only slowly, their residual action was generally of short duration especially on the sorptive Babati bricks. On the less sorptive Magugu bricks at 80% relative humidity the compounds of low volatility—3,5-dimethyl-4-methylthiophenyl *N*-methylcarbamate [OMS-93] and 3,4-dimethyl-6-chlorophenyl *N*-methyl carbamate [OMS-174]—were effective at a dosage of 2 g/m² for about three months, owing largely to persistence of surface deposits, while 3-isopropylphenyl *N*-methylcarbamate [OMS-162] was effective for four to five months owing to its high intrinsic toxicity and activity in the sorbed state.

To summarize the results, therefore, deposits of wettable powder formulations of a number of phosphorothionates and carbamates would be expected to have a long residual action on relatively non-porous, chemically inert building materials, and a very much shorter one on porous dried mud.

The relationship between chemical structure and intrinsic toxicity has been investigated for derivatives of phenyl *N*-methylcarbamate, and has been the subject of a separate report.[f] The physical properties of compounds also exert a considerable influence on the contact toxicity of deposits to adult mosquitos. Deposits from a wettable powder formulation of a liquid insecticide on a relatively non-porous substrate are generally more effective than those of a solid insecticide of comparable intrinsic toxicity, and this is probably due to ease of pick-up and penetration through the insect cuticle. Several instances have been referred to in this report of solid insecticides showing a lower contact action than would be expected from their intrinsic toxicity as measured by topical applications of solutions. Generally, the compounds are characterized by low lipoid solubility. Further investigations on the relationship between physical properties of compounds and their contact action are in progress and will be described in due course.

* * *

We extend our thanks to the following firms for their generosity in providing samples of their technical and formulated products: American Cyanamid Co., USA; California Chemical Co., USA; Cela, Germany; Dow Chemical Corporation, USA; Farbenfabriken Bayer AG, Germany; Geigy Chemical Co., Switzerland; Hercules Powder Co., USA; Hooker Chemical Corporation, USA; Shell Development Co., USA; Stauffer Chemical Co. USA; Sumitomo Chemical Co., Japan; Union Carbide Co., USA; and the Upjohn Co., USA.

Technique for Infecting Larvae of the *Culex pipiens* Complex with a Mermithid Nematode and for Culturing the Latter in the Laboratory

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An as yet undetermined mermithid nematode [a] parasite of mosquito larvae was discovered by the writer in Zambia (then Northern Rhodesia) some twenty years ago [b] and was successfully cultured in the laboratory.[c] When cultured in association with an unknown number of species of other organisms the method is termed "xenical".[d]

Until December 1962 the mermithid had not been seen again for many years, but by a rather lucky chance it was rediscovered in the same locality, where it parasitizes most but not all species of tree-hole-breeding culicines that occur there. But two of the commonest *Aedes* species appear to be entirely resistant to or not favoured by the nematode.

In view of the anti-filariasis campaigns being organized by the World Health Organization and other bodies in various parts of the world, it was decided to ascertain the susceptibility of one of the most important vectors—namely, *Culex pipiens fatigans* Wiedemann. It was found that not only is the larva of this species very much favoured by the mermithids, but they develop in it as well as or better than in larvae of *Aedes aegypti* L., which was the original laboratory host. In addition, the living

[a] Considered by Dr H. E. Welch (personal communication) to be referable to *Romanomermis* Coman.

[b] Muspratt, J. (1945) *J. ent. Soc. S. Afr.*, **8**, 13.

[c] Muspratt, J. (1946) *J. ent. Soc. S. Afr.*, **10**, 131.

[d] Welch, H. E. (1963) *Nematode infections*. In: Steinhaus, E. A., ed., *Insect pathology, an advanced treatise*, New York and London, Academic Press, vol. 2, p. 385.

larvae are more easily examined under the microscope than those of the latter species owing to their less violent wriggling.

In field tests a large number of laboratory-bred *C. fatigans* were introduced into the tree-hole habitat and when about half-grown were examined microscopically for the presence of the eelworm. Parasitized larvae were reared in the laboratory and the worms which emerged from the larval host were collected in a jar containing sand and water so as to establish a laboratory culture.

This as yet undescribed mermithid merits serious consideration as a candidate biological control agent for use against *C. p. fatigans* and related mosquitos. Further investigations, however, remain to be undertaken before field trials can be considered, and such research will, of course, call for widespread collaborative efforts.

In the meantime it is felt that improvements to the original culture method [e] should be made known to those interested.[e] As noted, larvae of *Aedes aegypti* L. initially served as host. These may indeed still have their uses when *C. pipiens* complex larvae are unavailable. However, the method now described concerns three culicine hosts which have been used in the laboratory or (rarely) found infected in nature—namely, *Culex pipiens pipiens*, *C. p. fatigans* and *C. torrentium*. It is considered that additional members of the *C. pipiens* complex should also be exposed to the parasite to determine their susceptibility to infection.

Material and methods

Infection technique. If one starts with eggs which have been deposited by the adult female nematodes in damp sand in a small jar, the first step is to introduce water and add a number of second- or early third-instar larvae (see "Discussion" below). When hatching of the pre-parasite stage of the mermithid is well under way (which may take a few days following the addition of water) it is an essential part of the writer's technique to examine the small mosquito larvae under the low power of the microscope [f] in order to detect the presence of the earliest parasitic stages (Fig. 1). These are less than 1 mm in length before and just after entry into the haemocoele of the mosquito larva, and are usually easily distinguishable by virtue of their active whip-like movements. Normally they enter (*via* the cuticle) and remain in the abdominal segments, but they may occasionally be found in the thorax and elsewhere. They may, however, be difficult to distinguish when among the Malpighian tubules, which are not dissimilar in appearance (Fig. 1). The normal movements of the posterior part of the intestinal tract can also cause some confusion, particularly the contractile loop of the small intestine in the 7th segment (ileum, Fig. 1). This rather closely resembles the sluggish transparent early parasitic worm in the stage after the first whip-like form (Fig. 2).

Living larvae of most species of *Culex* may be examined with comparative ease under low-power objectives for they remain reasonably quiet in contrast to those of *Aedes (Stegomyia)*. A viscid aqueous solution of methyl cellulose may, however, be used to reduce the movement even more. Examination of *Stegomyia* larvae demands more patience and practice. Larvae of the *C. pipiens* complex are also usually very transparent in the earlier stages and, as noted above, the actively moving first parasitic stage of the mermithid is readily detectable if the larva is withdrawn from the infection jar with a pipette of 6-mm glass tubing with a rubber teat, the tubing being about 7 cm in length and tapering gradually to an internal diameter of 3 mm. Small larvae can be placed with this on a depression slide and stranded by withdrawing the superfluous water (either with the same pipette or, preferably, with another tapering to an internal diameter of 2 mm). A slide having a large hollow about 2 cm in diameter has proved very suitable for the purpose in practice.

The frequency with which the small *Culex* larvae are examined will depend on the incidence of infection in the jar, the object being to remove a parasitized larva to a rearing vessel when it contains only one or two small worms, as the presence of several may very soon kill it. It should be noted, too, that as the parasites begin to develop in the larval haemocoele they become very sluggish and transparent, and therefore much more difficult to see (Fig. 2). Furthermore, any mosquito larvae which do not contain a developing worm by the time they are half grown should be discarded and replaced by others in the second or early third instar, the number introduced being in direct proportion to the rate of infection.

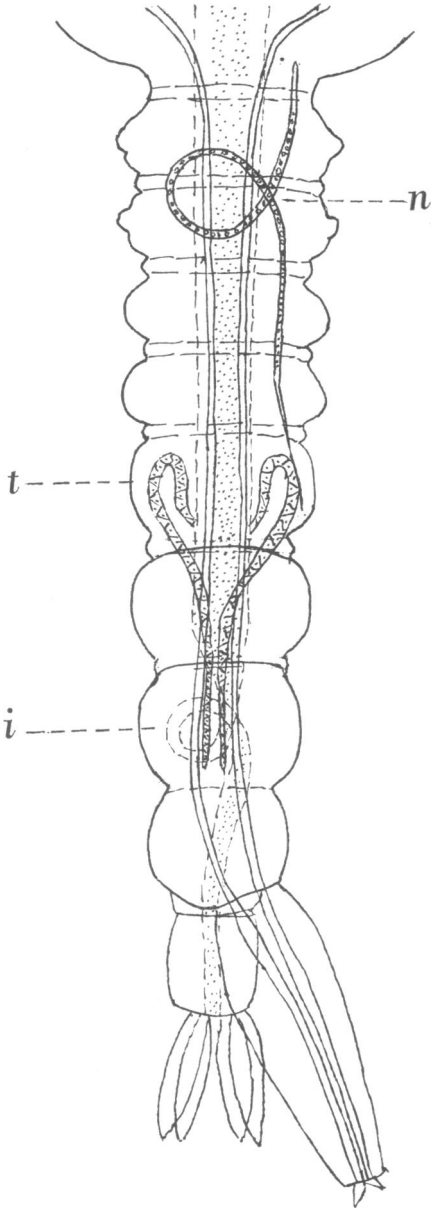
Rearing of parasitized mosquito larvae. This takes place in a separate container, and in general the procedure usual for the laboratory colonization of

[e] This improved method has not been published but was described in a working document prepared for WHO.

[f] Optimum: monocular ×80 using daylight and coarse adjustment focusing,

FIG. 1

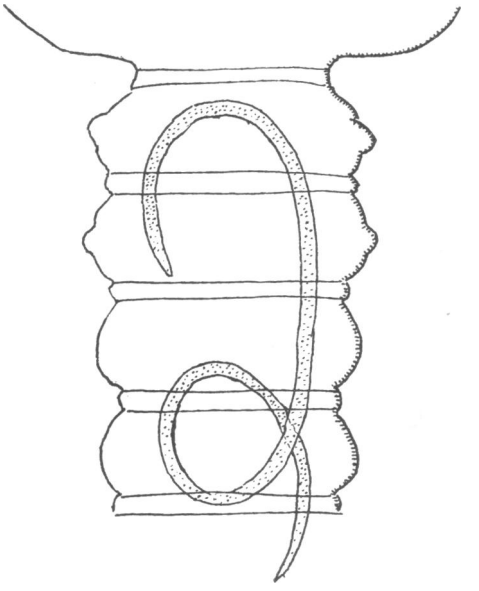
INTERIOR (VIEWED THROUGH DORSUM) OF PART OF AN EARLY THIRD-INSTAR LARVA OF THE *C. PIPIENS* COMPLEX, SHOWING TRACHEAL TRUNKS, ALIMENTARY CANAL (*i* = ILEUM), DORSO-LATERAL PAIR OF MALPIGHIAN TUBULES (*t*) AND A NEWLY ENTERED NEMATODE (*n*)



Length of the abdominal segments plus siphon of living larva = 1.7 mm (approx.).

FIG. 2

EARLY PARASITIC STAGE OF NEMATODE IN UPPER ABDOMEN OF LARGER LARVA



the species concerned is followed. There are, however, one or two pitfalls to guard against if the maximum degree of mermithid emergence is to be achieved. Too much stimulation of the larvae, either by high temperature or by a food rich in vitamin content, may result in the culicine larvae being able to pupate in spite of the presence of their parasite. If the larvae become infected when nearly half grown they should be kept on a sparse food supply for about a week, so as to allow the worm time to grow. The diet can then be enriched by degrees.

It has been found that for the three mosquitos mentioned a temperature of about 70°F (21°C) is satisfactory. The larvae are then reared in tap water with the addition of one or two dead poplar leaves (*Populus* sp.). Clear jars or beakers are used as rearing vessels so that the turbidity of the water can be observed. When the water in a container becomes distinctly cloudy it may be necessary to take out the leaves for a day or two until it becomes clear again, after which they are replaced. Removal of the leaves is less necessary for *C. fatigans*, but often advisable in the case of *C. pipiens* and *C. torrentium*, with which it may have to be repeated several times. The addition of yeast from tablet preparations, except in very minute quantities, was found to

stimulate larval growth too much, and should only supplement the diet when the worms are near emergence from the larval host.

The host larvae may remain alive for two or three times the length of the normal larval span. When the immature mermithids finally emerge it is usually *via* the anal segment. Large specimens average about 25 mm in length. An exceptionally large one which emerged from a larva of *C. torrentium*, however, attained a length of 37 mm (or about 4½ times the length of the host).

Culture of the immature nematodes. When the immature nematode larvae start to emerge, the contents of the rearing jar should be decanted daily into a glass dish and placed on a darkish surface such as the laboratory bench. In this way the parasites stand out clearly, appearing whitish against the dark background. They can then be transferred to the culture jar with a pipette.

The screw-top culture jars employed were about 6.5 cm in diameter, and proved suitable for about 50-60 of the larger worms. Into these are put 5-8 cm of sand (see " Discussion " below) and water is then poured in so as to have 3-5 cm of water over the sand. The worms are dropped into the water with a pipette and almost immediately burrow down into the sand. Sand which has been dry for some time, and therefore nearly sterile, appears not to be entirely satisfactory for the mermithids. In one instance when tap water only was used in beginning a new culture the immature worms burrowed down but immediately came out of the sand again. It is therefore recommended that water derived from an infusion of dead poplar leaves be used for filling up the sand culture jar.

When the requisite number of worms has been collected the water may be left in the jar for three or four weeks (or longer) before the drying-out process is started. At this stage, the standing water is first withdrawn, preferably by suction, so as not to disturb the sand. The jar is then packed with absorbent cloth (mutton cloth and cheese cloth are both very suitable) and the lid screwed on. Every two or three weeks the cloth is taken out, dried and then put back again, and the lid replaced. In this way the moisture is gradually withdrawn until the sand becomes very nearly dry. This can be tested by scratching the surface, and by the smell, which should be slightly musty. When the desired point has been reached the lid is screwed on for the last time and the jars are left until required for reinfection of small larvae.

Discussion

This paper is concerned chiefly with the establishment of laboratory cultures of this species of nematode from rather limited material. The worm's effect on populations of the *C. pipiens* complex in natural breeding-places and the means of establishing it are subjects for future study. If laboratory cultures are increased for this purpose, and not merely maintained, it will probably be necessary to modify the examination of the larvae exposed to the infection, as it would be too time-consuming to examine all larvae exposed.

It has been found that if first-instar larvae are exposed to the pre-parasitic form the entry of it into the haemocoele may cause high percentage mortality. Second-stage or even early third-stage larvae should therefore be used and usually fed rather sparingly for a few days after removal from the infection jar (this depends, however, on the size of the larvae and rate of growth). In the case of expanded cultures about 15-20 of the larvae in the infection jar can be examined microscopically daily, or more frequently if necessary, and when it is found that about 80% contain one or two worms the entire batch can be transferred to a rearing vessel, the pupae resulting from those which had escaped infection being removed on appearance. The writer has employed this method when circumstances necessitated using *Aedes aegypti* larvae, owing to the difficulty of examining a large number rapidly on account of their motility. In all cases it is desirable to keep controls of normal larvae, so as to check mortality due to parasitization.

It is not known just how long this species takes to reach maturity. Welch (personal communication) states that the period varies for different mermithids. Thus certain species parasitizing chironomids require only 24 hours or even less, but some terrestrial mermithids need up to 18 months. Again, it was found by Welch that with a mermithid (*Hydromermis churchillensis*) parasitizing a subarctic mosquito the time elapsing between emergence from the host and sexual maturity is 10-15 days, while in two blackfly mermithids it varies from 5 to 12 days.

The experiments outlined herein indicate that the mermithid under discussion matures within four months, although this length of time may not be mandatory. While it is not absolutely certain when mating occurs in nature, it would obviously have to be when there is water in the tree holes. The eggs, as well as the worms themselves, probably withstand the partial desiccation of the sand. In a former

experiment [c] the partially dried culture was left for several months before reculturing, and it is suggested that when it is merely desired to maintain a culture the sand could be dried out over a period of four to six months and reculturing performed about twice a year. It should be mentioned that it is not yet known for certain whether the worms can mature when continuously submerged, or if partial desiccation is a stimulus to both mating and oviposition.

The sand used in the culturing was taken from the foot of the tree in a cavity of which the nematode infection was discovered at Livingstone, Zambia. This was a softwood tree, *Ricinodendron rautenii*. The tree-hole was of large dimensions, being estimated to hold about a bucket of water. Sand and dead leaves constituted the débris at the bottom. It seemed that during the dry season the sand was carried up into the dried-out rot-hole and on to other parts of the tree by termites. In the absence of this particular sand, a fairly fine sand mixed with some broken-up dead leaves or a little compost would probably be the best substitute. But sand sifted through a sieve of 210-μ openings proved to be quite unsuitable, as the worms could not or would not burrow into it.

It is interesting to speculate on the biochemistry of this nematode. As has been noted, a surplus of food rich in vitamins, such as yeast or dog-biscuits, may stimulate the larvae to pupate despite the presence of the parasite. Questions which are considered to merit investigation are: Does the parasite have secretions which actually inhibit larval metamorphosis, or is such inhibition simply the result of interference on the part of the mermithid with the haemolymph of the host? The fact that on some occasions at least immature worms are carried through the pupal stage, emerging when fully grown from the adult mosquito, may be a pointer to the parasite's means of dispersal in nature.

Conclusions

Welch [f] and Weiser [g] have remarked on the possibility that mermithid nematodes may prove of real significance in the biological control of mosquitos and other insect vectors of medical importance, such as blackflies.[h] Furthermore, Weiser [g] emphasizes the necessity for mass culture of the possible control agent as a first step in the assessment of its potentialities.

* * *

I am grateful for facilities given me both at the South African Institute for Medical Research, Johannesburg, and at Bristol University, England, where culture experiments were carried out in addition to those at Livingstone.

[f] Welch, H. E. (1960) *Potentialities of nematodes in the biological control of insects of medical importance.* In: [*Proceedings of a conference on the*] *Biological control of insects of medical importance*, Washington, D.C., American Institute of Biological Sciences, pp. 67-76.

[g] Weiser, J. (1963) *Bull. Wld Hlth Org.*, **29**, suppl., pp. 107-113.

[h] Anderson, J. R. & DeFoliart, G. R. (1962) *Ann. ent. Soc. Amer.*, **55**, 542.

Non-Ovicidal Molluscicides in the Control of Bilharziasis

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In the search for molluscicides, WL 8008 (*N*-trityl morpholine) was found to be highly toxic to adult snails, but for all practical purposes this compound is non-ovicidal. We have therefore examined the possibilities of using a non-ovicidal compound in control measures.

The effect of multiple applications of a non-ovicidal molluscicide

It is important to remember that it is the transmission of bilharziasis that must be broken. Molluscicidal applications, by killing the snail intermediate hosts, interfere with the development of the parasites from the miracidial to the cercarial stage and are really aimed at preventing cercariae from being shed into the water. Thus, although a single application of a non-ovicidal compound might only have a transient effect upon the adult snail population, the young snails hatching out from the eggs take some time to become infected with the parasite and a further period of several weeks before they start to shed cercariae into the water.

A series of single applications could therefore break transmission of bilharziasis without necessarily