JOURNAL OF NEMATOLOGY
VOLUME 14 APRIL 1982 NUMBER 2

Anhydrobiosis in Nematodes II: Carbohydrate and Lipid Analysis in Undesiccated and Desiccate Nematodes

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Abstract: Glycogen, trehalose, glucose, and total lipid contents of six nematode species were studied. Anhydrobiotic *Anguina tritici* and *Ditylenchus dipsaci* stored trehalose in preference to glycogen and only small amounts of glucose were detected. Glycogen content was also reduced in anhydrobiotic *Aphelenchus avenae*. Conversely, *Panagrellus redivivus* and *Turbatrix aceti* contained large amounts of glycogen, appreciable amounts of glucose, and minimal amounts of trehalose. *Ditylenchus myceliophagous* "curds" contained low amounts of glycogen and very little trehalose; total lipid was 60% of that in fresh samples. The lipid contents of fresh samples of *P. redivivus, T. aceti,* and *A. avenae* were high (23.1, 21.9, and 36.7% dry weight, respectively), but in anhydrobiotic *A. avenae* larvae the level was reduced by over 60%. In contrast, lipid levels remained high in anhydrobiotic *A. tritici* and *D. dipsaci* larvae (40.6 and 38.3%, respectively). Analysis of lipid composition in anhydrobiotic *A. tritici* and *A. avenae* did not indicate any specific metabolic adaptations to desiccation survival. *Key words:* physiology, Anguina tritici, Ditylenchus dipsaci, D. myceliophagous, Panagrellus redivivus, Turbatrix aceti.

Journal of Nematology 14(2):145-153. 1982.

It is well established that a number of microorganisms are capable of withstanding prolonged periods of desiccation by entering into a state of anhydrobiosis. Some of the most successful multicellular anhydrobiotes are in the phylum Nematoda, particularly *Ditylenchus dipsaci* and *Anguina tritici,* both of which parasitize the ariel parts of plants (27,28). Similarly, the free-living mycophagous nematode *Aphelenchus avenae* has demonstrated an ability to survive periods of extreme dehydration (9). Anhydrobiotic survival in these organisms is supported by physical, structural, behavioral, and biochemical adaptations, all of which are closely interrelated (13,40).

Recently, efforts have focused on the biochemical adaptations employed by anhydrobiotes, specifically the role of carbohydrate and, to a lesser extent, lipid reserves. The major energy reserve of plant-parasitic and free-living nematodes is lipid (2), and it has been suggested that the high lipid content of *D. dipsaci* could contribute towards its successful survival during long periods of desiccation (37). Krusberg (19) and Krusberg et al. (22), however, could find no correlation between lipid content and desiccation survival, although no analyses were made on nematodes in the desiccated state. Conversely, Demeure et al. (11) showed a relationship between the quantity of stored carbohydrate and lipid reserves and desiccation survival in *Helicotylenchus dihysteria* and *Scutellonema cavenessi;* opaque females (those presumably containing more lipid and carbohydrate) survived desiccation far better than transparent females.

Studies on *A. avenae* larvae have shown that trehalose and glycerol levels rise significantly at the onset of desiccation at the expense of glycogen and lipid reserves (25) and that on revival glycogen is resynthesized, but lipid continues to be degraded (10). Womersley and Smith (41) also found a significant increase in trehalose content in anhydrobiotic *D. dipsaci* and *A. tritici* larvae, but lipid and glycogen contents were not determined.

The present study was undertaken to compare the storage carbohydrate and total lipid content of three nematode anhydrobiotes—*A. avenae, A. tritici,* and *D. dipsaci*—with those of three species which survive poorly—*Panagrellus redivivus, Turbatrix aceti,* and *Ditylenchus myceliophagous.* In

Received for publication 10 August 1981.
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The authors are indebted to Dr. E. G. Platzer for allowing the use of his unpublished results on the biochemical composition of fresh *A. avenae* larvae.

addition, analyses of the lipid composition of anhydrobiotic *A. avenae* and *A. tritici* larvae were undertaken for comparison with analyses already made on fresh samples of other free-living and plant-parasitic nematodes (15,19,20,21,22,31).

MATERIALS AND METHODS

Experimental material: Desiccation-resistant fourth-stage larvae of *D. dipsaci* were obtained in the anhydrobiotic state from "eelworm wool" (narcissus strain, 1977 material) and resistant anhydrobiotic second-stage larvae of *A. tritici* were obtained from wheat (41). *D. myceliophagous* was cultured as described previously (27); *P. redivivus* was maintained on brown flour cultures (12); *T. aceti* was obtained from vinegar cultures (38). Extraction and washing procedures were identical to those previously reported (41); nematode samples were lyophilized overnight then stored at 5 C until used. *A. avenae* was cultured in a manner similar to that of Cooper and Van Gundy (8) and harvested after the method of Crowe and Madin (9). Fresh worms were stored at –22 C until used. Pellets containing 100 mg of *A. avenae* larvae were prepared by the method of Crowe and Madin (9) and stored over phosphorus pentoxide in a vacuum desiccator prior to analysis.

Carbohydrate analyses: The alkali-stable carbohydrate fraction of fresh *A. avenae* samples was prepared by digestion of homogenized nematodes with 30% potassium hydroxide for 1 h (nematodes were homogenized by suspending in distilled water and passing twice through a cold Aminco French pressure cell at 19,000–20,000 psi). Glycogen was precipitated from the alkali-stable fraction by the addition of 1.2 vol 95% ethanol (14). Both fractions were analyzed quantitatively by the anthrone method of Mokrasch (26). Trehalose contents were determined by the subtraction of glycogen from the alkali-stable fraction. Glucose concentrations were determined after the method of Schmidt and Platzer (30). Glucose and trehalose in all other samples were extracted and purified after the method of Womersley (39) and analyzed by gas-liquid chromatography (GLC) of their trimethylsilyl derivatives (41). The glycogen content of these samples was determined by the anthrone method of Roe and Dailey (28) as reported by Womersley (38).

Lipid contents: Samples (10 mg) of lyophilized nematode tissue were sonicated for 15 min at 22 kc in 1 ml methanol: chloroform (1:1); the total lipid fraction was then purified and quantified gravimetrically (16).

Lipid composition: Total lipids of anhydrobiotic *A. avenae* and *A. tritici* larvae were extracted by homogenization in methanol:chloroform (2:1 v/v) and isolated by the method of Bligh and Dyer (4). Total lipid extracts were separated into individual lipid classes by two-dimensional thin-layer chromotography on 20 × 20 cm × 250μm thick silica gel G plates. Plates were activated at 110 C and developed in hexane: diethylether (80:20) in the first direction and hexane:diethylether:methanol (70:20: 10) in the second direction (35). Glacial acetic acid (1%) was included in each solvent system to reduce tailing. Identifications were made by co-chromatography of standards. Lipids were detected by spraying developed plates with 0.2% ethanolic 2, 7-dichlorofluorescein and visualized under UV light. Lipids were quantified by spraying developed plates with 50% H_2SO_4, charring at 125 C for 5 min and subsequent densitometric scanning with a KM3 Carl Zeiss scanning spectrometer.

Phospholipids were analyzed by spotting aliquots of total lipid extracts onto similar but nonactivated silica gel G plates. Plates were developed in chloroform:methanol:7N NH_4OH (60:35:5) in the first direction and chloroform:methanol:7N NH_4OH (35:60:5) in the second direction (32).

For fatty acid analysis, total lipid extracts were saponified (23) and the resultant free fatty acid mixture esterified with diazomethane (29). The methyl esters were analyzed by GLC on a Hewlett-Packard 58-30A gas chromatograph equipped with a 2-m column containing 15% diethylene glycol succinate on Chromosorb W (AW), 60/80 mesh (36). Nitrogen was the carrier gas (30 ml/min) and the column oven temperature was maintained at 175 C. The injection port temperature was 220 C and that of the flame ionization detector was 260 C. Standard solutions of known proportions of long-chain fatty acid methyl

esters (myristic [14:0], palmitic [16:0], palmitoleic [16:1] stearic [18:0], oleic [18:1], linoleic [18:2], linolenic [18:3]) were run before and after each sample to facilitate identification and quantitative calculations. The degree of unsaturation of the lipid was calculated in terms of the number of double bonds per mole (34).

RESULTS

Glycogen, trehalose, and glucose contents are presented in Table 1. The glycogen content of anhydrobiotic *D. dipsaci* could not be analyzed due to lack of material; trehalose and glucose contents of anhydrobiotic *A. avenae* larvae were not analyzed because of the adequate work of Madin and Crowe (25). The glycogen contents of anhydrobiotic *A. tritici* and *A. avenae* larvae were extremely low. These low levels were not distinctly different from the amount found in fresh *A. avenae* samples. Although the glycogen content of *D. myceliophagous* (6.35%) was low compared to that of *P. redivivus* and *T. aceti,* it was appreciably higher than that of fresh *A. avenae* samples. In contrast, anhydrobiotic larvae of *A. tritici* and *D. dipsaci* contained comparatively high levels of trehalose, whereas the trehalose contents of *D. myceliophagous, P. redivivus,* and *T. aceti* were low. The trehalose content of fresh *A. avenae* was higher than that of the latter three species and was approximately 50% of that found in anhydrobiotic *D. dipsaci* larvae. Free glucose contents varied greatly, with lowest levels being found in the anhydrobiotic larvae of *A. tritici* and *D. dipsaci.* The glucose contents of fresh *A. avenae* larvae and *D. myceliophagous* "curd" larvae were, in comparison, much higher. Highest concentrations of free glucose were found in *P. redivivus* and *T. aceti* samples; the free glucose content of individual *T. aceti* samples showed a high degree of variation.

The total lipid contents of *P. redivivus* and *T. aceti* were very similar (Table 2). Much higher levels of lipid were found in anhydrobiotic *D. dipsaci* and *A. tritici* larvae. Fresh *A. avenae* contained a high level of lipid, but this was reduced by approximately 60% in anhydrobiotic larvae. A similar situation occurred in *D. myceliophagous,* where curds contained 60% of the lipid content of fresh nematodes.

The total lipid compositions of anhydrobiotic *A. tritici* and *A. avenae* larvae are presented in Table 3 and a representative chromatogram is presented in Fig. 1. The major neutral lipid of both *A. tritici* and *A. avenae* was triglyceride, which accounted for 85.3% and 79.0% of the total lipid, respectively. Phospholipids comprised 14.6% of the total lipid in *A. tritici,* and free sterol, free fatty acid, and hydrocarbons were present in trace amounts. Diglycerides and sterol esters were not detected. The phospholipid level was not as high as in *A. tritici* (i.e., 8.2% of total lipid), but the free fatty acid fraction comprised 12.8% of the total lipid. Trace components of *A. avenae* lipid included free sterol, diglyceride, sterol ester, and hydrocarbon.

Phosphatidyl choline and phosphatidyl ethanolamine were the most abundant

Table 1. The free glucose, trehalose and glycogen contents of six species of nematode worms.*

Species	Type of sample†		Glycogen	Trehalose‡	Glucose
Anguina tritici	L_2	A	1.11 ± 0.16 (4)	9.1 ± 2.1 (5)	0.16 ± 0.11 (6)
Ditylenchus dipsaci	L_4	A	—	4.63 ± 1.63 (5)	0.36 ± 0.07 (4)
Aphelenchus avenae	L_4, Ad.	F	2.13 ± 0.7 (4)	2.84 ± 0.77 (4)	1.12 ± 0.21 (4)
			1.24 ± 0.16 (2)	—	—
Ditylenchus myceliophagous	Lm, Ad.	C	6.35 ± 0.37 (6)	0.53 ± 0.09 (4)	1.79 ± 0.09 (5)
Panagrellus redivivus	MAd.	F	25.23 ± 1.65 (12)	0.43 ± 0.1 (5)	2.55 ± 0.3 (5)
Turbatrix aceti	Lm, Ad.	F	22.73 ± 0.73 (12)	0.67 ± 0.12 (6)	5.87 ± 1.6 (5)

*Results expressed as percentage of dry weight ± standard deviation. Number of samples analyzed are included in parentheses.

†L_2 = second-stage larvae; L_4 = fourth-stage larvae; L_4, Ad. = fourth-stage larvae and adults; Lm, Ad. = mixed larval stages and adults; MAd. = mainly adult larvae; A = Anhydrobiotic; F = fresh; C = curds.

‡Womersley and Smith, 1981.

Table 2. The total lipid content of six species of nematodes.*

Species	Type of sample†		Total lipid
Anguina tritici	L_2	A	40.6 ± 2.64 (4)
Ditylenchus dipsaci	L_4	A	38.3 ± 2.4 (5)
Aphelenchus avenae	L_4, Ad.	F	36.65 ± 1.12 (6)
		A	14.0 ± 2.5 (2)
Ditylenchus myceliophagous	Lm, Ad.	F	30.76 ± 2.45 (5)
		C	18.0 ± 0.4 (2)
Panagrellus redivivus	MAd.	F	23.14 ± 2.27 (7)
Turbatrix aceti	Lm, Ad.	F	21.9 ± 2.58 (4)

*Results are expressed as percentage dry weight ± one standard deviation. Numbers of samples analyzed are included in parentheses.

†L_2 = second-stage larvae; L_4 = fourth-stage larvae; L_4, Ad. = fourth-stage larvae and adults; Lm, Ad. = mixed larval stages and adults; MAd. = mainly adult larvae; A = anhydrobiotic; F = fresh; C = curds.

Table 3. Composition of the total lipids of anhydrobiotic *Anguina tritici* and *Aphelenchus avenae* larvae separated by thin-layer chromatography.*

Lipid	*A. tritici*	*A. avenae*
Diglyceride	...	Trace
Free sterol	Trace	Trace
Free fatty acid	Trace	12.8
Triglyceride	85.3	79.0
Sterol ester	...	Trace
Hydrocarbon	Trace	Trace
Phospholipid	14.6	8.2

*Values expressed as a percentage of the total lipid.

phospholipids in both *A. tritici* and *A. avenae* (Table 4, Fig. 2). Phosphatidyl inositol and phosphatidyl serine were not adequately separated by the chromatographic system to determine their individual amounts; the combined fraction constituted the third most abundant phospholipid in *A. tritici* and *A. avenae*. In both species lysophosphatide was a minor component; the chromatographic system employed was unable to separate lysophosphatidyl choline and lysophosphatidyl ethanolamine. Phosphatidic acid was present in trace amounts but was only tentatively identified by its chromatographic behavior.

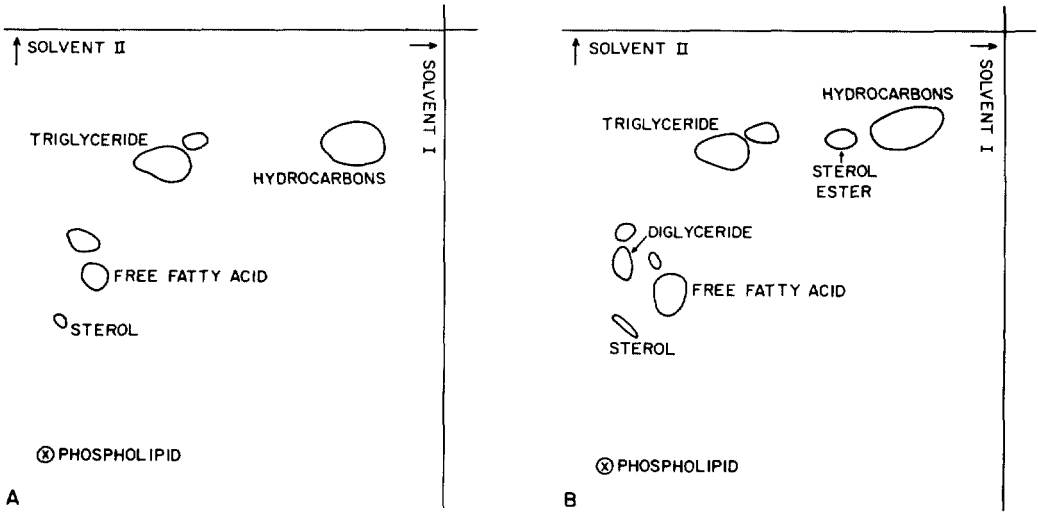

Fig. 1. Diagrammatic representation of two-dimensional thin-layer chromatograms of the total lipids from anhydrobiotic larvae of *Anguina tritici* (a) and *Aphelenchus avenae* (b). Solvent systems were hexane: diethylether:acetic acid (80:20:1) in the first direction followed by hexane:diethylether:methanol:acetic acid (70:20:10:1) in the second.

Table 4. Phospholipid composition of anhydrobiotic *Anguina tritici* and *Aphelenchus avenae* larvae separated by thin-layer chromatography.*

Phospholipid	*A. tritici*	*A. avenae*
Phosphatidyl choline	37.8	38.8
Phosphatidyl ethanolamine	36.4	41.9
Phosphatidyl serine/inositol	18.4	13.2
Lysophosphatide	7.4	6.1
Phosphatidic acid	Trace	Trace

*Values expressed as a percentage of the total phospholipid fraction.

The major fatty acids of anhydrobiotic *A. tritici* and *A. avenae* larvae are shown in Table 5; gas chromatograms are presented in Fig. 3. Approximately 88% of the total fatty acids in *A. tritici* and 74% of those in *A. avenae* were unsaturated. However, most

Table 5. Major fatty acid composition and degree of unsaturation of total lipid from anhydrobiotic larvae of *Anguina tritici* and *Aphelenchus avenae*.*

Fatty acid	*A. tritici*	*A. avenae*
14:0	2.8	3.2
Unknown	1.1	3.6
16:0	4.9	7.8
16:1	5.7	2.0
18:0	1.4	4.7
18:1	73.6	31.2
18:2	6.5	40.9
18:3	2.8	Trace
Unknown	1.0	4.5
Degree of unsaturation	1.01	1.15

*Results are expressed as relative percentage of total fatty acid.

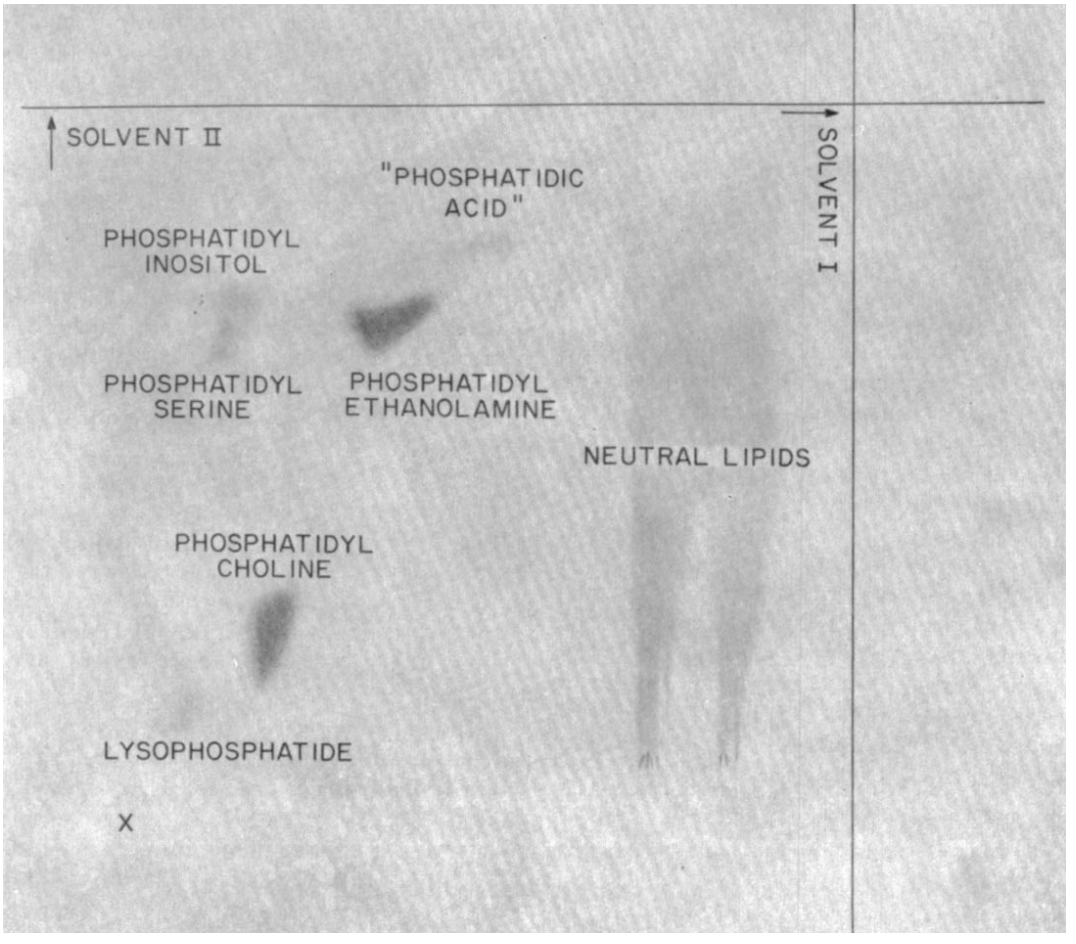

Fig. 2. A two-dimensional thin-layer chromatogram of the phospholipid composition of anhydrobiotic *Anguina tritici*. Solvent systems were chloroform:methanol:7N NH_4OH (60:35:5) in the first direction followed by chloroform:methanol:7N NH_4OH (35:60:5) in the second.

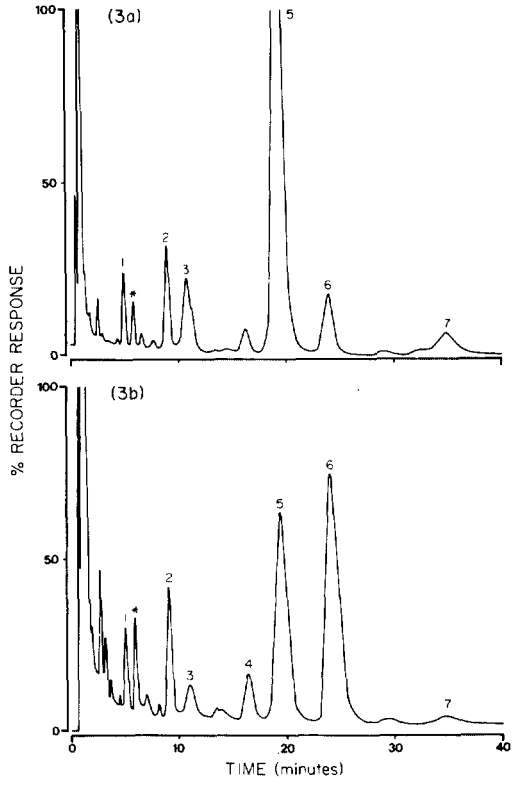

Fig. 3. Gas chromatograms on a column containing 15% diethylene glycol succinate of the total fatty acids from anhydrobiotic larvae of *Anguina tritici* (a) and *Aphelenchus avenae* (b).

of the unsaturated fatty acids in *A. tritici* were mono-unsaturates, whereas aproximately equal amounts of mono-unsaturates and di-unsaturates occurred in *A. avenae*. Thus, the degree of unsaturation in the two nematodes was almost identical (Table 5). The major fatty acid in *A. tritici* was 18:1, but 18:1 and 18:2 occurred in similar proportions in *A. avenae*. Although traces of a number of unidentified fatty acids were detected, two unknown peaks, one occurring directly after the appearance of 14:0 and one appearing over 30 min after 18:3, were regarded of sufficient importance to be included in Table 5.

DISCUSSION

Womersley and Smith (41) observed that anhydrobiotic *D. dipsaci* and *A. tritici* larvae stored appreciable amounts of trehalose, but they did not compare its levels to those of other storage carbohydrates. The present work shows that, with respect to amounts found in nematodes incapable of desiccation survival, *D. dipsaci* and *A. tritici* contain comparatively high levels of trehalose and extremely low levels of glycogen and glucose. This was not unexpected, since Madin and Crowe (25) demonstrated that upon entering anhydrobiosis, *A. avenae* increased its trehalose level while glycogen was reduced. The levels of glycogen found in anhydrobiotic *A. avenae* larvae during the present work compared favorably with those previously reported (32), but the glycogen content of fresh *A. avenae* larvae was low. This can be attributed to the method of storage before analysis, in which metabolism under anaerobic conditions may have occurred and resulted in a reduction in glycogen content (8).

In direct contrast to the above, the main storage carbohydrate of the free-living nematodes *P. redivivus* and *T. aceti* was glycogen, with appreciable amounts of glucose also occuring. The glycogen content of *T. aceti* was similar to that already reported (3). Barrett et al. (3) recorded a glycogen content of 6.5% of dry weight for *P. redivivus* when freshly extracted and 3.7% of dry weight when starved for 10 days; we found a glycogen content of 25.23% of dry weight for freshly extracted *P. redivivus*. This discrepancy may be attributed to the fact that they harvested *P. redivivus* from petri-dish lids where the worms had aggregated far from the culture medium and thus may already have been starved. A similar situation may have occurred in the *D. myceliophagous* samples analyzed from curds. These samples were collected when the culture medium became fouled and the worms aggregated to form the characteristic curds. That partial starvation had occurred is indicated by the reduced glycogen and lipid contents compared to freshly extracted worms.

It would appear from the observations made on storage carbohydrates that nematode anhydrobiotes, like other anhydrobiotes (40), store trehalose instead of glycogen and that this phenomenon is closely associated with the ability to survive desiccation. Madin and Crowe (25) suggested, and it was later confirmed (24), that preferential storage of trehalose reduces the chance of a "browning" reaction occurring

between the reducing sugars and free amino groups of dry proteins. Reductions in free glucose would also aid in the elimination of this reaction (41).

In the present work, each species studied contained high lipid content with respect to the animal-parasitic nematodes. The total lipid contents of *P. redivivus* and *T. aceti* agreed with the findings of both Sivapalan and Jenkins (31) and Barrett et al. (3). Similarly, fresh *D. myceliophagous* had a high lipid content (30.76% of dry weight), but this amount was drastically reduced in nematodes from curds and thus indicated that these nematodes were indeed starved. During periods of starvation the total lipid contents of *P. redivivus* and *T. aceti* are also reduced (3). Similarly, the lipid content of anhydrobiotic larvae of *A. avenae* was reduced by 60%. However, Madin and Crowe (25) suggested that lipid utilization in *A. avenae* at the induction of anhydrobiosis is geared not to the production of immediate energy, but rather to the synthesis of trehalose and glycerol and thus must be considered an important facet in the survival ability of this nematode. This situation would not appear to be the case in anhydrobiotic larvae of *D. dipsaci* and *A. tritici,* where high lipid levels (38.3 and 40.6%) were maintained and were identical to those previously reported for fresh samples of *D. dipsaci* (19). This observation suggests that in *D. dipsaci* and *A. tritici,* trehalose is synthesized at the expense of glycogen and not lipid and that the lipids in these nematodes remain unchanged as they pass from the active to the anhydrobiotic phase.

Our analyses of the lipid composition of *A. avenae* and *A. tritici* did not indicate any specific adaptations that could be primarily associated with anhydrobiotic survival. Both nematodes contained large amounts of neutral lipid, mainly in the form of triglyceride, as is the case in *T. aceti* (15) and *P. redivivus* (3). An appreciable amount of free-fatty acid (12.8% of total lipid) was found in *A. avenae,* but this was less than that found in *T. aceti* (15) and may be related to differences in host tissue lipids rather than direct synthesis (3). Except for traces of other lipid classes, phospholipid comprised the remaining total lipid in both species. Womersley and Smith (41) suggested that phospholipids, particularly phosphatidyl inositol, may be important in membrane stablization during desiccation. Our present results do not support this view. The levels of phospholipid found in *A. tritici* and *A. avenae,* although higher than that reported for *M. javanica* females (6), were low compared with the amounts found in *T. aceti* (7,15) and *P. redivivus* (31). The major phospholipids of *A. avenae* and *A. tritici* were phosphatidyl ethanolamine and phosphatidyl choline, which are also the most abundant phospholipids in *T. aceti* (7), *P. redivivus* (31), *M. javanica* (6), and the animal parasitic nematodes *Ancylostoma caninum* (18), *Ascaris lumbricoides* (33), *Dirofilaria immitis* (17), *Setaria cervi* (1), and *Trichinella spiralis* (5). Although comparatively high levels of phosphatidyl serine plus phosphatidyl inositol were detected in both species, it was impossible to determine the amounts of each due to lack of separation on the chromatograph. Comparison with other species (6) suggests that phosphatidyl serine may be the major contributor.

The total fatty acid compositions of *A. avenae* and *A. tritici,* although distinctly different in type, conformed to the findings in other nematodes (15,19,22,31) in that most of the fatty acids were unsaturated 18-carbon acids. The two major fatty acids in *A. avenae* were 18:1 and 18:2 (72% of total). The fatty acid composition of *A. tritici* was similar to that of *D. dipsaci* (19), *M. incognita,* and *M. arenaria* (22), in that the major fatty acid was 18:1, which comprised 73.6% of the total. No differentiation was made between the major isomers of 18:1 (vaccenic and oleic acid), although Krusberg et al. (22) have shown that in *Ditylenchus* and *Meloidogyne* species, the greater proportion of 18:1 is composed of vaccenic acid ($18:1^{\omega 7}$). The "unknown" fatty acid component reported in both *A. tritici* and *A. avenae* had a retention time within the range expected for 20-carbon polyunsaturates. In addition, two lesser peaks were also evident after 18:3 in *A. avenae* but were too small for quantification. Twenty-carbon polyunsaturates occur commonly in free-living and plant-parasitic nematodes and have been reported on numerous occasions, generally at levels

of 1–10% (6,19). Further analysis, however, was not carried out during the present study to confirm their presence or identity. Additional study is therefore necessary to characterize the 20-carbon fatty acid composition of these species.

Overall, the lipid compositions of *A. avenae* and *A. tritici* are similar to those of other free-living and plant-parasitic nematodes. Research does suggest a specific role for lipid in anhydrobiotic and revived *A. avenae* larvae (10,25), but its role in the survival of *D. dipsaci* and *A. tritici* is still uncertain. Ultrastructural studies on lipid in *A. tritici* (41) have detected alterations in cuticular lipid composition which could help reduce evaporative water loss. Womersley (40) has suggested that lipid could play an important role in survival by maintaining the spatial distribution of tissues in the absence of bulk water. Furthermore, because *A. avenae* cannot utilize lipid under anaerobic conditions (8), he postulated that the high levels of lipid in *D. dipsaci* and *A. tritici* could be a combined effect of anhydrobiosis plus anoxybiosis which would occur at the onset of anhydrobiosis.

In conclusion, it seems that the main purpose of lipid in free-living and plant-parasitic nematodes is the provision of food reserves which can either be utilized during periods of environmental stress or, as in the case of nematode anhydrobiotes, be utilized at the induction of, and revival from, the anhydrobiotic state.

LITERATURE CITED

1. Ansari, A. A., A. N. K. Yusufi, and M. Siddiqi. 1973. Lipid composition of the filarial parasite Setaria cervi. J. Parasitol. 59:939-940.

2. Barrett, J. 1977. Energy metabolism in nematodes. Pp. 11-70 *in* N. A. Croll, ed. The organisation of nematodes. New York: Academic Press.

3. Barrett, J., C. W. Ward, and D. Fairbairn. 1971. Lipid metabolism in the free-living nematodes Panagrellus redivivus and Turbatrix aceti. Comp. Biochem. Physiol. 38B:279-284.

4. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Physiol. 37:911-917.

5. Castro, G. A., and D. Fairbairn. 1969. Carbohydrates and lipids in Trichinella spiralis larvae and their utilisation in vitro. J. Parasitol. 55:151-158.

6. Chitwood, D. J., and L. R. Krusberg. 1981. Diacyl, alkylacyl, and alkenylacyl phospholipids of Meloidogyne javanica females. J. Nematol. 13(2): 105-111.

7. Chitwood, D. J., and L. R. Krusberg. 1981. Diacyl, alklacyl and alkenylacyl phospholipids of the nematode Turbatrix aceti. Comp. Biochem. Physiol. 69B:115-120.

8. Cooper, A. F., 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(4):305-315.

9. Crowe, J. H., and K. A. C. Madin. 1975. Anhydrobiosis in nematodes: evaporation, water loss and survival. J. Exp. Zool. 193:323-334.

10. Crowe, J. H., K. A. C. Madin, and S. H. Loomis. 1977. Anhydrobiosis in nematodes: metabolism during resumption of activity. J. Exp. Zool. 201:57-64.

11. Demeure, Y., G. Reversat, S. D. Van Gundy, and D. W. Freckman. 1978. The relationship between nematode reserves and their survival to desiccation. Nematropica 8(2):7-8.

12. Ellenby, C., and L. Smith. 1966. Observations on Panagrellus redivivus, Goodey, 1945. J. Helminth 40:323-330.

13. Evans, A. A. F., and R. N. Perry. 1976. Survival strategies in nematodes. Pp. 383-424 *in* N. A. Croll, ed. The organization of nematodes. New York, London: Academic Press.

14. Fairbairn, D., G. Wertheim, R. P. Harpur, and E. L. Schiller. 1961. Biochemistry of normal and irradiated strains of Hymenolepis diminuta. Exp. Parasitol. 11:248-263.

15. Fletcher, C. L., and L. R. Krusberg. 1973. Investigations of some lipids from Turbatrix aceti. Comp. Biochem. Physiol. 45B:159-165.

16. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

17. Hutchison, W. F., A. C. Turner, D. P. Grayson, and H. B. White. 1976. Lipid analysis of the adult heartworm, Dirofilaria immitis. Comp. Biochem. Physiol. 53B:495-497.

18. Khuller, G. K., S. K. Jain, and V. K. Vinayak. 1977. On the phospholipids of adult Ancylostoma caninum. Expereintia 33:1585.

19. Krusberg, L. R. 1967. Analyses of total lipids and fatty acids of plant-parasitic nematodes and host tissues. Comp. Biochem. Physiol. 21:83-90.

20. Krusberg, L. R. 1971. Chemical composition of nematodes. Pp. 213-234 *in* B. M. Zuckerman, W. F., Mai, and R. A. Rohde, eds. Plant-parasitic nematodes. Vol. 2. New York: Academic Press.

21. Krusberg, L. R. 1972. Fatty acid composition of Turbatrix aceti and its culture medium. Comp. Biochem. Physiol. 41B:89-98.

22. Krusberg, L. R., R. S. Hussey, and C. L. Fletcher. 1973. Lipid and fatty acid composition of females and eggs of Meliodogyne (Java) incognita and M. arenaria. Comp. Biochem. Physiol. 45(2): 335-341.

23. Lepper, H. A., editor. 1950. Methods of analysis. Washington, DC: Association of Official Agricultural Chemists.

24. Loomis, S. H., S. J. O'Dell, and J. H. Crowe. 1979. Anhydrobiosis in nematodes: inhibition of the browning reaction of reducing sugars with dry pro-

tein. J. Exp. Zool. 208:355-360.

25. Madin, K. A. C., and J. H. Crowe. 1975. Anhydrobiosis in nematodes: carbohydrate and lipid metabolism during dehydration. J. Exp. Zool. 193: 335-342.

26. Mokrasch, L. C. 1954. Analysis of hexose phosphates and sugar mixtures with the anthrone reagent. J. Biol. Chem. 208:55-59.

27. Perry, R. N. 1977. Desiccation survival of larval and adult stages of Ditylenchus dipsaci and D. myceliophagus. Parasitology 74:139-148.

28. Roe, J. H., and R. E. Dailey. 1966. Determination of glycogen with the anthrone reagent. Anal. Biochem. 15:245-250.

29. Schlenk, H., and J. L. Gellerman. 1960. Esterification of fatty acids with diazomethane on a small scale. Anal. Chem. 32:1412-1414.

30. Schmidt, S. P., and E. G. Platzer. 1980. Changes in body tissues and haemolymph composition of Culex pipiens in response to infection by Romanomermis culicivorax. J. Invert. Pathol. 36: 240-254.

31. Sivapalan, P., and W. R. Jenkins. 1966. Phospholipid and long-chain fatty acid composition of the nematode Panagrellus redivivus. Proc. Helminth Soc. Wash. 33:149-157.

32. Skidmore, W. D., and C. Entenman. 1962. Two-dimensional thin-layer chromatography of rat liver phosphatides. J. Lipid Res. 3:471-475.

33. Subrahmanyam, D., and S. Venkatesan. 1968. On the phospholipids of Ascaris lumbricoides. Comp. Biochem. Physiol. 25:733-737.

34. Sumner, J. L., and E. D. Morgan. 1969. The fatty acid composition of sporangiospores and vegetative mycelium of temperature-adapted fungi in the order Mucorales. J. Gen. Microbiol. 59:215-221.

35. Thompson, S. N. 1979. The effect of dietary carbohydrate on larval development and lipogenesis in the parasite, Exeristes roborator (Fabricius) (Hymenoptera: Ichneumonidae). J. Parasitol. 65(6): 849-854.

36. Thompson, S. N., and J. Johnson. 1978. Further studies on lipid metabolism in the insect parasite, Exeristes roborator (Fabricius). J. Parasitol. 64(4):731-740.

37. Van Gundy, S. D. 1965. Factors in survival of nematodes. Ann. Rev. Phytopathol. 3:43-68.

38. Womersley, C. 1978. Physiological and biochemical aspects of anhydrobiosis in some plant-parasitic and free-living nematodes. Newcastle Upon Tyne, England: The University; PhD Thesis.

39. Womersley, C. 1981. A micromethod for the extraction and quantitative analysis of 'free' carbohydrates in nematode tissue. Anal. Biochem. 112: 182-189.

40. Womersley, C. 1981. Biochemical and physiological aspects of anhydrobiosis. Comp. Biochem. Physiol. 70B:669-678.

41. Womersley, C., and L. Smith. 1981. Anhydrobiosis in nematodes I: the role of glycerol, myo-inositol and trehalose during desiccation. Comp. Biochem. Physiol. 70B:579-586.