficial layer and the cortical layer appear to be formed by a scleroproteid with a high content of SS and SH groups. The matrix of the lateral ledge is penetrated by fine branching fibrils from a fibrillar layer (Plate I, Fig. 4) and these react to methods demonstrating proteins with SS groups and amino acids (tyrosine and arginine) and contain also a small amount of silver-reducing substances. This layer, however, could not be demonstrated with any of the remaining methods. The basic content of the superficial part of the cuticular ledge consisted of acid mucosubstances. The underlying homogeneous layer consisted mainly of tyrosine, arginine and a certain amount of mucosubstances. The basal portion of the ledge was formed by a ribbonlike fibrillar layer with a dominance of reticular fibrils similar as in the inner fibrillar layer of the cuticle (Plate I, Fig. 3). Both layers reacted feebly for tyrosine and arginine, and

Table 1. Results of histological and histochemical reactions in the cuticle of sexually mature females

Methods	Superficia layer	Outer cortical layer	Inner cortical layer	Fibrillar intermediary layer	Homogeneous layer	Fibrillar layers			Basal membrane
						outer	middle	inner	
Sudan B	++++	–	++	–	+(+)	+	–	+	black
Luxol blue	–	–	++++	–	+	–	–	–	–
PAA AF	++++	–	++++	++ blue	+	blue	–	+	–
PFA AB	++++	++	+++	–	–	++	–	++	–
DDD (SH)	+++(+)	–	++	–	–	–	–	–	–
DDD (SS)	++++	–	+++	–	–	++	–	+	–
Morel Sisley	–	–	–	–	++++	+	–	++	–
DMAB	–	–	–	+++	–	–	+++	–	–
TK	–	–	–	–	++++	++	–	++	–
Sakaguchi	–	–	–	–	++–	+	–	+	–
DNFB	++	–	++	–	–	+	–	+	–
Bromphenol blue	–	+	+++	–	++	+	–	+	–
Gomori	–	–	++(+)	–	–	++++	–	++++	++
Gomori-PAS	–	–	black	pink	gray	blue	red	black	–
PAS	–	–	–	++	–	–	++++	–	–
PAS-saliva	–	–	–	–	–	–	–	–	–
PAS-acetylation	–	–	–	–	–	–	–	–	–
PAS-desacetylation	–	–	–	red	–	–	red	–	–
AB pH 2.5	refraction	–	–	–	+?	+	–	+	–
AB-methylation	–	–	–	–	–	–	–	–	–
AB-demethylation	blue	–	–	–	light blue	+	–	+	–
Hale	blue	–	–	–	bluish green	–	–	–	–
Toluidin blue	blue	–	–	–	violet	–	–	–	–
Mallory PTAH	–	–	blue	–	red	red	–	red	–
Van Gieson	–	–	–	–	pink	red	–	red	–

++++ strongly positive reaction, +++ positive reaction, ++ moderate reaction, + weak reaction

Table 2. Histological and histochemical reactions in the cuticular lateral ledge

Methods	Superficial layer		Inner content of the ledge			Basal portion of the ledge		
						middle		inner
	outer layer	cortex	anterior fibrillar portion	matrix	distal homogeneous portion	ribbonlike fibrillar layer	homo-geneous layer	fibrillar layer
Sudan B	++++	++	–		+(+)	–	–	–
Luxol blue	–	+++	–		+	–	–	–
PAA-AF	++++	+++	blue +++		–	++	–	+
PFA AB	++++	+++	blue +++		–	++	–	+
DDD(SH)	++	++	–		–	–	–	–
DDD(SS)	++++	+++	red ++		–	+++	–	+
Morel Sisley	–	–	+		++++	++	–	++
DMAB	–	–	–		–	–	+++	–
TK	–	–	++		++++	++	–	++
Sakaguchi	–	–	+		+++	+	–	+
DNFB	++	+	–		–	–	–	–
Bromphenol blue	–	++	+		++	+	–	+
Gomori	–	++	+		–	+++	–	+++
Gomori-PAS	–	black	gray		gray	black	red	black
PAS	–	–	–		–	–	++++	–
PAS-saliva	–	–	–		–	–	–	–
PAS-acetylation	–	–	–		–	–	–	–
AB pH 2.5	refraction	–	–	++++	–	–	+	–
Halo	blue ++	–	–	++++	bluish green	–	–	–
Toluidine blue	refraction	–	–	+++	violet	–	–	–
Mallory	–	–	–	–	red	red	–	red
Van Gieson	–	–	–	–	pink	red	–	red

the presence of proteins with SS groups was also disclosed mainly in the ribbonlike layer. The presence of lipids and acid mucosubstances could not be confirmed. The middle homogeneous layer situated in the basal portion of the ledge contained tryptophan and neutral polysaccharides.

Employing the physical method of electron microanalysis, we observed on the screen of the cathode-ray tube in a tangential section through the surface of the cortical layer an image of interchanging rings and grooves. On the stereoscopic image (Plate II, Fig. 1), the centre of the rings was light and elevated above the surface, the margins of the rings, and the grooves were dark and sunken between the rings. The grooves appeared to be of a finer structure, this being in accord with the histological and histochemical structure of the cortical layer. Measurements of the intensity of the spectral line with the x-ray spectrometer disclosed that this was the spectral line of sulphur (Sk alpha); we studied, therefore, the spatial distribution of sulphur. The passing beam of light marked spots of light on the cathode-ray tube indicating the distribution and density of sulphur atoms (Plate II, Fig. 2). As evident from Fig. 2 the spatial distribution of sulphur in the cortical layer is not uniform being denser in some parts and less dense in others. The distribution of sulphur in these parts seemed to be consistent with that in the rings and around the grooves between the rings, i.e., the density of sulphur was higher in the more massive parts of the rings, lower in the grooves and the parts adjoining the rings. Measurements of the intensity of the spectral line performed with the x-ray spectrometer in a tangential section disclosed also a minute

amount of phosphorus in the cortical layer. From the same section we obtained a stereoscopic image (Plate II, Fig. 3) and an image of the spatial distribution of phosphorus and a lineal scan showing minimum and maximum values of the phosphorus content. The presence of a minimum amount of P in the rings of the cuticle and a higher amount of P in the grooves of the cortical layer was signalized by spots of light. It is of interest that even the maximum amount of phosphorus was considerably lower than the values of sulphur (Plate II, Fig. 4). The diagram of the lineal distribution of P indicated always the presence of two curves showing the maximum in the grooves; the same situation seemed to occur with the content of phosphorus present in the contact areas between the rings and the grooves. It seemed most probable that phosphorus was not present in the rings of the cortical layer (Fig. 4).

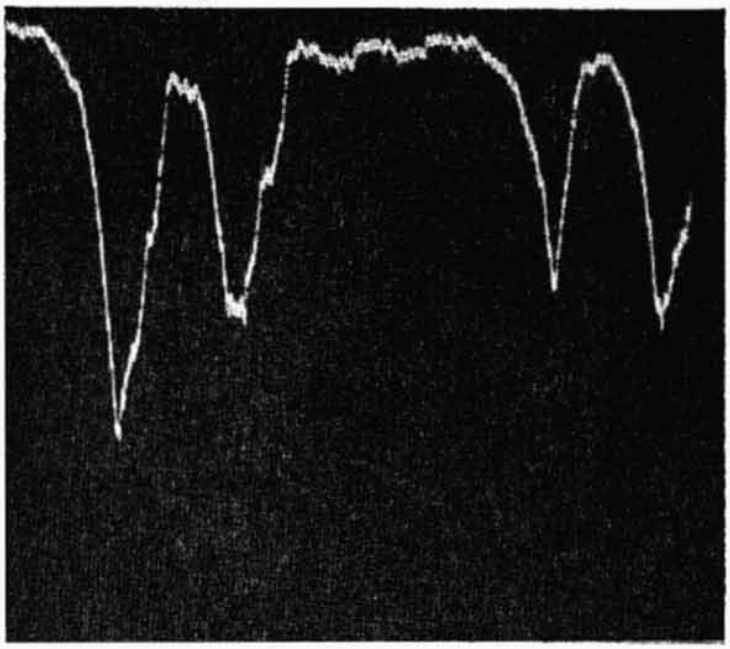

Fig. 4. A lineal analysis of phosphorus in a longitudinal section through the cortical layer. The micrograph indicates clearly that the maximum occurs always at the site of transition of the rings into the grooves, its minimum in the peaks of the rings and grooves. (× 4.170; the height of the frame has an intensity of 10 cps).

DISCUSSION AND CONCLUSIONS

The pattern of the cuticle as indicated by our findings is that: A superficial refractive membrane covers also the grooves; it is generally 0.1 μm thick. The grooves in a moderately stretched wall are 1.5 μm deep and responsible for the annulation of the cortical layer. The cortical layer is 1.3 μm thick under the centre of the ring. Its division into an outer and inner cortical layer is similar to that in rings of a extremely bent wall. A different non-homogeneous layer could be revealed by several methods in the bottom part of the cortical layer; this was higher under the centre of the rings and very attenuated under the grooves. This ununiform intermediary layer between the cortical and the homogeneous layer could frequently not be distinguished with various staining methods. It may well be that this layer is not differentiated in all parts of the body. The homogeneous layer and the underlying fibrillar layer are 1.5 μm thick. The ununiform character of the fibrillar layer was revealed with several staining methods. The distal border of the cuticle is formed by the basal layer (thickness 0.1 μm). The average

thickness of the cuticle in a normally extended wall is about 4 μm. According to Bogoyavlenskiy and Drynochkina (1967) the cuticle of the pinworm consists of 6 layers, i.e., the outer and inner cortical layer, the homogeneous layer, the "lamellate" layer, the basal layer and the basal membrane. The authors studied the division of the cuticle in histological sections stained with haematoxylin eosin and ferric haematoxylin only. This explains why they did not observe layers of different staining properties such as the superficial layer and the intermediary layer between the cortical and the homogeneous layer, which stain with special methods only. The thickness of the cuticle as recorded by these authors (2.4—2.6 μm) is approximately the same as that measured by us in a transverse section through the worm's body, but is slightly thicker than the cuticle of the dorsal side, which is extremely stretched and in which the grooves are shallow. In a longitudinal section through a normally stretched cuticle, its thickness was 4 μm, but slightly higher in, e.g., the vulval area on the ventral side of the body. The "lamellate" layer referred to by the two authors appeared to be highly argirophilic in our observation. This suggests that the fibrils of this layer are of the reticular type, this being particularly noticeable at the point of entrance of the outer layer into the inner content (matrix) of the ledge. Between this outer fibrillar layer and the inner layer of the same composition lies a histochemically differentiated intermediary layer forming the afore mentioned homogeneous layer in the basal part of the lateral ledge (Fig. 3). We compared our results with those obtained by other authors who had studied the structure of the cuticle of different nematode species (Inatomi et al. 1963, Inglis 1964, Lee 1965, Anya 1966) and agree with Inglis (1964) in that the general structure of the cuticle of various nematodes was always a variation of the *Ascaris* type. This concept is acceptable also as regards the histochemical structure of the pinworm cuticle. The accumulation of SS and SH groups in the cortical layer of the cuticle of *E. vermicularis* was observed also in the cuticle of other nematode species by, e.g., Brown 1950; Carbonell and Apitz 1961; Nagasawa 1961; Watson 1958; Šlais 1964. A similar situation occurs with the character of the fibrillar structure in the fibrillar layer of the pinworm cuticle. In its histochemical reaction, it resembles fibrils of the collagenous and reticular type described from *Ascaris* and other nematodes by Lee (1965), Johri and Smyth (1956), Monné (1955), Nagasawa (1961) etc. The identification of pinworm remnants in pathological material, e.g., oxyuric granulomas, has to be based on a sound knowledge of the histology and histochemistry of the pinworm's cuticle. Our knowledge obtained from a detailed study of the histology and histochemistry of the cuticular ledge which has not been described as yet in the literature, may help to elucidate this problem. The inner content of the ledge, the matrix, contains a large amount of acid mucosubstances. This fact was observed by Šlais (1964) when diagnosing pinworm remnants in oxyuric granulomas.

The method of a microanalysis of the elements with the electron microanalyser JXA-5 has not been used before in Czechoslovakia on biological material. We are convinced that the employment of this method will disclose new information on the surfaces of the tissue in the stereoscopic image, and on the content and spatial distribution of minute amounts of elements present in the chemical compounds forming the tissue. The spatial distribution of sulphur in the cortical layer, which is very uneven, suggests the dependence of this distribution on the morphological structure of this layer which may vary in the individual nematode species. The annulation of the cuticle of *Enterobius vermicularis* is far from being uniform, but the thickness of the outer and inner cortical layer in which sulphur has been found, does not change. This indicates that the high amount of cystine disclosed with histochemical methods by numerous authors, refers mainly to the cortical layer in the middle of the rings and not to that in the grooves between them; in these, hardly any cystine was detected with the

method of microanalysis and also the thickness of the outer cortical layer was minute. Phosphorus was confirmed in the grooves in spite of the fact that the amount of P was minute; it was present mainly in the contact areas, i.e., at the point of entrance of the rings into the grooves. This suggests the presence of a certain low percentage of phospholipids in the cortical layer, which were confirmed histochemically also by Anya (1964).

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ГИСТОЛОГИЯ И ГИСТОХИМИЯ КУТИКУЛЫ У САМОК *ENTEROBIUS VERMICULARIS*

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Резюме. Кутикула острицы изучалась гистологическим и гистохимическим методами и также с помощью электронного микроанализа. Результаты показали, что кутикула приблизительно 4μм толщины и что она состоит из 8 слоев: внешнего и внутреннего коркового слоя, промежуточного слоя, однородного слоя, внешнего, среднего и внутреннего фибриллярного слоя, основного слоя и преломляющего свет слоя на поверхности, толщиной 0,1 μм. Все слои переходят в латеральный кутикулярный выступ. В содержимом передней части выступа обнаружены кислые мукосубстанции. Гистохимически выступ представляет собою дифференцированное кутикулярное образование. Наличие белков с группами дисульфидов (SS) и сульфгидрилов (SH) и наличие фосфолипидов продемонстрировано в кутикуле гистохимическим путем и изучено до подробностей с помощью электронного микроанализатора JXA-5, с применением метода микроанализа элементов. Таким образом можно было точно обнаружить субстанции в содержимом и показать пространственное распределение серы и фосфора в кутикуле.

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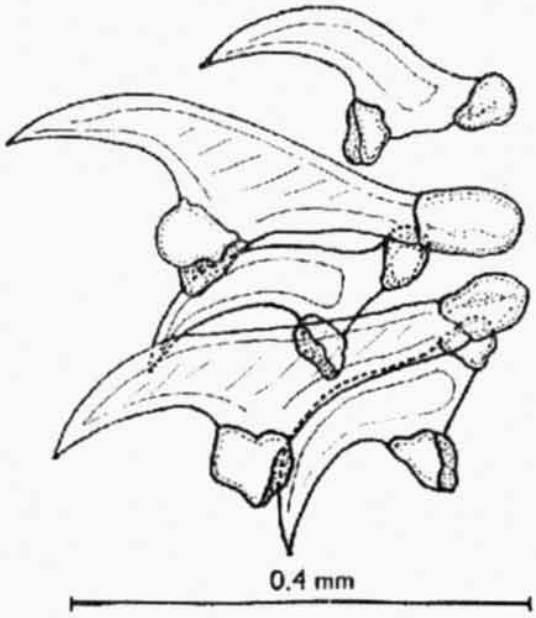
UNUSUAL FINDING OF LARVAL STAGES OF THE CESTODE HYDATIGERA TAENIAEFORMIS (BATSCH, 1786) IN THE PHEASANT



Cysticerci of the cestode family Taeniidae, recovered from 3 pheasants by Dr. Lípová, District Diagnostic Veterinary Institute, Karlovy Vary, during parasitological investigations in West-Bohemia were sent to our Institute for identification. The species concerned was *Strobilocercus fasciolaris*, the larva of the cestode *Hydatigera taeniaeformis* (Batsch, 1786). The strobilocerci were placed in oval or spherical bladders, diameter 6—9 mm. Length of larva from 4—7 cm. Diameter of scolex from 1.5—1.8 mm; four suckers, diameter 0.420—0.460 mm. Rostellum armed with 28—30 hooks arranged in two rows. Length of hooks of the first row 0.440—0.462, of the second row 0.280—0.292 mm The number, shape and measurements of the hooks coincides with the data given for this species by K. I. Abuladze (in: Taeniids—tapeworms of animals and man. Osnovy cestodologii, T. IV., Moscow 1964, pp. 1—530, in Russian). The finding of these cestode larvae in birds is very surprizing. According to Abuladze (1964), the intermediate hosts utilized by this cestode species are, generally, rodents of the families Muridae and Cricetidae and, very exceptionally, cat and gibbon. The utilization of birds as intermediate hosts of the cestode *Hydatigera taeniaeformis* has not been recorded as yet in the literature. The presence of strobilocerci in the pheasants is exceptional; evidently, these birds had come into contact with a large number of mature cestode eggs, which developed in their livers into mature strobilocerci.

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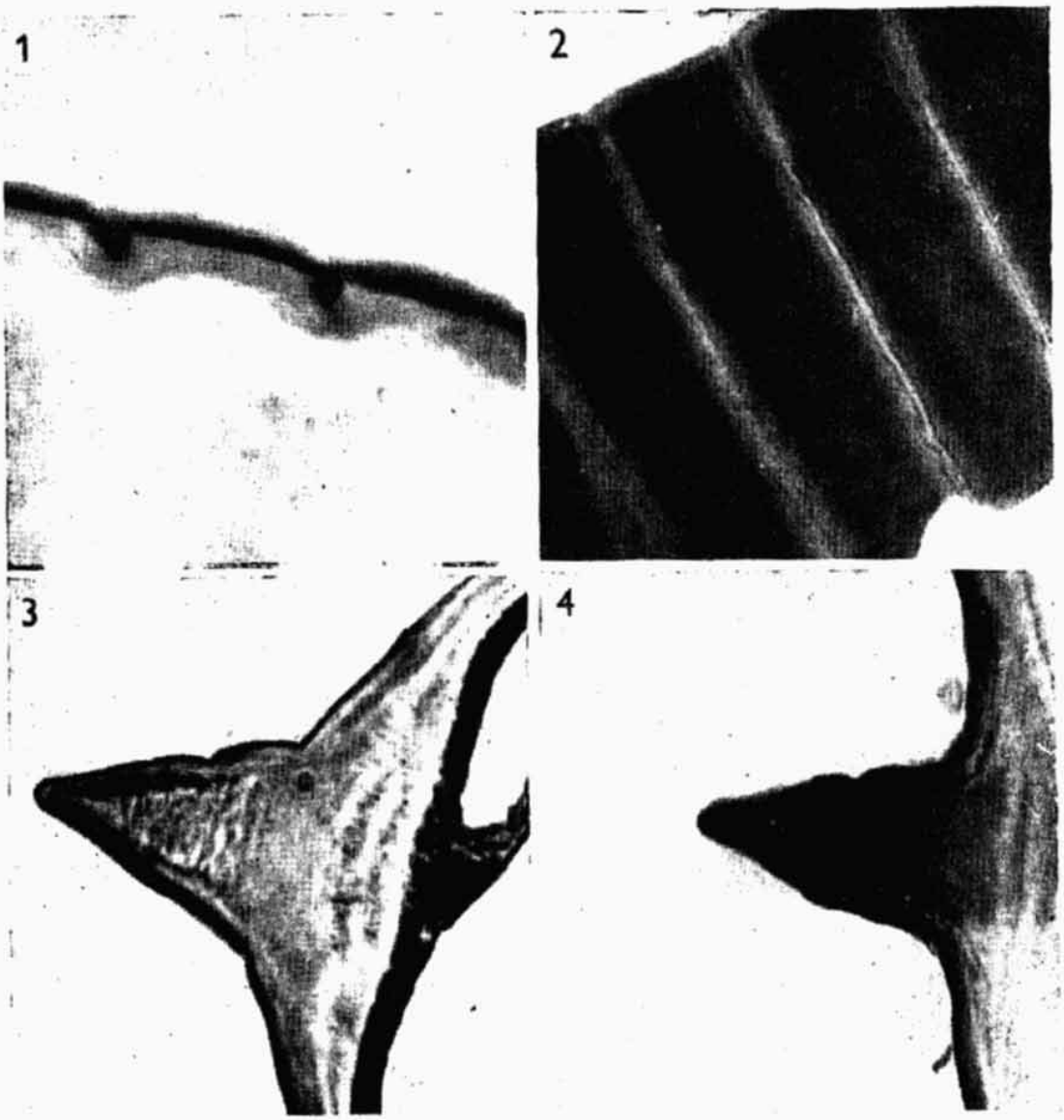

Plate I

Fig. 1. Demonstration of cystine in the cortical layer of the cuticle in a longitudinal section with the PFA AB method. The superficial layer and the cortex stained a bluish green and so did feebly also the fibrils of the outer and inner fibrillar layer (×1,000).

Fig. 2. Demonstration of SS groups of proteins with the PFA AB method in the cortical layer on a tangential section (×600).

Fig. 3. Demonstration of lipids with Sudan black B in a transverse section through the cuticular lateral ledge. The branches of the fibrillar layer between the cortical and homogeneous layer are visible in the inner content of the ledge (×1,000)

Fig. 4. The lateral cuticular ledge with the superficial cortical layer and the inner content, stained with PAA AF (×1,000).

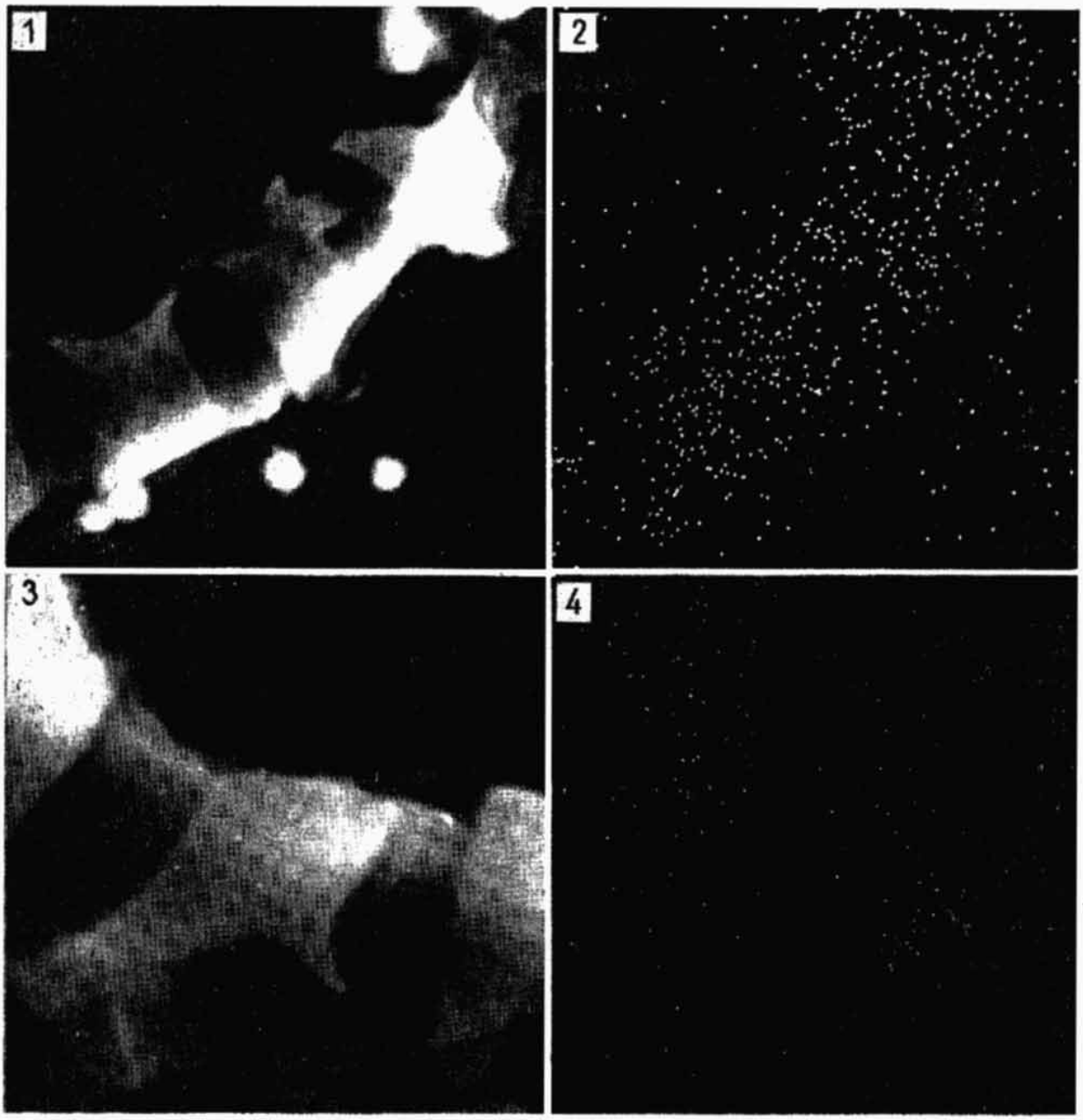

Plate II

Fig. 1. Tangential section through the superficial cortical layer studied with the electron microanalyser JXA-5. (Secondary electrons; directs magnification 2,500×; accelerating voltage 15 kV; current intensity 1.10^{-9} A; metal-coating C). The screen of the cathode-ray tube showed the cuticular rings and grooves. The light stripes in the image represent an increased content of sulphur. The dark stripes illustrate the transition of the cortical layer of the rings into the grooves.

Fig. 2. The spatial distribution of sulphur studied in the same section showed the higher density of sulphur in the rings and, particularly, in their middle parts. The density of sulphur was lower in the marginal parts of the rings. (Crystal PET; line Sk alpha; direct magnification 2,500×; metal-coating C, current intensity $3 . 10^{-9}$ A; accelerating voltage 17.5 kV).

Fig. 3. Stereoscopic image of a tangential section through the cortical layer inspected for the presence of phosphorus. (Metal-coating Au, direct magnification 4,170×; current intensity $3 . 10^{-9}$ A; absorption electrons 20 KV).

Fig. 4. Spatial distribution of phosphorus in a tangential section through the cuticle. Spots of light indicate the feeble distribution of P atoms at the sites of the grooves. (Direct magnification 4,170×; current intensity $3 . 10^{-9}$ A).