

Metabolism of Glycogen and Neutral Lipids by *Aphelenchus avenae* and *Caenorhabditis* sp. in Aerobic, Microaerobic, and Anaerobic Environments

A. F. COOPER, JR. AND S. D. VAN GUNDY¹

Abstract: Starving *Aphelenchus avenae* survived 3–4 weeks in microaerobic and anaerobic environments, but *Caenorhabditis* sp. survived less than 80 hr. Aerobically, both nematodes metabolize neutral lipid reserves: there was no microaerobic (< 5% O₂) or anaerobic neutral lipid catabolism. Early in anaerobiosis both nematodes utilized endogenous glycogen. *Caenorhabditis* sp. depleted the glycogen and died. *A. avenae* under oxygen stress longer than 120 hr entered cryptobiosis, during which there was neither measurable O₂ uptake nor glycogen or neutral lipid utilization. Only when re-aerated, did *A. avenae* recover and resume "normal" metabolism. **Key Words:** Aeration, Glycogen metabolism, Neutral lipid catabolism, *Aphelenchus avenae*, *Caenorhabditis* sp., Cryptobiosis.

Several reviews on nematode physiology (2, 8, 19, 32, 35) include information on oxygen effects, mostly upon economically important animal parasites. Inferences may be drawn and applied in studies of soil-inhabiting nematodes but differences reflecting ecological specialization can be expected.

Animal parasites, which often experience low O₂ tensions (2, 6, 9, 32), accumulated up to 80% (dry wt basis) storage glycogen and only 1–15% neutral lipids (2, 6, 8, 11, 15, 22, 32). Terrestrial forms generally encounter relatively high O₂ tensions (35, 37) and contain 30–40% (dry wt basis) neutral lipids and only 3–8% glycogen (18, 19, 32, 37).

Survival of nematode larvae in eggs and other nonfeeding situations requires endogenous energy reserve accumulation (21, 22, 24); lipids have often been mentioned (8, 9, 29, 37) but very few quantitative lipid balance data have been presented. Histochemical studies have shown accumulation of both lipid and glycogen reserves in earlier stages (3, 11, 15, 40) and a correlation of reserve depletion with decreased activity and infectivity of *Ancylostoma caninum* (4, 26), *Ascaridia galli* (6), *Haemonchus contortus* (27), and *Nippostrongylus brasiliensis* (13, 40).

Wilson (40) demonstrated 0.9% per day lipid depletion in infective *N. brasiliensis* larvae held 8 days at 25 C; glycogen was absent, carbohydrate level was stable and more non-lipids than lipids were consumed, suggesting lipids are not the only stored energy reserve affecting survival. Barrett (1) proved that *Strongylus ratti* third-stage larvae rely entirely upon reserves accumulated by the first and second stages. He showed linear decrease in lipids (comprised of 82% neutral lipids and 18% phospholipids) from 25% to 9% of dry body wt during 12 days of aerobic metabolism at 20 C. The depletion was more rapid during the first four days and involved only neutral lipids which agrees with data for *Panagrellus redivivus* (33). Both results support the generally accepted view that phospholipids have a structural rather than a storage reserve function.

As in other animals, endogenous neutral lipid metabolism in nematodes requires oxygen (9, 12). Van Gundy *et al.* (38) observed a rapid depletion of lipids in *Meloidogyne javanica* and *Tylenchulus semipenetrans* incubated at 27 C in oxygenated suspensions. Conversely, lipid was conserved at lower temperatures and decreased O₂ tensions. Likewise, Payne (22) starved *Trichinella spiralis* larvae under anaerobic conditions and found stored lipids were metabolized only after

Received for publication 19 January 1970.

¹ Department of Nematology, University of California, Riverside, California 92502.

oxygen was admitted. Little else is known about catabolism of lipids by nematodes in microaerobic or anaerobic environments.

Some data has been reported on the aerobic or anaerobic glycogen metabolism of free-living and plant-parasitic nematodes (8). Nevertheless the ability of many soil-inhabiting nematodes to survive low O₂ stress for extended periods suggests that their survival is due to either tolerance (passive survival) or possibly altered metabolism (active survival).

The purpose of the present study was to measure and compare the effects of various O₂ concentrations upon utilization of neutral storage lipids and glycogen by a mycophagous nematode (*Aphelenchus avenae*) and a microphagous nematode (*Caenorhabditis* sp.).

MATERIALS AND METHODS

TEST ORGANISMS: *Aphelenchus avenae* and *Caenorhabditis* sp. nematodes were chosen because both multiply rapidly and are easily cultured in the laboratory. Both populations originated from single females isolated from field soil. After taxonomic investigation the *Caenorhabditis* sp. was called an "undescribed species"; preserved specimens and prepared slides were deposited in the permanent nematode collection, University of California at Riverside.

Aphelenchus avenae were cultured by a modification of Evans' method (7). One hundred grams of wheat seed and 150 ml tap water were autoclaved at 16–17 psi for 1.5 hr in a 1-qt wide-mouth Mason jar covered with a petri dish lid. When cool, the medium was inoculated with a pure culture of *Rhizoctonia solani*, incubated 3 days at room temp (23–27 C), then 200–500 surface sterilized *A. avenae* were introduced. After 30 days at room temperature the entire culture was harvested by rinsing the substrate and mycelium on nested 32-mesh and 250-mesh screens. The nematodes on the 250-mesh

screen were washed an additional 30 min with tap water to remove eggs and second and third-stage larvae; those retained were primarily fourth-stage larvae and adults. The nematodes were transferred to wet-strength tissue (~150-mesh) supported in shallow water on a concave screen for 8 hr. Total harvest time was 9.5 hr. The average yield was 1 gm (wet weight) per jar.

Caenorhabditis sp. was cultured on a mixed bacterial culture growing on a mixture of 1 g oatmeal and 5 ml water in a 90 × 15 mm petri dish bottom floated on 30 ml of water in a closed container at 26 C for 21 days. Fourth-stage larvae and adults were segregated by the 32-mesh/250-mesh washing procedure outlined for *A. avenae* except that eggs and newly-hatched larvae were removed from the suspension by a final 20-min wash of the 8-hr separation on a 250-mesh screen just before the treatments were applied. Uniform populations of third-stage larvae were isolated by allowing migration from crowded cultures from the floating petri dish into the supporting water. Screening (250-mesh) and separation on wet-strength tissue was essentially as outlined for *A. avenae*. Total harvest time was 30 hr. Yields averaged 5,000 third-stage larvae per plate per day and 200,000 fourth-stage larvae and adults per plate.

The nematode suspensions for starvation trials, respiration tests and chemical analysis were prepared from nematodes stored for short periods at 4 C, washed 5 min in 120 ml sterile distilled water at 10 C, and settled five times by centrifugation (5 min at 460 g).

Nematodes for neutral lipid and glycogen analyses were suspended in 50 ml sterile distilled water and kept at 4 C while ultrasonically disrupted at 90,000 cycles/sec for 20 min in a Raytheon DF-101 Sonic Oscillator. Each sample was then individually shell-frozen at –60 C (dry ice and acetone) in a 150 ml round-bottom flask, lyophilized 10–

12 hr down to 15–20 microns of vacuum, then weighed to the nearest 0.1 mg.

RESPIRATION: Rates of respiration under various controlled atmospheres were estimated by standard Warburg techniques (36). For both *A. avenae* and *Caenorhabditis* sp., groups of six replicate flasks each containing 1 ml of nematode suspension (~75,000 nematodes), 1 ml of sterile distilled water and 1 ml of "AB/AF" (antibacterial/antifungal mixture, 10 ppm aretan, 45 ppm aureomycin and 150 ppm streptomycin) were flushed continuously either with air, nitrogen, 98% N₂ + 2% O₂ or 95% N₂ + 5% O₂ while shaken 87 strokes per min at 28 C for periods ranging from 1 hr to 10 days. At the start of each treatment six aliquant nematode + AB/AF suspension/solutions were ultrasonically disrupted, lyophilized, weighed and stored for glycogen and lipid analysis. When each treatment was completed, the nematodes were allowed to settle approximately 15 min and 2 ml of supernatant solution was removed by vacuum pipette. One ml of sterile tap water and 1 ml of AB/AF were added and 0.3 ml of 20% (w/v) KOH was added to the center well of one of each pair of flasks. The flasks were returned to the respirometer, equilibrated 15 min, then post-exposure oxygen uptake readings were taken every 15 min for the first 8 hr and every 30 min for the next 6 hr. The flasks were opened for 12 hr, fresh KOH was added and the flasks again closed and readings taken every 2 hr for the next 10 hr. In experiments involving longer exposures (2 to 10 days), readings were taken every 30 min for the first 3 hr and every 2 hr for the next 12 hr. Upon completion of respiration measurements the nematodes were disrupted ultrasonically, lyophilized, weighed and stored at -6 C for analysis.

Comparative measurements of O₂ uptake in atmospheric air were all conducted concurrently. Respirometer readings were re-

corded every 15 min for 4 hr then every 30 min for 12 hr, opened for 12 hr, fresh KOH introduced and readings taken hourly for the next 10 hr.

The paired-flask method was used to determine the respiratory quotient (RQ), with the necessary gas exchange data taken at the same time respiration of treated populations were measured. Six identical respirometer flasks were prepared as outlined above except that only three flasks received KOH. Manometer readings were recorded every 30 min for 20 hr, opened for 12 hr and the KOH changed and readings again recorded every 30 min for the next 8 hr. The respiratory quotient equals the volume of CO₂ produced per volume of O₂ consumed (36).

NEUTRAL LIPID AND GLYCOGEN CATABOLISM: Incubation Treatments.—Aerobic incubation of freshly-harvested starving (non-feeding) *A. avenae* from concentrated suspensions containing ~300,000 nematodes per ml was conducted as follows (i) Two ml of suspension plus 28 ml sterile tap water and 10 ml of AB/AF bubbled in a 100 ml beaker with filtered, 100% humidified atmospheric air flowing at 50 ml/min for up to 240 hr with the temperature regulated at 27 C. (ii) Five ml of suspension plus 345 ml sterile tap water and 150 ml AB/AF mixture similarly bubbled with air at 27 C in a 600 ml beaker. The purpose of this treatment was to dilute toxic metabolites potentially capable of altering endogenous metabolism. (iii) two ml of suspension plus 28 ml sterile tap water and 10 ml of AB/AF were incubated in a covered 160 mm petri dish (4 mm liquid layer) without supplemental aeration at 27 C. The purpose here was to minimize loss of volatile metabolites. 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 treatment was replicated six times and all tests were repeated at least three times.

Anaerobic and microaerobic incubation was carried out in commercially prepared gas mixtures (Matheson) of 90% N₂ + 10% O₂, 95% N₂ + 5% O₂, 96% N₂ + 4% O₂, 98% N₂ + 2% and pure N₂. The incubation solutions were prepared as above, except that just prior to use the sterile tap water and AB/AF mixture was bubbled with its gaseous component for 20 min preliminary equilibration. Incubation solutions (i) and (ii) were bubbled with the desired gas mixture at 20 ml/min (at 27 C) up to 240 hr. In order to reduce the loss of volatile metabolites incubation solution (iii) was placed in a sealed desiccator containing the desired gaseous component. Four times the desiccator was evacuated and refilled with the test gas mixture and incubated at 27 C for up to 240 hr. Each exposure time was replicated six times and all tests were repeated at least twice.

The degree of microbial contamination of each treatment (aerobic, microaerobic and anaerobic) was determined at the end of the treatment period by pipetting 1 ml of solution onto a PDA plate and into a tube of melted (45 C) NA, which was mixed and poured into a petri dish. Following incubation for 48 hr at 28 C, the resulting colonies were counted. Fewer than 150 colonies per plate were arbitrarily considered the threshold of significant interference from microbial metabolites.

Neutral Lipid and Glycogen Analysis.—Neutral lipids were isolated using the techniques of Folch *et al.* (10). Three hundred milligrams of sonically disrupted, lyophilized nematode sample were suspended in 3 ml distilled water and its total lipid extracted with 10 ml of chloroform-methanol (2:1 v/v). After being washed in chloroform, taken to dryness in vacuum at 40 C and redissolved in 10 ml chloroform, the total lipid extract was layered on a silicic acid column (2 × 50 cm) and the neutral lipids eluted with 100 ml of chloroform. This fraction was vacuum dried

at 40 C, weighed to the nearest 0.1 mg and redissolved in 10 ml of chloroform. The neutral lipid components were first resolved by thin-layer chromatography (34) using known standards on either 2" × 8" or 8" × 8" silica gel "G" plates (0.25 mm thick) with hexane/diethyl ether/acetic acid (80:20:1). The plates were sprayed with 50% aqueous H₂SO₄ (v/v) saturated with K(CrO₄)₂ and developed by charring at 160 C. Mono-, di-, and triglycerides were identified by comparison with simultaneously run, known standards. Specific neutral lipids were eluted from a 1-cm diameter column containing 5 g of Bio-Sil A silicic acid (Calbiochem). A 50-mg portion of mixed neutral lipids was fractionated by sequential passage of the following volumes of hexane/diethyl ether (v/v) mixtures; 40 ml of 99/1, 55 ml of 96/4, 60 ml of 92/8, 25 ml of 88/12, 35 ml of 75/25, 15 ml of 50/50 and a final 15 ml of diethyl ether to remove any material remaining on the column. Methyl esters of the neutral lipid fractions were prepared by refluxing with 5% H₂SO₄ in methanol for 5 hr at 70 C then gas chromatographed at 150 C on a 1/8 inch 10 ft stainless steel column of 60/70 mesh Chromosorb W coated with 10% diethylene glycol succinate (DEGS) using nitrogen carrier gas and flame ionization detection. The eluted esters were identified by their relative retention times and the percent concentration calculated from areas under their curves related to those of internal standards.

The presence of unsaturation was further verified by hydrogenation of the fatty acid mixture in presence of palladium catalyst and chromatographing the methyl esters.

Glycogen was isolated by the trichloroacetic acid technique (3). Weighed amounts of lyophilized nematodes were suspended in 0.9 ml cold (0–2 C) distilled water per 100 mg dry wt of material and 10% trichloroacetic (1 ml/100 mg dry wt of nema-

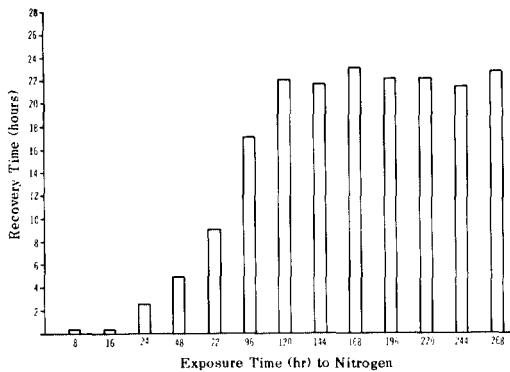


FIG. 1. Post-anaerobic exposure recovery time required for nonfeeding *Aphelenchus avenae* to resume initial oxygen uptake after various exposures to N_2 at 27 C.

tode sample = 1 volume) was added with stirring for 5 min. The extraction mixture was centrifuged 10 min at 8000 g and 0–2 C, the supernatant was decanted into a graduated cylinder and the precipitate was stirred back into one volume of 5% trichloroacetic acid at 0–2 C. This mixture was centrifuged 10 min at 8000 g, the supernatant was combined with the first. Two volumes of cold (0–2 C) 95% ethanol were added and the mixture allowed to stand ~30 min (until flocculation was complete) then centrifuged 5 min at 3000 g. The supernatant was discarded, the precipitate dissolved in 6 ml distilled water and reprecipitated in 12 ml of 95% ethanol. The precipitate was washed in 5 ml of 95% ethanol, then in 5 ml 100% ethanol and in 5 ml of diethyl ether, then dried at 60 C and weighed to the nearest of 0.1 mg. That the product was glycogen was verified by enzymatic hydrolysis with β -amylase and the anthrone procedure of Mokrasch (20).

RESULTS

RESPIRATORY RESPONSES OF *A. AVENAE* AND *CAENORHABDITIS* SP. TO INCUBATION IN MODIFIED GAS MIXTURES: In atmospheric air the O_2 uptake of *A. avenae* was 5.6 to

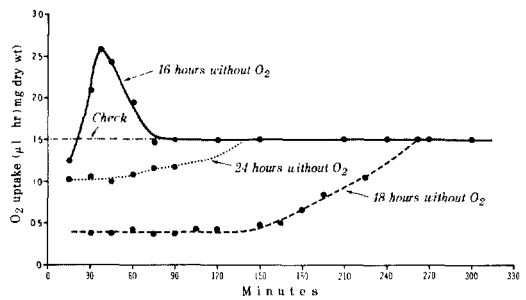


FIG. 2. Post-anaerobic oxygen uptake for nonfeeding *Aphelenchus avenae* and *Caenorhabditis* sp. at 27 C (Note oxygen debt after 16 hr N_2 exposure).

5.8 $\mu\text{l}/\text{mg}$ dry wt/hr and that of *Caenorhabditis* sp. 6.1–6.3 $\mu\text{l}/\text{mg}$ dry wt/hr. If groups of either nematode were kept in the same bathing solution, the Q_{O_2} slowly declined after 48 hr; if, however, the bathing solution was changed every 24 hr, initial O_2/CO_2 exchange rates were maintained for at least 144 hr. Long-term starvation (> 10 days) of nematodes produced a gradual decrease in Q_{O_2} which was not restored by changing the incubation fluid. Simultaneously, neutral storage lipid content decreased from 33–36% of dry body wt to 5–8%. Decreased respiration during long-term starvation appeared to be independent of complete depletion of endogenous food reserves. In the controls, there was no O_2 uptake of heat-killed nematodes and the AB/AF mixture.

Exposure of *A. avenae* to pure N_2 longer than 8 hr caused progressively longer times to resume initial oxygen consumption (Fig. 1) but after 16 hr of N_2 exposure initially fresh, unstarved *A. avenae* and *Caenorhabditis* sp. both exhibited a slight post-anaerobic oxygen debt (see Fig. 2). Starved (16–20 hr nonfeeding) *A. avenae* or *Caenorhabditis* sp. did not exhibit this. Exposure to anaerobiosis longer than 80 hr killed *Caenorhabditis* sp. but *A. avenae* endured it at least 15 days without decreased viability. After 120 hr in pure N_2 *A. avenae* entered cryptobiosis (39),

TABLE 1. Percentage (dry weight basis) of glycogen and neutral lipids, in starving *Aphelenchus avenae* after various periods of aerobic, microaerobic and anaerobic exposure at 27C.

Date	Aerobic			Microaerobic			Anaerobic
	20% O ₂ ^a	10% O ₂ ^b	5% O ₂	4% O ₂	2% O ₂	1% O ₂	0% O ₂
Glycogen							
0	8.0	8.2	7.9	8.1	8.1	7.8	8.0
1	7.9	7.7	7.9	7.1	6.7	6.9	6.9
2	8.0	8.0	8.1	5.5	5.1	5.6	5.6
3	8.1	7.9	8.2	4.5	3.9	4.7	4.2
4	8.0	7.9	7.9	3.6	2.9	3.9	3.1
5	7.8	8.1	7.7	2.8	2.1	2.4	2.4
6	7.9	7.8	8.0	2.1	1.7	1.8	1.8
7	8.0	7.9	7.9	1.8	1.8	1.9	1.8
8	8.1	8.1	8.0	1.9	1.8	1.9	1.8
9	8.0	7.9	7.8	1.8	1.7	1.8	1.8
10	8.0	8.0	8.1	1.8	1.9	1.7	1.8
Neutral Lipids ^c							
0	32.4	33.6	32.8	34.0	33.1	34.1	33.1
1	29.6	28.7	31.4	32.8	29.8	33.7	32.6
2	27.1	26.4	30.1	33.4	31.6	30.9	32.9
3	25.2	23.9	28.7	33.4	32.0	29.9	32.7
4	22.8	22.7	27.4	33.9	33.0	31.4	31.1
5	19.1	20.4	25.6	34.1	31.8	32.6	32.8
6	16.9	17.1	24.2	29.9	29.6	33.0	32.9
7	14.8	14.3	23.9	31.6	31.4	32.8	33.0
8	13.1	12.6	22.0	31.9	31.6	33.1	33.3
9	11.9	11.0	21.6	30.2	33.2	30.6	32.7
10	10.3	9.7	21.1	33.3	31.9	31.9	33.0

^a Air^b Remainder N₂^c Triglycerides of fatty acids and free fatty acids

a state in which no respiration was detectable (even when 20 to 100 times the initial number of nematodes were present) and normal respiration resumed within 24 hr when 10 ml/min of air was bubbled through the incubation solution.

A mixture of 98% N₂ + 2% O₂ caused the same effects as 100% N₂ but 95% N₂ + 5% O₂ caused no decrease of post-exposure oxygen consumption.

The respiratory quotient of fresh, unstarved *A. avenae* or *Caenorhabditis* sp. was .94-.96 for the first 10-18 hr, after which it shifted to .41-.43. An RQ of .94-.96 was never observed in starved nematodes of either species; therefore, the onset of starvation was physiologically defined when the RQ shifted from 0.94-0.96 to 0.41-0.43.

NEUTRAL STORAGE LIPID CATABOLISM DURING INCUBATION IN MODIFIED GAS MIXTURES: Fresh *A. avenae* and *Caenorhabditis* sp. had an initial neutral lipid content of 33% and 36%, respectively (Table 1 and 2) and were generally characterized at 90% triglycerides, 7-8% diglycerides, and 2-3% monoglycerides. The major fatty acids of *A. avenae* were stearic, oleic, palmitic and linoleic and those of *Caenorhabditis* sp. were oleic and linoleic.

While starving under aerobic (21% O₂) conditions, at 27 C, both nematodes used lipids linearly during the entire 10-day test period (Table 1 and 2). Comparable neutral lipid catabolism is maintained in oxygen tensions down to 10%, at 5% it was reduced one-half and below that ceased altogether.

TABLE 2. Percentage (dry weight basis) of glycogen and neutral lipids in starving *Caenorhabditis* sp. after various aerobic, microaerobic and anaerobic exposures at 27C.

Date	Aerobic			Microaerobic			Anaerobic
	20% O ₂ ^a	10% O ₂ ^b	5% O ₂	4% O ₂	2% O ₂	1% O ₂	0% O ₂
Glycogen							
0	3.3	3.4	3.3	3.1	3.4	3.0	3.3
1	2.4	2.6	2.1	0.9	1.2	1.7	1.0
2	1.6	1.9	1.3	0.1	trace	0.4	trace
3	1.3	1.0	0.4	trace	trace	trace	trace
4	.9	0.7	trace	— ^c	—	—	—
5	trace	trace	trace	—	—	—	—
6	trace	trace	trace	—	—	—	—
7	trace	trace	trace	—	—	—	—
8	trace	trace	trace	—	—	—	—
9	trace	trace	trace	—	—	—	—
10	trace	trace	trace	—	—	—	—
Neutral Lipids ^d							
0	36.0	34.7	36.1	35.1	36.0	33.8	36.2
1	32.9	33.1	34.8	36.2	36.1	36.1	36.2
2	30.2	29.8	29.9	34.8	34.8	35.0	24.9
3	28.1	27.4	28.4	35.3	31.9	34.9	35.1
4	25.8	26.1	27.7	— ^c	—	—	—
5	22.9	33.4	24.9	—	—	—	—
6	20.1	20.2	23.1	—	—	—	—
7	18.3	19.1	20.7	—	—	—	—
8	16.3	16.4	19.1	—	—	—	—
9	14.5	13.9	16.4	—	—	—	—
10	10.6	11.2	14.8	—	—	—	—

^a Air^b Remainder nitrogen^c After 80 hr these nematodes died^d Triglycerides of fatty acids and free fatty acids

Losses in total dry weight were 37% for *A. avenae* and 43% for *Caenorhabditis* sp. Approximately 60–65% of this loss was neutral storage lipids. Under anaerobic conditions *A. avenae* showed an 8–9% decrease in the total dry weight during 8 days. *Caenorhabditis* sp. total dry weight loss for 48 hr was 5–7%. In both cases there was no loss in neutral lipids.

GLYCOGEN CATABOLISM DURING INCUBATION IN MODIFIED GAS MIXTURES: The results of aerobic, microaerobic and anaerobic exposures are given in Table 1 and 2. Under atmospheric aeration (21% O₂) at 27 C *A. avenae* maintains a constant level of 8% glycogen (dry wt basis). Fresh unstarved *Caenorhabditis* sp. had an initial concentra-

tion of 3% which decreased linearly until only trace amounts were detectable after 96 hr. Aerating with gaseous mixture of 90% N₂ + 10% O₂ and 95% N₂ + 5% O₂ gave comparable results and the nematodes remained active throughout the test periods.

Under microaerobic (less than 4% O₂) and anaerobic conditions, glycogen catabolism of *A. avenae* was linear for 4 days then gradually decreased until at 6 days it ceased and the glycogen level remained constant at 1.8%. At this point the nematodes apparently entered cryptobiosis; no gas exchange or neutral lipid or glycogen catabolism was detectable until the nematodes were again well-aerated. *A. avenae* exposed to anaerobic conditions remained motile 6–8 hr, metaboli-

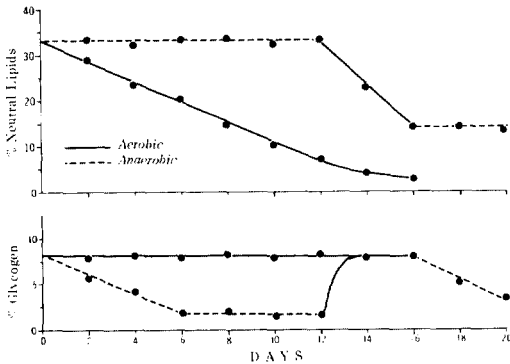


FIG. 3. Relationships between glycogen metabolism and neutral lipid catabolism in *Aphelenchus avenae* after various aerobic and anaerobic exposures.

cally active up to 6 days, and survived with 90–95% recovery for at least 20 days. *Caenorhabditis* sp. aerobic glycogen utilization rate was higher, and only trace amounts remained after 48 hr. Under anaerobiosis, however, movement ceased after 6 hr. Fresh unstarved *Caenorhabditis* sp. survived anoxobiosis for 80 hr while starved (nonfeeding 16–20 hr prior to anaerobic exposure) survived for only 30 hr.

RELATIONSHIP BETWEEN ENDOGENOUS NEUTRAL LIPID AND GLYCOGEN METABOLISM: In *A. avenae* glycogen was not catabolized when O_2 exceeded 5% and neutral lipids were not when O_2 was less than 5%. Glycogen utilization proceeded until approximately 1.8% (dry wt basis) remained. When returned to aerobic conditions, *A. avenae* rapidly anabolized glycogen from neutral lipids until the “normal” 8% found in aerated nematodes was present (see Fig. 3). The process was reversible (three times in this study) depending only upon the presence of sufficient neutral lipid reserve.

In *Caenorhabditis* sp. glycogen was not mobilized from neutral lipid reserves by aerobic/anaerobic cycling. Feeding was required to restore glycogen to former levels.

Both *A. avenae* and *Caenorhabditis* sp. required feeding to replenish exhausted neutral lipid reserves.

DISCUSSION

Several species of nematodes are facultative anaerobes, completing their life cycle most efficiently with oxidative metabolism but capable of surviving various periods of microaerobic or anaerobic oxygen stress. Their survival, therefore, implies either tolerance (passive survival) or metabolic adaptation (active survival). Growth and reproduction of facultative anaerobes has generally been considered to be dependent upon a continuously abundant oxygen supply (8, 21, 35, 37, 39).

In many biological systems respiration and the resulting respiratory quotient (RQ) have been utilized as an indicator of the kind of substrate being utilized and the efficiency of the oxidation (14, 16, 25, 28, 31). An RQ = 1.0 indicates oxidation of carbohydrates and RQ = 0.7 suggests lipid catabolism. An RQ < 0.7 is more difficult to interpret but may mean lipids are being incompletely oxidized (16). The observed shift in RQ from 0.94–0.96 to 0.41–0.43 probably indicates depletion of carbohydrate-supplying material in the gut and transition to incomplete oxidation of endogenous neutral storage lipid. This shift was not observed in starved nematodes. The low RQ may also be due to the presence of a glyoxalate cycle, as reported in *Caenorhabditis briggsae* (30). This pathway, however, was not investigated in these studies.

The respiration rates of free-living larvae and third-stage larvae (28) of *Nippostrongylus muris* decreased up to 12 days. Van Gundy *et al.* (38) noted similar decreases in respiration in second-stage *M. javanica* larvae over 8 days. Barrett (1) reported respiration of nonfeeding infective *S. ratti* larvae decreased exponentially and also

measured a corresponding drop in substrate utilization. Infectivity and motility in second-stage larvae of *T. semipenetrans* were not correlated with decreased oxygen uptake with time (38). In those studies there was a rapid initial reduction (24–48 hr) after which the respiration remained constant for 6 days. Substrate utilization, however, did decrease with time. In our studies with *A. avenae* and *Caenorhabditis* sp. there was neither an initial drop in oxygen consumption nor a decreased lipid utilization with time up to 10 days providing the bathing solution in the flask was changed every 24 hr. This suggests the many reports of decreased respiration of nematodes removed from their natural environment may have been an artifact.

The detection of a post-anaerobic oxygen debt in *A. avenae* indicated glycogen may be the major energy source during anoxybiosis.

At oxygen concentrations above 5% *A. avenae* catabolized neutral lipids without net change in the glycogen content. Conversely, at oxygen concentrations below 5%, glycogen catabolism occurred without utilization of neutral lipid. *Caenorhabditis* sp. behaved in a similar manner except that glycogen was utilized regardless of the oxygen concentration, which is observed among animal parasitic nematodes.

These two nematode species were metabolically (at least relative to glycogen) functional in microaerobic and anaerobic environments and capable of surviving glycolytic catabolism for limited periods. However, with *A. avenae*, anoxybiosis longer than 120 hr resulted in cryptobiosis (17, 37) from which 90–95% of the individuals recovered after 90 days. This appears to be the first demonstration of anoxybiosis-induced cryptobiosis in a soil-inhabiting nematode. *Caenorhabditis* sp. did not survive prolonged (more than 80 hr) anoxybiosis. Why *A. avenae* can survive prolonged anoxybiosis

and *Caenorhabditis* sp. cannot, is unknown. It may, however, be related to differences in glycogen usage during the early periods of anoxybiosis. Kostük (17) suggested glycolysis of simple sugars enabled *Anguina tritici*, *Ditylenchus allii*, *Aphelenchoides besseyi* and *Heteroderma major* to survive prolonged cryptobiosis (induced by desiccation). *Caenorhabditis* sp. utilizes all of its endogenous glycogen very early in anaerobiosis and may not retain sufficient nutrients to support even greatly reduced metabolic activity, so cannot survive or metabolically recover from extended periods of low oxygen. *Aphelenchus avenae* appears to convert neutral lipids into glycogen (carbohydrate) after anoxybiosis is terminated. While starving in aerobic environments there was no net change in the endogenous glycogen. The neutral storage lipids decreased. During anoxybiosis glycogen was used without any lipid. However, when this nematode was returned to aerobic environment, they rapidly anabolized more glycogen, to a level found in fresh unstarved nematodes, at the expense of their neutral lipids.

In a previous study on the influence of fluctuating aeration on the reproduction of soil-inhabiting nematodes, 5% oxygen concentration appeared to be a critical level (5). Oxygen concentrations above 5% were necessary for the reproduction of *A. avenae* and *Caenorhabditis* sp. Oxygen concentrations between 0–5% were termed microaerobic as contrasted to anaerobic (0%) and aerobic (5–21%). In the present study 5% oxygen was also critical to the physiology of these nematodes and confirms in more detail that nematodes function metabolically below 5% O₂ as if in an anaerobic environment.

The capacity of these nematodes for both oxidative and fermentative catabolism certainly enhances survival in environments with fluctuating aeration, such as may occur in

soil. *Aphelenchus avenae* survival is further enhanced by the ability to enter into cryptobiosis when oxygen concentrations remain unfavorable for long periods of time.

LITERATURE CITED

1. BARRETT, J. 1969. The effect of aging on the metabolism of the infective larvae of *Strongyloides ratti* Sandground, 1925. *Parasitology* 59:343-347.
2. VON BRAND, T. 1966. *Biochemistry of Parasites*. Academic Press, N. Y., 429 pp.
3. CHOWDHURY, A. B., H. N. RAY AND N. V. BHADURI. 1958. Polysaccharides in the hookworm larvae. *Bull. Calcutta School Trop. Med.* 6:59.
4. CLARK, F. E. 1969. *Ancylostoma caninum*: Food reserves and changes in chemical composition with age in third-stage larvae. *Exp. Parasitol.* 24:1-8.
5. COOPER, A. F., S. D. VAN GUNDY AND L. H. STOLZY. 1970. Nematode reproduction in environments of fluctuating aeration. *J. Nematol.* 2:182-188.
6. ELLIOT, A. 1954. Relationship of aging, food reserves, and infectivity of larvae of *Ascaridia galli*. *Exp. Parasitol.* 3:307-320.
7. EVANS, A. A. F. 1970. Mass culture of mycophagous nematodes. *J. Nematol.* 2:99-100.
8. FAIRBAIRN, D. 1960. The physiology and biochemistry of nematodes. In J. N. Sasser and W. R. Jenkins (eds.). *Nematology*. Chapel Hill, Univ. of N. Carolina 267-296 pp.
9. FAIRBAIRN, D. 1969. Lipid components and metabolism of Acanthocephala and Nematoda. In M. Florin and B. T. Scheer (eds.). *Chemical Zoology III*. Academic Press, N. Y. 361-378 pp.
10. FOLCH, J., M. LEES AND G. H. SLOANESTANLEY. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* 226:497-509.
11. GEOVANNOLA, A. 1936. Energy and food reserves in the development of nematodes. *J. Parasitol.* 22:207-218.
12. GREEN, D. E. AND S. J. WAKIL. 1960. Enzymatic mechanisms of fatty acid oxidation and synthesis. In K. Bloch (ed.). *Lipid Metabolism*. J. Wiley and Sons, Inc., N. Y. 1-40 pp.
13. HALEY, A. J. AND C. M. CLIFFORD. 1960. Age and infectivity of the filariform larvae of the rat nematode *Nippostrongylus brasiliensis* (Travassos, 1914). *J. Parasitol.* 46:579-582.
14. HAMMEN, C. S. 1967. Respiratory quotients of *Panagrellus redivivus*. *Nematologica* 13:599-604.
15. JONES, C. A. 1955. On the occurrence of glycogen and phosphate esters in filariform. *J. Parasitol.* 41:48 (Abstr.)
16. KLEIBER, M. 1961. *The Fire of Life*. An introduction to animal energetics. Wiley and Sons, Inc., N. Y. 454 pp.
17. KOSTÜK, N. A. 1965. The study of anabiosis in some phytohelminths. *Trudy Glen Lab. Akad Nauk USSR* 16:55-57.
18. KRUSBERG, L. R. 1967. Analysis of total lipids and fatty acids of plant-parasitic nematodes and host tissues. *Comp. Biochem. Physiol.* 21:83-90.
19. LEE, D. L. 1965. *The physiology of nematodes*. Oliver and Boyd, Great Britain. 154 pp.
20. MOKRASCH, L. C. 1954. Analysis of hexose phosphate and sugar mixtures with the anthrone reagent. *J. Biol. Chem.* 208:55-59.
21. PASSEY, R. F. AND D. FAIRBAIRN. 1957. The conversion of fat to carbohydrate during embryonation of *Ascaris* eggs. *Can. J. Biochem. Physiol.* 35:511-525.
22. PAYNE, F. K. 1923. Investigations on the control of hookworm diseases XXX. Studies on factors involved in migration of hookworm larvae in soil. *Amer. J. Hyg.* 3:547-583.
23. PAYNE, F. K. 1923. Investigations on the control of hookworm diseases XXXI. The relation of physiological age of hookworm larvae to their ability to infect the human host. *Amer. J. Hyg.* 3:584-597.
24. POINAR, G. O. AND RUTH LEUTENEGGER. 1968. Anatomy of the infective and normal third-stage juveniles of *Neoplectana carpocephalae* Weiser (Steinernematidae: Nematoda). *J. Parasitol.* 54:340-350.
25. RHOADES, H. L. AND M. B. LINFORD. 1959. Molting of preadult nematodes of the genus *Paratylenchus* stimulated by root diffusates. *Science* 130:1476-1477.
26. ROGERS, W. P. 1939. The physiological aging of ancylostome larvae. *J. Helminthol.* 17:195-202.
27. ROGERS, W. P. 1940. The physiological aging of the infective larvae of *Haemonchus contortus*. *J. Helminthol.* 18:183-192.
28. ROGERS, W. P. 1948. The respiratory metabolism of parasitic nematodes. *Parasitology* 39:105-109.
29. ROGERS, W. P. 1962. *The Nature of Parasitism*. Acad. Press, N. Y. 287 pp.
30. ROTHSTEIN, M. AND H. MAYOH. 1966. Nem-

- atode Biochemistry VIII. Malate Synthetase. *Comp. Biochem. Physiol.* 17:1181-1193.
31. SANTMEYER, P. H. 1956. Studies on the metabolism of *Panagrellus redivivus* (Nematoda, Cephalobidae). *Proc. Helminthol. Soc. Wash.* 27:121-123.
 32. SAZ, H. J. 1969. Carbohydrate and energy metabolism of nematodes and *Acanthocephala*. In M. Florkin and Scheer (eds.) *Chemical Zoology III*. Academic Press, N. Y. 329-359 pp.
 33. SIVAPALAN, P. AND W. R. JENKINS. 1966. Phospholipid and long-chain fatty acid composition of the nematode *Panagrellus redivivus*. *Proc. Helminthol. Soc. Wash.* 33: 149-157.
 34. STAHL, E. 1965. Thin-layer Chromatography. *A Laboratory Handbook*. Academic Press, N. Y. 553 pp.
 35. STOLZY, L. H. AND S. D. VAN GUNDY. 1968. The soil as an environment for microflora and microfauna. *Phytopathology* 58:889-899.
 36. UMBRIET, W. W., R. H. BURRIS AND J. F. STAUFFER. 1957. *Manometric Techniques*. Burgess Pub. Co., Minneapolis. 338 pp.
 37. VAN GUNDY, S. D. 1965. Factors in survival of nematodes. *Ann. Rev. Phytopathol.* 3:43-68.
 38. VAN GUNDY, S. D., A. F. BIRD AND H. R. WALLACE. 1967. Aging and starvation in larvae of *Meloidogyne javanica* and *Tylenchulus semipenetrans*. *Phytopathology* 57: 559-571.
 39. WALLACE, H. R. 1964. *The biology of plant parasitic nematodes*. St. Martin's Press, Inc., N. Y. 280 pp.
 40. WILSON, P. A. G. 1965. Changes in lipid and nitrogen content of *Nippostrongylus brasiliensis* infective larvae aged at constant temperature. *Exp. Parasitol.* 16:190-194.