J. exp. Biol. **114**, 685–689 (1985)
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SHORT COMMUNICATION
AN IMPROVED CHAMBER FOR THE SHORT-CIRCUITING OF EPITHELIA

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Accepted 4 July 1984

Since the pioneering work of Ussing & Zerahn (1951), the design of short-circuit apparatus has been modified only slightly. Such chambers generally consist of two halves, between which the epithelium is stretched. The assembly is clamped together and filled with saline, which is circulated in each half chamber by gas lift pumps, usually connected to the main chamber with plastic pipes. There are two current electrodes, one at each end of the chamber, and – usually – two voltage-sensing electrodes close to the tissue. The current passed through the chamber is adjusted until the p.d. between the sensing electrodes becomes zero. Under such conditions, only actively transported ions move across the tissue, and the short-circuit current is a precise measure of the net flux of charged species. Short-circuiting is thus a method of choice for investigating transport phenomena in epithelia.

In this paper, we describe a chamber which differs significantly from the classical design, and illustrate its performance with specific reference to a tissue containing a potent electrogenic pump, the midgut of the lepidopteran larva, *Manduca sexta* (Harvey, Cioffi, Dow & Wolfersberger, 1983).

The chamber (Fig. 1) consists of a short cylinder of Perspex, drilled axially with an 8-mm hole. Current electrodes of thin silver sheet are held against either end by Perspex endplates (Fig. 1), which are held to the main chamber with screws. The central bore is divided into two chamber 'halves' by a milled slot, into which the tissue-bearing aperture (Fig. 1) is inserted. Three voltage-sensing electrodes (Ag/AgCl wires, insulated to 1 mm below the tip, for normal use, or agar bridges to calomel electrodes for Ag^+-sensitive tissues) are inserted into the chamber, through rubber seals, on either side of the aperture (*A–C* in Fig. 1). The three electrode arrangement compensates for the resistivity of the saline, when this low resistivity tissue ($100\,\Omega cm^2$) is short-circuited (Wood & Moreton, 1978). Circulation of fluid is accomplished with gas-lift pumps, which are integral within the main chamber block. Gas (oxygen or nitrogen) is supplied at constant pressure to the two gas inlets (*F,G,* Fig. 1).

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Key words: Potassium transport, *Manduca sexta*, midgut.

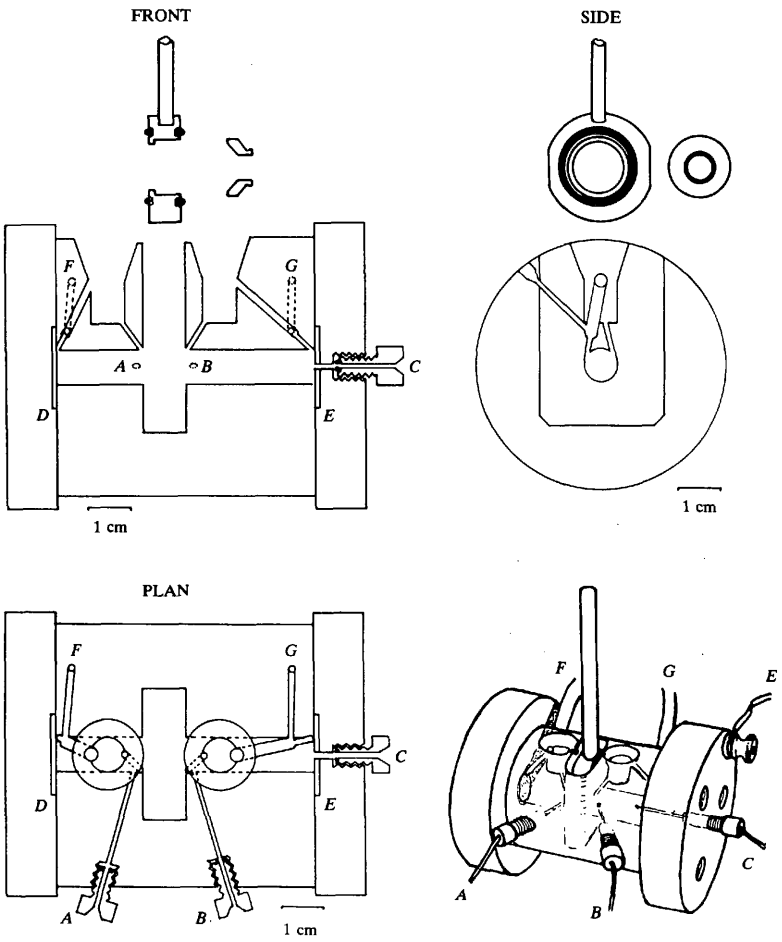

Fig. 1. Diagrams of the short-circuit chamber: front view, plan, end view and perspective sketch. The voltage-sensing electrodes (*A,B,C*), current electrodes (*D,E*) and gas inlet pipes (*F,G*) are labelled.

The fluid inlet and outlet pipes insert obliquely at either end of the chamber halves. This prevents air bubbles from lodging in corners, and ensures that there are no unstirred 'dead spaces' in the chamber. The tissue is tied onto an aperture in a Perspex disc (Fig. 1), a procedure which reduces edge damage, caused by

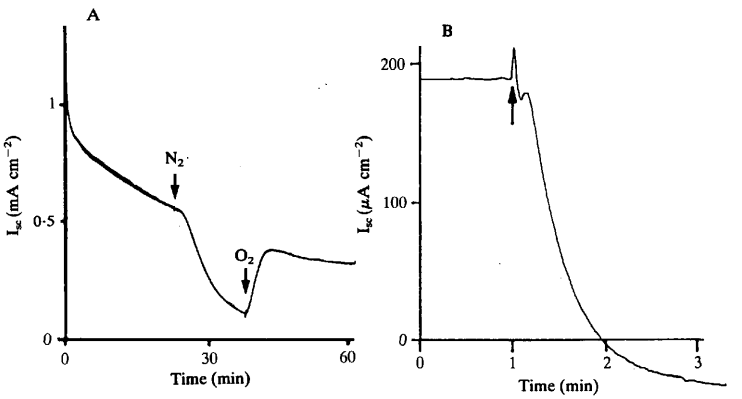

Fig. 2. Effect of (A) anoxia and (B) 1 mmol l^{-1} sodium azide, on the short-circuit current of *Manduca* midguts. In (A), the gut was short-circuited immediately upon assembly of the chamber, 2·8 min after the start of dissection. In (B), the time scale is expanded, so that the time course of the effect can be seen.

crushing the clamped circumference of the tissue (Wood & Moreton, 1978). The active area of tissue used here is 0·5 cm^2, corresponding to a diameter of 8 mm; to assure uniform current distribution, this diameter should match the bore of the chamber, and should be as large as the size of the tissue allows, to reduce edge effects. However, Thomas & May (1984) seem to have obtained good results with lepidopteran midgut, using an active area of only 0·03 cm^2. The disc is then pressed home into a holder, or 'lollipop' (Fig. 1). The assembly is inserted into the main chamber, which has been previously filled with saline. A tight, sliding fit is assured by applying high-vacuum grease to the two O-ring seals on the holder. The leakage pathway through the seal was found to have a resistance far in excess of 1 MΩ, which should prove adequate for most tissues. As the holder is inserted into the slot, displaced saline wells up into the reservoirs and funnels. The U-shaped channels between the funnels and the slot allow this saline to displace air from the holder assembly as it is inserted, ensuring that the assembly of the system, without trapping air, is very quick and straightforward. Thus, in a recent series of experiments, it was possible for short-circuit to begin 2·75 ± 0·2 min ($N = 4$) from the start of the dissection (5–8 min would be typical for the previous design). However, the shape of the short-circuit curve is similar under both conditions, suggesting that the rise of the short-circuit current over the first 5 min, then the subsequent decline, represents an adaptation to short-circuit, rather than tissue deterioration triggered by dissection and mounting (Schultz & Jungreis, 1977; Wolfersberger & Giangiacomo, 1983). Once the holder is in place, excess saline is drawn from the central slot, and the level in the reservoirs brought down to the bottom of the funnels; this provides a calibration level for flux studies, in which the chamber volumes must be accurately reproducible. Although there is a certain

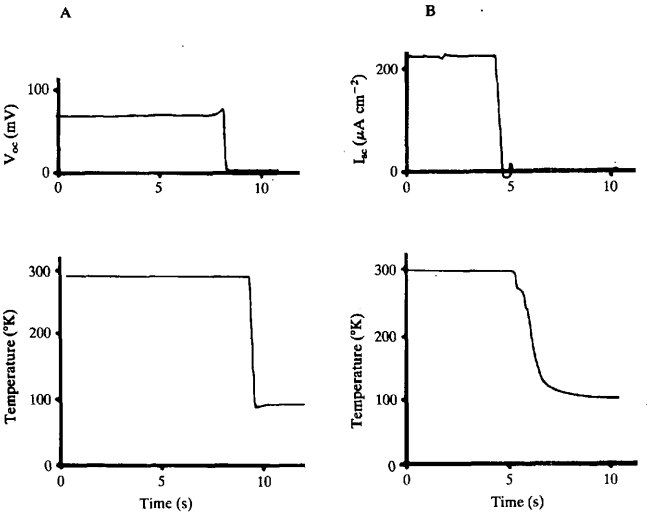

Fig. 3. Time course of quench freezing of a short-circuited tissue under open-circuit (A) or short-circuit (B) conditions. The upper traces show the open-circuit current (A) or short-circuit potential (B), recorded from the chamber; the lower traces show the temperature recorded by a thermocouple inserted into a fold in the epithelium.

amount of spray caused by gas bubbles bursting in the reservoirs, the funnels machined above the reservoirs serve to catch the spray, rendering the chamber suitable for flux measurements with radioactive isotopes. As the pressure head on the gut is less than 2 cmH_2O, the tissue remains completely still in the chamber, even though the saline circulates rapidly. The small volume of circulating saline (under 3 ml in each chamber half) ensures very rapid mixing times, and thus the speed of current inhibition by anoxia (Fig. 2A) is greatly increased. The tissue begins to respond within 17 s (compared with 2–3 min for the previous design). Thus the slower responses previously observed are more a function of slow gas exchange, than a gradual exhaustion of glycolytic or oxidative metabolic pathways, as has previously been suggested (Mandel, Riddle & Storey, 1980).

The rapid mixing time is even more impressive when azide is used to block oxidative metabolism. Here the only delays are the circulation time of the chamber halves, and the time for azide to penetrate the tissue. As shown in Fig. 2B, the current is affected within 2 s, and the half-time for inhibition is 29 s. This confirms that oxidative phosphorylation dominates the normal energy supply for the pump. The low basal rate of transport which sometimes remains after oxidative phosphorylation has been inhibited may represent the contribution of glycolytic metabolism, other transport processes, or a residual error in short-circuiting.

The rapid accessibility of the tissue allows fast removal and quenching, a procedure which has already been employed in electron probe X-ray micro-

analytical studies of midgut function (Dow, Gupta, Hall & Harvey, 1984; Gupta, Dow, Hall & Harvey, 1985). Fig. 3 demonstrates that the tissue can be quenched within 1·0 s of short-circuiting within the chamber. Similarly the ability to transfer the tissue repeatedly from one chamber to another, perhaps containing different salines, opens exciting possibilities for tracer flux or ion-substitution experiments.

This work was supported by NIH grant AI-09503. JATD was supported by a Harkness Fellowship of the Commonwealth Fund, and is currently a Research Fellow of St Catharine's College, Cambridge.

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