

A PASSIVE TWO LAYER PERMEABILITY-WATER CONTENT MODEL FOR *PERIPLANETA* CUTICLE

BY J. MACHIN AND G. J. LAMPERT

*Department of Zoology, University of Toronto, Toronto, Ontario, Canada
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SUMMARY

Two layers in *Periplaneta* pronotum, endocuticle and combined epicuticle and exocuticle were functionally distinguished by their permeabilities and water affinities. A passive model combining the permeabilities of these layers and the water content of the endocuticle was designed to predict pronotal water contents in a variety of ambient activities at 20°C.

The model predicted cuticle water contents with acceptable accuracy above ambient activities of 0.56 but overestimated them in drier conditions. Despite universally high water activities in the endocuticle, differences in water content related to ambient activity were predicted. Cuticle water contents below haemolymph equilibrated values, represented in the literature as evidence of active water regulation, were largely attributed to passive activity gradients combined with evaporation errors.

INTRODUCTION

Mass gains, observed when excised cuticle samples are exposed to atmospheres of the same water activity as the haemolymph, have been put forward as evidence that the insect epidermis is capable of actively regulating cuticle water content (Winston & Beament, 1969). We suggest that Winston & Beament's (1969) active model might have arisen from faulty assumptions about the influence of water activity gradients on cuticle hydration, especially the inner, water-rich layers. Since the mass gains in question are small and the standard errors are relatively high, Winston & Beament could also have underestimated the importance of evaporative loss during preparation of cuticle samples, by dismissing the time taken as 'only a few seconds'.

It is therefore appropriate to examine the evidence supporting a greater epidermal role more closely. Both the data in the preceding paper (Machin, Lampert & O'Donnell, 1985) and those of Winston & Beament (1969) are suitable for this purpose because these studies employed essentially the same methods of preparing *Periplaneta* pronotal discs. However they came to widely differing conclusions about the forces governing the distribution of cuticular water.

Resolution of these diverging interpretations will rest on two different

investigations. First, by reconstructing Winston & Beament's (1969) cuticle excision procedures, it has been possible to assess the magnitude of evaporation errors and their impact on subsequent rehydration. Second, the need to understand the forces governing the distribution of cuticular water at a quantitative level has led to the development of a passive model determined by ambient activity and the water affinities and permeabilities of component layers of the cuticle. The model interprets the water-holding characteristics of the cuticle components *in vitro* measured at equilibrium, in such a way that intact cuticular water content could be predicted for the range of water activity gradients experienced by living cockroaches. Most importantly the model can be used to simulate Winston & Beament's (1969) key experiment by predicting the amount of water the cuticle would possibly gain when returned to an atmosphere of the same water activity as the haemolymph.

METHOD AND RESULTS

Assessment of evaporation errors

The phases of preparing epidermis-free discs of cuticle from *Periplaneta pronotum* described by Machin *et al.* (1985) were timed with a stopwatch. As far as can be judged the technique was virtually identical to that used by Winston & Beament (1969), except that they wrapped their samples in foil prior to weighing on a torsion balance. To estimate the evaporative losses prior to wrapping, the masses of freshly excised discs were continuously recorded on a Mettler ME22 microbalance for several minutes. Air activity in the room was either 0.85 or 0.925 at a temperature of 22 °C. Winston & Beament prepared their cuticle samples in a humidified chamber with activities between 0.85 and 0.90. They are not clear on this point, but it seems likely that the chamber was at room temperature, presumably not greatly different from 22 °C.

Representative preparation times together with typical disc mass loss curves are shown in Fig. 1. The exponential equations best describing these curves are also indicated. Such equations were used to extrapolate the mass at the beginning of evaporation, considered to be the point at which epidermal and associated tissues were removed. As a result of our experience we estimate that the delay from the start of evaporation to the foil wrapping was probably between 20 and 40 s. This delay would account for a subsequent mass gain over Ringer to a minimum error (at 20 s delay at 0.90 a_w) (a_w , water activity) of 0.70 and a maximum (at 40 s delay at 0.85 a_w) of 1.83 % initial wet mass. Such gains represent a significant proportion of the gains interpreted by Winston & Beament (1969) as evidence of active regulation of cuticular water.

A passive model of cuticle water relations

Epidermal permeability has been shown to be insignificant so the model will concentrate on cuticle alone and is based on the two cuticular layers distinguished as endocuticle and combined exocuticle and epicuticle (Machin *et al.* 1985). The water content of any layer depends on its water affinity, expressed in terms of mass or volume, and the prevailing water activity. Water activity is determined, in turn, by the permeability of given layer in relation to the permeabilities of the others. If the

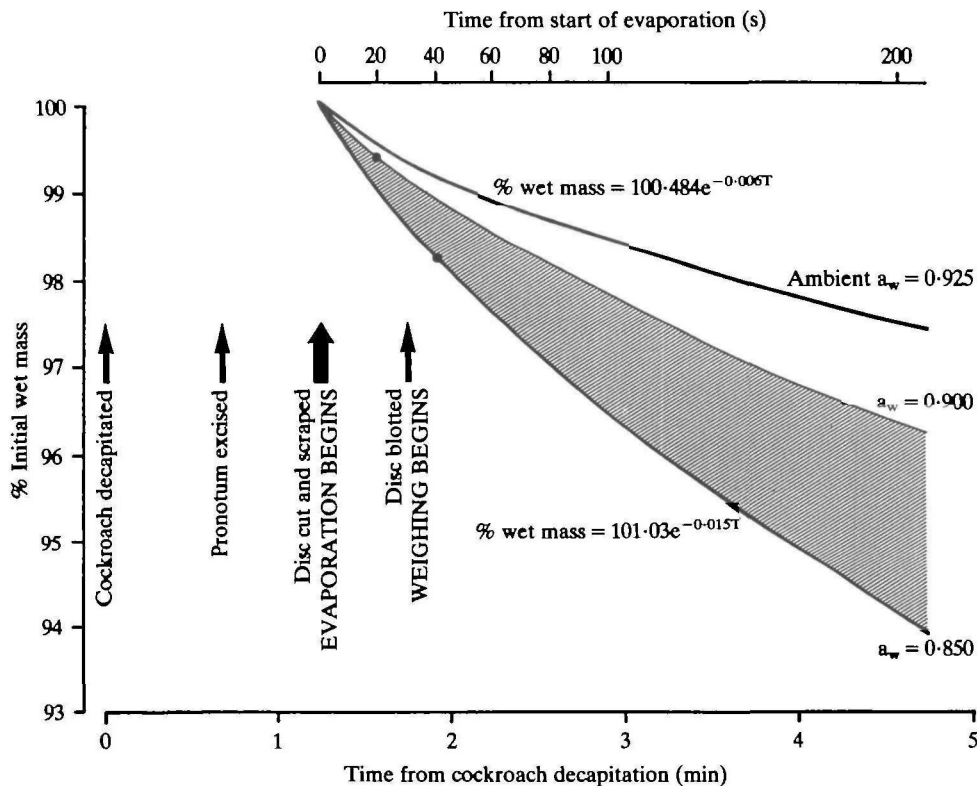


Fig. 1. Diagram to show the timing of the phases of disc preparation superimposed on observed pronotal water loss curves (heavy lines) at ambient water activities of 0.85 and 0.925 at 22 °C. Initial wet mass refers to fully hydrated cuticle discs. The parts of the curves preceding weighing were extrapolated using the least squares fitted exponential equations as indicated. The curves were used to construct a third (thin line) for 0.90 activity. The estimated range of potential evaporation error in conditions used by Winston & Beament (1969) is indicated by the shaded area. The closed circles indicate minimum and maximum errors at 20 and 40 s delay in weighing, respectively.

properties within each of the two layers are uniform, the driving force of water flux, assumed to be vapour pressure or activity (a_w), would fall linearly across each layer.

For systems containing two barriers in series, the following general relationship enables a component permeability (P_2) to be calculated, knowing the overall permeability (P_0) and the permeability of the other component, (P_1):

$$\frac{1}{P_0} = \frac{1}{P_1} + \frac{1}{P_2}. \quad (1)$$

The gradients (Δa_w) for each layer in any conditions can then be obtained from their permeabilities by rearrangement of Fick's equation describing passive diffusion:

$$\Delta a_w = \frac{J}{PA}, \quad (2)$$

where J is equal to the total flux across the cuticle, A the area and P the permeability

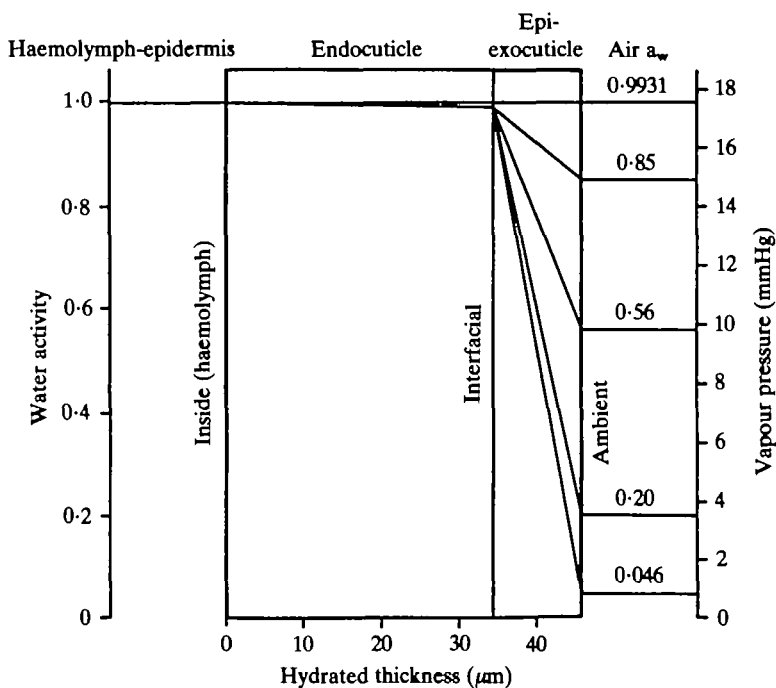


Fig. 2. Diagram showing steady state, *in vitro* water activity gradients across the cuticle in different ambient activities. The gradients were constructed by drawing straight lines between three fixed activities. Inside and ambient activities were measured and the steady state interfacial value between the two layers compatible with equal fluxes was calculated by progressive approximation.

of the layer in question. The total water held by the cuticle is then obtained by integrating water contents over the activity range existing in each layer.

Component permeabilities

Unfortunately, construction of a model is rather more difficult than the above account suggests because of the complex interdependence of permeability, water

Table 1. *Modelled in vitro, steady state permeabilities and interfacial vapour pressures at different ambient activities*

Ambient	Water activity		Endocuticle	Permeabilities ($\text{mg h}^{-1} \text{cm}^{-2} \text{mm Hg}^{-1}$)	Total
	Interfacial			Epi-exocuticle	
0.85	0.9929		74.283	0.127	0.127
0.56	0.9927		72.692	0.067	0.067
0.20	0.9925		70.771	0.053	0.053
0.046	0.9924		69.873	0.050	0.050

Inside a_w was 0.9931, vapour pressure = 17.427 mmHg.

content and activity gradient in each of the layers (Machin *et al.* 1985). We would expect activity gradients within each of the layers to lead automatically to local differences in water content, and presumably permeability. Steady state conditions of regional equality of water flux would therefore require the activity gradient to be curved not linear. A workable solution to these problems appears to be offered by empirical permeability equations which take no account of the complexities within each layer. To determine how the properties of component layers combine in the intact cuticle, it is first necessary to determine the interfacial activity at the border of the endocuticle and epi-exocuticle so that the gradients across each layer can be determined separately. A calculator programme was used to determine which interfacial activity was compatible with a steady state from a series of approximations starting from any arbitrarily chosen value. The programme then averaged high and low interfacial values progressively until equality between endocuticle and epi-exocuticle water fluxes was reached. Fluxes, calculated from equation 2, were considered equal when they agreed to within 1 %. Under these steady state conditions no further changes to the water content of a layer or its permeability could take place.

The calculations were based upon the following empirical equations relating endocuticle and whole cuticle permeability with the reciprocal of vapour pressure lowering ($vp_s - vp_a$) at different ambient activities, obtained by Machin *et al.* (1985):

$$\text{endocuticle } P = -0.892 + \frac{9.424}{vp_s - vp_a} \quad (3)$$

$$\text{epi-exocuticle } P = 0.0355 + \frac{0.263}{vp_s - vp_a}. \quad (4)$$

In vitro interfacial activities and component permeabilities calculated in this way are summarized in Table 1. Note that the very high endocuticle permeabilities are consistent with rapid, short-term evaporative loss in excised samples. In Fig. 2, which illustrates the corresponding linear activity gradients, it can be seen that all activity gradients in the endocuticle which fall within the width of the drawn line, are very close to the Ringer equilibrated ($a_w = 0.9931$) line. Since interfacial activities are based directly on empirical results they are known with some certainty.

It is worth noting that the model confirms the traditional arrangement of an external water barrier maintaining the inner endocuticle in a hydrated state (Beament, 1961). Endocuticle hydration exaggerated the permeability difference of the two layers to a factor of between 550 and 1400. Beament (1961) speculated that lipid removal from the cuticle might result in a 100- to 300-fold permeability increase. The higher values for endocuticle are presumably due to corrections for unstirred layers which have not been made previously.

Water contents

Cuticle water contents (per gram dry mass of cuticle or per 0.633 g endocuticle) were calculated from endocuticle gradients alone since no significant difference was found between the water affinities of whole cuticle and of endocuticle (Machin *et al.* 1985). In Fig. 3 the calculated water affinity regression line for the whole cuticle, together with the data on which it was based, has been re-drawn with water activity

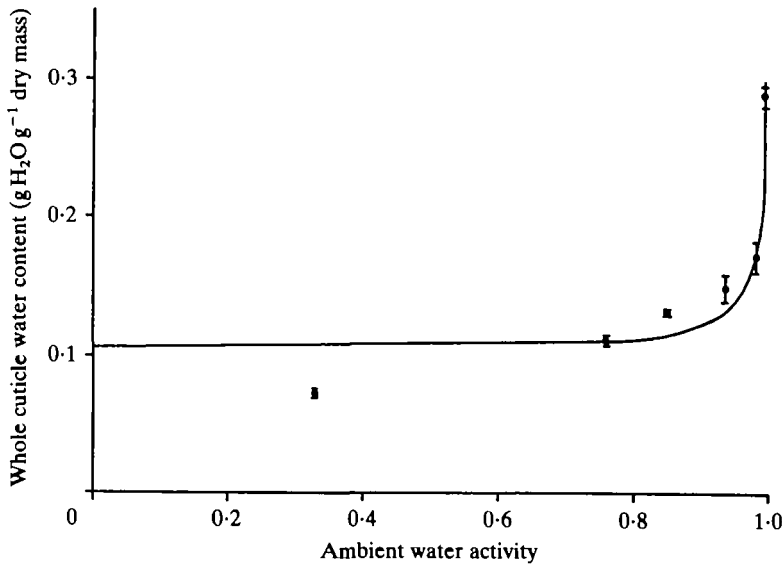


Fig. 3. Graph showing the relationship between whole cuticle water content and ambient water activity for *Periplaneta* pronotum at equilibrium. The line is the best least squares fit to the data of Machin, Lampert & O'Donnell (1985).

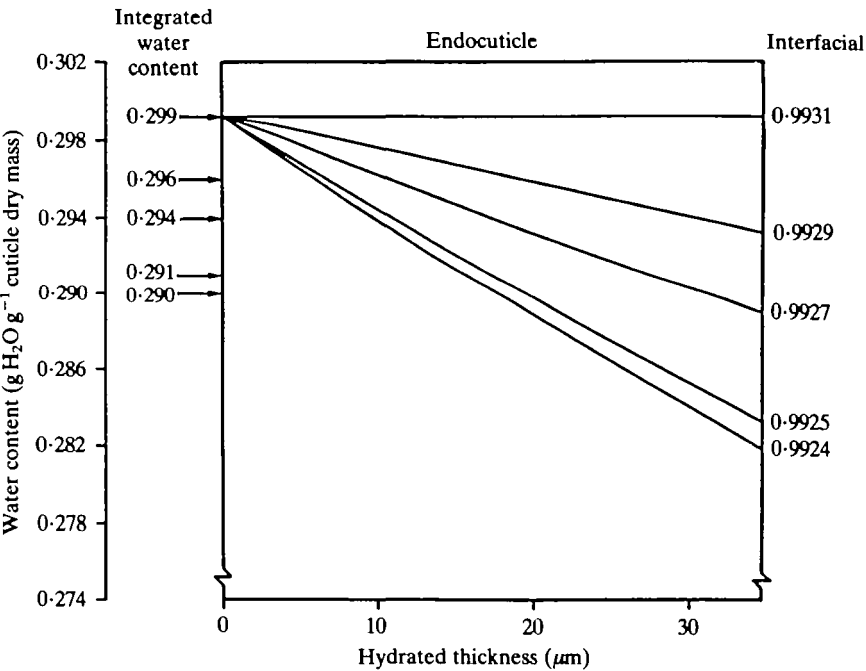


Fig. 4. Diagram demonstrating that endocuticle *in vitro* shows water content deficits even though activity gradients are very slight. The integrated total water contents determined by the model are also indicated on the left.

in place of the reciprocal of vapour pressure lowering on the abscissa. Total cuticle water contents were obtained by integrating the water content over the activity range existing across the endocuticle using the linear form of the water affinity relationship:

$$\text{whole cuticle water content (g g}^{-1} \text{ dry mass)} = 0.106 + \frac{0.0234}{vp_s - vp_a} \quad (5)$$

When the actual distribution of water across the endocuticle is obtained from activity gradients (Fig. 4) it can be seen that considerable differences occur. The small differences in activity gradient in Fig. 2 are in fact magnified by the steepness of the curve in Fig. 3 for very high activity values close to one. It has been argued above that steady state conditions within the endocuticle require that activity gradients be curved. However calculations show that the curvature may be neglected since predicted water contents fall within 1 % of the straight line values, presumably because endocuticle activity gradients are so slight. Machin *et al.* (1985) have presented evidence for a small, irreversible decrease in cuticle water affinity following initial water content measurement. Since the discrepancy in cuticular water content extends to samples equilibrated at haemolymph activities, *in vivo* water contents can be modelled by assuming that the same general *in vitro* form of the water affinity relationship applies, except that the *in vivo* slope is slightly greater. Modelled cuticular water contents are compared with observed values in Table 2. Data of Machin *et al.* (1985) were measured at 20°C with a haemolymph or Ringer

Table 2. Comparison of observed and modelled cuticular water contents under gradient conditions

Ambient a_w	N	Cuticle water contents (g H ₂ O g ⁻¹ dry mass)	
		Observed \pm s.e.	Modelled
Machin, Lampert & O'Donnell (1985)			
<i>In vitro</i>			
0.9931 (Ringer)	16	0.299 \pm 0.006	[0.299]
0.85	18	0.307 \pm 0.008	0.296
0.56	17	0.295 \pm 0.012	0.294
0.20	18	0.243 \pm 0.006	0.291
0.046	18	0.220 \pm 0.008	0.290
<i>In vivo</i>			
0.9931 (haemolymph)	16	0.366 \pm 0.009	[0.366]
0.85	18	0.357 \pm 0.011	0.362
0.56	17	0.357 \pm 0.014	0.359
0.20	18	0.286 \pm 0.006	0.356
0.046	18	0.281 \pm 0.012	0.354
Winston & Beament (1969)			
<i>In vivo</i>			
1.00	14	0.299 \pm 0.090	0.330
0.85	28	0.251 \pm 0.028	0.326
0.75	20	0.351 \pm 0.028	0.325
0.42	5	0.330 \pm 0.021	0.321
0.01	24	0.299 \pm 0.043	0.317

Values in square brackets are equilibrated values on which the model values are based.

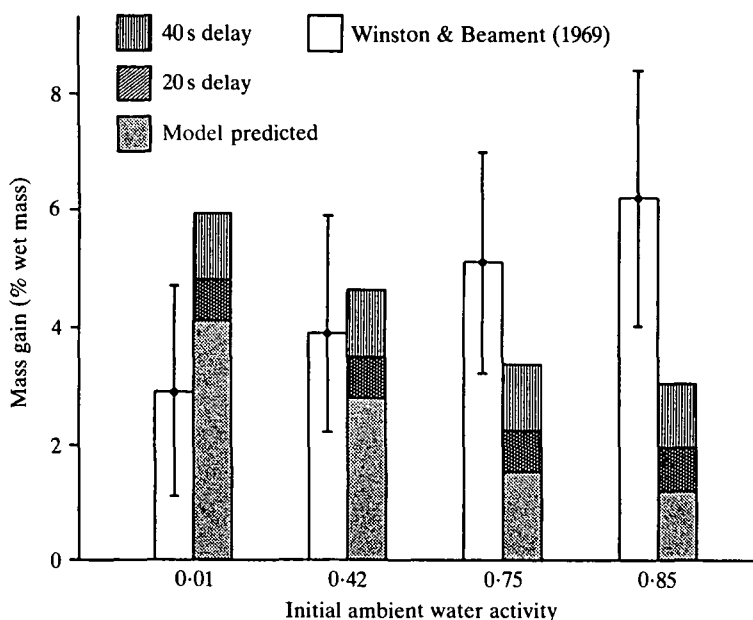


Fig. 5. Histograms comparing mean pronotal mass gains (\pm s.e.) following exposure to haemolymph water activities observed by Winston & Beament (1969) and those attributed by the present study to modelled cuticle activity gradients and evaporation errors. Cuticles were initially under gradient conditions with different ambient activities as indicated.

activity of 0.9931 (383 mosmol l^{-1}) while Winston & Beament (1969) made their determinations at 25 °C with activities of 0.9941 (328 mosmol l^{-1}). Model calculations adjust for the different activities but are based on permeabilities and water affinities measured at 20 °C. It can be seen that the agreement between observed and modelled water contents is good at high ambient activities and poor at activities below 0.20. The fit with Winston & Beament's (1969) values is good.

DISCUSSION

We interpret the results of modelling as evidence that cuticle water contents can be largely accounted for by purely passive mechanisms. This conclusion is based on the prediction by the model of the significant drop in cuticle water contents in ambient activities below that of the haemolymph. Since modelled water contents all show a decline with decreasing ambient activity from maximum values equilibrated to Ringer or haemolymph, it is reasonable to conclude that the occasional departure from this trend in observed values is due to measuring error.

The model has been shown to overestimate water contents at low ambient activities both *in vivo* and *in vitro*. It is important to point out, however, that this cannot be attributed to the poor fit of the water content equation at low activity because modelled values are based entirely on the narrow range of extremely high activities characteristic of the endocuticle. Over this range the fit of the equation to the observed

points is very good. Nevertheless, despite deficiencies in the model at low ambient activity, modelled water contents still appear to account for most of Winston & Beament's (1969) observations by passive mechanisms. Modelled water contents can therefore be used to simulate their mass measurement experiment following re-exposure of cuticle samples to water activities equivalent to the haemolymph. Modelled mass gains, expressed in their paper as a percentage of wet mass, were calculated by assuming that the cuticle in different gradient conditions would regain the water contents observed when equilibrated over Ringer. Water absorption would be further increased if cuticle samples had been previously subjected to significant evaporation. The estimated range of these errors has also been included in Fig. 5. The histograms show there is a considerable degree of correspondence between the percentage mass gains observed by Winston & Beament (1969) and those derived from modelled values plus estimated evaporation errors. Mass gains based on observed *in vivo* and *in vitro* values of Machin *et al.* (1985) and corresponding modelled values show essentially the same results. A discrepancy does however exist between the 0.85 activity values. The trend of the modelled values and those observed by Riddle (1981) is opposite to those of Winston & Beament (1969). Their large overlapping standard errors suggest that their differences may not be significant.

Thus it can be concluded that cuticular mass gains over Ringer can largely be attributed to the effects of passively generated activity gradients in the cuticle together with evaporation errors. We suggest Winston & Beament may have been deceived by the relative insensitivity of water content to ambient activity because their values were expressed as a percentage of water mass. In addition, without investigating the actual form of the water affinity relationship, they automatically assumed that small differences in vapour pressure would lead to small differences in water content in the hydrated endocuticle. Quite to the contrary, the non-linear nature of water affinity demonstrates that water content is very sensitive to small vapour pressure differences particularly at the high activities, close to one, found in the endocuticle. It follows, therefore, that significant water activity deficits should be expected whenever the ambient activity falls below the haemolymph level, even when only passive forces are in operation.

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