solutions were cleaned using Chelex-100. Previous results have shown that contaminating iron does not exceed a concentration of about 1 μM in such a system [22]. Nevertheless, the residual iron present seems to be sufficient to catalyze the lipid peroxidation observed: addition of the iron chelators EDTA and DETAPAC effectively inhibited the process. One may assume that cytochrome P-450 either directly, or indirectly via generation of superoxide anions, reduces non-heme iron that may act as initiator of the lipid peroxidation after binding of oxygen.

In conclusion, the results presented indicate that cytochrome P-450 may contribute to initiation of lipid peroxidation, although the extent of contribution of P-450 to the lipid peroxidation by its peroxidative properties requires further work.

Present studies in our laboratory focus on the mechanism of the cytochrome P-450-dependent lipid peroxidation.

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The influence of some anthelmintics on the bioenergetic metabolism of *Trichinella spiralis* and *Trichinella pseudospiralis*

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The goal of this investigation was to identify the drug sensitivities of two related nematodes: *Trichinella spiralis* and *Trichinella pseudospiralis*. The focus was upon the bioenergetic metabolism of *T. spiralis* and *T. pseudospiralis* [1]. Some of the results presented in this paper have been published as abstracts [2, 3].

Of the drugs effective in the therapy of intestinal nematodes and tapeworms, the most frequently investigated have been levamisole*, mebendazole† and praziquantel‡. Early studies demonstrated the inhibition of succinate dehydrogenase-fumarate reductase (SDH-FR) complex by thiabendazole§, levamizole and praziquantel. Today, the mode of action of these drugs is considered to be more complex [4]. For example, levamisole affects the neuromuscular system of Ascaris [5] and, in high doses, inhibits fumarate reductase [6]. Immature and adult nematodes kept in tetramisole and levamisole solutions show spastic contraction followed by tonic paralysis. The effect could be either reversible or irreversible according to the worm species. It has been suggested [6] that, in some nematodes, the irreversibility of this neuromuscular blocking activity may be related to the inhibition of the fumarate reductase.

Köhler and Bachman [7] localized the site of the inhibitory action in Ascaris of levamisole, thiabendazole, praziquantel and chloroquine in the electron transport chain between a quinone and NADH-dehydrogenase.

These drugs, except thiabendazole, did not affect succinate oxidation and reduction. Recently, Prichard *et al.* [8] concluded that the sustained release of endogenous Ca^{2-} by the drugs may affect the sequence of excitation-contraction coupling, and cause observed contraction of *H. dimunta*. The biochemical and neuropharmacological changes due to the praziquantel (in conc. of 10^{-7}M) were

* Levamisole is the laboratory isomer of 2,3,5,6-tetrahydro-6-phenyl imidazo(2,1-b) thiazole (the racemic mixture is known as tetramisole).

† Mebendazole is methyl-5(6)-benzoly-2-benzimidazole carbamate.

‡ Praziquantel is 2-cyclohexylcarbonyl-1,3,4,6,7,11b-hexahydro-2H-pyrazino(2,1-a)isoquinoline-4-one.

§ Thiabendazole is 2-(thiazol-4′-yl)benzimidazole.

suggested by Terada *et al.* [9] as a secondary effect of the drug through the action of this compound on ATP-ase's function in worms.

Materials and methods

Trichinella spiralis and *Trichinella pseudospiralis* were isolated from the flesh of experimentally-invaded rats [10]. Twenty percent homogenates of nematodes or 10% homogenates of rat control tissue were prepared in medium consisting of sucrose 0.25 M; EDTA 0.1 mM; Tris buffer 0.020 M, pH 7.3 (fortified in the case of nematodes homogenates with 0.5% of bovine serum albumin, fatty acid free, fraction V).

For the measurement of the activity of SDH-FR complex and of Mg^{2+}-stimulated ATP-ase activity, mitochondria were obtained by the differential centrifugation [11].

Mitochondrial preparations for the estimation of cytochrome-c reductases were incubated in 0.05 M phosphate buffer (pH 7.2) for 15 min at 0°. The activity of succinate-cytochrome oxidoreductase (SC-1.3.99.1) and NADH-cytochrome-c oxidoreductase (NC-EC1.6.99.3) was determined according to Green and Ziegler [12] and Mg^{2+} stimulated ATP-ase activity as the release of inorganic phosphate by the Fiske/Subbarow method [13].

The activity of succinate dehydrogenase (SDH) was determined in mitochondrial preparation after freezing and thawing 3 times, according to King [14] in the following medium: 2,6-dichloro-indophenol (0.05 mM), phosphate buffer (pH 7.6–7.8; 20 mM), KCN (1.5 mM), BSA (0.1%), phenosinemethosulphate (1.8 mM) and succinate (0.5; 50 mM). Activity of fumarate reductase (FR) was measured according to Prichard [15] in the following medium: phosphate buffer (pH 6.9; 400 mM), calcium chloride (33 mM), magnesium chloride (10 mM), NADH (120 mM) and fumarate (0.2–50 mM).

Oxygen uptake with succinate (succinate oxidase SOX) was measured polarographically using the Oxygraph (Yellow Spring Instrument Co.) in total volume of 1.0 ml using the following incubation medium: Tris buffer (pH 7.2; 20 mM), magnesium chloride (6 mM), potassium chloride (125 mM), inorganic phosphate (5 mM), bovine serum albumin (0.5%), succinate (20 mM) and EDTA (0.1 mM). Respiratory control index (RCI) was calculated according to Estabrook [16].

During the measurements of the influence of the drugs on the enzymes of electron transport chain, the following drugs were incubated with mitochondrial preparations for 15 min at room temperature (control preparation in the same conditions without the drug): (a) levamisole—dissolved in water (Janssen Pharmaceutica R-12564); (b) thiabendazole—suspended as 20% suspension in water (Merck Sharp and Dome Ltd.); and (c) mebenazole—micronized, suspended in water (Janssen Pharmaceutica B-1763).

For calculations of chemotherapeutical biochemical index [17], the inhibition constants were established for both the rat liver and *Trichinella* enzymes by Dixon's method [18]. Protein content was estimated by the Lowry method [19].

Results and discussion

It was shown that all anthelmintics tested in this investigation were inhibitors of some enzymes of the electron transport chain (Table 1). However, the high value of the inhibition constant for the SDH-FR complex makes it unlikely that this is a primary site of action of levamisole. On the other hand, the chemotherapeutic index (the higher the value, the less toxic the drug) is high enough (about 32) to assume that the drug sample has low toxicity.

The results indicate that levamisole acts as an uncoupler with mitochondria of *Trichinella pseudospiralis*. The drug enhances the Mg^{2+}-stimulated ATP-ase in coupled mitochondrial preparations. The strong stimulation of mitochondrial ATP-ase by levamisole (7.3×10^{-3} M) was similar to that of 2,4-dinitrophenol at a concentration of 1 mM. This suggests that another mode of action of levamisole could be that of an uncoupler.

If we compare the inhibition constant obtained with levamisole in *T. pseudospiralis* with those of Köhler and Bachman for Ascaris [7], it is evident that with both nematodes a chemotherapeutic target point of levamisole action is probably located in an NADH-oxidation pathway.

Microscopic examination during incubation at room temperature of *T. spiralis* and *T. pseudospiralis* with levamisole indicated an irreversible paralysis of both *Trichinella* nematodes in about 15 min. The larvae after 2 days (even with 10 min of boiling of larvae suspension in 0.9% NaCl) appeared as "tightly rolled spirals" in contrast to untreated worms, which always displayed a "C" shape when dead.

After about 10–15 min of incubation in 0.9% NaCl solution with different concentrations of drug (ranging from 100 to 1000 μM), some of the worms were tightly rolled and, after about 90 min, all of the worms were rolled. The ED_{50} value was calculated and estimated as 0.048 mM from the curve relating the levamisole concentration to the mobility of parasites (the norm was taken as the mobility

Table 1. Influence of anthelmintics: levamisole (L), thiabendazole (T), and mebendazole (M) on the electron transport enzymes and mitochondrial ATP-ase in *Trichinella spiralis* and *Trichinella pseudospiralis* mitochondria

		T. spiralis		*Trichinella pseudospiralis*								
									SOX		ATP-ase	
Drug		SDH	FR	SDH	FR	SC	NC	NOX	C(mM)	Stim(%)	C(mM)	Stim(%)
L	K_i	—	—	3.0 (2.8–3.1)	3.5 (2.9–4.0)	3.0 (3.9–3.0)	0.47 (0.40–0.54)	0.40 —	3.0	420 (300–500)	2.2	300 (100–300)
	Q			31.7							7.3	600
T	K_i	—	—	—	—	0.52 (0.48–0.56)	0.64 (0.60–0.65)	1.0		—		—
M	K_i	1.2 (1.0–1.4)	1.3 (0.9–1.5)	1.0 (0.8–1.2)	2.3 (1.8–2.8)	—	—	—		—		—
	Q	8.8	10.5	10.5								

All results are the mean from 3 experiments except NOX (a mean from two exp. only). SDH—succinate dehydrogenase, FR—fumarate reductase, SC—succinate: cyt.c oxidoreductase, NC—NADH: cyt.c oxidoreductase, NOX—NADH-oxidase, SOX—succinate oxidase, ATP-ase—mitochondrial, Mg^{2+}-stimulated ATP-ase. ATP-ase activity in coupled mitochondrial preparations (RCI between 3.0 and 7.0) without drug = 20 nA of P_i/min/mg of protein. SOX and NOX activity = 9.6 and 9.7 nmoles of O_2/min/mg of protein, respectively.

K_i values were calculated according to Dixon plots (SDH and FR) or from the curves relating % of inhibition to inhibitor concentration (both reductases and NOX). K_i—inhibition constant, Q—biochemical chemotherapeutic index = ratio of K_i for particular enzyme in rat liver to K_i value of the same enzyme in nematodes mitochondria. K_i value for rat liver mitochondria with levamisole was 95.0 and with mebendazole 10.5.

Table 2. Sensitivity of *Trichinella* metabolism to levamisole (L) and thiabendazole (T)

	L	T	Reaction
ED_{50}	about 50 μM	—	neuromuscular paralysis
K_i	470 μM	649 μM	NADH-cyt.c reductase
	400 μM	1000 μM	NADH oxidase
	3000 μM	—	SDH-FR complex
	3000 μM	520 μM	succinate-cyt.c reductase
	1600 μM	—	succinate oxidase
	about 7000 μM	—	oxidative phosphorylation

of the worms incubated at the same temperature for the same length of time without the drug). All measurements were repeated 4 times.

Levamisole, as in Ascaris [5], caused neuromuscular paralysis in *Trichinella* nematodes. Although it is difficult to compare the ED_{50} of this paralysis with the K_i values obtained in the measurements on electron transport enzymes, it seems possible that the neuromuscular system is the "sensitive point" of the chemotherapeutic action of this drug. Probably the effects are related to the contraction of the muscle and its energetic supply [4].

Table 2 summarizes the sensitivity of *Trichinella* metabolism to levamisole (L) and thiabendazole (T).

Earlier investigations on *H. contortus* demonstrated no inhibitory effects of mebendazole on FR activity, but this could be due to the solubility of the drug [15]. McCraken [21] demonstrated the high potency of mebendazole in rat trichinellosis, and suggested the influence of this drug on FR in *T. spiralis*. Boczon has shown the inhibitory effect of mebendazole on *T. spiralis* SDH-FR complex *in vitro* [3]. This paper presents the same inhibitory effect in *T. pseudospiralis* (Table 1). It is worthy to mention, however, that in these investigations on the influence of medendazole on *T. pseudospiralis*, a micronized drug sample was used.

In summary, levamisole was shown to have multiple sites of chemotherapeutic attack on *Trichinella spiralis* and *Trichinella pseudospiralis* larvae. These were: (a) irreversible paralysis (by low concentration of the drug, ED_{50} = 48 μM) of the neuromuscular system in both nematodes; (b) inhibition of the NADH oxidation and reduction pathway in *T. pseudospiralis* (K_i between 0.4 and 0.6 mM); (c) inhibition of the succinate dehydrogenase-fumarate reductase complex in both nematodes (K_i = 2 and 3 mH, respectively); and (d) uncoupling of the oxidative phosphorylation in *T. pseudospiralis* (K_i about 7 mM). Mebendazole also inhibited the succinate dehydrogenase-fumarate reductase complex in mitochondria of both nematodes.

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