Critical Reviews in Biochemistry and Molecular Biology, 34(4):273–284 (1999)

Biochemistry and Function of Nematode Steroids

David J. Chitwood

CONTENTS

I. INTRODUCTION

Although largely hidden from public view, nematodes are among the most numerous and ecologically diverse multicellular organisms inhabiting the planet. Although most species are microbivorous, numerous species are economically or medically important parasites of plants and animals. As Cobb[1] wrote 80 years ago, "If all the matter in the universe except the nematodes were swept away, our world would still be dimly recognizable, and if, as disembodied spirits, we could then investigate it, we should find its mountains, hills, vales, rivers, lakes and oceans represented by a film of nematodes. The locations of towns would be decipherable, since for every massing of human beings there would be a corresponding massing of certain nematodes. The location of the various plants and animals would still be decipherable, and, had we sufficient knowledge, in many cases their species could be determined by an examination of their erstwhile nematode parasites."

As more has been learned about nematodes in the 80 years since Cobb wrote these remarks, the importance of animal- and plant-parasitic nematodes has increased. Many scientists have attempted to discover and exploit some fundamental biochemical difference between the biochemistry or physiology of these parasites and their hosts in order to develop compounds useful for nematode control. One of the few major biochemical differences between vertebrate- and plant-parasitic nematodes and their hosts is that nematodes (like insects) are incapable of *de novo* sterol biosynthesis and therefore possess a nutritional requirement for sterol. This review focuses upon the metabolism of sterols by nematodes, the effects of sterol metabolism inhibitors on nematode biochemistry and biology, and the isolation and identification of steroid hormones from nematodes. Some of these specific areas or related ones have been the subject of other reviews.[2–9]

II. NUTRITIONAL REQUIREMENT FOR STEROL

Nutritional studies of nematodes are limited by the fact that plant- and vertebrate-parasitic species have not been successfully cultured apart from their hosts. The requirement for a dietary sterol was discovered by Dutky et al.[10] in the entomopathogenic nematode *Steinernema carpocapsae* and by Hieb and Rothstein[11] in the microbivorous nematode *Caenorhabditis elegans.* Both species reproduced only when their bacterial culture systems were supplemented with exogenous sterols. The results of these nutritional experiments were supported by concurrent investigations in which nematodes failed to produce radiolabeled sterols from radiolabeled sterol precursors such as acetate or mevalonate.[12,13] Although

occasional reports of *de novo* production of radiolabeled sterols by nematodes have appeared in the literature, these experiments have lacked the cultural sterility or analytical rigor necessary to establish such an ability.

Determination of the specificity of the nutritional requirement for sterol is difficult because of the necessity to propagate nematodes either upon hosts that biosynthesize and metabolize sterols or else upon complex culture media that often contain endogenous sterols. Generally, most 4-desmethylsterols with a *trans*-A/B ring system and an intact, nonhydroxylated side chain can satisfy the sterol requirement in nematodes. For example, cholesterol, cholestanol, sitosterol, stigmastanol, dihydrobrassicasterol, 7-dehydrocholesterol, or lathosterol (but not stigmasterol or ergosterol) supported the reproduction of *Neoaplectana carpocapsae* in a bacteria-containing diet.[10] In a different bacterial culture system, growth and reproduction of the same species were excellent when cholesterol, sitosterol, cholesteryl oleate, cholestanol, stigmastanol, lathosterol, or ergosterol was added. In stigmasterol-supplemented medium, growth was slower and growth did not occur when either lanosterol or 7-dehydrocholesterol was the supplemented sterol.[14] When *C. elegans* was propagated upon *Escherichia coli*, cholesterol, sitosterol, 7-dehydrocholesterol, ergosterol, or stigmasterol satisfied the nutritional requirement.[11] In the microbivorous nematode *Turbatrix aceti* (the vinegar eelworm), the nutritional requirement was satisfied by cholesterol, desmosterol, lathosterol, 7-dehydrocholesterol, cholestanol, 25-norcholesterol, 24-methylenecholesterol, sitosterol, fucosterol, cholest-4-en-3-one, or cholest-5-en-3-one; only coprostanol and coprost-7-enol were unsuccessful dietary sterols.[15] Eggs of the rat parasite *Nippostrongylus brasiliensis* developed to third-stage juveniles in a formaldehyde-killed bacterial culture system supplemented with ergosterol, sitosterol, stigmasterol, lanosterol, 7-dehydrocholesterol, or cholestane.[16] Cholesterol, lathosterol, 7-dehydrocholesterol, campesterol, ergosterol, sitosterol, stigmasterol, stigmastanol, isofucosterol, or cholesteryl acetate supported growth and reproduction of *C. elegans* growing in a sterile, defined medium coprost-7-enol, 4α-methylcholest-8(14)-enol, lanosterol, 22,23-dihydroxysitosterol, progesterone, ecdysone, or 20-hydroxyecdysone did not.[17]

Exactly which enzyme or enzymes in the *de novo* sterol biosynthesis pathways present in other organisms are lacking in nematodes is poorly understood. The detection of radiolabeled lanosterol in the microbivorous species *Panagrellus redivivus* incubated with radiolabeled 2,3-oxidosqualene led to the conclusion that this species contains 2,3-oxidosqualene lanosterol cyclase.[18] A metabolic block between farnesol and squalene was concluded based upon nutritional experiments in which squalene, lanosterol, or cholesterol but not mevalonolactone and farnesol supported growth of *C. elegans* and *T. aceti*.[19] Similarly, squalene and lanosterol (but not farnesol or mevalonate) supported development of eggs to third-stage juveniles in a formalin-killed *E. coli* culture system for *N. brasiliensis*.[16] In contrast, squalene did not support growth and reproduction in *C. elegans*,[11] and lanosterol did not support development of *C. elegans* in a defined medium[17] or in *S. carpocapsae.*[14] Additional research with radiolabeled sterol precursors in nutritionally defined, sterile culture systems is necessary to resolve these discrepancies and elucidate the specific blocks in the *de novo* biosynthetic pathway.

III. STEROLS OF ANIMAL-PARASITIC NEMATODES

Because of the present inability to culture plant- and animal-parasitic nematodes away from their hosts, investigation of sterol biochemistry in these parasites has been limited primarily to comparison of sterol compositions of host and nematode. Additionally, the small sterol content of many nematodes (particularly phytoparasitic species) has impeded the determination of C-24 stereochemical orientation in parasitic nematode sterols. Therefore, sterols identified in parasitic nematodes will be described by their systematic names in this review, e.g., 24α-methylcholesterol instead of campesterol.

A. VERTEBRATE PARASITES

In comparison to sterols of other groups of nematodes, the sterols of vertebrate-parasitic nematodes have been poorly characterized. The sterol content of several vertebrate-parasitic nematodes has been reported as 0.1–0.3%.[20–26] The only species examined that is not a digestive tract parasite is *Trichinella spiralis*, which contained cholesterol as its only sterol.[27] In contrast, the sterols of *Ascaris suum* have been reported as 39–46% cholesterol, 20–30% cholestanol, 4–8% 24-methylcholesterol, 7–10% 24-methylcholestanol, 7–15% 24-ethylcholesterol, and 8–11% 24-ethylcholestanol,[20,28,29] depending on the tissue and sex examined. Presumedly, the 24-alkylsterols originate in the host diet, and either *A. suum* saturates the sterol nucleus of the unsaturated phytosterols or else this conversion is performed by the gut microflora.

In contrast, stanols were not found in a gut parasite of chickens, *Ascaridia galli*.[30] In this species, cholesterol comprised 45% of the total sterol, with the remainder being 24-methylcholesterol, 24-ethylcholesta-5,22(*E*)-dienol, 24-ethylcholesterol, and 24-ethylidenelathosterol.

Few experiments have been performed with radiolabeled sterols administered to vertebrate-parasitic nematodes. When radiolabeled sitosterol was injected into *A. suum*, the lack of recovery of radiolabeled cholesterol from nematodes indicated that *A. suum* may be unable to dealkylate plant sterols,[28] unlike the microbivorous species subsequently described. *Nippostrongylus brasiliensis* did esterify radiolabeled cholesterol added to culture medium.[16]

In an investigation of sterol transport across the intestine of *A. suum*, the movement of cholesterol, although slow, was twice that of sitosterol.[31] Because cholesterol but not sitosterol accumulated in the epithelial cells, a suggestion was made that selective transport was caused by epithelial cell specificity. In another study of sterol uptake, a double cannulation apparatus was employed for continuous perfusion of the pseudocoelom of adult *A. suum*.[32] Occlusion of the digestive tract did not interfere with cholesterol uptake, which was primarily through the cuticular and muscular tissues.

B. ENTOMOPATHOGENIC NEMATODES

Because the nematode species that are presently being investigated as biological control agents of pest insects kill insects via the symbiotic bacteria vectored by the nematodes, these species are more properly described as entomogenous or entomopathogenic nematodes, not insect parasites. Comparative studies of host and nematode sterols have provided clues about the metabolic capabilities of entomopathogenic nematodes. In one such comparison, *S. carpocapsae* contained lathosterol (62% of total sterol) and cholesterol (38%) as its major sterols; the wax moth larval host (*Galleria mellonella*) contained cholesterol and traces of 24-methylcholesterol and 24-ethylcholesterol.[33] When insect larvae were injected with radiolabeled cholesterol, *S. carpocapsae* contained radiolabeled cholesterol and lathosterol with the same specific activity as that which had been injected. Therefore, it was concluded that nematodes could reduce the Δ^5-bond of the cholesterol found in host insects and introduce a Δ^7-bond to form lathosterol.

Many years later, *S. carpocapsae* was propagated in corn earworm larvae (*Heliothis zea*) fed diets supplemented with various sterols.[34] This experimental system is somewhat complex because insects are also incapable of de novo sterol biosynthesis and metabolize the dietary sterols in addition to the nematodes. When *H. zea* was fed cholesterol, 95% of its sterol was cholesterol; yet cholesterol comprised but 55% of the sterol in *S. carpocapsae*, with the remainder being lathosterol. Cholestanol-fed *H. zea* contained cholestanol as its major sterol (84% of total sterol); *S. carpocapsae* contained cholestanol (70%) and lathosterol (30%). When campestanol was fed to *H. zea*, 51% of its tissue sterol was campestanol and *S. carpocapsae* contained 43% cholestanol and 34% lathosterol. Finally, ergosterol-fed *H. zea* contained 7-dehydrocholesterol as its major sterol, yet *S. carpocapsae* contained lathosterol as its principal sterol. These experiments clearly demonstrate that *S. carpocapsae* can reduce Δ^5-bonds and introduce double bonds at C-7. Interestingly, although the growth of *H. zea* was influenced by alterations in the sterol composition of its diet, the nematodes grew and reproduced very well in all experiments.

IV. STEROLS OF PLANT-PARASITIC NEMATODES

Partly because of the need for development of biorational chemical nematicides, the sterols in plant-parasitic nematodes have received greater attention than those in animal parasites. Evidence for possible dealkylation of sterols at C-24 by phytoparasitic nematodes was first provided by Cole and Krusberg,[35] who compared the sterols of the stem and bulb nematode, *Ditylenchus dipsaci*, with those of the alfalfa callus tissues used to propagate the nematodes. Cholesterol and lathosterol were the major nematode sterols, whereas the plant tissue sterols were 24-ethylcholesta-7,22-dienol and 24-ethyllathosterol. Similarly, because the reniform nematode (*Rotylenchulus reniformis*) contained 24-ethylcholesterol and cholesterol as its major sterols but host roots (cotton) contained 24-ethylcholesterol and 24-ethylcholesta-5,22-dienol as the major sterols, the suggestion was that *R. reniformis* dealkylates plant sterols at C-24 to produce cholesterol.[36]

Another aspect of plant-parasitic nematode sterol metabolism was discovered during investigation of the tobacco cyst nematode *Globodera tabacum solanacearum*.[37] The sterols of this species were primarily devoid of nuclear unsaturation and included 43% 24-methylcholestanol, 20% 24-ethylcholest-22(*E*)-enol, 12% cholesterol, 9% 24-methylcholesterol, and 8% 24-ethylcholesterol. Although host plant roots were

not directly analyzed, the fact that higher plants seldom contain more than traces of stanols led to the suggestion that *G. tabacum solanacearum* can hydrogenate Δ^5-bonds.

The development of capillary gas chromatography-mass spectrometry (GC-MS) accelerated the discovery of additional sterols in phytoparasitic nematodes; the mass spectra of 63 different sterols identified in nematodes by this method have been published.[38] In the first such study, 14 sterols were discovered in females of the corn cyst nematode, *Heterodera zeae*, and compared directly to corn roots.[39] The roots contained 24-ethylcholesta-5,22-dienol, 24-methylcholesterol, 24-ethylcholesterol, and cycloartenol as the major sterols, whereas the principal sterols of *H. zeae* were 24-ethylcholesterol, 24-ethylcholesta-5,22-dienol, 24-methylcholesterol, and cholesterol. In contrast to the tobacco cyst nematode, in which the majority of sterols were stanols, only a small percentage (about 5%) of the sterols in *H. zeae* were stanols. The abundance of cholesterol in *H. zeae* and *R. reniformis* indicates that these species probably dealkylate phytosterols, although the fact that selective uptake mechanisms for sterols occur in insects[40] indicates that a similar process could exist in nematodes. Experiments with radiolabeled phytosterols are needed to clearly establish the presence of C-24 dealkylation in plant-parasitic nematodes.

Sterol compositions of two other genera of phytoparasitic nematodes have been determined with GC-MS. First, eggs of the root-knot nematodes *Meloidogyne incognita* and *M. arenaria* had qualitatively identical and quantitatively similar sterol compositions.[41] The major nematode sterols were 24-ethylcholesterol (35–39%), 24-ethylcholestanol (18–24%), 24-methylcholesterol (9–12%), 24-methylcholestanol (8–9%), 24-ethylcholesta-5,22(*E*)-dienol (3–4%), 24-ethylcholest-22(*E*)-enol (2%), cholesterol (6–12%), and cholestanol (3–4%). Because host plant (eggplant) roots were not analyzed and because cholesterol is often a significant component of the sterols of the Solanaceae (the plant family that includes eggplant),[42,43] the existence of dealkylation in *Meloidogyne* spp. could not be evaluated. However, because of the abundance of stanols in these root-knot nematodes, it was concluded that *Meloidogyne* females or eggs saturate the nucleus of phytosterols like *G. tabacum solanacearum* (and subsequently described microbivorous nematodes), although to a lesser extent. In contrast, in a comparison of sterols from resistant and susceptible cotton roots and *M. incognita* eggs, Hedin et al.[44] did not detect stanols. Instead, the major nematode sterols were 22-ethylcholesterol (39%), cholesterol (24%), 22-ethylcholesta-5,22(*E*)-dienol (19%), isofucosterol (12%), and 24-methylcholesterol (5%). Cotton root sterols included 22-ethylcholesterol (30–37%), cholesterol (1.3–1.8%), 22-ethylcholesta-5,22(*E*)-dienol (43–56%), isofucosterol (1.2–3.0%), and 24-methylcholesterol (9–14%).

In a GC-MS study comparing the sterols of a migratory phytoparasitic nematode to those of host plant roots, the sterols of corn root cultures propagated on an agar medium were compared with the root lesion nematode *Pratylenchus agilis.*[45] The major sterols of corn root cultures consisted of 24-ethylcholesta-5,22(*E*)-dienol (60%), 24-methylcholesterol (14%), 24-ethylcholesterol (9%), and isofucosterol (4%). The principal sterols of *P. agilis* included unmetabolized host 24-ethylcholesta- 5,22-dienol (24%) and 24-ethylcholesterol (5%) plus the nuclear-saturated homologues of the four major root sterols, i.e., 24-ethylcholest-22(*E*)-enol (30%), 24-methylcholestanol (9%), 24-ethylcholestanol (11%), and isofucostanol (5%). Thus, existence of Δ^5-bond saturation in this species seems likely. Interestingly, *P. agilis* contained a much lower relative proportion of cholesterol (0.4%) than any other nematode species investigated to date and therefore probably lacks the ability to dealkylate plant sterols. Finally, the presence in *P. agilis* of small quantities (0.3–0.5%) of the 4α-methyl derivatives of its four major sterol metabolic products (i.e., 24-ethylcholest-22(*E*)-enol, 24-methylcholestanol, 24-ethylcholestanol, and isofucostanol) and absence of these compounds in the corn root cultures indicates that this nematode may directly methylate the sterol nucleus. The significance of 4α-methylation is subsequently described for microbivorous nematodes.

V. STEROL METABOLISM IN MICROBIVOROUS NEMATODES

Because many microbivorous nematode species can be easily propagated in sterile media, much has been learned about their sterol metabolism. Many experiments have been performed with dietary sterols of synthetic origin and often with known stereochemistry at C-24. Generally, it has been assumed that the compounds from free-living nematodes possess the same stereochemistry as the supplemented dietary sterols.

In the first investigation of sterol composition in a free-living nematode species, *T. aceti* contained cholesterol, 7-dehydrocholesterol, and lathosterol when cultured in a sterile aqueous medium of yeast extract, soy peptone, acetic acid, and liver extract.[12] When this medium was supplemented with

radiolabeled cholesterol or sitosterol, the 7-dehydrocholesterol from *T. aceti* was radiolabeled, indicating that the nematode metabolized the dietary sterols by introducing a Δ^7-bond (as well as dealkylating the sitosterol at C-24). The Δ^{24}-sterol reductase inhibitor triparanol succinate was not inhibitory to *T. aceti* but did induce an accumulation of desmosterol, as similar inhibitors induce in insects.[40]

Many years later, these experiments were extended to involve a sterile culture medium consisting of solvent-extracted yeast extract, hemoglobin and glucose and unextracted soy peptone and supplemented with a specific dietary sterol in a Tween 80 solution. Solvent extraction minimized the contribution of endogenous sterol contaminants within the medium components. When *C. elegans* was propagated in such a medium supplemented with radiolabeled sitosterol, its major 4-desmethylsterols were 7-dehydrocholesterol (56% of total sterol), cholesterol (8%), lathosterol (6%), and unmetabolized dietary sitosterol (18%).[46] Addition of a known inhibitor of Δ^{24}-sterol reductase in insects, 25-azacoprostane hydrochloride, resulted in over 96% of the total nematode sterol (excluding the dietary sitosterol) consisting of Δ^{24}- or $\Delta^{24(28)}$-sterols such as cholesta-5,7,24-trienol, desmosterol, cholesta-7,24-dienol, and fucosterol (Figure 1). These latter sterols were detected in inhibitor-untreated nematodes in trace quantities at most. The fact that all nematode sterols had the same specific activity as the dietary sitosterol indicated that the compounds were metabolic products of *C. elegans* and that the azacoprostane inhibited the Δ^{24}-sterol reductase of *C. elegans*.

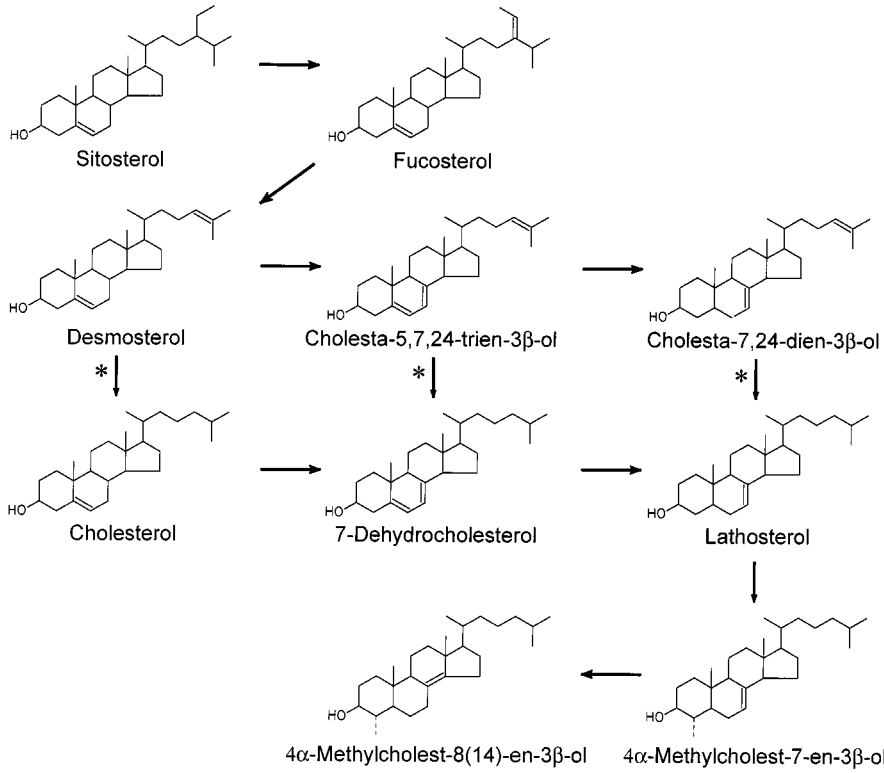

Figure 1 Pathway for metabolism of sitosterol by *Caenorhabditis elegans*. The most abundant nematode sterol is 7-dehydrocholesterol. Sites of inhibition of Δ^{24}-sterol reductase by 25-azacoprostane hydrochloride are marked with asterisks.

These experiments were repeated with *C. elegans* propagated in media supplemented with several C_{27} sterols.[47] This nematode species was able to modify the sterol nucleus in several ways, including C-5 hydrogenation, Δ^7- and $\Delta^{9(11)}$-bond formation, and $\Delta^7 \rightarrow \Delta^{8(14)}$-bond isomerization. Similarly, the absence or near absence of cholesterol in stigmastanol-, cholestanol-, lathosterol-, or 7-dehydrocholesterol-fed *C. elegans* indicated that it could not introduce Δ^5-bonds or hydrogenate Δ^7-bonds.[47,48] The most interesting nuclear transformation of sterols discovered in *C. elegans* was its production of substantial quantities (5–15% of total sterol) of 4α-methylcholest-8(14)-enol radiolabeled with the same specific activity as the original dietary sterol. Because 4α-methylation is a remarkable phenomenon, experiments

were performed to determine if the 4α-methylsterols were artifacts, e.g., addition of antibiotics to medium or incubation of nematode-free medium. All results were consistent with the existence of a pathway for direct 4α-methylation in *C. elegans*. This is a major pathway in which nematodes differ not only from higher animals and plants but also insects.

The production of 4α-methylsterols and other sterols arising from metabolism of sitosterol by *C. elegans* is illustrated in Figure 1. Disappointingly, similar 4α-methylsterols were not detected in the cyst and root-knot nematodes subsequently analyzed,[39,41] although traces were found in *P. agilis*, as previously discussed.[45] Failure to detect any 4α-methylsterols in the cyst and root-knot nematodes could have resulted from either the improved analytical methodology or expertise employed in the subsequent *P. agilis* studies, existence of higher amounts of 4α-methylsterols in vermiform nematodes than in sedentary nematodes or eggs, or true biochemical or phylogenic differences.

Other previously known inhibitors of Δ^{24}-sterol reductase activity in insects were evaluated for their biological and biochemical effects in *C. elegans*. From the perspective of both biological activity and inhibition of Δ^{24}-sterol reductase, several simple aliphatic amines were weaker against *C. elegans* than 25-azacoprostane. Additionally, biological activity and Δ^{24}-sterol reductase inhibition were not correlated. Among the amines and a related amide evaluated (*N,N*-dimethyldodecanamide, *N,N*-dimethyldodecanamine, *N,N*-dimethyltetradecanamine, *N,N*-dimethylhexadecanamine, and *N,N*-dimethyl-3,7,11-trimethyldodecanamine), the latter inhibited Δ^{24}-sterol reductase the greatest yet had the weakest inhibition of nematode growth and reproduction. The amide was as toxic to *C. elegans* as the homologous amine but did not inhibit Δ^{24}-sterol reductase.

Because *N,N*-dimethyldodecanamine was the strongest alkylamine inhibitor of the reductase, this amine was subsequently utilized as a metabolic probe for elucidation of pathways involved in dealkylation of other phytosterols such as campesterol and stigmasterol.[50] This amine inhibited stigmasterol metabolism in *C. elegans* and resulted in the accumulation of a stigmasta-5,22(*E*),24(28)-trienol intermediate. Dealkylation occurred before the Δ^{22}-bond was reduced, and Δ^{22}-bond reduction preceded reduction of the Δ^{24}-bond. In similar experiments with campesterol, the C_{12} amine also induced accumulation of several Δ^{24}-sterols. Because campesterol metabolites with intact side chains but modified nuclei (e.g., 4α-methylsterols or $\Delta^{5,7}$- or Δ^{7}-bond systems) were significant components in both inhibitor-treated and untreated nematodes, the enzymes in *C. elegans* that metabolize the sterol nucleus may exclude 24-ethylsterols but may be somewhat leaky with respect to 24-methylsterols.

Comparative investigations of other microbivorous species in the same taxonomic order as *C. elegans* revealed that *T. aceti* and *P. redivivus* also dealkylate phytosterols.[51–53] These species varied in their metabolism of the sterol nucleus, however. Like *C. elegans*, *T. aceti* contained significant C-7 dehydrogenase activity but differed from *C. elegans* in production of 4α-methylcholestanol and a small quantity of cholestanol. Each of the three species methylated the sterol nucleus. *Panagrellus redivivus* lacked the C-7 dehydrogenase activity present in the other species but instead possessed substantial Δ^{5}-sterol reductase activity, as cholestanol and not 7-dehydrocholesterol was produced in abundance from Δ^{5}-sterols. In addition, lathosterol was the major metabolic product of 7-dehydrocholesterol. The major 4-methylsterol in *P. redivivus* fed Δ^{5}-sterols was 4α-methylcholestanol. Lack of significant C-7 dehydrogenase activity and production of stanols is a feature shared by *P. redivivus* and many plant-parasitic nematodes.

In the sole investigation of the ability of nematodes to degrade the sterol nucleus, neither *T. aceti* nor *C. elegans* converted [^{14}C]cholesterol to [$^{14}CO_2$].[13]

VI. BIOLOGICAL ACTIVITY OF INHIBITORS OF STEROL METABOLISM

Numerous attempts have been made to evaluate the biological activity of various steroids or inhibitors of steroid metabolism upon nematodes. The purpose of these investigations is not only to shed light upon the physiological roles of steroids in nematodes but also to assist in the biorational design of compounds antagonistic towards nematodes. In some experiments, elucidation of the specificity of the observed activity is obscured by the presence of host organisms or tissues. Again, experiments with microbivorous nematode species have assisted the biochemist in precisely assessing the antinematodal activity.

The greatest research attention has probably centered upon the azasteroids and structurally related long-chain alkylamines. As described in the previous section, many of these compounds inhibit Δ^{24}-sterol reductase in *C. elegans*, and the same 5.0 μg/ml concentration of 25-azacoprostane hydrochloride that inhibited enzyme activity strongly inhibited growth and reproduction.[46] The same azasteroid inhibited

growth and egg deposition in *Caenorhabditis briggsae* and *P. redivivus*.[54] When cholesterol was added with the inhibitor, these inhibitory effects still occurred in *C. briggsae* but did not in *P. redivivus.*

The structurally simpler (and thus more inexpensive) aliphatic amines inhibited growth and reproduction in *C. elegans*, but to a lesser extent than 25-azacoprostane.[49] In addition, one of the most conspicuous effects of amine treatment was paralysis or other aberrations in movement. Several aliphatic alkylamines were toxic to *P. redivivus*, and some of these killed root-knot nematode juveniles at 20 µg/ml.[55] A large number of alkylamines were examined for activity against the pinewood nematode, *Bursaphelenchus xylophilus* octadecylamine and *N,N*-dimethyloleylamine were the most active compounds, with effects observed as low as 2.0 µg/ml.[56,57]

Similar experiments have been performed with numerous animal-parasitic nematodes. Seven alkylamines were active against the medium stomach worm of cattle, *Ostertagia ostertagi*, and sublethal concentrations often inhibited molting.[58] Because an anthelmintic did not interfere with nematode molting at sublethal concentrations, the conclusion was that these azasteroids and alkylamines could have antihormonal activity in nematodes. Against *N. brasiliensis* and *N. dubius*, 25-azacoprostane or 25-azacholestane at 10 µg/ml inhibited the development of first-stage to third-stage juveniles, and inhibited juveniles had abnormal anatomical features similar to those in sterol-deprived nematodes.[59] When added to culture medium, cholesterol reversed the azasteroid-induced developmental inhibition. Although these two azasteroids did not affect hatching of these two species, *N,N*-dimethyldodecanamide or *N*-ethyldodecanamine at 10 µg/ml inhibited egg hatching by halting embryogenesis.[60] At 5.0 µg/ml, two azasteroids inhibited microfilarial release by *Brugia pahangi*, and 25-azacoprostane also had macrofilaricidal activity.[61] Several alkylamines had greater activity, as some inhibited microfilarial production at 0.5 µg/ml and were macrofilaricidal at 1.0 µg/ml. An investigation of 50 alkylamines or alkylpyrrolidines revealed that several were toxic at concentrations of 3–6 µg/ml against *A. suum* and the dog hookworm, *Ancylostoma caninum.*[62] Finally, over 100 alkylamines, alkylamides and their derivatives were toxic to juveniles of the dog intestinal roundworm *Toxocara canis*, and activity sometimes occurred as low as 2.0 µg/ml.[63]

The only specific biochemical mode of action of the azasteroids, alkylamines, and amides in nematodes is inhibition of Δ^{24}-sterol reductase. Because these compounds not only inhibit Δ^{24}-sterol reductase but also affect ecdysteroid metabolism in insects, other enzymes involved in nematode steroid metabolism could be affected by these inhibitors. Given that all attempts to demonstrate conversion of radiolabeled cholesterol to steroid hormones by nematodes have been unsuccessful,[2,26,64] discovery of such enzymes may prove difficult. Also, because of the frequently described paralytic effects of the amines and amides, the suggestion has been made that toxicity may result from a direct effect on membranes.[6,63]

VII. ROLE OF STEROLS IN NEMATODE MEMBRANES

Few functional studies of nematode sterols have been performed. One would expect sterols to be located in the plasma membranes of nematode cells, as occurs in other organisms. Only one study to determine the cellular localization of sterols within nematodes has been performed, wherein ultrastructural analysis of *T. aceti* by filipin freeze-fracture electron microscopy revealed filipin-sterol complexes to occur in the vacuolar membranes rather than the plasma membranes.[65] The conclusion was that nematode cells may not possess a specific requirement for sterol as a structural component of membranes, except for cells needed to produce regulatory metabolites of sterols, such as steroid hormones.[65] Perhaps this lack of a major structural role for sterols in nematode membranes is reflected in the small quantities of sterol (0.01–0.06% of dry weight) in many phytoparasitic nematode species.[35–37,39,41,45]

VIII. STEROID HORMONES IN NEMATODES

A. ECDYSTEROIDS

An assumption of many researchers has been that because nematodes and insects molt, molting in nematodes must be governed by the same hormones that regulate molting in insects, i.e., the ecdysteroids. Ecdysteroids have been identified in nematodes through the use of radioimmunoassay or enzyme immunoassay coupled to high performance liquid chromatography,[66–69] and GC-MS has revealed the presence of ecdysone, 20-hydroxyecdysone, and/or 20,26-dihydroxyecdysone in *C. elegans*, *A. suum*, *D. immitis*, the fish parasite *Anasakis simplex*, and the human filarial parasite *Onchocerca volvulus*.[70–74] When injected with radiolabeled ecdysone, the equine parasite *Parascaris equorum* produced radiolabeled ecdysone 25-*O*-β-D-glucopyranoside, ecdysonoic acid, and ecdysone 22-phosphate,[75] the latter

of which also was produced by *C. elegans* cultured in the presence of radiolabeled ecdysone.[71] Although metabolism of ecdysteroids occurs in nematodes, experiments with four species of nematodes in three different laboratories have failed to successfully demonstrate conversion of radiolabeled sterols to ecdysone.[64,74,76] This failure, coupled with the widespread occurrence of ecdysteroids in plants, indicates that it is possible that the ecdysteroids isolated from nematodes could have their origin in the nematode diet, host, or host diet.

Although initial experiments indicated that nematode infections could be diagnosed in mammalian hosts via ecdysteroid analysis of host serum or urine, later research indicated that ecdysteroid-like material occurs in the serum and urine of healthy humans.[77–80] *Parascaris equorum* injected with radiolabeled ecdysone secreted or excreted radiolabeled glucosides of ecdysone and 20-hydroxyecdysone,[75,81] but *C. elegans* cultured in the presence of radiolabeled cholesterol did not secrete radiolabeled ecdysteroids.[64]

Many investigators have applied exogenous ecdysteroids in hope of learning about the physiological function of these compounds in nematodes. The stimulation or inhibition of various developmental processes (including molting, growth, and reproduction) by ecdysteroids has been described in reviews[2,5] and in more recent papers.[82–84] These experiments have usually been interpreted with the proper caution, as true hormones are identified by the application of analytical chemistry to a well-defined bioassay. However, even if ecdysteroids may not be endogenously biosynthesized by nematodes, these compounds may still possess a critically important regulatory function in nematode development.[2]

B. VERTEBRATE STEROIDS AND RECEPTORS

Vertebrate steroid hormones, which typically lack a complete side chain, have been found in several animal-parasitic and microbivorous nematodes. For example, progesterone, testosterone, estrone, and estriol were identified with GC-MS and nuclear magnetic resonance spectrometry (NMR) in samples consisting of mixed sexes of an intestinal parasite from goats, *Trichostrongylus colubriformis*.[26,85] Because the amounts of progesterone and testosterone isolated from nematodes were correlated with the sex of the host, the suggestion was that the nematode obtained the steroids from its host.[26] Although host intestinal contents were devoid of the four steroids, radiolabeled cholesterol was not converted to them by nematode homogenate supernatants. The supernatants did hydroxylate progesterone. In a related study, progesterone, testosterone, and estrone were identified in *Turbatrix aceti* by GC-MS and NMR.[86]

Proteins that bind testosterone, estradiol, and progesterone have been identified in *T. colubriformis* and *N. brasiliensis*.[87,88] In the latter species, several steroid hormones or analogs inhibited the binding.

Experiments involving the biological effects of vertebrate steroids upon nematodes have been reviewed[6] as well as performed more recently.[89,90] Some of these experiments have been performed directly upon nematodes others have involved host organisms. Observations include inhibition or stimulation of reproduction, growth, or molting, inhibition of feeding, embryonation, or movement, and alteration in sex ratio. As with similar experiments evaluating the effects of ecdysteroids upon nematodes, interpretation is often clouded by the presence of host physiological systems, the specificity of the observed responses, the lack of adequate control experiments, or the high concentrations of steroids occasionally used. Nonetheless, the mere observation of such effects has revealed the prospect for exploiting these results in development of novel means for controlling parasitic nematodes.

IX. SUMMARY AND CONCLUSIONS

Compared to other organisms, nematodes have been the subject of relatively few investigations into their steroid biochemistry. Nutritional experiments have clearly demonstrated a dietary requirement for sterol that results from the inability of nematodes to biosynthesize steroids *de novo*. Although the specificity of the nutritional requirement varies somewhat among nematodes, most 4-desmethylsterols can be directly utilized by nematodes or else metabolized to sterols better suited for nematode growth and development.

Much knowledge has been obtained about the abilities of microbivorous nematodes to metabolize sterols. Various species can remove ethyl and methyl substituents at C-24, introduce double bonds at C-7, C-23, and C-24(28), reduce Δ^5-, Δ^{22}-, and Δ^{24}-bonds, isomerize Δ^7- to $\Delta^{8(14)}$-bonds, esterify fatty acids at C-3, and directly methylate the sterol nucleus at C-4 by a process unique to nematodes. Moreover, azasteroids and structurally related alkylamines and alkylamides inhibit the activity of nematode Δ^{24}-sterol reductase and also disrupt the life cycles of these species as well as parasitic species. Less is known, however, about sterol metabolism in parasitic nematodes because of the inability to culture them apart

from their hosts. Most plant-parasitic nematodes appear to dealkylate plant sterols at C-24 and modify the sterol nucleus, particularly by reduction of Δ^5-bonds. Sterol metabolism in mammalian parasites has been studied to a very limited extent only.

Except for a structural presence of sterols in membranes of nematodes, the function of these compounds in nematodes is poorly understood. Although nematodes do contain steroids that have hormonal functions in other organisms, attempts to demonstrate endogenous biosynthesis of these steroids by nematodes have been unsuccessful and elucidation of the function of these compounds within nematodes warrants immediate investigation. Other areas of nematode steroid biochemistry requiring further research include investigation of the metabolism of sterols by vertebrate-parasitic nematodes, the purification and characterization of enzymes involved in nematode steroid metabolism, evaluation of a broader array of sterol biosynthesis inhibitors upon nematodes, and elucidation of the specific metabolic steps and the function of the 4-methylation pathway. Because *C. elegans* will be the first animal whose entire genome is sequenced, additional questions will be raised by the discovery of genes with sequences similar to those for steroid biosynthetic enzymes or receptors. All of these research fronts could accelerate the development of novel means for controlling parasitic nematodes.

REFERENCES

1. **Cobb, N. A.,** Nematodes and their relationships, in *Yearbook of Agriculture for 1914*, Government Printing Office, Washington, D.C., 1915, 457.
2. **Barker, G. C., Chitwood, D. J., and Rees, H. H.,** Ecdysteroids in helminths and annelids, *Invert. Reprod. Dev.*, 18, 1, 1990.
3. **Barker, G. C., Mercer, J. G., Delves, C. J., O'Hanlon, G. M., Howells, R. E., and Rees, H. H.,** Occurrence and possible biochemical significance of developmental hormones in nematodes, in *Progress in Comparative Endocrinology*, Epple, A., Scanes, G. C., and Stetson, M. H., Eds., Wiley-Liss, New York, 1990, 710.
4. **Bolla, R.,** Developmental nutrition of nematodes: the biochemical role of sterols, heme compounds, and lysosomal enzymes, *J. Nematol*., 11, 250, 1979.
5. **Chitwood, D. J.,** Inhibition of steroid or hormone action in nematodes, in *Vistas on Nematology*, Veech, J. A. and Dickson, D. W., Eds., Society of Nematologists, Hyattsville, MD, 1987, 122.
6. **Chitwood, D. J.,** Nematode sterol biochemistry, in *Physiology and Biochemistry of Sterols*, Patterson, G. W. and Nes, W. D., Eds., American Oil Chemists' Society, Champaign, IL, 1991, 257.
7. **Chitwood, D. J. and Lusby, W. R.,** Metabolism of plant sterols by nematodes, *Lipids*, 26, 619, 1991.
8. **Davey, K. G.,** Endocrinology of nematodes, in *Endocrinology of Selected Invertebrate Types*, Laufer, H., Ed., Alan R. Liss, New York, 1988, 63.
9. **Rees, H. H. and Mercer, J. G.,** Occurrence and fate of parasitic helminth ecdysteroids, in *Advances in Invertebrate Reproduction, Vol.4*, Porchet, M., Andries, J. C., and Dhainaut, A., Eds., Elsevier Science Publishers, Amsterdam, 1986, 173.
10. **Dutky, S. R., Robbins, W. E., and Thompson, J. V.,** The demonstration of sterols as requirements for the growth, development, and reproduction of the DD-136 nematode, *Nematologica*, 13, 140, 1967.
11. **Hieb, W. F. and Rothstein, M.,** Sterol requirement for reproduction of a free-living nematode, *Science*, 160, 778, 1968.
12. **Cole, R. J. and Krusberg, L. R.,** Sterol metabolism in *Turbatrix aceti*, *Life Sci.*, 7, 713, 1967.
13. **Rothstein, M.,** Lack of sterol biosynthesis in free-living nematodes, *Comp. Biochem. Physiol*., 27, 309, 1972.
14. **Ritter, K. S.,** *Steinernema feltiae* (= *Neoaplectana carpocapsae*): effect of sterols and hypolipidemic agents on development, *Exp. Parasitol.*, 67, 257, 1988.
15. **Cole, R. J. and Dutky, S. R.,** A sterol requirement in *Turbatrix aceti* and *Panagrellus redivivus*, *J. Nematol*., 1, 72, 1969.
16. **Bolla, R. I., Weinstein, P. P., and Lou, C.,** In vitro nutritional requirements of *Nippostrongylus brasiliensis*. I. Effects of sterols, sterol derivatives, and heme compounds on the free-living stages, *Comp. Biochem. Physiol.*, 43B, 487, 1972.
17. **Chitwood, D. J., Lozano, R., Lusby, W. R., Thompson, M. J., and Svoboda, J. A.,** Metabolism and function of sterols in nematodes, in *Ecology and Metabolism of Plant Lipids*, Fuller, G. and Nes, W. D., Eds., American Chemical Society, Washington, D.C., 1986, 200.
18. **Willett, J. D. and Downey, W. L.,** Sterol biosynthesis in the free-living nematode, *Panagrellus redivivus*, *Biochem. J*., 138, 233, 1974.
19. **Lu, N. C., Newton, C., and Stokstad, E. L. R.,** The requirement of sterol and various sterol precursors in free-living nematodes, *Nematologica*, 23, 57, 1977.
20. **Cole, R. J. and Krusberg, L. R.,** Sterols in *Ascaris lumbricoides*, *Comp. Biochem. Physiol*., 21, 109, 1967.
21. **Aisien, S. O. and Oneyeari, F.,** Comparative studies on the lipid composition of some parasitic nematodes of the agama lizard, *Int. J. Parasitol*., 18, 553, 1988.

22. **Barrett, J.,** Lipids of the infective and parasitic stages of some nematodes, *Nature*, 218, 1267, 1968.
23. **Barrett, J.,** The effect of ageing on the metabolism of the infective larvae of *Strongyloides ratti* Sandground, *Parasitology*, 59, 3, 1969.
24. **Ansari, A. A., Yusufi, A. N. K., and Siddiqi, M.,** Lipid composition of the filarial parasite *Setaria cervi*, *J. Parasitol*., 59, 939, 1973.
25. **Chatterjee, S. K. and Majumdar, G.,** Cholesterol and fatty acid values in *Stongyluris bengalensis* (Nematoda: Heterakidae), *Riv. Parassitol*., 42, 159, 1981.
26. **Chung, W.-L., Parish, E. J., and Bone, L. W.,** Sex steroid content and metabolism in *Trichostrongylus colubriformis* (Nematoda), *J. Parasitol*., 72, 326, 1986.
27. **Castro, G. A. and Fairbairn, D.,** Carbohydrates and lipids in *Trichinella spiralis* larvae and their utilization in vitro, *J. Parasitol*., 55, 51, 1969.
28. **Barrett, J., Cain, G. D., and Fairbairn, D.,** Sterols in *Ascaris lumbricoides* (Nematoda), *Macracanthorhynchus hirudinaceus* and *Moniliformis dubius* (Acanthocephala), and *Echinostoma revolutum* (Trematoda), *J. Parasitol*., 56, 1004, 1970.
29. **Tarr, G. E. and Fairbairn, D.,** Glycerides, waxes and sterols in ovaries of *Ascaris lumbricoides* (Nematoda), *Lipids*, 8, 303, 1973.
30. **Fulk, W. K. and Shorb, M. S.,** Lipids of male and female *Ascaridia galli*, *J. Parasitol*., 57, 840, 1971.
31. **Beames, C. G., Bailey, H. H., Rock, C. O., and Schanbacher, L. M.,** Movement of cholesterol and β-sitosterol across the intestine of *Ascaris suum*, *Comp. Biochem. Physiol*., 47A, 881, 1974.
32. **Fleming, M. W. and Fetterer, R. H.,** *Ascaris suum:* continuous perfusion of the pseudocoelom and nutrient absorption, *Exp. Parasitol*., 57, 142, 1984.
33. **Dutky, S. R., Kaplanis, J. N., Thompson, M. J., and Robbins, W. E.,** The isolation and identification of the sterols of the DD-136 insect parasitic nematode and their derivation from its insect hosts, Nematologica, 13, 139, 1967.
34. **Morrison, A. H. and Ritter, K. S.,** Effect of host insect sterols on the development and sterol composition of *Steinernema feltiae*, *Mol. Biochem. Parasitol.*, 19, 135, 1986.
35. **Cole, R. J. and Krusberg, L. R.,** Sterol composition of the nematodes *Ditylenchus triformis* and *Ditylenchus dipsaci*, *Exp. Parasitol.*, 21, 22, 1967.
36. **Svoboda, J. A. and Rebois, R. V.,** Sterol composition of *Rotylenchulus reniformis*, *J. Nematol.*, 9, 286, 1977.
37. **Orcutt, D. M., Fox, J. A., and Jake, C. A.,** The sterol, fatty acid, and hydrocarbon composition of *Globodera solanacearum*, *J. Nematol.*, 10, 264, 1978.
38. **Chitwood, D. J. and Lusby, W. R.,** Metabolism of plant sterols by nematodes, *Lipids*, 26, 619, 1991.
39. **Chitwood, D. J., Hutzell, P. A., and Lusby, W. R.,** Sterol composition of the corn cyst nematode, *Heterodera zeae*, and corn roots, *J. Nematol.*, 17, 64, 1985.
40. **Svoboda, J. A. and Thompson, M. J.,** Steroids, in *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Kerkut, G. A., and Gilbert, L. I., Eds., Pergamon, Oxford, 1985, 137.
41. **Chitwood, D. J., McClure, M. A., Feldlaufer, M. F., Lusby, W. R., and Oliver, J. E.,** Sterol composition and ecdysteroid content of eggs of the root-knot nematodes *Meloidogyne incognita* and *M. arenaria*, *J. Nematol.*, 19, 352, 1987.
42. **Itoh, T., Tamura, T., and Matsumoto, T.,** 4-Desmethylsterols in the seeds of Solanaceae, *Steroids*, 30, 425, 1977.
43. **Tso, T. C., Cheng, A. L. S., Sorokin, T., and Tso, E.,** Phytosterols and polyphenols in reciprocally grafted tobacco-tomato plants, *Phytochemistry*, 13, 1667, 1974.
44. **Hedin, P. A., Calahan, F. E., Dollar, D. A., and Creech, R. G.,** Total sterols in root-knot nematode *Meloidogyne incognita* infected cotton *Gossypium hirsutum* (L.) plant roots, *Comp. Biochem. Physiol.*, 111B, 447, 1995.
45. **Chitwood, D. J. and Lusby, W. R.,** Sterol composition of the corn root lesion nematode, *Pratylenchus agilis*, and corn root cultures, *J. Helminthol. Soc. Wash.*, 58, 43, 1991.
46. **Chitwood, D. J., Lusby, W. R., Lozano, R., Thompson, M. J., and Svoboda, J. A.,** Sterol metabolism in the nematode *Caenorhabditis elegans*, *Lipids*, 19, 500, 1984.
47. **Lozano, R., Salt, T. A., Chitwood, D. J., Lusby, W. R., and Thompson, M. J.,** Metabolism of sterols of varying ring unsaturation and methylation by *Caenorhabditis elegans*, *Lipids*, 22, 84, 1987.
48. **Lozano, R., Lusby, W. R., Chitwood, D. J., and Svoboda, J. A.,** Dealkylation of various 24-alkylsterols by the nematode *Caenorhabditis elegans*, *Lipids*, 20, 102, 1985.
49. **Lozano, R., Chitwood, D. J., Lusby, W. R., Thompson, M. J., Svoboda, J. A., and Patterson, G. W.,** Comparative effects of growth inhibitors on sterol metabolism in the nematode *Caenorhabditis elegans*, *Comp. Biochem. Physiol*., 79C, 21, 1984.
50. **Lozano, R., Lusby, W. R., Chitwood, D. J., Thompson, M. J., and Svoboda, J. A.,** Inhibition of C_{28} and C_{29} phytosterol metabolism by *N,N*-dimethyldodecanamine in the nematode *Caenorhabditis elegans*, *Lipids*, 20, 158, 1985.
51. **Chitwood, D. J., Lusby, W. R., and Salt, T. A.,** Sterol metabolism in the nematodes *Panagrellus redivivus*, *Turbatrix aceti* and *Caenorhabditis elegans*, *Comp. Biochem. Physiol*., 86B, 103, 1987.
52. **Salt, T. A., Lozano, R., Lusby, W. R., Chitwood, D. J., and Thompson, M. J.,** 24-Methyl-23-dehydrocholesterol: a new sterol intermediate in C-24 demethylation from the nematodes *Panagrellus redivivus* and *Caenorhabditis elegans*, *Steroids*, 48, 451, 1986.

53. **Salt, T. A., Chitwood, D. J., and Lusby, W. R.,** Sterol metabolism in the nematode *Panagrellus redivivus*, *Lipids*, 24, 325, 1989.
54. **Bottjer, K. P., Weinstein, P. P., and Thompson, M. J.,** Effects of an azasteroid on growth, development and reproduction of the free-living nematodes *Caenorhabditis briggsae* and *Panagrellus redivivus*, *Comp. Biochem. Physiol.*, 82B, 99, 1985.
55. **Feldmesser, J., Thompson, M. J., Robbins, W. E., and Sponaugle, R. P.,** Nematicidal activity of secondary and tertiary alkyl amides and amines. *Experientia*, 32, 466, 1976.
56. **Nagase, A., Kuwahara, Y., Tominaga, Y., and Sugawara, R.,** Nematicidal activity of alkylamine against the pine wood nematode, *Bursaphelenchus lignicolus*, *Agric. Biol. Chem.*, 46, 167, 1982.
57. **Nagase, A., Kuwahara, Y., Tominaga, Y., and Sugawara, R.,** Nematicidal activity of *N*-substituted and *N*,*N*-disubstituted alkylamines against the pine wood nematode, *Bursaphelenchus xylophilus*, *Agric. Biol. Chem.*, 47, 53, 1983.
58. **Douvres, F. W., Thompson, M. J., and Robbins, W. E.,** In vitro cultivation of *Ostertagia ostertagi*, the medium stomach worm of cattle: effect of insect-growth-disrupting amines and amides on development, *Vet. Parasitol*., 7, 195, 1980.
59. **Bottjer, K. P., Weinstein, P. P., and Thompson, M. J.,** Effects of azasteroids on growth and development of the free-living stages of *Nippostrongylus brasiliensis* and *Nematospiroides dubius*, *Comp. Biochem. Physiol.*, 78B, 805, 1984.
60. **Bottjer, K. P., Weinstein, P. P., and Thompson, M. J.,** Effect of insect growth inhibitors upon hatching of the nematodes *Nippostrongylus brasiliensis* and *Nematospiroides dubius*, *Int. J. Invertebr. Reprod. Dev.*, 11, 235, 1987.
61. **Barker, G. C., Mercer, J. G., Svoboda, J. A., Thompson, M. J., Rees, H. H., and Howells, R. E.,** Effects of potential inhibitors on *Brugia pahangi* in vitro: macrofilaricidal action and inhibition of microfilarial production, *Parasitology*, 99, 409, 1989.
62. **Kunitomo, M. and Nagakura, T.,** On the antiparasitic effects of long-chain alkylamine compounds, *Yakugaku Zasshi*, 91, 198, 1972.
63. **Kiuchi, F., Nishizawa, S., Kawanishi, H., Kinoshita, S., Ohsima, H., Uchitani, A., Sekino, N., Ishida, M., Kondo, K., and Tsuda, Y.,** Nematocidal activity of long alkyl chain amides, amines, and their derivatives on dog roundworm larvae, *Chem. Pharm. Bull.*, 40, 3234, 1992.
64. **Chitwood, D. J. and Feldlaufer, M. F.,** Ecdysteroids in axenically propagated *Caenorhabditis elegans* and culture medium, *J. Nematol.*, 22, 598, 1990.
65. **Silberkang, M., Havel, C. M., Friend, D. S., McCarthy, B. J., and Watson, J. A.,** Isoprene synthesis in isolated embryonic *Drosophila* cells. I. Sterol-deficient eukaryotic cells, *J. Biol. Chem.*, 258, 8503, 1983.
66. **Fleming, M. W.,** *Ascaris suum:* role of ecdysteroids in molting, *Exp. Parasitol.*, 60, 207, 1985.
67. **Fleming, M. W.,** Ecdysteroids during embryonation of eggs of *Ascaris suum*, *Comp. Biochem. Physiol*., 87A, 803, 1987.
68. **Fleming, M. W.,** Ecdysteroids during development in the ovine parasitic nematode, *Haemonchus contortus*, *Comp. Biochem. Physiol*., 104B, 803, 1987.
69. **Nembo, B., Duie, P., Garcia, M., Breton, P., Gayral, P., Porcheron, P., and Goudey-Perriere, F.,** Levels of ecdysteroid-like material in adults of *Nippostrongylus brasiliensis* during the intestinal phase, *J. Helminthol*., 67, 305, 1993.
70. **Mendis, A. H. W., Rose, M. E., Rees, H. H., and Goodwin, T. W.,** Ecdysteroids in adults of the nematode, *Dirofilaria immitis*, *Mol. Biochem. Parasitol.*, 9, 209, 1983.
71. **Mercer, J. G., Munn, A. E., and Rees, H. H.,** *Caenorhabditis elegans:* occurrence and metabolism of ecdysteroids in adults and dauer larvae, *Comp. Biochem. Physiol.*, 90B, 261, 1988.
72. **Cleator, M., Delves, C. J., Howells, R. E., and Rees, H. H.,** Identity and tissue localization of free and conjugated ecdysteroids in adults of *Dirofilaria immitis* and *Ascaris lumbricoides*, *Mol. Biochem. Parasitol.*, 27, 93, 1988.
73. **Evershed, R. P., Mercer, J. G., and Rees, H. H.,** Capillary gas chromatography-mass spectrometry of ecdysteroids, *J. Chromatogr*., 390, 357, 1987.
74. **Mercer, J. G., Barker, G. C., Howells, R. E., and Rees, H. H.,** Analysis of ecdysteroids in *Onchocerca gibsoni*, *O. volvulus* and nodule tissues, *Trop. Med. Parasitol.*, 40, 434, 1989.
75. **O'Hanlon, G. M., Cleator, M., Mercer, J. G., Howells, R. E., and Rees, H. H.,** Metabolism and fate of ecdysteroids in the nematodes *Ascaris suum* and *Parascaris equorum*, *Mol. Biochem. Parasitol.*, 47, 179, 1991.
76. **Koolman, J., Walter, J., and Zahner, H.,** Ecdysteroids in helminths, in *Biosynthesis, Metabolism, and Mode of Action of Invertebrate Hormones*, Hoffmann, J. and Porchet, M., Eds., Springer-Verlag, Berlin, 1984, 323.
77. **Koolman, J. and Moeller, H.,** Diagnosis of major helminth infections by RIA detection of ecdysteroids in urine and serum, *Insect Biochem.*, 16, 287, 1984.
78. **Simon, P. and Koolman, J.,** Ecdysteroids in vertebrates: pharmacological aspects, in *Ecdysone*, Koolman, J., Ed., Georg Thieme Verlag, Stuttgart, 1989, 254.
79. **Landsoud-Soukate, L., Gharib, B., Baswaid, S., Capron, A., and de Reggi, M.,** Ecdysteroid-like compounds in the serum and urine of African patients infected with *Loa loa* and *Mansonella perstans* microfilariae, *Trans R. Soc. Trop. Med. Hyg.*, 84, 269, 1990.
80. **Gharib, B., Baswaid, S., Quilici, M., and de Reggi, M.,** Ecdysteroid-like compounds in human urine: they can occur in the absence of any parasitic infection, *Clin. Chim. Acta*, 199, 159, 1991.

81. **O'Hanlon, G. M., Mercer, J. G., and Rees, H. H.,** Evidence for the release of 20-hydroxyecdysone 25-glucoside by adult *Parascaris equorum* (Nematoda) in vitro, *Parasitol. Res.*, 77, 271, 1991.
82. **Barker, G. C., Mercer, J. G., Rees, H. H., and Howells, R. E.,** The effect of ecdysteroids on the microfilarial production of *Brugia pahangi* and the control of meiotic reinitiation in the oocytes of *Dirofilaria immitis*, *Parasitol. Res.*, 77, 65, 1991.
83. **Goudey-Perriere, F., Simo, B. F., Maccario, J., Perriere, C., and Gayral, P.,** Effects of ecdysteroids on reproductive physiology of *Nippostrongylus brasiliensis* (Nematoda) in vivo, *Comp. Biochem. Physiol.*, 103C, 105, 1992.
84. **Warbrick, E. V., Barker, G. C., Rees, H. H., and Howells, R. E.,** The effect of invertebrate hormones and potential hormone inhibitors on the third larval moult of the filarial nematode, *Dirofilaria immitis*, in vitro, *Parasitology*, 107, 459, 1993.
85. **Lee, H.-M., Parish, E. J., and Bone, L. W.,** The occurrence of estrone and estriol in *Trichostrongylus colubriformis* (Nematoda), *Lipids*, 24, 903, 1989.
86. **Lee, H.-M., Parish, E. J., and Bone, L. W.,** Occurrence of mammalian sex steroids in the free-living nematode, *Turbatrix aceti*, *Comp. Biochem. Physiol.*, 97A, 115, 1990.
87. **Kiser, C. S., Parish, E. J., and Bone, L. W.,** Binding of steroidal sex hormones by supernatant from *Trichostrongylus colubriformis* (Nematoda), *Comp. Biochem. Physiol.*, 83B, 787, 1986.
88. **Majundar, T. K., Parish, E. J., and Bone, L. W.,** Steroid hormone analogs inhibit hormone binding by an extract from *Nippostrongylus brasiliensis* larvae, *Comp. Biochem. Physiol.*, 88B, 81, 1987.
89. **Richardson, T. L. and MacKinnon, B. M.,** *Heligmosoides polygyrus:* effect of exogenous steroid hormones on egg output in vitro, *J. Helminthol.*, 64, 123, 1990.
90. **Harder, A., Wunderlich, F., and Marinovski, P.,** Effects of testosterone on *Heterakis spumosa* infections in mice, *Parasitology*, 105, 335, 1992.