A Sterol Requirement in *Turbatrix aceti* and *Panagrellus redivivus*

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Abstract: A nutritional requirement for sterol was demonstrated for *Turbatrix aceti* and *Panagrellus redivivus* with a sterol deficient test system that consisted of endospores of *Bacillus subtilis* on peptone glucose agar slants. Neither nematode species would sustain growth unless cholesterol was supplemented to the test system. At least 0.1 μg of cholesterol supplement was required to support growth of *Turbatrix*. However, 1.0 and 10.0 μg supplements usually produced better growth as measured by total nematodes produced per agar slant. Several highly purified sterols representing considerable structural diversity supported growth of *Turbatrix* in our test system. However, some specificity existed since two sterols of the coprostane series failed to support growth of this nematode.

In extensive studies of sterol metabolism in insects, it has been well documented, both biochemically and nutritionally, that insects rely on exogenous sterol for normal growth and development. This requirement is manifested by an apparent lack of *de novo* sterol biosynthesis. Also, Dutky *et al.* (5) reported that the DD-136 insect-parasitic nematode required exogenous sterol for normal growth and development and were able to demonstrate the sterol requirement with a monoxenic culture of the nematode and its associated bacterium. The source of sterol for the nematode was the insect host, and the nematode failed to develop and produce fertile ova in the monoxenic test system unless the system was supplemented with the appropriate sterol. Rothstein and Hieb (6) reported that *Caenorhabditis briggsae* (Dougherty and Nigon) required sterol for reproduction. They used a monoxenic test system with *Escherichia coli* (Migula) as the nutrient source.

Biochemical studies by Cole and Krusberg (4) indicated that *Turbatrix aceti* (Müller) could not biosynthesize sterols from simple precursors and therefore probably relied on exogenous sterol for normal growth and development. Since both *T. aceti* and *Panagrellus redivivus* (L.) could be cultured monoxenically with a number of different bacteria, we used the same technique as used with DD-136 to evaluate their sterol requirement.

The purpose of the present study was to obtain nutritional confirmation of an exogenous sterol requirement in *T. aceti* and *P. redivivus*.

MATERIALS AND METHODS

The sterol-deficient test system consisted of endospores of *Bacillus subtilis* Cohn (red stain 10R) previously grown on tryptose phosphate agar slants supplemented with 20 μg manganous sulfate per slant and subsequently transferred to peptone-glucose agar slants (test system). About two billion endospores were added to each slant. Sterols in methanol solution and the bacterium were each added aseptically to the slants by bacteriological loop, and the nematodes were added aseptically either by bacteriological loop or individually with a fine wire. Incubation temperatures were 30 C for *T. aceti* and 25 C for *P. redivivus*. The initial pH of the test system (peptone-glucose agar slants) was 5.0. After the test, it was 5.2 to 5.4 (about 14 days).

All components of the test system were analyzed for sterol content as previously described (3). Also, the validity of the test system was further challenged by determining whether the supplemented sterol was

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TABLE 1. Demonstration that cholesterol is required for growth and reproduction of *Turbatrix aceti.*

Treatment*	Average total living nematodes/agar slant after 14 days
Nematode	about 50
Nematode + sterol	about 50
Nematode + bacterium	0
Nematode + sterol + bacterium	250,000

* Inoculum was about 100 larvae and adults per peptone glucose agar slant.

biologically altered by the bacteria as follows: several of the peptone-glucose agar slants were supplemented with 100 μg cholesterol-4-^{14}C. Then one-half were inoculated with spores of *B. subtilis,* and the other half was left uninoculated but otherwise treated the same. Finally, after 14 days incubation at 30 C, the sterol was isolated and purified from both sets of tubes, and both the total mass and the radioactivity recovered from each were compared. In addition, the extracts were examined for radioactive polar metabolites of the supplemented sterol by partition against n-butanol and subsequent radioassay of these extracts by liquid scintillation counting. A second approach was to suspend the endospores in the agar medium containing the radioactive sterol in an effort to produce a number of different microenvironments similar to those that might occur in the gut of the nematode; a second set of uninoculated tubes served as the control.

RESULTS

Nutritional studies with the sterol-deficient bacterial test system conclusively demonstrated that neither species of nematode would sustain growth and reproduction in the absence of supplemented sterol (Tables 1 and 2).

For *Turbatrix,* the sterol requirement was evident about one week after the nematode was transferred to the sterol-deficient test system. For *Panagrellus,* a second serial transfer was necessary before the sterol requirement was clearly demonstrated (Table 2).

TABLE 2. Demonstration that cholesterol is required for growth and reproduction of *Panagrellus redivivus.*

Source of inoculum	Test system*	No. living nematodes/ slant after 12 days
1. Axenic liver culture	a. Sterol-supplemented	272,000
Axenic liver culture	b. Sterol-deficient	42,000
2. Sterol-deficient culture (1 b)	a. Sterol-supplemented	175,000
Sterol-deficient culture (1 b)	b. Sterol-deficient	less than 500

* Inoculum was 4 gravid females per peptone glucose agar slant supplemented with 2 billion spores of *B. subtilis.* Sterol supplemented contained 20 μg of cholesterol.

The minimum amount of cholesterol required to support growth of *Turbatrix* in our test system was 0.1 μg per slant; however, 1.0 and 10 μg supplements often produced higher yields. Concentrations of 0.01, 0.1, 1.0, 10, and 100 μg were tested.

A number of highly purified sterols with diverse structures were tested by this method for support of growth and reproduction of *Turbatrix* including Δ^7-cholestenol, cholestanol, β-sitosterol, fucosterol, 24-methylenecholesterol, 25-norcholesterol, cholest-4-ene-3-one, cholest-5-ene-3-one, campesterol, stigmasterol, desmosterol, coprostanol, coprost-7-ene-ol, and 7-dehydrocholesterol. All compounds except coprostanol and coprost-7-ene-ol, could replace cholesterol in supporting growth and reproduction. The 2 exceptions differed from the other compounds by the conformation of rings A and B of the steroid nucleus.

The validity of the bacterial test system was challenged by (i) determining the amount of sterol present and (ii) determining whether the supplemented cholesterol was biologically altered by the bacterium. Small amounts of

two sterols tentatively identified as cholesterol and β-sitosterol were found in the bacterial endospores. Also, endospores (3.8×10^{11}) from 56 slants contained about 3 to 4 μg of sterol, mostly cholesterol, and 22 μg of sterol was found in the tryptose phosphate medium (0.4 μg per slant) after the bacterial spores were removed. The source of the β-sitosterol was probably the cotton plugs. The approximate amount of sterol added to the test system (peptone-glucose agar slants) in the bacterial inoculum (2.0×10^9 spores) was 0.02 μg per slant. The quantity of sterol present in the test system was about 0.15 μg per slant. The amount of ^{14}C-labelled cholesterol recovered from the tests in the presence and absence of the bacteria were in excellent agreement. Recoveries of radioactivity and mass of cholesterol (determined by gas liquid chromatography) were always quantitative. Also no radioactivity was detected in the n-butanol extracts that would contain the polar metabolites of the radioactive cholesterol.

Discussion

The nutritional demonstration that both *T. aceti* and *P. redivivus* require exogenous sterol for normal growth and reproduction confirms the lack of *de novo* sterol biosynthesis in these nematodes. This demonstration also confirms that sterols and/or their metabolites perform a vital function(s) in these nematodes.

In insects, sterols are said to perform a dual role (2). They are required for a structural function, and a metabolic function which includes their role as precursors to the insect steroid molting hormones (ecdysones). Since nematodes undergo a series of molts during their development to the adult, sterols may serve as precursors to nematode molting hormones in addition to a probable structural function. If this proves true, it will be interesting to determine whether these hormones are the same or similar to the insect molting hormones.

As noted, with *Turbatrix,* the sterol requirement was clearly evident about one week after the nematode was transferred to the sterol-deficient test system. With *Panagrellus*, a second serial transfer to a sterol-deficient system was necessary before the sterol requirement was clearly demonstrated. Thus, *Panagrellus* can either utilize stored and available sterol in an extremely efficient manner, or its threshold requirement for growth is just above the amount of sterol available in our test system.

The bacterial test system was challenged and found to be valid since (i) the bacteria did not biologically alter supplemented radioactive cholesterol in two types of experiments and (ii) the test system contained only trace amounts of sterol, only a portion of which was available to the nematode via the bacteria.

Several highly purified sterols supported growth of *Turbatrix,* though some specificity existed, since two sterols of the coprostane series did not. Biochemical studies previously showed that *Turbatrix* can metabolize the phytosterols fucosterol and β-sitosterol to cholesterol and 7-dehydrocholesterol (4). However, the ability of other sterols to support growth and reproduction in the monoxenic test system does not provide conclusive proof that they are metabolized to nematode sterols. Small quantities of cholesterol from the test system may satisfy any requirement for nematode hormone precursors. Supplemented sterols could then serve to "spare" any structural function in the absence of a sufficient source of cholesterol as occurs with certain insects (1). Also, only cholesterol was challenged in the test system. Conclusive proof that

sterols other than cholesterol were not biologically altered by the bacterium is therefore lacking.

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