THE TRANSPIRATION OF TERRESTRIAL ISOPODS

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INTRODUCTION

The transpiration of terrestrial arthropods has been the subject of some attention during recent years. It is evident from the work of Ramsay (1935*b*), Wigglesworth (1945) and Beament (1945) that in insects the effective barrier to evaporation is a thin layer of epicuticular lipoids. In view of the similarity between crustacean and insect cuticles (Pryor, 1940; Dennell, 1947) it would be of interest to determine whether isopod permeability is restricted by similar means.

MATERIALS AND METHODS

Representatives from three different genera were studied, *Oniscus asellus* Linné, *Porcellio dilatatus* Brandt and *Armadillidium vulgare* Latreille. Most of the experiments were carried out with *Oniscus*, and results will refer to this animal unless otherwise specified.

Transpiration rates were determined by suspending animals singly in a desiccation chamber. This was maintained at constant temperature, and relative humidities were controlled with potassium hydroxide solutions (Buxton & Mellanby, 1934). Animals were weighed at regular intervals on a 200 mg. torsion balance. The loss of solid material during the course of an experiment was shown to be negligible compared with the loss of water. Transpiration rates could thus be calculated on the basis of recorded weight loss.

Ramsay (1935*a*) has pointed out that certain sources of error may attend experiments carried out in still air. Tests were made to estimate the magnitude of such errors by comparing transpiration rates obtained in the desiccation chamber with values obtained in a current of air. Since no significant difference could be established it appears that the present results are not critically affected by these errors. Nevertheless, it would be useful to have some standard with which experimental results could be compared. The rate of evaporation from a free water surface was accordingly measured (see Table 1).

The relation between surface area and weight was determined so that transpiration rates could be expressed in terms of permeability.

RESULTS

(1) *The transpiration of isopods*

To establish the general level of permeability in woodlice the transpiration of *Oniscus*, *Porcellio* and *Armadillidium* was determined under different conditions of saturation deficiency. The loss of water from isopods takes place in a very charac-

teristic manner, as shown in Fig. 1, where a few typical transpiration curves are plotted.

There is a marked fall in the rate of water loss during exposure to unsaturated air. This decrease is most rapid during the early stages of desiccation; but even after

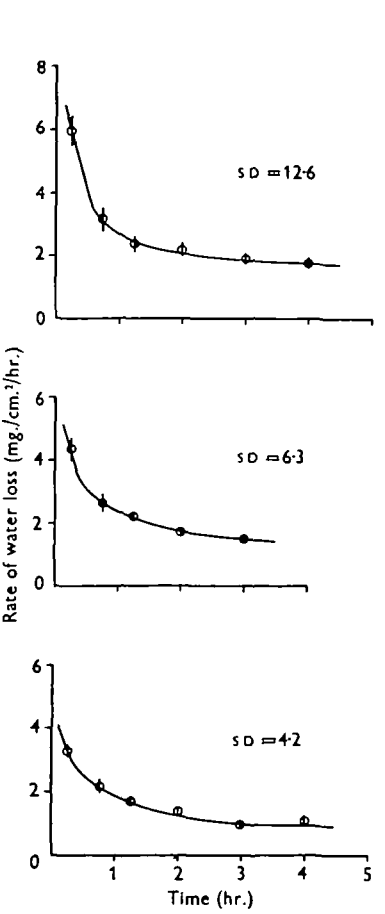

Fig. 1.

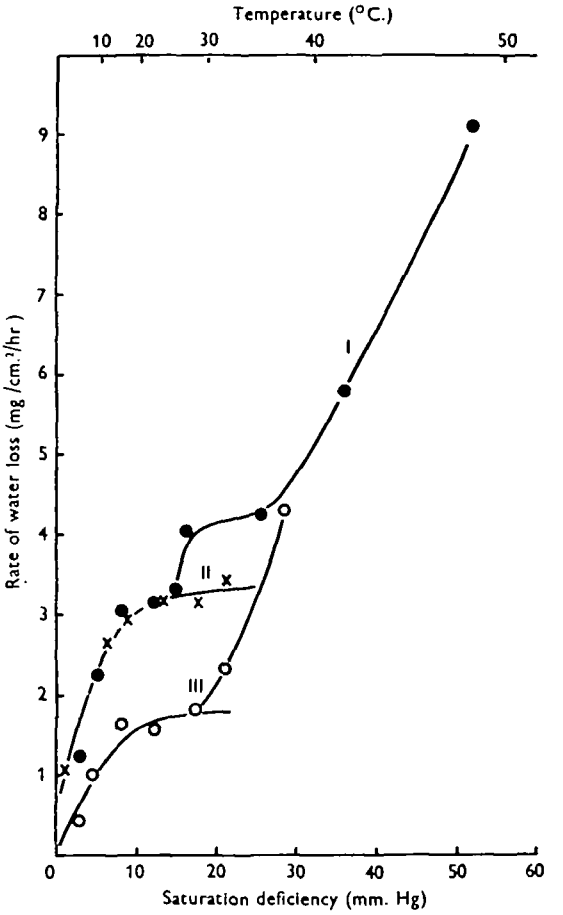

Fig. 2.

Fig. 1. The rate of water loss during exposure to different saturation deficiencies. Lines have been drawn to the fiducial limits of the means ($P=0{\cdot}05$).

Fig. 2. The effect of temperature on the rate of water loss of woodlice. I: (●), *Oniscus* exposed to different temperatures at constant relative humidity (41 %). II: (×), *Oniscus* exposed to different relative humidities at constant temperature (23° C.). III: (○), *Porcellio* exposed to different temperatures at constant relative humidity (41 %).

2–3 hr. the decline is appreciable, and it continues after the death of the animal. The phenomenon has been demonstrated for all three species over a wide range of saturation deficiencies. It seems to be a fundamental characteristic of transpiration in terrestrial isopods.

Another constant feature of isopod transpiration is illustrated in Fig. 2 (curve II),

where the rate of water loss is plotted against saturation deficiency at constant temperature (see also Table 2). Over the lower part of the range the rate of transpiration increases rapidly, but at higher saturation deficiencies the rate of increase becomes smaller and smaller and the curve tends to flatten out. In other words, the rate of water loss per unit of saturation deficiency is not constant but decreases with increasing saturation deficiency. It will be shown that this departure from the expected rectilinear relation is a function of the decrease in the rate of water loss with time.

For comparison with other arthropod groups the transpiration rates of isopods were calculated from the water loss during the third hour of desiccation. It will be shown that the rate of water loss during initial stages of exposure does not give a true reflexion of cuticular permeability. The figures for insects and ticks were obtained from the publications of Wigglesworth (1945) and Lees (1947). Loss of water from a free water surface is included in Table 1 for comparison with transpiration values.

Table 1. *The transpiration rates of some arthropods*

Temp. (° C.)	Saturation deficiency (mm. Hg)	Animal	Rate of water loss (mg./cm.²/hr./mm. Hg)
		Insects:	
20	17·5	*Tenebrio*	0·013
30	31·8	*Agriotes*	0·028
20	17·5	*Bibio*	0·046
20	17·5	*Pieris*	0·069
		Ticks:	
57	140	*Ornithodorus*	0·006
29	30·1	*Ixodes*	0·043
		Isopods:	
23	12·5	*Armadillidium*	0·056
23	12·5	*Porcellio*	0·088
23	12·5	*Oniscus*	0·176
23	12·5	Free water surface	6·16

The level of permeability is approximately the same in these different groups of arthropods. The transpiration rates of woodlice are somewhat greater than those of most adult insects and ticks. But this difference is negligible compared with the enormous difference between the water loss from isopods and that from a free surface of water. The cuticle of terrestrial Crustacea thus constitutes a highly efficient barrier to the penetration of water, only slightly inferior to the cuticles of insects and ticks in this respect.

(2) *Cuticular lipoids*

(i) *The effect of temperature on transpiration*

The low permeability of isopod cuticles suggests that lipoids may form an important constituent of the integument. In order to test this possibility the effect of temperature on the rate of transpiration was determined. The results for *Oniscus* are shown in Fig. 2 (curve I).

There is very good agreement between transpiration rates determined at constant temperature (×) and at rising temperatures (●) up to 25° C. But above this temperature there is an abrupt break in the curve indicating that an increase in permeability has taken place. Transpiration levels off to a second plateau and a second transition occurs above 35° C., after which the curve rises more steeply to high rates of water loss.

Fig. 2 shows the transpiration during the second half hour of exposure. Numerical data are given in Table 2, and it is seen that similar breaks occur during the first and third half hours.

Table 2. *The effect of humidity and temperature on the transpiration rate of* Oniscus asellus *and* Porcellio dilatatus

I. *Oniscus*

(*a*) Constant temperature (23° C.)

Saturation deficiency mm. Hg	Time (min.)		
	0–30	30–60	60–90
	Rate of water loss (mg./cm.²/hr.)		
1·1	1·12 ± 0·04	1·08 ± 0·06	1·06 ± 0·08
4·2	3·28 ± 0·11	2·16 ± 0·13	1·70 ± 0·09
6·3	4·37 ± 0·19	2·65 ± 0·15	2·21 ± 0·10
8·4	5·43 ± 0·21	3·04 ± 0·16	2·14 ± 0·11
12·6	5·94 ± 0·24	3·16 ± 0·19	2·34 ± 0·13
17·8	5·96 ± 0·21	3·16 ± 0·11	3·14 ± 0·11
21·1	6·08 ± 0·18	3·46 ± 0·11	3·04 ± 0·05

(*b*) Constant relative humidity (41 %)

Temp. (° C.)	Saturation deficiency mm. Hg	Time (min.)		
		0–30	30–60	60–90
		Rate of water loss (mg./cm.²/hr.)		
1·5	3·1	2·16 ± 0·17	1·24 ± 0·08	1·09 ± 0·10
9·5	5·3	3·88 ± 0·10	2·26 ± 0·18	1·64 ± 0·22
16·0	8·1	5·43 ± 0·21	3·04 ± 0·25	2·14 ± 0·19
23·0	12·3	5·94 ± 0·24	3·16 ± 0·19	2·34 ± 0·13
26·2	15·0	6·67 ± 0·43	3·32 ± 0·20	2·94 ± 0·07
27·5	16·3	7·12 ± 0·40	4·07 ± 0·39	3·22 ± 0·21
35·5	25·6	7·63 ± 0·33	4·26 ± 0·21	3·65 ± 0·15
42·0	36·3	8·10 ± 0·19	5·80 ± 0·23	5·52 ± 0·18
49·0	51·9	11·40 ± 0·41	9·10 ± 0·40	8·30 ± 0·36

II. *Porcellio*

Constant relative humidity (41 %)

Temp. (° C.)	Saturation deficiency mm. Hg	Time (min.)		
		0–30	30–60	60–90
		Rate of water loss (mg./cm.²/hr.)		
1·5	3·1	0·89 ± 0·09	0·42 ± 0·09	0·51 ± 0·10
7·2	4·6	1·39 ± 0·21	1·00 ± 0·18	0·38 ± 0·20
16·0	8·1	3·46 ± 0·15	1·63 ± 0·13	1·30 ± 0·12
23·1	12·5	4·33 ± 0·28	1·56 ± 0·19	1·25 ± 0·06
28·7	17·5	4·81 ± 0·24	1·82 ± 0·12	1·48 ± 0·13
32·0	21·1	6·30 ± 0·21	2·31 ± 0·11	1·78 ± 0·08
37·5	28·6	8·98 ± 0·37	4·32 ± 0·27	2·75 ± 0·15

The occurrence of two critical temperatures can be shown by subjecting a group of animals to progressively rising temperatures. As shown in Table 3 the rate of water loss decreases during the course of desiccation in the same way as for animals at constant temperature (see Fig. 1). But when the temperature is raised above 26° C. there is a rapid increase in transpiration rate, indicating that a change in cuticular permeability has taken place; the rate falls again as the temperature is raised further until at temperatures above 38° C. a second transition point is reached and the rate of water loss increases progressively.

Table 3. *The effect of a progressive rise in temperature on the rate of transpiration of* Oniscus asellus

(Relative humidity = 60%.)

Time (min.)	Temp. (° C.)	Rate of water loss (mg./cm.²/hr.)
0–30	17	4·73 ± 0·14
30–60	21	3·18 ± 0·16
60–90	24	2·82 ± 0·12
90–120	26	2·73 ± 0·08
120–150	31	3·10 ± 0·10
150–180	35	2·82 ± 0·10
180–210	38	2·64 ± 0·12
210–240	41	3·00 ± 0·18
240–270	43	3·37 ± 0·12
270–300	45	4·08 ± 0·16

To establish that the transition points are based on some passive property of the cuticle, experiments were carried out with groups of dead animals. These were killed by exposure to ammonia vapour before transpiration was determined. The humidity to which each group was exposed was chosen with reference to the temperature so that saturation deficiency was the same in all cases. Differences in the rate of water loss would be due to changes in cuticular permeability since evaporating power was constant. The results are shown in Table 4.

Table 4. *The effect of temperature on the rate of transpiration of* Oniscus *at constant saturation deficiency*

Temp. (° C.)	R.H. (%)	Saturation deficiency mm. Hg	Time (min.)			
			0–30	30–60	60–90	90–150
			Rate of water loss (mg./cm.²/hr.)			
23	15	17·8	4·02 ± 0·16	2·26 ± 0·12	2·03 ± 0·08	1·95 ± 0·09
30	44	17·8	5·50 ± 0·32	3·14 ± 0·16	2·98 ± 0·25	3·14 ± 0·22
40	68	17·8	4·98 ± 0·28	4·05 ± 0·18	3·66 ± 0·22	4·30 ± 0·24

The rate of evaporation at 30° C. is greater than at 23° C., and the rate at 40° C. is greater than both except during the first half hour. With this exception also the differences are statistically significant and show that an increase in permeability has

occurred at the higher temperatures. It is probable that these changes in permeability correspond to the appearance of transition points in the curve of Fig. 2.

The relation between temperature and water loss has been investigated for *Armadillidium* and *Porcellio* also, both of which have critical temperatures at about 28° C. The curve for *Porcellio* is shown in Fig. 2 (III), with numerical data in Table 2.

The water loss/temperature relations presented differ considerably from those of other arthropods. The transition point of the isopod curve represents a change from a progressively decreasing to a progressively increasing slope. In insects and ticks it represents a change from a negligible to a very steep slope (Wigglesworth, 1945; Lees, 1947). But there can be little doubt that the underlying mechanism of these permeability changes is the same in the two cases. The critical temperature of the isopod cuticle probably reflects a change in the state of cuticular lipoids.

(ii) *The extraction of lipoids*

An attempt was made to isolate the lipoids of *Oniscus* cuticle by extraction with fat solvents. Cast skins were used as it was difficult to prepare fresh cuticle free from adhering tissue fragments.

A number of moulted cuticles were collected and washed with water. They were dried and treated with boiling chloroform for 6 hr. under a reflux condenser. When the solvent was allowed to evaporate a small fatty residue was left at the bottom of the container. Under the microscope this residue was seen to consist of two distinct components; one formed a homogeneous matrix in which crystals of the other were embedded. When observed with polarized light the homogeneous ground substance showed a faint birefringence, while the crystalline fraction stood out brilliantly against this background.

It was not possible to stain this material effectively with the usual fat stains. Even at high temperatures the Sudan dyes were only sparsely soluble. But the extract was very effective in reducing osmium tetroxide which turned intensely black after a short period of exposure.

In an attempt to establish melting-points for the extracted lipoids advantage was taken of their birefringence. A small sample was sealed between two cover-slips, and these were placed in a flat-sided glass tube, through which water of known temperature could be circulated. The lipoids could be viewed in polarized light with a low-power objective, and a distinction could be made between the two components of the system.

The slight birefringence of the non-crystalline fraction faded at about 23° C. The lipoid crystals showed intense birefringence up to a temperature of 36° C.; above 37° C. the double refraction faded, and the crystals ceased to exist as such.

There is a fairly close correspondence between the critical temperature of the transpiration curves and the temperature at which melting of the two components occurs in the isolated lipoids. It seems probable that the lipoids which have been described in this section constitute the material bases of the permeability changes recorded above.

(iii) *The distribution of lipoids in the cuticle*

Since the isolated lipoids are capable of reducing osmium tetroxide it should be possible to demonstrate their presence in the cuticle by means of this reagent.

Pieces of fresh cuticle were immersed in a solution of osmium tetroxide, and sections were cut on the freezing microtome. The results showed that both epicuticle and endocuticle had caused intense reduction of osmium tetroxide, while the exocuticle showed no signs of staining.

If the material is extracted with boiling chloroform before treatment with osmium tetroxide, the reducing capacity of the endocuticle is greatly decreased, but it is not completely suppressed. A completely negative reaction is obtained only if the cuticle is decalcified with weak acid prior to extraction with chloroform.

The results show that materials occur in the endocuticle which are capable of causing the reduction of osmium tetroxide, and these materials are largely removed by extraction with fat solvents. They can undoubtedly be identified with the lipoids previously isolated by means of chloroform extraction.

The reduction which occurs in the epicuticle may be due to the epicuticular fats described by Dennell (1947); or to chemically combined lipoid. But some experiments, done with newly moulted animals, indicate that the epicuticle is not responsible for the low permeability of the integument.

If animals are tested soon after completion of a moult the rate of transpiration is found to be more than twice as high as that of normal controls. Drach & Lafon (1942) have shown that when the old cuticle is shed the epi- and exocuticles of the new integument are complete, but deeper layers have not yet been elaborated. Thus, in the absence of endocuticular layers, the integument offers comparatively little resistance to the diffusion of water.

The conclusion that a deep-seated lipoid impregnation constitutes the limiting barrier is confirmed by determining the effect of inert dusts. Even severe abrasion with dusts has no significant effect on the rate of transpiration. If the impermeable layers were superficial, as in insects, this treatment would result in a very substantial increase in the rate of water loss (Beament, 1945).

(3) *The decrease in transpiration rate with time*

The deep-seated nature of impermeable layers will in some part account for the characteristic form of isopod transpiration curves. On exposure to unsaturated air a gradient of vapour pressure will be set up across the cuticle, and this will entail a rapid loss of water from the relatively permeable outer layers of the integument. The rate of water loss will decrease rapidly as the gradient becomes established, and until this happens transpiration will not give a true indication of cuticular permeability.

But after the rapid decline there follows a steady decrease in the rate of transpiration which cannot be accounted for on this basis. This secondary decrease would suggest that a change is taking place in the permeability of the cuticle, and some experiments were carried out to determine the nature and mechanism of this change.

(i) *The relation between water content and permeability*

A decrease in permeability could be due to a progressive drying up of the outer layers of the cuticle. King (1945) and Gluckauf (1944), working with keratin membranes, have shown that the rate of diffusion of water vapour is dependent on the degree of hydration of the keratin. A similar phenomenon has been shown to be a general property of hygroscopic materials (Babbitt, 1940). But preliminary experiments with *Oniscus* suggested that permeability was a function of the extent of desiccation, rather than of the degree of hydration of the cuticle. To explore this possibility the effect of desiccation on water loss was determined using animals which had been brought into equilibrium with saturated air, so that their cuticles were fully hydrated.

The animals were exposed to dry air for different lengths of time. They were then left in 100 % R.H. for 5–6 hr. during which time a certain amount of water was absorbed. This absorption was presumably associated with the disappearance of a vapour pressure gradient across the cuticle. Equalization of vapour pressures within the cuticle would be brought about by diffusion of water into regions of low from regions of high vapour pressure, that is, by diffusion into the outer layers both from the blood and from the saturated atmosphere. The proportion of water deriving from these possible sources would be determined in part by the permeability of the inner layers of the cuticle, and in part by the hygroscopic properties of the cuticular substance. When the permeability is low a considerable amount of water will be taken up from the atmosphere. This would appear to be the case with *Oniscus*. The uptake is rapid to begin with but declines sharply during the first half hour, being complete after about an hour. Thus after 5–6 hr. in saturated air the cuticle may be assumed to have regained its maximal degree of hydration.

The animals were then exposed to some test humidity, and the rate of transpiration during the first half hour was determined. Water content was used as a measure of the extent of previous desiccation, and the transpiration rate of each animal was expressed as a percentage of mean control rates for undesiccated animals. The results of all experiments are summarized in Fig. 3, and it is clear that the rate of transpiration is a function of the extent of previous desiccation. The lower the water content of the animal the lower is the rate of evaporation.

The regression of transpiration rate on water content is of the right order of magnitude to account for the observed decrease in transpiration rate with time. For example, during exposure to 0 % R.H. for 1½ hr. the water content of *Oniscus* drops from 66 to 60 %; on the basis of Fig. 3 the water loss should decrease to 47 % of its original rate. The observed decrease is from 7·0 to 3·1 mg./cm.²/hr., that is to 44 % of the original rate. In spite of this close correspondence the possibility cannot be excluded that a decrease in permeability brought about by drying out of the cuticle may contribute to the fall in transpiration rate with time.

These experiments show that a decrease in permeability occurs which is independent of cuticular hydration. It seemed possible that this decrease could depend on changes in the concentration of fluids which bathe the transpiring

surfaces, and it was necessary to establish whether such changes occurred during desiccation.

ii) *The effect of desiccation on the salt concentration of body fluids*

The apparatus for dealing with this problem was put at my disposal through the kindness of Dr J. A. Ramsay. The method depends on determination of the freezing-point of very small quantities of fluid and has been described elsewhere (Ramsay, 1949).

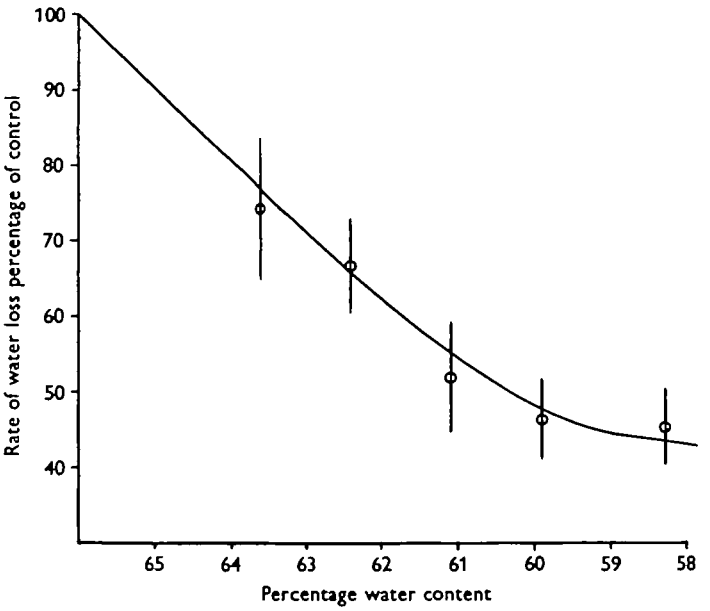

Fig. 3. The effect of previous desiccation on the rate of water loss. The data have been grouped for water content and are plotted against the mean value for each of the five groups. Lines have been drawn to the fiducial limits of the means ($P=0{\cdot}05$). The correlation coefficient calculated from the ungrouped data is 0·55 ($P=0{\cdot}01$).

By the use of a simple capillary pipette it was possible to withdraw several samples of blood from the dorsal blood sinus of *Oniscus* without apparent deleterious effect. The freezing-points of successive samples from animals kept on moist filter-paper showed no significant variation.

Table 5 shows the changes in blood concentration during desiccation. Animals were weighed before and after sampling, and the blood concentration could thus be expressed as a function of water content.

It is clear that during desiccation there is a marked increase in the salt concentration of body fluids. The concentration of blood from undesiccated animals is 1·59%; values calculated on the assumption that the water lost has been withdrawn from a 1·59% salt solution of volume equal to the volume of water originally present in the animal agree well with experimental figures. This correspondence shows the absence of an active regulation of total blood concentration.

The concentration of osmotically active substances in the blood increases as the animal loses water; there is a simultaneous decrease in cuticular permeability. It seemed possible that these two phenomena might be causally related, and that the increased salt concentration of the blood in some way modifies the structure of the cuticle so as to cause a decrease in permeability. The crustacean cuticle contains considerable quantities of protein (Lafon, 1943, 1948), and an interaction between these and their ionic environment might produce effects of this kind.

Table 5. *Changes in the blood concentration of* Oniscus *during desiccation*

Mean water content (%)	Blood concentration (% NaCl)	Blood concentration calculated, (see text)	No. of determinations N
66·0	1·59	—	13
64·2	1·73	1·74	5
62·6	1·91	1·88	5
60·0	2·00	2·09	11

(iii) *The effect of salt concentration on the structure of the cuticle*

(*a*) *Macroscopic changes.* Changes in the permeability of the cuticle would presumably be associated with changes in submicroscopic structure. It seemed possible that such changes might be reflected in corresponding changes of macroscopic dimension. To investigate this possibility a perfusion cell was made which enabled fresh sections of cuticle, cut on the freezing microtome, to be studied continuously under a high-power objective. The solutions bathing the section could be changed rapidly without disturbing the preparation. Outline drawings of a projected image of the section were made, and it was possible to establish changes amounting to 0·5–1·0% of the thickness of the cuticle with certainty.

Cuticle thickness was measured at a series of reference points and a mean value was calculated; this was expressed as a percentage of the thickness in 0·275N sodium chloride, the concentration of the blood of undesiccated animals.

The results are shown in Fig. 4*a*. It is clear that sodium chloride concentration has a marked effect on the thickness of the cuticle. The changes indicate that as the salt concentration is raised from 0·100 to 0·275 N the cuticle swells; with a further increase in ionic strength shrinkage occurs up to concentrations of 2·000 N.

(*b*) *Submicroscopic changes.* Another way in which changes in cuticular structure may be demonstrated depends on the sorption of water vapour. If a dry piece of cuticle is exposed to saturated air a certain amount of water vapour will be adsorbed. The actual quantity will depend on the nature, the extent and the disposition of adsorbing surfaces in the cuticle. Any change in structure may be reflected in a difference in the amount of water vapour which is taken up.

To study this possibility pieces of moulted cuticle were immersed in solutions of different ionic strengths. In order to facilitate penetration exposure was carried out at 60° C. After 2 hr. the cuticle was rapidly washed free of adhering salt solution by a 1 min. immersion in cold tap water. It was blotted and dried to constant

weight. On subsequent exposure to 100% relative humidity the amount of water vapour adsorbed at equilibrium was determined. The results are expressed as a percentage of the dry weight (% regain) and are summarized in Fig. 4*b*.

The relation between salt concentration and percentage regain is similar to that shown for cuticle thickness in Fig. 4*a*. The high adsorption after exposure to

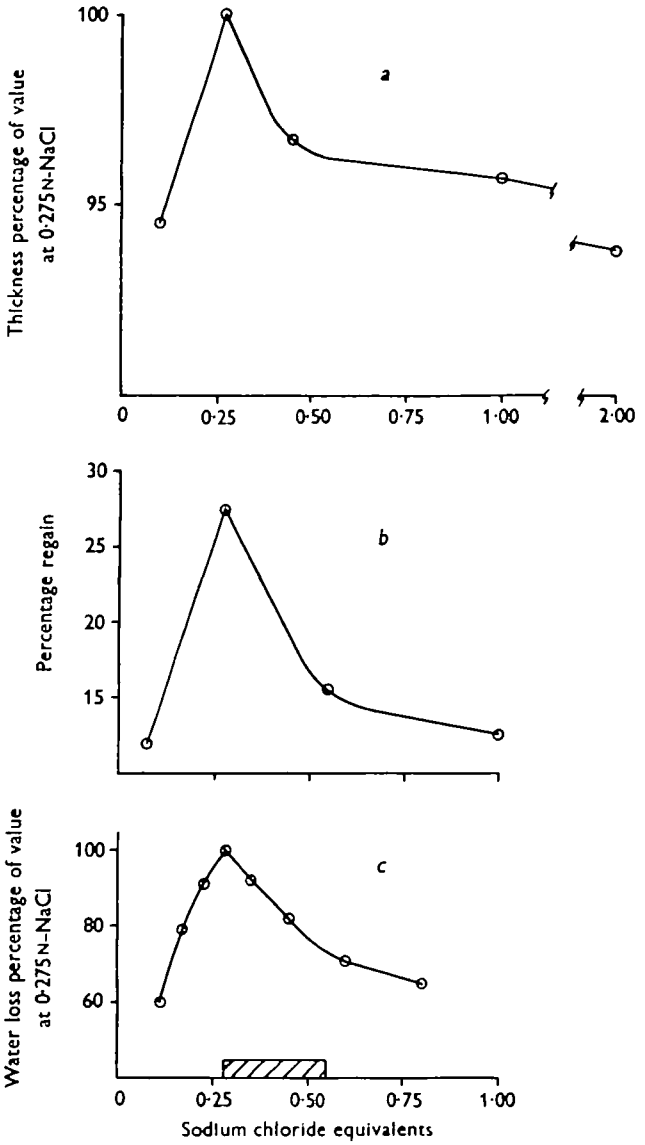

Fig. 4. The effect of salt concentration on cuticular structure. (*a*) Effect on the thickness of the cuticle. (*b*) Effect on the adsorption of water by moulted cuticle. (*c*) Effect on permeability of perfused cuticle. The range of blood concentrations observed during desiccation of *Oniscus* is marked on the abscissa.

0·275 N sodium chloride is probably an expression of a comparatively open submicroscopic structure. The surface area available for adsorption would be relatively large and so would the capacity of any capillary spaces which may exist in the fine structure of the membrane (Barrer, 1941; Bull, 1944; Sponsler, Bath & Ellis, 1940). With higher or lower salt concentrations the cuticle becomes more compact, and there is a corresponding decrease in the water-holding capacity.

(*c*) *Permeability changes.* To see whether these changes in the structure of the cuticle would cause corresponding changes in cuticular permeability it was necessary to study the rate of water loss through isolated cuticle which could be bathed with solutions of known concentration. A number of attempts were made to find a method of dealing with this problem. The calcareous nature of the cuticle proved the main obstacle as it rendered the integument very liable to cracking.

The method finally adopted was to remove head and first body segment which come off together with the gut and digestive glands. The body cavity was scraped with a fine metal hook and washed with sodium chloride solution to remove as much tissue as possible. The preparation was then mounted on two fine glass capillaries inserted through the second body segment, and the body cavity was sealed off at anterior and posterior ends with cellulose paint.

The capillary tubes were connected to a perfusion set, so arranged that hydrostatic pressure and rate of flow of perfusing liquid could be accurately controlled. The solution flowing through the preparation could be rapidly changed by switching to one or other of two reservoirs.

The preparation could be inserted into a desiccation chamber containing a small tray of calcium chloride suspended on a torsion balance, so that the amount of water passing through the cuticle could be measured at short intervals. The air inside the chamber was kept in circulation by a small fan operated from the outside by a rotating magnet. The whole was enclosed in a water-jacket through which water from heating coils immersed in a water-bath could be circulated. A thermoregulator in the desiccation chamber controlled the temperature of the water-bath, and by this means the temperature inside the chamber was maintained at 22·0 ± 0·5° C.

With this apparatus a measure of permeability could be obtained in 30 min., so that a number of determinations could be made in a single day. This made it possible to check the constancy of the preparation by determining the rate of water loss under standard conditions at the beginning and end of each series.

A serious drawback to the method is that determination of transpiration rates is empirical, since it is impossible to estimate the extent of the cuticle which is actually being perfused. This means that observations have only relative value; but if results are expressed with reference to transpiration under a standard set of conditions the data obtained with different preparations will be comparable.

In the first series of experiments solutions containing a variety of ions were employed. But it was found that the rate of water loss was the same whether a balanced solution was used, or a solution of pure sodium chloride, provided ionic concentration and pH were the same. So for the sake of simplicity pure sodium chloride was subsequently used. The solutions were buffered with Na_2HPO_4 and

the pH adjusted to 7·5 with sodium hydroxide. This value corresponds with the pH of *Oniscus* blood as determined roughly with indicators (Gorvett, 1950, records a higher value for isopods).

The results of all experiments on the effect of salt concentration on permeability are shown in Fig. 4*c*. With distilled water (and possibly at extremely low salt concentrations) an irreversible change in cuticular permeability takes place, and the integument becomes freely permeable to water.* At a salt concentration of 0·110 N cuticular permeability is very low; it rises with increasing concentration to a peak at 0·275 N; subsequently there is a sharp drop and the curve tends to level off above concentrations of 0·600 N.

The relation was tested at different values of pH. The shape of the curve was found to be independent of the reaction of the perfusing medium, but the level of permeability varied greatly (see below).

The curve is very similar to those showing the effect of salt concentration on cuticle thickness and adsorption; there can be little doubt that the three phenomena represent different aspects of an interaction between cuticular elements and ionic medium. The nature of this interaction cannot be discussed in detail. The curves show certain similarities to the solubility curves of protein in solutions of different salt content (Cohn, 1932). Such resemblances indicate that salting-in and salting-out of endocuticular proteins may lie at the base of the effects described. But a detailed study of the problem lies outside the scope of the present investigation.

The data presented suggest that changes in the salt concentration of the body fluids of woodlice are likely to produce changes in the permeability of the integument. Over the range of concentrations observed with *Oniscus* (marked on the abscissa in Fig. 4*c*) there is a progressive decrease in cuticular permeability with increasing sodium chloride concentration. It is probable that the decrease in permeability which has been described for living animals may be an expression of this phenomenon. During exposure to dry air the water content of the animal decreases; this causes a concentration of body fluids. The consequent shrinkage of the endocuticle brings about a closer packing of the impregnating fat molecules and a fall in permeability.

That proteins of the endocuticle in particular are involved in these effects is indicated by the effect of pH on the permeability and on cuticle thickness. Both are at a minimum when the pH is about 4; this value corresponds closely with the isoelectric point of decapod endocuticle as determined by Yonge (1932) and confirmed for *Oniscus* during the present investigations.† A correspondence between the pH of isoelectric point and the pH of minimal swelling is shown by most protein gels (Jordan Lloyd & Shore, 1938).

* Richards & Korda (1948), working with the electron microscope show that distilled water causes the appearance of a coarse fibrous network in the cuticle of arthropods; there appears to have been some sort of precipitation of constituent molecules.

† The isoelectric point of the epicuticle is above pH 5 (Yonge, 1932; Dennell, 1946).

CONCLUSION

On the basis of the foregoing results it is possible to offer an explanation of the fall in the rate of transpiration with time which is so characteristic a feature of desiccation in isopods. On exposure to dry air a gradient of vapour pressure is established across the cuticle, and this entails a rapid loss of water from exo- and epicuticles. The initial stages of desiccation are therefore characterized by very high rates of transpiration, which fall rapidly as the gradient approaches stable values. Simultaneously with the loss of water from the outer layers there is a diffusion of water from the blood to the surface along the gradient of vapour pressure. The consequent loss of water from the blood causes a progressive shrinkage of the cuticle with a decrease in permeability, and hence there is a steady decrease in the rate of water loss from the animal as a whole. It is possible that a decrease in cuticular hydration may contribute to this compacting of the cuticle.

Attention has been drawn to the apparent decrease in the permeability with increasing saturation deficiency (see Fig. 2; noted also by Edney, 1951). This anomaly finds interpretation in the light of later findings. It has been shown that cuticular permeability is proportional to water content (Fig. 3). For a given length of exposure animals which have been in high saturation deficiencies will have lost more water than animals which have been in low saturation deficiencies; their water content will be less and so will their permeability. Since transpiration rates at different saturation deficiencies are compared after equal durations of desiccation there will be an apparent decrease in permeability in drier air. Unless rates of water loss are compared not after equal duration, but after equal extent of desiccation, a rectilinear relation between transpiration rate and saturation deficiency cannot be expected.

DISCUSSION

A close similarity has been shown to exist between crustacean and insect cuticles as regards their general structure (Dennell, 1947). This similarity does not extend to the means by which the integument is waterproofed in the two groups. In insects the cuticle is rendered impermeable by a very thin layer of lipoid situated near the surface of the cuticle. In terrestrial isopods the diffusion of water is limited by a lipoid impregnation of endocuticular layers separated from the surface by exo- and epicuticles. Although these layers are much more permeable than is the endocuticle they may play an important role in the mechanism of waterproofing by preventing vapour pressure gradients from exerting their full force across the lipoid barrier.

Differences in the organization of lipoid are probably responsible for differences as regards the water loss/temperature relations of arthropod groups. Breaks in the isopod curves are of a gradual nature, while in insects and ticks the transition points are usually sharply defined. An exception to this general rule has been reported by Beament (1949); transpiration through the secondary wax layer of *Rhodnius* egg shell is characterized by a gradual transition point; but the lipoids concerned are

disposed in the form of an impregnation of the fertilization membrane rather than as a discrete layer.

Another difference between isopods on the one hand, and insects and ticks on the other, is that in isopods the transpiration curve above the critical temperature is not nearly so steep as in the other groups (see Edney, 1951, fig. 3, where *Blatella* is plotted on the same graph as a group of isopods). The low rate of increase of transpiration with temperature for woodlice is probably correlated with the presence of impregnating rather than free lipoids. The thermal agitation of lipoid molecules above critical temperatures would be restricted by the non-lipoid structural framework, and the membrane would possess appreciable waterproofing capacity even at high temperatures. With insects and ticks, disorganization of the oriented lipoid is free to increase progressively as the temperature is raised, until the rate of transpiration approximates to evaporation from a free water surface.

This interpretation is borne out by the work of Lees & Beament (1948), who found that in the transpiration curve for the egg of *Ornithodorus moubata* the steepness of the slope above the critical temperature decreases greatly in the course of development; and the change is apparently associated with a gradual infiltration of lipoids, at first superficial, into the substance of the egg shell.

The egg of *Rhodnius* may provide another example of this phenomenon; the slope of the curve for eggs possessing the primary wax layer only is much steeper than that which obtains after the secondary wax impregnation has been laid down.

The presence of two distinct types of lipoid, one a low melting-point grease and the other a crystalline wax, has been demonstrated in the egg of *Ornithodorus* (Lees & Beament, 1948). The critical temperature of the egg membrane is intermediate between the melting-points of these two lipoids; this is contrary to the condition in *Oniscus* where each lipoid imposes its characteristic transition point on the water loss/temperature curve. This discrepancy may depend on a difference of molecular organization in the two cases. The mixture of lipoids in the case of the tick egg may be of an extremely intimate nature, approaching the limiting condition where molecules of the two types alternate in the oriented monolayer. Under these conditions a 'mixed' transition point, such as that observed, would result. In *Oniscus* the system may have the nature of a mosaic; multimolecular aggregates of one lipoid species may adjoin similar aggregates of the other. In this case there is reason to suppose that two separate transition points might characterize the impregnation.

Simultaneously with the present investigations a comparative study of transpiration in woodlice was in progress. A preliminary note was published by Edney (1949) and the full results have subsequently become available (Edney, 1951). Edney finds no evidence for transition points in the water loss/temperature curves, and concludes from his determination of transpiration rate that there is no efficient check to evaporation in terrestrial isopods. These conclusions are at variance with the ones reached in the present account, but there is no contradiction in experimental results. It is clear from Fig. 2 above that if determinations had been made at 10° C. intervals as in Edney's investigations, the points would fit fairly well on

a straight line. The critical temperatures only become apparent when the relative decrease in the rate of water loss with increasing saturation deficiency is taken into account, and when determinations are made at intermediate temperatures.

It has been indicated that during initial stages of desiccation the water loss derives largely from water held in the exo- and epicuticles, and its rate cannot be taken as a reflexion of cuticular permeability. If this initial water loss is included in estimates of transpiration, as in Edney's experiments, abnormally high transpiration rates are recorded, which suggest that the integument is very inadequately waterproofed.

The subsequent investigations of Edney (1953) have shown that rapid loss of water from terrestrial isopods may be of great biological importance during exposure to insolation. But it is conceivable that if such exposure were prolonged the benefit conferred by ability to cool the tissues would be offset by the danger of desiccation. The presence of a substantial exocuticle with considerable water-holding capacity, and of an endocuticle whose permeability decreases with desiccation would seem particularly well suited to provide against the possibility of overheating on the one hand, and of desiccation on the other.

The concepts arrived at during the present investigation stand in marked contrast to the conclusions reached by Yonge with regard to the relative permeability of crustacean epi- and endocuticles (Yonge, 1936, 1946). A study of the lining of the foregut in *Homarus* and of *Homarus* egg-shells suggested that permeability is controlled entirely by the epicuticle. The endocuticle played no part in regulating the diffusion of substances in and out through the integument. Objections have been raised to Yonge's experiments by Richards & Korda (1948); these authors state that purified membranes of the type employed are without biological significance for studies on permeability, since membrane structure is extensively altered by treatment. Present experiments with perfused cuticle showed that even distilled water may cause irreversible damage to the endocuticle; but the epicuticle shows a striking resistance to the action of strong chemical reagents. It seems possible that under the conditions of Yonge's experiments the endocuticle had suffered extensive damage with consequent destruction of semipermeability, while the epicuticle had remained comparatively unchanged. The permeability of the integument as a whole would then reflect the permeability of the epicuticle alone.

Heeley (1941) attributed the imperviousness of isopod cuticle largely to calcification, and this view is shared by Gorvett (1946) and Lafon (1948). The present work has shown that the waterproofing mechanism is based on a lipoid impregnation of the endocuticle. Calcium may play an important role as a component of the substrate for this impregnation, but it is not itself concerned with limiting the diffusion of water. The permeability of different species cannot be correlated with different degrees of calcification; thus the integument of *Porcellio dilatatus* is very soft compared with that of *Oniscus*, yet its permeability is much lower. And the calcification of egg-bearing females of *Oniscus* is less extensive than that of normal adults, but their transpiration rates are no different. A similar independence of cuticular hardness and permeability has been demonstrated for insects (Wigglesworth, 1948;

Eder, 1940); in this case the hardness is an expression of sclerotization rather than of calcification.

The permeability of *Oniscus* cuticle has been shown to depend on the nature of the solution in contact with its inner surface (see Fig. 4*c*). Analogous phenomena have been reported for the permeability of various natural membranes to water (Brauner & Brauner, 1943; Orru, 1939). The rate of penetration was found to be minimal at the isoelectric point of the membrane proteins, as with *Oniscus*. The permeability changes can be interpreted on the basis of an interaction between protein molecules and ionic medium, leading to changes in dispersion of the proteins and consequent alteration of membrane structure. In the case of woodlice the proteins are associated with chitin and these constituents form a substrate for the impregnation of lipoids. Shrinkage of the cuticle will lead to condensation of the ground substance, and hence to a closer packing of lipoid molecules; the resistance to aqueous diffusion will consequently increase. This system bears some resemblance to the scheme put forward by Hurst (1948) in an attempt to account for the asymmetrical permeability of *Calliphora* larval cuticle. In both cases the packing of impregnating lipoid molecules is influenced by changes in the organization of structural proteins.

SUMMARY

1. The transpiration of three species of Isopoda has been investigated; *Armadillidium* shows the highest resistance to desiccation, *Oniscus* the least, and *Porcellio* is intermediate.

2. The permeability of isopod cuticle is limited by lipoids which impregnate the endocuticle. If the temperature is raised above the lipoid melting-point a marked increase in permeability results.

3. Isopods show a characteristic decline in transpiration rate during exposure to desiccating atmospheres. The high initial rate of evaporation is due mainly to loss of water from layers of the cuticle external to the lipoid barrier.

4. There is a progressive decrease in the permeability of the integument during desiccation. This is brought about by a shrinkage of the cuticle, which leads to a closer packing of lipoid molecules. The mechanism of this structural alteration is based on an interaction between endocuticular proteins and the ionic constituents of body fluids; in the course of desiccation the blood concentration increases and there is a corresponding decrease in the dispersion of endocuticular elements.

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