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With 11 *figures in the text*

Changes in cuticular permeability associated with recovery from anhydrobiosis in the plant parasitic nematode, *Ditylenchus dipsaci*

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SUMMARY

The cuticular permeability of anhydrobiotic 4th-stage juveniles of *Ditylenchus dipsaci* decreases during rehydration, as indicated by staining with osmium tetroxide, the measurement of permeability coefficients by the rate of uptake of tritiated water from a $^{3}HOH/^{14}C$-inulin bathing solution and by an increased ability to slow down the rate of water loss and to survive subsequent desiccation. The initial decrease in permeability coefficient is rapid, suggesting a physical effect due to the rehydration of the cuticle, followed by a slower reduction over the subsequent 1–4 h which is dependent upon metabolic activity. The re-establishment of the permeability barrier is sensitive to metabolic inhibitors which affect enzyme activity and post-transcriptional protein synthesis. Sodium iodoacetamide was the only inhibitor which affected maintenance of the permeability barrier. An Arrhenius plot of changes in cuticular permeability with temperature indicated a sharp increase in permeability at 40–50 °C and brief exposure to diethyl ether resulted in loss of the permeability barrier, suggesting that a superficial layer, probably the epicuticle, is responsible for controlling cuticular permeability.

INTRODUCTION

Some species of nematode can lose almost all of their body water and enter into an ametabolic state of anhydrobiosis (Barrett, 1982). The 4th-stage juveniles of the plant parasitic nematode *Ditylenchus dipsaci* can survive in a desiccated state for many years and yet, upon immersion in water, recover activity after a lag phase of 2–3 h. During the lag phase there is a slow and ordered series of morphological changes which may represent membrane repair prior to recovery (Wharton & Barrett, 1985; Wharton, Barrett & Perry, 1985). In anhydrobiotic *Aphelenchus avenae* and *D. dipsaci* leakage of ions and metabolites during the initial phases of rehydration indicates that desiccation results in disruption of the cuticular permeability barrier (Crowe, O'Dell & Armstrong, 1979; Womersley, 1981). However, rehydrated *D. dipsaci* have a very low permeability to fixatives (Wharton & Barrett, 1985). Anhydrobiotic nematodes provide a system in which permeability is disrupted during desiccation and repaired during rehydration and may prove a useful model for the study of the maintenance and repair of the nematode cuticular permeability barrier. We have therefore investigated the changes in the permeability of the cuticle of anhydrobiotic 4th-stage juveniles of *D. dipsaci* during rehydration.

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MATERIALS AND METHODS

Ditylenchus dipsaci giant race 4th-stage juveniles were grown on field beans (*Vicia fabia*) and stored dry in the infected plant material at room temperature. Rehydrated nematodes were obtained by immersing the plant material in water and allowing the juveniles to migrate through tissue paper. Anhydrobiotic juveniles were induced by exposing rehydrated juveniles to 50% relative humidity over glycerol solution for 24 h at 22 °C (Grover & Nicol, 1940), followed by 0% relative humidity over freshly activated silica gel for 24 h at 22 °C. The anhydrobiotic juveniles were stored at 0% relative humidity over silica gel at 22 °C until required. Rehydrations of anhydrobiotic juveniles were conducted at room temperature using artificial tap water (ATW) (Greenaway, 1970).

The desiccation-susceptible nematode, *Panagrellus redivivus* was cultured on autoclaved oat grains at 22 °C.

Osmium staining

Staining with osmium tetroxide has been used to reveal changes in the permeability of nematode egg-shells (Bird & McClure, 1976). Anhydrobiotic juveniles were rehydrated in ATW for various periods and then immersed in 1% osmium tetroxide in 0·2M sodium cacodylate buffer (pH 7·2) for 1 h. The numbers of juveniles stained completely black were counted.

Desiccation survival

Anhydrobiotic juveniles were rehydrated for various periods in ATW and then exposed to 0% relative humidity over silica gel at 22 °C for 24 h. The juveniles were then given a second rehydration in ATW and the numbers active after 3 h were counted.

Ability to control the rate of water loss

Anhydrobiotic juveniles were rehydrated for various periods and then exposed to 0% relative humidity over silica gel at 22 °C for 5 min. The water contents were determined by interference microscopy (Ellenby, 1968), using the techniques described by Perry (1977*a*).

Measurement of permeability coefficients

The permeability coefficients of rehydrated *D. dipsaci*, anhydrobiotic *D. dipsaci* and hydrated *P. redivivus* were measured by the rate of uptake of tritiated water from a $^{3}HOH/^{14}C$-inulin bathing solution (Amersham International), giving $2{\cdot}4 \times 10^{7}$ disintegrations/min/ml (d.p.m./ml) of ^{3}H and $1{\cdot}6 \times 10^{5}$ d.p.m./ml of ^{14}C. Inulin does not penetrate cell membranes and was used to check that most water uptake occurred via the cuticle, rather than the body orifices. After exposure to the bathing solution the nematode sample was transferred to a millipore filter, washed rapidly in distilled water, immersed in 1 ml of distilled water in a scintillation vial and left for 3 days to allow diffusion of the radio-isotope label into the water prior to counting. A vol. of 10 ml of scintillation cocktail was added to each vial (ReadySolv MP - Beckman Ltd, High Wycombe, UK) and a double ^{14}C, ^{3}H count was performed with a LKB Rackbeta II scintillation counter, with appropriate quench corrections and corrections for radioactive decay. ^{14}C counts were low, indicating that uptake occurred mainly via the cuticle, and the ^{14}C counts were used to provide a correction factor to allow for incomplete washing after exposure to the bathing solution.

Counts were made after various periods of exposure to the bathing solution to determine the ^{3}HOH uptake curve. Volumes and surface areas were calculated from length and diameter measurements (Wharton *et al.* 1985) and the permeability coefficient was calculated using the formulae given by Reisin & de Falla (1984) after an appropriate period of exposure to the bathing solution (5 min for rehydrated *D. dipsaci* and hydrated *P. redivivus*, 0·5 min for anhydrobiotic *D. dipsaci*). Details of the calculations involved have been given by Preston & Bird (1987).

Changes in permeability coefficient during rehydration

Anhydrobiotic juveniles were rehydrated for various periods and the permeability coefficient calculated from the ^{3}HOH uptake after exposure to the bathing solution for 5 min.

Temperature effects

Anhydrobiotic juveniles were rehydrated for 24 h at room temperature. They were then incubated at various temperatures from 25 to 70 °C for 30 min, cooled to room temperature, then the permeability coefficient determined by exposure to the ^{3}HOH/^{14}C-inulin bathing solution, as before. An Arrhenius plot of log permeability against the reciprocal of temperature was used to indicate phase changes in cuticular lipids (Barrett, 1981).

Effects of metabolic inhibitors on permeability changes

Maintenance of the permeability barrier

Anhydrobiotic juveniles were rehydrated for 24 h and then incubated for 30 min in a solution of one of the following inhibitors: rotenone (2·5 mg/ml), chloromercuribenzoate (CMB, 10 mM); sodium iodoacetamide (SIA, 10 mM), puromycin (10 mM) and cycloheximide (10 mM). Anhydrobiotic juveniles were also exposed to the following solvents; diethylether and chloroform/methanol (2:1, v/v) for 5 sec. Brief exposure to organic solvents did not affect worm motility. Following exposure to inhibitors or solvents, nematodes were washed briefly in ATW then exposed to the ^{3}HOH/^{14}C-inulin bathing solution.

Re-establishment of the permeability barrier

Anhydrobiotic juveniles were rehydrated for 2 or 24 h in the presence of chloramphenicol (10 mM) and other inhibitors (as above), washed briefly in ATW then exposed to the ^{3}HOH/^{14}C-inulin bathing solution as before.

RESULTS

Staining of *D. dipsaci* 4th-stage juveniles after exposure to 1% osmium tetroxide decreased as they were rehydrated; indicating a decrease in permeability to osmium tetroxide (Fig. 1). The permeability barrier to osmium tetroxide was re-established after 1–2 h rehydration. The ability of *D. dipsaci* 4th-stage juveniles to survive exposure to subsequent desiccation increased during rehydration (Fig. 2) and the water content after exposure to 0% relative humidity at 22 °C for 5 min increased during

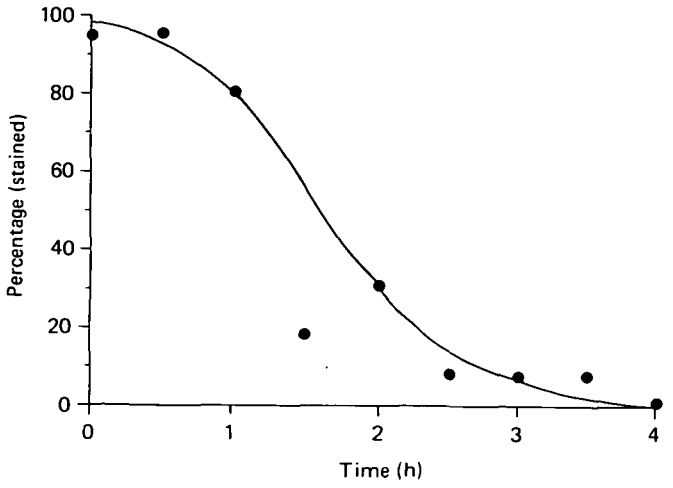

Fig. 1. Percentage of *Ditylenchus dipsaci* 4th-stage juveniles stained completely black after various periods of rehydration of anhydrobiotic juveniles in artificial tap water before exposure to 1% osmium tetroxide in 0·2 M sodium cacodylate buffer (pH 7·2) for 1 h.

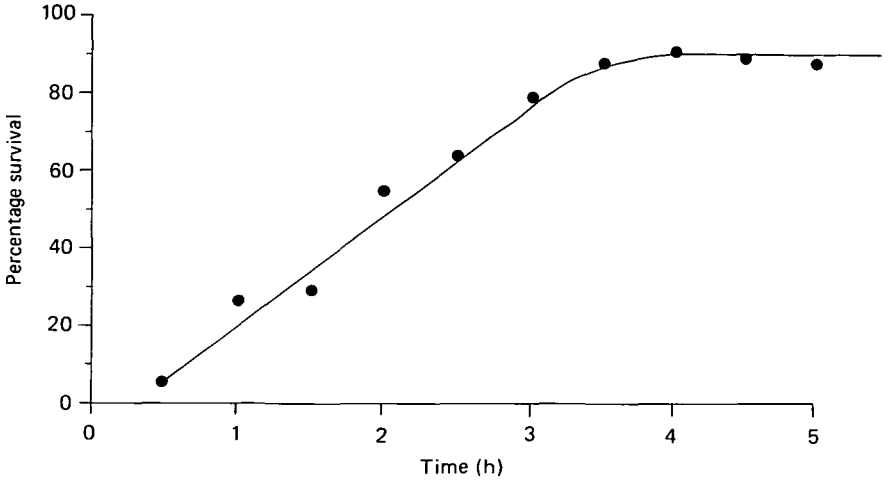

Fig. 2. Survival of *Ditylenchus dipsaci* 4th-stage juveniles after various periods of rehydration of anhydrobiotic juveniles in artificial tap water before exposure to 0% R.H., 22 °C for 24 h, followed by a second rehydration under similar conditions for 3 h.

the rehydration period (Fig. 3). Juveniles which had been rehydrated for periods between 0·5 and 1·5 h showed little ability to control water loss upon subsequent exposure to desiccation. Juveniles rehydrated for 2·5 h or longer were more able to control subsequent water loss, suggesting the re-establishment of a barrier to water loss after between 1·5 and 2·5 h rehydration. These differences do not reflect variations in the initial water content after the various periods of rehydration as water uptake is rapid, and the difference in water content between 0·5 h and 5·0 h is less than 10% (Wharton *et al.* 1985). Analysis of variance, using the refractive index determinations from which water content values are obtained (Ellenby & Perry, 1976), gives a significant F ratio for time ($F = 12{\cdot}66$; D.F. $= 171, 8$; $P < 0{\cdot}01$). Least significant difference tests

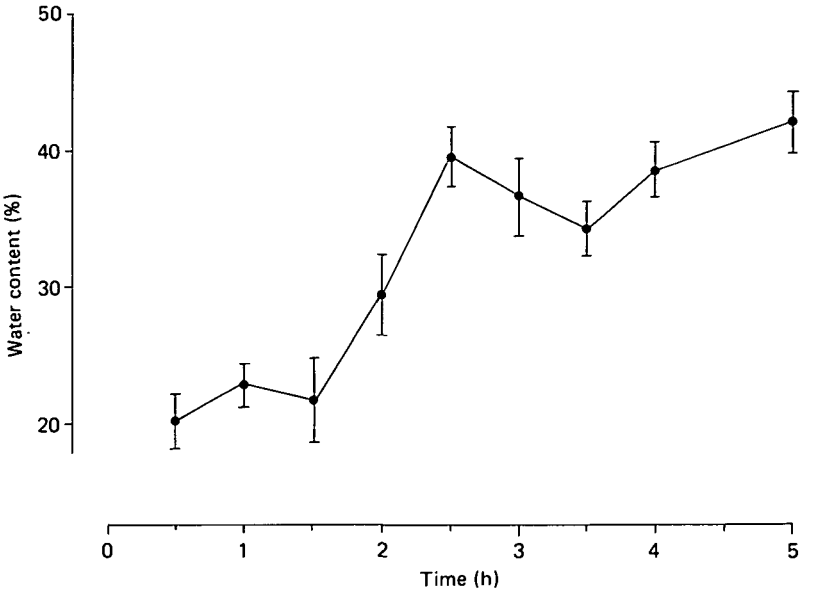

Fig. 3. The water content of *Ditylenchus dipsaci* 4th-stage juveniles after various periods of rehydration of anhydrobiotic juveniles in artificial tap water before exposure to 0% R.H., 22 °C for 5 min. Vertical bars represent the standard error of the mean (n = 8–10).

(LSD = 0·012) show that the results fall into three sections. Means within the first group (0·5–1·5 h rehydration) are not significantly different. Similarly, means within the third group (2·5–5·0 h rehydration) do not differ significantly. However, the overall means for these two groups are significantly different; indicating a change in cuticular permeability. The mean water content after 2 h rehydration and 5 min desiccation was significantly different from those after 1·5 h and 2·5 h rehydration, indicating a change in permeability to water loss during rehydration.

The ^{3}HOH uptake curve of rehydrated *D. dipsaci* 4th-stage juveniles is shown in Fig. 4. Uptake was slow, taking approximately 5 h to reach equilibrium. The permeability coefficient, measured after 5 min, was $4{\cdot}53 \times 10^{-5}$ cm/sec. In contrast, anhydrobiotic juveniles took up water rapidly with a ^{3}H count of $13{\cdot}5 \times 10^3$ d.p.m./mg after 0·5 min exposure to the bathing solution (Fig. 5), giving a permeability coefficient of $2{\cdot}7 \times 10^{-3}$ cm/sec. After the initial rapid uptake the ^{3}H count fell during the first 10 min and then showed a steady increase, not reaching equilibrium even after 3 h exposure. The tritium uptake curve of *P. redivivus* reached equilibrium after 15 min, giving a permeability coefficient of $4{\cdot}96 \times 10^{-4}$ cm/sec, measured after 5 min (Fig. 6).

The permeability coefficients of 4th-stage juveniles changed during rehydration (Fig. 7). The permeability coefficient, measured after exposure to the bathing solution for 5 min, increased during the first 1 h of rehydration; followed by a decline to 20·7% of its maximum value after 24 h rehydration. Most of this decline occurred after 1–2 h rehydration. The initial rise in permeability coefficient may be an artifact resulting from the rapid decrease in permeability during the initial phase of rehydration – from $2{\cdot}7 \times 10^{-3}$ cm/sec after 0 min rehydration, 0·5 min exposure to label to $1{\cdot}498 \times 10^{-4}$ cm/sec after 15 min rehydration, 5 min exposure to label.

In 4th-stage juveniles exposed to increasing temperatures a sharp increase in permeability coefficient was observed at 40–50 °C, from $0{\cdot}402 \times 10^{-4}$ cm/sec at 40 °C to

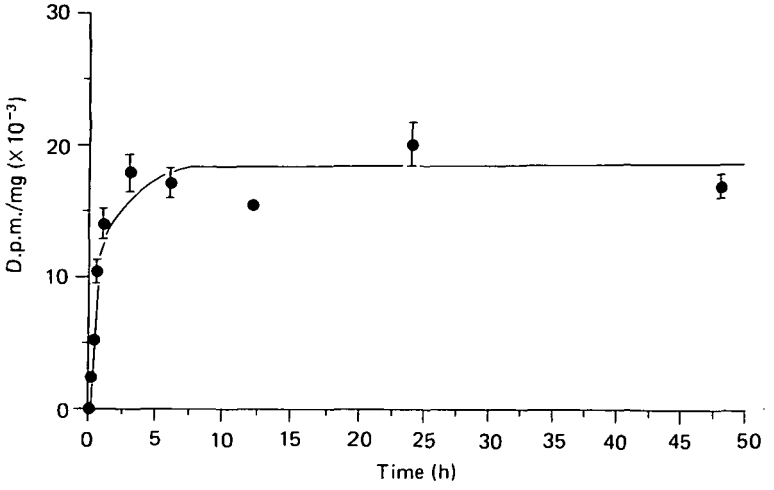

Fig. 4. ^{3}HOH uptake curve of rehydrated *Ditylenchus dipsaci* 4th-stage juveniles. Vertical bars represent the standard error of the mean ($n = 6$).

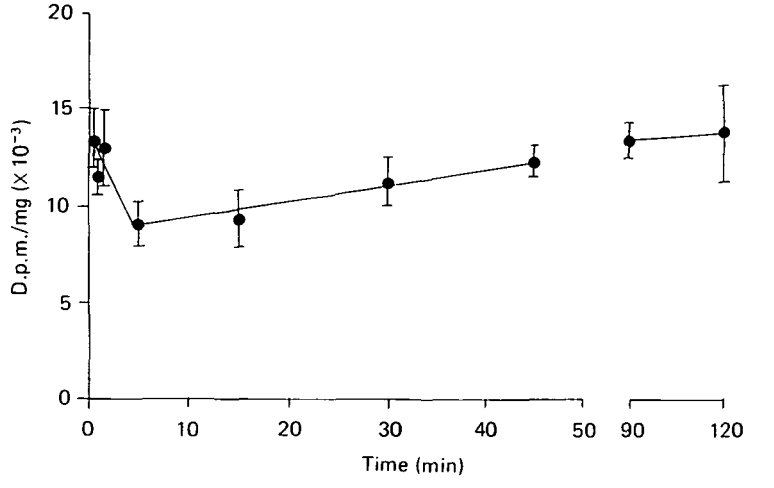

Fig. 5. ^{3}HOH uptake curve of anhydrobiotic *Ditylenchus dipsaci* 4th-stage juveniles. Vertical bars represent the standard error of the mean ($n = 6$).

$1{\cdot}614 \times 10^{-4}$ cm/sec at 50 °C, indicating a phase change in the cuticular lipids (Fig. 8). A further increase was observed at 55–70 °C, perhaps as a result of the death of the nematodes at this temperature.

The effect of metabolic inhibitors on the maintenance of the cuticular permeability barrier is shown in Fig. 9. Rotenone, CMB, puromycin and cycloheximide had little effect on cuticular permeability, whereas treatment with SIA resulted in a significant change of permeability (t test: $t = -13{\cdot}4$, D.F. $= 14$, $P < 0{\cdot}001$) doubling the permeability coefficient compared to that of fully hydrated (24 h) controls. Exposure to diethylether for 5 sec resulted in an increase in permeability coefficient compared to that of hydrated controls ($t = -22{\cdot}28$, $P < 0{\cdot}001$) and was not significantly different to desiccated (0 min) controls ($t = -0{\cdot}493$, $P > 0{\cdot}1$). In contrast, incubation with chloro-

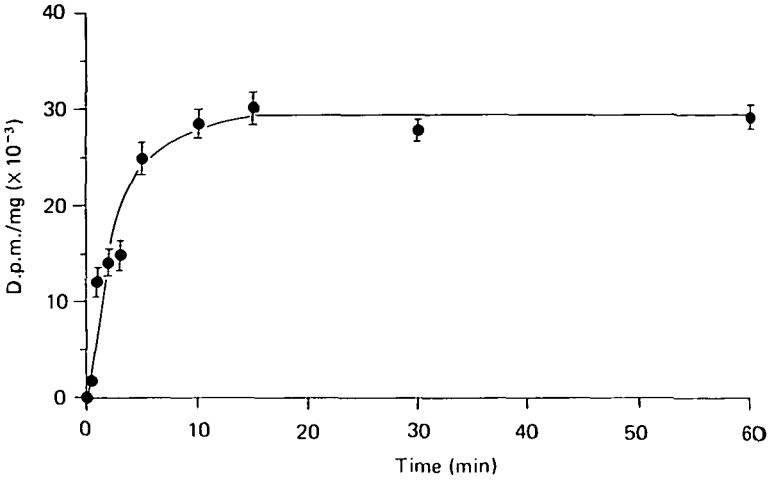

Fig. 6. ^{3}HOH uptake curve of hydrated *Panagrellus redivivus*. Vertical bars represent the standard error of the mean ($n = 6$).

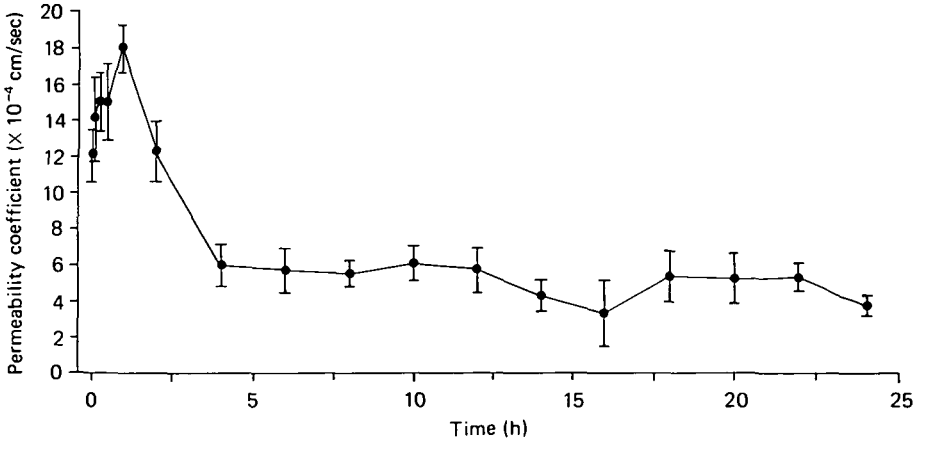

Fig. 7. Changes in cuticular permeability coefficient of *Ditylenchus dipsaci* 4th-stage juveniles during recovery from anhydrobiosis. The permeability coefficient was measured by a 5 min pulse label of ^{3}HOH/^{14}C-inulin after various periods of rehydration of anhydrobiotic juveniles in artificial tap water. Vertical bars represent one standard deviation ($n = 8$).

form/methanol had little effect on cuticular permeability, the permeability coefficient values being close to that of fully-hydrated controls (Fig. 9).

All the inhibitors reduced the ability of *D. dipsaci* 4th-stage juveniles to re-establish the cuticular permeability barrier during rehydration (Fig. 10). Rehydration in the presence of inhibitors resulted in permeability coefficients that were significantly higher than the ATW (24 h) control (rotenone: $t = -3{\cdot}56$, $P < 0{\cdot}01$; CMB: $t = -9{\cdot}81$, $P < 0{\cdot}001$; SIA: $t = -8{\cdot}99$, $P < 0{\cdot}001$; puromycin: $t = -18{\cdot}41$, $P < 0{\cdot}001$; cycloheximide: $t = -12{\cdot}46$, $P < 0{\cdot}001$). The effect of rotenone, however, was considerably greater when the permeability coefficient was measured after incubation in the presence of the inhibitor for 2 h (Fig. 11).

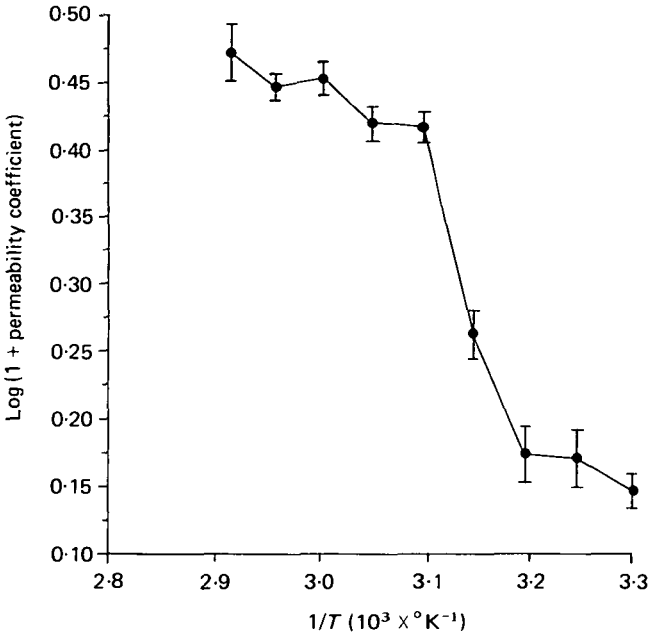

Fig. 8. Arrhenius plot of cuticular permeability of *Ditylenchus dipsaci* 4th-stage juveniles. Vertical bars represent one standard deviation ($n = 8$).

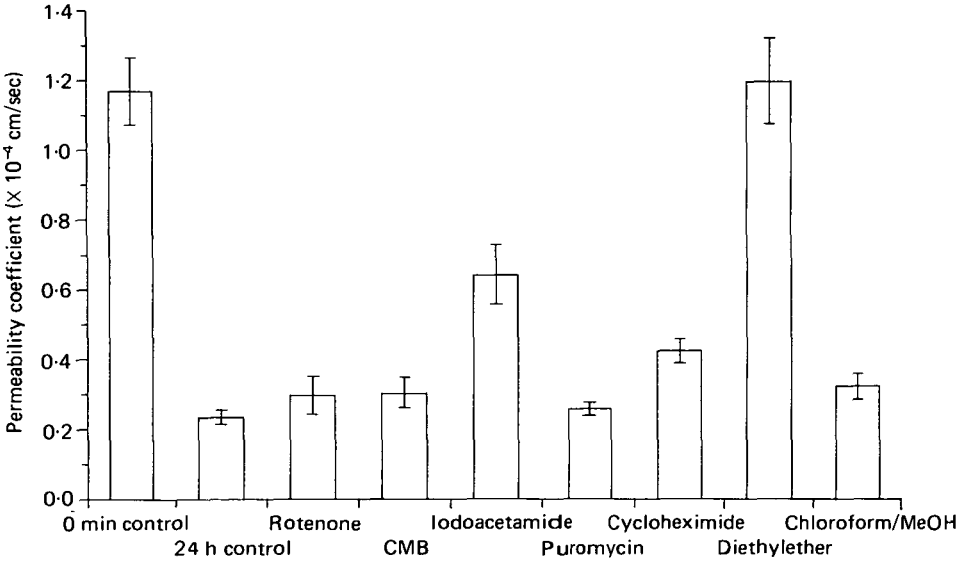

Fig. 9. Effect of inhibitors on the maintenance of the cuticular permeability barrier of *Ditylenchus dipsaci* 4th-stage juveniles. Anhydrobiotic juveniles were rehydrated in artificial tap water for 24 h, exposed to inhibitor for 30 min, washed in artificial tap water and the permeability coefficient measured by a 5 min pulse label of $^{3}HOH/^{14}C$-inulin. CMB, chloromercuribenzoate. Exposure to diethyl ether and chloroform/methanol was for 5 sec. Vertical bars represent one standard deviation ($n = 8$).

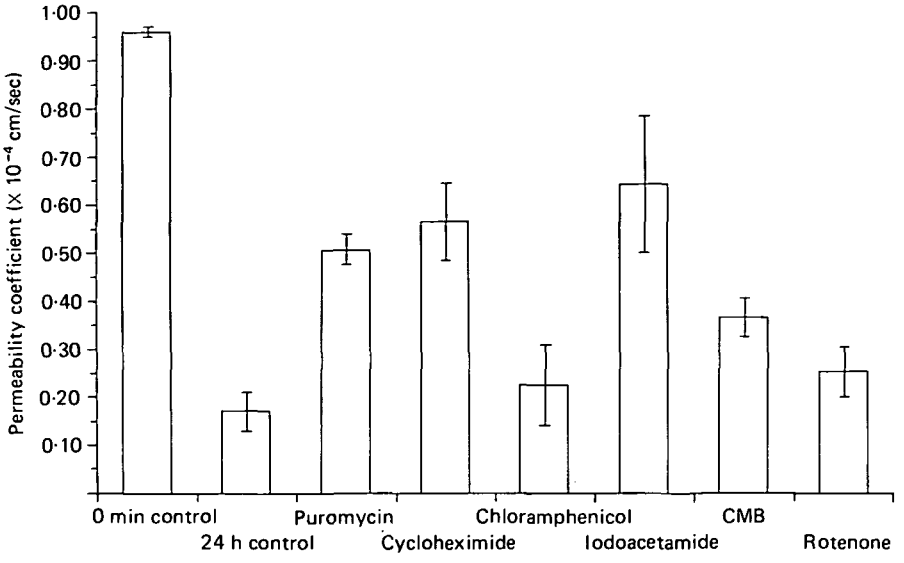

Fig. 10. Effect of inhibitors on the re-establishment of the cuticular permeability barrier of *Ditylenchus dipsaci* 4th-stage juveniles. Anhydrobiotic juveniles were rehydrated in artificial tap water for 24 h containing various metabolic and protein synthesis inhibitors and the permeability coefficient measured by a 5 min pulse label of $^{3}HOH/^{14}C$-inulin. CMB, chloromercuribenzoate. Vertical bars represent one standard deviation ($n = 8$).

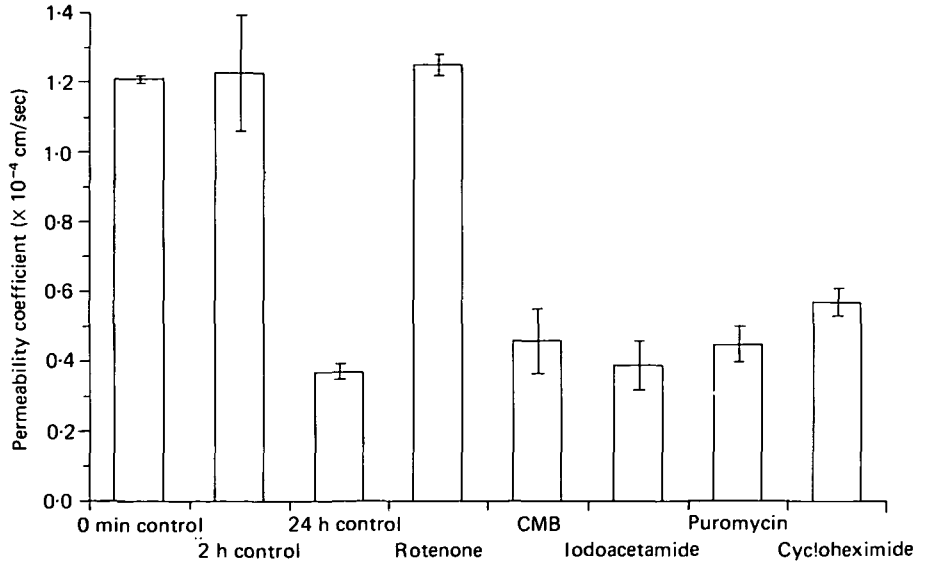

Fig. 11. Effect of inhibitors on the re-establishment of the cuticular permeability barrier of *Ditylenchus dipsaci* 4th-stage juveniles. Details as for Fig. 10 but rehydrated in artificial tap water containing inhibitors for 2 h. Vertical bars represent one standard deviation ($n = 4$).

DISCUSSION

Staining with osmium tetroxide, the survival of subsequent desiccation, the ability to control water loss and the measurement of permeability coefficients by ^{3}HOH uptake

all indicate that the cuticular permeability of *D. dipsaci* 4th-stage juveniles decreases during rehydration of anhydrobiotic juveniles. The initial decrease in permeability coefficient is rapid from $2{\cdot}7 \times 10^{-3}$ cm/sec after 0 min rehydration, 0·5 min exposure to label, to $1{\cdot}498 \times 10^{-4}$ cm/sec after 15 min rehydration, 5 min exposure to label (a fall of 94·5%); suggesting a physical effect due to the rehydration of the cuticle. This is followed by a slower decline after 1–4 h rehydration of a further 79·3% decrease in permeability coefficient. Osmium staining and the ability to control the rate of water loss also indicate a decrease in cuticular permeability after 1–2 h rehydration. The increased ability to survive subsequent desiccation after various periods of rehydration also suggests a decrease in cuticular permeability; since a slow rate of water loss is important in the ability to survive desiccation (Perry, 1977*a*). A similar decrease in cuticular permeability during rehydration has been observed in the 2nd-stage infective juvenile of *Anguina agrostis* (Preston & Bird, 1987).

During the initial phases of rehydration anhydrobiotic juveniles are inactive and only resume activity after a lag phase of 2–3 h (Wharton *et al.* 1985). Metabolic activity during the lag phase, however, rapidly returns to normal levels (Barrett, 1982). Metabolic inhibitors limit the reduction in permeability coefficient. These observations suggest that the decrease in cuticular permeability is divided into two phases: firstly a physical effect due to the rehydration of the cuticle and secondly a metabolic phase during which, presumably, some sort of repair of the permeability barrier occurs. Dead *D. dipsaci* 4th-stage juveniles are freely-permeable to water both during rehydration and exposure to desiccation (Perry, 1977*b*), suggesting metabolic involvement in maintaining the cuticular permeability barrier.

The re-establishment of the cuticular permeability barrier is dependent upon the operation of metabolic pathways. An indication of a requirement for protein synthesis was suggested by a higher permeability coefficient following incubation with puromycin and cycloheximide. Both of these compounds act at the translational level of protein synthesis, suggesting that the disruption of post-transcriptional control of cuticle protein synthesis has an adverse effect on the re-establishment of the cuticular permeability barrier or of the enzymes involved in membrane repair. The greater effect of these inhibitors when present at the beginning of rehydration may be due to greater uptake into the nematode as a result of higher cuticular permeability. The slightly greater effect of cycloheximide compared with puromycin may reflect the specificity of the former in inhibiting protein synthesis in eukaryotes only, whereas puromycin inhibits both pro- and eukaryotic systems. Transcriptional inhibitors were not investigated in this study as collagen synthesis in nematodes is known to be unaffected by inhibitors of RNA synthesis (Pasternak & Leushner, 1975).

Rotenone, CMB and SIA all had significant effects on the re-establishment of the cuticular permeability barrier. Rotenone blocks NADH dehydrogenase and CMB and SIA are general enzyme inhibitors. The effect of rotenone was considerably greater when applied after 2 h than after 24 h of rehydration. This could be due to the higher permeability of the cuticle at the start of the rehydration period, allowing more rotenone to penetrate to internal target sites, or the lesser effect in a 24 h incubation may reflect the action of detoxification systems capable of inactivating the inhibitor taken into the nematode during the early permeable phase. Despite the effect of inhibitors on the re-establishment of the cuticular permeability barrier, the anhydrobiotic nematodes can recover normally in the presence of inhibitors of protein and RNA synthesis (Barrett, 1982), suggesting that repair of the cuticle is not essential for the initial resumption of activity. There are no reports of rotenone, CMB or SIA having

Table 1. *Permeability coefficients of various nematodes*

Species	Habitat	Desiccation survival	Permeability coefficient (cm/sec)	Reference
Ditylenchus dipsaci	Plant parasite*	Good	$4{\cdot}53 \times 10^{-5}$	Present work
Panagrellus redivivus	Free-living	Poor	$4{\cdot}96 \times 10^{-4}$	Present work
Anguina agrostis	Plant parasite*	Good	$1{\cdot}27 \times 10^{-4}$	Preston & Bird (1987)
Enoplus brevis and *E. communis*	Marine	Poor?	$3{\cdot}4 \times 10^{-4}$	Wright & Newall (1980)
Aphelenchus avenae	Free-living	Good	$1{\cdot}3 \times 10^{-6}$	Wright & Newall (1980)
Caenorhabditis briggsae	Free-living	?	$2{\cdot}0 \times 10^{-6}$	Wright & Newall (1980)
Nippostrongylus muris	Animal parasitic*	?	$6{\cdot}1 \times 10^{-6}$†	Wright & Newall (1980)
Ancylostoma caninum	Animal parasitic*	?	$0{\cdot}8 \times 10^{-6}$†	Wright & Newall (1980)
Haemonchus contortus	Animal parasitic*	Good	$1{\cdot}11 \times 10^{-5}$	Calculated from Davey & Rogers (1982)

* Infective juvenile. † Estimated. ? Unknown.

direct effects on membrane permeability (Webb, 1963), but this possibility cannot be entirely discounted.

SIA was the only inhibitor which had a significant effect on the maintenance of the cuticular permeability barrier, doubling the permeability coefficient compared to that of controls. It was not possible to determine if these effects were due to different metabolic actions or to the differential penetration of the inhibitors into the nematodes. Rotenone does not inhibit mitochondrial enzymes of *Panagrellus redivivus in vivo* but will inhibit *in vitro* systems (D. Burke and M. R. Samoiloff, unpublished observations), whereas SIA readily penetrates tissues.

A restricted cuticular permeability may be partly responsible for the slow rate of water loss which is essential for nematodes to survive desiccation. The permeability coefficient of rehydrated *D. dipsaci* is approximately one tenth of that of the desiccation-susceptible nematode *P. redivivus*. Permeability coefficients have only been measured in a few species (Table 1). Desiccation-susceptible species do tend to have high cuticular permeabilities (*P. redivivus*, *Enoplus brevis*, *E. communis*), whereas desiccation-resistant species have low cuticular permeabilities (*D. dipsaci*, *A. agrostis*, *Aphelenchus avenae*, *Haemonchus contortus*). The desiccation-survival abilities of *Caenorhabditis briggsae*, *Nippostrongylus muris* and *Ancylostoma caninum* are not known; although their low permeability coefficients suggest that they would be desiccation resistant. The permeability coefficients of *N. muris* and *A. caninum* were, however, estimated from the pulsation rate of the excretory ampulla. This may give an underestimate of water flux across the cuticle.

The effects of temperature and organic solvents suggest the involvement of a lipid layer in the cuticular permeability barrier. A sharp increase in permeability coefficient was observed in 4th-stage juveniles incubated at 40–50 °C compared to room tempera-

ture controls. Similar 'transition' or 'critical' temperature phenomena in arthropods have been interpreted as being due to a change in phase of the cuticular lipids (Edney, 1977); although Gilby (1980) has suggested that this is an artifact due to the failure to measure the cuticle temperature accurately. The further increase in permeability coefficient at 55–70 °C may have been due to the death of the nematodes. Searcy, Kisiel & Zuckerman (1976) found no difference between the permeability of live and heat-killed *Caenorhabditis briggsae*. *D. dipsaci* 4th-stage juveniles, however, become permeable to 1% acid fuchsin after heat treatment (Wharton, unpublished observations) and the selective permeability of the cuticle of *Aphelenchus avenae* is lost after heat treatment or killing with potassium cyanide (Marks, Thomason & Castro, 1968). Dead *D. dipsaci* 4th-stage juveniles are freely-permeable to water (Perry, 1977*b*).

Exposure to diethyl ether for 5 sec resulted in an increase in permeability coefficient compared to that of fully-hydrated controls. In contrast, incubation with chloroform/methanol had little effect on permeability; the permeability coefficient being close to that of fully hydrated controls. The different effects observed with these two solvent systems suggests that the lipid component which was extracted was relatively non-polar, for example sterol esters or long chain hydrocarbons. Lysophospholipids are also known not to be readily extracted with chloroform/methanol (Christie, 1982). The movement of non-polar solutes into or out of isolated cuticle preparations of *Ascaris suum* and into intact *A. suum* and *H. contortus* infective juveniles also indicates the presence of a lipoidal barrier to diffusion (Fetterer, 1986).

The brief exposure to diethylether required to disrupt the cuticular permeability barrier implies that the lipid layer is superficial and resides in the cuticle. The epicuticle is lipoprotein in composition and is the outermost layer of the cuticle, providing the interface between the nematode and the environment. It is likely to be the main barrier to diffusion in the cuticle. Differences in the ultrastructure of the epicuticle of anhydrobiotic and rehydrated *D. dipsaci* 4th-stage juveniles have been observed (Wharton & Barrett, 1985) and may reflect repair of the permeability barrier. There has been some controversy in recent years regarding the nature of the epicuticle. Some authors consider it to be a highly modified cell membrane, whilst others argue that it is an extracellular envelope with little in common with a cell membrane (Bird, 1987). Whatever its nature, there is evidence that the epicuticle is physiologically active and is not just an inert covering. Shedding of antibody complexes to surface antigens implies that the material forming the epicuticle is continually being replaced, at least in some nematodes (Wharton, 1986). The epicuticle can also rapidly respond to the adhesion of microorganisms (Bird, 1987) and changes in its ultrastructure are associated with entry into and recovery from anhydrobiosis (Bird & Buttrose, 1974; Wharton & Barrett, 1985) and with the formation of dauer larvae (Bird, 1980). There is, however, no loss of surface label from adults and 4th-stage juveniles of *Brugia pahangi* (Marshall & Howells, 1986) and although there is a turnover of label in 3rd-stage juveniles, the label may not be confined to the epicuticle. Fluorescence recovery after photobleaching suggests that the epicuticular lipids of *Trichinella spiralis* and *Toxocara canis* have an extremely limited lateral mobility, at least over short time-scales (Kennedy, Foley, Kuo, Kusel & Garland, 1987).

Changes in the permeability coefficient of *D. dipsaci* 4th-stage juveniles provide further evidence for the dynamic nature of the epicuticle. The action of metabolic inhibitors suggest that it is repaired by underlying cells; presumably by the hypodermis. This repair occurs over a fairly long time-scale. Anhydrobiotic nematodes provide a system in which the cuticular permeability barrier is damaged by desiccation

and repaired during rehydration. They should therefore prove useful models for the study of how this important function of the nematode cuticle is maintained.

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