The Water Relations and Cuticle of *Paradesmus gracilis* (Diplopoda, Strongylosomidae)

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SUMMARY

Water-loss in *Paradesmus gracilis* depends upon temperature and humidity, and is directly related to saturation deficiency. There is no evidence of any 'critical point' to indicate an epicuticular wax layer. Water is readily lost and taken up through the cuticle, the effect of the spiracles and of excretion being negligible. Despite great sensitivity to desiccation, there is nevertheless some degree of impermeability.

The cuticle is in many ways similar to that of an insect: it is composed of a 'cuticulin' epicuticle, a 'tanned' chitinous exocuticle, and a laminated endocuticle of two optically distinguishable layers. The outer endocuticle is strongly calcified. The cuticle is penetrated by pore canals and the ducts of dermal glands. The latter are concerned with the production of exo- and endocuticle, and the secretion of the polyphenols which tan the protein of the exocuticle. The double hardening is probably a specialized condition of millipedes.

Transpiration is almost quadrupled by extraction with hot, but not cold chloroform, as the exocuticle is impregnated with lipoids which reduce permeability. These are secreted by epidermal and dermal gland cells, and pass up the pore canals and gland ducts.

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INTRODUCTION

Investigation of their sensory physiology and behaviour indicates that millipedes are extremely susceptible to the effects of desiccation, and that moisture is to them the most important environmental factor. The physiology of their water relations has therefore been considered in relation to the histology of their cuticle.

The integument of Arthropoda has been the subject of much recent research, and the extensive literature concerning the insect cuticle has been...
reviewed by Wigglesworth (1948b). The other classes have not been neglected, and recent work such as that of Yonge (1932), Drach (1939), and Dennell (1947a, b) emphasize the similarity of the integument of all arthropod classes. It consists normally of three layers, the most convenient terminology for which is that proposed by Campbell (1929) who refers to them as 'epicuticle', 'exocuticle', and 'endocuticle'.

The myriapod cuticle, however, has as yet been little investigated. The early accounts of Verhoeff (1902–25, 1926–8) do not include precise histochemical details, whilst that of Langner (1937) is also not comparable with recent work in other groups. Lafon (1943) distinguished two types of exoskeleton: 'organic' (Insecta, Arachnida, Chilopoda) and calcareous (Crustacea, Diplopoda). He did not believe that two types could occur in the same animal; but Dennell (1947b) has since demonstrated the presence of phenolic tanning in Decapod Crustacea, and established the homology of the crustacean cuticle with that of insects; while Hackman, Pryor, and Todd (1948) showed by means of the argentaffin reaction that phenolic substances occur in the cuticle of the Diplopod Tachypodoiulus niger. Edney (1949) demonstrated the absence of a wax layer in the cuticle of Glomeris, and Cloudsley-Thompson (1950) showed that an epicuticle of 'cuticulin' is present in both Diplopoda and Chilopoda.

Blower (1949), who has investigated the histology of the integument of Chilopoda and Diplopoda, has been kind enough to let me see his unpublished manuscript. He concludes that the cuticle in both groups is similar in that it consists of two optically distinguishable layers: an outer homogeneous, highly refractile, and usually pigmented exocuticle which appears to be a product of sclerotization as in insects, and an inner colourless, laminated endocuticle. Both layers are basically composed of chitin, and no evidence was obtained of an outer, non-chitinous epicuticle.

The principal occupants of the adult integument are the dermal gland cells which send ducts through the cuticle, opening on to the surface. These glands secrete the lipoid material which impregnates the exocuticle, where presumably it undergoes some change which reduces its staining properties with fat stains. It was pointed out that, despite the presence of lipoid materials in their cuticle, myriapods are very susceptible to desiccation.

According to Blower, therefore, the cuticle of myriapods could, in some respects, be compared with that of the spider, Tegenaria atrica, in which Browning (1942) claims that there are but two layers of the integument: an exocuticle and an endocuticle which resemble the impregnated and non-impregnated cuticle of Rhodnius where variation in hardness is accompanied by the presence or absence of impregnation rather than by relative thickness. Browning found not only no epicuticle, but no pore canals, and no evidence of lipoids or waxes in the integument of spiders. He presumed that the exocuticle serves the function of the epicuticle at least in regard to permeability and desiccation. It has since been shown, however, that a 'cuticulin' epicuticle is found in most, if not all, arthropods (including spiders) whether additional
Cuticle of Paradesmus

epicuticular layers are present as in insects (Wigglesworth, 1948b), and ticks (Lees, 1947), or not (Cloudsley-Thompson, 1950).

TEMPERATURE AND EVAPORATION FROM THE CUTICLE

Preliminary Experiments

A number of living Paradesmus gracilis (Koch) were placed in an arena, the floor of which was composed of zinc gauze covered with fine voile. Sulphuric acid mixtures and distilled water were used to control the humidity at 50, 90, 20°C.

![Text-fig. 1. Relationship between water-loss and temperature and humidity.](image)

and 100 per cent. respectively (Buxton and Mellanby, 1934) at temperatures of 20° C. and 25° C. The millipedes were weighed at hourly intervals, and some of the results are given in Text-figs. 1 and 2. They show that water-loss is dependent not only upon the temperature and humidity at which the experiments were carried out, but also upon the initial weight and therefore surface area of the millipedes, from which it is inferred that evaporation takes place through the cuticle as well as through the spiracles. It is important to notice that the rate of water-loss remains almost constant from the outset of the experiment until it ceases altogether when the animals have lost about 50 per cent. of their weight and are completely desiccated. Possible differences in the initial state of the water-balance of various individuals do not therefore affect their rate of water-loss. Some slight decrease is to be expected under the influence of depletion of water in the body, as in Agriotes larvae (Wigglesworth, 1945), and in non-living membranes (King, 1944). This is usually very small, and is not apparent until the final stages of desiccation.
are reached. The sensitivity of the animals to desiccation is indicated by the fact that they lose weight even over a bath of distilled water.

The fact that no significant loss in weight was noted in 10 *Paradesmus* which were kept in a medium of damp asbestos wool for 10 days, during which time a colourless liquid was excreted in place of the usual black faecal pellets, suggests that the effect of starvation is negligible.

![Text-fig. 2. Relationship between rates of water-loss and relative humidity at different temperatures.](image)

**Effect of Temperature on Transpiration**

The rate of water-loss per hour in dry air was ascertained by suspending weighed millipedes over anhydrous phosphorus pentoxide in conical flasks immersed in a water bath; the rate of loss being expressed as mg./cm.²/hr.

Estimation of effective surface is even more difficult in a millipede than in an insect; for not only are there innumerable fine irregularities, but the cuticle is calcified and cannot be spread out on squared paper. Instead, the relation between the width of the dorsal surface and that of the remaining lateral and ventral surfaces was measured on an enlarged photograph of a transverse section, by means of a map measurer (= 0.035). From this, the total surface area (S) was obtained by measuring the length and breadth of a millipede of known weight, and calculating the approximate surface area of the 62 legs. From this figure the value of k in the formula $S = k \times W^{3/4}$ was found, and the surface area of any other member of the same species could be calculated from its weight (W). The value of k employed = 15, which compares with *Agriotes* larva, 11.0; *Pieris* larva, 9.8; and *Tenebrio* larva, 8.4 (Wigglesworth, 1945). The value of k need be only approximate because specific differences in evaporation are so great even between different species.
of insects, that an error of 50 per cent. in the value of $k$ will not affect the conclusions drawn (Wigglesworth, 1945).

The results obtained are shown in Text-fig. 3, where water-loss per hour has been plotted against temperature. Each point represents a mean value of four individuals. At temperatures over 50°C the water-loss was measured over a period of $\frac{1}{2}$ hour only. Loss from an open water surface, and from *Pieris* larvae (from Wigglesworth, 1945) are added for comparison. They indicate that although *Paradesmus* is very susceptible to desiccation when compared with insects, the cuticle nevertheless possesses a certain degree of impermeability. No significant difference was noted between the water-loss of recently moulted, pale and more mature, darker individuals.

The absence of a critical point, and therefore of a wax layer, is seen even more clearly when water-loss is considered in relation to temperature and saturation deficiency (Text-fig. 4), where figures for *Glomeris* (from Edney, 1949) and *Pieris* larva (based on Wigglesworth's 1945 data) are added for purposes of comparison. The direct linear relationship is clear evidence of the absence of any discrete wax layer showing a critical temperature effect.

**Water Excretion and Uptake**

**Effect of spiracles.** In view of the impracticability of blocking the spiracles in the experiments outlined above, not only on account of their large numbers but because of the hard and shiny surface of the cuticle, it was thought possible that this difficulty might be overcome by carrying out experiments on living millipedes both in air and in an atmosphere containing a proportion
of carbon dioxide gas. Wigglesworth (1935) showed that in insects, concentrations of $CO_2$ above 2 per cent. cause the spiracles to remain permanently open; and it seemed possible that below the critical point of a wax layer, if present, and at all temperatures if not, animals in flasks containing $CO_2$ would lose water more rapidly than controls. No difference was noted, however, after forty comparisons; and it was concluded that, if the spiracles do respond to carbon dioxide, the water-loss takes place so readily through the cuticle at all temperatures that the effect of the spiracles is negligible. Removal of the legs caused a marked increase in the rate of water-loss.

Effect of Excretion. Under normal conditions millipedes excrete moist black faecal pads. When kept under conditions of starvation, a colourless liquid is excreted. At higher temperatures they may be observed drinking from droplets of condensed water. When the mouth, anus, or both were blocked with cellulose paint, the gain or loss in weight per sq. cm. of surface area on alternate exposure to moist, and to dry surfaces in dry air, did not differ significantly from that of control animals. It was concluded, therefore, that although millipedes may drink and normally excrete water, moisture is absorbed and lost so readily through the cuticle that the effect of the former is masked.
THE STRUCTURE OF THE CUTICLE

General

The sclerites of millipedes are dorsally telescoped into one another when the animals are extended, but are placed more or less end to end when in spiral reflex. Consequently in transverse sections, the posterior half (metazonite) of one segment is seen to lie outside the prozonite of the succeeding segment, and the arthrodial membrane can be seen connecting the two. Hairs may be present, and are particularly numerous in Polyxenidae (Text-fig. 7).

The cuticle of a diplopod as seen in transverse section consists of two layers: an outer, homogeneous highly refractile exocuticle, and an inner colourless laminate endocuticle. The endocuticle consists of two regions which can be distinguished optically and chemically: an outer, translucent layer with closely spaced, ill-defined horizontal striae, which is impregnated with calcium salts (Lafon, 1943); and an inner transparent and slightly refractile layer with fewer conspicuous, well-spaced horizontal striae (Blower, 1949).

Langner (1937), too, describes two endocuticular layers: an outer 'Platten-schicht', and an inner 'Balkenschicht', and Verhoeff (1902–25) mentioned an endocuticle of two layers. Blower (1949) suggests that the presence of an outermost 'epicuticle' (Grenzhautchen) as recorded by Fuhrmann (1921) and Verhoeff (1902–25) in Chilopoda, and by Langner (1937) in Diplopoda, based on staining reactions and appearance alone, can be attributed to optical effects and possibly a difference in the extent of sclerotization.

Histological methods. Millipedes are difficult to section on account of their extremely hard, calcareous cuticle, and soft internal anatomy. Satisfactory results were obtained, however, using ester wax. The animals were chloro-formed for a few seconds, straightened with needle and brush, and placed in Henning's fixative. After 24 hours they were transferred to 70 per cent. alcoholic iodide, and left overnight. Next they were washed thrice in 90 per cent. alcohol, and placed in cellosolve. The following day they were washed twice in cellosolve, and left in an oven at 50°C. in a mixture of 50 per cent. cellosolve, 50 per cent. ester wax. Next morning they were transferred to ester wax which was replaced in the evening. After a week in this, they were embedded, cut in 8μ serial sections, and stained with Mallory's triple dye. In other cases normal wax sections were cut, but this involved considerable wastage.

Dermal gland cells. In Paradesmus, gland cells are more numerous where the cuticle is thicker, for example, at the lateral carinae (Text-fig. 5). In ventral areas the exocuticle is very thin or absent, and the gland cells and ducts are present in very small numbers, or else absent too. The cuticle of Glomeris is much thicker than that of Paradesmus (50μ instead of 20μ approx.), and the dermal gland ducts can be seen in great numbers (Text-fig. 6). Owing to pigmentation, their path cannot easily be seen through the exocuticle, but they appear to project beyond this, presumably on account of excreted materials deposited at their openings.
Pore canals. Blower (1949) was unable to distinguish pore canals because these are usually not visible in histological sections. They can readily be seen, however, in air-dried wax sections, in frozen sections, and occasionally in sections stained with Heidenhain's iron haematoxylin, and are shown in Text-figs. 5, 6, and 7, leading from the epidermal cells to the outer surface of the exocuticle.

Experimental

Effect of liquid paraffin. Liquid paraffin has a similar effect on the cuticle of millipedes as of insects (Hurst, 1940; Wigglesworth, 1941, 1942). Within

15 minutes of submersion, small droplets of water appeared all over the antennae of Blaniulus, and after 20 minutes all parts of the body were covered. An hour and a half later, the animal was completely coated with fine bubbles. The same process occurred, but more slowly and to a lesser degree, in Paradesmus. The oil presumably dissolves in the lipoids which impregnate the cuticle, and so displaces the water in its deeper layers.

Inert dust abrasives. The rate of water-loss per sq. cm. at various temperatures did not differ significantly from that of controls in 28 Paradesmus which had been kept in an abrasive medium of damp alumina dust for several days (cf. Rhodnius; Wigglesworth, 1945). Hence there can be no epicuticular wax layer to be abraded.

Argentaffin reaction. Both in controls and in millipedes rubbed with alumina dust, the only parts stained black were the openings of the ducts of the dermal glands. Blower (1949) obtained similar results, and explained them by suggesting that the outer layer of the exocuticle is inert 'sclerotin' which protects a reactive region of incipient 'pro-sclerotin'. However, polyphenols frequently do not reduce silver oxide solution unless they are fresh, except in sections where a vastly increased reactive surface is exposed. Similar results
are obtained in the epicuticle of newly moulted insects before tanning has taken place (Wigglesworth, 1947), but in this case the polyphenol layer has not yet been formed. In sections the exocuticle only can be seen to reduce 10 per cent. silver oxide: the epicuticle cannot be distinguished. Hackman, Pryor, and Todd (1948) obtained similar results with Tachypodoiulus although they designated the exocuticle as ‘epicuticle’.

**Effect of wax solvents.** Although no epicuticular wax layer has been demonstrated in *Paradesmus*, it was thought possible that the lipoids which evidently impregnate the exocuticle might be removed with chloroform, which is a good general solvent for oils and waxes. Batches of four millipedes were exposed to chloroform vapour and extracted with chloroform at different temperatures before their rate of water-loss at 30° C. was measured. Half an hour was allowed to elapse after extraction to ensure that every trace of chloroform had evaporated, before weighing. The results are summarized in Table I. Comparative figures for *Rhodnus*, calculated from Wigglesworth’s (1945) data, suggest that the wax is more easily removed from the cuticles of insects than of millipedes.

**Chitosan test.** This was positive to exocuticle and endocuticle of *Paradesmus gracilis*.

**Lipoid colouring-agents.** A number of *Paradesmus* were embedded in gelatine using Baker’s method (Pantin, 1946); sectioned with a freezing microtome, and coloured with osmium tetroxide, or sudan black and Mayer’s carmalum. There was some evidence that the pore canals and epidermal gland ducts took up the stain. Most of the exocuticle appeared to be stained also. No results were obtained with sudan IV.
TABLE I. Loss of Water in mg./cm.²/hr. at 30° C. from Paradesmus and Rhodnius

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<th>Paradesmus</th>
<th>Rhodnius</th>
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<td>A. Dead, normal controls</td>
<td>3.0</td>
<td>0.0001</td>
</tr>
<tr>
<td>B. After exposure to chloroform vapour for 1 hour at 20° C.</td>
<td>3.0</td>
<td>0.0009</td>
</tr>
<tr>
<td>C. After extraction with chloroform for 15 min. at 20° C.</td>
<td>3.0</td>
<td>0.13</td>
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<tr>
<td>D. After extraction with chloroform for 15 min. at 50° C.</td>
<td>10.7</td>
<td>1.7</td>
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Blower (1949) also found some evidence that in Schizophyllum the dermal gland ducts, and the epidermal cells in the neighbourhood of the dermal glands, were stained. He believed that lipoids passed up the gland ducts. Langner (1937) claimed that the epidermal glands and their ducts stained with sudan III in Sphaerothrix. She did not record any staining of the exocuticle, but Blower suggests that perhaps this was because sudan III is less efficient than sudan B.

**Sodium pyrogallate.** The presence of calcium was ascertained by placing frozen sections in alkaline pyrogallol (Lison, 1936). The calcium replaces the sodium ions in situ as insoluble brown calcium pyrogallate. Calcification appeared to be restricted chiefly to the outer endocuticle, a result in agreement with that of Blower (1949). Incineration of sections at 600° C. and the action of dilute mineral acids lend support to this view.

**Concentrated mineral acids.** The calcified outer endocuticle dissolves rapidly in concentrated hydrochloric acid, followed by the inner endocuticle. The
pore canals are more resistant, and their fine filaments take longer to dissolve. The brown exocuticle does not dissolve, probably because it is tanned. It is soluble, however, in chlorated nitric acid, leaving a thin colourless epicuticle which dissolves on heating, forming oily droplets. It is insoluble in thioglucronic acid. An epicuticle has not previously been conclusively demonstrated in myriapods. Similarly results were obtained with Blaniulus and Glomeris. The thickness of the epicuticle does not exceed 1 μ: it is quite invisible in most histological sections, which is why it has not previously been discovered with any certainty (Cloudsley-Thompson, 1950). An epicuticle is easily seen, however, in sections of the large Natal species Doratogonus setosus (Vog.).

Penetration of stains. A simple technique, based on Beament's (1946, 1948, 1949) work, was devised to investigate the presence of wax in the cuticle. Living millipedes were beheaded, and their posterior segments and alimentary canals removed. The posterior ends were sealed with paraffin wax and the body-cavities filled from the head end, by means of a fine waxed pipette, with Delafield's haematoxylin. These openings were then sealed with paraffin wax also, and the animals left in water for 3 days. Other animals were treated in a similar manner but filled with water and left to soak in stain. Both sets were then embedded in wax, and sectioned. In neither case was any part of the cuticle stained. The experiment was repeated after fixing with trichloracetic acid, and it was found that all layers of the cuticle took up the stain, particularly those of the endocuticle.

DISCUSSION

Transpiration experiments show that water-loss depends upon temperature and humidity, being directly related to saturation deficiency. There is no evidence of any 'critical point' which would indicate the presence of an epicuticular wax layer as obtains in insects and ticks; and water is readily taken up and lost through the cuticle, possibly via the pore canals, the effect of the spiracles and of excretion being negligible. Although the sensitivity of the animals to desiccation is so great that they lose weight even over a bath of distilled water, the cuticle nevertheless possesses a certain degree of impermeability.

Histological experiments show that the cuticle of Paradesmus is in many ways similar to that of an insect, and is penetrated by pore canals and the ducts of dermal glands. There is an outer 'cuticulin' epicuticle, an amber-coloured exocuticle, and an endocuticle composed of two optically distinguishable layers, the outer of which is strongly calcified. Both exo- and endocuticle are chitinous: the former contains polyphenols and is 'tanned', as in insects. The contents of the gland ducts reduce silver oxide, suggesting that the dermal glands are concerned with the secretion of polyphenols. At the same time, concentrations of dermal gland cells when the cuticle is thickened indicate that these are also concerned with the secretion of exo- and endocuticle.

Hardening both by tanning of the exocuticle and by calcification of the outer endocuticle explains the extreme hardness of the diplopod integument.
It is possible that the condition is a primitive one since both the two methods of hardening employed by other arthropods are used; but since there is little phenolic tanning in Crustacea it would seem more likely that the millipede condition is a specialized one.

The absence of an epicuticular wax layer is no doubt correlated with the humid environment in which the creatures live. At the same time the cuticle is impregnated with lipoids which reduce permeability to some extent. Transpiration is almost quadrupled by extraction with hot but not cold chloroform, hence these lipoids are more difficult to remove than those of the insect epicuticle. They appear to be secreted both by epidermal and dermal gland cells, and pass up the pore canals and gland ducts. It has not been possible to obtain suitable material for studying the moulting cycle, and thereby proving the above hypothesis.

ACKNOWLEDGEMENTS

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