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Membrane potential and resistance in rabbit gallbladder epithelial cells

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Fisiologia. — *Membrane potential and resistance in rabbit gall bladder epithelial cells* (*). Nota di SILVIO HÉNIN, DARIO CREMASCHI e ARNALDO FERRONI, presentata (**) dal Corrisp. V. CAPRARO.

RIASSUNTO. — Il profilo di potenziale elettrico nelle cellule epiteliali di cistifellea di coniglio presenta due brusche variazioni, una mucosale ed una serosale, entrambe pari a $-41{,}9 \pm 2{,}3$ mV (153 esp.). Le resistenze elettriche corrispondenti alle due membrane cellulari (mucosale e serosale) sono pari a $26{,}2 \pm 2{,}7$, $41{,}1 \pm 6{,}9$ MΩ (6 esp.).

INTRODUCTION

Intracellular electric profile measurements were carried out on the following epithelia: small intestine [1], colon [1], renal tubules and collecting ducts [2], toad and turtle urinary bladder [3, 4] and frog skin [5]. This article reports the techniques adopted and the preliminary observations on the electrical characteristics of rabbit gall bladder.

METHODS

Gall bladders were excised from ordinary rabbits, washed free from bile with bicarbonate Krebs–Henseleit solution and opened lengthwise. The tissue was held horizontally between two lucite chambers (fig. 1). The perfusion medium was Krebs–Henseleit bicarbonate solution bubbled with 95 % O_2–5 % CO_2 at 27° C ± 1. 8 ml of solution were present in the serosal chamber and 2 ml in the mucosal one; the latter was renewed continuously to avoid concentration changes with time.

Recording microelectrodes were filled with 3 M KCl. Microelectrode impedance measurements were performed in Krebs–Henseleit solution with A.C. current (15 Hz) by an automatic device [6], adapted from a circuit of O. Schanne [7]. Impedance values were obtained comparing the potential drop between the microelectrode and ground with a potential drop at the ends of a standard resistance. The error was less than 0.05 % in a range of resistance values 0 to 70 MΩ. Microelectrodes with 30 MΩ impedance were used. Block diagram of the experimental arrangement is shown in fig. 1. Intracellular potential measurements with respect to mucosal (V_m) and serosal (V_s) media could be performed using a switch.

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(**) Nella seduta dell'8 aprile 1972.

Tissue and cell membrane impedance measurements were carried out by a combination of two techniques:

1) the impedance between ground and microelectrode was measured before and during impalement; the difference of these values represented the impedance of mucosal (R_m) and serosal (R_s) membranes in parallel;

2) an anodic and cathodic D.C. current (200 μA, 1 sec pulses) was passed across the tissue during impalement using Ag/AgCl electrodes. P.D. between serosal and mucosal Agar bridges and between these and the microelectrode was measured.

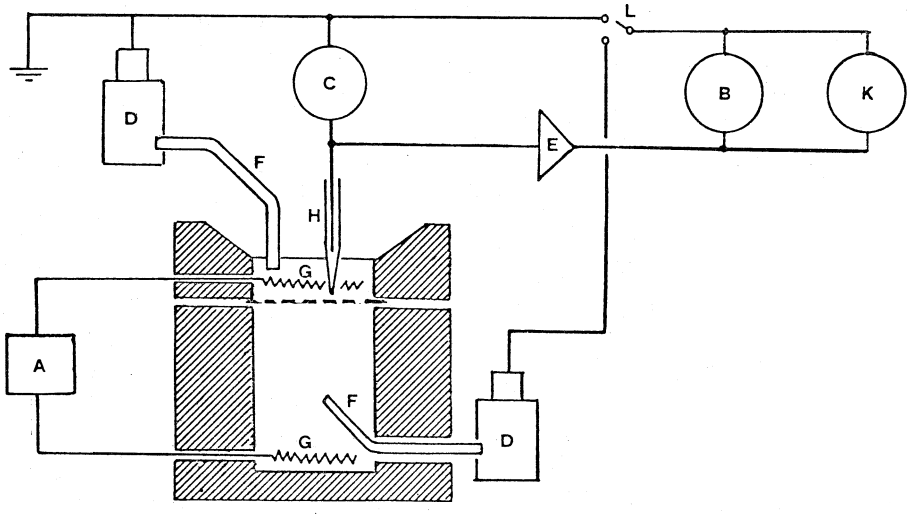

Fig. 1. – Block diagram of the circuit used for micropuncture of Rabbit gallbladder epithelium. The microelectrode (H) is connected by an Ag/AgCl wire and probe (E) (I.L. 181) to the differential input of a dual beam oscilloscope (B) (Tektronix R 5030) and to a chart-recorder (K) (Varian G 1000). Reference electrodes are matched calomel half cells (D), connected by agar 3 M KCl bridges (F) to serosal and mucosal media. The impedance recorder (C) is connected in parallel with the probe to the microelectrode. P.D. measurements with respect to mucosal and serosal media can be performed using a switch (L). D.C. Pulses (Generator A) are passed through the tissue by two Ag/AgCl electrodes (G).

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 microelectrode and serosal bridge after penetrating through the serosal membrane, and the P.D. between mucosal and serosal bridge without the tissue.

We will refer to the corrected value as ΔV_m, ΔV_s, ΔV_t. Penetration through the serosal membrane was denoted by a return of the V_s to the baseline (cfr. fig. 3). We obtained the ratio R_m/R_s from the $\Delta V_m/\Delta V_s$ values recorded with method 2. The absolute values R_m and R_s are then calculated by equating the above ratio with the results from method 1.

RESULTS AND DISCUSSION

Measurements were accepted according to the following criteria:

a) an abrupt P.D. change and impedance increase between microelectrode and ground on advancing;

b) a constant P.D. during impalement of at least 10 sec. The intracellular potential remains constant for impalements lasting also several minutes;

c) an abrupt return to the baseline for both potential and impedance on withdrawl.

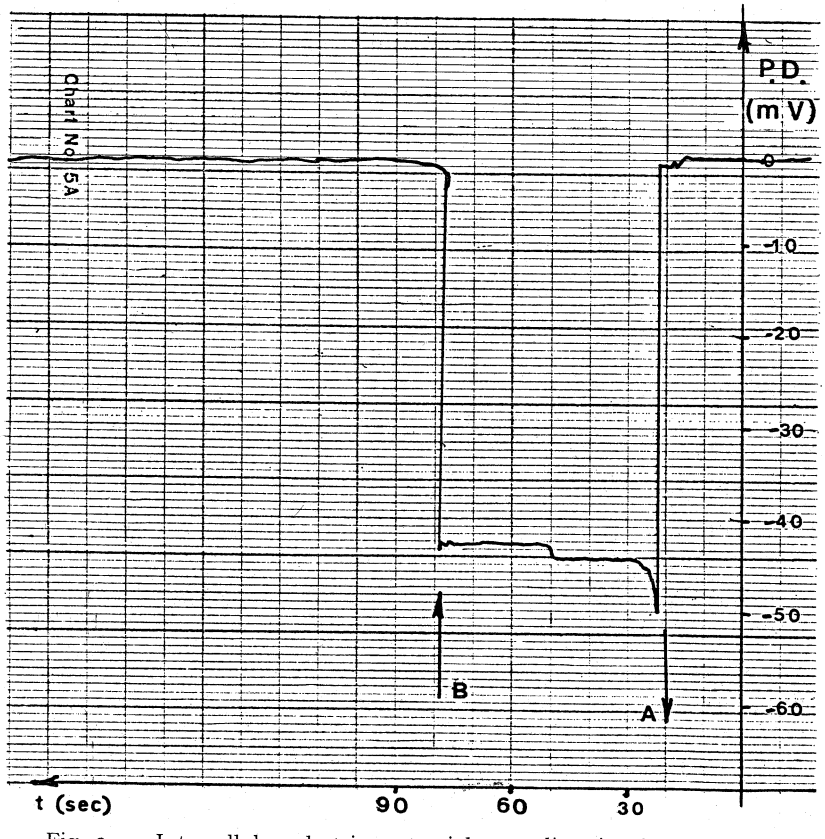

Fig. 2. – Intracellular electric potential recording in the epithelial cells of rabbit gallbladder. Explanation in the text.

The recording of an intracellular potential measure is shown in fig. 2. On penetration (A) an abrupt potential drop of — 50 mV is noted which rapidly declines and stabilizes at — 45 mV. The microelectrode is removed after 1 min and the potential returns to the baseline (B).

In the measurements accepted the intracellular potential decline was 0–10 % of its initial value and is probably due to an incomplete sealing of

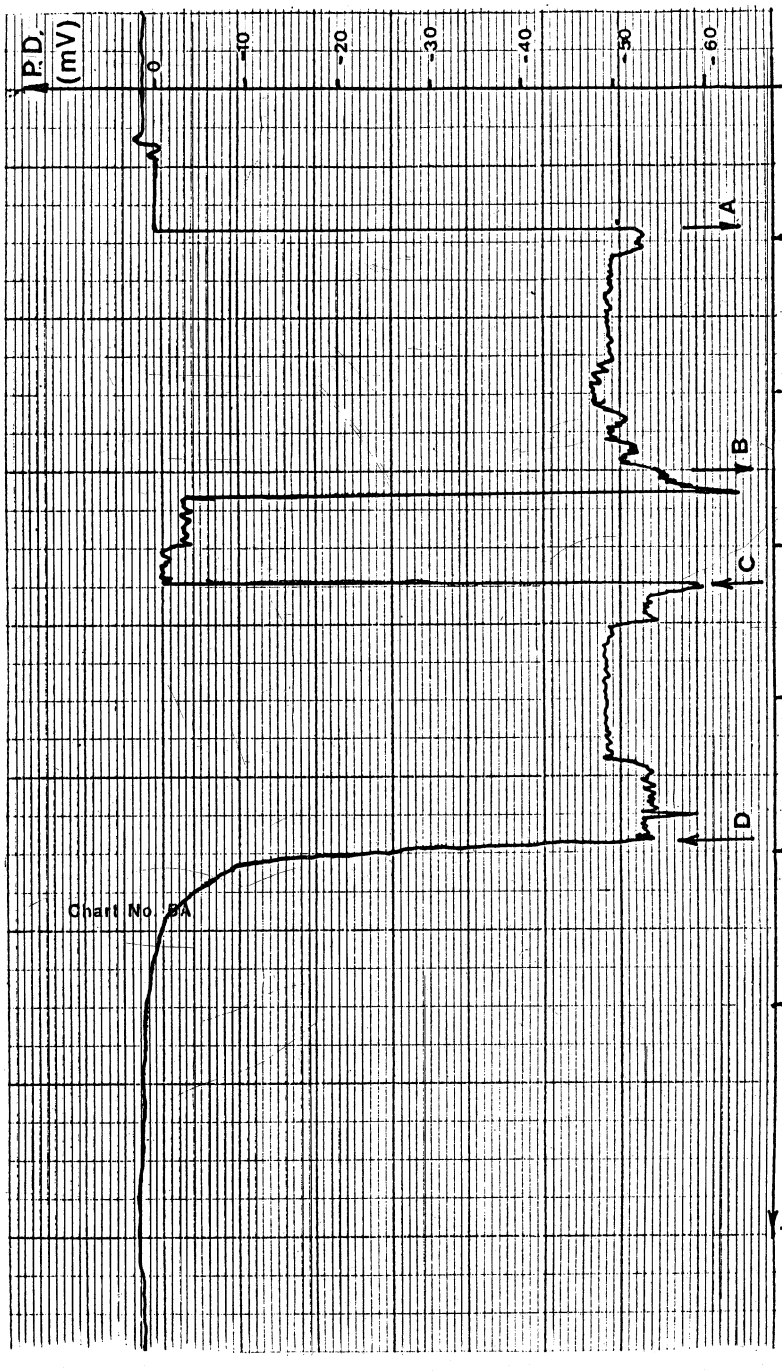

Fig. 3. – Intracellular electric potential recording in the epithelial cells of rabbit gallbladder. Explanation in the text.

the membrane around the electrode. In fact occasionally the potential returned slowly to the original value.

In some experiments (e.g. fig. 3), after recording the intracellular P.D. (A–B) we moved the microelectrode further in and recorded a return to zero by the potential (B–C). Then we slowly drew the microelectrode and recorded a P.D. similar to that of advancement (C–D). Since in gallbladder epithelium the transmural potential is approximatively zero [8] we can deduce that:

1) the abrupt return of the P.D. to the baseline (B) indicates that the cell has been pierced right through;

2) the cell has not been injured by microelectrode tip;

3) there are no electric potential gradients within the cytoplasm.

TABLE I.

Intracellular electric potential of rabbit gallbladder epithelial cells.

Gallbladder n.	P. D. mV Mean±S.E.	N. of Impalements
1	43.8 ± 4.7	9
2	32.1 ± 1.9	13
3	39.5 ± 1.1	34
4	37.8 ± 1.9	6
5	43.1 ± 1.7	33
6	35.7 ± 1.7	7
7	48.5 ± 2.6	17
8	53.8 ± 1.1	14
9	50.5 ± 2.2	13
10	39.0 ± 1.2	7
	41.9 ± 2.3	

In Table I intracellular potential measurements are reported. An average of 41.9±2.3 mV was obtained by impaling 153 cells from 10 gallbladders. The intracellular potential is always negative with respect to the mucosal and serosal medium. This value should not have been affected by tip potential changes when going from the perfusion to the intracellular medium. In fact the tip potential changes recorded on passing to a solution resembling the intracellular medium (1) was —0.5±1.1 mV (3 exp.) (2).

(1) Cytrate as a large anion 14 mM, Cl^- 83 mM, HCO_3^- 24.9 mM, Na^+ 113 mM, K^+ 38.9 mM, pH 7.4. Direct measurements of intracellular Na^+ and Cl^- concentrations were carried out using Inulin ^{14}C as an extracellular space marker.

(2) Reference electrode: H^+ glass electrode.

TABLE II.

Electric resistances in the rabbit gallbladder.

R_w (Ω cm^2)	R_e (Ω cm^2)	R_m (MΩ)	R_s (MΩ)	R_m/R_s
32.6 (*)	22.0 (*)	26.2 (*)	41.1 (*)	0.71 (*)
$\pm$ 5.0	$\pm$ 2.3	$\pm$ 2.7	$\pm$ 6.9	$\pm$ 0.14
(8)	(9)	(6)	(6)	(8)

R_w: resistance of the gallbladder wall. R_e: resistance of the gallbladder epithelium. R_s: resistance of the serosal barrier in the epithelial cell. R_m: resistance of the mucosal barrier in the epithelial cell. (*) Mean $\pm$ S.E., *n* of experiments in brackets.

The electric resistance values of the gallbladder wall, of the epithelium and of the cellular membranes are reported in Table II. The resistance of the serosal plasma membrane (R_s) is larger than that of the mucosal one (R_m).

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