

THE EFFECT OF TEMPERATURE ON THE WATER-PROOFING MECHANISM OF AN INSECT

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INTRODUCTION

Ever since Ramsay (1935) first demonstrated that drops of water placed on the surface of a cockroach were waterproofed by a grease spreading from the cuticle, information has been sought on the role of cuticular lipoid, and on the mechanism of waterproofing. The extension of Ramsay's work to insects in general, stems principally from the researches of Wigglesworth (1945) and Beament (1945). Wigglesworth showed that in a large number of terrestrial species the cuticle contains a waterproofing barrier located superficially in the epicuticle; its lipoid nature was revealed by the effect of solvents and of emulsifiers. Beament demonstrated that much of the waterproofing properties could be simulated by making artificial 'cuticles' using waxes extracted from cast skins; he concluded that the essential part of the waterproofing mechanism was a discrete layer of wax. Subsequently, Wigglesworth (1947, 1948) followed the deposition of the cuticle in *Rhodnius* and *Tenebrio* and showed the relationship of the wax to the other cuticular components. Ramsay (1935) also tested the effect of temperature on the permeability of the cockroach. He suggested that at lower temperatures, the cuticle was impermeable and little affected by temperature, but that—at about 30° C.—there occurred a sharp transformation to a system of high permeability, increasing steeply with temperature. He illustrated the effect by drawing two straight lines through the plot of permeability values at various temperatures, but adduced to a constant saturation deficiency.

Consequently, transpiration/temperature investigations figured largely in Wigglesworth's and Beament's work. As well as the correspondence between such properties for an intact cuticle and for its wax on an artificial membrane, the observations suggested that each species exhibited a 'critical temperature' at which the permeability of the wax increased quite sharply. Beament's measurements of the thickness of wax in cuticle indicated that the permeability was remarkably low, even for such a substance as wax, so he considered that this was due to an organized molecular structure within the wax, which changed at the critical temperature or 'transition point'.

Subsequently, a number of workers—particularly Edney (1951)—pointed out that since the saturation deficiency of dry air increases exponentially with temperature, the rate of water loss from the cuticle might also be expected to change in this

way, thus giving a plot with the appearance of a sudden steepening of gradient, but not implying a discontinuous change in the waterproofing mechanism. Later, Holdgate & Seal (1956) made an extensive study of the cuticles of *Rhodnius* and *Tenebrio*. They plotted their results in a way which they suggested would eliminate the effect of increasing temperature on saturation deficiency, and could find little evidence for a sudden breakdown in the waterproofing mechanism at a particular temperature. On the experimental evidence which they presented, their conclusions were certainly justified, and they suggested that the permeability of the cuticle could be explained in terms of diffusion through a membrane with capillaries. However, the author has concluded that the experimental methods on which all the previous workers—himself included—have based their arguments, are so inadequate as to render invalid the interpretations of the structure and the physical chemistry of the waterproofing process based on these methods. For this reason, he has developed the techniques elaborated below, and started a reinvestigation of the problem of cuticle permeability; this paper deals with the permeability of the cuticle of the cockroach, for it was with this insect that Ramsay (1935) made the first investigation in this field.

THE MEASUREMENT OF CUTICLE PERMEABILITY

The term 'saturation deficiency' is used to denote the difference between the pressure of water vapour at saturation and that actually obtaining at the same temperature. It will be expressed in mm. Hg and represented by 's.d.'. Percentage relative humidity will be represented by 'R.H.'.

In the original experiments of Wigglesworth (1945) and Beament (1945) water loss was promoted by the simple process of placing a quantity of the drying agent, phosphorous pentoxide, in a large conical flask and suspending the specimen in the air in it. Temperature control was obtained either by immersing the flask in a large water-bath, or by placing it in a crude oven. An ordinary mercury thermometer in the air revealed temperature, and water loss was measured by removing the specimen at intervals to a chemical balance and determining the loss in weight. The authors appreciated that their methods left much to be desired: in particular, experiments were usually severely limited because the duration of desiccation was governed by the water content of the specimen. While small improvements were added by Edney (1951) who used a continuous air stream, and Holdgate (1956) who stirred the air in an enclosed space, subsequent workers have in general adopted this type of experimental method; at best, there has been the virtue that a direct comparison of the results of the various workers is possible.

The physico-chemical controversy which followed, arose out of the interpretation of evidence which purports to show the temperature/permeability relationship of the cuticle membrane. This evidence contains a grossly invalid assumption. All the experimental results so far produced relate to the evaporation from the cuticle at various *air* temperatures; while, ecologically, the evidence is worth its face value as indicating the amount of water which an insect in similar environmental

circumstances in the field might be expected to lose, as evidence of cuticle permeability for physico-chemical interpretation it is useless. What is wanted is the relationship of cuticle permeability to *cuticle* temperature, which must necessarily be less than the temperature of the surrounding air.

Consider an insect of 100 mg. suspended in a stream of dry air. Evaporation from it involves a loss of latent heat, and without an external heat source its temperature would fall by 5°C. for every mg. evaporated. However, as soon as it is cooled, heat flows in from the surroundings by conduction and convection. A thermal equilibrium is thus established between evaporative heat loss and heat gain through temperature difference. Since it has been shown (Jeffreys, 1918) that the laws governing molecular conductivity and diffusivity are the same, and since it is clear from the work of Pasquill (1949) that similar relationships hold when one considers eddy diffusivity, it follows that as the permeability of the cuticle increases, and thus the rate of evaporation, so the temperature difference between cuticle and air must increase in strict proportion. Thus (Fig. 1 A) to each evaporation rate, and

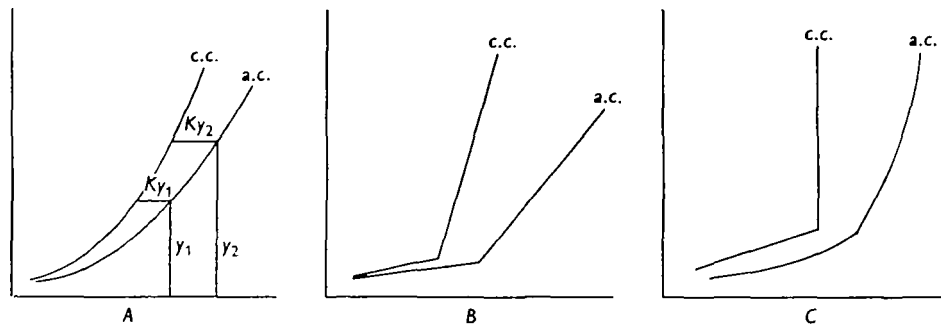


Fig. 1. Possible relationships between the 'air' temperature curve (*a.c.*) of water loss and the 'cuticle' temperature curve (*c.c.*) of water loss. For explanation, see text. Ordinate, rate of water loss; abscissa, temperature.

corresponding air temperature, there is also a cuticle temperature which is less than the former by an amount proportional to the evaporation rate. If we could obtain this proportionality, and work under ideal conditions, the cuticle temperature curve could be derived from the air temperature data; a rough calculation shows that the difference is likely to be of the order of 1°C. when water is evaporating from the insect at 0.1 mg./min. Such differences are therefore not to be ignored.

A knowledge of the temperature of the cuticle is important for other reasons, besides that of assessing the true temperature coefficient of cuticle permeability. It is obvious from Fig. 1 A that if the plot of the rate of evaporation against air temperature (the 'air' curve) is a smooth curve, so also will be the plot of rate of evaporation against cuticle temperature (the 'cuticle' curve), although it will be everywhere steeper than the 'air' curve. Where the 'air' curve shows a discontinuity, however, it is clear from Fig. 1 B that the 'cuticle' curve will also show that discontinuity and will define its position more clearly because of the greater slope.

Indeed, it is even possible that an 'air' curve with a finite slope could correspond to a 'cuticle' curve taking the form of a vertical line (Fig. 1 C).

Turning our attention to the actual experimental methods used by previous workers (see above) several possible sources of error can be suggested, many of which have been demonstrated experimentally by the present author.

(1) Fluctuations of $\pm 1^{\circ}\text{C}$. have been recorded in the air in conical flasks surrounded by a water-bath, while an ordinary mercury thermometer shows no change. The sharpness of a discontinuity will not be revealed by experiments conducted in fluctuating conditions, because each determination will be a mean value for a temperature range.

(2) The equilibrium between an evaporating surface, air, and a drying agent is established very slowly. Thus, for a large part of the time occupied by a typical water loss determination, the s.d. is not maximal and the measured rate of loss will be low. Even so, this method will never give conditions equivalent to putting the insect in dry air. Consequently, previous authors have tended to over-compensate for the change of s.d. with temperature.

(3) To ensure conditions in which it is the membrane which limits the rate of water loss, it is essential to ventilate the surface. With insect cuticle at higher temperatures considerable wind speeds must be used, or the recorded losses will be low.

(4) Apart from the disturbance of stable conditions within the flask when it is opened to withdraw the insect, significant errors arise either from weighing the insect while hot, or from allowing it to cool (and to gain or lose water) before weighing.

Experimental methods

A large programme of technical development has been carried out in order to overcome as fully as possible the objections to previous work, outlined above.

Temperature control. The control of air temperatures, especially in small spaces, is a very difficult proposition and commercially available equipment for this purpose does not appear to exist (Beament, 1958*a*). However, Beament & Machin (1958*a, b*) have developed a method which is ideally suited to this purpose. Very briefly, the source of heat which is used is of continuously variable power dissipation, instead of the typical heater which is either 'on' or 'off', and the output is controlled according to requirement by electronic methods. The temperature-sensitive element is a thermistor of minute thermal capacity. Now it is generally true that the best temperature control in an enclosed box is obtained at the site of the thermostat; in this equipment the element is movable and can be placed next to the specimen under investigation. The device also has the useful characteristic that the heater current remains almost constant when control has been achieved, provided that circulation over the element is adequate; but when objects in the box interfere materially with the air circulation the heater current fluctuates appreciably in a cyclical fashion, and this can be used to determine the most satisfactory arrangement of the contents of the box. The device accommodates very rapidly either to changes in thermal loading, or to new temperatures; it is

possible to obtain control to $\pm 1/50^\circ$ C. over a temperature range $15-35^\circ$ C. and to $\pm 1/25^\circ$ C. over the range $35-55^\circ$ C. The layout of the equipment is shown in Figs. 2 and 3.

Measurement of air temperature. This was also carried out by the use of a thermistor (Beament & Machin, 1958a); the temperature of the air near the specimen can be obtained with a sensitivity at least equal to that of the control element. Information is presented remotely as a reading on a large meter, which can follow quite rapid fluctuations in temperature. The heat dissipation of the sensitive element (an important characteristic of all electrical resistance thermometers) is so small that it is not sensibly affected by the wind-speed over its surface.

Measurement of loss of water. The rate of water loss of an intact dead insect is best measured as rate of weight loss, and for reasons given above the arrangements for weighing should not require either the opening of the box or the transfer of the insect to a balance. The order of weight loss to be detected may be as little as 0.2 mg., so that a delicate weighing method is needed; the specimen may be 500 mg. or more, on the other hand, so that even though a torsion method might be the most convenient way of detecting such small weight changes, it cannot be used directly to give a sensitivity of $1/2500$, such as this experiment requires.

The method eventually chosen used a combined beam and torsion principle. The main bulk of the specimen was counterpoised on a beam balance, to which a torsion balance of high sensitivity and smaller range was attached (Figs. 2, 3); thus the loss in weight of the specimen was countered by applying suitable force from the torsion spring to bring the beam back to its original position. The centre of gravity of the loaded beam must be arranged to produce a sensitivity of the same order as that of the torsion balance; this does not mean, however, that the position of the equivalent fulcrum of the beam need be fixed, nor that the suspension should be free of torque. All that matters is that both should be constant at the one chosen null-point position. Both these considerations have very important consequences or the method of measuring cuticular temperature.

Measurement of cuticle temperature. The device for measuring cuticle temperature must not only be comparable in sensitivity with that for measuring air temperature; it must be carried on the beam and it must also be extremely small, to equilibrate with a surface temperature. For these reasons, thermocouples were used. Both junctions were attached to the specimen end of the beam, one arranged conveniently for insertion into the specimen, the other lying alongside the thermistor element measuring air temperature. The current generated in the thermocouple system thus measured the temperature difference between the cuticle and the air, the air temperature being already measured absolutely. The suspension of the beam was made of two parallel nichrome wires, 0.001 in. in diameter, attached to an insulating cross-member at the centre of the beam (Fig. 2) and to similar insulating units in the roof of the box; these wires also served to carry the thermocouple current to a suitable meter.

The circulation of air. The provision of circulating air is necessary both for temperature control and also for the removal of water vapour from the evaporating

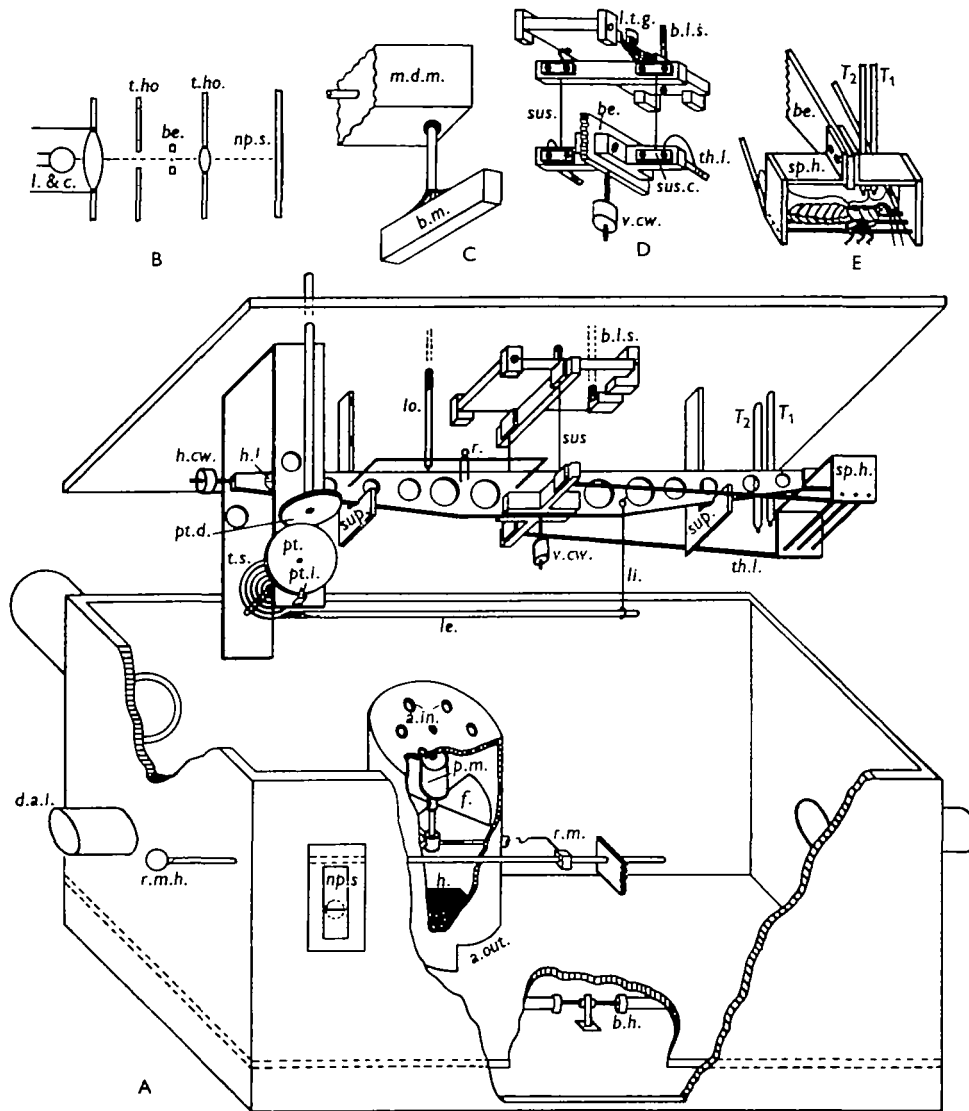


Fig. 2. Detail of equipment described in text; A, orthogonal projection of polystyrene box containing air heating and circulation equipment, with lid raised to show weighing mechanism suspended from it. B, section of optical attachment to project position of beam on to scale. C, magnetic drive for circulating fan, lowered over lid when box is closed. D, projection of beam suspension seen from end of box, showing method of conducting thermocouple current out through suspension. E, projection of specimen holder seen from end of box, showing cockroach in position and thermocouples: one junction under thoracic fold and one junction positioned between the two thermistors. *a.in.* and *a.out.*, air inlet and outlet to heater-circulator unit; *be.*, balance beam; *b.m.*, bar-magnet driving fan; *b.h.*, background heater; *b.l.s.*, beam lowering screw; *d.a.i.*, dry air inlet and outlet; *f.*, fan; *h.*, heater; *h.cw.*, horizontal counterweight; *h.l.*, hair-line index in beam; *l.&c.*, lamp and collimator; *le.*, lever between torsion spring and beam; *li.*, link between lever and beam; *lo.*, beam locking screw; *l.t.g.*, lead to galvanometer; *m.d.m.*, mechanism driving magnet; *np.s.*, scale showing projection of beam for null-point; *pt.*, protractor recording torsion of hairspring; *pt.i.*, protractor index; *pt.d.*, remotely operated drive on protractor; *r.*, rider; *r.m.*, rider manipulator; *r.m.h.*, rider manipulating handle; *sp.h.*, specimen holder; *sup.*, supports for beam when clamped; *sus.*, fine suspension wires of beam; *sus.c.*, clamp for suspension wires, also connected to galvanometer circuit; T_1 and T_2 , air-temperature measuring and controlling thermistors; *th.l.*, thermocouple main leads; *t.ho.*, torsion-spring housing, seen in section; *t.s.*, torsion spring; *v.cw.*, vertical counterweight.

surface, and preferably, the rate of circulation should be variable at will. But the amount of heat emitted by any motor would be far too great for the size of enclosure we are using, and with this in view the small fan in the heater shroud was magnetically coupled to a variable speed motor outside the box.

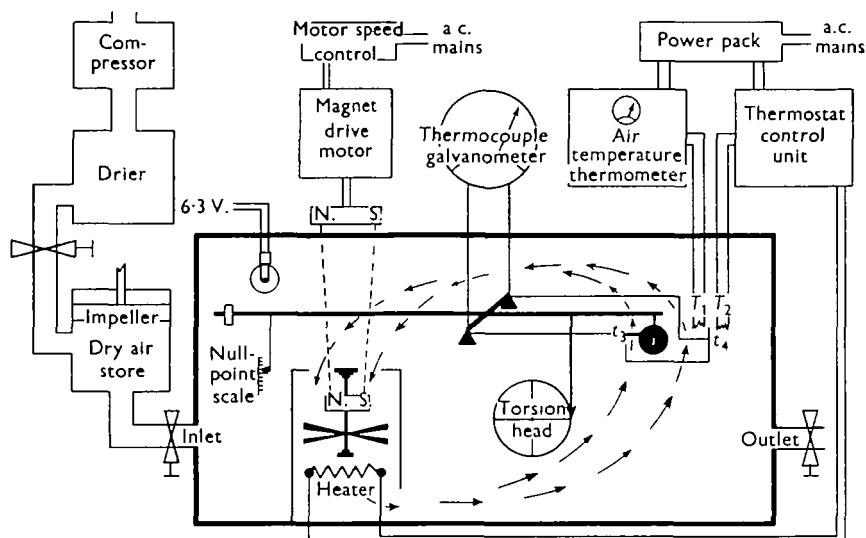


Fig. 3. Schematic drawing of entire equipment. T_1 thermistor measuring air temperature; T_2 thermistor controlling air temperature; t_3 cuticle temperature thermocouple junction; t_4 air temperature thermocouple junction.

Desiccation. Humidity—and especially a fluctuating humidity—cannot be measured accurately (Beament, 1958*b*). Further, Beament (1954) has pointed out that drying agents or saturated aqueous solutions do not give reliable equilibrium atmospheres for $\frac{1}{2}$ hr. or so after any of the many conditions affecting their operation have been disturbed. Because of this, and of preliminary investigations made into the conditions in conical flasks containing a drying agent, a new approach was made. The information needed in these experiments is the actual s.d. of the air in the box. Chemically dried air can be readily obtained, and the actual s.d. can be calculated if one knows the temperature and the amount of water introduced into a known volume of dry air, provided that no adsorptive surface is present. Water vapour is adsorbed on to glass and most other materials, but certain plastics, of which polystyrene is particularly convenient, have no measurable water adsorption even when steeped in liquid water. A vessel of such material is thus ideally suited to enclose a volume of dry air in which a known R.H. can be obtained by admixture of a known quantity of water. Further, a cement of distrene in benzene can be used to coat metals and other components of apparatus inside such a vessel, and when it has dried thoroughly it gives them a similarly non-adsorptive surface.

The impossible aim of maintaining any particular constant humidity inside the box was therefore abandoned; instead, it was proposed to start with the box filled

with chemically dried air and to ensure that the only surface exchanging water vapour with the air was the transpiring insect under study. The recorded weight loss of the specimen is in fact the amount of water being added to the air originally dry, so that, provided the volume of the container is known, the actual s.d. at any point in the experiment can readily be calculated.

Construction of equipment. The container (Figs. 2, 3) was a polystyrene box having an internal capacity of 1520 ml. This plastic is, additionally, a remarkably fine thermal insulator, so that few further precautions were needed in this respect. A portion of a second similar box was used to form an air-jacket round the bottom of the main container, and this space included a number of carbon resistors, such that it was possible to dissipate about 5 W. 'background' heat for work at higher temperatures. Leads to the heater element were brought through air-tight seals in the base of the box; the outlet from the fan-heater shroud was aimed to provide the most efficient circulation over the specimen and the three temperature-sensitive elements. Non-magnetic materials were used throughout to avoid any interaction with the magnetic field driving the fan. The balance beam was of Elektron metal and extensively lightened by perforation; its suspension and the whole of the accessory mechanism associated with weighing were attached to the lid of the box, which was consequently strengthened by brass girders to prevent distortion when hot. To support the specimen, a grid of phosphor-bronze wires was constructed, thus to provide for the maximum of air circulation. This specimen holder was also strengthened by bronze rods running to the centre of the beam, and these rods carried the thermocouple current to the suspension. The thermocouples were of 44 s.w.g. copper-constantan, pot-brazed with silver-cadmium solder. All parts of the thermocouple circuit, except for the junctions, were heavily coated with distrene cement to prevent local heat conduction through the wires.

The suspension was attached to the lid of the box so that the unit clamping the upper ends of the nichrome wires could be raised and lowered by a screw passing through the lid; thus the whole beam could be raised to swing free, or lowered on to two V-rests, one at each end, in which position it could be clamped by a further screw. The specimen could thus be mounted without risk to the fine suspension. The other end of the beam carried an adjustable counterweight and a graduated slide for small 'rider' weights; these latter could be manipulated by a lever passing out through the side of the box. Below this end of the beam also, but attached to the lid, a plastic cage carried a coiled hair-spring, from which a fine glass capillary applied tension through a pivot-link to the 'specimen side' of the beam. The shaft bearing the torsion spring also carried a 360° graduated protractor, from which the degree of torsion applied by rotating the shaft could be obtained; rotation was transmitted by a friction-drive wheel on a vertical shaft through the lid of the box.

A horizontal cross-wire was mounted in one of the holes in the beam, and an image of this was projected on to a graduated screen on the side of the box; this allowed the accurate location of the beam in its zero position during an experiment. The beam also carried adjusting screws, in order that it could be set to appropriate

sensitivity. With the exception of the wire of the heater element, the torsion spring and the balance suspension wires, all materials which were not polystyrene were covered with distrene cement.

Plastic tubes were let into the ends of the box, each with sealing devices, so that the enclosure could be flushed through with dry air; the lid was carefully ground to fit the seating on the box, and a small quantity of silicone grease used as a seal. Finally, a minute capillary was left open to the atmosphere to allow for the escape of air during sealing the box, and for thermal expansion (see p. 503).

Calibration. The torsion balance was calibrated against standard weights and riders; in the model used in these experiments, it was arranged that 10° rotation of the torsion spring corresponded to 1 mg. weight change at the specimen. The thermocouples were calibrated in the usual way against Beckmann thermometers, and the galvanometer gave a deflexion of *ca.* 2 cm./ $^\circ$ C. difference.

Experimental procedure

The specimen, a large nymph of *Periplaneta americana*, was very lightly anaesthetized with carbon dioxide, and immediately placed in hydrogen sulphide gas for 20 min.; unless narcotized, this species voids faeces violently when first sensing the poison, usually contaminating its surface, and sometimes even rupturing the hindgut. The dead specimen was temporarily stored over phosphorous pentoxide in a large vessel to bring the surface towards a condition of equilibrium with dry air. A store of air at less than 1% R.H. was obtained by passing air at 15 lb./sq.in. through a long column of silica gel and allowing it to escape slowly through a sinter-glass funnel immersed in 98% sulphuric acid. A large store of such dry air was held at atmospheric pressure in large polythene bags. Where the experiment was to be started appreciably above room temperature, the box was also 'warmed up' at this stage; the temperature regulator was then disconnected and the lid bearing the balance was jacked up; the specimen was placed on the holder and the 'cold' thermocouple junction placed under a meso- or meta-thoracic wing-bud, so as to be in intimate contact with the cuticle surface; great care was exercised not to damage the cuticle. A rough balance was obtained, using the counterweight and heavy riders, but ensuring also that if high rates of water loss were expected, a suitable rider had sufficient traverse to offset the bulk of this loss by moving it during the experiment, for the weight loss which the torsion spring could accommodate was limited. The lid was then lowered into the enclosure, the beam was allowed to swing completely freely, and a rider was manipulated to such a position that the image of the cross-wire came to a convenient scale position, with a full traverse of the torsion spring available.

The box was then swept through with a sufficient volume of dry air to replace its previous atmosphere. From this point onwards it is essential to record the total weight loss of the specimen, whether the box is at constant temperature or changing to a new temperature, for until it is reflushed with dry air, *all* losses contribute to the humidity of the enclosed air. The box was then sealed, its air temperature was brought to the required value, and readings were taken at intervals of air temperature,

cuticle-air temperature difference and weight loss of the specimen. The box was then brought to a new temperature and the procedure was repeated. According to the temperature excursion and the duration of the experiment, limits are set to the permissible amount of evaporation into the air contained in the box; immediately following a selected reading for water loss, the box was refushed with air and a further measurement of evaporation rate taken *at the same temperature* as the previous one. The ratio of the two readings at one temperature thus obtained should be the ratio of the two s.d.'s respectively obtaining, which gives a useful check on the experimental procedure. It was found that, providing one was not working close to the maximum temperature obtainable (i.e. with the heater at capacity load) the polystyrene insulation of the box kept it and its contents so well insulated that the heater was easily capable of bringing freshly introduced air up to the required temperature almost at once and closely controlled temperature conditions were restored in a matter of seconds after the box was resealed.

One experimental problem remains: air expands on heating, and to allow for this, a fine capillary leak was provided. But the assessment of the s.d. at any point in the experiment depends on a knowledge of the quantity of moisture evaporated into a known volume of air. In the first place, the experiments with one filling of air were always carried out at a constant temperature, or with increasing temperatures, so that any leakage would always be outwards; thus humid air from the ambient would not enter the box. Since it was found experimentally that there was always an increased rate of evaporation at higher temperatures, frequent air replacement was necessary at higher temperatures, whereas low temperature experiments had to be run for much longer periods in order to get measurable water loss. This circumstance in itself helps to keep down the error involved. In practice the greatest excursion of temperature with one filling of air was well under 10°C ., and heating through this interval would cause a volume expansion of 3%. Suppose a maximum of 3 mg. water is allowed to evaporate before the air is changed; in the air volume under consideration, this water would be distributed at 2 mg./l., and over the experimental range we are considering 2 mg./l. is equivalent to 2 mm. Hg vapour pressure. Suppose in the course of one run the air is heated from 25°C . to 35°C .; the s.d. of dry air at 35°C . is 42 mm. Hg which by evaporation from the specimen has become $42 - 2 = 40$ mm. Hg provided no loss has occurred. With 3% of leakage, the actual vapour pressure due to evaporation might be only 97% of 2 mm. so that the error in calculating the actual s.d. at 35°C . is actually 3% of 2 in 40, i.e. 0.2% which is, of course, negligible.

RESULTS

In Fig. 4 the actual water loss in mg. from a large cockroach nymph is plotted against the air temperature and against the corresponding cuticle temperature. The extent of the temperature difference revealed by the thermocouples is thus the horizontal intercept between the curves. The wind speed was constant throughout. Thus the 'air' curve is closely comparable with the curves obtained by previous

workers, for in the present experiments the R.H. has not exceeded 10%, and previous workers would have been fortunate to have achieved as close control as this in their experiments using drying agents. The temperature scale is an open one, but no amount of compression of this axis would give the curve any appearance other than of a smooth change of gradient in which no sudden break can be detected. The absence of any discontinuity therefore upholds the objections of Holdgate & Seal (1956).

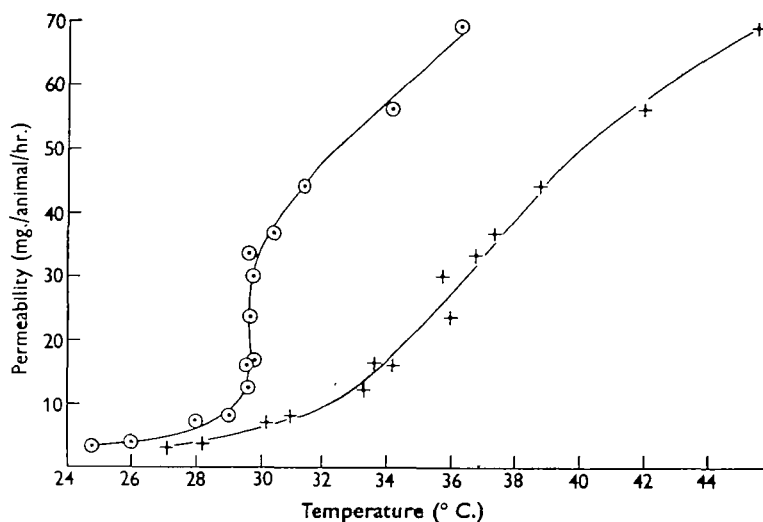


Fig. 4. Graph showing water loss of a large cockroach nymph about 1 week after moulting at various temperatures. +, air temperature; O, cuticle temperature. Humidity (known accurately) varying between 0 and 10% R.H.

However, when the same readings of rate of evaporation are plotted against cuticle temperature, an entirely different picture is obtained. There is a slow, gradual increase in the rate of evaporation up to a temperature of about 29.7° C above which the rate of evaporation increases steeply, while the cuticle temperature remains almost constant. Then the temperature of the cuticle again increases with increased rate of evaporation, but with an ever increasing gap between the cuticle and air temperatures. It is immediately obvious that a sharply defined phenomenon in the 'cuticle' curve is completely masked in the 'air' curve.

In order to remove the effect of a changing s.d., due to the changing temperature of 'dry air', Holdgate & Seal (1956) plotted the logarithm of transpiration against the reciprocal of absolute temperature; they thus assumed that in their experiments s.d. increased exponentially with temperature. But the data at our disposal enables us to calculate the actual mean s.d. obtaining at each determination of water loss. Now, under conditions where the permeability of the membrane is limiting, the actual amount of water passing through the cuticle at any temperature will be proportional to the permeability of the membrane at that temperature and to the s.d. Thus, if we divide each determination by the corresponding mean s.d. of the

air, we obtain the permeability per unit of s.d. This transformation has been carried out on the data of Fig. 4 to give Fig. 5. This curve gives the true relationship between permeability and temperature, and has the great advantage that it presents the data on a linear scale.

The 'air' curve in Fig. 5 does not give any real evidence of a discontinuity either; when we examine the 'cuticle' curve, however, we find a clearly delineated phenomenon. Below 29.7° C. we have a membrane with a finite temperature coefficient of permeability; above that temperature, although the permeability is greatly increased, there is a similar temperature coefficient. When the membrane is at 29.7° C. the distribution of points justifies only the drawing of a vertical line, the

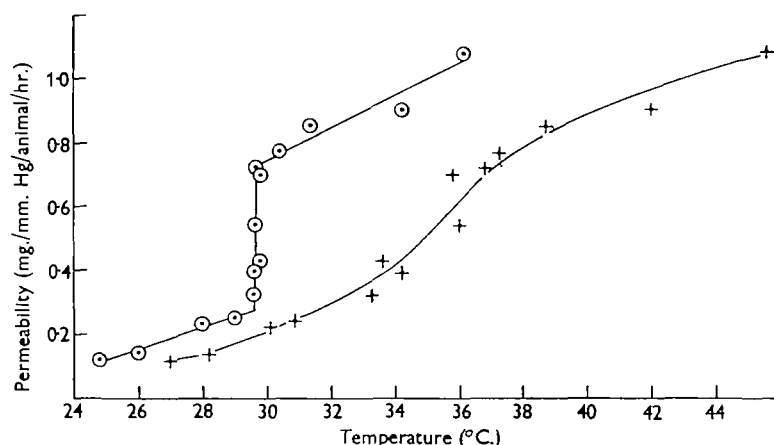


Fig. 5. Graph showing results of Fig. 4 corrected to water loss per unit saturation deficiency of the air at each experimental determination. Symbols as in Fig. 4.

simplest interpretation of which is that the permeability of the cuticle has increased suddenly. It must be realized that the vertical portion of the curve is 'real' (i.e. points have been determined along its length). Over those portions of the curve with finite slope, we know the removal of water vapour to be adequate, so that the limiting factor is the permeability of the cuticle. Over the vertical portion it is still true that ventilation is adequate, but the rate of loss we record is here limited by the rate of heat transfer from the air to the cuticle—a process which determines the amount of latent heat available for evaporation.

The significance of Fig. 5 will be discussed in more detail after the properties of isolated grease films (p. 507) have been described. It is perhaps worth noting that the particular experiment described above was the last of the series reported in this paper; it involved such high air temperatures as to cause certain damage to the equipment, and though it was essential to obtain such information, the danger was foreseen, and for this reason all previous experiments (which are reported below) were discontinued before the higher temperatures were reached.

It is clear, then, that the cuticle of the cockroach goes through a transition at a sharply defined temperature, at which its permeability increases suddenly. Though

previous workers are mostly agreed that the cuticular lipoids are responsible for waterproofing, it would be unwise, in view of the unreliability of their experimental methods, immediately to attribute the transition to any one part of such a complex system as the cuticle; yet it would be surprising if it proved to be the result of interaction of a large number of components, and if we can show a change in one component at the temperature in question it is virtually certain that that component is responsible for the change in the permeability of the cuticle.

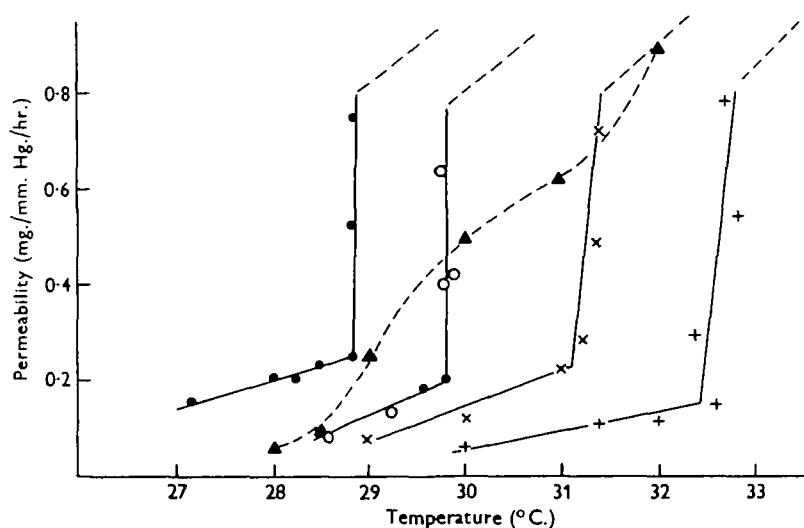


Fig. 6. Graph showing permeability of the cuticle of large cockroach nymphs of various ages within the instar at various cuticle temperatures.

●, nymph 2-3 days after moulting; ○, nymph 1 week after moulting; ×, nymph 2 weeks after moulting; ⊕, nymph 2-3 days before next moult; ▲, and broken line, mean permeability of all four insects considered as a homogeneous sample.

Now Ramsay (1935), in the original investigation, suggested that the temperature of breakdown in waterproofing was around 30° C. If we were to plot the 'air' curve, uncorrected for S.D. on an abscissa with a more compressed scale, and draw two straight lines through the points as he did, the intersection would certainly lie above 29.7° C. Ramsay drew two straight lines to indicate a change of conditions and certainly not to imply two linear functions. Nevertheless, it is important, for many reasons, to establish the uniqueness or otherwise of 29.7° C. for the species *P. americana*. If it is not unique to the species, experiments with a group of specimens which yield only average results could mask the sharpness of a phenomenon nevertheless clearly defined in each. Wigglesworth (1945) showed that the rate of water loss for *Rhodnius* nymphs was very constant for any one temperature, and Beament (1946, 1951) pointed out the sharp collapse at a fairly constant temperature of eggs heated in hypertonic solutions. On the other hand, Holdgate & Seal (1956) showed a change in the temperature characteristics of waterproofing with the age of *Rhodnius* and *Tenebrio* nymphs within the instar. In Fig. 6 the permeabilities of

four large nymphs, considered individually, are displayed; the individuals were of the same order of size, but form a series from about 48 hr. after a moult, to within 2-3 days of the next ecdysis. (It is difficult to predict, even with most closely controlled culture conditions, the time of moulting of a cockroach nymph, though it can be estimated roughly by examining the wing buds.) It will be seen that while the form of the curve is similar in all these nymphs, the transition temperature advances with the age of the individual within the instar, by as much as 5° C. The dotted curve shows the mean reading which would be obtained from the four individuals together and although an experimenter would be unlucky (or unwise) to use quite so heterogeneous a group, the necessity for conducting experiments on individuals is clearly shown.

On the other hand, when cockroaches all of approximately the same age within the moulting cycle are compared, their transition temperatures would seem to agree more closely. Thus for five animals, each about 48 hr. after moult, the transition temperature lies between 28.2 and 30° C. When five nymphs, each believed to be within 2-3 days of the next moult are considered, the transition temperatures lie between 33.2 and 36.1° C.; the variation here is greater than for animals at the beginning of the instar. It is clear that the cuticles are more uniform in respect of the temperature-sensitive characteristic of waterproofing when first formed, but that this characteristic may change during the instar.

EXPERIMENTS WITH WATER DROPS

As is well known (Ramsay, 1935; Beament, 1955, etc.) a drop of water placed on the cuticle of a cockroach is waterproofed against evaporation by the spreading of the cuticular grease over it. The grease spreads so rapidly that one can certainly rely on complete coverage over the surface of a drop of 1 mm.³ within 5 sec. of applying it. It is important to compare the temperature characteristics of water loss of the intact animal, as demonstrated above, with those of droplets covered with a film of grease, since a close parallel in the two cases would provide the strongest evidence for the function of the grease on the intact cuticle. It is not, however, at all easy to make measurements on drops placed on the surface of cuticle. Water loss cannot be detected by weighing, for one cannot distinguish between the loss from the drop and from the animal. The drop has a surface of contact with the animal through which water exchange can take place as the author has found in another connexion. Experiments of this kind are therefore much more satisfactorily performed on an artificial surface. The conditions for success are fairly critical. Beament (1955) has already outlined some crude experiments of this kind. Briefly, it is necessary to have a surface with a contact angle of 90° or greater to pure water, otherwise the water spreads out into a thin film when the grease is applied to it, presumably because of the lower 'surface tension' of the combined surface.

Now it is quite simple to measure by thermocouples the temperature difference between a small drop of water and the surrounding air while evaporation is taking place. It is not at all satisfactory to do the same thing when the drop is covered

with grease, for the following reasons. When one measures the rate of evaporation from the surface of an intact cockroach one assumes with some justification that the surface area of the animal remains constant, at least for about 5% loss of its contained water. Estimation of the surface area of the animal is extremely difficult, but the form of the curves here presented is not altered by expression in units of surface area, and provided that only single animals are used in each determination it is perfectly satisfactory to express the recorded water loss in mg./animal. But when a water drop—particularly a small one—is placed on a surface and proceeds to evaporate, its exposed surface area changes substantially; even its initial surface area depends on its surface tension (including the effect of any grease film), its size and the nature of the surface on which it is resting. By inserting an object, such as a thermocouple, into the drop the surface is deformed, and even neglecting the difficult technical problem of ensuring that the probe does not interfere with the ‘waterproofedness’ of the film and that water does not creep up the probe and evaporate from its surface, the determination of the exposed surface area is made excessively difficult if it is of complex shape.

In fact, the form of the profile of a large drop of liquid on a surface cannot be evaluated by rational mathematics (Rayleigh, 1896). If the drop is sufficiently small it may be possible to assume that it takes the form of a cap of a sphere, in which case the calculation of its exposed surface area from simply measured parameters is a straightforward matter.

Experimental method

If a drop, under suitable conditions of illumination, is placed on a horizontal surface and observed from a direction in the plane of the surface through a microscope with a micrometer eyepiece, it is easy to measure both the maximal height and the diameter of the plane of contact of the drop. With a 360° protractor in the eyepiece (in these experiments one was placed in one of the eyepieces of a binocular microscope) and a fixed reference index, the angle of contact of the drop to the surface can also be obtained. A variety of surfaces of different contact angles to water were enclosed in a transparent box, suitably illuminated. The humidity in the box was maintained at about 96% R.H. to keep evaporation to negligible proportions. Water drops of various sizes were applied to the surfaces by an ‘Agla’ micrometer syringe, and the parameters—maximum height (a), radius of the plane of contact (b) and angle of contact (ϕ)—were measured. The volume (V) of the drop was calculated from the formula:

$$V = \pi \frac{1}{8} a (a^2 + 3b^2).$$

The calculated volumes were compared with those measured by delivery of the syringe. The agreement was better than 3% provided the drop was of 1 mm.³ or less, though if it is below 0.1 mm.³ the parameters cannot be measured sufficiently accurately. It is of interest to note that a drop of 1 mm.³ which weighs 1 mg. cannot readily be weighed to 3% and certainly cannot be so weighed if one is trying to detect 0.03 mg. in the combined weight of the drop and a supporting surface of

several mg. weight. It was therefore concluded that such optical methods would give the most accurate means of assessing the rate of evaporation from drops. The evaporating surface area (A) is given by

$$A = \pi (a^2 + b^2)$$

and the contact angle by

$$\sin \phi = \frac{2ab}{a^2 + b^2}.$$

The observed contact angle was used as a quick check on the measurements, but since the assessment of contact angles by placing a tangent (methods of Beament, 1944; Holdgate, 1955) is a subjective measurement liable to considerable inaccuracy, calculation by this formula is likely to be more accurate.

For measurement of evaporation through grease films on a water surface a temperature-controlled plastic box as described above was used, but with the balance arm replaced by a specimen holder on a universal joint. Plastic sleeves were let into the lid of the box, through which a micrometer syringe cannula and a needle holder could be respectively inserted, in order to deposit drops of water on to the specimen holder and to add grease to them. A clean thick sheet of polystyrene was placed horizontally on the holder, and a binocular microscope with one micrometer squared eyepiece and one micrometer protractor eyepiece was lined up on it. The temperature was allowed to become stable, the box was swept with dry air, and two drops of distilled water, each 1 mm.³, were deposited rapidly approximately 1 cm. apart, the line joining their centres being at right angles to the direction of flow of the circulating air. The needle, which had previously been stroked over the surface of a large cockroach nymph, within 1 week of having moulted, was rapidly touched against the surface of one of the drops. The necessary parameters of the drops were recorded at suitable intervals of time. The procedure was then repeated at a higher temperature using a fresh surface of polystyrene. As polystyrene is an extremely poor conductor of heat, a considerable time must elapse before the surface to which the drops are applied is at air temperature, and of course it is essential to use a clean surface for each recording. It may be added that the reason for having the cannula in the box is so that the water delivered to the plate shall be initially at the same temperature as the air and the surface. The curve for evaporation per unit surface area against air temperature obtained in this way was of smooth 'exponential' type, and in view of the arguments put forward earlier it was suspected that it suffered from the disadvantages of all the earlier work—in this case, that the polystyrene was such a good heat insulator that the temperature of the drop was well below that of the air. Experiments with unwaterproofed drops and thermocouples confirmed this. But if drops of clean water were placed on a large sheet of a good conductor, such as aluminium, measurements with thermocouples indicated that during evaporation the water temperature fell by only a small part of one degree—the drop can obtain heat from its surface in contact with the metal, and the metal is not only a very good conductor but it also has such a large surface exposed to the surrounding air that it can easily make good the heat lost to the drop. Unfortunately, a metal is readily wetted by water, and cannot

therefore be used for experiments with waterproofed drops. It was found that a suitable surface could be obtained by coating the metal with a minutely thin film of silicones, using the solution D.C. 1107 and subsequently baking it. The film was so thin (it is probably of molecular dimensions) that it did not appear to interfere with the transmission of heat from the plate to the drop.

The rates of evaporation per unit surface area are plotted against temperature in Fig. 7, in which the selected data are based on the observed rates of evaporation between the volume limits of 0.95 and 0.75 mm.³, for drops initially of 1.0 mm.³. This precaution kept down the effect of evaporation on the s.d. of the enclosure, and, more important, to some extent limited the suspected effects of ageing of the film with exposure. In view of the individual differences in nymphs already demonstrated, all the data for any one graph was obtained with grease from one nymph; for Figs. 7 and 8 the animal was about 7 days post-moulting. The airspeed was held constant throughout the experiments and, though adequate, was slow, in order to prevent vibration and distortion of the droplet profile.

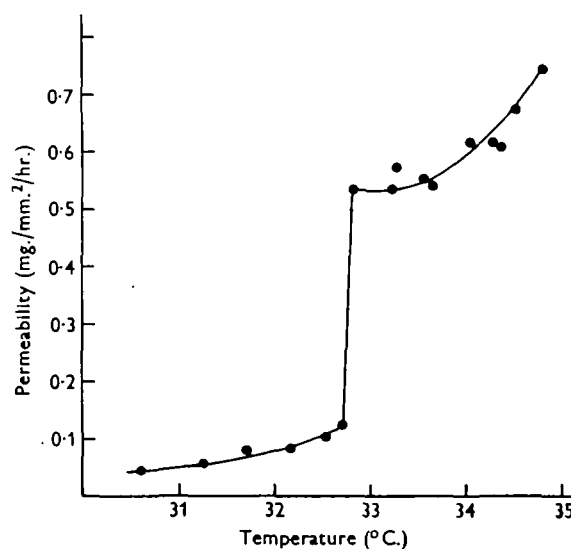


Fig. 7. Graph showing rate of evaporation of water through a thin film of cockroach cuticular grease plotted against temperature, when evaporating system is maintained at air temperature. Humidity between 0 and 5 % R.H.

From Fig. 7 it is at once clear that a sudden breakdown in waterproofing occurs; in the experiment illustrated, this takes place at 32.7° C. The observations parallel closely the corresponding measurements on the intact animal (Fig. 4), while Fig. 8 (for permeability per unit s.d.) can be compared with Fig. 5. The permeability does not appear to be temperature-dependent below its transition, but it is probably significantly so above it; the consequences of this are outlined in the Discussion. Two obvious differences are seen: with the whole animal, the vertical part of the curve is 'real' with several determinations along it; the isolated film changes from

one permeability characteristic to another and by drawing a vertical line between the two one does not imply that one could determine readings on it—in fact one failed to do so. Secondly, the relative permeability increase at the transition temperature is considerably greater for the isolated film. The vertical part of Fig. 5 is determined by rate of heat transfer, as explained above. But here, where we are dealing with a simpler physical system in which temperature is not being determined by evaporation but is being kept close to that of the air, we should expect the transition to be so represented.

The fact that the two phenomena are so strikingly similar can only mean that, of all the many components in cuticle, the grease is the significant factor limiting water loss.

DISCUSSION

While experiments with artificially waxed membranes (Beament, 1945) provided evidence for believing that the impermeability of insect cuticle is due to an intact layer of freely extractable wax, these models have not been entirely satisfactory for explaining the very high order of impermeability of the cuticle in so far as (*a*) that they have never achieved quite the impermeability of their corresponding natural cuticles, and (*b*) that they have been, like the cuticle, the centre of much controversy over physico-chemical interpretations. The discrete film of grease on water, which from Beament's (1955) study of the composition of cockroach grease seems likely to be of the order of one molecule thick, is in every sense a more satisfactory model; it is likely, in the absence of dust, to be a uniform and completely imperforate layer.

In order to compare directly the properties of the film on water with those of the thick grease on the animal, it is desirable to reduce the observations to common units of absolute permeability, and those selected by Barrer (1941) will be used. The values which we now derive are based on the means of several careful determinations: for the film on water, the measured permeability at 25° C. is quite consistent, and the accuracy of the absolute permeability is therefore higher than that derived for the thick grease on whole animals, since not only have we to contend with typical biological variations but also we have to assume that the thickness of grease on the animals is uniform and we have to estimate the surface area of the nymph.

The mean permeability of the grease film at 25° C. is 2.5 mg./cm.²/hr. which gives an absolute permeability of 0.40×10^{-8} ml. water vapour at N.T.P./sec./cm.²/mm. Hg/mm. thickness. In this calculation it is assumed (Beament, 1955) that the film will be of the order of 100 Å thick, i.e. one average molecular length. The absolute permeability of the film is thus extremely low compared with any of the substances cited by Barrer (1941). The average permeability of large cockroach nymphs at 25° C. is 3.5 mg./animal/hr. which gives an absolute permeability of 1.65×10^{-8} absolute units. For this calculation we have assumed that the nymph has a surface area of 7.5 cm.² and a thickness of grease of 0.25 μ. This is still below the value of absolute permeability of the most impermeable natural materials described in the literature; Taylor, Hermann & Kemp (1936) give $2 - 2.1 \times 10^{-8}$ for measurements on slabs of wax, with which our figures can be compared.

Let us then propose a structure for the grease layer and suggest a physico-chemical change at the transition point, and let us subject our hypothesis to a number of tests based on information we can calculate from the absolute permeabilities and from the graphs above. We suggest that, below the transition temperature, the grease layer on the water droplet surface is organized, by the attraction of the polar endings of the long-chain molecules of the grease for the water, to form a solid, tightly packed orientated 'monolayer'. It is this structure which accounts for the very great absolute impermeability of the film. If this is so, then the lowermost portion of the thick grease on the animal, which is also against a wet surface, must also be orientated and organized into a layer similar to the film on water. The remainder of the thick grease, which is without such a polarizing influence, must presumably be a fluid with the typical random lack of organization of the molecules of liquids. Since we know the permeabilities of the organized film and of the film plus the thick grease and since we also know the thickness involved, it is a simple matter to calculate the absolute permeability of the thick unorganized portion alone. From the thicknesses used in the calculations above, the 'monolayer' to thick grease stands in the ratio 1:24. If we convert permeability into resistivity by dividing thickness by permeability we obtain properties which can be added thus

$$\frac{d_1}{p_1} + \frac{d_2}{p_2} = \frac{d_3}{p_3}.$$

The thicknesses (d) are as 1:24; 25; the permeabilities p_1 (0.4×10^{-8}) and p_3 (1.65×10^{-8}) for film and total grease on the animal can be substituted to find p_2 , the absolute permeability of the unorganized fluid grease at $25^\circ \text{C}.$:

$$\frac{1}{0.4} + \frac{24}{p_2} = \frac{25}{1.65} \quad (\text{all divided by } 10^{-8}).$$

From this, p_2 is 1.85×10^{-8} ; it can be compared with Taylor, Herrmann & Kemp's figure of $2-2.1 \times 10^{-8}$ for pieces of wax which also are presumably without molecular organization.

Secondly, we propose that at the transition temperature, the organized structure of the 'monolayer' breaks down—possibly because of the accumulation of thermal energy in its molecules which eventually overcomes the forces attracting the polar groups to the water. All the molecules would then assume the random configuration of a fluid. The absolute permeability of the film on water would thus jump at the transition temperature from that of organized layers to that of the fluid portion of the thick grease which we have just determined (presuming the figures to be of the correct order at transition temperatures as well as at $25^\circ \text{C}.$ which will not involve great errors). The ratio of these two values, $0.4 \times 10^{-8} : 1.85 \times 10^{-8}$, gives approximately a fivefold increase, and on examination of Fig. 8 we find that this is precisely what does happen.

Thirdly, we can predict the complete curve for the whole animal. It is well known that once all the grease has been removed by solvent action from cockroach cuticle, the water permeability of the remaining cuticle is so great that, whatever

temperature dependency it may have, it cannot have had any effect on the temperature dependency of the impermeable grease layer, for the cuticle is several orders more permeable at all temperatures. From Fig. 5 we have already noted the considerable temperature dependency of the permeability of thick grease, both below and above transition; in Fig. 8 the 'monolayer' film certainly has temperature dependency above transition, but significantly little below it. It is, therefore, proposed that in the absence of an 'organizing' substrate, such as water or a wet surface, the grease will be in the molecularly unorganized state at temperatures both above *and below* the critical temperature. Once again it is necessary to work

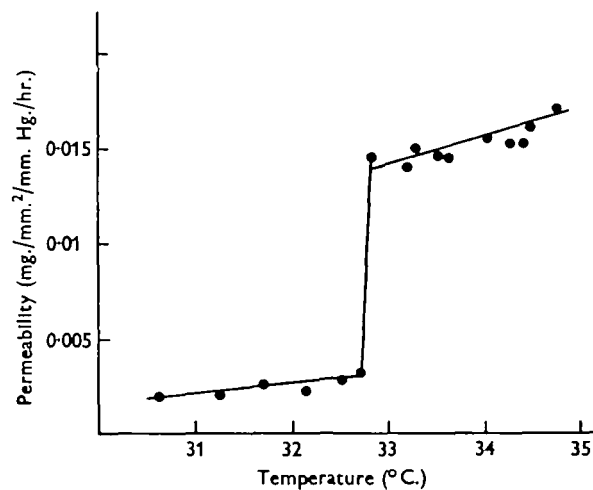


Fig. 8. Graph showing the results of Fig. 7 corrected to permeability per unit saturation deficiency of the air at each experimental determination.

in 'resistivity' rather than impermeability units. In Fig. 9a, we plot the reciprocal of the absolute permeability of the monolayer below the transition—assuming it to remain constant with temperature—and we plot above the transition the reciprocal of the permeability of a fluid grease layer of equivalent thickness. From the slope of the cuticle temperature curve above transition in Fig. 5 we can obtain an estimate of the temperature dependency of permeability of grease entirely in the fluid state. We assume that such a temperature coefficient of permeability holds also for grease in the molecularly unorganized state *below* the critical temperature. If due account is taken of scales and units, the order of temperature dependency of the 'monolayer' film on water above the transition region is similar. We can then, in Fig. 9a, also plot the theoretical curve for the resistance at various temperatures of a layer of grease (without molecular organization) which is twenty-four times as thick as the 'monolayer', basing our values only on the calculated absolute permeability of unorganized grease at 25° C., and the apparent temperature dependence.

Now these two 'resistance' characteristics can be added giving the uppermost curve in Fig. 9a, representing the change of resistance with temperature for the

combined layers, i.e. the whole waterproofing system of the natural cuticle. The reciprocal of this curve, plotted in Fig. 9*b*, thus gives the theoretical picture for permeability of the whole cuticle against temperature and can be compared with the 'cuticle' curve of Fig. 5. It will be seen that in *pattern* they are very similar indeed; in detail, the magnitude of the vertical excursion at the transition temperature is appreciably greater in the experimental curve. It must, however, be remembered that one has no idea of the amount of grease on the individual on

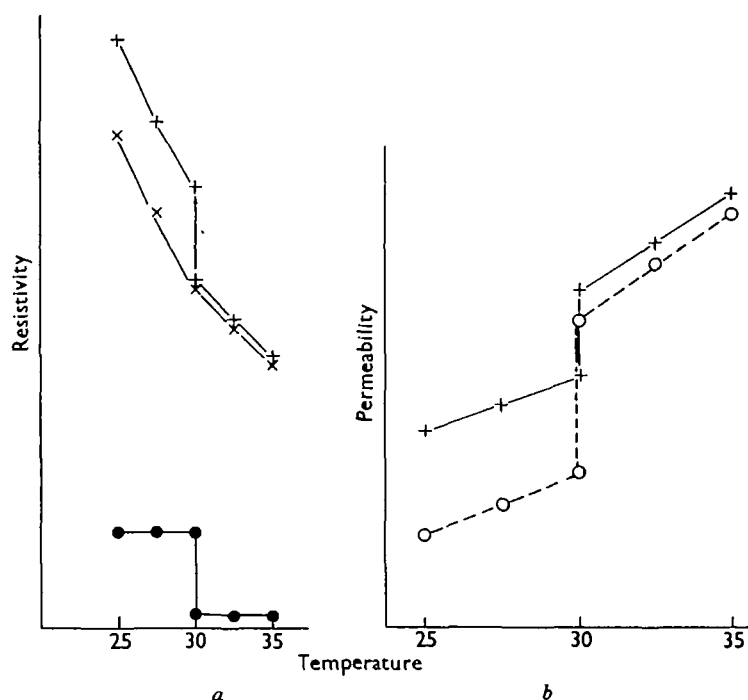


Fig. 9. Theoretical derivation of the form of the transpiration/temperature curve for intact animals. 9*a*. ●, resistivity (thickness divided by absolute permeability) of a 'monolayer' of grease on an aqueous substrate below a transition point at 30° C. and changing to unorganized grease above 30° C. ×, resistivity of a layer of unorganized grease molecules twenty-four times the thickness of the 'monolayer'. ⊕, combined resistivity of a system made up of these two layers, obtained by adding the two previous curves. 9*b*. ⊕, theoretical permeability/temperature curve obtained by taking the reciprocal of the combined resistivity curve of 9*a*. ○, and broken line; the curve of Fig. 5. relating to cuticle temperature converted to the same scale.

which the experiments illustrated in Figs. 4 and 5 were carried out; all our calculations above have been made on the assumption that the mean thickness of grease on intact animals is 0.25μ , but if in fact this individual had less total grease, then this would cause the relative magnitude of the vertical region to be greater, and thus to depart more from the theoretical curve we have derived, though without in any way altering the general pattern of the curve (see Fig. 9*b*).

These three examples, in which calculations based upon a theoretical picture of the physico-chemical form of the grease and of its changes with temperature

have been compared quantitatively with the practical measurements, show—and are only expected to show—agreement to within an order of magnitude. They help to confirm, rather than form the basis for, the proposed explanation. If, as it would appear, the permeability of the 'bulk' grease changes continuously and quite appreciably with temperature, and the fundamental cause of this change is thermal agitation of the molecules of the fluid, then it is similarly likely that the forces causing the grease molecules to be organized against a hydrophilic surface would resist such agitation, and that in consequence the 'monolayer' would have little temperature dependence. What one would like to do is to measure the true permeability of grease entirely in the unorganized state, at a temperature well below the transition, but the way to do this is not obvious. There is of course also the possibility that the portion of the thick grease immediately over the organized layer would itself be to some extent organized, so that the 'monolayer' effect in a thick grease system would be greater than our simplest explanation would suggest.

The existence of an organized layer of grease molecules at the water interface certainly explains some observations made during this study, and which it is proposed to report in detail elsewhere. If the permeability of a grease-covered drop is determined in dry air at a temperature well below transition, and the drop is then immediately exposed to a humidity of, for example, 60% R.H. at the same temperature, its rate of water loss may decrease, or it may increase to two or three times its value in dry air. Fortunately for the experiments reported earlier, the behaviour of all waterproofed droplets is consistent at 0% R.H. The random behaviour of droplets at higher humidities proved difficult to explain, but it was eventually discovered that the recorded rate of evaporation was always proportional to S.D., if the measurements were made in dust-free air. It would appear that dust particles—and especially those of material capable of adsorbing water—can materially interfere with the resistance to water movement through a grease film. The organization of the grease layer we have presumed to depend on the affinity for water of polar endings, such as the hydroxyl groups at the end of long-chain alcohols. A hydrophylic surface above the 'monolayer' could likewise attract these polar endings and compete for them with the water droplet's surface. In dry air particles of dust on the grease film will be dry and so no competition can take place; but at higher humidities, water could be adsorbed on the surface of the dust, and produce a surface attracting some of the polar groups away from the droplet. If this causes such local disorientation, there would be a form of pinhole diffusion through areas of high permeability, so that the magnitude of the observed effect is not altogether surprising.

If then we accept the theory that an organized and orientated 'monolayer' covers the aqueous substrate of the cuticle surface, a physico-chemical issue of some importance arises; because of this, the term 'monolayer' has heretofore been used in parentheses. Whereas in 1945, Beament suggested that the high impermeability of the hard waxes of typical cuticle was due to the existence of a layer of molecularly organized wax, whose increased permeability at transition was caused by a Müller (1932) transformation in the intermolecular spacings, the author now postulates,

for the soft grease of the cockroach, something rather more drastic in disorganization; both ideas demand a 'monolayer' type of organization in a substance which is chemically a complex mixture. But it has been suggested on many occasions that mixtures do not in general form organized monolayers in the sense that pure substances do (see, e.g. Adam (1941)). It is quite clear (Beament, 1955) that cockroach grease is a mixture of very special properties and that the natural solvents in it have unique effects; yet the hard waxy components which comprise the other part of the grease are substantially the same as the hard cuticular wax of other insects which have so far been examined. The criticism that complex mixtures do not form 'monolayers' and show changes in crystal form at critical temperatures has not been based only on theoretical or general grounds. Holdgate & Seal (1956) for example, using X-ray and electron-diffraction techniques, failed to find any evidence of sudden changes in samples of wax heated through appropriate temperature ranges; but it has already been pointed out (Beament, 1954), and still is suggested, that organization of this kind is only formed against, and in the presence of, a water substrate which clearly cannot be present in samples studied by these methods. Preliminary tests which the author has made with electrical techniques are, however, promising and free from such objection.

When we now consider the shift in transition point with the age of the cockroach nymph within the instar (p. 506), it is evident that some progressive change affecting the determination of the transition temperature itself is going on. Beament (1955) suggested that by driving off the solvent from the grease one could get samples with successively higher critical temperature up to about 40° C., using a rather crude waterproofed droplet technique. Eventually a hard wax melting between 50 and 55° C. was obtained. It now seems even more likely that as the solvent naturally evaporates from the nymph, during an instar, so the accumulation of higher proportions of the long-chain components in the grease leads to the increased transition temperatures. What now becomes clear is not only that the 'monolayer' is the region of extreme impermeability, but also that its stability and eventual breakdown may be directly governed by the actual proportion of the components in it. In this light Table 4 of Beament (1955) assumes considerably greater importance. Of the insects so far examined the cockroach is unique in that after heating to regions of high permeability and then cooling to ordinary temperatures it alone seems to recover its full impermeability; below the critical temperature there is no reason why a 'monolayer' should not be reformed under the influence of the wet substrate. On the other hand, polar solvents such as chloroform, which could interfere with 'monolayer' formation, certainly do cause permanent damage to waterproofing—damage which can only be made good experimentally by causing the chloroform to evaporate and then using octane-octyl alcohol vapour to replace the natural solvent. The natural solvent, in fact, seems essential for the stabilization of the 'monolayer', and there is (Table 4, Beament 1955) a very strong suggestion that this solvent can cause high impermeability to be restored in cuticles with hard wax (such as those of *Rhodnius*) which have previously been damaged by heat or polar solvent action. But again it must be remembered that none of these things can

happen with dead, dry cuticle; it is essential apparently that the surface of the cuticle against the wax, where we may imagine a polarized layer to be formed, should be fully hydrated, once again pointing to the extreme importance of the hydrophilic surface of the cuticle.

CONCLUSION

The waterproofing grease on the cuticle of *Periplaneta americana* would seem to consist mainly of molecularly unorganized lipoids, but (at lower temperatures) to have a layer immediately against the aqueous substrate of the cuticle which is extremely impermeable to water, even though its thickness is of molecular dimensions. At a certain temperature, which undoubtedly depends on the age of the nymph since it last moulted and thus on the relative proportion of volatile and non-volatile components in the grease, this impermeable layer breaks down, causing a sudden increase in the overall water loss of the animal, and the order of change in permeability is consistent with the transition of this organized layer into a state of molecular disorganization similar to that in the main body of grease above it.

The waterproofing of insects having hard cuticular waxes (i.e. the majority of insects), will be investigated as soon as suitable high-temperature techniques have been developed; but it is clear from the form of the curves already published by Wigglesworth, Edney, Holdgate, Beament, etc., that when proper physical measurements are made, the true temperature characteristics of permeability of the hard-waxed cuticles could reveal discontinuities of a similar nature.

SUMMARY

1. Previous methods for determining the temperature characteristics of the permeability to water of insect cuticle are criticized on five fundamental points, and in particular it is shown that they do not measure the temperature of the cuticle which will be substantially different from that of the surrounding air.
2. The physical principles for accurate measurement are outlined, involving the accurate control of air temperature and air circulation, the measurement of cuticle temperature and of water loss within a sealed enclosure, and the assessment of true saturation deficiency. Apparatus for meeting these requirements and making these measurements is described.
3. Determinations have been made of the permeability of the cuticle of *Periplaneta americana* nymphs. When the permeability is plotted against cuticle temperature a sudden and substantial increase in permeability is seen to take place at around 30° C. The temperature at which this change occurs is higher the longer the time which has elapsed since the nymph previously moulted.
4. A method of measuring the permeability of the cuticular grease, when spread as a layer over an isolated water droplet, is described. This layer, whose thickness is of molecular dimensions, has much greater absolute impermeability to water than the thick grease on the insect. It also exhibits a sudden increase in permeability at about 30° C., and the order of change is considerably greater than that found with intact cuticle.

5. The findings are quantitatively compatible with the postulate that, at lower temperatures, the grease on the cockroach consists of an orientated 'monolayer' organized by the attraction of its polar groupings for the aqueous cuticle surface underlying it, together with a much greater thickness of unorganized grease above it. In absolute units, the grease is about five times as permeable to water as is the 'monolayer', but at the transition temperature the measurements agree with the supposition that the molecular organization of the 'monolayer' breaks down into the disorganized state of the major part of the grease with a corresponding increase in permeability.

6. The suggestion that films of grease on water, and the lowermost portion of the grease on the animal, have orientated molecular organization is supported by some observations on the effect of dust particles on the films and by information previously published.

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