

Ethanol Production and Utilization by *Aphelenchus avenae* and *Caenorhabditis* sp.¹

A. F. COOPER, JR.² AND S. D. VAN GUNDY³

Abstract: In microaerobic and anaerobic environments the principal glycolytic end-product of *A. avenae* and *Caenorhabditis* sp. was lactic acid during the first 12–16 hr, after which it was ethanol. Upon return to aerobiosis, ¹⁴C-labeled ethanol in the medium was utilized by the nematodes; ¹⁴CO₂ and some ¹⁴C-labeled glycogen was detected. Total dry weight loss of non-feeding nematodes was 25% greater in the absence of alcohol than in the presence of ethanol or *n*-propanol. Physical movement and respiration increased and reproduction was extended by alcohol in the bathing solution. **Key Words:** Lactic acid, Acetaldehyde, Succinic acid, Aerobic, Anaerobic, Microaerobic, Glycolysis, Alcohol dehydrogenase, Lactic dehydrogenase.

In mammals, anaerobic glycolysis yields lactic acid and generates nicotinic adenine dinucleotide (NAD⁺). In anaerobic fermentation by animal-parasitic nematodes, however, succinic acid production and carbon dioxide fixation are common. Of the many genera investigated, only *Litomosoides*, *Dracunculus*, and *Dirofilaria* excreted appreciable lactic acid (11); a few others excrete trace amounts (11). In *Ascaris*, lactic dehydrogenase activity is low and only traces of lactic acid have been detected. The anaerobic end-products were primarily succinic acid (3) and smaller amounts of α -methylbutyric and α -methylvaleric acids (9).

Schiebel and Saz (14) identified cytochrome oxidase in the rat tapeworm, *Hymenolepis diminuta* (a cestode), but found its metabolism to be essentially anaerobic. They suggested succinate formation is commonly present in helminth and flatworm metabolism and that the excreted organic acids were produced primarily by carbon dioxide fixation.

Bryant and Nicholas (2) demonstrated

glycolysis of glucose, succinate, and acetate as well as transamination and other syntheses of the tricarboxylic acid cycle mediated by body wall fragments of *Moniliformis dubius*. They judged this to be consistent with the known glucose→pyruvate→malate-succinic acid-oxalacetic acid pathways. Ward and Crompton (15) reported ethanol to be a major end-product of aerobic and anaerobic glycolysis in *M. dubius*. Neither aerobic nor anaerobic ethanol production had previously been reported in nematodes.

Dunagan (6) found succinic and lactic acids were only minor end-products of aerobic and anaerobic fermentation in *Neochinorhynchus* sp. and suggested (without evidence) that acetic acid might be the major one.

Cooper and Van Gundy (4) reported aerobic utilization of neutral storage lipids by nonfeeding *Aphelenchus avenae* and *Caenorhabditis* sp., both free-living, soil-inhabiting nematodes. Anaerobically these nematodes utilized no lipids but survived on endogenous glycogen.

Ells and Read (7) found that intact *Turbatrix aceti* utilized acetate and oxaloacetate. Citrate was synthesized from acetate but no respiratory activity was stimulated by exogenously supplied citrate, malate, or succinate. Lack of malate dehydrogenase activity suggested succinate may not be in the central

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² Present address: Department of Biological Sciences, California State Polytechnic College, San Luis Obispo, California 93401.

³ Department of Nematology, University of California, Riverside, California 92502.

respiratory pathway of *T. aceti*. No lactate dehydrogenase (LDH) or alcohol dehydrogenase (ADH) activity was found in *T. aceti* (15), and Ells (8) further suggested that the low levels of glycolytic enzymes detected may perform only synthetic functions.

Krusberg (10) found that glycolytic and pentose phosphate pathway enzymes were uniformly present in *Ditylenchus trififormis* but could not detect LDH in *D. dipsaci*. Myers and Krusberg (12) reported no lactic acid excretion by five plant-parasitic nematodes.

The purpose of the study reported here was to add to our knowledge of the aerobic and anaerobic metabolism of lipids and glycogen in *Aphelenchus avenae* and *Caenorhabditis* sp. (soil-inhabiting nematodes) and to define their role in the survival of non-feeding nematodes.

MATERIALS AND METHODS

The culture, harvest and general procedures for handling the test organisms, *Aphelenchus avenae* (a mycophagous nematode) and *Caenorhabditis* sp. (a microphagous nematode), were described earlier (4).

FERMENTATIVE END-PRODUCT AND ENZYME DETERMINATIONS: Incubation solutions and homogenates of *A. avenae* and *Caenorhabditis* sp. were analyzed for organic acids, alcohols, aldehydes, glycols, ketones, alcohol dehydrogenase and lactic dehydrogenase after exposure to controlled aerobic, microaerobic and anaerobic conditions.

Freshly-harvested nonfeeding *A. avenae* suspensions containing 300,000 nematodes per ml were incubated aerobically as follows: (i) Two ml of suspension, 28 ml of sterile tap water, and 10 ml of "AB/AF" (an antibacterial/antifungal mixture; 10 ppm aretan, 45 ppm aureomycin and 150 ppm streptomycin) were bubbled in a 100 ml beaker with 50 cc/min of filtered, 100% humidified air at 27 C for up to 24 hr; (ii) Five ml of sus-

pension, 345 ml of sterile tap water, and 150 ml of AB/AF mixture similarly bubbled up to 24 hr with air at 27 C in a 600 ml beaker; (iii) To favor detection of volatile metabolites, two ml of suspension, 28 ml of sterile tap water, and 10 ml of AB/AF were incubated in a 160-mm petri dish (4-mm liquid layer) contained in a sealed desiccator evacuated and flushed four times with the desired gas mixture and maintained without supplemental aeration at 27 C up to 24 hr. The incubation treatments of *Caenorhabditis* sp. were essentially the same except the number of nematodes was fewer by a factor of four. Each exposure time was replicated six times and each test was repeated at least three times.

Anaerobic and microaerobic atmospheres were commercially prepared (Matheson) gas mixtures: 90% N₂ + 10% O₂, 95% N₂ + 5% O₂, 96% N₂ + 4% O₂, 98% N₂ + 2% O₂ and 100% N₂. The incubation solutions were prepared as above, except that just prior to the addition of the sterile tap water and AB/AF mixture, they were bubbled with the corresponding gases for 20 min to hasten equilibration. The gaseous environment was changed every 8 hr during the incubation period. Each exposure time was replicated six times and all tests were repeated at least four times.

Because the nematodes used were not cultured axenically (due to the large numbers required) there was always the chance that the respiratory activity of associated microorganisms might have influenced the results. The degree of microbial contamination within each treatment group (aerobic, microaerobic and anaerobic) was determined at the end of the treatment period by pipetting 1 ml of solution onto a PDA plate and 1 ml into melted (45 C) NA and poured into a petri dish. After incubation 48 hr at 28 C, the resulting colonies were counted. Fewer than 150 colonies per plate was arbitrarily con-

sidered the threshold beneath which significant interference from microbial metabolites would be unlikely. Assay for anaerobic bacterial contaminants was conducted in thio-glycollate agar containing indicators (Difco). Any treatments with contamination were discarded. The use of axenically-propagated nematodes would obviate the need for the AB/AF and its possible influence.

The nematodes were removed from the incubation solutions by filtration and sonically homogenized in 20 ml of distilled water (4). The cellular debris was removed by centrifugation 1 hr at 20,000 g. The resulting supernatant and original incubation solution were analyzed in the same manner.

Organic acids.—Analyses for ether-soluble nonvolatile organic acids followed the methods of Myers and Krusberg (12) except that thin-layer chromatography (TLC), with 0.25-mm silica-gel G, was substituted for paper chromatography.

Volatile aliphatic acids were isolated from 25 ml of incubation solution on Rexyn AG-50-NH₄⁺ and Amberlite IRA-400-CO₃⁻ resin columns (12). The aliphatic acids were eluted from the Amberlite resin with 75 ml 2N NH₄OH and the eluate was evaporated to 20 ml in a rotary evaporator at 30 C. The eluate was then adjusted to pH 3.0 with 1N HCl and immediately extracted three times with 10 ml of peroxide-free ethyl ether. One ml aqueous 0.5% (NH₄)₂SO₄ was added to the combined ether extracts and the solution was concentrated to 10 ml with a stream of nitrogen. Qualitative and quantitative analyses (vs known standards) were by gas-liquid chromatography using a Varian Aerograph 600D (with flame-ionization) fitted with an 3.2 mm × 182.9 cm (1/8-inch diameter × 6 ft) column of 10% diethylene glycol succinate on Chromosorb-W® (60–80 mesh) or Porapak-T® (80–100 mesh) with 6% FFAP at 150 C with nitrogen carrier gas.

Alcohols (C₁-C₅), Aldehydes (C₁-C₅),

Glycols, and Ketones.—These compounds were analyzed directly from the aqueous phase of the incubation solution or supernatant fluid of the nematode homogenate by gas-solid chromatography on a 3.2 mm × 182.9 cm (1/8-inch × 6-ft) column containing Porapak-Q® at 200 C with flame-ionization and nitrogen carrier gas. This was possible because water elutes more rapidly than the compounds of interest. Qualitative and quantitative measurements were based on retention times and areas under the curve compared with known internal standards. Final confirming identification of ethanol was determined by preparing a 3,5-dinitrobenzoyl derivative and measuring its melting point and nuclear magnetic resonance spectrum.

Alcohol Dehydrogenase (ADH) and Lactic Dehydrogenase (LDH).—Enzyme activities were determined after exposure of the nematodes to aerobic, microaerobic, and anaerobic environments. For LDH analysis 3 ml of nematode suspension (300,000 nematodes/ml or 270 mg of dry nematodes per 3 ml) were suspended in 27 ml of 0.1M Tris-buffer (pH 8.0) and homogenized by sonic disruption for 20 min at 1–3 C. The homogenate was centrifuged at 35,000 g for 8 hr at 0–3 C. This isolation of LDH parallels that of ADH except 0.1 M phosphate buffer (pH 7.2) was substituted for Tris-buffer. The supernatant fluid was considered the “crude” enzyme preparation (Prep 1). Twenty ml of the “crude” prep was dialyzed against 3 liters of Tris-buffer (pH 8.0) for 24 hr (Prep 2). Ten ml of Prep 2 were precipitated with 30 ml cold (–15 C) acetone and centrifuged (–4 to 0 C) at 10,000 g for 15 min. The precipitate was dissolved in ice-cold 0.1 M Na₂EDTA (pH 8.0) and dialyzed against two changes of 2 liters of Tris-buffer (pH 8.0) each for 24 hr at 1–3 C (Prep 3).

The enzyme assays were conducted by following the reduction of NAD⁺ in the presence of substrate at 340 nm wavelength

on a Beckman Kintrac VII. Cuvettes were set up containing the following mixtures: (i) 1.0 ml enzyme prep (Preps 1, 2, or 3), 0.5 ml buffer (for ADH 0.1 M Tris-buffer pH 8.8; for LDH 0.1 M phosphate buffer pH 7.5), 1.0 ml 25×10^{-3} M NAD^+ and 0.5 ml lactic acid were added to start the reaction; (ii) 1.0 ml enzyme prep, 1.5 ml buffer, and 0.5 ml substrate; (iii) 1.0 ml autoclaved (6.8 kg/15 min) enzyme prep, 0.5 ml buffer, 1.0 ml NAD^+ , and 0.5 ml substrate; (iv) 1.0 ml buffer, 1.0 ml substrate, and 1.0 ml NAD^+ (the blank). Optical density was recorded every 1.5 min.

EFFECTS OF ETHANOL ON RESPIRATION: Standard Warburg techniques (4) were used to compare the effects of 125, 250, 500, 750 and 1000 $\mu\text{g}/\text{ml}$ ethanol on the aerobic (atm air) respiration rates of each nematode species. About 75,000 fourth-stage larvae and adult nematodes (surfaced-sterilized in sterile tap water and AB/AF for 15 min) were added in 1 ml sterile tap water to each 15-ml Warburg flask containing 2 ml sterile tap water and AB/AF mixture (v/v). The required amount of 95% ethanol was added via microsyringe and 0.3 ml 20% KOH was placed in the center well. Four replicates were used per test for each nematode. The flasks were shaken 87 strokes/min at 28 C. Data were recorded every 30 min during the first 12 hr and then at 1-hr intervals for 8 hr. All tests with the same ethanol concentrations were conducted concurrently.

ETHANOL UTILIZATION: Radio-labeled ethanol-1- ^{14}C (specific activity = 200,000 cpm/mg ethanol-1- ^{14}C , New England Nuclear) was added at 200 $\mu\text{g}/\text{ml}$ (4.3×10^{-3} M) to 3 ml of nematodes (300,000/ml) plus 22 ml of 1:1 (v/v) sterile tap water and AB/AF mixture. Ten ml/min air was passed through the incubation solution then through two cold traps (dry ice in propanol) and finally bubbled through 5 ml of 1.0 M cold hydroxide of Hyamine (Rohm and Haas)

solution that was changed every 30 min for 20 hr. The treatments were replicated four times.

The ^{14}C -labeled carbon dioxide in each change of hydroxide of Hyamine solutions was measured with a Packard Tri-Carb Model 3214 liquid scintillation spectrometer at 2 C. The scintillator solutions consisted of 3 ml of the hydroxide of Hyamine added to 12 ml toluene containing 5 g/l of 2,5-diphenyloxazole (PPO) and 0.3 g/l of Dimethyl POPOP-1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene.

The ^{14}C -labeled and unlabeled glycogen was isolated from the treatment of nematodes after 20 hr. The glycogen was isolated from each replicate as described earlier (4). There were 3 g of nematodes/rep with *A. avenae* yielding 24 mg glycogen/rep and *Caenorhabditis* sp. yielding 9 mg/rep. Eight mg of glycogen were dissolved in 2 ml of water and this was added to 12 ml of Dioxane (Malinckrodt) and then counted.

All samples were counted a sufficient number of times to achieve a 2% error. The actual counting rate (dpm) was determined by comparison with an internal standard.

EFFECT OF ETHANOL AND PROPANOL ON NEUTRAL LIPID USAGE AND NEMATODE SURVIVAL: The ethanol and *n*-propanol treatments were as follows: (i) To one set (3 reps per set) of incubation solutions 95% ethanol was added, via a pipet, to give a concentration of 1000 ppm ethanol; (ii) To a second set, 100% *n*-propanol was added to give a concentration of 1000 ppm *n*-propanol; (iii) To a third set 95% ethanol plus 100% *n*-propanol was added to give a 1000 ppm concentration for each alcohol; (iv) The fourth (control) set received no additions. Each replicate received nematodes from suspensions of freshly-harvested starving (= non-feeding) *A. avenae* containing $\sim 300,000$ fourth-stage and adults per ml as follows: 5 ml of suspension plus 345 ml sterile tap

water and 150 ml of an AB/AF mixture (300 ppm streptomycin, 90 ppm aureomycin and 12 ppm aretan) were bubbled in a 1000-ml beaker with filtered, 100% humidified atmospheric air flowing at 150 ml/min for 5, 10 and 15 days at 26 C. Aeration air was humidified by bubbling it first through 4000 ml of sterile tap water plus AB/AF mixture containing the same ethanol and/or *n*-propanol concentrations as the treatment solutions. These were also monitored with the gas-chromatograph and the alcohol concentrations maintained within 500–1000 ppm. Incubation and humidification solutions were replaced every 48 hr in order to avoid the possible build-up of toxic compounds. All tests were repeated three times. Alcohol concentrations were monitored periodically on a gas-chromatograph, at 200 C.

The survival experiments were conducted in the same manner but separate from the neutral lipid usage experiments. Movement, respiration and reproduction were the criteria used to measure survival after 0, 20, 40 and 60 days exposure to the treatments. Physical movement was determined by 10 min observations of 200 nematodes under a dissecting microscope (20 \times). Respiration of 75,000 nematodes per 3 ml of sterile water and AB/AF mixture was measured using standard Warburg techniques (4). Reproduction was measured by placing 50 nematodes on three-day-old cultures of *Rhizoctonia solani* growing on 4% PDA supplemented with 1% yeast extract and incubating for 21 days at 26 C.

RESULTS

FERMENTATIVE END-PRODUCT AND ENZYME DETERMINATIONS: The major anaerobic fermentative end-product of *A. avenae* was ethanol and its production was directly correlated with glycogen catabolism (Fig. 1). Quantitative determinations (4) indicated that 75–80% of the catabolized glycogen was converted to ethanol. In the first 12 hr no

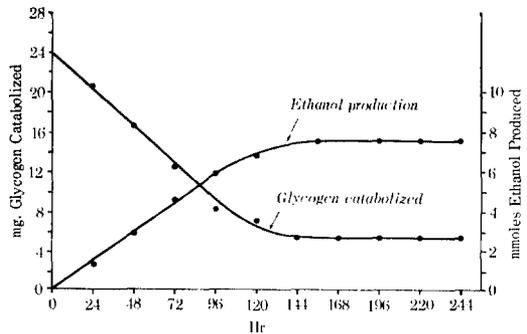


FIG. 1. Anaerobic endogenous glycogen utilization vs. ethanol production for nonfeeding *Aphelenchus avenae* (300 ± 1 mg dry weight) at 26 C. [Glycogen utilization data from (6).]

ethanol was detected in the incubation solution (Table 1). From the 12th to the 40th hr, however, ethanol gradually increased until the concentrations in the nematode homogenates and the incubation solution were equal. Ethanol production then decreased as glycogen catabolism decreased. There was no aerobic fermentation of ethanol. No acetic acid or acetaldehyde, precursors of ethanol, were detected under anaerobic, microaerobic (0–4% oxygen) (5) or aerobic conditions. Anaerobic lactic acid production was observed in the nematode homogenates up to 18 hr after which there was a decrease. Lactic acid was not detected in the incubation solution. There was no aerobic lactic acid production. Succinic acid was excreted under aerobic, microaerobic, and anaerobic conditions, however, there was no increase under anaerobic conditions.

The major anaerobic fermentative end-products of *Caenorhabditis* sp. were ethanol, acetaldehyde, and an unidentified 4-carbon alcohol (Table 1). Ethanol and acetaldehyde production accounted for 50–60% of the catabolized glycogen. Acetaldehyde was detected during the first 12–16 hr and then decreased to nondetectable levels after 30 hr, after which only ethanol was detected. For the first 8–10 hr ethanol was only found in

TABLE 1. Glycolysis end-product determination* for *Aphelenchus avenae* and *Caenorhabditis* sp. after various periods of exposure to aerobiosis and anaerobiosis at 27 C.

Chemical groups	Specific chemical	Presence of (+)								
		Aerobic (hr)				Anaerobic (hr)				
		16,	24,	48,	96	16,	24,	48,	96	
Organic acids (C ₁ -C ₆)		+	+	+	+	+	+	+	+	Yes
	Lactic acid	-	-	-	-	+	-	-	-	Yes ^{b,e}
	Succinic acid	+	+	+	+	+	+	+	+	None
	Acetic acid	-	-	-	-	-	-	-	-	None
Alcohols (C ₁ -C ₆)		-	-	-	-	+	+	+	+	None ^d
	Ethanol	-	-	-	-	+	+	+	+	Yes
Aldehydes (C ₁ -C ₅)		-	-	-	-	+	+	-	-	Yes ^e
	Acetaldehyde	-	-	-	-	+	+	-	-	Yes ^f
Glycols ^g		-	-	-	-	-	-	-	-	None
Ketones ^g		-	-	-	-	-	-	-	-	None

* Combined data for incubation solution and nematode homogenate.

^b For *A. avenae* it increased under anaerobic conditions until 18 hr after which it decreased until nondetectable at 40 hr.

^c For *Caenorhabditis* sp. it increased under anaerobic conditions until 10 hr after which it decreased until nondetectable after 21 hr.

^d Trace amount of an unidentified 4-carbon alcohol in *Caenorhabditis* sp.

^e No aldehydes detected in *A. avenae*.

^f Acetaldehyde found in *Caenorhabditis* sp. for 30 hr after which it decreased.

^g May be present in concentrations not detectable in these tests.

the nematode homogenate, while the majority of acetaldehyde was found in the incubation solution. The ethanol in the incubation solution and the nematode homogenate came to equilibrium after 34 hr. An unidentified 4-carbon alcohol accounted for 5–10% of the catabolized glycogen. Anaerobically-produced lactic acid, found only in nematode homogenates, was detected until 12–18 hr after which the amount decreased. No aerobic lactic acid production was detected.

Apparently the major end-products of aerobic TCA glycolysis in both *A. avenae* and *Caenorhabditis* sp. are carbon dioxide and water. Alcohol dehydrogenase and lactic dehydrogenase activities were detected in *A. avenae* and *Caenorhabditis* sp. under aerobic, microaerobic, and anaerobic conditions. The results for ADH and LDH activities are given in Fig. 2A, B, C, D.

EFFECTS OF ETHANOL ON RESPIRATION: At the concentrations tested, ethanol had no

major influence on the aerobic (21% O₂) oxygen consumption of either nematode species. There was a 10–15% decrease in oxygen uptake for the first 15 min followed by a 20–25% increase for the next 45 min with 500, 750 and 1000 ppm ethanol after which the oxygen uptake remained the same as the controls. At 100 and 250 ppm ethanol there was a slight increase in oxygen uptake for the first 30 min, after which there were no detectable differences from those nematodes with no ethanol. The oxygen consumption levels remained similar for 43 hr.

ETHANOL UTILIZATION: *A. avenae* and *Caenorhabditis* sp. were capable of aerobic catabolism of ethanol $-1-^{14}\text{C}$ (Fig. 3). *A. avenae* converted 55–60% of the radio labeled ethanol to $^{14}\text{CO}_2$ and 5–8% of the ^{14}C label was detected in the glycogen after 20 hr. *Caenorhabditis* sp. almost quantitatively converted ethanol $1-1-^{14}\text{C}$ to $^{14}\text{CO}_2$. No ^{14}C was detected in the glycogen of *Caenorhab-*

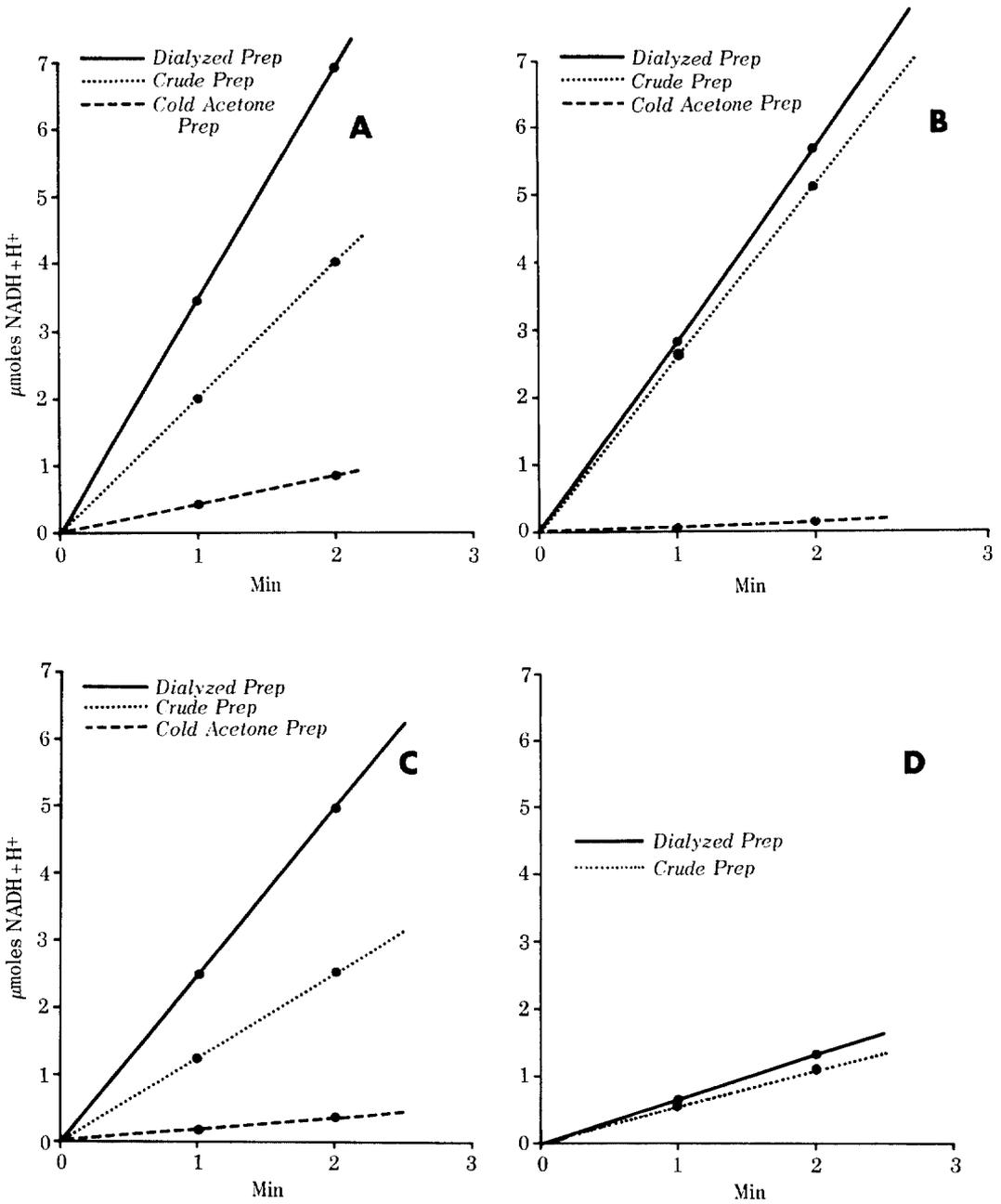


FIG. 2. Alcohol dehydrogenase (A and C) and lactic dehydrogenase (B and D) activities in homogenates of *Aphelenchus avenae* (A and B) and *Caenorhabditis* sp. (C and D). Homogenates contained 300 ± 1 mg (dry wt) of nematode tissue and activity measurements were made after 24 hr anaerobic incubation at 27 C.

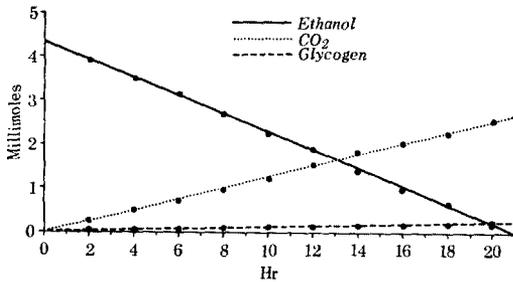


FIG. 3. *Aphelenchus avenae*. Ethanol-1-¹⁴C catabolism vs. carbon dioxide-¹⁴C production under aerobic conditions at 26 C. Data based on measurements of a homogenate containing 300 ± 1 mg dry weight of nematodes.

ditis sp. The rate of ethanol -1-¹⁴C catabolism by the nematode species differed widely when equal weights of *A. avenae* and *Caenorhabditis* were tested. No ethanol-1-¹⁴C was detected after 20 hr incubation with *A. avenae* while 80-85% remained in the *Caenorhabditis* sp. incubation solution. There was no microaerobic or anaerobic ethanol utilization.

EFFECTS OF ETHANOL AND *N*-PROPANOL ON NEUTRAL LIPID USAGE AND SURVIVAL: The influence of ethanol and *n*-propanol on total dry weight loss and neutral lipid loss of nonfeeding *A. avenae* is given in Fig. 4 and 5. After 15 days total dry weight loss was 25% greater in the absence of alcohol than in the presence of the alcohols. Approximately 50% of the loss in total dry weight was accountable to neutral lipid loss. There was no significant difference between the use of ethanol or propanol as a substrate.

Movement, respiration and reproduction of *A. avenae* were extended for 20 days in the presence of alcohols in the incubation solution (Tables 2, 3, 4).

DISCUSSION

Scheibel and Saz (14) judged carbon dioxide fixation and succinate production to

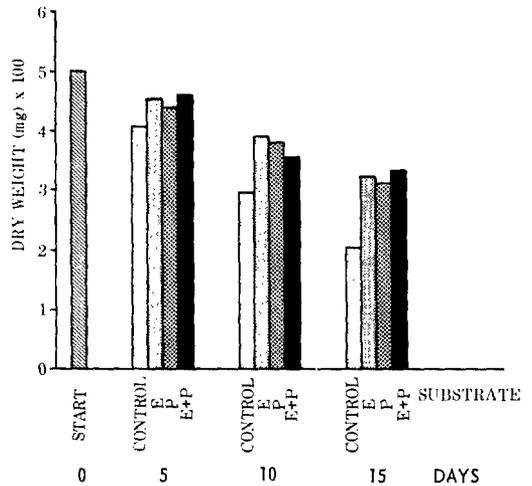


FIG. 4. Effect of 1000 µg/ml of ethanol and/or propanol on loss of dry weight after 15 days of starvation at 26 C.

be a general feature of glycolysis in helminths and flatworms. This was not so in the nematodes *A. avenae* and *Caenorhabditis* sp. The lack of carbon dioxide in the environment did not appear to affect respiration (4, 5) and although they produced succinate in aerobic, microaerobic and anaerobic environments, it was not the major glycolytic end-

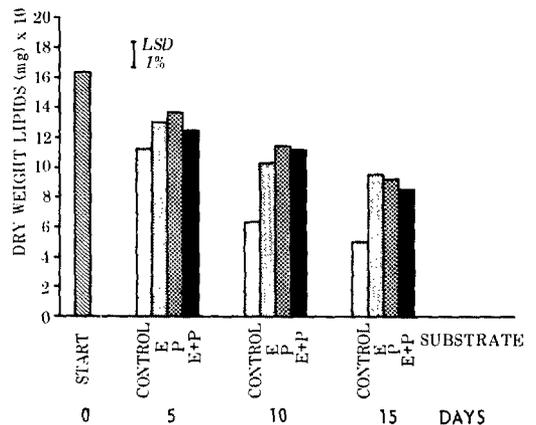


FIG. 5. Effect of 1000 µg/ml of ethanol and/or propanol on loss of dry weight of neutral lipids after 15 days of starvation at 26 C.

TABLE 2. Motility† of nonfeeding *Aphelenchus avenae* after exposure (days) to 1000 µg/ml of ethanol and/or *n*-propanol.

Days	Control	Ethanol	Propanol	Ethanol and Propanol
0	100%	100%	100%	100%
20	~98%	~98%	~98%	~98%
40	~30%	~65%	~55%	~55%
60	~0.1%	~40%	~35%	~40%

† Based on 10-min visual observation of ~200 nematodes at 20X.

product. The major metabolite of both microaerobic and anaerobic glycolysis was ethanol. Approximately 75–80% and 55–60% of the catabolized glycogen was converted to ethanol in *A. avenae* and *Caenorhabditis* sp., respectively. Carbon dioxide fixation is not required for ethanol production in other organisms (1); this may explain why the lack of carbon dioxide did not affect respiration.

Ethanol was excreted into the incubation solution very rapidly. Selective permeability of excretory organelles may favor release of ethanol in preference to lactic or succinic acids. For the first few hours of anoxybiosis lactic acid is a major fermentative end-product. When anoxybiosis is prolonged, however, lactic acid accumulates inside the nematodes and may become toxic. Probably the more easily-excreted ethanol is then produced, alleviating toxic levels of lactic acid. Ethanol production, like that of lactic acid, converts $\text{NADH}+\text{H}^+$ to NAD^+ causing no net energy loss. On the other hand, succinic acid production requires energy (1) that these nematodes may not be able to expend during anaerobiosis. Organisms (nematodes, cestodes and trematodes) (13) which excrete large quantities of succinate also have large stores of glycogen.

Ells (8) working with *Turbatrix aceti*, suggested ADH or LDH synthesis may be induced by anaerobic conditions. With *A. avenae* and *Caenorhabditis* sp., LDH and

TABLE 3. Oxygen uptake ($\mu\text{l O}_2$ per hr per mg of nematode dry wt) of nonfeeding *Aphelenchus avenae* after exposure (days) to 1000 ppm ethanol and/or propanol.

Days	Control	Ethanol	Propanol	Ethanol and Propanol
0	5.9	5.8	5.9	5.7
20	3.1	3.6	3.1	3.3
40	0.9	2.1	2.0	1.8
60	0.0	1.4	1.1	1.2

ADH activities were detected regardless of the gaseous environment or length of exposure time. Even after anaerobic lactic acid production disappeared, the LDH activity of the nematode homogenate remained fairly high. The mechanism(s) and cause(s) for the shift from lactate to ethanol production after 14–18 hr is as yet unknown.

The importance of ethanol catabolism in the test nematodes upon resumption of aerobic respiration is not fully understood but it leads to interesting speculation on nutrition and survival of nematodes. Survival of certain stylet-bearing nematodes between hosts is enhanced by an ability to become quiescent and conserve food reserves and an ability to obtain exogenous energy from other sources. It has already been demonstrated that in anaerobic and microaerobic environments *A. avenae* goes into cryptobiosis after about 5 days due to the production and accumulation of ethanol from glycolysis. Respiration drops below present detection levels and no food

TABLE 4. Reproduction† of nonfeeding *Aphelenchus avenae* after exposure (days) to 1000 ppm ethanol and/or propanol.

Days	Control	Ethanol	Propanol	Ethanol and Propanol
0	+	+	+	+
20	+	+	+	+
40	(+)‡	+	+	+
60	-	+	+	+

† Three reps (50 individuals per rep).

‡ Only one rep showed reproduction.

reserves are used during the period of cryptobiosis. When the environment becomes aerobic, alcohol in the incubation solution is used by the nematode to supply energy for metabolic and physical activity. Most of the alcohol is oxidized to CO₂ and water, however, some of it is reconverted to glycogen (stored energy).

The use of alcohols as an energy source suggests that certain nematodes may be able to obtain energy-yielding short-chain organic molecules from metabolites of soil microorganisms by direct absorption through the cuticle. Results of the present studies indicate that *A. avenae* is capable of metabolizing ethanol and *n*-propanol which enables a measurable conservation of endogenous lipid reserves and increased survival.

In summary, alcohols may at times be important energy sources in the nutrition and survival of certain nematodes *in vitro* and *in vivo*.

LITERATURE CITED

1. BRAND, T. VON. 1966. *Biochemistry of Parasites*. Academic Press, N. Y. 429 pp.
2. BRYANT, C. AND W. L. NICHOLAS. 1965. Intermediary metabolism in *Moniliformis dubius* (Acanthocephala). *Comp. Biochem. Physiol.* 15:103-112.
3. BUEDING, E. AND G. W. FARROW. 1965. Identification of succinic acid as a constituent of the perientic fluid of *Ascaris lumbricoides*. *Exp. Parasitol.* 16:345-349.
4. COOPER, A. F., JR. AND S. D. VAN GUNDY. 1970. Metabolism of glycogen and neutral lipids by *Aphelenchus avenae* and *Caenorhabditis* sp. in aerobic, microaerobic and anaerobic environments. *J. Nematol.* 2: 305-315.
5. COOPER, A. F., JR., S. D. VAN GUNDY AND L. H. STOLZY. 1970. Nematode reproduction in environments of fluctuating aeration. *J. Nematol.* 2:182-188.
6. DUNAGAN, T. T. 1964. Studies on the carbohydrate metabolism of *Neochinorhynchus* sp. (Acanthocephala). *Proc. Helm. Soc. Wash.* 166:57-64.
7. ELLS, H. A. AND C. P. READ. 1961. Physiology of the vinegar eel, *Turbatrix aceti* (Nematoda). I. Observations on respiratory metabolism. *Biol. Bull.* 120:326-336.
8. ELLS, H. A. 1969. Physiology of the vinegar eel, *Turbatrix aceti* (Nematoda)—II. Enzymes of the glycolytic sequence. *Comp. Biochem. Physiol.* 29:689-701.
9. HARPUR, R. P. 1969. The nematode intestine and organic acid excretion: Volatile acids in *Ascaris lumbricoides* feces. *Comp. Biochem. Physiol.* 28:805-876.
10. KRUSBERG, L. R. 1960. Hydrolytic and respiratory enzymes of species of *Ditylenchus* and *Pratylenchus*. *Phytopathology* 50: 9-22.
11. LEE, D. L. 1964. *The Physiology of Nematodes*. Oliver and Boyd, Great Britain. 154 pp.
12. MYERS, R. F. AND L. KRUSBERG. 1965. Organic substances discharged by plant-parasitic nematodes. *Phytopathology* 55: 429-437.
13. SAZ, H. J. 1969. Carbohydrate and energy metabolism of nematodes and Acanthocephala. p. 329-359. *In: M. Florkin and B. T. Scheer (eds). Chemical Zoology III. Echinodermata, Nematoda, and Acanthocephala*. Academic Press, N. Y.
14. SCHEIBEL, L. W. AND H. J. SAZ. 1966. The pathway for anaerobic carbohydrate dissimulation in *Hymenolepis diminuta*. *Comp. Biochem. Physiol.* 13:151-163.
15. WARD, P. F. V. AND D. W. T. CROMPTON. 1969. The alcoholic fermentation of glucose by *Moniliformis dubius* (Acanthocephala) *in vitro*. *Proc. Royal Soc., Ser. B., Biol. Sci.* 172:65-79.