SHORT COMMUNICATION

C. ELLENBY and L. SMITH 1): *A narcotic and an immersion medium for living nematodes with some observations on the refractive index of the cuticle.*

The visibility of an unstained object under the microscope depends on differences in refractive index between the mounting medium and the object and on differences in refractive index within the object. Thus Franklin & Hooper (1962) found that observation of the cuticle of living specimens of *Bursaphelenchus* was aided by mounting the specimens in immersion oil, that is, by increasing the contrast between cuticle and medium. Now, although nematodes are very transparent, the refractile nature of the cuticle limits visibility of the internal organs; and the use of phase contrast is disappointing for the same reason. For some years we have examined living nematodes in this laboratory in solutions of Bovine ox plasma, "matched" to the refractive index of the cuticle. Internal organs were more easily seen, but, due to the movement of the animals, there was not a great deal to be gained. However, we have now found a suitable narcotic to use with it. The use of neither the plasma nor the narcotic is original but their combined use for nematodes is so helpful that it was thought desirable to publish this note.

Owen (1955) first used the bacteriocide propylene phenoxetol as a narcotic for mollusca. It has since been found to be more generally useful (Bagenal, 1963). A 0.5 p.c. tap-water solution will immobilise specimens of both *Panagrellus redivivus* and *Heterodera rostochiensis* in about an 1/2 hour; they will recover after about 2 hours in the narcotic. More dilute solutions are also effective and the animals survive better. J. L. Townshend informs us (personal communication) that the drug is also effective with *Aphelenchus asparagi* (Propylene phenoxetol is obtainable from Nipa Laboratories, Cardiff).

Barer & Joseph (1955) used solutions of Bovine Ox Plasma, Fraction V, as an immersion medium. Due to the large molecules it contains, even concentrated solutions have negligible osmotic effect. Nematodes survive in it very well: moreover, as evaporation takes place at the edge of the cover glass, preparations are "self-sealing" and last for some hours. (Bovine ox plasma, fraction V is obtainable from Armour Laboratories, Folkestone: about £ 2 for 10 g).

It is most convenient to prepare the plasma in solution of narcotic of the appropriate strength. We find that a 30 p.c. solution of plasma gives a near match to the cuticles of both *Panagrellus* and *Heterodera* species and it has been our practice to prepare a solution of this strength in 1 p.c. propylene phenoxetol in tap-water; slight adjustment can be made subsequently, if necessary. Worms are immobilised very quickly in the higher concentration of narcotic although they do not survive so well.

Under phase contrast illumination the improvement in contrast is most striking. Not all of this is manifest in photographs: but those presented in Fig. 1 of second-stage *Heterodera rostochiensis* larvae clearly show a number of structures which, as far as we know, have not been previously recorded. The magnification in the print is approx. 3,000.

The Refractive index of the cuticle.

Specimens of *Panagrellus* and *Heterodera* larvae were mounted in a series of dilution of ox plasma in tap-water-narcotic solution until a dilution was found in which the cuticle became invisible. In practice we found it most convenient to "match-up" to the tail. The refractive index of the matching medium was then measured with a Bellingham and Stanley hand refractometer. A series of determinations gave values all in the region of 1.38. This value would be considered low for a cuticle of solid protein and suggests a spongy structure; it is, in fact, consistent with the view of the cuticle as a mesh of interlocking protein fibres (Picken, Pryor & Swann, 1947) and would help to explain its permeability.

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Fig. 1. Second-stage larvae of *Heterodera rostochiensis* mounted in 29 p.c. Bovine ox plasma in 1 p.c. propylene phenoxetol in tap water. Phase contrast illumination. All at same magnification, approx. X 3000. Arrows indicate points of interest. A. Ampulla at end of duct from ant. oesophageal gland. Gland is also shown. B. Duct opening into lumen of oesophageal bulb. Gland faintly shown behind it. C. Ampulla on excretory duct.