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Metabolic end products of helminths: their degradation and excretion by the host

Biochemical approach to the diagnosis of ascaridiasis and other helminthiases

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Summary

Accumulation, degradation and excretion by the host of helminth metabolic end products, such as branched-chain volatile fatty acids are poorly studied although they play an appreciable role in host-parasite interactions and, being detectable in excreta, provide a reliable diagnostic test for at least some helminthiases. The amount and the structure of the end products of intestinal Nematoda metabolism depend on pH, rH, pCO_2, pO_2, and composition of the media. In the human intestine *Ascaris* excretes 40–60 mmol volatile fatty acids/100 g wet weight daily. α-methylbutyric, α-methylvaleric and isovaleric acids are slowly degraded. In concentrations of 10^{-3}–10^{-4} M they exert an inhibitory effect on O_2 consumption, oxydative phosphorylation and Mg^{2+}-ATP'ase activity in mitochondria. Given by mouth to laboratory animals or injected intraperitoneally, ^{14}C-labeled branched-chain volatile fatty acids rapidly appear in saliva and urine and are detectable by chromatography and IR spectrography.

Key words: Ascaris; metabolism; volatile fatty acids; diagnostic; saliva.

Introduction

Metabolic pathways of helminths differ in many aspects from the pathways of their hosts (Bueding, 1966; von Brand, 1972; van den Bossche, 1972; Soprunov, 1978). Some end products of helminth metabolism are unusual for the host (Moyle and Baldwin, 1952; Saz and Gerson, 1962; Bueding, 1966; Greichus and Greichus, 1966), for example the branched-chain volatile fatty

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Table 1. End products of helminths

Species	End products (acids)	References
Nematodes:		
Ascaris lumbricoides (Ascaris suum)	Formic (C_1), acetic (C_2), propionic (C_3), butyric (C_4), α-methyl-butyric, valeric (C_5), α-methyl-caproic, lactic, carbonic (ethyl alcohol)	Flury, 1912; Schulte, 1917; Epps et al., 1950; Bueding and Yale, 1951; Moyle and Baldwin, 1952; Bueding, 1953; Ellison et al., 1960; Harpur and Waters, 1960; Ueno, 1960; Pushkarev, 1965; Greichus and Greichus, 1966; Harpur and Leigh-Brown, 1971; Laynis, 1971, 1975
Parascaris equorum	Propionic, valeric, lactic	Toryu, 1933
Angiostrongylus cantonensis	Lactic	Yanagisawa and von Brand, 1965
Mecistocirrus digitatus	Acetic, propionic, α-methyl-butyric, lactic	Shestak, 1973
Litomosoides carinii	Acetic, lactic, acetyl-methyl-carbinol	Bueding, 1949
Dracunculus insignis	Lactic	Bueding and Oliver-Gonzales, 1950
Heterakis gallinae	Acetic, propionic, lactic, pyruvic, long-chain fatty acids	Glockin and Fairbairn, 1952; Fairbairn, 1954
Dirofilaria uniformis	Lactic	von Brand and Browman, 1963
Trichuris vulpis	Formic, acetic, propionic, butyric, valeric, α-methyl-valeric, succinic, lactic, carbonic	Kmetec and Bueding, 1965; Bueding, 1966
Ancylostoma caninum	Acetic, propionic, iso-butyric, iso-caproic, carbonic	Crowley and Warren, 1963; Warren and Poole, 1970
Trichostrongylus colubriformis (larvae)	Acetic, propionic, butyric	Prichard and Rothwell, 1972
Trichinella spiralis	Formic, acetic, propionic, butyric, valeric, caproic (C_6)	Prichard and Rothwell, 1972; von Brand, 1952; Castro and Fairbairn, 1969
Dictyocaulus viviparus	Acetic	Vaastra, 1969

Table 1 (continued)

Trematodes:		
Fasciola hepatica	Acetic, propionic, butyric, iso-butyric, α-methyl-valeric, valeric, lactic	Mansour, 1959; Moss, 1970; Senutayte, 1970; Lahoud et al., 1971
Fasciola gigantica	Lactic	Goil, 1957, 1961
Cestodes:		
Hymenolepis diminuta	Lactic, succinic	Read, 1956; Fairbairn et al., 1961
Moniezia expansa	Lactic, succinic, long-chain fatty acids	von Brand, 1933; Smyth, 1947
Echinococcus granulosus	Acetic, propionic, valeric, sussinic, carbonic, long-chain fatty acids, ethyl alcohol	Agosin, 1957

acids (VFA) excreted by many species (Table 1). These end products are found in the body of vertebrates only if these harbour helminths (Karnaukhov and Soprunova, 1976; Alieva et al., 1980).

Accumulation, degradation and excretion of end products of parasites by the host are poorly studied although the identification of VFA in excreta of the host could serve as a highly sensitive and reliable diagnostic test for at least some forms of helminthiasis.

Materials and methods

Amounts and structure of end products excreted by *Ascaris lumbricoides, A. suum, Ascaridia galli, Fasciola hepatica* and some other parasitic worms have been investigated in our laboratory during the last 15 years (Pushkarev, 1966; Laynis, 1971; Senutayte, 1971; Lazdyna et al., 1976). The fate of these end products in the body of the host, their possible damaging action on the tissues and their excretion rate were extensively studied (Soprunova, 1968, 1971; Soprunova and Andreeva, 1968; Lazdynya and Buykis, 1970 a/1970 b; Laynis, 1971, 1975; Soprunova and Lurje, 1972; Soprunova et al., 1973; Karnaukhov and Soprunova, 1976; Alieva and Lurje, 1979; Alieva and Soprunov, 1979; Alieva et al., 1980). This work was carried out in laboratory animals (rats, rabbits, pigs and chickens) and in infected patients in our clinic and in foci of helminthic diseases.

Compensative potentiometry was used to follow the excretion of acid end products by the worms maintained in Hedon-Fleig medium at 37° C; the isolation of VFA from biological material was effected by distillation with steam and specific extraction with ether, benzene and other organic solvents. Individual VFA were identified by paper chromatography of hydroxamate derivatives, gas chromatography and infra-red spectroscopy; isotopic labeling was used for following VFA transformation and excretion and polarography in closed cells with rotating platinum electrode for the registration of the inhibitory effect of VFA on O_2 consumption and oxidative phosphorylation in isolated mitochondria (medium for polarography: 0.02 M KH_2PO_4, 0.05 M tris-buffer-pH-7.5, 0.001 M $MgCl_2$, 0.15 M KCl, 0.15 M glucose + 0.5 mg hexokinase, 0.001 M ATP, 0.001 M EDTA; concentration of substrates – 0.025 M; inhibitor – FNa); the micro-Astrup method was employed for determining the shift of physico-chemical properties in saliva. For details see previous publications (Pushkarev, 1966; Soprunova, 1968; Laynis, 1971; Soprunova and Lurje, 1972; Alieva and Lurje, 1979; Alieva et al., 1980).

Results

End products of Ascaris

Carbohydrate degradation pathways of intestinal worms are to some extent labile. Amounts and chemical structure of excreted VFA closely depend on the properties of the medium (e. g. pH, pCO_2, pO_2, concentration of glucose and mineral salts). For the effect of the redox potential, see Soprunov (1978, p. 28). Table 2 shows the effect of oxygen on VFA production.

Under conditions close to those prevailing in the lumen of the intestine the daily excretion of VFA by *Ascaris* is of the order of magnitude of 20–60 mmol/100 g wet weight. The structure of these metabolic end products is presumably similar to the VFA extracted from the hemolymph and tissues of fresh collected worms (Table 3).

Table 2. Amount of VFA excreted by *Ascaris suum* in dependence of medium conditions

Medium conditions for *Ascaris*	Total amount mmol/(10 g · 24 h)	VFA (mol %)					
		C_1	C_2	C_3	C_4	C_5	C_6
Aerobic	1.89 ± 0.32	4.7	27.5	18.5	4.3	27.0	18.0
Anaerobic (CO_2)	2.01 ± 0.37	4.6	20.2	13.8	3.4	35.6	22.4
Anaerobic (N_2)	2.17 ± 0.47	8.0	21.8	16.1	4.1	30.0	20.0

Table 3. VFA in haemolymph and tissues of *A. suum*

VFA	VFA (mol %)	
	in tissues of *Ascaris*	in haemolymph of *Ascaris*
C_2	9.8 ± 0.7	13.7 ± 1.7
C_3	4.0 ± 0.1	5.0 ± 0.3
iso-C_4	1.2 ± 0.2	1.1 ± 0.3
n-C_4	2.2 ± 0.1	2.4 ± 0.1
α-Me-C_4 / iso-C_5	41.7 ± 1.4	40.0 ± 2.3
n-C_5	9.2 ± 1.1	5.2 ± 0.9
α-Me-C_5	31.2 ± 2.1	32.4 ± 1.4
iso-C_6 / n-C_6	0.7 ± 0.2	0.2 ± 0.1

A reliable diagnostic test for ascaridiasis can be based on the identification of α-methylbutyric, α-methylvaleric and isovaleric acids in the excreta of the host.

Damaging action of VFA on the host

Table 4 shows the inhibitory effect of ascaris VFA on O_2 consumption and on oxidative phosphorylation in isolated mitochondria. Table 5 shows the influence of VFA on ATP-ase activity.

We have found that as much as 60–80% of the total amount of VFA, excreted by the worms, are retained and metabolized in the body of the host (piglets). Structure and damaging effect of presumable intermediary products are unknown.

Excretion of VFA by the host

Given by mouth to laboratory animals (rats) or injected intraperitoneally, VFA rapidly appeared in saliva and urine (Table 6). On pigs experimentally invaded with *A. suum* it was shown that VFA appeared in urine before the worms reached maturity (before ova were found in faeces). In two pigs harbouring at autopsy only 1 and 2 male worms the urine test was positive. In urine and

Table 4. Influence of VFA ($1 \cdot 10^{-3}$ M) on O_2 consumption and oxidative phosphorylation in isolated liver mitochondria of rats (%)

VFA	Δ O	Δ P	Δ (P:O)
C_2	−2.8	−23.3	−20.9
C_3	−2.8	−6.0	−2.7
n-C_4	−0.2	−18.7	−19.7
iso-C_4	−8.1	−26.5	−24.3
n-C_5	+2.2	−15.1	−18.0
iso-C_5	+3.2	−22.6	−26.5
α-Me-C_4	+5.0	+1.0	−3.5
n-C_6	−3.6	−12.0	−11.6
iso-C_6	−0.7	−28.0	−27.7
VFA from *A. suum* tissues	+0.5	−14.7	−11.4
VFA from *A. suum* haemolymph	−14.0	−25.9	−13.6

Table 5. Influence of VFA ($5 \cdot 10^{-3}$ M) on Mg^{2+}-dependent ATP-ase activity in rat liver mitochondria

VFA	ATP-ase activity μg P/(mg of protein · 30 min)	Stimulation (%)
Without VFA	2.55 ± 0.07	0
C_2	4.64 ± 0.11	+82.4
C_3	4.92 ± 0.14	+93.4
n-C_4	4.73 ± 0.14	+85.6
iso-C_4	3.65 ± 0.13	+43.0
n-C_5	4.31 ± 0.16	+69.0
iso-C_5	3.50 ± 0.12	+37.2
α-Me-C_4	3.32 ± 0.16	+30.3
Me_3-C_2	3.20 ± 0.08	+25.0
n-C_6	4.85 ± 0.07	+90.0
iso-C_6	4.34 ± 0.10	+69.8
α-Me-C_5	3.26 ± 0.10	+27.7
VFA from *A. suum* tissues	3.60 ± 0.14	+41.0
VFA from *A. suum* haemolymph	3.83 ± 0.13	+50.0

saliva of patients with ascaridiasis VFA are readily detected by paper and gas chromatography after extraction with suitable organic solvents (Soprunova, 1968).

In our experiments the quantity of VFA detected in urine, saliva and faeces did not exceed 10–20% of the injected amount or the amount presumably excreted by the worms in the intestine. Excretion through the lungs and the skin was not studied.

Table 6. Excretion of VFA in rat urine

VFA given p.o.	Amount of VFA detected in urine, mmol/(m · 24 h)	VFA (%)					
		C_2	C_3	C_4	α-Me-C_4	C_5	α-Me-C_5
α-Me-C_4 (0.25 mg/g of animal weight)	0.24 ± 0.05	57.3 ± 2.2	22.8 ± 7.2	7.0 ± 2.3	12.9 ± 4.1	–	–
α-Me-C_5 (0.25 mg/g)	0.20 ± 0.04	51.7 ± 2.8	23.4 ± 5.0	9.2 ± 2.1	–	traces	15.7 ± 4.1
Volatile fraction from coeloma fluid of *A. suum* (0.50 mg/g)	0.25 ± 0.09	47.0 ± 14.3	19.5 ± 2.6	7.0 ± 4.4	11.5 ± 3.7	traces	15.0 ± 3.9
Without VFA	0.15 ± 0.08	57.5 ± 6.2	32.5 ± 5.7	10.0 ± 2.3	–	traces	–

Table 7. Comparative data of coproovoscopy and biochemical tests

Colour test in urine*		Coproovoscopy	Test in urine
106	patients with acute and chronic diseases (hepatitis, gastritis and others)	106 –	106 –
14	patients with ascaridiasis	14 +	14 +
76	patients with other helminthiasis (opistorchosis, trichocephalosis, enterobiosis and others)	76 +	76 –
Test in saliva**		**Coproovoscopy**	**Δ pH**
309	healthy children	309 –	1.04 ± 0.02
69	children with ascaridiasis***	69 +	1.37 ± 0.02
32	children with mixed invasion (ascaridiasis + other helminths)	32 +	1.44 ± 0.02
32	children with other helminthiases (children harbouring *Trichocephalus trichiuris, Strongyloides stercoralis, Enterobius vermicularis, Hymenolepis nana, Taeniarhynchus saginatus* and *Diphyllobothrium latum*)	32 +	1.25 – 1.34

* Patients in the clinic of the institute
** School children in foci of helminthiases
*** In cases of heavy ascaridiasis the mean value was 1.55 ± 0.09.

Simple diagnostic tests for VFA detection

Excess of NaOH is added to collected samples of urine or saliva. After drying on a water bath, residues are sent to the laboratory for chromatographic determination of VFA. In the absence of facilities for chromatography, the following tests may be useful:

a) Colour test in urine

1. Put 10 ml urine into a tube with a glass stopper containing 5 g anhydrous $MgSO_4$. Shake up to dissolution.
2. Add 1 ml H_2SO_4 conc. and 12 ml C_6H_6. Shake vigorously by hand for 4–5 min.
3. After separation into two layers transfer 10 ml of the upper benzene extract into a tube with 10 ml 3 mM NaOH. (N. B. Be careful not to contaminate the pipette.) Shake vigorously by hand for 4–5 min.
4. Discharge the upper layer. Transfer the alcaline solution (10 ml) into a clean tube.
5. Titrate the re-extract with the indicator solution up to colour change: blue → orange. The reaction is considered positive if $\leqslant 6$ drops are needed for colour change.

To prepare the indicator-solution dissolve 0.1 g bromthymol blue in 20 ml of hot ethanol and dilute up to 100 ml with 0.1 N H_2SO_4. The colour changes when 9–10 drops (0.3 ml) of this indicator are added to 10 ml of 3 mM NaOH sol.

b) Test in saliva

Collected samples of saliva in capillary tubes (80 microl). Close tubes rapidly at both ends with mastic (BMA-102) and send to laboratory in refrigerated box. Diluted samples 1/10 with physiol. sol. and introduce in vessels of p-CO_2-microelectrodes (membrane-Silicon fume D-606; sensitivity 10^{-4}–10^{-5} mM H^+). Registrate drop of pH (Δ pH) after 2 minutes.

Evaluation:

	Δ pH	<1.08	reaction negative (no VFA in saliva)
1.08 $<$	Δ pH	<1.16	reaction negative (doubtful)
	Δ pH	>1.16	reaction positive (VFA in saliva)

Biochemical methods are used for mass examination in USSR, Roumania and Poland. Some results and comparative data are given in Table 7. Biochemical methods have been used for routine mass examination. As a rule they give a higher percentage of positive findings than coproovoscopy (Karnaukhov and Soprunova, 1976, Alieva et al., 1980).

Discussion

Amounts and structure of specific metabolic end products of *Ascaris* and their dependance on the culture medium have been studied by many authors. In this respect our data seem to roughly resemble those published previously.

However, there is a great discrepancy between the results in Table 2 and the results given in a previous publication (Soprunov, 1978, p. 28). Even if one takes into consideration that CO_2 accounts for 10 to 15% of the total amount of organic acids excreted by *Ascaris* at a redox potential of –400 mV and pH 8.0, it is obvious that the excretion rate of VFA in buffered alkaline flowing media with a low oxidative potential is 10 to 20 times higher (45.5 micromol/h/g wet weight) than in media where an accumulation of VFA takes place at a neutral pH (Table 2). The properties of the medium and the conditions in our previous experiments are rather close to those usually existing in the lumen of the human intestine and consequently the excretion of VFA by *Ascaris* in vivo must be much higher than usually expected.

To our knowledge, no data have been previously published about an accumulation of specific metabolic end products of helminths in the tissues of the host and no research has been carried out on the host's degradation pathways and the excretion of these products. We have found that at least 20 to 40% of the

VFA produced by *Ascaris* are excreted unchanged by the host through urine and saliva. This phenomenon can be used for diagnostic purposes.

We have developed simple, sensitive and reliable diagnostic methods which are much easier to use than microscopic or immunological assays. They may be useful, where microscopy fails. Polish authors (Pirog and Pietron, 1974) found the biochemical method to be 3 times more effective than copro-ovoscopy. The colour test proved to be sensitive and reliable (Roman et al., 1970; Pirog and Pietron, 1974).

Since the metabolic end products of helminths vary from species to species, it seems reasonable to assume that an analysis of the composition of VFA in saliva and urine should allow the development of differential diagnostic tests for various forms of helminthic infections.

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