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# DESICCATION SURVIVAL OF THE INFECTIVE LARVA OF *HAEMONCHUS CONTORTUS*

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## INTRODUCTION

The infective stage of *Haemonchus contortus*, the third-stage larva, retains the second-stage larval cuticle. Exsheathment takes place in the rumen of the host (Somerville, 1954), under the influence of a number of factors, chiefly unionized carbonic acid and dissolved gaseous carbon dioxide (Rogers, 1960). The ensheathed larva survives desiccation, but, if caused to exsheathe by appropriate treatment, it soon succumbs if dried. This aspect of sheath function is therefore clear; but the mechanism by which survival is improved is obscure. Rogers & Sommerville (1960) showed that the sheath is permeable to water and they therefore consider (1963) that it 'seems unlikely to prevent the larva from becoming desiccated'.

The infective larva of the potato-root eelworm *Heterodera rostochiensis* survives prolonged desiccation inside the egg-shell, but it is very susceptible to drying once it has hatched (Ellenby, 1968*b*). The free larva loses water much more rapidly than the enclosed larva; it was therefore suggested that the egg-shell, freely permeable when wet, becomes impermeable as it dries and that the reduction in the rate at which the larva dries helps it to survive. The hypothesis that the same sort of mechanism may operate in *Haemonchus contortus* is examined in the present work.

## MATERIALS AND METHODS

In general, techniques were similar to those already described (Ellenby, 1968*a*, *b*).

Experiments were carried out on ensheathed larvae which arrived, at fortnightly intervals, from the Moredun Institute, Edinburgh. They were stored at 5° C. and survived for several months; however, in all tests, animals not more than a fortnight old were used. They were allowed to come into equilibrium with room temperature for at least an hour before an experiment. Ensheathed larvae were obtained by incubating the ensheathed forms overnight at 37° C. in $CO_2$-saturated 50% Ringer. All animals were passed through distilled water before use.

Desiccation survival was examined on batches of 10–100 individuals set to dry inside petroleum jelly-ring cells (Ellenby, 1943) on microscope slides. Under a stereomicroscope, all superficial water was removed from the animals. The slides were then transferred to racks in 11 cm. diam. × 3 cm. chambers maintained at 47% relative humidity (R.H.) by a solution of glycerol (Grover & Nichol, 1940). The humidity chambers were kept at 18 ± 0·5° C. At intervals, of hours for the exsheathed larvae, and of days for the ensheathed forms, slides were removed from the humidity chambers and tap water was added to the cells. They were then set aside in moist chambers and

the revival of larvae was checked from time to time until it reached a maximum. It was sometimes 4–5 days before maximum revival was reached; the time which the larvae took to revive appeared to increase with the length of the drying period, although this was not examined critically.

Water content was estimated by interferometry (Ellenby, 1968*a*, *b*) and rate of water loss, for ensheathed and exsheathed larvae, from measurements on individuals of both sorts dried on microscope slides for various times at 47% R.H. and 18° C. Specimens in liquid paraffin (mineral oil), were photographed with a reflex camera using monochromatic light ($\lambda = 0{\cdot}546\mu$) and measurements of optical displacement and specimen thickness were made subsequently on enlarged prints. Estimates of total solids, in g./ml., were derived from specimen refractive index using the value for the specific refractive increment generally employed, viz. 0·0018 (Davies & Wilkins, 1952; Barer, 1953).

In the present work and elsewhere (Ellenby, 1968*a*, *b*) water content, %, has been derived by subtracting estimated solids/100 ml., from 100. This is only valid if the solids have a specific gravity of 1, that is, if they occupy the same volume as water. Barer & Joseph (1954) state that the specific gravity of dry protein is 1·33, but it is not certain whether this is also true when the protein is in solution. If it is assumed that the 'solids' are protein of this specific gravity, a nematode with about 25% solids would have a specific gravity of about 1·06. Our own preliminary estimates, however (C. Ellenby & L. Smith, unpublished).show that the specific gravity of the nematodes we have examined is much nearer to 1; if it is assumed to be 1, the underestimation of water content is likely to be slight, except with very dry specimens. In fact, values for water content estimated in this way agree very well with determinations by more conventional methods (Fairbairn, 1956; Myers, 1966; C. Ellenby & L. Smith, unpublished). The interference method for estimating water content clearly has quantitative limitations and it is important that they should be borne in mind; however, where it is used, as in the present case, in work of a comparative nature, the limitations are of less moment.

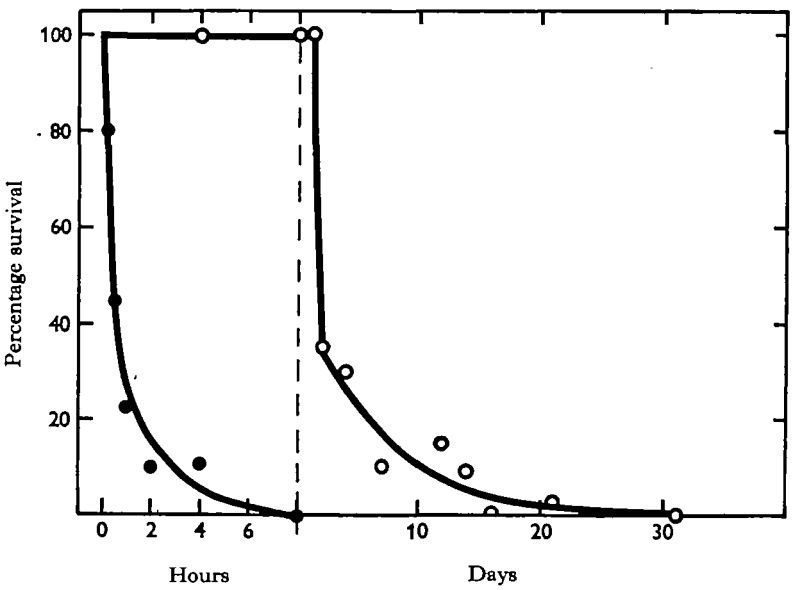
Water uptake was estimated by somewhat similar methods. Individual larvae of both sorts, on microscope slides, were dried overnight at 47% R.H. at 18° C. An aqueous solution of bovine ox plasma was then added (Barer & Joseph, 1954), the specimen was quickly covered, and the water uptake was followed with the interference microscope; photographs were taken at appropriate intervals and from these water content was subsequently estimated. Both an ensheathed and an exsheathed form were included in some preparations so that the two forms could be examined under precisely similar conditions. Glass fibres were sometimes included with preparations to provide a check on changing refractive index of the immersion medium. Due to the self-sealing properties of the medium, the changes were very slight at the centre of the preparation, even after 2 days.

Tests were also carried out on ensheathed larvae which had been dried for 2 hr. only.

## RESULTS

### *Desiccation survival*

The results, presented in Text-fig. 1, show clearly that the ensheathed larva survives desiccation far better than the exsheathed form. After only an hour's exposure to 47% R.H., survival of exsheathed larva is poor, and after 8 hr. there is no recovery; on the other hand, the ensheathed larvae all recover after 30 hr. exposure, and, even after 3 weeks, some worms recover.




Text-fig. 1. Percentage survival of exsheathed (●) and ensheathed (○) larvae of *H. contortus* exposed to 47% R.H. at 18° C. Broken line indicates change in scale of exposure time.

### *The rate of water loss in the ensheathed and exsheathed larva*

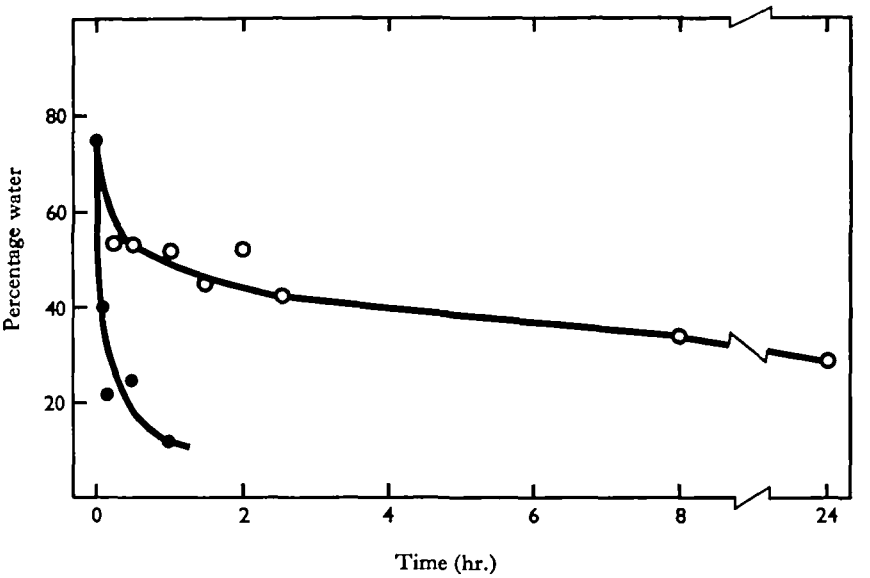
Living fully hydrated larvae, ensheathed or exsheathed, were found to have a water content of about 75%.

Text-fig. 2 shows that the exsheathed larva dries very rapidly; after an hour its water content is about 10%. The ensheathed larva also dries rapidly at first, losing about half of its water in the first 15 min.; thereafter the rate of water loss slows and, even after 24 hr., the ensheathed larva still contains about 30% of water.

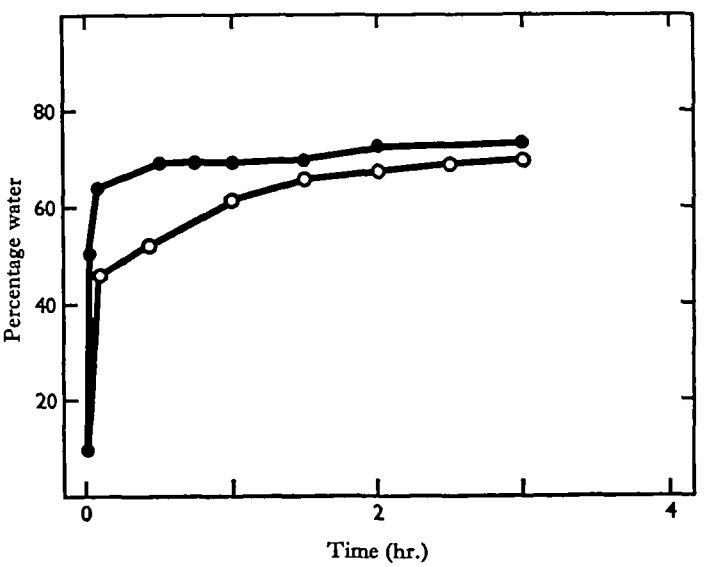
Measurement of the water content of dry larvae is difficult as the body is frequently distorted. The sheath is an additional problem; after about 2 hr. at 47% R.H., the larva begins to contract away from the sheath and, at the places where this has taken place, measurement is difficult. In spite of these difficulties, with care, good agreement can be obtained in the determinations. For example, measurement of 11, 2 hr.-dried, ensheathed larvae, gave a mean value for refractive index of $1{\cdot}428 \pm 0{\cdot}006$ or an estimated water content of about $50 \pm 3\%$; this compares favourably with the accuracy of conventional methods for determining water content of any animal.

*The rate of water uptake of the ensheathed and exsheathed larva*

The results, presented in Text-fig. 3, show that the ensheathed larva, dried overnight, is freely permeable to water; however, its rate of water uptake is substantially lower than that of the similarly dried exsheathed form. Apart from the complications arising from the presence of the sheath, *Haemonchus* is not such good material as the




Text-fig. 2. Water loss of exsheathed (●) and ensheathed (○) larvae of *H. contortus*. $\alpha$ is assumed = 0·0018 for all figures.




Text-fig. 3. Rate of water uptake of dry exsheathed (●) and ensheathed (○) larvae of *H. contortus*. Based on the water uptake of single larvae followed continuously.

species previously studied (Ellenby, 1968*a*, *b*) and measurements are therefore less accurate. But there is no doubt about the general validity of this conclusion; and tests conducted with specimens of each sort in the same preparation confirm it—water always entered the exsheathed dry larva more rapidly than the ensheathed form (Pl. 1 *A*).

The experiments on desiccation survival showed that, after a few hours exposure to 47% R.H., all exsheathed larvae died and all ensheathed larvae survived; this may account, to some extent, for the higher rate of water uptake of the dry exsheathed larvae. It is probable, however, that the contraction of the drying ensheathed larva away from the sheath is of greater importance.

As already mentioned, the ensheathed larva begins to contract away from the sheath after about 2 hr. at 47% R.H., when its water content is about 50%. Examination of 2 hr. dried ensheathed larvae showed that water enters them very rapidly indeed: these dry larvae, in fact, appear to 'leap' into activity on the instant that they make contact with water. Attempts to record the rate of water uptake of the 2 hr. dried ensheathed larva, using fast film, showed that the ensheathed larva becomes fully hydrated almost immediately; even a measurement only 20 sec. after the addition of water showed a completely hydrated larva. Indeed, water enters so rapidly that it would not be surprising if the cuticle proved to be exceptionally permeable.

### *The drying of the second-stage cuticle*

Tests with the 2 hr. dried ensheathed larva show that the sheath is freely permeable to water; nevertheless, the ensheathed larva loses water less rapidly than the exsheathed form. This strongly suggests that, like the egg-shell of *Heterodera rostochiensis*, the sheath may become less permeable as it dries. Close examination of dry larvae under the interference microscope shows that this is certainly the case.

Plate 1B is a photograph of an ensheathed larva dried for 15 min. at 47% R.H. The liquid paraffin in which it is mounted has a refractive index of 1·478; if the specimen matched it in refractive index, it would have a water content of about 20%. The first two interference fringes, displaced to the right by the tail, show that the second-stage larval tail is very dry; it has a water content of less than 10%. The next fringe is also displaced to the right at the periphery, that is, in the sheath; but it then turns back to the left as it traverses the breadth of the animal. This clearly shows that the larva itself has a far higher water content than the sheath; in fact, the water content of the larva is more than 50%.

## DISCUSSION

The results show that retention of the second-stage cuticle enables the infective larva to survive desiccation. The cuticle itself is freely permeable to water yet, nevertheless, the ensheathed larva loses water far more slowly than the exsheathed form.

In *Heterodera rostochiensis* the egg-shell is freely permeable to water when wet; it becomes impermeable as it dries and slows the rate at which the contained larva dries (Ellenby, 1968*b*). This enables the larva to survive; it is known that, for reasons which are not understood, slowing the rate of drying increases survival to desiccation (Lees, 1953). The same sort of mechanism may operate in the infective larva of *Ditylenchus dipsaci*, both in the 'eelworm wool' larval aggregations and in individual isolated larvae (Ellenby, 1968*b*). Individuals on the outside of an aggregation dry faster than

those in the centre and they also die first. The case was not so clear for isolated individuals. After a few minutes exposure, their rate of drying slowed: it was suggested that their drying cuticle reduced the rate of water loss, like the egg-shell of *Heterodera*, and the outside individuals in the *Ditylenchus* aggregates. In fact, the interference microscope provided some evidence that in dry specimens of *Ditylenchus* the cuticle was drier than the rest of the animal. Unfortunately, these animals frequently contain globules of highly refractive material which, in dry specimens, could produce the same effect. Generally, the globules are large, and easily avoided; but if present in minute droplets, their influence on measurements might be more insidious. It is therefore very gratifying that the results with the infective larva of *Haemonchus* are so clearly in keeping with the hypothesis: when exposed to the air, the sheath dries first; this slows the rate of drying and enables the larva to survive.

It seems, then, that desiccation survival in nematodes is always associated with mechanisms to slow the rate of water loss; but, clearly, many other factors must be involved. The ensheathed *Haemonchus contortus* larva, for example, dries less rapidly than the infective larva of *Ditylenchus dipsaci* (Ellenby, 1968*b*), but *Ditylenchus* is far better at surviving desiccation.

## SUMMARY

1. The ensheathed larva of *Haemonchus contortus* is far better at surviving desiccation than the exsheathed form.

2. Using interference microscopy, it is shown that, although the sheath is freely permeable to water, the ensheathed larva loses water much more slowly than the exsheathed larva.

3. Close inspection of partially dry ensheathed larvae shows that the sheath becomes dry first and that this slows the rate at which the larva it contains loses water.

4. The results are discussed in relation to other examples among nematodes where desiccation survival appears to be associated with mechanisms to slow the rate of drying.

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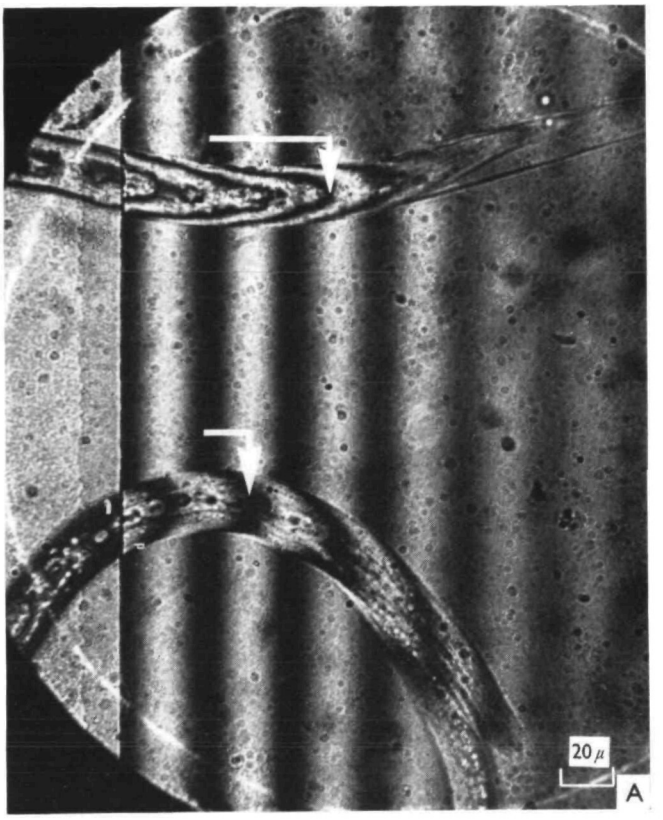
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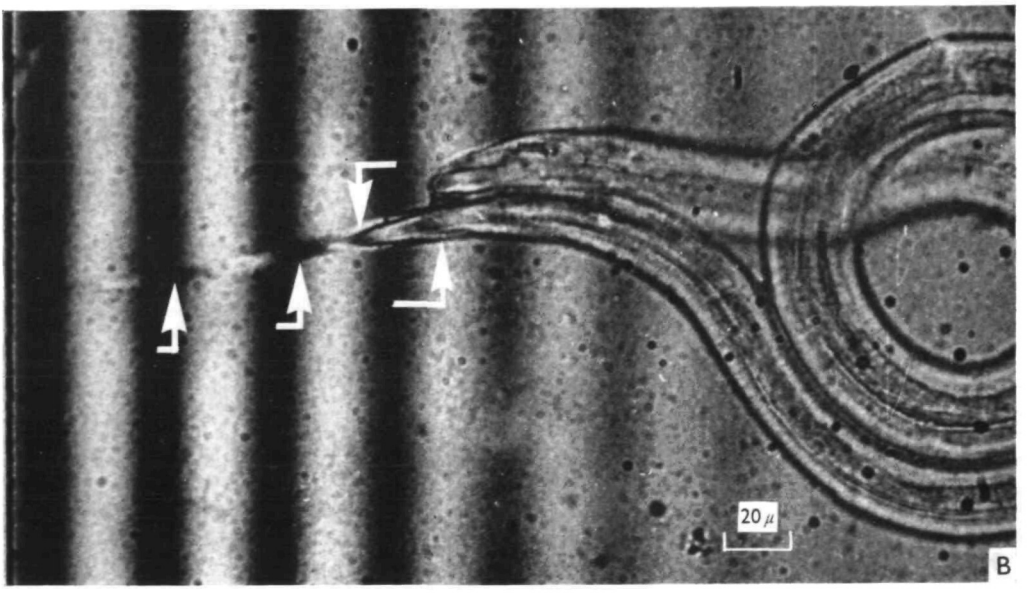
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## EXPLANATION OF PLATE

A. Water uptake of very dry ensheathed and exsheathed larvae of *H. contortus*. Arrows show that, 1 hr. after immersion in aqueous bovine ox plasma, the optical displacement is far less for the exsheathed form (lower specimen) than for the ensheathed form (upper specimen) indicating that far more water has entered the former.

B. Ensheathed larva of *H. contortus* dried for 15 min. at 47% R.H.; mounted in liquid paraffin. Greater refractive index (less water) is shown by displacement to the right. The second-stage cuticle has a higher refractive index than the medium at the tail (left and central lower arrows) and also where it ensheaths the third-stage larva (right lower arrow); the larva itself, however, has a lower refractive index than the medium (note displacement to left indicated by upper arrow) showing its higher water content.