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Ion Transport across the Early Chick Embryo: II. Characterization and pH Sensitivity of the Transembryonic Short-Circuit Current

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Abstract. The ectoderm of the one-day chick embryo generates dorsoventrally oriented short-circuit current (I_{sc}) entirely dependent on extracellular sodium.

At the dorsal cell membrane, the I_{sc} was modified reversibly and in a concentration-dependent manner by: amiloride (60% decrease at 1 mM, with 2 apparent IC_{50}s: 0.13 and 48 μM), phlorizin (0.1 mM) or removal of glucose (30% decrease, additive to that of amiloride), SITS (1 mM, 13% decrease). Acidification or alkalinization of the dorsal (but not ventral) superfusate produced, respectively, decrease or increase of I_{sc} with a pH_{50} of 7.64.

Ba^{2+} (0.1–1 mM) from either side of the ectoderm decreased the I_{sc} by 30%. Anthracene-9-carboxylic acid, furosemide and inducers of cAMP had no effect on electrophysiological properties of the blastoderm.

The chick ectoderm is therefore a highly polarized epithelium containing, at the dorsal membrane, the high and low affinity amiloride-sensitive Na^+ channels, Na^+-glucose cotransporter, K^+ channels and pH sensitivity, and, at the ventral membrane, the Na^+, K^+-ATPase and K^+ channels. The Na^+ transport reacts to pH, but lacks the cAMP regulatory system, well known in many epithelia.

The active Na^+ transport drives glucose and fluid into the intraembryonic space, across and around the blastoderm which, in the absence of blood circulation, could secure renewal of extracellular fluid and disposal of wastes and thus maintain the cell homeostasis.

Key words: Chick embryo — Ussing conditions — Amiloride — Phlorizin — SITS — pH

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Introduction

In a preceding paper (Kučera, Abriel & Katz, 1994), we have studied the one-day chick blastoderm cultured in vitro under Ussing conditions by using the voltage clamp technique and demonstrated that the blastoderm consists of two regions with different electrophysiological properties. The values of the open-circuit potential (V_{oc}), short-circuit current (I_{sc}) and total resistance (R_{tot}) were significantly higher in the extraembryonic *area opaca* than in the embryonic *area pellucida*. Using radio-isotopic tracers of sodium and chloride, it was found that the net Na^+ transblastodermal flux fully accounted for the measured short-circuit current. The Cl^-, however, was shown to diffuse passively across the blastoderm mainly through the paracellular conductance. Moreover, the I_{sc} was to a great extent sensitive to the ventral application of ouabain and completely dependent on the presence of sodium in the dorsal extracellular fluid.

In anterior studies, the chick blastoderm has already been shown to transport sodium (Stern & Mackenzie, 1983) and water (New, 1956; Elias, 1964; Stern, Manning & Gillespie, 1985) in the dorsoventral direction. Similar transporting properties have been described also in mammalian blastocoels (*for a review, see* Biggers, Baltz & Lechene, 1991). However, a study of the transporters involved in functional polarity of the chick blastoderm have not yet been attempted.

In the present study, we have analyzed the apical and basolateral membranes of the embryonic ectoderm in terms of the transporters involved in the electrical activity using specific inhibitors. In addition, the influence of the extracellular pH on the I_{sc} has been studied.

Some of the data had been previously published as an abstract (Abriel, Ksontini & Kučera, 1992).

Table. Composition of the solutions used

(mM) / Solution number	Explantation 1	Control 2	Glucose free 3	Δ pH 4[a]
Na^+	108.6	108.6	108.6	108.6
K^+	2.3	2.3	2.3	2.3
Mg^{2+}	0.8	0.8	0.8	0.8
Ca^{2+}	1.4	1.4	1.4	1.4
Cl^-	109.0	95.0	95.0	95.0
$H_2PO_4^-$	0.3	0.3	0.3	0.3
HCO_3^-	6.0	20.0	20.0	0.3–37.2
% CO_2 in air	Air	5	5	0.04–10
Measured pH	8.40	7.35	7.35	5.6–9.5
Glucose	7.6	7.6		7.6
Osmolarity (mOsm)	236	235	235	215–240

Solutions 1 to 4 were buffered with bicarbonate and equilibrated with CO_2 at various concentrations. [a]Solution 4 was buffered using combinations of different concentrations of bicarbonate (from 0.3 to 37.2 mM) and CO_2 (0.04 to 10% in air). The pH of the tested solutions ranged from 5.6 to 9.5.

Materials and Methods

Preparation of the Embryo and Electrical Measurements

The procedure of explantation and mounting of the embryonic preparation has been described in detail in a previous paper (Kučera et al., 1994). In brief, whole chick blastoderms (Warren strain) at stage 4 according to Hamburger and Hamilton (1951) were obtained from eggs incubated for 20 hr at 37.5°C and 90% relative humidity. The blastoderms were dissected from the egg and cleaned of adhering yolk particles. The explantation procedure was carried out at room temperature in a Tyrode solution (Table, solution 1).

Each blastoderm was mounted in a culture chamber modified to accommodate with Ussing conditions (*see* Kučera et al., 1994). The blastoderm separated the chamber into dorsal and ventral compartments of equal volumes (1 ml). The two compartments were perfused with solution 1 or other solutions according to the experimental protocol (Table), using peristaltic pumps. The chamber was fixed onto the table of an inverted microscope (Leitz) placed in a thermostabilized air box (37.5 ± 0.5°C) and the preparation was photographed every hour.

The area of the preparation in contact with the two compartments was 0.125 cm^2 and consisted of the *area pellucida* and the surrounding part of the *area opaca*. The dorsal and the ventral compartments of the chamber were connected via agar bridges (2% agar in 1 M KCl) to Ag/AgCl electrodes according to Ussing conditions (Ussing & Zerahn, 1951). The open-circuit potential (V_{oc}) and short-circuit current (I_{sc}) were measured using an automatic voltage clamp device manufactured by Van Driessche, Louvain, Belgium. The total electrical conductance (G_{tot}) was determined as the ratio I_{sc}:V_{oc}, or from the current responses to externally applied square voltage pulses (1.5 to 4.5 mV for 1.5 sec every 12 sec). The electrical parameters were simultaneously recorded on a paper recorder and stored in a microcomputer. The electrodes were regularly checked for polarization effects.

Solutions and Inhibitors

The control and experimental solutions (Table, solutions 1–4) with various inhibitors were superfused at 0.4 ml/min.

The following inhibitors were used: amiloride and ethylisopropylamiloride (EIPA) (blockers of Na^+-channels and Na^+/H^+ exchanger), furosemide (Na^+,K^+,2 Cl^- cotransporter), anthracene-9-carboxylic acid (9-AC) (Cl^- channels), barium chloride (K^+-channels), phlorizin (Na^+-glucose cotransporter), 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonate (SITS) (anion exchangers). Moreover, cAMP inducers such as N^6,2′-*O*-dibutyryladenosine 3′:5′-cyclic monophosphate (dibu-cAMP), forskolin and theophylline were also tested. All these substances were from Sigma GmbH, Deisenhofen, Germany, except EIPA which was a gift from Merck Sharp and Dohme Chibret AG, Glattbrugg, Switzerland. Amiloride, EIPA and SITS were dissolved in dimethylsulfoxide at a concentration of 10 mM and diluted to the desired concentration with the Tyrode solution.

The pH of the superfusate was changed by using different concentrations of bicarbonate or different partial pressures of CO_2 in the equilibration gas (Table, solution 4).

All experimental conditions were applied to both sides of the embryo.

Statistical Analysis

All values are given as arithmetical means and standard deviations. Bilateral Student's *t*-test (paired or unpaired) was used to compare the data.

Results

Electrical Parameters

At room temperature and in open-circuit conditions, the dorsal side of the blastoderm was slightly negative (about −2 to −5 mV) with respect to the ventral side. In short-circuit conditions, the I_{sc} increased during warming and reached a value around which it oscillated regularly (*see* Figs. 1, 3, 4, 6). After stabilization, the electrical parameters measured were: I_{sc} = 20.4 ± 6.0 μA/cm^2, G_{tot} = 2.5 ± 0.8 mS/cm^2 and V_{oc} = −8.9 ± 3.0 mV (n = 31). These values are not significant-

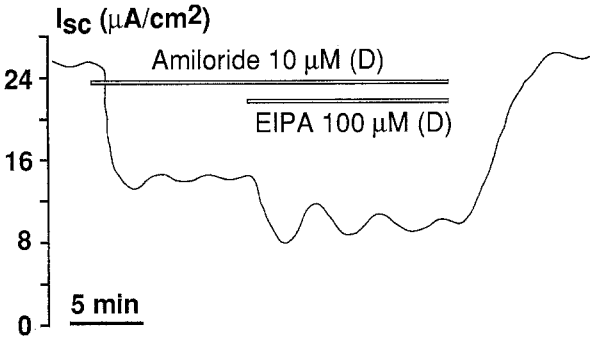

Fig. 1. The effect of amiloride and EIPA on the I_{sc}. A record showing the decrease of I_{sc} after administration of amiloride (10 μM) in the dorsal superfusate (*D*). Additional decrease was observed upon adjunction of EIPA (100 μM) to the dorsal solution. The effect of both compounds was fully reversible at these concentrations.

ly different from those reported in the preceding paper (Kučera et al., 1994).

Modifications of Electrical Parameters Induced Exclusively from the Dorsal Side

Amiloride decreased the I_{sc} down to 40% of the control value (Figs. 1 and 2, $n = 4$). The inhibition was completely reversible. The decrease was concentration dependent beginning at 0.01 μM and reaching a maximal inhibition at 1 mM (Fig. 2*A*). The linearization of the concentration-response curve using the Hanes plot (Höfer, 1981) showed two apparent IC_{50}s: 0.13 and 48 μM (Fig. 2*B*).

EIPA, an analogue of amiloride, completely suppressed the I_{sc}. This effect was concentration dependent with an IC_{50} of 0.21 mM ($n = 4$, Fig. 2*A*) and additive to the effect of amiloride (Fig. 1, $n = 3$). Neither amiloride nor EIPA were able to abolish the oscillations of I_{sc} (Fig. 1). However, the effect of EIPA at concentrations higher than 0.1 mM was not reversible.

Phlorizin, applied dorsally, decreased the I_{sc} (Fig. 3*A*) between 10 μM and 1 mM (*not shown*). At maximal inhibition, the I_{sc} was 67 ± 6% of the control value ($n = 5$). The effect of phlorizin was fully reversible. The actions of phlorizin (0.1 mM) and amiloride (1 mM) were additive, decreasing the I_{sc} to about 15% of the control value ($n = 4$, *not shown*).

Removal of glucose from the dorsal superfusate induced a significant and reversible decrease of the I_{sc} (−21 ± 5%, $n = 4$) and was the only maneuver suppressing the oscillations of the I_{sc} (Fig. 3*B*). Absence of glucose in the ventral superfusate caused a slight (5–10%, $n = 3$) increase of the I_{sc} (Fig. 3*B*).

SITS, at 1 mM, decreased the I_{sc} by 13 ± 3% ($n = 6$) as illustrated in Fig. 4. The effect was significant ($P < 0.0001$, paired *t*-test) and completely reversible.

Extracellular pH was varied using different bicar-

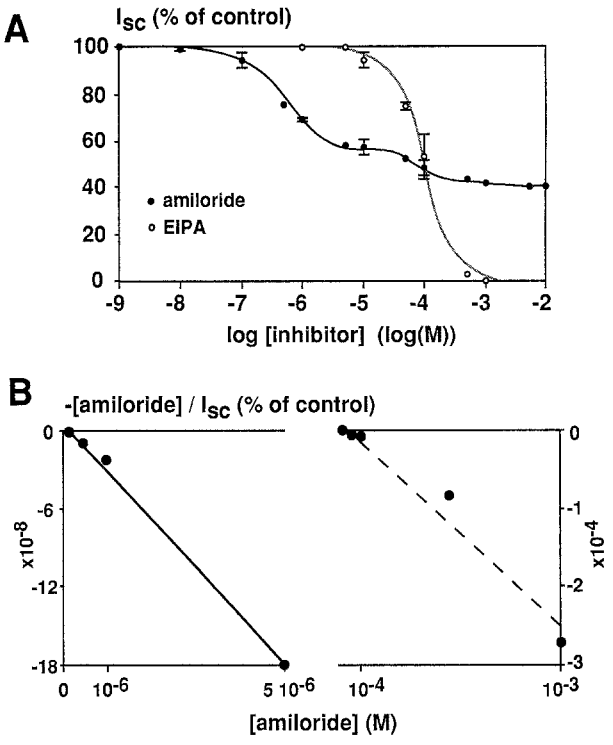

Fig. 2. Concentration response curve of the effect of amiloride and EIPA on the I_{sc}. (*A*) Concentration-response relationships obtained with amiloride and EIPA (each point represents 1–4 determinations, mean ± SD) on four independent preparations for each compound. Both kinetics were linearized using the Hanes plot. The calculated IC_{50} of EIPA is 0.21 mM. (*B*) Hanes plot of the amiloride data points. Notice the two different ordinate scales. *Left:* low-concentration (μM) range of the linearization *Right:* high-concentration (mM) range of the same points. According to the linearization, a double site kinetic was postulated. The unbroken line is the regression ($r^2 = 0.99$) of the 10 mM–5 μM range and the dotted line ($r^2 = 0.96$) the regression of the 5 μM–1 mM range. The two calculated IC_{50}s are 0.13 and 48 μM, respectively.

bonate or CO_2 concentrations in the superfusate (Table, solution 4). Variations of bicarbonate in the dorsal (but not ventral) superfusate induced significant changes: alkalinization increased ($n = 10$) and acidification decreased ($n = 11$) the I_{sc} to new steady-state values. Figure 5 shows the I_{sc}-pH relationship fitted according to Hanes (in Höfer, 1981) and giving a pH_{50} of 7.64. A typical response to acidification as well as absence of sensitivity to pH at the ventral side are illustrated in Fig. 6.

Acidification by increasing the CO_2 to 10% caused a decrease of I_{sc} (Fig. 5). The responses were either stable ($n = 7$) or transient and followed by a new plateau ($n = 3$). The average decrease was −18 ± 7% ($n = 10$). However, alkalinization by decreasing the CO_2 concentration induced only transient increases in the I_{sc} (+19 ± 10% for 1% CO_2) followed by a return to the control value ($n = 5$). These variations were not observed in response to modification of the sole ventral so-

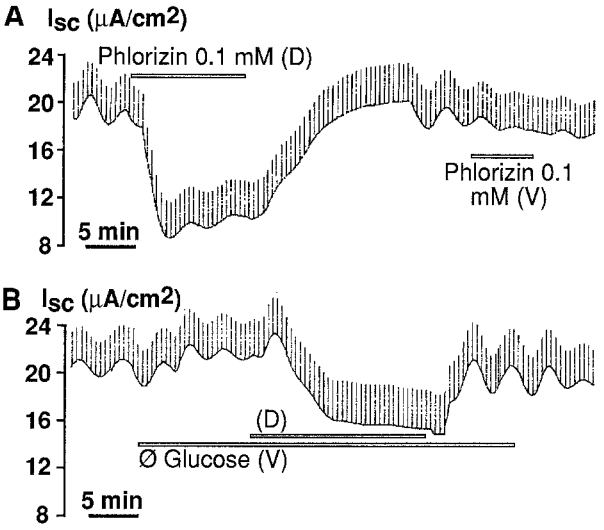

Fig. 3. The effect of glucose and phlorizin on the I_{sc}. (*A*) A record showing the decrease of I_{sc} in the presence of phlorizin (0.1 mM) in the dorsal (*D*) superfusate. Phlorizin had no effect from the ventral side (*V*). The vertical lines are deflections of I_{sc} in response to short electrical pulses of +1.5 mV. (*B*) The effect of removal of glucose from the ventral (*V*) and dorsal (*D*) superfusate of the blastoderm.

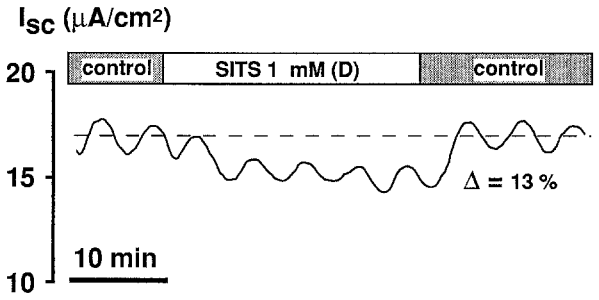

Fig. 4. The effects of SITS on the I_{sc}. A record showing reversible decrease of I_{sc} (by about 13%) in the presence of SITS (1 mM) in the dorsal (*D*) superfusate (pH = 7.35, solution 2 in the Table).

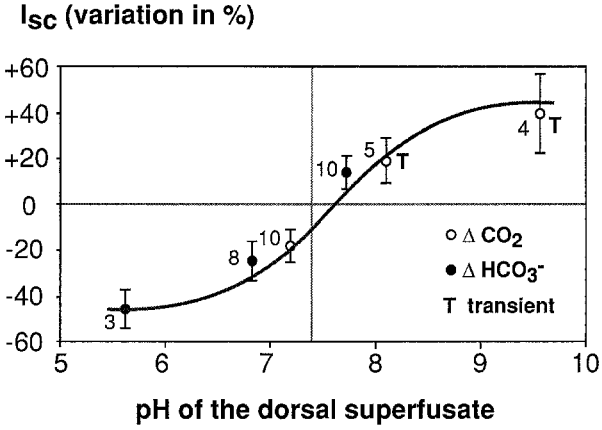

Fig. 5. The effect of dorsal pH on the I_{sc}. Variation of the I_{sc} (%) with respect to the pH of the dorsal superfusate. The control value corresponds to pH of 7.35 (Table, solution 2). The open circles are values obtained by changing the CO_2 tension only (bicarbonate remained at 20 mM). The filled circles are values obtained by changing the bicarbonate concentration only (CO_2 remained at 5%). The curve was calculated according to a single site, noncooperative kinetic model. The calculated pH_{50} (from the Hanes plot) is 7.64. The values (means ± SD) are given with the number of experiments. The letters *T* indicate the maxima of transient responses (*see text*).

lution but only when the dorsal, or both ventral and dorsal solutions were modified.

EIPA (1 μM), applied at the dorsal side, had no significant effect on the variations of I_{sc} induced by pH modifications (*not shown*).

Modifications of Electrical Parameters Induced from Both Sides of the Blastoderm

Whereas the effects described above were strictly linked to the cell polarity, Barium at 0.1–1 mM, either from the ventral or dorsal sides of the blastoderm ($n = 6$), slowly decreased the I_{sc} to about 60–80% of the control value (*not shown*).

Conditions without Any Effect on Electrical Parameters

Furosemide (1–100 μM), 9-AC (1–100 μM), dibu-cAMP (1 mM), forskolin (10 μM) and theophylline (1 mM) were ineffective regardless of the side of administration.

Discussion

Sodium Pathways

Apical Entry of Sodium

In the one-day chick embryo, up to 60% of the I_{sc} could be blocked by amiloride. This suggests the presence at the apical membrane of the amiloride-sensitive Na^+-channel (Fig. 7*A*, Benos et al., 1992). The apparent IC_{50} values obtained from the concentration-response curve, i.e., 0.13 and 48 μM, indicate that the blocking sites might be of two types, corresponding to the high and low affinity amiloride-sensitive Na^+ channels according to Benos et al. (1992).

In the older chick embryo (stage 14 HH), the transectodermal potential difference is still sensitive to amiloride, although much less than in the stage 4 HH studied in the present work (Abriel & Nuccitelli, 1992).

Sodium transport in embryonic tissues was first described across the trophectoderm of the rabbit blastocyst by Smith (1970), Cross and Brinster (1970) and Cross (1973), and its amiloride sensitivity by Powers, Borland and Biggers (1977). Amiloride-sensitive Na^+ uptake by the late blastula was also described in the newt (Komazaki & Takada, 1988). In the mouse blas-

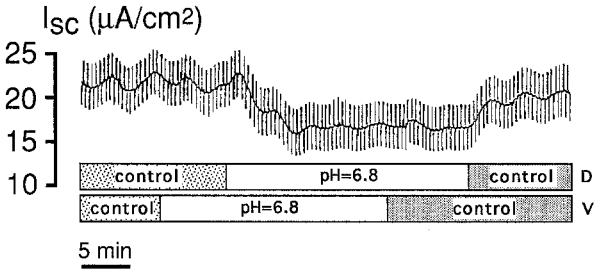

Fig. 6. Dorsal sensitivity to pH. A record showing the decrease of I_{sc} upon acidification of the dorsal (*D*) superfusate (4.7 mM bicarbonate, 5% CO_2, pH 6.8). This effect was reversible and could not be observed when the ventral (*V*) superfusate only was acidified. The control buffer solution (Table solution 2) contained 20 mM bicarbonate and 5% CO_2 (pH 7.35). The vertical lines are deflections due to the short electrical pulses to ±1.5 mV applied to the blastoderm.

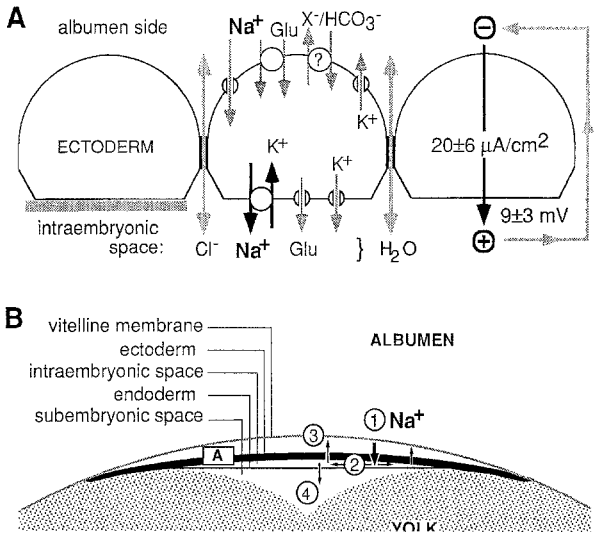

Fig. 7. Transports in the one-day chick blastoderm. (*A*) Schematic drawing of the functional polarity of ectodermal cells. In reality, all the transporters may not be present in all cells. The apical (dorsal) membrane contains the amiloride-sensitive Na^+ channel, Na^+-Glucose (Glu) symport, and possibly a SITS-sensitive exchanger of bicarbonate (*X:* as yet undetermined anion). The basolateral (ventral) membrane contains the Na^+,K^+-ATPase, K^+ channel and a glucose permease. The active dorsoventral transport of Na^+ charges positively the intraembryonic space and induces paracellular flux of chloride and water. The average values of transectodermal short-circuit and open-circuit potential difference are indicated on the right. Under open-circuit conditions, the transblastodermal electrochemical gradient drives extracellular currents through regions of low electrical resistance (grey arrows). (*B*) Schematic drawing of a meridional section of the blastoderm attached to the vitelline membrane. The rectangle *A* indicates the approximate location of the cells depicted in *A*. On the left, the sequence of compartments and barriers between the albumen and yolk. On the right, the proposed movements of electrolytes in the blastoderm: the active transfer of Na^+ into the intraembryonic space (*1*) is followed by passive inflow of anions and water. The resulting transectodermal electrochemical and pressure gradients drive fluid transfer within the intraembryonic space (*2*) and filtration through the more leaky *area pellucida* (*3*) and endoderm (*4*). These fluid movements may participate in the formation and renewal of the intraembryonic milieu, facilitate the substrate availability and allow for disposal of wastes in the subembryonic space.

tocyst, amiloride-sensitive channels were claimed to be less important than the Na^+/H^+ exchanger as the Na^+ flux was more sensitive to EIPA than to amiloride (Manejwala, Cragoe & Schultz, 1989). However, Robinson et al. (1991), using throphectodermal rabbit cells, proposed that such a low sensitivity to amiloride could be explained by the presence of low affinity amiloride-sensitive Na^+ channels, which is supported by our experiments. In the chick embryo, EIPA decreased the I_{sc} with a potency 1,600-fold lower than amiloride acting on the high affinity site. Such a difference in affinity of the two compounds has been documented for the Na^+ channel (Kleyman & Cragoe, 1988). The total and irreversible suppression of I_{sc} with high concentrations of EIPA was probably due to intracellular toxic effects of this more lipophilic compound (Kleyman & Cragoe, 1988; Benos et al., 1992).

In the one-day chick embryo, about 30% of the I_{sc} was also reduced by phlorizin (10–100 μM) or glucose removal. This indicates the presence at the apical membrane of the Na^+-glucose cotransport (Fig. 7*A*) usually inhibited by phlorizin at IC_{50} of 5 μM (Elsas & Longo, 1992; Wright, 1993).

In older chick embryo (stage 14 HH), the transectodermal potential difference becomes more sensitive to phlorizin than to amiloride (Abriel & Nuccitelli, 1992).

In the rabbit blastocyst (Benos, 1981; Robinson et al., 1990) and mouse embryo (Powers & Tupper, 1977), the uptake of glucose is not coupled to Na^+. Although phlorizin can interfere with the accumulation of blastocoelic fluid at the blastula stage (Wiley & Obasaju, 1989), there is no conclusive evidence for a Na^+-coupled glucose transport (Wiley et al., 1991). Thus, the chick embryo seems to be unique in its early expression of this transport mechanism.

The presence of both the Na^+ channel and Na^+-glucose symporter in the blastoderm is interesting. The amiloride-sensitive Na^+ channel is characteristic for tight epithelia, e.g., frog skin, toad urinary bladder and the kidney collecting duct, while the Na^+-glucose symport is usually found in leaky epithelia, e.g., mammalian kidney proximal tubules and small intestines enterocytes (Stein, 1990). In the chick ectoderm, both pathways for the apical entry of sodium are present in significant proportions (i.e., 60% for Na^+ channel and 30% for Na^+-glucose symporter). Preliminary results (P. Kučera and U. Katz, *unpublished*) show that both the *area opaca* and *area pellucida* are sensitive to both amiloride and phlorizin. Whether the two pathways are located in the same or different cells could be studied by using RNA probes for Na^+-glucose cotransporter (Hwang, Hirayama & Wright, 1991).

Basal extrusion of sodium is provided by the

Na^+,K^+-ATPase which is sensitive to 85% to ouabain (Kučera et al., 1994).

Chloride Movement

The chloride does not seem to participate in the active transcellular transport as indicated by the fact that replacement of Cl^- by impermeable anions (Kučera et al., 1994) and use of 9-AC or furosemide were without effects on the measured I_{sc}. The Cl^-, however, does diffuse easily across the blastoderm, mainly through the paracellular conductance, as shown in the radio-isotopes flux experiments (Kučera et al., 1994). Thus, the Cl^- can passively equilibrate across the blastoderm (Fig. 7*A*). It is also possible that Cl^- can enter the ectodermal cells across the dorsal membrane in exchange against bicarbonate (*see* effect of SITS discussed below).

Potassium Pathways

Barium decreased the I_{sc} whether administered on ventral or dorsal sides of the preparation. Most probably, the block of the K^+ conductance depolarized the ectodermal cells and decreased the apical Na^+ entry and, consequently, the I_{sc}. Thus, K^+ can leave the ectodermal cells through both the apical and basolateral membranes (Fig. 7*A*), depending on the respective electrochemical gradients. Recently, two types of K^+-channel were found on the apical side of the chick blastoderm using the patch clamp technique (Prod'hom & Kučera, 1992).

pH Sensitivity at the Apical Membrane

The I_{sc} was influenced by the pH, exclusively from the dorsal side (Figs. 5 and 6). As the intracellular pH was not measured in these experiments, we cannot but speculate that such an apical pH sensitivity is either due to direct influence of the proton on the extracellular part of the membrane channels (Van Driessche & Zeiske, 1985) or to intracellular effects of pH on the conductance of membrane channels (Palmer & Frindt, 1987; Harvey & Ehrenfeld, 1988). Thus, the change of I_{sc} could be explained by decrease or increase of conductance of the apical Na^+ channels due to intracellular acidification or alkalinization. How the proton (or hydroxyl) would cross the dorsal membrane is not clear. The Na^+/H^+ exchanger does not seem to be present as the responses to pH variations were not modified by EIPA (although the exchange is electroneutral the conductance of Na^+ channels should vary differently in the presence of inhibitor). That the increase of CO_2 solely in the ventral solution did not produce a significant response, unless the dorsal side was also acidified, suggests the presence on the apical membrane of a regulatory mechanism which could be a SITS-sensitive bicarbonate transport (*for reviews,* Aronson, 1989; Alper, 1991). In the chick embryo, SITS inhibited the I_{sc} by about 13%. This may suggest the presence at the apical membrane of an electrogenic transport carrying either net positive charges into the cell or negative charges out of the cell. In somitic cells isolated from older chick embryo, Gillespie and Greenwell (1988) postulated the presence of an electrogenic Na^+/HCO_3^--cotransporter which was blocked by stilbene derivatives. However, such a transporter has not been reported in apical membranes of epithelia (Aronson, 1989; Alper, 1991). Alternatively, a SITS-sensitive electroneutral "base-loader" (Alper, 1991) present at the apical membrane could provide the entry of bicarbonate and its inhibition would produce a decrease of intracellular pH with a consequent decrease of the conductance of membrane channels (Fig. 7*A*). Experiments combining the effects of SITS in the presence and absence of chloride are necessary to precise if the anion exchanged for bicarbonate is the chloride.

Absence of cAMP Modulation

We were unable to observe any effect related to a cAMP regulatory system. Thus, inducers of intracellular cAMP did not change the transblastodermal I_{sc} nor the G_{tot}. This was quite surprising as it is known that in fluid transporting epithelia, cAMP is a second messenger often involved in Na^+ flux regulation (Hall et al., 1976; Duffey et al., 1981; Van Driessche & Zeiske, 1985). Hormones acting via cAMP increase the apical Na^+ influx perhaps by recruiting Na^+ channels (Benos et al., 1992). Interestingly, such stimulation was shown to be effective in the mouse blastocyst where cAMP analogues increased the blastocoel Na^+ uptake and its expansion (Manejwala & Schultz, 1989). Our observations suggest that, in the early period of development of the chick embryo, the regulatory mechanisms may differentiate later than or be temporally separated from the transport machinery.

Physiological Considerations

Under conditions of open-circuit and possibly *in ovo*, because of the regional differences in electrical properties of the blastoderm (Kučera et al., 1994), the transectodermal electrochemical gradients resulting from the active Na^+ transport must be dissipated by extracellular fluxes (Figs. 7*A* and *B*). On the one hand, electrical currents, characteristically organized in space, flow from the ventral side to the dorsal side of the blastoderm (Kučera & de Ribaupierre, 1989). On the other hand, fluid movements must be presented in the embryo. According to the sodium flux values (Kučera et

al., 1994) and assuming isosmotic flow, the volume of the fluid transported with the NaCl into the intraembryonic space would be about 8 μl/hr. Such a transfer would constantly increase the hydraulic pressure and dilate the intraembryonic space [the latter must be less than 1 μl: according to Romanoff (1967), the wet weight of the whole chick blastoderm is less than 1 mg]. As electron microscopy does not show dilatation of intercellular spaces it might be that (i) the fluid helps to create new intraembryonic space (arrow 2 in Fig. 7*B*) [Manejwala et al., (1989) reported a decrease of mouse blastocyst expansion in the presence of EIPA], and/or, (ii) the fluid is filtered across regions of high hydraulic conductance, e.g., the leaky *area pellucida* and endoderm (Kučera et al., 1994). The former transport (arrow 3 in Fig. 7*B*) would allow for renewal of interstitial fluid and availability of substrates, the latter transport (arrow 4 in Fig. 7*B*) would allow for formation of the subembryonic fluid (New, 1956; Howard, 1957; Romanoff, 1967) and rejection of metabolic products.

In the one-day chick embryo, glucose is the primary energy substrate (Kučera, Raddatz & Baroffio, 1984). The glucose Na^+-dependent apical entry is most probably followed by a basolateral Na^+-independent exit (Fig. 7*A*). Indeed, removal of glucose from the ventral solution (increase of the outward basolateral glucose gradient) produces a slight increase of the I_{sc} which indicates an increased glucose and Na^+ apical entry (Fig. 3*B*). Interestingly, the value of the glucose cotransported (up to 210 nmol/cm^2/hr) covers the glucose consumption at this period (about 120 nmol/cm^2/hr, Kučera et al., 1984).

In conclusion, the present study shows that the morphogenetic events such as cell proliferation and migration coexist with a rather differentiated and organized supracellular function, i.e., a system of hydroelectrolytic transports coupled to the active transport of sodium. In the early embryogenesis, when blood circulation is not yet present, these transports could secure the renewal of the extracellular environment and facilitate the exchanges by diffusion and cell functions. In this manner, both morphogenesis and transports might be closely interrelated.

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References

Abriel, H., Ksontini, R., Kučera, P. 1993. Influence of the extracellular pH on the active transports in the chick embryo. *Experientia* **49:**A47 (*Abstr.*)

Abriel, H., Nuccitelli, R. 1992. Measurement of the endogenous electric field and transectodermal potential in living chick embryo. *Experientia* **48:**A33 (*Abstr.*)

Alper, S.L. 1991. The band 3-related anion exchanger (AE) gene family. *Annu. Rev. Physiol.* **53:**549–564

Aronson, P.S. 1989. The renal proximal tubule: a model for diversity of anion exchangers and stilbene-sensitive anion transporters. *Annu. Rev. Physiol.* **51:**419–441

Benos, D.J. 1981. Developmental changes in epithelial transport characteristics of preimplantation rabbit blastocysts. *J. Physiol.* **316:** 191–202

Benos, D.J., Cunningham, S., Baker, R.R., Beason, K.B., Oh, Y., Smith, P.R. 1992. Molecular characteristics of amiloride-sensitive sodium channels. *Rev. Physiol. Biochem. Pharmacol.* **120:**31–113

Biggers, J.D., Baltz, J.M., Lechene, C. 1991. Ions and preimplantation development. *In:* Current Communications in Cell and Molecular Biology 4. Animal Applications of Research in Mammalian Development. R.A. Pedersen, A. McLaren, N.L. First, pp. 121–146. Cold Spring Harbor Laboratory NY

Cross, M.H. 1973. Active sodium and chloride transport across the rabbit blastocoele wall. *Biol. Reprod.* **8:**566–575

Cross, M.H., Brinster, R.L. 1970. Influence of ions, inhibitors and anoxia on transtrophoblast potential of rabbit blastocyst. *Exp. Cell Res.* **62:**303–309

Duffey, M.E., Hainau, B., Ho, S., Bentzel, C.J. 1981. Regulation of epithelial tight junction permeability by cyclic AMP. *Nature* **294:** 451–453

Elias, S. 1964. The subembryonic liquid in the hen's egg: Formation and biochemistry. *Rev. Roum. Embr. Cytol.* **1:**165–192

Elsas, L.J., Longo, N. 1992. Glucose transporters. *Annu. Rev. Med.* **43:**377–393

Gillespie, J.I., Greenwell, J.R. 1988. Changes in intracellular pH and pH regulating mechanisms in somitic cells of the early chick embryo: a study using fluorescent pH-sensitive dye. *J. Physiology* **405:**385–395

Hall, W.J., O'Donoghue, J.P., O'Regan, M.G., Penny, W.H. 1976. Endogenous prostaglandins, adenosine 3′, 5′-monophosphate and sodium transport across isolated frog skin. *J. Physiol.* **258:**731–753

Hamburger, V., Hamilton, H. 1951. A series of normal stages in the development of the chick embryo. *J. Morphol.* **88:**49–92

Harvey, B.J., Ehrenfeld, J. 1988. Proton passage across cell membranes. pp. 139–164. Wiley, Chichester (Ciba Foundation Symposium 139)

Höfer, M. 1981. Transport across biological membranes. pp. 133–145. Pitman, London, Marshfield

Howard, E. 1957. Ontogenetic changes in the freezing point and sodium and potassium content of the subgerminal fluid and blood plasma of the chick embryo. *J. Comp. Physiol.* **50:**451–470

Hwang, E.-S., Hirayama, B.A., Wright, E.M. 1991. Distribution of the SGLT1 Na^+/glucose cotransporter and mRNA along the crypt-villus axis of rabbit small intestine. *Biochem. Biophys. Res. Commun.* **181:**1208–1217

Kleyman, T.R., Cragoe, E.J., Jr. 1988. Amiloride and its analogs as tools in the study of ion transport. *J. Membrane Biol.* **105:**1–21

Komazaki, S., Takada, M. 1988. Amiloride-sensitive potential difference across the blastocoelic wall of early embryos of the newt, *Cynops pyrrhogaster*. *Comp. Biochem. Physiol.* **91A:**129–133

Kučera, P., Abriel, H., Katz, U. 1994. Ion transport across the early chick embryo: I. Electrical measurements, ionic fluxes and regional heterogeneity. *J. Membrane Biol.* **141:** 149–157

Kučera, P., de Ribaupierre, Y. 1989. Extracellular electrical currents in the chick blastoderm. *Biol. Bull.* **176(S):**118–122

Kučera, P., Raddatz, E., Baroffio, A. 1984. Oxygen and glucose uptakes in the early chick embryo. *In:* Respiration and metabolism

of embryonic vertebrates. W. Seymour, editor. pp. 299–309. Junk publ., Dordrecht, Boston, London

Manejwala, F.M., Cragoe, E.J., Schultz, R.M. 1989. Blastocoel expansion in the preimplantation mouse embryo: role of extracellular sodium and chloride and possible apical routes of their entry. *Dev. Biol.* **133:**210–220

Manejwala, F.M., Schultz, R.M. 1989. Blastocoel expansion in the preimplantation mouse embryo: stimulation of sodium uptake by cAMP and possible involvement of cAMP-dependent protein kinase. *Dev. Biol.* **136:**560–563

New, D.A.T. 1956. The formation of sub-blastodermic fluid in hen's eggs. *J. Embryol. Exp. Morph.* **4:**221–227

Palmer, L.G., Frindt, G. 1987. Effects of cell Ca and pH on Na channels from rat cortical collecting tubule. *Am. J. Physiol.* **253:**F333–F339

Powers, R.D., Borland, R.W., Biggers, J.D. 1977. Amiloride-sensitive rheogenic Na^+ transport in rabbit blastocyst. *Nature* **270:**603–604

Powers, R.D., Tupper, J.T. 1977. Developmental changes in membrane transport and permeability in the early mouse embryo. *Dev. Biol.* **56:**306–315

Prod'hom, B., Kučera, P. 1992. Ion channels in the chick embryonic ectoderm. *Experientia* **48:**A33 (*Abstr.*)

Robinson, D.H., Smith, P.R., Benos, D.J. 1990. Hexose transport in preimplantation rabbit blastocysts. *J. Reprod. Fert.* **89:**1–11

Robinson, D.H., Bubien, J.K., Smith, P.R., Benos, D.J. 1991. Epithelial sodium conductance in rabbit preimplantation trophectodermal cells. *Dev. Biol.* **147:**313–321

Romanoff, A.L. 1967. Biochemistry of the Avian Egg. pp. 295–329. Wiley, New York

Smith, M.W. 1970. Active transport in the rabbit blastocyst. *Experientia* **26:**736–738

Stein, W.D. 1990. Channels, Carriers, and Pumps: An Introduction to Membrane Transport. pp. 286–305. Academic, San Diego, London

Stern, C.D., MacKenzie, D.O. 1983. Sodium transport and the control of epiblast polarity in the early chick embryo. *J. Embryol. Exp. Morphol.* **77:**73–98

Stern, C.D., Manning, S., Gillespie, J.I. 1985. Fluid transport across the epiblast of the chick embryo. *J. Embryol. Exp. Morphol.* **88:**365–384

Ussing, H.H., Zerahn, K. 1951. Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta Physiol. Scand.* **23:**110–127

Van Driessche, W., Zeiske, W. 1985. Ionic channels in epithelial cell membranes. *Physiol. Rev.* **65:**833–903

Wiley, L.M., Obasaju, M.F. 1989. Effects of phlorizin and ouabain on the polarity of mouse 4-cell/16-cell stage blastomere heterokaryons. *Dev. Biol.* **133:**375–384

Wiley, L.M., Lever, J.E., Pape, C., Kidder, G. 1991. Antibodies to a renal Na^+/glucose cotransport system localize to the apical plasma membrane domain of polar mouse embryo blastomeres. *Dev. Biol.* **143:**149–161

Wright, E.M. 1993. The intestinal Na^+/glucose cotransporter. *Annu. Rev. Physiol.* **55:**575–589