Electrical Potential Profile of the Toad Skin Epithelium

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ABSTRACT The electrical potential profile of the isolated toad skin was recorded, *in vitro*, by impalement with micropipette-electrodes, when both sides of the skin were bathed with sulfate-Ringer. The outer side of the skin was some 110 mv negative with respect to the inner side. Upon impalement from the outer side, two main positive steps of 40 to 70 mv each were found to form the skin potential. The site of measurement of each potential difference was permanently marked in the tissue during recording, by deposition of carmine from the micropipette tip using iontophoresis. Serial histological sections of the skin were prepared and search was then made of the carmine deposits 2 to 6 μ in size, under phase contrast microscopy. By this method the main steps were located at the outer and the inner sides of the stratum germinativum cells. The DC resistances between the micropipette tip and the bathing solutions were measured during the recording of each potential difference. The resistance at the outer side of the stratum germinativum cells, of 1.09 kilohm. cm^2, was larger than that at their inner side, of 0.30 kilohm. cm^2. The stratum germinativum cells maintained a potential difference of -34 mv during short-circuiting of the skin.

Amphibian skins are convenient for the study of several processes involved in ion transport. For better interpretation of these processes further knowledge of the differences in electrical potential, one of the forces driving ions across the skin structures, is necessary. It is well known that the outer side of the skin is negative with respect to the inner side (24). Recent recordings with micropipettes indicate that the skin electrical potential profile is formed by steps (19, 5, 20), but disagree in their location and number. Thus Ottoson *et al.* (19) recorded one electrical potential step and interpreted it as localized at the basement membrane; Engbaek and Hoshiko (5, 8) found two steps and suggested that one was located at the outer and one at the inner face of the stratum germinativum cells; and Scheer and Mumbach (20) described one step at the epithelium and one at the tela subcutanea. Frazier (6) working with the toad bladder, a preparation that behaves in many ways as the skin,

also found two steps and interpreted them as located at the two surfaces of the epithelial cell layer of the bladder. To establish the site of origin of the electrical potential steps, and thereby settle these controversial reports, use of a better technique for the localization of the micropipette tip during potential difference recording appears necessary. Consequently, the following types of experiment have been performed: (I) the skin potential profile was recorded with micropipettes. (II) The position of their tips was permanently marked, during the recording of each potential level, by deposi-

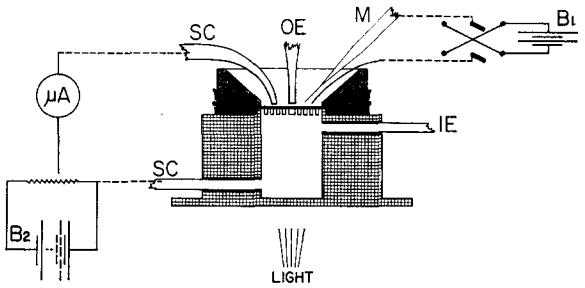


FIGURE 1. Experimental set-up. The skin was tied onto a lucite cylinder and placed over a perforated disk with its outer side facing upwards, thus separating two chambers containing sulfate solutions that bathed the two sides of the skin. The circular area exposed between chambers was 0.785 cm^2. Two openings (not shown) in the lower chamber allowed circulation of the inside solution to and from a reservoir by means of a stream of oxygen. *OE* is the outer side electrode-agar bridge, *IE* is the inner side electrode-agar bridge, and *M* is the carmine-filled micropipette-electrode, used for the potential difference measurements. The independent circuit shown to the right was used to apply a voltage from battery B_1 to *M*, in order to deposit the carmine into the tissue. To the left, *SC* are carbon electrodes which were connected *via* agar bridges to the outer and inner side bathing solutions to supply current to the skin, from battery B_2, through microammeter μA, by means of the voltage divider.

tion in the tissue of carmine from the micropipette. The carmine spot was localized by histological examination afterwards (18). (III) The DC resistances between the micropipette tip and the solutions bathing the skin were measured during impalements to ascertain whether the observed potentials were maintained across cell membranes, since these membranes are known to possess resistances higher than the cytoplasm.

We have found in agreement with Engbaek and Hoshiko (5), that as the micropipette is advanced from the outer side, the skin potential difference is formed by two main positive steps of 40 to 70 mv each. The present work shows that these steps are located at the outer and inner side of the stratum germinativum cells, across high resistances. The DC resistance at the outer side of the cells is higher than at their inner side. These cells are able to maintain a potential difference of about 34 mv, negative as referred to the outside,

during short-circuit. A preliminary report of part of these data has already been presented (29).

I. ELECTRICAL POTENTIAL PROFILE

Experimental Method

The experiments were carried out at 21 ± 1°C in a Faraday screening cage. The abdominal skin was removed from pithed *Bufo marinus*, cleaned of muscle and adherences, stretched out, tied tightly to the end of a cylinder to diminish deformation during impalement (Fig. 1), and mounted on a chamber with the outer side facing upwards. Both sides of the skin were bathed in a solution (16) containing in mM: Na_2SO_4, 56; K_2SO_4, 2.4; $NaHCO_3$, 2.4; calcium gluconate, 1; and glucose, 5; pH 8.0 to 8.5, giving a total osmolarity of 156 mOsM/liter, as determined by measurement of the freezing point depression. No significant difference was observed between steady-state measurements with skins bathed in this solution and those with skins in a similar solution made isotonic with toad extracellular fluid by addition of the requisite amounts of sucrose.

SKIN HISTOLOGY. (See Fig. 3.) The skins used in our experiments were 80 to 200 μ thick, measured with a micrometer after being tied to the lucite cylinder. The epithelium, 40 to 60 μ in thickness, is formed by two or three layers of cells; above the basement membrane is the stratum germinativum, formed by cylindrical cells 30 to 40 μ thick; then comes an intermediate layer with some cells resembling those of the stratum germinativum, while others are partially keratinized; finally nearest to the outer surface is the stratum corneum, a layer of fully keratinized flat cells. Below the basement membrane is the corium, 40 to 150 μ thick, built up of connective tissue. No continuous layer like the tela subcutanea, a thin cellular layer separating the corium from the subcutaneous lymph spaces, could be identified in our histological sections, possibly because in the toads we have used, strong adherences to the abdominal muscular wall had to be sectioned to excise the skin. However, we have recorded skin potential differences as high as −150 mv and short-circuit currents as high as 120 $\mu a/cm^2$.

ELECTRODES Symmetrical calomel half-cells, connected *via* agar-solution bridges to the solutions bathing the outer and inner sides of the skin respectively, were used to measure the skin potential difference (Fig. 1). For measurements within the skin, one glass micropipette (15) held in a micromanipulator was connected *via* a 3 M KCl bridge to a third calomel half-cell. The micropipettes were drawn on a puller (2). Their tips were filled with water by capillarity (3); a 3 M KCl solution saturated with carmine lithium (18, 26, 28) was injected in the lumen of the micropipette and the air bubbles were dislodged with a fine glass probe.

By means of a switch, we could record between the following points: (*a*) between outer side and inner side solution, to measure the skin potential difference; (*b*) between micropipette and inner side solution, to record the skin potential profile; the inner side electrode was taken as reference (controls during impalements indicated that the skin potential was always the algebraic sum of the potential difference recorded between the outer side solution and the micropipette, plus that recorded be-

tween the micropipette and the inner side solution); and (*c*) between micropipette and outer side solution to measure the micropipette tip potential and resistance in the outer side solution before and after each impalement. The criteria for acceptance of a micropipette were (1): tip resistance between 3 to 30 megohm, tip potential between 0 to −5 mv. The observations were rejected if after impalement a significant change was observed in the micropipette characteristics. The experimental results were not corrected for the tip potential.

Two voltmeters (model 200-B, Keithley Instruments Inc., Cleveland, Ohio, 10^{14} ohm input impedance) were used, one to record the total skin potential as in (*a*) and a second one to record the skin potential profile or the micropipette characteristics as in (*b*) or (*c*). When continuous graphs of the potential profile were necessary, leads from the voltmeter output were taken to a galvanometer with a spot follower (photodyne, Sefram, Paris). A known voltage applied between the micropipette tip and the reference electrode was used as calibration.

RECORDING THE ELECTRICAL POTENTIAL PROFILE In contrast to other authors (19, 20) who used skins with low potential differences, we have employed a sulfate solution (12) with adequate potassium concentration (10) at an alkaline pH (21) in order to work with skins showing their highest potential difference and thus obtain a clearer record of their electrical potential profile. Skins were used only if their potential difference and short-circuit current values were greater than −80 mv and 25 μa/cm^2 respectively. The impalements were started 1 hour after the skin potential difference had stabilized. The experiments lasted at the most 3 hours. Penetrations with the micropipette oblique to the skin were preferred in order to follow the advance of the carmine-colored tip under the dissecting microscope.

The micropipette was advanced 1 to 2 μ at a time, at intervals of several seconds, to permit stabilization of the micropipette tip in the tissue. We considered that a new potential level was reached when the measured potential difference remained stable within a range of 5 mv over a period longer than 30 seconds, instead of changing or showing unsteadiness. In several impalements, measurements were continued for several minutes to demonstrate the stability of the potential levels with proper penetration.

It is known that the low potentials sometimes recorded may be due to large holes with imperfect sealing around the micropipette. We have indeed observed low potentials when impalements were defective. They were unstable and the resistance between micropipette and outer side solution was markedly lower than usual (see also reference 8). Such measurements were rejected.

No difference was observed in the potentials measured with micropipettes filled with 3 M KCl or with 3 M KCl–carmine lithium (26, 28).

RESULTS

Most of our efforts were concentrated in recording the potential profile obtained as the micropipette was advanced from the outer side, through the epithelial layer, until it had passed the epithelium, since practically no potential difference was recorded across the corium. Three profiles are illustrated in Fig. 2. The mean values of fifty-three electrical potential profiles

performed successfully in thirteen skins, agree with those of Engbaek and Hoshiko (5). First, the micropipette in the outer side solution recorded the skin potential difference T, with a mean value of -107 ± 15 mv (SD), the inner side solution taken as reference. Upon impalement, when the micropipette had barely penetrated the skin, the potential difference increased a mean of 11 mv, but sometimes as much as 40 mv, and an unstable potential

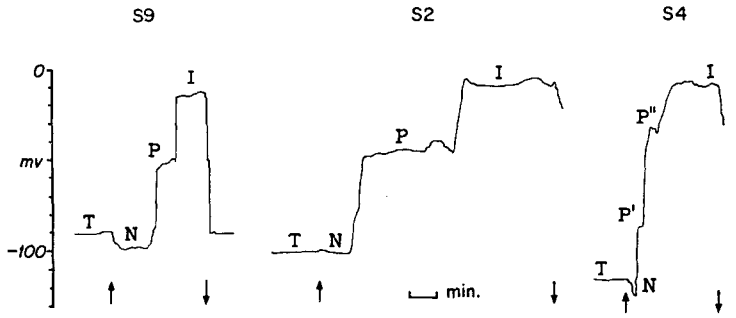


FIGURE 2. Toad skin electrical potential profile. Skins impaled from their outer side. The inner side solution was taken as reference. The micropipette in the outer side solution records the skin potential difference T; upon impalement, potential level N, and potential level P are recorded, before reaching I, the potential difference across the corium. The arrows shown at the bottom indicate micropipette penetration (point upwards) and withdrawal (point downwards).

Skin 9 (S9) had a total potential of -90 mv; upon penetration, levels N, P, and I are recorded. Upon withdrawal the potential difference returns to -90 mv; the small notch at -50 mv corresponds to level P recorded before.

Skin 2 (S2) had a total potential of -100 mv. This impalement illustrates a very slow penetration. After level P was stable for about 3 minutes penetration was continued and I was recorded.

Skin 4 (S4) had a total potential of -118 mv. Upon impalement, after level N, two levels which have been labeled P' (-80 mv) and P'' (-30 mv) were recorded, before reaching I (-10 mv).

level N, with a mean value of -118 ± 13 mv (SD), was recorded in forty-five out of fifty-three impalements. With further penetration a sudden positive change in potential was recorded in forty-nine out of the fifty-three impalements, from N to a highly stable potential level P, which could be recorded for several minutes (Fig. 2) with a mean value of -58 ± 7 mv (SD). Finally, on further penetration a sudden positive change to level I with a mean value of -7 ± 5 mv (SD) was observed in all the fifty-three impalements. In half the impalements this level was practically zero. In the others it had values between -10 and -20 mv; with further penetration these values changed continuously towards zero. Level I had values not different from zero in impalements performed in a region in which some of the corium was removed

over a restricted area; the removal did not cause the skin potential to change significantly.

Two other levels were observed less frequently: one with a mean value of −104 mv, similar to the skin potential, recorded in nineteen out of the fifty-three impalements, between levels *N* and *P*; another with a mean value of −30 mv, which was recorded in sixteen out of the fifty-three impalements, before reaching *I* (see *P″*, in S4, Fig. 2).

TABLE I

PERMANENT MARKING OF ELECTRICAL POTENTIAL LEVELS

Total skin potential	Level marked	Electrical potential	Recording site
*mv**		*mv**	
−150	*N*	−180	Stratum corneum
−115	*N*	−135	Stratum corneum
−80	*N*	−107, −105‡	Stratum corneum
−110	*N*	−133	Stratum corneum
−115	*P*	−56	Stratum germinativum
−110	*P*	−65, −62‡	Stratum germinativum§
−120	*P*	−55	Stratum germinativum
−110	*I*	−10, −15‡	Subepithelial
−100	*I*	−25, −2‡	Subepithelial

* Referred to the inner side solution.

‡ Two marks were left in this skin.

§ One of the spots was in the cell layer immediately above the one in contact with the basement membrane.

II. SITE OF RECORDING OF THE POTENTIAL LEVELS

The micromanipulator advance during impalement is only an indirect index of the micropipette tip position within the skin, since skin distortion is variable during penetration (5, 20). Consequently we resorted to Mitarai's (18) histological localization technique, which has definite advantages over other methods (20), since carmine spots, 2 to 6 μ or less in diameter, may be deposited without further color development or counterstaining. The immediate fixation of the tissue with Bouin's solution precludes spreading of the spots, since carmine is insoluble at acid pH. Villegas' circuit in parallel (26) avoids polarization of the calomel electrodes, and permits measurements of each potential level before and after marking, thus reducing the ambiguity of the potential localized (28).

Experimental Method

After skin impalement and recording of a potential level, as described in section I, a minute amount of carmine was transferred to the tissue by iontophoresis, by means of the independent circuit (26) shown to the right of Fig. 1. Immediately after, the

potential difference was recorded again without moving the micropipette, and then the micropipette was withdrawn and its characteristics checked in the outer side bathing solution. Experiments were discarded when the potential difference measured after the iontophoresis was not within 5 mv of the previous measurements or when the micropipette characteristics had changed significantly. The skin was then fixed in Bouin's solution. A segment containing the carmine spot was embedded in paraffin, and about three hundred sections, 7 to 10 μ thick, were prepared per skin and examined, unstained, with phase contrast microscopy at magnification $\times$ 480 or 756 to locate the carmine spot. A single potential level was marked in each skin. The procedure was repeated in several skins.

RESULTS

The sites of recording and the values of the potential levels marked successfully may be seen in Table I. (*a*) Five spots, deposited in four skins, corresponding to level *N*, were found and located in the stratum corneum (see Fig. 3 *A*). Four spots could not be found. (*b*) Four spots, deposited in three skins, corresponding to level *P*, were found and located within the stratum germinativum cells (Fig. 3 *B*). Two other spots could not be found. (*c*) The four spots corresponding to level *I* were found and located in the subepithelial region between the basement membrane and the melanophores (Fig. 3 *C*).

Therefore, our experiments localize, beyond reasonable doubt, two main electrical potential steps: one of 40 to 70 mv maintained across the outer side of the stratum germinativum cells (the interior of the cell being positive with respect to its outer side), and a second step of 40 to 70 mv maintained across the inner side of the stratum germinativum cells (the inner side solution being positive with respect to the cell interior). These two steps add up to give the skin potential which ranged in our experiments between 80 and 150 mv.

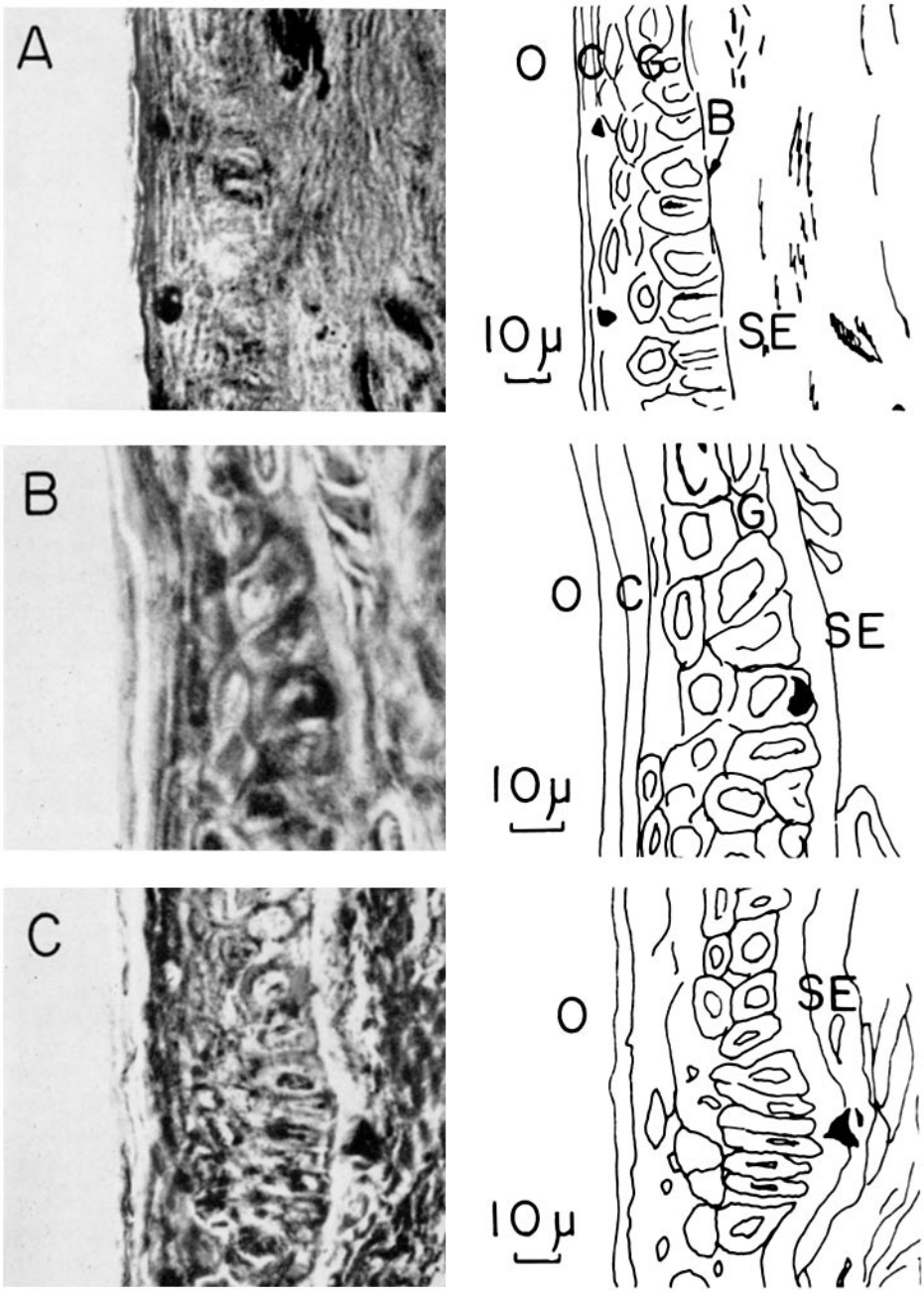
We tried, without success, to label the levels found less frequently, with averages of -104 mv and -30 mv. The potentials measured after labeling were markedly different from those measured before, and the experiments had to be discarded.

III. MEASUREMENTS OF DC RESISTANCES

Experimental Method

After impaling the skin and recording a potential level as described in section I, current from a battery was supplied to the outer and inner side of the skin, through carbon electrodes (*SC*, Fig. 1). The total skin potential and the potential difference between micropipette and inner side solution were recorded (simultaneously with the two voltmeters), and by moving the voltage divider, variations in the potential differences as a function of the current steps supplied to the skins were obtained. Plots of the potential differences *vs.* current gave straight lines within the errors of

measurement. The total skin DC resistance and that between micropipette and inner side solution were computed from the slopes ($\Delta v/\Delta I$, Fig. 4), on the assumption that the current density through any skin region was the same as through the whole skin.



However, the real surface area of the stratum germinativum cells and the resistance of the lateral walls of the cells and of the intercellular spaces remain uncertain.

These measurements were completed within 5 seconds. No significant difference was observed when the procedure lasted 10 or 15 seconds, nor if one point was redetermined after curves similar to those of Fig. 4 were completed. Influence of the skin capacitance should be minimal, since the skin time constant may be calculated to be less than 10 milliseconds from Teorell's measurements of the skin resistance and capacitance (23). The variation of the short-circuit current with the position of the outer side electrode bridge used to record the skin potential was less than 5 per cent, so that the field over the skin was reasonably uniform in spite of the eccentric position of the outer side electrode bridge. In view of these considerations, our measurements of DC resistances are to be taken as a first approximation. For comparative purposes the resistances were transformed into specific transverse DC resistances (kilohm. cm^2) by multiplying by the skin area exposed in our preparation, 0.785 cm^2. The specific transverse resistance of our circuit measured with the same experimental set-up, but without skin, was of 5 ohm. cm^2.

RESULTS

Measurements were performed in twelve skins (Table II). The mean value for the skin potential was -102 mv. The short-circuit current density (current supplied to 1 cm^2 of skin to drop its potential difference to zero) was 69 ± 15 $\mu a/cm^2$ (mean $\pm$ SD). Some plots of potential difference *vs.* current when the micropipette was recording the potential levels *N*, *P*, and *I* may be seen in Fig. 4.

The mean value of the total skin DC resistance was 1.53 ± 0.42 kilohm. cm^2 (SD). The mean value of the DC resistances between the recordings sites of the various electrical potential levels may be evaluated from Table II. The micropipette crossed practically no resistance when it was made to penetrate from the outer side solution into the stratum corneum to record the

FIGURE 3. Micrographs of unstained sections of toad skin showing carmine spots. Schematic drawings are shown to the right of each micrograph. From left to right, appear in each photograph, the skin outer side *O*, stratum corneum *C*, stratum germinativum *G*, basement membrane *B*, and the subepithelial region *SE*, with the melanophore layer. In black and white phase contrast micrographs, structures other than the carmine spot may appear as dark marks. However, the carmine spots cannot be confused when the histological section itself is observed.

Light micrograph A shows two carmine spots in *C*; -107 and -105 mv respectively were recorded from the micropipette. Skin potential -80 mv.

Phase contrast micrograph B shows the carmine spot inside a cell of *G*; -55 and -53 mv were recorded from the micropipette before and after marking. Skin potential -120 mv.

Phase contrast micrograph C shows the carmine spot in *SE*, past *B*. -10 and -6 mv were recorded from the micropipette before and after marking. Skin potential -110 mv.

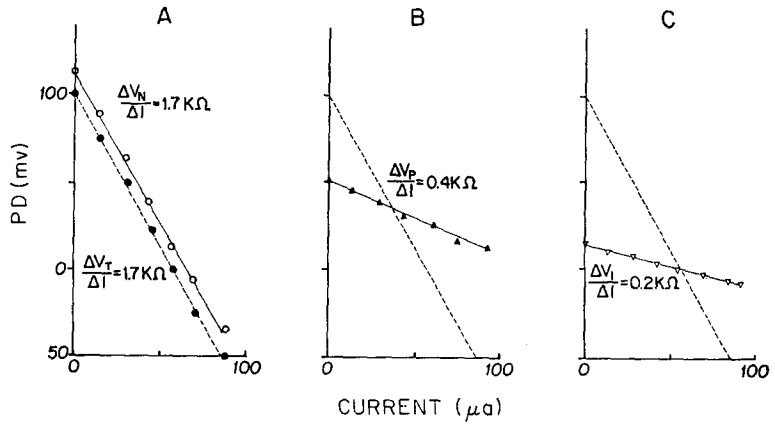


FIGURE 4. Toad skin DC resistances. The dashed lines are the plots of the skin potential T (filled circles) as a function of the current supplied to 0.785 cm^2 of skin area. The continuous lines are plots of the electrical potential levels N (open circles), P (filled triangles), and I (open triangles) as a function of the current supplied to the same skin. DC resistances are obtained from the slopes of these lines, $\Delta v/\Delta I$.

FIGURE 4A. T was -100 mv. Upon penetration from the outer side the micropipette records level N from the stratum corneum with a value of -112 mv. The two slopes are practically equal. Consequently the micropipette has crossed no significant resistance.

FIGURE 4B. The micropipette records level P from within a stratum germinativum cell with a value of -50 mv. The slope of the values of P is 0.4 kilohm, and the slope of the values of T is 1.7 kilohm. Consequently, the micropipette has crossed a resistance of $\sim$1.3 kilohm to go through the outer side of the stratum germinativum cell. Note that level P is ~ -30 mv when the skin potential is 0 (short-circuit).

FIGURE 4C. After passing the basement membrane, the micropipette records level I with a value of ~ -10 mv. The slope of the values of I is $\sim$0.2 kilohm. Consequently the micropipette has crossed a resistance of $\sim$0.2 kilohm to go through the inner side of the stratum germinativum cell.

TABLE II

DC RESISTANCE MEASUREMENTS IN TOAD SKIN*

Electrical potential level	Recording site	Electrical potential	Specific resistance	
			Inner side‡	Outer side§
		mv	kΩ cm^2	kΩ cm^2
T	Outer side solution	−102±7	1.53±0.42	—
N	Stratum corneum	−115±10	1.53±0.42	0
P	Stratum germinativum	−55±3	0.44±0.17	1.09
I	Subepithelial	−12±7	0.14±0.10	1.39

* Mean ±SD (twelve skins).

‡ Between micropipette tip and inner side solution.

§ Between micropipette tip and outer side solution.

potential level *N*. From this region, the micropipette penetrated the outer side of the stratum germinativum cell to record the potential level *P*, crossing a mean DC resistance of 1.09 kilohm. cm². Then the micropipette penetrated the inner side of the stratum germinativum cells, to record the potential level *I* from the subepithelial region, crossing a mean DC resistance of 0.30 kilohm. cm². Finally, the mean value of the DC resistance left between the corium and the inner side solution was 0.14 kilohm. cm². Measurements performed after scraping off the epithelium were approximately in the same range.

IV. SHORT-CIRCUIT EXPERIMENTS

The magnitudes of the potential levels *N*, *P*, and *I* were measured while the skin was short-circuited (25). Experiments were similar to those used to evaluate the skin resistances. Once the micropipette recorded a potential level, the skin was short-circuited for less than 5 seconds and the new value for the potential level was recorded. The circuit was opened again and the potential level was again recorded. Experiments were rejected when the post-short–circuit measurement was not within 5 mv of the pre-short–circuit measurement. The mean short-circuit values obtained in ten measurements, were -12 ± 5 mv (SD) for level *N*, -34 ± 8 mv (SD) for level *P*, and -1 ± 2 mv (SD) for level *I*. One would expect some drift in the potential recorded from within the stratum germinativum cells as a function of time after the onset of short-circuiting since the alteration in the electrical conditions should result in changes in the ionic concentrations within the cells. To study this possibility, observations were performed during prolonged short-circuiting of the skin, while the micropipette was left *in situ* recording level *P*. At the beginning a rather rapid drift towards a lower value was observed, reaching a mean of -27 mv in about 3 minutes. A practically steady value was reached after about 5 minutes. It was difficult to prolong this type of observation since the micropipette would hardly stay within a cell. Consequently measurements of the stratum germinativum cell potential were performed after 30 to 60 minutes of short-circuiting by means of a new impalement. A mean of -24 ± 8 mv (SD, ten values) was obtained.

DISCUSSION

Role of the Skin Structures in the Origin of the Electrical Potentials

STRATUM CORNEUM Our marking experiments show that level *N* is recorded from the stratum corneum. We found that the relative negativity of level *N* was maintained even when the skin potential was reduced to values near zero, either by short-circuiting the skin or by addition to the bathing solutions of 2,4-dinitrophenol or of 0.1 N HCl. Furthermore, the skin potentials and profiles recorded by impaling an area where the stratum corneum

had been removed were not different from those recorded when this *stratum* was present, except for the absence of level *N*. Consequently level *N* appears to be superimposed upon the skin potential, and the stratum corneum does not seem necessary for the existence of either the skin potential or of level *P*. It has been suggested that level *N* represents the membrane potential of the stratum corneum cells (5). This seems rather unlikely since their outer side resistance is practically zero. Another possibility is that this potential is due to some ion exchange character of the stratum corneum or to mechanical distortion of the micropipettes, since their tips bent and quite often broke when pushed through the tough stratum corneum. Penetration through the rest of the epithelium was easier without any distortion of the micropipette.

THE INTERCELLULAR SPACES AND THE BASEMENT MEMBRANE Micropipette recordings, using chloride-Ringer as bathing solution, indicated that the epithelial cell potential became zero by short-circuiting the skin (19). On these grounds it was suggested that the bulk of salt and water movement occurs between the epithelial cells and not through them, and that the basement membrane is the site of active sodium transport (14). This conclusion cannot be maintained since we have recorded level *P* while the skin was bathed in chloride-Ringer, obtaining values between −15 to −25 mv during short-circuit; besides, the stratum germinativum cell membranes and not the basement membrane determine passage by size of non-electrolytes (27) and of electrolytes (16).

STRATUM GERMINATIVUM The present experiments clearly show that the micropipette records a positive step of 40 to 70 mv across the stratum germinativum cell outer side, with a resistance of 1.09 kilohm. cm², before reaching the cell interior, and across the inner cell border, another positive step of 40 to 70 mv with a resistance of 0.30 kilohm. cm². Systematic impalements performed a few micra apart, to obtain profiles of presumably neighboring cells, indicate that the cells are arranged in parallel.

Only one carmine spot was found within one of the cells of the layer immediately above the stratum germinativum (see Table I), with a potential difference between −65 and −62 mv. On the other hand we did not succeed in marking the third level (mean −30 mv) that was found in about a third of the impalements. Its mean value during short-circuit was −20 ± 4 mv (SD, six measurements). The resistances between micropipette and outer side and micropipette and inner side solutions were comparable with those found for the stratum germinativum cells. Therefore it seems likely that this level was also recorded from within a cell. At the present time it is not possible to decide whether this level arises from a different cell type. The absolute values of the cellular potentials in a given skin are variable enough so that the micropipette might enter through one stratum germinativum cell and pass

across the lateral border into another one before finally passing the basement membrane, thus giving rise to a series of steps.

Our observations of the existence of two main resistances in the skin support a number of experiments suggesting more than one diffusion barrier in the skin epithelium (22, 17, 11). They indicate that ion movements are more restricted at the outer side, since current flow in electrolyte solutions is due to ions (7, 13). They support the observation that the rate-limiting step for sodium transport across the skin occurs before sodium enters the "pool" (4). The outer side membrane of the stratum germinativum cells has also been found to be more restrictive than the inner one to the passage of water (16, 27) and of non-electrolytes (27).

In Koefoed-Johnsen and Ussing's (12) model for ion transport across the skin, the stratum germinativum cells working in parallel are proposed as the transporting cells. These cells are markedly asymmetrical. The skin potential is the sum of (*a*) a sodium diffusion potential (cell positive with respect to the outer side solution) arising across the cell's outer border, and (*b*) a potassium diffusion potential (inner side solution positive with respect to the cell) arising across the cell's inner border. The net transcellular sodium movement occurs in two steps: passive entry from the outer side solution into the cells across its outer side, and extrusion from the cells, into the inner side solution, as an active transport performed by a pump, situated towards the cell's inner side.

The present experiments show that the main electrical potential steps of the skin are located as envisaged in that model (12). Our measurements of level *P* while the skin was short-circuited (mean, −24 mv) indicate that the stratum germinativum cells generate their own electrical potential and maintain it at a significant level across the high resistance of their membranes even though current is being drawn from the skin. During short-circuit active sodium transport continues from the outer towards the inner side, the two bathing solutions having equal electrochemical potentials (25). Therefore, sodium moves against an electrochemical potential gradient only at the inner cell border, since the stratum germinativum cells have at most half the concentration of the bathing solutions, during short-circuit (9, 4). However, this argument would hold for even higher cellular sodium concentrations. In open circuit conditions the electrical potential step is of about 40 to 70 mv at the outer side of the stratum germinativum cell, in the presence of an impermeable anion. If sodium were the only diffusing cation, a cellular sodium concentration of about one-fifth to one-twelfth of the sodium concentration in the bathing solution would be required for a passive entry of sodium into the cell.

In view of the complex nature of the skin potential, demonstrated in the

present work, some of the conclusions that appear in the literature based exclusively on skin potential changes should be cautiously interpreted.

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REFERENCES

1. ADRIAN, R. H., *J. Physiol.*, 1956, **133**, 631.
2. ALEXANDER, J. T., and NASTUK, W. L., *Rev. Scient. Instru.*, 1953, **24**, 528.
3. CALDWELL, P. C., and DOWNING, A. C., *J. Physiol.*, 1955, **128**, 31P.
4. CURRAN, P. F., HERRERA, F. C., and FLANIGAN, W. J., *J. Gen. Physiol.*, 1963, **46**, 1011.
5. ENGBAEK, L., and HOSHIKO, T., *Acta Physiol. Scand.*, 1957, **39**, 348.
6. FRAZIER, H. S., *J. Gen. Physiol.*, 1962, **45**, 515.
7. HARNED, H. S., and OWEN, B. B., The Physical Chemistry of Electrolytic Solutions, New York, Reinhold Publishing Corporation, 1958.
8. HOSHIKO, T., *in* Biophysics of Physiological and Pharmacological Actions, (A. Shanes, editor), Washington, American Association for the Advancement of Science, 1961, 31.
9. HOSHIKO, T., and USSING, H. H., *Acta Physiol. Scand.*, 1960, **49**, 74.
10. HUF, E. G., WILLS, J. P., and ARRIGHI, M. F., *J. Gen. Physiol.*, 1955, **38**, 867.
11. KARGER, W., and KRAUSE, D., *Arch. ges. Physiol.*, 1962, **274**, 340.
12. KOEFOED-JOHNSEN, V., and USSING, H. H., *Acta Physiol. Scand.*, 1958, **42**, 298.
13. KORTÜM, G., and BOCKRIS, J. O'M., Textbook of Electrochemistry, New York, Elsevier Publishing Co., 1951.
14. LINDERHOLM, H., *Acta Physiol. Scand.*, 1954, **31**, 36.
15. LING, G., and GERARD, R. W., *J. Cell. and Comp. Physiol.*, 1949, **34**, 383.
16. MACROBBIE, E. A. C., and USSING, H. H., *Acta Physiol. Scand.*, 1961, **53**, 348.
17. MEYER, K. H., and BERNFELD, P., *J. Gen. Physiol.*, 1946, **29**, 353.
18. MITARAI, G., *J. Gen. Physiol.*, 1960, **43**, No. 6, suppl., 95.
19. OTTÖSON, D., SJÖSTRAND, F. S., STENSTRÖM, S., and SVAETICHIN, G., *Acta Physiol. Scand.*, 1953, **29**, suppl. 106, 611.
20. SCHEER, B. T., and MUMBACH, M. W., *J. Cell. and Comp. Physiol.*, 1960, **55**, 259.
21. SCHOFFENIELS, E., *Arch. Internat. Physiol. et Biochem.*, 1955, **63**, 513.
22. STEINBACH, H. B., *J. Cell. and Comp. Physiol.*, 1933, **3**, 1.
23. TEORELL, T., *Acta Physiol. Scand.*, 1946, **12**, 235.
24. USSING, H. H., *in* The Alkali Metal Ions in Biology, by H. H. Ussing, P. Kruhøffer, J. H. Thaysen, and N. A. Thorn, Berlin, Springer-Verlag, 1960, 112.
25. USSING, H. H., and ZERAHN, K., *Acta Physiol. Scand.*, 1951, **23**, 110.
26. VILLEGAS, L., *Biochim. et Biophysica Acta* ,1962, **64**, 359.
27. WHITTEMBURY, G., *J. Gen. Physiol.*, 1962, **46**, 117.
28. WHITTEMBURY, G., *Am. J. Physiol.*, 1963, **204**, 401.
29. WHITTEMBURY, G., and HERRERA, F. C., Seventh Annual Meeting of the Biophysical Society, New York, 1963, abstract TC3.