ELECTROPHYSIOLOGY OF MOSQUITO ANAL PAPILLAE

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The anal papillae of mosquito larvae are classic transporting epithelia that can absorb inorganic ions from extremely dilute external media. Since the original investigations of Wigglesworth (1933*a,b,c*) and Koch (1938) studies have been made of the ultrastructure of the papillae (Copeland, 1964; Sohal & Copeland, 1965; Meredith & Phillips, 1973*a,b*), of radio-cation fluxes (Treherne, 1954; Stobbart, 1959, 1960, 1965, 1967, 1971), of their water permeability (Nicolson & Leader, 1974) and their role in osmoregulation (Edwards, 1979). Quantitative understanding of epithelial salt transporting mechanisms, however, requires knowledge of the appropriate electrochemical gradients. Only one attempt has so far been made to provide this information for mosquito larvae (Stobbart, 1974). These measurements were made by recording the potentials between the larval haemolymph and a drop of fluid surrounding the papillae. However, the potentials measured in this way were found to be very unstable and of variable polarity. This communication describes an attempt to measure the electrical properties of the papilla epithelium using isolated anal papillae of *Aedes aegypti*.

In these experiments the three posterior abdominal segments were removed from fourth instar larvae and cannulated close to the base of the anal papillae. An inner cannula was used to perfuse the preparation. Electrical contact with the haemolymph was made with a chloridized wire inside the cannula. The whole preparation was put into a divided bath of the type described by Berridge & Prince (1972). Unstable potentials of variable polarity (+56 mV to −68 mV maximum variation) were obtained as Stobbart (1974) had found. It seems probable that these fluctuations do not reflect changes in transepithelial potential of the anal papillae. The fluid within the papillae is not in free communication with the haemolymph. When preparations were perfused with saline containing the dye amaranth, none could be detected within the papillae. It might be argued that because the papillae are blind sacs there would be no flow of dye into them; however, cutting the tips from the papillae does not lead to dye flowing through them. Only when magnesium ions were added to the perfusate was dye observed to enter the papillae, an effect which might be expected to result from muscular relaxation. An electron microscope study (Edwards, 1979) has revealed the presence of a thin ring of circular muscle at the base of each papilla which could, when contracted, effectively isolate the fluid within the papillae from the haemolymph in the general body cavity. The reversals of potential, measured between the haemolymph and a drop of saline surrounding the papillae (Stobbart, 1974) may

therefore, reflect opening and closing of bases of the papillae and not transepithelial potential changes.

The above possibility is also supported by resistance measurements made in this preparation. The measurements were made by introducing a current supply unit and two Keithley electrometers into the recording circuit. Recordings of resistance were taken from preparations in which no dye entered the papillae ('preparation' resistances) and preparations where the papillae were believed to be in free communication with the haemolymph judged by the presence of amaranth after perfusion ('papilla' resistances). The relation between the resistance of the 'papillae' and that of the 'preparation' revealed two classes of data (Fig. 1). Discriminant analysis (Rothamsted Genstat Programme) showed that the data could be divided into two classes with a probability of 0·9 of the points being in the group to which they were assigned. The results provide a rough estimate of papilla resistance of 2–45 KΩ for the situation in which the haemolymph and the papilla lumen would be in free communication. The resistances in the range of 60–120 KΩ would thus correspond to the resistance at the papilla base, if the papilla was isolated from the haemolymph.

Internal treatment of papillae with 5 mM-sodium azide, 0·1 mM-ouabain, 1 mM-acetazolamide, or 0·1 mM-5-hydroxytryptamine produced no measurable change in resistance. The epithelium appears to be insensitive to classical transport inhibitors.

Despite the lack of effects of the above inhibitors on papilla resistance, essentially conventional potential profiles were measured across the epithelial cells of the papillae (Fig. 2). These measurements were made by impaling isolated, ligatured, papillae from fourth instar larvae reared in 2 % sea water, with conventional KCl filled microelectrodes. The papillae were impaled and then bathed in 2 % sea water, sodium-free 2 % sea water or chloride-free 2 % sea water to see if there is coupled ion transport in this system. In all cases the haemocoel is negative with respect to the exterior and the epithelium is negative with respect to both. Therefore the uptake of chloride is against its electrical gradient and also against a steep chemical gradient as haemolymph chloride is maintained at 50–60 mM even in media containing only 0·1 mM-Cl. Sodium could enter the epithelium passively down an electrical gradient but would then encounter adverse electrical (Fig. 2) and chemical (haemolymph Na 90–130 mM, Edwards, 1979) gradients in moving from there to the haemocoel of the papilla.
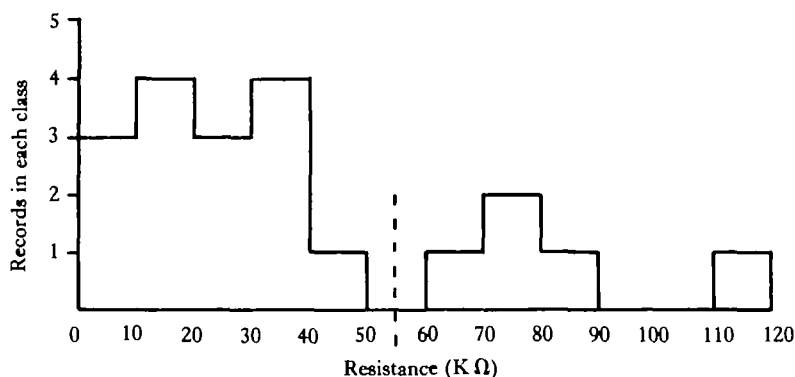

Fig. 1. Shows resistances measured at the papilla base. The dotted line indicates the division of the data into two distinct classes.
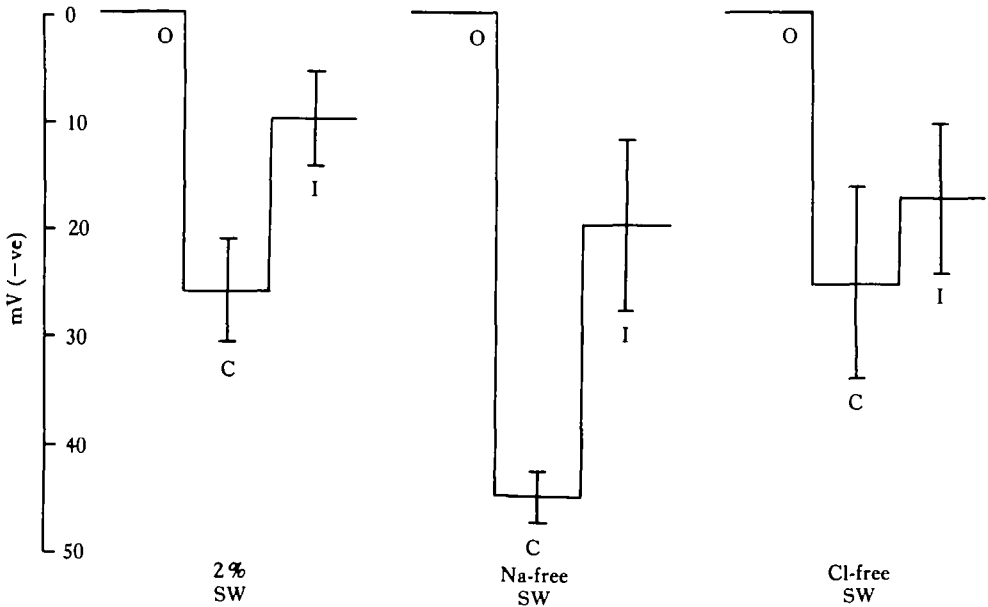
Fig. 2. Epithelial potentials across papillae in 2% sea water, 2% sodium-free sea water and 2% chloride-free sea water recorded with 3 M-KCl glass microelectrodes. O, the outside medium is the baseline, C is the cell interior and I the haemolymph in the papilla lumen; n = 4.

The specific resistances of many epithelia have been measured. Frömter & Diamond (1972) list a number of vertebrate tissues, Wood & Moreton (1978) give a figure of $150 \, \Omega \, cm^2$ for *Cecropia* midgut, which falls well within the range of Fromter & Diamond's figures and is directly comparable with Karnaky & Kinter's (1977) figures for the killifish opercular skin. The resistance of another insect hindgut transporting tissue has been calculated, based on the figures of Leader & Green (1978), for the rectum of the larva of the dragonfly, *Uropetala*; its specific resistance is $117 \cdot 8 \, \Omega$ cm^2. If we assume that the mean figure of $21 \cdot 9 \, K\Omega$ is nearest the true resistance of the papillae and take a value of $5 \, mm^2$ for the total surface area then the specific resistance is $1095 \, \Omega \, cm^2$. Frömter & Diamond (1972) distinguish 'tight' from 'leaky' epithelia; tight epithelia have specific resistances in the range $400-2000 \, \Omega \, cm^2$ and are characterized by their ability to maintain high concentration gradients. The value for papillar resistance is well within Frömter & Diamond's value for tight epithelia.

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