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Na^+ and Cl^- transports in gallbladder: a neutral or an electrical coupling?

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Fisiologia. — Na^+ *and* Cl^- *transports in gallbladder: a neutral or an electrical coupling?* (*). Nota di DARIO CREMASCHI e SILVIO HÉNIN, presentata (**) dal Corrisp. V. CAPRARO.

RIASSUNTO. — Nelle cellule epiteliali di cistifellea di coniglio la conduttanza per il Cl^- sembra essere trascurabile sia attraverso la membrana plasmatica luminale sia attraverso quella basolaterale. Malgrado ciò la cellula contiene Cl^- in elevata concentrazione. Esso è marcabile con $^{36}Cl^-$, ma solo dal lato luminale e solo in presenza di Na^+. Ciò dimostra che l'entrata del Cl^- in cellula (lume → cellula) avviene mediante un cotrasporto neutro col Na^+. L'uscita (cellula → serosa) dovrebbe essere a sua volta ascritta ad un trasporto neutro, ma non è escluso che possa avvenire per accoppiamento elettrico con il trasporto di Na^+.

For many years Na^+ and Cl^- were thought to be actively transported, on the same carrier in one to one ratio, by gallbladder epithelium; thus the pump itself was not considered to be electrogenic [4, 5, 16]. In short, experimental evidences supporting such a view are three: i) electrical potential difference (p.d.) in fish and rabbit gallbladder is nearly zero or slightly negative (serosa negative); ii) replacement of transported anions (Cl^-, HCO_3^-) by large and poorly permeable anions (e.g. SO_4^{2-}) or of the transported cation (Na^+) by large and poorly permeable cations (choline, tetraethylammonium) does not affect transepithelial p.d.; iii) Cl^- epithelial conductance is too small to account for a rapid shortcircuit of a p.d. created by an electrogenic Na^+ pump.

Recently, however, tight junctions have been demonstrated to have a very small electrical resistance in comparison to that of cells [6]. Then a possible p.d. due to an electrogenic pump, could be shortcircuited. Obviously this is not a direct evidence for the electrogenic pump, but it can explain why we cannot measure a transepithelial p.d., if such a pump exists.

Moreover, with extension of p.d. measurements to gallbladders of many other species, it has been observed that in several cases the p.d. is positive up to 7–8 mV; it is abolished by ouabain and by Na^+ replacement with choline [7, 13]; it increases when electrical resistance of the tight junctions is artificially increased [11].

Furthermore Na^+–K^+ activated ATPase has been detected also in gallbladder epithelial cells [15].

All these facts, added to the poor selectivity of the transport with respect to anions (Na^+ is transported together with Cl^- as well as HCO_3^-, Br^-, propionate, acetate pivalate and DMO) [4, 17] suggest that an electrogenic Na^+

(*) These results have been presented in many Congresses of the Soc. Ital. Biol. Sper. (Palermo 1971, Roma 1972, Trieste 1973, Riva del Garda 1974), in the Joint Meeting of Società Italiana di Fisiologia and Physiological Society (Varenna, 1972), in the 2nd International Symposium on Bioelectrochemistry and Bioenergetics (Pont à Mousson 1973).

(**) Nella seduta dell'8 marzo 1975.

pump, related to the enzymatic system of the Na^+–K^+ activated ATPase, is responsible for the Na^+ salt transport. Cl^- could cross the cell passively, following Na^+ without any direct coupling on a carrier.

This paper deals with these problems which are approached by both electrophysiological and radioactive tracer technique.

Gallbladders were excised from ordinary rabbits, washed free from bile with bicarbonate Krebs-Henseleit solution.

The tissue, opened lengthwise, was held horizontally between two lucite chambers (fig. 1). A volume of 8 cm^3 of solution was present in the serosal chamber and 2 cm^3 in the mucosal one; the latter was renewed continuously to avoid concentration changes with time.

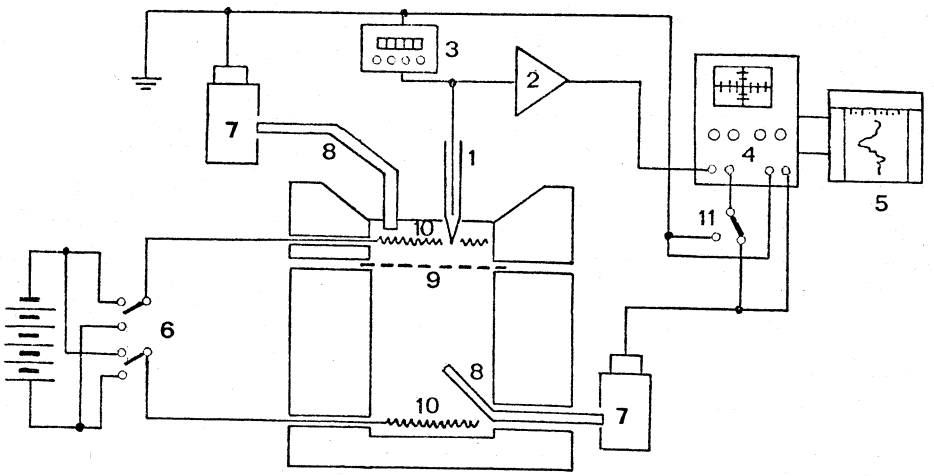

Fig. 1. – Block diagram of the circuit used for micropuncture of rabbit gallbladder epithelium [9]. The microelectrode [1] is connected by an Ag/AgCl wire and a probe [2] (I. L. 181) to the first channel input of the dual beam oscilloscope [4] (Tektronix R 5030) and to a chart recorder [5] (Varian G 1000). Reference electrodes are matched calomel half cells [7] connected by agar 3M KCl bridges [8] to serosal and mucosal media. The impedance meter [3] is connected, in parallel with the probe input, to the microelectrode. P. D. measurements with respect to mucosal or serosal media can be performed using a switch [11]. D. C. pulses (generator 6) are passed through the tissue by two Ag/AgCl electrodes [10].

Recording microelectrodes were filled with 3 M KCl. Microelectrode impedance measurements were made in Krebs-Henseleit solution with a.c. (frequency of 15 Hz) by an automatic device [3, 8]. Microelectrodes with 30 MΩ impedance were used. The block diagram of the experimental lay-out is shown in fig. 1. Intracellular potential measurements with respect to mucosal (V_{mc} and serosal (V_{sc}) media could be performed using a switch. Transepithelial potential difference was continuously monitored during the experiment using the second channel of the dual beam oscilloscope.

An anodic and cathodic d.c. (200 μA/cm^2, 1 sec pulses) was passed through the tissue during impalement using Ag/AgCl electrodes. The potential diffe-

rence (p.d.) between serosal and mucosal agar-KCl bridges and between these and the microelectrode was measured. A correction to estimate the actual potential drop across the membranes was obtained by measuring the p.d. between mucosal bridge and microelectrode before impalement, the p.d. between serosal bridge and microelectrode after penetrating through the serosal membrane and the p.d. between mucosal and serosal bridge without the tissue. We calculated the ratio R_m/R_s (i.e. the ratio between electrical resistances of mucosal and serosal cell barriers) from the ratio of the actual potential drops across the two membranes, so obtained.

Cell Cl^- concentration was measured both by chemical and radiochemical titration, with $^{36}Cl^-$ placed either in both (tissue cut open in a flat sheet) or in one (intact sac) of the two media bathing the mucosal and the serosal side. The incubation time of one hour has been demonstrated to be largely sufficient for the isotope equilibration and this was the time used in all the experiments [1]. Extracellular space was measured by ^3H-sucrose (1 $\mu C/cm^3$, The Radiochemical Center, Amersham). The mucosa was slightly blotted and scraped using a cover-slip. Wet weight was determined and 2 cm^3 of bi-distilled water added. The suspension was frozen and thawed, boiled for 15 min and centrifuged. Cl^- was determined using a modified diphenyl-carbazone method. ^3H-sucrose and $^{36}Cl^-$ were counted in a TRICARB scintillation spectrometer. The dry weight of the mucosa was measured in each case and the total mucosa water was calculated from the difference between wet and dry mucosa weight. The technique used is more precisely reported in ref. [2] and in a manuscript under preparation.

Bicarbonate Krebs-Henseleit solution had the following composition (mM): Na^+ 142.9; Cl^- 127.7; HCO_3^- 24.9; K^+ 5.9; Ca^{2+} 2.5; $H_2PO_4^-$ 1.2; SO_4^{2-} 1.2; Mg^{2+} 1.2. Cl^- and HCO_3^- were replaced by SO_4^{2-} or J^- in SO_4^{2-} and J^- solutions respectively. Mannitol was added to SO_4^{2-} solution to keep constant osmolarity. Na^+ was replaced by choline in choline solution. Bicarbonate Krebs-Henseleit and choline solutions were bubbled with 95 % O_2 + 5 % CO_2; SO_4^{2-} and J^- solutions were bubbled with 100 % O_2.

All experiments were carried out at 27 ± 1 °C.

A first approach to the problem is an electrophysiological one. Intracellular potential measured in rabbit gallbladder is —58.2 ± 0.2 mV (mean ± ± S.E.M. of 1082 impalements, 120 gallbladders). When SO_4^{2-} or J^- are substituted for Cl^- and HCO_3^- in the incubation media, the intracellular potential is not modified (Table I) [2]. The replacement is performed in both

(1) The complete equilibration is reached in 30 minutes.

(2) It is to emphasize that the intracellular Cl^- concentration is about 84 mM and the rapididly exchangeable pool for this anion is 68 mM, when tissue is incubated in Krebs-Henseleit solution. Thus Cl^- is largely far from its electrochemical equilibrium and its replacement by poorly permeable anions (SO_4^{2-} or J^-) should affect the intracellular potential.

mucosal and serosal medium to avoid diffusion potentials across tight junctions; measurements are taken 10 min after the solution changes, to allow ions to equilibrate in the large serosal unstirred layers. This result suggests that anions are not involved in intracellular potential origin, both across luminal and serosal membrane.

TABLE I.

Effect of HCO_3^- *and* Cl^- *replacement by* SO_4^{2-} *or* J^- *on intracellular electric potential* (V_{mc}).

Solution	V_{mc} (mV) mean ± S.E.M.	Number of impalements	Number of Gallbladders
Krebs	−55.4±0.8	74	7
SO_4^{2-}	−56.1±0.6	77	7
Krebs	−58.9±0.9	45	4
J^-	−58.7±1.1	41	4

A second electrophysiological approach confirms this conclusion. When K^+ concentration in the mucosal medium is varied, immediate and large intracellular potential changes are observed. Transepithelial p.d. is negligibly affected by the imposed changes in luminal K^+ concentration, so that interferences due to diffusion potentials across tight junctions are negligible. It is also important to emphasize that Cl^- is maintained at a constant concentration in the incubation media, when K^+ concentration is varied, so that the product $[Cl^-]\cdot[K^+]$ is not constant. Measured intracellular potential changes across the luminal barrier (ΔV_{mc}) can be corrected to eliminate the effect of the tight junction shunt, by the following equation (for derivation see ref. 9)

$$\Delta E_{mc} = \Delta V_{mc}\left(1 + \frac{R_m}{R_s}\right).$$

R_m/R_s, i.e. the ratio between electrical resistances of mucosal and serosal cell barriers, is measured in each solution [9]. Fig. 2 reports the plot of the corrected potential changes across mucosal barrier (ΔE_{mc}) vs. the logarithm of mucosal K^+ concentration. $[K^+]_m$, increased from the physiological value (5.9 mM) to 29.4 mM, causes a depolarization of the mucosal electromotive force (E_{mc}) and the slope is 59.5 mV/decade. This slope decreases when $[K^+]_m$ is decreased under 5.9 mM and E_{mc} hyperpolarizes. The same curve is obtained when SO_4^{2-} is substituted for Cl^- and HCO_3^- in the bathing media. On the contrary the slope is constantly 59.5 mV/decade at any $[K^+]_m$, when incu-

bation media do not contain Na^+ (replaced by choline). These results suggest that E_{mc} is largely due to K^+ and slightly to Na^+ diffusion; conversely Cl^- conductance through the luminal barrier seems completely negligible.

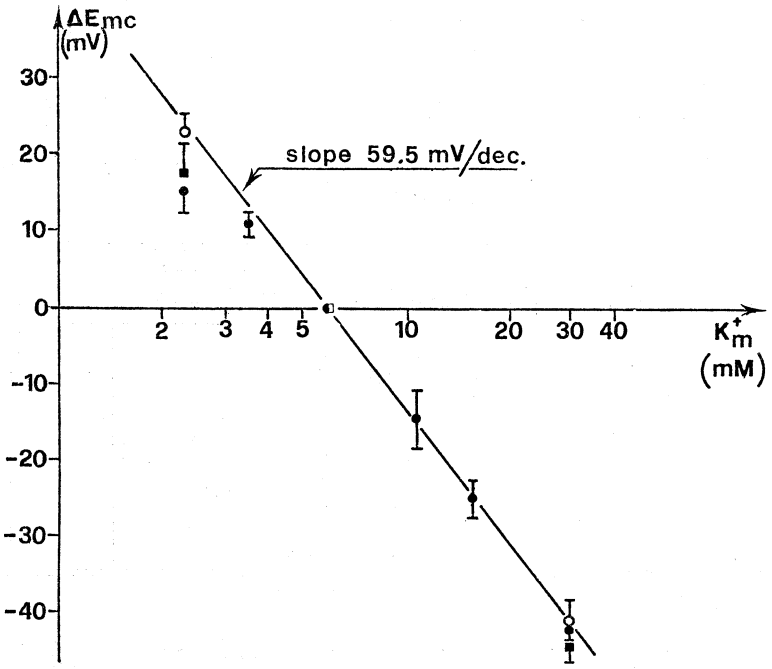

Fig. 2. – Effect of luminal K^+ concentration $[K^+]_m$ on the electromotive force (e.m.f.) of the luminal membrane. ΔE_{mc} are e.m.f. variations. The experiments were carried out in Krebs (●) or in SO_4^{2-} (■) or in choline (○) solution.

As a conclusion of the two electrophysiological approaches, we deduce that Cl^- conductance through both luminal and basolateral cell membranes is nearly zero.

When the tissue, cut open in a flat sheet, is incubated in Krebs-Henseleit solution labelled with $^{36}Cl^-$ or when the intact sac is incubated in a Krebs solution with $^{36}Cl^-$ only in the lumen, no significant differences in the intracellular Cl^- labelling are observed (see Table II). This result suggests that permeability of the basolateral barrier either is zero or, at least, is negligible with respect to that of the luminal barrier. Such a conclusion is confirmed when the sac is in contact with ^{36}Cl only on the blood side: after 1 hour incubation the isotope is not detected in the cell.

As shown above, not only the basolateral but also the luminal Cl^- conductance seems about zero. Therefore, we suggest [3] that Cl^- influx into the

(3) We suggested it in the 2nd International Symposium on Bioelectrochemistry and Bioenergetics (Pont à Mousson, 1973).

cell from the lumen is due to a coupling with Na^+ on a carrier as it is for intestine [12]. In fact, when choline is substituted for Na^+ and $^{36}Cl^-$ is in the lumen for 1 hr, intracellular Cl^- labelling is very low (a small Na^+ concentration is always present in the lumen: even after three washings it is 2–5 mM).

TABLE II.

Ratios between intracellular Cl^- *concentrations measured by chemical* (Cl_i^-) *and radiochemical* ($^*Cl_i^-$) *methods.*

Luminal and serosal solution	$^{36}Cl^-$ in	$[^*Cl^-]_i/[Cl^-]_i$ mean ± S.E.M.	Number of Experiments
Krebs	both media	0.82±0.13	9
Krebs	luminal medium	0.73±0.08	5
Krebs	serosal medium	0.09±0.06	5
Choline	luminal medium	0.16±0.05	8
Choline	serosal medium	—0.04±0.03	10

We have now the possibility to experimentally reduce the luminal Cl^- permeability near to zero and to demonstrate that basolateral Cl^- permeability is not only relatively low, but also negligible as absolute value. We incubate the sac for 1 hr in choline solution in luminal and serosal media, with $^{36}Cl^-$ in contact with blood side.

In such an experiment $^{36}Cl^-$ cannot be largely lost in the lumen through the mucosal cell membrane; it should be detected within the cell if it enters through the basolateral membrane. Nevertheless we do not find $^{36}Cl^-$ in the cell.

The experiments reported demonstrate that the luminal membrane potential is largely due to K^+ and it is negligibly shortcircuited by Na^+ influx. A second fraction of Na^+ influx is neutrally coupled to Cl^-. This cotransport is not necessarily an active one: the electrochemical potential gradient for Na^+ (142.9 mM outside the cell, 50 mM of rapidly exchangeable pool inside the cell, —58 mV the electrical potential) and for Cl^- (127.7 mM outside the cell, 68 mM of rapidly exchangeable pool inside the cell). Evidence for a similar process is reported also for intestine [12], in which, however, it accounts only for 20 % of the total influx.

The basolateral membrane potential seems mainly due to K^+ [9]. K^+ is taken up into the cell by the Na^+–K^+ activated ATPase system [15] and Na^+ is extruded to the blood side. Our demonstration that Cl^- conductance is negligible and that $^{36}Cl^-$ does not enter the basolateral membrane seems

to rule out the possibility that Cl^- passively leaves the cell according to its favourable electrochemical potential gradient. It is, however, true that cell labelling by $^{36}Cl^-$ through the serosal barrier may be largely reduced by the negative intracellular potential; and cell labelling would be further reduced, if a site with a low conductance for Cl^- is localized at the apical end of the intercellular spaces, on the lateral cell membrane. Moreover, in this case, the conductance of the whole basolateral cell membrane would be likely found negligible. So the present data are not sufficient to conclude if Cl^- outflux (cell $\rightarrow$ serosa) is electrically coupled with Na^+ outflux or if Cl^- must be neutrally transported together with Na^+, owing to an impermeability of the serosal barrier.

On the contrary, it seems possible to exclude that Cl^- is coupled with Na^+ transport via tight junctions. In fact, Cl^- conductance in the tight junctions is very low in spite of the large total conductance ($G_{Cl}/G_{tot} = 0.074$) [1].

Thus, a transepithelial p.d. of some ten mV (serosa positive) is necessary to account for the actual net Cl^- transport. However, transepithelial p.d. in rabbit gallbladder is negative ($-0.7 \div -2.4$ mV) [10] and the transport should also take place against the concentration gradient between intercellular channels and lumen.

A large positive p.d. across tight junctions could not be hidden by an opposite p.d. across the channel length. These two p.d.'s should be caused by current loops due to an e.m.f. localized at the apical end of the intercellular channels. However, in the transporting gallbladder the channels are open and the resistance is negligible in respect to the tight junction resistance [14]. This makes negligible the potential drop on the electrical resistance of the channels.

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