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THE TRANSEPITHELIAL POTENTIAL AND OSMOTIC REGULATION IN THE GREEN *HYDRA*

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SUMMARY

The properties of the ion-transporting systems in the green *Hydra* were investigated by measuring the trans-epithelial potential and the potential profile across the body wall. The major potential step occurs across the inner ectodermal membrane, and is produced by an inwardly directed electrogenic sodium pump, which is sensitive to ethacrynic acid but not to ouabain. Calcium ions inhibit sodium transport across the epithelium. Calculations using data for the short-circuit current across the epithelium show that sodium transport requires a relatively high rate of energy expenditure, as would be expected 'a priori' for a small organism.

INTRODUCTION

The ectodermal epithelium of the fresh water *Hydra* is the main site of ionic regulation in these organisms. It actively transports sodium ions from the environment into the enteron and extracellular fluids, the two milieux which act as these animals' only 'internal environment'. This process has been investigated by measurements of both ionic concentrations and fluxes (Benos & Prusch, 1972; Prusch, Benos & Ritter, 1976; Benos *et al.* 1977) and by studying the trans-epithelial potential created by these ionic movements (Macklin, 1967; Josephson & Macklin, 1969; Macklin & Josephson, 1971). However, many details of the generation of this potential remain unknown, and it is as yet difficult to compare the properties of this transporting system to those of the more well-known vertebrate epithelia, especially the isolated frog skin preparation. Such a comparison would be of particular interest in view of the very great size difference which exists between *Hydra* and the smaller vertebrates, which is in the region of two orders of magnitude for the linear dimensions. Such a scaling factor must have important consequences for any process of internal homeostasis because of the resulting shift in volume/surface area ratio.

The present study was therefore carried out as an extension of the original experiments of Josephson & Macklin (1969), using a variant of their technique to measure the transepithelial potential in the green *Hydra*. The results obtained will be discussed in terms of possible models for epithelial transport in *Hydra*, and in relation to the energetic requirements of osmotic regulation in small organisms.

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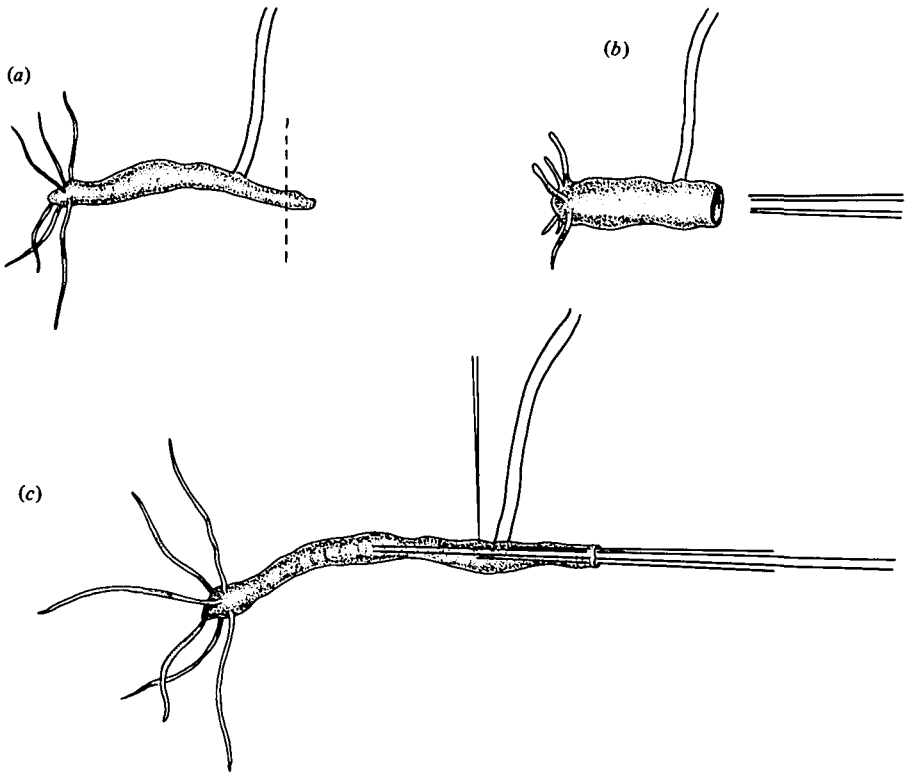

Fig. 1. The procedure for setting up *Hydra* for recording. (*a*) The *Hydra* is held horizontal on the end of a suction electrode, and the base is cut off. (*b*) The *Hydra* contracts, and is manipulated onto the end of the holding electrode. (*c*) The *Hydra* re-extends and attaches itself to the holding electrode. The recording microelectrode and the perfusion micropipette are also shown.

METHODS

The method used to record from the green *Hydra* is shown in Fig. 1 (cf. Josephson & Macklin, 1969). The animals were held horizontal with a suction electrode mounted on a micromanipulator. The base of the *Hydra* was cut off and as the animal contracted vigorously, the *Hydra* could be manipulated over the end of a broken-off microelectrode, filled with culture fluid, previously inserted through a hole in the side of the perspex bath and stuck in place. The animal was then left to recover. In the majority of cases, it slowly re-extended, with the basal part creeping up the glass tube and simultaneously contracting radially around it. The basal cells continuously secrete a sticky mucus substance normally used for adhering to the substratum, and within 15–30 min the base of the *Hydra* will be tightly sealed around the supporting electrode and stuck to it by its own mucus. The body of the *Hydra* is thus free to contract, but the base is held sufficiently firmly to enable the transepithelial potential (between the gut and the external solution) to be recorded by conventional glass capillary microelectrodes (filled with 3 M-KCl) introduced through the gut wall as shown in Fig. 1. In addition, this method of mounting the *Hydra* allowed microelectrode recordings

Table 1. *Parameters of the resting epithelium*

		S.E.	*n*
Resting potential (mV)	42	0·9	16
Short-circuit current (nA)	58	3·8	13
Epithelial resistance (KΩ) (V/I)	740	—	—
Relative resistance of outer ectodermal membrane (%)	11	2·3	6
Surface area (mm^2)	0·83	0·1	10
Specific short-circuit current ($\mu A/cm^2$)	7	—	—
Epithelial specific resistance ($K\Omega/cm^2$)	6·1	—	—

the potential of the ectodermal cells themselves. Tip resistances of greater than 30 MΩ were necessary to obtain stable penetrations. Tip potentials were large (10–15 mV) because of the extremely low ionic strength of the medium (Adrian, 1956; Agin & Holtzman, 1966). These potentials were independent of the concentrations of monovalent ions in the medium, within the range used in the experiments; they were, however, rather sensitive to alterations in the calcium and magnesium levels, behaving to some extent as divalent ion-selective electrodes.

The possibility of artifact in measuring resting potentials must therefore be borne in mind, especially when considering experiments involving changes in the levels of these ions.

The potential was recorded via a d.c. amplifier (20 × gain) and displayed on a Telequipment DM 63 storage oscilloscope. The traces were photographed with a polaroid CR-9 Land Camera. The output from the amplifier was also passed through a transient store and recorded at fast and slow speeds on two Servoscribe pen recorders. One recorder was used as a continuous monitor of the transepithelial potential and the other to look at the profiles of individual action potentials (see Chain, 1979).

While recording the transepithelial potential, it was possible to perfuse the enteron via a micropipette introduced into the gut through the holding electrode (Fig. 1). Fluid flowed into the enteron from a 25 μl syringe, driven by a micrometer screw-gauge, and flowed back through the holding electrode. In order to pass current across the epithelium, the micropipette was replaced by a fine silver wire, which protruded into the enteron from the end of the holding electrode, and was chloridized 'in situ'. A current amplifier in the earth lead measured the total current passed.

The green *Hydra* were cultured according to the methods of Loomis & Lenhoff (1956).

Recordings were made in a small perspex chamber (*ca.* 200 μl), which permitted rapid exchange (less than 1 min) of the external solution. Fresh solutions flowed continuously into the chamber via a multi-way non-return valve, and were removed by suction. The control solution contained 1 mM NaCl, 1 mM $CaCl_2$, 1 mM $KHCO_3$ and 1 mM Hepes buffer, pH 7·4.

RESULTS

A number of basic electrical and anatomical parameters for the preparation are given in Table 1. The surface area measurements were calculated from direct microscopic observation and from photographs, by approximating the *Hydra* body to a

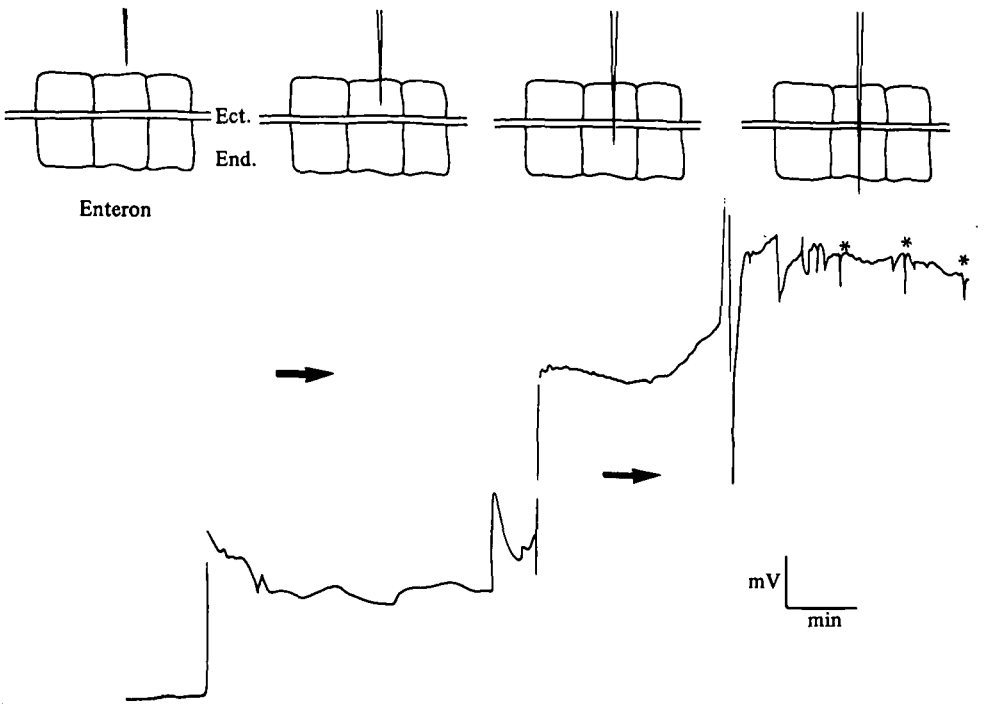

Fig. 2. Chart recording of a potential profile obtained during penetration of the body wall. 'Contraction pulses' were recorded when the electrode was in the enteron. The recording reads from left to right. Insets show the supposed position of the electrode tip corresponding to each voltage step. *Contraction pulse (attenuated). Scale: vertical 5 mV; horizontal 1 min.

regular cylinder and measuring the average radius and the length. The parameters will be discussed in more detail in the various sections below, but are given here for easier reference.

Potential profiles

Various potential profiles can be obtained during microelectrode penetration of the epithelium. These are dependent primarily on the precise position of the electrode in relation to the body of the animal. The most common type of profile (Fig. 2) was obtained during penetration in the central region of the column, as seen from above.

As the electrode approaches and touches the surface of the epithelium, small transient negative potentials are sometimes seen, associated in some cases with visible deformation of the membrane. Provided the electrode resistance is sufficiently high (> 30 MΩ) this is followed immediately by a sharp positive d.c. shift of approximately 10–15 mV. This potential is stable and can be maintained for several minutes. It is rather insensitive to the exact position of the electrode tip, which one can advance by 30 μm or more without significant effect. On further advance, however, more positive steps occur. The final potential is stable even during considerable vertical movement of the electrode tip, and corresponds to a region in which contraction pulses, the active responses associated with contraction, can be recorded as shown in Fig. 2. In all cases the profile consists essentially of two or more distinct positive steps. A seco

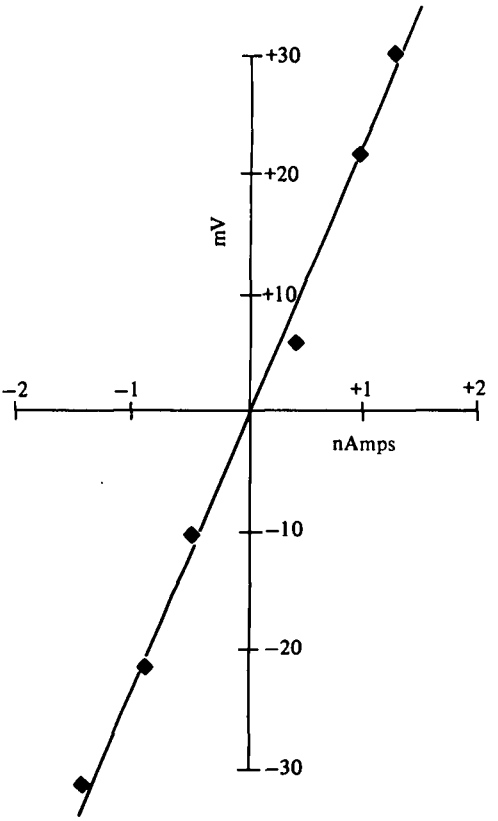

Fig. 3. Voltage/current relationships of a typical ectodermal cell. The slope at the origin was taken as the input impedance of the cell. The values were obtained using a single-electrode technique, with a Wheatstone bridge current injection circuit, as described in the text.

type of profile observed was obtained when the electrode was advanced through the marginal region of the column. The presence of the green algae which line the basal edge of the endodermal cells (just below the mesogloea) provide clear demarcation between two cell layers in green *Hydra*, and it was therefore possible to introduce the electrode through a region in which only ectodermal tissue would be traversed. In such a case the potential profile was usually a single positive step of 10–12 mV. In some cases, however, a second step was observed, reaching the value of the trans-epithelial potential even though the tip remained clearly outside the gastrodermal layer. The significance of this observation will be discussed below.

The initial positive potential change is associated with a sharp change in input impedance of the electrode. This impedance was measured using a current-injection Wheatstone bridge circuit incorporated in the d.c. amplifier. The resistance of the electrode was balanced when the tip was outside the cell so that current pulses in the range ± 1 nA produced no change in the electrode potential. The tip was then advanced until the first positive step was observed. Varying current pulses were again injected (over the same range) and the resulting potential deflexions measured. The electrode was then withdrawn to check that the tip potential and impedance remained

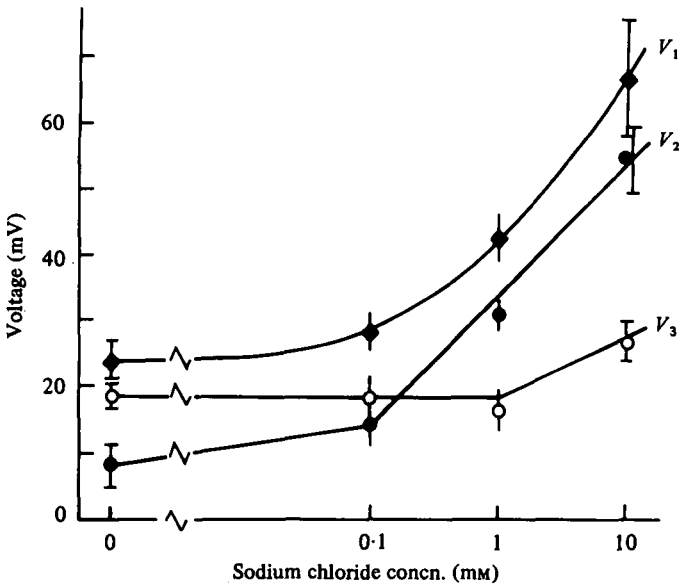

Fig. 4. The transepithelial potential (V_1), the measured potential across the outer ectodermal membrane (V_3) and the calculated potential across the inner ectodermal membrane ($V_2 = V_1 - V_3$) as a function of the external concentration of sodium chloride.

unchanged (i.e. that the bridge circuit was still balanced). Resulting I/V curves are linear in the region ± 20 mV though occasional deviations occur at the extreme ends of the range. The slope of the I/V curve in the region of 0 V can be taken as a measure of the apparent input impedance. The average value obtained was 22 MΩ (S.E. = 0·2, $n = 10$).

The relative values of impedance elements associated with each potential step during penetration were measured by recording the voltage deflexions produced by current pulses passed through the body wall with a chloridized silver wire introduced into the enteron (see Methods). The current pulses were long enough for any capacitative transients to be ignored. Average values for total transepithelial impedance and the relative contributions of each resistive barrier are shown in Table 1. The calculated value for the specific impedance (6·1 KΩ cm^2) agrees closely with the figure of 5·1 KΩ cm^2 obtained by Josephson & Macklin (1969).

A comparison of the figure for the specific resistance of the outer membrane (0·67 KΩ cm^2) with the average input of the ectodermal cells (22 MΩ) suggests that the current flows out over a membrane area of approximately 3000 μm^2 (ignoring the internal cytoplasmic resistance), which would correspond to the outer surface area of 1–5 cells. This result would indicate that there may be a certain amount of intercellular current flow within the epithelium.

Transepithelial resting potential

External ion substitution

(*a*) *Sodium.* The ionic basis of the transepithelial resting potential in the green *Hydra* appears to be broadly similar to that of *H. oligactis* (Macklin & Josephson

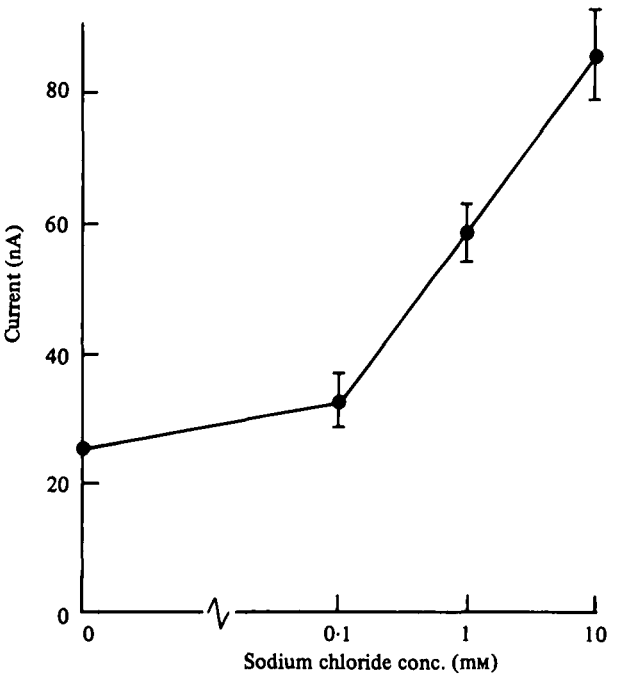

Fig. 5. The short-circuit current across the epithelium, as a function of the external concentration of sodium chloride. [Bars represent ± S.E.M., $n = 5$.]

1971). The potential is primarily sodium-dependent, varying with the external concentration over the range 0·1–10 mM (Fig. 4). The results obtained were essentially identical whether the sodium chloride was replaced by Tris/Tris Cl or KCl. To increase the concentration above that of the standard solution, additional sodium chloride was simply added to the bathing medium, since changes in the total osmotic strength over this range were shown to be without any effect (see below).

The precise site of sodium-dependent electrogenesis in the epithelium was investigated by carrying out potential profiles at the different ionic concentrations (Fig. 4). The outer ectodermal membrane is relatively insensitive to the sodium concentration. The potential across the inner ectodermal membrane cannot be measured directly but can be estimated as being equal to E(total)—Eo (outer ectodermal membrane), since the gastrodermis is leaky and does not contribute any measurable potential to the total (see profile studies above). It is obvious that the inner ectodermal membrane constitutes the major site of electrogenesis. The potential across it varies linearly with the logarithm of the external sodium concentration over the range 0·1–10 mM, with a slope of 22 mV per decade change in concentration.

The short-circuit current across the epithelium also varies with the external sodium concentration and in fact closely mirrors the potential changes across the inner ectodermal membrane (Fig. 5). The residual current and voltage in zero Na^+ is as yet unexplained.

(*b*) *Other ions.* The addition of potassium chloride, Tris buffer and potassium bicarbonate in concentrations up to 10 mM had no effect on the preparations. The

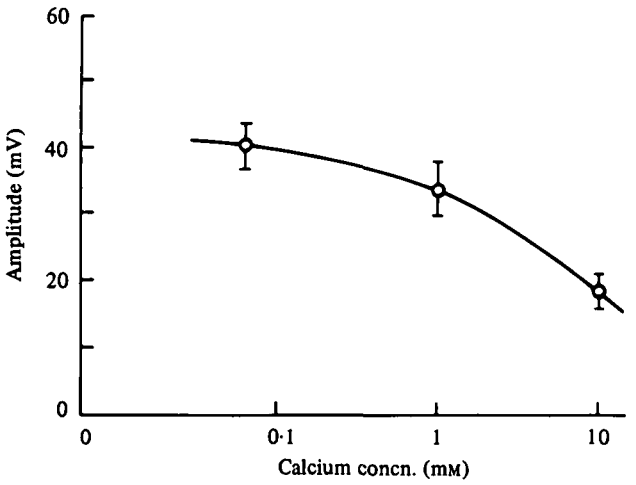

Fig. 6. The transepithelial resting potential, as a function of the external concentration of calcium chloride. [Bars represent ± S.E.M., $n = 5$.]

epithelium therefore seems to be insensitive to all these cations and anions, and is also unaffected, at least in the short term, by an increase of osmotic strength up to 20 mM. This latter result had previously been demonstrated also for *H. oligactis* (Macklin & Josephson, 1971). Preliminary experiments did, however, suggest that the epithelial potential was sensitive to pH.

The most interesting effect was that of varying the external calcium concentration: increased calcium markedly decreased the transepithelial potential (Fig. 6). This effect was relatively slow, suggesting that the calcium does not act at the outer surface of the epithelium, but the precise site of action could not be located. A similar effect of calcium has been demonstrated in a number of other sodium-transporting epithelia, where the phenomenon has been shown to be due to an inhibition of the sodium transport (e.g. Curran & Gill, 1962; Benos *et al.* 1976). This would certainly appear to be the most probable, though not the only possible cause in the epithelia of the green *Hydra*.

(*c*) *Inhibitors*

(i) Ouabain is a well-known inhibitor of Na^+ transport in a very wide range of tissues, both vertebrate and invertebrate (see Glynn & Karlish, 1975). However, when applied externally at a concentration of 1 mM it was found to increase the transepithelial potential (Table 2). About 3 min were required for the full effect. Owing to the enormous range in sensitivity to this drug found for different tissues it is difficult to know whether the concentrations used were 'physiological'.

It remains difficult, in any case, to give a simple explanation of the phenomenon in terms of the classic action of ouabain. A number of more recent studies have suggested that ouabain can also inhibit electrogenic chloride transport (e.g. Cooperstein, 1959; Candia, 1972). A possible small component of such transport may exist in this epithelium since Macklin & Josephson (1971) have shown that chloride-free solutions

Table 2. *The effects of potassium and some metabolic inhibitors on the transepithelial potential*

	Relative epithelial potential	Probability of significant difference
10 mM potassium chloride (internal perfusion)	0·97 ± 0·03 (5)	0·2
30 mM potassium chloride (internal perfusion)	0·95 ± 0·04 (4)	0·2
1 mM ouabain (external solution)	1·12 ± 0·03 (6)	0·02
0·1 mM ethacrynic acid (external solution)	0·83 ± 0·03 (8)	0·001
0·1 mM ethacrynic acid (internal perfusion)	0·75 ± 0·05 (5)	0·01

The data are presented as the relative transepithelial potential in the presence of the test substances (the potential in Normal solution being taken as unity) ± the standard error of the mean. The number of experiments are given in parenthesis.

increase the potential in *H. oligactis*. The precise nature of any ouabain effect must, nevertheless, remain very speculative.

(ii) Amiloride, an inhibitor of passive sodium entry in frog skin (Bentley, 1968) was without effect in concentrations up to 10^{-4} M.

(iii) Ethacrynic acid inhibits electrogenic sodium transport in a variety of tissues (see Pichon & Treherne, 1974). In the present case, it partially abolished the transepithelial potential when applied externally at a concentration of 10^{-4} M (Table 2). Its effect was rather slow under these conditions, once again suggesting that the outer ectodermal membrane is not the major site of sodium transport in this tissue.

Internal perfusion

Very few substances were found to affect the transepithelial resting potential when perfused through the enteron. The normal external solution could be perfused for long periods without any effect. This result is perhaps to be expected, in view of the periods which *Hydra* normally spent with their mouth 'gaping' during which free exchange of solution between the gut and the surrounding medium must occur. More surprisingly, perhaps, perfusion with high potassium solutions (10 or 30 mM) were likewise without any depolarizing effect (Table 2), although under these conditions the concentration of potassium in the gut approaches that measured in the cells. The inner surface of the ectoderm is therefore insensitive to both potassium and chloride. Ouabain had no measurable effect on the transepithelial potential.

Ethacrynic acid inhibited the potential; the inhibition was much more rapid than with external application, but was still only partial (Table 2). The effects of internal calcium were not investigated in detail because the known sensitivity of the electrode tip potential to divalent ions made it difficult to distinguish possible artifacts.

The interpretation of the internal perfusion experiments is not entirely straightforward, since it is difficult to ascertain the degree to which the solutions in the gut penetrate to the ectoderm (cf. Macklin & Josephson, 1971). However, the rapid effect of ethacrynic acid, and the known structure of the body wall (see *Discussion*) suggest that no significant barrier exists between the enteron and the transporting epithelium, and that the absence of any effect of potassium ions and ouabain reflects a real insensitivity to these ions.

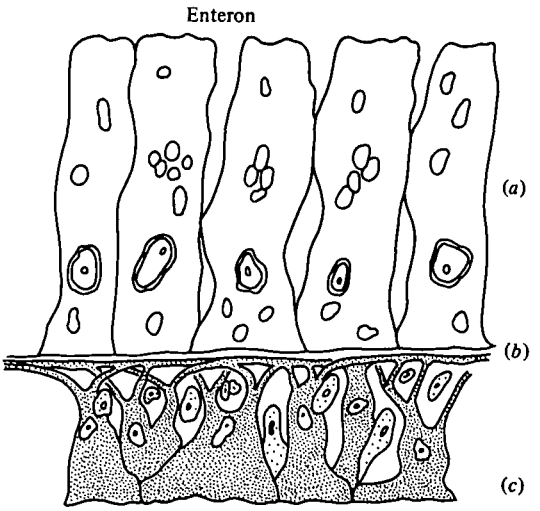

Fig. 7. Semi-diagrammatic drawing of a section through the body wall of a typical polyp-phase hydroid. Nerve cells and interstitial cells are found mainly within the extensive system of extra-cellular spaces in the ectoderm (drawn after Kleinenberg, 1872; Schulze, 1871). (*a*) endoderm, (*b*) mesogloea, (*c*) ectoderm.

DISCUSSION

The structure of the body wall of *Hydra* is shown semi-diagrammatically in Fig. 7. The endodermal layer is 'leaky' (Benos *et al.* 1977; Chain, 1979) and the mesogloea and the inner ectodermal membrane are therefore in direct contact with the enteron. The suggested interpretation of the potential profile in terms of structural elements in the epithelium is shown in Fig. 2. The first positive potential step seems to be associated with penetration of the outer ectodermal membrane. A true intracellular localization of the electrode at this point is suggested by the sharpness and stability of the potential change observed, and the simultaneous increase in the input resistance of the electrode.

The final potential reached during penetration is that of the gut lumen. The intermediate potential steps observed are either those of the inner cell layer (the gastrodermis) or potential artifacts obtained as the electrode tip crosses this layer. It was of interest that potentials identical to those of the enteron were sometimes observed *outside* the gastrodermal layer. These presumably correspond to the potential of the extracellular spaces in the ectoderm, and confirm the supposition that these spaces are in direct communication with the enteron.

Conflicting reports exist in the literature dealing with the potential profiles of species of brown *Hydra* (Macklin, 1967; Benos *et al.* 1977; Kass-Simon & Diesl, 1977). The principal disagreement between these various studies concerns the polarity across the outer ectodermal membrane; the original measurements (Macklin, 1967) agree with those described above in recording the initial step as positive, but two other reports of negative potentials across this outer barrier have since appeared. In view of the uncertainties caused by the large tip potentials, it would be unwise to draw too definite conclusions from any set of experiments; the results may reflect simply

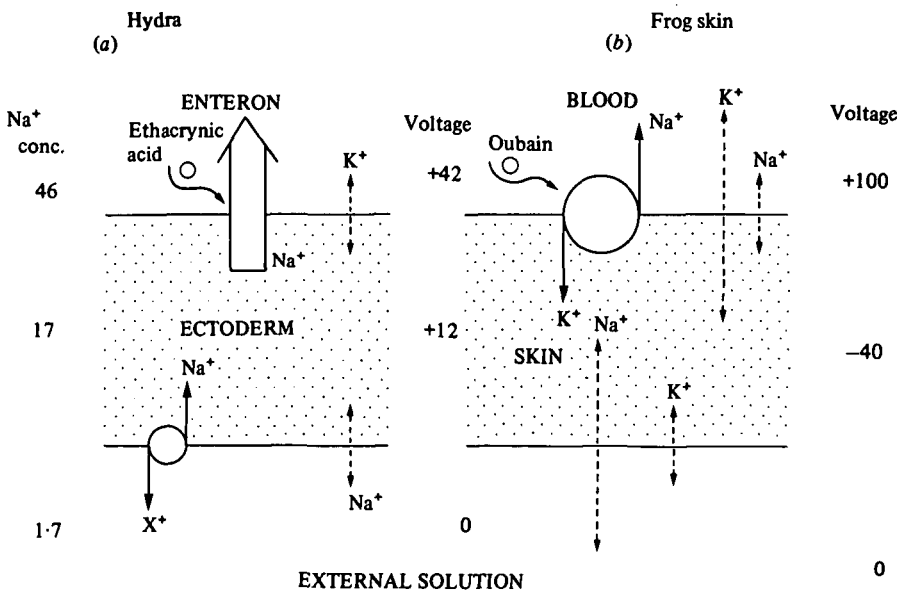

Fig. 8. (*a*) The principal features of transepithelial transport in *Hydra*. The figures for the sodium concentration are taken from Benos *et al.* (1977). The precise site of calcium inhibition is as yet undetermined. The sodium exchange pumps on the outer surface of the ectoderm are hypothetical, and are included merely in order to account for the active sodium uptake into the ectodermal cells, which is apparently an electroneutral process.

(*b*) The principal features of the generally accepted model for transepithelial transport in the frog skin. The general properties of this model, first suggested by Koefoed-Johnsen and Ussing (1958), have been substantiated by the more recent studies of Nagel (1976) and Herman *et al.* (1979). ——, Electrogenic pumps; – – – –, passive exchange; ⊖, inhibition.

differences in experimental technique, or real interspecific variations. In any case it appears fairly certain that the major potential difference occurs between the ectodermal cells and the enteron, and more specifically across the inner ectodermal membrane. The ectodermal cells, therefore, have conventional negative resting potentials of 40–50 mV, relative to the enteron.

The principal features of transepithelial transport in the ectoderm of *Hydra* are shown in Fig. 8, together with the generally accepted model for the frog skin. In both models, the major site of active sodium transport is the inner membrane. As a result, the transporting cells move sodium *out* of the cytoplasm, and thus maintain an inwardly directed sodium gradient at the inner membrane. Such a gradient is an almost universal feature of living cells. Furthermore, this arrangement greatly facilitates the movement of water, which must enter the cells from the very dilute medium through the epithelium into the body cavities, since it decreases the adverse osmotic gradient between the cytoplasm and the internal medium. The other major similarity between the two preparations is the inhibitory action of calcium ions on sodium transport (Curran & Gill, 1962). Although the biological significance of this phenomenon is not clear, its presence in two such different animals suggests that this antagonistic action of two of the major cations of the freshwater environment may well play an important role in their osmoregulatory processes. In contrast to these two

general similarities, the details of the transport process in the two preparations are entirely different. Na^+/K^+ exchange pumps (ouabain-sensitive) do not appear to play a major part in transepithelial transport in *Hydra*, though there is a certain amount of evidence for their presence within the tissue (Benos *et al.* 1977). The inner membrane of the ectoderm is also entirely insensitive to changes in potassium concentration within the enteron, suggesting a low permeability to this ion. Because of the very low concentrations of potassium in the enteron and the rapid flow of water through the epithelium (Benos & Prusch, 1972), uptake of potassium is presumably very energetically expensive and is kept to a minimum.

In conclusion it is of interest to compare briefly the energetics of osmotic regulation in these two fresh water animals. The total short-circuit current of 58 nA given above (Table 1) corresponds to an uptake of about 0·6 pM s^{-1} (an increase in the enteron concentration of about 1 mM min^{-1}). The energy required to take up one mole of sodium ions is given by:

$$\Delta G = RT \ln (Na)_{in}/(Na)_{out} + FV,$$

where $(Na)_{in}$ is the concentration in the enteron, $(Na)_{out}$ is the concentration in the medium and V is the potential across the epithelium. Using the data given in Table 1:

$$\Delta G = 12 \text{ KJ/mole}.$$

The total energy utilization is given by:

$$E = \Delta G \times (\text{short-circuit current}) = 7{\cdot}2 \text{ nJ } s^{-1}.$$

The average weight of a green *Hydra* is approximately 80 μg (unpublished observation), so the energy utilization per unit weight is given by:

$$E' = E/(\text{weight}) = 0{\cdot}3 \text{ J } g^{-1} h^{-1}.$$

It is possible to calculate a similar figure for the energy cost per unit weight of the whole animal, of sodium uptake through the skin of the frog, when the animal is immersed in fresh water. Using the data of Scheer *et al.* (1974) one obtains a value of 8×10^{-3} $Jh^{-1} g^{-1}$, and data from a number of other studies yield relatively similar results (see Shoemaker & Nagy, 1977).

The general requirements of ionic regulation in a dilute environment have led to a broadly similar strategy in the arrangement of ionic transport in the outer epithelium of *Hydra* and the frog skin. As would be expected, however, in view of their totally different positions within the animal kingdom, this similarity is not reflected in the details of the process at the cellular level. Furthermore, while for the frog osmoregulation is an insignificant drain on the animal's energy sources, these same processes are likely to be a major factor in the metabolic balance of the fresh water *Hydra*.

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