

REFINEMENTS IN THE SHORT-CIRCUIT TECHNIQUE AND ITS APPLICATION TO ACTIVE POTASSIUM TRANSPORT ACROSS THE CECROPIA MIDGUT

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(Received 10 March, 1978)

SUMMARY

1. The conventional, two-electrode method for measuring potential difference across an epithelium is subject to error due to potential gradients caused by current flow in the bathing medium. Mathematical analysis shows that the error in measuring short-circuit current is proportional to the resistivity of the bathing medium and to the separation of the two recording electrodes. It is particularly serious for the insect larval midgut, where the resistivity of the medium is high, and that of the tissue is low.

2. A system has been devised, which uses a third recording electrode to monitor directly the potential gradient in the bathing medium. By suitable electrical connexions, the gradient can be automatically compensated, leaving a residual error which depends on the thickness of the tissue, but not on the electrode separation. Because the thicknesses of most epithelia are smaller than the smallest practical electrode spacing, this error is smaller than that inherent in a two-electrode system.

3. Since voltage-gradients are automatically compensated, it is possible to obtain continuous readings of potential and current. A 'voltage-clamp' circuit is described, which allows the time-course of the short-circuit current to be studied.

4. The three-electrode system has been used to study the larval midgut of *Hyalophora cecropia*. The average results from five experiments were: initial potential difference (open-circuit): 98 ± 11 mV (S.E.M.); short-circuit current at time 60 min: 498 ± 160 μ A cm⁻²; 'steady-state' resistance at 60 min: 150 ± 26 Ω cm². The current is equivalent to a net potassium transport of 18.6 μ -equiv cm⁻² h⁻¹.

5. The electrical parameters of the midgut change rapidly with time. The potential difference decays with a half-time of about 158 min, the resistance increases with a half-time of about 16 min, and the short-circuit current decays as the sum of two exponential terms, with half-times of about 16 and 158 min respectively. In addition, potential and short-circuit current show transient responses to step changes.

6. The properties of the midgut are compared with those of other trans-

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porting epithelia, and their dependence on the degree of folding of the preparation is discussed. Their time-dependence is discussed in the context of changes in potassium content of the tissue, and the implications for measurements depending on the assumption of a steady state are outlined.

INTRODUCTION

The short-circuit technique devised by Ussing & Zerahn (1951) for the frog skin is a powerful tool for analysing the movements of ions across epithelia. In this technique a tissue is isolated, mounted in a chamber and bathed with identical physiological solutions on both sides. The chamber contains two pairs of electrodes used respectively for passing current and for recording the electrostatic potential difference across the tissue. By passing a suitable current, the potential difference is reduced to zero. This current is termed the 'short-circuit current' (I_{sc}), and since all electrochemical gradients across the tissue have been abolished, it is equal to the sum of the net charges transferred by all ions actively transported across the tissue in unit time.

Practical application of the short-circuit technique requires that the potential difference across the tissue be accurately measured, independently of the current flow. In particular, the potential measurements must not be disturbed by potential gradients due to current flow in the saline between the tissue and the recording electrodes. In the case of the frog skin, where the resistivity of the tissue is much greater than that of the bathing medium, this is very nearly true. For many other transporting epithelia, however, serious errors can be caused as the resistivity of the epithelium is generally lower (requiring higher short-circuit current for a given value of the open-circuit potential) and that of the bathing medium is often higher, than in the case of the frog skin. The present object is to analyse mathematically the possible sources of error in determining short-circuit currents, and to present a method of compensating for potential gradients in the bathing medium, using a three-electrode recording system. A device will also be described for automatic short-circuiting, to allow continuous monitoring of I_{sc} . The methods will be illustrated by analysing the electrical properties of the *Hyalophora cecropia* midgut, which actively transports potassium from the blood-side to the lumen-side (Harvey & Nedergaard, 1964; see review by Harvey & Zerahn, 1972).

THEORY

Figure 1*a* is a schematic diagram of the conventional short-circuit chamber (cf. Clarkson & Toole, 1964), with paired current and recording electrodes. Potential profiles in various hypothetical situations are shown below. (No assumptions are made about the potential distribution within the tissue, which is shown dotted). Profile (i) is the 'open-circuit' case, with no net current flow, and a potential difference V_0 developed across the tissue. The resistivity of the bathing medium is, of course, unimportant here, since no current flows in it. Profile (ii) is the ideal 'short-circuit', where the resistivity of the medium is very low, so that current flow produces potential changes only in the tissue. By reducing to zero the potential difference ($V_B - V_A$) between the recording electrodes, the tissue is accurately short-circuited. In (iii) is shown a practical 'short-circuit', in which ($V_B - V_A$) is again at zero. But because of the potential gradient due to current flow in the saline between the tissue and the

Recording electrodes, the tissue is not accurately short-circuited, but develops a small, residual potential difference V_e . The magnitude of this may be found from Ohm's Law as follows:

If the total current path through saline between the recording electrodes is x_s (equal to the distance between the electrodes, less the thickness of the tissue), the chamber cross-sectional area is A , and the resistivity of the saline is r_s , then the resistance of the saline current path is

$$R_s = \frac{r_s x_s}{A}. \quad (1)$$

Writing I'_{sc} for the apparent short-circuit current, examination of profile (iii) shows that the error in the potential is

$$V_e = \frac{I'_{sc} r_s x_s}{A}. \quad (2)$$

To compare I'_{sc} with the true short-circuit current I_{sc} , we use the resistance of the tissue, defined by

$$R_m = \left[\frac{dV}{dI} \right]_{I=I'_{sc}}. \quad (3)$$

Provided the I/V curve is reasonably linear around I'_{sc} we can thus write

$$I_{sc} - I'_{sc} = \frac{V_e}{R_m} = \frac{I'_{sc} r_s x_s}{R_m A} \quad (4)$$

whence the fractional error in the short-circuit is

$$\frac{I_{sc} - I'_{sc}}{I_{sc}} = \frac{1}{1 + R_m A / x_s r_s}. \quad (5)$$

The true short-circuit current is thus underestimated, to an extent which increases with the resistivity of the medium and the separation of the electrodes, but decreases with increasing resistivity of the tissue. Of these three factors, neither R_m nor r_s is under the control of the experimenter, since the saline composition is closely dictated by physiological considerations. The electrode separation x_s must be large enough to prevent mechanical damage to the tissue, or interference with current distribution and stirring of the medium. For the midgut, the smallest practical value for x_s is about 0.1 cm, the resistivity of the standard bathing medium is 180 Ω cm, and the resistivity of the tissue ($R_m A$) is about 150 Ω cm² (Table 1), giving an error in I_{sc} of some 10.7%. For the frog skin (Linderholm, 1952) the resistivities of tissue and saline are 1000–2000 Ω cm² and 95 Ω cm respectively, so that the same electrode spacing would give an error in I_{sc} of only 0.5 to 1%.

For low-resistance epithelia it is therefore necessary to make some compensation for this source of error. Two semi-empirical methods have been published: the first requires a direct measurement of the resistance of the saline between the recording electrodes, made in the absence of the tissue (Ussing & Zerahn, 1951; Harvey, Haskell & Zerahn, 1967). A voltage-current plot is constructed for the 'empty' chamber, and used to correct the short-circuit current by successive approximations. The method is slow, and requires separate plots for each different saline used in the

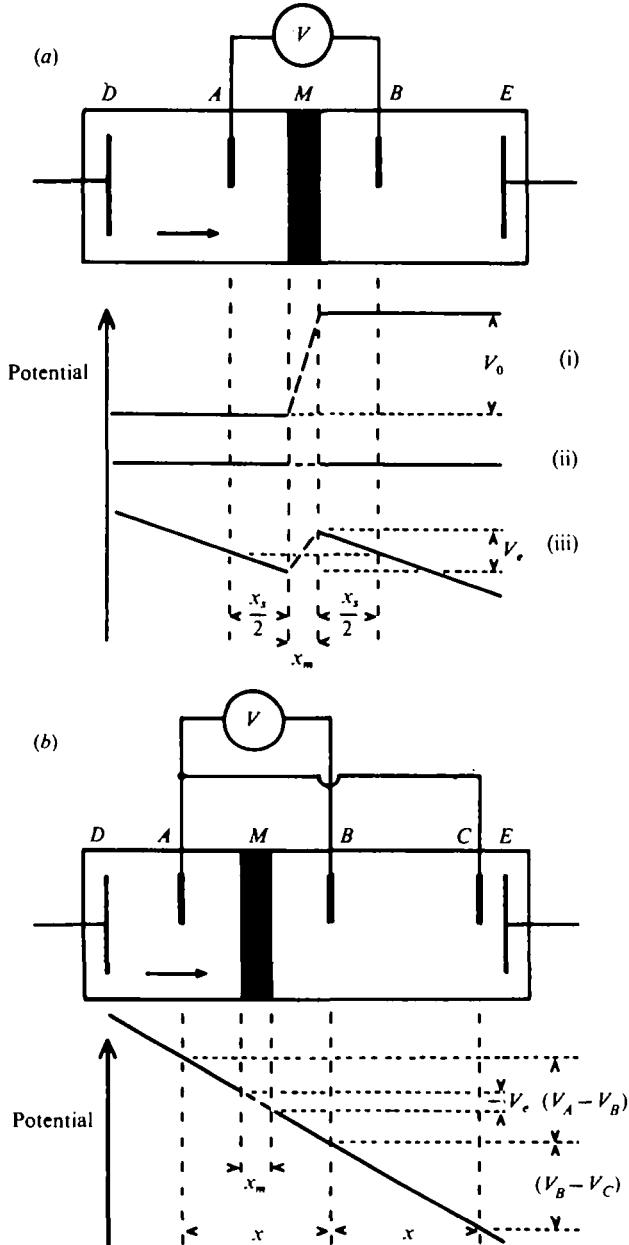


Fig. 1. (a) Schematic diagram of the 'conventional' short-circuit chamber (Ussing & Zerahn, 1951). *D, E* - current electrodes; *A, B* - recording electrodes; *M* - tissue; arrow indicates direction of current flow through the chamber during short-circuiting. Potential profiles in three hypothetical situations are shown below. (i) open-circuit, with no current flow, and potential difference V_0 across the tissue; (ii) idealized short-circuit, with current I_∞ flowing, but a bathing medium of high conductivity, so that potential gradients in it are negligible; (iii) practical short-circuit, showing potential gradient due to current flow in the medium. Electrodes *A* and *B* are at the same potential, but a residual potential difference V_e remains across the tissue. x_m and x_s are defined in the text. (b) Chamber as described in the text, with a third recording electrode (*C*). The meter records $V_B - \frac{1}{2}(V_A + V_C)$. In the short-circuit condition, as shown below, the meter reads zero, and a small, reversed potential difference ($-V_e$) remains across the tissue. Because the tissue is thinner than the distance between electrodes *A* and *B*, V_e in case (b) is smaller than the corresponding error in case (a). x is the electrode separation.

Experiment. A second method (Clarkson & Toole, 1964) provides a reading of I_{sc} in one step, but assumes that the I/V relationship of the tissue is linear and constant with time, an assumption which clearly does not hold over long periods (see Discussion). Neither method makes any allowance for the finite volume of bathing medium displaced by the tissue.

Figure 1*b* shows a system devised to allow automatic compensation for the potential gradient in the saline, irrespective of the electrical properties of saline or tissue. The potential difference across the tissue is read by electrodes *A* and *B*, as before, but a third electrode, *C*, is added to monitor the potential gradient in the saline. The three electrodes are exactly equally spaced so that, in the absence of the tissue

$$V_B - V_C = V_A - V_B = I \frac{x r_s}{A}, \quad (6)$$

where x is the electrode spacing. For recording purposes, the electrodes are connected (Fig. 3) so that the meter reads

$$V_B - \frac{1}{2}(V_A + V_C) = \frac{1}{2}[(V_B - V_C) - (V_A - V_B)]. \quad (7)$$

Without the tissue, the output will thus be zero (which provides a means of adjusting the electrode spacing). With the tissue present, and a current I'_{sc} flowing,

$$V_A - V_B = V_e + I'_{sc} \frac{x_s r_s}{A}, \quad (8)$$

where V_e is, as before, the residual potential difference across the tissue, and the x in equation (6) has been replaced by x_s , since the tissue has displaced some of the saline. Using $V_B - V_C$ from (6), the meter reading is now obtained as

$$\frac{1}{2}[(V_B - V_C) - (V_A - V_B)] = \frac{1}{2}I'_{sc} \frac{(x - x_s) r_s}{A} + V_e. \quad (9)$$

Setting the meter reading to zero, we thus find that the potential difference across the tissue is now slightly reversed, as

$$V_e = -I'_{sc} \frac{(x - x_s) r_s}{A} \quad (10)$$

to find the error in I'_{sc} , we use V_e from (4) above, and note that

$$x - x_s = x_m \quad (11)$$

the thickness of the tissue, so that

$$\frac{I_{sc} - I'_{sc}}{I_{sc}} = \frac{1}{1 - R_m A / x_m r_s}. \quad (12)$$

The short-circuit current is thus overestimated by an amount which now depends, not on the electrode separation, but on the thickness of the tissue. The complex, folded structure of the midgut makes estimation of its effective thickness difficult, but examination of sections suggests values from 0.01 to 0.08 cm, depending on whether the distance is measured from the basal lamella to the microvilli or from the valley to the ridge of the corrugation folds (Anderson & Harvey, 1966; Wood, 1972). With respect to electric current flow, where stirring of the medium is of minor importance

(except in so far as local accumulation of ions may alter the resistivity (Rehm, 1968)) the lower figure may be more appropriate. The wet weight of the tissue (Table 1) corresponds to thicknesses from 0.03 to 0.1 cm, though again one does not know how much of this is trapped saline which is electrically part of the bathing medium. The error in I_{gc} from the three-electrode system may thus be as low as 1.2%, although the largest estimate of thickness would bring it up to 10.6%, no better than the best obtainable from a simple, two-electrode system. On the other hand, the error is now independent of electrode separation, which can thus be made larger, to minimize disturbance to the tissue. Since I_{gc} is read instantaneously, without the need for manual corrections, the system lends itself well to continuous monitoring experiments using a 'voltage-clamp' to maintain short-circuit conditions. With thinner epithelia, the improvement in accuracy would be proportionately greater.

METHODS AND MATERIALS

Insects

Larvae of *Hyalophora cecropia* (L.) were reared on artificial diet (Riddiford, 1968). Midguts were isolated from healthy, mature, fifth instar larvae (Haskell, Clemons & Harvey, 1965) by the procedure described by Nedergaard & Harvey (1968), except that the larvae were chilled under ice at 0 °C for 1 h, instead of using dry ice. The isolated midguts were mounted in the chamber described below.

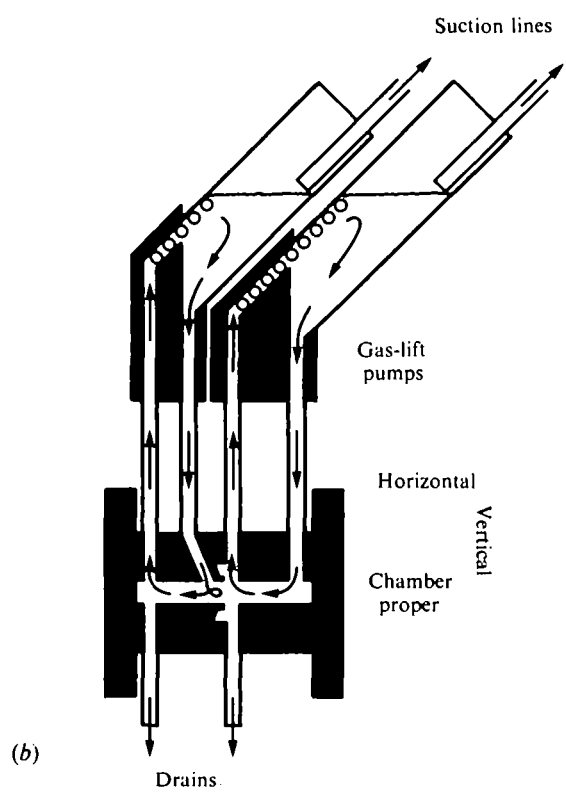
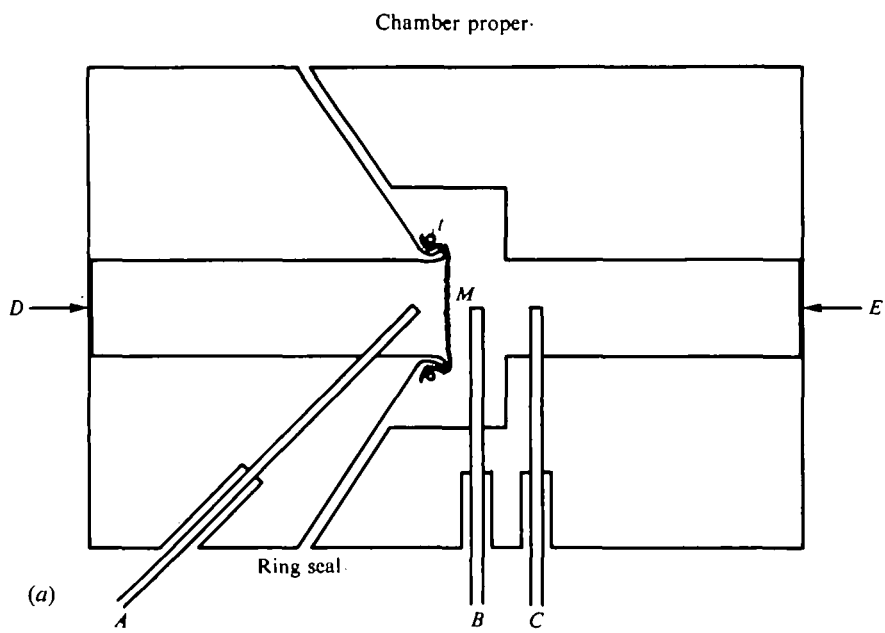
Bathing solutions

The standard bathing solution was chosen as 32K-S-Tris after Harvey & Nedergaard (1964), except that Tris-(hydroxymethyl) aminomethane chloride was substituted for HCO_3^- as the buffer. The solution thus contained (mM): KCl 32, CaCl_2 5, MgCl_2 5, Trizma base 5, HCl 1.5, sucrose 166; pH was 8.3. AR grade chemicals and glass double-distilled water were used throughout.

Chamber

The isolated midgut was mounted as a flat sheet, which provides optimum geometry for current distribution (Wood, 1972), in a chamber (Fig. 2a) very similar in design to that of Ussing & Zerahn (1951), except that the tissue (area 0.5 cm²) was tied over a flange with loops of cotton thread by the method developed by Wood, Farrand &

Fig. 2. (a) Perspex chamber. The midgut (*M*) is attached to the flange of the left-hand compartment with loops of cotton thread (*t*). The exposed tissue lies in a plane, and has an area of 0.5 cm². The right hand compartment was counter-bored to avoid mechanical contact with the tissue. The two halves of the chamber are clamped together with three brass bolts (not shown), and sealed with a rubber washer. The three potential bridges (*A*, *B* and *C*) are equally spaced 0.5 cm apart along the chamber. Each is held in place by a brass chuck, which is sealed off from the solution by a rubber gasket (not shown in figure). The two silver/silver chloride current electrodes (*D*, *E*) are placed symmetrically 3.0 cm from the midgut. (b) Bathing solutions are stirred and oxygenated by two gaslift pumps. The descending flow of solution from the lefthand pump is aimed at the blood-side of the midgut, to increase stirring and oxygen supply. Normally, the solutions are continuously re-circulated, with about 10 ml in each compartment. Solutions can be changed by adding ten volumes of the new solution, the correct level being maintained by the suction lines (normally closed). Drains (normally closed) allow for complete removal of solutions. The apparatus can be operated with the chamber in the horizontal (normal) or the vertical position.



Harvey (1969; see also Forte, Adams & Davies, 1965), to accommodate the delicate nature of the midgut tissue. Flat Ag/AgCl current electrodes were positioned on each side, parallel to the tissue and 3 cm from it. Internal current electrodes were used as giving the most uniform current distribution: toxicity of silver to the midgut is not a problem (Harvey & Nedergaard, 1964; Harvey *et al.* 1967). The three potential electrodes were connected to agar bridges (3% agar in bathing medium) equally spaced 0.5 cm apart along the axis of the chamber. For accurate location, the polyethylene tubing of each bridge was terminated by 5 cm of capillary glass (1 mm O.D.), located in the chamber wall by a threaded brass chuck, with a small rubber gasket to separate the brass from the solution.

The bathing solutions in the two chamber halves were continuously recirculated by separate gas-lift pumps (Fig. 2*b*). This method of stirring is inherently discontinuous and it was found that pumps in which the gas rose at an angle of 45° to the vertical provided the smoothest stirring (Ussing & Zerahn, 1951). The rate of stirring is regulated by the total gas flow rate (5 ml min⁻¹), and the oxygen tension is determined by the composition of the gas (normally 100% oxygen).

Electrical methods

The complete circuitry for measuring the potential difference and providing current for short-circuiting is illustrated in Fig. 3. Calomel cells are used for potential measurement because of their high d.c. stability and absence of polarization by small currents. The outputs of the three electrodes were summed in a *Y*-circuit (see legend to Fig. 3), monitored with an electrometer (Keithley 602) and recorded on a chart recorder (Servoscribe RE 541, Smiths Industries). The automatic current supply was constructed by Bionics Instruments, Bala Cynwyd, Pa., in consultation with one of the authors (J. L. W.). The short-circuit current was measured with an ammeter (630-NA, Triplet Corp.) and recorded on another chart recorder. To align the bridges, the voltage reading at zero current and with no tissue present (asymmetry potential) was first noted. A current of ± 2 mA cm⁻² was then passed, and bridge *A* was moved until the electrometer returned to the original reading. To short-circuit the tissue, the current supply was simply set to clamp the meter reading at the asymmetry potential; potential gradients in the solution were then automatically compensated.

RESULTS

Electrical parameters of the midgut

The electrical parameters of five individual midguts are summarized in Table 1. The values vary widely: the initial, open-circuit potential difference ranged from 72 to 130 mV, with a mean of 98 ± 11 mV (S.E.M.), the lumen side being always positive to the blood side. The 'steady-state' current-voltage relationship (see below) was linear over a wide range of currents (Fig. 4), slight departures from linearity being shown for very large currents ($2 \times I_{sc}$). The resistance measured from the slope at time $t = 60$ min after isolation varied from 98 to 238 Ω cm², with a mean of 150 ± 26 Ω cm². The short-circuit current at this time ranged from 122 to 1018 μ A cm⁻², with a mean of 498 ± 160 μ A cm⁻², which is equivalent to a net potassium transport of 18.6 μ -equiv cm⁻² h⁻¹. The resistance and short-circuit current depended strongly on

Table 1. Variations of the electrical parameters with the wet weights of the tissue

Tissue wet wt (mg)	PD° (mV)	R_m^{90} ($\Omega \text{ cm}^2$)	I_{sc}^{90} ($\mu\text{A cm}^{-2}$)
16.2	75	238	122
28.6	99	180	304
31.6	116	109	702
32.6	72	125	342
52.0	130	98	1018
Mean \pm S.E.M.	98 \pm 11	150 \pm 26	498 \pm 160

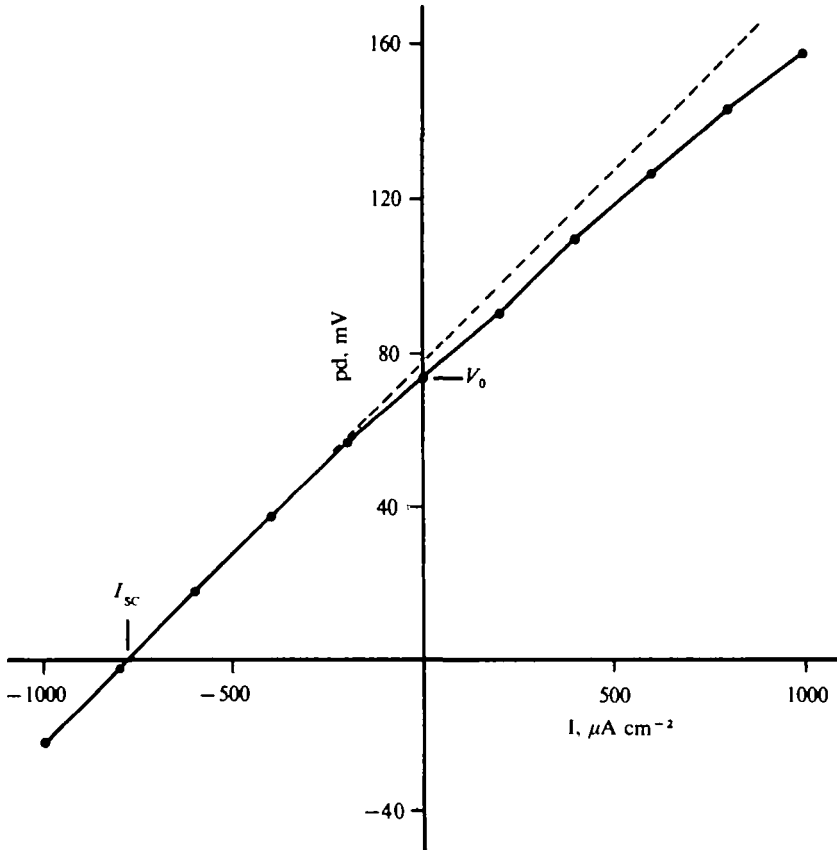


Fig. 4. Voltage-current relationship, obtained from the ascending, two-minute current steps of the experiment in Fig. 6. The dotted line shows results corrected for the spontaneous decay in the potential (cf. Fig. 5). The slope of the resulting linear relationship corresponds to a resistance (R_m) of $98 \Omega \text{ cm}^2$.

the wet-weight of tissue exposed in the chamber (Table 1), but the open-circuit potential showed less dependence on the weight.

The performance of the midgut is not constant with time. Fig. 5 shows the time-course of short-circuit current, potential and resistance of a midgut during the first 3 h after isolation from the insect. The potential difference (V_0) declines on an approximately exponential time-course. The 'steady-state' resistance (R_m) increases exponentially during the first hour, then remains constant for some hours, after which it

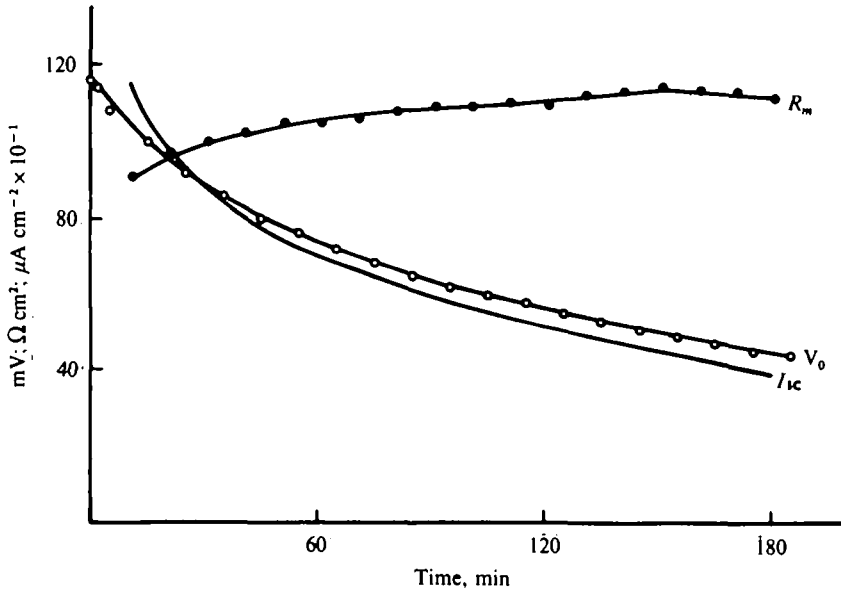


Fig. 5. Time-course of the electrical parameters of a typical midgut. The potential difference (V_0) decreases at a slow, exponential rate (○), the resistance (R_m) increases exponentially (●), and the short-circuit current (I_{sc}) decays following a double-exponential time-course.

begins to decline slowly (not shown in the figure), but never reaches zero (one preparation had a resistance of $46 \Omega \text{ cm}^2$ after 24 h, long after the potential had disappeared). The short-circuit current (I_{sc}) decays on a double exponential time-course, which can be fitted by the following empirical equation.

$$I_{sc}(t) = 1358[0.37 \exp(-0.043t) + 0.63 \exp(-0.0044t)], \quad (13)$$

where t is the time in minutes and $1358 \mu\text{A cm}^{-2}$ is the extrapolated initial I_{sc} . The two time-constants correspond to half-times of 16 and 158 min respectively, the shorter time-constant accounting for 37% of the decay.

The choice of 60 min after isolation as the time to record the comparative data in Table 1, represents a compromise between relative freshness of the tissue and reasonable constancy of the current and resistance with time.

Potential and current transients

Measurement of parameters other than the open-circuit potential is complicated by transient components in the response of the midgut to step changes in the applied current. Fig. 6 shows an experiment in which a series of current or voltage steps was imposed. During the early part of the experiments, the potential response to a current step consisted of an immediate (< 1 sec) jump, comprising about 90% of the final change, followed by a gradual approach to the final value over some minutes. The duration and time-course of the transient depended on the magnitude and direction of the current step. Conversely, clamping the potential at zero required a short-circuit current which was initially larger, but declined over a few minutes to the value expected from the double-exponential decay curve described above.

At longer times (> 60 min) after isolation of the tissue, the transients were reversed,

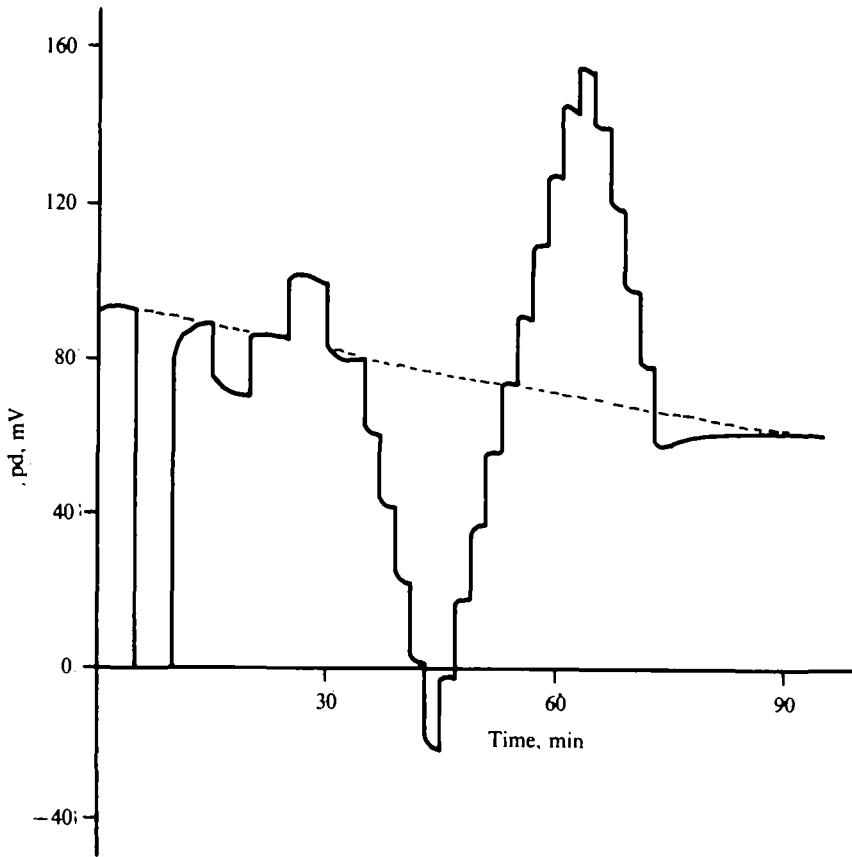


Fig. 6. Transients resulting from short-circuiting ($1050 \mu\text{A cm}^{-2}$) and from 5 min pulses ($\pm 200 \mu\text{A cm}^{-2}$) and 2 min pulses (± 200 , ± 400 , ± 300 and $\pm 100 \mu\text{A cm}^{-2}$) of applied current. The dotted curve shows the projected time-course of the potential difference. Hyperpolarising currents cause the potential to undershoot the projected value during recovery.

the potential response showing a small overshoot, and the I_{sc} showing a gradual increase, before resuming its slow, exponential decay (Fig. 6).

The influence of these transients on the measurement of transepithelial resistance will depend on their origin. If, as is probable, they represent short-term adjustments in membrane permeabilities or ion distribution within the epithelium, in response to changes in the electrostatic potential gradient, then clearly the instantaneous I/V relationship is of most significance in determining the membrane resistance (see Blankemeyer, 1976). On the other hand, in assessing the accuracy of the short-circuit technique, the 'steady-state' values are more important. Since, however, the properties of the midgut are never truly constant with time, true steady-state values can never be obtained. A reasonable compromise can be achieved by allowing two minutes for the decay of transients after each current step, before reading the voltage. The values for resistance quoted in Table I were obtained in this way. The short-circuit currents, however, were taken from the double-exponential decay curve, which is as near to a steady state as can be.

DISCUSSION

Advantages of the three-electrode system have been outlined in the theoretical section above. The principal aim in devising the system was to reduce the error in short-circuiting, due to voltage gradients in the bathing medium – an error which has been shown to be particularly serious in the case of the midgut. In this it is partially successful, although a residual error remains in that, as with other methods that have been used, no allowance is made for the volume of saline displaced by the tissue. The result is a slight over-compensation, so that readings of the short-circuit current are a little too high. (If a two-electrode system is used without compensation, they are too low.)

The magnitude of the residual error depends on the thickness of the tissue: with thin epithelia it will be small, and with thick ones it can be calculated, if the thickness is known. The midgut appears to be a thick epithelium in these terms – wet weights per unit area (cf. Table 1) indicate thickness ranging from 0.03 to 0.10 cm, with a mean of 0.06 cm – but it is also highly folded (Anderson & Harvey, 1966), and much of the variation in weight is evidently due to differing degrees of folding, since the resistance is nearly inversely proportional to the total mass of exposed tissue (Table 1). In fixed, sectioned material (Anderson & Harvey, 1966), the height of the cells from basement membrane to microvilli is only about 0.01 cm (100 μ m), although the total thickness from base to apex of the folds may be as much as 0.08 cm. The extent to which saline can be occluded in the folds and, by becoming electrically distinct from the bathing medium, contribute to the error in short-circuiting, is unknown. The relation between mass and resistance in Table 1 suggests that it is not too large. A pessimistic estimate might put the effective thickness at about 0.03 cm, in which case the error in short-circuit current is about 3% (see Theory), as compared with the minimum of 10.6% which can be obtained with two electrodes.

Apart from an improvement in accuracy, the three-electrode method offers other important advantages. The compensation for voltage gradients is automatic, so that readings are obtained immediately. This is particularly useful for the midgut, whose parameters change quite rapidly with time (Fig. 5), and for the study of transients (Fig. 6). Two-electrode methods (Ussing & Zerahn, 1951) can give the same accuracy with careful calculation of the errors (from previous calibration of the chamber), but the method of successive approximations leads to loss of time-resolution. Three-electrode monitoring also allows the use of automatic circuitry to provide a continuous 'voltage-clamp', so that the current for any imposed potential can be studied as a function of time.

The residual error is a function of tissue thickness but is independent of the separation of the two recording electrodes (as represented by the tips of the agar bridges (Fig. 2)). This is a practical advantage in that the electrodes can be placed far enough from the tissue to ensure a minimal possibility of mechanical damage, or of interference with stirring of the adjacent bathing medium, without loss of accuracy.

Examples of the application of the three-electrode system to the midgut include the important study eliminating electrical errors as a source of the measured discrepancy between the net flux of potassium and the short-circuit current (Wood & Harvey, unpublished; Wood, 1972), which led to the discovery of active magnesium (Wood,

Table 2. *Properties of some transporting epithelia*

Tissue	Potential difference mV	Resistance $\Omega \text{ cm}^2$	Transport rate $\mu\text{-equiv (cm}^{-2} \text{ h}^{-1})$	Classification*	Source
<i>H. cecropia</i> midgut	98	150	18.6		Table I
<i>A. pernyi</i> midgut	106	159	29.3		Wood (1972)
Rat jejunum	11	30	14	'Leaky'	Barry <i>et al.</i> (1965)
Turtle urinary bladder	70	650	4.5	'Tight'	Solinger <i>et al.</i> (1968)
Frog skin	100	2000	1.5	'Tight'	Ussing (1960)

* After Frömter & Diamond (1972).

Jungreis & Harvey, 1975) and calcium transport (Wood & Harvey, 1976); and the demonstration of long isotope mixing times which indicate the presence of a large 'transport pool' of potassium in the tissue (Wood, 1972; Harvey & Wood, 1972, 1973; Wood & Harvey, 1975; Wood, 1978).

Electrical parameters of the midgut

The electrical properties of the *Hyalophora cecropia* larval midgut are almost identical with those of the midgut from *Antheraea pernyi* (Guérin-Meneville) (Wood, 1972; Table 2). For purposes of wider comparison, transporting epithelia in general may be divided into two classes (Frömter & Diamond, 1972): 'leaky' preparations are characterized by low values of resistance and open-circuit potential, and transport rates which may be high or low, whereas 'tight' preparations have high resistance and potential, but low transport rates. Table 2 compares the properties of the larval midgut (*A. pernyi* from Wood (1972); *H. cecropia* average values from Table 1 above) with those of three other epithelia. It will be seen that the midgut has a resistance and transport rate more characteristic of 'leaky' epithelia, but a potential more characteristic of 'tight' preparations. This unusual combination of features illustrates the specialized nature of the midgut tissue, whose potassium transport is unaccompanied by active movement of any counter-ion (Harvey, Haskell & Nedergaard, 1968).

In terms of the properties of individual cells, however, straightforward comparisons with other epithelia can be misleading, owing to the highly folded nature of the midgut epithelium. The corrugation folds and plication folds (Anderson & Harvey, 1966) are estimated to increase the surface area of plasma membranes facing the bathing medium by some 400 times (Wood, 1972). On this basis, the transport rate of $18.6 \mu\text{-equiv cm}^{-2} \text{ h}^{-1}$ is reduced to $0.047 \mu\text{-equiv cm}^{-2} \text{ h}^{-1}$ of actual plasma membranes, which resembles, for example, the maximal rates of sodium extrusion from squid giant axons (Hodgkin & Keynes, 1954). The net transepithelial resistance is similarly increased to some $6 \times 10^4 \Omega \text{ cm}^2$ of plasma membrane. Microelectrode (Wood *et al.* 1969) and tracer measurements (Harvey & Zerahn, 1969) have indicated that about $\frac{1}{3}$ of this resistance can be attributed to the lumen-side membranes, indicating a resistance for the non-junctional, apical cell membranes of some $5 \times 10^4 \Omega \text{ cm}^2$, somewhat higher than that of most other cell membranes (e.g. *Helix* neurons (Maiskii, 1964)), and much higher than that of junctional pathways between cells (Loewenstein, 1966). The potential difference across the apical membranes is also high, reaching 187 mV (Wood *et al.* 1969).

The wide range of electrical parameters among different midgut preparations (Table 1) is presumably due to variations in the amount of tissue exposed, which in turn varies with the degree of folding of the epithelium. In support of this, the short-circuit current and the resistance vary as expected. The potential also tends to vary, but less widely: it is possible that the lightest preparations, having been more stretched, were slightly more subject to damage than the heavier, more folded ones. Variations with season (Wood, 1972) and diet (Jungreis, Jatlow & Wyatt, 1973) have also been reported.

The linear current-voltage relationship of the midgut (Fig. 4), while it is convenient for calculation purposes (e.g. Clarkson & Toole, 1964), is an unexpected feature, since the active transport rate has been shown to be potential-dependent (Harvey *et al.* 1967). Presumably the true resistance of the epithelium changes in parallel with the active transport rate in such a way as to compensate.

The electrical parameters of the midgut are not constant with time (Fig. 5). The progressive decay in current and potential is accompanied by loss of potassium from the cells (Wood, 1972; Harvey & Zerahn, 1969, Table 4), possibly resulting from the combination of a reduced potential difference across the luminal membrane (due to short-circuiting), and a luminal potassium concentration much below that encountered *in vivo* (Quatralé, 1966). The rate of potassium loss depends on the concentration in the bathing medium: the half-time in 50 mM-K⁺ is about 33 min, but in 2 mM-K⁺ it is 20 min (Harvey & Wood, 1972). The half-time for decay of the 'fast' component of the short-circuit current is about 16 min in 32 mM-K⁺ (equation 13), suggesting that this component, together with the initial increase in trans-epithelial resistance (Fig. 5), may result directly from the loss of cell potassium. The cause of the slow component remains unknown, although some obvious factors such as oxygen tension, pH and carbohydrate supply have been ruled out (Wood, 1972).

The correlation between loss of cell potassium and the rapid changes in the short-circuit current supports the idea that a major part of the intracellular potassium lies effectively in series with the transport pathway, and can form the 'transport pool' which has been demonstrated from tracer studies (Wood, 1972; Harvey & Wood, 1972, 1973; Wood & Harvey, 1975; Wood, 1978).

Like the initial decay of the short-circuit current, the transient components of the response to step changes in current or potential difference across the midgut are also probably related to changes in ion distribution within and around the cells (Spring, 1973). A model for such changes was proposed by Kidder & Rehm (1970), with reference to the frog skin. The essential requirement for the model was the existence of two permeability barriers with different properties, in series. The midgut fulfils this requirement, the lumen-side barrier being much less permeable to potassium than the blood-side barrier (Wood *et al.* 1969; Harvey & Zerahn, 1969). Further studies are needed to establish the precise cause of the transients.

If changes in current flow across the midgut do cause significant changes in ion distribution, then a true figure for the resistance across the epithelium will only be obtained if the instantaneous current-voltage relation can be studied (Blankemeyer, 1976). On the other hand, for the purpose of assessing the accuracy of the short-circuiting method, the 'steady-state' resistance should be used. In practice, transients account for about 10% of the response to any current step (Fig. 6), so that the two-

minute readings of resistance in Table 1 are all about 10% too high. A better figure for the average instantaneous resistance would be about $135 \Omega \text{ cm}^{-2}$.

Even after allowing time for the decay of transients, the progressive changes in potential and current throughout each experiment show that the midgut preparation is never in a true steady-state. This is particularly true during the first 90 min after isolation, while cell potassium is rapidly being lost to the bathing medium: indeed Zerahn (1973) has argued that the tissue is virtually dead by the end of this time. However, the long-term performance of the tissue when mounted as a flat sheet is better than that in the spherical set-up of Harvey *et al.* (1967), and midguts mounted flat retain their responsiveness to such factors as stirring, oxygen tension and potassium concentration for periods of several hours (Wood & Harvey unpublished). Thus the period from 90 min to 3 h probably represents the best approximation to a steady-state, as the short-circuit current is then decaying only slowly. For experimental purposes, the closeness of the approximation depends on the parameters being measured. In measuring 'steady-state' tracer fluxes, for example, it can be quite good: taking an average figure of 50 m-equiv kg^{-1} wet weight for the potassium content (Harvey *et al.* 1975), and an average weight of 32 mg (Table 1), the total potassium content of the tissue in the present experiments averages $1.6 \mu\text{-equiv}$. At a mean short-circuit current of $500 \mu\text{A cm}^{-2}$, this amount of potassium will be transported *across* the tissue in about 10 min, during which time the current will have decayed by only 3.2% (equation 13). Changes in tissue potassium by asymmetrical loss to one or other bathing medium can thus contribute only a small fraction to the short-circuit current.

In cases where the tissue is not fully equilibrated with the tracer, as for example when attempting to measure compartments within the transport pathway, changes in the current become more significant, and must be allowed for (Wood, 1972; Harvey & Wood, 1972; Wood & Harvey, 1975). Detailed consideration of this point will be given in a succeeding paper (Wood, 1978).

In summary, a method has been devised for measuring current and potential, which is of general application in electrical studies of transporting epithelia, and is particularly useful for epithelia of low resistance and high transport-rate. The method has been used to measure the electrical parameters of the *Hyalophora cecropia* midgut more accurately than has hitherto been possible, and also to follow their time-course continuously throughout each experiment. The time-course of the 'natural decay' in short-circuit current is described, and the response of the tissue to step-changes in the applied current has been investigated.

Part of this research was conducted while one of the authors (J. L. W.) was a Ph.D student at Cambridge University, under the supervision of Professor Weis-Fogh, Professor Ramsay and Dr Moreton. We thank Dr W. R. Harvey for critical discussions of the manuscript. This research was supported in part by a research grant (A1-09503) from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

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