Biophysical Chemistry 14 (1981) 65–80
Elsevier/North-Holland Biomedical Press

OSCILLATION OF THE ELECTRICAL POTENTIAL OF THE FROG SKIN UNDER THE EFFECT OF Li^+: THEORETICAL FORMULATION

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Received 29 October 1980
Revised manuscript received 29 April 1981

A theoretical model of oscillation is proposed. It is based on the non-linearity introduced in the functioning of the active pump by the presence of lithium. Other plausible causes of oscillation are shown not to interfere in this case. The oscillation is of the local type. Synchronization between the local oscillators is not achieved by diffusional, but by electrical coupling. Numerical calculation shows that the model fits reasonably well to the experimental data.

1. Introduction

Our group and others [1–5] have shown that Li^+ in the external medium of an isolated frog skin induces oscillatory processes. Each 'point' on the surface of the frog skin can generate oscillations [2]. First, we shall study the possible mechanism generating these local oscillations, then we shall investigate the manner in which the local oscillators synchronize themselves to produce an overall oscillation of the skin.

2. The epithelium model

2.1. General characteristics

The transport properties of epithelia for Na^+ and Li^+ have already been studied [6–20]. We shall summarize the cation transport in frog skin as follows (fig. 1): when the active 'pump' is fully efficient (Na^+ pumping in the absence of any inhibitor), the first cellular layer is enough to pump out practically all of the Na^+. Consequently, everything occurs as if this first layer were the only one possessing ionic pumps. Conversely, when the active pump is not fully efficient (Li^+ pumping, or Na^+ pumping in the presence of an inhibitor), only a fraction of the considered ions are expelled at the level of the first cellular layer of the epithelium. Remaining ions can then diffuse to

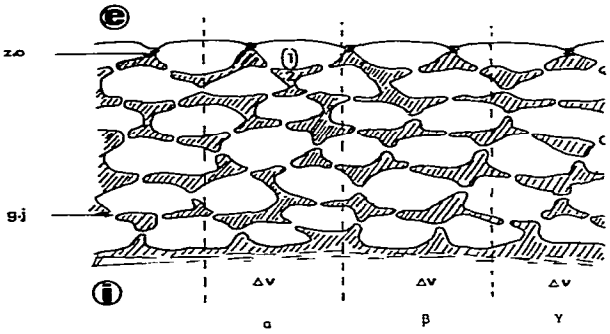

Fig. 1. Schematic representation of the epithelium. (e) and (i), external and internal media; zo, *zonulae occludentes*; gj, 'gap junction' between neighbouring cells; (1) and (2), cellular media (1) and (2); ΔV local volume of epithelium at various points (α, β, γ, etc.) of the skin. For the sake of clarity, the drawing has been made here as if compartment (1) was the cell cytoplasm and compartment (2) the intercellular spaces. Things are, however, probably more complicated in the actual system, with compartment (2) possibly including a part of the cytoplasm (endoplasmic cisternae), or corresponding only to a part of the intercellular spaces.

0301-4622/81/0000-0000/$02.50

the other cellular layers which will therefore contribute to their active extrusion. With regard to cation transport, the cellular medium is assumed to be in two compartments, (1) and (2), (fig. 1, ref. [11]), although the precise location of compartments and membranes is not always very clear [21–24].

2.2. The two-compartment assumption

Compartments (1) and (2) defined in fig. 1 extend throughout both the surface and the thickness of the epithelium; hence, the ionic concentrations may vary in these macroscopic compartments. A simplification occurs if one considers time variations of the concentrations slow enough that diffusion is sufficient to homogenize these concentrations over distances comparable to the thickness of the epithelium (*hypothesis h1*). This enables us to consider the frog skin as a set of 'points' ($\alpha, \beta \ldots$), each 'point' being an oscillator. The structure of this oscillator has to be compatible with the scheme in fig. 2, which shows the more general assumptions of the model of cation transport in frog skin.

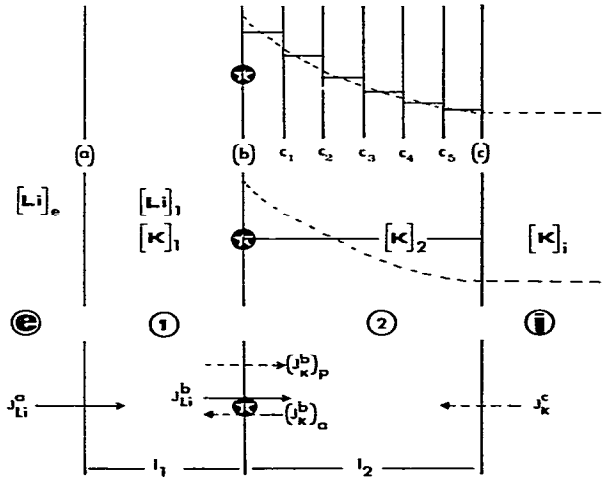

Fig. 2. Local model of epithelium. Membrane (a) and (b) delineate the internal medium (e) and the cellular compartment (1); membranes (b) and (c) delineate medium (2) and (i). Variations of concentration throughout the depth of the epithelium are shown on compartment (2). The upper part of the figure indicates how such variations are approximated: a series of homogeneous steps are separated by virtual barriers c_1, $c_2 \ldots$. The central part of the figure corresponds to slow time variations of concentration: not noticeable variation of the concentration is observed throughout the thickness of the epithelium. In such a case, the internal medium (i) corresponds not only to the internal solution, but also to the extracellular spaces of the epithelium. The lower figure gives the ionic characteristics of the model: J_{Li}^a, J_{Li}^b, J_K^b and J_K^c are the ionic fluxes (mol cm^{-2} s^{-1}) of Li^+ and K^+ through the various membranes, and $(J_K^b)_a$ and $(J_K^b)_p$ hold for the active and passive components, respectively; $[Li]_e$, $[Li]_1$, $[K]_2$ and $[K]_i$ are the ionic concentrations in the various compartments; l_1 and l_2 (cm) are the volumes of compartments (1) and (2) per unit of area of the epithelium. Symbol ☆ represents the active pump.

2.3. The external medium (e)

Based on the experimental data [2], we can assume that in the external medium, the main factor controlling the oscillation is the concentration of Li^+ $[Li]_e$ (*hypothesis h2*).

2.4. Membrane (a)

The permeability to K^+ of membrane (a), P_K^a, is negligible [25] compared to that of Na^+ or Li^+ [12,26]. We shall use for the Li^+ flux through (a), J_{Li}^a, a classic formulation (see Appendix B):

$$J_{Li}^a = P_{Li}^a([Li]_e - [Li]_1) + (t_{Li}^a/F)I \qquad (1)$$

where $[Li]_1$ is the Li^+ concentration in compartment (1) (see section 2.5), t_{Li}^a the transport number of Li^+ for membrane (a) and I the density of the externally imposed current (A cm^{-2}). As a matter of fact, P_{Li}^a and t_{Li}^a might vary quite significantly for varying values of the Li^+ concentration. However, in the case of oscillations with a moderate amplitude, we can assume constant values for the permeabilities and transport numbers (*hypothesis h3*).

Eq. (1) implies also that Li^+ flux through membrane (a) is not coupled to any metabolic process. This seems to be a reasonable assumption for the high concentrations [27–29] used to induce the oscillations.

2.5. Compartment (1)

According to section 2.1 and *hypothesis h1*, there is a single homogeneous compartment (1) in

each 'point' of the frog skin. It has been shown [30] that the accumulation of Li^+ in the cells is accompanied by a decrease in the cellular concentration of K^+. However, given the fact that K^+ is far and away the dominant cation in the cells, the relative variations of the K^+ concentration in the oscillating epithelium must remain small (for further details see Appendix B). Therefore, we shall assume that the K^+ concentration in compartment (1) remains constant (*hypothesis h4*). We shall also assume that Cl^- activity remains constant throughout the epithelium during the electrical oscillations, or that a modification of the cellular concentration of Cl^- has no major effect on the oscillatory process (*hypothesis h5*). This is justified by the facts that (i) K^+ being the main cellular cation and Cl^- the main cellular anion, the Cl^- concentration cannot vary much more than that of K^+; (ii) the osmotic pressure, as seen in the experimental results [2], has little effect on the oscillation (for further details see Appendix A), and (iii) Cl^- is without effect on the cation transport [4]. Moreover, it was shown [2] that oscillations occur even when there is no Na^+ in the external medium.

Thus, according to *hypotheses h2* and *h4*, the only variable which is significant in compartment (1) is the Li^+ concentration $[Li]_1$. We shall state

$$[Li]_1 \equiv X \tag{2}$$

2.6. Membrane (b)

The exact position of membrane (b) in the epithelium is still controversial; but there is general agreement that this membrane is permeable to K^+ and not to Na^+, and that is bears the sites of active pumping. A number of general reviews have been devoted to the process of active pumping [31–34] and several models have been proposed to relate it to ATPase activities [45,36]. The active pump is most often assumed to be a Na^+/K^+ exchange pump [37], somewhat comparable to that of the nerve cell as the former is also inhibited by ouabain. The Na^+/K^+ coupling has been extensively studied [31,38–44] and the ability of the active pump to transport Li^+ compared to the way it acts on Na^+ has been disputed [16,30,45–48]. Data on erythrocytes [49–51], ATPase studies [52–56], as well as studies of interactions between Li^+ and K^+ or Na^+ [57–59] may, however, be interpreted as follows: (i) Li^+ is actively transported; (ii) each active pump on membrane (b) is less efficient for Li^+ than it is for Na^+, especially when Li^+ interferes on the K^+ sites of the pump; (iii) given the syncytial structure of the epithelium, the total number of pumps engaged in Li^+ transport is much greater than that engaged in Na^+ transport (see section 2.1); (iv) as a consequence of these two opposing effects, it is likely that the overall transepithelial flux densities obtained for Li^+ and Na^- are not too different from one another ; (v) the active step is a poorly electrogenic process. This can be summarized by writting as a new assumption (*hypothesis h6*)

$$\left(J_K^b\right)_{\text{active}} = r J_{Li}^b; \quad r \div 1 \tag{3}$$

where r is the equivalent of a stoichiometric coefficient, since it relates flux densities (see fig. 2).

2.7. Compartment (2), barrier (c) and internal medium (i)

As the position of membrane (b) is still controversial, the exact nature of compartment (2) also remains questionable.

However, even if compartment (2) is not separated from the internal medium by an actual membrane, according to *hypothesis h1*, we shall represent for each 'point' of the epithelium a homogeneous compartment (2) delineated by a non-selective barrier (c) (*hypothesis h7*).

The variations of either $[Li]_i$ or $[Na]_i$ are unable to induce or perturb the oscillation [1,2]; and we have seen that $[K]_i$ was the main factor in the internal medium controlling the oscillation [2]. Given the fact that $[K]_i$ ($\div 2$ mM) is much smaller than $[Na]_i$ ($\div 115$ mM), relative variations of K^- will be much more significant than those of Na^+ in disturbing the active step [34]. Hence, $[K]_2$ can be taken as the main variable in compartment (2). We shall state

$$[K]_2 \equiv Y \tag{4}$$

2.8. Important implications of the model

According to the original model of Koefoed-Johnsen and Ussing [42], the main implications of which remain quite valid [23.60], the electric potential difference across the skin

$$\Delta\phi = \phi_i - \phi_e \tag{5}$$

was considered as the algebraic sum of two diffusion potentials, $\Delta^a\psi$ and $\Delta^b\psi$, through membranes (a) and (b), respectively. According to *hypothesis h7*, barrier (c) gives rise to negligible diffusion potentials and the expression of $\Delta\phi$ does not need to be changed.

Experiments performed on the separated epithelium [61] have shown that modifying $[Na]_e$ perturbed the electric potential difference of the skin significantly faster than did a modification of $[K]_i$. Let us define a characteristic time, τ_2, corresponding to K^+ modifications in compartment (2) in the same manner as a characteristic time, τ_1, has been defined above for the Na^+ (or Li^+) variations in compartment (1). The adimensional parameter

$$\alpha \equiv \frac{\tau_1}{\tau_2} \tag{6}$$

will thus obey the relation

$$0 < \alpha < 1 \tag{7}$$

3. The local oscillator model

3.1. Statement of the problem

We know from the experimental data [2] that the oscillation is of the local type. The theoretical treatment must therefore begin by attempting to formulate the oscillation at a 'point' of the epithelium.

It has been shown [2] that, when two epithelia oscillating with similar periods were coupled electrically, this induced synchronization of both epithelia but did not lead to any phenomenon of 'beats'. This is characteristic of a non-linear oscillator. When the passive fluxes may be described by linear equations as in eq. (1), it leaves the origin of the non-linearity to the functioning of the active pump.

3.2. Kinetics of Li^+ transport through membrane (b)

The flux of Li^+ through membrane (b), J^b_{Li}, is written as a function, v_p, of the two main variables, $[Li]_1$ and $[K]_2$

$$J^b_{Li} \equiv v_p(X, Y) \tag{8}$$

where index p recalls that the considered flux depends upon the functioning of an active pump. Such a formula considers implicitly that the activity of the pump is not very sensitive to the value of the electric potential through (b), which is not unreasonable for a pump that is poorly electrogenic.

According to ref. [62], variables can be separated in eq. (8). Since the K^+ concentration is low in compartment (2), the ATPase activity remains proportional to its value [62]. Hence

$$v_p(X, Y) \approx Y f_1(X) \tag{9}$$

We shall see later (section 3.4) that a necessary condition to obtain oscillations is that values of X exist such that

$$\frac{\partial f_1}{\partial X} < 0 \tag{10}$$

Using eqs. (9) and (10) and generalizing the Michaelis-Menten equation to the case where several sites are involved, we shall write

$$J^b_{Li} = VY \frac{X^k}{\sum_j K_j X^j} \tag{11}$$

where V, k and K_j are constants.

3.3. Use of dimensionless equations

Let M_{Li} and M_K be the masses of Li^+ and K^+, respectively, per unit of surface area of the skin, and t_r be the physical time (s). By writing the mass conservation for Li^+ and K^+, one obtains (fig. 2)

$$\frac{dM_{Li}}{dt_r} = J^a_{Li} - J^b_{Li} \tag{12}$$

$$\frac{dM_K}{dt_r} = J_K^c - (J_K^b)_{active} + (J_K^b)_{passive} \quad (13)$$

where indices 'active' and 'passive' refer to active and passive fluxes through membrane (b); and

$$M_{Li} = l_1[Li]_1; \quad M_K = l_2[K]_2 \quad (14)$$

where l_1 and l_2 are the volumes of compartments (1) and (2) per unit of surface area of the epithelium. The passive flux of K^+ through (b) is written as

$$(J_K^b)_{passive} = P_K^b([K]_1 - [K]_2) \quad (15)$$

In a like manner we have the K^+ flux through (c)

$$J_K^c = P_K^c([K]_i - [K]_2) \quad (16)$$

As (c) is only a diffusional barrier we can assume that

$$P_K^c \gg P_K^b \quad (17)$$

Defining

$$[K]_r \div [K]_i\{1 + (P_K^b[K]_1/P_K^c[K]_i)\} \quad (18)$$

we shall introduce the dimensionless variables

$$x = \frac{[Li]_1}{[Li]_c} = \frac{X}{[Li]_c} \text{ and } y = \frac{[K]_2}{[K]_r} = \frac{Y}{[K]_r} \quad (19)$$

Using eq. (19) to rewrite eq. (11), we shall define two new functions $f(x)$ and $\theta(x)$ by

$$J_{Li}^b = [K]_r f_{max} y\theta(x) \quad (20)$$

x_{max} is the value of x for which f attains its maximum value, f_{max}, and the dimensionless function $\theta(x)$ is given by

$$\theta(x) = \frac{f}{f_{max}} \quad (21)$$

Let us now consider a 'point' in the epithelium when there is no externally imposed electric current. We specify the characteristic times, τ_1 and τ_2, which have been introduced above, by

$$\tau_1 \equiv l_1/P_{Li}^a \text{ and } \tau_2 \equiv l_2/P_K^c \quad (22)$$

and define a dimensionless time t by

$$t \equiv t_r/\tau_1 \quad (23)$$

We can express eqs. (12) and (13) as

$$\frac{dx}{dt} = 1 - x - \rho y\theta(x) \quad (24)$$

$$\frac{dy}{dt} = \alpha\{1 - y - uy\theta(x)\} \quad (25)$$

where α is the dimensionless parameter already defined by eq. (6), and where ρ and u are two other dimensionless parameters defined respectively by

$$\rho \equiv \frac{f_{max}}{P_{Li}^a}\frac{[K]_r}{[Li]_c} \text{ and } u \equiv \frac{rf_{max}}{P_K^c} \quad (26)$$

The biological significance of parameters u and ρ is to express the relative importance of the active to the passive fluxes. Therefore, the active fluxes being usually much larger than the passive ones, one can state

$$u > 1 \text{ and } \rho > 1 \quad (27)$$

From the relaxation experiments [11] it appeared that τ_1 was of the order of magnitude

$$1 \text{ min} \lesssim \tau_1 \lesssim 5 \text{ min} \quad (28)$$

while the period of oscillation, T_r, was found [2] to be of the order of magnitude

$$5 \text{ min} \lesssim T_r \lesssim 10 \text{ min} \quad (29)$$

The dimensionless period of oscillation

$$T \equiv T_r/\tau_1 \quad (30)$$

thus obeys the relation

$$1 \lesssim T \lesssim 10 \quad (31)$$

3.4. Analytical study of the equations

Consider the general case of a system of two variables, x and y, depending on time t and such that the derivatives are functions, R and Q, of x and y:

$$\frac{dx}{dt} = R(x,y) \qquad \frac{dy}{dt} = Q(x,y) \quad (32)$$

Bendixon's criterion [63] states that sustained oscillations are possible only if the quantity

$$\frac{\partial R}{\partial x} + \frac{\partial Q}{\partial y} = -1 - \rho y\frac{d\theta(x)}{dx} - \alpha - \alpha u\theta(x) \quad (33)$$

can change sign on the domain of variation of the variables. Given all the contraints on the system, this implies

$$\frac{d\theta}{dx} < 0 \tag{34}$$

for some values of x. We can recognize in eq. (34) the dimensionless eq. (10) which we stated above to obtain an analytical formulation of the kinetics of the pump.

Other information can be obtained on the system, even when an exact knowledge of function $\theta(x)$ is lacking, by application of the method of the 'normal modes' [64] to eqs. (24) and (25).

There is no absolute criterion governing the occurrence of an oscillatory behaviour or of a limit cycle, but there is a good chance of obtaining oscillations if the secular equation has complex roots with a real positive part [65]. In the case of eqs. (24) and (25), the latter requirement is fulfilled only when

$$\alpha < 1 - y_{st} \tag{35}$$

that is, α smaller than 1. It is noteworthy that the experimental approach indeed indicated that α was always less than unity in our system (see eq. 7).

3.5. Numerical studies

As already stated, one has no precise knowledge of $\theta(x)$. Let us try a simplified case of the general analytical form previously suggested in ref. (11)

$$\theta(x) \equiv \left(\frac{x}{1+\beta x + Kx^n}\right) \Big/ \left(\frac{x_{max}}{1+\beta x_{max} + Kx_{max}^n}\right) \tag{36}$$

where β, K and n are kinetic parameters. Fig. 3 gives the dependence of $\theta(x)$ on x, for a series of values of x_{max} when β and n have been fixed at the values

$$\beta = 5 \text{ and } n = 3 \tag{37}$$

Oscillatory solutions of eqs. (24) and (25) have then been investigated in the domains of the parameters

$$\begin{cases} 0.1 \leqslant \alpha < 1 \\ 1 < u, \rho < 150 \\ 0.04 < x_{max} < 0.2 \end{cases} \tag{38}$$

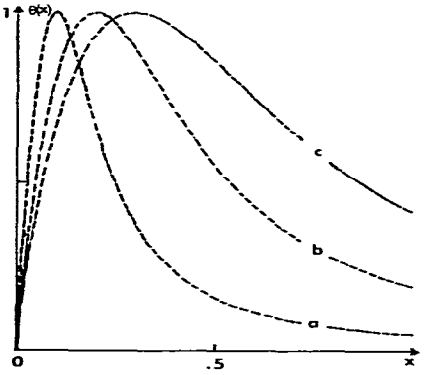

Fig. 3. Numerical calculation of $\theta(x)$ with respect to x, according to eq. (36). The values of the main parameters are $n=3$, $\beta=5$, and $x_{max}=0.1$ (a), 0.2 (b), or 0.3 (c).

One might wonder if such a study bears any general significance. Results have been obtained in the particular cases when eqs. (36) and (37) are satisfied, while a priori, there is an infinite number of possible analytical forms for $\theta(x)$. However, a certain number of results remained practically unchanged over the very broad scale of numerical values (relation 38). They can thus be taken generally. The main ones are as follows: first, when the model indicates that oscillations can be anticipated

$$\rho \lesssim u \lesssim 2\rho \tag{39}$$

i.e., u and ρ are of the same order of magnitude. A second interesting point is that the calculated dimensionless period of oscillation of $[Li]_1$ and $[K]_2$ remains restricted to the domain of values

$$4 \lesssim T \lesssim 15 \tag{40}$$

Fig. 4 gives an example of such calculated oscillations.

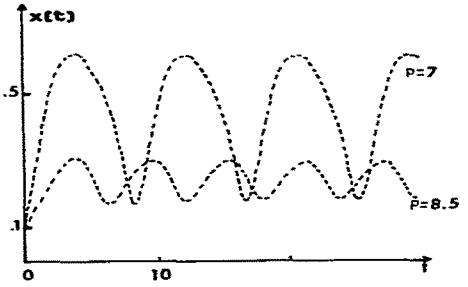

Fig. 4. Effect of a variation of parameter ρ on the calculated oscillation of $x(t)$. The values of the other parameters were $\alpha=0.2$, $\beta=5$, $u=10$, $x_{max}=0.14$. For $\rho=7$, one obtains $T=8.5$, $x_{st}=0.25$ and $y_{st}=0.12$. For $\rho=8.5$ the values become $T=5.8$, $x_{st}=0.46$ and $y_{st}=0.23$.

3.6. Comparison of theoretical predictions with experimental data

The calculated range of values for T given by eq. (40) is compatible with the experimental range (31). We can also calculate the ratio of important quantities by using the values of fig. 4 with eq. (26) and from the fact that there is no net flux of K^+ between compartments (2) and (i) over the period of the oscillation. We get

$$\frac{P_K^b}{P_K^c} \ll 1 \qquad \frac{P_{Li}^a}{P_K^c} \ll 1 \qquad \frac{l_1}{l_2} \ll 1 \tag{41}$$

Our model of oscillations thus implies that compartment (2) is much larger than compartment (1). It is noteworthy that this might help in making a choice about the various possible modes of transport of Na^+ through the epithelium which have been proposed in the literature (see section 2.1). For instance, it does not support the hypothesis that compartment (1) would correspond to most of the cytoplasmic volume, while compartment (2) would be restricted to a few endoplasmic cisternae. On the contrary, it concurs very well with the idea of compartment (1) corresponding to a few cytoplasmic vacuoles transporting Na^+ (or Li^+) and compartment (2) being part or all of the intercellular spaces in the epithelium (and perhaps also some endoplasmic cisternae), while most of the cell cytoplasm would remain inactive in the Na^+ (or Li^+) transport. The latter hypothesis does not require that the whole of the cytoplasm of the epithelial cells which are engaged in the active pumping must be invaded by Na^+. This is a strong argument in its favour. Indeed (see any review article on halophily, for instance, ref. [66]), it has never been found, except perhaps for a few halophilic bacteria, that the actual cytoplasmic matter could contain much Na^+, while it has often been noted that vacuoles might become significantly enriched in Na^+.

4. Synchronization of the local oscillations

4.1. Statement of the problem

Until now, we have considered only the local problem, i.e., we have limited the theoretical study to that of a small group of cells considered as the fundamental oscillator. We have seen also that the range of values of the parameters compatible with the induction of the oscillation was rather limited. Hence, given the possible fluctuations of the parameters all over the frog skin, it is likely that the skin behaves as a 'mosaic', where, in some points, an oscillation is possible and others where it is not. The problem is now to understand how all these local oscillators can synchronize themselves in the form of the overall electric oscillation of the skin which is experimentally observed.

Different interpretations of the synchronization can be considered [67–72], but it is seen in the experimental results [2] that synchronization of two epithelia is achieved by a purely electrical way, without any diffusion between the cells.

4.2. The 'mean field' equations

Let us assume that the n oscillators of the skin are synchronous and give rise to a macroscopic oscillator. The problem is to express the 'mean' characteristics of this oscillator, as functions of the local variables $x_j(t)$ and $y_j(t)$, for each point j. This can be done by using the 'mean field' or 'molecular field' assumption [71,73,74]. The complex interactions between n oscillators are replaced

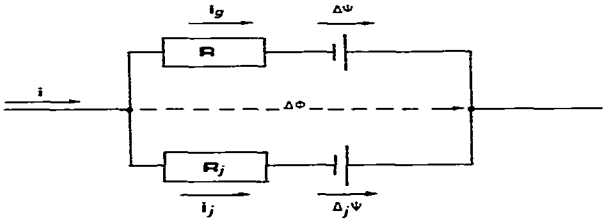

Fig. 5. Interaction between a domain j and the $(n-1)$ other domains on the epithelium. When the ionic content of j is different from the mean ionic content of the epithelium, diffusion potentials appear which generate an electric current i_j between domain j and the rest of the epithelium. Symbols: i_j, electric current (A) through j; i_g, electric current through the $(n-1)$ other domains; i, external current (A); $\Delta\psi$, mean diffusion potential of epithelium; $\Delta_j\psi$, diffusion potential of domains j; R, electrical resistance of the epithelium; R_j, electrical resistance of domain j; $\Delta\phi$, transmembrane electric potential difference.

by the interactions between one given oscillator and a 'mean' oscillator. Both are represented in fig. 5 by their ohmic resistance and their diffusional potential. It is easy to understand the mechanism of coupling between cells. Even in the absence of an externally applied current, one can see that when the diffusional potential $\Delta_j\psi$ of a given oscillator differs from the mean, $\Delta\psi$, a current arises. It changes the ionic content in j, hence disturbing the kinetics of cation transport at this point. This in its turn changes $\Delta_j\psi$.

Let us express Ohm's law using the notations of fig. 5. The electric current at a point j may be written

$$i_j = i\frac{R}{R_j} + \frac{\partial_j\Delta\psi}{R_j} \tag{42}$$

with

$$\partial_j\Delta\psi \equiv \Delta_j\psi - \Delta\psi \tag{43}$$

The total current i, is the sum of all the i_j

$$i = \sum_1^n i_j \tag{44}$$

Using eqs. (42)–(44) and the expression for the diffusional potential, given in Appendix B, then

$$\partial_j\Delta\psi = -t_{\mathrm{Li}}^{\mathrm{a}}\frac{RT}{F}\cdot\ln\frac{x_j}{x} - t_{\mathrm{K}}^{\mathrm{b}}\frac{RT}{F}\cdot\ln\frac{y_j}{y} \tag{45}$$

Eqs. (42)–(45) express the relation between local and mean variables. The recorded potential (see eq. 78 in Appendix B) can then be expressed with these mean variables x and y. For the numerical calculations, in order to show in a simple way the mechanism of the coupling, we shall discard the second term in the right-hand side of eq. (45). This is perfectly valid only if the transport number $t_{\mathrm{K}}^{\mathrm{b}}$ is much smaller than $t_{\mathrm{Li}}^{\mathrm{a}}$. In this case, for oscillators with physically identical characteristics we get

$$t_{\mathrm{K}}^{\mathrm{b}} \ll t_{\mathrm{Li}}^{\mathrm{a}} \Rightarrow x = \left(\prod_1^n x_j\right)^{1/n} \tag{46}$$

and eqs. (24) and (25) can be rewritten by expressing in eqs. (12) and (13) the part of the coupling current which corresponds to an Li^+ flux in compartment (1)

$$\frac{dx_j}{dt} = 1 - x_j - \rho y_j\theta(x_j) + K + \delta'\cdot\ln\frac{x}{x_j} \tag{47}$$

$$\frac{dy_j}{dt} = \alpha_j\{1 - y_j - uy_j\theta(x_j)\} \tag{48}$$

$$x = \left(\prod_1^n x_j\right)^{1/n}, \quad j = 1, 2 \ldots n \tag{49}$$

In these equations,

$$K \equiv \frac{t_{\mathrm{Li}}^{\mathrm{a}}}{F}\cdot\frac{I}{P_{\mathrm{Li}}^{\mathrm{a}}[\mathrm{Li}]_{\mathrm{e}}} \tag{50}$$

expresses the effect of an externally applied current I, and

$$\delta' \equiv 2.3\frac{RT}{F^2}\cdot\frac{(t_{\mathrm{Li}}^{\mathrm{a}})^2}{P_{\mathrm{Li}}^{\mathrm{a}}[\mathrm{Li}]_{\mathrm{e}}}\cdot\frac{1}{R_u} \tag{51}$$

expresses coupling between the cells. R_u (Ω cm^2) is the resistance of the unit of surface area of the skin.

When eq. (46) is not valid eq. (48) must be expressed analogously to eq. (47). But even if eq. (46) is valid, for physically different oscillators, the exponent in the mean value of x given by eq. (46) is different. In particular, according to Teorell [4], the resistance of the frog skin is also subject to rhythmical changes. This results in a varying exponent in eq. (46). Moreover, changes in osmotic

pressure, which also produce changes in resistance, do not lead to oscillations (Appendix A). Hence, it will be assumed from now on that these changes are not the primary phenomena; and the resistance of the epithelium will be considered constant (*hypothesis h8*).

4.3. The coupling of two epithelia

In the preceding section, we have introduced parameter δ' which represents the coupling of two elementary domains of the epithelium. There is, however, no direct experimental access to δ'. What has been studied in the experimental part [2] was the coupling between a first and a second epithelium, made of $n^{(1)}$ and $n^{(2)}$ elementary domains, respectively. Such a coupling experiment is represented in fig. 6. In fact, it is easy to modify eqs. (47) and (48) by considering that interaction between the two epithelia generates an electrical current between them. This is due to structural dissymmetry, phase differences in the oscillations, etc.

Introducing

$$r_t \equiv 1 + \frac{R^2}{R^1} + \frac{R_c^e + R_c^i}{R^1} \tag{52}$$

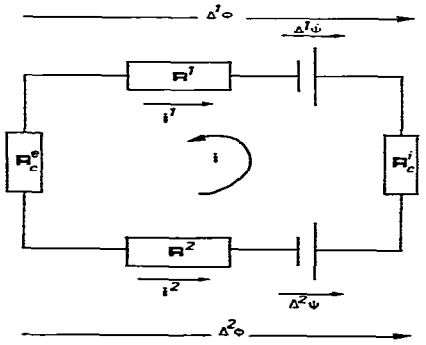

Fig. 6. Equivalent scheme of the coupling between two epithelia. Symbols: R_c^e and R_c^i, coupling resistances, between external and internal faces respectively; R^1 and R^2, electrical resistances of epithelia (1) and (2); i^1 and i^2, electrical currents through epithelia (1) and (2); $\Delta\phi$, electrical potential differences; $\Delta\psi$, diffusion potential differences.

and

$$\delta^1 \equiv \frac{\delta'}{r_t}; \quad \delta^2 \equiv \frac{\delta'}{r_t} \cdot \frac{R^2}{R^1} \tag{53}$$

the same reasoning as used to obtain eqs. (47) and (48) gives for epithelium (1)

$$\begin{cases} \dfrac{dx_j}{dt} = 1 - x_j - \rho y_j \theta(x_j) + \delta' \cdot \ln \dfrac{x^1}{x_j} - \dfrac{\delta'}{r_t} \cdot \ln \dfrac{x^1}{x^2} \\ \dfrac{dy_j}{dt} = \alpha_j \{1 - y_j - u y_j \theta(x_j)\} \end{cases} \tag{54}$$

and for epithelium (2)

$$\begin{cases} \dfrac{dx_k}{dt} = 1 - x_k - \rho y_k \theta(x_k) \\ \qquad + \delta' \cdot \ln \dfrac{x^2}{x_k} + \dfrac{\delta'}{r_t} \cdot \dfrac{R^2}{R^1} \cdot \ln \dfrac{x^1}{x^2} \\ \dfrac{dy_k}{dt} = \alpha_k \{1 - y_k - u y_k \theta(x_k)\} \end{cases} \tag{55}$$

where x^1 and x^2 are the mean values of x for epithelia (1) and (2).

4.4 Numerical study

We shall first estimate the numerical values assumed by δ', K, δ^1 and δ^2 under experimental conditions where we know [2] that a skin oscillates. Then we shall calculate the theoretical values of δ', K, δ^1 and δ^2 allowing the oscillations to occur or not, according to the model. Comparison of both sets of data (experimental and theoretical) will be a test of the internal consistency of our approach.

Assume the approximate values

$$\begin{cases} [\mathrm{Li}]_e \div 10^{-4}\ \mathrm{mol\ cm^{-3}} \\ t_{\mathrm{Li}}^a \div 0.7 \\ P_{\mathrm{Li}}^a \div 0.8\ 10^{-5}\ \mathrm{cm\ s^{-1}} \\ R_u \div 10^{-3}\ \Omega\ \mathrm{cm^2} \end{cases} \tag{56}$$

$[Li]_c$ corresponds to a usual concentration of Li^+ in (e). Under these experimental conditions, most of the electric current is carried by Li^+; hence the choice (somewhat arbitrary, though) of 0.7 for t^a_{Li}. The value of P^a_{Li} is assumed to be similar to P^a_{Na} [12]. R_u has been estimated [1] to lie between 0.5 and 1.5 kΩ cm^2. At the ambient temperature, application of eq. (51) leads to an estimate of δ'

$$\delta' \div 0.4 \tag{57}$$

Estimation of K by eq. (50) requires a knowledge of the value of the externally imposed electric current I. We have performed the calculation in two different situations

$$\begin{cases} I \div 10\ \mu A\ cm^{-2} \Rightarrow & K = 0.1 \\ I \div 50\ \mu A\ cm^{-2} \Rightarrow & K = 0.5 \end{cases} \tag{58}$$

as it is known [2] that synchronization is possible in the first situation, while it generally vanishes in the second. A poor coupling between two epithelia occurs when the coupling resistances become infinite, hence

$$\delta^1 \to 0; \quad \delta^2 \to 0 \tag{59}$$

For low values of these coupling resistances, when the coupled epithelia have identical electrical properties

$$\delta^1 = \delta^2 = \frac{\delta}{2} \tag{60}$$

Experimentally, we have seen [2] that synchronization between two epithelia occurred when

$$\begin{cases} R^1 = R^2 \div 10^3\ \Omega \\ R^e_c = R^i_c \div 10^3\ \Omega \end{cases} \tag{61}$$

which implies

$$\delta^1 = \delta^2 = \delta'/4 \div 0.1 \tag{62}$$

Conversely, synchronization was impossible in the case when

$$\begin{cases} R^1 = R^2 \div 10^3\ \Omega \\ R^e_c = R^i_c \div 6 \times 10^3\ \Omega \end{cases} \tag{63}$$

which leads to

$$\delta^1 = \delta^2 = \delta'/14 = 0.03 \tag{64}$$

Fig. 7 gives the simulation of coupling between 10 elementary domains. No appreciable difference was observed in the results when the averaging of x was effected geometrically or arithmetically. One sees that synchronization occurs when

$$\delta' = 0.5 \tag{65}$$

while it does not when

$$\delta' = 0.1 \tag{66}$$

This means that coupling can occur only when δ' is not too small. It should be emphasized that the

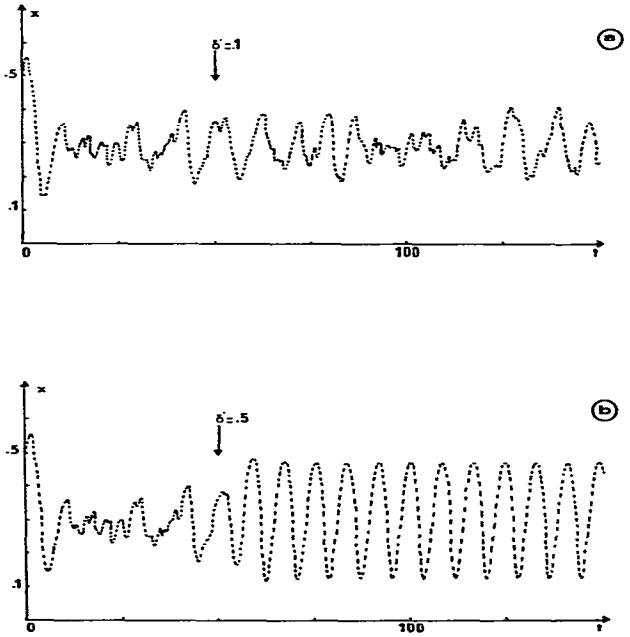

Fig. 7. Influence of δ' on the synchronization of different domains of the skin. The numerical calculation was performed with 10 elementary domains characterized by their values of α: $\alpha_1 = 0.24$; $\alpha_2 = 0.15$; $\alpha_3 = 0.26$; $\alpha_4 = 0.10$; $\alpha_5 = 0.28$; $\alpha_6 = 0.12$; $\alpha_7 = 0.22$; $\alpha_8 = 0.18$; $\alpha_9 = 0.20$; $\alpha_{10} = 0.14$. The other parameters were $\rho = 8$; $u = 10$; $\beta = 5$; $x_{max} = 0.14$ for all the domains. The averaging was performed here using the geometrical mean, but the results were similar when using the arithmetical mean (a) $\delta' = 0.1$, no synchronization between the elementary domains; (b) $\delta' = 0.5$, synchronization occurs, and the mean values of the concentrations (x for instance) start regularly oscillating.

value of δ' corresponding to our experimental conditions (0.4 according to eq. (57)) lies just between the two values which have been shown here to permit or prevent synchronization of the local oscillators. This indicates that the oscillating epithelia were working near the limit of desynchronization, which fits in quite well with the observation [2] that a skin oscillation might spontaneously vanish or reappear, even when media (e) and (i) are left unchanged. When an external current is imposed, there is a satisfactory agreement between the actual experimental situation (eq. 58) and the quantitative data given in fig. 8. When considering the coupling between two similar epithelia, using the arithmetical mean to average x, we found that synchronization occurred for

$$\delta^1 = \delta^2 \gtrsim 0.1 \tag{67}$$

while it did not for

$$\delta^1 = \delta^2 \lesssim 0.03 \tag{68}$$

In this case, the results obtained when using the geometrical mean instead of the arithmetical one were less clear. One must therefore remain cautious about the exact transition value of the coupling parameters. As they are, however, the above results of the calculations (eqs. (67) and (68)) are consistent with those obtained from the experiment (eqs. (62) and (64)).

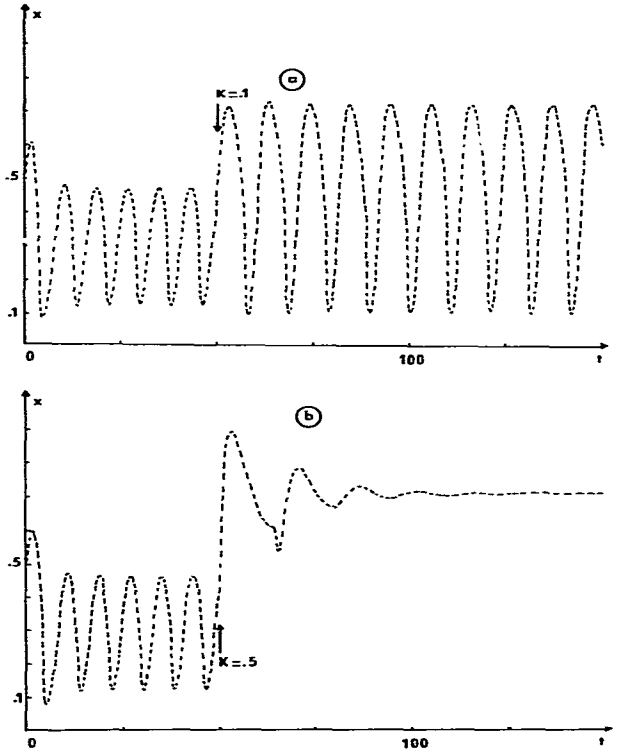

Fig. 8. Influence of an externally imposed current on a set of synchronized oscillators. Ten elementary domains have been considered in the calculation, with the same characteristics as already indicated in fig. 7. The averaging was performed using the geometrical mean, but similar results have been obtained when using the arithmetical mean. (a) $K=0.1$, the externally imposed electric current modifies the amplitude of the oscillation but synchronization is maintained; (b) $K=0.5$, the oscillation is rapidly damped.

5. Appendix A: discussion of some other plausible models of oscillation

Our model of oscillation has been built on the idea that the non-linearity responsible for the induction of the oscillations arises from the response of the pump to Li^+, according to eq. (10). This was done because the experimental data [2] strongly suggested such an interpretation. However, is it possible that some other sources of non-linearity might play an important part in the phenomenon? We examine below the three possibilities which seem the most likely.

5.1. Non-linearity originating from an Na^+/Li^+ competition

It has been found that the oscillations generated by Li^+ occurred even when there was no Na^+ in the external medium [2]; hence, it seems quite improbable that oscillations could originate from a simple competition between Na^+ and Li^+ on the pump. However, we can study, from a theoretical point of view, the effect of the Na^+/Li^+ competition.

Consider the dimensionless concentration of Li^+, x, in compartment (1), and in like manner,

the dimensionless concentration of Na^+, z, in the same compartment. Let $F(x,z)$ and $G(x,z)$ be the functions describing, respectively, the dimensionless Li^+ and Na^+ fluxes carried through the pump. Functions F and G thus generalize function f as it appeared in our basic model. Generalization of eqs. (24) and (25) gives

$$\begin{cases} \dfrac{dz}{dt} = 1 - z - \rho G(x,z) \\ \dfrac{dx}{dt} = \alpha\{1 - x - uF(x,z)\} \end{cases} \tag{69}$$

A competitive interaction, in the absence of any inhibition by an excess of Na^+ or Li^+, is written

$$\frac{\partial G}{\partial z} > 0; \quad \frac{\partial G}{\partial x} < 0; \quad \frac{\partial F}{\partial z} < 0; \quad \frac{\partial F}{\partial x} > 0 \tag{70}$$

Applying Bendixon's criterion (section 3.4) one can see that there can exist no instability in the stationary states. Hence no oscillation can be generated by a simple Li^+/Na^+ competition on the pump.

5.2. Interference of the osmotic pressure

A priori, osmotic pressure might interfere with the oscillatory process at various levels. A direct effect on the kinetics of the pump is unlikely, as it is not evident why it would promote oscillations only in the presence of Li^+. An indirect, mechanical effect, though, might be considered. The cellular network of the epithelium possesses some elasticity, and oscillations have been obtained, at least in artificial membranes, by coupling an externally imposed current to a pressure gradient [75,76]. However, this cannot be the explanation in our case, as we have seen [2] that oscillations occurred in the absence of any imposed electric current (oscillations of the spontaneous potential difference of the skin).

One could also consider taking into account the volume changes in compartments (1) or (2) which are due to the modifications of osmotic pressure. Indeed, the compartment volumes are considered to be roughly proportional to the activity, v_p, of the ionic pumps [77]. Consider again l_1 and l_2, the volumes of compartments (1) and (2) per unit of surface area of the epithelium. To make calculations simple, let us consider that only l_2 varies. According to the kinetics of the pumps (20) we get

$$l_1 = \text{constant} \tag{71}$$

$$l \equiv \frac{l_2}{l_1} = Cy\theta(x) \tag{72}$$

C being a constant. Eq. (24) remains valid, while, with eq. (72), eq. (25) must be modified to

$$\frac{dy}{dt} = \frac{\alpha_m}{2y\theta}(1 - y - uy\theta) - \frac{y}{2\theta}\frac{d\theta}{dx}(1 - x - \rho y\theta) \tag{73}$$

with

$$\alpha_m = \frac{\tau_1 P_K^c}{Cl_1} \tag{74}$$

Applying again Bendixon's criterion (section 3.4) to eqs. (24) and (73), we conclude that no oscillation can be generated, when $d\theta/dx$ remains positive.

5.3. Non-linearity at the level of the apical membrane

In the basic model, we have assumed that the passive fluxes through apical membrane (a) were expressed at a constant permeability (*hypothesis h2* and eq. (1)). However, it is well known that the apical permeability is not actually constant [12,78], and a 'diode' effect has even been observed for the ionic fluxes [45]. One might thus wonder if such a non-linear behaviour at the level of the apical membrane could not be responsible for the induction of oscillations.

The study of these types of non-linearity has shown that they remained monotonic, i.e., that the derivatives kept a constant sign. They are in fact non-linearities which Glansdorff and Prigogine [68] call 'linear in the broad sense', and which cannot in themselves lead to instability. In the case of the small regular, quasi-sinusoidal oscillations which are often observed experimentally [2,5], it can thus be assumed that variations of P_{Li}^a cannot be the determining factor in the induction of the oscillation. However, for the complicated oscillations

which sometimes occur [2,5], it cannot be discounted that variations of P^{a}_{Li} might contribute to modulating the phenomenon (Nicolis, personal communication).

6. Appendix B: derivation of the simplified expressions of the transmembrane electric potential and ionic fluxes

6.1. General expression of the electrical terms

Consider a membrane (m) and its electrical characteristics defined as indicated in fig. 9. The density of imposed electric current, I, is written

$$I = -\frac{\Delta\phi}{R_u} + \frac{\Delta\psi}{R_u} + I^x \tag{75}$$

In the right-hand side of eq. (75), the first term, $\Delta\phi/R_u$, contributes to a possible potential difference, $\Delta\phi$, externally applied. The second term, $\Delta\psi/R_u$, corresponds to the diffusional potential which is created by the passive transport of the various permeating species through the membrane, while the last term stands for a possible electrogenic current associated with the functioning of the active pump.

The detailed expression of $\Delta\psi$ is a rather complex function of the transport numbers of membrane (m) [79]. Assuming constant transport numbers, t_j^m, it simplifies to

$$\Delta\psi = -\sum_j \frac{t_j^m}{z_j F}\,\Delta\mu_j \tag{76}$$

where j holds for the permeant species (chemical potentials μ_j), and where each transport number, t_j^m, corresponds to the fraction of the electric current which is carried through (m) by species j in the absence of any chemical gradient through (m).

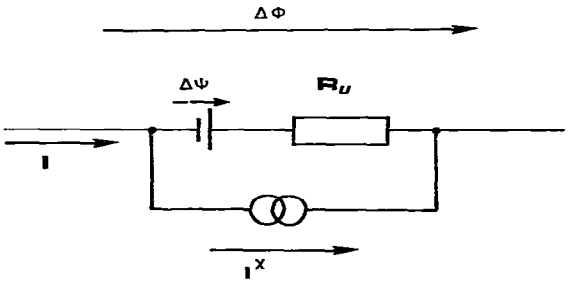

Fig. 9. Equivalent electrical circuit for a membrane (m). Symbols: $\Delta\psi \equiv \psi_i - \psi_e$ diffusion potential difference between media (i) and (e), taking into account the ionic distributions through (m); R_u, electrical resistance per unit surface area of (m); I^x, generator of electric current symbolizing an electrogenic metabolic transport through (m); I, density of imposed electric current (A cm^{-2}).

6.2. Simplified expression of the transepithelial potential difference

According to the model of Koefoed-Johnsen and Ussing [42], the transepithelial potential $\Delta\phi$, is the sum of two diffusional potentials. Taking into account that, when there is no Na^- in the external medium, the only cations important in the generation of the electric potential are K^- diffusing through (b) and Li^+ diffusing through (a), one obtains

$$\Delta^a\psi + \Delta^b\psi = -\frac{t^a_{Li}}{F}\Delta^a\mu_{Li} - \frac{t^b_K}{F}\Delta^b\mu_K \tag{77}$$

It is known [30] that the cellular content of K^+ decreases while Li^+ accumulates in the cells, when Na^- is replaced by Li^+ in the external medium. Under conditions corresponding approximately to our usual experimental conditions, Li^+ was thus found to accumulate up to 25–50 μequiv. per g of fresh epithelium, while the K^+ concentration fell from 370 to about 300 μequiv. per g of fresh epithelium. This shows that, in the course of the oscillations, the relative variations of $[Li]_i$ are likely to be much greater than those of $[K]_i$. Hence, we may consider that the variations of $[K]_i$ with respect to time remain negligible. This leads, using the conventional expression of the chemical potential [80], to the following expression for eq. (77)

$$\Delta\psi(t) = \Delta\psi_o - t^a_{Li}\frac{KT}{F}\cdot\ln x(t) - t^b_K\frac{RT}{F}\cdot\ln y(t) \tag{78}$$

where $\Delta\psi_o$ corresponds to the sum of all the constant terms.

6.3. Simplified expression of the passive fluxes

Assuming that the cations and anions follow different pathways through a membrane (m), the chemical coupling between both types of ions can be considered as negligible. For the small electrochemical gradients, the passive flux, J_j, of species j can be expressed as being proportional to the electrochemical potential difference of j, $\Delta\tilde{\mu}_j$ [80]:

$$J_j = L_j \Delta\tilde{\mu}_j \tag{79}$$

where L_j is the phenomenological coefficient.

Using Bockris notations [81], one can write

$$J_j = P_j \Delta[X_j] + \frac{t_j^m}{z_j F} I \tag{80}$$

where Pj is the passive permeability of species j, $\Delta[X_j]$ the transmembrane difference of concentration j, and t_j^m the transport number of species j [81].

7. Discussion and conclusions

The numerical calculations have been based on several assumptions. First, we have considered a 'one-dimensional problem' by neglecting the thickness of the epithelium (*hypothesis h1*): according to Leblanc [11] the characteristic time for compartment (1) is of the order of several minutes, while the period of the oscillations is about 10 min. It is thus a rather crude assumption to neglect the possibility of 'concentration waves' through the depth of this epithelium. In the same manner, we have used the simplifying eq. (46) to express the coupling; and we have neglected the variations in the resistance of the epithelium (*hypothesis h8*). It is difficult to take into account the influence of these simplifications. For instance, it is well known from studies on non-linear oscillators [63] that increasing the number of variables can drastically change the model. However, all these simplifying assumptions were formulated keeping in mind that the basic non-linearity inducing oscillations is the effect of Li^+ on the active pump. This led us to a theoretical description allowing numerical calculations which cope quite well with the quantitative experimental data. Theory and experiment are consistent with parameter α being less than 1, with synchronization between two epithelia occurring only when the electric current is small, and with osmotic pressure not being a determining factor in the induction of the oscillation. The period of oscillation has the right order of magnitude and the model states that a direct relation exists between the time course of the electric oscillations and that of the ionic ones (eqs. (75) and (78)); experiment shows that the oscillations of electric potential and of Na^+ flux have approximately the same period [2]. The model of oscillation is a local one, with a synchronization of the local oscillators by the electric current; experiment shows that varying the surface area of the studied skin has no systematic effect on the period of oscillation, and synchronization of two epithelium fragments is achieved with purely electrical bridges (without any possible diffusion). Moreover, quantitative agreement between experimental and theoretical estimates is satisfactory. The model considers that the skin works at the limit of desynchronization, which is consistent with the occurrence of spontaneous desynchronization [2], and with the fact that some skins oscillate while others do not, under apparently identical experimental conditions, etc.

There remains now the study of which of the models ('falsifiability' [82]) will allow the taking into account of some particular features of the oscillatory process (for instance the fact that the oscillations might become complex instead of being quasi-sinusoidal). Such an approach might be very helpful for a better understanding of the structural and physiological characteristics of complex systems like frog skin.

It is also possible that our approach is not limited to the case of the frog skin, but can be generalized to other systems. For instance, it is known that the functioning of the central nervous system is accompanied by the emission of a complex set of electrical signals. Hence, there is the possibility of coupling interactions between these different signals, which might play a part in the psychiatric role of Li^+ [83–85]. While this remains largely speculative at present, it can however be underlined that some authors [86,87] have already advocated the relevance of the coupling between oscillators in the brain.

Acknowledgements

This work was carried out with the help of the C.N.R.S. (L.A. 203 and A.T.P. 98147) and of the D.G.R.S.T. (grant 77 7 1272). We thank Professors Ussing, Nicolis and Matricon for fruitful discussions.

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