Effects of Anticestodal Agents on Mitochondria from the Nematode, *Ascaris lumbricoides*

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SUMMARY

Several anticestodal drugs were shown previously to inhibit the mitochondrial ^{32}P-ATP exchange reaction in the cestode *Hymenolepis diminuta*. It was postulated, on the basis of similarities in their energy metabolism, that the same anticestodal agents should also inhibit the exchange reaction in the mitochondria of the nematode, *Ascaris lumbricoides*. Most of these compounds did effectively inhibit the reaction in the nematode mitochondria, indicating that the selective toxicity for cestodes is a result of differences in permeability between the two groups of helminths.

Scheibel, Saz, and Bueding (1) reported that the anaerobic incorporation of ^{32}P into ATP in the intact cestode, *Hymenolepis diminuta*, is inhibited by classical uncouplers of oxidative phosphorylation as well as by a number of anticestodal agents. The same authors also showed that these compounds inhibit the ^{32}P-ATP exchange reaction in mitochondria prepared from the tapeworm. The anticestodal drugs studied have no chemotherapeutic effect on the nematode, *Ascaris lumbricoides*. This is of interest, since the energy metabolism of this roundworm closely resembles that of *H. diminuta* (2–4). Both helminths appear to be anaerobes which form succinate by CO_2 fixation followed by reduction of the dicarboxylic acid formed (5); cytochrome oxidase activity is not demonstrable (1, 6, 7), and phosphorylation associated with anaerobic electron transport has been reported to occur in both helminths (1, 8–10).

Two explanations may be offered for the differences in susceptibility of nematodes and cestodes to these anticestodal agents. Either biochemical variations exist between the two groups of parasites at the site of drug action, or the permeability characteristics of the nematode prevent the inhibitors from reaching these sites. Results of the following experiments indicate that the latter is the case. As in *H. diminuta*, mitochondria from *Ascaris* catalyze the ^{32}P-ATP exchange reaction, which, in turn, is inhibited markedly by most of the anticestodal agents investigated.

Adult female *A. lumbricoides* var. *suis* were collected at a local slaughterhouse. Muscle was obtained by dissection in the cold, and each gram of tissue was minced and homogenized in 10 ml of 0.24 M sucrose containing 0.005 M ethylenediaminetetraacetate (pH 7.4) and 0.15% (w/v) crystalline bovine serum albumin. Homogenization was performed in a Potter-Elvehjem homogenizer with a Teflon pestle. Cell debris was removed by centrifugation at 128 *g* for 10 min. Mitochondria were obtained from the supernatant by centrifugation at 9000 *g* for 30 min, and the pellet was washed once with the suspending fluid. All operations were carried out at 2–4°.

Incubations were terminated after 10 min by the addition of perchloric acid to a final concentration of 3% (w/v), and the

precipitated protein was removed by centrifugation. A 10-min incubation period was chosen, since time course experiments had shown that isotopic equilibrium between ATP and ^{32}P was not attained by this time. Nucleotides were separated from inorganic ^{32}P in the supernatant solution essentially as described by Scheibel *et al.* (1) by adsorbing the nucleotides on charcoal, washing, and eluting. ATP concentrations were determined in an aliquot of the eluate, and another aliquot was subjected to thin layer chromatography. Complete separation of residual inorganic ^{32}P from nucleotides was verified by monitoring the thin layer chromatograms with a strip scanner. Areas corresponding to the nucleotides were scraped from the thin layer chromatogram plates directly into scintillation fluid (11) and assayed for radioactivity. ATP was assayed spectrophotometrically according to the procedure of Lamprecht and Trautschold (12) as modified by Bueding *et al.* (13).

Uncouplers and anticestodal agents were solubilized as described previously (1). Where indicated, the 5-nitrofurfurylidene hydrazide of 3,5-dinitrosalicylic acid[1] (Salsbury Laboratories, compound RT-6912) was dissolved in methanol prior to addition to the incubation mixture. When compounds in ethanol or methanol were used, control vessels were incubated that contained equivalent quantities of the solvents without solute. The concentrations of ethanol added had no significant effect upon the exchange reaction. Methanol, on the other hand, was inhibitory.

In the absence of exogenous substrate, *Ascaris* mitochondria catalyze a rapid incorporation of inorganic ^{32}P into added ATP (Table 1). This exchange reaction is inhibited by dinitrophenol and carbonyl cyanide *m*-chlorophenylhydrazone, both classical uncouplers of oxidative phosphorylation. Desaspidin, chlorsalicylamide (Yomesan), 2,4-dihexanoyl-6-methylphloroglucinol (Smith Kline and French, compound 90,625) and *N,N*-diheptyl-4-pentyloxynaphthamidine (Burroughs Wellcome, compound 61-435) are all anticestodal agents which also inhibit the exchange reaction in *Ascaris* mitochondria (Table 1), in the same concentrations previously tested with the *H. diminuta* mitochondrial system. Dichlorophen, on the other hand, had no effect on the incorporation of ^{32}P in the nematode system. It is of interest that this anthelmintic was the least potent inhibitor of the mitochondrial exchange reaction in cestodes, as reported by Scheibel *et al.* (1). The 5-nitrofurfurylidine hydrazide of 3,5-dinitrosalicylic acid has no anticestodal activity. This nitrophenol derivative also has no significant inhibitory effect upon the *Ascaris* mitochondrial system, indicating some degree of specificity of the inhibitors. In all experiments, the quantities of ATP recovered after incubation with the inhibitors were essentially the same as the control values, indicating that the observed effects did not result from stimulation of ATPase.

With the exception of dichlorophen, therefore, all of the anticestodal compounds reported to inhibit the exchange of ^{32}P into ATP in the cestode *H. diminuta* have the same inhibitory action in mitochondria from the nematode *A. lumbricoides*. These findings are in agreement with the supposition that the ^{32}P-ATP exchange reaction, which is presumably associated with the coupling mechanism of electron transport-mediated phosphorylation, occurs in both the cestode and the nematode, but that permeability barriers prevent the anticestodal drugs from inhibiting the intact nematode. On this basis, it appears likely that inhibitors of the ^{32}P-ATP exchange reaction would have antinematodal as well as anticestodal activities if they were capable of penetrating the tissues of the respective helminths.

[1] The structures of compounds used in these experiments are listed in Table 1.

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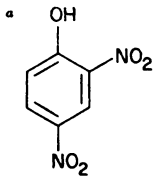
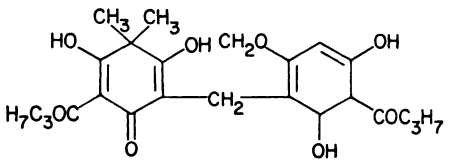
TABLE 1

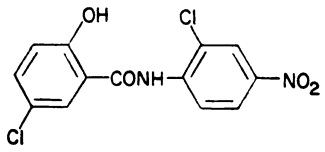
Effect of anticestodal agents and uncouplers of oxidative phosphorylation on the ^{32}P-ATP exchange reaction in Ascaris mitochondria

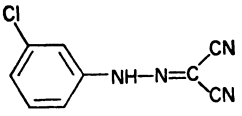
In experiments 1 and 2, mitochondria prepared from 5 g of *Ascaris* muscle were suspended in 7 ml of medium. Then 0.5 ml of mitochondrial suspension was added to 2.5 μmoles of ATP, 0.99 μmole of potassium phosphate buffer (pH 7.4) containing 0.1 mC of ^{32}P, and the indicated concentrations of inhibitors in a final volume of 1.0 ml. Tubes were incubated at 25° for 10 min in air. Conditions were the same in experiments 3, 4, and 5 except that the mitochondrial suspension added was twice as concentrated, being prepared from 10 g of *Ascaris* muscle.

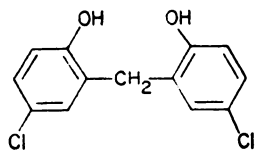
Expt.	Inhibitor	Concentration of inhibitor (μM)	Specific activity of ATP (cpm × 10^{-3}/ μmole)	Inhibition (%)
1	None		68.3	
	Dinitrophenol[a]	100	33.5	51
	Desaspidin[b]	10	27.1	60
	Chlorsalicylamide[c]	50	16.5	76
2	None		31.6	
	CCP[d]	10	20.0	37
	Dichlorophen[e]	100	32.1	0
	Salsbury, compound RT-6912[f]	400	28.7	9
3	None		127.3	
	BW compound 61-435[g]	100	58.8	54
	SKF compound 90,625[h]	10	47.2	63
4	Methanol control		86.7	
	Salsbury, compound RT-6912 in methanol	100	82.9	4
5	None		336.1	
	Dichlorophen	100	337.9	0
	Chlorsalicylamide	50	49.2	85
	CCP	10	62.6	81

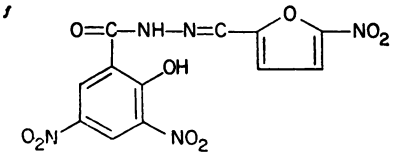
[a] 

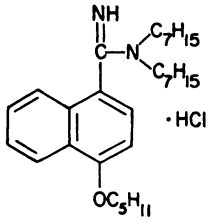
[b] 

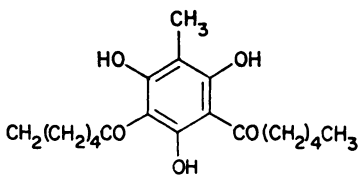
[c] 

[d] Carbonyl cyanide *m*-chlorophenylhydrazone: 

[e] 

[f] 

[g] 

[h] 

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REFERENCES

1. L. W. Scheibel, H. J. Saz and E. Bueding, *J. Biol. Chem.* **243,** 2229 (1968).
2. H. J. Saz and E. Bueding, *Pharmacol. Rev.* **18,** 871 (1966).
3. D. Fairbairn, G. Wertheim, R. P. Harpur and E. L. Schiller, *Exptl. Parasitol.* **11,** 248 (1961).
4. L. W. Scheibel and H. J. Saz, *Comp. Biochem. Physiol.* **18,** 151 (1966).
5. H. J. Saz and A. Vidrine, Jr., *J. Biol. Chem.* **234,** 2001 (1959).
6. E. Bueding and B. Charms, *J. Biol. Chem.* **196,** 615 (1952).
7. B. Chance and D. F. Parsons, *Science* **142,** 1176 (1963).
8. C. H. Chin and E. Bueding, *Biochim. Biophys. Acta* **13,** 331 (1954).
9. E. Kmetec and E. Bueding, *J. Biol. Chem.* **236,** 584 (1961).
10. I. Seidman and N. Entner, *J. Biol. Chem.* **236,** 915 (1961).
11. G. A. Bray, *Anal. Biochem.* **1,** 279 (1960).
12. W. Lamprecht and I. Trautschold, *in* "Methods of Enzymatic Analysis" (H. U. Bergmeyer, ed.), p. 543. Academic Press, New York, 1963.
13. E. Bueding, E. Bülbring, G. Gercken, J. H. Hawkins and H. Kuriyama, *J. Physiol. (London)* **193,** 187 (1967).