

SHORT COMMUNICATION

CONTROL OF CUTICULAR WATER PERMEABILITY IN INSECTS

BY P. C. CROGHAN AND J. NOBLE-NESBITT

School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ

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In a recent note (Croghan and Noble-Nesbitt, 1989), it was shown that water permeability of the cockroach epicuticle could not be explained in terms of what was known about the properties of the components of the system. Additionally, there are further observations in need of explanation. Coenen-Stass and Kloft (1976), using both weight loss and tritiated water, showed that the rate of water loss from the cockroach (*Periplaneta*) was lower in living animals than in dead animals and suggested that this could indicate an active reduction of cuticular permeability. There is evidence that cuticular water loss is under endocrine control (Treherne and Willmer, 1975; Noble-Nesbitt and Al-Shukur, 1987) and the progressive reduction in cuticular water permeability under desiccating conditions has been interpreted as an expression of this control (Noble-Nesbitt and Al-Shukur, 1987). It is not immediately obvious how the epicuticular permeability could be controlled. Also, Winston and Beament (1969) have shown that, in pieces of cuticle removed from the living cockroach and locust, the chemical potential of water is significantly less than in the blood. In the cockroach, the cuticular water phase was in equilibrium with a solution with an osmotic pressure of 1940 kPa. This is greater than that of the blood, which has an osmotic pressure of 900 kPa. This conclusion has been criticised by Machin and Lampert (1985, 1987), who suggested that evaporation errors during the preparation stage could account for the increase in weight subsequently observed. However, this takes no account of the careful controls of Winston and Beament (1969), which discount the possibility that evaporation errors might explain the subsequent weight increases. It has already been suggested that a lowered chemical potential of water in insect cuticle might play a role in restraining water loss (Noble-Nesbitt, 1970). It will be suggested that the observation of Winston and Beament (1969) is crucial to an understanding of the permeability properties of the cuticle.

The chemical potential of water can be expressed in terms of the hydrostatic pressure and osmotic pressure (Dainty, 1963):

$$\Delta\mu_w = \bar{V}_w\Delta P - \bar{V}_w\Delta\Pi, \quad (1)$$

where μ_w is the chemical potential of water, P is the hydrostatic pressure, Π is the

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osmotic pressure and \bar{V}_w is the partial molar volume of water. The difference signs indicate the value of the parameter in a phase minus the value in a reference phase. It is stressed that the hydrostatic pressure is the hydrostatic pressure of the water in the phase and this avoids the problem of the so-called matric potential (Passioura, 1980). If the water is in equilibrium between the phases, $\Delta\mu_w$ is zero. The situation described by Winston and Beament (1969) could arise in two ways. The epithelial cells could transport solute into the water phase of the procuticle, increasing its osmotic pressure to 1940 kPa. However, a consequence of this would be that the water phase of the procuticle *in situ* would have a hydrostatic pressure of 1040 kPa relative to the blood, and it is difficult to imagine how structural integrity could be maintained, as this hydrostatic pressure might be expected to separate the epicuticle from the procuticle and the procuticle from the epithelium. Further, Machin and Lampert (1987) found very low concentrations of Na^+ and Cl^- in the cuticle. Removal of water from the procuticle provides an alternative and more plausible explanation and, if the water phase in the procuticle is pure water, the hydrostatic pressure would be -1940 kPa relative to the blood. Negative hydrostatic pressures would not be unexpected in a relatively rigid, porous matrix and negative pressures of considerably greater magnitudes than this have been measured in the xylem of plants (Scholander *et al.* 1965).

The data of Winston and Beament (1969) could be interpreted as indicating active transport of water out of the procuticle. However, there is evidence that water transport is a passive process consequent upon active solute transport (House, 1974). If the underlying epithelial cells transport solute across their apical membrane so that the concentration of solute in the water phase of the procuticle tends to zero, water would move until the chemical potential was the same in the procuticle as in the epithelial cells and the hydrostatic pressure in the procuticle would be -900 kPa relative to the blood. This is insufficient to explain the data of Winston and Beament (1969). It is necessary to seek a much more concentrated phase with which the water phase in the procuticle could equilibrate. Diamond and Bossert (1967) showed how the transport of solute into small spaces could lead to the generation of standing gradients of osmotic pressure that could explain water transport. These ideas can be applied to the generation of the negative hydrostatic pressure in insect cuticle. The apical face of the epithelial cells is extended as numerous narrow cytoplasmic filaments in pore canals running across the procuticle. In the cockroach these have a diameter of $0.15 \mu\text{m}$ and the density is about $1 \mu\text{m}^{-2}$ of epithelial cell (Richards and Anderson, 1942). If there is active transport of solute from the procuticular water phase into the filament water phase, the filaments provide a possible site, intracellular in this case, for the production of a hyperosmotic fluid.

The mathematical treatment of Diamond and Bossert (1967) is quite complex and requires numerical solution. However, to illustrate how the system could operate in insect procuticle, a number of simplifying assumptions can be made. Consider a cross-section at any point along a filament (Fig. 1). The flux of solute (J_s) across this section can be written in terms of a convection and diffusion term:

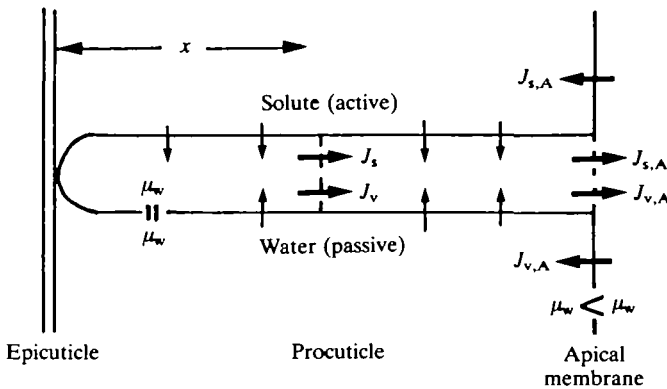


Fig. 1. Diagram of the pore-canal filament and apical cell membrane system. For an explanation of the symbols see text.

$$J_s = J_v C_F - DA \frac{dC_F}{dx}, \tag{2}$$

where J_v is the volume flux across the section, C_F is the solute concentration in the filament at that place, D is the solute diffusion coefficient, A is the area of the cross-section and x is the distance from the closed distal end of the filaments. An assumption will be made that the water permeability of the filament membrane is so great that there is no difference between the chemical potential of water in the procuticle and in the filament. This assumption is aided by the very high surface area/volume ratio of the filament. In the original Diamond–Bossert model, this assumption results in no solute concentration difference across the tubule wall. In the present model, if there is a difference of hydrostatic pressure across the filament wall, there will be a difference of solute concentration. From equation 1:

$$C_F = C_C - \frac{\Delta P}{RT}, \tag{3}$$

where C_C is the concentration of solute in the procuticular water, ΔP is the hydrostatic pressure of the procuticular water relative to the blood, R is the gas constant and T is the absolute temperature. It can be seen that the solute concentration in the filament is constant throughout its length, with a step change in concentration where the filament joins the underlying epithelial cell. Thus, equation 2 is greatly simplified:

$$J_s = J_v \left(C_C - \frac{\Delta P}{RT} \right). \tag{4}$$

Under steady-state conditions, the fluxes across the proximal section of a filament into the body of the epithelial cell equal the fluxes into the procuticle across the area of apical membrane corresponding to one filament. These apical fluxes are

regarded as passive and can be expressed in terms of appropriate flux equations. Solute flux ($J_{s,A}$) can be expressed using the Fick equation:

$$J_{s,A} = P_s A (C_B - C_C), \quad (5)$$

where P_s is the solute permeability coefficient of the apical epithelial cell membrane, A is the area of apical membrane across which diffusion is occurring and C_B is the solute concentration in the epithelial cell, taken to be the same as in the blood. Water flux ($J_{v,A}$) can be expressed in terms of an osmotic permeability coefficient (Dainty, 1963):

$$J_{v,A} = -P_{os} A \frac{\bar{V}_w}{RT} (\Delta P - \Delta \Pi), \quad (6)$$

where P_{os} is the osmotic permeability coefficient of the apical epithelial cell membrane, and $\Delta \Pi$ is the osmotic pressure of the procuticular water relative to the epithelial cell, taken to be the same as the blood.

Making the appropriate substitutions into equation 4:

$$P_s \Delta \Pi = P_{os} \frac{\bar{V}_w}{RT} (\Delta P - \Delta \Pi) (\Pi_C - \Delta P), \quad (7)$$

where Π_C is the osmotic pressure of the procuticular water phase. The hydrostatic pressure of the procuticular water phase is determined by the ratio P_s/P_{os} for the apical membrane of the epithelial cell. The rate at which the pump transporting solute from the procuticular water phase into the filament water phase must operate is defined by equation 5 and thus will influence the value of Π_C . Many epithelia can take up ions from very dilute media (Lockwood *et al.* 1976) and, if the pump can operate so that Π_C tends to zero:

$$P_s \Pi_B = P_{os} \frac{\bar{V}_w}{RT} (\Delta P + \Pi_B) \Delta P, \quad (8)$$

where Π_B is the osmotic pressure of the blood. Taking $\Delta P = -1940$ kPa and $\Pi_B = 900$ kPa, the ratio of the permeabilities (P_s/P_{os}) required to generate this hydrostatic pressure is 0.016. There is an enormous range of values for water and ion permeabilities of cell membranes but ion permeability is usually considerably less than water permeability. For example, Hodgkin and Horowitz (1959) give data for chloride and water for frog muscle cell membrane. The ratio P_{Cl}/P_{os} calculated from these data is only 1.8×10^{-4} . In contrast, the data of Smith (1969) for the gill epithelium of *Artemia* give a ratio P_{Na}/P_{os} of 0.29. If this ratio applied to the apical membrane, the predicted hydrostatic pressure would be -6500 kPa. This is taken as evidence that the model is a plausible explanation of the data of Winston and Beament (1969).

The reduction of hydrostatic pressure in the procuticle, although it may appear impressive, is equivalent to only a small reduction in water vapour pressure (Denbigh, 1961) and thus will have a negligible direct effect on the loss of water across the epicuticle. Croghan and Noble-Nesbitt (1989) have reinterpreted

the data of Beament (1958) and show that the diffusion of water through a solid packed layer derived from the fluid grease layer of the cockroach is much less than would be expected for a liquid grease. A negative hydrostatic pressure in the procuticle results in a compressive force acting on the epicuticle and it is suggested that this could result in an increase of order at the molecular level that could substantially reduce water permeability. It is suggested that this is the cause of the lower-than-expected water permeability of cockroach epicuticle. This could also explain the mechanism of control of water permeability. The hydrostatic pressure of the procuticular water phase is a function of transport processes in the epithelial cell membrane and, in many cases, such processes are known to be under endocrine control. Thus, the model provides a basis for the interpretation of cuticular water loss in insects.

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