HISTOLOGICAL AND HISTOCHEMICAL STUDIES ON
THE BODY WALL OF PSEUDOPROLEPTUS KHERAI
(NEMATODA)

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Abstract. The body wall of the mature Pseudoproleptus kherai has been studied employing various
histological and histochemical techniques. The outer cortex is basically proteinous, the proteins
comprising mainly tryptophan, arginine and other amino acids having —NH₂ and —SH groups,
whereas the inner cortex is more rich in carbohydrates, and also shows RNA activity. The matrix is
phospholipoidal and fairly pyroninophilic. The fibrillar layer is collagenous. Hypodermis is rich in
proteins, carbohydrates and lipids, and also shows RNA activity. The muscles contain proteins,
lipids and carbohydrates including glycogen. Tyrosine and neutral lipids are exclusively absent
from the body wall. The outer cortex is elastic in nature. The functional significance of the various
components has been discussed.

Pseudoproleptus kherai is an intestinal parasite of fresh-water fish. The histological and
cytochemical studies on this nematode have not been carried out so far. The cuticle
is an important organ in nematodes since it provides the defensive mechanism against
the action of the various hydrolytic enzymes constituting its ecological niche. During
the present investigations an attempt has been made to work out the preliminary
nature of the body wall of P. kherai.

MATERIAL AND METHODS

The mature specimens of P. kherai were obtained from the gut of a fresh-water fish, Mastacembelus
armatus, collected locally. The worms were fixed in various fixatives including Zenker, Bouin,
normal formalin, formaldehyde calcium and weak Bouin, and processed for histological studies and
for the histochemical localization of proteins, carbohydrates, lipids and nucleic acids according to
the procedures detailed in Pearse (1968).

RESULTS

The body wall of P. kherai consists of an external cuticle, a hypodermis and a single
layer of longitudinal muscles (Plate I, Figs. 1,2). The cuticle comprises an outer cortex
averaging 0.002 mm in thickness, an inner cortex which also averages 0.002 mm in
thickness, a 0.006 mm thick matrix layer, and a fibrillar layer which is 0.008 mm thick.
The cuticle is lined by hypodermis on its inner side. The hypodermis is 0.003 mm thick
and is followed by a layer of longitudinal muscles. The muscles show two distinct
sub-regions — the proximal broader fibrillar zone that lies towards the hypodermis,
and an inner distal nuclear zone which harbours a nucleus. The various regions of the
cuticle comprise layers and no cellular entity is observed.
1. CUTICLE

a) Outer cortex. The outer cortex which is the outermost limiting layer of the cuticle, is basically proteinous (collagenous) in nature (Plate I, Figs. 3, 4); the proteins comprise tryptophan, arginine and other amino acids having —NH$_2$ and —SH groups as revealed by coupled tetrazonium reaction (positive after dinitrofluorobenzene and performic acid controls), ninhydin—Schiff and ferric ferricyanide reactions respectively. Alkaline tetrazolium and performic acid —Schiff reactions reveal that traces of proteins carrying —SS groups are also present in the outer cortex. However, tyrosine is completely lacking since the tissue fails to stain in Millon’s test.

The outer cortex is moderately sudanophilic (lipidal) (Plate I, Fig. 5), the sudanophila being exclusively due to phospholipids as is revealed by blue colouration after acid haematein and Nile blue sulphate stainings (negative after pyridine extraction). The lipids are present in conjugation with proteins as lipo-protein complexes since the tissue stains black in acetone — Sudan black B solution (Barenbaum’s technique).

It also reveals the limited amount of acid mucopolysaccharides and has β- metachromatic properties. The outer cortex is decisively elastic in nature (Plate I, Fig. 6).

b) Inner cortex. The inner cortex possesses more carbohydrates and less proteins (Plate I, Figs. 3, 4), and also shows RNA activity (Plate II, Fig. 2). The carbohydrates comprise neutral and acid mucopolysaccharides in bulk and glycogen in traces (Plate II, Fig. 9) as revealed after periodic acid—Schiff, Alcian blue and Best’s carmine stainings respectively. The proteins are primarily having —NH$_2$, —SH and also —SS groups in their amino acids (Plate II, Fig. 4).

Inner cortex is lightly sudanophilic due to the presence of phospholipids (Plate I, Fig. 5). It is basophilic (Plate I, Figs. 1, 2), the basophilia being due to pyroninophilia, i.e., the presence of RNA activity (Plate II, Fig. 2) since the pyroninophilia is labile after perchloric acid digestion. The inner cortex appears to be elastic.

c) Matrix layer. The matrix is primarily lipidal in nature (Plate I, Fig. 5). only phospholipids account for sudanophilic. It stains lightly in mercuric bromphenol bue for general proteins (Plate I, Figs. 3, 4). The proteins are limited to the amino acids with bound amide group, sulphhydryl and disulphide groups. However, it fails to show periodic acid—Schiff reactivity, thereby suggesting an absence of carbohydrates (Plate II, Fig. 1), and is non-elastic. Matrix is fairly pyroninophilic due to the presence of RNA (Plate II, Fig. 2).

d) Fibrillar layer. The fibrillar layer is quite thick and is demarcated into three sub-regions. The outer and inner sub-regions are moderately pyroninophilic whereas the middle lacks RNA activity. It is distinctly proteinous in nature (Plate I, Figs. 3, 4) comprising collagenous proteins. The inner layer which is adjacent to hypodermis also shows elastic nature (Plate I, Fig. 6). The proteins are composed of basic proteins (chiefly histones and protamines), arginine, tryptophan, histidine and other amino acids with —NH$_2$, —SH and —SS groups.

Traces of glycogen and acid mucopolysaccharides account for whatever amount of carbohydrates are present in the fibrillar layer. This zone is non-lipidal (Plate I, Fig. 5).

2. HYPODERMIS

Hypodermis is a layer between the cuticle and the longitudinal muscles. It is rich in proteins (Plate I, Figs. 3, 4), carbohydrates (Plate II, Fig. 1) and lipids (Plate I, Fig. 5), and also shows RNA activity (Plate II, Fig. 2). Tyrosine is absent whereas arginine, tryptophan and other amino acids with —NH$_2$, —SH and —SS groups are present. Carbohydrates comprise neutral mucopolysaccharides, and sulphated and
non-sulphated acid mucopolysaccharides. Glycogen could not be demonstrated histo-
chemically in this layer. Phospholipids are the sole constituent of the lipid moiety,
the triglycerides being absent.

3. LONGITUDINAL MUSCULAR LAYER

The muscles, as stated earlier, show two sub-regions, (i) the fibrillar zone, and (ii)
the nuclear zone.

The fibrillar zone is richly proteinous and lipidal. It is also rich in carbohydrates
present as neutral and acid mucopolysaccharides. The fibrillar region tapers into nuclear
zone where the nucleus is lodged. The nucleus possesses a prominent nucleolus which is
intensely pyroninophilic due to the presence of RNA (Plate II, Fig. 2), and also stains
for general proteins. The nuclear chromatin stains green in methyl green—pyronin G,
blue in azure A and pink to violet in Feulgen for DNA. It also comprises basic proteins
(chiefly histones and protamines) as it stains blue green in alkaline fast green. The
cytoplasm in the nuclear zone shows the presence of glycogen (Plate II, Fig. 3), and also
reveals an intense staining in the various reactions for localizing proteins, carbohydrates
and lipids.

During present investigations cholesterols were not observed after the usual histo-
chemical techniques (Schultz’ and Okamoto’s techniques) in the body wall of P. kherai.

DISCUSSION

The gross histomorphological architecture of the body wall of the nematode, P.
kherai, resembles that of other nematodes inasmuch as it consists of various layers,
viz, external cuticle, hypodermis and longitudinal muscles (Chitwood and Chitwood

The present histochemical observations reveal that essentially proteins and carbo-
hydrates account for the chemical make up of the body wall with lipids also playing
a significant role in binding the various constituents together. Since the body wall
consists of layers showing nuclei only in the longitudinal muscles, the presence of DNA
could be located only in them. However, the RNA activity was observed in many
different layers, thereby suggesting that the body wall is a living structure and not a mere
body covering.

The outer and inner cortices differ in their chemical nature in P. kherai. The outer
cortex is basically proteinous and lipidal; the lipids are present as lipo-proteins. Traces
of acid mucopolysaccharides are also present. The proteins are apparently elastic and
collagenous in nature, and consist of tryptophan, arginine and other amino acids
having —NH₂ and —SH groups. Small quantities of amino acids with —SS groups
(cystine) are also observed in outer cortex. Homanberg and Peana sky (1976) have
recorded that tyrosine is absent in the cuticle of Ascaris. In conformity with these work-
ers tyrosine has not been observed histochemically in P. kherai.

The sulphydryl and disulphide linkages observed in the cuticle of P. kherai lend
strength to the structural proteins and may make the worm invulnerable to the proteo-
lytic enzymes present in the intestine of the fish it inhabits. The elastic nature of the
outer cortex helps in contractibility and thus the adaptability of the worm. The phos-
pholipids in the outer cortex also lend stability to the structural proteins forming
phospholipoprotein complexes. The presence of lipids in the cuticle has also been
reported by Bird (1957), Beames (1964), Reznik (1971) and Sood and Kalra (1977).
in various nematodes. Phospholipids are also important since they are essential constituents of all the biological membranes.

The presence of acid mucopolysaccharides in outer cortex, and neutral as well as acid mucopolysaccharides in inner cortex also appear to be of significance as they regulate the permeability of nematode cuticle as suggested by Wright (1968).

The inner cortex in *P. kherai* reveals RNA activity, and it is quite possible that this layer itself is responsible for the synthesis of at least some of the proteins constituting the cuticle. This is in conformity with the findings of Anya (1966) and Sood and Kalra (1977). However, this function of inner cortex appears to be under the control of hypodermis since during moult the entire cuticle is shed and the new cuticle is formed directly above the hypodermis. Thus the proteins synthesised in inner cortex are of additive nature.

The matrix is primarily lipidal in the present material. However, Sood and Kalra (1977) observed this layer to be devoid of lipids in *Haemonchus contortus*. In conformity with these workers the matrix in *P. kherai* was observed to be free of carbohydrates. However, matrix appears to be metabolically active in the synthesis of cuticular proteins as it is rich in RNA activity.

The present observations reveal that the fibrillar layer essentially comprises collagenous proteins in *P. kherai*. Dimitrova (1962) has also observed lipids in this layer. However, the present studies do not confirm his findings. Small amount of carbohydrates has also been observed in fibrillar layer of *P. kherai* as previously reported by Kan and Davey (1968) in *Phocanema*. On the contrary, Naura (1969) recorded that the carbohydrates are present only in the cortical and matrix layers, and not in fibrillar layer.

Various authors (Any, 1966, Lee 1966) opine that the hypodermis is the seat of protein synthesis. In *P. kherai* the protein synthesis appears to be a collective function of inner cortex, matrix and hypodermis. Hypodermis gives an intense reaction for proteins, carbohydrates and lipids in conformity with the observations of Sood and Kalra (1977), and thus it can be compared with the basement membrane of other tissues that supports the cuticle on outside and lines the muscles on its inner face. However, its role in protein synthesis cannot be denied since it shows RNA activity. Sood and Kalra (1977) have also proposed it to be a storage organ. The presence of various chemical moieties in this layer suggests it to be metabolically very active.

The present studies reveal that the cuticle in *P. kherai* contains small amount of cystine but it is definitely non-keratinized (keratin is notable for its high contents of cystine). The non-keratinized nature of the cuticle has also been advocated by Fairbairn (1957). The observations of Colley (1970), Singh and Khera (1972) and Sood and Kalra (1977) confirm the present observations that the muscles are rich in carbohydrates (including glycogen). The muscles also show the presence of lipids in *P. kherai* in conformity with the findings of Beames (1964) and Sood and Kalra (1977). Thus the muscles act as store-house where assimilated food is stored as carbohydrates (glycogen) and lipids (exclusively phospholipids). No protein synthesis appears to take place in muscles.

In *P. kherai*, the protein synthesis takes place in the various layers of the body wall. The protein synthesis is a function of RNA under the direction of DNA — the master molecule. The various regions of the body wall, barring muscles, show no indication of either organized nucleus or free DNA histochemically. Chandler and Read (1961) and Levine (1970) have concluded that the cuticle is secreted by hypodermis. According to Chandler and Read (1961) the hypodermis is a syncytial layer where the nuclei are confined to the four thickened chords or lines, one dorsal, one ventral and two lateral. This explains the role of hypodermis in secreting the cuticle (at the direction of nuclear
DNA in the chords). However, the present authors, in spite of their best efforts, could not observe nuclei in any layer of the body wall excepting the muscular layer, the latter appearing to be non-secretory in function. Lee (1966) also proposes that the various chemical constituents are secreted by hypodermis and later these migrate to the various cuticular layers. However, if the nuclei are absent in hypodermis, as apperas to be in P. kherai, it should act only as a basement membrane controlling the passage of the chemical constituents of the cuticle formed elsewhere, through it. Alternatively, the transcription of RNA may not be taking place in the developed worm itself, but the RNA may be present as informosomes formed some time during the early stages of cell differentiation, which are then called upon to act during the later life cycle of the worm. However, the authors suggest a more intensive research in these directions utilizing the various microscopical and biochemical approaches so that the physiology of the cuticle, and the worm as a whole is understood in a better way.

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The present monograph on the adult mosquitoes of Poland was compiled by a prominent specialist in systematics and ecology of this family, the late Docent Dr. Barbara Skierska. In 1971 this author published in the same series, issue 9 a, a treatise with a similar title devoted to larvae and pupae of the family Culicidae. Unlike most monographs of recent date the family Culicidae here is conceived after former views, so that both families of non-parasitic representatives, i.e. according to the author’s concept the subfamilies Dixinae and Chaoborinae, are also treated in detail.

The general part deals with the characteristics of the family, morphology of imagoes, survey of their bionomy, their medical and veterinary importance, collecting, preservation and culturing. The systematic part includes a survey of important species found in Poland. It reveals that the subfamily Dixinae in Poland numbers 22 species (possible occurrence of three more species), the subfamily Chaoborinae includes 7 species (one more species possible), the subfamily Culicinae — 47 species (ten more species possible).

Keys to the genera and species are arranged for both sexes. Particular species are provided with detailed morphological descriptions, with brief notes on distribution in the world and in Poland, and the bionomy of each species is also summarized to the point. The text is supplemented with numerous pen-drawings, either done by the author, or adopted from other European monographs, including recent ones. Together with the previous issue 9 a the treatise makes up an excellent monograph on Culicidae in Poland which will surely be used for the identification of material by all entomologists studying this family. The volume is a valuable contribution to the monographic literature dealing with European representatives of the family Culicidae.

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Histology of the body wall. Fig. 1. Zenker (haematoxylin eosin) (990). Fig. 2. Zenker (haematoxylin eosin) (× 1520). Histochemical nature of the various regions of the body wall. Fig. 3. Zenker mercuric bromphenol blue (× 610). Fig. 4. Zenker mercuric bromphenol blue (× 990). Fig. 5. formaldehyde calcium (post-chromed) Sudan black B (× 1520). Fig. 6. Zenker aldehyde fuchsin (for elastin) (× 990).