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# Oscillation of the Electric Potential of Frog Skin under the Effect of $Li^+$: Experimental Approach

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**Summary.** When a frog skin is used to separate two compartments, and lithium is added to the external medium, transmembrane electric potential oscillations frequently occur. When no external current is imposed, sustained oscillations, with a period of about 10 min, are maintained for several hours. An oscillation of the $Na^+$ influx accompanies the electric oscillation, though the two oscillations are out of phase to a greater or less extent.

Theophyllin promotes a significant decrease in the mean electric potential of the skin, but it does not affect very much the characteristics of the oscillation. Important factors influencing the oscillation are temperature, permeability of the external membrane to lithium, and potassium concentration in the internal medium. No correlation can be detected between oscillation characteristics and skin area. This suggests that the oscillation is of a local nature, possibly originating at the cellular level. Occurrence of macroscopic oscillations implies coupling between local oscillators. Coupling between two epithelia has been studied under diverse conditions. The coupling is of an electrical nature: by varying the value of the coupling resistance, it is possible to control synchronization of the oscillations.

Since Dubois-Raymond discovered in 1848 [5] the electromotive force of the frog skin, many studies have shown that the presence, in the external medium, of either $Na^+$ or $Li^+$, was absolutely necessary to maintain the epithelium potential [7, 11, 20]. $K^+$, $Cs^+$, $Rb^+$ or $NH_4^+$ are ineffective [33]. It was first considered that $Li^+$ merely replaced $Na^+$ in the electrogenic process, but it soon became apparent that the presence of $Li^+$ in the external medium was also capable of inducing an oscillation of the transepithelial potential [13]. Since then, the phenomenon has been studied in more detail [6, 32, 33, 35] and a number of experimental data have been obtained. However, up to now, no comprehensive theoretical interpretation of the phenomenon has been proposed.

We aim to derive such an interpretation by using the formulation of the nonstationary states, as developed by the Brussels school [10, 21]. The idea is that the epithelium attains a stationary state when no lithium is present in the external medium, but that addition of lithium would promote, in the flow-force relations of the epithelium, one of those nonlinearities that can lead to instability and oscillations. In the chronological development of our approach, the experiments and the theoretical considerations have been elaborated simultaneously. For the sake of clarity, we have divided our work into several parts. The present paper gives only our original experimental data, i.e., data the necessity for which has emerged in the course of the theoretical elaboration and which we have not found in the literature. The details of the theoretical treatment will be explained subsequently in two steps: (i) building of a local model of oscillations and (ii) explanation of how the local oscillators synchronize themselves in the form of an overall oscillation of the electric potential.

## 1. Materials and Methods

The frogs were male *Rana esculenta* originating from two different suppliers (Animalabo, 75014 Paris, France; and Guy Conetard, Le Pissot 85270 St Hiller de Riez, France), and referred to as batches *A* and *B* in the following text. Between arrival at the laboratory and being used in the experiments, the frogs were kept in a pond maintained at constant temperature 10 °C. For the experiments, the animals were decapitated and the skin of the abdomen removed. It was mounted in an experimental device [30] modified from that of Ussing [38], between two compartments *e* and *i* corresponding, respectively, to its external and internal faces. The compartments were filled with various solutions of mineral salts or organic substances, including the conventional Ringer solution

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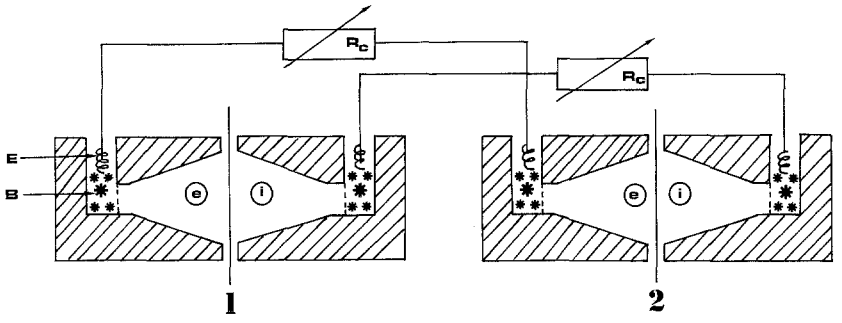


**Fig. 1.** Scheme of the apparatus used for producing electrical coupling between two frog skins. ⓔ and ⓘ: external and internal media for both frog skins *1* and *2*. $R_c$: salt bridge, with a variable resistance between the external or the internal compartments. *E:* saturated KCl electrodes allowing transepithelial potential measurements. *B:* Ag/AgCl electrodes allowing injection of an imposed electric current.

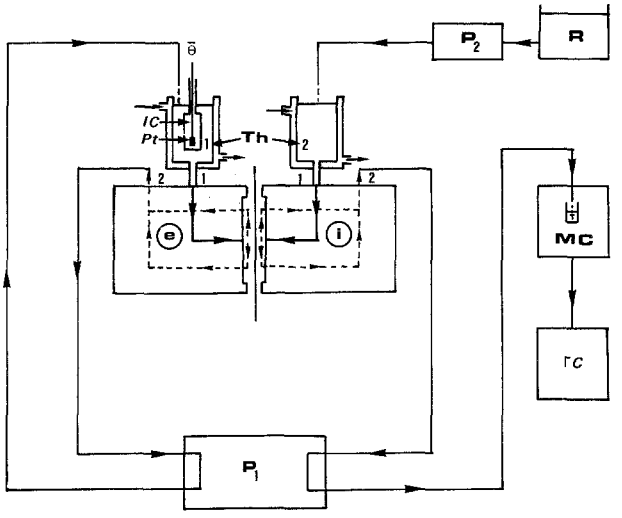


**Fig. 2.** Scheme of the apparatus used for flux measurements. ⓔ and ⓘ: external and internal media. $\bar{\theta}$: mean temperature of the compartment. *Pt:* platinum probe for temperature measurements, contained in an inox chamber (*IC*). *Th 1* and *2:* thermostatically controlled chambers (the labeling radioisotope is in *Th 1*). $P_1$: pump circulating the external solution in compartment *e*. *MC:* microcollector sampling solutions from compartment *i* for radioactive measurements. *R:* reservoir with unlabeled Ringer solution. $P_2$: pump supplying thermostated chamber *Th 2* with Ringer solution taken from reservoir R. *ΓC* Gamma Spectrometer.

(NaCl, 112 mM; KCl, 2 mM; $NaHCO_3$, 2.4 mM; $CaCl_2$, 2 mM) or more or less modified variants of the above basic Ringer solution. The solutions in compartment *e* and *i* are, respectively, referred to as "external" and "internal" solutions. According to the experiment, the area of skin separating both compartments varied between about 0.3 and 3 $cm^2$. Prior to beginning any type of treatment, the skin was equilibrated by filling both compartments with conventional Ringer solution and left until stable electric parameters were obtained. This took from one to several hours, depending on the sample.

The measurements of the transepithelial electric potentials were made with KCl-saturated electrodes. Agar bridges (Agar-Agar 2%) with concentrated NaCl (1 M) were used to link the electrodes to the solutions in the compartments. The potentials to be measured lying between 10 to 100 mV and the epithelium impedance being at the most a few kΩ, a conventional potential recorder was suitable for such measurements. We used a 12-way recorder which allowed several experiments to be carried out simultaneously. It was possible to impose an external current of fixed value through the skin by using a battery; and, when necessary, a reinjection circuit [30] imposed across the skin any desired value of electric potential difference, with a transepithelial electric current of up to 50 μA $cm^{-2}$. The reinjection electrodes were of the Ag/AgCl type. A particular case of such a situation was that of the short circuited conditions, when the imposed potential difference was maintained at zero [39]. The compartments used in those conditions were given a conic shape [30] so that the equipotential surfaces were quasi-parallel to the skin. A continuous injection of air bubbles ensured both homogenization of the solutions in the compartments and oxygenation of the skin. Electrical linkage of two different epithelia was achieved by using agar bridges (*see* Fig. 1) with NaCl concentration chosen such that the bridges assumed any desired value of electrical resistance.

Estimation of unidirectional fluxes of sodium were performed as usual [38] with the aid of the radioactive isotope $^{24}Na$. However, the experimental device was modified in such a way that these measurements could be made precise enough to detect possible oscillations of the sodium fluxes. For that purpose (*see* Fig. 2), the volumes of both compartments were reduced as much as possible (down to 3 ml), which increased the values of the specific radioactivities to be measured. The depth of fluid above each face of the skin was then no more than 0.8 mm. Teflon netting was extended over both sides of the skin in order to prevent it from being pressed towards the rear wall of either compartment. A continuous flow of solution was obtained in each compartment by the use of a Minipuls pump. The solutions arrived, from thermostabilized reservoirs, at the level of the skin; then they progressed to the periphery of the compartment through 35 holes 0.6 mm in diameter, and were finally collected with an automatic sample collector. The volume of liquid outside the compartments was not more than 1 ml. Liquid flow was adjusted to 5 ml $min^{-1}$, which was enough to ensure a sufficient oxygenation of the skin, thus rendering injection of air bubbles unnecessary and at the same time avoiding the development of excessive pressure gradients liable to damage the skin. Each liquid sample corresponded to one minute's functioning of the device, hence to 5 ml solution. The radioactivity measurements were made with a gamma spectrometer (Gammatic SAIP, France). In each solution reservoir, an inox chamber allowed measurement of the mean temperature with a platinum probe. Control experiments, made with the aid of thermistors (Fenwal electronic, USA), showed that the temperature change of the solution did not exceed 0.5 °C during flow along the skin, the reservoir temperature being 10 °C and the ambient temperature 20 °C. This shows that the temperature at the level of the skin can be estimated by that of the reservoir with a precision better than 1 °C.

Most of the experiments were performed on the entire skin. However, in a few cases, only the separated epithelium was used. Separation of the epithelium from the underlying cellular layers of the skin was achieved by an appropriate enzymatic treatment [25].

In some instances both compartments, *e* and *i*, were subcom-

partmentalized ($e_1$, $i_1$; $e_2$, $i_2$; etc.), thus allowing the simultaneous study of different areas of a single skin.

## 2. Results

### *2.1. Evidence of Oscillations: General Features of the Phenomenon*

Consider a skin pre-equilibrated with a Ringer solution bathing both faces, followed by a change in the external medium composition, in which lithium is incorporated. Frequently under such conditions, the spontaneous potential of the skin will begin to oscillate with a period of a few minutes. Figure 3*a* gives an example of such a phenomenon. The oscillatory process exhibited at least two fundamental characteristics: (i) no condition other than the presence of $Li^+$ in the external medium was able to induce such oscillations; (ii) when it occurred, the oscillation started almost immediately after modification of the external medium. There was no visible lag-phase.

The phenomenon was rather variable in character. For instance, for batch of frog *A*, under the ionic conditions "Ringer solution in the internal medium" and "115 mM LiCl in the external medium," the smallest and the largest values of the periods measured in 10 comparable experiments were 3.5 and 12.5 min, respectively. Similar variations of the period value were observed under other experimental conditions. Table 1 gives the mean values and standard deviations of the period in the different experimental situations. The amplitude was even more variable (not shown in the table). There was also a significant difference between the two batches of frogs, although they were of the same species (*see* Table 1). For a given skin in a given ionic situation, both the period and the amplitude varied with time. This is not indicated in the table, though we have previously given [35] an example of skin oscillation lasting more than 20 hr, with the period progressively changing from 8 min at the beginning of the oscillation to 18 min at the end. This is the reason why, in most of the figures and tables, we give only the mean values of the period over each whole experiment (duration of the oscillation divided by the number of oscillations). The phenomenon was also seasonally dependent. The seasons the most favorable were autumn and winter. At the end of spring the oscillation became less obvious, and during summer it proved impossible to obtain any oscillation, even when the experiments were performed in thermostabilized conditions. During winter, some skins started oscillating for $Li^+/Na^+$ ratios not greater than 0.1 or 0.2, while most needed much higher $Li^+/Na^+$ ratios ($>0.5$), and a few wouldn't oscillate at all, even when all of the sodium was replaced by lithium in the external medium.

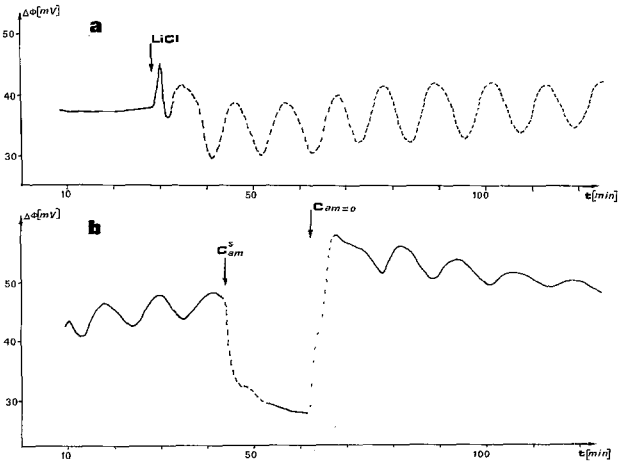


**Fig. 3.** Oscillation of the transepithelial electric potential $\Delta\phi$ (relative to external medium). (*a*): Initiation of the oscillation and its stability with time. Apparatus used was that described in Fig. 1. The internal medium was conventional Ringer solution throughout the whole experiment. The external medium was conventional Ringer solution at the beginning of the experiment, when the skin potential difference was stationary. At the time indicated by the arrow, the external Ringer solution was changed for a 115 mM LiCl solution: oscillations started almost immediately, and were sustained during several hours. (*b*): Effect of amiloride in the external medium. A skin, oscillating under the same conditions as described above, was treated with 25 μM amiloride at the time indicated by the first arrow ($C^s_{am}$): the oscillation immediately vanished without the potential falling completely to zero. Amiloride was removed at the time indicated by the second arrow ($C_{am}=0$): the oscillation was almost immediately restored.

**Table 1.** Period of the oscillation for various external media[a]

| External medium | Frog batch | Number of experiments | Period of oscillations (min) | |
|---|---|---|---|---|
| | | | Mean value | Standard deviation |
| LiCl 115 mM | A | 10 | 5.9 | 2.4 |
| | B | 7 | 10.4 | 1.5 |
| Varied | A | 59 | 6.3 | 3.2 |
| | B | 18 | 10.5 | 1.5 |

[a] The internal medium was the conventional Ringer solution. The external medium was either a 115 mM LiCl solution or solutions more complex and varying from one experiment to another (diluted LiCl solutions or solutions of total concentration 115 mM but containing one or several other substances such as NaCl, KCl, $CaCl_2$, choline, or sucrose besides lithium).

Table 2 shows that, after a transient modification of the composition of the bathing media, the reversibility of the phenomenon was not very good: 5.9 min for the mean period in initial conditions, 5.2 for that corresponding to changes in the media, and

**Table 2.** Effect of a transitory modification of the ionic conditions[a]

| Reference No. | Date of experiment | External medium (mM) | Internal medium (mM) | Period (min) |
|---|---|---|---|---|
| 2–1 | 23–2–76 | LiCl: 69; KCl: 46 | Ringer | 4.6 |
| | | LiCl: 115 | Ringer | 4.6 |
| | | LiCl: 69; KCl: 46 | Ringer | 4 |
| 2–2 | 23–1–76 | LiCl: 23; KCl: 92 | Ringer | 7.6 |
| | | LiCl: 23; KCl: 92 | Ringer (KCl:10) | 4.6 damped |
| | | LiCl: 23; KCl: 92 | Ringer | 7.5 |
| 2–3 | 20–1–76 | LiCl: 23; Chol: 92 | Ringer | 5.5 |
| | | LiCl: 11.5; Chol: 103.5 | Ringer | 6.6 |
| | | LiCl: 23; Chol: 92 | Ringer | 7.5 |
| 2–4 | 26–1–76 | LiCl: 23; KCl: 92 | Ringer | 5.1 |
| | | LiCl: 69; KCl: 46 | Ringer | 3.7 |
| | | LiCl: 23; KCl: 92 | Ringer | 4.3 |
| 2–5 | 21–1–76 | LiCl: 23; Chol: 92 | Ringer | 4 |
| | | LiCl: 23; Chol: 92 | Ringer (KCl:10) | 4.6 |
| | | LiCl: 23; Chol: 92 | Ringer | 4.9 |
| 2–6 | 14–1–76 | LiCl: 115 | Ringer | 5.6 |
| | | LiCl: 57.5; NaCl: 57.5 | Ringer | 6 |
| | | LiCl: 115 | Ringer | 8.2 |
| 2–7 | 10–2–76 | LiCl: 23; KCl: 92 | Ringer | 8.6 |
| | | LiCl: 46; KCl: 69 | Ringer | 6.5 |
| | | LiCl: 23; KCl: 92 | Ringer | 8.3 |
| Mean value in initial conditions | | | | 5.9 |
| Standard deviation in initial conditions | | | | 1.7 |
| Mean value during transitory conditions | | | | 5.2 |
| Standard deviation during transitory conditions | | | | 1.1 |
| Mean value when back to initial conditions | | | | 6.4 |
| Standard deviation when back to initial conditions | | | | 1.9 |

[a] The experiments were always carried out by starting the oscillation in given ionic conditions, then changing either the external or the internal medium, and ultimately coming back to the initial situation. Chol = choline; Ringer = conventional Ringer solution; Ringer (KCl:10) = Ringer solution where part of the NaCl was replaced by KCl in order to bring the KCl concentration to 10 mM. All of the frogs belonged to batch *A*.

6.4 when coming back to conditions analogous to the initial ones.

The shapes of the oscillations were also somewhat variable. They varied from very simple, quasi-sinusoidal oscillations to highly complex, irregular ones. In a previous paper [35], we showed that appearance of the complex type of oscillation was favored by using an external medium modified from a Ringer solution by retaining only 2% of its original $Na^+$ and replacing the rest by choline and 10 to 20% $Li^+$. This, however, cannot be taken as a general rule, as we have now found some examples of complex oscillations in the absence of choline, and of quasi-sinusoidal oscillations in the presence of choline Cases were even noted where, in the course of an experiment, the oscillation spontaneously changed from a quasi-sinusoidal to a complex one or, conversely, without any modification of the bathing media.

### *2.2. Electric Potential Oscillations of the Isolated Epithelium*

Experimenting with the isolated epithelium was rather cumbersome because the latter is mechanically fragile. It was especially difficult to ensure a sufficient oxygenation by injection of air bubbles without also tearing the epithelium. Figure 4 gives, however, an example of the results obtained with isolated epithelia. Lithium induced an oscillation of the electric potential. The oscillation was rather irregular and it damped rapidly, while the period (5 to 6 min) was of the same order of magnitude as for the oscillations obtained with the entire skin.

For the sake of experimental simplicity, in what now follows all the experiments were performed with entire skins.

### *2.3. Effect of the Composition of the External Medium*

The induction of the oscillatory process depended mainly on the existence of a sufficiently high Li concentration in the external medium, though it did not appear to depend very much on the presence of other substances in the external medium. Indeed, in Table 1, calculation of the mean value and of the standard deviation of the period was carried out for external media of widely varying composition, including 115 mM LiCl. The results are remarkably similar, for frog batches A and B. It is notable also that

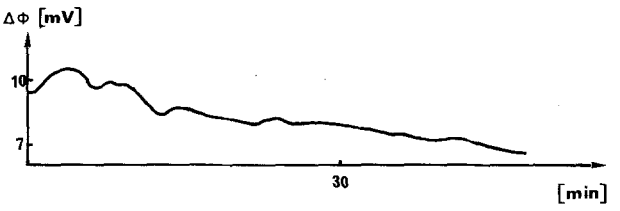


**Fig. 4.** Oscillation of the spontaneous electric potential difference of the separated epithelium. The internal medium was a conventional Ringer solution; the external medium was a Ringer solution where 98% of $Na^+$ was replaced by $Li^+$.

the osmotic pressure had certainly not the same value in the different external media, even at the same total concentration 115 mM, because the different compensating substances (NaCl, KCl, choline, sucrose) have not the same ionization properties. Hence, at least on this scale of values, the osmotic pressure of the external medium does not influence the period of the oscillation very much.

### *2.4. Effect of Various Inhibitors*

Theophylline is known to induce an accumulation of cyclic AMP in the cells via inhibition of the phosphodiesterase [15, 23]. Table 3 describes the effect of this drug on the electric potential of the skin in three different experimental situations: skin nonoscillating because there was no lithium in the external medium (experiment 3–1), skin nonoscillating although lithium was present in the external medium (experiment 3–2), and skin oscillating in the presence of lithium in the external medium (experiments 3–3, 3–4, and 3–5). Theophylline, at least when added to the internal medium, clearly affected the mean electric potential of the skin. Indeed, in all the experimental situations (whether the skin was oscillating or not), addition of theophylline to the internal medium, at doses of the order of magnitude of 1 mM, caused a large decrease of the mean skin potential. Subsequent washing out of theophylline in the internal medium was always followed by a re-establishment of the mean potential of the skin. The effect on the oscillation was, however, much less clear. When the skin was not oscillating, even with a large concentration of lithium in the external medium (experiment 3–2), addition of 1 mM theophylline to the internal medium did not help to induce oscillation. When the skin was oscillating in the presence of lithium (experiments 3–3 to 3–5), addition of theophylline to the internal medium seemed to increase the period and decrease the amplitude of the oscillation. However, the effect was very small and not always reproducible, especially as regards the amplitude.

Several other drugs, known to modify the permeability of the external membrane of the skin, have been tested: amphotericin B [22] and parachloromercuribenzoic acid, on the one hand (supposed to increase the passive permeability of external membrane to sodium), and amiloride, on the other hand (supposed to decrease this permeability) [19]. The oscillations vanished in the presence of any of these substances. Clearly reversible results were obtained with amiloride only (Fig. 3b), and even when the oscillation was blocked, the skin was able to maintain a non-negligible potential difference which was hardly likely to be due to passive diffusion only. The minimum dose of amiloride required to suppress the oscillation was found to be in the region of 5 to 10 μM. Here again, the value of the period found after elimination of amiloride appeared to be always somewhat longer than that observed before addition of the poison. The mean drift, calculated from 7 different experiments, was about 12%.

### *2.5. Modification of the Composition and Osmotic Pressure of the Internal Medium*

It is more difficult to modify the internal medium than the external medium, because the epithelium seems to be very sensitive to perturbations on the internal side, which can damage it irreversibly. For instance, some experiments have been performed with the internal medium provided with the same solutions (where part of the sodium was replaced by lithium) as given to the external medium. This did not make the skin oscillate better. When up to 20% (and sometimes up to 50%) of $Na^+$ was replaced by $Li^+$ in the internal medium, in the most favorable case, the results were identical to those obtained in comparable conditions with no $Li^+$ in the internal medium. In most cases, using a $Li^+$ modified Ringer solution in the internal medium led to various and nonreproducible perturbations, such as big drifts of the mean electric potential of the skin or rapid damping of the electric potential oscillations. When all of the internal sodium was replaced by lithium, not only was the oscillation blocked, but the skin potential collapsed irreversibly. In most of our oscillation experiments therefore, no lithium was added to the internal medium. We also assumed that the lithium transported through the skin from the external medium did not contribute a significant amount of lithium to the internal medium, and did not therefore disturb the experiment.

We were able to modify the osmotic pressure of the internal medium, by diluting the Ringer solution from which it was made, without irreversibly damaging the skin. This, in itself, never induced an oscillation when no lithium was present in the external medium. There were cases, though, of skins which did not truly oscillate although lithium was present in

**Table 3.** Theophylline effect on the oscillation of the electric potential of the skin[a]

| Experiment No. | Ionic composition of external medium | Theophylline (mM) | | Mean skin potential (mV) | Period of the oscillation (min) | | Amplitude of the oscillation (mV) | |
|---|---|---|---|---|---|---|---|---|
| | | Internal medium | External Medium | | Mean value | SD | Mean value | SD |
| | | 0 | 0 | 27 | – | – | – | – |
| | | 0.1 | 0 | 28 | – | – | – | – |
| | | 1 | 0 | 22 | – | – | – | – |
| 3-1 | Ringer | 0 | 0 | 29 | – | – | – | – |
| | | 0 | 0.1 | 30 | – | – | – | – |
| | | 0 | 1 | 33 | – | – | – | – |
| | | 1 | 1 | 25 | – | – | – | – |
| 3-2 | Ringer | 0 | 0 | 18 | – | – | – | – |
| | (90% Li) | 1 | 0 | 9 | – | – | – | – |
| 3-3 | Ringer | 0 | 0 | 14 | 11.1 | 1.4 | 1.4 | 0.3 |
| | (70% Li) | 1.25 | 1.25 | 10 | 14.4 | 6.5 | 0.5 | 0.3 |
| | | 0 | 0 | 24 | 11.8 | 1.0 | 12.1 | 0.0 |
| 3-4 | Ringer | 0 | 0 | 41 | 9.4 | 4.1 | 3.2 | 0.9 |
| | (70% Li, | 0.1 | 0 | 33 | 9.9 | 0.2 | 4.2 | 0.8 |
| | 28% choline) | 0.2 | 0 | 31 | 10.2 | 0.9 | 7.2 | 2.4 |
| | | 0.5 | 0 | 25 | 11.3 | 0.3 | 4.0 | 1.2 |
| | | 1 | 0 | 22 | 12.4 | 0.4 | 2.2 | 0.4 |
| | | 0 | 0 | 25 | 11.3 | 0.7 | 6.4 | 1.0 |
| | | 1 | 0 | 22 | 13.8 | 0.5 | 1.9 | 0.3 |
| | | 2 | 1 | 21 | 16.3 | 2.1 | 1.0 | 0.2 |
| 3-5 | Ringer | 0 | 0 | 25 | 7.0 | 0.4 | 2.3 | 0.6 |
| | (70% Li, | 0.1 | 0 | 25 | 9.8 | 0.5 | 8.7 | 2.4 |
| | 28% choline) | 1 | 0 | 15 | 12.0 | 0.5 | 3.6 | 1.1 |
| | | 0 | 0 | 38 | 10.3 | 0.4 | 7.1 | 0.8 |
| | | 0 | 1 | 29 | 12.3 | 0.9 | 15.6 | 1.4 |
| | | 0 | 0 | 28 | 10.0 | 1.4 | 3.0 | 2.5 |

[a] Internal medium was always the conventional Ringer solution; external medium was a Ringer solution where part of $Na^+$ was possibly replaced by $Li^+$ or choline. When no values of period and amplitude are given, it means that the skin did not oscillate.

their external medium, and which began to oscillate following a 20% diminution of the internal osmotic pressure. This effect, however, was not fully reproducible as it was obtained only 5 times in 12 comparable experiments.

The effect of the internal concentration of potassium was more interesting. The experiments were carried out by modifying the $K^+/Na^+$ ratio (at roughly constant total ionic concentration) in the Ringer solution on the internal face of the epithelium. Figure 5b shows a typical result obtained. Increasing the internal potassium blocked the oscillation, which was restored when the right concentration of potassium was re-established. The experiment was not always perfectly reproducible. However, from 14 experiments comparable to that of Fig. 5*b*, it appeared that, even when increasing the internal $K^+$-concentration did not completely suppress the oscillation, it enhanced the period of the oscillation in most cases. Moreover, increasing the internal potassium concentration also changed the shape of the oscillation curve, which then tended to look like a relaxation process instead of remaining quasi-sinusoidal.

### *2.6. Sustained Character of the Oscillation*

Several authors [6, 33] have described oscillations of the spontaneous potential which were not sustained for more than 1 or 2 hr. We have sometimes obtained the same type of result, but we have also observed oscillations sustained for more than 20 hr. When the oscillation vanished after a few hours it often looked like the disconnection of an oscillator rather than like the passive damping of an oscillation. Furthermore, the oscillation sometimes vanished, then started again without any modification of the external medium (Fig. 5*a*). Such a phenomenon was observed 12 times over 133 experiments. Once, the phenomenon even occurred three times successively during

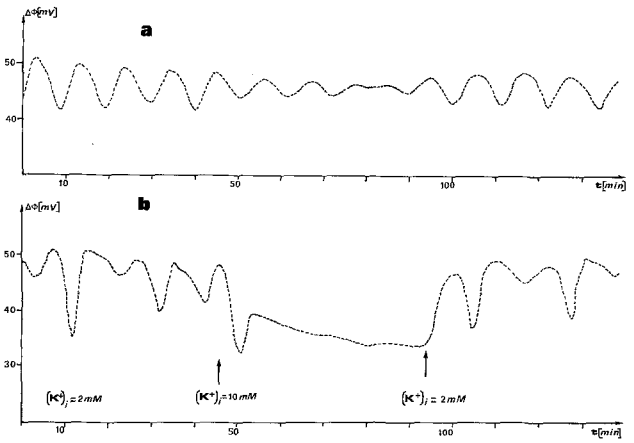


**Fig. 5.** Oscillation of the transepithelial electric potential $\Delta\phi$ (relative to external medium). (*a*): A complex type of behavior was sometimes encountered. External and internal media were, respectively, 115 mM LiCl and conventional Ringer solutions throughout the whole experiment. The oscillations seem to vanish spontaneously and start again. The shape of the oscillation curve suggests the occurrence of an interference between several individual oscillatory processes. (*b*): Effect of the internal concentration of potassium. The skin was oscillating in the same conditions as above. At the time indicated by the first arrow ($K_i^+ = 10$ mM), the $K^+$ concentration in the internal Ringer solution was changed from 2 to 10 mM. The oscillation rapidly vanished, with only a slight decrease of the mean electrical potential difference of the skin. When conventional Ringer solution was replaced in the internal medium, at the time indicated by the second arrow ($K_i^+ = 2$ mM), the oscillation was almost immediately restored. Note that, although the composition of the media were comparable for experiments *a* and *b* during the first 45 min, the shapes of the oscillations were quite different.

three hours in a single experiment. This suggests the occurrence of an oscillator working at the limit of disconnection, rather than that of the regular passive damping of a system.

In short-circuited conditions, the occurrence of oscillations of the electric current under the effect of lithium has been mentioned, here and there, without a great concern by the authors for the phenomenon [18, 26, 40]. We also have observed oscillations for a variety of conditions of imposed potential, including short-circuited conditions [35]. In most cases the amplitude of the oscillations diminished rapidly with time. However, Table 4 shows that the oscillations may be sustained, so long as the reinjection current was not too large. The result depends, in fact, on the physiological state of the skin. For instance, a reinjection current of 5 $\mu A\ cm^{-2}$ tended to suppress the oscillation when the epithelium had already been working for several hours (experiment 4–3), whereas it remained uneffective with freshly prepared epithelia (experiments 4–1 and 4–2). Under the short-circuited conditions, the order of magnitude of the reinjected current was 30 $\mu A\ cm^{-2}$, and, under

**Table 4.** Influence of a reinjected electric current on the oscillation[a]

| Reference No. | Date of experiment | Reinjection electric current ($\mu A\ cm^{-2}$) | Period of oscillation (min) | Remarks |
|---|---|---|---|---|
| | | 0 | 11.2 | |
| | | + 2 | 12.5 | |
| 4–1 | 13–3–78 | + 7 | 14.5 | |
| | | +12.5 | 12.8 | |
| | | −12.5 | 14.5 | |
| | | 0 | 8.5 | |
| | | + 5.5 | 10.5 | |
| 4–2 | 23–3–78 | +12 | 13.5 | |
| | | − 6 | 12 | |
| | | +12 | 12 | |
| 4–3 | 14–3–78 | 0 | 12 | |
| | | + 5 | (rapidly damped) | Skin potential collapses |
| | | 0 | 8.3 | |
| 4–4 | 11–5–78 | +12.5 | 8.5 Damped | |
| | | 0 | 13.1 | |

[a] The epithelia were subjected to different densities of electric current, either from internal to external medium (negative values) or vice versa (positive values). All the frogs belonged to batch *B*. Internal medium was a conventional Ringer solution, and external medium was a 115 mM LiCl solution.

the usual conditions of recording, the oscillations always seemed to be rapidly damped; but when working with a sufficient magnification, it appeared that the oscillations were, in fact, sustained (with a very small amplitude though) for up to 1 hr or more. Furthermore, when the oscillations had almost vanished, renewal of the external medium (even without changing its composition) was enough to reinitiate the oscillation with a large amplitude.

### *2.7. Temperature Effect*

As shown in Fig. 6, oscillation vanished when the temperature was reduced from 28 to 15 °C, and was restored when the initial temperature was once more reinstated. The same experiment has been reproduced 5 times with the same result, except for the fact that the oscillation has not always been restored exactly as it was before the temperature change took place.

### *2.8. Sodium Flux Measurements*

With a period of a few minutes, it is very unlikely that the oscillation can be interpreted by purely capacitive effects. Besides, different authors [6, 33] have

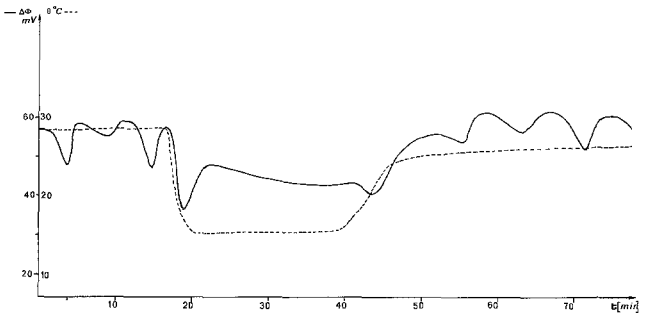

**Fig. 6.** Temperature effect on the oscillation. External and internal media were, respectively, 115 mM LiCl and conventional Ringer solutions. ------ = skin temperature (C); ——— = electrical potential difference of the skin relative to external medium ($\Delta\phi$ mV).

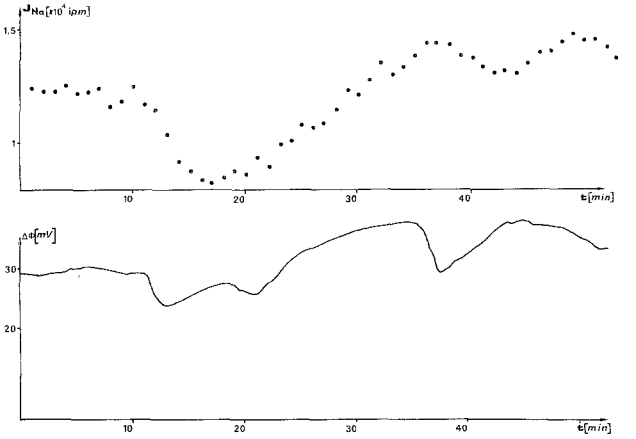

**Fig. 7.** Sodium influx measurement through the frog skin. The internal medium was conventional Ringer solution. The external medium was a Ringer solution, labeled with $^{24}$Na 33 $10^{11}$ *ipm* mole$^{-1}$, where 80 mM $Na^+$ had been replaced by $Li^+$. Although the oscillation is here not of the quasi-sinusoidal type, it is evident that sodium influx $J_{Na}$ (•••••) oscillates and is correlated with the transepithelial electrical potential difference ($\Delta\phi$, relative to external medium).

shown that the oscillations of the skin electric potential were accompanied by oscillations of the electrical resistance of the skin, which suggests that oscillation of the ionic fluxes might accompany the potential oscillation; hence the idea of following the influx of sodium during an oscillation induced by lithium.

Standardization of the experimental device was performed in a preliminary experiment, with aid of a nonoscillating artificial ion exchange membrane (here a cation exchanger) separating two different solutions. The first solution (*e*) was conventional Ringer solution, the second one (*i*) was the Ringer solution diluted five times. Solution (*e*), 5 ml in volume, was labeled with 30 μCi $^{24}$Na. The flux of sodium from *e* to *i*, according to the concentration gradient of the salt, was measured using exactly the same device as the one measuring the unidirectional fluxes of sodium through the skin (*see* p. 108). The scattering of the points, which is due to the nature of the experimental device, proved to be small enough to allow the calculation of Na-flux values with a maximum possible error of 8%. This precision was sufficient to show that the oscillation of the electric potential of a frog skin was actually accompanied by an oscillation of the $Na^+$ influx (Fig 7). From five successive experiments carried out under comparable conditions, it appears that both oscillations (electric potential and $Na^+$ influx) had the same period, though they were sometimes almost in phase and sometimes clearly out of phase.

In another experiment, the sodium influx was measured for varying values of the $Li^+/Na^+$ ratio in the external medium (Fig. 8). The mean potential of the epithelium remained approximately constant (about 52 mV) throughout the Experiment. Meanwhile the influx of sodium diminished from the value 73 pmol cm$^{-2}$ sec$^{-1}$ for the ratio $Li^+/Na^+ = 2$ to the value 39 pmol cm$^{-2}$ sec$^{-1}$ for the ratio $Li^+/Na^+ = 10$.

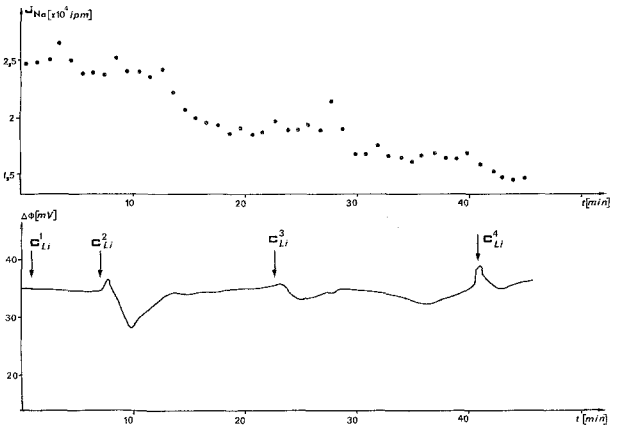

**Fig. 8.** Sodium influx and transepithelial electrical potential difference (relative to external medium) for varying values of the external LiCl concentration. The internal medium was conventional Ringer solution. The external medium contained 6 mM NaCl to which were added various concentrations of LiCl at the times indicated by the arrows: 11.5 mM ($C^1_{Li}$); 23 mM ($C^2_{Li}$); 34.5 mM ($C^3_{Li}$); and 57.5 mM ($C^4_{Li}$). In all these cases, the skin showed negligible oscillation, even for the largest value of LiCl concentration. It appears that sodium influx $J_{Na}$ (•••••) is much reduced by increasing lithium concentration, while transepithelial electrical potential difference (———) remains almost unaffected. The external medium was a Ringer solution, labeled with $^{24}$Na 17.7 $10^{11}$ *ipm* mole$^{-1}$.

### 2.9. *Influence of the Area of the Studied Skin*

Table 5 gives the result of a series of experiments carried out to study the oscillations obtained for different surface areas of skin under constant ionic conditions (experiments 5–1 to 5–5). In order to minimize

**Table 5.** Influence of the skin area on the period of oscillation[a]

| Reference No. | Date of experiment | Value of the period (min) for skin areas ($cm^2$) originating of a single frog for each experiment | | |
|---|---|---|---|---|
| | | 0.28 | 0.8 | 2 |
| 5–1 | 16–11–78 | 5.7 | 5.6 | 5.3 |
| 5–2 | 17–11–78 | 4.6 | 5 | 4.2 |
| 5–3 | 20–11–78 | 4.3 | 5.6 | 6.3 |
| 5–4 | 20–11–78 | 4 | 4 | 5.3 |
| 5–5 | 11–12–78 | 12 | 9.6 | 10.6 |
| Mean value | | 6.11 | 5.96 | 6.34 |
| SD | | 3.35 | 2.14 | 2.45 |

[a] The frogs belonged to batch *B*. The internal medium was a conventional Ringer solution, while external medium was a 115 mM LiCl solution

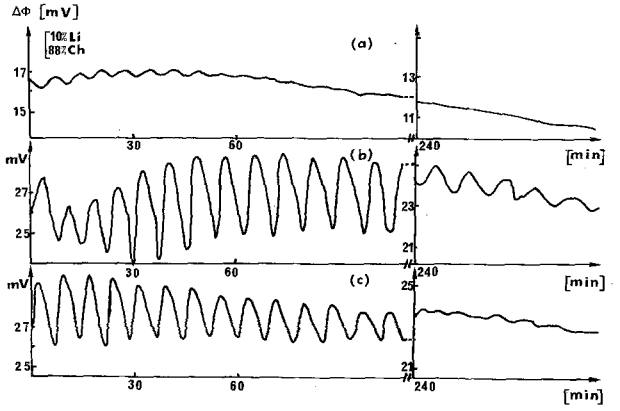


**Fig. 9.** Simultaneous study of the spontaneous potential differences of three different zones of a single skin by virtue of subcompartmentalized chambers. Internal medium was the conventional Ringer solution; external medium was a Ringer solution where 10% $Na^+$ had been replaced by $Li^+$ and 88% by choline.

variations, animals of the same batch were used and experiments performed within a short period of time (16/11/78 to 11/12/78). Statistical calculations with the aid of the *F*-test (*see*, for instance, Heller [14]), did not reveal any significant effect of skin area on the period of oscillation. Moreover, examination of the values of periods obtained by other authors [6, 26, 32, 33, 40] for variation of skin area between 0.3 and 7 $cm^2$, does not show any significant correlation.

### *2.10. Simultaneous Study of Different Zones of the Same Skin*

Figure 9 gives the result of an experiment performed with subcompartmentalized chambers, thus allowing

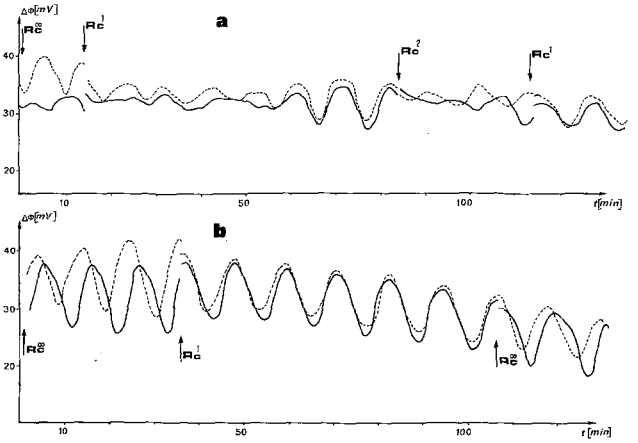


**Fig. 10.** Synchronization of electric potential oscillations between two epithelia ($\Delta\phi$ relative to external medium). *Rc* is the resistance of the bridge between the two epithelia (*see* Fig. 1 for technical details): $R_c^\infty$, infinite resistance (no bridge at all); $R_c^1$, 1.2 kΩ; $R_c^2$, 6 kΩ. The internal medium is conventional Ringer solution; the external medium is a 115 mM LiCl solution. (*a*): Two skins were oscillating independently: coupling with a low resistance ($R_c^1$) synchronized the oscillations; coupling with a large resistance ($R_c^2$) allowed nonsynchroneous oscillations to continue; reintroducing the low-resistance bridge ($R_c^1$) immediately restored synchronization. (*b*): Here also, two skins oscillating independently were synchronized with aid of a bridge with a low resistance ($R_c^1$), and desynchronization occurred immediately after removal of the bridge ($R_c^\infty$).

simultaneous study of three different parts of a single skin. The different parts of the skin were subjected to identical ionic conditions: (i) internal compartments, standard Ringer solution; (ii) external compartments, Ringer solution modified with $Li^+$ (10%) and choline (88%) replacing a part of $Na^+$. All three parts of the skin exhibited oscillations of their electric potential difference, but these oscillations were out of phase with each other. Moreover, the amplitude of these oscillations, and the times during which each of them was sustained, varied very significantly from one zone of the skin to another. The period itself was not completely identical for the three cases, although it always remained of the same order of magnitude as that obtained for the full areas of skin. The experiment has been reproduced 3 times with comparable results.

### *2.11. Electrical Coupling of Skin Fragments*

Consider a skin devided into fragments (two fragments, for example), each one oscillating by itself. Such a situation can be achieved either by using, as above (§ 2.10), subcompartmentalized cells on the full area of skin or by cutting the skin into pieces and mounting each piece between two conventional chambers. Whichever method is used, one can electrically couple the skin fragments (as indicated in Mate-

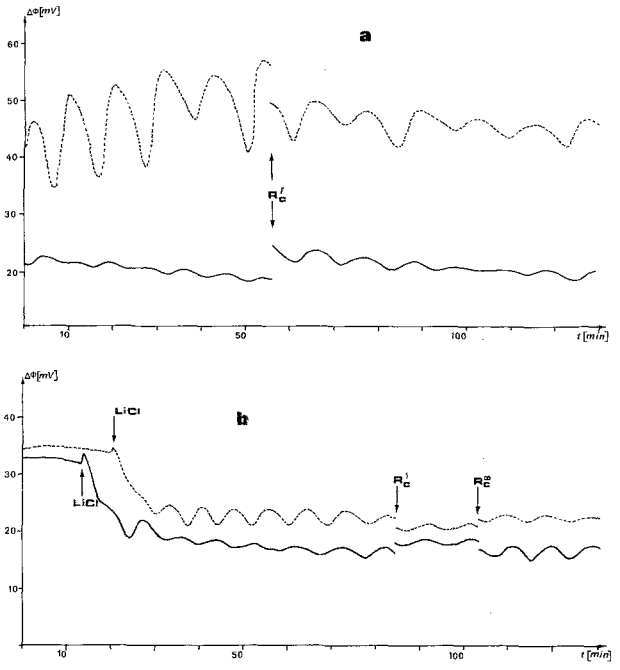


**Fig. 11.** Coupling of two skins: effect of skin area and spontaneous potential difference on the efficiency of synchronization. (*a*): The two epithelia (----- and ——) had the same area but they differed in their mean spontaneous electrical potential (about 45 and 20 mV, respectively). Coupling, even with a low-resistance bridge ($R_c^1$) did not achieve perfect synchronization of both oscillations. (*b*): The two epithelia had similar spontaneous potential values (about 20 mV), but different areas, 0.4 $cm^2$ (——) and 0.8 $cm^2$ (-----). Coupling by the low-resistance bridge ($R_c^1$) effectively synchronized the oscillations, while removal of the bridge allowed immediate desynchronization. In both experiments, the internal medium was 115 mM LiCl. In *b*, the arrows indicate the time when the external medium was changed from conventional Ringer solution to 115 mM LiCl in order to initiate the oscillation.

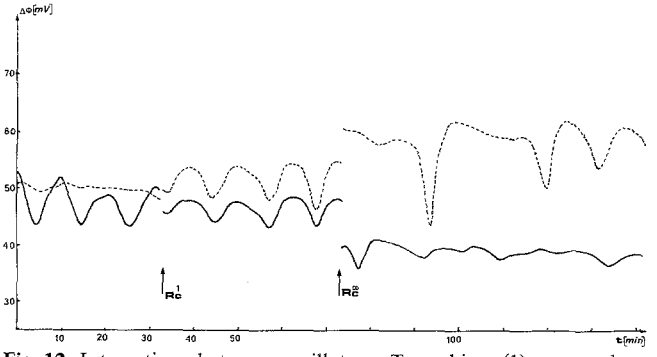


**Fig. 12.** Interactions between oscillators. Two skins, (1) ...., and (2) ——, were oscillating independently under comparable ionic conditions (conventional Ringer solution inside and 115 mM LiCl outside). Oscillation of skin 2 was very clear, whereas that of skin 1 was almost nonexistent. Coupling with the low-resistance ($R_c^1$) made both skins oscillate in phase. After removal of the coupling bridge ($R_c^\infty$), the oscillations desynchronized, but skin 1 then showed a strong oscillation while the amplitude of oscillation was very small for skin 2.

rials and Methods) with the linking resistance adjustable to any desired value. A series of such experiments has been performed (Fig. 10). When the mean potentials of the coupled fragments were comparable, synchronization was always achieved, providing the value of the linking resistance was sufficiently low (1.2 kΩ), while it was never achieved when the resistance was sufficiently high (6.5 kΩ). For values of the linking resistance between 1.2 and 6.5 kΩ, the results were more or less intermediate but not always as clear-cut. An interesting observation was that of an experiment (Fig. 11*a*) where coupling was not achieved, although the linking resistance was 1.2 kΩ. This was a case where the mean value of the potential was very different for the two fragments of skin; the current in the coupling resistance was 16 μA $cm^{-2}$ instead of being about 5 μA $cm^{-2}$, as in the other cases. This result is consistent with the observation that high external currents also tended to damp the oscillation of the electric current under short-circuited conditions (§ 2.6). Conversely, Fig. 11*b* shows that coupling is possible between two epithelia with different areas, as long as they have similar electrical characteristics.

Another aspect of the coupling is shown by Fig. 12. A poorly oscillating fragment was forced to oscillate by coupling to a strongly oscillating one. Furthermore, it is seen that, in this case, once the coupling was released, the previously poorly oscillating fragment was eventually the one oscillating best. Such a kind of "exchange of stabilities" has been frequently encountered with physical oscillators where nonlinear behavior occurred.

## 3. Discussion and Conclusions

The thorough formulation of the oscillatory process in such a complex system as frog skin would require an almost infinite number of variables: enzymatic reactions, transmembrane transports, diffusion within each cellular compartment, etc. Indeed, any of these variables might act more or less, directly or not, on the induction, the sustaining, and the modulation of the oscillation. The calculations would, however, soon become inextricable if one endeavored to treat the problem directly with all its possible variables. We must therefore begin by attempting to pick out, from the set of possible variables, the few "main" ones, i.e., those having the determining effect in the induction of the oscillation. As a first approximation of the model, only these main variables will be used, while all the others, e.g., those merely modulating the phenomenon, can be provisionally neglected.

In the case of the physical oscillators, the study of phase relations has often been very helpful in arriving at a better understanding of the system. One might thus think of looking for various skin characteristics oscillating in connection with the electric potential, and studying the phase relations. Unfortunately, it is doubtful whether such an approach would actually help in this case. Diffusion, especially in the very tortuous intercellular spaces, can cause delays which will always be very difficult to estimate and to take into account. It is not reproducible from one skin to another. This is illustrated by the conductance measurements which have been performed independently be Teorell [33] and Finkelstein [6] on electric potential oscillating skins. The former has found that electric potential and conductance oscillated out of phase, while the latter has found that they were in phase... phase relations such as that shown on Fig. 7 or Fig. 8 between ionic fluxes and voltage cannot be a reliable basis on which to formulate a model of the skin oscillations.

In the same manner, again by analogy with the usual physical oscillators, one might be tempted to look for variables capable of acting biunivocally on the period of the oscillation. But we have underlined the substantial variability of the period from one experiment to another and the poor reproducibility of the period when returning to the initial situation after a perturbation. This clearly makes it hopeless to attempt a systematic study of the period of oscillation with respect to the experimental conditions. However, the domain of the variable values where oscillation is possible is probably quite small, as oscillation occurs in winter but not in summer, and, even in winter, some skins oscillate and others do not under comparable experimental conditions. Therefore, to arrive at the main variables, the best that we can do consists of finding which variables $x$ are such that a sufficient increment $\Delta x$ prevents the oscillation, while the reverse increment $-\Delta x$ restores it.

The first question is whether or not an "active," metabolic step must be taken into consideration among the main variables. There is the possibility that the induction of oscillations by $Li^+$ would be a mere passive effect. The skin would go from an initial stationary state, without lithium, to a final one, with lithium, by damped oscillations. Such phenomena are known to occur in numerous physical or physicochemical systems, and previous authors [6, 32, 33] have indeed reported that the Li-induced electric potential oscillations of the skin were more or less rapidly damped. But we have seen here that the oscillation could actually be sustained for several hours. There is the result by Takenaka [32] that anesthetics prevent the oscillation. We have seen (Fig. 6) that a temperature change from 28 to 15 °C was enough to delete the oscillation, while it did not completely abolish the spontaneous electric potential of the skin. All this is consistent with the oscillation, depending on metabolism.

Lithium is known to act on a number of metabolic processes [9, 16]. For instance, the literature contains indications that $Li^+$ ions might inhibit the ADH-stimulated adenylate cyclase [4, 12, 31] or other enzymes [3]; lithium affects the stability of multiproteic organeles, such as ribosomes or virus particles [1]; the presence of lithium modifies the cellular content of potassium [18], which in turn might disturb the activity of a number of proteins; the hydrated ion of lithium has properties somewhat comparable to those of magnesium, which means that lithium might interfere with the balance of the divalent cations [28] and, consequently, with most of the cellular processes; etc. Any of these properties of $Li^+$ could lead to straightforward feedback systems from which oscillations could be expected. We have seen that theophylline did not promote any large and reproducible modification of the characteristics of the oscillation, even at the concentrations which affected the mean electric potential of the skin. This is not in favor of the first of the hypotheses given above. We have not yet tested the other possible hypotheses, but, in fact, even if some correlations were to be observed with the oscillation, it would remain very controversial to determine what is the cause and what the consequence. However, from the information already available, it seems at least that the process of active ionic pumping of the skin is implicated in the oscillation: lithium, the only ion which can replace sodium to maintain the spontaneous potential of the skin [33] is also the only one whose presence in the external medium can induce the oscillation. Under the short-circuited conditions $Li^+$ induces oscillations of the electric current, which, then, corresponds to pumped ions only [33]. We have seen here that, at constant $Na^+$ concentration, increasing the $Li^+$-concentration in the external medium systematically decreased the influx of $Na^+$ through the skin. Furthermore, an oscillation of the $Na^+$-influx accompanied the oscillation of the electric potential of the skin with a period of similar value, and the oscillations of the electric potential occurred with the separated epithelium as well as with the entire skin. Direct measurements of unidirectional fluxes of lithium, with stable isotopes $^6Li$ and $^7Li$, have also shown us that Li is actively transported by the ion pump [34]. Other authors, with more indirect methods, have reached the same conclusion [24, 26, 40]. As there is no visible lag-phase between the addition of $Li^+$ and the beginning of the oscillation, one can assume that there

are probably not very many metabolic steps between the primary site of lithium action and the sodium pump. But it is not known whether the effect of lithium on the pump is actually direct or not. Whatever the case, the activity of the pump can be taken as an important variable for the model.

Lithium is a requisite for the oscillation, but is ineffective when supplied to the internal medium. However, as we have shown with the stable Li-isotopes [8, 34, 36], $Li^+$ can pass through the skin from the internal to the external medium. When it is supplied to the external medium at a sufficient concentration ($>20$–$30$ mM), lithium is effective in inducing the oscillation. Amiloride, which is known to decrease the apical conductance for sodium [19] and thus probably also for lithium [27] reversibly suppresses the oscillation long before totally cancelling the mean electric potential of the skin. A structural model of the epithelium cells [41] slightly modified from that of Ussing [17, 37, 38, 39] might help in interpreting those data: the epithelium cells would have at least two ionic compartments, *1* and *2*, with compartment *1* being situated before the sites of active pumping, while compartment *2* would be after; moreover compartment *1* would be easily accessible from the external medium, and compartment *2* from the internal one. Our results would then show that the concentration of lithium in cellular compartment *1* is a main variable for the theoretical model of oscillations, whereas that in compartment *2* is not. In like manner, we have seen that $K^+$-concentration in the external medium had no effect on the oscillation, while a small increase of the $K^+$-concentration in the internal medium reversibly blocked the oscillation. This suggests that the $K^+$-concentration in compartment *2* is also a main variable. The oscillations have been shown to be neither pH-dependent between pH 4.7 and pH 10 [32] nor affected by the nature of the anion accompanying $Li^+$ [32, 33]. We have seen here that a modification of the external medium by various substances ($Ca^{++}$, choline, etc.), or a change of the osmotic pressure in the internal medium, might possibly modulate the shape of the oscillation or affect the amplitude, but could not induce an oscillation in the absence of $Li^+$ in the external medium. The corresponding variables can thus be discarded from the set of the main ones.

Changing the area of the oscillating skin did not change the period of oscillation. This leads to the important conclusion that the skin oscillator is of the "local" type, i.e., that it is made of a population of microscopic individual oscillators which secondarily tend to synchronize with each other when a sufficient "coupling-effect" occurs. This conclusion is also supported by more indirect arguments such as the following: the different parts of a single skin oscillate independently from each other, but with periods of the same order of magnitude, when the skin is studied with the subcompartmentalized chambers (§ 2.10). Spontaneous and reversible desynchronizations of a single oscillating skin are sometimes observed in the course of the oscillation (§ 2.6.), and in any case, the single oscillating skin generally ceases to oscillate long before its mean electric potential has fallen to zero, which can be interpreted as the local oscillators ending in desynchronizing themselves, thus making the overall oscillation of the skin statistically disappear. Coupling experiments between two skins have also emphasized the role of the external electric current as corresponding to the main "coupling-effect", and discounted the possibility that synchronization of the local oscillators might occur by diffusion of chemical species.

A theoretical model of the oscillation will be developed subsequently from these bases. It can be briefly outlined as follows. The first step will consist in building a local model of oscillator at the level of a single epithelial cell or of a group of a few cells. Two main variables will be considered: $Li^+$ concentration in compartment *1* and $K^+$ concentration in compartment *2*. The nonlinearity responsible for the induction of the oscillation could originate from Li effect on apical permeability or on the ionic pump or both. We have seen, above, a number of arguments indicating that the active step of the ionic transport was implicated in the induction of the oscillation and, starting from experimental results [2], it will be shown that the nonlinearity due to varying apical permeability can probably be neglected. In agreement with recent data [29] which suggest that lithium is both actively transported and acts as a poison on the skin, we can thus assume an effect of lithium on the ion pump of the type "inhibition by excess of substrate." Such an effect is indeed a well-known cause of instability [21]. Writing the law of mass conservation for the two main variables can then give the set of partial differential equations whose periodic solutions will describe the local oscillation. As second step of the theoretical treatment, synchronization between the individual oscillators can ultimately be formalized by taking into account the local currents generated by the dispersion of the electric characteristics of the cells.

From the biological point of view, the important point of this approach is that it will introduce a certain number of parameters ($\alpha$, $\rho$, $u$, etc.) related to the cellular properties of the system, and periodic solutions of the partial differential equations will exist for only some values of those parameters. This, in turn, can serve as a guide to determine the cellular

properties associated with the ionic transport, which, we have seen (p. 116) would be very difficult to determine directly.

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