CELL COMMUNICATION IN THE BASAL CELLS OF THE HUMAN EPIDERMIS

J. SIEGENBEEK VAN HEUKELOM, D. W. SLAAF, *and* J. C. VAN DER LEUN

From the Department of Medical Physics, Physical Laboratory, State University of Utrecht, Utrecht, The Netherlands. Dr. Siegenbeek van Heukelom's present address is the Department of Animal Physiology, University of Amsterdam, Amsterdam, The Netherlands. Dr. van der Leun's present address is the Institute of Dermatology, State University of Utrecht, Utrecht, The Netherlands.

ABSTRACT Electrotonic spread can be measured in the basal cells of the human epidermis. The communication between neighboring cells is high, whereas no leak to the intercellular spaces could be detected. The specific resistance of the membranes between the cells is about 10 Ωcm^2. This finding suggests that for those particles that are able to pass the cell membrane the intracellular path through the epidermis is at least as suitable as the path through the intercellular spaces.

INTRODUCTION

Present day knowledge of cell communication and other coupling phenomena in various cell systems (Furshpan and Potter, 1968; Loewenstein and Kanno, 1964; Loewenstein et al., 1965, 1969; Loewenstein and Penn, 1967; Penn, 1966; Siegenbeek van Heukelom et al., 1970) makes it fairly likely that cell coupling should exist in the basal cells of the human epidermis. A technique, first introduced by Slowey and Leider for making suction blisters (Slowey and Leider, 1961), enables us to handle the epidermis in such a way that impalement of the basal cells with glass microelectrodes under microscopic control is feasible, and cell coupling in the basal layer could be investigated. Cell communication in the human epidermis is detected by means of these two techniques: make a suction blister, dissect the blister roof, mount it for measurement, and measure electrotonic spread with microelectrodes under microscopic control. The results obtained are in good agreement with the findings of other investigators with different cell types. (Loewenstein, 1966; Penn, 1966; Siegenbeek van Heukelom et al., 1970).

With the help of an idealized model of the cell configuration (see Fig. 4) a more detailed description and interpretation can be given, which results in a value for the "apparent" specific resistance of the membranes between the cells. Alternative

models referring to more complicated situations were considered, too. As for the general conclusions the model turned out to be not critical enough for setting all characteristic parameters but sufficient for determining the resistance between two adjacent cells.

Finally an attempt was made to relate our results to measurements concerning diffusion of molecules in the skin (Halprin and Ohkawara, 1967; van der Leun, 1966; Malkinson and Kirschenbaum, 1963; Scheuplein, 1967). Although quite a few assumptions are made, the conclusion seems justified that, disregarding active processes, the over-all diffusivity of the viable layer of the human epidermis for small molecules and ions is about 100–1000 times less than the corresponding diffusivity in water.

MATERIALS AND METHODS

The method for making a suction blister has been fully described by Kiistala (1968). By applying underpressure to a patch of skin (in our case on the flexor side of the forearm) the basal cell layer is detached from the dermis. The gap developing between the epidermis and the dermis is filled with blister fluid. The time of formation and growth of the blisters depends upon several factors; the three most important are: the amount of underpressure (here, 175 mm Hg), the temperature of the skin (here, 39 $\pm$0.5°C), and the properties of the skin (usually the donor was the same [*S*], but no remarkable systematical differences could be obtained by a few runs with other subjects).

After about 3 hr the blister obtains its full diameter (about 2 cm). The lapse of time between the blistering and the electrical measurements seems to have some influence on the results (see Discussion). So, after a short time (about 1 hr) the blister is dissected with a scalpel and placed over a small Perspex ring, the basal layer upwards. A second, larger ring is placed over it sticking slightly, the outer diameter of the first ring is 50 μ smaller than the inner diameter of the second. The edges of both rings are rounded and smoothed. With these two rings the epidermis is fixed and stretched. Because of forces acting during blistering and by the stretching of the epidermis between the rings, the cells are stretched, too (see Fig. 1). This reshaping will be included in the results.

The preparation so obtained is placed in a shallow holder filled with Hanks' solution (Oxoid Ltd., London) under the phase-contrast microscope. Visibility was often poor because of the thickness of the preparation (see Fig. 2). The temperature in the holder was not verified but was approximately 25°C. The bathing fluid in the holder was electrically grounded with an Ag-AgCl electrode.

With two microelectrodes the electrotonic spread was measured: one electrode injected a symmetrical square wave current (10^{-9} A $\leqq I_{stim} \leqq$ 10^{-8} A) into one of the basal cells. The voltage changes in the basal cells due to the injected current were recorded by the second electrode. Only these voltage changes were used for the calculations. The microelectrodes were filled with 3 M KCl and had a top potential always smaller than 10 mv (average 5 mv). This tip potential is not found to influence the voltage changes measured. The electrical equipment was conventional for this type of electrophysiological work with a flat frequency response up to 3 kHz. The square wave current and the voltage changes were recorded by means of an ultraviolet (UV) oscillograph with a flat frequency response up to 2 kHz and a recording inaccuracy smaller than 5%.

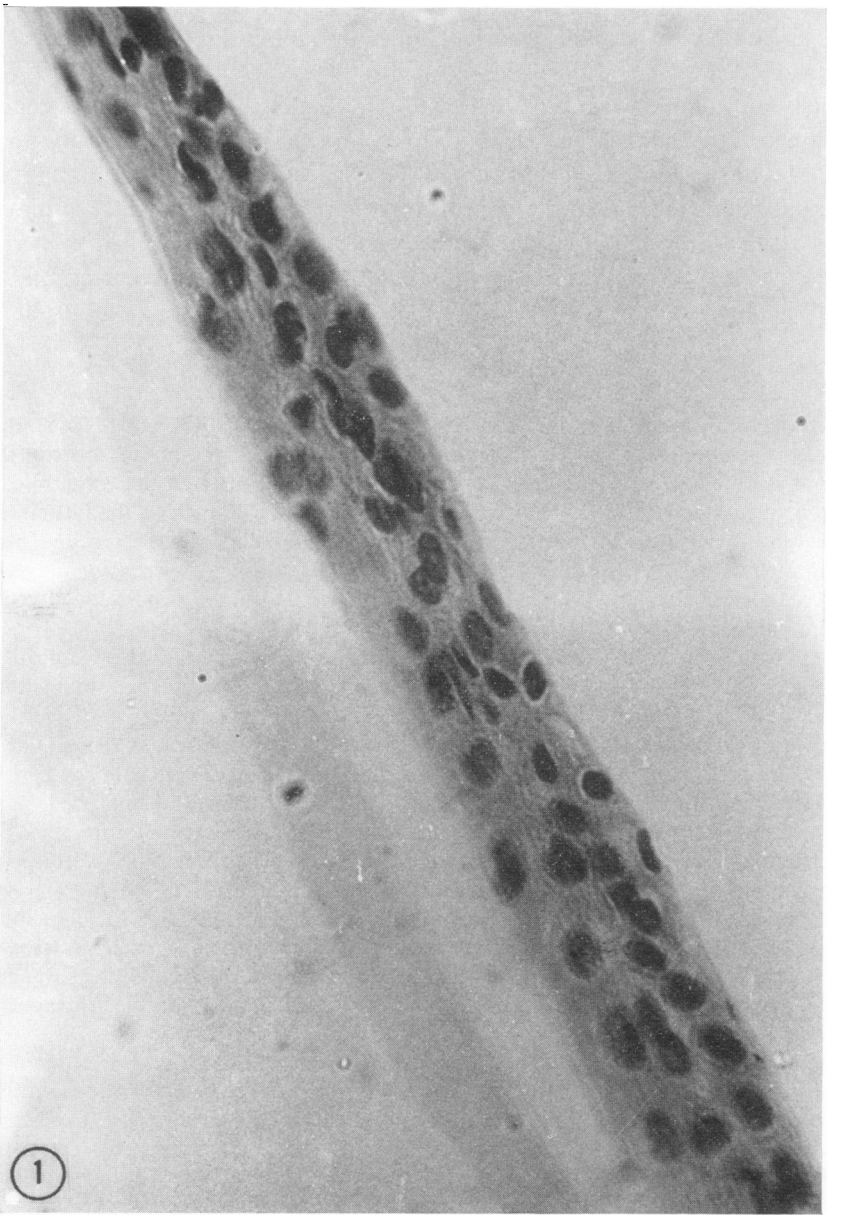

FIGURE 1 Photomicrograph of section through the stretched epidermis as used in the electrophysiological experiment; the diameter of a cell is about 10 μ. $\times$ 1000.

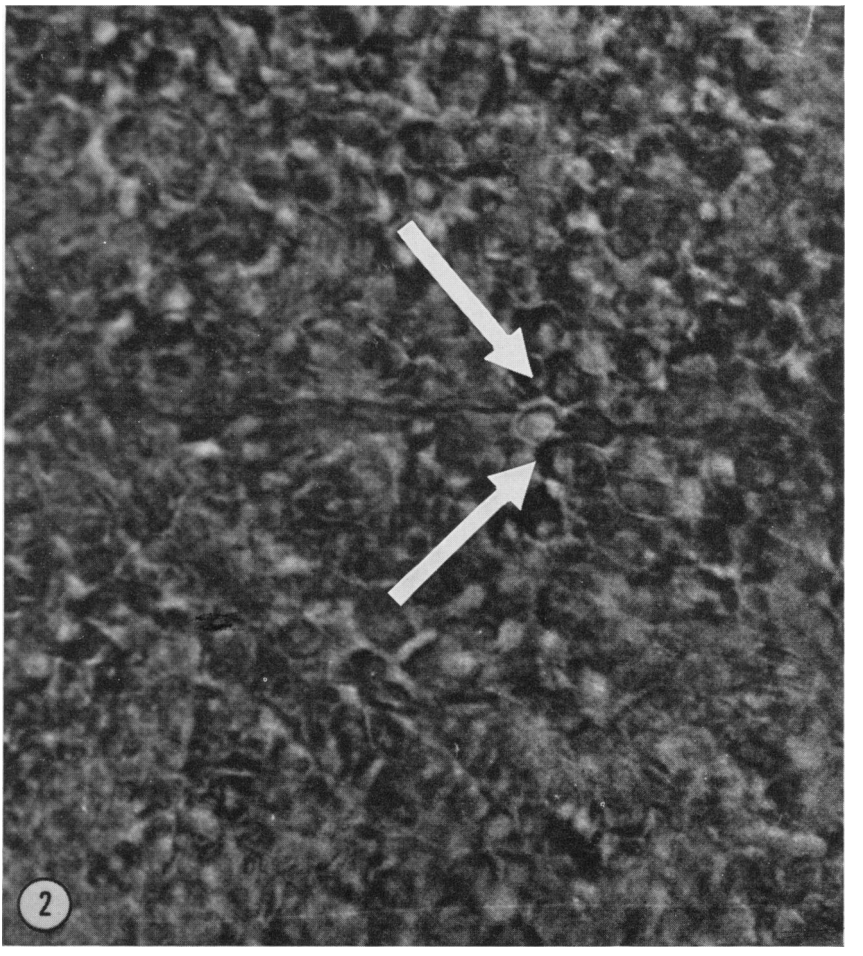

FIGURE 2 Photomicrograph of epidermis together with two electrodes (arrows) in zero-order cell ($m = 0$). × 750.

RESULTS

A. *Measuring Results*

The time after the blistering seemed to influence the intracellular potential V_M. The greatest value of V_M we measured was 30 mv. The average value measured was 10 mv, but we could not find that cell coupling was seriously influenced by the actual value of V_M, and so the value of V_M does not provide serious repercussions in the given results as these are determined from voltage differences. The same argument holds for the tip potential, and with the low currents used possible polarizing effects at the tip will be small, too.

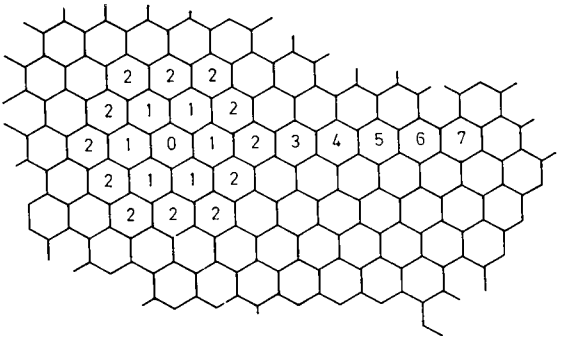

FIGURE 3

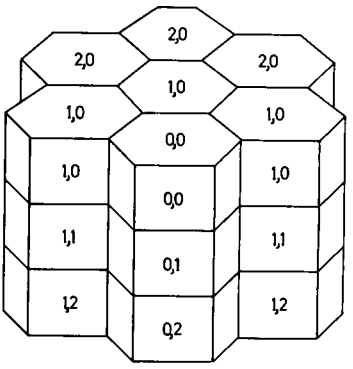

FIGURE 4

FIGURE 3 Honeycomb idealization of the cells in one layer with order numbers.
FIGURE 4 Multilayered honeycomb structure as an idealization of the stretched epidermis with cells numbered (m, q) with order number m ranging from 0 to 2 and layer number q ranging from 0 to 2.

The only criterion used for good and bad impalement was the stability of V_M. Often nonstable values of V_M were found, decreasing in time roughly exponentially to zero. Measurements in cells with nonstable V_M were discarded.

A second indication for a reasonable impalement is the increase in the electrode resistance on impalement (average 15 MΩ). Impaling an electrode deeper than the first cell layer (the other electrode remaining inside the original cell that it was stitched in) sometimes a point could be found (the intercellular space) where no electrotonic spread could be detected; this strongly supports the conclusion that there exists no external membrane over the whole system, isolating the system as a whole from the bathing fluid and playing the part of a nonjunctional membrane. A little deeper a second cell could often be impaled (see Discussion and Appendix), but the difficulty with the determination of the exact position of the cell made these measurements less useful.

No clear nonlinear behavior was observed in the voltage-current relation. The recorded voltage changes were normalized to a stimulating current $I_{stim} = 10^{-8}$ A.

To each cell in the basal layer an order number was ascribed: the cell with the stimulating electrode was numbered zero ($m = 0$).The cells around this one were numbered one ($m = 1$), the next ring number two ($m = 2$), etc. ($m = 3, 4, \ldots$ see Fig. 3). The average measuring results $V_{m,q}$ are produced in columns II, III, and IV in Table I. The second index q of $V_{m,q}$ refers to the cell layer, zero being the basal layer. $V_{m+1,q}/V_{m,q}$ for $m = 0$, $q = 0$ can now be calculated, analogous to the communication ratios mentioned by Loewenstein (1966). (Their V_{II}/V_I is equivalent to our V_1/V_0.) The average ratio found here is 0.35.

B. *Calculation of* ρ_i

To infer more from these findings a more elaborated description of our cell system was developed, as illustrated in Fig. 4. The cells were numbered (m, q) where m is

the order number in one layer and q the number of the layer. Indeed, a high degree of idealization was introduced by this description, but the Discussion will deal with the influences of this form of idealization and possible objections that can be made on grounds of ultrastructural findings.

In the model it is assumed that the surface area of the membrane between two adjacent cells in the same layer is dh where d is the hexagon side and h the height of the idealized cells (see Fig. 4); the hexagon surface area is $3\sqrt{3}\,d^2/2$. The ratio between these two is called

$$\alpha = 3\sqrt{3}\,d/2h. \tag{1}$$

The value of α is derived from the configuration in the basal layer; variation of this value through the epidermis is not considered in the model (see Discussion B).

The membranes between two cells are assumed to be fully junctional (This assumption will be reconsidered in the Appendix.) The specific resistances are defined as: ρ_i (ohms $\times$ square centimeters), the specific resistance of the junctional membranes; ρ_m (ohms $\times$ square centimeters), the specific resistance of the nonjunctional membranes; ρ_c (ohms $\times$ centimeters), the specific fluid resistance of the cytoplasm.

The quantity ρ_m is attributed only to the membranes in contact with the bathing fluid. Its determination is difficult and not of much importance, as will be explained in the Discussion. Also the quantity ρ_c is of less importance, as will be shown in section D.

The value of ρ_i is assumed to be the same for any membrane between two cells. This assumption can easily be abandoned when information can be found about the change of ρ_i through the epidermis. This information, however, cannot be obtained, neither from the literature nor from our experiments, but mathematically the complication, introduced then, can be met (see further Discussion B).

It should be kept in mind that throughout this chapter ρ_i and ρ_m are given as specific resistances in the framework of this model. Lack of conclusive microscopic data concerning foldings and other particularities of the membranes makes impossible the translation from the phenomenological values ρ_i and ρ_m given here to data more pertinent to the membranes as such.

For each ring with a given value of m and q an equation can be given with the help of Kirchhoff's first law. Cells with the same indices m and q are supposed to have the same voltage. These equations form together a set of coupled algebraic equations which can be solved by computer when boundary conditions are introduced: (*a*) a known current I_{stim} is injected into the cell numbered (0, 0); (*b*) the cells form junctions, and the voltage outside the cellular space $V_{ex} = 0$; (*c*) for a certain value p of q the current to $p + 1$ is assumed to be zero (horny layer); (*d*) for a certain value N of m the potential V is assumed to be zero: $V_{N,P} = 0$.

For convenience we introduce the quantity:

$$\beta = 4 + \frac{3}{2}\sqrt{3}\,\frac{d\rho_i}{h\rho_m} = 4 + \alpha\,\frac{\rho_i}{\rho_m}. \tag{2}$$

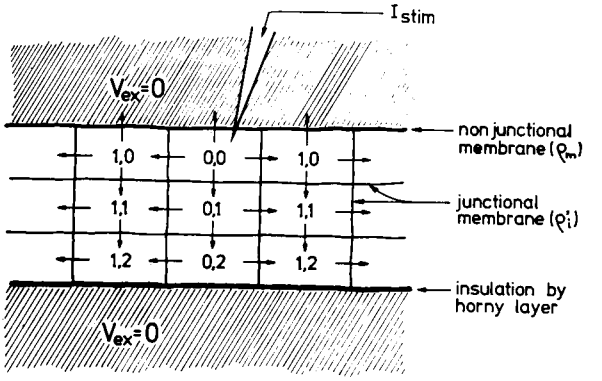

FIGURE 5 Illustration for the derivation of formulas 3–8: the potential in a ring $V_{m,q}$ is constant, and arrows indicate currents considered with Kirchhoff: $\sum i = 0$. I_{stim} is injected through a microelectrode in cell (0, 0).

So one gets (see Fig. 5):

$q = 0$ and $m \geqq 1$,

$$V_{m+1,0} = \frac{m}{2m+1}(\alpha + \beta)V_{m,0} - \frac{2m-1}{2m+1}V_{m-1,0} - \frac{m}{2m+1}\alpha V_{m,1}; \quad (3)$$

$0 < q < q$ and $m \geqq 1$,

$$V_{m+1,q} = \frac{m}{2m+1}(4 + 2\alpha)V_{m,q} - \frac{2m-1}{2m+1}V_{m-1,q} - \frac{m}{2m+1}\alpha V_{m,q-1} - \frac{m}{2m+1}\alpha V_{m,q+1}; \quad (4)$$

$q = p$ and $m \geqq 1$,

$$V_{m+1,p} = \frac{m}{2m+1}(4 + \alpha)V_{m,p} - \frac{2m-1}{2m+1}V_{m-1,p} - \frac{m}{2m+1}\alpha V_{m,p-1}; \quad (5)$$

$0 < q < p$ and $m = 0$,

$$V_{1,q} = \frac{1}{6}(6 + 2\alpha)\,V_{0,q} - \frac{\alpha}{6}V_{0,q-1} - \frac{\alpha}{6}V_{0,q+1}; \quad (6)$$

$q = p$ and $m = 0$,

$$V_{1,p} = \frac{1}{6}(6 + \alpha)\,V_{0,p} - \frac{\alpha}{6}V_{0,p-1}; \quad (7)$$

$q = 0$ and $m = 0$,

$$\rho_i = \frac{dh[6(V_{0,0} - V_{1,0}) + \alpha(V_{0,0} - V_{0,1})]}{I_{stim} - V_{0,0}\frac{3}{2}\sqrt{3}\frac{d^2}{f_m}} . \quad (8)$$

It turns out (see Discussion A) that the second term in the denominator of equation 6 is small compared with I_{stim} ; so in approximation:

$$\rho_i = \frac{dh}{I_{stim}} [6(V_{0,0} - V_{1,0}) + \alpha(V_{0,0} - V_{0,1})] . \quad (9)$$

From the microphotographs of some preparations the values for d and h were determined (see Fig. 1):

$$d = 4.0 \pm 0.5\ \mu,$$

$$h = 5.0 \pm 0.5\ \mu.$$

From these one obtains $\alpha = 2.1 \pm 0.4$ (see equation 1); $V_{0,0}$ and $V_{1,0}$ can be obtained by measurement (see Table I). This does not apply to $V_{0,1}$. If one computes $V_{0,1}$ for

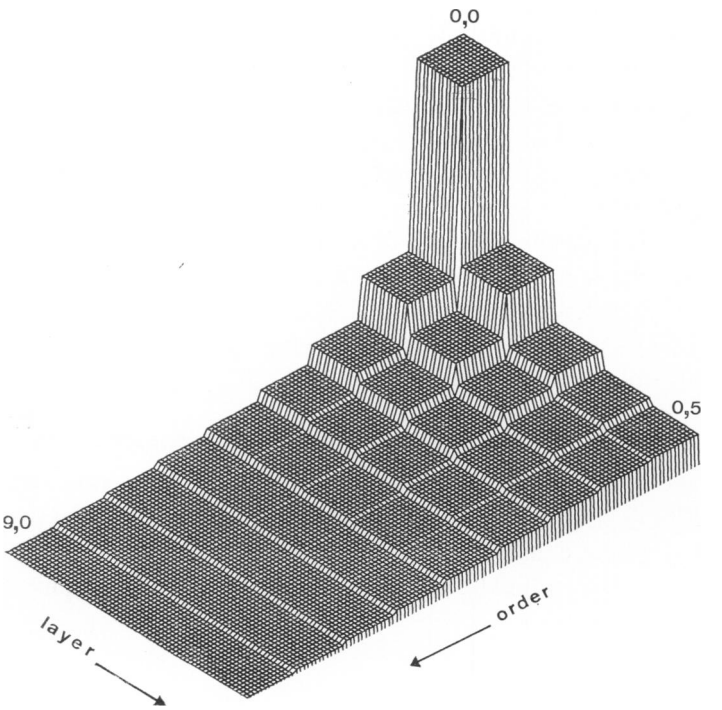

FIGURE 6 Results of the computer model of the epidermis: $V_{m,q}$ as a function of m and q with m (the order number) ranging from 0 to 9 ($N = 9$) and q (the layer number) ranging from 0 to 5 ($p = 5$); $\beta - 4 = 0.05$ and $\alpha = 2.0$.

TABLE I

SURVEY OF THE RESULTS

I Subject	II* $V_{0,0}$	III* $V_{1,0}$	IV* $V_{2,0}$	V‡ $V_{2,0}$ (computed)	VI§ ρ_i	VII¶ ρ_i (corrected)
	mv	*mv*	*mv*	*mv*	Ωcm^2	Ωcm^2
S	64	27	18	17	6	4
B	175	85	69	58	14	10
S	102	32	—	16	11	8
S	108	30	14	13	12	9
S	69	27	19	16	7	5
B	105	27	14	10	12	9
S	240	73	35	36	26	20

* Averaged values in one preparation when the injected current I_{stim} is normalized to 10^{-8} A.
‡ $V_{2,0}$ as computed with equation 10 from $V_{0,0}$ and $V_{1,0}$.
§ The apparent specific resistance ρ_i calculated with equation 8.
¶ Here ρ_i is corrected for d and h values in the normal epidermis.

different values α, ρ_i, and ρ_m, however, it turns out that, as long as ρ_m is at least several times greater than ρ_i, the relation $V_{1,0}/V_{0,1} \approx 0.8$ approximately holds (see Fig. 6 and Fig. 11) (For justification of the assumption $\rho_m \gg \rho_i$ see Discussion A.) Now ρ_i can be evaluated; the results are given in Table I (column VI); the values range from 6 to 26 Ωcm^2 with an average of $\rho_i = 13$ Ωcm^2.

The inaccuracies in the values of $V_{0,0}$ and $V_{1,0}$ can be estimated from different experiments in comparable situations in the same preparation. The inaccuracy in $V_{1,0}$ was estimated to be about 5 mv; the inaccuracy in $V_{0,0}$ was estimated to be about 20 mv, reflecting the difficulties encountered. There are two reasons for this. The first is that the cell (0, 0) should tolerate the impalement of two electrodes; the second is that the voltage gradient in cell (0, 0) can not be neglected.

The inaccuracies mentioned here together with the inaccuracies given a few pages back enable us to calculate the inaccuracy in ρ_i. It turns out to be about 4 Ωcm^2.

C. *Comparison of the Measured Value of $V_{2,0}$ with the Computed $V_{2,0}$*

A check on the model can be made by comparing the $V_{2,0}$ experimentally found and the computed $V_{2,0}$. This comparison can also be made by inserting the values of α, $V_{1,0}$, and $V_{0,0}$ in the equation for $V_{1,0}$ (see equation 3 with $m = 1$) and taking $V_{1,1} = 1.38 \times V_{2,0}$ (see Fig. 11), which approximately holds according to our model computations. Then:

$$V_{2,0} = \frac{1}{3 + 1.38\alpha} [(\alpha + \beta)V_{1,0} - V_{0,0}] . \qquad (10)$$

With the data of Table I the influence of the inaccuracy of α in $V_{2,0}$ can be calculated to be smaller than 15%.

The results computed differ about 10% from the experimental ones, suggesting that the model is quite satisfactory, but the comparison of both $V_{2,0}$ can not be used as a proof that $\rho_i/\rho_m \ll 1$.

D. *Computation of the Apparent Specific Fluid Resistance in the Epidermis: ρ_v (ohms × centimeters)*

Once accepted the multilayered honeycomb structure will serve in translating the results of the discrete model in terms of a continuous model. One can distinguish in the model three main axes (see Fig. 7): (*a*) axis 1, perpendicular to a honeycomb side in a layer; (*b*) axis 2, parallel to a honeycomb side in a layer; (*c*) axis 3, perpendicular to the layers.

In each unit the contributions to the total resistance of the unit by the resistances of the junctional membranes and the cytoplasm can be calculated (see Fig. 7). The apparent resistance of the total contents of one unit in the direction of an axis can be expressed in terms of ρ_i, ρ_m, d, and h and is (in ohms × centimeters):

$$\text{axis 1, } \rho_{v_I} = \rho_c + (\rho_i/d\sqrt{3}); \tag{11}$$

$$\text{axis 2, } \rho_{v_{II}} = \rho_c + (\rho_i/d\sqrt{3}); \tag{12}$$

$$\text{axis 3, } \rho_{v_{III}} = \rho_c + (\rho_i/h). \tag{13}$$

It appears that the specific fluid resistance along axis 1 and axis 2 are the same. Axis 1 can be realized in six different directions in the plane; the same is true for

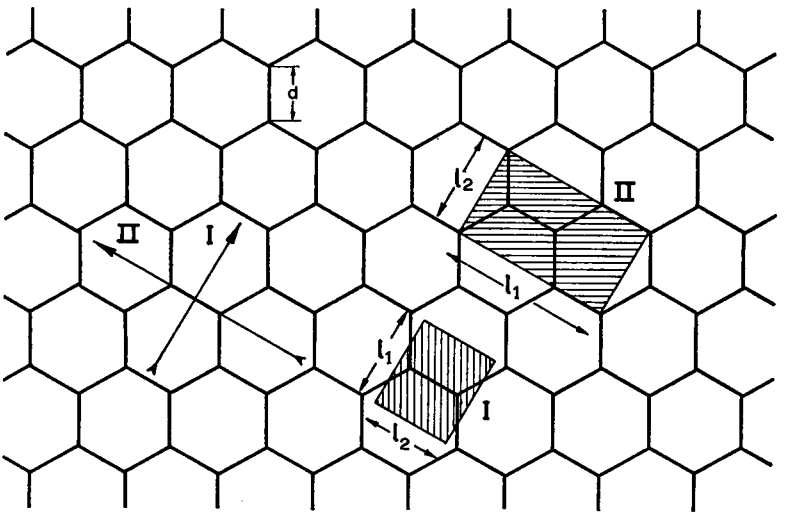

FIGURE 7 Part of the honeycomb structure with two different repeating units; d is the hexagon side; l_1 the long axes and l_2 the short axes are defined as illustrated in the figure.

axis 2. So in 12 different directions $\rho_{v_I} = \rho_{v_{II}}$ is found in one layer, and the assumption $\rho_{v_m} = \rho_{v_I} = \rho_{v_{II}}$ being the fluid resistance in one layer is not too farfetched. The contribution of ρ_c and ρ_v is small and may be neglected, as will be shown. So:

$$\rho_{v_I} = \rho_{v_{II}} \approx (\rho_i/d\sqrt{3}) \equiv \rho_{v_m}, \qquad (11\,a)$$

and

$$\rho_{v_{III}} \approx (\rho_i/h) \equiv \rho_{v_q}. \qquad (13\,a)$$

In our blister roofs average results are: $\rho_{v_m} = 20 \cdot 10^3$ Ωcm, $\rho_{v_q} = 26 \cdot 10^3$ Ωcm.

A more interesting value is found by using values from the unstretched skin for d and h. Comparing the values found for d and h in our preparation with d and h values for the normal, unstretched epidermis we found for the stretched epidermis $d/h = 0.8$ and for the normal epidermis $d/h = 0.5$. As the volume $(3/2\sqrt{3}\,d^2h)$ is the same, one will find with our blister unstretched: $d = 3.5\ \mu$ and $h = 7.0\ \mu$. Also ρ_i is changed, for by stretching, no new membrane area is formed; so the contribution of ρ_i in ρ_{v_q} (13 and 13 *a*) changes with d^2, and the contribution of ρ_i in ρ_{v_m} (11 and 11 *a*) changes with d^{-1}.

With the changes introduced thus in d and h as well as in ρ_i one obtains:

$$\rho_{v_m} = 28 \cdot 10^3\ \Omega\text{cm},$$

$$\rho_{v_q} = 13 \cdot 10^3\ \Omega\text{cm}.$$

The true value of ρ_c can not be measured by our method in this preparation. But it is most likely that the value of ρ_c falls within the range of values normally found (smaller than 500 Ωcm [Schanne, 1969]). It then contributes less than 5% in ρ_v, irrespective of the true value of ρ_c. This enables us to calculate ρ_v with only a slightly reduced accuracy.

DISCUSSION

A. *The Physiological State of the Dissected Epidermis*

One may question the physiological condition of the epidermis when it is dissected from the blister, but some arguments support the idea that the epidermis does still exhibit a condition resembling the normal one. After blistering the epidermis reattaches itself spontaneously to the dermis if the suction blisters are small. If after dissecting one tries to flatten out the removed epidermis in a physiological saline solution it tends to curl inwards in such a pronounced way that it is often difficult to stretch it. This phenomenon decreases with time and after about 2 hr it has disappeared. Our measurements were performed in a shorter period. Finally, after removal of the blister roof from the skin, the epidermis cells are still viable and may be grown in culture (Ingemansson-Nordquist et al., 1967).

During blistering the basal layer is detached from the dermis; a general argument for assuming that during our measurements the membrane surface formed by this blistering has a specific resistance ρ_m greater than ρ_i is mainly based on findings of Loewenstein et al. (1965; Loewenstein, 1967; Jamakosmanović and Loewenstein, 1968; see especially the recent work of Oliveira-Castro and Loewenstein, 1971). Their results show clearly that when the skin is wounded, junctional membranes will appear to be converted in the edge of the wound from a low resistance state to a high resistance state that resembles the nonjunctional membrane. The concentration of Ca^{++} also influences the resistance of the cell membrane; due to the fact that in our bathing medium the concentration of the Ca^{++} ions is 1.3 mmole/liter this concentration is sufficient to seal off the cells. This, combined with the first argument and the detection of cell communication, formed the basis for assuming that $\rho_m \gg \rho_i$. A value $\rho_m > 60\ \Omega cm^2$ suffices to make the second term in the denominator in equation 8 6%, as small as the measuring inaccuracy in I_{stim}.

A problem closely related is the question of how the junctions are brought about. In their articles Loewenstein et al. (1964, 1969; Loewenstein and Penn, 1967; and Loewenstein, 1966) discriminate three types of resistances ρ_m, ρ_i, and a third, the perijunctional resistance ρ_e relative to an insulation around the possible channels between the coupled cells. Their calculations (Loewenstein et al., 1965) show that the separate determination of ρ_e is extremely difficult if not impossible. Nevertheless, there are convincing arguments for the existence of ρ_e (Loewenstein and Kanno, 1964).

On inspection of electromicrographs only desmosomes are revealed (Breathnach 1971). The conclusion that these desmosomes are the places of good communication, is premature, but, if this would be so, from Breathnach's pictures it can be concluded that roughly 10% of the membranes between two cells are truly junctional (this conclusion is based on stereological considerations, see Weibel, et al., 1966). The possible organization of desmosomes is not known either.

Because of these difficulties and uncertainties no attempt was made in our analysis to introduce a more detailed description of the junctional membranes in the computational model. Possible extensions of the model relative to these problems are discussed in the Appendix.

The capacity of the detached membranes of the basal cells in contact with the bathing fluid was not determined: rise times were too small to record them accurately. Besides, the value would not have any physiological significance in relation to the normal intact skin.

B. *The Introduced Idealizations*

Another point of discussion is the idealizations introduced on several levels in the derivation of ρ_i. This was mainly done to make the model not too complicated for handling it. The value of ρ_i as given in this article is taken as final result rather than

the resistance per junctional membrane (ρ_i/dh), because in this way electron microscopic findings could be compared more easily with our findings. It is well known that the basal layer is undulated, while the horny layer upon the epidermis is rather flat. The number of cells between the basal layer and the horny layer fluctuates. By stretching the epidermis this fluctuation is reduced (see Fig. 1), and the assumption of two parallel planes formed by the horny layer and the basal layer seems plausible. Besides, the results obtained with computer models showed that the number of cells between these two planes has only a slight influence upon the electrotonic spread as measured in the basal layer (see Fig. 9).

Another related idealization is the multilayered honeycomb structure. This idealization is mainly taken on the one hand for its usefulness for calculation and on the other for the general resemblance with the factual situation. The final results will certainly depend on the different types of idealization, for other idealizations, see for instance, Thomson (1917). Quite a range of models, however, can be simulated in our computer model by changing the values α and ρ_i/ρ_m.

Generally the membranes between cells are folded, as is revealed by the electron microscope (Wood, 1967; Breathnach, 1971); the interpretation of which can be disputed (see Discussion D). These foldings would introduce a form factor which can be included in α, changing the found values of ρ_i and ρ_m without disrupting the computational model. In general the influences of the boundary conditions and the setting of α and ρ_i/ρ_m are not independent. An impression of the influence of the several parameters is given in Figs. 8–12.

The approximation $(V_{1,0}/V_{0,1}) \simeq 0.8$ introduces an inaccuracy smaller than 10%, contributing an inaccuracy of about 3% in the calculation of ρ_i with $\alpha = 2.1$.

The value for d and h will change in the epidermis, resulting in a variation in the value of α through the epidermis. Such a variation, when its value becomes known, can easily be included in the model.

C. *Relation of the Obtained Resistance ρ_v to Values for Diffusion in the Human Epidermis Found Macroscopically*

The ratio between ρ_v and ρ_e would give information about the effective diffusion of small ions through the cellular space formed by the coupled cells (see A. L. Hodgkin in appendix to Weidmann, 1966). Without knowledge of ρ_e one can only try to find values limiting the possible ratio ρ_v/ρ_e. Normally the value of ρ_e is found to be in between 100 and 500 Ωcm (Schanne, 1969); this would give values of ρ_v/ρ_e between 130 and 30. This ratio must resemble the ratio between the diffusion coefficient in water containing the same amount of ions as the cytoplasm of the cells and the diffusion coefficient in the epidermal cells together (this ratio is called here Δ).

It is of interest to compare this result with diffusion coefficients in the human epidermis determined macroscopically. Malkinson (1958) measured the penetration of ^{14}C-labeled steroids into the living layers of the epidermis. From his data on the

penetration of testosterone (mol wt 362), van der Leun (1966) calculated an average value of $\Delta = 1400$. From data (Malkinson and Kirschenbaum, 1963) on the blanching of skin by triamcinolone acetonide (mol wt 434) a value $\Delta > 1200$ was found (van der Leun, 1966). Halprin and Ohkawara (1967) studied the entrance of glucose (mol wt 180) into slices of human epidermis from a medium. The entrance proceeded in approximately 40 min. Diffusion of glucose into a layer of water of the same thickness would take approximately 4 sec. For glucose this leads to $\Delta = 600$.

These findings cannot exactly be compared with our data, as in all cases mentioned the penetrating substances were applied extracellularly, whereas we determined the diffusion of ions injected intracellularly. The difference between our results and the other suggests that the intracellular path through the epidermis is a reasonable alternative for those particles that can pass the cell membranes from the exterior to the interior of the cell.

D. *The Influence of the Intercellular Spaces*

The intercellular spaces in the epidermis have been seen, photographed, described, and discussed thousands of times. Yet, in searching the literature for the width of these spaces, one encounters serious difficulties. Measurements reported by electron microscopists differ considerably from values found under the light microscope.

A typical value measured in light microscopic sections is 1.5 μ; this particular number was determined from two clear pictures of epidermal cells in the normal human skin (Rothman, 1959; Montagna, 1962). The ratio of the average width of the intercellular spaces and the average diameter of the cells in the two pictures was 0.14 and 0.16, respectively, giving a mean ratio of 0.15. This leads, on the basis of an average size of the cells of 10 μ, to the value of 1.5 μ mentioned. Values reported by electron microscopists range, however, from 0.015 to 0.020 μ (Wood, 1967; Breathnach, 1971).

These electron microscopic values are smaller than the light microscopic values by two orders of magnitude. In discussions, this discrepancy is usually ascribed to artifacts arising during the preparation of the sections. There is no general agreement, however, on the question which of the two techniques is more likely to produce serious errors.

A few data independent of these techniques are available. Ehring developed light microscopy of the living skin, where preparation of the tissue does not play any role. He succeeded in making visible the living epidermal cells, with nuclei and even intercellular bridges (Ehring and Schumann, 1966). He measured, on the request of one of us, the width of the intercellular spaces in his pictures, and found 2.5 to 3.0 μ. The method was limited to the proximal nail fold of fingers.

An additional value, independent of any microscopic result, may be found from a determination of the intercellular volume. Halprin and Ohkawara (1967) found, from a study on the entry of glucose-6-phosphate into epidermal specimen, that the

intercellular volume approximates 16% of the total epidermal volume. This fractional volume F may be related to the size of the cells and the width of the intercellular spaces in simplified models. In a regular arrangement of cubical cells with edge e and intercellular spaces with width i, the relation $F = (3i)/e$ holds, to a good approximation if $i \ll e$; the constant 3 does not depend much on the precise shape and arrangement of the cells. The relation gives, with $e = 10\ \mu$ and $F = 0.16$, $i = 0.5\ \mu$.

The two independent data seem to corroborate, at least in order of magnitude, the values found in conventional light microscopy. For similar problems with the smooth muscle and a more detailed discussion of the possible faults, see Burnstock (1970).

APPENDIX

With equations 3–8 the values for $V_{m,q}$ are calculated by means of an iteration process on a digital computer (CDC 1700) starting with: $V_{m,q} = 0$ with $m = 0, \ldots N$ and $q = 0, \ldots p$ except $V_{0,0} = 1$.

A parameter S_i is introduced, defined at each time:

$$S_i = \sum_{m=0}^{N} \sum_{q=0}^{p} V_{m,q}; \qquad (A\ 1)$$

During iteration the values S_i form a monotonic increasing series, and iteration is stopped as soon as some value S_j holds $S_j < S_{j-1}$ caused by the absolute inaccuracy of the computer.

Throughout this paper several possible factors are discussed that will influence the final results. Some of them are shown schematically in Fig. 8. It is not possible to produce quantities for correcting the values of ρ_i found in this work, as these quantities can not be found in the literature. It is nevertheless clear that intercellular spaces exist, and that they are likely

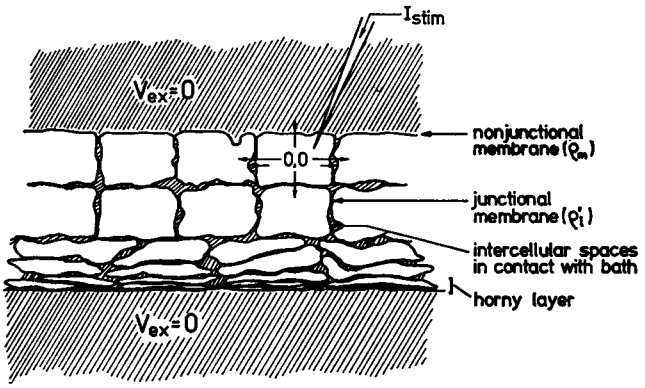

FIGURE 8 Fig. 5 redrawn with intercellular spaces, transition from basal layer to horny layer, and currents [arrows leaving cell (0, 0)]. Other possible particularities of the preparation are not shown. The membranes of the cells in contact with the intercellular spaces have a specific resistance ρ_m.

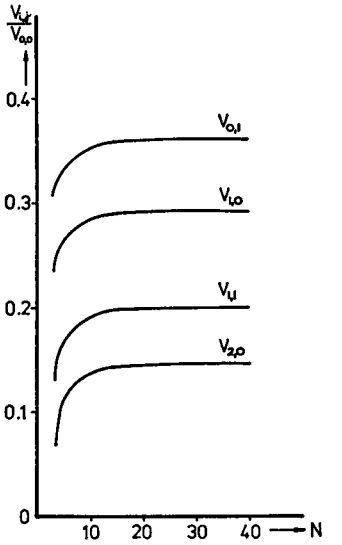

FIGURE 9 Dependence of $V_{1,0}$, $V_{0,1}$, $V_{1,1}$, and $V_{2,0}$ on N in $V_{N,p} = 0$ with $V_{0,0} = 1$, $p = 5$, $\alpha = 2.1$, $\gamma = 1$ and $\rho_i/\rho_m = 0.1$.

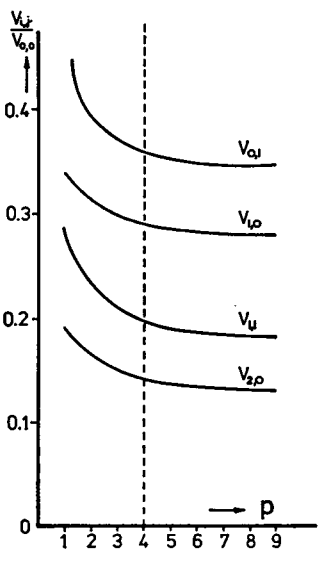

FIGURE 10

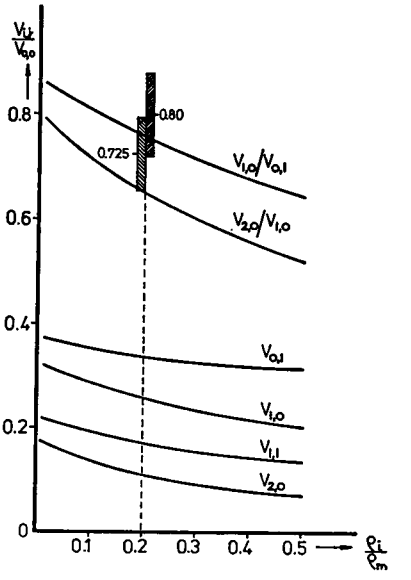

FIGURE 11

FIGURE 10 Dependence of $V_{1,0}$, $V_{0,1}$, $V_{1,1}$, and $V_{2,0}$ on p with $N = 10$, $V_{0,0} = 1$, $\alpha = 2.1$, $\gamma = 1$, and $\rho_i/\rho_m = 0.1$.

FIGURE 11 Dependence of $V_{1,0}$, $V_{0,1}$, $V_{1,1}$, and $V_{2,0}$ on the ratio ρ_i/ρ_m ($\beta - 4 = 2.6 \times \rho_i/\rho_m$) with $N = 10$, $p = 5$, $V_{0,0} = 1$, $\alpha = 2.1$ and $\gamma = 1$. Also given are the ratios $V_{1,0}/V_{0,1}$ and $V_{2,0}/V_{1,1}$; indicated are the values used for computations together with 10% deviation.

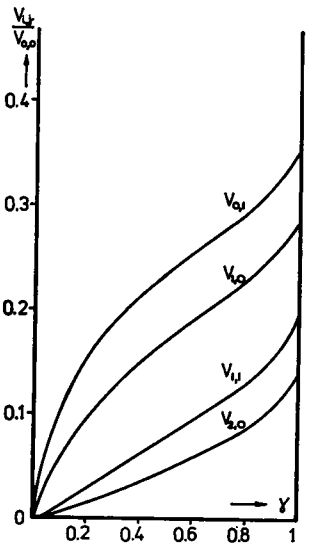

FIGURE 12

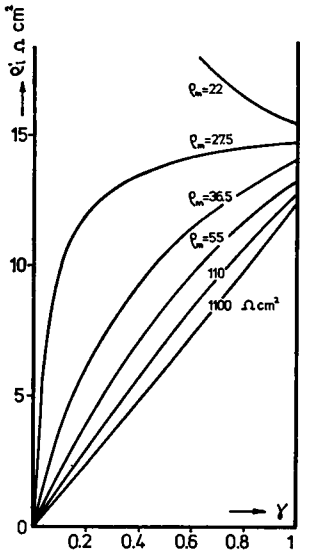

FIGURE 13

FIGURE 12 Dependence of $V_{1,0}$, $V_{0,1}$, $V_{1,1}$, and $V_{2,0}$ on the ratio between the truly junctional and total common surface area of two adjacent cells γ with $N = 10$, $p = 5$, $V_{0,0} = 1$, $\alpha = 2.1$, and $\rho_i/\rho_m = 0.1$.

FIGURE 13 Dependence of the specific resistance of the truly junctional part of the membrane between two adjacent cells ρ_i' on the ratio between the areas of the truly junctional complex and the total area of the common membrane complex between two adjacent cells γ with $N = 10$, $p = 5$, $V_{0,0} = 108$ mv, $V_{1,0} = 30$ mv, $\alpha = 2.1$, $d = 4\ \mu$, $h = 5\ \mu$. $V_{0,1}$ is obtained from $V_{1,0}$ by multiplying the last value by the ratio found from Fig. 12. Several curves with different value of ρ_m are drawn. The curve with $\rho = 22\ \Omega\text{cm}^2$ goes to infinity as the denominator of equation A 3 becomes zero.

to be the most important factor to be considered. Therefore an extension was made in the model for studying the functional dependence of ρ_i on the intercellular space.

If one assumes that at each interface between two cells a fraction γ of the juxtaposed membranes acts as truly junctional and the other fraction $(1 - \gamma)$ as nonjunctional connected to the intercellular space with potential $V = 0$, this assumption introduces an alteration in the equations. This alteration is easily introduced: in the equations 3–7 in the first term on the right-hand side an extra term in the parentheses is introduced:

$$\frac{\rho_i'}{\rho_m}\left(\frac{1-\gamma}{\gamma}\right)\{2\alpha + 6\}, \qquad (A\,2)$$

ρ_i' being the specific resistance of the truly junctional part of membranes between two cells.

Equation 8 becomes

$$\frac{\rho_i'}{\gamma} = \frac{dh[6(V_{0,0} - V_{1,0}) + \alpha(V_{0,0} - V_{0,1})]}{I_{stim} - \frac{V_{0,0}}{\rho_m}\left\{\frac{3}{2}\sqrt{3}d^2 + (1-\gamma)3\sqrt{3}d^2 + 6dh(1-\gamma)\right\}}. \qquad (A\,3)$$

The term A 2 introduced in the first term of the right-hand side of equations 3–7 is zero for $\gamma = 1$ and becomes infinite for $\gamma = 0$. We calculated the influence for several values of γ.

In Figs. 9–13 some results are demonstrated as obtained by computation. Fig. 9, the influence of the values of N in the boundary condition $V_{N,p} = 0$ on the potentials was found; Fig. 10, the influence of the thickness of the epidermis on the potentials was found; Fig. 11, the influence of ρ_i/ρ_m on the potentials was found; Fig. 12, the influence of the ratio junctional/total area γ on the potentials was found; Fig. 13, the influence of γ on the specific resistance of the truly junctional part of the membranes between two adjacent cells was found. In Figs. 9–12 only one parameter was changed at a time with the other parameters constant: $N = 10$; $p = 5$; $\alpha = 2.1$; $\gamma = 1$; $\rho_i/\rho_m = 0.1$ and $V_{0,0} = 1$. In Fig. 13 a set of graphs is given with different values of ρ_m. Besides, for computation of these curves with equation A 3 the ratio $V_{1,0}/V_{0,1}$ was taken from Fig. 11. It can be seen from Fig. 13 that with ρ_m too low with respect to ρ_i unrealistic values result from equation A 3 as the denominator becomes zero.

We wish to thank Dr. J. J. Denier van der Gon for his valuable advice on several crucial points in this article, Thijs Zoethout for producing the computer program and Fig. 6, and Ank van Wees for her unfailing assistance during the experiments.

Received for publication 1 March 1971 and in revised form 8 September 1971.

REFERENCES

BREATHNACH, A. S. 1971. *In* An Atlas of the Ultrastructure of Human Skin. J. P. Churchill Ltd., London. 17, 19.

BURNSTOCK, G. 1970. *In* Smooth Muscle. E. Bülbring, A. F. Brading, and A. W. Jones, editors. Edward Arnold (Publishers) Ltd., London. 1–69.

EHRING, F., and J. SCHUMANN. 1966. *Arch. Klin. Exp. Dermatol.* **227**:328.

FURSHPAN, E. J., and D. D. POTTER. 1968. *Curr. Top. Dev. Biol.* **3**:95.

HALPRIN, K. M., and A. OHKAWARA. 1967. *J. Invest. Dermatol.* **49**:561.

INGEMANSSON-NORDQUIST, B., U. KIISTALA, and H. RORSMAN. 1967. *Acta Derm.-Venereol.* **47**:237.

JAMAKOSMANOVIĆ, A., and W. R. LOEWENSTEIN. 1968. *J. Cell Biol.* **38**:556.

KIISTALA, U. 1968. *J. Invest. Dermatol.* **50**:129.

LOEWENSTEIN, W. R. 1966. *Ann. N. Y. Acad. Sci.* **137**:441.

LOEWENSTEIN, W. R. 1967. *J. Colloid Interface Sci.* **25**:34.

LOEWENSTEIN, W. R., and Y. KANNO. 1964. *J. Cell Biol.* **22**:565.

LOEWENSTEIN, W. R., M. NAKAS, and S. J. SOCOLAR. 1969. *J. Gen. Physiol.* **50**:1865.

LOEWENSTEIN, W. R. and R. D. PENN. 1967. *J. Cell Biol.* **33**:235.

LOEWENSTEIN, W. R., S. J. SOCOLAR, S. HIGASHINO, Y. KANNO, and N. DAVIDSON. 1965. *Science (Wash. D.C.).* **149**:215.

MALKINSON, F. D. 1958. *J. Invest. Dermatol.* **31**:19.

MALKINSON, F. D., and M. D. KIRSCHENBAUM. 1963. *Arch. Dermatol.* **88**:427.

MONTAGNA, W., editor. 1962. *In* The Structure and Function of Skin. Academic Press, Inc., New York. 2nd edition. 30.

PENN, R. D. 1966. *J. Cell Biol.* **29**:171.

OLIVEIRA-CASTRO, G. M., and W. R. LOEWENSTEIN. 1971. *J. Membrane Biol.* **5**:51.

ROTHMAN, S., editor. 1959. *In* The Human Integument. American Association for the Advancement of Science, Washington D. C. 12.

SCHANNE, O. F. 1969. *In* Glass Microelectrodes. M. Lavallée, O. F. Schanne, and N. C. Hébert, editors. John Wiley and Sons, Inc., New York. 299–321.

SCHEUPLEIN, R. J. 1967. *J. Invest. Dermatol.* **48**:79.

SIEGENBEEK VAN HEUKELOM, J., J. J. DENIER VAN DER GON, and F. J. A. PROP. 1970. *Biophys. Biochem. Acta.* **211**:98.

SLOWEY, S., and M. LEIDER. 1961. *Arch. Dermatol.* **83**:1029.

THOMSON, D'ARCY. 1917. *In* On Growth and Form. Cambridge University Press, Cambridge, England. 465–565.

VAN DER LEUN, J. C. 1966. Ultraviolet erythema, a study on diffusion processes in human skin. Ph. D. Thesis. University of Utrecht, Utrecht, The Netherlands.

WEIBEL, E. R., G. S. KISTLER, and N. F. SCHERLE. 1966. *J. Cell Biol.* **30**:23.

WEIDMANN, S. 1966. (With an appendix by A. L. Hodgkin). *J. Physiol.* (*Lond.*). **187**:323.

WOOD, R. L. 1967. *In* Ultrastructure of Normal and Abnormal Skin. A. S. Zelickson, editor. Lea and Febiger, Philadelphia. 32.