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A compartmental analysis of the effect of osmotically induced permeability changes in rabbit gallbladder epithelial cells

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Fisiologia. — *A compartmental analysis of the effect of osmotically induced permeability changes in rabbit gallbladder epithelial cells.* Nota di FRANCESCO ANDRIETTI (*) presentata (**) dal Corrisp. V. CAPRARO.

RIASSUNTO. — È stato eseguito uno studio compartimentale di un modello di epitelio di cistifellea di coniglio con lo scopo di determinare il percorso attraverso il tessuto effettuato da parte di sostanze che non sono in grado di attraversare le « tight junctions » che uniscono le cellule epiteliali.

Le relazioni trovate sono state applicate ai dati sperimentali di Smulders, Tormey e Wright [6] riguardanti l'effetto di gradienti osmotici sui flussi dell'1,4-butandiolo.

I loro risultati sperimentali possono essere spiegati, almeno qualitativamente, alla luce della presente analisi, ritenendo che il passaggio avvenga sia a livello della parte basale della membrana basolaterale delle cellule epiteliali nello spazio sottoepiteliale che attraverso la parte laterale della membrana basolaterale negli spazi intercellulari.

INTRODUCTION

In the following we will develop a compartmental analysis of the tracer fluxes across the rabbit gallbladder in steady-state conditions. We will show that, with the only assumption of a first order kinetic for the tracer crossing the different membranes of the system, we may predict, at least qualitatively, some observed experimental results.

The theory that we have developed is not confined to the case of the gallbladder, but could be used as well for other epithelial membranes where intercellular spaces are present, such as intestine, renal tubule or frog skin. The only restriction to the applicability of the model is the knowledge of the geometrical features of the system under study.

THEORY

Let us consider a cylindrical epithelial cell of radius r and length l, and the intercellular space between the membrane of the given cell and those of the other cells surrounding it (figs. 1 and 2).

The intercellular space has constant width a. The connective tissue will not be considered as a compartment, because its structure allows substances to diffuse with a velocity not too different from that of free diffusion in water [1].

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The cell constitutes a " homogeneous compartment ", i.e. the velocity of diffusion of the tracer in its interior is such as to maintain negligible differences in concentrations.

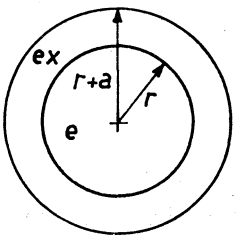

Fig. 1. – Representation of a cross section of the model of a cylindrical epithelial cell (*e*) of radius r surrounded by an intercellular space (*ex*) of width a.

Cells and intercellular spaces are in contact with two other compartments corresponding respectively to the mucosal and serosal spaces that we call m and s, with concentrations $\overline{C}_m$ and $\overline{C}_s$ respectively. Their dimensions are such that their concentrations do not vary in time. The intercellular space is open on s. We will make the hypothesis that the movement of the tracer follows a first order kinetics, i.e. that the quantity of radioactive isotope flowing in the unit time from the i-component to the j-component through a given surface $A_{i,j}$ is proportional, in every point, to the difference of tracer concentration through the same surface. We will call *permeability* [1] of surface $A_{i,j}$ (in the direction from i to j) such a constant of proportionality. It will be indicated with $k_{i,j}$ and its value will be considered constant in every point of $A_{i,j}$. Moreover we will not consider the possible coupling of tracer movements with other fluxes.

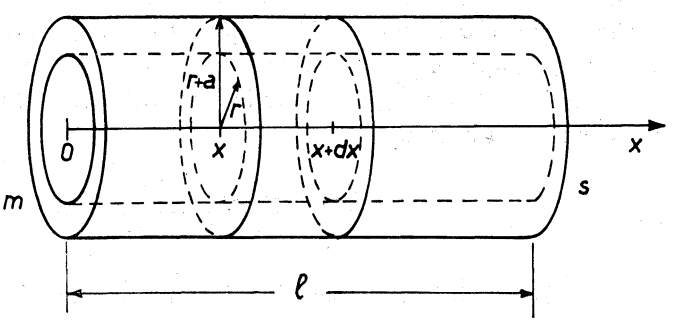

Fig. 2. – Side view of an epithelial cylindrical cell; m and s are the mucosal and the serosal space, respectively; l is the cell height. Two cross sections at a distance x and $x+dx$ from the origin are represented.

Let us now take the axis of the cylinder as a coordinate axis x, with a positive direction from m to s and the origin on the base in contact with m. The tracer concentration in the intercellular space will vary only in the direction of the x-axis, i.e. it will remain constant on every section orthogonal to the axis.

(1) This definition of permeability is that usually given in biology when one considers only diffusive forces.

We will have

$$(1)\qquad \frac{dX_e}{dt} = k_{m,e} A_{m,e} \overline{C}_m - (k_{e,m} A_{e,m} + k_{e,s} A_{e,s} + 2\pi r l k_{e,ex}) C_e(t) + \\ + 2\pi r k_{ex,e} \int_0^l C_{ex}(x,t)\, dx + k_{s,e} A_{e,s} \overline{C}_s$$

where $k_{i,j}$ and $A_{i,j}$ are the permeability constants and the surfaces of separation between the compartments, as they have been defined above; dX_e/dt is the variation in the unit time of the quantity of tracer present in the epithelial cell, function of time t; $C_e(t)$ the tracer concentration in the same compartment; $C_{ex}(x,t)$ the tracer concentration in the intercellular space, function of distance x and of t. If we indicate with V_e the volume of the epithelial cell, it will be $C_e(t) = \frac{X_e(t)}{V_e}$, and in a steady-state situation

$$(2)\qquad \frac{dC_e(t)}{dt} = \frac{dX_e(t)}{dt} = 0,$$

$$(3)\qquad \frac{\partial C_{ex}(x,t)}{\partial t} = 0.$$

Let us now consider the infinitesimal volume of intercellular space obtained with the intersection of two planes orthogonal to the coordinate axis, at a distance x and $x + dx$ from the origin, with the intercellular space (fig. 2). If the value of a is small with respect to r, a section A of the intercellular space will be approximated by $2\pi ra$. In this case the quantity of tracer flowing in the unit time through the section of the intercellular space with the first plane will be given by

$$(4)\qquad -D \left(\frac{\partial C_{ex}(x,t)}{\partial x}\right)_x 2\pi ra$$

where D is the diffusion coefficient (in the water) of the tracer.

The quantity of tracer flowing in the unit time through the section of the intercellular space with the second plane will be

$$(4')\qquad -D \left(\frac{\partial C_{ex}(x,t)}{\partial x}\right)_{x+dx} 2\pi ra$$

Their difference will be given by

$$(5)\qquad -D \left[\left(\frac{\partial C_{ex}(x,t)}{\partial x}\right)_x - \left(\frac{\partial C_{ex}(x,t)}{\partial x}\right)_{x+dx}\right] 2\pi ra = D\, 2\pi ra \frac{\partial^2 C_{ex}(x,t)}{\partial x^2}\, dx.$$

At last the quantity of tracer flowing through the lateral surface of the infinitesimal volume of intercellular space in the unit time will be approximately

$$4\pi r\,[k_{e,ex}\,C_e(t) - k_{ex,e}\,C_{ex}(x,t)]\,dx. \tag{6}$$

From (5) and (6) we obtain the variation of the concentration in the infinitesimal volume of space

$$\frac{\partial C_{ex}(x,t)}{\partial t} = \frac{1}{2\pi ra}\left[D\frac{\partial^2 C_{ex}(x,t)}{\partial x^2}\,2\pi ra + 4\pi r\,(k_{e,ex}C_e(t) - k_{ex,e}C_{ex}(x,t)\right]. \tag{7}$$

In steady-state, by (2) and (3), equation (7) becomes

$$D\frac{d^2 C_{ex}(x)}{dx^2}\,a + 2\,[k_{e,ex}\,C_e - k_{ex,e}\,C_{ex}(x)] = 0. \tag{8}$$

where C_e and $C_{ex}(x)$ are now the asymptotic concentrations in the epithelial cell and in the intercellular space.

Let us now look at the homogeneous equation

$$D\frac{d^2 C_{ex}(x)}{dx^2}\,a - 2\,k_{ex,e}\,C_{ex}(x) = 0. \tag{8'}$$

Its characteristic equation will be given by

$$D\lambda^2 a - 2k_{ex,e} = 0 \tag{9}$$

whose roots are:

$$\lambda_1 = \lambda = \sqrt{\frac{2k_{ex,e}}{Da}} \tag{10}$$

$$\lambda_2 = -\lambda = -\sqrt{\frac{2k_{ex,e}}{Da}}. \tag{11}$$

On the other hand a particular solution of (8) will be

$$C_{ex}(x) = \text{cost} = C_e\frac{k_{e,ex}}{k_{ex,e}}. \tag{12}$$

So we may conclude that the wanted solution of (8) will be

$$C_{ex}(x) = C_e\frac{k_{e,ex}}{k_{ex,e}} + a_1 e^{\lambda x} + a_2 e^{-\lambda x} \tag{13}$$

where the coefficients a_1 and a_2 are determined by means of the boundary con-

ditions. It is immediate to find one of them because, taking into account the fact that the intercellular space is opened on s, it must be

$$C_{ex}(l) = \overline{C}_s \,. \tag{14}$$

For the second boundary condition let us observe that, as we are dealing with steady-state phenomena, the same quantity of substance will flow through a section of the intercellular space at a given distance x or through the lateral walls and the base of it.

So it will be

$$\int_0^x 4\pi r[k_{e,ex} C_e - k_{ex,e} C_{ex}(x)]\, dx + \tag{15}$$

$$+ 2\pi ra\,[k_{m,ex}\overline{C}_m - k_{ex,m} C_{ex}(0)] = -2\pi ra\, D \frac{dC_{ex}(x)}{dx}$$

where $k_{m,ex}$ and $k_{ex,m}$ are the permeabilities of the closed end of the intercellular space.

From (15), when $x = 0$, we have

$$D\left(\frac{dC_{ex}(x)}{dx}\right)_{x=0} = k_{ex,m} C_{ex}(0) - k_{m,ex}\overline{C}_m \,. \tag{16}$$

From (13), (14) and (16) we obtain

$$C_{ex}(l) = C_e \frac{k_{e,ex}}{k_{ex,e}} + a_1 e^{\lambda l} + a_2 e^{-\lambda l} = \overline{C}_s \tag{17}$$

$$C'_{ex}(0) = a_1\lambda - a_2\lambda = \frac{I}{D}[k_{ex,m} C_{ex}(0) - k_{m,ex}\overline{C}_m]. \tag{18}$$

When $k_{m,ex} = k_{ex,m} = 0$ we have

$$C'_{ex}(0) = a_1\lambda - a_2\lambda = 0 \tag{18'}$$

so that

$$a_1 = a_2 \,. \tag{19}$$

From (17) and (19) we finally find

$$a_1 = a_2 = \frac{\overline{C}_s - C_e \dfrac{k_{e,ex}}{k_{ex,e}}}{e^{\lambda l} + e^{-\lambda l}} \,. \tag{20}$$

Then, by (13) and (20)

$$C_{ex}(x) = C_e \frac{k_{e,ex}}{k_{ex,e}} + \frac{\bar{C}_s - C_e \frac{k_{e,ex}}{k_{ex,e}}}{e^{\lambda l} + e^{-\lambda l}} (e^{\lambda x} + e^{-\lambda x}). \tag{21}$$

In steady-state, taking into account (2) and (21), equation (1) becomes

$$k_{m,e} A_{m,e} \bar{C}_m - [k_{e,m} A_{e,m} + k_{e,s} A_{e,s} + 2\pi r l k_{e,ex}] C_e + \tag{22}$$

$$+ 2\pi r k_{ex,e} \int_0^l \left[C_e \frac{k_{e,ex}}{k_{ex,e}} + \frac{\bar{C}_s - C_e \frac{k_{e,ex}}{k_{ex,e}}}{e^{\lambda l} + e^{-\lambda l}} (e^{\lambda x} + e^{-\lambda x}) \right] dx + k_{s,e} A_{e,s} \bar{C}_s = 0.$$

Calculating the definite integral appearing in (22) we obtain for C_e

$$C_e = \frac{k_{m,e} A_{m,e} \bar{C}_m + (k_{s,e} A_{e,s} + k_{ex,e} P) \bar{C}_s}{k_{e,m} A_{e,m} + k_{e,s} A_{e,s} + k_{e,ex} P} \tag{23}$$

where

$$P = 2\pi r \int_0^l \frac{e^{\lambda x} + e^{-\lambda x}}{e^{\lambda l} + e^{-\lambda l}} dx = \frac{2\pi r}{\lambda} \tanh \lambda l \tag{24}$$

Let us now consider two different possibilities

(*a*) $\bar{C}_m = \bar{C}$, $\bar{C}_s = 0$;

(*b*) $\bar{C}_m = 0$, $\bar{C}_s = \bar{C}$.

We will indicate with $J_{m,s}(\bar{C})$ the quantity of tracer flowing in the unit time from m to s in case (*a*); analogously $J_{s,m}(\bar{C})$ will be the quantity of tracer flowing in the unit time from s to m in case (*b*).

Given that the compartment s is connected to the intercellular space and to the epithelial cell, it will be

$$J_{m,s}(\bar{C}) = J_{e,s}(\bar{C}) + J_{ex,s}(\bar{C}) = k_{e,s} A_{e,s} C_e + \tag{25}$$

$$+ 2\pi r \int_0^l [k_{e,ex} C_e - k_{ex,e} C_{ex}(x)] dx$$

where $J_{e,s}$ and $J_{ex,s}$ [2] are respectively the fluxes crossing the basal membrane of the epithelial cell and the intercellular spaces.

(2) Let us remark that the intercellular space shares the membrane of *two* cells, so its contribution to $J_{ex,s}$ has to be divided by 2.

Using the values of $C_{ex}(x)$ and C_e given by (21) and (23) with $\overline{C}_m = \overline{C}$, $\overline{C}_s = 0$, we obtain

$$J_{m,s}(\overline{C}) = \frac{k_{m,e} A_{m,e} \overline{C}}{k_{e,m} A_{e,m} + k_{e,s} A_{e,s} + k_{e,ex} P} (k_{e,ex} P + k_{e,s} A_{e,s}) . \tag{26}$$

In case (*b*) we will have

$$J_{s,m}(\overline{C}) = k_{e,m} A_{m,e} C_e \tag{27}$$

where C_e will be given by (23) with $\overline{C}_m = 0$, $\overline{C}_s = \overline{C}$.

Then it will be

$$J_{s,m}(\overline{C}) = \frac{k_{e,m} A_{m,e} \overline{C}}{k_{e,m} A_{e,m} + k_{e,s} A_{e,s} + k_{e,ex} P} (k_{ex,e} P + k_{s,e} A_{e,s}) . \tag{26'}$$

It is interesting to compare the values given by (26) and (26′) with those obtained from a two compartment system, where the intercellular space constitutes a homogeneous compartment with the same tracer concentration as compartment *s*. In this case, following the method given in [2] (reduced to the case of a two compartment system), we would find

$$J^*_{m,s}(\overline{C}) = \frac{k_{m,e} A_{m,e} \overline{C}}{k_{e,m} A_{e,m} + k_{e,s} A_{e,s} + k_{e,ex} 2\pi rl} (k_{e,ex} 2\pi rl + k_{e,s} A_{e,s}) \tag{28}$$

$$J^*_{s,m}(\overline{C}) = \frac{k_{e,m} A_{m,e} \overline{C}}{k_{e,m} A_{e,m} + k_{e,s} A_{e,s} + k_{e,ex} 2\pi rl} (k_{ex,e} 2\pi rl + k_{s,e} A_{e,s}) \tag{28'}$$

where now $J^*_{m,s}$ and $J^*_{s,m}$ represent the quantity of tracer flowing in the unit time through the homogeneous compartmental system.

When $k_{m,e} = k_{e,m}$, $k_{e,ex} = k_{ex,e}$, $k_{e,s} = k_{s,e}$

$$J_{m,s}(\overline{C}) = J_{s,m}(\overline{C}) . \tag{29}$$

The result may be easily extended to the case of ionic substances, when we take into account the difference of potential between membranes. In fact, provided that there is *no* difference of potential between *m* and *s* [3], and that no other forces except chemical and electrical gradients are present, the coefficients of *k* in the numerator of (26) and (26′) have to be multiplied by the same numbers (the " Goldman's coefficients ") so that (29) will still hold.

(3) See for example [5] for a recording of the intercellular potential in the epithelial cells of rabbit gallbladder.

Relation (28) may also be found by the use of classical thermodynamics [3] or of that of irreversible processes [4]. Here we have given a kinetic proof, valid in a system of parallel and series membranes.

DISCUSSION

We will use the results of the preceding section to discuss some experimental results regarding the permeation pathways of 1.4-butanediol across the rabbit gallbladder. It has been found [6] that the increase of the tonicity of the mucosal solution reduces the total flux of 1.4-butanediol and at the same time has the effect of changing the structure of the intercellular spaces. These changes must play a major role in the explanation of the experimental results, because other effects, as solvent-solute interactions, predict results that are contrary to the ones observed [6].

We will assume, as in [6], that no flux passes across the tight junctions between epithelial cells and intercellular spaces. This fact is likely, although not completely proved, for lipid soluble nonelectrolytes such as 1.4-butanediol [7]. With the above assumptions $k_{ex,m} = k_{m,ex} = 0$, and we may use eqn.'s (26) and (26') with appropriate parameters. Experiments were performed in [6] with identical mucosal and serosal solutions ($\Delta\pi = 0$), with 50 mM ($\Delta\pi = 50$) and 300 mM ($\Delta\pi = 300$) sucrose added to the mucosal solution. Corresponding changes in the structure of epithelial cells and intercellular spaces were also measured.

From the data of [6], we may compute about $5 \cdot 10^6$ epithelial cells for *every* cm^2 of rabbit gallbladder epithelial area.

Using the data of [6] we have

$$r = 2.5 \cdot 10^{-4} \text{ cm.}$$

$$l = 30 \cdot 10^{-4} \text{ cm } (\Delta\pi = 0)\,,\ 60 \cdot 10^{-4} \text{ cm } (\Delta\pi = 50)\,,\ 60 \cdot 10^{-4} \text{ cm } (\Delta\pi = 300).$$

$$a = 300 \cdot 10^{-7} \text{ cm } (\Delta\pi = 0)\,,\ 18 \cdot 10^{-7} \text{ cm } (\Delta\pi = 50)\,,\ 10 \cdot 10^{-7} \text{ cm } (\Delta\pi = 300).$$

The epithelial surface area is 1.5 times greater than the serosal surface area. We will assume that the permeability coefficients $k_{e,m}$ and $k_{e,ex}$ are the same (same assumption as in [6]) and moreover they are equal to $k_{e,s}$. We will call k the common value of all permeability coefficients.

The free diffusion coefficient in water of 1.4-butanediol will be taken as $7.5 \cdot 10^{-6}$ cm/sec, an intermediate value between that of butanol ($7.7 \cdot 10^{-6}$ cm/sec) and that of glycerol ($7.2 \cdot 10^{-6}$ cm/sec).

With the above values and making use of (10) we find

$$\lambda\,(\Delta\pi = 0) = 0.94 \cdot 10^5 \sqrt{k} \text{ (1/cm)};$$

$$\lambda\,(\Delta\pi = 50) = 3.85 \cdot 10^5 \sqrt{k} \text{ (1/cm)};$$

$$\lambda\,(\Delta\pi = 300) = 5.16 \cdot 10^5 \sqrt{k} \text{ (1/cm)}.$$

Making use of (26), for what we have said above, we will have for the flux crossing a square centimeter of the mucosal surface

$$(30) \qquad J_{m,s}(\bar{C}) = \frac{k\bar{C}}{1 + 0.66 + 5 \cdot 10^6 P}(5 \cdot 10^6 P + 0.66).$$

When $\Delta\pi = 0$, the experimental value of $J_{m,s}(\bar{C})/\bar{C}$ given by [6] is 2.3 (cm/sec $\times 10^5$). The corresponding estimated value of k from (30) will be 2.45 (cm/sec $\times 10^5$).

Using this value of k we may compute the value of $5 \cdot 10^6 P$ from (24) for different values of $\Delta\pi$. It will be

$$5 \cdot 10^6 P \ (\Delta\pi = 0) = 14.60 \text{ cm};$$

$$5 \cdot 10^6 P \ (\Delta\pi = 50) = 4.00 \text{ cm};$$

$$5 \cdot 10^6 P \ (\Delta\pi = 300) = 2.98 \text{ cm}.$$

In [6] it is assumed that the flux crossing the basal membrane of the epithelial cell is *always* negligible with respect to the flux passing across the intercellular spaces. As the ratio between the two fluxes is given by $0.66/(5 \cdot 10^6 P)$, we see that this may be true for $\Delta\pi = 0$ and for $\Delta\pi = 50$ but it is not so for $\Delta\pi = 300$.

The assumption made in [6] is based on the grounds that the surface of the intercellular spaces is at least ten times larger than that of the epithelial cell basal membrane.

This in fact is true, as may be easily verified, but in the intercellular space we have a concentration of the tracer that reduces the tracer efflux. The effect of having a nonhomogeneous compartment is not so important for $\Delta\pi = 0$, but it increases for $\Delta\pi = 50$ and $\Delta\pi = 300$.

Using the value of $k = 2.45 \cdot 10^{-5}$ cm/sec computed above, we find

$$\frac{J_{m,s}(\bar{C})}{\bar{C}} \ (\Delta\pi = 50) = 2.02 \cdot 10^{-5} \text{ cm/sec}$$

$$\frac{J_{m,s}(\bar{C})}{\bar{C}} \ (\Delta\pi = 300) = 1.93 \cdot 10^{-5} \text{ cm/sec}.$$

The values given in Table I show the observed experimental results, the results predicted with the use of LePage and Seely's formula ([8] quoted in [6]) and those predicted by us.

CONCLUSIONS

Table I shows that our results are not so far from those of [6] for $\Delta\pi = 50$, but they become very different when $\Delta\pi = 300$. In any case they are higher than those of [6].

TABLE I

The effect of osmotic gradients on the permeability of the gallbladder to 1.4-butanediol; experimental results (exp.), *theoretical results as predicted by* [6] ($pred_1$) *and by us* ($pred_2$)

Osmotic gradient (mosm)	$P_{1.4\text{-butanediol}}$ (cm/sec × 10^5)		
$\Delta\pi = 0$		2.3	
	$pred_1$	$pred_2$	exp
$\Delta\pi = 50$	1.7	2.02	1.5
$\Delta\pi = 300$	1.1	1.93	0.7

We want to remark here that we did not use any *ad hoc* assumption regarding the geometry of the system: instead we used the same assumptions used by [6] to explain the experimental results on the basis of a different model. Moreover our results indicate an upper boundary for the value of $\frac{J_{m,s}(\bar{C})}{\bar{C}}$. Many factors may contribute to a decrease of $\frac{J_{m,s}(\bar{C})}{\bar{C}}$. For example a restriction of the diffusion coefficients in the intercellular spaces, as an effect of the proximity of lateral membranes [9] or a partial occlusion of them when they become narrower, are all factors that may contribute to diminishing the value of $\frac{J_{m,s}(\bar{C})}{\bar{C}}$. A complete occlusion of the intercellular spaces, for example, would give a predicted value of about $1.0 \cdot 10^{-5}$ cm/sec for $\frac{J_{m,s}(\bar{C})}{\bar{C}}$, a value not too far from the observed experimental value of $0.7 \cdot 10^{-5}$ cm/sec when $\Delta\pi = 300$.

Moreover, as in [6], we did not take into account the collapse of mucosal folds in the presence of an increase of the mucosal tonicity.

Anyway we believe that the major restriction to the applicability of the theory consists in the uncertainty of the geometrical changes in the structure of the intercellular spaces, and in the assumption that no flux is passing through the tight junctions.

For these reasons we think that we could not expect, from our model, more than a qualitative prediction of the actually measured changes in the fluxes of 1.4-butanediol or other lipid soluble nonelectrolytes.

As a proposal for further experimental work we would suggest a better description of the structural changes of the intercellular spaces and the use of substances such as TAP (2.4.6-triaminopyrimidinium) able to block the passage through the tight junctions [10].

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