

THE EFFECT OF TEMPERATURE UPON THE PERMEABILITY TO WATER OF ARTHROPOD CUTICLES

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INTRODUCTION

Earlier work on the effect of temperature upon the rate of transpiration from arthropods appeared to show a relatively sudden increase in this rate from most insects, spiders and ticks at what has become known as the 'critical' or 'transition temperature'; this change has been ascribed to a correspondingly sudden change in the permeability of a wax layer at, or near to, the surface of the epicuticle (Beament, 1945; Wigglesworth, 1945; Lees, 1947; Davies & Edney, 1952). For the most part measurements have been made in dry air, so that the effect of rising saturation deficit upon the drying power of the air, and the effect of temperature upon the permeability of the cuticle itself, have both contributed to the observed rise in transpiration rate with temperature.

There are two ways of dealing with this effect of increasing saturation deficit; measurements may be made in dry air and the results reduced to rate per unit vapour-pressure difference across the cuticle, or measurements may be made in air whose relative humidity at the different experimental temperatures is so adjusted that the saturation deficit remains constant. Holdgate (1956) and Holdgate & Seal (1956) have recently employed, indirectly, the first method. In the present work the second method has been employed as well; this has not been done with insect material before.

MATERIALS AND METHODS

The greater part of the work was carried out on larvae of *Tenebrio molitor* L., adult *Glossina palpalis* (R.-D.), adult *Calliphora erythrocephala* Mg., and adult *Periplaneta americana* (L.). A few comparative measurements were made on adult *Blatta orientalis* L., the woodlouse, *Oniscus asellus* L., and the centipede, *Lithobius* sp.

The mealworms, blowflies and cockroaches were taken from departmental stocks and had continuous access to food before experiments. The tsetse flies were obtained as pupae from the West African Institute for Trypanosomiasis Research. A small breeding stock was maintained for several months by feeding the flies on rabbit's blood. Woodlice and centipedes were obtained locally and kept until required in large Petri dishes on moist sand together with a few pieces of clean bark and potato.

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The experimental technique used in the present work will be described in some detail, as certain discrepancies that have appeared in earlier work by various authors may be the result of variations in experimental methods. The apparatus used was designed to pass a slow stream of air, at the appropriate temperature and saturation deficit, through an exposure chamber in which the material could be suspended. The value of the saturation deficit chosen was 31.8 mm. Hg, which is the saturation deficit of dry air at 30° C. The use of a stream of air virtually maintains a surface of constant concentration of water vapour at a fixed small distance from the evaporating surface. A steep concentration gradient of water vapour results, and it is reasonable to assume that the rate of evaporation is not affected by the shape of the exposure chamber. However, the effective agent of evaporation over this gradient is diffusion, and the rate at which molecules diffuse is approximately proportional to the square of the absolute temperature, T . This means that irrespective of the value of the saturation deficit the rate of evaporation may be expected to increase with temperature by a factor proportional to T^2 . The effect of the increasing rate of diffusion is considerable; the calculated increase in rate at 60° C. as against that at 30° C. is 21%. Corrections have been applied to all appropriate experimental data for this effect by multiplying by the factor T_1^2/T_2^2 , where T_1 was 303° absolute (the lowest experimental temperature of the series) and T_2 was the temperature (degrees absolute) at which the experimental value was obtained.

Dry air was obtained by passing air in a continuous circuit over phosphorus pentoxide and calcium chloride. All other humidities necessary for the provision of the required constant saturation deficit were obtained by bubbling the air stream through solutions of sulphuric acid of appropriate concentrations. A unit of the apparatus using sulphuric acid solution for humidity control is illustrated diagrammatically in Fig. 1; a separate unit was required for each temperature. Air, circulated by a small electric pump, was bubbled through the acid in a large gas jar and thence, via a trap to remove any droplets of acid, to the exposure chamber. The material, suitably mounted, was introduced into the exposure chamber through a wide tube which could be sealed by the constriction of a piece of flexible rubber tubing. The unit providing dry air differed in that phosphorus pentoxide replaced acid at the bottom of the exposure chamber and a long tube of granular, anhydrous calcium chloride replaced the gas jar.

The temperature of the air stream was controlled to within $\pm 0.25^\circ$ C. by immersing the units of apparatus in a water-bath. Condensation from rising, warm, moist air tended to occur on the inner walls of the glass entry tube above the water-bath surface in the experiments at higher temperatures, when the air stream had a high water content. This condensation was prevented by warming the tube with an electrically heated jacket. The air flow was about 1.5 l./min., a rate equivalent to one complete circulation a minute. Ramsay (1935) has shown that the rate of transpiration from the body surface of the cockroach, unlike that from the tracheal system, is not greatly influenced by variations in the wind velocity; consequently any small variations in wind velocity that occurred within the different units probably caused negligible errors in the results obtained for the rates of transpiration.

Freshly killed animals were used in all experiments. The use of dead material was convenient because support was easier and also because no defaecation occurred during the exposure. It has been shown by Ramsay (1935) for *Periplaneta*, Wigglesworth (1945) for several species of insects and Edney (1951) for several species of woodlice that the rate of loss of water through the cuticle, during short exposures, is effectively the same whether the arthropod is alive or recently dead. Loss of weight was taken as a measure of loss of water, a close approximation in experiments of relatively short duration. Cyanide was used as the killing agent, there being no evidence that it caused any damage to the waterproofing properties of the cuticle as do wax solvents, etc. (Beament, 1945). The spiracles of the insects and centipedes were blocked with cellulose paint to prevent loss of water from the tracheal system.

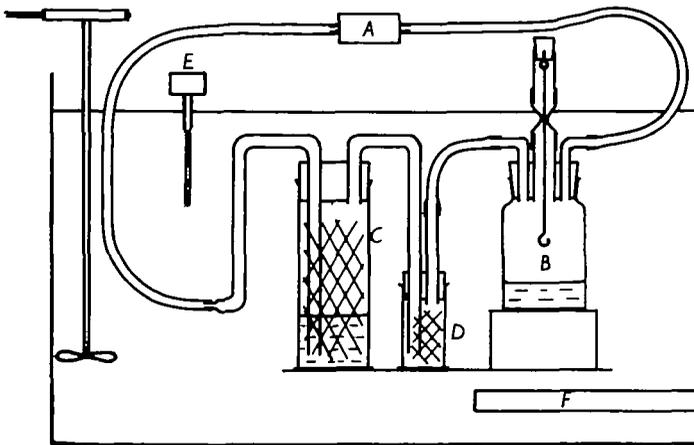


Fig. 1. Diagram of a unit of the apparatus used to provide a stream of air of controlled humidity and temperature. *A*, air pump; *B*, exposure chamber containing sulphuric acid; *C*, humidifier containing sulphuric acid and glass wool; *D*, spray trap containing glass wool; *E*, thermostat; *F*, heater.

In order to compare the rates of transpiration of different species it was convenient to express the losses in terms of surface area, viz. mg. of water/cm.² surface area/hr. The surface areas were obtained from the relationship: surface area = $K \times \text{mass}^{\frac{2}{3}}$, values for *K* being as follows;

<i>Tenebrio</i>	8.4	Wigglesworth (1945)
<i>Oniscus</i>	13.6	Edney (1951)
<i>Calliphora</i>	10.0	Measured in course of present work.
<i>Lithobius</i>	12.0	Estimated
<i>Glossina</i>	10.0	Estimated
<i>Periplaneta</i>	10.0	Estimated
<i>Blatta</i>	10.0	Estimated

Weighings were made with an aperiodic balance reading to 0.1 mg. The minimum measurable rate of loss in terms of mg./cm.²/hr. depends upon the surface area and the duration of the experiment; in the present experiments at constant saturation deficit it was 0.2 for *Oniscus* and *Lithobius* and 0.1 or less for the insects.

EXPERIMENTAL RESULTS

(a) Preliminary experiments

Evaporation, from a simple physical system providing a free water surface, into the supposed constant saturation deficit atmospheres was measured. The results, shown in Table 1, indicate that the apparatus provided atmospheres of constant drying power at the six experimental temperatures. The rates of evaporation from the water surface into dry air at a number of temperatures contrast sharply with this constancy, the rate at 60° C. being four times the rate at 30° C.

Table 1. *The rate of evaporation of water, in mg./hr., from a free water surface into a constant saturation deficit of 31.8 mm. Hg, and into dry air, at a number of temperatures*

Temperature (° C.)	Rate of evaporation	
	Constant saturation deficit	Dry
30	5.3	5.3
35	5.23	—
40	5.11	8.7
45	5.23	—
50	5.38	15.9
55	5.24	—
60	—	22.8

Whilst it is probable that at any one temperature the same physico-chemical systems control the movement of water through and from the cuticle in all normal individuals of a species, it is likely that there will be a certain individual variation in the absolute permeability of the cuticle within the species. If this is the case, and the effect of temperature alone upon these physico-chemical systems, as far as waterproofing is concerned, is not to be obscured by individual variations in the levels of water loss, it is preferable to expose each individual to the full range of experimental temperatures rather than use a different individual at each temperature. A difficulty that has frequently been referred to is a progressive decline in the rate of transpiration from a specimen in constant conditions. This was the case with *Periplaneta* (Ramsay, 1935), *Agriotes* larvae (Wigglesworth, 1945) and woodlice (Bursell, 1955). Measurements were therefore made of the consistency in rate of transpiration from various arthropods when exposed to one temperature and saturation deficit until desiccation was well advanced. In *Locusta migratoria*, the rate of transpiration into dry air at 30° C. had decreased to less than a quarter of the initial rate after 14 hr., although the water content was reduced by less than 20%. The

fall in rate was rapid at first but after a few hours much more gradual. This asymptotic type of decrease was not found in *Glossina palpalis* or *Calliphora erythrocephala*, where the rate was more nearly constant. By the time the water content had been reduced to 50% the rate was only about 10% below the initial rate. As a result of these measurements locusts were regarded as unsuitable material for the purposes of the present studies.

It is clearly necessary to design the experiments in order to keep the total loss of water from the material during the experiment to a minimum, and thereby prevent any marked decline in transpiration rate associated with increasing desiccation during the later exposures. The use of a fixed saturation deficit at all experimental temperatures is valuable in that the very high rates of water loss (and attendant more rapid desiccation) associated with transpiration into dry air at high temperatures are eliminated. In the experiments at constant saturation deficit described below, total water loss was very much less than 50%, so that with the insect material it was hoped that transpiration was little affected by desiccation. Woodlice and centipedes have cuticles considerably more permeable than those of terrestrial insects, and at higher saturation deficits the rate of transpiration was found to fall off rapidly with time, and special allowance was made for this in the design of some of the experiments with these animals (see § (b) (1) below).

(b) *The relation between rate of transpiration and temperature*

(1) *In Oniscus asellus*. When freshly killed woodlice were exposed for $\frac{1}{4}$ hr. periods to the constant saturation deficit atmospheres of 31.8 mm. Hg at six temperatures in the order 30, 40, 50, 55, 45, 35 and 30° C., it was found that there was a small rise in the rate of transpiration during the first three exposures, but then the rates decreased and the results for the intermediate temperatures did not fall on the same line as the first three. As it was not practicable to reduce the period of exposure, each individual was exposed at only three temperatures, either 30, 40 and 50° C. or 35, 45 and 55° C. The order of exposure to these temperatures was varied so that some individuals were exposed, first, to the highest temperature, some to the intermediate one and some to the lowest. This served to compensate for any falling off in rate caused by desiccation as the experiment progressed. The results are shown in Table 2 and are plotted against temperature in Fig. 2. There are two curves on Fig. 2 relating to *Oniscus*, one for each 'temperature group'. Both are approximately straight and rise only slightly, which indicates a very small increase in permeability with temperature. Edney (1951) reports a similar result for *Porcellio scaber* Latr. at constant saturation deficit.

(2) *In Lithobius sp.* Measurements were made of the rate of transpiration, at three temperatures and a saturation deficit of 31.8 mm. Hg, from freshly killed centipedes with all spiracles occluded. The order of exposure was varied as with *Oniscus*. The results are given in Table 2 and plotted against temperature in Fig. 2. There is an increase in rate with temperature, but not nearly so great an increase as that found in insects. The individual variation in rate was considerable, as

Table 2. *The rate of transpiration, in mg./cm.²/hr., from certain arthropods into a slow stream of dry air, and into air at a constant saturation deficit of 31.8 mm. Hg. Each entry is a mean and is followed by the standard error*

Material and Temperature (° C.)	Constant saturation deficit (31.8 mm. Hg)			Dry air		
	Mean rate and S.E.	Mean rate corrected for diffusion	$\log \frac{R \times 10^6}{pT^2}$	Mean rate and S.E.	Mean rate corrected for diffusion	$\log \frac{R \times 10^6}{pT^2}$
<i>Calliphora</i>	(Mean of 9)			(Mean of 3)		
30	1.62 (0.15)	1.62	1.74	1.2 (0.09)	1.2	1.61
35	2.07 (0.14)	2.00	1.84	2.1 (0.17)	2.0	1.72
40	2.54 (0.20)	2.38	1.91	3.0 (0.38)	2.8	1.74
45	2.90 (0.16)	2.63	1.96	5.2 (0.72)	4.7	1.85
50	4.05 (0.25)	3.56	0.09	9.4 (0.72)	8.3	1.99
55	4.75 (0.27)	4.05	0.14	15.5 (1.13)	13.2	0.09
<i>Glossina</i>	(Mean of 9)			(Mean of 3)		
30	0.40 (0.04)	0.40	1.14	0.53 (0.02)	0.53	1.26
35	0.67 (0.06)	0.65	1.36	0.90 (0.04)	0.87	1.35
40	0.96 (0.08)	0.90	1.49	1.35 (0.13)	1.26	1.40
45	1.30 (0.08)	1.18	1.61	2.00 (0.00)	1.82	1.44
50	1.82 (0.10)	1.60	1.74	3.00 (0.21)	2.64	1.49
55	2.08 (0.12)	1.77	1.78	—	—	—
60	—	—	—	7.00 (0.85)	5.82	1.63
<i>Tenebrio</i>	(Mean of 23)			(Mean of 3)		
30	0.18 (0.01)	0.18	2.79	0.24 (0.01)	0.24	2.91
35	0.20 (0.02)	0.19	2.82	0.24 (0.01)	0.23	2.78
40	0.25 (0.02)	0.23	2.90	0.33 (0.01)	0.31	2.78
45	0.38 (0.03)	0.35	1.07	0.61 (0.03)	0.55	2.92
50	0.99 (0.14)	0.87	1.47	1.39 (0.09)	1.22	1.16
55	1.90 (0.21)	1.62	1.74	2.81 (0.33)	2.40	1.35
60	—	—	—	6.26 (0.87)	5.19	1.58
<i>Periplaneta</i>	(Mean of 6)			(Mean of 6)		
20	—	—	—	0.47 (0.03)	0.49	1.49
30	1.56 (0.23)	1.56	1.73	1.72 (0.09)	1.72	1.77
35	3.09 (0.31)	2.99	0.01	4.13 (0.34)	3.99	0.01
40	4.49 (0.29)	4.21	0.16	6.75 (0.21)	6.32	0.10
45	5.18 (0.33)	4.70	0.21	11.15 (0.53)	10.12	0.19
50	7.21 (0.40)	6.34	0.34	19.04 (0.65)	16.76	0.30
55	5.97 (0.50)	5.09	0.24	24.52 (0.63)	20.92	0.29
<i>Blatta</i>	(Mean of 3)					
30	1.54 (0.16)	1.54	1.72			
35	2.85 (0.15)	2.76	1.98			
40	4.07 (0.42)	3.81	0.12			
45	6.35 (0.21)	5.77	0.30			
50	8.04 (0.05)	7.08	0.38			
55	6.35 (0.26)	5.42	0.27			
<i>Omiscus</i>	(Mean of 6)					
30	3.69 (0.17)	3.69	0.10			
40	4.28 (0.16)	4.01	0.14			
50	5.00 (0.12)	4.40	0.18			
35	4.19 (0.19)	4.05	0.14			
45	4.63 (0.21)	4.20	0.16			
55	4.96 (0.20)	4.23	0.16			
<i>Lithobius</i>	(Mean of 12)			(Mean of 5)		
20	—	—	—	3.7 (0.36)	3.9	0.39
25	—	—	—	5.0 (0.40)	5.2	0.37
30	8.5 (0.67)	8.5	0.46	6.8 (0.40)	6.8	0.37
35	—	—	—	9.5 (0.57)	9.2	0.38
40	11.2 (0.87)	10.5	0.56	12.4 (0.58)	11.6	0.36
45	—	—	—	15.0 (0.59)	13.6	0.31
50	13.7 (1.05)	12.1	0.62	16.6 (0.81)	14.6	0.24

shown by the high standard errors, but the trend was consistent with all twelve individuals. A statistical analysis showed that the difference in rates at 30 and 40° C. and at 40 and 50° C. was significant. ($P < 0.001$ and $< 0.01 > 0.001$ respectively.)

Results were also obtained for the rate of transpiration from each of five individuals into dry air at 5° C. intervals from 20 to 50° C. They are included in Table 2 and Fig. 3. In this particular experiment the values for the higher temperatures were probably too low owing to a decreased 'availability' of water as desiccation proceeded, otherwise the curve is close to a saturation deficit/temperature curve.

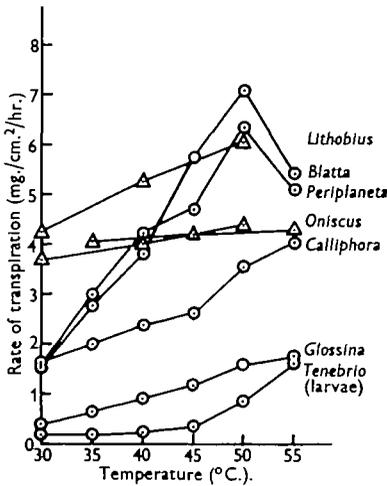


Fig. 2

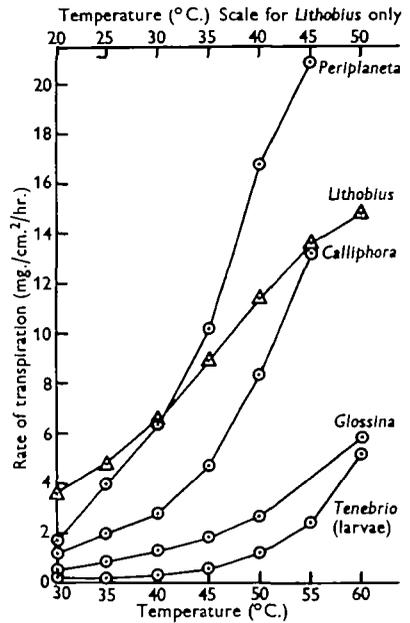


Fig. 3

Fig. 2. The rate of transpiration from certain arthropods into a constant saturation deficit of 31.8 mm. Hg at various temperatures. *Lithobius* plotted as rate $\times \frac{1}{2}$.

Fig. 3. The rate of transpiration from certain arthropods into dry air at various temperatures.

(3) *In Tenebrio molitor larvae*. The rate of transpiration was measured from recently dead *Tenebrio* larvae, with occluded spiracles, when exposed to a saturation deficit of 31.8 mm. Hg at six temperatures, and to dry air at seven temperatures. The results are included in Table 2 and Figs. 2 and 3. The curve obtained from measurements at constant saturation deficit and shown in Fig. 2 indicates a gradual, but progressive increase in permeability of the cuticle over the temperature range studied, 30–55° C.

(4) *In Glossina palpalis and Calliphora erythrocephala*. The rates of transpiration from dead *Glossina* and *Calliphora*, with thoracic spiracles occluded, into dry air and air at constant saturation deficit, are given in Table 2. They are shown plotted against temperature in Figs. 2 and 3. It will be seen that *Glossina* cuticle is relatively

less permeable than that of *Calliphora*. The graph of rate of transpiration, measured at constant saturation deficit, against temperature (Fig. 2) for *Glossina* is sloping and approximately linear, whilst that for *Calliphora* is sloping but slightly curved; the apparent cusp at 45° C. is not statistically significant. The cuticles of both species are thus shown to become progressively more permeable as the temperature rises from 30 to 55° C.

(5) *In Periplaneta americana and Blatta orientalis*. Measurements were made of the rate of transpiration, into air at constant saturation deficit and at six temperatures, from each of six individuals of *Periplaneta* and three of *Blatta* when freshly killed and with spiracles blocked. Six other *Periplaneta* were exposed to dry air at a range of temperatures from 20 to 55° C. The results are included in Table 2 and shown graphed on Figs. 2 and 3. The two species show very similar permeability changes; it is important to notice the marked and progressive increase in permeability over the range 30–50° C. Both species show an apparent decrease in permeability at 55° C.; whilst this could be the result of increasing desiccation, it may result from some hygroscopic effect at the high relative humidity (73%) necessary at 55° C. in the constant saturation deficit experiments.

Table 3. *The rate of transpiration, in mg./cm.²/hr., from adult Periplaneta americana, into a slow stream of air at a constant saturation deficit of 31.8 mm. Hg before, and after, treatment with chloroform to remove the cuticular wax. Each entry is the mean of six measurements and is followed by the standard error*

Temperature (° C.)	Before de-waxing			After de-waxing		
	Mean rate and s.e.	Mean rate corrected for diffusion	$\log \frac{R \times 10^6}{pT^2}$	Mean rate and s.e.	Mean rate corrected for diffusion	$\log \frac{R \times 10^6}{pT^2}$
30	1.42 (0.16)	1.42	1.69	14.29 (0.95)	14.29	0.69
40	4.49 (0.46)	4.21	0.16	14.24 (1.55)	13.34	0.66
50	7.68 (0.98)	6.76	0.32	15.31 (1.45)	13.47	0.67

An experiment was performed to investigate whether this progressive increase in permeability of insect cuticle, with increase of temperature, is entirely dependent upon the presence of the wax of the epicuticle. This is difficult to prove by direct experiment with the majority of insects, as the wax is normally protected by an outer layer of cement that is not removable without drastic treatment, such as boiling in chloroform. In the cockroach, however, the wax is freely exposed on the surface of the cuticle, the cement layer being embedded in wax rather than being entirely superficial (Kramer & Wigglesworth, 1950; Dennell & Malek, 1955). This allows much of the soft waxy material of the epicuticle to be removed readily by brief washing in cold chloroform. The permeability of the de-waxed cuticle may then be investigated in the usual manner.

Six freshly killed individuals of *P. americana* were used, each with spiracles and body openings occluded. The rate of transpiration from each was measured during

$\frac{1}{4}$ hr. exposures to the constant saturation deficit of 31.8 mm. Hg at 30, 40 and 50° C. The specimens were then washed in cold chloroform for 3-4 min., and transferred to a moist atmosphere for $\frac{1}{2}$ hr. to allow the adherent chloroform to evaporate. When they were free from chloroform odour their weight had decreased by about 100 mg. (c. 1% of the original body weight) owing to some evaporation of water. They were then re-exposed at the same temperatures and saturation deficit as used before de-waxing.

The results are summarized in Table 3. The permeability of the cuticle increases fivefold between 30 and 50° C. before de-waxing, but is unaffected by temperature afterwards. All the progressive change in cuticle permeability is thus dependent upon the presence of cuticular wax. It will be seen from the data in Tables 2 and 3 that the cockroach cuticle without its soft wax covering has a permeability even greater than that of the cuticles of the woodlice and centipedes.

(c) *The relation between the rate of transpiration and saturation deficit at high temperatures*

If the limiting factor for the rate of transpiration from an insect is the drying power of the air, it is to be expected that the rate of transpiration per unit saturation deficit will be constant at one temperature, whatever the value of the saturation deficit used during the measurement. Comparison of the rates measured in a saturation deficit of 31.8 mm. Hg and in dry air indicate that, in *Tenebrio* larvae and *Glossina*, the rate is relatively greater when determined at the lower saturation deficit. This may be caused by desiccation, the effects of which will normally appear sooner in experiments carried out with dry air than in those using moist air, but may also indicate that some other factor limits the rate of evaporation at high saturation deficits.

The difference is much less apparent in the results obtained for *Calliphora*. Measurements were made of the rates of transpiration from each of eighteen individuals of *Calliphora* into saturation deficits of 31.8, 65.8 and 118.0 mm. Hg at 55° C. The ratios of loss per unit saturation deficit were 1.0:1.0:0.93, which is reasonably close to a direct proportionality between rate of transpiration and saturation deficit at this one temperature.

DISCUSSION

We may conclude that the cuticle permeability of *Oniscus* and *Lithobius* is little affected by temperature, over the range studied, as their rates of transpiration at constant saturation deficit do not increase greatly. This agrees with the conclusions of Edney (1951) and Cloudsley-Thompson (1950). The absolute permeability of the centipede and woodlouse cuticle is high compared with that of most terrestrial insects that have been studied. These two characteristics, high absolute permeability and low increase of permeability with temperature, are expected with species which apparently lack a continuous wax layer to the cuticle.

The present data for insects show certain irregularities that may derive from some unaccounted experimental hazard. For example, the measured rate of transpiration from *Calliphora* at constant saturation deficit and 45° C. is too low to allow the construction of an otherwise smooth curve when the rate of transpiration/temperature relationship is drawn. Similarly, the rates at 55° C. and 31.8 mm. Hg saturation deficit for *Blatta* and *Periplaneta* are exceptionally low. We must recognize that certain experimental errors arise with the usual methods for the determination of cuticle permeability and should not place too great a reliance upon small apparent changes in permeability from temperature to temperature; marked trends, however, are valid and may allow certain definite conclusions to be drawn.

The data obtained for the rates of transpiration into dry air at various temperatures from insects (Wigglesworth, 1945), ticks (Lees, 1947) and spiders (Davies & Edney, 1952) led to the conclusion that at a fairly definite temperature, dependent upon the species, the cuticle permeability greatly increased. This increase was believed to result from some transition in a discrete wax layer of the epicuticle. When Beament (1945) extracted this wax and deposited it upon suitable membranes he found that the permeability of these membranes showed temperature-dependent changes generally similar to those of the intact insect. He regarded the basis of these permeability changes to be a single change in the wax, probably a 'crystalline transition' of the type Müller (1932) found to occur in certain long-chain normal paraffins (C₂₄-C₄₄) a few degrees below their melting-points. It is possible to envisage that such a transition, producing an abrupt change in the dimensions and spacings of the wax molecules, in a suitably orientated wax layer could lead to a sharp increase in permeability to water of the system. Such a single change could explain the existence of a certain degree of permeability below the transition temperature and a higher one above it, but not a continuous rise in permeability above this temperature. The measurements made at constant saturation deficit clearly indicate that there is a real, continuous change with temperature in the permeability of the cuticle. This was so over the whole experimental range of 30-55° C. for *Glossina* and *Calliphora*, from 30 to 50° C. for *Blatta* and *Periplaneta*, and from at least 40 to 55° C. for *Tenebrio* larvae. The use of dry air at all temperatures, with its necessarily increasing drying power, has generally tended to obscure the existence of this continuous change which produces a real, progressive increase in cuticle permeability with temperature.

A single transition of the Müllerian type cannot explain continuous permeability changes. Chibnall, Piper, Pollard, Williams & Sahai (1934) have shown that the cuticular waxes of insects are complex mixtures of long-chain paraffins, primary alcohols, fatty acids, etc. Beament (1955) found that shorter-chain, volatile paraffins, alcohols, etc., are additionally present in the soft wax of *Periplaneta*. Such complex mixtures of waxy materials will not possess sharply defined physical characteristics. Recently, Holdgate & Seal (1956) have found that no 'crystalline transition' is demonstrable in the chloroform-extracted, primary cuticular wax of *Tenebrio* pupae. This primary wax showed a very diffuse melting-point with parts

melting from about 35 to 55° C., although most melted by 42° C. It is possible that physical changes in the cuticular waxes, which have such complex chemical composition and variable spatial distribution within the epicuticle, could produce a continuous change in permeability over a fairly wide temperature range.

Holdgate & Seal (1956) believe that the cuticle permeability changes are basically exponentially related to the temperature, as is the movement of water vapour and certain gases by activated diffusion through some organic membranes (Barrer, 1939; Doty, Aiken, & Hermann, 1944). To test this hypothesis with the present results values of $\log_{10} \frac{R}{pT^2} \times 10^6$ were calculated, and these values are included in Table 2. (R is the rate of transpiration, in mg./cm.²/hr., measured at a saturation deficit of p mm. Hg and an absolute temperature of T° .) The values of $\frac{R}{pT^2}$ represent, on an arbitrary scale, the rate of transpiration per unit saturation deficit, with correction for the increasing rate of diffusion with rising temperature. These values therefore give an indication of the permeabilities of the cuticles concerned. If the cuticle permeability is strictly exponentially dependent upon temperature the graphs of log permeability against temperature, or the reciprocal of temperature should be linear. Fig. 4 shows the graphs of log 'permeability' ($\log_{10} \frac{R}{pT^2}$) against $1/T$ (using the data obtained with the insect material at constant saturation deficit). The graphs approximate to linearity, with the exception of that for *Tenebrio* larvae, which is decidedly curved. (It is unlikely that this difference in shape can be correlated with the presence of an outer cement layer in the cuticle of *Tenebrio*, and with its absence, as such a layer, in the other species used, since Holdgate & Seal obtained similar curved graphs for freshly moulted *Tenebrio* pupae and *Rhodnius* nymphs which both lack a cement layer at that stage.) The linearity of the majority of the plots of log 'permeability' against $1/T$ may indicate that the cuticle permeability of several species of insects is exponentially dependent upon temperature, and may obey the relationship $P = P_0 e^{-E/T}$, where P is the permeability constant and P_0 and E are constants; this is a relationship characteristic of transmission by activated diffusion (see Barrer, 1939).

It seems that at the present time there are still too many unknown factors to enable us to come to any final conclusions as regards the physical basis of the temperature-dependent changes in the permeability of insect cuticle. There is a certain inconsistency in results obtained by different workers, which might be overcome by refinement and standardization of technique; but even in the hands of one worker there are interspecific differences of such a nature as to indicate either a very considerable experimental error or a complex interaction of factors determining the effect of temperature upon cuticle permeability. Thus the curves for log permeability against temperature are sometimes approximately linear, sometimes curved upwards or downwards. Broadly speaking, there appear to be two possible mechanisms that might lead to an approximately exponential relationship between temperature and permeability: activated diffusion through a cuticle

that does not itself undergo physical change, and progressive change towards greater permeability of a complex wax layer in the cuticle. Between these two mechanisms, or various combinations of the two, it is impossible to distinguish using the present techniques, and a new method of approach is required.

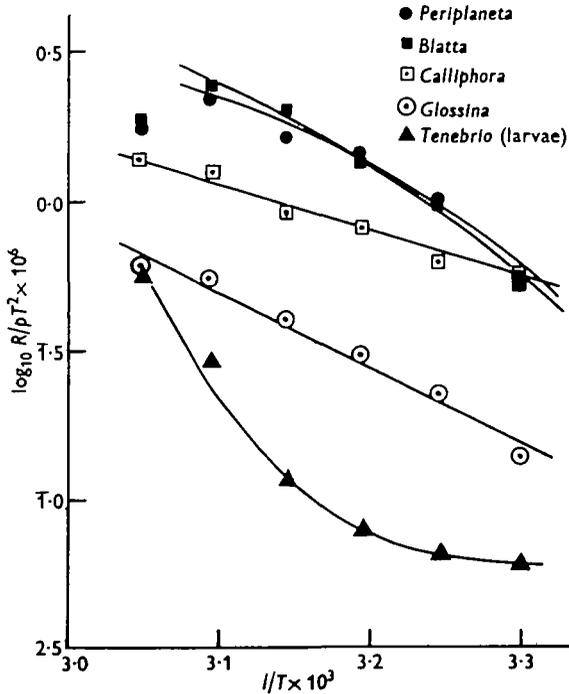


Fig. 4. Graphs of $\log_{10} (R/pT^2) \times 10^6$ against $1/T$ for a number of insects. Derived from measurements of rate of transpiration into a constant saturation deficit of 31.8 mm. Hg.

SUMMARY

1. The effect of temperature alone upon the permeability of arthropod cuticles has been investigated by measuring the rate of transpiration into a constant saturation deficit at a number of temperatures.

2. The permeability of the cuticles of *Oniscus asellus* and *Lithobius* sp. is little affected by temperature.

3. The cuticles of *Calliphora erythrocephala*, *Glossina palpalis*, *Blatta orientalis* and *Periplaneta americana* become progressively more permeable as the temperature is raised from 30 to 50, or 55° C. That of *Tenebrio molitor* larvae behaves similarly, but the increase in permeability is less marked until the temperature rises above 40° C.

4. The progressive increase in permeability of the cuticle of *Periplaneta* is entirely eliminated if the soft wax coating is removed.

5. The increase in permeability of the insect cuticle with temperature is approximately exponential and cannot result from a single, abrupt change in a wax layer at a 'critical temperature'. Possible physical bases for a continuous change are discussed.

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