The Journal of Experimental Biology **198**, 249–261 (1995)
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AN ELECTRICAL MODEL FOR *PERIPLANETA AMERICANA* PRONOTAL INTEGUMENT: AN EPIDERMAL LOCATION FOR HYDRATION-DEPENDENT RESISTANCE

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Accepted 18 August 1994

Summary

Measurements of electrical resistance appear to be useful indicators of porosity and related water permeability in insect cuticle. To develop an adequate understanding of how such measurements relate to the physical and structural properties of the integument, we made detailed determinations of resistance and impedance values for pronotal cuticle in adult male *Periplaneta americana*. The most consistent estimates were obtained by averaging measurements across the integument on both sides of the midline at several intervals starting 15 min after electrode application. Pronotal resistance varied inversely with water content, from about 10 kΩ cm^2 in hydrated cockroaches to about 40 kΩ cm^2 in dehydrated insects. Though the dermal gland canals appear to act as the main conductive pathway across the cuticle, the variable 'barrier' is located in the epidermal layer, since removal of the epidermis from isolated pronota also removes most of the variable component of the integumental resistance. Comparison of measurements between two external electrodes with single-electrode measurements revealed a 'shunt' pathway parallel to the cuticle surface; modelling this shunt suggested that it was variable and located mostly internal to the cuticle, supporting an epidermal location for a variable barrier. Impedance measurements over a range of frequencies showed the integument to be electrically complex, and a model is proposed to account for its properties

Key words: cockroach, integument, cuticle, permeability, electrical resistance, epidermal barrier, dermal glands, *Periplaneta americana*.

Introduction

In an investigation of the mechanism of cuticular water permeability changes in response to alterations in the water reserves of the cockroach *Periplaneta americana*, Machin *et al.* (1994) have found that electrical techniques provide useful means of gathering information about the state of the integument. By modifying the techniques developed by Scheie and Smyth (1967, 1968), they confirmed and extended earlier observations showing that the resistances (or conductances) of different cuticular plates in *Periplaneta* vary consistently. Moreover Machin *et al.* (1994) have demonstrated that, in all cuticular areas apart from the antennae of males, conductance decreases with level of hydration, to a degree that apparently depends on the density of perforations in the cuticle. Machin *et al.* (1994) link at least some of the regional, sexual and developmental differences in integumental conductance to pheromone-releasing glands and provide evidence that dermal gland canals represent an important pathway for conducting electricity.

Measurements of conductance (or resistance) are often carried out using alternating current (a.c.) instead of direct current (d.c.) in order to overcome problems due to polarization of electrodes and the presence of voltage sources in the tissue. However, use of alternating current adds another dimension to the analysis because of the presence in tissue of elements that behave differently in response to alternating current. The capacitance associated with cell membranes in particular causes changes in apparent resistance with frequency. Studying the electrical properties of this integument using different frequencies of alternating current should, therefore, provide additional information about structure and may contribute to our understanding of functional changes.

Scheie and Smyth (1967) suggested that the electrical properties of the integument could be represented by a simple resistive–capacitative network of two resistors and one capacitor. However, the structure of the cuticle and the underlying epidermis is probably more complex than this, consisting not only of insulating epicuticle, hydrated endocuticle, both perforated by dermal gland canals, pore canals and perforations of chemosensory sensilla, but also of a cell layer with epidermal cells and various types of gland cell. These structures form a network of parallel and series elements, some of which may vary with the state of dehydration of the animal. In this paper, we extend the analysis of the resistive and reactive characteristics of the cockroach

integument and develop new models embodying these properties. Such models may help reveal which parts of the integument are hydration-dependent. For instance, the region where the dermal gland canals meet the gland cells in the epidermis is structurally complex, and we hope that an electrical model of this area will lead to a more precise localization of the putative duct-closing mechanism and a better understanding of how it works. Better models will also aid in understanding the link between electrical conductance and water permeability, not only of the integument as a whole but of its various components. Such an understanding will provide a basis for using electrical measurements as a tool for the investigation of water permeability and its possible control.

Materials and methods

Animals

Adult male *Periplaneta americana* (L.) were selected from a culture described by Machin *et al.* (1994). To obtain as wide a range of water contents as possible, animals were selected directly from the culture and from another group, removed from the culture and kept over silica gel for 4–6 days. When calculation of water content was required, animals were weighed prior to measuring and dry masses were determined at the end of the experiment (Machin *et al.* 1991).

Electrical measurements

We chose the pronotum as the test area for our studies for several reasons. It is large and easily removed, and there is a wealth of data pertaining to this structure from earlier studies, including those of Scheie and Smyth (1967, 1968). Machin *et al.* (1994) obtained evidence that the male abdominal tergites and the pronota of both sexes have the highest dermal gland densities and, as such, show the greatest conductance decreases upon dehydration. It will be seen below that pronotal water permeability is representative of that of the cuticle as a whole. Because Machin *et al.* (1994) found a significant sexual difference in pronotal resistance, we worked exclusively on males.

To determine the resistance or impedance (for a simple explanation of impedance and reactance, see Appendix) of the cuticle, we used the current-clamping amplifier described by Machin *et al.* (1994). This device produces an output voltage directly proportional to the resistance of the preparation. The input signal was usually a variable-frequency sine wave; any reactance in the preparation produces a phase shift at the output of the operational amplifier. Thus, the output voltage gives the preparation impedance, and the phase shift (relative to the input signal) allows this to be broken into resistive and reactive components (see Appendix). At very low frequencies (1 Hz or less), reactance was negligible, and measurements approximated true d.c. measurements of resistance while still avoiding polarization effects.

For impedances at frequencies at and above 10 Hz, output voltages were measured with a precision voltmeter (Keithley 179 TRMS Digital Multimeter; 10 Hz is the lower limit of the useful range for a.c. measurement). Below 5 Hz, the signal could be accurately recorded on a chart recorder, and the peak-to-peak voltage was measured from the amplitude of the recorded traces. Peak-to-peak voltages were also measured on an oscilloscope screen in experiments involving capacitance determinations. Phase shifts were determined by using a variable-phase oscillator (General Radio Company type 1305-A) with a frequency range of 0.01 Hz to 1 kHz; this oscillator allows the input signal to the current-clamping amplifier to be varied relative to a reference signal. Output and reference signals were displayed on the *x*- and *y*-axes of an oscilloscope, the phase was adjusted to produce a straight-line Lissajous figure showing the two signals to be now in phase, and the phase shift was read from the dial setting on the oscillator. In this way, phase shifts could be determined to within $\pm 2^{\circ}$ of the frequency range of the oscillator. For scraped cuticle, higher-frequency measurements were necessary and were made by measuring the time delay between the peaks of signals displayed on a dual-beam oscilloscope; this method is not as accurate and was not used for intact integument.

Cockroaches were prepared and mounted for measurement as described in Machin *et al.* (1994). Electrical connection to the preparation was made with two 1 mm diameter external electrodes filled with cockroach Ringer (Machin *et al.* 1985) and one internal electrode. For *in vivo* measurements, the internal electrode was a piece of chloridized silver wire inserted about 1 cm anteriorly into the dorsal abdomen, usually between the fifth and sixth abdominal tergites. The 'internal' electrode for *in vitro* measurements consisted of a coiled piece of chloridized silver wire positioned at the base of a Ringer-filled well, made of wax, on a glass slide.

Resistance values determined with a single external electrode will be the sum of the integumental resistance (R_i), the electrode resistances and any resistance due to the electrolyte-rich contents of the body between the recording site and the internal electrode in the abdomen. By short-circuiting two external electrodes in a drop of Ringer, we found their resistance of about 20 kΩ to be negligible compared with the typical values of 0.7–4 MΩ we read with the single-electrode technique; similarly, the internal electrode and body resistances were negligible. Thus, the 'single-electrode' resistance, which will be referred to as R_{single}, should be a close approximation to the integumental resistance. An alternative method of measuring is to use two external electrodes; this allows non-invasive *in vivo* measurements and tends to cancel any d.c. potential differences across the cuticle. With this 'double-electrode' configuration, the integument is traversed twice by the current, and thus the measured value, assuming negligible resistance internal to and parallel to the integument, should equal twice $R_{single.}$ Half the integumental resistance measured in this way is referred to as R_{double}. Experiments variously used combinations of external and internal electrodes, and the amplifier was designed to permit easy switching between any combination of two out of three electrodes.

The output voltage-to-resistance conversion factors for the current-clamping amplifier were determined using measured resistors; all readings of integumental resistance were standardized for differences in electrode contact area and are therefore expressed as a resistance for one square centimetre of integument ($\Omega\,cm^2$).

Means of electrical and other measurements are presented ±1 standard error of the mean (S.E.M.).

Experimental treatments

To analyse the electrical properties of the components of the integument, resistance measurements were made on the intact animal and then after the following treatments. The first treatment consisted of severing the anterior thorax containing the pronotum from the head and body, using single scissor cuts through the neck and through the thorax posterior to the pronotum. The second measurement was made with the internal electrode placed in the thoracic tissue between the pronotum and the remaining ventral cuticle. For the third measurement, the pronotal preparation was removed from the chamber used for the intact animal and placed on a piece of Ringer-soaked tissue over the 'internal' electrode in the wax well. The fourth measurement was made in the same apparatus after 'peeling', i.e. very carefully removing any remaining ventral cuticle and as much soft tissue as possible from the inside surface of the pronotum. The fifth measurement was made after 'scraping' the inside surface with a scalpel blade to remove all adhering tissue. Finally, the waxy epicuticle was removed by wiping it with chloroform:methanol (2:1) before making the final resistance measurement. Each of these manipulations took approximately 2–3 min and required repositioning of the external electrodes. Care was taken to replace the electrodes in the same area of cuticle.

Circuit modelling

It was quickly apparent that we needed to model the integument using circuits more complex than the simple two-resistor, one-capacitor circuits described by Scheie and Smyth (1967). To facilitate determination of the properties of such circuits, we either built them using standard resistors and capacitors of appropriate value, then inserted the circuit in place of the preparation into the measuring equipment, or used a computer circuit-simulation program (Electronics Workbench, Interactive Images Technology, Toronto, Ontario, Canada). The latter proved particularly effective, since components could be easily altered, and the behaviour of the resulting circuit could be computed in a few seconds. The strategy for deriving the models was twofold: the 'try it and see' approach, where values were changed and components added or subtracted to see whether the circuit behaved more like the integument; and the 'first principles' approach, where we attempted to construct a circuit using electrical analogues of actual structures, with starting values determined from reasonable guesses (e.g. $1\,\mu F\,cm^{-2}$ for a cell membrane, the common figure used in neurobiology).

Results

Measurement characteristics

The readiness with which stable electrical contact could be established with minute pore structures surrounded by relatively large areas of highly hydrophobic material puzzled us at first. We naively supposed that pore structures such as dermal gland canals, though hydrophilic at the bottom, were largely air-filled, requiring the displacement of an air bubble by electrode electrolyte before electrical contact could be established. This picture was inaccurate; our observations suggest that the dermal gland canals are fluid-filled. Assuming that the canal lining is minimally hydrophilic, surface tension would generate enough force to fill the canals many times over (see Appendix). We presume that fluid fills the canals to the point of contact with the hydrophobic epicuticle at the open distal end. Because of its contact with the underlying subcuticular space and epidermis, it seems likely that the canal fluid is in osmotic equilibrium with the epidermal cells and is electrically conductive. Even if this were not so, equilibration with the fluid in the electrodes would be very rapid. We calculate that it would take less than 1 s for NaCl to diffuse to the bottom of the dermal gland canal from the Ringer in the electrodes (see Appendix).

Initial experiments had shown considerable variability in resistance measurements; consequently, various factors were examined to determine the sources of this variability and whether they could be controlled. The area of contact between the cuticle and the external electrodes was typically under $1\,mm^2$, considerably smaller than that in the study by Scheie and Smyth (1967), where each drop of electrolyte was about 3 mm in diameter. Consequently, the position on the pronotum might be critical if R_i (integumental resistance) varies in different regions. Single-electrode readings were therefore taken at 0.1 Hz at 14 different sites on the pronotum. Analysis of variance (ANOVA) showed no significant effect of position, though the mean resistance of the six most lateral sites was lower in both hydrated and dehydrated animals (hydrated medial $24.8\pm1.3\,k\Omega\,cm^2$, hydrated lateral $21.8\pm2.3\,k\Omega\,cm^2$; dehydrated medial $33.6\pm1.6\,k\Omega\,cm^2$, dehydrated lateral $30.7\pm2.5\,k\Omega\,cm^2$). Subsequent measurements were therefore confined to two medial sites placed symmetrically about the midline, and the value used was the mean of the two single measurements at these sites.

Initial experiments had also involved variable periods between the application of electrodes and measurements, partly because of the time necessary to establish a reading at 0.1 Hz. Therefore, we examined the effect of time on measurements of R_i by making repeated pronotal resistance measurements on three hydrated cockroaches. To shorten measurement time, a frequency of 1 Hz was used. Readings were taken at 5 min intervals up to 60 min, the electrodes being maintained in place for the duration of the experiment. Fig. 1 shows a slight decrease in resistance with time, particularly in the first 20–25 min. Though the correlation was not significant, we took subsequent measurements, where possible, starting at least

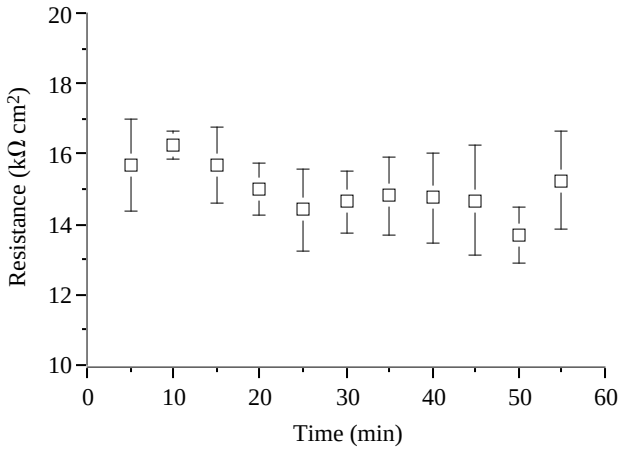

Fig. 1. Changes of resistance of intact pronotal integument of hydrated adult male *Periplaneta americana* with time. Each point is the mean ± S.E.M. for three measurements on each of three animals at each time.

15 min after electrode contact, and at three subsequent 5-min intervals; each resistance value was then the mean of the resulting eight values (four times, two sites).

Relationship between resistance and water permeability

Fig. 2 shows that there is a strong negative correlation between the water content of adult *P. americana* and their transintegumental pronotal resistance (R_i), with dehydrated cockroaches having higher resistances than hydrated ones. This finding extends that of Machin *et al.* (1994), who reported that the conductance decreases upon dehydration in pronota, abdominal sternites and tergites, and in wings in nymphs and adults of both sexes, and that the conductance decrease is correlated with the initial hydrated conductance. Here, resistances were measured for a broad range of hydrated states in one area (pronotum) and in one sex (males). Whereas dermal gland densities vary in the different regions tested earlier, here this is a constant. Resistance rather than conductance is used here. At present, we have no theoretical basis for fitting a straight line to either quantity; however, if the two 'outliers' in Fig. 2 are excluded, the best fit is to resistance (r^2=0.931 compared with 0.805 for conductance). Why the two outliers do not fit is unknown. It was found in Machin *et al.* (1994) that the regression line for conductance change *versus* initial hydrated conductance suggested a minimal conductance in all regions of the integument, in both nymphs and adults, of about 25 μS cm^{-2} (equivalent to about 40 kΩ cm^2).

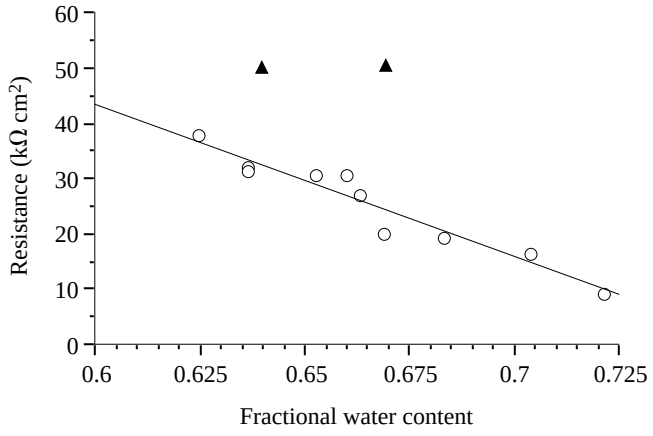

Fig. 2. Pronotal *in vivo* resistances increase with decreasing water content of adult male cockroaches (y=208.5−275.2x, where y is resistance and x is water content). Each point is the mean of two readings taken on either side of the midline in a different cockroach whose water content, expressed as a fraction of *in vivo* mass, was subsequently determined. The two triangles mark 'outliers' that were excluded from the analysis.

Manipulation of the integument

Resistance measurements were made at each stage after isolating the pronotum, then stripping the epidermis, scraping the inner surface of the cuticle and applying solvent to remove the epicuticle; in initial studies, we found that all these treatments reduced the resistance.

Using a frequency of 1 Hz, with one internal electrode and two external electrodes placed on either side of the pronotal midline, we measured the mean single-electrode resistances after various treatments of the pronotal cuticle. Readings were taken after the electrodes had been in place for 15 min to minimize possible time-dependent decreases. In Fig. 3 we plot

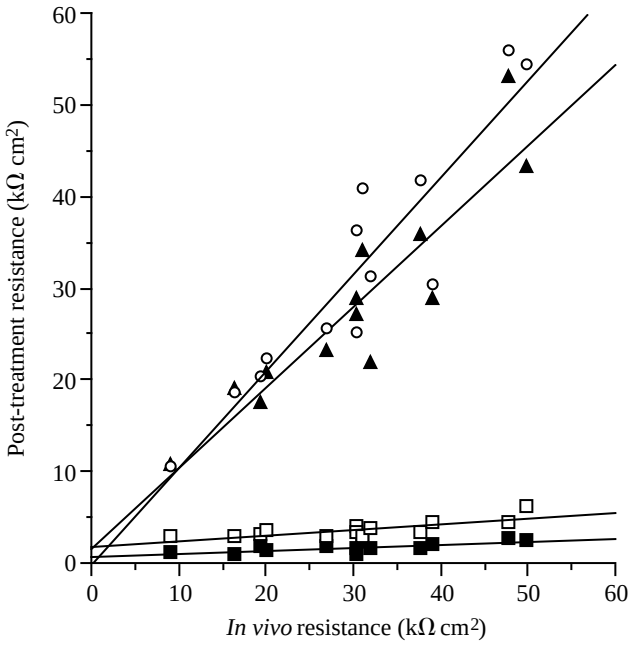

Fig. 3. The effect of progressive treatments on pronotal electrical resistance. Open circles, removal of head and abdomen; filled triangles, placing the pronotum over Ringer; open squares, removal of the epidermis by peeling; filled squares, scraping off the inner surface of the cuticle.

the post-treatment resistances against their initial values. A regression line of slope 1 would represent no effect of the treatment. Removal of the head and abdomen had no significant effect (slope of regression line=0.986±0.14), although it appeared to lower the resistance in preliminary experiments. Removal of the pronotal integument and placing it on Ringer had a significant effect (slope=0.877±0.117, P=0.05), whose magnitude depended on the initial resistance of the integument. The fitted regression line intersects the no-effect line at about 10 kΩ cm^2. We interpret this to mean that the *in vivo* integumental resistance consists of two parts, a hydration-dependent part affected by the treatment, and a fixed part, not dependent on hydration, with a d.c. value of about 10 kΩ cm^2. The third treatment, peeling the epidermis from the cuticle, causes a large drop in resistance down to a mean value of about 3.5 kΩ cm^2; this residual resistance is only slightly dependent on hydration (slope=0.062±0.015), indicating that peeling has removed almost all the hydration-dependent barrier and about 6.5 kΩ cm^2 of the fixed part of the resistance as well. Mechanical scraping of the inner surface of the cuticle removes a further 2 kΩ cm^2, leaving a mean value of about 1.6 kΩ cm^2 for the remaining endo- and epicuticle. This value is still slightly dependent on hydration (slope=0.035±0.010). Solvent treatment of the remaining cuticle reduced this to about 800 Ω cm^2 (data not shown in Fig. 3), presumably by removing an insulating layer of epicuticle.

These results show that the majority of the electrical resistance presented by the integument to ionic current flow lies in the epidermis rather than in the cuticle; the cuticle itself has a relatively low resistance of about 1.6 kΩ cm^2 with intact epicuticle. They also show that the hydration-dependent components of the integumental resistance are entirely, or almost entirely, associated with the cellular layer of the epidermis rather than with the cuticle, and that this 'variable barrier' is highly sensitive to experimental manipulation: careful excision of the pronotal integument and floating it on Ringer has an appreciable effect on its resistance.

Resistive model

The results of the above experiment can be represented by a resistance network (Fig. 4). The resistances of 7 (rounded from 6.5) and 2 kΩ cm^2 are components revealed by peeling and scraping respectively. The variable barrier is the hydration-dependent component. The cuticle is modelled by two parallel resistors: one represents the normally conducting porous structures of the intact cuticle, established above at about 1.6 kΩ cm^2, and the other represents the rest of the endocuticle. This should include another parallel resistance representing remaining conductive pathways, such as open pore canals (Machin *et al.* 1994), of about 31 kΩ cm^2 (40 kΩ cm^2 minus the 7+2 kΩ cm^2 internal to the cuticle). Since this is high compared with the 1.6 kΩ cm^2 resistance and makes only a negligible difference to the model, we have not included it. Removal of the waxy epicuticle allows current also to pass through the mass of endocuticle (closing the switch in Fig. 4). A drop in cuticular resistance to about 800 Ω cm^2 would be achieved by a parallel resistance also of about 1.6 kΩ cm^2. Values for these two resistances in the model have been reduced and rounded to 1 kΩ cm^2 in part to account for the resistance (340 Ω cm^2) of the internal electrode and body, which becomes important at low values. The resistance representing the variable barrier is placed between the inner cuticular resistance and the fixed epidermal resistance; although this position is somewhat arbitrary, it reflects the likelihood that a mechanism affecting pore conductance may be located close to the pore entrances.

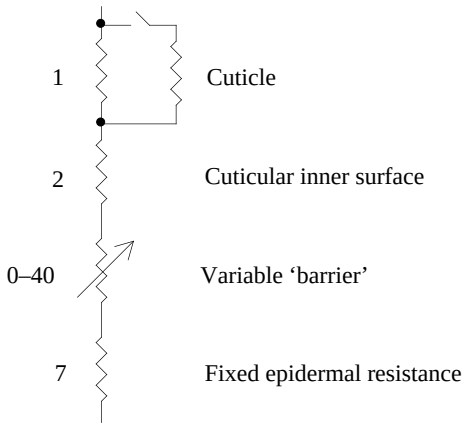

Fig. 4. Resistive model of the integument, values in kΩ cm^2. For explanation, see text.

An integumental shunt

In order to measure resistance in intact animals, Scheie and Smyth (1968) used two external electrodes. They found no detectable difference between resistances determined by halving the double external measurement and those measured between a single external and an internal electrode. Since our recording system appeared to give greater precision, we wanted to check this conclusion. We therefore took readings that had been made with two external and one internal electrode on seven hydrated and 18 dehydrated intact cockroaches, and compared the mean value for the two resistances measured between the external and the internal electrodes (the 'mean single' resistance, R_{ms}) and half the value measured between the two external electrodes (R_{double}). For both hydrated and dehydrated cockroaches, R_{double} was lower than R_{ms} (Table 1). Paired t-tests showed the differences were highly significant (P<0.0001).

Table 1. *Resistances for single and double external electrodes applied to pronotal cuticle in hydrated and dehydrated cockroaches*

State	R_{ms} (Ω)	R_{double} (Ω)	N	ΔR (Ω)
Hydrated	16 607±505	15 330±472	52	1277±79
Dehydrated	50 136±1226	44 121±1079	72	6015±283

Values are mean ± S.E.M.

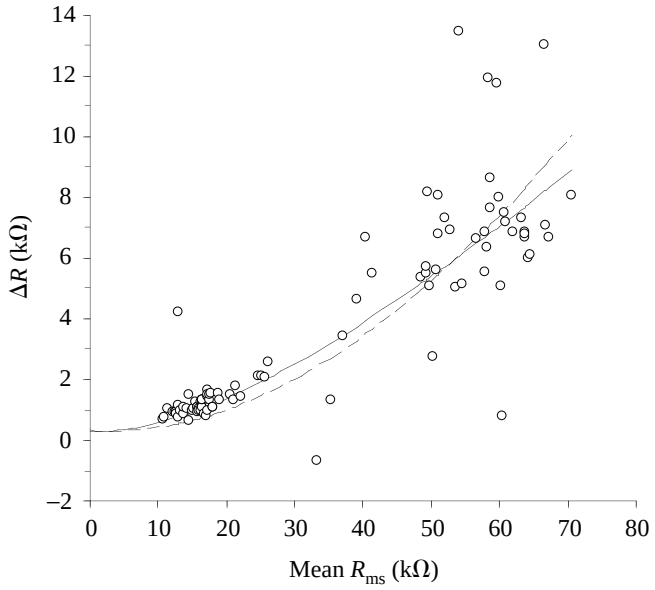

Fig. 5. The difference (ΔR) between the transintegumental resistance determined with a single external electrode and half the resistance measured between two external electrodes, plotted against the mean single reading, R_{ms}. The lines are fitted to optimise r^2 using equations derived from two models of a cuticular shunt (see text). Resistance values are for 1 cm^2.

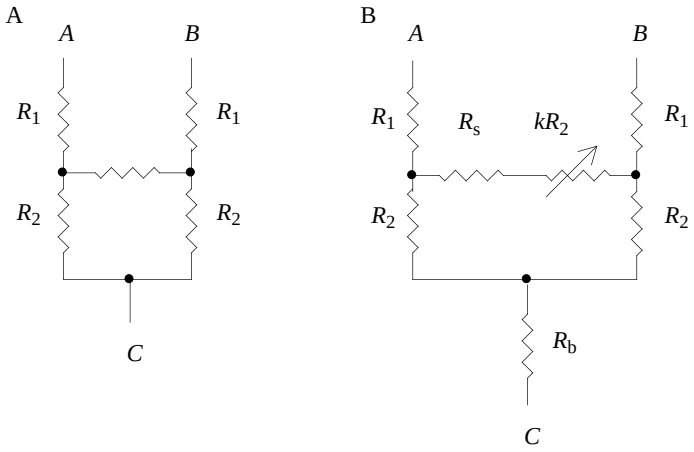

Fig. 6. Model circuits representing an integumental 'shunt' pathway. R_1 and R_2 represent the portions of the transintegumental resistance R_i external and internal to the shunt, respectively. R_s is the 'shunt', the resistance of a pathway for current inside the integument and parallel to its surface. *A* and *B* represent connection points for external electrodes, and *C* for the internal electrode. Thus *AC* and *BC* would represent R_{single}, the 'single-electrode' resistance, and *AB*/2 is R_{double}, half the resistance between the external electrodes. (A) Simple model. (B) Modified model incorporating R_b, the resistance of the body cavity and the internal electrode, and kR_2, a variable part of the shunt pathway whose resistance is hydration-dependent.

Values for ΔR, the difference between R_{ms} and R_{double}, are plotted as a function of R_{ms} in Fig. 5. Clearly, the difference in the two measurements is a function of the resistance of the cuticle, with ΔR increasing with increasing values for the integumental resistance. The difference suggests a 'shunt' pathway within the integument, i.e. that not all the current flows through all the components between the exterior and the haemolymph under the epidermis. A simple model of such a shunt is shown in Fig. 6A. For such a circuit, the value for R_{double} (i.e. half the resistance between the two external electrodes, *A* and *B*) is given by:

$$R_{double} = R_1 + \frac{0.5}{(1/R_s) + (1/2R_2)} ,$$

where R_1 and R_2 represent the portions of the transintegumental resistance R_i external and internal to the shunt, respectively, and R_s is the shunt resistance. The single resistance is given by:

$$R_{single} = R_1 + R_2$$

and thus the difference, ΔR, is:

$$\Delta R = R_{single} - R_{double}$$
$$= R_2 - \frac{0.5}{(1/R_s) + (1/2R_2)} . \quad (1)$$

Note that the value for ΔR is independent of the value of R_1 and depends only on the shunt resistance and integumental resistances internal to that point. Since ΔR is dependent on R_{single}, which is in turn dependent on hydration state, it follows that the hydration-dependent variable resistance (the 'barrier') must be at least partly located internal to the shunt, that is R_2 is variable and dependent on the hydration state.

Using values derived from the previous model in equation 1, we can attempt to fit a line to the data in Fig. 5. Assuming that the shunt is located internal to the cuticle, including the component removed by scraping (both resistances are assumed to be independent of hydration state), R_1=3 kΩ cm^2 and R_2=R_{single}−R_1. Using trial-and-error values for the shunt resistance R_s, the best-fitting line (not shown in Fig. 5) goes consistently below the low-resistance points, but otherwise fits the general trend. However, the measured value for R_{single} includes a component that is usually ignored, the resistance of the body and internal electrode (R_b):

$$R_{single} = R_1 + R_2 + R_b$$

and equation 1 should be modified to:

$$\Delta R = R_2 + R_b - \frac{0.5}{(1/R_s) + (1/2R_2)} . \quad (2)$$

By inserting external electrodes through a hole made in the cuticle and underlying epidermis, thereby bypassing R_i, we established that this value was about 18 kΩ in a dehydrated animal and 15 kΩ in a hydrated one; if these values are adjusted by the same factor used to convert actual measurements to resistances for 1 cm^2 (average value about 0.017), then R_b has a value of about 250–300 Ω cm^2. The dashed line in Fig. 5 is the best-fitting line (determined by eye) using this value for R_b

and 800 kΩ cm^2 for R_s in equation 2; however, it is still below the low-resistance data points.

The model in Fig. 6A may be misleading in that it implies a single pathway for the shunt resistance. In fact, the shunt represents the sum of all the paths that current may take between the two electrodes external to the haemolymph, i.e. through cuticle and/or epidermis parallel to the surface. Thus, it is possible that a significant fraction of the shunt is through components whose resistance varies with hydration state (parts of the 'barrier'). To model this, we added a second resistance in series with the shunt, whose value depends on the value of R_2, with a proportionality constant of k (Fig. 6B). Equation 2 then becomes:

$$\Delta R = R_2 + R_b - \frac{0.5}{[1/(R_s + kR_2)] + (1/2R_2)}. \quad (3)$$

Using equation 3 with values of 500 kΩ cm^2 for R_s and 7 for k, and reducing R_1 to 1 kΩ cm^2 gives a much better fit to the data, shown by the solid line in Fig. 5. Thus, the model suggests that some of the current between the two external electrodes passes parallel to the surface of the cuticle through structures whose resistance has a fixed component of about 500 kΩ cm^2 plus a component that varies, depending on hydration state, roughly between 50 kΩ cm^2 and 350 kΩ cm^2.

The model also suggests that most of the shunt is internal to the cuticle, with little of the pathway *via* the cuticle. The resistance of a cuticular path may be estimated as follows. Unlike the epicuticle, the endocuticle is fairly hydrated, containing 0.256 g H_2O g^{-1} dry mass, about 18 % of hydrated volume (Machin *et al.* 1985). The pronotum contains 7.4×10^{-8} equiv Na^+ g^{-1} dry mass (Machin and Lampert, 1987), about twice the value reported by Hyatt and Marshall (1985) for the cuticle as a whole. Doubling the pronotal value to take into account an equivalent amount of anion (assumed to be Cl^-), we can obtain an electrolyte concentration, assuming both to be dissolved in the measured pronotal water. Using standard values (CRC Handbook; see Weast, 1969), we estimate this concentration of NaCl to have a conductivity at 20 °C of about 30 μS cm^{-1}. Making corrections for thickness and cross-sectional area, assuming the conducting pathway to be the same proportion of total area as the water content (18 %), and assuming no ionic binding, we estimate that the resistance across the endocuticle should be about 930 Ω cm^2. This is very close to the transcuticular resistance of about 1 kΩ cm^2 suggested by the results of solvent treatment (Fig. 4). For current in the plane of the cuticle, the resistance of a 1 mm wide strip of 50 μm thick cuticle between the electrodes, which are about 2 mm apart, should be about 72 MΩ. Applying an electrode area correction factor of about 60 (see Materials and methods) gives a theoretical shunt through the cuticle of about 1.2 MΩ cm^2, which, given all the assumptions made above, is in reasonable agreement to the 550–850 kΩ cm^2 arrived at in the model.

Reactive properties

Using frequencies between 0.1 and 100 Hz, impedance and

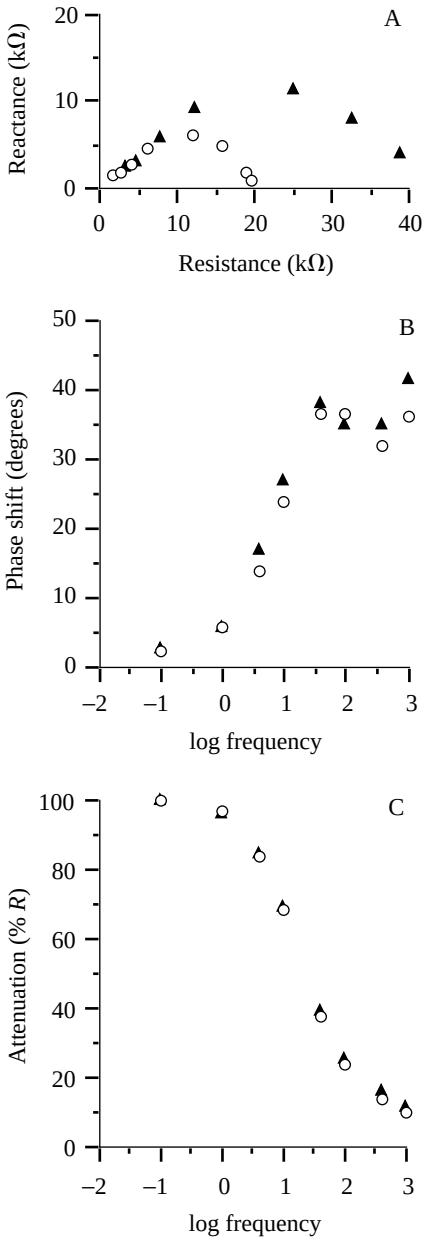

Fig. 7. Representative impedance plots for 1 cm^2 of pronotal integument from a cockroach in both the hydrated and dehydrated states (see Appendix for explanation of such plots). Measurements were made *in vivo*. Open circles, hydrated; filled triangles, dehydrated. (A) Reactance plotted against resistance for different frequencies. Note that the points do not lie on semicircles, as would be the case for a simple one-capacitor, two-resistor circuit, and there appears to be a flattened 'tail' at the low-resistance (high-frequency) end of both sets of points. (B). Phase shift plotted against the logarithm of frequency (in Hz). For both hydrated and dehydrated values, the points fit curves with at least two peaks, indicating more than one capacitative element. Note, however, that dehydration produces only a small change in the shape of the curve and the position of its first peak, despite the doubling of d.c. resistance. (C) Attenuation, *versus* the logarithm of frequency. Attenuation is the impedance at each frequency expressed as a percentage of the 'd.c.' value (actually 0.1 Hz). Again, little change is seen with dehydration.

phase measurements were taken on intact *in vivo* integument, freshly removed integument and cuticle stripped of its epidermis. Fig. 7 shows representative impedance and phase and attenuation plots for hydrated and dehydrated intact integument. In all cases, both the measured impedance and the phase angle varied with frequency. Scheie and Smyth (1967) explained such results with the reasonable assumption that the cuticle has capacitative as well as resistive properties. However, their simple one-capacitor, two-resistor model of the cuticle did not fit their data fully: the graph of reactance against resistance of such a circuit should be a semicircle with its centre on the resistance axis, whereas the semicircle they fitted had its centre below the axis.

Such departures from the predicted values had been noted in other tissues and cell suspensions; they are discussed by Cole (1933), who invoked 'polarization elements' whose reactance and resistance change appropriately with frequency, but failed to identify what such elements might be. Though cell membranes are electrically complex, with resistive properties that can exhibit rectification and voltage-dependence, we wondered whether the departure from circularity in the present preparation might be better explained by assuming a more complex circuit. For instance, a two-capacitor, three-resistor circuit can produce an impedance plot that is no longer semicircular (Fig. 8). Thus, it seems reasonable that real integument, which is a complex distributed three-dimensional structure and not a circuit of discrete elements, might act similarly.

Accordingly, we attempted to model the integument with more complex circuits, using resistance values determined earlier. The cuticle stripped of its epidermis should be the simplest to model, and indeed its impedance plot appears semicircular within the range of our measurements (Fig. 9). Since the waxy epicuticle acts as an insulator in series with the bulk of the endocuticle, according to our resistive model, it should also have capacitance. Adjusting its value empirically in a model circuit, we obtained a good match to the data for cuticle (Fig. 9, inset). Is the value of $20\,\mathrm{nF\,cm^{-2}}$ reasonable? The capacitance of a parallel-plate capacitor is given by $C=KA/2\pi d$, where K is the dielectric constant of the material, A is the area and d is the thickness; if A and d are in centimetres, the capacitance is in $\mu\mathrm{F\,cm^{-2}}$. Natural waxes have dielectric constants of about 2.5–3 (CRC Handbook); assuming a thickness of epicuticle of about $0.25\,\mu\mathrm{m}$ (Croghan and Noble-Nesbitt, 1989), the capacitance is calculated to between 8 and $10\,\mathrm{nF\,cm^{-2}}$, about half the value determined from the hydrated cuticle data. The difference might easily be accounted for by a higher dielectric constant or a thinner epicuticle. Thus, the simple electrical model seems reasonable.

Modelling the intact integument is more difficult. The flattened low-resistance, high-frequency end of the reactance–resistance plot of intact integument (Fig. 7) suggests at least two resistive–capacitative (RC) elements. We arrived at a plausible circuit (Fig. 10) by considering the anatomy of the integument. The cuticle is modelled by a resistance R_{dg} representing the resistance of the dermal-gland canal pathway,

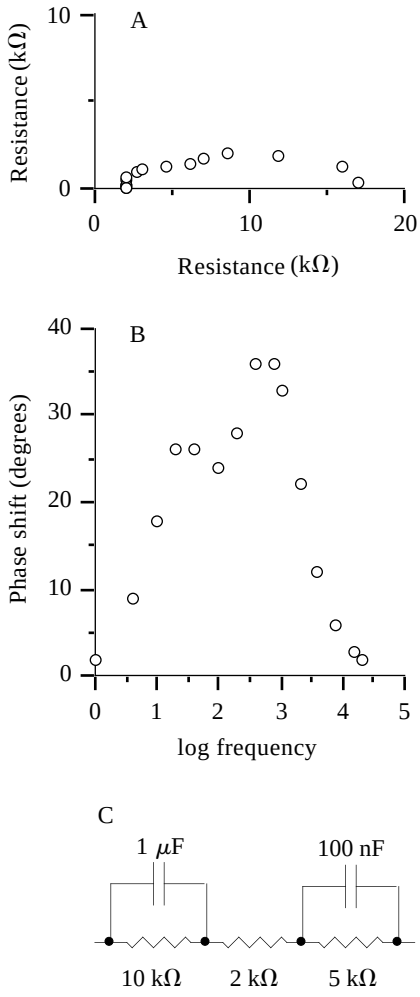

Fig. 8. Non-semicircular plots of reactance *versus* resistance (A), with a high-frequency 'tail', and double-peaked phase plots (B), are produced by circuits with two capacitative elements such as that in C.

in parallel with the endocuticular resistance R_c, itself in series with the capacitance of the epicuticle, C_e. These are the elements modelled in Fig. 9; however, they are no longer immediately connected, since the inside of the cuticle is appressed to the cellular layer of the epidermis, not in contact with low-resistance Ringer. The endocuticular resistance instead connects to elements representing the cell layer of the epidermis; each element models one layer of cell membranes, with capacitative and resistive properties. Thus, the left-hand side of the model circuit represents the pathway through the mass of endocuticle, normally non-conducting for direct current because of the insulating epicuticle. The dermal gland pathway is modelled by the right-hand side of the diagram. The conducting dermal gland canals connect to separate cellular elements in the epidermis, possibly the dermal gland cells. The elements R_v and C_v represent structures, presumably including membranes, separating the lumen of the dermal gland canals from the interior or the surrounding cells. A connection is made from here to the left-hand side of the circuit, representing a

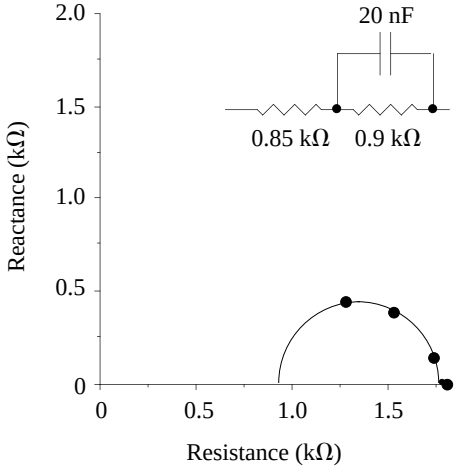

Fig. 9. Reactance plot for 1 cm^2 of scraped pronotal cuticle. Impedance measurements were made in this case up to 10 kHz. The fitted semicircle corresponds to the resistance values in the circuit shown above. The position of a point on the semicircle at a given frequency depends only on the value of the capacitance; the experimentally determined values correspond to a capacitance of 20 nF.

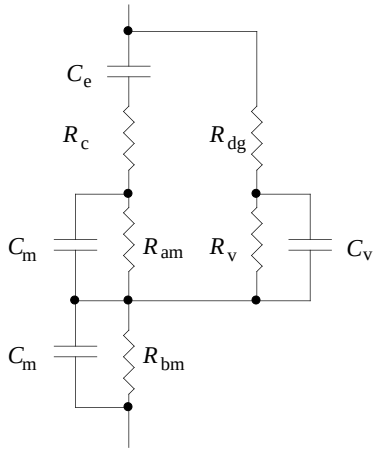

Fig. 10. Possible circuit corresponding to intact pronotal integument. For explanation and terminology, see text.

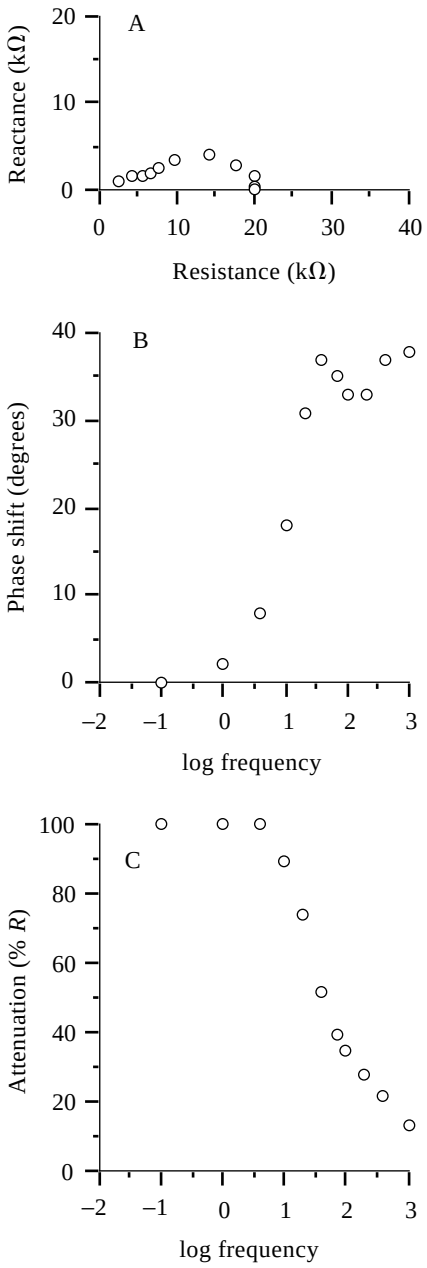

Fig. 11. Impedance plots for the circuit in Fig. 10, a model for 1 cm^2 of hydrated pronotal integument (compare pronotal plots in Fig. 7). Values in the circuit were C_e, 20 nF; R_c, 1 kΩ; C_m, 1 μF; R_{am}, 10 kΩ; R_{bm}, 7 kΩ; R_{dg}, 1 kΩ; R_v, 2 kΩ; C_v, 200 nF.

shared final pathway through the basal membranes of the epidermal cell layer. In this model, R_v includes the variable-resistance hydration-dependent barrier.

Initial values for 1 cm^2 of model integument were chosen as follows. We assumed a 'standard' value of about 1 μF cm^{-2} for the cell membrane C_m. Values for C_e and R_c were determined earlier. R_{dg}, in fact, represents the approximately 1 kΩ cm^2 of the conductive pathway in the scraped cuticle, plus any additional resistance internal to the cuticle not in parallel to the capacitance C_v; some of this could be hydration-dependent. R_v is the remainder of the variable barrier, 0 to a few kΩ cm^2 in the hydrated integument. R_{bm}, the resistance of the epidermal basal cell membrane layer, must be equal to the remaining fixed resistance in the d.c. model of Fig. 4, i.e. about 7 kΩ cm^2. R_{am}, the resistance of the apical cell membranes, was given a similar value arbitrarily rounded to 10 kΩ cm^2.

Starting with these values, and adjusting R_{dg}, R_v and C_v by trial and error, our computer-simulated model circuit could be made to perform reasonably like the hydrated integument (Fig. 11; compare with Fig. 7). Values here are plausible: 2 kΩ for R_v and 200 nF for C_v would be consistent with a cell membrane with an area of about 0.2 cm^2, suggesting that the

structures controlling access to the dermal gland canals might have an effective area of one-fifth of the area of the integument as a whole. However, the model was overly sensitive to changes in these values and showed none of the robustness of the curves obtained from actual integuments. Furthermore, increasing the values of R_v and R_{dg} to approximate dehydrated values changed the shape of the phase–frequency plot dramatically, whereas that of the real integument changed very little (Fig. 7). The present model does account for some of the observed electrical properties and identifies components that may be experimentally manipulated; nevertheless, it needs further modification to account for the hydration-dependent changes.

Interpretation

Sources of integumental conductance

Scheie and Smyth (1967) considered the setae to be implausible candidates for significant conductance through the integument. Pronotal setae are sparse and unevenly distributed, and our gentle preparatory treatment probably broke very few; thus, if broken setae were the principal route, the variability between preparations would be much higher than observed. Scheie and Smyth (1967) estimated the resistances of pore canals, dermal gland canals and broken setae, assuming they became filled with the 0.1 mol l^{-1} NaCl used to make electrical contact with the cuticle. For pore canals, the estimated resistance for 1 cm^2 of cuticle was too low by at least an order of magnitude to account for observed cuticular values, suggesting that the pore canals are probably wax-filled and thus largely non-conductive. However, Machin *et al.* (1994) found that correlations between dermal gland density and conductance and with the change in conductance following dehydration, suggested a constant residual conductance in addition to that of the dermal glands of about 25 $\mu S\,cm^{-2}$. This translates to a resistance of about 40 $k\Omega\,cm^2$. Machin *et al.* 1994 speculated that the residual conductivity might represent a pathway *via* a small number of pore canals remaining open or incompletely blocked.

However, dermal gland canals are different. Acceptable correlations between integumental conductances of some cuticular plates and their dermal gland densities have already been established by Scheie and Smyth (1968) and Machin *et al.* (1994). The present paper has shown that most of the transintegumental resistance lies in the epidermis, not in the cuticle. Our measurements of cuticular resistance, using values for scraped cuticle calculated from the regression equations for the data in Fig. 3, are 1186 $\Omega\,cm^2$ for a hydrated insect (R_i=15 $k\Omega\,cm^2$) and 1893 $\Omega\,cm^2$ for a dehydrated insect (R_i=40 $k\Omega\,cm^2$), giving a mean of 1539 $\Omega\,cm^2$. To investigate whether this may reasonably be attributed to dermal gland canals, we must first determine whether the canals can be viewed as a series of straight uniform cylinders. Using a microscope to focus through the depth of pronotal cuticle suggests that the canals are relatively straight and perpendicular to the surface and thus cannot be more that 1.5 times the thickness of the pronotal cuticle, which is about 50 μm (Machin *et al.* 1985). Using this upper limit of 75 μm, the conductivity of the cockroach Ringer used in our electrodes (16.3 $mS\,cm^{-1}$) and Scheie and Smyth's (1968) dermal gland densities for the pronotum ($2.4\times10^5\,cm^{-2}$), canal diameter should be about 0.4 μm, giving a resistivity of about 1500 $\Omega\,cm^2$. Our own (J. J. B. Smith, J. Machin and G. J. Lampert, unpublished results) scanning electron micrographs indicate typical external canal diameters of 1.0 μm. This suggests that their minimum diameters are less than this and that they may taper inwards. Scheie and Smyth (1968), who used diameters of 10 μm in their calculations, seem to have greatly overestimated canal diameter.

Given that every open dermal gland canal is filled with fluid and that diffusion from the electrodes into them is so rapid, another explanation for the approximately 15 % drop in resistance over a 15 min period must be sought. It is possible that this change represents a much slower accommodation of the cuticle surrounding the canal, or the closing mechanism itself, to the new ionic environment imposed by the application of external electrodes.

Relationship between conductance and water permeability

Variations in integumental resistance that are correlated with the physiological state of the animal suggest that our measurements have some biological meaning. In other words, even though our measurement techniques may introduce foreign materials into dermal gland canals, there appears to be no serious alteration to their structure. Thus, our measurements should reflect dermal gland canals as they are *in vivo*. The calculations given above have shown that there is a reasonable correspondence between the conductance of excised cuticle and the number and dimensions of the dermal gland canals in the pronotum. Since cuticle thickness in a given region is reasonably consistent from animal to animal, so presumably is canal length. Thus, electrical conductance in other parts of the cuticle should be directly related to the number and, hence, the total cross-sectional area of the dermal gland canals, i.e. to the area of integument not protected by the cuticle.

Two different models would explain the observed conductance decreases resulting from animal dehydration, one continuously variable and the other quantal. A reduction in conductance could be brought about by a progressive constriction in the dermal gland canal; present evidence suggests that this constriction would be proximal rather than distal. Alternatively, reduced conductance in the cuticle could be achieved by an all-or-none closing mechanism operating through changes in the relative numbers of open and closed canals. However, changes in evaporation from the canals would only change in the second proposed mechanism, because only complete closing would result in their drying out. In partly open canals, capillarity would ensure that they remained filled with fluid (see Appendix) and therefore that the exposed fluid area would remain constant.

In the case of the quantal model, changes in integumental conductance with hydration may be used to determine the

relative magnitude of porous and non-porous water pathways. Unfortunately, pronotal water permeabilities, required for the calculation, are only available for dehydrated cockroaches. At a mean water content of 64.8±0.3 %, dehydrated pronotal water permeability (P_d) was 0.92×10^{-10} m s^{-1} (Machin *et al.* 1992). This compares with an overall cuticular permeability of 0.96×10^{-10} m s^{-1} at a mean water content of 65.1±0.7 % (Machin *et al.* 1991). This similarity suggests that pronotal permeability is representative of the water-conducting properties of the cuticle as a whole. In the calculations below, we have therefore taken the overall cuticular permeability of hydrated cockroaches (water content=76.6±0.6 %) to represent pronotal permeability, $P_h=1.61\times10^{-10}$ m s^{-1}. Overall pronotal integumental conductance corresponding to a dehydrated water content of 64.8 % can be derived from the regression equation for the data in Fig. 2, and is 35.9 μS cm^{-2}. This conductance comprises both the dermal gland canal conductance and the conductance through the rest of the integument. The latter value was estimated by Machin *et al.* (1994) to be about 25.4 μS cm^{-2}. Consequently, the dehydrated dermal gland canal conductance $g_d=35.9-25.4=10.5$ μS cm^{-2}. The hydrated conductance at 76.6 % water content cannot be so easily estimated, since the regression equation from Fig. 2 would predict a negative resistance. However, Fig. 3 was interpreted to mean that in hydrated animals there is a lower limit to resistance, when the variable component is minimal, of about 10 kΩ cm^2, equivalent to an overall conductance of 100 μS cm^{-2}. Again subtracting the non-canal conductance, the hydrated dermal gland canal conductance $g_h=100-25.4=76.6$ μS cm^{-2}.

If a is the permeability of non-porous integument, which we shall assume to be constant at different hydration levels, and b is the permeability of the porous pathways when dehydrated, it follows that, when hydrated, the porous permeability becomes $(g_h/g_d)b$. Thus, the ratio of overall pronotal permeabilities in hydrated and dehydrated animals is given by:

$$\frac{P_h}{P_d} = \frac{a + (g_h/g_d)b}{a + b} .$$

Using the data above, and solving for b, we calculate that the porous permeability (b) is 0.13a when dehydrated and 0.98a when hydrated. Thus, when the animal is hydrated, the water lost through the canals is nearly equal (49.5 % of the total) to that flowing through the non-porous integument. When the animal is dehydrated, total flux decreases to about 60 % of its hydrated value. To account for this, the pore flux must have declined by a factor of about 7, to 6.8 % of the total hydrated value. Because the pores occupy only 0.55 % of total area, they are 200 times as conductive as the non-porous integument when hydrated and about 30 times as conductive when dehydrated.

Although it seems clear that the porous pathway is *via* the dermal gland canals, the evidence presented in this paper indicates that *changes* to their electrical conductance, and thus presumably to their water permeability, take place not in the canals as they cross the cuticle, but either at their entrances or in the structures underlying them. Thus, we conclude that the dehydration-dependent barrier is probably a property of the apical secretory surfaces of the dermal gland cells or of the overlying cellular and canal material connecting the cells to the cuticular pores, both well placed for possible hormonal control.

This work has shown the usefulness of electrical measurements both as indicators of cuticular porosity and in refining structural and functional models of the integument. Although such measurements relate principally to pathways that conduct ions, and do not reveal routes for water in the absence of ions, it is clear that such pathways contribute substantially to water loss through the integument. Furthermore, these techniques provide a ready means of monitoring changes in porosity and should facilitate experiments on the mechanism of its physiological regulation.

Appendix

Resistive–capacitative circuits

Living tissue acts electrically as a conducting fluid in which ionic current travels in complicated paths with both resistive and capacitative properties. There are, of course, also voltage sources (resting membrane potentials, action potentials), but these are not involved in the present study. Analysing the electrical properties of living tissues requires some understanding of resistive–capacitative (RC) circuits. Although the level of analysis used in this paper is covered by most elementary physics or electrical engineering textbooks, such sources may not be too accessible to many readers; for this reason, we summarise here the relevant material. A more extensive treatment of the ‘impedance-locus’ method is given in Scheie and Smyth (1967); the use of impedance measurement in studies of living tissue is also reviewed in Geddes and Baker (1989).

The effect of resistance on current and voltage is given by Ohm’s law, $I=V/R$, where I is current in amps, V is voltage in volts and R is resistance in ohms. Conductance is the inverse of resistance, and Ohm’s law can also be expressed as $I=gV$, where g is conductance in siemens (reciprocal ohms). Elements in a circuit with capacitance introduce a complication. Unlike the situation with pure resistive elements, the current associated with a capacitor is no longer in phase with the voltage. Initially, the current is high and the voltage zero; the voltage then rises as the capacitor accumulates charge and, since the charge opposes further current, the current declines. In other words, the voltage lags behind the current or the current leads the voltage. For direct current, the current declines to zero, and thus the capacitor presents an effective resistance of infinity. For alternating current, the higher the frequency, the lower is the charge built up for each cycle, and hence the lower is the opposing voltage. Consequently, the effective resistance of the capacitor declines. This frequency-dependent ‘resistance’ is called *reactance*, its value (in ohms) being given by $1/2\pi fC$, where f is the frequency in hertz and C is the capacitance in farads. For a ‘pure’ capacitative

reactance, the current leads the voltage for sinusoidal alternating current by 90°.

Current in circuits containing both resistive and reactive elements can be related to voltages if the concept of resistance is replaced by impedance. Conventionally, resistance and reactance are represented by two perpendicular dimensions, as in a graph where resistance is the *x*-axis and reactance the *y*-axis. A point (*x*,*y*) on this graph will the represent a particular resistance (*x*) and a particular reactance (*y*); the impedance is the length of the line connecting the point to the origin. The phase shift produced by such an impedance is the angle made by this line with the *x*- (resistive) axis.

In the present experiments, the constant-current amplifier produces an output whose voltage amplitude is directly proportional to the impedance (*Z*) of the preparation. The output also exhibits a phase lag (ϕ) if there is reactance in the preparation, and this is measured (in degrees) with a variable-phase oscillator. The resistive and reactive components (the '*x*' and '*y*' values) can then easily be calculated with simple trigonometry, as resistance = $Z\cos\phi$ and reactance = $Z\sin\phi$.

Various plots can be used to describe the electrical properties of circuits with both resistive and reactive elements. Cole (1933) plotted reactance against resistance as described above. A circuit containing a capacitor with one series and one parallel resistance gives a semicircular plot when impedances are measured over a range of frequencies. The circle intersects the resistance axis (i.e. reactance is zero) at two points. The lower value, obtained at very high frequencies, equals the value for the series resistance, since the zero effective resistance of the capacitor effectively short-circuits the parallel resistance. The higher value is obtained with direct current (zero frequency) and represents the sum of the two resistances. Here, the capacitor is effectively an open circuit, and all current flows through the series and parallel resistors.

The form of the resistance/reactance plot of such a circuit is independent of the value of the capacitance; the points for any frequencies simply shift along the curve as the capacitance varies. Thus, other types of plot are better for highlighting capacitative components. We have used two: phase shift and attenuation plotted as a function of frequency. Phase shifts are zero at zero and infinite frequencies, but peak at frequencies highly dependent on capacitance. Attenuation is the decline in effective resistance (impedance) as frequency rises, and is expressed as the ratio of the impedance at each frequency to the direct current impedance (resistance).

Capillarity in dermal gland canals

The height to which an aqueous solution (*h*) will rise in a vertical tube due to capillarity is given by the following equation (Hallet *et al.* 1977):

$$h = (2\gamma\cos\theta/\rho \boldsymbol{g} r),$$

where *r* is the radius of the tube (about 5×10^{-7} m), γ is the surface tension of the solution, ρ is its density (about 10^3 kg m^{-3} for the Ringer in our electrodes), $\boldsymbol{g}$ is the acceleration due to gravity (9.8 m s^{-2}) and θ is the contact angle with the tube wall. Using a value for γ of 72.9×10^{-3} N m^{-1} for 0.6% NaCl at 20°C, and 80° for θ (assuming the canal to be only slightly hydrophilic), the calculated height is about 5 m. Since the canals are estimated to be 75 μm long, there would be ample force to fill them. For non-vertical canals, less force is needed.

Electrolyte diffusion into dermal gland canals

The time (*t*) required for one-third of the sodium and chloride ions in Ringer to diffuse at least the length of the dermal gland canals can be estimated from the the following equation for mean square displacement of diffusing molecules from a starting point (Hallet *et al.* 1977):

$$\overline{l}^2 = 6Dt,$$

where *l* is the displacement (in m), *t* is time (in s) and *D* is the diffusion coefficient (in m^2 s^{-1}). If *l*=75 μm, the canal length, and *D* for NaCl at 20°C is 1.48×10^{-9} m^2 s^{-1}, the time for at least one-third of the diffusing molecules to reach the end of the canal is about 0.6 s.

Financial support for this research from the National Science and Engineering Council of Canada in operating grants to J.M. and J.J.B.S. is gratefully acknowledged, as is advice on the use of the current-clamping amplifier from Jan van der Pers.

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