Lithium-Induced Oscillations of Potential and Resistance in Isolated Frog Skin

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ABSTRACT The rhythmical variations of electrical potential and DC resistance resulting from the exposure of the anatomical outside of isolated frog skin to a concentration of lithium ion greater than 20 millinormal were reinvestigated. In general, the potential and resistance changes were in phase, although in some skins, a phase shift occurred after the first few waves. The mean level of the resistance declined during the exposure to lithium, returning to its former level upon reintroduction of sodium in place of lithium. The oscillations, with a period of from 3 to 15 minutes, could last for 2 hours or more before damping out; the amplitude of the waves could be altered during this time by the passage of direct current or by the introduction of a hydrostatic pressure difference across the skin. Even after the oscillations damped out, the system remained "excitable," responding to a step of direct current or hydrostatic pressure with an oscillatory train. The nature and magnitude of the response to current and pressure were dependent upon the "polarity" of the applied perturbation. Direct observation of the skin revealed no evidence of oscillatory water movement concomitant with the electrical events.

INTRODUCTION

The phenomenon of the oscillations of the electrical potential of the isolated frog skin in response to lithium ion was first observed by Takenaka (1937) and was further studied by Teorell (1954). Despite extensive research over the years directed toward an elucidation of the nature of the frog skin potential, no other investigations have been made of the lithium-induced oscillations. (In a paper dealing with the "short circuit" current of the frog skin in the presence of lithium ion, Zerahn (1955) noted that he had observed oscillations in the magnitude of the current.) The problem seemed of sufficient interest to warrant a reinvestigation of the system with particular attention to the parameters affecting its behavior. It was hoped that the information gained from such a study would contribute to an understanding of the mechanism of this oscillatory response and perhaps shed light on the general electromotive properties of frog skin. The present paper describes the results of this study.

METHOD

These experiments were performed from February to June on the abdominal skin of both males and females of the frog *Rana temporaria*.[1] The abdominal skin was dissected and immediately mounted with Ringer's solution as modified by Gray[2] on both sides in a double chamber identical with that previously described by Teorell (1954). Each chamber consisted of two compartments filled with the appropriate solution and separated from each other by 0.38 cm^2 of frog skin The skin in each chamber came from the same frog, so that one chamber acted as a control for the other. The solutions were changed frequently during the first half-hour, and a steady state of the potential and impedance was obtained, generally in 1 or 2 hours after mounting. The potential was measured across the skin by calomel electrodes through 10 per cent methyl carboxy-cellulose (saturated with KCl) junctions. The impedance electrodes were made of platinum blackened with Pt-sponge. The electrodes for pulsing the skin with direct current were silver-silver chloride, these replacing the platinum electrodes in such experiments; the constant current source was a dry cell with a large resistance in series. For those experiments in which a difference of hydrostatic pressure was applied across the skin, a chamber in which the liquid level could be raised on either side was used instead of the double chamber. In these experiments the skin was placed between lucite disks with finely drilled holes, so that stretching or bulging of the skin under the difference in hydrostatic pressure was negligible. In all experiments the solutions on both sides of the skin were continually bubbled by a 97 per cent O_2, 3 per cent CO_2 gas mixture.

The recording apparatus consisted of a relay system which at 10 second intervals closed successively one of four circuits to a DC amplifier (input resistance of 5 megohms) connected to a speedomax pen recorder which printed the record on a moving paper chart. When one circuit was closed the other three were automatically open in order to avoid interference. In general, one circuit measured the potential across the skin in one-half of the double chamber, one measured the impedance of the skin in this chamber, one measured the skin potential in the other half of the chamber (control), and one recorded the base line. When impedance was not measured, two circuits were devoted to the potential in the experimental half of the chamber in order to give values at shorter time intervals. Potentials were measured to the nearest 0.5 mv and impedance to ± 7.5 ohms.

The impedance measurements were made by feeding the unbalance of the bridge (Fig. 1) through an AC amplifier and rectifier to the DC amplifier of the recording system. The bridge was fed by an AC oscillator at 20 CPS, but AC flowed through the skin only while the impedance circuit was recording, a relay switching the skin in for this time only.

A few comments may be made concerning the impedance measurements. The current density through the skin (5 $\mu a/cm^2$) was deliberately made low so as to assure

[1] The oscillations obtained in experiments wih *Rana pipiens* have generally been of poorer quality and short duration.

[2] 71.75 mM NaCl; 5.00 mM KCl; 2.60 mM $CaCl_2$; 1.60 mM $MgCl_2$; 20.00 mM $NaHCO_3$; 1.03 mM Na_2HPO_4; 0.12 mM NaH_2PO_4; 0.26 mM glucose.

that its effect on the skin would be negligible. The 8 μf condensers were inserted to block any direct current that might flow between the platinum electrodes. The reference arm of the bridge consisted of a capacitance and resistance in parallel since the impedance characteristic of the frog skin has the behavior of such a circuit (Teorell, 1949). (It may be noted here that the "capacitance" of the skin (2.5–4 μf/cm^2) remained constant in all experiments so that the impedance changes presented in the following section represent changes in the resistive element of the skin.) Before addition of LiCl to the outside of the skin, the bridge was balanced, thus giving the capacitance and resting resistance. The bridge was then unbalanced by reducing the resistance in the reference arm to one-third of the balanced value, LiCl was applied to the skin, and the unbalance of the bridge was recorded throughout the remainder of the experiment. At the conclusion of the experiment, the unbalance of the bridge was calibrated by substituting for the frog skin a dummy element consisting of a capacitance equal to that of the frog skin in parallel with a variable resistor. Finally, the reason for using a 20 CPS current should be mentioned. Because of the "capacity element" in the frog

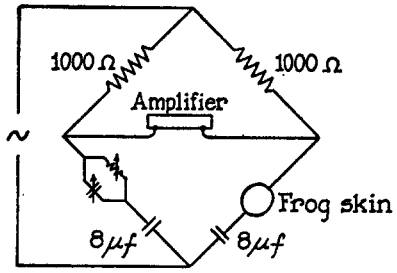

FIGURE 1.
Bridge for resistance measurements.

skin, a high frequency current would tend to be shunted across this element and hence make it somewhat difficult to observe the relatively small resistance changes that occur. The use of a direct current on the other hand would introduce the possibility of troublesome time-delayed rectification effects. A 20 CPS frequency offers a satisfactory compromise between these two extremes. (A similar discussion on the use of a low frequency current is given by Teorell (1954).)

RESULTS

A. *Application of Lithium Ion* Within 2 hours after mounting of the skin a steady-state potential (30 to 100 mv) and resistance (1.3 to 4 kilo Ω; *i.e.*, 0.5–1.5 k Ω/cm^2) were obtained between the anatomical inside bathed with Ringer's solution and the anatomical outside bathed with either Ringer's or 0.1 N NaCl—the inside potential being positive relative to the outside. Upon substitution of 0.1 N LiCl for the outer solution, the potential and resistance of the skin proceeded to oscillate rhythmically with a relatively constant period of from 3 to 15 minutes. Fig. 2 shows the results from one such experiment. The amplitudes of the potential swings, which might

initially be as high as 25 mv, were in general damped, dying out in from 15 minutes to 2 hours. At times, however, the oscillations would settle down after two or three large swings into an undamped train of about 10 mv amplitude which lasted for 2 hours or more. As a general rule the periods of the highly damped oscillations were shorter than those of the relatively undamped trains. The wave form of the oscillations is of some interest. For those of small amplitude (1 to 5 mv), the shape was more or less sinusoidal in form; those of larger amplitude, however, displayed markedly the characteristics of a "relaxation" oscillation. A particularly good example of this is

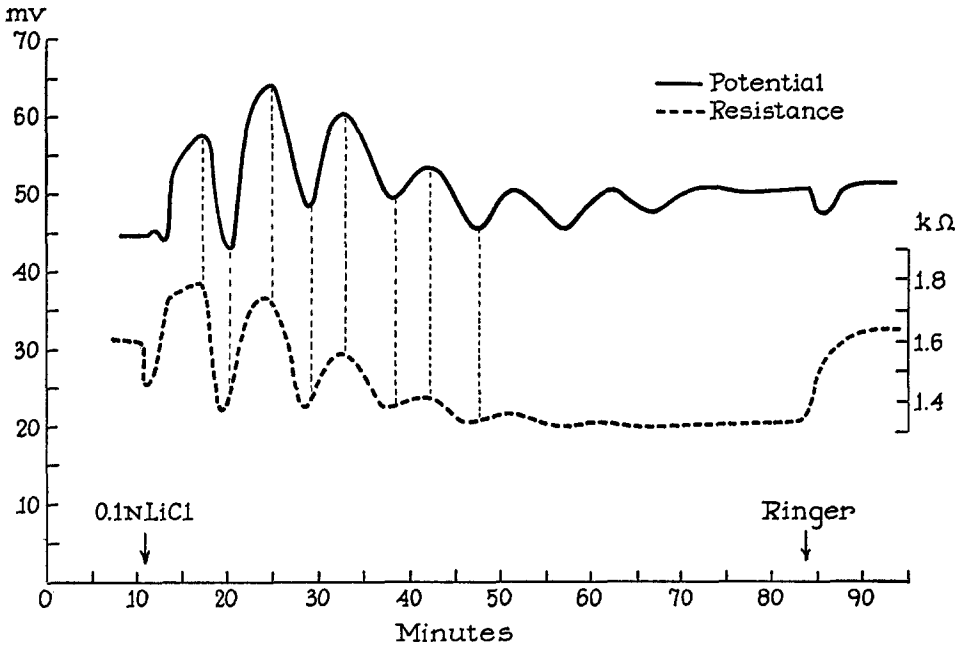

FIGURE 2. Response to the application of 0.1 N LiCl on the anatomical outside of frog skin. Ringer's solution bathes the anatomical inside.

seen in Fig. 3. In most experiments the resistance oscillations were in phase with those of the potential; *i.e.*, resistance maxima appeared with potential maxima and resistance minima with potential minima. This is in contrast to the results reported previously by Teorell (1954). In a few skins the potential and resistance oscillations began in phase but then shifted with the former leading the latter by from 45–90°.

Following the introduction of 0.1 N LiCl to the outside of the skin, the resistance initially dropped by 50 to 150 ohms following which the *mean* level of the oscillations continued to decline during the next 30 minutes to a level several hundred ohms below the value prior to introduction of the lithium

chloride. In some skins the resistance dropped so rapidly that the oscillations were obscured, appearing as slight shoulders on a descending curve. The initial behavior of the potential was somewhat less predictable. When the lithium chloride was first applied, the potential rose in some skins and declined in others; generally, however, the first "peak" was above the potential prior to introduction of the lithium chloride. The mean level of the potential then declined to a steady-state value which might be greater than, equal to, or less than the potential existing before the lithium chloride was applied. Similarly, when Ringer's or 0.1 N NaCl solution was returned to the outside, the potential change was variable, but in all cases the resistance rose continuously for some time (Fig. 2) before attaining a steady-state value approxi-

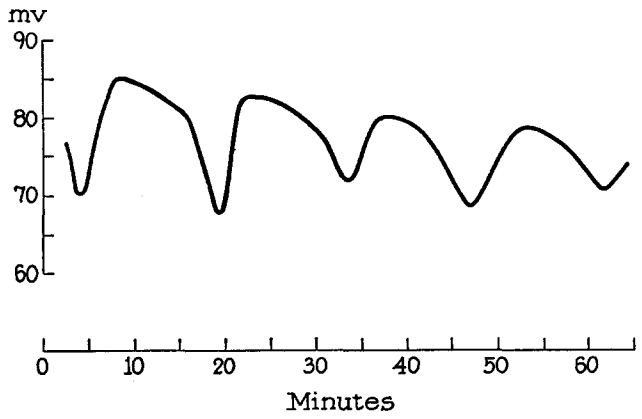

FIGURE 3. Typical potential oscillations of the "relaxation" type.

mately the same as that which it possessed earlier. In a few skins the return of sodium chloride produced a short train of three or four oscillations.

The above cycle, Ringer's, lithium chloride, Ringer's could be repeated several times, each time obtaining an oscillatory response to the lithium solution provided some time (15 minutes or more) had elapsed before reapplication of the LiCl. In this sense the system was reversible, although the subsequent responses were generally of progressively smaller magnitude and shorter duration. It was also found that lithium sulfate apparently acted as well as lithium chloride, a result which is consistent with Takenaka's findings that the oscillatory state was achieved with a variety of lithium salts. The amplitude of the oscillations did not appreciably depend upon lithium concentrations between 0.1 N and 0.05 N, but below this level it declined sharply, becoming barely detectable at 0.02 N. Application of lithium ion to the anatomical inside of the skin produced no effect nor did it alter the response to the introduction of lithium to the outside.

B. *Response to Direct Current*[3] After the lithium-induced oscillations have died out, the skin potential (and resistance) remains at a constant level for several hours; *i.e.*, with Ringer's solution on the inside and lithium chloride solution on the outside. In this state, however, the system is "excitable," capable of responding to certain perturbations with a short train of oscillations. In Fig. 4 *a* are reproduced typical responses to square waves of direct current. Several features of this type of response deserve comment. In the

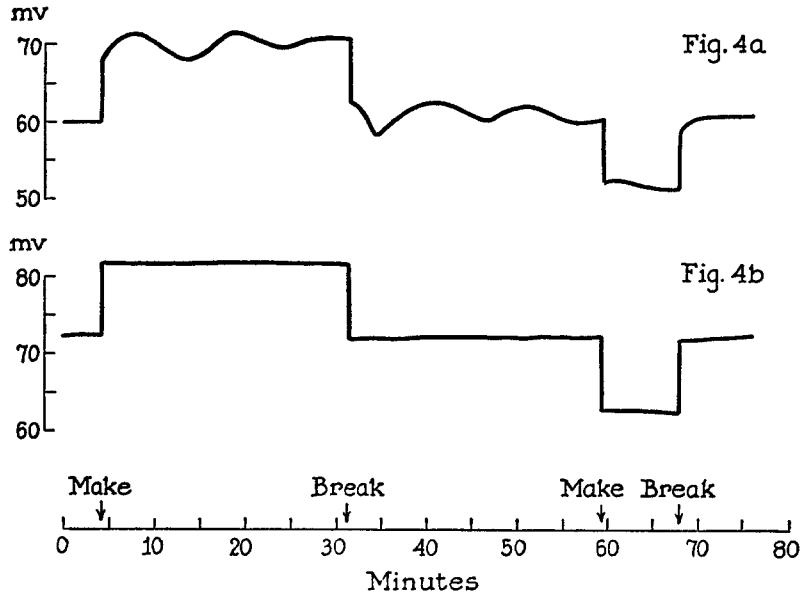

FIGURE 4 *a*. Response to square waves of current with 0.1 N LiCl on the outside and Ringer's solution on the inside. The oscillations have previously died out and the skin potential is now stable. Current strength, 5 μa (13 μa/cm^2).

FIGURE 4 *b*. Response to square waves of current of the same skin as depicted in 4 *a* except that 0.1 N NaCl is on the outside instead of 0.1 N LiCl.

first place, the direction of current flow is of crucial importance; an outward current (anode inside, cathode outside) inducing a rhythmical response, an inward current producing no such effect. Second, although not shown in the figure, after the oscillatory train has died out, a further increase of the imposed current will induce another oscillatory burst, and so on as successive steps of current are applied. Third, a "break" response occurs following

[3] It should be clearly understood that the responses discussed in this section, and for that matter in this entire paper, are occurring on a time scale measured in seconds and minutes. Recent work in this laboratory has shown that frog skin with Ringer's solution on both sides will respond actively to current pulses of sufficient magnitude of approximately 30 milliseconds duration. A note on this phenomenon is in press in *Nature*.

removal of the current (or lowering of the current level). Finally, comparison of Fig. 4 *a*, in which 0.1 N LiCl is on the outside, with Fig. 4 *b*, in which 0.1 N NaCl is on the outside of the same skin, illustrates the fact that "electrical excitability" is not a general property of frog skin, but occurs only in the presence of lithium ion.

Resistance measurements were not made while current was being passed through the skin, electrode polarization effects obscuring the results. It was nevertheless possible to demonstrate that resistance changes were associated with the potential variations, particularly with the first swing. This result is

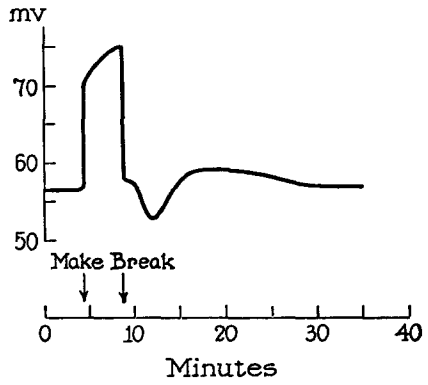

FIGURE 5. "Make" of current step followed by "break" at the peak of the first oscillation. Note that the potential drop at the break is greater than the potential rise at the make, demonstrating an increase in the skin resistance. Also note that the potential level immediately following the break is greater than the potential level preceding the make (0.1 N LiCl outside, Ringer's solution inside). Current strength; 15 μa (39 μa/cm^2).

illustrated in Fig. 5 where the resistance at the break is seen to be greater than that at the "make." This follows from the fact that the initial rise in potential upon application of the current is less than the fall in potential upon removal of the current, and barring very rapid changes in the resistance, the initial potential change upon make and break of the current i is simply the iR drop across the membrane at that time. It should also be noted, however, that the potential does not immediately return to its former level, so that the slow rise of potential following application of the current pulse is not entirely accounted for directly by the change of R with time but must also involve a change in the skin potential.

Another example of the sensitivity of the lithium system to direct current (and its polarity) is given in Fig. 6. In this experiment the skin in both halves of the double chamber was treated with 0.1 N LiCl, whereupon the usual oscillatory response was obtained in each. After several cycles a constant hyperpolarizing current was applied to one and an equal depolarizing current

to the other. The hyperpolarized skin showed some diminution in the wave amplitude, but in the depolarized skin the oscillations were effectively abolished. Upon removal of the current, the oscillations in the previously depolarized skin reappeared. In other experiments it was sometimes found that the wave amplitude during hyperpolarization would increase as a function of current up to a certain magnitude of current and then decrease

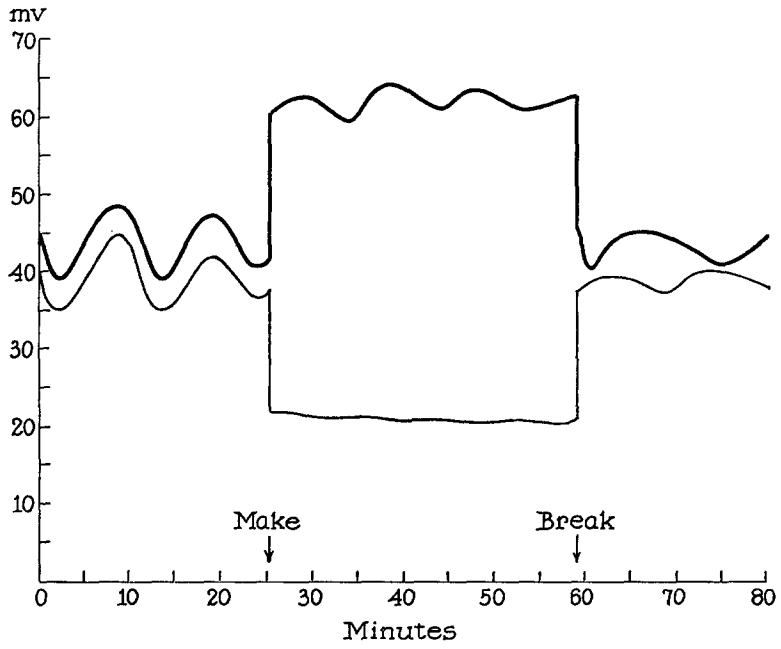

FIGURE 6. Response to square wave of current during oscillatory train induced by 0.1 N LiCl on the outside (Ringer's solution on the inside). Current strength; 15 μa (39 μa/cm^2). The skin in one-half of the double chamber was hyperpolarized (upper curve); the skin in the other half of the chamber was depolarized (lower curve).

with higher currents. In all cases depolarization produced a decrease in amplitude, although not necessarily abolition as in Fig. 6.

C. *Response to Hydrostatic Pressure*[4] In Fig. 7 *a* the results of the application of square pulses of hydrostatic pressure to a skin bathed with 0.1 N LiCl on the outside and Ringer's solution on the inside are seen. As in the case of the record in Fig. 4 *a*, the oscillations produced upon introduction of the lithium chloride had damped out and the skin was in a steady state prior to the pressure "shock." It is interesting to note that the "polarity" of the pressure step is significant just as is the direction of current flow. Thus,

[4] Resistance was not measured in these experiments.

with no initial pressure difference across the skin, elevation of the pressure on the inner surface (or lowering of the pressure on the outer surface) caused a rise in potential followed by an oscillatory train, while elevation of the pressure on the outer surface (or lowering of the pressure on the inner surface) produced little or no change in potential. As with the square pulse of current, the pressure pulse induced both a make and a break response, and successively increasing steps of pressure produced successive oscillatory trains. A step of pressure during an oscillatory train resulted in variable responses, increasing

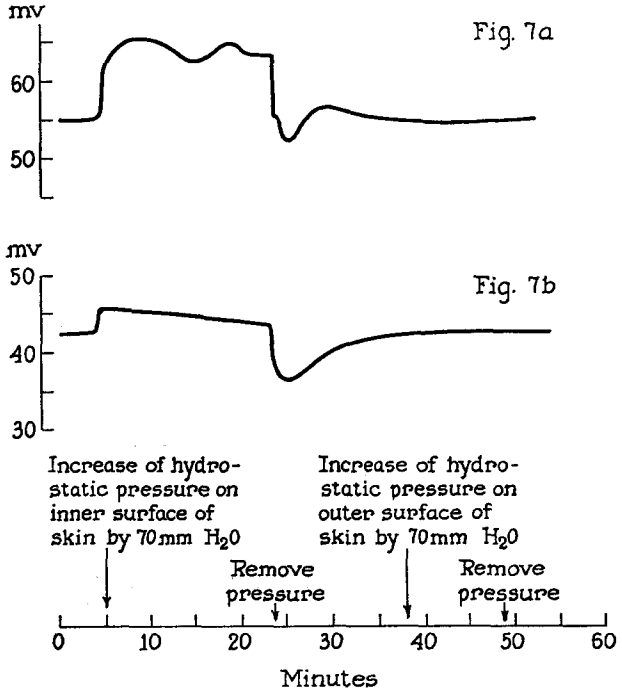

FIGURE 7*a*. Response to square waves of pressure when the outside is bathed by 0.1 N LiCl and the inside by Ringer's solution.

FIGURE 7 *b*. Response to square waves of pressure when the outside is bathed by 0.1 N NaCl and the inside by Ringer's solution.

the wave amplitude in some cases and reducing it in others. If the pressure on both the outer and inner surface were elevated or lowered simultaneously, no potential changes occurred, thus demonstrating that pressure difference and not absolute values of pressure is the effective parameter. It is of interest that the potential of the skin, even in the absence of lithium ion, is sensitive to pressure differences across it as is shown in Fig. 7 *b*, although lithium is necessary for the occurrence of an oscillatory response.

That the two parameters, pressure and direct current, are interrelated was most clearly seen in experiments in which the pressure step was applied to a skin that had attained a steady state with a constant direct current flowing across it. It was found that the pressure response was diminished or completely inhibited in the presence of a depolarizing current, while it was generally enhanced in the presence of a hyperpolarizing current.

D. *KCl Shock* As was mentioned in section A, upon return of 0.1 N NaCl to the outside of the skin previously exposed to 0.1 N LiCl, the potential change was somewhat variable, and the resistance rose gradually to the resting level. Furthermore, at least 15 minutes must have elapsed before

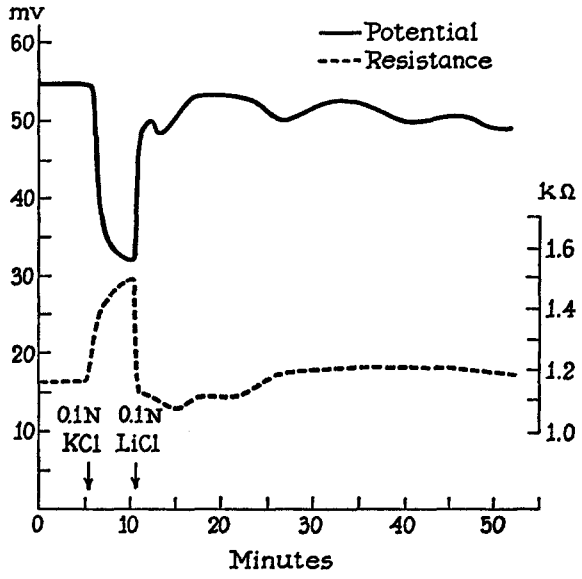

FIGURE 8. Result of substitution on the outside of 0.1 N KCl for 0.1 N LiCl after oscillations have ceased, and then resubstitution of 0.1 N LiCl for the KCl after a short time (Ringer's solution on the inside).

reapplication of lithium evoked an oscillatory response. A much more dramatic change occurred, however, when 0.1 N KCl replaced the lithium solution, as demonstrated in Fig. 8. There occurred a sharp drop of potential and rise in resistance (see also Teorell, 1949) following application of the KCl solution. The removal of the KCl and return of LiCl produced an immediate rise in potential and drop in resistance, followed by an oscillatory train. These results are presented to illustrate the difference in response of the frog skin to the application of lithium, sodium, and potassium solutions to the outer surface.

DISCUSSION

The above results demonstrate that in the presence of lithium ion (concentration > 20 millinormal) bathing its outer surface, frog skin becomes an excitable structure (see footnote 3), exhibiting rhythmical changes of its potential accompanied by similar oscillations of its resistance and responding rhythmically to changes of direct current and/or hydrostatic pressure across it. At present there appears to be no satisfactory explanation for this unusual behavior in response to lithium. Recently, in two interesting papers, Teorell (1959) has discussed the properties of a model "membrane oscillator," the basis for this system being a coupling of electroendosmosis with water movement produced by a pressure gradient. Since both current and hydrostatic pressure are also effective in the "frog skin oscillator," it would be tempting to relate the two systems. Direct observation of the frog skin with a water-immersion lens, however, has failed to reveal any rhythmical change in skin thickness during the course of the oscillations, so that further speculation concerning water movement would appear to be unwarranted at this time. Nor is it particularly obvious how the action of lithium ion is to be interpreted in terms of the present ideas concerning the cause of the potential difference existing across frog skin. According to Koefoed-Johnsen and Ussing (1958) this potential results from a double membrane system, the outer facing membrane being primarily permeable only to sodium and lithium while the inner membrane is primarily potassium-permeable. (The rise in resistance and drop in potential as seen in Fig. 8 upon substitution of potassium for sodium in the solution bathing the outer surface are explainable on the basis of the relative impermeability of the outer membrane to potassium.) Due to the action of the so called sodium pump at the inner membrane, the solution between these membranes is low in sodium and high in potassium. The over-all potential difference across the skin is then the sum of a sodium concentration potential across the outer membrane and a potassium concentration potential across the inner membrane. If this be correct, the lithium ion might be supposed to produce a rhythmical change in the selectivity of the outer membrane, but this is hardly what one would call an explanation of the mechanism by which lithium acts.

It is a pleasure for me to express my gratitude to Professor Torsten Teorell for having suggested this problem, for having made available to me the facilities of his laboratory, and for many stimulating and enlightening discussions during the course of this investigation.

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REFERENCES

1. KOEFOED-JOHNSEN, V., and USSING, H. H., *Acta Physiol. Scand.*, 1958, **42**, 298.
2. TAKENAKA, S., *Japan. J. Med. Sc. III. Biophys.*, 1937, **4**, 143, 283.
3. TEORELL, T., *Arch. sc. physiol.*, 1949, **3**, 205.
4. TEORELL, T., *Acta Physiol. Scand.*, 1954, **31**, 268.
5. TEORELL, T., *J. Gen. Physiol.*, 1959, **42**, 831, 847.
6. ZERAHN, K., *Acta Physiol. Scand.*, 1955, **33**, 347.