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Electrical Parameters in Gallbladders of Different Species. Their Contribution to the Origin of the Transmural Potential Difference

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Summary. Amphotericin B enhances Na^+ conductance of the mucosal membrane of gallbladder epithelial cells and in such a way it modifies the brush border electromotive force. On this basis a method to measure cell and shunt resistances by comparing changes of the mucosal membrane potential (V_m) and of the transmural p.d. (V_{ms}) is developed. This method is applied in gallbladders of different vertebrate species (i.e. rabbit, guinea pig, goose, tortoise, toad, trout). The two tested mammals, rabbit and guinea pig, exhibited a lower shunting percentage (89–93%) than the nonmammals (96–97%), but this fact did not bring about a homogeneous positive V_{ms}. This means that shunting percent contributes, but it is not the only source of differences in V_{ms}, in accordance with that reported by Gelarden and Rose (*J. Membrane Biol.* **19**:37, 1974). Moreover, mammals exhibited a lower luminal resistance and a lower ratio between luminal and basolateral resistance than nonmammals. Possible causes of these differences are discussed.

It is well known that gallbladders of different species exhibit different transepithelial p.d.'s, either serosa negative, or positive or equal to zero [4, 13, 17]. The negative p.d., measured across rabbit gallbladder, has been explained as a negative backdiffusion potential due to Na^+ salts moving from the lateral spaces into the lumen: Na^+ mobility is increased with respect to the anion's by the neutral/negative fixed charges at the tight junctions [17, 28]. Then a positive p.d. could be explained by positive fixed charges at the junctions and by positive backdiffusion potentials [13]. However, the study of tight-junction selectivity in different animal species, by artificially imposed diffusion potentials, has ruled out this hypothesis: fixed charges are always neutral/negative, never positive [13]. It has been suggested that different serosa positive p.d.'s developed by the cell (asymmetry of the serosal and mucosal membrane potential,

due perhaps to an electrogenic Na^+ pump at the serosal barrier) and different serosa negative backdiffusion p.d.'s, added together, explain all the found transepithelial p.d.'s [13]. It is also possible that equal electrical asymmetries of the two cell membranes are differently short-circuited through tight junctions, in different species.

The aim of this work is to study whether or not a correlation exists between shunting and transepithelial p.d., i.e. to see if positive transepithelial p.d.'s are present in those species in which the shunting effect is smaller. The examined animal species (rabbit, guinea pig, goose, tortoise, toad, trout) were of different vertebrate classes. The histology of the correspondent gallbladders appeared very different with different foldings and microvilli. None of these epithelia was a flat sheet. Thus, it was impossible to use the flat cable theory in order to measure tight-junction and cell resistances. We have thus taken advantage of amphotericin B capability to modify the luminal cell resistance and electromotive force, without altering other electrical parameters, at least in the first 10-min period of treatment [5]. Cell and tight-junction resistances can be evaluated by comparing luminal membrane potential change with transepithelial p.d. change.

Theoretical Considerations

Let us consider for gallbladder epithelium the electrical model reported in Fig. 1. We define: R_m, R_s: the mucosal and serosal cell resistances; R_{sh}: the shunt resistance; R_c: the cell resistance; R_{ep}: the epithelium resistance; α: the R_m/R_s ratio; E_m, E_s: the mucosal and serosal electromotive forces; E_{sh}: the electromotive force in the shunt; V_m, V_s: the mucosal and serosal membrane potentials; V_{ms}: the transepithelial p.d.

Let us suppose that in the first 10-min period of its action amphotericin B modifies only R_m and E_m so that R_s, E_s and E_{sh} remain constant. About the validity of this supposition *see* the first paper of this series. [5]. We call R'_m the changed mucosal resistance, and α' the R'_m/R_s ratio, R'_c and R'_{ep} the changed cellular and epithelial resistances, R'_{sh} the shunt resistance during treatment. The prefix Δ indicates changes due to the antibiotic addition. So we have:

$$\Delta V_m = \Delta E_m - \Delta E_m \frac{R'_m}{R'_m + R_s + R'_{sh}}$$

$$= \Delta E_m \left(1 - \frac{R'_m}{R'_m + R_s + R'_{sh}}\right) = \Delta E_m \left(\frac{R_s + R'_{sh}}{R'_m + R_s + R'_{sh}}\right) \qquad (1)$$

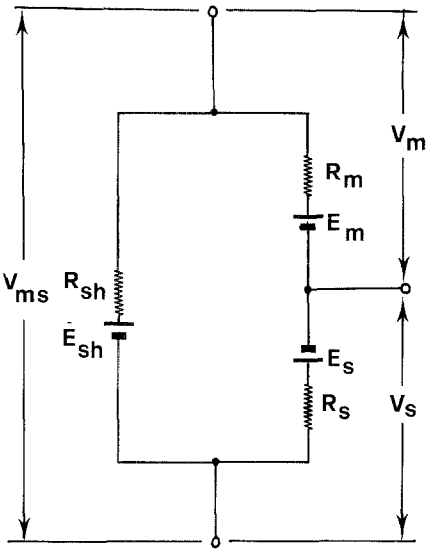

Fig. 1. Electrical circuit correspondent to the cell and to the shunt pathways of the epithelial layer. For symbol explanation *see* text

$$\Delta V_{ms} = \Delta E_m \frac{R'_{sh}}{R'_m + R_s + R'_{sh}} \tag{2}$$

$$\frac{\Delta V_m}{\Delta V_{ms}} = \frac{R_s + R'_{sh}}{R'_{sh}} = \frac{R_s}{R'_{sh}} + 1 \tag{3}$$

$$R_s = \left(\frac{\Delta V_m}{\Delta V_{ms}} - 1\right) R'_{sh}. \tag{4}$$

Since $R'_c = R'_m + R_s$, we have:

$$R'_m = \alpha' R_s \tag{5}$$

$$R'_c = \alpha' R_s + R_s = R_s(1 + \alpha') \tag{6}$$

$$R'_c = \left(\frac{\Delta V_m}{\Delta V_{ms}} - 1\right) R'_{sh}(1 + \alpha'). \tag{7}$$

On this basis the resistance ratio between cellular and extracellular pathway is:

$$\frac{R'_c}{R'_{sh}} = \left(\frac{\Delta V_m}{\Delta V_{ms}} - 1\right)(1 + \alpha'). \tag{8}$$

Since $1/R'_{ep} = 1/R'_c + 1/R'_{sh}$ we have:

$$\frac{R'_{sh}}{R'_{ep}} = \frac{R'_{sh}}{R'_c} + 1 \tag{9}$$

$$R'_{sh} = R'_{ep}\left(\frac{R'_{sh}}{R'_c} + 1\right) = R'_{ep}\left(\frac{1}{\left(\frac{\Delta V_m}{\Delta V_{ms}} - 1\right)(1+\alpha')} + 1\right). \tag{10}$$

Since R'_{ep}, ΔV_m, ΔV_{ms} and α' can be measured, on the basis of Eqs. (8) and (10) R'_{sh} is calculated. R_s is then evaluated from Eq. (4).

The tissue physiological parameters in the absence of the antibiotic are determined on the assumption that R_s is not modified by the antibiotic and by considering that:

$$R_m = \alpha R_s \tag{11}$$

$$R_c = R_m + R_s \tag{12}$$

$$\frac{1}{R_{sh}} = \frac{1}{R_{ep}} - \frac{1}{R_c} = \frac{R_c - R_{ep}}{R_{ep} R_c} \tag{13}$$

$$R_{sh} = \frac{R_{ep} R_c}{R_c - R_{ep}}. \tag{14}$$

Shunt conductance as a per cent of the epithelium conductance is:

$$\frac{G_{sh}}{G_{ep}}\,\% = \left(\frac{1}{R_{sh}} \Big/ \frac{1}{R_{ep}}\right) 100. \tag{15}$$

Thus, the measurements needed for these calculations are: *i*) R_m/R_s, R_{ep}, V_m and V_{ms} under control conditions and *ii*) R'_m/R_s, R'_{ep}, V'_m and V'_{m_S} under treatment with the polyene.

Finally, it is to be emphasized that the experiment must be discarded if R_{ep} increases after amphotericin B treatment. In fact, this would correspond to the situation in which lateral spaces have closed, so that a no longer negligible channel resistance is added to tight-junction resistance [23]. Then the serosal resistance would also increase, as the lateral surface of the cell is no longer easily available for current transport. So, one of the two main assumptions, R_s constant, fails. The experiment must also be discarded when the calculated R'_{sh} is less than R_{sh}: in this case E_{sh} could be changed. This point was crucial for guinea pig. Three experiments were discarded for this reason.

Materials and Methods

Electrophysiological Experiments

Gallbladders from ordinary rabbits were excised, washed free from bile with the appropriate saline and opened lengthwise. The flat sheet held horizontally between two lucite chambers, with the epithelium upwards. The serosal and mucosal chambers were filled with 4 and 2 ml of incubation solution, respectively; both solutions were continuously renewed. An incubation solution added with amphotericin B (40 μg/ml) could flow in the mucosal chamber when desired. For microelectrode preparation and the electrical lay-out of the set up *see* refs. [5] and [14]. The saline used for mammals and trout [8] was Krebs-Henseleit solution and had the following composition (mM): Na^+ 142.9; K^+ 5.9; Ca^{2+} 2.5; Mg^{2+} 1.2; Cl^- 127.7; HCO_3^- 24.9; $H_2PO_4^-$ 1.2; SO_4^{2-} 1.2. It was bubbled with 95% O_2+5% CO_2; pH was 7.4. The saline used for goose was (mM): Na^+ 142.9; K^+ 5.9; Ca^{2+} 2.5; Mg^{2+} 1.2; Cl^- 151.9; SO_4^{2-} 1.2; phosphate buffer 2.6 pH 7.2; glucose 5.5. It was bubbled with 100% O_2. The saline used for toad was the following (mM): Na^+ 116; K^+ 2; Ca^{2+} 1; Cl^- 114; HPO_4^{2-} 0.85; $H_2PO_4^-$ 2.1; pH 7.2. It was bubbled with air. The saline used for tortoise had the following composition (mM): Na^+ 118.8; K^+ 2.0; Ca^{2+} 2.5; HPO_4^{2-} 3.7; $H_2PO_4^-$ 1.7; pH 7. It was bubbled with air.

Rabbits and guinea pigs were fed with pellets (ClR, P.A.L.A.T.A. Spa, S. Agata Bolognese, Italy); the incubation temperature for their gallbladders was 27 °C. Geese were fed with pellets, bread, water and their gallbladders were incubated at 27 °C. Tortoises (*Testudo graeca*) were kept at room temperature (27 °C, July) and fed with lettuce; incubation temperature was 27 °C. Toads (*Bufo bufo*) were kept at room temperature (23 °C, March–May) and fed with fly larvae; incubation temperature was 23 °C. Trouts (*Salmo gairdneri*) were kept in an aquarium bubbled with air, at 15 °C; incubation temperature was 15 °C.

Microscopy

Isolated gallbladders of the different species were incubated in the appropriate solutions (as described above) at the appropriate temperature for 30 min. The tissue was opened as a flat sheet and fixed for electron-microscopy. The specimens have been immersed in 2% gluteraldehyde in 0.1 M cacodylate buffer and immediately cut in small pieces. After a brief washing in buffer, the specimens were postfixed in cacodylate buffered osmium tetroxide. For transmission electron-microscopy (TEM), after an "en bloc" prestain in 1% aqueous uranyl acetate, the cuttings were rapidly dehydrated in a graded ethanol series and embedded in an Epon 812-Araldite mixture. Sections were obtained with an LKB Ultratome III, collected on uncoated grids, stained with lead citrate and observed with a Hitachi HU 11 ES electron-microscope. Semi-thin sections, collected on microscope slides, have been stained according to Sato and Shamoto [21] and photographed with a Zeiss Axiomat optical microscope.

For scanning electron-microscope (SEM) the specimens, after osmium fixation, were treated by the technique of Kelly *et al.* [15], dehydrated by a graded ethanol series, air-dried and observed with a Hitachi SS M2 Scanscope.

Results

Tissue Electrical Parameters

The six studied animals represent five different vertebrate classes. Their gallbladder transepithelial p.d.'s (V_{ms}) are very slight (Table 1),

negative in some species (rabbit and trout), positive in others (guinea pig and goose), distributed around zero in the remaining species (tortoise and toad)[1]. In rabbit we measured a V_{ms} equal to -0.1–0.2 mV, a value which is smaller than that measured by Machen and Diamond, i.e. -1.7 mV [17], but the lower incubation temperature (27 °C against 37 °C) can explain this discrepancy; also Gelarden and Rose [13] measured -0.1 ± 0.1 mV (4 exp.) at 22 °C and even -0.2 ± 0.1 mV (9 exp.) at 37 °C.

In guinea pig, V_{ms} measured 15 min after tissue isolation, was some mV large, but rapidly decreased to some hundred microvolts at 30–40 min: in all of the animals studied it was serosa positive (13 exp.).

In the same way, in all the geese studied V_{ms} was positive (8 exp.), but its value was 0.6–0.7 mV throughout the experiment. This value, measured at 27 °C, is between those reported by Gelarden and Rose [13] for 22 °C (+0.4 mV) and 37 °C (+3.6 mV). In tortoise and toad some animals exhibited negative and some positive V_{ms}. In trout V_{ms} was negative of some mV in all the examined animals and increased its negativity with time.

The luminal membrane potential (V_m) is negative of some 10 mV in all the studied species (about -60 mV, in rabbit and toad, about -50 mV in goose, tortoise and trout, and about -40 mV in guinea pig).

The transmural resistance is equal to some $10 \Omega\,cm^2$ in all of the species studied. The percentage of tissue resistance related to subepithelial layers is very different with species and, in the same species largely dependent on the filling of the gallbladder with bile before the isolation. This last fact was especially true for goose in which the resistance of subepithelial layers (R_{se}) amounted to 43% of R_t in the filled gallbladders (Table 1), but to 80% of R_t in the emptied organs. Wright and Diamond

1 Such differences in transmural potentials can be affected not only by species differences, but also by differences in temperature and saline composition. On the other hand it is unjustified to use the same temperature for omeotherms and eterotherms. Concerning saline composition, the presence of bicarbonate and CO_2, which stimulate Na^+ salt transport, could affect the transmural p.d. In rabbit the absence of bicarbonate reduces the negativity of transmural p.d. by reducing the back diffusion potential from the lateral spaces to the lumen [17]. In goose bicarbonate addition to the incubation medium reverses the p.d. from positive to negative values [13]. For discussion of bicarbonate effects in gallbladders of other species *see* ref. [13]. In our case we have used bicarbonate Krebs-Henseleit solution for the two mammal species and for trout. Bicarbonate was not present in the saline used for goose as we preferred to employ a solution as similar as possible to the solution used by Gelarden and Rose [13]. As for toad, the addition of 2.3 mM HCO_3^- did not significantly change the transmural p.d.

Table 1. Gallbladder electrical parameters

Animal	V_{ms}		V_m	R_t	R_{ep}	R_m/R_s
	10–20 min	30–40 min				
Rabbit	−0.1 ±0.1 (34)	−0.2 ±0.1 (27)	−59.0 ±0.9 (115-10)	30.4 ±1.8 (13)	19.3 ±2.6 (6)	1.09 ±0.07 (44-6)
Guinea pig	+2.4 ±0.5 (12)	+0.8 ±0.2 (13)	−42.3 ±0.8 (149-13)	50.3 ±5.8 (12)	44.4 ±6.2 (8)	0.99 ±0.05 (98-10)
Goose	+0.7 ±0.1 (8)	+0.6 ±0.2 (6)	−48.7 ±0.8 (118-9)	51.5 ±10.4 (4)	28.4 ±2.0 (8)	1.76 ±0.08 (93-9)
Tortoise	+1.7 ±0.9 (4)	+1.2 ±1.3 (4)	−50.3 ±1.2 (79-4)	106.1 ±7.0 (4)	94.5 ±13.9 (4)	1.70 ±0.12 (35-4)
Toad	–	+0.1 ±0.3 (9)	−62.2 ±0.6 (230-7)	87.7 ±10.0 (10)	65.6 ±7.6 (10)	2.55 ±0.21 (61-6)
Trout	−2.7 ±0.8 (8)	−3.5 ±0.6 (7)	−49.9 ±1.1 (103-9)	41.9 ±3.0 (7)	28.2 ±1.4 (5)	2.68 ±0.21 (50-5)

The transepithelial p.d. (V_{ms}) was measured 10–20 min and 30–40 min after the organ isolation. The luminal membrane potential (V_m) and the ratio between mucosal and serosal cell resistances (R_m/R_s) were steady a few minutes after the isolation, over a period of many hours. The whole tissue (R_t) and the epithelium (R_{ep}) resistances were measured 30–40 min after isolation.

V_{ms} and V_m are reported as mV (mean ± SEM). R_t and R_{ep} are reported as Ω cm^2 (mean ± SEM). The figure alone or the second in parentheses indicates the number of animals examined; the first figure in parentheses indicates the number of cells tested.

[29] reported for rabbit a R_{se} equal to 6% of the tissue resistance, a value much lower than ours. It is to be pointed out, however, that in their case value was obtained after destroying the epithelium with chloroform or with a scalpel, techniques which can damage the subepithelial layers. In our case R_{se} was measured by piercing the epithelium from the mucosal side, with the voltage-recording microelectrode: the crossing of the epithelium was acknowledged by the p.d. return to the baseline [14]. In any case, our data are in good agreement with those of Gelarden and Rose [13].

R_m/R_s ratios are nearly 1 in the two mammals and larger than 1 for the animals of all the other classes tested.

Study of the Tissue with Amphotericin B

In ref. [4] effects of amphotericin B on V_{ms} and R_t, similar in different animal species, are reported. We can now confirm these results, i.e. the antibiotic has qualitatively equal effects in the different gallbladders tested here. V_{ms} in any case rapidly changes and becomes positive (or more positive) by several mV; V_m and R_m/R_s in parallel largely decrease.

The epithelial resistance generally remains nearly equal or slightly decreases; this is in accordance with the fact that R_{ep} is dominated by the shunt pathway, which is not affected by the antibiotic in the first minutes of action (as in Table 2, shown by the two columns R_{sh} and R'_{sh} and reported in ref. [5]). All of the data reported in Table 2 were in fact measured in the first 10 min after addition of amphotericin B.

In the toad gallbladder the antibiotic generated similar V_{ms} and V_m

Table 2. Electrical parameters used for the study of the tissue with amphotericin B

Animal	ΔV_{msA}	ΔV_{mA}	R_m/R_s	R'_m/R_s	R_{ep}	R'_{ep}	R_{sh}	R'_{sh}
Rabbit	+3.9 ±0.6 (6)	−31.3 ±3.2 (6)	1.09 ±0.07 (46-6)	0.44 ±0.05 (39-6)	19.3 ±2.6 (6)	16.9 ±1.7 (6)	20.8 ±2.8 (6)	18.4 ±1.9 (6)
Guinea pig	+2.1	−10.3	0.95 ±0.07	0.48 ±0.07	62.2	65.6	69.7	77.8
	(1)	(1)	(12-1)	(6-1)	(1)	(1)	(1)	(1)
Goose	+1.7 ±0.3 (4)	−22.1 ±4.3 (4)	1.73 ±0.11 (44-4)	0.56 ±0.11 (20-4)	29.0 ±2.7 (4)	29.0 ±2.9 (4)	29.9 ±2.8 (4)	30.5 ±2.9 (4)
Tortoise	+3.0 ±0.7 (4)	−29.8 ±1.9 (4)	1.70 ±0.12 (35-4)	0.49 ±0.04 (26-4)	94.5 ±13.9 (4)	87.6 ±11.6 (4)	99.1 ±15.6 (4)	95.4 ±13.8 (4)
Trout	+3.4 ±0.8 (5)	−24.5 ±3.7 (5)	2.68 ±0.21 (50-5)	1.30 ±0.12 (50-5)	28.2 ±1.4 (5)	26.9 ±3.1 (5)	29.4 ±1.5 (5)	28.7 ±3.5 (5)

ΔV_{ms} and ΔV_m, reported as mean ± SEM (mV) with the number of animals tested in parentheses, are the variations of the transepithelial and of the luminal membrane p.d.'s induced by the antibiotic. R_{ep} and R_m/R_s are the epithelium resistance and the ratio between mucosal and serosal cell resistances just before treatment; conversely, R'_{ep} and R'_m/R_s are the same parameters measured 10 min after treatment.

R_{sh} and R'_{sh} are the shunt resistances (both calculated by the amphotericin B method) before and after treatment respectively. R_{ep}, R'_{ep}, R_{sh}, R'_{sh} are expressed as $\Omega\,cm^2$. Values are reported as mean ± SEM; a figure alone or the second in parentheses indicates the number of animals examined; the first figure in parentheses indicates the number of cells tested.

changes as well as in the other species. However, the assumptions for the analysis were respected only for so few minutes that it was impossible to correctly measure R'_m/R_s. For this reason no data obtained from toad gallbladders are reported in Tables 2 and 3.

Cell and Shunt Resistances

The results of calculations of cell and shunt resistances, on the basis of the data of Table 2, are reported in Table 3. A comparison between different species is possible only for the ratio between the shunt and the epithelium conductance $[(G_{sh}/G_{ep})100]$. As a matter of fact, all of the other parameters are expressed not for real but for apparent area and it is clear from the micrographs reported that a square centimeter of apparent area corresponds to different square centimeters of real area in the different species. Thus, R_c, R_m, R_s, and R_{sh} cannot be compared without further corrections.

It is noteworthy that in the two mammals, $(G_{sh}/G_{ep})100$ is less than 95%, whereas that of all the other species is larger than 95%. Thus, electrical asymmetries of the two cell barriers are less short-circuited in guinea pig and rabbit (89–93%) than in goose, tortoise and trout

Table 3. Cell and shunt resistances calculated with the amphotericin B method

Animal	R_c	R_m	R_s	R_{sh}	$\frac{G_{sh}}{G_{ep}} \cdot 100$
Rabbit (6 exp)	299 ± 50	156 ± 27	143 ± 24	20.8 ± 2.8	93.1 ± 0.8
Guinea pig (1 exp)	575	280	295	69.7	89.2
Goose (4 exp)	1003 ± 79	625 ± 74	378 ± 19	29.9 ± 2.8	97.0 ± 0.2
Tortoise (4 exp)	2468 ± 268	1607 ± 253	861 ± 94	99.1 ± 15.6	95.8 ± 1.1
Trout (5 exp)	740 ± 81	528 ± 72	212 ± 33	29.4 ± 1.5	96.0 ± 0.5

The mucosal and serosal membrane (R_m, R_s), cell (R_c), shunt (R_{sh}) resistances are reported as Ω cm^2 (mean ± SEM).

$\frac{G_{sh}}{G_{ep}} \cdot 100$ indicates the ratio between shunt and epithelium conductances referred to 100.

Figures in parentheses represent the number of animals tested.

(96–97%). For *Necturus* gallbladder in which these calculations are based on a voltage spread analysis, Frömter [11] reports 96% in very good agreement with our data for nonmammals.

R_m Correction

In order to compare R_m, R_s, R_c, and R_{sh} in the different species it is necessary to know how large is the real area with respect to the apparent area. This is possible with good approximation for the luminal

Table 4. Correction of the exposed apparent area of the epithelium concerning mucosa foldings (FCF)

Animals	Cell apical diameter (μm)	Tall foldings				Low Foldings				FCF
		Length (mm/mm²)	Cell number in the cross-section	Cross-section length (mm)	CF	Length (mm/mm²)	Cell number in the cross-section	Cross-section length (mm)	CF	
Rabbit	7.0	5.4	80	0.56	3.0	5.8	46	0.32	1.9	4.9
Guinea pig	8.7	3.9	44	0.38	1.5	3.7	1.7	0.15	0.6	2.1
Goose	4.7	1.9	320	1.50	2.9	–	–	–	–	2.9
Tortoise	5.4	5.4	65	0.35	1.9	–	–	–	–	1.9
Toad	8.0	6.0	30	0.24	1.5	–	–	–	–	1,5
Trout	4.8	6.6	45	0.22	1.4	–	–	–	–	1.4

In mammals tall and low foldings were observed. In the nonmammals it was possible to take into account a mean folding. The correction factor due to the foldings is reported in the last column (FCF): in mammals it is obtained by the sum of the two partial correction factors (CF) for tall and low foldings (5th and 9th columns). FCF is the real area of the foldings per mm² of apparent area.

The folding real area is calculated from the product of the folding total length per mm² of apparent area (2nd and 6th columns) and the cross-section length (4th and 8th columns). The cross-section length is calculated by multiplying the cell number of the cross-section (3rd and 7th columns) and the apical diameter of the cell (1st column).

All of the reported data are means of many measurements taken on different regions of the tissue.

Fig. 2. Foldings, cells and microvilli of rabbit (*a, b, c, d*) and guinea pig (*e, f, g, h*) gallbladders. (*a, e*) Folding cross-section: ordinary light microscopy (12.5×). (*b, f*) Cells and microvilli: transmission electron-microscopy ($b=4200\times$; $f=3000\times$ for cells; 27,000× and 42,000× are the correspondent magnifications for inserts with microvilli). (*c, g*) Apical cell surface: scanning electron-microscopy ($c=3750\times$; $g=2500\times$). (*d, h*) Epithelium surface: scanning electron-microscopy (100×). From many micrographs of the *a, b, c, d* type, the mean measurements reported in Tables 4 and 5 were calculated

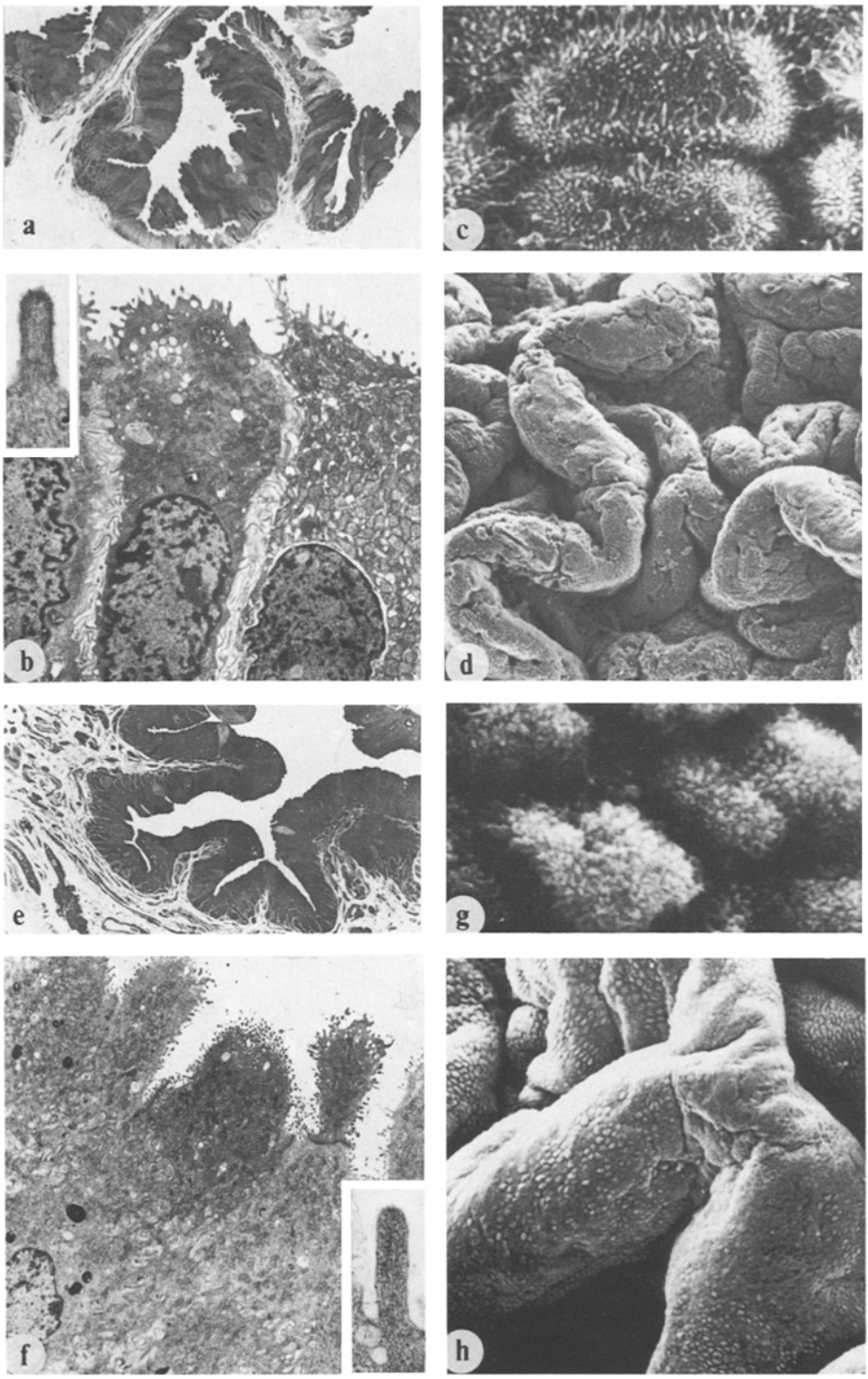

Fig. 2

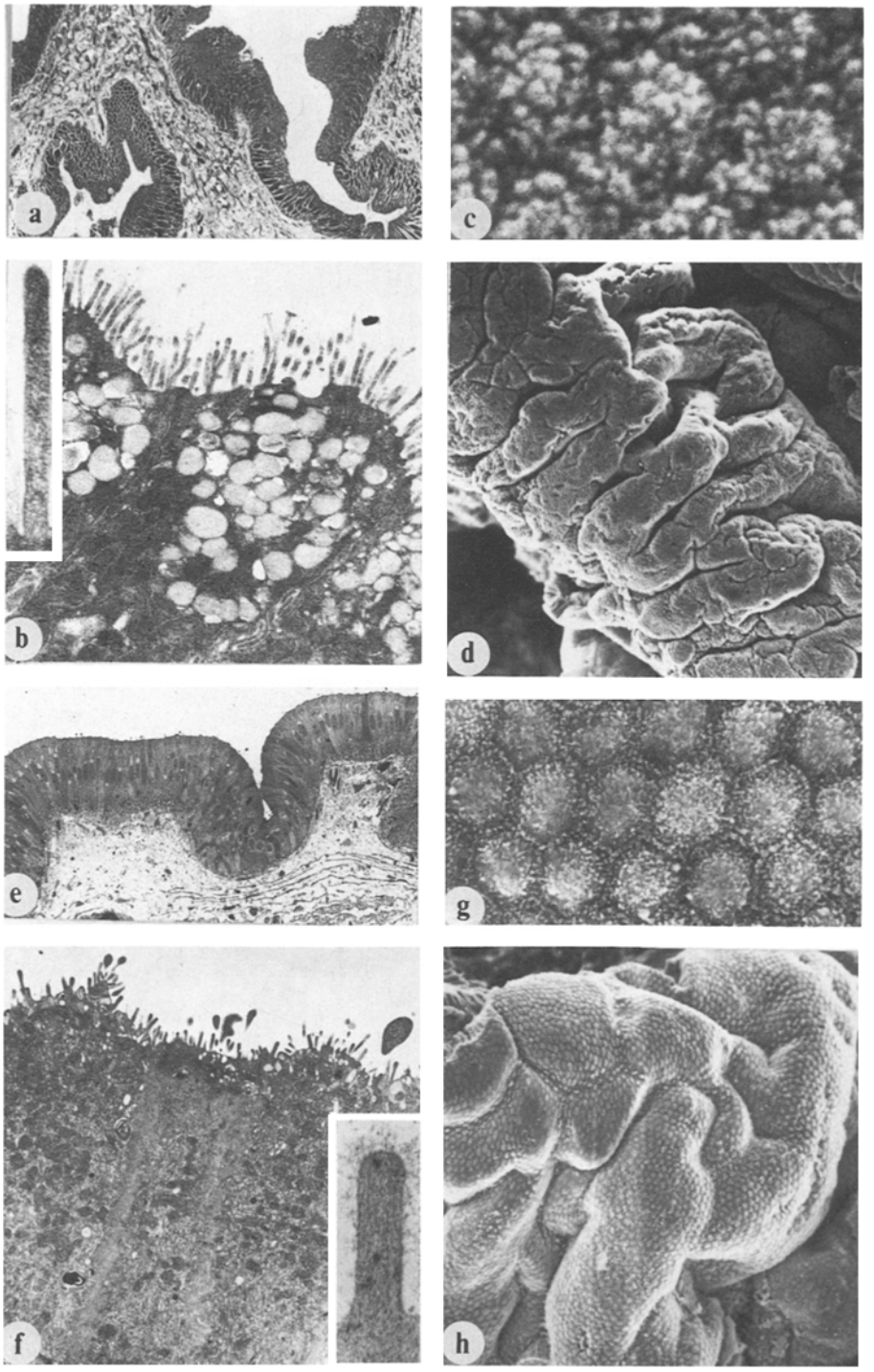

Fig. 3. Foldings, cells and microvilli of goose (*a, b, c, d*) and of tortoise (*e, f, g, h*) gallbladders. (*a, e*) Folding cross-section (12.5×). (*b, f*) Cells ($b=7500\times$; $f=3500\times$) and microvilli ($b=34{,}500\times$; $f=42{,}000\times$). (*c, g*) Apical cell surface ($c=5000\times$; $g=2000+$). (*d, h*) Epithelium surface ($d=100\times$; $h=200\times$). For further details *see* Fig. 2

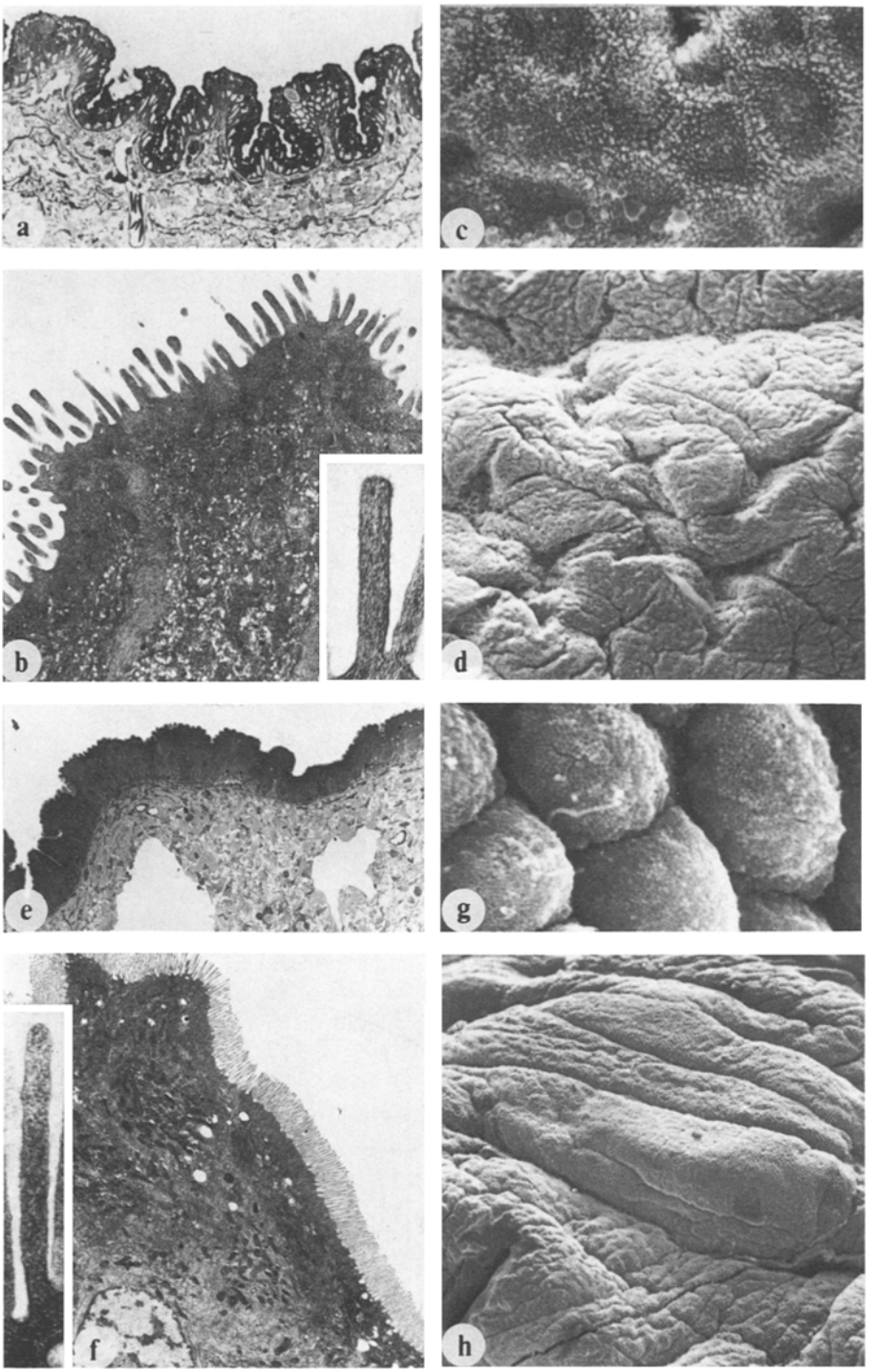

Fig. 4. Foldings, cells and microvilli of toad (*a, b, c, d*) and trout (*e, f, g, h*) gallbladders. (*a, e*) Folding cross-section (12.5×). (*b, f*) Cells ($b=10{,}500\times$; $f=3000\times$) and microvilli (42,000×). (*c, g*) Apical cell surface ($c=2000\times$; $g=5000\times$). (*d, h*) Epithelium surface ($d=150\times$; $h=100\times$). For further details *see* Fig. 2

plasma membrane of the cell; it is, however, quite difficult for the basolateral membrane which exhibits much more irregular plications. Thus, we have tried to calculate correction factors only for the former. A first correction is related to the mucosa folding (Table 4). The total length of the plications was measured per mm^2 in the micrographs obtained by scanning electron-microscopy (SEM); the mean number of cells per folding section was counted on a cross-section of the tissue (ordinary light microscopy); the mean diameter of the apical part of the epithelial cell was measured on micrographs obtained by transmission electron-microscopy (TEM) (Figs. 2, 3, 4). The diameter times the cell number gave the length of the cross-section. The folding area was obtained by the folding total length/mm^2 times the section length. So, the ratio between the real area of the folding and the apparent area was measured (folding correction factor = FCF). In rabbit and guinea pig it was impossible to take into account a single type of plication; so low and tall plication were considered and the respective FCF added to each other.

The second correction is related to microvilli (Table 5). Their mean length and diameter were measured on micrographs obtained by TEM (Figs. 2, 3, 4). From these measurements the lateral area was calculated, by considering microvilli as a cylinder. Microvilli number per square micrometer were measured on micrographs taken with SEM (Figs. 2, 3, 4). In such a way the total lateral area per μm^2 was calculated.

Table 5. Correction of the exposed apparent area of the epithelium concerning microvilli (MCF)

Animal	Length (μm)	Diameter (μm)	Lateral surface (μm^2)	Number / (μm^2)	Total lateral suface (μm^2)	MCF
Rabbit	0.54	0.11	0.19	20	3.80	4.80
Guinea pig	0.42	0.10	0.13	11	1.40	2.40
Goose	1.21	0.07	0.27	25	6.70	7.70
Tortoise	0.55	0.16	0.28	14	3.90	4.90
Toad	0.65	0.11	0.22	14	3.10	4.10
Trout	1.30	0.08	0.33	74	24.40	25.40

The correction factor due to microvilli (MCF) is calculated by considering microvilli as cylinders. The total real area of the apical surface of the cell per μm^2 is equal to the sum of the total lateral surface of microvilli per μm^2 plus 1 μm^2. All of the reported data are means of many measurements taken on different regions of the tissue.

Then the real area is: (total lateral area/μm^2)+ 1 μm^2. The microvilli correction factor (MCF) is: total real area/apparent area.

In Table 6 MCF and FCF are reported and TCF (total correction factor for microvilli and foldings) is calculated from their product.

From the reported FCF it is evident that more complicated foldings are present in the omeotherms with respect to eterotherms. On the contrary, it is impossible to assume any rule from the reported MCF. In fact, microvilli are about 0.5 μm tall in rabbit, guinea pig, tortoise and toad, but they are a bit taller in goose and trout (1.2–1.3 μm); their diameter is about 0.1 μm in rabbit, guinea pig and toad, but it is much larger in tortoise and thinner in goose and trout. Microvilli number per μm^2 is 11–14 in guinea pig, tortoise and toad, but it is higher in rabbit and goose (20–25) and enormously large in trout (74). In conclusion, goose and trout especially have peculiar microvilli, higher, thinner and more frequent; and in trout the frequency is so high that MCF becomes really anomalous.

The corrected R_m values are of 3–4000 Ω cm^2 for mammals and 5 times larger for the animals of the other classes. In *Necturus* Frömter reports 4470 Ω cm^2 [11]. We do not know how much larger the MCF is for *Necturus* gallbladder; if it is similar to the toad one ($\sim$4) R_m for *Necturus* would be equal to about 17,800 Ω cm^2, which is in good agreement with our data for nonmammals.

Finally, Smulders [22] reports a value equal to 14 as TCF, a value which is in some way lower than ours. By applying this TCF, rabbit R_m becomes 2184 Ω cm^2 and this emphasizes even more the difference between mammals and nonmammals.

Table 6. Correction of the mucosal membrane resistance from Ω cm^2 of apparent area to Ω cm^2 of real area

Animal	TCF	Corrected R_m (Ω cm^2)
Rabbit	23.5	3,700
Guinea pig	5.0	1,400
Goose	22.3	13,900
Tortoise	9.3	14,900
Toad	6.1	–
Trout	35.6	18,800

The total correction factor (TCF) to calculate the real area from the apparent area is the product of FCF (Table 4) and MCF (Table 5). The corrected R_m is obtained by multiplying the R_m's of Table 3 with the respective TCF.

Discussion

In tight epithelia [12] transepithelial p.d. is of many mV or many tens of mV positive (e.g. *see* refs. [16, 25]). In leaky epithelia [12] transepithelial p.d. is generally a few mV positive (e.g. *see* refs. [2, 20]). The small positive p.d. is what appears across the whole epithelium of the cellular emf ($E_m + E_s$) short-circuited by tight-junctions. A contribution to it is also given by the negative emf localized in tight junctions (E_{sh}). In the gallbladder of some animal species transepithelial p.d. is even zero or negative: it is clear that in these species E_{sh} is equal to or larger than the short-circuited cellular emf.

Since E_{sh} arises from the diffusion p.d. due to Na^+ salt moving back from the intercellular channels into the lumen, different E_{sh} can be brought about by two factors: *i*) differences in tight-junction selectivity for Cl^- and Na^+ (i.e. different P_{Cl}/P_{Na}), and *ii*) differences in NaCl concentrations in the lateral spaces due to different rates of NaCl pumping through the lateral cell membrane and due to different geometries of the channels.

About factor *i*) it is noteworthy that very different P_{Cl}/P_{Na} were observed by Gelarden and Rose [13] and that the highest positive V_{ms} (+7.6 mV) was measured in man gallbladder which exhibits a small tight-junction selectivity ($P_{Cl}/P_{Na} = 0.76$) and, thus, should have a small backdiffusion p.d. However, dog with a similarly low tight-junction selectivity shows a V_{ms} nearly zero and species with the same P_{Cl}/P_{Na} ($\simeq 0.3$) exhibit negative (rabbit) and differently positive (*Macaca mulatta, Cercopithecus aethiops,* goose) V_{ms}. As a conclusion, factor *i*) is really a source of differences in V_{ms}, but cannot be the only one.

Concerning factor *ii*), it is well known that different transport rates of NaCl are present in different species (*see* refs. [9, 26] for rabbit and guinea pig, ref. [7] for toad, ref. [1] for bullfrog, refs. [6, 8] for fishes). Differences in conformations of intercellular channels, especially due to the number and structure of the projections from the cell lateral membranes, can be observed from the micrographs reported in ref. [24] for rabbit and in ref. [6] for goldfish (many projections are present in rabbit, very few in goldfish). In the micrographs presented here toad lateral spaces appear especially noticeable, owing to the enormous development of the projections; this is quite similar to that reported in ref. [1] for bullfrog. As a conclusion, factors *i*) and *ii*) together can explain different E_{sh} and V_{ms}.

Other sources of differences in V_{ms} are different cell emf's and different

shuntings. Cell emf is given by the algebraic sum of E_m and E_s. In gallbladder E_m and E_s are opposite, as they are largely due to K^+ diffusion out of the cell [14, 18, 19, 27]. So, if E_m and E_s are equal, the net emf across the cell is zero: this seems to be nearly the case for rabbit gallbladder [14] in which E_s and E_m seem to be very near to the values of an equilibrium potential for K^+ (a very slight Na^+ conductance is revealed only through the luminal membrane). An asymmetry between E_m and E_s due to a consistent Na^+ conductance through the luminal membrane is conversely present in *Necturus* [19, 27]. Thus, also different cell emf's are real causes of different V_{ms}.

In this paper the shunting per cent of five species is reported; to these values one can add that reported by Frömter for *Necturus* [11], i.e. 96%. It is evident that in all the nonmammals the shunting value is noticeably constant, i.e. 96–97%: this means that cell resistance is 24–32 times larger than shunt resistance. In the two tested mammals the shunting value is lower, i.e. 89–93% which means a cell resistance 8–13 times larger than shunt resistance. Since in the nonmammals we find species with positive (goose, *Necturus*), with nearly zero (toad, tortoise) and with negative V_{ms} (trout), these different V_{ms} should be mainly explained with differences in (E_m, E_s) asymmetries and in E_{sh} rather than in $(G_{sh}/G_{ep})100$. On the other hand, trout which exhibits the most negative V_{ms} has a $(G_{sh}/G_{ep})100$ equal to 96% and guinea pig with the most positive V_{ms} is short-circuited of only 89%. Thus, these data indicate that in some cases also different shunting effects have to be taken into account to explain V_{ms}.

A last point to emphasize is that many electrical parameters are sharply distributed in two classes on the quantitative point of view, one concerning mammals, the second nonmammals. The two tested mammals have homogeneous values of R_m/R_s ($\simeq 1$), of R_m (1,000–4,000 Ω cm^2 of real area), and of $(G_{sh}/G_{ep})100$ (89–93%); the nonmammals have $R_m/R_s > 1$, R_m between 10,000 and 20,000 Ω cm^2 of real area and a shunting value of 96–97%.

The reason for the higher luminal conductance in mammals could be suggested as due to the higher rate of Na^+ salt transport which is measured across their gallbladders. However, in rabbit the majority of NaCl influx into cells seems to be an electrically neutral process [3, 10, 14]. Thus, a higher K^+ conductance seems the probable explanation, even if the reason for this higher conductance is obscure. In any case, a smaller R_m could explain an R_m/R_s reduced to about 1 from 1.7–2.7 and the decrease in shunting percentage.

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