Permeability of Luminal Surface of Intestinal Mucosal Cells

B. LINDEMANN and A. K. SOLOMON

From the Biophysical Laboratory, Harvard Medical School, Boston

ABSTRACT A method has been devised to measure the permeability characteristics of the intestinal mucosal cells in the rat. The method makes use of an electrical recording balance to register changes in weight when the mucosal face of a small strip of intestine is exposed to anisotonic solutions. The permeability coefficient of the luminal surface of intestinal mucosal cells to water is measured as 0.15 cm^4/Osm, sec. and reasons are adduced to suggest that the true value might be higher than this. The equivalent pore radius of the luminal face of the tissue, measured in experiments in which lipid-insoluble non-electrolytes have been used according to the method of Goldstein and Solomon, appears to be 4.0 Å.

The easy accessibility of the mucosal surface of the small intestine offers favorable geometrical conditions for investigation of the permeability characteristics of a single face of the mucosal cell. The permeability of the luminal face to water has been determined from changes in the weight of a tissue strip consequent to exposure to anisotonic salt solutions. The equivalent pore radius has also been estimated in the luminal face from measurements of water movement under an osmotic pressure gradient created by a number of lipid-insoluble non-electrolytes.

EXPERIMENTAL METHOD

The procedure is mainly based upon the zero time method of Goldstein and Solomon (1) which requires determination of the initial rate of cell swelling as a function of the concentration of a number of lipid-insoluble non-electrolyte test molecules. An electrical recording balance was used to register changes in tissue weight resulting from exposure of the tissue to solutions containing the test molecules.[1] A standard analytical balance was equipped with a Fisher automatic recording unit (Model 75), which made use of an inductive restoring torque to keep the position of the balance arms constant. The current required to produce the restoring torque was

[1] We are indebted to Dr. Ernest Page for suggesting this method of measurement of cell swelling.

amplified by a Keithley DC electrometer (600 A) and displayed on a Varian recorder (G 11A). A weight change of 13.7 mg resulted in full scale deflection at the highest sensitivity (0.93 cm/gm); the sensitivity chosen for the measurements was 0.46 cm/mg. Following a sudden displacement by a 10 mg weight, 2.4 seconds were required to establish half the final recorded deflection.

Male Sprague-Dawley rats (250–350 gm) were anesthetized by injection of 20 to 25 mg nembutal intraperitoneally. A cannula was tied into the small intestine and the intestinal lumen was washed with an oxygenated salt solution (*control* solution) of the following composition (millimolar): Na, 188; K, 6; Ca, 0.5; Mg, 0.5; Cl, 196; glucose, 5.6; total, 340 mOsM.

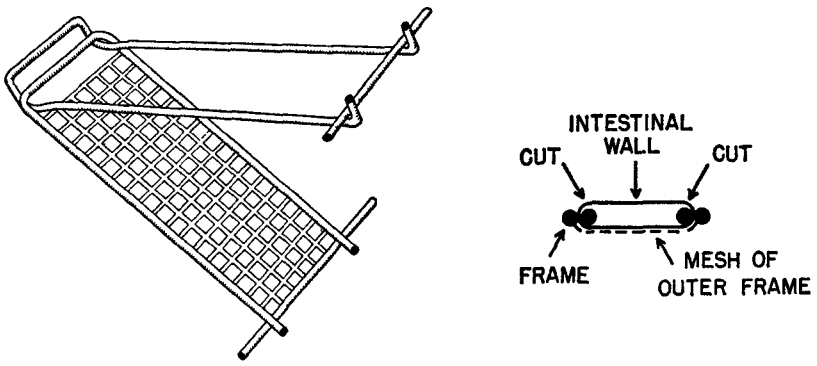

FIGURE 1. Schematic drawing showing wire frame for mounting tissue strip.

When the tissue was prepared for measurement, the apparatus illustrated in Fig. 1 was used: (*a*) to minimize the effects of smooth muscle contractions on the exposed surface area; and (*b*) to expose the maximal number of epithelial cells. A 2 cm length of jejunum was slipped over an inner frame of wire, and stretched by a 26 gm weight. The inner frame was clamped into an outer frame and held to it by rubber bands. The upper half of the intestine was then cut away above the frame so that the mucosal surface of the lower half became accessible. This procedure was completed within 2 to 3 minutes after interruption of the blood supply. The tissue was immersed in oxygenated control solution at room temperature while these manipulations were carried out.

Fig. 2 shows the arrangement by which the solutions were directed onto the exposed mucosal surface. A constant rapid flow was ensured by keeping the 2 liter solution bottles under 4 to 5 pounds/in^2 of oxygen. Solutions could be switched rapidly by the use of toggle switches (2), half a second being required to clear the tubing of previous solution. After the jet of streaming fluid impinged on the tissue, it flowed down the exposed mucosal tissue surface. The 50–60° angle of inclination of the tissue to the vertical ensured that the whole mucosal surface was covered by the solution, which did not come in contact with the serosal face. At the lower end of the tissue, a liquid bridge was formed to a stationary cotton wick whose effluent dropped into a funnel and was sucked away by a water pump. Since the restoring torque applied

to the balance kept the tissue support a constant distance from the cotton wick, the length of the fluid bridge remained constant, independent of the weight of the tissue. To compensate for the forces arising from the pressure of the jet at the upper end and the surface tension of the fluid bridge at the lower end of the tissue strip, the balance was counterweighted with a coarse and a fine balance chain. Before each experiment the effect of viscosity differences among solutions was eliminated by adjusting the heights of the storage bottles so that no significant base line changes were introduced when switching from one solution to another, using a filter paper dummy instead of tissue.

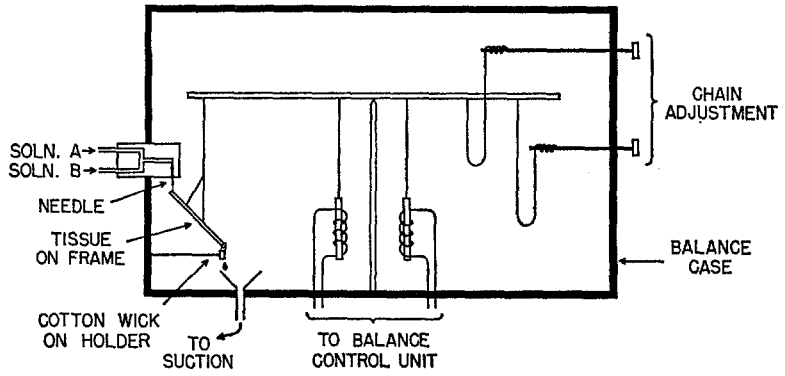

FIGURE 2. Schematic drawing showing mounting of tissue frame on automatic recording balance.

The lipid-insoluble non-electrolyte molecules (reagent grade), sucrose, mannitol, erythritol, urea, ethylene glycol, and formamide, replaced 230 mOsM of the NaCl in the control solution. All solutions were made up from a *base* solution containing the following components (millimolar): Na, 50.5; K, 6; Ca, 0.5; Mg, 0.5; Cl, 58.5; glucose, 5.6. The osmolarity of the electrolyte solutions was checked by freezing point determinations. Non-electrolyte was added to this base solution up to a maximal total osmolarity of 660 mOsM. To determine the water permeability all constituents in the base solution were unchanged except for NaCl, which was varied to achieve total salt concentrations from 100 to 1000 mOsM.

Detailed Experimental Procedure

As soon as the tissue was mounted, the stream of control solution was turned on, the balance was equilibrated by adjusting the chains, and the recorder was started. A constant tissue weight was recorded, usually within 1 minute, though occasionally 3 to 4 minutes were required. When osmotic equilibrium was reached, as indicated by constant tissue weight for 30 to 60 seconds, the streaming medium was changed. Within 10 to 15 seconds after switching, a constant rate of weight change was observed, which diminished after 30 seconds to 2 minutes, according to the composition of the new medium. Upon return to the control solution the direction of weight change

was reversed, but frequently the original base line was not reached; nor did a second exposure of the tissue to the test solution necessarily show the same rate of weight change. Generally, the response of the tissue diminished with increasing number of trials. Consequently, each piece of tissue served to determine only a single experimental point.

Contractions in the smooth muscle layer of the intestinal tissue complicated many of the measurements. Contractions tended to decrease, and relaxations to increase the amount of fluid covering the tissue. Therefore, solutions were switched during a moment of relative inactivity, and all records were rejected in which major contrac-

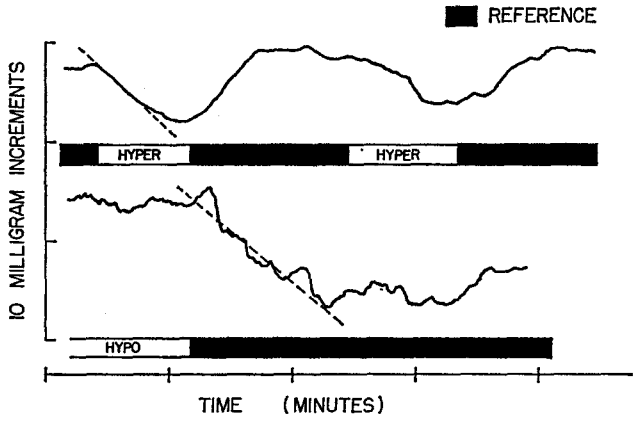

FIGURE 3. Typical recordings of tissue weight changes as a function of the osmolarity of the test solution. The top record is typical of a tissue strip with small contractions. The bottom record shows a tissue strip with major contractions; data showing these large contractions were rejected.

tions tended to obscure the linear rate of weight change. Fig. 3 shows examples of small and large contractions in typical records.

When the experiment was complete, those parts of tissue that had been exposed to the fluid were cut free from the frame and dried to constant weight. In three experiments on rats whose intestinal mucosa had not been stretched, an average dry weight of 28.8 mg/(cm length) (30.0, 28.5, and 27.8 mg/cm) was obtained. When this figure is combined with Fisher and Parsons' value (3) for the surface area in rat jejunum, 7 cm^2/(cm length), the weight change may be expressed in terms of the rate of volume change (v in cm^3) per unit area (A in cm^2) as $[(dv/dt)/A]$ with units of cm/sec. (1 cm/sec. = 1.46×10^4 mg/min., mg dry weight). This conversion assumes that the weight change is caused by movement of fluid with the density of water, which is taken as 1.

In a set of preliminary investigations, osmotic responses were also studied in rat stomach and colon. No clear cut results were obtained in these tissues, an observation which has been tentatively ascribed to the inaccessibility of many of the epithelial cells to the test solutions. These findings are in agreement with previous observations that short exposure to distilled water is without effect in the colon (4) though it

impedes further absorption of salts from the small intestine (5, 6). Magee and Reid (7) in 1931 showed that changes in luminal osmotic pressure influenced the appearance, mobility, and absorptive capacity of the small intestinal villi. In 1940, Dennis (8) made microscopic observations of the *in vivo* effect of distilled water on the mucosa in dog small intestine and colon. He also studied histological slices obtained from stomach, duodenum, jejunum, ileum, and colon. The small intestine alone showed a clear increase in epithelial cell volume. Dennis observed that the epithelial layer in this tissue doubled in thickness within 1 or 2 minutes after washing with distilled water. He described "a thickening of the epithelial cells near the ends of the villi where contact with the distilled water had been most intimate, while deeper in the crypts and on most of the sides of the villi, the cells still looked normal." This observa-

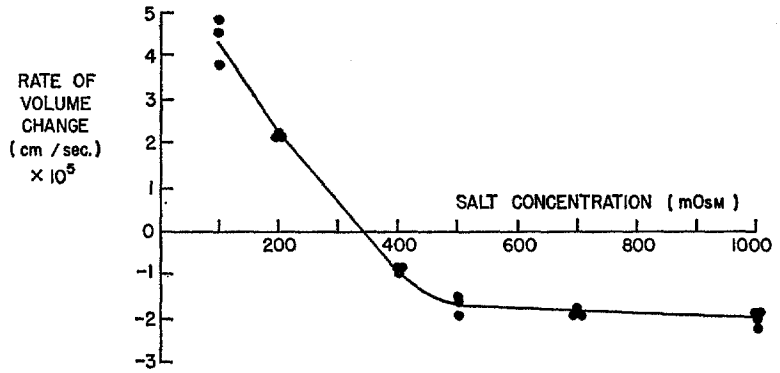

FIGURE 4. Rate of volume change of tissue as a function of the concentration of salt solution (base solution plus NaCl).

tion reflects the degree of contact of the mucosal cells with the test solution, and suggests that the degree of swelling is by no means uniform for all the cells in the epithelial membrane. When the tissue is exposed to distilled water, it might be possible for the cells at the tip of the villi to burst before those in the crypts had swollen appreciably. In consequence, we have restricted our observations to solutions no more dilute than 100 mOsM and have made our studies with the least possible distortion of cell volume.

RESULTS AND DISCUSSION

It was first necessary to establish the response of the tissue to changes in NaCl concentration. The results presented in Fig. 4 show that the intitial rate of volume change is approximately linear between 100 and 400 mOsM; above 500 mOsM the rate remains approximately constant.

The question arises whether the observed weight change may be attributed to the mucosal cells alone or whether it involves the entire tissue. In order to examine this point, experiments were carried out in which the luminal surface of the tissue was exposed to a hypotonic urea test solution for a period of about

1 minute, which represents the approximate time of observation for our experiments. At the close of this period, the tissue was blotted and the mucosa separated from the muscularis by the method of Dickens and Weil-Malherbe (9). The two segments of the tissue were then weighed separately on a quartz helix balance (Misco). To correct for evaporation a stop-watch was started

TABLE I

COMPARISON OF WATER MOVEMENT INTO MUCOSA AND MUSCULARIS

Composition of solution*		Wet weight‡ / Dry weight	
Salt	Urea	Mucosa	Muscularis
mOsM			
340		4.25 ± 0.08 (9)	4.08 ± 0.10 (10)
110	40	4.72 ± 0.04 (13)	3.95 ± 0.07 (8)

* The top line refers to the control solution; the second line refers to base solution plus urea.
‡ Errors are standard errors of the mean. Numbers of experiments are in parentheses.

TABLE II

P_w FOR MUCOSAL CELLS

Concentration difference*	Initial rate of water flow	P_w
$Os_M/cm^3 \times 10^4$	$cm/sec. \times 10^5$	$cm^4/Os_M\ sec.$
+1.40	+2.20	0.157
+1.40	+2.20	0.157
+1.40	+2.26	0.161
−0.60	−0.82	0.134
−0.60	−0.82	0.134
−0.60	−0.96	0.160
		Mean 0.151 ± 0.005

* Concentration of control solution minus concentration of test solution.

during the blotting procedure and stopped at the time of weighing; further drying was then measured during a subsequent 1 minute period and appropriate corrections were made. The dry weight was obtained on the same balance after drying to constant weight under an infrared lamp. The data in Table I show that the change in wet to dry weight ratio is restricted to the mucosal cells, the water content of the muscularis remaining constant during the experiment. This result indicates that the observed weight changes can be attributed to the mucosal cells alone.

The permeability coefficient of the tissue to water has been determined from the data closest to zero volume flow when the tissue was exposed to

slightly anisotonic solutions (base solution plus NaCl). Table II gives the data from which a mean water permeability coefficient, P_w = 0.15 cm⁴/OSM, sec. may be calculated. Since the stretching procedure does not expose all the cells in the mucosa equally to the salt solution, the coefficient probably represents a lower limit to the real value. Nonetheless, it is interesting to observe that the permeability coefficient is very little lower than the value of 0.23 cm⁴/OSM, sec. given by Sidel and Solomon (2) for human red cells, which Jacobs (10) some years ago considered particularly large. Dick (11) gives a comparison of permeability coefficients for a variety of tissues.

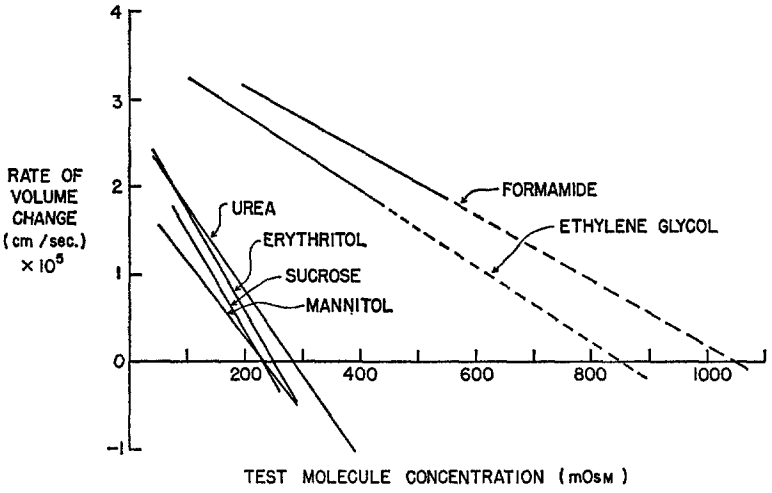

FIGURE 5. Rate of volume change for the test molecules as a function of the concentration of the test molecule (excluding the contribution of the salts in the base solution). The lines are regression lines drawn through the data from the number of experiments shown in Table III. The dotted lines are extrapolations in those cases in which there were no experiments in hypertonic solutions.

Data for determination of the equivalent pore radius are derived from the values for the interpolated or extrapolated concentrations at which the lipid-insoluble non-electrolyte test molecules caused zero water movement at zero time as determined from the regression lines in Fig. 5 (Table III, column 2). These experiments were restricted to the concentration range in which the rate of weight change bears an approximately linear relation to salt concentration (Fig. 4). The test molecule concentrations were chosen in order to allow for the most accurate interpolation (or extrapolation) through the zero rate of weight change. At least three experiments were carried out with each test molecule at each concentration. The Staverman reflection coefficient, σ, for the test molecules may be calculated from the data presented in Table III using Equation 1 of Goldstein and Solomon (1). For this purpose, it is neces-

sary to divide the NaCl osmolarity difference between control and base solution (230 mOsM) by the concentration of test solute that causes zero initial weight change. Table III also includes available data on the olive oil-water partition coefficients of the test molecules and estimates of the molecular radius as determined from Stuart-Briegleb models (12).

The calculation of σ for the test molecules assumes that the major electrolyte in the control solution, NaCl, behaves like an impermeable probing molecule, as far as osmotic balance is concerned. This assumption is supported by the observation that the zero weight change concentrations for mannitol and sucrose are 232 and 233 mOsM when they replace 230 mOsM of control solution NaCl.

TABLE III

DETERMINATION OF σ FOR LIPID-INSOLUBLE NON-ELECTROLYTES

Molecule	Concentration of test molecule for zero water movement	σ	Molecular radius	Olive oil/water partition coeficient
	mM		Å	$\times 10^3$
Sucrose	232 ± 15 (17)	0.99	4.5	—
Mannitol	233 ± 11 (20)	0.99	4.0	—
Erythritol	248 ± 9 (27)	0.93	3.2	0.03
Urea	284 ± 8 (29)	0.81	2.3	0.15
Ethylene glycol	850 ± 320 (11)	0.27	2.3	0.49
Formamide	1050 ± 170 (20)	0.22	2.2	0.8

Changes in medium Cl concentration which occur when the control solution is replaced by the test solution may be expected to cause shifts in cell volume, as is well known in the case of the red blood cell (13). In order to minimize this effect, 58.5 mM Cl was present in all the test solutions. The agreement between the values for zero weight change for mannitol and sucrose and that for NaCl suggests that the Cl shift probably does not cause a significant shift in the intercept. On the other hand, the slopes of the regression lines in Fig. 5 appear to be sensitive to these large changes in electrolyte concentration. Permeability coefficients for water, calculated from these slopes for urea, erythritol, and sucrose, lie in the range of 0.11 to 0.12 cm^4/OsM, sec., after correction for the appropriate reflection coefficients. These values are well below the figure of 0.15 cm^4/OsM, sec. obtained with NaCl. The fact that the coefficients obtained with three such diverse organic molecules lie so close together supports the conclusion that the effect is related to the shift in total electrolyte concentration. It may be caused by the instantaneous water shifts consequent to the displacement in medium Cl concentration or by changes in membrane permeability due to changes in ionic strength or Na concentration. Though the permeability coefficient calculated from the mannitol data, 0.087 cm^4/OsM, sec. lies below that obtained with the other non-electrolytes, the

standard error in this case is 0.02 cm⁴/OSM, sec., and the difference is hardly significant. In view of these considerations, the water permeability coefficient has been determined with NaCl rather than with non-electrolyte molecules.

The data in Table III may be used to calculate an equivalent pore radius on the basis of the assumptions discussed by Goldstein and Solomon (1). As Fig. 6 shows, the four largest molecules studied would suggest an assignment of 4 Å for the equivalent pore radius. Examination of Table III shows that the values of σ for both mannitol and sucrose, molecules with radii of 4 Å or greater, are not different from 1, whereas all the smaller molecules are characterized by σ's that appear to differ from 1. In consequence, we should expect the equivalent pore radius to lie between the 3.2 Å radius of erythritol and the

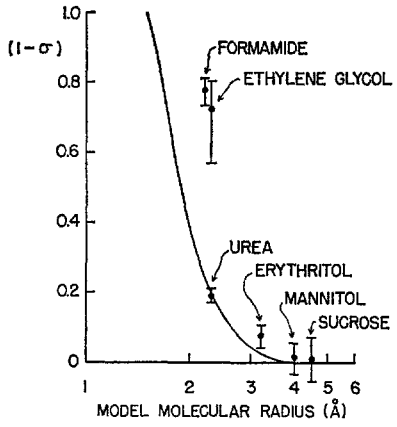

FIGURE 6. $1-\sigma$ (mean and standard error) as a function of the molecular radius of the test molecules. The curve has been drawn for a 4.0 Å equivalent pore radius.

4.0 Å radius of mannitol. The assignment of a 4 Å equivalent pore radius is therefore in agreement with the data, independent of the assumptions inherent in the treatment of Goldstein and Solomon.

Two of the molecules studied, formamide and ethylene glycol, have values of σ which differ markedly from the predicted values according to the curves given by Goldstein and Solomon. Results with these molecules show much more variability than do the other molecules studied as can be seen from the standard errors given in Table III. The reason for this behavior is not clear, particularly since σ for ethylene glycol in the human red cell membrane lies in the expected range. Examination of the last column in Table III reveals that ethylene glycol and formamide have the highest lipid solubility of all the molecules used. Though the olive oil–water partition coefficient for ethylene glycol is only 0.00049, it is three times higher than that of the next most lipid-soluble molecule used. The fraction of the membrane area occupied by pores can be quite small, lying in the range of 0.01 to 1 per cent in human red cells (14). As the fractional equivalent pore area is made smaller, the lipid solubility of a probing molecule becomes progressively more critical. For the

more lipid-soluble molecules, the question of the relevance of olive oil/water partition coefficients to actual membrane solubility should also be taken into account. As the molecules become progressively less lipid-soluble, entrance through the pores completely overshadows the route through the membrane and the exact determination of the true membrane solubility becomes unimportant. Thus it would seem possible that the aberrant behavior of ethylene glycol and formamide reflects their solubility in the cellular membrane.

In sum, a method has been devised to measure the permeability to water of the luminal surface of the intestinal mucosa in the rat and to characterize this face of the cell by an equivalent pore radius. The water permeability coefficient is quite high which would accord well with the function of the intestinal mucosa in conserving the water balance in the body. The 4 Å equivalent pore radius lies in the same range with values of this parameter measured in other tissues (1, 15, 16).

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BIBLIOGRAPHY

1. GOLDSTEIN, D. A., and SOLOMON, A. K., *J. Gen. Physiol.*, 1960, **44**, 1.
2. SIDEL, V. W., and SOLOMON, A. K., *J. Gen. Physiol.*, 1957, **41**, 243.
3. FISHER, R. B., and PARSONS, D. S., *J. Anat.*, 1950, **84**, 272.
4. GOLDSCHMIDT, S., and DAYTON, A. B., *Am. J. Physiol.*, 1919, **48**, 459.
5. REID, E. W., *J. Physiol.*, 1898, **22**, lvi.
6. BLICKENSTAFF, D. D., *Am. J. Physiol.*, 1954, **179**, 467.
7. MAGEE, H. E., and REID, E., *J. Physiol.*, 1931, **73**, 163.
8. DENNIS, C., *Am. J. Physiol.*, 1940, **129**, 171.
9. DICKENS, F., and WEIL-MALHERBE, H., *Biochem. J.*, 1941, **35**, 7.
10. JACOBS, M. H., *Ann. New York Acad. Sc.*, 1950, **50**, 824.
11. DICK, D. A. T., *Internat. Rev. Cytol.*, 1959, **8**, 387.
12. SCHULTZ, S. G., and SOLOMON, A. K., *J. Gen. Physiol.*, 1961, **44**, 1189.
13. JACOBS, M. H., *J. Appl. Physics*, 1938, **9**, 81.
14. PAGANELLI, C. V., and SOLOMON, A. K., *J. Gen. Physiol.*, 1957, **41**, 259.
15. WHITTEMBURY, G., SUGINO, N., and SOLOMON, A. K., *J. Gen. Physiol.*, 1961, **44**, 689.
16. VILLEGAS, R., and BARNOLA, F. V., *J. Gen. Physiol.*, 1961, **44**, 963.