THE ELECTRICAL PROPERTIES OF ISOLATED FROG'S SKIN

III. THE EFFECT ON THE POTENTIAL OF THE PASSAGE OF SMALL CURRENTS ACROSS THE SKIN

IV. THE SPECIFIC EFFECT OF POTASSIUM IONS

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(With Nine Text-figures.)

In two previous papers in this *Journal* (Francis and Pumphrey, 1933; Francis, 1934) the relation between the potential across pieces of isolated frog's skin and various environmental factors has been described and discussed. In the work there described the potential was measured by means which were virtually electrostatic and no appreciable work was done by the skin. In this paper is described a method by which the potential can be measured during and after the performance of electrical work on or by the skin. Certain features are common to all the electrical phenomena manifested by cells and tissues and it seems likely that in all cases the basic mechanism is the same. It is probable therefore that the results here described will eventually be of service in the interpretation of the electrical behaviour of nerve and other excitable tissues, where on account of the structure of the tissue such experiments are difficult or impossible of performance.

METHOD.

The material and its preparation have been described in a previous paper (*loc. cit.*). Pieces of skin were mounted between the flanges of two glass half-cells whose construction is shown in Fig. 1.

A disc-shaped electrode of platinum coated with platinum black was fused into each half-cell; these electrodes were connected in series with a reversing key, a high-tension battery, a high variable resistance and a microammeter, and were used for passing across the skin a current whose magnitude and direction could be altered at will. It has been assumed that the lines of current flow across the skin were nearly parallel. Each half-cell also had a thin-walled capillary tube running axially through the middle of the platinum electrodes and ending within about 0·1 cm. of the skin. The distal end of the capillary was connected externally with a siphon tube and beaker containing the same solution as the half-cell. The beakers were connected by means of saturated calomel electrodes to a potentiometer and a Einthoven string galvanometer. With this circuit the potential between two points in close approximation to the two sides of the skin can be measured. It was found that the potential drop across the liquid between the end of the capillary and the surface of the skin could be neglected.

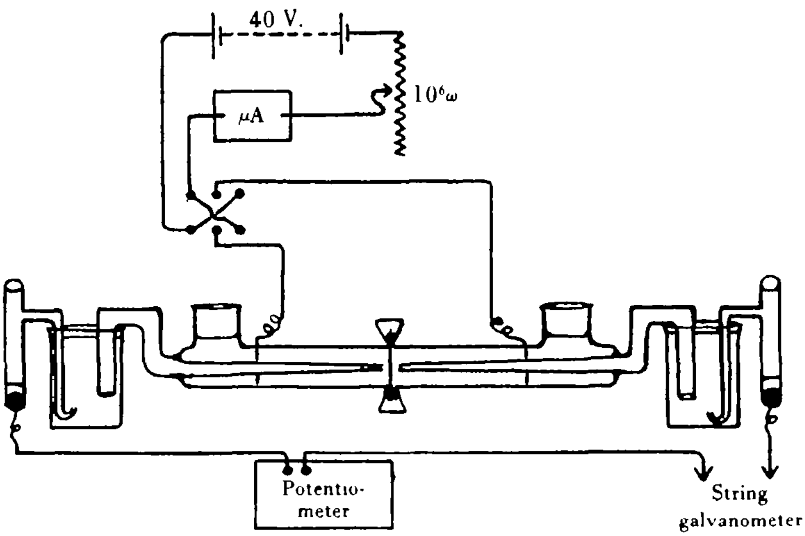

Fig. 1.

RESULTS.

PART I. WITH RINGER SOLUTION IN CONTACT WITH BOTH SIDES OF THE SKIN.

In the first series of experiments a current increasing in steps was passed across the skin and the potential across the skin was measured as soon as it had become steady. The inside of the skin on open circuit is always positive. The magnitude of the potential differs from skin to skin, but 50 mv. is a fair average for healthy skins. When the current is passed across the skin so that positive ions tend to pass through it from the inside to the outside the potential across the skin during the passage of current is increased by an amount proportional to the magnitude of the current, *i.e.* dI/dV is constant. A current in this sense is spoken of as a polarising current. Representative results are shown in Fig. 2.

With a current in the opposite sense (spoken of here as a depolarising current) the results are different. With small current densities the decrease of potential is still proportional to the increase of current (*i.e.* if I in this sense is taken as negative, dI/dV has the same value as for a polarising current).

When the current is increased further, however, the potential falls rapidly and changes sign (Fig. 3). Still greater increase of current dI/dV after becoming a minimum increases again to or near its original value. The points on the curves, it must be emphasised, represent the values of the potential after it has become steady.

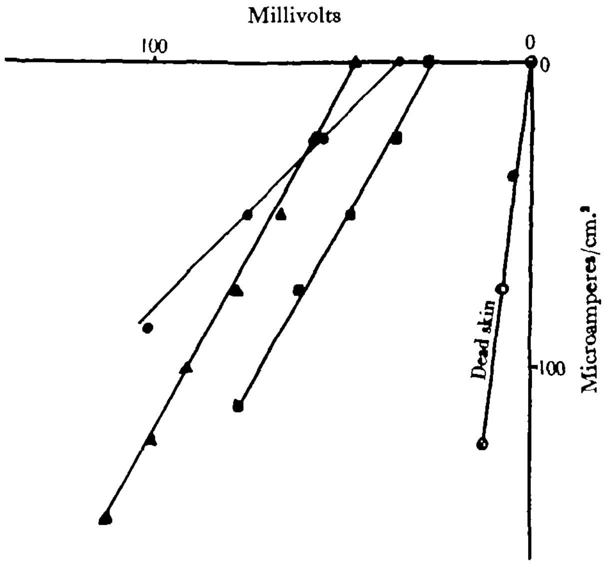

Fig. 2.

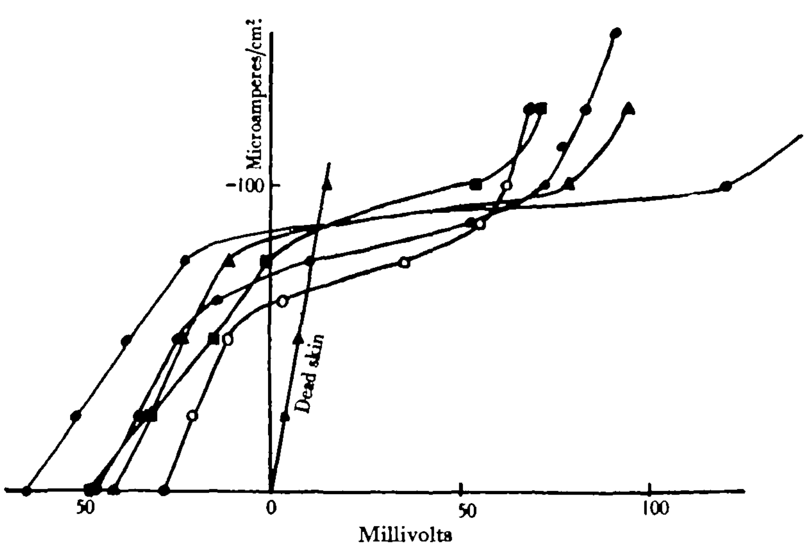

Fig. 3.

With a polarising current these values do not differ appreciably from the values obtained at the moment when the current is made. With a depolarising current, however, this is not the case.

Change of potential with time during the passage of a depolarising current.

Both the rate of change and the total change of potential vary with the magnitude of the current. With a depolarising current below about 50 μA/cm.[2] the change with time is insignificant, as it is with a polarising current. With a depolarising current of about 80 μA/cm.[2] the total change is considerable and the rate of change quite small, so that it takes several minutes to reach a steady state and it is easy to extrapolate to find the potential at the moment the current was made. As the depolarising current is made greater still the total change of potential increased and the rate of change increased greatly so that extrapolation becomes less accurate (Fig. 4).

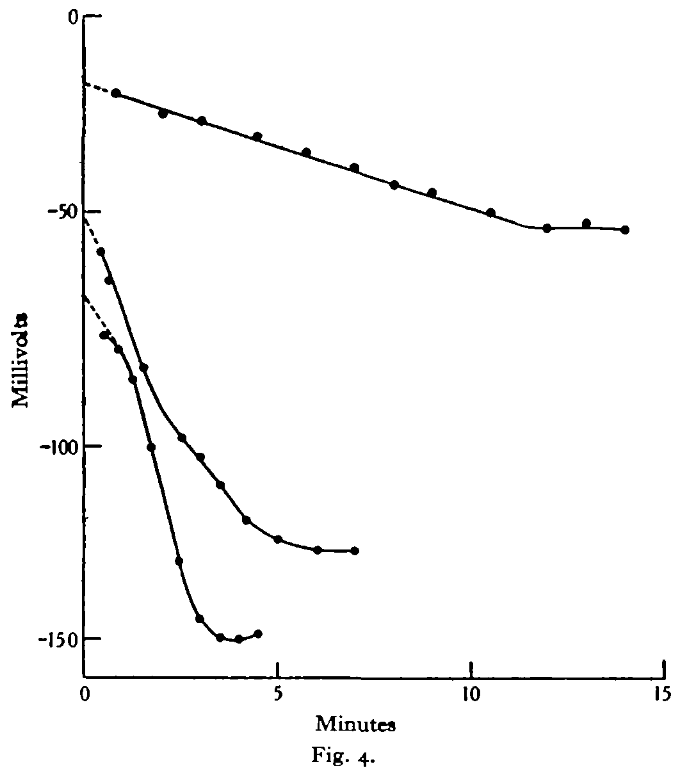

Fig. 4.

In Fig. 5 both the final steady and the extrapolated initial values have been plotted against the current density for a single skin. It can be seen that within the limits of experimental error all the initial values fall along a straight line.

The potential on open circuit after the passage of current through the skin.

When the circuit is opened after the passage of polarising or of small (less than 40 μA/cm.[2]) depolarising currents, a steady potential is reached immediately which does not differ by more than a few millivolts from the initial potential on open circuit. With polarising currents it is generally slightly lower, with depolarising currents slightly higher than the initial potential. With depolarising currents of 80 μA/cm.[2] or higher there is a marked after-effect. In Fig. 6 is shown a case in

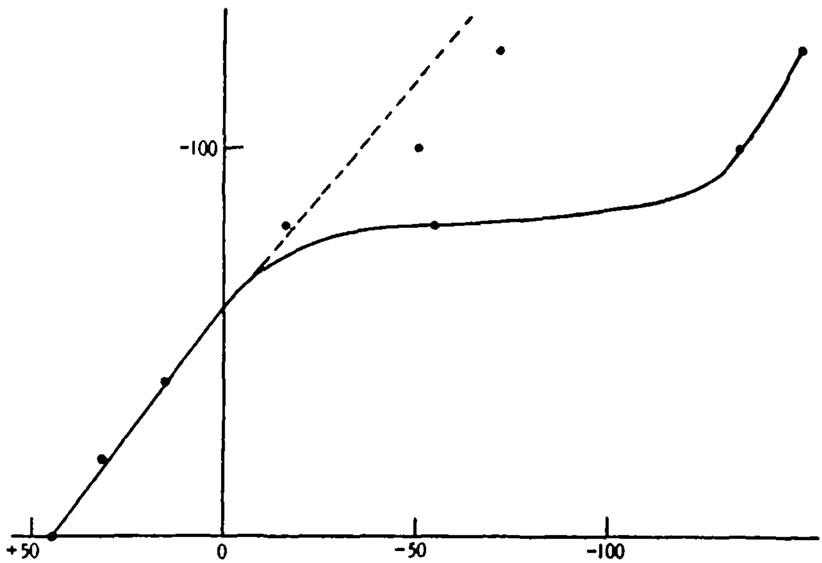

Fig. 5.

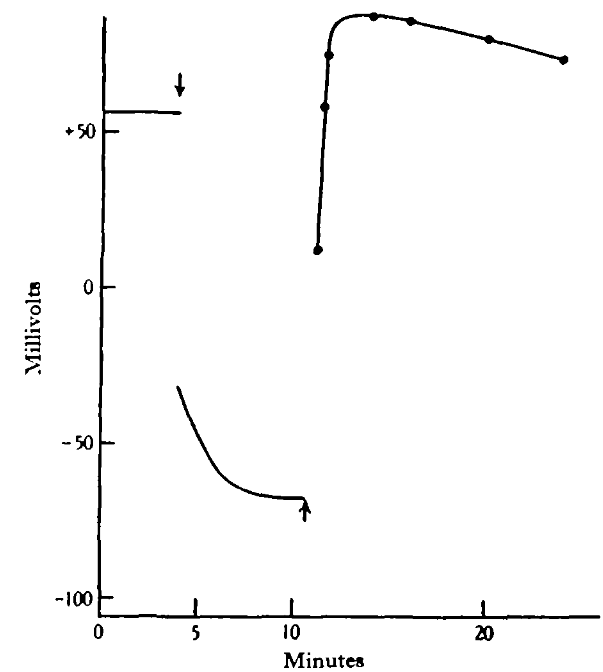

Fig. 6. A depolarising current of 80 $\mu A/cm.^2$ is made at the first arrow and broken at the second.

which a depolarising current was passed till a steady value was reached and then broken. The potential measured as soon as possible after the break was low but rising rapidly. It reached a maximum considerably higher than the initial resting value. It then fell slowly towards the initial value, reaching it in about 20 min. This is typical of all the results obtained provided the depolarising current is not too great. Currents in the region of 140 μA/cm.^{2} frequently seem to have an injurious effect. The initial rise is not pronounced and recovery to the initial potential may not take place.

Discussion.

These results seem to indicate that the skin behaves in a manner comparable with a polarised electrode such as a hydrogen electrode. In the resting skin the potential is maintained as has been shown in earlier papers by an oxidative mechanism. Since with a polarising current the relation between current and potential is linear it is reasonable to assume that what may be called the electrode potential of the skin is unaffected and that which is measured is the sum of this potential + the potential gradient due to the resistance of the skin. The correctness of this assumption is supported by the fact that fair agreement is found between the resistance deduced from the slope of the graphs in Fig. 1 and the resistance measured in an A.C. bridge. In the parallel case of the hydrogen electrode the potential is not altered by the deposition of more hydrogen ions on the electrode provided the current is not too large for equilibrium with the hydrogen gas to be maintained.

With a depolarising current of sufficient magnitude, however, the electrode potential of the skin is altered, being decreased and finally reversed in sign. It is as if the mechanism in the skin which maintains the potential were no longer able to keep pace with the drain on it. It is comparable to a hydrogen electrode from which hydrogen ions were being withdrawn faster than the supply of gaseous hydrogen would allow, resulting finally in the formation of an oxygen electrode. At the same time it is evident that depolarisation of the skin accelerates the potential-maintaining reactions since on breaking the current the skin rapidly recovers to a potential higher than the normal potential on open circuit.

It is interesting to compare the effect of the depolarising current with the effects of brief oxygen starvation, of the application of urethane and KCN and of stimulation of the skin by an alternating current. All these cause an abrupt fall in the potential of the skin followed on renewal of the oxygen supply, the removal of the drug or the cessation of the stimulus, by a rapid rise to a potential higher than the normal resting potential. Our results with urethane have recently been confirmed and extended by Boell and Taylor (1933). The results of electrical stimulation are not yet published.

These phenomena seem to indicate that, however the lowering of the skin potential is caused, the lowering in itself is a sufficient cause of an acceleration of the process by which the potential is normally maintained. This would be explicable if this process were reversible and of the form

$$A+B+C+\ldots \rightleftharpoons X+Y+Z+\ldots$$

and the temporary check allowed an accumulation of the active substances *A*, *B*, etc., with a corresponding increase in the velocity of the reaction from left to right when the check was removed.

Blinks (1929–30) has demonstrated comparable phenomena in the protoplasmic membrane of *Valonia* and *Nitella*. He showed that the high apparent resistance of the protoplasmic film to a constant small electromotive force was due to polarisation resulting in an opposed E.M.F. nearly equal to the applied E.M.F., and also that the protoplasmic film was asymmetrical so that the degree of polarisation for any

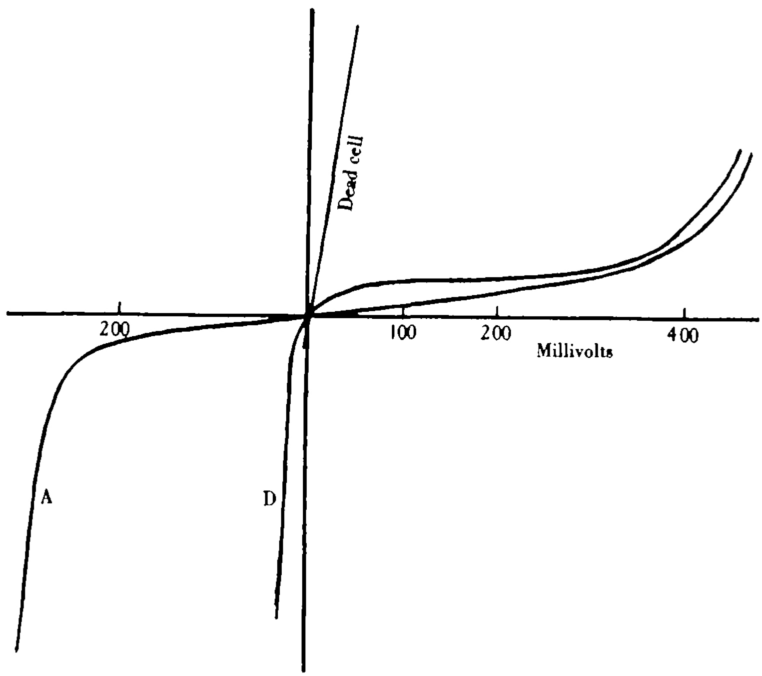

Fig. 7. Taken from the figure in Blinks' paper in which the resistance (in arbitrary units) across the protoplasmic membrane of the *Valonia* cell is plotted against the applied potential. In this figure current (in arbitrary units) is plotted against potential.

applied E.M.F. and consequently the apparent resistance varied according to whether the positive electrode was inside or outside the film.

On replotting one of his figures (*J. Gen. Physiol.* **13**, 802) in terms of current and potential the similarity with the frog's skin is evident.

IV. THE SPECIFIC EFFECT OF POTASSIUM IONS.

In the experiments described above and in previous papers Ringer solution has been used in contact with both sides of the skin in order to make the system as symmetrical as possible. The skin is intolerant of great changes in the composition and concentration of the solution in contact with its inner surface. The experiments of Alcock (1906) indicate, however, that the seat of the potential difference is and the temporary check allowed an accumulation of the active substances *A*, *B*, etc., with a corresponding increase in the velocity of the reaction from left to right when the check was removed.

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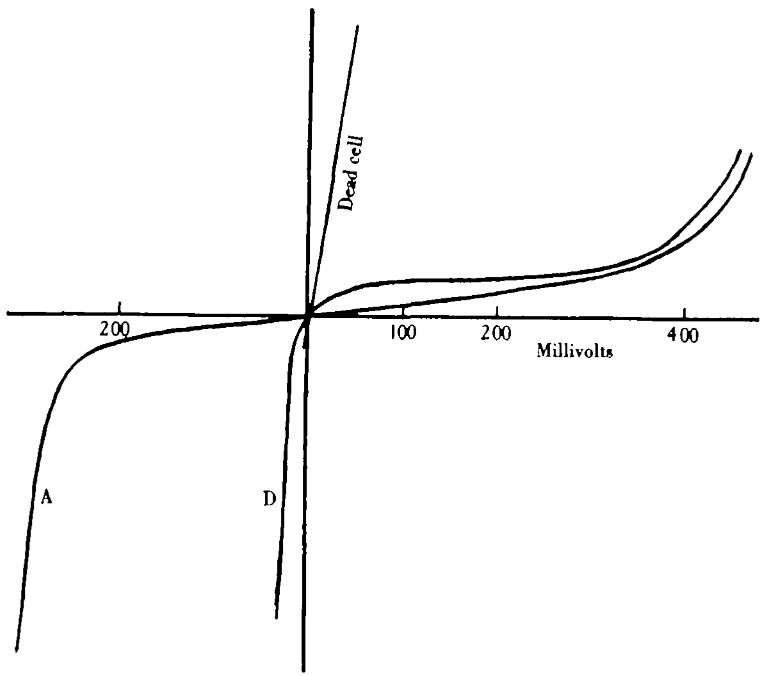

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Results.

Substitution of $M/10$ sodium chloride for Ringer in contact with the external surface of the skin either had no effect or caused a slight rise (5–10 mv.) in the potential on open circuit. When a current was passed across the skin the current-potential-relation was indistinguishable from that obtained with Ringer solution on

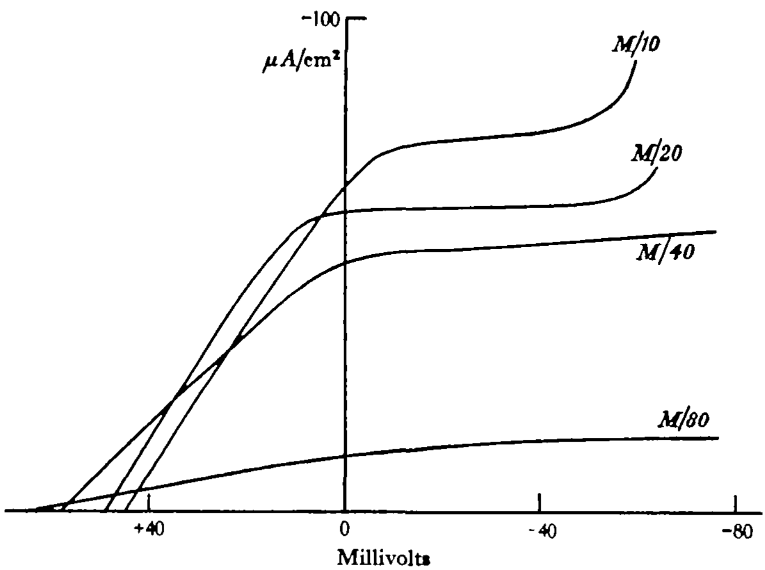

Fig. 8.

both sides. Repetition of these experiments with sodium acetate and $M/20$ di-sodium sulphate gave exactly the same results so that the anion does not appear to be important.

With $M/10$ $CaCl_2$ the potential on open circuit was somewhat lower than with Ringer. The relation of potential to current where the current was passed in either direction was of the same form and showed a similar inflexion as with Ringer (Fig. 8).

$M/10$ KCl caused a marked fall in the potential which did not become constant till after several washes with the same solution. On passing a current across the skin a straight line with no trace of inflexion was obtained and further trial showed that this was the case with all concentrations of KCl though the slope of the graph decreased (*i.e.* the resistance of the skin increased) with decreasing concentration (Fig. 9).

It was further found that a straight line was obtained using Ringer solution containing ten times the usual concentration of potassium chloride. These effects of potassium ions are completely reversible, the skin behaving normally on returning to Ringer solution.

Discussion.

These results seem to show that the skin behaves like an electrode reversible for potassium but not for sodium or calcium ions. The same result was obtained by Blinks with *Nitella* (1930, p. 505). It is probable that the specific effect of the potassium ion is dependent on its high mobility and on its relatively high concentration in the protoplasm of the cells. A steep concentration gradient of potassium ions across the cell surface seems necessary for the maintenance of normal polarisation, cf. the reversible inactivation of nerve by increasing the concentration of

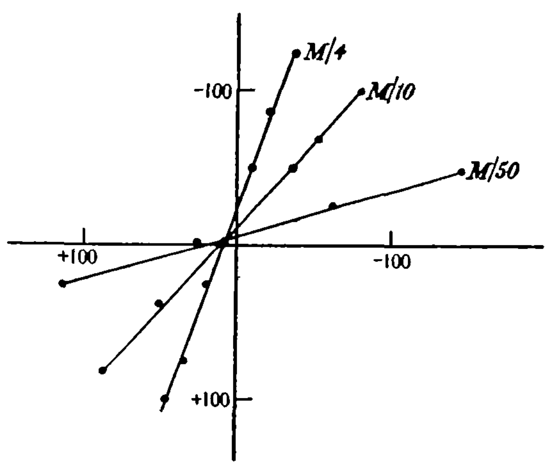

Fig. 9.

potassium in contact with it and the fall of the injury potential in the same circumstances.

In a recent paper by Steinbach the effects of salts on the potential on open circuit have been investigated and considered at some length. I am in agreement with his main experimental results as regards the potential between the inside and the outside of the skin. The paper contains, however, a remarkable misapprehension which leads the author to draw conclusions about the electrical structure of the frog's skin which are certainly unjustifiable on other grounds. His method, which must be discussed in some detail, is (unless I have misunderstood him) as follows. Contact with the external and internal surfaces of a piece of isolated skin was made with Ringer or other electrolyte solutions in the usual manner. A third point of contact was made with the edge of the skin in Ringer solution and by this means the author believes he has established an indifferent lead to the skin itself. Neglecting the extreme improbability of this assumption, he observed that the algebraic sum of the potential differences measured from this third electrode to the solutions in contact with the inner and outer surfaces was always equal to the potential difference between the latter, and from this observation alone he derives a theory about the nature of the skin. But it is obvious that in any system whatever containing three points A, B and C the sum of the differences in potential AB, BC and CA must be zero. This is a truism and indicates merely that the measurements were correctly made. It allows no conclusions to be drawn about the nature of this system.

Whether any significance is to be attached to measurements from his so-called lead to the skin itself is another question. The present writer believes that it is not. Immersion of the edge of the skin in an electrolyte solution clearly constitutes a local short circuit between the inner and outer surfaces at that point and it seems probable that the potential there relative to the other two electrodes is determined by the respective resistances of the paths to them. This view is supported by the figures for the potential which are given in the paper.

SUMMARY.

A method is described by which the potential difference between the inner and outer surfaces of pieces of isolated frog's skin can be measured during and after the passage of small currents across the skin in either direction.

It is found that the behaviour of the skin is comparable to that of an electrode reversible for potassium but not for sodium or calcium ions.

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