

CUTANEOUS RESPIRATION IN WOODLICE

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INTRODUCTION

There is experimental evidence, reviewed by Mödlinger (1931) and by Edney (1954), that oxygen is absorbed by woodlice through flat, biramous abdominal appendages known as pleopods. In the more advanced species, the exopodites of either the first two or of all five pairs of pleopods each possess an internal tuft of tubules (pseudotracheae) opening to the exterior by a pore, and it is these species which, in general, are associated with drier habitats. The main purpose of the present work was to find whether cutaneous respiration (meaning respiration through the general body surface apart from the pleopods) also occurs, and if so to define its extent. The fact that the integument of woodlice is relatively permeable to water suggests that such respiration is not unlikely: the possibility has been briefly referred to by Verrier (1932) and Macagno (1938, 1939), but it has never been critically investigated.

MATERIAL AND METHODS

Four species of woodlice were used, and they were chosen to cover, so far as possible, the range of habitats and of modification of the pleopods in the British species. *Ligia oceanica* Linn. is a littoral species; it is larger than the others (up to 3 cm. long) and possesses unmodified pleopods. *Oniscus asellus* Linn. is also without pseudotracheae, but the exopodites are excavated near their outer margins to form a number of simple air spaces. *Porcellio scaber* Latr. is capable of surviving, at least for short periods, in somewhat drier regions than *Oniscus*, and possesses well-developed pseudotracheae on the exopodites of the 1st and 2nd pairs of pleopods. *Armadillidium vulgare* Latr. is the most terrestrial of all the species here studied and also possesses two pairs of pseudotracheae. These species also stand in the above order as regards rate of water transpiration through the integument (from most to least rapid) (Edney, 1951).

The procedure adopted was to compare the rates of oxygen absorption and carbon dioxide output in normal animals with those in animals whose pleopods were blocked. Two coats of an emulsion paint were used to block the pleopods. This substance is harmless to the animals (they live indefinitely if painted on the back), it flows easily over the hygrophilic surface of the pleopods, and on drying is nearly or quite impermeable to oxygen.

The permeability of this substance to oxygen was investigated in two ways. In the first method, a small glass container with a well-fitting rubber bung was half

filled with a slightly acid solution of pyrogallol. The bung possessed two holes, through one of which a glass tube from a nitrogen supply entered; the other was covered with a small piece of fine muslin. Oxygen was then removed from the solution and the air above it by bubbling nitrogen through for 1 hr. Excess alkali was then added to the pyrogallol by means of a hypodermic needle through the rubber bung. If the muslin covering the second hole was left untreated, the pyrogallol turned brown within a few minutes, but if the muslin had previously been painted with two coats of emulsion paint, the solution remained colourless for at least 12 hr. and then began to turn brown very slowly.

In the second method, reduced indigo was injected into living woodlice. When normal animals were used, the pleopods turned deep blue (the colour of oxidized indigo), but if the pleopods had been painted, and the paint allowed to dry, no blueing of these organs was observed. If the pleopods on one side only were painted, they alone remained colourless, those on the opposite side turning deep blue.

It may therefore be accepted that a layer of dried emulsion paint is sufficiently impermeable to oxygen to act as a block for the purposes of the present experiments.

Respiration was measured by means of Dixon's (1934) modification of the Barcroft manometer, at 22° C., and the results reduced to standard temperature and pressure. When oxygen uptake was being measured, 4% potassium hydroxide on Whatman's no. 40 filter-paper was used to absorb the carbon dioxide; when carbon dioxide output was being measured, the filter-paper was moistened with 0.5 ml. of 1% sulphuric acid, and the necessary correction for solubility was made. The air in the respiration flasks was therefore nearly saturated with water vapour. When respiration in dry air was being measured for comparison extra precautions had to be taken, and these will be described below.

The respiration rates obtained have been expressed in terms of surface area rather than body weight, since Ellenby (1951) found the rate of oxygen absorption in *Ligia oceanica* to be more nearly proportional to surface area than to weight. In practice, the surface area of a woodlouse is not easy to determine, and the values obtained previously (Edney, 1951) for k in the expression $S = kW^{\frac{1}{2}}$ were again employed. The error in absolute terms may be considerable, but at least it is consistent between individuals of one species. For some purposes it may be more convenient to have the results in terms of unit weight ($\text{mm.}^3/\text{mg.}/\text{hr.}$), and this may readily be obtained, for one size of animal, by multiplying the present figures by a factor which varies from one species to another. These factors, which apply to animals of a size representative of those used in the present work (where very large and very small animals were excluded) are as follows: *Ligia* (0.8 g.), $\times 1.24$; *Oniscus* (0.1 g.), $\times 2.86$; *Porcellio* (0.1 g.), $\times 2.63$; *Armadillidium* (0.12 g.), $\times 2.35$.

The numbers of individuals used in each determination were: *Ligia*, 1; *Oniscus* and *Porcellio*, 10; *Armadillidium*, 10 or 5. The manometer scale, and the surface area of the animals, were such that measurements could be made to the nearest $0.005 \text{ mm.}^3/\text{mm.}^2$.

EXPERIMENTS

(a) Respiration of normal and blocked animals in moist air

Preliminary experiments having shown no significant difference between the respiration rates of male and female animals (provided the latter were without brood pouches) the two sexes were used indiscriminately. Each determination was carried out for 1 hr., during which readings were taken every 10 min. Twenty such determinations were made of oxygen uptake by normal animals of each of the four species. After each oxygen determination, the same animals were immediately used for carbon dioxide determination. Further experiments were made in which carbon dioxide output was measured first, followed by oxygen absorption in the same animals, and the results showed that the rates measured were in no way affected by the order in which they were obtained. Five further determinations were carried out with each species over 6 hr. periods, and these showed that both oxygen absorption and carbon dioxide excretion remained remarkably steady over this longer period.

Experiments were then carried out with animals in which the pleopods had been blocked as described above. Twenty determinations were made of the oxygen absorption in each species, and twenty more of the carbon dioxide output, but in this case the two determinations were made on different groups of animals because carbon dioxide output did not remain steady, but was always lower during the second hour than during the first.

At intervals during these determinations control experiments were run. Woodlice of the appropriate species, either normal or blocked, were killed by immersion for a minute in water at 90° C. They were then dried on filter-paper and subsequently treated in the same way as the experimental animals. No measurable oxygen consumption or carbon dioxide output was found in these controls, which therefore showed that the apparatus was working satisfactorily and that no spurious results were being caused by application of the emulsion paint used for blocking the pleopods.

The main results described above are shown in Table 1, and may now be considered in more detail. The mean rates of oxygen consumption by normal animals were (in $\text{mm.}^3/\text{mm.}^2/\text{hr.}$): *Ligia*, 0.155; *Oniscus*, 0.075; *Porcellio*, 0.084; *Armadillidium*, 0.086; so that oxygen consumption in these terms is considerably higher in *Ligia* than in the other species. If these same results are expressed in terms of body weight, the figures are (in $\text{mm.}^3/\text{mg.}/\text{hr.}$): 0.192, 0.214, 0.221 and 0.202 respectively, and the difference between *Ligia* and the rest is much smaller. This suggests that when different species are compared, oxygen consumption is more nearly proportional to weight than to surface area. In earlier experiments, much variation was encountered in the oxygen consumption of animals from different sources which were presumably in different physiological conditions, and it was only by obtaining a large number of animals from one source and using them for the main experimental runs, that more consistent results were obtained. Even so, the variation, as shown by the standard error figures included in Table 1, was considerable. In addition, the factors applied for obtaining surface area from body weight are not very reliable,

so that little significance can be attached to the differences between the species, except in the case of *Ligia*, whose relatively high oxygen consumption in terms of surface area has already been mentioned.

Table 1. *The rates of respiration in mm.³/mm.²/hr.* of woodlice with normal and blocked pleopods in moist air*

(Each entry is a mean (\pm standard error of the mean) of twenty determinations. Calculation of R.Q. values is explained in the text.)

		Oxygen uptake	Carbon dioxide output	R.Q.
<i>Ligia oceanica</i>	Normal	0.155 \pm 0.0058	0.128 \pm 0.0048	0.82
	Pleopods blocked	0.081 \pm 0.0051	0.113 \pm 0.0043	1.40
<i>Oniscus asellus</i>	Normal	0.075 \pm 0.0030	0.064 \pm 0.0030	0.88
	Pleopods blocked	0.039 \pm 0.0024	0.052 \pm 0.0025	1.36
<i>Porcellio scaber</i>	Normal	0.084 \pm 0.0029	0.070 \pm 0.0028	0.85
	Pleopods blocked	0.029 \pm 0.0019	0.053 \pm 0.0031	1.69
<i>Armadillidium vulgare</i>	Normal	0.086 \pm 0.0033	0.077 \pm 0.0032	0.90
	Pleopods blocked	0.022 \pm 0.0018	0.036 \pm 0.0029	1.64

* For conversion of these figures to mm.³/mg./hr., see p. 257.

Ellenby (1951) obtained a mean oxygen consumption for *Ligia* weighing 0.8 g. of about 0.21 mm.³/mg./hr. at 25° C., with which the present results compare well. Other measurements of respiration in terrestrial isopods are those of Reinders (1933), who obtained a mean oxygen consumption for *Porcellio* of 0.15 mm.³/mg./hr. at 16° C., Morrison (1946): 0.35 mm.³/mg./hr. (reliability unknown) for *Oniscus* at 25° C., and Edwards (1946): 0.348 mm.³/mg./hr. (reliability unknown) for *Oniscus* at 17° C. Allowing for the difference in temperature, Reinders's figures are consistent with the present results; those of the other two authors are not very well established.

Carbon dioxide output by normal animals remained steady during the present determinations, and the respiratory quotients, calculated as means of the R.Q.'s for each pair of determinations, were reasonably consistent, varying from 0.82 (*Ligia*) to 0.90 (*Armadillidium*).

As regards the effect of blocking the pleopods, the results show a marked decrease in oxygen absorption, but it appears that all four species are nevertheless capable of absorbing some oxygen through the rest of the integument. The amount so absorbed is greatest in *Ligia* and *Oniscus* (52% of normal), less in *Porcellio* (34% of normal) and least in *Armadillidium* (26% of normal), and with the exception of *Ligia* the animals stand in the same order in this respect as they do in respect of permeability of the integument to water. The cutaneous absorption of oxygen by *Ligia* appears on this assumption to be too low, but there is no reason to expect a strict correspondence for there may well be factors other than permeability of the integument to water which limit cutaneous absorption.

As mentioned above, carbon dioxide output by animals with blocked pleopods was by no means steady. It fell more or less gradually from something like the

normal figure at the beginning of the first hour to a lower value. Experiments with blocked *Armadillidium* and *Porcellio* could not be carried out for more than 2 hr. because they began to suffer from lack of oxygen and soon died (see p. 264), but during the second hour the mean carbon dioxide output remained above the mean oxygen uptake figure, so that the R.Q. remained greater than 1. Blocked *Oniscus* and *Ligia* lived longer, but even in these species the R.Q. usually remained greater than 1 for 6 hr.; but the behaviour in this respect was very variable. The mean carbon dioxide outputs during the first hour after blocking and drying are shown in Table 1.

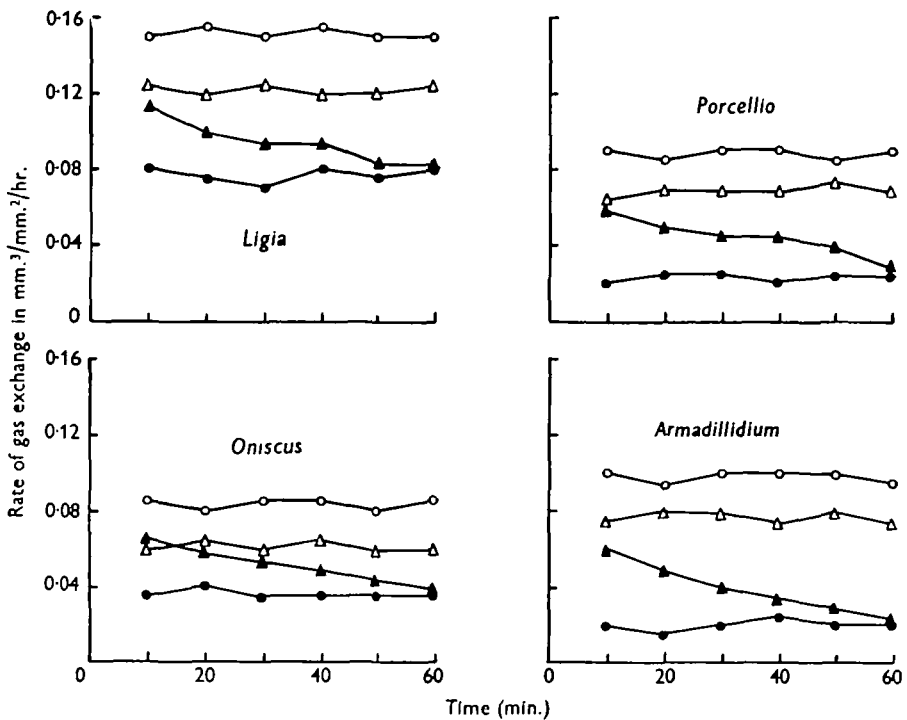


Fig. 1. The rate of oxygen uptake (circles) and carbon dioxide output (triangles) in four species of woodlice. Open symbols, normal animals; black symbols, animals with the pleopods blocked.

It must be remembered, however, that the rate of production was falling during this time, so that the R.Q.'s, calculated in this case from the means of the oxygen and carbon dioxide values, are of limited significance. Fig. 1 contains examples of some individual records of oxygen uptake and carbon dioxide output in normal and blocked animals.

The measurements of carbon dioxide output in these animals commenced 30 min. after the application of the first coat of emulsion paint to the pleopods. We do not know how the rate varied during this period, but since the initial measured rates were not far short of the rates for normal animals it seems reasonable to conclude that blocking the pleopods had but little immediate effect on carbon dioxide excretion: the subsequent drop would follow naturally as a result of reduced oxygen

uptake. We do not know the permeability of dry emulsion paint to carbon dioxide, but it is unlikely to be as permeable as a moist integument. The simplest explanation of the above results may therefore be that carbon dioxide is normally excreted through the whole integument, including the pleopodal surfaces. This conclusion is consistent with the results of work by Thorpe (1928) and others on insects.

Reinders (1933) found the rate of carbon dioxide output in *Porcellio scaber* to be unaffected by blocking the pseudotracheae (which are on the first two pairs of pleopods only) with paraffin. Oxygen consumption was, however, reduced, and he found an R.Q. of 1.46, as compared with the normal R.Q. of 0.72. He, too, explained the high R.Q. by assuming that carbon dioxide is normally excreted by the whole integument.

There are, in fact, many records of increased R.Q.'s in animals wholly or partly deprived of oxygen (refs. in Prosser, 1952), and these are generally thought to indicate the use of oxygen already present in the tissues or body fluids of the animals concerned, together with the excretion of carbon dioxide already formed. No doubt such processes explain the present results in part, but it is rather difficult to understand why carbon dioxide excretion remains higher than oxygen uptake for so long. Bosworth, O'Brien & Amberson (1936) found a similar sustained rise in the R.Q. of the decapod *Homarus* in low oxygen tensions. These authors used the Van Slyke method of gas analysis, and their animals were, of course, in water. They were able to demonstrate that the high CO₂ figures they obtained were caused by respiratory CO₂ reacting with the calcium carbonate of the integument (from outside) to liberate twice as much bicarbonate. Such an explanation is not applicable to the present results, which must remain unexplained for the time being.

(b) *Respiration in dry air*

The use of any manometric method for the measurement of respiration in dry air leads to false results unless the effect of evaporation of water from the respiring animal is allowed for. In the present work the difficulty was overcome by running control experiments with dead animals whose respiratory enzymes had been destroyed by heat. It was established that the rate of evaporation of water from such animals did not differ significantly from normal. An equal number of control and experimental determinations were made for each set of conditions, and the difference between the mean value for the controls and the mean for the experimental determinations was taken as the true respiration rate in the conditions concerned.

In these experiments the procedure before placing the animals in the respirometer cups was as follows: they were weighed, painted if necessary (two coats separated by 15 min. in dry air over phosphorus pentoxide) placed in a slowly moving stream of dry air at 25° C. for 30 min. to make sure that all surface moisture was removed, and tipped straight into the respirometer cup which contained a layer of calcium chloride on the floor and through which a stream of dry air had been passing up to the moment when the animals were introduced. This thorough preliminary drying was found to be important, for the respiration rate of animals placed directly in still dry air without preliminary drying was found not to differ

from that of animals in moist air. This point is referred to again in the discussion on p. 266. During measurement the animals were allowed to come into contact with the calcium chloride. Contact could be prevented by enclosing them in gauze cages, but this was laborious and made no difference to the respiration rate, so that it was discontinued.

When oxygen absorption was to be measured, a saturated solution of potassium hydroxide on filter-paper was used to absorb the carbon dioxide, such a solution comes into equilibrium with a water vapour pressure of about 2.6 mm. Hg (15% R.H. at 20°C.). (This was of course allowed for by the control experiments.) When carbon dioxide was to be measured, potassium hydroxide was omitted. Measurements were again made for 1 hr. periods, but oxygen and carbon dioxide measurements had to be made on separate groups of animals, since survival was greatly curtailed.

Table 2. *The rates of respiration in mm.³/mm.²/hr.* of woodlice with normal and blocked pleopods in dry air after previous drying in moving dry air at 25°C. for 30 min.*

(Each entry is a mean (\pm standard error of the mean) of five determinations.)

		Oxygen uptake	Carbon dioxide output	R.Q.
<i>Ligia oceanica</i>	Normal	0.020 \pm 0.0031	0.020 \pm 0.0031	1.00
	Pleopods blocked	< 0.005 —	0.036 \pm 0.0040	Very high
<i>Oniscus asellus</i>	Normal	0.023 \pm 0.0038	0.029 \pm 0.0037	1.26
	Pleopods blocked	0.005 \pm 0.0019	0.032 \pm 0.0041	6.40
<i>Porcellio scaber</i>	Normal	0.075 \pm 0.0057	0.084 \pm 0.0112	1.12
	Pleopods blocked	< 0.005 —	0.045 \pm 0.0035	Very high
<i>Armadillidium vulgare</i>	Normal	0.081 \pm 0.0064	0.094 \pm 0.0143	1.16
	Pleopods blocked	< 0.005 —	0.067 \pm 0.0072	Very high

* See footnote to Table 1.

The results of these experiments are shown in Table 2. Five determinations were made for each species in each condition; the figures obtained were very variable (which is not unexpected, for the animals were often approaching exhaustion), but certain main conclusions may be drawn from them.

So far as intact animals are concerned, the rate of oxygen absorption was reduced; to a great extent in *Ligia* and *Oniscus*, and to a lesser extent in *Porcellio* and *Armadillidium*. This confirms the suggestion made by several authors on grounds other than direct measurement, that the presence of pseudotracheae is an advantage so far as respiration in dry air is concerned. The R.Q. of intact animals taken over the whole hour was higher than normal, but since oxygen uptake and carbon dioxide output had to be measured on different animals, and since the variability of each measure was high, little reliance can be placed on these R.Q. values.

All these animals were alive at the end of the experiments (although *Ligia* and *Oniscus* usually appeared sluggish) and if they were removed to moist air, they recovered permanently.

In the blocked animals, oxygen uptake was found to be reduced almost to vanishing point. Carbon dioxide output was also low, but always measurable. R.Q.'s for these animals were often extremely high, but this is simply a reflexion of the very low oxygen uptake. From the fact that oxygen uptake by blocked animals is very much lower in dry air than in moist, it may be concluded that dry air inhibits cutaneous uptake.

Now a few of the blocked animals in dry air, although alive when the measurements started, did not survive the hour in the respirometer cups, and this raises the question as to whether loss of vitality was caused by lack of oxygen, or by some other factor associated with dehydration. This question will be discussed below, after further evidence has been presented.

(c) *The effect of high oxygen tension on respiration in dry air*

The oxygen uptake of normal and blocked animals was measured in a thoroughly dry mixture of air and oxygen in approximately equal proportions. A stream of this mixture was caused to flow through the respirometer cups for a few minutes immediately before their attachment to the respirometer. In all other respects the conditions were the same as those for respiration in dry air described in the preceding section.

The results (Table 3) show that with the exception of normal *Armadillidium* there was a greater oxygen uptake in these conditions than there was in dry air, and this is true for both normal and blocked animals. In the former, absorption may have occurred, of course, through the pleopods and/or pseudotracheae; in the latter, absorption can only have occurred through the integument. All these animals, both normal and blocked, were alive at the end of the experiment.

Table 3. *Oxygen uptake in mm.³/mm.²/hr.* by woodlice in a dry 50% air/oxygen mixture after previous exposure to moving dry air at 25° C. for 30 min.*

(Each entry is a mean (\pm standard error of the mean) of five determinations.)

	Normal	Pleopods blocked
<i>Ligia oceanica</i>	0.102 \pm 0.0053	0.084 \pm 0.0072
<i>Oniscus asellus</i>	0.045 \pm 0.0030	0.037 \pm 0.0033
<i>Porcellio scaber</i>	0.080 \pm 0.0052	0.053 \pm 0.0046
<i>Armadillidium vulgare</i>	0.081 \pm 0.0083	0.040 \pm 0.0114

* See footnote to Table 1.

(d) *Survival of normal and blocked animals in moist and dry conditions*

The experiments already reported have shown that oxygen may be absorbed through the general integument with greater or less facility by all four species examined. The following experiments were designed to find out for how long such cutaneous respiration is capable of supporting life.

All four species were exposed in the same sets of conditions as those used previously, to determine the period of survival (except that the animals were finally placed in desiccators rather than respirometer cups); the survival of blocked

animals being calculated from the time of application of the first coat of paint to the pleopods. In addition, blocked and normal animals were exposed to moist nitrogen.

At least twenty animals were used to determine the mean survival period in each condition, and the results are shown in Table 4. Variability was again rather great, but the main effects of the various treatments are clear enough. All species survived

Table 4. *Survival times (in hr.) of normal and blocked woodlice when subjected to various conditions*

(Each entry is a mean (\pm standard error of the mean) of at least twenty records).

	Moist nitrogen	Moist air	Dry air	Dry 50% air oxygen mixture
Normal				
<i>Ligia oceanica</i>	1.6 \pm 0.20	*	7.6 \pm 0.51	11.0 \pm 0.74
<i>Oniscus asellus</i>	1.7 \pm 0.21	*	4.5 \pm 0.31	9.3 \pm 0.51
<i>Porcellio scaber</i>	0.8 \pm 0.08	*	10.1 \pm 0.83	14.4 \pm 1.5
<i>Armadillidium vulgare</i>	0.9 \pm 0.07	*	12.3 \pm 1.2	18.0 \pm 1.3
Pleopods blocked				
<i>Ligia oceanica</i>	1.8 \pm 0.09	60 \pm 5.3	3.5 \pm 0.17	8.0 \pm 0.43
<i>Oniscus asellus</i>	1.7 \pm 0.12	48 \pm 3.7	2.5 \pm 0.19	3.3 \pm 0.12
<i>Porcellio scaber</i>	0.5 \pm 0.02	5.0 \pm 0.30	1.9 \pm 0.08	6.0 \pm 0.40
<i>Armadillidium vulgare</i>	0.5 \pm 0.02	4.5 \pm 0.25	2.6 \pm 0.22	7.1 \pm 0.53

* Indefinitely

significantly ($P < 0.01$) longer in moist air than in moist nitrogen when the pleopods were blocked, and this is consistent with the conclusion drawn above that oxygen may be absorbed through the integument. But no animals survived indefinitely if the absorption of oxygen through their pleopods was prevented. In other words, cutaneous absorption was insufficient to supply the minimum oxygen requirements.

In dry air, survival was significantly curtailed in all species, but if the oxygen tension was raised, survival was again prolonged. (Oxygen absorption, it will be recalled, was also found by direct measurement to be greater if the oxygen tension was raised.)

When blocked animals belonging to the different species are compared, there appears to be a general correspondence between period of survival and facility for cutaneous uptake of oxygen. Thus in moist air, blocked *Ligia* survived for a mean period of 60 hr., and *Oniscus* for 48 hr. In sharp contrast with these are *Porcellio* and *Armadillidium*, which survived for 5.0 and 4.5 hr. respectively.

In dry air, there is less difference between the survival times of blocked animals of the four species than there is in moist air, and this is consistent with the fact that in dry air cutaneous absorption is almost entirely inhibited in all species.

(e) *Does cutaneous absorption of oxygen occur in natural conditions?*

The results considered above leave little doubt that cutaneous respiration is possible in certain circumstances, but there is no means of telling whether, when oxygen is not in short supply, it enters through the general integument as well as

through the pleopods. Such entry has so far been demonstrated only when there is a shortage of oxygen in the tissues and therefore a steep gradient in oxygen tension across the integument.

An attempt was therefore made to demonstrate cutaneous absorption of oxygen in intact animals by injecting reduced indigo, which turns blue in the presence of oxygen. Remy (1925) used this method for several invertebrates, including woodlice. He obtained distinct blueing of the pleopods but he did not record blueing elsewhere. In our experiments, injected animals were fixed in alcohol, embedded in ester wax and sectioned at $15\ \mu$. Counterstaining with carmalum provided a pink ground against which the blueing could be more easily seen. There was intense

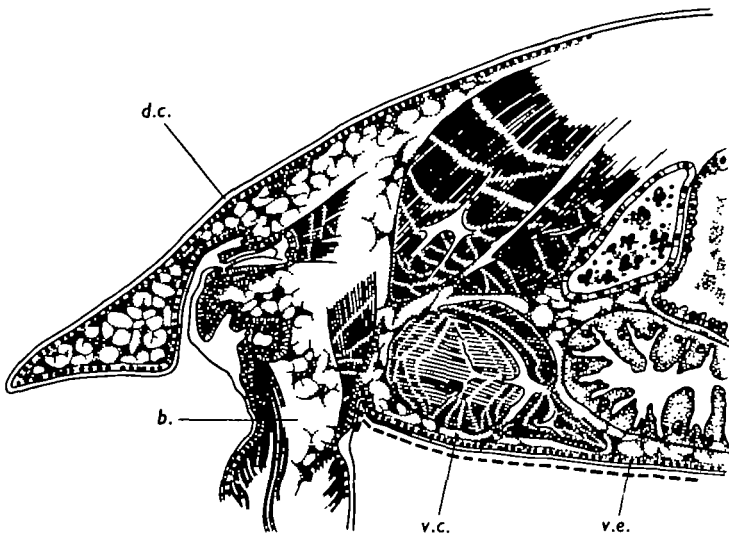


Fig. 2. Transverse section of the left half of the pereon of *Ligia oceanica* previously injected with reduced indigo. The broken line below the ventral integument indicates the region in which the epidermis was blue. *b.*, base of leg; *d.c.*, thick dorsal cuticle; *v.c.*, thin ventral cuticle; *v.e.*, ventral epidermis.

blueing of the exopodites of *Ligia*, the pseudotracheae of *Porcellio* and the endopodites of *Oniscus*. (*Armadillidium* was not used in these experiments.) There was also a less intense, but distinct, blueing of the epidermis of the ventral surface and of the base of the legs in *Ligia*, and of the ventral surface of the abdomen in *Oniscus* and *Porcellio*. Fig. 2 shows, semi-diagrammatically, the area of blueing in *Ligia*. It may be concluded, then, that cutaneous absorption of oxygen does occur in natural conditions. That the amount of blueing observed was not great is hardly surprising, for these animals were intact, and oxygen was being absorbed normally through the pleopods at the time of injection; furthermore, the total area of the integument is large compared with that of the pleopods, and the relatively small amount of oxygen absorbed is likely to be spread out over this area, so that an intense blueing, associated with a high concentration of oxygen in a small area, as in the pleopods, was not to be expected.

DISCUSSION

Cutaneous respiration is by no means uncommon in arthropods. It has been demonstrated in several aquatic forms, and Thorpe & Crisp (1947) found that in the 5th-instar nymph of the bug *Aphelocheirus* oxygen uptake was entirely cuticular. Fraenkel & Herford (1938) found that in the larvae of *Calliphora erythrocephala*, *Chaerocampa elpenor* and *Tenebrio molitor*, oxygen uptake was possible through the general integument when the spiracles were blocked, and amounted to about 25% of the normal basal values. In *Culex* larvae, the amount of oxygen so absorbed was higher still.

The literature on respiration in woodlice contains very few references to the possibility of non-pleopodal absorption of oxygen. Verrier (1932) believed that the epipodial sacs attached to the mouthparts of *Hemilepistus reaumuri* had a respiratory function, but this was not confirmed when Macagno (1938) investigated the matter experimentally by injection of reduced indigo. Macagno (1939) also claimed that in *Tracheoniscus* the pleopods are the only sites of oxygen absorption, on the grounds that no other area turned blue on injection of reduced indigo. We have in the present work observed blueing in regions other than the pleopods, but only when the animal has been exposed to very moist air, and exposure of the animals in Macagno's experiments to dry air is a possible explanation of the difference between his results and ours. Reinders (1933) measured the oxygen uptake of *Porcellio* and found that if the four pseudotracheae were blocked (by paraffin) this was reduced to 39% of normal, thus allowing for the possibility of absorption through the general integument as well as through the other pleopods.

The present work has shown that, in moist air, woodlice are capable of absorbing oxygen through the general integument, and the question raised above (p. 262) concerning respiration in dry air must now be considered. The rates of oxygen uptake and carbon dioxide output are both unaffected if blocked animals are exposed to relatively gentle desiccation in still, dry air, and this may be interpreted as meaning that water lost from the integument is replaced from within rapidly enough to maintain permeability of the integument to oxygen. But after more vigorous desiccation, in a stream of warm dry air, oxygen absorption is greatly reduced, there is apparent loss of vitality, and indeed some of the animals died during exposure. The question is whether the observed loss of vitality was caused by a lack of oxygen or by something else, such as dehydration of the tissues, after which the fall in oxygen absorption followed as a result.

There is evidence that such early loss of vitality would not have occurred if oxygen had been more freely available in the tissues: thus blocked animals, when in dry air with a high oxygen tension, survived longer and absorbed more oxygen. Normal animals showed the same response. Again, blocked animals can hardly have suffered greater desiccation than those of the same species with free pleopods when both were subjected to the same dry conditions, yet the latter absorbed more oxygen than the former and lived longer. The implication of this is that the blocked animals would have used more oxygen if it had been available in the body. It can

therefore be said that these animals in dry air died because they could not obtain sufficient oxygen. On the other hand, it might also be argued that if more water had been available they might have lived longer (or suffered less loss of vitality) even with the same low oxygen uptake. The question as to which of the two factors caused death is not a very useful one: both are involved, and the level of oxygen deficit necessary to cause death will vary according to the degree of dehydration, and vice versa.

Nevertheless, it may properly be said that if they are exposed to sufficiently vigorous desiccation, woodlice are not able to absorb as much oxygen as they need and as they otherwise would. This reduction may well be the result of drying of the integument, making it less permeable to oxygen. But this is not the only possible explanation: there might, for instance, be an active secretion of oxygen across the epidermis which breaks down if the epidermal cells suffer dehydration.

The fact that desiccated woodlice absorb less oxygen than they need, and in this sense at least may be said to die from asphyxia, is relevant to experiments designed to test the relation between survival and humidity. Thus Waloff (1941) did not find a linear relation between period of survival and humidity in *Oniscus*, *Porcellio* or *Armadillidium*, all of which lived for a shorter time in low humidities than would be expected if such a relation were true. This may perhaps be put down to the fact that in low humidities evaporation occurs rapidly, drying the integument and pleopods, so that lack of oxygen may have contributed to the animals' death. Again, Webb-Fowler (unpublished) has found that when woodlice are desiccated, the amount of water which they lose before death is not constant but depends upon the speed of desiccation; and in this case too, lack of oxygen probably contributes to the death of the rapidly desiccated animals.

It has been known for a long time that woodlice are very sensitive to desiccation; more so, in fact, than most insects. The present work has shown one way in which desiccation affects them, namely, by inhibiting the absorption of oxygen. There is evidence in the present work that pleopodal respiration is supplemented by cutaneous respiration to a considerable extent in *Ligia*, and to a lesser extent even in animals with less permeable integuments such as *Porcellio*. In the absence of efficient internal respiratory surfaces (such as those found in insects) a moist integument, general as well as pleopodal, is necessary, and the animals are therefore confined to moist surroundings not only to prevent undue depletion of the water content, but also to ensure efficient respiration.

As regards the evolution of the group, there may have been two factors favouring the retention of a permeable cuticle: first, the ability to reduce body temperature in an ecological crisis (Edney, 1953); and secondly, the need to absorb oxygen. These two factors would be balanced against the obvious advantages of an impermeable cuticle, but it is only after the problems of efficient respiration and tolerance of high temperatures have been solved that the integument can become impermeable, and a true exploitation of the terrestrial environment can begin.

SUMMARY

1. Oxygen uptake and carbon dioxide output were measured in the woodlice *Ligia oceanica*, *Oniscus asellus*, *Porcellio scaber* and *Armadillidium vulgare*, in moist and in dry air, using normal animals and animals whose pleopods had been blocked by emulsion paint.

2. In moist air, the rate of oxygen uptake which occurred through the general integument in animals whose pleopods had been blocked was about 50% of normal in *Ligia* and *Oniscus*, but less than this in *Porcellio* and *Armadillidium*.

3. After exposure to moving dry air, oxygen uptake in dry air was below normal in intact animals and nearly ceased in animals with blocked pleopods. Both showed a somewhat higher uptake if the oxygen tension was raised.

4. Carbon dioxide output in moist air was not immediately inhibited by blocking the pleopods, suggesting free diffusion through the integument. Output fell during the first hour, but the R.Q. remained above 1 in all species.

5. Survival times of blocked animals in moist air corresponded qualitatively with rates of oxygen absorption in the four species. In dry air survival was further curtailed, but prolonged again by an increased oxygen tension. Blocked animals of all species survived for a shorter time in moist nitrogen than in moist air.

6. Injection of reduced indigo showed that oxygen was absorbed by intact animals through the thin ventral integument and bases of the legs in *Ligia*, and to a lesser extent, through the ventral abdominal integument in *Oniscus* and *Porcellio*.

7. The ecological implications of these results are discussed, particularly with reference to the relation between survival and humidity. The dehydration death point is probably affected by the level of oxygen deficit and vice versa. A water-permeable integument is of value for respiration as well as for temperature control, but restricts the habitat range of terrestrial animals.

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