THE ELECTRICAL PROPERTIES OF ISOLATED FROG SKIN

PART II. THE RELATION OF THE SKIN POTENTIAL TO OXYGEN CONSUMPTION, AND TO THE OXYGEN CONCENTRATION OF THE MEDIUM

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(*Received 8th May*, 1933.)

(With Eight Text-figures.)

INTRODUCTION.

THE oxygen consumption of cells and tissues and the utilisation of the energy of metabolism are related factors in many vital processes. The dependence of functional activity on oxygen supply and on inhibitors of oxidative processes has been established by studies on muscular contraction (Fletcher and Hopkins, 1917), on the electrical response of nerve to stimulation (Gerard, 1930), and on the movement of cilia (Gray, 1928) and amoeba (Pantin, 1930). It is established in these cases that activity is associated with anaerobic reactions, while oxygen is only used for the restoration of the subject to a state apt for activity and its maintenance in that condition.

It was shown in a previous paper (Francis and Pumphrey, 1933) that the potential across an isolated piece of frog skin is maintained in a physiologically balanced salt solution by oxidative processes which can only proceed in the presence of dissolved oxygen. When once the potential is destroyed by complete oxygen starvation it cannot be revived by readmission of oxygen. This suggests, in contrast with the cases cited above, that the potential is not maintained by anaerobic reactions but is an accompaniment of the consumption of oxygen and ceases when oxygen consumption ceases. It is therefore of interest to trace more closely the relation of the potential to the respiratory processes of the skin and to the concentration of dissolved oxygen in the solution surrounding the skin.

APPARATUS AND METHODS.

Two methods were used for mounting the skin. In one case all gases except in solution were excluded from the skin. In the other the liquid volume was reduced to a minimum and the composition and pressure of the gas having access to the skin could be controlled. The glass cell used for the former method as well as the

means of potential measurement have been previously described (Francis and Pumphrey, 1933).

In the second method of mounting, the piece of skin is held by a rubber ring across the end of a short length of glass tubing hung vertically in an inverted bell-jar (Fig. 1) which stands in vacuum-tight contact with a ground-glass plate (*g*). The tube is attached by another rubber ring (*R*) to the lower end of a spiral (*S*) of silver wire which serves as electrode within the tube. The upper end of the wire is soldered to the tip of a steel plunger (*P*) which slides in a tight-fitting collar (*C*) mounted in the rubber stopper of the jar which also carries inlet and outlet tubes for gas flow (not shown). The plunger and collar fit to less than $\frac{1}{1000}$ in. and are lubricated with

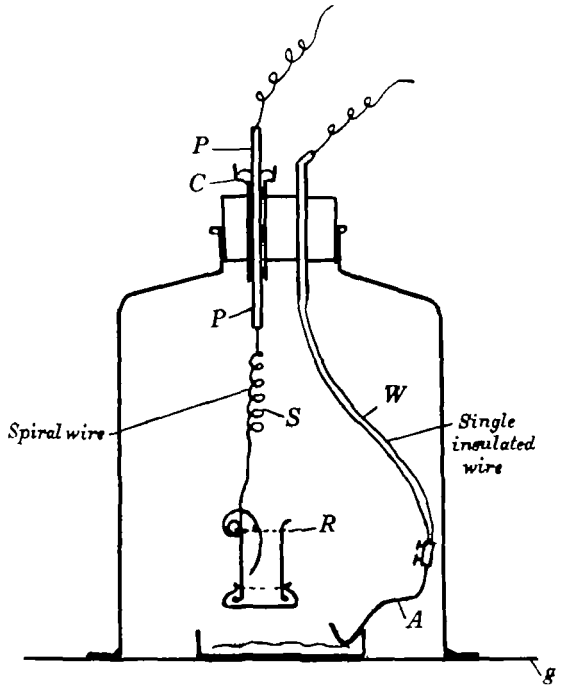

Fig. 1.

thick grease. A mercury seal is poured into the top of the collar. Connection to the second electrode (a strip of silver, *A*) is made by the rubber-covered wire (*W*) cemented into a length of glass tubing through the stopper. The apparatus leaks less than 1 cm. Hg per hour when holding gas at 4 cm. Hg.

Electrical connection is made to the inner side of the skin by a piece of cotton-wool soaked in Ringer solution which is also touching the end of the silver wire. The top of the plunger is depressed by a screw device until the outer surface of the skin descends on to a pad of filter-paper soaked in Ringer solution with which the second electrode rests in contact. When no skin is mounted the silver electrodes show a potential difference of a few millivolts. The constancy of this zero error is verified by measurements before and after an experiment with the skin.

The gaseous mixture to be used for saturating the Ringer solution or for admission to the bell-jar was previously prepared in known proportions in an aspirator. Oxygen and nitrogen from cylinders or air were used without purification to make up the mixtures. The oxygen content of the nitrogen and, at times, of the experimental mixtures was determined by analysis in a form of Haldane apparatus which had an experimental error of 0·07 per cent. The measurement of the lowest oxygen content of the mixtures used, 0·7 per cent., is thus accurate to ± 10 per cent. The error is less in proportion for higher oxygen contents.

THE RELATION BETWEEN THE POTENTIAL AND THE OXYGEN CONCENTRATION.

Experiments were made with both types of apparatus described. Results typical of experiments with many skins are shown in Fig. 2. The character of the response

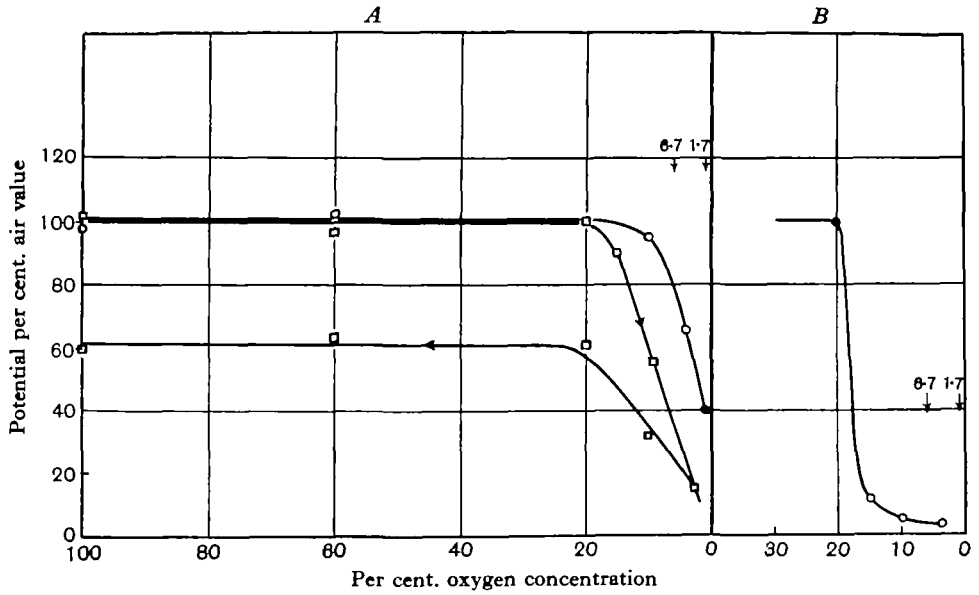

Fig. 2, *A* and *B*. Relation between oxygen concentration and skin potential.

of the potential to changes in oxygen concentration is the same if different solutions of different oxygen content are used as if only the composition of the gas in the bell-jar (Fig. 1) is varied. The majority of readings were carried out with the small glass cell using different solutions saturated by bubbling for ¾ hour with the requisite gas mixture.

It is found that the potential with glucose Ringer solution on both sides of the skin is independent of the oxygen content until a partial pressure of 15–8 cm. Hg is reached. The potential is the same in air or oxygen-saturated solutions. Individual skins differ in the value of the critical partial pressure of oxygen below which the potential falls, but the shape of curves from different skins is the same (Fig. 2, *A*). Occasional skins were found for which the potential decreased abruptly as soon as

the oxygen concentration fell below the air value (Fig. 2, *B*). With such skins the potential-oxygen concentration curves showed a point of inflection between the air value and zero of oxygen concentration.

When the oxygen supply to the skin is reduced the first response is a temporary rise in potential. The effect is smaller than that noted previously at the start of complete oxygen starvation. It occurs using either the solution or the gas technique and is therefore not due to a mechanical stimulus given to the skin or to the washing away of diffusible substances. The corresponding initial rise on substituting oxygen-rich solutions is less pronounced than in the anaerobiosis experiments. The readjustment of the potential to a new steady value when the oxygen content of the solution is changed takes place slowly, 15–50 min. for different skins. In the earlier experiments, when the potential had reached a steady value, the solutions were renewed in order to replace the oxygen absorbed by the skin. Such renewal was found to be unnecessary. Even with solutions of the least oxygen content used the renewal did not shift the potential by more than ± 1 mv. It is found that the reversibility of the potential response to partial oxygen starvation is incomplete. The same shape of curve is retraced, but a lower potential in air-saturated solution is reached. The longer the exposure and the lower the oxygen content the smaller the subsequent recovery in air.

RELATION BETWEEN POTENTIAL AND GAS PRESSURE.

The experiments were performed with the bell-jar apparatus, the gas present being air or oxygen. The initial effect of reduction in pressure is one of stimulation. If a skin is mounted in air and the pressure then reduced to, say, 5 cm. Hg, the potential rises at first and then falls. If the pressure is raised again to one atmosphere before the potential fall begins, the potential is stabilised at a new high value. This effect of stimulation by reduction of pressure is comparable with that previously found for oxygen impoverishment at atmospheric pressure but of longer duration. Newly mounted skins can withstand an air pressure of 5 cm. Hg without the potential falling off for 1–2 hours. In studying the effect of reducing pressure on the potential the initial atmospheric value was obtained by alternate applications of vacuum (5 cm. Hg) and atmosphere to the skin till no further stimulation occurred. Then it was found that lower steady values of the potential were obtained at each reduction of pressure.

The relation between potential and pressure typical of results with several skins is shown in Fig. 3 and is similar to that found for changing the oxygen concentration in the last section. The fall in potential as soon as the air pressure is reduced below atmospheric pressure is parallel with the fall in potential at atmospheric pressure when the oxygen concentration is reduced below its normal air value. Below about 7 cm. Hg the potential falls steadily but slowly to zero at the end of several hours and is not revived by admission of air. Provided the potential has not fallen below *ca.* 10 mv. the form of the curve is recoverable on readmission of air but recovery of potential is not complete.

When oxygen is initially present instead of air, a fall in potential does not occur until lower pressures are reached. The potential-pressure curves are of the same shape as with air but displaced towards the ordinate axis. The steady decay of the potential at the lowest pressure used (4 cm. Hg) is slower in oxygen than in air at the same pressure.

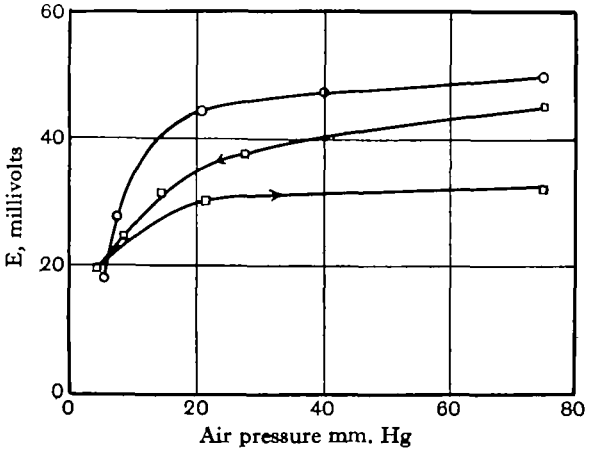

Fig. 3. Relation of potential to air pressure.

THE RELATION BETWEEN RATE OF RESPIRATION AND OXYGEN CONCENTRATION.

The respiration of frog skin was also measured in various gaseous mixtures using bicarbonate-free glucose Ringer solution. Four Barcroft differential manometers were used, the improvements in technique suggested by Dixon and Elliot (1930) being adopted. The temperature was 17° C. ± 0·2. The experiments were done in July and August. A dorsal or ventral piece of skin of dry weight 0·02—0·06 gm. cut into four or six pieces was used for each determination. At the end of an experiment the skin was left in a P_2O_5 desiccator overnight and its dry weight found. In every case the rate of respiration in air was first observed; the altered gas mixture was admitted after evacuation to 180 mm. Hg and was replaced three times as recommended by Keilin (1929). In the first half hour after removal from the frog, the skin showed a high rate of respiration probably due to injury. This becomes steady and the rate of respiration falls off about 3 per cent. per hour afterwards. The distribution of the steady values for respiration in air obtained from 43 experiments with skin from 32 frogs is indicated in Fig. 4. The mean value for the respiration of the skin of a summer frog from these experiments is 18 c.mm. oxygen (dry at N.T.P.) per minute per gram dry weight of skin (compare results summarised by Adolph, 1929).

The rate of respiration was very slightly less (2 per cent.) in air than in oxygen. When the partial pressure of oxygen is less than the air value the rate of respiration is substantially less. After 1 hour's exposure to oxygen-poor gas the

rate of respiration when air was readmitted did not recover its original air value. Therefore, for the sake of comparison, each determination at a new oxygen concentration was performed with fresh frog skin and was preceded by a determination in air. The rate of respiration is expressed as a percentage of the value found in air (Fig. 5). The vertical lines embrace the scatter of at least six determinations at each oxygen concentration.

A comparison of the curves in Figs. 2 and 5 reveals a close similarity between the dependence of potential and respiration on oxygen concentration. This gives further support to the conclusion previously reached from the results of complete oxygen starvation that the skin potential is a direct accompaniment of the respiration processes. If the rate of respiration falls off there is no reserve material or auxiliary process by which the potential can be maintained in spite of oxygen lack.

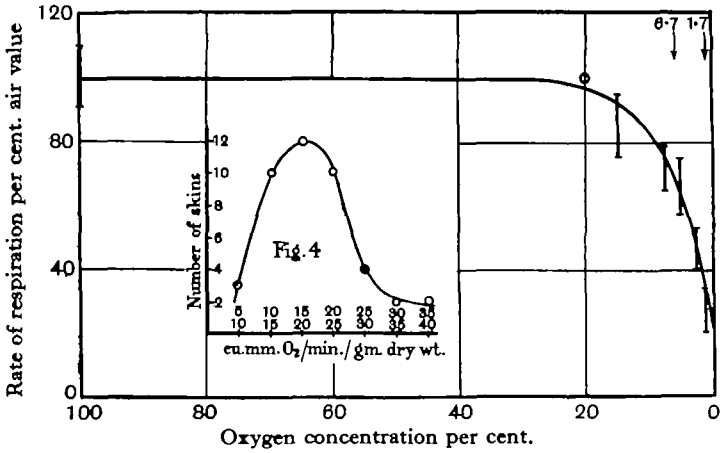

Fig. 4. Variation in respiratory rate of different skins.

Fig. 5. Effect of oxygen concentration on rate of respiration of skin.

The dependence of respiration on oxygen concentration has been determined for the skin of *Rana pipiens* by Lund (1928) and Adolph (1929). Lund, using unstirred solutions, found an almost linear relation between oxygen concentration and respiration with an air value 33 per cent. of the rate in pure oxygen. The corresponding value for air compared with oxygen found by Adolph was 92 per cent. (in this work 98 per cent.). The latter only worked at one pressure of oxygen below the air value.

Lund established a qualitative correlation between electrical potential and rate of respiration in solutions of different oxygen content. His values of potential at any one oxygen concentration are not steady, however. In the experiments here reported it was found that with glucose present the potential reached a steady potential at each oxygen concentration down to 4 per cent. with 80 per cent. of the skins used.

It is desirable to know what part, if any, is played by diffusion in the fall of respiration during oxygen impoverishment. This can be decided by the use of the equation (Hill, 1929)

$$2b = \sqrt{\frac{2Ky}{a}},$$

where b = thickness of skin in air, K = diffusion constant of oxygen in the skin, y = oxygen pressure, and a = rate of oxygen consumption per c.c. of tissue per minute. The thickness of the ventral skins was found by the method of Krogh (1919) to average 0·2 mm. and of the dorsal 0·4 mm. $K = 1{\cdot}13 \times 10^{-5}$ for connective tissue at 20° C. (Krogh, 1918) and $a = 0{\cdot}0038$ c.c. per minute since the dry weight value at 17° C. was 0·018 c.c. per minute and the temperature rise in respiration rate between 17 and 20° C. is approximately 5 per cent. (The dry weight was found to be 20 per cent. of the wet weight.) Substituting these values in the equation gives the following oxygen pressures at which diffusion would cease to be able to supply the requirements of the skin—for the ventral skin 0·017 atmosphere, for the dorsal skin 0·067 atmosphere of oxygen (*i.e.* 1·7 and 6·7 per cent.). These pressures are indicated by vertical arrows in Figs. 2 and 5. It will be seen that the potential and respiration fall off before these low pressures are reached. Diffusion is therefore not a limiting factor under the conditions of the experiment. The rate of respiration and correspondingly the value of the potential across the skin are a function of the oxygen concentration even when the oxygen supply is in excess of the normal requirements of the skin.

THE EFFECT OF INHIBITORS OF RESPIRATION ON THE POTENTIAL.

The foregoing work has established a close relation between the electrical potential and respiratory processes of the skin. In recent years considerable insight has been gained into the mechanism of the respiratory processes. It is of interest therefore to see to what extent agents which are known to inhibit the stages of respiration can also influence the skin potential. A common type of respiratory process (Keilin, 1929) involves the functional relationship of dehydrases, intracellular haematin compounds (*e.g.* cytochrome) and oxidases. Respiration can be arrested by inhibiting oxidase or dehydrase activity. NaCN, Na_2S, and CO inhibit oxidase activity. Urethane and aliphatic alcohols and aldehydes inhibit dehydrase activity. The effect of these inhibitors on the skin potential was investigated.

Glass cells of the form previously described (Part I) were used. Solutions of glucose Ringer, *p*H 8, were prepared 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} molar with respect to NaCN. Four separate pieces of skin were taken and the normal potentials of the skins were found first in cyanide-free glucose Ringer by waiting till the potentials rose to steady values. This took at least an hour after the removal of the skin from the frog. The four cyanide solutions were then run in and the fall of potential with time observed for each skin. The same procedure was adopted in studying the effect of sodium sulphide. The results which were repeated several times are shown in Figs. 6 and 7. In concentrations above 10^{-4} molar, cyanide and sulphide cause an immediate drop of potential. Cyanide is ultimately more toxic than sulphide perhaps because of the oxidation of the latter by the skin. If, during the course of the potential drop, normal glucose Ringer is replaced in the cells the potential shows a partial recovery. The recovery is smaller the lower the potential has sunk and does not

occur at all if the potential has fallen below 5 mv. The toxic effect of cyanide and sulphide is therefore incompletely reversible. These findings with respect to

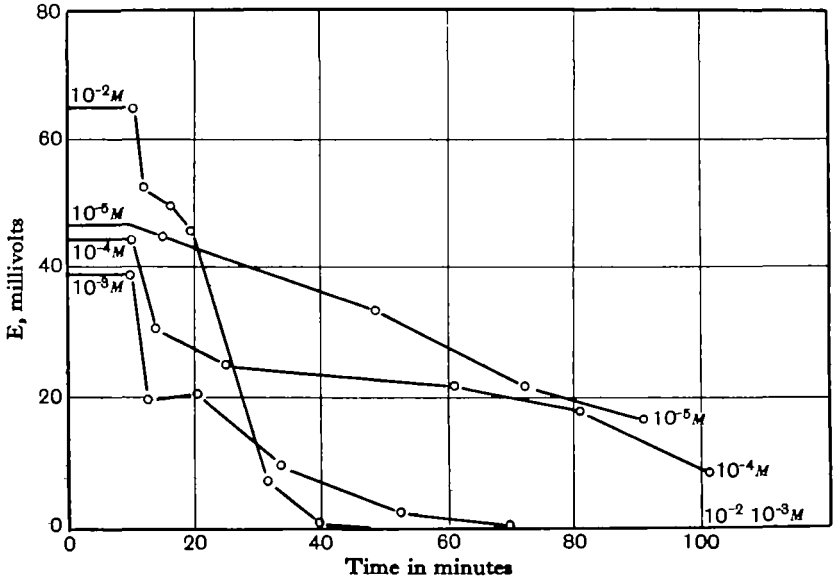

Fig. 6. Effect of sodium cyanide on skin potential.

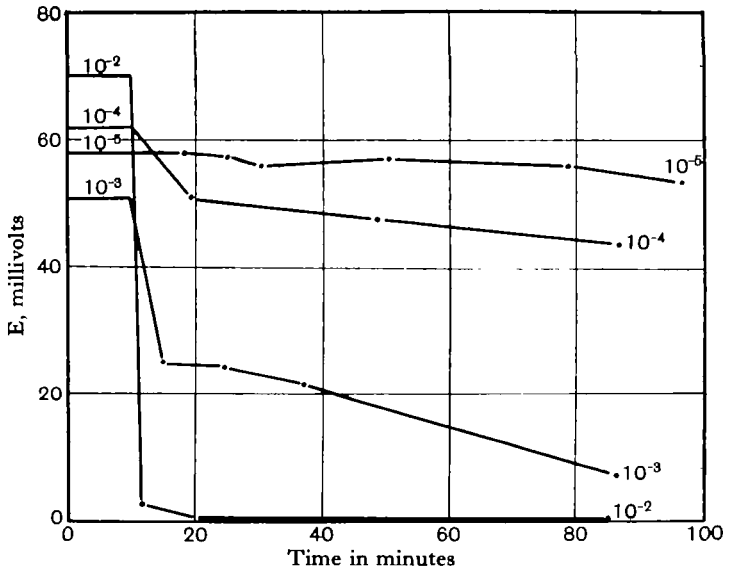

Fig. 7. Effect of sodium sulphide on skin potential.

cyanide agree substantially with the work of Lund (1926) who has reported the reversible inhibition of the potential across the skin of *Rana pipiens* by solutions of $M/1000$KCN.

The effect of CO was studied in the bell-jar apparatus by surrounding the skins in an atmosphere containing four-fifths CO and one-fifth O_2 and also by mounting the skins in glucose Ringer solutions previously saturated with the same gaseous mixture. A lower concentration of oxygen would depress the potential even in the absence of CO. Under these conditions CO is found to have no effect on the potential in the light but a depressing effect in the dark. The fall of potential is greater the higher the initial potential, but in no case exceeds 50 per cent. of the original value even when the skin is left in the dark for some hours. The fall of potential is arrested by illumination and partial recovery takes place. Observations typical of several skins are illustrated in Fig. 8.

These experiments indicate that agents which inhibit the oxidase activity in the chain of respiratory processes also depress the potential of the skin. The effect of dehydrase inhibitors on the potential although definite is not as great.

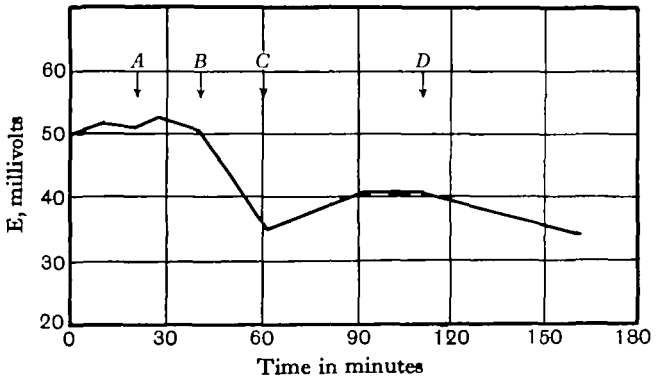

Fig. 8. Effect of carbon monoxide on skin potential. *A*, CO-Ringer; *B*, darkness; *C*, light; *D*, darkness.

The addition to the glucose Ringer of butyl alcohol $M/1000$ and $M/100$ caused less than 10 per cent. drop in the potential after 30 min. treatment. Amyl alcohol in the same concentrations had a greater effect, causing a 20 per cent. fall in $M/100$ solution and complete annihilation in 5 min. in saturated solution, and urethane in concentrations from $M/2$ up to saturation reduced the potential to 40–60 per cent. of its original value. Thus the complete annihilation of potential observed with NaCN and Na_2S is not also brought about by the common dehydrase inhibitors.

DISCUSSION.

The above work indicates that a close relationship exists between the maintenance of potential and the consumption of oxygen by the skin of *Rana temporaria*. The curves (Figs. 2 and 5) relating potential and rate of respiration to oxygen concentration are almost identical and show that when the ionic composition of the solutions is kept constant the processes in the skin responsible for the potential and

respiration are closely related and may be the same. The effects on the potential of substances known to inhibit respiration (Figs. 6 and 7) support this conclusion though the evidence here is not direct as the oxygen consumption of the skin in the presence of these inhibitors has not been determined. In this connection the work of Lund (1928) with the skin of *Rana pipiens* is relevant. He showed by collateral measurements that in some cases both the oxygen consumption and the potential of the skin showed the same percentage decrease in the presence of KCN solutions. This supports the close relationship of the potential and respiratory processes. On the other hand he emphasises elsewhere (Lund and Moorman, 1931) that the same increase in oxygen concentration may accelerate respiration by 1600 per cent. and yet only increase the potential by 100 per cent. Disparity of this kind has never been found in the work described in this paper. Neither have the measurements recorded above nor those of Adolph (1929) confirmed the linear relationship between oxygen consumption and oxygen concentration found by Lund and the consequent rapid acceleration of respiration throughout the range of increasing oxygen concentration. This may be because in measuring the oxygen uptake of the skin Lund did not stir the solutions.

Before considering more fully the rôle of oxidation in maintaining the potential of frog's skin it should be emphasised that the oxygen concentration in the medium is not the only factor governing the value of the potential. It will be shown more fully in a later paper that the potential is affected by the nature and concentration of the ions present in the solution (see also Francis and Pumphrey, 1933). It was found that the potential decayed when the skin was mounted in NaCl solutions isotonic with a Ringer solution in which the rate of oxygen consumption was unaffected (Adolph, 1929). Uhlenbruck (1924) has also demonstrated the effect on the potential of changing the ionic content of the solutions in contact with the skin. Another observation which emphasises that rate of oxygen uptake is not the only factor in maintaining the potential is the following. In the work reported here it is found that ventral skins give constantly higher potentials than dorsal skins although there is no systematic difference in their rates of respiration, per gm. dry weight. Further, dorsal skins are on the average twice as thick as ventral skins, so that for equal areas of skin the dorsal skin, having twice the oxygen consumption, gives a less potential than the ventral skin. It will be of interest to see if any difference is revealed between the electrical properties of dorsal and ventral skins when the ionic content of the solution is changed. In view of these facts a consideration of the lines along which the frog skin potential may be interpreted seems desirable at this stage.

The interpretation of the potential.

In the past thirty years the physico-chemical interpretation of bioelectric potentials has focussed attention on the properties of the plasmatic membrane in relation to the nature and concentration of the ions present in the solution with which the cell or tissue is in contact. It is likely that the normal properties of the cell membranes are maintained by intracellular oxidation processes (Hill, 1928). As long as oxidation proceeds normally, the bioelectric properties of the cell are a function of

the plasmatic membrane in relation to the composition of the external medium. If the oxidation is inhibited the properties of the membrane are modified and the potential difference across it is altered. Hill (1933) has drawn attention to the dependence of the injury potential of a crab's nerve both on the presence of oxygen and on the concentration of potassium ions in the solution bathing the nerve.

It is desirable therefore in interpreting the electrical properties of frog skin to consider two factors, the influence of the electrolytes present in solution and the rôle of oxidation. The first factor will be fully considered in a later paper. Lund has set aside this factor in his theoretical treatment of the frog skin potential.

He interprets the frog skin potential and bioelectric potentials in general as due to "the flux equilibrium of the oxidation-reduction systems" in the cells. There are, however, grave difficulties in considering bioelectric potentials as oxidation-reduction potentials. Potentials characteristic of the ratio of the concentration of the oxidised and reduced state of a substance can only be measured by the use of an inert metal electrode which can act as an electron donor or acceptor to the system. The metal electrode must be immersed in the solution containing the redox system and the difference in potential between this electrode and a standard electrode determined. The standard electrode is a similar metal electrode immersed in a solution in which the ratio of the concentration of the oxidant to reductant is unity. Instead of this a reversible electrode (*e.g.* calomel electrode) may be used provided the potential difference between the two latter electrodes is known. The experimental difficulties attending the insertion of platinum or gold electrodes into the cell interior has led to the use of redox dyes as indicators of the oxidation-reduction potential of the cell interior (summary in Würmser, 1930). These are introduced into the cell, and from the colour developed the oxidation-reduction potential of the cell interior may be approximately found.

The dyes themselves must be calibrated before use so that the relation between colour and potential is known. It is possible, therefore, with reservations, to give a value for the "oxidation-reduction potential of a cell." But this expression means the potential difference that would exist between an inert metal electrode and a standard electrode if both could be immersed in the *interior* of the cell without injury. The relation of this intracellular potential to the potential difference, measured by external electrodes between different parts of the cell exterior is unlikely to be one of identity. In any case it must involve a knowledge of the properties and function of the surface membranes of the cells. The same objections apply to the formulation of the potential difference between opposite sides of a membranous tissue (*e.g.* frog skin) in terms of the oxidation-reduction potentials of the individual cells. The properties of the plasmatic membranes of the individual cells will affect the total potential difference across the skin.

The system in which the frog skin potential has been measured in the work under discussion is the following:

Calomel electrode	Ringer solution	Frog skin	Ringer solution	Calomel electrode

The potential is maintained by the consumption of oxygen. It is measured between calomel electrodes which cannot measure directly an oxygen tension nor an oxidation-reduction potential but which measure changes in the state of ionic distribution in the solutions between the electrodes. The state of ionic distribution in this case is the direct result of a chemical action in which oxygen is consumed and diffusible ions are produced by the oxidation of a non-electrolyte substance.

If, in a transverse plane in the electrolyte solutions between the electrodes, any process can give rise to a partial separation of oppositely charged ions, a double layer of ions of opposite sign is formed across which a potential difference exists. This potential is measured by the calomel electrodes. If several of such double layers exist, the potential measured is the algebraic sum of the separate double layer potentials. A partial separation of oppositely charged ions can occur (i) by the existence of a concentration gradient, (ii) by the presence of a semi-permeable membrane and (iii) by the distribution of dissolved salts between two immiscible solvents. The value of the potential across the double layer in the first two cases depends on the nature and relative mobilities of the ions present and on the magnitude of the concentration gradient.

The simplest view to take at this stage is that the respiratory processes afford a constant supply of diffusible ions. These ions in escaping across the cell membranes may set up modified diffusion potentials. Alternatively they may bring about a concentration gradient of ions to which the membrane is specifically semi-permeable (compare Osterhout, 1932), in which case again a potential difference is established. The summation of these potentials through the tissue of the skin gives the total skin potential. Until the experimental work on the change in potential caused by changes in the ionic composition of the solution in contact with the skin is available, the specific ions concerned in the maintenance of potential are unknown and a detailed interpretation must be deferred.

It will appear that oxidation processes may serve two purposes: (1) to act as a source of ions the distribution of which across the membranes in the tissue gives rise to a potential difference, and (2) to preserve intact the properties of the membranes in virtue of which this ionic distribution is maintained.

SUMMARY.

The relation of the electrical potential across isolated frog skin in Ringer to oxygen concentration and oxygen pressure has been experimentally investigated. The dependence of the respiration of isolated frog skin in Ringer on oxygen concentration has also been determined. The potential and respiration depend in the same way on the oxygen concentration. The potential is diminished by inhibitors of respiratory processes.

The rôle of oxygen consumption in maintaining the skin potential is discussed.

The author is indebted to the Department of Scientific and Industrial Research for a Senior Research Award. He is indebted to Prof. J. Barcroft, F.R.S., and Prof.

D. Keilin, F.R.S., for helpful advice. The respiration experiments were carried out in the Department of Colloid Science, and thanks are due to Prof. Rideal, F.R.S., for his interest.

REFERENCES.

ADOLPH, E. F. (1929). *Journ. Exp. Zool.* **53**, 313.
DIXON, M. and ELLIOT, K. A. C. (1930). *Biol. Journ.* **24**, 820.
FLETCHER, W. M. and HOPKINS, F. G. (1917). *Proc. Roy. Soc.* B, **89**, 444.
FRANCIS, W. L. and PUMPHREY, R. J. (1933). *Journ. Exp. Biol.* **10**, 379.
GERARD, R. W. (1930). *Amer. Journ. Physiol.* **92**, 498.
GRAY, J. (1928). *Ciliary Movement.* Cambridge University Press.
HILL, A. V. (1928). *Proc. Roy. Soc.* B, **104**, 39.
—— (1929). *Boyle Lecture* (1928). H. Milford.
—— (1933). *Nature*, **131**, 501.
KEILIN, D. (1929). *Proc. Roy. Soc.* B, **104**, 206.
KROGH, A. (1919). *Journ. Physiol.* **52**, 391.
LUND, E. J. (1926). *Journ. Exp. Zool.* **44**, 383.
—— (1928). *Journ. Exp. Zool.* **61**, 265, 291, 327.
LUND, E. J. and MOORMAN, J. B. (1931). *Journ. Exp. Zool.* **61**, 249.
OSTERHOUT, W. J. V. (1932). *Journ. Gen. Physiol.* **15**, 667.
PANTIN, C. F. A. (1930). *Proc. Roy. Soc.* B, **105**, 538.
UHLENBRUCK, P. (1924). *Zeit. f. Biol.* **82**, 225.
WÜRMSER, R. (1930). *Oxydations et Réductions*, p. 315.