

## THE INFLUENCE OF DIFFERENT ANTHELMINTICS ON THE INTESTINAL EPITHELIAL TISSUE OF *TOXOCARA CANIS* (NEMATODA)

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**Summary.** Twenty-five puppies naturally infected with *Toxocara canis* were selected by faecal egg counts for the experiment. Five of them were treated with pyrantel pamoate (14.4 mg/kg BW), five – with albendazole (30 mg/kg BW), five – with levamisole (7.5 mg/kg BW) and five – with nitroscanate (50 mg/kg BW), respectively, and remaining five puppies were served as untreated control. For histological and histochemical investigation all excreted nematodes were collected and the standard technique for investigation of intestinal epithelial tissue was used.

The epithelial tissue of *T. canis* intestine under the action of pyrantel pamoate and nitroscanate changed significantly. The changes were expressed by the appearance of vacuoles in the cytoplasm and by a total disintegration of intestinal epithelial cells. Under the influence of albendazole and levamisole the changes of enterocytes were less significant. The swelling of basal membrane, toddle cytoplasm and blending of fibers in the apical cytoplasm of epithelial cells were registered.

The glycogen inclusions and neutral lipids in treated tissue under the action of all used anthelmintics have changed. After treatment with pyrantel pamoate, albendazole and nitroscanate the accumulation of the glycogen deposits in enterocytes lowered gradually and finally dissapeared . Further, after treatment with levamisole the glicogen deposits from enterocytes dissapeared, however, a distinct positive PAS reaction was repeatedly observed at the end of experiment.

After anthelmintic treatment was registered a distinct infiltration of the neutral lipids in the epithelial cells of *T. canis* intestine. It should be mentioned, that significant accumulations of neutral lipids were observed after treatment with albendazole. The significant fat dystrophy was expressed by a number of fat agregates that fulfilled the cell cytoplasm.

Basing on the obtained data it was concluded that anthelmintic treatment caused significant micro- morphological changes in the epithelial tissue of *T. canis* intestine and destroyed the metabolism of glycogen and neutral lipids. Moreover, highly significant degeneration was noted under the action of pyrantel pamoate and nitroscanate.

**Keywords:** *Toxocara canis*, histology of nematodes, glycogen, neutral lipids, pyrantel pamoate, albendazole, levamisole, nitroscanate.

## KAI KURIŲ ANTIHELMINTIKŲ POVEIKIO *TOXOCARA CANIS* (NEMATODA) ŽARNOS EPITELIUI TYRIMAI

**Santrauka.** Straipsnyje pateikti šunų nematodų *Toxocara canis* žarnos epitelinio audinio tyrimų rezultatai, gauti po šuniukų gydymo skirtingais antihelmintiniais preparatais. Penki eksperimentiniai šuniukai buvo gydyti pirantelio pamoatu (14,4 mg/kg), penki - albendazoliu (30 mg/kg), penki - levamizoliu (7,5 mg/kg) ir penki - nitroskanatu (50 mg/kg). Penki užsikrėtę nematodais šuniukai nebuvo dehelmintizuoti, iš jų plonojo žarnyno surinkti nematodai buvo panaudoti kontroliniams tyrimams. Visi surinkti nematodai analizei buvo paruošti taikant standartinius histologijos ir histochemijos metodus.

Tirtų *T. canis* žarnos epitelis ženkliai pakito veikiamas pirantelio pamoato ir nitroskanato. Pokyčiai pasireiškė gausiomis vakuolėmis enterocituose eksperimento pradžioje ir visišku epitelinį ląstelių suirimu eksperimento pabaigoje. Albendazolio bei levamizolio poveikis nematodų žarnos struktūroms nebuvo toks ryškus ir pasireiškė pamatinės membranos išbrinkimu, citoplazmos drumstumu ir mikrofilamentų sulipimu enterocitų apikalinėje dalyje.

Glikogeno tarpai ir neutraliųjų lipidų sankaupos tirtame nematodų audinyje pakito veikiami visų taikytų antihelmintikų. Pirantelio pamoato, albendazolio ir nitroskanato veikiami glikogeno tarpai enterocituose mažėjo nuosekliai, iki visiškai išnyko. Neigiama PAS reakcija nustatyta ir veikiant levamizoliui, tačiau šio eksperimento pabaigoje nematodų enterocituose glikogeno tarpai vėl buvo gausūs.

Tirti antihelmintikai *T. canis* nematodų žarnos epitelyje sukėlė ryškią riebalinę infiltraciją, kuri pasireiškė gausiais riebaliniais lašeliais, užpildančiais visą ląstelių citoplazmą. Itin gausios, laikinos neutraliųjų lipidų sankaupos buvo nustatytos veikiant albendazoliui.

Apibendrinant tyrimų rezultatus galima teigti, kad veikiant antihelmintikams pakito ne tik *T. canis* žarnos epitelinės dangos mikrostruktūra, bet ir glikogeno bei neutraliųjų lipidų metabolizmas. Itin ženkli šio audinio degeneracija nustatyta veikiant pirantelio pamoatui ir nitroskanatui.

**Raktažodžiai:** *Toxocara canis*, nematodų histologija, glikogenas, neutralieji lipidai, pirantelis, albendazolis, levamizolis, nitroskanatas.

**Introduction.** One of the most important objects of modern parasitology is a detailed micro morphological investigation of the helminthes tissues. This kind of investigation is important not only in searching the answers about parasite systematic and phylogeny. So they have a wide biological meaning, explaining the complicated aspects of host-parasite relationship. The results of micro morphological investigations form the base for the further physiological and biochemical examinations of helminthes. The data concerning the micro morphology of helminthes is necessary for explaining the action of anthelmintic on the organism of a parasite.

The tissues of many nematodes (Nematoda, Ascaridida) are thoroughly investigated. It is worth mentioning the fundamental works of A. Bird and J. Bird (1991), J. Vogojavlenskis (Богоявленский, 1998), K. Wright (1987) and others, which present the results of micro morphological investigations of the nematode tissues from that order. The great importance while investigating the microstructure of nematodes was attached to *Ascaris*, *Parascaris*, *Nippostrongylus*, *Trichuris*, *Trichinella* and to the representatives from other genus. However, nematodes from the *Toxocara* genus were less investigated (Рачковская, 1985; Mackenstedt et al., 1993; Bruňanská, 1997).

*Toxocara canis* primarily is a dog parasite, whose larva causes *visceral larva migrans*, the infection sometimes being referred to as *toxocariasis*. Ingesting infective eggs of *T. canis* infects humans. The larvae, which hatch in the gut, migrate through the tissues and become trapped in the lungs, liver, eyes and other organs. It is especially dangerous for children since they get the inflammations of lungs, liver and eyes, the central nervous system can be destroyed as well. Therefore the micro morphological investigations of *T. canis* tissues side by side with a wild biological meaning are also important in the applied aspect, evaluating the order of other nematocides' action on the parasites and on their individual tissues. However the influence of nematocides on *T. canis* tissues *in vivo* is studied insufficiently.

The importance of the above-mentioned questions prompted us to undertake the researches on micro morphology of canine nematode *Toxocara canis* (Nematoda, Ascaridida) tissues and to investigate the dynamics of their changes under the action of various anthelmintics. The data of anthelmintic action on parasitic worm tissues can be useful in pharmacology by several aspects: in the originating new drugs combinations, selecting the effective drug doses and others. The material for these investigations was gathered during the complicated experiments *in vivo*.

**The objective of this study** was to investigate the pathology of *T. canis* intestinal epithelium under the action of some nematocides *in vivo* using micro morphological and histochemical methods of research. This article will discuss only some anthelmintics now in common use, those still in use, but likely to be replaced by newer better drugs.

**Material and methods.** *T. canis* nematodes were collected from the 25 one-month-old hybrid puppies that were obtained from homeless animal quarantine station in Vilnius. The efficiency of *T. canis* invasion in the

experimental puppies was pre-determined by coproscopic examinations applying standard Fulleborn method.

20 experimental puppies were treated with pyrantel pamoate, albendazole, levamisole, order nitroscanate. Standard therapeutic doses of the pyrantel pamoate (14.4 mg/kg), albendazole (30 mg/kg), levamisole (7.5 mg/kg), order nitroscanate (50 mg/kg) were used. Five infected puppies were left untreated.

Pyrantel pamoate, albendazole, levamisole, order nitroscanate were administered to each puppy individually. 96 hours onset the anthelmintic treatment we observed the behaviour of puppies, their appetite and defecation. We collected their feces and examined them with respect to *T. canis* helminthes also. All alive (that were moving order were twisted) nematodes were quickly washed in saline solution, divided into two parts (for the fixative material quicker reaches all tissues) and immediately fixed in 10% neutral formaldehyde for microstructure and neutral lipids examination and in Karnua solution for glycogen deposits examination. All (treated and control) puppies were euthanised at the 96<sup>th</sup> hour post treatment. *T. canis* nematodes from untreated puppies were collected and fixed the same way for the control histological and histochemical investigation of their intestinal epithelium.

Fixed *T. canis* fragments were prepared standard for histological investigation and were stained according to standard hematoxylin and eosin principles. Some of washed from formaldehyde helminthes were not dehydrated for the neutral lipid examination. These fragments were cut using refrigerating microtome and cuts were stained according to Sudan III-IV reaction. For histochemical examination of glycogen in Karnua fixed helminthes were dehydrated in graded alcohol and embedded in paraffin. Semi thin sections were stained according periodic acid- Schiff (PAS) stain.

**Results.** The results showed the control *T. canis* intestinal epithelial tissue structure is similar to that from Ascaridida order (Aukštikalnienė et al., 2000). Enterocytes lied on the distinct basal membrane, were more or less cylindrical and formed rather long microvilli. The interior of these cells was closely filled with microfilaments and few granules inside the cytoplasm were observed. The ovoid nucleus lied in the basal pole of the cells and appeared relatively pale with a non-central nucleolus.

The histochemical research of control *T. canis* intestine showed very bright, PAS positive, epithelial cells and diffusely located pale straw-colored droplets of neutral lipids.

H. Mehlhorn and A. Harder (1997) have described similar microstructure of intestinal epithelial cells of *Heterakis spumosa*. D. Lee (1965) and I. Rackovskaja (Рачковская, 1985) described similar location of glycogen and neutral lipids in intestinal tissues of other nematode species.

*T. canis* elimination from puppies intestine after the deworming occurred in very different intervals in all experiments. The first nematodes were expelled only after 4 hours post treatment with levamisole and nitroscanate, and after 8 hours after pyrantel pamoate treatment. When it take 19 hours to eliminate the helminthes after the administering albendazole. The elimination of *T. canis*

canceled after 36 hours post treatment with pyrantel pamoate, but it took even 50-53 hours after the treatment with all other treated drugs. The micro morphological and histochemical investigation of the all eliminated nematodes intestinal epithelial tissue was carried out after the grouping worms according conditional time of their elimination. That is till 10<sup>th</sup>, 20<sup>th</sup>, 30<sup>th</sup>, 40<sup>th</sup> and 50<sup>th</sup> hours after the anthelmintic administration, correspondingly 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> periods of experiments.

The changes of *T. canis* intestinal epithelium under the action of pyrantel pamoate. The results show that the applied anthelmintic cause serious changes in the structure of enterocytes. The changes may be described as destructive, degenerative and necrotic processes. At the 24<sup>th</sup> hour onset the treatment there was observed a total degeneration of striated border of epithelial tissue. The cells were filled out with vacuoles and granules, their nuclei were picnotic and some of them looked swollen (Fig. 1a). The epithelium of intestine was completely destroyed at the 36<sup>th</sup> hour.

The changes of *T. canis* intestinal epithelium under the action of albendazole. The first helminthes eliminated in 19 hours. According to our histological investigation microfibrils of the intestinal epithelial cells were blended together in their apical cytoplasm by the first (19-21) hours of the experiment. However, cell shape remained

unchanged during all the period of the experiment. There have not been established any changes of the microvilli layer of the cells. Their cytoplasm was lightly porous with various-shaped vacuoles and granules, and very basophilic. Basal membrane was divided from enterocytes and very thick (Fig. 1c).

The changes of *T. canis* intestinal epithelium under the action of levamisole. The changes in the enterocytes during the first period (the 4-10<sup>th</sup> hour) of experiment were expressed like blending of their fibrils in the apical cytoplasm. 25 hours onset the treatment epithelial cells showed darker then in control. There was eosinophilic, dark layer in the apical poles of the cells with small rare vacuoles visible in it. 46 hours onset the treatment we also established the vacuolization and slight swelling of these cells.

The changes of *T. canis* intestinal epithelium under the action of nitroscanate. Enterocytes have changed by the 4<sup>th</sup> hour under the action of nitroscanate. These cells looked swelled. The blending of fibers in the apical cytoplasm was determined during all periods of the investigation. Distinct granulation of the cytoplasm and the disruption of microvilli layer were noticed at the 27<sup>th</sup> hour. At the 48<sup>th</sup> hour the epithelial tissue of intestine was totally degenerated (Fig. 1b).

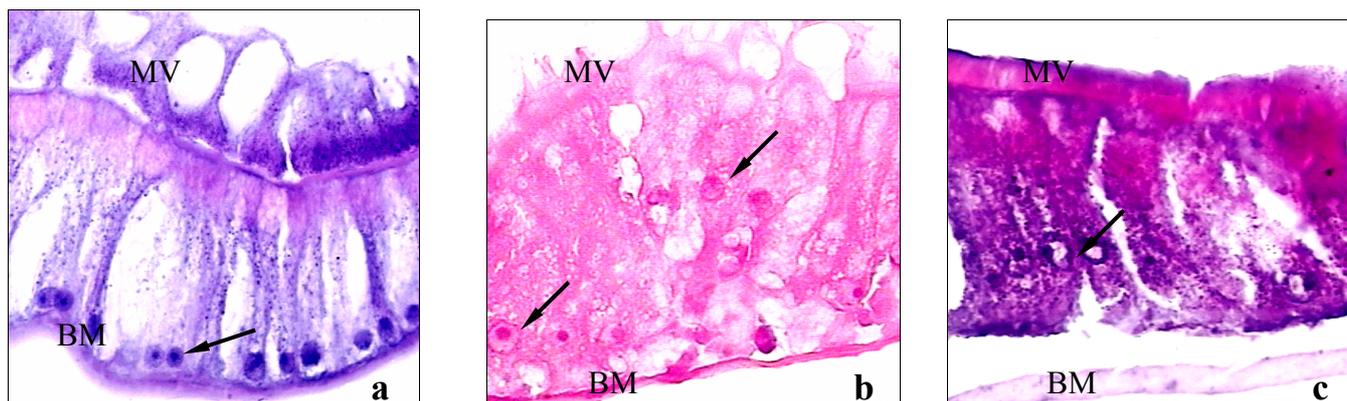


Fig. 1. The enterocytes of *T. canis* under the action of anthelmintics: a) after pyrantel pamoate treatment; b) after nitroscanate treatment; c) after albendazole treatment. MV – microvilli, BM – basal membrane, arrows – nucleus. 10x40

The influences of pyrantel pamoate, albendazole, levamisole and nitroscanate on glycogen deposits in the intestinal epithelial tissue of *T. canis* are presented in Table 1. The influences of mentioned drugs on neutral lipids in the same tissue of *T. canis* are presented in Table 2.

**Discussions.** Nematodes are of significant importance for human health and pose severe problems in the rearing of companion and farm animals. In industrialized countries it is estimated that about 100 mln dogs represent a considerable reservoir of potential worm infection (Mehlhorn and Harder, 1997). In most countries farm livestock are devormed regularly to avoid endemic outbreaks of parasitic diseases that result in major economic losses. In companion animals such as dogs prevention is often neglected. The reason is mainly the lack of awareness of the problem. This may become dangerous since prophylaxis is particularly important in

view of the often very close contact between humans and dogs, being especially hazardous for younger children.

A large list of drugs is available worldwide for the control of diseases caused by nematodes, including *T. canis*. However, their efficacy ranges are quite different and depend on the dose, the mode of application, the times and periods of administration, and the susceptibility of different worm species and/or their developing stages (Raether, 1988).

Our study showed that all our treated anthelmintics (pyrantel pamoate, albendazole, levamisole and nitroscanate) are reliably effective against *T. canis*. We didn't find any worm at puppy's necropsy seven days after treatment. With a single oral dose of mentioned above drugs *T. canis* nematodes were completely eliminated from the dog gut.

Discussing the changes of microstructure of *T. canis* intestinal epithelial tissue it could be seen that it has

distinctly changed under the action of pyrantel pamoate and nitroscanate. These changes were expressed by the appearance of vacuoles in the cytoplasm and also by a total disintegration of these cells (Fig. 1a, 1b).

Pharmacological investigations of pyrantel pamoate (Atchinson et al., 1991) showed that this drug inhibits cholinesterase. That cause irreversible depolarisation of

myoneural junction and results in spastic paralysis of the worm. The micro morphological and histochemical data recorded after the treatment with pyrantel pamoate (Mackenstedt et al., 1993; Aukštikalnienė et al., 2000) proved that *T. canis* had taken up pyrantel pamoate via an oral route leading to the initial intestinal damage.

**Table 1. Glycogen deposits in the intestinal epithelial tissue of *T. canis* after the treatment with pyrantel pamoate, albendazole, levamisole and nitroscanate**

Administered drug	Glycogen deposits in the intestinal epithelial cells after treatment				
	till 10 <sup>th</sup> hour	till 20 <sup>th</sup> hour	till 30 <sup>th</sup> hour	till 40 <sup>th</sup> hour	till 50 <sup>th</sup> hour
Pyrantel pamoate	the apical parts of enterocytes are PAS negative	glycogen is located in the 2/3 part of the cells (Fig. 2a)	almost negative PAS reaction, only the remnants of glycogen are found around the nucleus	enterocytes are PAS negative, glycogen deposits are disappeared	-
Albendazole	-	a weak PAS reaction, there are only small glycogen deposits above the nucleus	almost negative PAS reaction, only tracks of glycogen (Fig. 2b)	enterocytes are PAS negative	enterocytes are PAS negative
Levamisole	glycogen deposits, like big flakes) are located in the center and basal poles of the cells (Fig. 2a)	the same like in 1 <sup>st</sup> period	enterocytes are PAS negative (Fig. 2c); only some worms showed tracks of glycogen on 25 <sup>th</sup> hour after the dehelminthization	-	a positive PAS reaction on the 46 <sup>th</sup> hour
Nitroscanate	the apical parts of enterocytes are PAS negative	a weak PAS reaction in the center cytoplasm	only tracks of glycogen above the nucleus (Fig. 2b)	enterocytes are PAS negative	enterocytes are PAS negative

**Table 2. Neutral lipids in the intestinal epithelial tissue of *T. canis* after the treatment with pyrantel pamoate, albendazole, levamisole and nitroscanate**

Administered drug	Neutral lipids in the intestinal epithelial cells after treatment				
	till 10 <sup>th</sup> hour	till 20 <sup>th</sup> hour	till 30 <sup>th</sup> hour	till 40 <sup>th</sup> hour	till 50 <sup>th</sup> hour
Pyrantel pamoate	bright neutral lipids drops around nucleus	cytoplasm and intercellular spaces are full of big lipid drops (Fig. 3b)	a fat infiltration	a fat dystrophy, denser in the basal pole of the cells	-
Albendazole	-	big neutral lipids drops around nucleus and in all cytoplasm (Fig. 3c)	the same as till 20 <sup>th</sup> hour	a fat dystrophy; huge, enormous fat aggregates fulfilled the cells	the diffusely located pale droplets of neutral lipids (Fig. 3a) very similar to controls
Levamisole	diffusely located lipid droplets mostly in the apical cytoplasm	big neutral lipids drops in all cytoplasm (Fig. 3c)	a fat dystrophy	-	a fat dystrophy
Nitroscanate	cytoplasm and intercellular spaces are full of various shaped lipid drops (Fig. 3b)	the cells fulfilled with big lipid drops	a fat dystrophy	a fat dystrophy	a fat dystrophy

U. Mackenstedt et al. (1993) has calculated the amount of pyrantel taken up by *T. canis*, and it provides the evidence that these nematodes can limit or even stop the uptake of this drug for 4 or 10 hours. According to these data we can presume, that in our experiment ingested the primary amount of this drug by the worm

caused insignificant micro morphological changes in their intestine. After the temporary few hours starving, nematodes began to feed and ingested the bigger amounts of food and the additional amounts of drug also. The repeated influence of pyrantel pamoate caused irreversible

changes of *T. canis* intestinal epithelial cells as it is showed in Fig. 1a.

Enterocytes of *T. canis* totally degenerated under the action of nitroscanate, also (Fig. 1b). Nitroscanate is a broad-spectrum anthelmintic drug used for the dog's treatment. If compare it with other anthelmintic drugs, his influence is exceptionally, because it is the only one drug effective against *T. canis*, *Toxascaris leonina*, *Ancylostoma caninum* and *Echinococcus granulosus* (Dorchies et al., 1981; Craig et al., 1991).

According to very limited data about the influence of nitroscanate on parasitic worms, in our opinion the elimination of *T. canis* on the 4<sup>th</sup> hour of this experiment could be based on the energy resources. Basing on the data by W. Campbel and R. Rew (1986), nitroscanate in the tissues of *Litomosoides carinii* and *Brugia paghangi* inhibits the ATP synthase. The generation of ATP is very essential not only for the survival, but for replication also. As showed our data under the action of nitroscanate enterocytes changed on the firsts hours after the dehelminthization (4-10 hour). The changes were expressed like cytoplasm vacuolization, picnosis of the nuclei, and the swelling of basal membrane. According to the data from other experiments (Campbel and Rew, 1986), the elimination of *T. canis* from intestine on the 4<sup>th</sup> hour of our experiment should be caused by the decrease of energy in the tissues, which is connected with the inhibition of ATP synthesis under the action of the drug.

Basing on our data under the action of albendazole and levamisole the changes of intestinal epithelial tissue was less significant. They were expressed by swelling of basal membrane and nuclei (Fig. 1c), the toddle cytoplasm and by the blending of the fibers together in the apical cytoplasm of these cells.

There were not available any publications concerning the changes of the intestinal helminthes tissues under of action of the albendazole neither *in vivo* nor *in vitro*. Therefore we applied the results of the investigations of benzimidazole anthelmintic for the evaluation and analysis of the micro morphological changes of *T. canis* nematode tissues under the action of albendazole *in vivo*.

Some authors described the mode of action of benzimidazoles on various nematode species. P. Köhler (1990) described the mode of action of mebendazole on *Ascaris suum* intestine, J. Comley (1980) - on *Aspicularis tetraptera*, K. Zintz and W. Frank (1982) - on *Heterakis spumosa*. According to their data, benzimidazoles destroy the cytoskeleton of the cells in that way disordering a number of the cell functions. During the linking process of benzimidazoles to a cell tubulin, the process of polymerisation of a tubulin to microtubules is definitely stopped. This not only disturbs the reproduction of a cell, but also its secretion, absorption and nutrition processes. That induces the processes of autolysis since lysis ferments that are found in the granules of endoplasmic reticulum are activated in the cytoplasm. The latter changes cause the death of a parasite (Zintz and Frank, 1982). According to W. Raether (1988), mebendazole first of all affects cytoskeleton of the intestine epithelial cells. Secondly, after the cytoskeleton disruption, starts a production of the secretion granules of Golgi complex and cell degeneration begins.

The disruption of the cell cytoskeleton as well as the appearance of a number of variously sized granules and vacuoles was also observed in the enterocytes of *T. canis* during our experiment of the *in vivo* action of albendazole. During the firsts hours of albendazole treatment in the intestine epithelial cells there already disappeared microfibrils and 46-hours onset the dehelminthization large vacuoles, filled with the disordered material, were evident in enterocytes. Nevertheless, the microvilli layer remained undamaged. Meanwhile, K. Zintz and W. Frank (1982) described mebendazole effect on the microvilli layer of the intestine epithelial cells of *H. spumosa*. According to their data, at the 36<sup>th</sup> hour after the dehelminthization, microvilli of the intestine epithelial layer of these nematodes were already at some places completely disrupted and there were some organelles dropped into the lumen of the intestine. Our micromorphological studies showed that the first changes in *T. canis* intestine immediately after their elimination from the host organism were considerably clear. Thus, it seems highly probable that albendazole gets into the worm organism through the mouth, is quickly absorbed into the intestine and is immediately spread over the tissues.

A number of diverse effects upon different systems have been attributed to levamisole. This includes inhibition of fumarate reductase (Sanderson, 1970), the rapid contraction and paralysis of nematode muscle (Roy et al., 1981), stimulation of glycogen syntase conversion (Komuniecky and Saz, 1982) and others. But no one examined the micro morphological changes in tissues of nematodes after the influence of levamisole.

H. L. Verhoeven et al (1976) has recently reported levamisole uptake through the cuticle of *Ascaris suum*. They presented that the cuticle-hypodermis-muscle (CHM) system contained much more levamisole and/or metabolites (calculated per unit body weight) than the pseudocoelomic fluid, intestine and reproductive system. This suggests that the uptake of levamisole occurs mainly, if not exclusively, through a transcuticular mechanism, thus reaching directly the nerve-muscle system and causing paralysis of nematode.

On the other hand, T. K. Roy and others (1981) presented the uptake of levamisole in the CHM system and gut of *Ascaridia galli* as a function of drug concentration and showed biphasic behaviors: uptake of levamisole in CHM system reaches peak at 120 min after which steep fall occurs. On the other hand in case of gut the uptake continues to rise up to 210 min of incubation.

Our obtained results in the present experiment indicate that there are no significant and good visible on light microscopy changes in epithelial tissue of *T. canis* intestine after levamisole treatment *in vivo*. We noticed some changes in enterocytes only after 25 hr of the administration of levamisole: we registered the swelling of these cells and the blending of microfilaments together inside the cell cytoplasm. Maybe this is the result of the prolonged uptake of levamisole by the enterocytes of *T. canis*. Correspondingly, levamisole passes quickly through the hosts gut and penetrates CHM system of nematodes, but very soon begins the release of levamisole from CHM system (Verhoeven et al., 1976) maybe not only into surrounding but also into the body cavity of the

worm. And then levamisole reaches the epithelial cells of the gut of *T. canis* and causes additional effect.

Summarizing our data and comparing it with the data obtained by other authors it is possible to presume that different reaction of *T. canis* intestinal epithelial tissue under the action of anthelmintics *in vivo* could be related not only to different nature of nematocides. It depends to the physiological condition of these nematodes also, first of their maturity, intensity of nutrition and other factors.

The glycogen inclusions and neutral lipids have changed in epithelial cells of *T. canis* intestine under the action of all our treated anthelmintic. The accumulation of the glycogen deposits in this tissue lowered gradually until they totally vanished by the last period under the action of pyrantel pamoate, albendazole and nitroscanate (table 1).

After the treatment with pyrantel pamoate we can conclude, that this drug affects carbohydrate metabolism in the epithelial cells of *T. canis* intestine. We can see the dynamical decrease of glycogen deposits in these cells in the table 1. As it was mentioned (Mackenstedt et al., 1993), nematodes can limit or even stop the uptake of this drug for some hours. This can explain a rather weak decrease of glycogen deposits in the intestinal cells of *T. canis* eliminated from the host intestine from 8<sup>th</sup> to 12<sup>th</sup> hour after the treatment with pyrantel pamoate (Fig. 2a). It is probable that the worms ingest large amounts of the drug beginning immediately after this period and show a high glycogen deposit decrease in the enterocytes on the 24<sup>th</sup> hour after the treatment.

We can observe an entirely different affect using albendazole. Albendazole could bloc the incorporation of glucose and its use by the parasite (Delgado, 1989). Inhibition of glucose uptake leads to glycogen depletion in the tissues of parasites, leading to a decreased

generation of ATP that is very essential for the survival and replication.

Our data show only a very low positive PAS reaction in the epithelial cells of the intestine of *T. canis*, eliminated from the host intestine 19 hours after the treatment with albendazole. There was only a little amount of glycogen deposits above the nucleus of the enterocytes. After 30 hours post treatment we can see only the remnants of glycogen (Fig. 2b). P. Köhler (1990) showed the dynamical decrease of glycogen in the intestinal epithelial cells of *Ascaris* spp. after the treatment with mebendazole *in vivo*, also.

According to the data from other experiments, the elimination of *T. canis* from the puppy's intestine on the 4<sup>th</sup> hour after the treatment with nitroscanate should be caused by the decrease of energy in the tissues. Which is connected with the inhibition of ATP synthesis under the action of the drug (Campbel and Rew, 1986). Basing on the data by W. Campbel and R. Rew (1986), the nitroscanate in the tissues of *L. carinii* and *B. paghangi* not only inhibits the ATP synthase but affects the uptake of glucose and its metabolism, also.

During the first period of experiment with nitroscanate we registered the glycogen disappearance from the apical part of intestinal cells of *T. canis*. On the 27<sup>th</sup> hour the negative PAS reaction was in all cell cytoplasm. There fore it can be concluded that in *T. canis*, like in other nematode species, enterocytes the uptake of glucose was inhibited. There for the glycogen in this tissue was used as the reserve carbohydrate.

Under the action of levamisole till the third period of the examination glycogen deposits vanished from the enterocytes of *T. canis* intestine, too (Fig. 2a, 2c). However a distinct positive PAS reaction was observed again at the end of this experiment (Table 1).

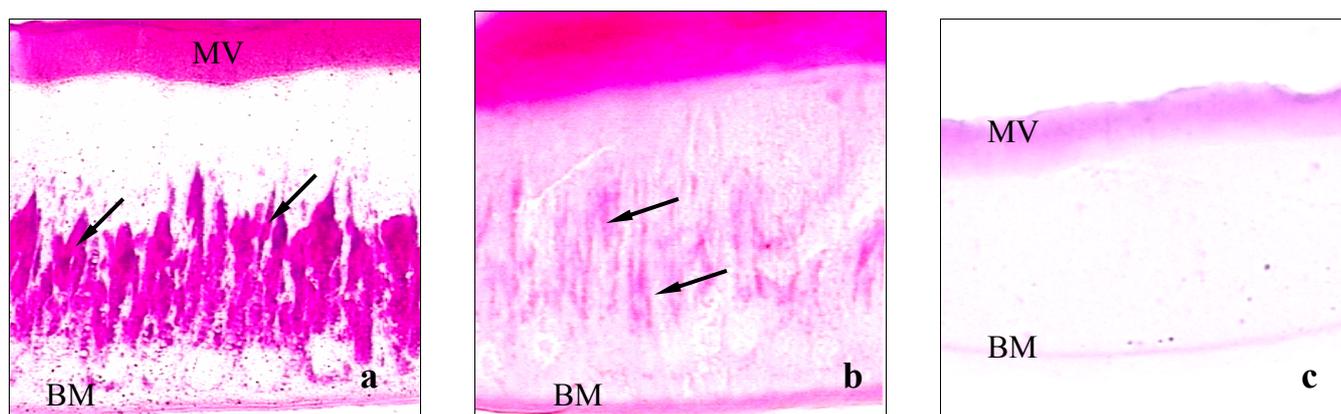


Fig. 2. PAS reaction in *T. canis* enterocytes under the action of anthelmintics: a) after pyrantel pamoate order levamisole treatment; b) after nitroscanate order albendazole treatment; c) PAS negative after levamisole treatment. MV – microvilli, BM – basal membrane, arrows – PAS positive glycogen deposits. 10x40

It is known, levamisole affects various systems in parasites that may or may not contribute to the ultimate chemotherapeutic effect (Roy et al., 1980; Komuniecky and Saz, 1982). It might be expected that, secondary to the levamisole paralysis of the parasite muscles, a change in the carbohydrate metabolism would be expected as a consequence of an altered energy requirement.

P. R. Komuniecki and H. J. Saz (1982) examined the effect of levamisole on glycogen metabolism in the adult filariid *L. carinii*. Incubation of helminthes for up to 6 hr in the presence of levamisole resulted in a fourfold increase in total worm glycogen levels. In accord with this observation, levamisole stimulated the incorporation of glucose into glycogen: they suggested that levamisole

could act to stimulate the glycogen syntase to more active form.

The decrease of glycogen in the enterocytes of *T. canis* we are trying to explain that levamisole not so fast reaches the epithelial cells of the gut. And during the firsts hours of experiment PAS reaction showed the decrease of glycogen, then at the end of experiment (46<sup>th</sup> hr) the enterocytes of *T. canis* were PAS positive, almost similar to controls. This may occur because begins the release of levamisole from nematodes CHM system (Verhoeven et al., 1976). As was mentioned above, maybe, levamisole is released not only into surrounding but also into the body cavity of the worm. Then levamisole reaches the epithelial cells of the gut, glycogen synthesis is activating, and the intensive PAS positive reaction shows the beginning of glucose synthesis to glycogen.

As it is known (Barrett, 1983) neutral lipids forms in the tissues of the worms living in the anaerobic conditions under the intensive glycolysis. In our experiment after the vanishing of the glycogen, neutral lipids occurred in epithelial cells of *T. canis* intestine (Fig. 3b, 3c; table 2).

All our treated nematocides caused a distinct infiltration of the neutral lipids in the enterocytes of *T. canis* intestine. It is important to note that particularly large accumulations of neutral lipids occurred under the

action of albendazole. The significant temporal fat dystrophy was expressed by a number of fat drops that fulfilled the cell cytoplasm. At the end of experiment we noticed the diffusely located pale droplets of neutral lipids very similar to controls (Fig. 3a; table2).

Although at the 46<sup>th</sup> hour onset the treatment with levamisole, the enterocytes of *T. canis* was plenty of glycogen inclusions, neutral lipids were distinct also (Fig. 3c). This can mean that carbohydrate metabolism products (neutral lipids) elimination from the cells has been disordered under the action of this drug.

Basing on the data of our results we can presume that all our treated anthelmintics caused more or less expressed micro morphological changes in the epithelial cells of *T. canis* intestine and destroyed the metabolism of glycogen and neutral lipids in it. Especially significant degeneration was noted under the action of pyrantel pamoate and nitroscanate.

It is worth to note that the micro morphological examinations of nematode tissues and their changes under the action of various agents *in vivo* are not sufficiently analyzed. Therefore we think that in order to explain the disorganization of the structure, energy and lipids' metabolism of *T. canis* more detailed further investigations based on the methods of biochemistry, autoradiography and electron microscope are necessary.

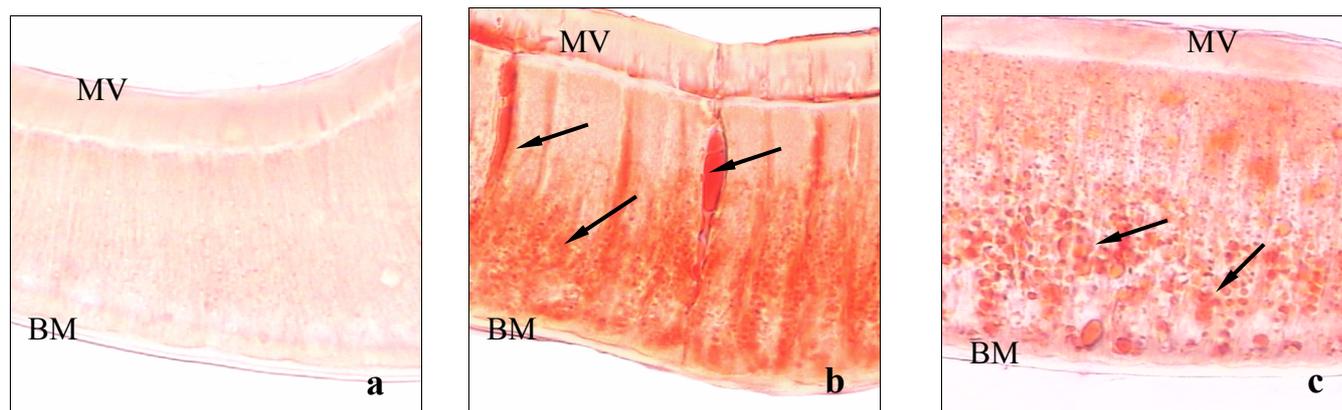


Fig. 3. Neutral lipids in *T. canis* enterocytes under the action of anthelmintics: a) after albendazole treatment; b) after pyrantel pamoate order nitroscanate treatment; c) after albendazole order levamisole treatment. MV – microvilli, BM – basal membrane, arrows – neutral lipids inclusions. 10x40

### Conclusions.

1. The epithelial cover of *T. canis* intestine totally disintegrated under the action of pyrantel pamoate and nitroscanate.
2. Levamisole caused insignificant morphological changes of *T. canis* enterocytes.
3. Significant vanishing of glycogen deposits from the epithelial cells, as well as fat dystrophy of various degrees was established under the action of all our treated anthelmintics.

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