Haemoglobin in a Marine Nematode

ALTHOUGH haemoglobin is present in numerous parasitic nematodes, it has never been recorded from a free-living form[1]. Even the recent demonstration of its presence in the free-living egg-laying female of *Mermis subnigrescens*[2] scarcely affects the generalization, for *Mermis* is parasitic in insects for most of its life-history. However, we have now found that it is present in abundance in the purely marine form *Enoplus brevis* and, to a lesser extent, in *E. communis*.

The haemoglobin of *Mermis* was present in the so-called "chromotrope". Since haemoglobin had not hitherto been implicated as a sensory pigment, the finding, in our view, cast doubt on the function hitherto attributed to this organ[3]. In addition to experimentation with *Mermis* itself, it seemed worthwhile to examine the pigments found in the eye-spots which are a not uncommon feature of certain marine nematodes. In all the examples examined, mostly Enoploidea, the eye-spot pigments have been found to be other than haemoglobin. However, during the course of our searches, we found specimens of a haemoglobin containing enoplid in the estuarine mud of Budle Bay, Northumberland; we are now satisfied that these are specimens of *Enoplus brevis* Bastian. As shown by spectroscopic and histochemical test, the haemoglobin is present in the pharynx of both male and female, and in the "copulatory muscles" of the male (Fig. 1); it is also present, in smaller quantity, in hypodermal chord material along the length of the animal.

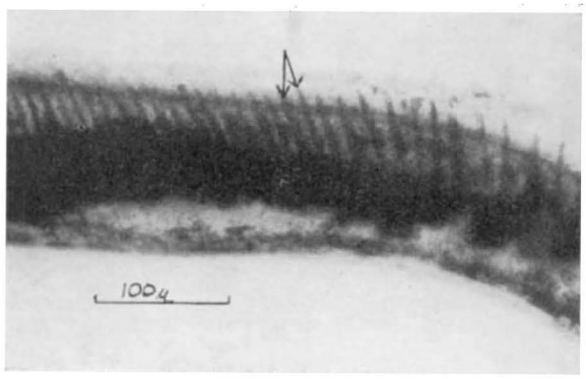

Fig. 1. Benzidine test on posterior end of male *E. brevis*. Haemoglobin is present in the series of "copulatory muscles" (arrows) which show heavy black

E. brevis has frequently been recorded from various localities[4] and it is, at first, puzzling that the presence of haemoglobin in such quantity should have gone undetected. Presumably, most other workers have only examined fixed material. We have confirmed that *E. brevis*, taken from two local habitats from which it has previously been recorded, have haemoglobin, distributed in the same way.

The haemoglobin in *E. communis* is present in much smaller quantity and, in this form, it occurs solely at intervals along the length of the hypodermal chords. Since it is present in this position also in *E. brevis*, and the tissue in which it is found in *Mermis* is hypodermal chordal in nature[5], the pigment may prove to be much more widely distributed in free-living forms.

C. ELLENBY
L. SMITH

Department of Zoology,
University of Newcastle upon Tyne.

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ENTOMOLOGY

Chemosterilization of *Aedes aegypti* (L.) by Pupal Treatment

THE concept of insect control through the liberation of sterile males[1] proved successful in the eradication of the screw-worm fly (*Cochliomyia hominivorax*) from the south-eastern United States[2]. Following this success, in which sterility was induced by γ-irradiation, interest was taken in the possibility of inducing sexual sterility by chemical means[3]. Screening of chemicals yielded several substances with a prospective sterilizing capacity[4,5], notably alkylating agents incorporating the aziridine moiety. In general they are less injurious to insects than radiation[6,7], and treatment of insects with chemicals is in certain respects more convenient than the procedures of irradiation.

In the first of the experiments carried out on chemosterilization of mosquitoes[8], the fertility of *Aedes aegypti* and *Anopheles quadrimaculatus* was reduced by feeding adults on the alkylating agents 'Tepa', aphomide or apholate dissolved in honey; other workers successfully sterilized both sexes by tarsal contact[9,10]. Sterilization of several mosquito species has also been obtained by immersion of larvae in solutions of the order of 10 p.p.m. of various aziridine compounds[11–13].

For practical applications of chemosterilization against natural mosquito populations, it seems logical to focus attention on sterilization of the mosquitoes in the aquatic stages. This is due to the fact that the principle of sterilization as a control method depends on sterilization of male insects and adult male mosquitoes cannot be readily found in nature, only females of troublesome or disease-carrying species being common as they come to feed, or at rest in the precincts of their hosts. By treatment of aquatic stages in their natural breeding sites, both sexes would be exposed to sterilization and the sterile males at emergence immediately available to produce sterile inseminations in females of other broods which had not been exposed. An alternative, and more acceptable, approach is to avoid dispersal of these mutagenic chemicals in the natural environment by treating the male insects in the laboratory and later releasing them at selected points in the area to be controlled so that they may disperse into the wild population to mate with normal wild females.

Although treatments of male insects for their subsequent release have been made by larval exposure, little attention has been given to the effectiveness of treatment of mosquitoes at the pupal stage, and this communication directs attention to certain advantages which pupal treatment appears to offer.

The toxic and sterilizing dosages of alkylating agents for mosquito larvae do not differ greatly, so that the dose required to ensure induction of complete sterility in the resultant adults also incurs mortality[9,12,14]. Investigations carried out in this laboratory on the sterilization of *Aedes aegypti* with the chemosterilant 'Thiotepa' (*tris* (1-aziridinyl) phosphine sulphide) show that this difficulty is avoided when the pupal stage is treated. Preliminary tests using aqueous solutions of this alkylating agent indicated that, although fertility was unaffected in adults which emerged from pupae subjected to doses of the same order as those which sterilized but also caused partial mortality of larvae, pupal mortality was negligible. Further experiments were conducted to determine what dosage pupae would tolerate and if sterility could be obtained with sub-lethal treatments. Immersion of pupae in 'Thiotepa' solutions as strong as 500 p.p.m. or more for 24 h proved highly satisfactory in inducing complete and permanent sterility in both sexes. It was also repeatedly found that this was far below the toxic dose for pupae, which could tolerate immersion in concentrations as high as 2,000 p.p.m. for 24 h. Adults