THE ELECTRICAL PROPERTIES OF FROG SKIN

PART I. INTRODUCTORY

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(With Four Text-figures.)

This paper is the first of a series in which the results of a study of the electrical properties of frog skin will be presented. Frog skin is a convenient material for bio-electric investigation. It is easily obtained, can be mounted as a membrane and gives high potentials (*ca.* 40–70 millivolts between opposite sides of the skin). On the other hand the complexity of structure of the skin tissue makes it difficult to determine which cells are the seat of the potential. Considerable insight has, however, been gained during this work into the chemical and physical processes by which the electrical potential is maintained. This is of interest in relation to the general problem of the nature and origin of bio-electric potentials.

The skin of the frog has a respiratory function (Krogh, 1904) and also serves to maintain a water equilibrium between the animal and its surroundings (Adolph, 1925). The existence of the frog skin potential has been known since 1857 and the literature summarised by Orbeli (1910). Much of the work has been done with preparations of the whole animal and is concerned with the nature of the response of the potential to stimulation of the nerve supply. In the following work only the steady resting potential is considered and the skin is separated from the frog before the experiment begins.

MATERIAL AND METHODS.

The skin of the frog *Rana temporaria* was used. Each frog provided two specimens of skin, dorsal and ventral. The skin is mounted between two flanged glass half-cells (Fig. 1) held together by three spring clips and the solutions run in either side (tube *A*). The internal diameter of the half-cells is 1–1·5 cm. and the cubic content of the whole cell is *ca.* 10 c.c. The solution in the cell can be changed without aeration of the skin or disturbance of the electrical connections. The siphon tubes dip into beakers containing the same solution as is in the cell. These beakers also receive the side tubes of two calomel electrodes.

The electrical potential across the skin is measured by connecting the calomel electrodes to a slide-wire potentiometer. As null instrument an Einthoven string galvanometer with a megohm in series with the fibre was used and for part of the

time a Lindemann electrometer (*vide* Pumphrey, 1931). The accuracy in the first case was ± 0·1 mv. and in the latter ± 0·25 mv. With either arrangement no appreciable current was drawn from the skin.

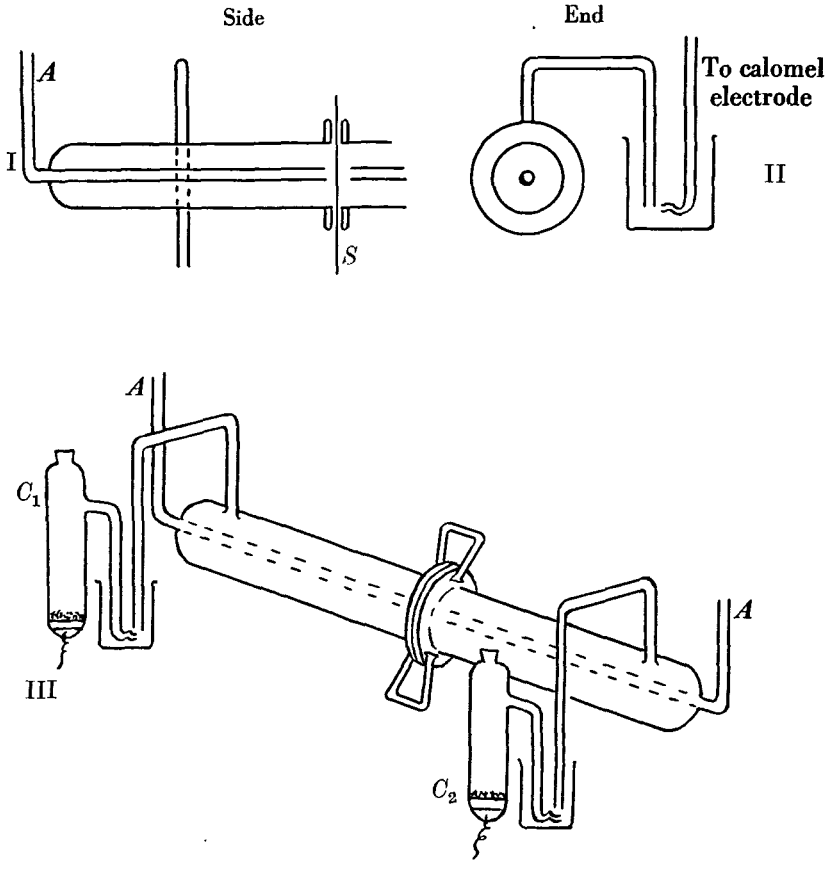

Fig. 1. Apparatus to measure potential of skin with calomel electrodes. I. Side view: *S*, skin; Solutions enter by *A*. II. End view showing siphon connection from cell to calomel electrode. III. Whole apparatus, skin and cell mounted with calomel electrodes C_1, C_2.

Optimum experimental conditions.

The first essential in an experimental study of the frog skin potential is to establish the conditions under which the potential remains steady for as long a time as possible. These conditions when found serve as a standard of comparison in studying the effect of changes in the composition of the solutions used and in the physical conditions of the measurement. The optimum conditions were found to be at room temperature (12–17° C.) in glucose Ringer solutions saturated with atmospheric air (NaCl 6·5, KCl 0·14, $CaCl_2$ 0·12, $NaHCO_3$ 0·2, NaH_2PO_4 0·01, glucose 2·0, water 1000, *p*H 8·3). Under these conditions the potential of a healthy skin remains constant to ± 1 mv. for several hours before falling off begins. The

room temperature during any one experiment did not alter more than $\pm 1°$ C. Decreasing the *p*H from 8·4 to 7·5 did not affect the survival time of the steady potentials although it slightly increased the values.

After mounting the skin in glucose-Ringer solution the steady value of the potential is not reached until 1–2 hours have elapsed. The initial value of the potential is always lower than the final steady value. Before an experiment can begin, therefore, it is necessary to wait until this standard "zero" value of the potential has been reached. When glucose is absent from the solution the time during which the potential remains steady is shorter though it may still be 2–3 hours in some cases. The falling off of the potential in a carbohydrate-free solution may be arrested by the addition of glucose. This point will be considered again later.

Ventral and dorsal skins show no difference in electrical properties except that the former give potentials 15–30 mv. higher than the latter. The potential is greater the bigger the frog and the shorter its period of captivity. With Ringer solution on both sides the morphologically inner side of the skin is positive to the external circuit. The potential of the skin of male frogs was 20–30 mv. in February and March and 40–80 mv. in May–December. The skin from females gave potentials 20–40 mv. higher than skin from males. This difference between the sexes was most marked from January to March.

The seat of the potential.

A section through the skin perpendicular to the surface is shown in Fig. 2. The potential has been ascribed to the activity of the glands in the skin by Engelmann (1872) because of the parallel effects on secretion and potential of mechanical and chemical stimulation. Bayliss (1886) decided that both the glands and epidermal cells contributed to the resting potential. These conclusions were for preparations of the whole animal. Alcock (1906) studying the effect of chloroform on the separated skin decided that the potential was due to the epidermal cells.

Potential differences due to glandular activity have only been observed during the process of secretion with the nerve supply to the glandular tissue still intact (Bayliss, 1924). The steady potential observed in our work over several hours, if of glandular origin, would require a corresponding steady secretory activity by the skin. There is no evidence for this with Ringer on both sides of the skin (Adolph, 1925). Also the following gland stimulants, pilocarpine, muscarine and adrenaline, were found to have no effect on the potential in concentrations from 1:5,000,000 to 1:5000. Therefore when the connection with the nervous system is severed the glands do not contribute to the skin potential.

Since the nerve supply to the skin is destroyed before mounting the skin, the significance of nerve injury potentials must be considered. These potentials would make the inside of the skin electrically negative to the outside in the external circuit since the injured cells are exposed on the inner side of the skin. The contrary sign of potential is always observed. The resting potential of the skin is therefore not an "injury potential." Further evidence for this conclusion is provided by experiment on the uninjured frog. An incision is made in the skin and

a capillary full of Ringer solution inserted. Connection is thus made from the inside of the skin to a calomel electrode, another calomel electrode dips into Ringer solution in contact with the outside of the skin and the potential is measured. The potential is of the same order and sign as with the isolated skin. It is possible that the gradual rise in the resting potential during the first 2 hours after mounting the isolated skin is due in part to the decay of the injury potential which exists at first in opposition to the surviving resting potential.

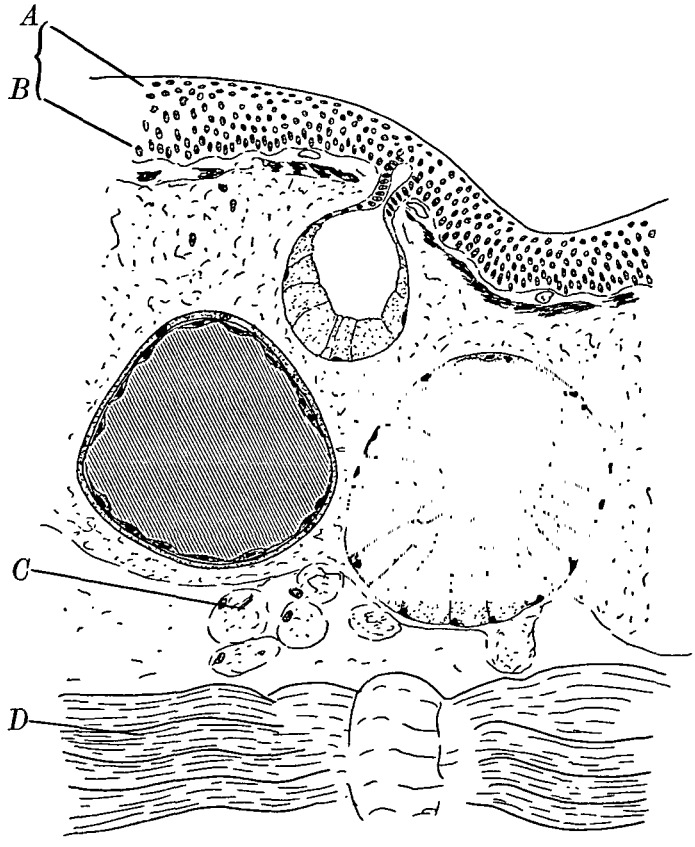

Fig. 2. Section through dorsal skin of frog. *A*, *B*, epidermis; *C*, capillaries; *D*, connective tissue. Three glands in different stages of secretory activity embedded in connective tissue.

It will be shown later that the potential is maintained by oxidative processes in the cells of the skin. The final location of the seat of the potential will therefore require histological examination to determine the relative rates of oxidation in the different kinds of cells in the skin and the position of the surfaces or membranes at which the chemical energy of oxidation appears as a potential difference.

The source of the potential.

The skin when mounted between solutions of identical composition maintains a steady potential difference for many hours. If the skin permits the diffusion of

ions to the least extent, (from thermodynamic considerations) it must be doing work. The source of this energy must be chemical and is likely to depend on the consumption of oxygen. Lund (1926) showed that the skin potential of *Rana pipiens* was decreased by oxygen starvation. We have confirmed this conclusion for *R. temporaria*, but our results differ from those of Lund in several points.

EXPERIMENTAL.

(*a*) *Effect of complete oxygen starvation.*

The Ringer solution was prepared in the following manner. The solids and distilled water requisite were contained in separate flasks. The water was boiled under reduced pressure for 1 hour and cooled in a stream of hydrogen which had

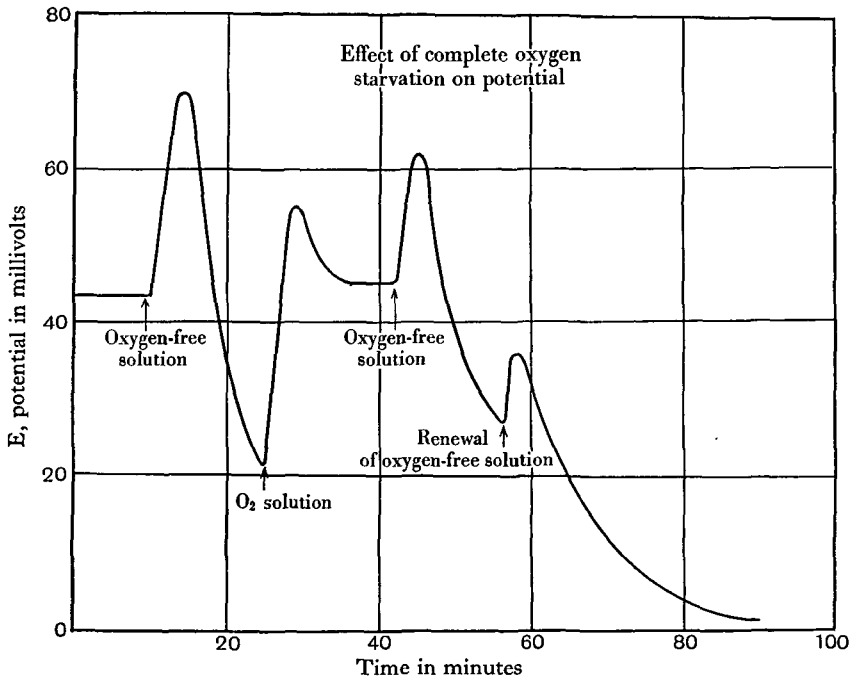

Fig. 3. Skin initially mounted in Ringer saturated with oxygen. The arrows indicate changes of the solution.

passed over heated palladinised copper. The flask containing the solids was evacuated and rinsed with hydrogen several times and the oxygen-free water siphoned in and shaken till solution was complete. The solution was then forced by hydrogen pressure through the cell containing the skin (Fig. 1).

The response of the potential to changing air-saturated solution for oxygen-free solution or *vice versa* was immediate and striking (Fig. 3). On changing from aerated to air-free solution the first effect is a rapid rise in the potential which attains a peak value 40–80 per cent. above the initial figure within 2–5 min. After this the potential falls rapidly to less than 5 mv. in 20–40 min., taking a longer

time the higher the initial potential. At this stage, readmission of aerated solutions brings about no revival in potential; the skin, electrically speaking, is dead. If aeration occurs at an earlier stage in the anaerobic decay, partial or complete recovery of the potential is seen according to the extent to which decay had proceeded. The recovery curve in its initial course resembles the decay curve in showing a sharp rise in potential.

These findings agree with those of Lund (1926) except that he records no potential rise at the beginning of oxygen starvation and the time for irreversible decay was found to be 3–5 hours. These differences may be due to the difference of material (*R. pipiens* instead of *R. temporaria*) or to a less thorough exclusion of oxygen in his experiments.

These results indicate that the presence of oxygen is essential to the maintenance of the potential and that during anaerobiosis the substances or reactions which

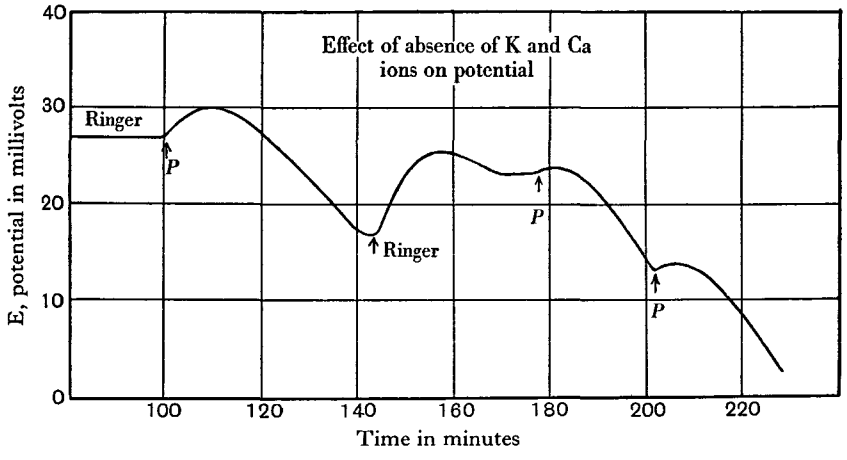

Fig. 4. Arrows indicate change of solution. *P* indicates NaCl phosphate buffer.

maintain the potential are soon exhausted or brought to a standstill. The dependence of the potential on the rate of oxygen consumption of the skin and on the oxygen concentration in the Ringer solution will be considered in Part II of this series.

(*b*) *Effect of electrolytes.*

The effect of oxygen starvation may be imitated by removing the potassium and calcium ions from the Ringer solution. The skin was mounted in glucose-sodium chloride solution buffered to *p*H 8·3 with *M*/100 sodium phosphate buffers and isotonic with the glucose-Ringer solution previously used. Potentials of the same order as before were obtained initially with this solution saturated with air but they did not survive. The potentials fell to *ca.* 5 mv. in 1–2 hours (Fig. 4), whereas in Ringer solution they rise at first and then are constant for many hours. Renewal of the solutions did not arrest the decay of the potential. Replacement of the impoverished solution by glucose Ringer brings about a partial recovery in

the potential. The greater the previous decay in potential the less the recovery on re-immersion in Ringer solution.

It is also found that in this physiologically unbalanced solution the response to oxygen starvation is far more rapid. An oxygen-free solution was prepared in the manner already described and the effect of substituting this for glucose Ringer was observed. The potential fell to 5 mv. in 10–20 min., half the time noted for a similar decay in the presence of calcium and potassium. The specific effect of these ions on the potential is receiving further attention.

SUMMARY.

Experimental methods are described for the investigation of the means by which the electrical potential across an isolated piece of frog skin is maintained. The best medium is glucose-Ringer solution *p*H 8·3 at room temperature (12–17° C.).

The potential is not due to glandular activity and is not an injury potential.

Complete oxygen starvation destroys the potential rapidly and irreversibly. Lack of potassium and calcium ions from the solution does the same but more slowly.

The frog skin potential is therefore maintained in a physiologically balanced salt solution by oxidative processes which can only proceed in the presence of dissolved oxygen.

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REFERENCES.

ADOLPH, E. F. (1925). *Amer. Journ. Physiol.* **73**, 85.
ALCOCK, N. H. (1906). *Proc. Roy. Soc.* B, **78**, 159.
BAYLISS, W. M. (1924). *Principles of General Physiology*, p. 350.
BAYLISS, W. M. and BRADFORD, J. R. (1886). *Journ. Physiol.* **7**, 217.
ENGELMANN, T. W. (1872). *Pflügers Arch.* **6**, 97.
KROGH, A. (1904). *Skand. Arch. f. Physiol.* **15**, 328.
LUND, E. J. (1926). *Journ. Exp. Zool.* **44**, 383.
ORBELI, A. L. (1910). *Zeitschr. f. Biol.* **54**, 329.
PUMPHREY, R. J. (1931). *Proc. Roy. Soc.* B, **107**, 511.