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Ion Permeability and Strength of Cell Contacts

Ion Permeability and Mechanical Properties of Cell Contacts in Small Intestine Epithelium

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Summary. The effects of several simple parameters (pH, concentration of bivalent cations, osmotic pressure, and temperature) on the ion permeability and mechanical properties of cell contacts have been investigated. It has been shown that the mechanical properties of a cell contact make it possible to describe it as a viscoelastic system. The main contribution to cell adhesion is made by the tight junction. Two populations of acidic centers have been identified on the cell membrane surface. One population interacts with bivalent cations to assure cell adhesion. The other population of weaker acidic centers regulating ion permeation is involved in the cell membrane's interaction of the repulsion type. An intimate correlation has been established between changes in passive transepithelial ion permeability and cell adhesion in response to changes in pH and in bivalent cation concentration. Such a correlation is possible if the tight junction is the principal contributor to the passive ion permeability and mechanical strength of the cell contacts.

Knowledge of the characteristics of passive transepithelial permeability of alkaline cations makes it possible to obtain information on cell contacts, since the latter provide the principal route for the passive permeation of alkaline cations through the "leaky" epithelium [6–7].

In the present study, this method of investigating cell contacts is combined with the use of adhesiometric techniques. The main experimental approach was to study the effects of cell contacts of such simple physical parameters as pH, concentration of bivalent cations, temperature, and osmotic pressure on the ion permeability and mechanical properties.

This study has confirmed and specified the participation of acidic centers in the realization of barrier and mechanical properties of intercellular contacts. In order to characterize and differentiate the populations of acidic centers which determine a given property, the kinetic features of the parameter's behavior as a function of exposure were

determined, and the selectivity series for mono- and bivalent cations were established.

Materials and Methods

Sac preparations 10 cm in length from the fourth part of the small intestine of white rats (150–200 g) were used [1].

The permeability to ions was evaluated by measuring the diffusion and bi-ionic potentials according to [2], with correction for junction potential.

Osmotic potential was measured as in [14]. Tonicity of the solutions was altered by adding sucrose, since it was shown that sucrose is impermeable for small intestinal epithelium and has a reflection coefficient $\sigma = 1$ [8]. The potentials were measured with an ED-05M electrometer through salt agar bridges (0.150 M NaCl with 4% agar), connected to silver chloride electrodes. The readings were recorded on a type MK automatic recorder (GDR). All measurements were made at room temperature, with oxygenation. The basic solution was as follows: 0.135 M NaCl, 0.010 M $NaHCO_3$, 0.0012 M NaH_2PO_4, 0.001 M $CaCl_2$, 0.0005 M $MgCl_2$, 0.0004 M $MgSO_4$, and 0.005 M KCl; pH = 7.4.

Both everted and uneverted sacs were employed.

The following buffer solutions were employed to vary pH: glycine – HCl: 2.2–3.6; KH-phtalate – HCl: 2.2–3.8; succinic acid – borax: 3.0–5.8; Na_2HPO_4 – citric acid: 2.2–8.0; $Na_2HPO_4 - NaH_2PO_4$: 5.8–8.0; $Na_2CO_3 - NaHCO_3$: 9.2–10.7; glycine – NaOH: 8.6–10.6.

Measurements at pH other than 7.4 were bracketed by two control measurements at pH 7.4 at the beginning and at the end of the experiments in order to assess the reversibility of the pH effect.

The effect of alkaline-earth cations on the diffusion potential was studied as in [13].

In most experiments, the alkaline-earth cations were removed from tissue using Na_2H_2 EDTA in the concentration of 10 mM.

The mechanical properties of cell contacts were evaluated with a simple method devised by the authors. A portion of the everted intestine 1 cm long was fixed in position on a cylindrical rod with a radius of 0.3 cm immersed in a small glass containing solution. The rod, together with the glass, was placed in an upright position and set in rotary motion, and the number of cells released to the solution under the action of the applied centrifugal force was calculated in the Gorjaev's counting chamber under microscope.

The measurements at pH other than 7.4 were continued for less than 3 min, since it has been shown in [13] and by us that irreversible destruction of the contact's constituents does not occur during that period. Several preparations from adjacent portions of the same intestine were used in most of the experiments to study the effects of Ca concentration and pH on cell contact permeability and mechanical properties.

The specimens exposed to centrifugation were fixed in formalin, dehydrated in ethanol of ascending strength, and embedded in paraffin. Sections 5 μm thick were stained with hematoxylin and eosin.

Results

1. The Number of Separated Cells as a Function of the Magnitude and the Time of Action of the Force

Fig. 1 shows the number of separated cells N as a function of the time of exposure to various centrifugal forces – at n from 0 to 12,000 rpm, which corresponds to accelerations from 0 to $500 \times g$. The number of cells

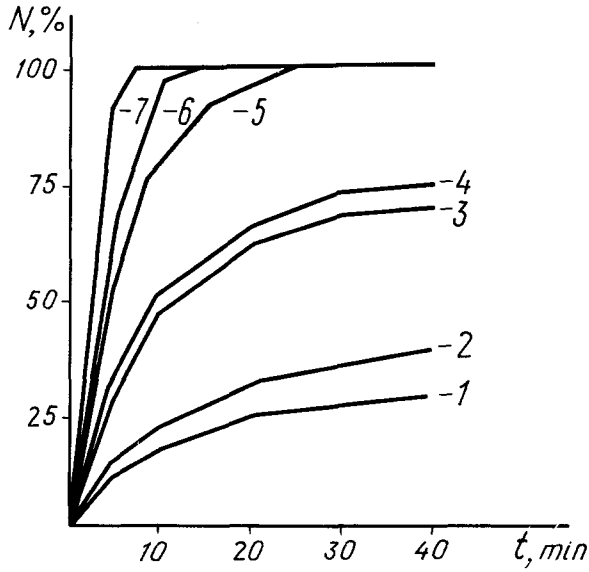

Fig. 1. The number of separated cells N as a function of centrifugation time at n = 3000 (*1*), 4500 (*2*), 5000 (*3*), 5500 (*4*), 6000 (*5*), 9000 (*6*) and 12,000 (*7*) rpm

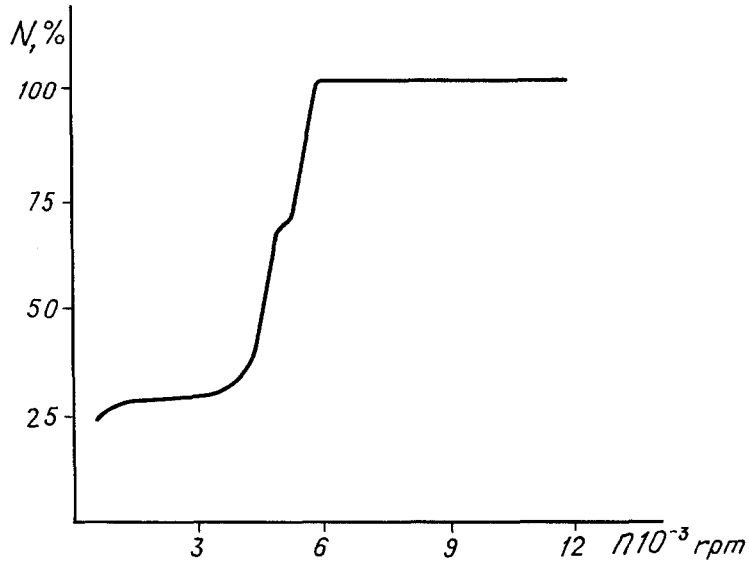

Fig. 2. The number of cells separated for 30 min as a function of n rpm

separated at the maximal force, was taken to be 100 %. Each of curves *1*, *2*, *3* and *4* in Fig. 1 tend to saturation while curves *5*, *6* and *7* have had a plateau. Morphological analysis showed that the plateau at n from 6000 to 12,000 rpm corresponds to the separation of nearly all epithelial cells

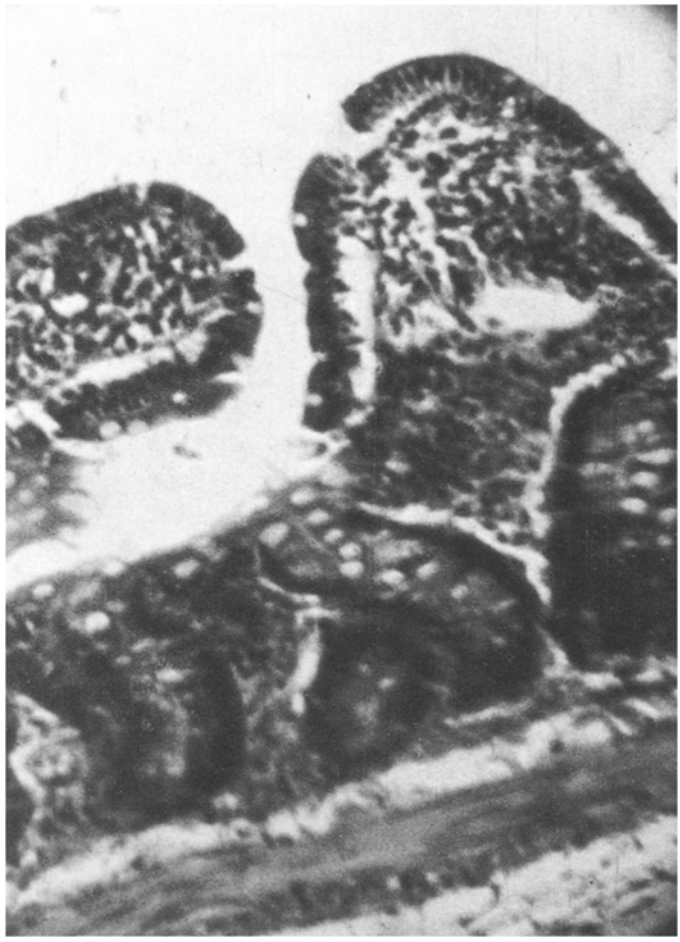

Fig. 3. Thin section of a small intestine preparation fixed after centrifugation at n = 3000 rpm for 5 min

excepting those located in crypts. In all experiments mainly single cells were observed (80–90 % of the total amount).

At smaller n, a large proportion of cells remained on villi, despite increased exposure time. Fig. 2 shows the maximum number of cells capable of separating for 30 min as a function of n. It will be seen that at n = 3000 rpm not more that 30 % of all cells are separated.

Histological study of the sections showed that cells separated from the top of villi. With increasing acceleration, a portion of the stratum detached from the basement membrane in the form of a tape engirdling the villus. The cells appear to have separated from the upper rim of that tape (Figs. 3–4).

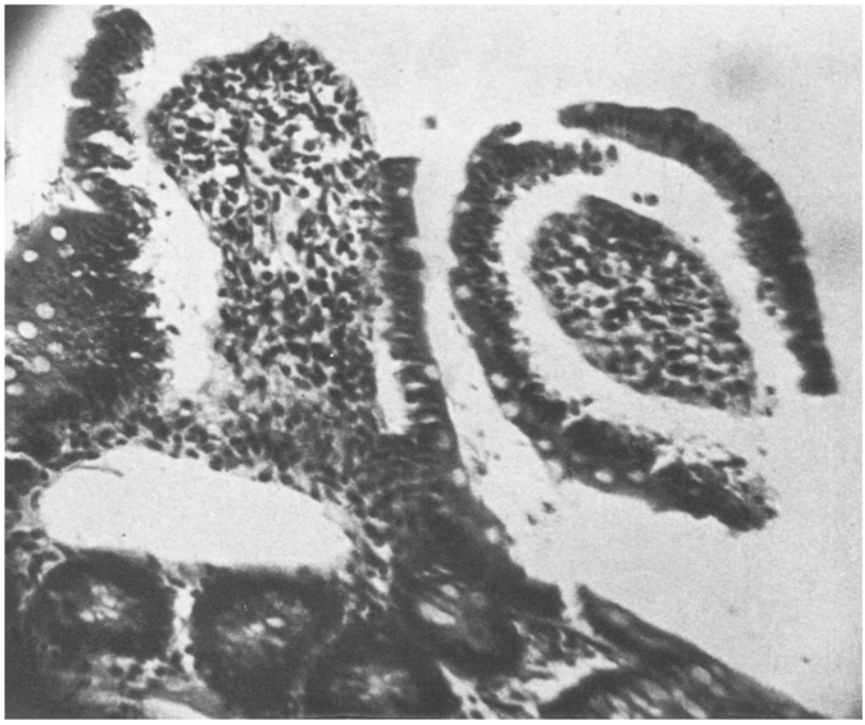

Fig. 4. Thin section of small intestine preparation fixed after centrifugation at n = 9000 rpm for 5 min

2. *Effects of pH and Alkaline-Earth Cations on the Separation of Cells by Centrifugation*

The number of cells N separated at fixed n and t, was strongly dependent upon pH. Fig. 5 shows N as a function of pH (n = 6000 rpm, $t = 3$ min). In the pH interval of 6–9, N did not change. The decrease of N at pH > 9 was due to rapid and irreversible destruction of the cells. Lowering pH below 6 also resulted in decreased N; this decrease, however, was not associated with cell destruction. The effect of pH in the acid region (up to pH = 2.0) on the cell adhesion was reversible. At pH = 3.0 N attained a minimum but again increased with further lowering of pH.

After incubation in 10 mM solution of Na_2H_2EDTA for 45 min, centrifugation at n = 3000 rpm for 5 min proved to be sufficient for the separation of all the cells lining the small intestinal surface. Subsequent addition of $CaCl_2$ restored the initial characteristics of the tissue. Other bivalent cations acted similarly to Ca^{2+}, their effectiveness having decreased in the order Mg > Ca > Sr > Ba (6 experiments), which corresponds

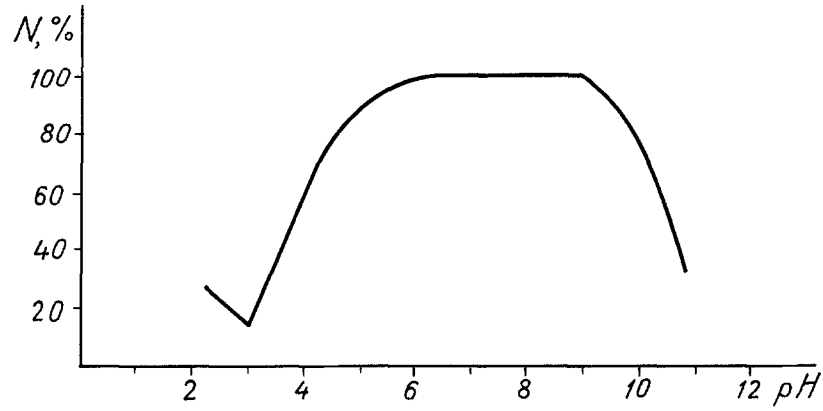

Fig. 5. The number of separated cells N as a function of pH. The number of cells separated at pH 7.4 was taken as 100%

to series *VII* of Sherry [11]. Raising the concentration of bivalent cations (up to 10 mM) decreased N somewhat (Fig. 13), and in this case the cations decreased their effectiveness in the order Ba > Sr > Ca > Mg (5 experiments) (Sherry's series *I*) or Sr > Ba > Ca > Mg (2 experiments), which differs from series *I* only in that Sr and Ba are transposed.

3. *Osmotic Potential Across Small Intestinal Epithelium*

When the tonicity of one of the solutions bathing the epithelial stratum was gradually increased, the transepithelial potential changes as shown in Fig. 6. The right branch of curve reflects the behavior of φ_{osm} in response to increasing osmotic pressure from the serosal side (P^s), while the left branch of the curve describes φ_{osm} as a function of osmotic pressure from the mucosal side (P^m). On the left branch of the $\varphi^m_{osm} = f(P^m)$ curve, the linear segment remains such up to 0.1 Osm, the maximum value of $\varphi^m_{osm} = 4$–5 mV being reached at P^m values in the range of 0.350–0.400 Osm (9 experiments). Further increases in pressure decreased the potential somewhat. This pattern is essentially repeated on the right branch of the $\varphi^s_{osm} = f(P^s)$ curve but the parameters are strongly different: the linear segment persists up to 0.050–0.060 Osm, and the maximum value of the potential does not exceed 0.8–0.1 mV at $P^s = 0.150$–0.200 Osm (9 experiments). With a further increase in pressure, φ^s_{osm} also somewhat decreases.

When the pressure was abruptly increased on the mucosal side, the potential rapidly attained a given value and remained stationary. An abrupt increase of osmotic pressure on the serosal side resulted in a complex

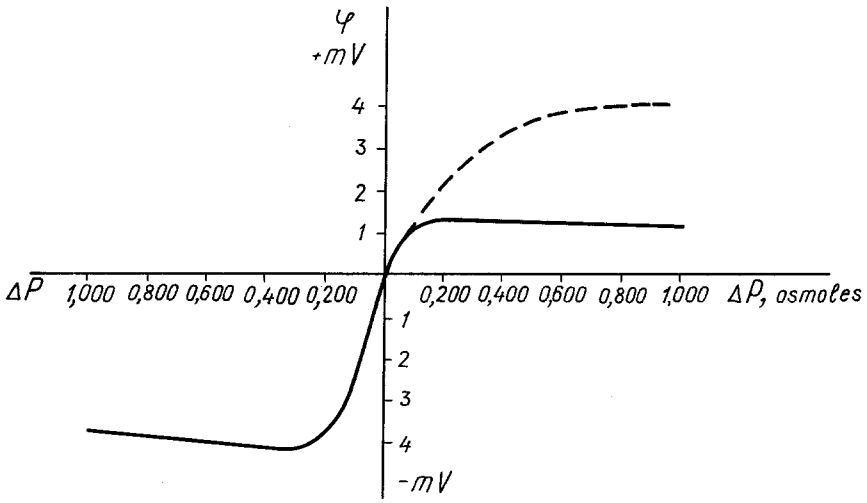

Fig. 6. Changes in transepithelial potential upon gradual increase of solution tonicity from the serosal (right) and mucosal (left) sides

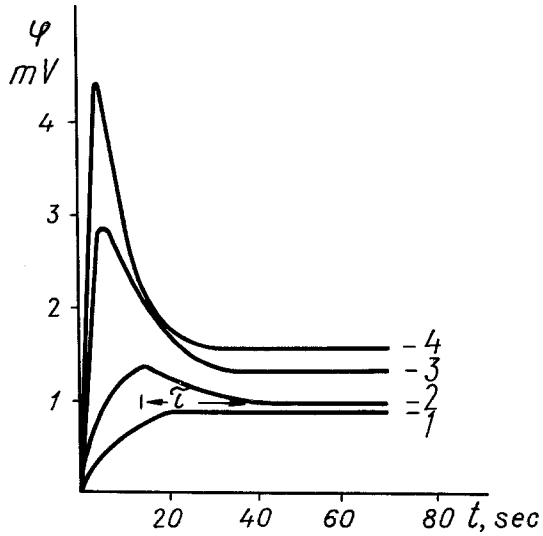

Fig. 7. Changes in the shape of transepithelial potential upon abrupt increases of solution tonicity from the serosal side by 0.050 (*1*), 0.100 (*2*), 0.500 (*3*) and 1.000 (*4*) Osm

behavior of the potential. At P^s values up to 0.050–0.060 Osm, the potential remained stationary upon reaching a given value but at higher P^s, it rapidly attained a maximum and then fell to a new, stationary value (Fig. 7). Both the time taken to reach the maximum and the complete time of establishing the stationary value (τ) decreased with rising P^s. In Fig. 6, the dashed line

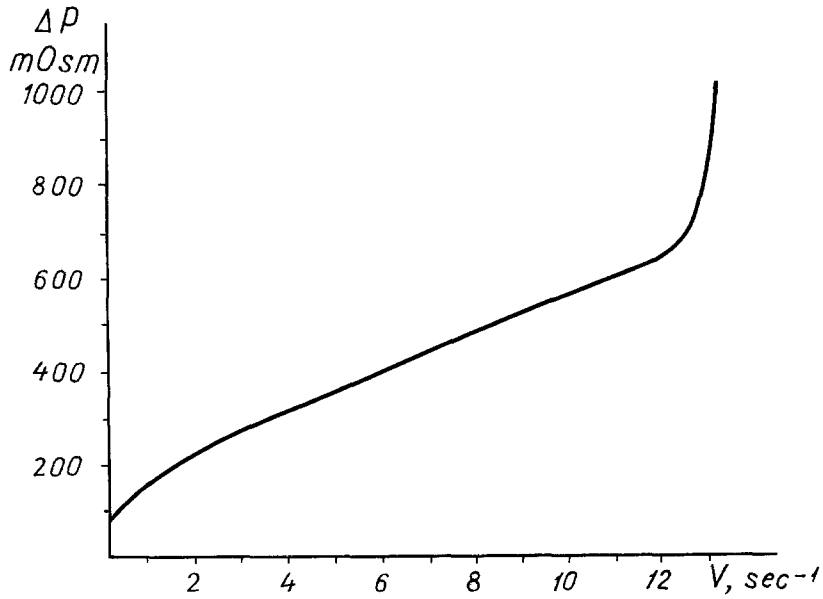

Fig. 8. The rate of fall (v) of transepithelial potential as a function of the osmotic pressure applied

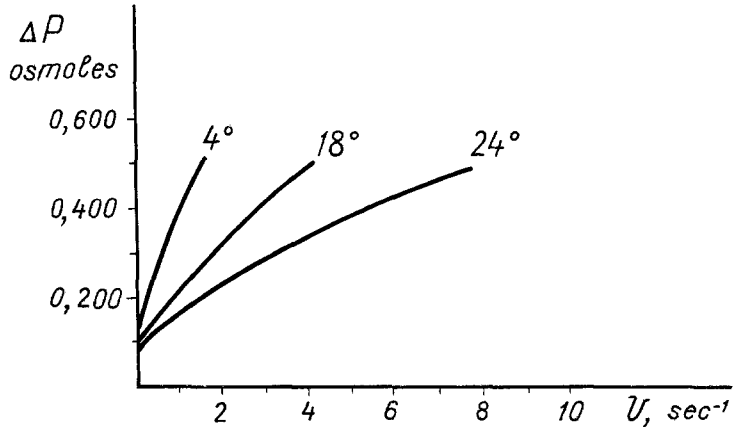

Fig. 9. Temperature dependence of the initial segment of the $v=f(P^s)$ curve

represents the maximum values of φ^s_{osm}, and the curve plotted from the established values nearly coincides with that obtained upon a gradual increase in pressure. The effects of increased pressure from both sides at short exposures (up to 2 min) and at P up to 1.0 Osm were completely reversible.

The rate of φ_{osm} decline to the stationary value may be described, as a first approximation, by the value v, which is the ratio of the semi-height of

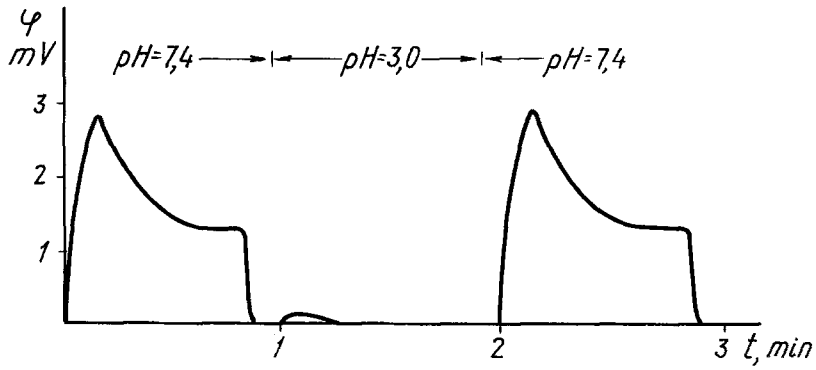

Fig. 10. The effect of the pH of the serosal solution on the osmotic potential induced by an abrupt (by 0.300 Osm) increase in P^s

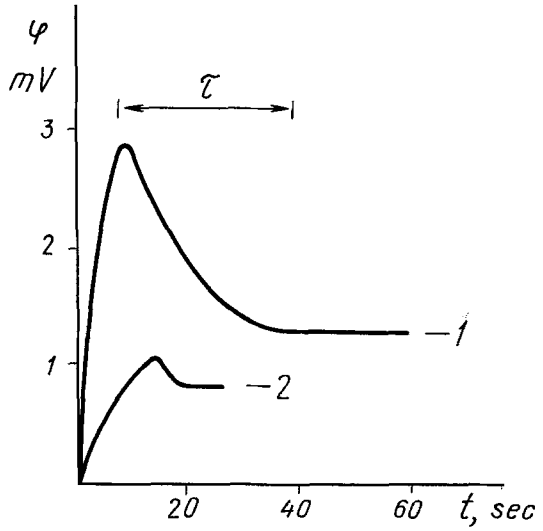

Fig. 11. The shape of osmotic potential: normal (*1*); after exposure to Na_2H_2EDTA (*2*) for 30 min

the maximum to its semi-width. The rate of fall in v as a function of pressure is shown in Fig. 8.

Lowering the temperature increases the slope of the initial segment of the $v=f(P^s)$ curve (Fig. 9).

4. Effects of pH and Na_2H_2EDTA on the Osmotic Potential Behavior

Decreasing pH toward acidity resulted in symmetrical decreases both φ^m_{osm} and φ^s_{osm}, these potentials attaining zero value at pH = 3.0. Fig. 10

shows changes in φ^s_{osm} at two pH values (3.0 and 7.4) by abrupt increases in pressure on the serosal side.

N_2H_2EDTA decreased these potentials and also altered the shape of the osmotic potential induced by abrupt increase serosal solution tonicity (Fig. 11).

5. *Effects of pH and Bivalent Cations on Spontaneous and Diffusion Potentials*

In most cases, the spontaneous potential φ_{sp} did not exceed 3–4 mV and remained at that level for 4–5 hr. Addition of Na_2H_2EDTA initially led to a fast (1–2 sec) change in φ_{sp}, increasing or decreasing it when Na_2H_2EDTA was added from the mucosal side or the serosal sides, respectively (Fig. 12). Further incubation in Na_2H_2EDTA progressively reduced φ_{sp}. Placing the mucosal or the serosal side of the specimen in Ca-free solutions did not alter the spontaneous potential at the initial moment, though the potential did decrease over a longer period (3–5 hr).

The effect of Na_2H_2EDTA on φ_{sp} was reversible when calcium was added. The diffusion potential φ_d 1 : 2 NaCl [2] remained within the range of 6–9 mV in most of the experiments, which corresponds to the range of relative permeability $P_{Cl}/P_{Na}=0.27$–0.45 (73 experiments). Addition of Na_2H_2EDTA reduced φ_d, the effect being greater on the mucosal side, i.e., the potential reached the same value on that side over a shorter period than on the serosal side. The effect of Na_2H_2EDTA was reversible upon subsequent addition of Ca^{2+}. Analysis of the time-course and reversibility of changes in permeability to ions following removal of bivalent cations enabled us to distinguish three phases of this process: the first phase, in which φ_d slightly increased at first followed by its return to initial level; the second phase, in which the potential gradually fell but restored its value when calcium was added; and the third phase, which was irreversible, when addition of Ca could not restore the potential or even prevent it from falling.

The time taken by the potential to reach its maximum in the first phase depended on the conditions of bivalent cation removal and varied from dozens of minutes to minutes, the more intensive the removal, the shorter the time. The first phase appears to have been due to a more rapid increase in permeability for Na than for Cl, which led to decreased P_{Cl}/P_{Na} ratio. The second phase reflected the subsequent increase in permeability for both cations and anions. The irreversibility of the third phase made it possible to suggest, as in [10], that during that phase the outer layers of the plasma membrane lost certain constituents necessary for normal functioning of the

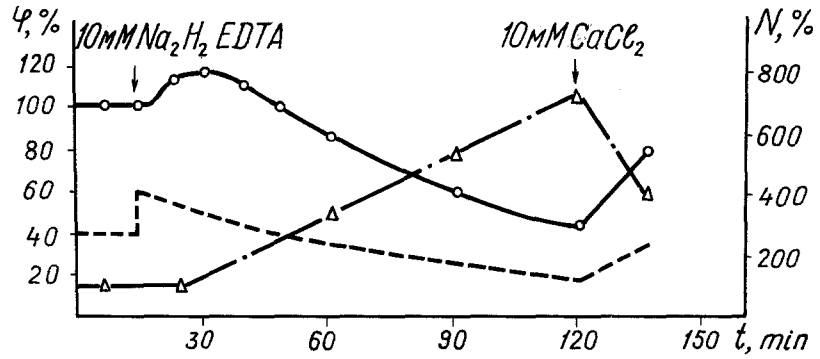

Fig. 12. Changes with time in diffusion (———) and spontaneous (------) potentials and in number of separated cells (— · — · —) upon exposure to N_2H_2EDTA and subsequent addition of $CaCl_2$ (everted sacs). The initial values of φ_d and N were taken as 100%

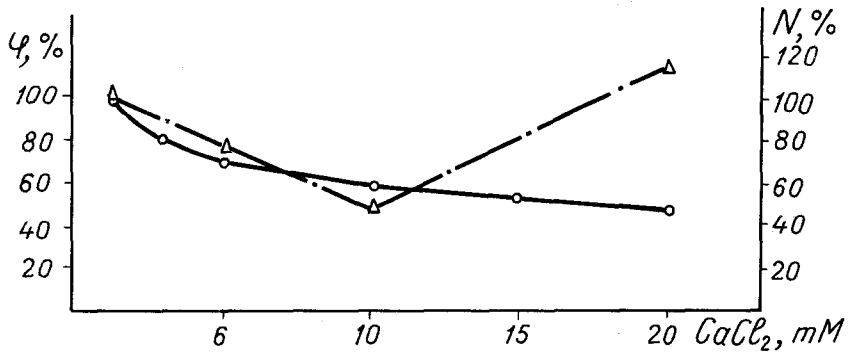

Fig. 13. The diffusion potential (———) and the number of separated cells (— · — · —) as a function of $CaCl_2$ concentration. The initial values of φ_d and of the number of separated cells were taken as 100%

cell contact. The diffusion potential remained unchanged for a long time (4–5 hr) at calcium concentrations on the order of 1.0 mM; at 1.0 mM, φ_d attained its maximum. Increasing calcium concentration above that value resulted in a fast (< 20 sec) lowering of φ_d. φ_d as a function of concentrations is shown in Fig. 13. This effect was readily and rapidly (< 60 sec) reversed: φ_d returned to the initial level as calcium concentration decreased to the physiological level. Excessive concentrations of other alkaline-earth cations affected φ_d similarly to calcium; in terms of effectiveness, they were arranged in the sequence Ca > Ba > Mg > Sr (5 experiments), which corresponds to Sherry's [11] series *IV*. Decreasing the mucosal solution pH reduced φ_d. At pH = 2.80 ± 0.1 φ_d reached zero and reversed its sign upon a further decrease in pH. Decreasing the pH of the serosal solution affected φ_d very slightly (Fig. 14).

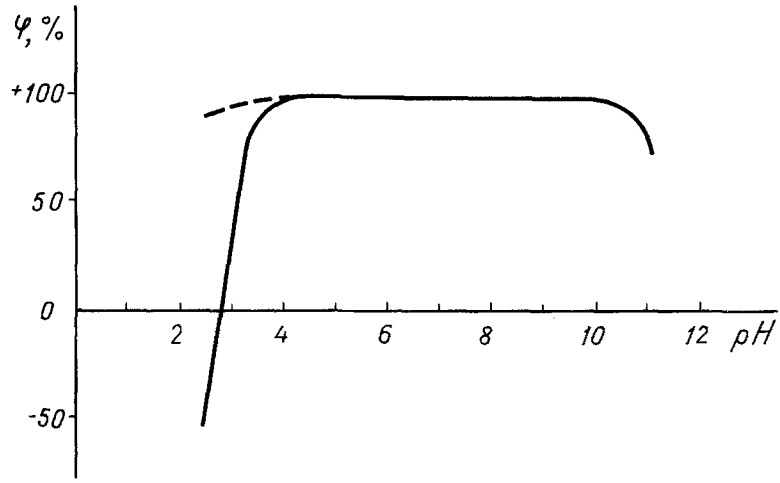

Fig. 14. The diffusion potential of 1:2 NaCl as a function of solution pH from the mucosal (——) and serosal (------) sides. φ_d at pH 7.4 was taken to be 100%

The pH value at which φ_d reversed its sign remained the same for all the buffer solutions used.

The relative permeability of alkaline cations was studied by measuring the bi-ionic potential. At pH = 7.4, the permeability of alkaline cations declined in the order $Cs \geqq Rb \geqq K > Na > Li$ (7 experiments) or $K \geqq Rb \geqq Cs > Na > Li$ (5 experiments), which corresponds to Eisenmann's series *1* and *4*, respectively. At pH = 2.80, only the first series was obtained, and the initial ratios of cation permeability – $P_{Cs}/P_{Na} = 1.72 \pm 0.07$, $P_{Rb}/P_{Na} = 1.70 \pm 0.05$, $P_K/P_{Na} = 1.62 \pm 0.07$, $P_{Li}/P_{Na} = 0.72 \pm 0.04$ – decreased. Na_2H_2EDTA also decreased the ratios of cation permeability.

Discussion

1. Work of Adhesion of Cellular Membranes in the Cell Contact Region

One of the important characteristics of a cell contact is the energy of membrane interaction (A). In order to evaluate this characteristic from experiments with contact destruction, it is necessary to know whether particular structures of the cell contact possess solid, viscoelastic or plastic properties. In other words, in order to evaluate the energy of membrane interaction (work of adhesion), it is necessary to know the dependence of A

or of the force separating two adjacent cells upon the contact destruction rate (v) as well as the location of the experimentally determined separating force on the resulting curve.

Study of the contact destruction process in relation to the magnitude and the time of action of the force applied (F) has shown that the cell contact behaves as a solid body when $F < F_{cr}$ and as a viscoelastic body when $F > F_{cr}$. The same measurements reveal the distribution of epithelial cell populations in relation to F_{cr} (Fig. 2). It appears that the process of destruction of such a viscoelastic system as a cell contact can be best approximated by a model of the peeling off of an adhesive tape. In this case, the work of adhesion can be expressed in terms of the acting force F and the width of the tape being peeled off: $b : A = F/b$ (erg/cm^2).

Our results indicate that the F depends on the rate of separation. True work of adhesion corresponds to the saturation range of the $F = f(v)$ curve, when the deformation rate of the system exceeds that of its relaxation [5]. Of the methods known in adhesiometry, this condition is best fulfilled by Coman's method [4], although in this case, too, the separation rate is not great enough. For this reason the value of 1.0 dyne/cell obtained by Coman's method, appears to be minimal for evaluating A.

Let us now make the minimal evaluation of the work of adhesion for cell membranes. Assuming the width of the cell contact to be equal to 10 μm, we obtain the work of adhesion equal to 10^3 erg/cm^2. Expressing this value in calories and assuming that all molecules of the cell surface are involved in the adhesive junction (and also assuming that the molecular density is 3×10^{14} moles/cm^2), we find that the adhesion energy is:

$$A = \frac{10^3 \cdot 0.24 \cdot 10^{-7}\ 6 \cdot 10^{23}}{3 \cdot 10^{14}} = 50{,}000 \text{ cal/mole} = 50 \text{ kcal/mole},$$

where $0.24 \times 10^{-}7$ and 6×10^{23} are the transition coefficient (erg/cal) and Avogadro's number, respectively.

It should be noted that during centrifugation cells separate at much smaller forces. In our conditions, they separated at centrifugal forces as low as $0.1–1.2 \times 10^{-4}$ dyne/cell over a period of $\sim 10^3$ sec. The above values of the separating force are by 3 to 4 orders smaller than the actual force acting during short-term exposure (< 1 sec), as in Coman's method. Moreover, in our case the magnitude of the applied force is strongly dependent upon the rate of destruction of cell contacts. These results point to a viscoelastic nature of the contacts.

2. *Differences in Mechanical Properties between Tight Junction and Underlying Intercellular Space* (IS)

The osmotic behavior of the small intestine also suggests a viscoelastic nature of the cell contacts.

The φ_{osm} resulting from the local transmembrane salt gradient has been shown to depend mainly on the rate of osmotic water flow, the thickness of unstirred layers, and the relative permeability P_{Cl}/P_{Na}. Alterations in φ_{osm} are chiefly caused by variations in the geometry of the cell contacts [9, 12, 15]. When the water flow is directed toward the solution bathing the serosal side of the epithelium, the sectional area of cell contacts progressively increases with increasing P^s. The water flow rate through the widened contact now decreases, and this in turn determines the nonlinear behavior of φ^s_{osm} (Fig. 6). When, on the other hand, the water flow is directed toward the mucosal solution, the intercellular spaces collapse and become more "rigid".

The collapse of the spaces results not only in greater absolute values of φ^m_{osm} but also in greater linearity. Pressures up to 0.300 Osm lead to disjunction of cell contact structures, except for one – the tight junction. At higher pressures, however, the tight junction also undergoes structural alterations [3], and this may affect ion permeability by increasing it. An increase in ion permeability should lead to a fall in φ_{osm}, and such a fall did occur in our experiments at $P^s = 0.200$–0.250 Osm and $P^m = 0.350$–0.400 Osm.

The existence of a correlation between the structural changes and the behavior of the osmotic potential suggests that the observed decline of φ^s_{osm} to the stationary value upon an abrupt increase in P^s is responsible for a decrease in water flow rate due to widening of the intercellular space and the resulting increase in the area of the unstirred serosal layer. Intercellular spaces are capable of widening only under the action a water flow induced by the $P_{cr} \geqq 0.050$ Osm. This latter value probably characterizes the adhesiveness of the cells along the entire length of the contact, excepting the tight junction. Then the v and τ should reflect, respectively, the rate and time of the restructuring of the contacts. Na_2H_2EDTA reduces cell adhesion and should result in decreased τ, and this was observed in our experiments (Fig. 9). The slope of the initial segment of the $P^s = f(v)$ curve changes with temperature similar to the viscosity curve. This circumstance serves to corroborate the suggestion that the v has the sense of the rate of contact deformation.

The fact that the pH of the serosal solution had no effect on the diffusion

potential appears to be due to difficulties of proton diffusion in the IS. If this is so, then the widening of the intercellular space under the effect of the water flow directed toward the serosa, may account for the pH effect on φ^s_{osm} caused by the hypertonicity of the serosal solution. These two effects combine to localize in the tight junction the population of the acidic centers controlling the ion permeability.

Comparison of the minimal critical loads necessary for the deformation of the IS ($P_{cr} \approx 0.050$ Osm) and tight junction ($P_{cr} \approx 0.300$ Osm) indicates that the contribution of the tight junction to the total cell junction is severalfold greater than that of the IS. The slope of the initial segment of the $P^s = f(v)$ curve is proportional to viscosity of the contact's constituents responsible for cell adhesion. The $P^s = f(v)$ curve is a typical adhesiogram of viscoelastic systems [5].

Thus, two completely independent methods (that of centrifugal separation and that of reversible deformation of contacts under the effect of P^s) indicate that the cell contact behaves as an solid body when the loads are less than critical and as a viscoelastic body when they exceed the critical value. The former method, in combination with Coman's method, makes it possible to evaluate the minimal work of adhesion, while the latter enables us to assess the adhesion properties of an IS. Both these methods reveal the same behavior of the slope of the initial segment of the curve describing the magnitude of the deforming force as a function of deformation rate with a change in temperature.

3. The Role of Acidic Centers in Cell Adhesiveness and in Passive Transepithelial Ion Permeability

A decrease in N with decreasing pH implies an increase in cell adhesion. Cell adhesion and φ_d similarly depend on pH. In both these cases the pH values of 2.80–3.0 are extreme points when the maximal adhesion of the cells and the minimal absolute value of φ_d are attained. At the same pH values, the selectivity series of alkaline cations are observed to shift toward Eisenmann's first series. The acidic centers which regulate cation permeation through the tight junction appear also to be involved in cell interaction of the repulsion type. If this is so, then their protonation should increase cell adhesiveness while the recharging of the cell surface in the pH region of < 2.8–3.0 should again lead to decreased cell adhesion, just as was observed in the experiments.

A prolonged (several scores of minutes) removal of alkaline-earth cations from the tissue with Na_2H_2EDTA leads to a sharp decrease in cell

adhesiveness and concurrent increase in ion permeability of the contacts. A concomitant decrease in permeability ratios points to additional hydration of the permeability route. Subsequent addition of alkaline-earth cations restores the initial levels of ion permeability and contact adhesion. The ability to restore these parameters declines in the seventh series obtained by Sherry [11] for strong acidic centers.

To maintain the permeability and adhesion of the contacts at initial levels, 1.0 mM of $CaCl_2$ proves to be sufficient. Increasing the concentration of alkaline-earth cations above 1.0 mM results in a slight increase of cell adhesion. The concomitant reduction in φ_d appears to be due to the blocking of negative charges in the contact and to decreased permeability for Na. The capacity for increasing cell adhesiveness and for reducing φ_d declines in selectivity series *1–4* obtained by Sherry for weaker acidic centers. Also, when present in excessive concentrations, the alkaline-earth cations are weakly connected with the cell membrane. Their removal does not require the presence of Na_2H_2EDTA and can be effected in a short period of time (<60 sec), by simply replacing the solution with increased concentration of a given cation by a solution free from that cation.

Considered together, the present results suggest that there are two populations of acidic centers in the tight junction. The first population is made up of acidic centers binding bivalent cations in the Sherry's 7th series in such a way that they can be removed with high concentrations of Na_2H_2EDTA over a prolonged period of time; these centers are involved in interaction of the attraction type. This population fully links with bivalent cations when these are present in the solution in concentrations of not more than 1.0 mM. The second population is that of acidic centers which bind bivalent cations in Sherry's series *1–4* in such a way that their removal does not require the presence of Na_2H_2EDTA and can be achieved by simple short-term incubation in solutions free from those cations; these centers are involved in membrane interaction of the repulsion type. This population is more numerous – sorption of Ca continues at concentrations of this cation higher than 20 mM (Fig. 13), and it regulates the permeation of alkaline cations through the tight junction.

The first population of acidic centers appears to interact with bivalent cations to form ion-dipole links between a bivalent cation and two acidic centers located on adjacent, opposite plasma membranes.

The totality of such links is capable of forming a double electrical layer in a tight junction and this layer may account for the observed increase in the work of adhesion, since electrostatic forces act at greater distances than

molecular ones despite the fact that the absolute value of electrostatic interaction may be smaller.

It should be noted that our experiments have revealed an intimate correlation between changes in passive transepithelial ion permeability and cell adhesiveness. Such a correlation is possible if the tight junction is the principal contributor to the passive ion permeability and to the mechanical strength of the cell contacts.

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