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Microscopical Determination of the Filtration Permeability of the Mucosal Surface of the Goldfish Intestinal Epithelium

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Summary. The rate of shrinkage of the mucosal folds of goldfish intestine in response to mucosal hypertonicity was measured by microscopic means. Because of the geometry of the intestinal folds the rate of shrinkage could be directly related to the loss of volume from the fold through the brush border membranes and tight junctions. Experimentally a wide range of velocities was observed, reflecting the difficulty of rapidly establishing a uniform osmotic gradient at the preparation's mucosal surface. The initial velocity of volume loss provided a measure of the filtration permeability (P_f) of the mucosal surface. From the highest velocities observed the filtration permeability was estimated to be approximately 14×10^{-3} cm/sec related to the folded mucosal surface and 65×10^{-3} cm/sec related to the straight serosal surface. Consideration of the experimental errors and unstirred layer effects make it probable that the latter value is still an underestimate of the true P_f. The series barriers of the epithelium cause the total tissue P_f to be less than the P_f of the mucosal surface alone. In addition the P_f measured in the presence of an osmotic gradient may differ substantially from the tissue filtration permeability which exists in the absence of a change in osmolarity.

Key words: intestine, mucosal hypertonicity, morphology, filtration permeability, (goldfish)

Introduction

In a recent review Diamond (1979) discussed the experimental difficulties of estimating the filtration permeabilities of epithelia by establishing osmotic gradients. The first one is that the osmotic gradient is never established instantaneously across the membrane due to the half-time (τ_D) needed by the osmoticant to diffuse through the unstirred layer. The second difficulty is that, in response to the osmotic gradient across the membrane, water flows through it to the hypertonic side, causing changes in the composition of the solutions at both sides of the membrane: i.e., there is "solute polarization" a phenomenon well described by Dainty (1963), Wedner and Diamond (1969), Wright, Smulders and Tormey (1972), Schafer, Patlak and Andreoli (1974), Diamond (1979) and recently Pedley and Fischbarg (1980). The half-time needed to establish a steady osmotic flow is called the sweeping away time (τ_{sweep}). As mentioned by Diamond (1979), it is desirable that τ_D should be shorter than τ_{sweep}; it is estimated that the unstirred layer should be less than 30 μm to establish the gradient sufficiently fast to fulfill this condition.

The measuring time resolution (τ_{meas}) should be clearly shorter than τ_D to observe the initial phase of the osmotic response; i.e. τ_{meas} should be a few seconds or less. The condition $\tau_D < \tau_{sweep}$ is difficult to meet in folded epithelia (Thomson & Dietschy, 1977) like rabbit gallbladder (Smulders, Tormey & Wright, 1972) and goldfish intestine (Albus & Siegenbeek van Heukelom, 1976). Attempts to stretch the epithelium (Loeschke, Eisenbach & Bentzel, 1977) have been made, but, generally, the unstirred layer thickness is still not sufficiently reduced.

In rabbit gallbladder optimal conditions were achieved by the use of a fast measuring apparatus (Wiedner, 1976) and high stirring rates. The time resolution for determination of transepithelial volume flow (J_v) was 5 sec (Van Os, Wiedner & Wright, 1979). These authors report that mucosal hypertonicity of more than 75 mosmol/liter produced a reduction in tissue weight of 22.5%. This can be explained only if water is drained out of the tissue faster than it is replenished from the serosal side. We also observed

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similar tissue shrinkage by the goldfish intestinal epithelium.

The preceding observations indicate that there is a barrier serosal to the epithelial cells that significantly impedes transepithelial water flow. A consequence is that the transmural determination of the steady-state volume flow in response to the transmural osmotic gradient does not provide direct information on the water permeability of the mucosal surface, as this surface is in series with another permeability barrier with lower water permeability. Only a dynamic experimental analysis can provide an estimation of the filtration permeability of the mucosal surface by extrapolation of the onset of water flow to the moment that hyperosmolarity is applied (i.e. $t=0$). At time zero the only membrane through which volume flows in response to mucosal hypertonicity is the mucosal surface. The initial velocity of volume loss thus measured is not dependent on other permeability barriers and might provide a direct measure of the mucosal filtration permeability P_f^m. We determined this parameter in the goldfish intestine by measurements of the initial rate of shrinkage of goldfish intestinal epithelial folds.

Since P_f is a phenomenological parameter related to a given surface, P_f^m can be related to the mucosal surface. P_f^s, the filtration permeability of the serosal surface, was related to the mucosal surface area as well, since the site of the serosal barrier was not evident.

Materials and Methods

Correlation Between Shrinkage and Volume Loss

The long laminar foldings of the goldfish intestinal epithelium exhibit the herringbone structure typical of this epithelium (Albus & Siegenbeek van Heukelom, 1976; Weinberg, 1976). This provides good conditions for the determination of P_f^m, because the length of the fold cannot change much during changes in volume of the epithelium. To explain how P_f^m is calculated Fig. 1 shows: *a)* a transmission light micrograph of a stripped and unfixed preparation as used during the experiments in the control Ringers; *b)* a transmission light micrograph of the same preparation with 87 mosmol/liter mannitol added to the mucosal solution; *c)* a cross-section perpendicular to the folds of the intestine stripped free of underlying tissue (for a full description of the stripping procedure *see* Albus, Groot and Siegenbeek van Heukelom, 1979, and Groot, Albus and Siegenbeek van Heukelom, 1979); *d)* an idealization of Fig. 1*c* drawn to scale and showing all morphological values needed for the following derivations; *e)* a cross-section as in *c)* but now with 145.5 mosmol/liter mucosal hyperosmolarity.

The diameter of a fold was measured in a control period and then during perfusion of a hypertonic solution across the mucosal surface. The decrease in fold diameter (*FD*) provided a measure of the volume loss through the mucosal surface *MF*. When V is the fold volume, S is the surface area through which the volume flows out of the fold and h the height of the noncurved part of the fold, the ratio Volume/Surface (V/S) of a fold can be calculated as

$$V/S=(\pi FD^2+8h\,FD)/(4\pi FD+16h). \tag{1}$$

The relation between the volume flow across the mucosal surface J_v^m, and the decrease in fold diameter, FD, is:

$$J_v^m=d(V/S)/dt=0.40\,d(FD)/dt, \tag{2}$$

if it is assumed that h does not change during shrinkage. Any decrease in h, due to mucosal hypertonicity, would increase J_v^m, as function of $d(FD)/dt$; the assumption that h does not change then gives the worst-case condition. We have taken the average fold diameter $FD=220$ μm, the height of the fold $h=450$ μm and the interfold distance $d=70$ μm. As shown in Fig. 1*d*, all curvature was approximated by cylindriform surfaces. The total height of the fold, h', normally measured in the experiments was $h'=h+FD/2$, neglecting the curved part at the crypt-end of the fold. In the extreme cases when $h=0$ then $V/S=0.25$ and when $h=\infty$ (infinitely high folds) then $V/S=0.50$. In the preparation h/FD varied from 1.5 to 5.5, and $d(V/S)/dt$ was 0.40 (± 0.05). $d(FD)/dt$. This mean value of 0.40 was used for the analysis of the data [see Eq. (2)]. The general equation for volume flow (J_v; cm/sec) through a membrane (*cf.* Katchalsky & Curran, 1967, and House, 1974) is:

$$J_v=L_P(\Delta P-\sigma\Delta\Pi) \tag{3}$$

where ΔP (atm) is the pressure difference across the membrane, $\Delta\Pi$ is the osmotic difference across the membrane, and σ is the reflection coefficient which equals 1 for nonpermeating solutes.

If no negative pressure with respect to the external solutions is generated in the cells by mucosal hypertonicity, and if the reflection coefficient of the added solute is one, this equation reduces for the volume flow through the MF J_v^m to:

$$-J_v^m=P_f^m\,V_w\,\Delta c \tag{4}$$

where $\Delta\Pi$ is related to the concentration difference Δc by $\Delta\Pi=RT\Delta c$ and R and T have their usual meaning. The hydraulic conductivity L_p (cm $\sec^{-1}$ atm^{-1}) is related to the filtration permeability, i.e. $P_f=L_pRT/\bar{V}_w$ (cm/sec) with $\bar{V}_w$ is the partial molar volume of water.

From the steady-state shrinkage the filtration permeabilities of the mucosal surface (P_f^m) and serosal barrier in the shrunken situation can be evaluated in the following way. In the steady state the relative fold diameter shrinkage is related to the relative volume change by:

$$\{\pi+16(h/FD)\}\cdot\Delta V/V=\{2\pi+16(h/FD)\}\cdot\Delta FD/FD. \tag{4a}$$

(The length of the fold cannot change much as is clear from Fig. 1*a*.)

Insertion of the ratio $h/FD=1.5$ to 5.5 gives:

$$\Delta V/V=1.08(\pm 0.04)\cdot\Delta FD/FD. \tag{4b}$$

If it is assumed that $P_f^s=0$, and the cells behave as perfect osmometers, shrinkage of the volume between the serosal barrier SB and the mucosal face MF, will be related to the mucosal hypertonicity (ΔOsm) by:

$$\Delta V/V=1-315/(315+\Delta Osm) \tag{5a}$$

where 315 is the osmolarity of the control solution in mosmol/liter. In that situation, shrinkage stops when the tonicity in the tissue compartment between the MF and SB equals the mucosal tonicity and volume loss is inversely related to the mucosal hypertonicity. When $P_f^s\neq 0$ a smaller volume loss will be found, approximately given by:

$$\Delta V/V=P_f^m\{1-315/(315+\Delta Osm)\}/(P_f^m+P_f^s). \tag{5b}$$

Preparation and Experimental Set-up

Goldfishes were bought through a local commercial dealer and kept for more than three weeks in thermostated (18 °C) low-copper

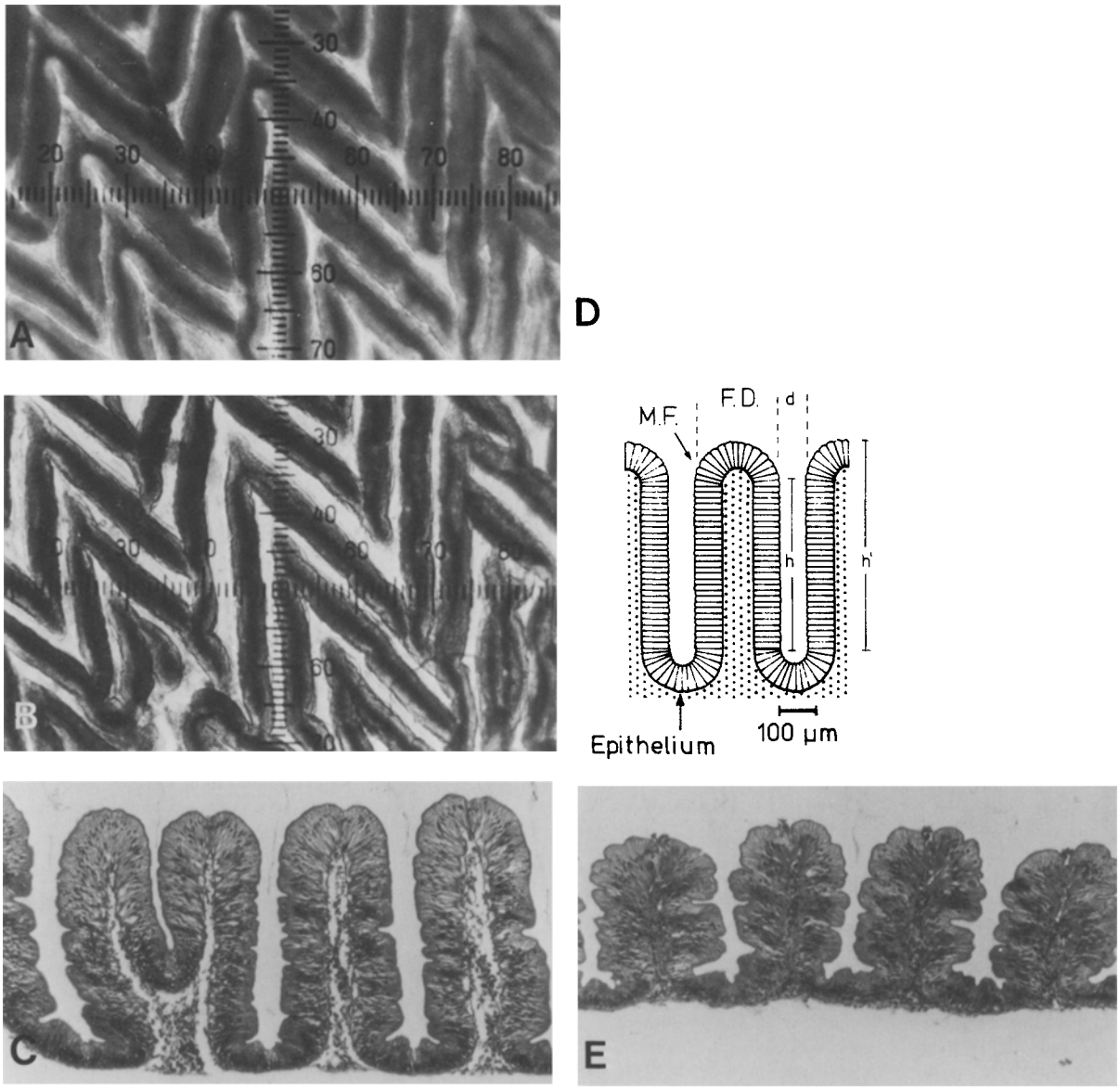

Fig. 1. The morphology of the goldfish intestinal epithelium: *a)* Appearance of the unfixed stripped epithelium in control situations as it was observed during the experiments; the average fold diameter is 220 µm, *b)* The same preparation 20 min after 87.3 mosmol/liter mannitol were added to the mucosal solution. *c)* Cross-section through the epithelium in control situation stained with haematoxylin and eosin; ×200. *d)* A curved idealization drawn to scale of Fig. 1 *c*: *MF*=Mucosal Face (having a filtration permeability P_f^m) composed of the brush border membranes and tight junctions. *FD*=fold diameter, *d*=interfold distance, *h*=height of the noncurved part of the fold, $h'=h+FD/2$. The amplification factor relating the *MF* to the straight serosal surface is 4.7×. *e)* Appearance of a mucosal strip from the same intestine as in *c* after incubation during 30 min in mucosal hypertonicity (+145.5 mosmol/liter). At that time the solutions were drained away and immediately replaced with similar solutions containing 5% formalin and stained as in *c* (200×). Alternatively lifting the epithelia out of the incubation media and fixation outside the measuring chambers produced no different results. The fold diameter decreased and the fold obtained a more lobed structure ("oak-leaf-structure"). The decrease in height is not representative as this depends on the angle of the section as well as the place in the tissue. Note that the shrinkage of the fold is also due to shrinkage of the lamina propria in the fold

tap water. The goldfish was sacrificed by cutting the spine just behind the gills and decerebration, and the intestines were removed. From the first 10 cm mucosal strips were obtained. A square piece of the strip of approx. 7×7 mm was mounted mucosa upwards in the measuring chamber.

A schematic drawing of the measuring chamber (made of perspex) is given in Fig. 2. In the middle a hollow voltage-measuring electrode serves as serosal inlet (flow approx. 1.0 ml/min). The rim, on which the epithelium is mounted is 1 mm higher than the height of the serosal inlet; the circular lumen of the rim (0.200 ± 0.005 cm^2) is taken as the effective surface of the epithelium. The rim itself is rounded off and the epithelium was fixed to the rim by an O-ring that was previously overstretched. Mounted thus the epithelium showed no edge-damage on histological examination

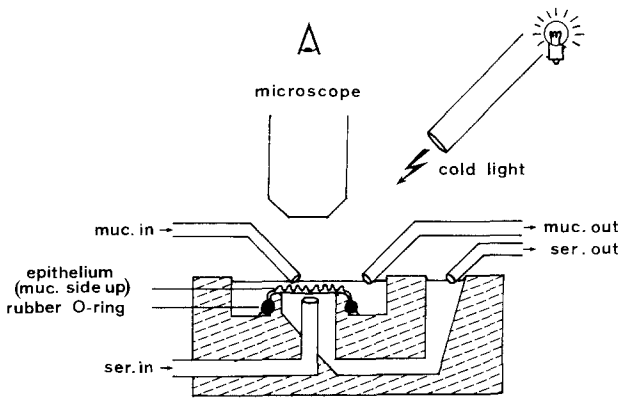

Fig. 2. Schematic drawing of the measuring chamber (made of Perspex) showing the location of the epithelium, the inlets and outlets of the mucosal and serosal baths as well as the position of the microscope objective and the indirect illuminator

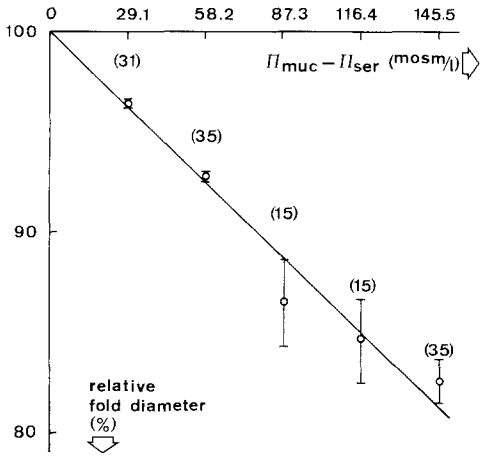

Fig. 3. Changes in the steady-state relative fold diameter as a function of the mucosal hypertonicity (±SEM; numbers of measurements in parentheses)

after the experiment. By virtue of the construction of the serosal outlet (*see* Fig. 2) pressure differences were always less than 1 mm H_2O. The ring-shaped serosal current electrode was mounted in the wall of the serosal compartment. Surrounding the central pedestal the mucosal compartment is a small cup in the wall on which the ring-shaped mucosal current electrode is placed. A small electrode was placed in the mucosal inlet, as the mucosal potential electrode. All electrodes were made of Ag–AgCl.

The solutions used as control contained (in mmol/liter): NaCl 117.5; KCl 5.7; $NaHCO_3$ 25.0; NaH_2PO_4 1.2; $CaCl_2$ 2.5; and $MgSO_4$ 1.2. Concentrations of glucose or mannitol were 27.8 mmol/liter. The osmolarity of all solutions was checked (Advanced Osmometer), the value for the control solution being 315± 5 mosmol/liter. Fluids were maintained at 20 °C and gassed with humidified 5% CO_2+95% O_2, pH 7.3. The osmolarity was increased by addition of mannitol to give solutions with increased osmolarities of 29.1±0.7; 58.2±0.5; 87.3±1.6; 116.4±0.7 and 145.5±0.9 mosmol/liter. In preliminary studies it was found that labeled mannitol enters the epithelium from the mucosal side more easily than labeled inulin, but at 30 min it does not amount to more than 4% of the tissue water content as compared to inulin 1%. This was taken as an indication that mannitol can be used as an appropriate solute for increasing the tonicity of the solution.

All experimental data are represented ± standard error of the mean (±SEM).

Morphological Measurements

The measuring chamber was placed on the stage of a microscope (Leitz Laborlux II; objective 2.5×; one eyepiece 10× and one filar micrometer eyepiece OSM (10×) of Olympus) and illuminated by reflected cold light (Schott KL 150 B). For steady-state measurements the eyepiece and the stage were adjusted so that at least five folds of the epithelium could be measured in a (more or less) perpendicular run of the adjustment of the micrometer eyepiece. The reading of the eyepiece micrometer was calibrated by use of an object micrometer.

In the Introduction it was mentioned that correct measurement of P_f^m requires that the diffusional delay (τ_D) should be as short as possible. For this reason a fold should be selected that satisfied the following requirements:

1. The fluid from the mucosal inlet reached the fold directly. The bore of the mucosal inlet was 0.5 mm so that only a part of the fold was subjected to a nearly instantaneous change of the osmolarity.
2. The fold did not touch neighboring folds.
3. There was not a mucous layer on top of the fold.

Adjusting of the eyepiece micrometer could be speeded up to approx. 8 sec for one diameter measurements (τ_{meas}). Adjusting errors in the reading were ±1 scale division (i.e. 2.5 μm) resulting in an average error in the diameter measurements of ±5 μm. Some of this error could be compensated for by a curve-fitting procedure (*see* Fig. 4), thereby giving a good indication of the observed transient. Occasionally, a 10× objective was used in an attempt to improve the accuracy of the diameter measurement, but a significant improvement was not found (e.g. registration "220.3 μm" in Panel 4*a* was made with a 10× objective).

All measurements were made perpendicular to the fold. The first change observable in the electrical recordings indicated the time that the hypertonic solution entered the mucosal compartment (*see* Fig. 5).

Estimation of the Water Content and Extracellular Volume of Mucosal Strips

During an experiment wet weight and dry weight were determined in six mucosal strips of one goldfish of which four strips were mounted in the experimental conditions and two in control conditions as described earlier (Groot et al., 1979). After 30 min the strips were punched out, gently blotted on Whatman no. 1 filter paper and placed in tared aluminium one-way combustion boats (Hereaus 5–8 mg). Wet weight (ww) was determined directly on a microbalance (Mettler ME 30), and dry weight (dw) after drying to constant weight at 105 °C during 2 hr. Tissue water per dry weight (tw/dw) was determined as (ww-dw)/dw.

The extracellular compartments of the tissues were estimated from the ratio of radioactivity in the tissues and in the incubation solutions. (Hydroxy-^{14}C-methyl) Inulin (The Radiochemical Centre) was used as a marker for the extracellular compartment. Radioactivity was measured using Instagell (Packard) as scintillation mixture and a Mark I (Nuclear Chicago) Liquid Scintillation Counter. Normally the inulin was added bilaterally more than 15 min after hypertonicity was applied.

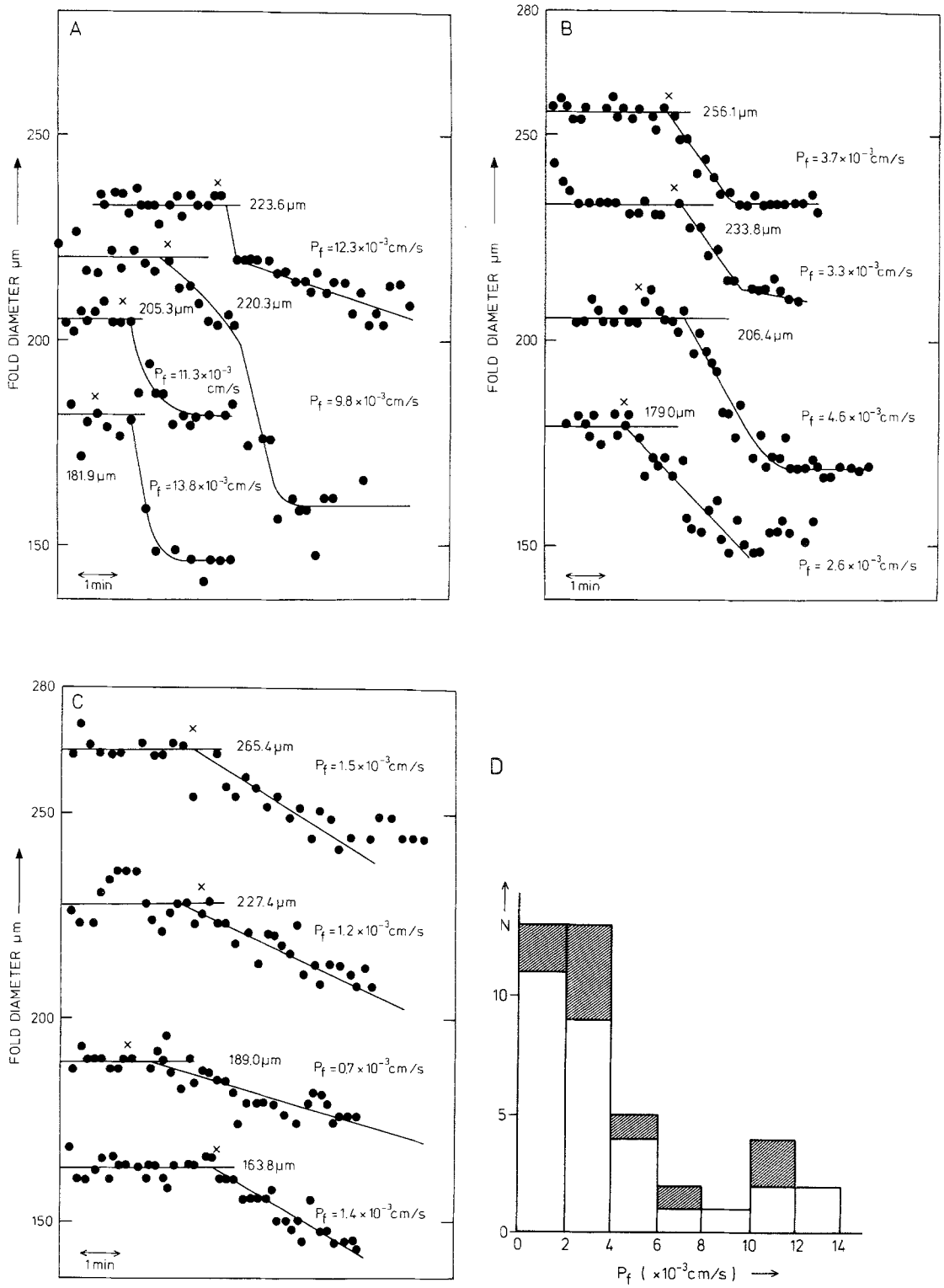

Fig. 4. Typical time courses of the shrinkage of folds due to mucosal hypertonicity (+145.5 mosmol/liter mannitol): *A)* fast responding folds; *B)* intermediate responses; *C)* slow responses. The diameter is expressed in µm; the recordings are identified by their average value before the onset of diameter changes. The onset of electrical responses is indicated by x. Lines are drawn by eye for the best fit of the slope. P_f^m is calculated with the formulae from the Materials and Methods section. Changes sometimes were not fitted with one slope, e.g. curve "220.3" in panel *A)* is fitted with two slopes; while the fastest is related with the P_f value presented, the slower is more equal to the slopes as presented in panel *B)*: in those cases two P_f values were found. In panel *D)* a histogram is presented of all recordings made for this paper. In 14 epithelial strips 45 time courses were determined: 35 are analyzed and presented in panel *d)*; if one record is analyzed with two slopes, both P_f values are presented shaded

Results

A. Steady-State Relation Between Fold Diameter and Mucosal Tonicity

As described in Materials and Methods the decrease in fold diameter was measured in the steady state more than 15 min after the change in tonicity. This was done for several hypertonic values and the results, as presented in Fig. 3, show a linear relationship between the mucosal hypertonicity and the decrease in fold diameter. During this series the height of the fold was sometimes also measured; only a small decrease was found which was of minor importance. In contrast to the fold diameter changes induced by mucosal hypertonicity an increase of serosal osmolarity of 145.5 mosmol/liter caused a $5 \pm 1\%$ ($n=6$) increase in fold diameter.

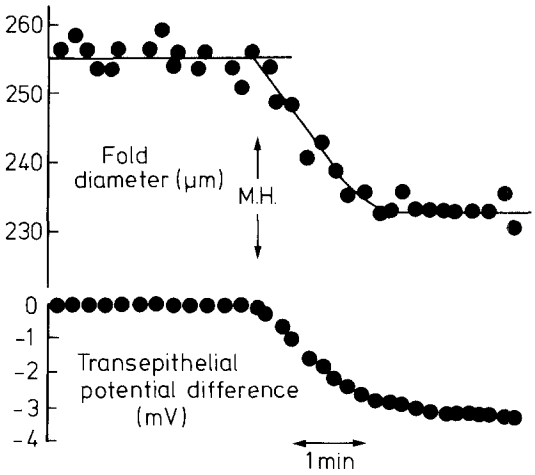

Fig. 5. Time courses of transepithelial potential difference and shrinkage of fold "256.1 μm" of Panel 4*b*. At *MH*, mucosal hypertonicity (145.5 mosmol/liter) is applied

B. Tissue Water and Inulin Space

Tissue water was determined with a mucosal hypertonicity of 145.5 mosmol/liter and a reduction of $23 \pm 5\%$ ($n=25$) was found. This is not significantly different from the decrease in fold diameter ($19 \pm 2\%$) presented in Fig. 3 or the fold volume change calculated from Eq. (4*b*) ($21 \pm 2\%$). Inulin space was measured in both normal conditions and with mucosal hypertonicity ($\Delta\Pi = +145.5$ mosmol/liter). The ratio (inulin space/tissue water) did not differ significantly ($P > 0.05$) in the two groups from 5 to 90 min after inulin addition. 45 min after inulin labeling the extracellular space was $16 \pm 1.5\%$ of the tissue water. These results suggest that during fold shrinkage water is drained from the cellular and extracellular space in the same ratio.

C. Dynamic Response of the Fold Diameter

The transient responses of the fold diameter to hyperosmotic mucosal solutions (+145.5 mosmol/liter) are shown in Fig. 4. In panel *A* four fast responses are shown; in panel *B* intermediate velocities and in panel *C* slow responses. The different curves are identified by their average fold diameter before shrinkage. The average fold diameter during the dynamic and steady-state measurements was 220 ± 5 μm ($n=170$) in control situations. In Fig. 4 x indicates the moments when the first electrical responses were observed as a negative deflection of the transepithelial PD (*see* Fig. 5).

For each registration the filtration permeability of the mucosal face P_f^m calculated with the formulae (2) and (4) are given in the Figure. In panel *D* of Fig. 4 a histogram is presented showing the distribution of the different P_f^m values found. The highest value found is $P_f^m = 14 \times 10^{-3}$ cm/sec; this value is related to the folded mucosal surface. If this value is to be related to the straight serosal surface it must be amplified by a factor of 4.7 representing the ratio of mucosal surface to serosal surface, as can be derived from Fig. 1*d*. This would provide a hypothetical $P_f^m = 65 \times 10^{-3}$ cm/sec related to the serosal surface.

Fig. 5 is an example of the transepithelial PD change (ψ_{ms}) together with a morphological response of the same tissue, showing the negative deflection of the PD. However, the transepithelial potential as such represents an average potential response of the individual cells of the epithelium, and cannot be related directly to the J_v measured in a fast reacting part of the preparation. Only the onset of the PD changes is representative for fast reacting parts. (A paper dealing more extensively with electrophysiological responses of the epithelium on mucosal hypertonicity is in preparation.)

D. Ratio of Mucosal and Serosal Filtration Permeabilities in Steady State

With Eq. (5b) the ratio between P_f^m and P_f^s under hypertonic conditions can be calculated. For + 145.5 mosmol/liter ΔFD is -19%, and ΔV is -21% while the loss in tissue weight is 23%; as average is taken to be 22%. The maximal volume loss according to Eq. (5a) is 32%. Thus in the steady state $P_f^m = 3.2\, P_f^s$. However, this ratio may not be valid in the undisturbed situation, because it is likely that P_f^s and P_f^m change during shrinkage of the fold.

Discussion

The P_f^m value of 14×10^{-3} cm/sec related to the mucosal surface corresponds to an osmotic permeability $P_{os} = 0.25 \times 10^{-3}$ cm/sec Osm. Related to the serosal surface the value becomes $P_{os} = 1.2 \times 10^{-3}$ cm/sec Osm. Corrections for solute polarization effects as described by Van Os et al. (1979) were not introduced.

Using arguments similar to those presented here, Lindemann and Solomon (1962) estimated the initial water flow through the mucosal surface from the rate of weight changes of rat jejunum in response to mucosal anisotonicity. They found a filtration permeability of 8.3×10^{-3} cm/sec with NaCl as osmoticant and 4.8×10^{-3} cm/sec with mannitol, somewhat lower than the values found here. In general our P_f values are higher than previous results for many epithelia (*cf.* Diamond, 1978, 1979). Only our highest permeabilities are physiologically meaningful; deviations are caused by experimental conditions which do not meet the requirements mentioned in the section entitled Morphological Measurements (*see* Materials and Methods). These high values may still be underestimates of the true P_f of the mucosal surface, because it cannot be proved that $\tau_{meas} < \tau_D < \tau_{sweep}$ (Diamond, 1979). Van Os et al. (1979) measured the P_f of the rabbit gallbladder and calculated that their underestimation came to a factor of 5 because of solute polarization effects. The degree of underestimation in the goldfish intestine, however, cannot be inferred from our data.

The results presented show that there exists a serosal barrier (*SB*) with filtration permeability P_f^s. So, in experiments measuring the overall P_f of the tissue by mucosal hypertonicity one does not measure P_f^m, but a filtration permeability which is the resultant of the series barriers, P_f^m and P_f^s. The initial velocity of volume change provides an estimation of P_f^m. The steady-state fold shrinkage shows that $P_f^s < P_f^m$ in that situation, but the value of P_f^s in the osmotically undisturbed situation cannot be determined from these results.

During the application of serosal hypertonicity, the fold diameter increases by only 5%. This change was so small that we did not attempt to measure the dynamic response. Formula (5b) does not apply to swelling of the fold. Most probably, the assumption that the cells behave as perfect osmometers does not hold anymore. This would imply that the serosal barrier is more permeable to the osmoticant than the mucosal surface, and that the cell interior becomes hypertonic with respect to the mucosal bath. We did not attempt analysis of these results because of the uncertainties in the data and the complex models required.

Our morphological observations are in agreement with those of others in rabbit gallbladder (Smulders et al., 1972), the frog intestine (Loeschke, Bentzel & Csáky, 1970) and *Necturus* gallbladder and intestine (Loeschke et al., 1977). We conclude that shrinkage might be observable in those tissues as well (Spring & Hope, 1978, 1979). It is uncertain both whether the low serosal filtration permeability found in our experiments is due to a reduction in the cross-sectional area available for volume flow (Wright et al., 1972) and whether it differs importantly from the P_f^s which exists in the absence of an osmotic gradient. The tissues proposed by Smulders et al. (1972) to be the serosal barrier (the underlying muscles and lamina propria) are not present in our preparation, which is stripped free of them. The size of the goldfish enterocytes (diameter approx. 5 μm and height 70 μm) are comparable to the values cited by Smulders et al. (1972). This fits in with the suggestion, made by Loeschke et al. (1977), on the basis of electrical measurements, that long small cells and a folded structure are prerequisites for the typical asymmetric behavior.

On the basis of the morphology one might consider four possible sites for the serosal barrier (*see* Fig. 1*e*):

1. The long cablelike connection through a narrow slit between the serosal compartment of the fold and the serosal bath.

   As it is observed that the total fold is reduced in diameter and not only the epithelial layer, this is the simplest explanation for the serosal barrier. This would imply that P_f^s, related to the surface of this narrow slit at the bottom of the fold, is much higher than the P_f^s value related to the mucosal face used in this paper, because the ratio of these two surfaces exceeds a factor of 10. As already mentioned, it is possible that osmotically induced structural changes might lead to a decrease of P_f^s but also to changes in other tissue parameters including P_f^m.
2. The basement membrane together with a collagen rich structure underneath the epithelial cells.

   This alternative also provides a good explanation as can be inferred from Fig. 1*e* that shows the typical oak-leaflike structure observed normally on histological examination of the epithelium during mucosal hypertonicity. If in the control situation the fold is distended, the force necessary for this distention is produced by a pressure built up in the epithelial layer during transport (*cf.* Ogilvie, McIntosh & Curran, 1963; Huss & Marsh, 1975). This pressure pushes the cells apart till elas-

tic forces counteract these dilating forces. In a previous paper (Albus et al., 1979) we suggested that the terminal web and the basement membrane produce these elastic forces. However, Fig. 1*e* indicates that elastic connections running through the fold into the epithelial layer also counteract the pressure-driven dilation of the epithelial cells. If this pressure is dissipated by mucosal hypertonicity, the folds collapse in an irregular way and the influence of these elastic connections becomes clearly visible as an oak-leaf appearance of the fold. The fluid is easily squeezed out of the lamina propria of the fold, resulting in the histology shown in Fig. 1*e*. A full morphological and histological analysis, which we have begun, is necessary to substantiate this explanation.

3. The walls of the capillaries in the fold.
4. The capillaries themselves, because they are easily collapsible and could function as valves.

Alternative 3 would comply with the analysis presented by Sackin and Boulpaep (1975); alternative 4 would identify a structure for reducing the transepithelial flow (Smulders et al., 1972). it is not clear, however, how either of these alternatives can be included in a description of an *in vitro* preparation like ours.

In conclusion: though the P_f found is high, there are several reasons to believe that it is still an underestimate:

1. The diffusion time τ_D and the measuring time τ_{meas} (approx. 8 sec) are not small enough to produce enough time resolution for accurately measuring the onset of volume flow through the mucosal surface (*see* Fig. 4*a*). Maybe a faster optical measuring technique with film or television (Spring & Hope, 1978, 1979) can increase the measuring speed, but a reduction of τ_D is unlikely to be achieved with present methods.
2. The filtration permeability of the serosal barrier is not zero; therefore the replenishment of water through the serosal barrier (i.e. J_v^s) will lead to an underestimation of J_v^m and consequently of the mucosal filtration permeability.

From the analysis presented here it is clear that measurements on the whole epithelial layer provide even larger underestimations of P_f due to the fact that the unstirred layers will be larger (*cf.* Thomson & Dietschy, 1977). Furthermore, we conclude that transepithelial measurements do not provide information on P_f^m alone but on the overall P_f. Especially when $\tau_{meas} \gg \tau_{sweep}$ it is likely that these permeability values are not similar to the values before the application of the osmotic gradient, which are the values necessary for a quantitative analysis of the standing osmotic gradient hypothesis (Diamond & Bossert, 1967).

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