Mermithid Nematodes: Physiological Relationships with their Insect Hosts[1]

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Abstract: This paper assesses our state of knowledge of physiological processes involved in the relationships between insects and their mermithid nematode parasites. Three major components of the host-parasite relationship(s) are reviewed: effects of mermithids on host physiology, effects of host physiology on mermithids, and the physiology of the nematodes themselves. Mermithids induce an array of changes in host physiology, and the effects on host metabolism and endocrinology are discussed at some length. Few studies have been done to ascertain the effects of the host on the parasites from a physiological standpoint. Whereas host immunity mechanisms against mermithids have been described at the ultrastructural level, the physiological basis of such responses is not known. Mermithids are atypical nematodes, both structurally and physiologically. In the absence of a functional gut, nutrients are absorbed across the outer cuticle and stored in a trophosome. The transcuticular mode of feeding, storage within the trophosome, and metabolism of storage products are discussed. The usefulness of physiological information toward expediting in vitro culture of these nematodes is discussed, and problems that need to be addressed are defined. *Key words:* cuticle, fat body, *Filipjevimermis leipsandra, Gastromermis boophthorae,* hemolymph, immunity, *Mermis nigrescens,* mermithid nematode, *Neomesomermis flumenalis, Romanomermis culicivorax,* trophosome.

Mermithid nematodes (Enoplida: Mermithidae) have considerable potential for biocontrol of insect pests. The insect host invariably dies when the juvenile nematode completes its parasitic development and exits from the host's hemocoel (the parasite's microenvironment). When adult female insects are infected, they are rendered sterile. The potential of mermithids for insect biocontrol, however, has yet to be realized. Only one species of mermithid, the mosquito parasite *Romanomermis culicivorax,* has been mass cultivated on the scale needed to permit field trials and for this an in vivo procedure was used (29). Several authorities (28,46) have advocated the establishment of in vitro cultivation procedures because they are potentially cheaper, more efficient, and easier to maintain than in vivo methods. However, attempts to culture mermithids in vitro (8) have met with limited success.

The paucity of information about the

Received for publication 9 February 1981.

[1]Symposium paper presented at the annual meeting of the Society of Nematologists, New Orleans, Louisiana, August 1980.

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Continuing financial support from the Natural Sciences and Engineering Research Council of Canada (Grant No. A6679) is gratefully acknowledged.

physiological interrelationships between insects and mermithids has hampered progress of in vitro culture of mermithids. Mermithids are atypical nematodes. They lack a functional gut and absorb nutrients from the host's hemolymph through their outer cuticle. The parasitic juveniles store these nutrients in a storage organ (trophosome) that almost completely fills the pseudocoelom. Storage reserves are utilized by the free-living stages which feed only minimally or not at all. Such adaptations suggest that mermithids are deeply committed to parasitism and rely heavily upon their host to provide nutritional requirements and, in all probability, stimuli for growth and development. For in vitro mermithid culture to be effective, these nutrients and stimuli have to be identified and incorporated into the culture media.

Severe overt effects of parasitism indicate the degree to which the physiology of the host is affected. Mermithids cause either gross degeneration or suppress the development of a number of host tissues (9) which in the normal insect fulfill vital metabolic roles. Recent studies by Wülker (49,50) have significantly advanced our understanding of the cytological changes that occur in the imaginal discs and gonads of chironomids (Diptera) as a result of mermithid parasitism. Mermithids induce a greater variety of developmental alterations of their hosts than do other entomogenous nematodes. They prevent molting and, in some instances, induce intersexuality (19,49), gynandromorphism (19), or formation of intercastes (26).

In 1927 Cobb et al. (4), intrigued by the discovery that the sex of mermithids is determined by factors inherent in the level of parasitism, called for physiological studies on the host-parasite relationships. However, little attention was paid to such physiological considerations until recently. Two parasites have constituted the primary focus for physiological studies: *Mermis nigrescens,* parasitic in grasshoppers and locusts, and *R. culicivorax,* parasitic in larval mosquitoes.

MERMITHIDS ON HOST PHYSIOLOGY

In 1940 Rempel (36) surmised from histological examinations of chironomids: "Very little or no food is abstracted from the haemolymph of the host, for in the parasitized forms there is always an increase, instead of a decrease, in the lipoid content of the blood." Rempel's contention that the hemolymph does not constitute the primary source of nutrients for the developing mermithid has since been shown to be incorrect. Whether or not blood lipid levels really were elevated cannot be ascertained from his study, but such an increase would not discount the hemolymph as a nutrient source.

Among mermithids, as among nematodes in general, there is considerable uniformity in morphology and general pattern of life cycle. It would seem reasonable to suggest that information obtained concerning the physiology of one species would, in principle, be applicable to the family as a whole. The same conclusions may not be drawn, however, when considering the physiology of hosts that are so distantly related as orthoptera and diptera. In the ensuing discussion, therefore, effects of mermithid infection on the two types of host are considered separately.

M. nigrescens in locusts: Jutsum and Goldsworthy (22) reported that relatively low infections (ca. three worms per host) of *M. nigrescens* caused pronounced reductions in the levels of proteins within the blood and fat body of adult male *Locusta migratoria.*

The physiological condition of the host influences the degree to which hemolymph and tissue metabolite levels are affected by infection. Electropherograms of hemolymph taken from adult female *Schistocerca gregaria* 3 wk after infection with heavy doses of *M. nigrescens* (40–50 worms per host) showed that almost all of the protein fractions were depleted through parasitism (12). These locusts had been infected soon after their imaginal molt, and the parasite prevented vitellogenesis. But in a study where infection was delayed until vitellogenesis commenced, the mermithid did not affect overall hemolymph protein levels (9). The initiation of infection was delayed in these experiments, so the parasitized host commenced vitellogenesis, but the yolk was subsequently resorbed into the hemolymph.

Such a resorption of vitellogenic proteins likely compensated for the reduction in hemolymph (protein) levels that would otherwise have occurred. The mermithid was found to cause a significant depletion of hemolymph proteins in fifth-instar *S. gregaria* (infected as fourth instars) as the nematode embarked upon its rapid growth phase (7). *Mermis nigrescens* modified protein turnover in *S. gregaria* in a stepwise fashion. The electrophoretic pattern of fat body soluble proteins was altered prior to comparable alterations in hemolymph protein fractions (12). Compared to noninfected control insects, proteins in fat bodies of parasitized insects were found to be depleted at 2 wk after infection; corresponding reductions in proteins were not recorded from the hemolymph until 1 wk later. The 3d wk of infection represented a period of maintenance and reconstitution of fat body soluble proteins, but corresponding effects were not found for hemolymph proteins until the 4th wk of infection.

Gordon and Webster (9) found that the overall level of amino acids in the hemolymph of adult female *S. gregaria* was not affected by mermithid parasitism, but the content of amino acids within the fat body was significantly reduced. In male hosts, Rutherford and Webster (41) recorded a doubling of hemolymph amino nitrogen levels toward the end of the period of the parasite's development as a result of significant increases in the levels of 11 amino acids. The finding that the hemolymph of infected insects was not depleted of amino acids led to the suggestion that the mermithid obtains dietary protein nitrogen by stimulating protein catabolism by the host fat body, thereby releasing amino acids into the hemolymph and bringing about the observed reductions in fat body proteins (12). Equally plausible hypotheses were advanced by Rutherford and Webster (41), who suggested that amino acids could accumulate in the hemolymph as a result of the host's impaired excretory capacity, via excretion of amino acids by the nematode and/or by secretion of an amino acid analog that inhibited the host's amino acid transport systems. The last hypothesis would explain the impaired capacity of fat bodies of parasitized insects to synthesize proteins (7), because the fat body tissue would be deprived of amino acid precursors. Though the mechanisms involved are matters for speculation, there would appear to be sufficient evidence to support the proposal that *M. nigrescens* acquires amino acids by inhibiting protein synthesis in addition to stimulating protein catabolism within the insect's fat body (7).

Parasitism of *L. migratoria* or *S. gregaria* by *M. nigrescens* is characterized by a drastic reduction in the total level of hemolymph carbohydrates (9,22). In *S. gregaria* the depletive effect of parasitism on blood carbohydrates is caused by a dramatic reduction in hemolymph trehalose levels that coincides with the nematode's rapid growth phase (41). The mermithid is able to utilize glucose, but not trehalose, and must, therefore, modify host carbohydrate metabolism to favor the production of glucose. Gordon et al. (10) showed that the fat bodies of infected locusts contained approximately half the concentration of glycogen as did those of controls, but fat body glycogen levels did not change during the 3rd wk of infection when the nematode grew most rapidly and completed parasitic development. The parasitism caused a progressive and total depletion of glycogen phosphorylases in the host fat body (10), an effect which blocks glycogenolysis and accounts for the low, but stable, glycogen levels. Fat body glycogen is not a nutrient source for the developing nematode and is depleted in parasitized locusts because its synthesis is impaired (41). Rutherford and Webster (41) found that trehalose was synthesized from injected glucose at a faster rate by infected insects than by controls; they suggested that the nematode acquires dietary glucose by stimulating the host fat body to hydrolyze trehalose.

The total level (22) and fatty acid composition (41) of hemolymph lipids in locusts are unaffected by mermithid parasitism, but hemolymph levels of the sterols cholesterol and chloestanol appear to be increased (40). The mermithid reduces the host's capacity for lipid mobilization, thereby rendering prolonged flight inefficient or impossible (22).

Excretion was not inhibited in lightly infected *L. migratoria* (22), but was impaired in heavily infected *S. gregaria* (9).

The hemolymph of infected hosts contained more than five times the concentration of uric acid found in controls, while the concentration of fecal uric acid was reduced to one-quarter of the control level in parasitized insects (5). Accumulation of uric acid within the hemolymph likely results from the host's reduced excretory capacity and, possibly, the postulated increased catabolism of fat body proteins.

Insects normally control such processes as protein metabolism, carbohydrate metabolism, lipid metabolism, and excretion via their endocrine system (18). Since these physiological processes are disrupted through mermithid parasitism, one might reasonably expect to find an altered hormonal status in the parasitized host. However, there is no direct evidence of such endocrinological changes in locusts. Jutsum and Goldsworthy (22) noted that sexual maturation of male locusts was not affected by parasitism and reasoned that the activity of the corpora allata must be unaffected also. These authors provided evidence that *M. nigrescens* does not interfere with the release of adipokinetic hormone from the host's corpora cardiaca. In response to injections of corpora cardiaca extracts, parasitized insects mobilized lipids to a lesser degree than did controls, suggesting that the mermithid interferes with lipid mobilization by reducing the response of the fat body to the hormone. Craig and Webster (7) found that ecdysone levels in locusts were unaffected by *M. nigrescens* parasitism, and they attributed the inhibition of molting in parasitized insects to depletion by the nematode of precursors required by the host for protein and cuticle synthesis. However, the fact that the ovaries of parasitized locusts were unable to sequester vitellogenic proteins available within the hemolymph (12), a process also endocrinologically controlled (18), suggests endocrine dysfunction in the host. Considering the degree to which *M. nigrescens* affects a variety of physiological and developmental processes in the host that are normally under endocrine control, it would be surprising, indeed, if the host's endocrinology were not disrupted. The parasite may secrete substances that directly affect host endocrine activity. More likely, nematode feeding activity would induce endocrinological disturbances in the host indirectly by disturbing the various hormonal feedback systems that serve to regulate metabolite levels.

Mermithids in larval diptera: Due to limitations imposed by the small size of the host in obtaining samples for analyses, physiological information on the effects of mermithids on larval diptera is less readily obtained than for grasshoppers and locusts. Studies have utilized "pooled" tissue or hemolymph samples collected from large numbers of insects.

Romanomermis culicivorax was found to deplete hemolymph proteins of *Culex pipiens* to one-sixth of the control levels (43). Using polyacrylamide gel electrophoresis, Gordon et al. (13) showed a similar exhaustive effect of mermithid *(Neomesomermis flumenalis)* parasitism on the hemolymph proteins of the blackflies *Prosimulium mixtum/fuscum* and *Simulium venustum* and, as in *C. pipiens* (43), the reduction affected all detected protein fractions. Wülker (48), on the other hand, recorded no differences in proteins between the paper electropherograms of the blood of mermithid-infected and uninfected chironomid larvae.

Mermithid parasites of larval diptera probably resemble *M. nigrescens* in obtaining dietary amino acids by stimulating the catabolism of proteins within the host fat body. Total amino acid levels in the hemolymph of parasitized *C. pipiens* larvae were found to be the same as in controls when the hosts were reared at pH 4.5, but were inexplicably reduced compared to controls when the rearing pH was raised to pH 7.4 (43). Studies on mermithid parasitized chironomids (24) indicated that levels of several hemolymph amino acids were increased substantially as a result of parasitism, while only a few amino acids decreased in concentration. In the simuliid *S. venustum* 14 of 32 ninhydrin positive substances detected in the hemolymph were found to be increased by parasitism, 10 substances decreased in concentration, and 8 substances were unaffected (13). The same study showed, by contrast, that mermithid parasitism in the simuliid *P. mixtum/fuscum* is characterized by a decrease in the majority of the hemolymph amino com-

pounds. Thus, as in locusts, mermithids do not appear to cause a general drain on the hemolymph amino acid pool of larval diptera; in many instances, individual and total amino acid levels were found to be unaffected or increased by parasitism. The concept outlined above to explain the manner by which the host fat body could provide *M. nigrescens* with amino acids fits the data available for these mermithids. In addition to the fat body, other tissues break down in parasitized *Aedes aegypti* (1) and undoubtedly provide nutriment for the developing nematode.

Wülker (49) noted that the hemolymph carbohydrate level was diminished in chironomids by mermithid parasitism. In parasitized simuliids such reductions in carbohydrate levels are caused by depletion of blood glucose but not trehalose (13). Schmidt and Platzer (43) found that levels of total carbohydrates in the hemolymph of *C. pipiens* were unchanged by *R. culicivorax* parasitism. However, additional experimental details are needed to interpret this observation. *Romanomermis culicivorax* was found to cause an almost complete exhaustion of Periodic Acid Schiff positive material within the fat body tissue of *A. aegypti* during the nematode's rapid growth phase (1). Glycogen reserves were shown to be similarly reduced in the fat bodies of mermithid parasitized simuliids (6). In contrast to what we found to be the case for *M. nigrescens,* mermithid parasites of mosquitoes and simuliids cause almost complete degeneration of host fat body tissue. All storage metabolites, including glycogen, within the fat body may, directly or indirectly, be utilized by the developing nematode.

Gordon et al. (14) found that mermithid parasitism did not significantly affect either the overall concentration of lipids or the relative proportions of the lipid fractions in the hemolymph of *A. aegypti.* The mermithid did, however, cause an increase in the myristic acid:palmitic acid ratio in the free fatty acid faction of the host's hemolymph.

Wülker (49) recorded ultrastructural abnormalities in the corpora allata of mermithid parasitized chironomid larvae and pupae. Using a combination of histochemistry and microspectrophotometry, Condon and Gordon (6) found that *N. flumenalis* disturbed the activity of the neuroendocrine system in *S. venustum* by significantly increasing the nuclear DNA/RNA activity of the corpus allatum as well as the volume and amount of stored neurosecretory material in the corpus cardiacum. Because the mermithid was found not to induce noticable changes in the endocrinology of *P. mixtum/fuscum,* it is unlikely that the endocrinological disturbances noted in parasitized *S. venustum* have any bearing upon the development of the nematode. In all likelihood, the mermithid is not actively manipulating the hormonal balance of its host to modify its own microenvironment. The disturbances in *S. venustum* endocrinology probably constitute one of several possible stressful symptoms of the parasitemia, induced by the nutritional disturbances caused by mermithid parasitism.

HOST PHYSIOLOGY ON MERMITHIDS

Little attention has been paid to the reverse effect; i.e., the possibility that differences in physiology within or among hosts influence the parasitic development of mermithids. This is unfortunate, since knowledge of such effects could provide important clues to the nature of the nematode's requirements for growth and development which would be applicable to in vitro culture.

From the host's standpoint, the most desirable response to an invading mermithid would be a successful defense mechanism. Mermithids may be either encapsulated by the host hemocytes (with or without melanin formation) or melanized by noncellular (humoral) components of the hemolymph. Present knowledge of immunity responses is limited to morphological considerations, derived largely from light microscope and ultrastructural studies (32). Studies have not been undertaken to ascertain the physiological events associated with the immunity mechanisms. Thus, we do not know, for example, what enables *Aedes triseriatus* to melanize *R. culicivorax* humorally, or *Culex territans* to encapsulate it (35), given the fact that this nematode will develop normally in more than 50 spe-

cies of mosquitoes (32). In susceptible host species some mermithids may be able to block the host's immunity mechanism(s) by inhibiting the melanization process. In chironomids parasitism by mermithids retarded and weakened the capacity of the hemolymph to melanize fungal parasites of the flies (45). Such an impaired capacity of the host's blood for melanization may be a consequence of the reduction in phenoloxidase activity that occurs in the blood of mermithid parasitized chironomids (25).

Certain host-parasite relationships offer particularly intriguing lines of physiological inquiry. Unless the mermithid *Filipjevimermis leipsandra* first enters and partially develops within a nerve ganglion of its coleopteran host before returning to the hemolymph, it is encapsulated (31). During its period of development within the host nerve ganglion, the parasite persumably changes physiologically and/or induces changes in host physiology to render itself compatible upon returning to the hemolymph. The head region of *A. triseriatus* provides a microenvironment where *R. culicivorax* can develop free from immunity reactions, whereas development in other body regions is prevented by humoral melanization (35).

Parasitic development may be modified according to the quality and/or quantity of the host's diet. In *R. culicivorax* the quantity of food available to the host affects the determination of sex (16,27). When parasitized *A. aegypti* were fed on a diet low in quantity and/or protein content, parasitic development became asynchronous and some of the nematodes from hosts infected by more than one nematode emerged before others had completed their development (16). Thus, substantially fewer postparasites were recovered from nutritionally deprived hosts.

PHYSIOLOGY OF MERMITHIDS

The nutrition of mermithids is considerably modified from the basic nematode pattern. There is general agreement that mermithids absorb nutrients from the host's hemolymph across their outer cuticle. The ability of parasitic *M. nigrescens* to take up ^{14}C-glucose was not lessened when the head of the nematode was positioned out of the incubation medium, and there was no significant difference in glucose uptake between anterior and posterior halves of totally immersed worms (39). Ultrastructural evidence for the transcuticular feeding process was provided by Poinar (33,34) who showed that *R. culicivorax* can absorb ferritin across its outer cuticle. The cuticle of mermithids would appear on ultrastructural grounds to be well adapted for nutrient absorption. The cuticle of the parasitic stage(s) of *Gastromermis boophthorae* is very thin, contains fewer layers than subsequent free-living stages, and overlies a hypodermis replete with organelles indicative of high metabolic activity; the hypodermal surface is of a microvillous nature (2). Cuticle ultrastructure may vary widely among mermithids. Pores are apparently present in the cuticle of *R. culicivorax* (33, 34) but not in parasitic *M. nigrescens* (47) or *G. boophthorae* (2).

There is some disagreement as to whether mermithids secrete enzymes to predigest host nutrients before absorbing them. Rubtsov (37,38) theorized that the cells of the longitudinal cords secrete enzymes to induce lysis of host fat body tissue, and a variety of digestive enzymes were found in homogenates and exudates of mermithids from chironomids (44).

It is highly unlikely that *M. nigrescens* secretes digestive enzymes. Radiotracer studies showed that the nematode is able only to absorb simple molecules such as glucose (39) or amino acids (11) across its cuticle. Uptake of glucose and amino acids occurs by mediated transport systems, which are not coupled to co-transport of sodium ions and are not energy dependent (42). Our recent studies (unpublished) have shown that palmitate, in *R. culicivorax,* is similarly absorbed by a carrier system(s). Uptake of large molecules, such as ferritin (33,34), may be confined to species with porous cuticles.

Once absorbed, nutrients are transferred to the nematode's trophosome for metabolic conversion. Nutrients may enter the trophosome of *G. boophthorae* through cytoplasmic bridges that connect it to the hypodermal cords and/or through the pseudocoelom, since the outer surface of the trophosome is structurally well equipped

for nutrient absorption (3). Batson (3) showed that the trophosome of *G. boophthorae* is syncytial, without lumen, and contains globules of high and low electron density as well as reticulate granular inclusions. Histochemical and analytical studies showed that lipids constitute the predominant storage metabolite in *R. culicivorax,* glycogen the next most abundant, and proteins quantitatively a minor storage product (21). Kaiser and Fachbach (23) demonstrated differences in the electrophoretic separation of trophosomal (soluble) proteins among several species of *Hexamermis* and suggested that electropherograms of trophosomes might be used as a taxonomic tool. Using both thin layer and gas chromatography, Gordon et al. (14) showed that the trophosomal lipids of *R. culicivorax* and *N. flumenalis* comprised in order of prevalence: triacylglycerols, phospholipids, free sterols, and sterol esters. The degree of unsaturation of fatty acids within the triacylglycerol and sterol ester moieties was found to be greater for the boreally adapted *N. flumenalis* than for *R. culicivorax* (14). It is possible that the higher proportion of unsaturated fatty acids in *N. flumenalis* is of adaptive significance in permitting maintenance of physical state and consequent enzymic activity at low temperatures. A variety of sterols were tentatively identified from the free sterol and sterol ester fractions of the trophosomes of *R. culicivorax* and *N. flumenalis* (15); a C26 sterol predominated in both nematodes but, surprisingly, cholesterol was present in relatively small amounts.

Trophosomal storage products are required for energy metabolism, egg development, and, undoubtedly, a variety of other processes as yet undetermined. In *R. culicivorax* postparasites there is a functional β-oxidation pathway (17), the presence of which correlates with the nematode's preferred storage of triacylglycerols. To what degree the mermithid uses the β-oxidation pathway under natural conditions is speculative, however, since details are lacking concerning the oxygen tensions prevailing within the nematode's microenvironment. Imbriani and Platzer (20) suggest that conditions within the substratum of the mermithid's natural habitat are anoxic; therefore, the nematode is a facultative anaerobe. Conditions on the surface of the substratum, however, would not be anoxic. It is not known how long a period of time is spent by the mermithid between emerging from the host and penetrating the substratum.

CONCLUSIONS

During the past 10 yr a substantial amount of base-line data has been gathered to provide a general understanding of how the physiology of insects is altered by mermithid parasitism. Details are still lacking, however. Thus, the manner by which mermithids induce changes in the metabolic processes of the host fat body is not known, neither has the physiological basis of parasitic castration been elucidated. Why are the oocytes of parasitized insects unable to sequester proteins? The effect(s) of parasitism on the host endocrine system is unclear and only in larval diptera have effects been demonstrated. But these small aquatic insects are not ideal for such endocrinological studies. A larger terrestrial insect, such as a locust, readily lends itself to endocrinological manipulations (through microsurgery, injections, etc.), and such studies would reveal whether the pronounced physiological disturbances induced in the host emanate from, or cause, endocrinological changes.

The effects of the host's physiology on the developing nematode constitutes an area that has been almost entirely neglected and yet, from the standpoint of relevance to in vitro culture, represents a highly rewarding field of study. What factor(s) within the host influence or control mermithid development? Does the parasite depend upon its host purely for a supply of nutrients, or is its development controlled by "physiological triggers" (e.g., hormones or specific metabolites) that would have to be supplied in artificial culture media?

More so than in most other nematodes, lipids constitute an extremely important nutritional requirement for mermithids. Studies ongoing in our laboratory are aimed at elucidating the requirements of mermithids for specific lipid classes and the manner by which such nutrients are absorbed. Such information could be of direct benefit to researchers involved in in vitro

culture attempts, especially when one considers that commercially available synthetic culture media used for culturing nematodes are singularly deficient in lipids.

Aside from the abovementioned problems that are directly relevant to in vitro culture, mermithid nematodes present many tantalizing problems for physiologists to sort out. What physiological mechanisms underly the host's defense mechanism and, in *F. leipsandra,* what role does the insect's nervous system play in averting immunity responses? The fate of trophosomal storage products, their incorporation into the developing eggs and utilization for other processes remains to be investigated. While the oxygen content of the environment of an aquatic mermithid such as *R. culicivorax* is in some doubt, a terrestrial mermithid such as *M. nigrescens* burrows into the soil up to a depth of 45 cm—hardly an aerobic situation. If, as seems likely, the nematode engages in facultative anaerobiosis, it probably does not use the phosphoenolpyruvate carboxykinase pathway characteristic of many other facultative anaerobes (30). How does it metabolize in such an environment, in which it may live for up to 3 yr?

As in any rewarding field of research, there appear to be more questions than answers!

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