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Comparison of Nonelectrolyte Permeability Patterns in Several Epithelia

Dickson J. Hingson and Jared M. Diamond

Physiology Department, University of California Medical Center, Los Angeles, California 90024

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Summary. Reflection coefficients (σ's) have been determined for 13 to 36 nonelectrolytes in goldfish gallbladder, bullfrog gallbladder, bullfrog intestine, and guinea-pig intestine. These results have been compared with the results of similar previous studies in rabbit gallbladder, bullfrog choroid plexus, and guinea-pig gallbladder to determine types of species variation, organ variation, and individual variation. Two principal types of variation were established: (1) Branched solutes are much less permeant than straight-chain analogues in gallbladders of all four species studied, whereas this effect is small in intestine and negligible in choroid plexus. This variation in degree of discrimination against branched solutes is attributed to variation in closeness of packing of membrane lipids. (2) In certain epithelia, small polar solutes are more permeant than expected from their bulk nonpolar-solvent/water partition coefficients and from their size. This feature is very marked in rabbit gallbladder, somewhat marked in guinea-pig gallbladder and intestine and in bullfrog choroid plexus, apparent mainly just for formamide (the smallest polar nonelectrolyte) in bullfrog intestine, and virtually absent in goldfish and bullfrog gallbladders. Analysis of individual variability in preparations of guinea-pig intestine and rabbit gallbladder from different animals shows covariation in σ's of small polar nonelectrolytes uncorrelated with variation in σ's of other solutes. Small polar solutes, in epithelia where their permeability is as expected from size and from nonpolar-solvent/water partition coefficients, resemble other solutes in having markedly temperature-dependent σ's (high apparent activation energies of permeation), but have nearly temperature-independent σ's (low activation energies, as for diffusion in aqueous solution) in epithelia where their permeability is enhanced. These findings suggest that additional size-restricted permeation pathways by-passing membrane lipid ("pores") are present in some tissues and smaller or virtually absent in others.

The general pattern of nonelectrolyte permeation through apparently all cell membranes is that most solutes permeate via membrane lipids, while a few small polar solutes may also permeate via polar regions of the membrane ("pores"). However, within this general scheme it is obvious that there are detailed differences between the permeability patterns of different membranes. For example, discrimination between branched and straight-chain

solutes is marked in rabbit gallbladder (Wright & Diamond, 1969*b*), but negligible in bullfrog choroid plexus (Wright & Prather, 1970); Collander (1954) found two individual cells of the alga *Nitella mucronata* which were exceptionally permeable to small polar solutes but showed normal permeabilities to other solutes; some plant species are exceptionally permeable to basic solutes but normal in other respects (Collander, 1937); and dog red cells are more permeable than human red cells to most nonelectrolytes tested (Rich, Sha'afi, Barton & Solomon, 1967; Sha'afi, Gary-Bobo & Solomon, 1971). Systematic study of this variation may assist in identifying the molecular features of membrane structure controlling nonelectrolyte permeation. In the present paper, we have therefore determined nonelectrolyte permeability patterns in four epithelia: goldfish gallbladder, bullfrog gallbladder, bullfrog intestine, and guinea-pig intestine. Previous studies by the same method exist for three other epithelia: rabbit gallbladder (Wright & Diamond, 1969*a*, 1969*b*), bullfrog choroid plexus (Wright & Prather, 1970), and guinea-pig gallbladder (H. B. Irvine, *personal communication*). Thus, the results permit comparisons between different epithelial organs in the same species, between the same organ in different species, and between the same organ in different individuals of the same species.

Theory

The principle of the experiments consists of determining so-called reflection coefficients (σ's) for nonelectrolytes by means of streaming potentials. The present section summarizes the underlying theory as background for interpreting the experimental curves of Fig. 3*a–g* and Fig. 4*a–e*. More detailed accounts of the method and theory were given by Wright and Diamond (1969*a*) and Smulders and Wright (1971).

It is found empirically that the rate of osmotic flow set up across a membrane by a solute concentration gradient is lower, the more permeant the solute; i.e., that osmotic flow is proportional to $\sigma RT\Delta C$, where ΔC is the concentration gradient, R the gas constant, T the absolute temperature, and σ the reflection coefficient. σ, a parameter characteristic of a particular solute in a particular membrane, equals 1.0 for an impermeant solute, and for increasingly permeant solutes becomes progressively less than 1 and closer to zero. A solute more permeant than water itself has a negative value of σ (i.e., net volume flow is from concentrated to dilute solution).

The theoretical basis for σ values less than 1.0 in perfectly stirred systems is twofold: the diffusion of a permeant solute down its concentration gradient from concentrated to dilute solution represents a volume flow opposite to the osmotic volume flow of water from dilute to concentrated solution; and the diffusing solute may drag some water from concentrated to dilute solution, if solute and water interact (e.g., frictionally) in crossing the membrane. Thus, the expression derived for the reflection coefficient from irreversible thermodynamics (Kedem & Katchalsky, 1958; Dainty & Ginzburg, 1963) is:

$$\sigma' = 1 - (P_s v_s / P_{\text{osm}}) - k f_{sw} / (f_{sw} + f_{sm})\, \phi_w \qquad (1)$$

where P_s is the solute permeability coefficient (cm/sec), v_s is the partial molar volume of solute (cc/mole), P_{osm} is the osmotic water permeability or hydraulic conductivity (cc/cm^2, sec, mole/cc), k is a partition coefficient, the f's are frictional coefficients between solute and water (f_{sw}) or solute and membrane (f_{sm}), ϕ_w is the volume fraction of water in the membrane, and the reflection coefficient measured under conditions of perfect stirring is written as σ' to distinguish it from the practical values discussed below. In Eq. (1), the term $P_s v_s/P_{osm}$ (often written $\omega v_s/L_p$ where $\omega\ RT \equiv P_s$, $RTL_p \equiv P_{osm}$) represents the reduction in σ below 1 due to the volume of diffusing solute, while $kf_{sw}/(f_{sw}+f_{sm})\phi_w$ represents the reduction in σ due to the volume of dragged water.

Empirically, measurements of $(1-\sigma)$ are found to yield virtually the same permeability sequences of most solutes as measurements of P_s (or $P_s v_s$) in *Nitella* (Collander, 1954, Table 4, columns 2 and 3) and in rabbit gallbladder (Smulders & Wright, 1971, Fig. 3). The same conclusion is implied by the close correlation between σ's and partition coefficients (Figs. 3 and 4) for most solutes in the four epithelia studied in the present paper. This means that, for most solutes in these membranes, the water-drag term $kf_{sw}/(f_{sw}+f_{sm})\phi_w$ is either 0 or else closely correlated with P_s. The former interpretation has been shown to be the correct one in the alga *Valonia ventricosa*, which by other criteria lacks pores and for which Eq. (1) holds (Gutknecht, 1968). However, the water-drag term is significant and bears a somewhat complex relation to P_s for most solutes (except for one with high solubility in the membrane matrix) in an artificial cellulose acetate membrane known to be porous (DiPolo, Sha'afi & Solomon, 1970, Fig. 3), and for three or four small polar solutes postulated to permeate through pores in human erythrocyte (Sha'afi *et al.*, 1971) and dog erythrocyte (as calculated by combining data in Sha'afi *et al.*, 1971, Table 1, and Rich *et al.*, 1967, Table 6). The significance of these facts for permeation studies in mosaic biological membranes is that $(1-\sigma)$ is a good measure of permeability for solutes permeating via membrane lipid, i.e., for most solutes; and that the σ values lower than expected from lipid/water partition coefficients and molecular size for small polar solutes, because of permeation through pores, may reflect not only a component of P_s that is independent of lipid/water partition coefficients, but also a significant water-drag component of σ.

Eq. (1) applies to systems with perfect stirring right up to the membrane. When (as is usually the case in practice) significant unstirred layers exist on both sides of the membrane, two unstirred-layer effects cause a further shift in σ towards zero. (1) Part of the solute concentration gradient is dissipated in the unstirred layers rather than across the membrane. It may be shown that if the total concentration drop between the bulk bathing solutions is ΔC, the actual drop across the membrane is only $(\Delta C)(1/P_s)(1/P_s+\delta/D)$, where δ is the sum of the thicknesses of the two unstirred layers, D the solute diffusion coefficient in the unstirred layers (assumed to have the same value in both unstirred layers), and P_s is still the solute permeability coefficient (the true one corrected for the unstirred-layer effect, not the uncorrected apparent value). Combining this expression with Eq. (1) and assuming the water-drag term to be negligible, one obtains an expression for apparent uncorrected σ's as modified by this unstirred-layer effect:

$$\sigma = \left(\frac{1}{P_s} - \frac{v_s}{P_{osm}}\right) \Big/ \left(\frac{1}{P_s} + \frac{\delta}{D}\right). \tag{2}$$

In Eq. (2), P_s and P_{osm} are both true membrane values corrected for unstirred-layer effects. (2) If the linear rate of volume flow V is sufficiently high, a "sweeping-away" effect in unstirred layers reduces the effective solute gradient at the membrane further, since the volume flow from dilute to concentrated solution dilutes the solute immediately adjacent to the membrane on the concentrated side by the factor $e^{V\delta/D}$ and concentrates the solute immediately adjacent to the membrane on the dilute side by the same factor.

This effect is negligible under the conditions used for σ determinations in rabbit gallbladder, as shown by the experimental findings that the same σ values are obtained in the presence and absence of flow (Wright & Diamond, 1969*a*), that the relation between gradient and flow is linear over the concentration range used, and that no sweeping-away effect can be detected even at higher flow rates (Smulders, Tormey & Wright, 1972). Eq. (2) is similar but not identical to an equation derived previously by Smulders and Wright [1971, Eq. (10)].[1] Even these equations provide only an approximate description of the unstirred-layer effects, since they assume a steady state, whereas σ values in the present study and in the previous epithelial studies cited for comparison have been obtained from peak streaming potentials under quasi-steady-state conditions, after initial equilibration of the mucosal unstirred layer but before significant equilibration of the serosal unstirred layer (p. 100).

Figs. 1 and 2 display the relation calculated from Eq. (2) between σ (uncorrected for unstirred-layer effects) and P_s (corrected for unstirred-layer effects), for different

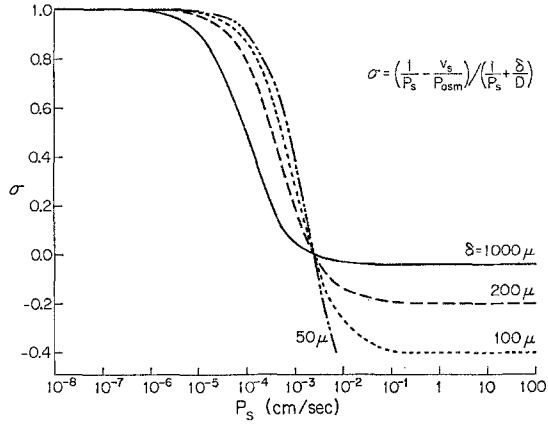


Fig. 1. Theoretical relation between σ (reflection coefficients uncorrected for unstirred-layer effects) and P_s (permeability coefficients corrected for unstirred-layer effects), as calculated from Eq. (2). Values of parameters are: solute diffusion coefficient $D = 10^{-5}$ cm^2/sec, solute partial molar volume $v_s = 100$ cc/mole, the approximate values expected for a nonelectrolyte of molecular weight 100 at room temperature; osmotic water permeability $P_{osm} = 0.25$ cc/cm^2, sec, mole/cc; unstirred-layer thickness $\delta = 50$, 100, 200, or 1,000 μ as indicated beside each curve. Note that with increasing δ the asymptotic value of σ for large P_s becomes less negative, the value of P_s at which σ equals 0.5 becomes smaller, and the curve becomes less steep

1 Smulders and Wright (1971, Appendix) obtain their Eq. (9) by combining their Eq. (6), which is the same as our Eq. (1) without the water-drag term, and their Eq. (8). Their Eqs. (6) and (8) are valid when their symbols σ, P, P_{H_2O}, P_s, and L_p in these equations stand for true membrane values corrected for unstirred layers and when Eq. (8) is generalized by multiplying the right-hand side by n where $n \geqq 1$ for porous membranes (Sha'afi, Rich, Sidel, Bossert & Solomon, 1967, p. 1394). Smulders and Wright implicitly assume that equations of the same form as Eqs. (6), (8), and (9) hold for experimental values uncorrected for unstirred layers [their symbol σ' in Eq. (10) and P in Eq. (2)] and substitute their Eq. (2) for water and for solute into this implicit Eq. (9) to arrive at their final Eq. (10). This assumption is not correct, and leads to their prediction of positive asymptotes.

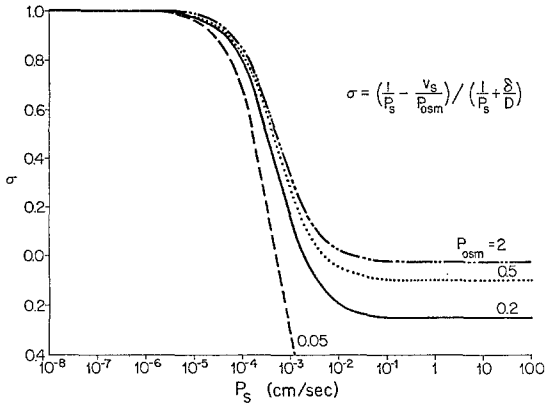


Fig. 2. As Fig. 1, except $\delta = 200\,\mu$; $P_{osm} = 0.05$, 0.2, 0.5, or 2 cc/cm², sec, mole/cc, as indicated beside each curve. Note that with increasing P_{osm} the asymptotic value of σ for large P_s becomes less negative, the value of P_s at which σ equals 0.5 becomes slightly larger, and the curve becomes less steep

values of δ (Fig. 1) or of P_{osm} (Fig. 2). These figures are similar to Figs. 6 and 7 of Smulders and Wright (1971), except that their equation and figures predict the possibility of positive asymptotic σ's and ours do not (*see* footnote 1). Reference will be made to these theoretical figures in comparing the experimental figures for different epithelia. σ equals 1.0 for low values of P_s, decreases with increasing P_s, and asymptotically approaches zero or a negative value for very high P_s. The existence of this asymptotic value of σ [$-v_s D/P_{osm}\delta$, from Eq. (2)] is caused by the rate-limiting effect of unstirred layers for highly permeant solutes; σ' (i.e., values corrected for unstirred layers) does not approach an asymptote but continues to go negative with increasing P_s. The asymptotic value is closer to zero and less negative for larger values of δ or of P_{osm}. The value of P_s at which σ equals 0.5 depends significantly upon the value of δ, but to a lesser extent upon the value of P_{osm}. Thus, if one measures σ's uncorrected for unstirred layers, differences between patterns for different membranes (e.g., Fig. 3*a–g* of this paper) may reflect differences not only in absolute magnitudes of permeability coefficients P_s, but also in δ and P_{osm}. For the epithelia used in the present study we have measured δ at the mucosal surface, but not P_{osm}, since the meaning of P_{osm} measurements in epithelia is presently uncertain (Wright, Smulders & Tormey, 1972), nor δ at the serosal surface, since σ's are measured before significant equilibration of the serosal unstirred layer. Without knowledge of P_{osm}, measurements of σ uncorrected for unstirred layers, as in the present study, permit no conclusions about possible differences in absolute permeability coefficients between different tissues, though they do permit conclusions about types of relative differences (pp. 112–116 and 122–128).

σ for a test molecule in a given membrane may be calculated as the quotient of the osmotic flow rate caused by a given gradient of the test solute divided by the osmotic flow rate caused by the same gradient of an impermeant solute. In epithelia such as the gallbladder, intestine, and choroid plexus, osmotic flow is associated with an electrical potential difference called a streaming potential, whose magnitude is related monotonically and (up to 0.1 M gradients) approximately linearly to the flow rate or effective osmotic gradient, and which arises principally as a boundary diffusion potential (Wedner & Diamond, 1969). Hence, σ can be rapidly determined as the quotient of the

streaming potential caused by a given gradient of the test solute divided by the streaming potential caused by the same gradient of an impermeant reference solute.[2]

Smulders and Wright (1971) showed that σ's obtained by this method in rabbit gallbladder correlated well with values of P_s measured with radioactive tracers and corrected for unstirred-layer effects, the form of the experimental relation being similar to the theoretical curves of Figs. 1 and 2. Smyth and Wright (1966) obtained virtually the same σ's in rat intestine by this electrical method as by a gravimetric procedure for measuring flow (Lindemann & Solomon, 1962). Collander (1954) found close agreement in *Nitella* between sequences of σ's obtained by an "osmotic-contraction" method similar in design to the present method and sequences of P_s values obtained by direct chemical analysis. Further discussion of the method may be found in papers by Wright and Diamond (1969*a*), Diamond and Wright (1969*b*), and Smulders and Wright (1971).

Materials and Methods

Dissection

Experimental material consisted of gallbladders and intestines from bullfrogs (*Rana catesbiana*) weighing ca. 350 g, gallbladders from goldfish (*Carassius auratus*) weighing ca. 225 g, and intestines from guinea-pigs weighing 300 to 500 g. Gallbladders were dissected, washed free of bile, everted, and cannulated by techniques described previously (Diamond, 1964; Diamond & Harrison, 1966). Small intestines were flushed *in situ* with a Ringer's solution, then a segment 3 cm long taken half-way between the start of the duodenum and the ileocaecal valve was excised, ligated at one end, everted with a glass rod, and cannulated with polyethylene tubing 0.22 cm in internal diameter. The intestine was tied to the cannula in a moderately stretched state so as to decrease the effective thickness of the unstirred layers and to minimize peristaltic contractions.

Experimental Techniques

Gallbladder and intestine in the *in-vivo* orientation consist of a single layer of epithelial cells facing the lumen (the so-called mucosal surface of the tissue), supported by a layer of connective tissue and muscle fibers on the outside (the so-called serosal surface). All experiments were performed in the everted orientation, in which the epithelial cells are in direct contact with the outer bathing solution. The outer solution was contained in a nearly full 30-ml beaker, was stirred by a stream of oxygen bubbles, and was changed by transferring the gallbladder or intestinal sac from one beaker of solution to the next, the procedure requiring less than 1 sec. The luminal solution remained unstirred. The electrical potential difference (abbreviated p.d.) between the

2 The usefulness of streaming potentials in σ measurements depends on their empirically observed relation to flow rates and is independent of their detailed mode of origin (Machen & Diamond, 1969; Wedner & Diamond, 1969; Wright *et al.*, 1972). The relation between flow rate and osmotic gradient becomes nonlinear for mucosa-hypertonic gradients of about 0.2 M or larger (Diamond, 1966; Wright *et al.*, 1972). The relation between streaming potential and flow rate becomes nonlinear for serosa-hypertonic gradients (Wright *et al.*, 1972), but only mucosa-hypertonic gradients were used in the present study. Streaming potentials caused by gradients of impermeant solutes in the gallbladder are stable with time in sac preparations but not in chamber preparations (Wright *et al.*, 1972); only sac preparations were used in the present study.

luminal and outer bathing solutions was measured to an accuracy of ± 0.01 mV by means of polyethylene bridges filled with 0.150 M NaCl in 4 % agar, connected to a Keithley 610B electrometer and Varian G11A recorder (Diamond & Harrison, 1966). Unless stated otherwise, experiments were conducted at room temperature of 21 to 24 °C, with the temperature held constant to ± 0.5 °C during any individual experiment. For determinations of σ's at different temperatures, the beaker containing the gallbladder or intestinal sac was placed in a water bath maintained at the desired temperature.

Solutions

The standard Ringer's solutions had the following molal compositions and osmolalities: guinea-pig, 0.148 M NaCl, 0.006 M KCl, 0.00025 M $CaCl_2$, 0.002125 M Na_2HPO_4, 0.000375 M NaH_2PO_4 (295 milliosmolal); bullfrog, 0.11 M NaCl, 0.001 M $CaCl_2$, 0.002125 M Na_2HPO_4, 0.000375 M NaH_2PO_4 (211 milliosmolal); goldfish, 0.114 M NaCl, 0.005 M KCl, 0.00025 M $CaCl_2$, 0.002125 M Na_2HPO_4, 0.000375 M NaH_2PO_4 (285 milliosmolal). All solutions were buffered at pH 7.3. Nonelectrolytes whose σ's were to be determined were added to the Ringer's solutions at a concentration of 100 millimolal. All compounds were obtained in the highest purity commercially available, generally from either Eastman Organic Chemicals or Aldrich Chemical Company. Previous experience (Wright & Diamond, 1969*a*, 1969*b*) was used to restrict the choice of solutes to those which were stable in aqueous solution, pure, and yielded expected values of pH and osmolality. The osmolalities of all solutions were checked with a Fiske osmometer, and the resulting values were used to correct all σ values for apparent osmotic coefficients, as discussed previously (Wright & Diamond, 1969*b*, pp. 228–229).

Procedure for σ Determinations

A small p.d. was observed across the epithelia studied at the outset of experiments, the outer ("mucosal") solution being negative to the luminal solution. The magnitude of this p.d. was 1.4 ± 0.9 mV in three goldfish gallbladders, 1.2 ± 0.5 mV in eight bullfrog gallbladders, 0.7 ± 0.4 mV in 13 bullfrog intestines, and 3.2 ± 0.4 mV in nine guinea-pig intestines (these and all other measures of variation are expressed as standard deviations). These initial p.d.'s usually declined to virtually zero within the first or second hour.

σ's were determined by recording the streaming potential for a 0.1 M gradient of the impermeant reference solute sucrose or mannitol, then for a 0.1 M gradient of the test solute, and finally for a 0.1 M gradient of sucrose or mannitol again, and calculating σ as the ratio of the test streaming potential to the average of the preceding and following reference streaming potentials. In practice, this meant maintaining the composition of the luminal solution as Ringer's solution, and taking the outer solution through the following sequence of changes: Ringer's, Ringer's + 0.1 M sucrose (or mannitol), Ringer's, Ringer's + 0.1 M test solute, Ringer's, Ringer's + 0.1 M sucrose (or mannitol), Ringer's. This procedure has been illustrated previously (Wright & Diamond, 1969*a*, Fig. 1; Wright & Prather, 1970, Fig. 1). Streaming potentials were generally sufficiently reproducible that the value for sucrose or mannitol was determined after every second test compound rather than after each compound. The reference solute was taken as sucrose in bullfrog and goldfish gallbladders and bullfrog intestine, but was taken as mannitol in guinea-pig intestine, to avoid the p.d. coupled to active Na^+ transport which the hydrolysis products of sucrose stimulate in mammalian small intestine (Barry, Dikstein, Matthews, Smyth & Wright, 1964; Schultz & Zalusky, 1964). The other

carbohydrates chosen as test solutes in the intestine are not transported nor hydrolyzed in the adult. Between 6 and 51 σ's were determined in each individual experiment, depending upon how well the preparation lasted. The luminal solution was renewed after every second σ determination. The peak value of each streaming potential was attained after a transient caused by the solute diffusional delay in the unstirred layer at the mucosal surface of the epithelium (Wright & Diamond, 1969*a*, Fig. 1; Wright & Prather, 1970, Fig. 1). Streaming potentials for sucrose, mannitol, and relatively impermeant test solutes remained constant at the peak value during continued application of the gradient, while streaming potentials for more permeant solutes slowly declined because of solute leakage across the epithelium (*cf.* Wright & Diamond, 1969*a*, pp. 210–221; Barry & Diamond, 1970, Fig. 11). Repeated σ determinations in a given preparation showed that, over the course of an experiment lasting several hours, σ values declined on the average by 0.02 in bullfrog intestine and bullfrog gallbladder, and by 0.05 in guinea-pig intestine and goldfish gallbladder. Since these changes with time were too small to be significant in interpreting the results, all σ values over the course of an experiment were averaged.

The reference solute sucrose (or mannitol in guinea-pig intestine) was assumed to have a σ of 1.0 in these epithelia; i.e., to be relatively impermeant. In rabbit gallbladder (Diamond, 1964; Smulders & Wright, 1971) and bullfrog choroid plexus (Wright & Prather, 1970) this assumption is justified by the facts that the tracer flux of sucrose is very low and attributable to shunts rather than to native membrane,[3] that the streaming potentials of sucrose and mannitol are stable with time instead of slowly decaying as with permeant solutes, that many other solutes (38 in rabbit gallbladder, 18 in bullfrog choroid plexus) expected on chemical grounds to be poorly permeant give the same streaming potential as sucrose and mannitol within experimental error, and that no solute gives a higher streaming potential (or calculated σ). Tracer fluxes have not been measured in the four epithelia used in the present study, but the assumption of low sucrose (or mannitol) permeability is supported by the facts that the sucrose or mannitol streaming potential is stable with time, that in each preparation two to eight other solutes expected on chemical grounds to be poorly permeant (such as maltose, pentaerythritol, erythritol, and malonamide) give virtually the same streaming potential as sucrose or mannitol, and that no solute gives a higher streaming potential.

Table 1 gives, for each preparation, the mean peak value of the streaming potential caused by a 0.1 M gradient of sucrose (or mannitol), the mean half time for build-up of this streaming potential, and the thickness of the unstirred layer at the mucosal surface of the epithelium as calculated from this half time. The unstirred layer is thicker for the two intestines than for the three gallbladders, as expected on anatomical grounds from the greater folding of the intestinal mucosal surface. In goldfish gallbladder and the two intestinal preparations, the time course of each streaming potential was qualitatively similar to that illustrated previously for rabbit gallbladder or for bullfrog choroid plexus. In bullfrog gallbladder the form differed in that, after a peak at about 40 sec, the p.d. did not remain constant but instead gradually began to rise further after a slight dip. This gradual rise was apparently caused by osmotic withdrawal of water from the luminal solution itself and hence an increase in luminal salt concentration, since the rise could be reversed by renewing the luminal solution. Streaming potentials in bullfrog gallbladder were therefore taken as the values of the initial peak p.d. and were

3 Since the sucrose and inulin fluxes in rabbit gallbladder are in the ratio of the free-solution diffusion coefficients (Smulders & Wright, 1971), σ's in the shunts giving rise to these low fluxes should be zero for small nonelectrolytes, so that solute gradients could not cause a streaming potential in these shunts and they could not affect σ determinations for the selective membrane.

Table 1. Streaming potentials in epithelia

Epithelium	No. of expts.	Streaming potential (mV)	$t_{1/2}$ (sec)	δ (μ)	$(K_{ether} M^{-1/2})_{\sigma=0.5}$
Bullfrog gallbladder	7	4.8 ± 0.7	8 ± 1	104	9×10^{-3}
Guinea-pig gallbladder	6	—	10 ± 2	117	7×10^{-3}
Rabbit gallbladder	10	3.6 ± 0.9	10	117	5×10^{-3}
Goldfish gallbladder	3	5.0 ± 1.6	$11 \pm 2 \rightarrow 23 \pm 13$	$123 \rightarrow 177$	1.5×10^{-3}
Bullfrog intestine	11	7.7 ± 0.8	$57 \pm 12 \rightarrow 76 \pm 19$	$279 \rightarrow 322$	5×10^{-4}
Bullfrog choroid plexus	14	1.9 ± 0.6	68 ± 14	305	2.5×10^{-4}
Guinea-pig intestine	9	5.7 ± 0.4	62 ± 16	338	4×10^{-4}

The third column is the streaming potential produced by addition of 0.1 M sucrose (mannitol in the case of guinea-pig intestine) to the mucosal solution. The fourth column is the time after addition of sucrose for the p.d. to rise to half its peak value. This half time is a measure of the diffusional delay for sucrose to diffuse through the unstirred layer at the mucosal surface of the epithelium. From the expression $t_{1/2} = 0.38\ \delta^2/D$, the values of mucosal unstirred-layer thickness in the fifth column were calculated, using the value of $t_{1/2}$ in the fourth column and taking D as 5.2×10^{-6} cm^2/sec for sucrose and 7.0×10^{-6} cm^2/sec for mannitol. For goldfish gallbladder and bullfrog intestine, $t_{1/2}$ and δ increased somewhat with time after dissection; the arrows in the fourth and fifth columns connect initial and final values in experiments lasting several hours. Data for rabbit gallbladder are taken from Diamond (1966) and Diamond and Harrison (1966), for bullfrog choroid plexus from Wright and Prather (1970), for guinea-pig gallbladder from H. B. Irvine (*personal communication*), and for the other epithelia from the present study. The last column gives the value of $K_{ether} M^{-1/2}$ at which σ equals 0.5, read off the main patterns of Fig. 3*a–g* (*see* p. 115 for discussion).

terminated as soon as it was clear that this value had been reached. Regarding long-term stability, repeated determinations of streaming potentials in guinea-pig gallbladder showed that they began to decline after 1 hr, and experiments were terminated after 3 hr. In the other epithelia, streaming potentials were slightly higher at 2 to 3 hr after dissection than at earlier times and remained constant for several hours longer.

Results

Values of σ

σ's were measured for 13 solutes in goldfish gallbladder, 36 in bullfrog gallbladder, 35 in bullfrog intestine, and 20 in guinea-pig intestine. The list of solutes tested in each epithelium was designed to include solutes covering as wide a range of partition coefficients and molecular weights as possible, to characterize the relation between these variables and σ; and to include

Table 2. Reflection coefficients in four epithelia

Compound/Formula		M	K_{ether}	K_{oil}	Fish gallbladder	Bullfrog gallbladder	Bullfrog intestine	Guinea-pig intestine
One-carbon compounds								
Methanol	H_3COH	32.0	0.14	0.0078	—	0.14 ± 0.04 (2)	0.00 ± 0.01 (3)	—
Formamide	$HC(=O)-NH_2$	45.0	0.0014	0.00076	0.61 ± 0.13 (3)	0.96 ± 0.03 (4)	0.11 ± 0.01 (3)	0.05 ± 0.02 (3)
Urea	$H_2N-C(=O)-NH_2$	60.1	0.00047	0.00015	0.98 ± 0.05 (3)	1.05 ± 0.07 (5)	0.96 ± 0.03 (11)	0.48 ± 0.19 (9)
Thiourea	$H_2N-C(=S)-NH_2$	76.1	0.0063	0.0012	—	—	0.81 ± 0.08 (4)	—
Two-carbon compounds								
Ethanol	H_3C-CH_2-OH	46.1	0.26	0.032	—	0.11 ± 0.03 (3)	—	—
Ethylene glycol	$HO-CH_2-CH_2-OH$	62.1	0.0053	0.00049	0.80 ± 0.01 (2)	0.98 ± 0.01 (2)	0.10 ± 0.01 (4)	0.18 ± 0.01 (2)
Acetamide	$H_3C-C(=O)-NH_2$	59.1	0.0025	0.00083	0.71 ± 0.04 (3)	1.00 ± 0.04 (4)	0.45 ± 0.04 (3)	0.09 ± 0.04 (2)
Methyl urea	$H_3C-NH-C(=O)-NH_2$	74.1	0.0012	0.00044	0.92 (1)	1.02 ± 0.03 (5)	0.82 ± 0.03 (4)	0.40 ± 0.13 (7)
Three-carbon compounds								
Acetone	$H_3C-C(=O)-CH_3$	58.1	0.62	0.08	—	0.01 ± 0.01 (3)	—	—
n-Propanol	$H_3C-CH_2-CH_2-OH$	60.1	1.9	0.13	—	0.04 ± 0.03 (2)	—	—

Table 2. Continued

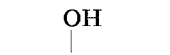
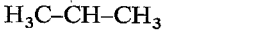
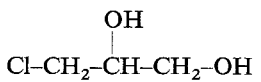
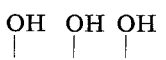
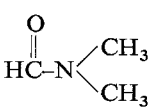
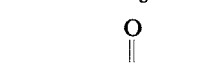
Compound/Formula	M	K_{ether}	K_{oil}	Fish gallbladder	Bullfrog gallbladder	Bullfrog intestine	Guinea-pig intestine
Isopropanol 	60.1	0.64	0.047	–	0.18 ± 0.03 (2)	–	–
1,3-Propanediol 	76.1	0.012	–	–	–	0.13 ± 0.02 (4)	–
3-Chloro-1,2-propanediol 	110.5	0.080	0.012	–	0.86 ± 0.02 (4)	–	–
Glycerol 	92.1	0.00066	0.00007	–	1.04 ± 0.01 (3)	0.64 ± 0.01 (3)	0.65 ± 0.06 (7)
Dimethyl formamide 	73.1	0.024	0.0049	–	–	0.05 ± 0.00 (4)	–
Proprionamide 	73.1	0.013	0.0036	0.49 ± 0.05 (2)	0.92 ± 0.01 (3)	0.22 ± 0.01 (3)	0.09 ± 0.05 (3)
Ethyl carbamate 	89.1	0.64	0.074	–	0.15 ± 0.05 (5)	–	–
sym-Dimethyl urea 	88.1	0.0029	–	–	–	0.41 ± 0.08 (4)	–
Ethyl urea 	88.1	0.0041	0.0017	–	–	0.60 ± 0.04 (4)	–

Table 2. Continued

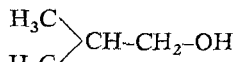
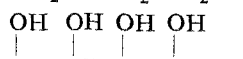
Compound/Formula	M	K_{ether}	K_{oil}	Fish gallbladder	Bullfrog gallbladder	Bullfrog intestine	Guinea-pig intestine
Malonamide 	102.1	0.00030	0.00008	—	1.09 ± 0.00 (4)	1.04 ± 0.03 (4)	1.01 ± 0.14 (3)
Four-carbon compounds							
n-Butanol $H_3C-CH_2-CH_2-CH_2-OH$	74.1	7.7	0.53	—	−0.03 ± 0.01 (3)	0.00 ± 0.01 (3)	−0.05 ± 0.03 (2)
Isobutanol 	74.1	6.9	0.44	—	—	—	—
Diethyl ether $H_3C-CH_2-O-CH_2-CH_3$	74.1	10	3.8	—	−0.04 (1)	—	—
Dioxane 	88.1	—	0.24	—	0.05 ± 0.01 (4)	0.00 ± 0.01 (3)	—
Ethyl acetate 	88.1	8.5	2.5	−0.04 (1)	−0.04 ± 0.01 (2)	−0.01 ± 0.01 (4)	—
2,3-Butanediol 	90.1	0.029	0.0034	—	0.92 ± 0.03 (3)	0.19 ± 0.02 (4)	0.11 ± 0.01 (2)
1,4-Butanediol $HO-CH_2-CH_2-CH_2-CH_2-OH$	90.1	0.019	0.0021	—	—	0.23 ± 0.01 (4)	—
Ethylene glycol monoethyl ether $HO-CH_2-CH_2-O-CH_2-CH_3$	90.1	0.20	0.019	0.03 (1)	0.21 ± 0.04 (3)	0.00 ± 0.01 (4)	—
Diethylene glycol $HO-CH_2-CH_2-O-CH_2-CH_2-OH$	106.1	0.004	0.005	—	—	0.21 ± 0.02 (4)	0.41 ± 0.03 (3)
Erythritol 	122.1	0.00011	0.00003	0.97 ± 0.01 (2)	1.02 ± 0.02 (4)	0.94 ± 0.03 (4)	0.96 ± 0.03 (3)

Table 2. Continued

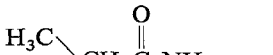
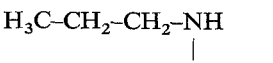
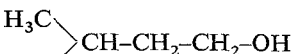
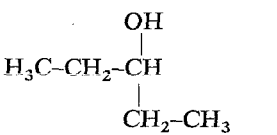
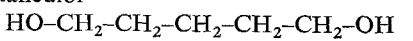
Compound/Formula	M	K_{ether}	K_{oil}	Fish gallbladder	Bullfrog gallbladder	Bullfrog intestine	Guinea-pig intestine
n-Butyramide 	87.1	0.058	0.0095	0.24 ± 0.04 (3)	0.71 ± 0.03 (3)	0.12 ± 0.01 (3)	–
Isobutyramide 	87.1	–	–	–	0.89 ± 0.02 (3)	0.22 ± 0.01 (3)	–
n-Propyl urea 	102.1	–	–	–	–	0.44 ± 0.05 (4)	–
Isopropyl urea 	102.1	–	–	–	–	0.59 ± 0.06 (4)	–
Five-carbon compounds							
Isoamyl alcohol 	88.1	19	1.8	–	−0.05 (1)	–	–
3-Pentanol 	88.1	–	–	–	−0.05 (1)	–	–
1,5-Pentanediol 	104.2	0.055	0.0061	0.48 ± 0.05 (2)	0.84 ± 0.01 (4)	0.25 ± 0.00 (3)	0.03 (1)

Table 2. Continued

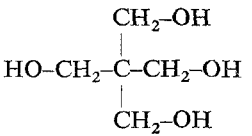
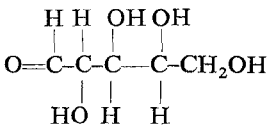
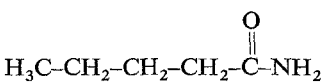
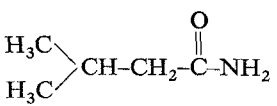
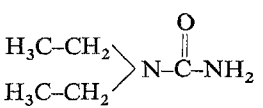
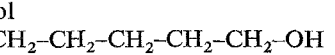
Compound/Formula	M	K_{ether}	K_{oil}	Fish gallbladder	Bullfrog gallbladder	Bullfrog intestine	Guinea-pig intestine
Pentaerythritol 	136.2	0.0003	—	—	—	—	1.01 ± 0.01 (1)
D-Arabinose 	150.1	0.000038	—	—	—	1.04 ± 0.04 (4)	1.13 ± 0.08 (2)
n-Valeramide 	101.2	—	—	—	0.41 ± 0.02 (4)	—	—
Isovaleramide 	101.2	0.17	0.023	—	0.72 ± 0.02 (4)	—	—
asym-Diethyl urea 	116.2	0.019	0.0076	—	0.92 ± 0.05 (4)	0.32 ± 0.06 (4)	—
Six-carbon compounds							
1,6-Hexanediol 	118.2	0.12	0.0068	0.18 (1)	0.63 ± 0.04 (3)	0.09 ± 0.01 (4)	—

Table 2. Continued

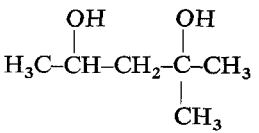
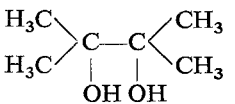
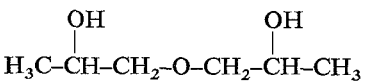
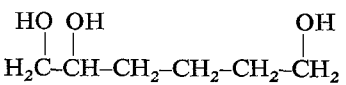
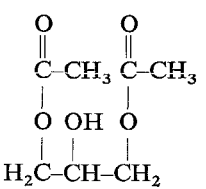
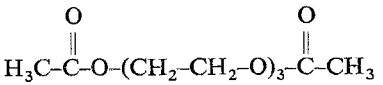
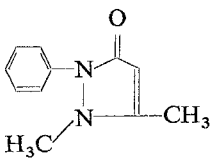
Compound/Formula	M	K_{ether}	K_{oil}	Fish gallbladder	Bullfrog gallbladder	Bullfrog intestine	Guinea-pig intestine
2-Methyl-2,4-pentanediol 	118.2	0.51	0.024	–	0.64 ± 0.05 (2)	–	–
Pinacol 	118.2	0.43	–	0.42 (1)	0.82 (1)	0.20 ± 0.05 (4)	0.10 ± 0.07 (4)
Dipropylene glycol 	134.2	0.035	0.0020	–	–	0.27 ± 0.05 (4)	–
1,2,6-Hexanetriol 	134.2	0.0031	–	–	–	0.50 ± 0.06 (4)	0.74 ± 0.05 (4)
Triethylene glycol $HO–CH_2–CH_2–O–CH_2–CH_2–O–CH_2–CH_2–OH$	150.2	0.0031	–	–	–	–	0.60 ± 0.01 (2)
Seven — or — more carbon compounds							
Diacetin 	176.2	0.22	0.071	–	0.64 ± 0.01 (2)	–	–

Table 2. Continued

Compound/Formula	M	K_{ether}	K_{oil}	Fish gallbladder	Bullfrog gallbladder	Bullfrog intestine	Guinea-pig intestine
Tetraethylene glycol $HO-(CH_2-CH_2-O)_3-CH_2-CH_2-OH$	194.2	0.0024	—	—	—	0.74 ± 0.02 (4)	0.72 ± 0.04 (4)
Triethylene glycol diacetate 	234.2	0.52	0.033	—	0.13 (1)	—	—
Antipyrine 	188.2	0.073	0.032	—	0.48 (1)	0.05 ± 0.01 (4)	—
Maltose	$C_{12}H_{22}O_{11} \cdot H_2O$	360.3	—	—	—	—	—	0.99 (1)

For each nonelectrolyte, the third column gives the molecular weight, the fourth column the ether/water partition coefficient (Collander, 1949), and the fifth column the olive-oil/water partition coefficient (mostly from Collander, 1954). The remaining columns give the average value, standard deviation, and total number of estimates of σ in each epithelium.

The order of solutes is as follows: Solutes are grouped initially according to the number of carbon atoms. Within each such carbon grouping, solutes are grouped according to the number of nitrogen atoms, those with no nitrogens coming first. Within each such nitrogen subgrouping, solutes are grouped according to the number of oxygen atoms, those with no oxygens coming first. Solutes in which sulfur replaces oxygen follow their oxygen analogues; solutes containing halogens follow their nonhalogenated analogues.

the four smallest highly polar nonelectrolytes with low partition coefficients (formamide, urea, acetamide, methyl urea) and at least one highly branched solute (pinacol, plus in some cases isovaleramide, 2-methyl-2,4-pentanediol, and triacetin).

The σ values obtained are summarized in Table 2, which lists solutes in the same sequence used previously in presenting σ values for rabbit gallbladder (Wright & Diamond, 1969*b*) and bullfrog choroid plexus (Wright & Prather, 1970), to facilitate comparison. For each solute we have given the chemical formula, molecular weight, ether/water partition coefficient (K_{ether}), olive-oil/water partition coefficient (K_{oil}), and average value, standard deviation, and number of determinations of σ in each epithelium.

Collander Plots

For preliminary analysis of the σ values we use a method introduced by Collander (1954; *see also* Collander & Bärlund, 1933) in his studies of values of permeability coefficients (P's), namely, to plot the values against some function of the form KM^a, where K is a solute's partition coefficient in some bulk nonaqueous/aqueous solvent system, M is the molecular weight of the solute, and a is a parameter characteristic of a given membrane. The reasoning behind these graphs is as follows. If the rate-limiting step for permeation through a homogeneous membrane is diffusion through the membrane interior rather than crossing the membrane/water interfaces[4], then P is given by the expression

$$P = KD/d \tag{3}$$

where d is the membrane thickness, D the diffusion coefficient of the solute in the membrane, and K the solute's membrane/water partition coefficient. Values of D for different solutes in a given solvent are approximately proportional to M^a, where a equals approximately -0.5 in water and -1 to -4 in long-chain polymers (Lieb & Stein, 1971). Hence, when one plots experimentally determined σ's against KM^a, one should obtain a monotonic curve of the same form as the σ-*vs.*-P graphs of Figs. 1 and 2. Values of K are unknown in biological membranes but have been determined by Collander (1949, 1950, 1954) and Collander & Bärlund (1933) for several

4 The good agreement between the experimental curves of Figs. 3 or 4 and the theoretical expectations based on this assumption does not, however, necessarily mean that this assumption is correct. If the rate-limiting step consists of crossing the interface from water into the membrane, the rate constant for this step is likely to depend on the same intermolecular forces that determine the partition coefficient, viz, the balance of forces between solute and water and between solute and membrane (*cf.* Diamond & Wright, 1969*b*, pp. 620–621).

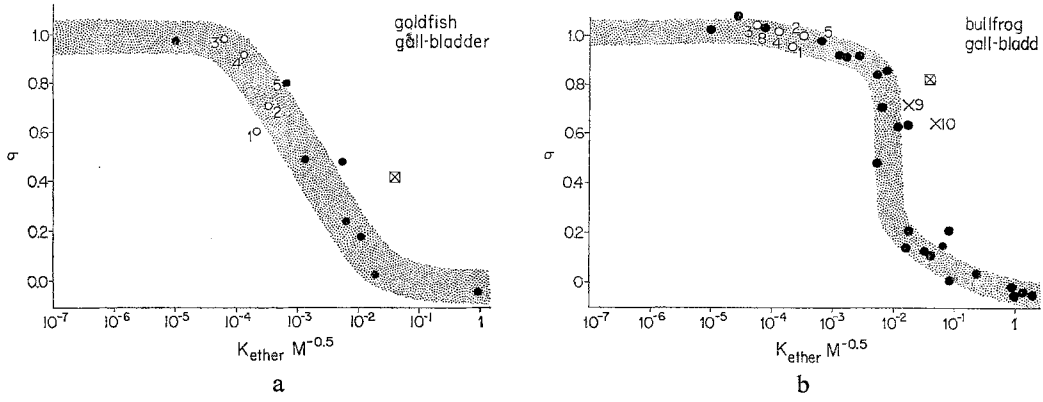


Fig. 3*a–g*. Collander plots for σ's in epithelia. *Ordinate:* average values of σ for nonelectrolytes in the indicated epithelium; *abscissa:* the ether/water partition coefficient divided by the square root of molecular weight of each nonelectrolyte. Points referring to small polar solutes, branched solutes, and glycerol are numbered or given a particular symbol: the smallest polar solutes (○), 1 = formamide, 2 = acetamide, 3 = urea, 4 = methyl urea; other small polar solutes, 5 = ethylene glycol, 6 = dimethyl urea, 7 = ethyl urea; 8 = glycerol; ⊠ pinacol, the most highly branched solute tested; other branched solutes (×), 9 = isovaleramide, 10 = 2-methyl-2,4-pentanediol, 11 = triacetin; 12 = hexamethylenetetramine, the most strongly basic solute tested. The shaded line is drawn to indicate the general pattern of the other points. Fig. 3*a–d* are based on the present study, Fig. 3*e* on Wright and Prather (1970), Fig. 3*f* on H. B. Irvine (*personal communication*), and Fig. 3*g* on Wright and Diamond (1969*b*)

nonpolar solvents relative to water (notably ether, isobutanol, olive-oil, and olive-oil +20% oleic acid), and K's for different nonpolar solvents are systematically interrelated (Collander, 1947, 1950, 1951). Differences in P values among the different solutes tested in the same membrane probably arise principally from differences in K and only secondarily from differences in D, since M varies over a range of only 11 but K's over a range of up to 500,000 for the solutes tested.

For each of the four epithelia used in the present study and the three epithelia studied previously, we prepared graphs of σ against $K_{ether}M^a$, $K_{oil}M^a$, and $K_{oil+acid}M^a$, where several alternative values were used for the exponent a. Some plots were also prepared with relative molecular volumes instead of molecular weights, taking critical volumes v_c (the molar volume of the pure substance at the critical point) from experimental values in the International Critical Tables or from values calculated by the Lydersen method (Reid & Sherwood, 1966, p. 28 and Table 2–1). Fig. 3*a–g* are the graphs of σ against $K_{ether}M^{-0.5}$ for each of the seven epithelia, while Fig. 4*a–e* are graphs of σ against K_{oil}, $K_{oil}M^{-2}$, $K_{oil+acid}$, K_{ether}, and $K_{isobutanol}$ for goldfish gallbladder. The value of $a = -0.5$ used in Fig. 3*a–g*

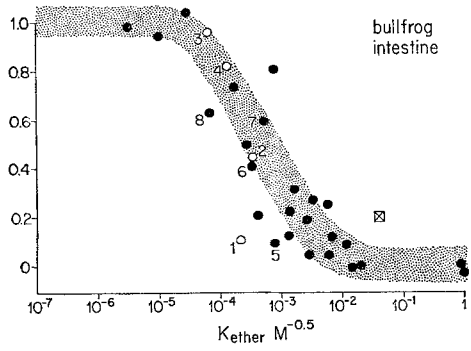


Fig. 3c

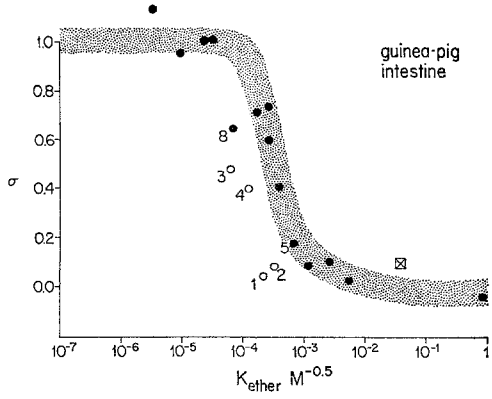


Fig. 3d

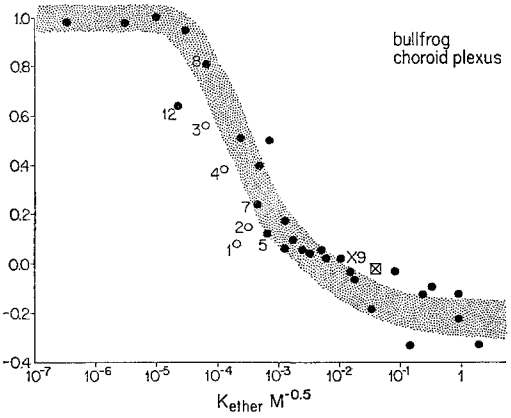


Fig. 3e

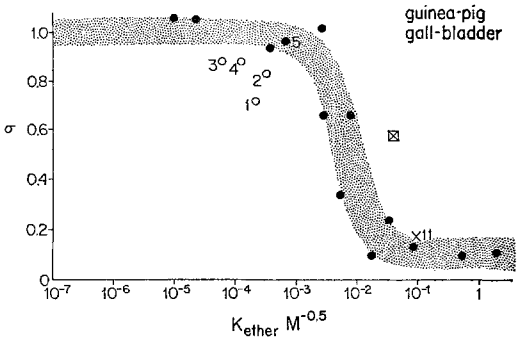


Fig. 3f

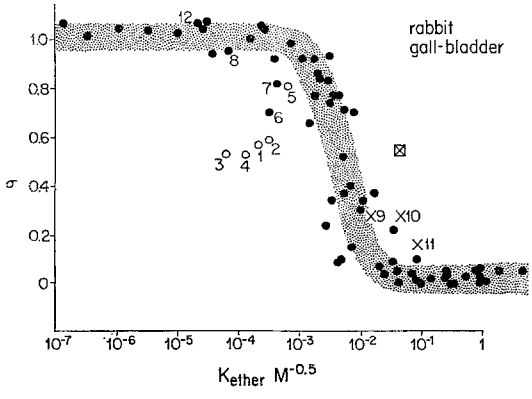


Fig. 3g

for display was selected because it is close to the value which Smulders and Wright (1971) determined experimentally for rabbit gallbladder from measurements of permeability coefficients. K_{ether} was used in Fig. 3*a–g* because values of K_{ether} were available for more of the solutes used than in the case of K_{oil} or $K_{isobutanol}$. The value of a is discussed further on p. 125, and different Collander plots in the same epithelium are compared on p. 116.

The forms of Figs. 3*a–g* and 4*a–e* are all similar to each other and to Figs. 1 and 2 in that σ is constant at 1.0 for low values of KM^a, decreases from 1.0 towards 0 over an intermediate range of KM^a, and asymptotically approaches a value near 0 for high values of KM^a. The meaning of this form is as follows. Solutes with very low partition coefficients have such low permeabilities that $P_s V_s/P_{osm} \sim 0$, $\sigma \sim 1$ [Eq. (2)], and an increase in KM^a sufficient to multiply the permeability coefficient P_s 10-fold still leaves σ experimentally indistinguishable from 1 (e.g., reduces it from 0.9999 to 0.9995, or from 0.999 to 0.995). For solutes with larger partition coefficients and permeabilities such that $P_s v_s/P_{osm} \ll 1$ no longer holds, an increase in KM^a by the same factor necessary to increase P_s 10-fold now reduces σ significantly (e.g., from 0.99 to 0.95, or from 0.90 to 0.50). For solutes with still higher partition coefficients and permeabilities, the unstirred layers become rate-limiting, and σ asymptotically approaches $-v_s D/P_{osm}\delta$ [Eq. (2)].

More detailed examination of Fig. 3*a–g* reveals the following seven types of differences among the seven epithelia studied:

(1) While there is some random scatter of the experimental σ's from the curve comprising the main pattern, probably because ether is not a perfect model for membrane lipids, there are four types of consistent deviations which possess significance. The most obvious of these deviations involves the smallest and most polar solutes such as urea, methyl urea, formamide, and acetamide ($M \leqq 74$, $K_{oil} \leqq 0.00083$, $K_{ether} \leqq 0.0025$), whose σ's (marked by open circles in Figs. 3*a–g* and 4*a–e*, and numbered 1 through 4) fall below and to the left of the general curve in some but not all epithelia. These deviations are most marked in rabbit gallbladder (Fig. 3*g*), where these four solutes have σ values of 0.53 to 0.59 instead of the values of 1.00 for other solutes with comparable values of $K_{ether} M^{-0.5}$, and where several other slightly larger or slightly less polar solutes (ethylene glycol, dimethyl urea, ethyl urea, etc.) also have low σ values. Similar but less marked deviations for these four solutes are present in guinea-pig intestine (Fig. 3*d*), guinea-pig gallbladder (Fig. 3*f*), and bullfrog choroid plexus (Fig. 3*e*). Formamide, the smallest of these solutes, has a deviant low σ in bullfrog intestine and barely so in goldfish gallbladder, where σ's for the other three of these solutes fall on the main pattern. At the other extreme, in the bullfrog gallbladders

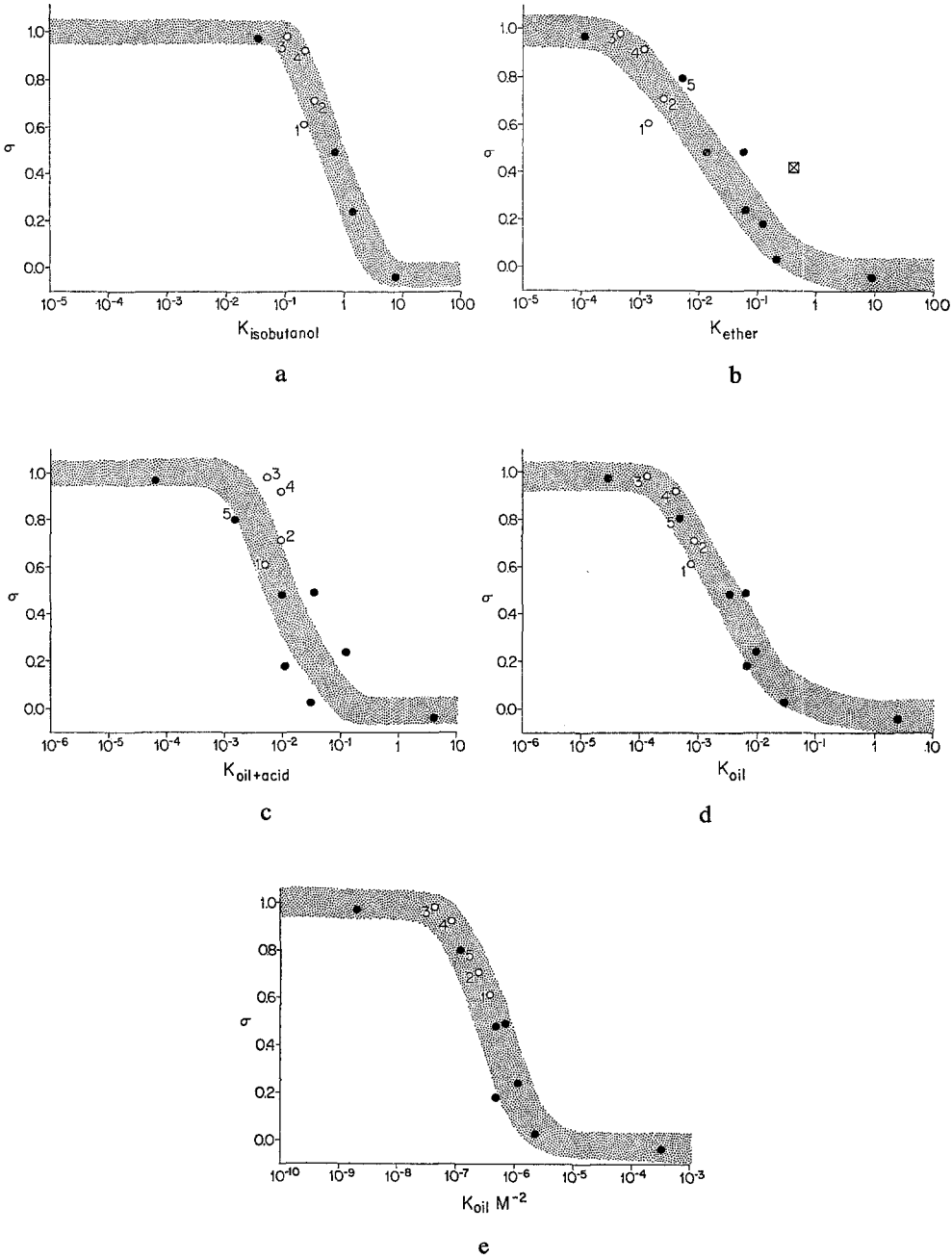
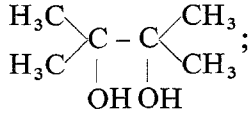


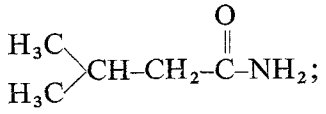
Fig. 4*a–e*. Collander plots for σ's in goldfish gallbladder. *Ordinate:* average values of σ for nonelectrolytes; *abscissa:* the isobutanol/water partition coefficient (Fig. 4*a*), ether/water partition coefficient (Fig. 4*b*), olive-oil plus 20 % oleic-acid/water partition coefficient (Fig. 4*c*), olive-oil/water partition coefficient (Fig. 4*d*), or olive-oil/water partition coefficient divided by the molecular weight squared (Fig. 4*e*). *Compare also* Fig. 3*a*. For meaning of numbers, particular symbols, and shaded line, *see* legend to Fig. 3

tested none of these four solutes is deviant: their σ's are 1.0 (meaning that they are impermeant) and fall on the main pattern[5]. A deviant low σ means that a solute is more permeant, or has a larger water-drag component of σ, or both, than expected from its partition coefficient and size. Collander (1954) showed by direct measurement of permeability coefficients (P's) in the alga *Nitella mucronata* that small polar solutes, including urea, methyl urea, formamide, and acetamide, have P's higher than expected from the main pattern of the *P-vs.-KM*^{a} graph in *Nitella*. In rabbit gallbladder several lines of evidence indicate that the enhanced permeability of these small polar solutes arises from their having access to a polar, size-limited permeation pathway that by-passes membrane lipid and that may be loosely termed a "pore" (Wright & Diamond, 1969*b*; Diamond & Wright, 1969*b*). The differences among Fig. 3*a–g*, therefore, suggest that the seven tissues differ in the number and/or size of membrane pores, with rabbit gallbladder having the most numerous and/or largest pores, and bullfrog gallbladder having no pores.

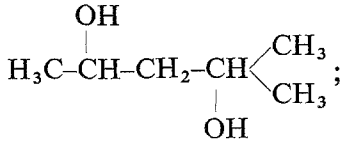
(2) The second class of deviations involves the highly branched solutes (marked ⊠ or × in Figs. 3*a–g* and 4*a–e*), whose σ's fall above and to the right of the general curve in some but not all epithelia. Although the K and M of the highly branched pinacol (structure



marked ⊠ in the figures) are such that the main patterns of all seven epithelia would yield σ near 0, its σ is actually 0.42 to 0.82 in gallbladders of the four species studied, 0.10 to 0.18 in intestines of the two species studied, and −0.02 in bullfrog choroid plexus. σ's of three other branched solutes tested (isovaleramide,

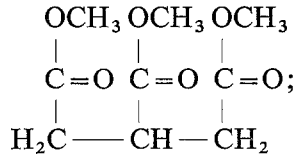


2-methyl-2,4-pentanediol,



5 In several gallbladders from a separate shipment of bullfrogs obtained at a different time of year for pilot experiments, values of σ_{urea} less than 0.7 were obtained, suggesting the possibility of large seasonal or hormonal variation.

triacetin,



marked × in the figures) are higher than the main pattern in rabbit and bullfrog gallbladders but fall on the main pattern in bullfrog choroid plexus. A deviant high σ means that a solute is less permeant than expected from its bulk-phase partition coefficients and size. Thus, there appears to be a difference that is characteristic of a given organ and crosses species lines: gallbladder discriminates markedly against the permeation of branched solutes compared to their straight-chain analogues, intestine similarly but less markedly, and choroid plexus not at all.

(3) In bullfrog choroid plexus σ for hexamethylene tetramine (marked 12 in the figures) equals 0.64 and falls below the main pattern, but in rabbit and guinea-pig gallbladders its σ is near 1.0 and falls on the main pattern. As noted by Wright and Prather (1970), the anomalous behavior of hexamethylene tetramine in choroid plexus is probably related to the fact that it is the most strongly basic nonelectrolyte tested (*see* p. 126 for further discussion).

(4) The remaining possible deviation involves glycerol (marked 8 in the figures; not tested in guinea-pig or goldfish gallbladders), whose σ falls on the main pattern in rabbit and bullfrog gallbladders and in bullfrog choroid plexus but falls slightly below the main pattern in bullfrog intestine and further below in guinea-pig intestine. Glycerol is known to permeate erythrocytes by means of a facilitated-diffusion carrier mechanism (Stein, 1962), and a similar mechanism might exist in the intestine. This would result in a σ value lower than expected from partition coefficients, as shown for facilitated diffusion of sugars in the choroid plexus by Wright and Prather (1970).

(5) The curves for some epithelia are shifted along the $K_{\text{ether}} M^{-0.5}$ axis with respect to other epithelia. Thus, a σ value of 0.5 on the main pattern falls approximately at $K_{\text{ether}} M^{-0.5} = 2.5 \times 10^{-4}$ for bullfrog choroid plexus, 4×10^{-4} for guinea-pig intestine, 5×10^{-4} for frog intestine, 1.5×10^{-3} for fish gallbladder, 5×10^{-3} for rabbit gallbladder, 7×10^{-3} for guinea-pig gallbladder, and 9×10^{-3} for bullfrog gallbladder. Essentially the same ranking is obtained when the abscissa is based on $K_{\text{isobutanol}}$ or K_{oil} rather than K_{ether} or uses a different power of M. The significance of these differences and the following two types of differences is discussed on p. 122.

(6) The epithelia differ in the steepness of the middle portion of the curve around $\sigma = 0.5$. For instance, comparison of Fig. 3*a* and *b* shows that σ is reduced from 0.8 to 0.2 by a 33-fold increase in $K_{\text{ether}} M^{-0.5}$ in fish gallbladder but by only a 1.7-fold increase in bullfrog gallbladder. Similar differences appear when the abscissa is taken as $K_{\text{isobutanol}}$ or K_{oil}, using other powers of M. The approximate sequence from the steepest to the least steep is bullfrog gallbladder > rabbit gallbladder, guinea-pig intestine > guinea-pig gallbladder > fish gallbladder, bullfrog intestine, bullfrog choroid plexus.

(7) The epithelia differ in the asymptotic value which σ approaches at high values of KM^a, where permeation becomes rate-limited by unstirred layers. The apparent value of this asymptote is approximately 0.00 in rabbit and goldfish gallbladders and in bullfrog and guinea-pig intestines, +0.1 in guinea-pig gallbladder, −0.05 in bullfrog gallbladder, and −0.2 in bullfrog choroid plexus. The most unequivocally distinct of these values is the strongly negative one of bullfrog choroid plexus.

Alternative Collander plots using different combinations of K and M^a for the abscissa have also been prepared for each epithelium, as illustrated in Figs. 3*a* and 4*a–e* for fish gallbladder. Regarding the effect of different sets of K, plots using $K_{\text{isobutanol}}$ (e.g., Fig. 4*a*) are steeper in the region of $\sigma = 0.8$ to 0.2 than plots using K_{ether} (e.g., Fig. 4*b*), K_{oil} (e.g., Fig. 4*d*), or $K_{\text{oil+acid}}$ (e.g., Fig. 4*c*) for a given epithelium, as follows immediately from the relation between $K_{\text{isobutanol}}$ and K_{oil} or K_{ether} (values of $K_{\text{isobutanol}}$ vary over a narrower range than do values of K_{ether} or K_{oil} [Collander, 1950]). There is no obvious difference in goodness of fit between plots using K_{ether}, K_{oil}, or $K_{\text{isobutanol}}$. However, plots using $K_{\text{oil+acid}}$ give conspicuously worse fits in all epithelia, because σ's for basic solutes such as hexamethylene tetramine, ethyl urea, diethyl urea, propionamide, butyramide, dimethyl formamide, and sometimes methyl urea deviate considerably to the right of and above the main pattern. This implies that relative membrane/water K's for these solutes (i.e., relative to K's of neutral solutes) are smaller than their relative values of $K_{\text{oil+acid}}$: that is, that the membranes studied are not notably "acidic" (*see* p. 126). Regarding the effect of the molecular weight exponent a, some plots using KM^0 ($= K$) show slightly less scatter than plots using KM^{-2}, but the differences are small. The reason why the choice of exponent for M has little effect is that the solutes used vary over a range of only 11 in M but of 2125 in $K_{\text{isobutanol}}$, 130,000 in K_{oil}, and 500,000 in K_{ether}. Most of the deviations discussed on pp. 112–115 remain even when the abscissa is taken as KM^{-2}. Plots using molecular volume and molecular weight do not differ conspicuously (e.g., Kv_c^{-2} *vs.* KM^{-2}), since the maximum deviation of v_c/M from the mean value among the compounds studied is only 26%.

Individual Variation

The preceding analysis concerns systematic differences between the same organ in different species or between different organs in the same species. The possible existence of systematic individual variation in the same organ from the same species was suggested by an observation made by Collander (1954) while measuring permeability coefficients in the alga *Nitella mucronata.* Two *Nitella* cells with otherwise normal permeability properties were found to yield permeability coefficients (P's) several times the normal values in the cases of urea, formamide, and acetamide, three of the small polar solutes with P's higher than expected from the main pattern of the *P-vs.-KM*[a] graph in *Nitella.* This implies that some distinct structural feature of *Nitella* membrane is responsible for the permeabilities of urea, formamide, and acetamide but not of other solutes (e.g., that these two particular cells had more numerous or larger "pores" but normal membrane lipid).

In the present experiments, significant individual variation was encountered in guinea-pig intestine. We could not analyze individual variation in bullfrog intestine or bullfrog gallbladder because of high reproducibility among different preparations (average standard deviation of σ's only 0.03), nor in goldfish gallbladder because of the small number of preparations tested.

Guinea-pig Intestine. Urea and methyl urea had the widest range of variation in σ (0.17 to 0.72 and 0.21 to 0.58, respectively) and highest coefficient of variation for σ (0.40 and 0.33, respectively), from animal to animal,

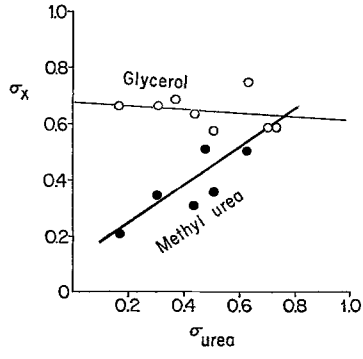


Fig. 5. σ's for urea, methyl urea, and glycerol were all determined in five guinea-pig intestines, σ's for urea and methyl urea both determined in two additional guinea-pig intestines, and σ's for urea and glycerol both determined in two additional guinea-pig intestines. The ordinate is σ for methyl urea (•) or for glycerol (○), while the abscissa is σ for urea in the same animal. The straight lines were calculated by least mean squares. Note that variations among animals in σ's for urea and for methyl urea show a strong positive correlation, while there is no correlation between the variations in σ's for urea and for glycerol

of the 20 solutes tested. This variation was inter-correlated; preparations with a high value of σ_{urea} generally had a high value of $\sigma_{methyl\ urea}$. As illustrated in Fig. 5, when $\sigma_{methyl\ urea}$ is plotted against σ_{urea} in the same preparation, a line is obtained with a slope of 0.63 and a high positive correlation coefficient (0.87). In contrast, σ's for the other four solutes providing sufficient data for analysis (glycerol, 1,2,6-hexanetriol, tetraethylene glycol, diethylene glycol) were much less variable (coefficients of variation between 0.06 and 0.09) and showed little correlation with σ_{urea}. This is illustrated by the graph of $\sigma_{glycerol}$ *vs.* σ_{urea} in Fig. 5, which is nearly a horizontal line (slope -0.06, correlation coefficient -0.20). Graphs of σ for hexanetriol, tetraethylene glycol, and diethylene glycol *vs.* σ_{urea} yielded similarly small slopes (0.18, -0.03, and 0.22, respectively).

Hexanetriol, tetraethylene glycol, and diethylene glycol have σ's falling on the main pattern in guinea-pig intestine and presumably permeate through membrane lipid. The deviant low σ's for urea and methyl urea, however, suggested that they permeate through "pores", and a carrier mechanism was suspected for glycerol. The analysis of individual variation suggests independently that urea and methyl urea share a common permeation mechanism which is not shared by glycerol nor by hexanetriol, tetraethylene glycol, and diethylene glycol in guinea-pig intestine.

Rabbit Gallbladder. The extensive σ determinations in rabbit gallbladder by Wright and Diamond (1969*b*) have been reexamined with respect to individual variation. Nineteen pairs of solutes were selected for analysis on the basis of three criteria: σ's for the pair had to have been measured together in at least four gallbladders; the coefficient of variation of σ based on all experiments had to exceed 0.1 for each member of the pair; and the average value of σ for each member of the pair had to fall between 0.3 and 0.7, the range in which measured σ values are most sensitive functions of permeability. The solutes in the pairs selected were classified as permeating in rabbit gallbladder predominantly via "pores" (urea, methyl urea, formamide, acetamide) or via membrane lipid (*n*-butyramide, propionamide, butyl urea, 2,5-hexanediol, 2,5-hexynediol, 1,6-hexanediol, antipyrine), on the basis of criteria discussed previously by Wright and Diamond (1969*b*) (σ much less than or else on the main pattern of the Collander plot, σ independent of or else inversely proportional to pH, σ independent of or else inversely proportional to temperature). The 19 pairs of solutes were in three cases both "pore" compounds, in ten cases both "lipid" compounds, and in six cases one a "pore" compound and the other a "lipid" compound (these designations of course imply only the principal, not the sole, route of permeation, and are valid only for rabbit gallbladder, not necessarily

Table 3. Co-variation of σ's in rabbit gallbladder

Solute$_x$/solute$_y$		r	Slope
Lipid$_x$/lipid$_y$			
butyl urea/butyramide	(5)	0.67	0.95
antipyrine/butyramide	(5)	0.61	0.33
2,5-hexanediol/butyramide	(7)	0.48	0.79
1,6-hexanediol/butyramide	(6)	0.92	0.96
2,5-hexynediol/butyramide	(5)	0.44	0.64
proprionamide/butyramide	(9)	0.84	0.60
	avg.	0.66	0.71
Pore$_x$/pore$_y$			
urea/acetamide	(6)	0.86	0.85
formamide/acetamide	(6)	0.91	0.79
methyl urea/urea	(4)	0.93	0.99
	avg.	0.90	0.88
Lipid$_x$/pore$_y$			
butyl urea/urea	(9)	−0.29	−0.24
antipyrine/urea	(5)	0.89	0.41
2,5-hexanediol/urea	(7)	0.61	0.54
1,6-hexanediol/urea	(6)	0.60	0.33
2,5-hexynediol/urea	(5)	0.71	0.58
proprionamide/urea	(7)	0.51	0.41
butyramide/urea	(61)	0.50	0.35
proprionamide/acetamide	(7)	0.62	0.68
butyramide/acetamide	(7)	0.51	0.08
butyl urea/acetamide	(5)	0.06	0.08
	avg.	0.47	0.34

To test whether individual variation in σ's for two solutes in different animals was intercorrelated, instances were assembled in which σ's for both solutes (x and y) had been determined in the same gallbladder, and a straight line was fitted to σ_y plotted against σ_x, as in Fig. 5. The table gives correlation coefficients (r) and slopes for 19 pairs of solutes, classified according to whether both solutes appear to permeate predominantly through membrane lipid, both solutes permeate predominantly through pores, or one permeates predominantly through lipid and the other through pores. Numbers in parentheses are the number of gallbladders in which both solutes of a given pair were determined. Note that there is a stronger correlation (more positive slope and correlation coefficient) between σ's for two "pore" solutes, or between σ's for two "lipid" solutes, than between σ's for a "pore" solute and a "lipid" solute. σ determinations are taken from the experiments of Wright and Diamond (1969*b*). *See* text, pp. 118–120, for further discussion.

for other tissues). For each pair, then, the slope and linear correlation coefficient were calculated when σ for one member of the pair was plotted against σ for the other member determined in the same experiment. As summarized in Table 3, the average slope was 0.88 for "pore/pore" comparisons and 0.71

for "lipid/lipid" comparisons but only 0.34 for "lipid/pore" comparisons, and the average correlation coefficient was higher in the former two cases than in the latter case. That is, rabbit gallbladders which are especially permeable to urea tend to be also especially permeable to other solutes thought to permeate through "pores" but not to butyramide and other solutes thought to permeate through membrane lipid; and vice versa. The fact that the slope for "lipid/pore" comparisons is 0.34 rather than 0 is probably because the osmotic water permeability P_{osm} and the unstirred-layer thickness δ affect all measured σ values, independent of the permeation mechanism [Eq. (2)]. Thus, analysis of individual variability supports the coexistence of distinct permeation mechanisms in rabbit gallbladder, as in guinea-pig intestine and the alga *Nitella*.

Effect of Temperature

In rabbit gallbladder, increasing temperature markedly decreases σ (increases the permeability) of solutes whose σ's fall on the main pattern of Collander plots and presumably permeate through membrane lipid, as expected for a process with a high activation energy. In contrast, small polar solutes with deviant low σ's and assumed to permeate through "pores" have σ's nearly independent of temperature (Wright & Diamond, 1969*b*). Apparent activation energies of permeation are 12 to 15 kcal/mole for the former solutes, but are only 5 to 7.5 kcal/mole for the latter solutes, scarcely larger than activation energies for diffusion of small nonelectrolytes in aqueous solution (Smulders & Wright, 1971).

Since the present study shows that a given small polar solute may have a deviant low σ suggestive of permeation via "pores" in one epithelium but a σ on the main pattern and suggestive of permeation via lipid in another epithelium, the following experiment was designed to examine whether there was a corresponding difference between the epithelia in the temperature dependence of σ of the same solute. The solute selected was formamide, since its σ falls far below the main pattern in bullfrog intestine (Fig. 3*c*) and rabbit gallbladder (Fig. 3*g*) but almost on the main pattern in goldfish gallbladder (Fig. 3*a*).

Fig. 6 depicts the results of an experiment in bullfrog intestine, in which σ's were determined first at 30 °C, then at 3 °C, and again at 30 °C for formamide, *n*-butyramide, 1,6-hexanediol, and dimethyl formamide. The three latter solutes differ from formamide in having σ's on or close to the main pattern in bullfrog intestine, but butyramide and hexanediol have nearly the same value of σ as formamide at room temperature. It is obvious

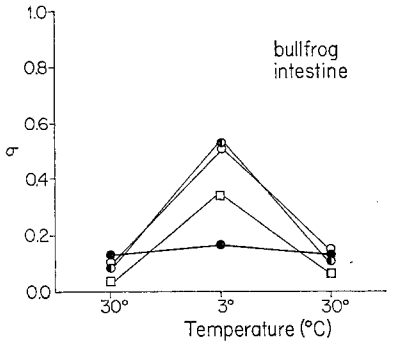


Fig. 6

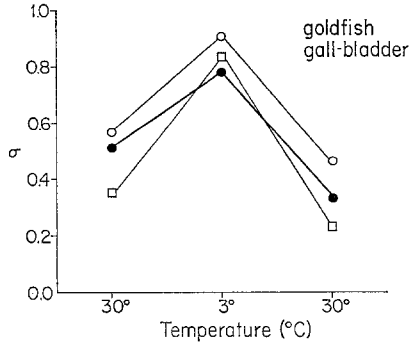


Fig. 7

Fig. 6. Effect of temperature on σ's in bullfrog intestine. σ's for formamide (•), *n*-butyramide (○), 1,6-hexanediol (◐), and dimethyl formamide (□) were determined at 30 °C, then at 3 °C, and finally again at 30 °C. Note that σ for formamide is virtually independent of temperature, while σ's for the other three solutes reversibly increase at low temperature

Fig. 7. Effect of temperature on σ's in goldfish gallbladder. σ's for formamide (•), acetamide (○), and propionamide (□) were determined at 30 °C, then at 3 °C, and finally again at 30 °C. Note that σ's for all three compounds increase at low temperatures

from Fig. 6 that σ for formamide is nearly independent of temperature but that σ's for the other three solutes undergo a large reversible increase at low temperature, such that their σ's are less than $\sigma_{\text{formamide}}$ at 30 °C but much larger than $\sigma_{\text{formamide}}$ at 3 °C. The same result was obtained for formamide in rabbit gallbladder (Wright & Diamond, 1969*b*).

A different result was obtained in goldfish gallbladder, using acetamide and propionamide as main-pattern solutes for comparison with formamide. As depicted in Fig. 7, σ's for all three solutes undergo a large reversible increase at low temperature, with the increase for formamide being only slightly less marked than that for the other two solutes.

Thus, σ for formamide is nearly independent of temperature in two epithelia where its deviation below the main pattern suggests permeation via "pores", but is markedly temperature-dependent in an epithelium where its agreement with the main pattern implies permeation via lipid. Similarly, $\sigma_{\text{acetamide}}$ shows more marked temperature dependence in goldfish gallbladder (Fig. 7) than in rabbit gallbladder (Wright & Diamond, 1969*b*), correlated with the fact that $\sigma_{\text{acetamide}}$ falls on the main pattern in the former epithelium and below it in the latter. Correspondingly, the apparent activation energy of permeation for acetamide is close to the free-solution diffusion value in rabbit gallbladder (Smulders & Wright, 1971) but considerably higher in goldfish gallbladder (E. M. Wright, *personal communication*).

Discussion

Five problems will be considered: the significance of the quantitative differences among the epithelial main patterns of Fig. 3*a–g*; permeation through pores; relations between molecular structure and permeability; preferential permeability to basic or acidic solutes; and the significance of discrimination against branched solutes.

Quantitative Differences in Epithelial Main Patterns

As discussed on pp. 115–116, the main patterns of the Collander plots for different epithelia, although similar qualitatively, differ quantitatively in three respects: the value of $KM^{-0.5}$ at which σ equals 0.5 [abbreviated $(KM^{-0.5})_{\sigma=0.5}$], a measure of the curve's displacement along the abscissa; the steepness; and the asymptotic value of σ for solutes with high partition coefficients and permeabilities (abbreviated $\sigma_{k=\infty}$). These parameters are determined by three factors: the unstirred-layer thickness δ, the osmotic water permeability P_{osm}, and the absolute magnitude of nonelectrolyte permeability coefficients. As illustrated in Fig. 1, an increase in δ tends to decrease $(KM^{-0.5})_{\sigma=0.5}$, decrease steepness, and make $\sigma_{k=\infty}$ closer to zero (less negative). As illustrated in Fig. 2, an increase in P_{osm} tends to increase $(KM^{-0.5})_{\sigma=0.5}$, decrease steepness, and make $\sigma_{k=\infty}$ closer to zero. An increased nonelectrolyte permeability (i.e., higher values of P for a given $KM^{-0.5}$ would decrease $(KM^{-0.5})_{\sigma=0.5}$. Of these factors, we have measured δ's at the mucosal surface, but neither P_{osm}'s, δ's at the serosal surface, nor absolute nonelectrolyte permeabilities. As summarized in Table 1 (columns 5 and 6), there is an inverse correlation between experimental values of mucosal δ's and $(KM^{-0.5})_{\sigma=0.5}$. Thus, differences in mucosal δ's are in the correct direction to explain qualitatively the left-right displacements of Collander plots for different epithelia along the abscissa, though we do not know if this explanation is quantitatively sufficient. Increasing steepness of the Collander plots (p. 116) also correlates approximately, though not perfectly, with decreasing δ.

Permeation through Pores

Among the epithelia studied there is conspicuous variability in σ's for small polar solutes relative to σ's for other solutes. In some, but not all, tissues these solutes apparently can permeate not only via membrane lipid but also via a separate route, whose permeability characteristics resemble

those of a pore. The evidence for this conclusion discussed previously in connection with rabbit gallbladder (Diamond & Wright, 1969*b*; Wright & Diamond, 1969*b*) was as follows: (1) σ's or P's for small polar nonelectrolytes deviate from Collander plots in the direction of enhanced permeability, the magnitude of the deviation being independent of the solute's partition coefficient but approximately inversely proportional to its size below some limiting size. (2) Decreasing pH markedly increases σ's (decreases permeability) of most solutes but not of the small, polar, deviant solutes. The origin of the pH effect on solutes permeating via membrane lipid may be associated with the fact that some lipids pack more closely at low pH than at neutral pH (van Deenen, Houtsmuller, de Haas & Mulder, 1962, pp. 435–436); this would lead to lower partition coefficients and diffusion coefficients at low pH for solutes permeating via lipid (*cf.* pp. 127–128 of this paper). A similar pH effect has been reported for permeation in rat intestine (Schiff, Small & Dietschy, 1972) and in toad urinary bladder (Rosen, Leaf & Schwartz, 1964). (3) Increasing temperature markedly decreases σ's (increases permeability) of most solutes but not of small, polar, deviant solutes, whose apparent activation energies of permeation are scarcely larger than those for diffusion in aqueous solution. The steep temperature dependence and high activation energies of solute permeation through membrane lipid in epithelia, as well as through artificial lipid bilayers and erythrocyte membranes (de Gier, Mandersloot, Hupkes, McElhaney & van Beek, 1971), probably arise from the breaking of hydrogen bonds which determine lipid/water partition coefficients (Collander, 1949; Diamond & Wright, 1969*a*, 1969*b*) or from the temperature dependence of diffusion in an ordered lipid membrane interior, or from both of these factors.

The types of co-variation noted in the present study provide additional evidence that small polar solutes have access to a distinct pathway in some tissues. For example, correlation coefficients among σ's in the same preparation from different individual animals show co-variation of σ's for urea and methyl urea in guinea-pig intestine, and of σ's for urea, methyl urea, formamide, and acetamide in rabbit gallbladder, independent of variation in σ's for other solutes. The differences between the same organ in different species, or between different organs in the same species, constitute similar co-variation. For instance, the most marked difference between σ's in rabbit gallbladder and bullfrog gallbladder is that σ's for urea, methyl urea, formamide and acetamide are all 0.39 to 0.52 lower in the former than in the latter species; the next most marked difference is that σ's for several branched solutes are 0.23 to 0.44 lower in the former species; and all other solutes have σ's differing by less than 0.30 between the two species.

Temperature experiments on the same solutes in different epithelia underscore the significance of this co-variation. Thus, formamide and acetamide have markedly temperature-dependent σ's in goldfish gallbladder, where σ's for both fall on the main pattern; but their σ's show much less temperature dependence than those of other solutes in rabbit gallbladder, where σ's for both deviate considerably below the main pattern.

The simplest interpretation of this co-variation, then, is that rabbit gallbladder behaves as if it had the most numerous or largest pores; guinea-pig gallbladder, guinea-pig intestine, and bullfrog choroid plexus, fewer pores; bullfrog small intestine, smaller pores through which only the smallest polar nonelectrolyte, formamide, is markedly permeant; and bullfrog gallbladder and goldfish gallbladder, essentially no pores (or only pores too small to pass even formamide). Similar co-variation appears in studies of nonelectrolyte permeation in single cells. For example, the alga *Valonia ventricosa* behaves as if it lacked pores (Gutknecht, 1968: σ's for small polar solutes near 1.0, P_{osm} and the diffusional water permeability P_d approximately equal, σ's equal $1 - P_s v_s / P_{osm}$ without a water-drag term); human erythrocyte behaves as if it had pores similar in size to those of rabbit gallbladder (Goldstein & Solomon, 1960, and Sha'afi *et al.*, 1967: $P_{osm} > P_d$, $\sigma < 1 - P_s v_s / P_{osm}$ implying a water-drag term, preferential permeability to small solutes up to the approximate size of malonamide) but smaller in size than those of dog erythrocyte (Rich *et al.*, 1967; Sha'afi *et al.*, 1971); and the bacterium *Beggiatoa mirabilis* (Ruhland & Hoffmann, 1925; Schönfelder, 1930), which is permeable to fully hydroxylated solutes up to the size of disaccharides, appears to have considerably larger pores than rabbit gallbladder.

A possible molecular basis for pores has been provided by recent studies (Holz & Finkelstein, 1970) on the antibiotics nystatin and amphotericin. These compounds cause a parallel increase in permeability to water, ions, and small polar nonelectrolytes up to the approximate size of glycerol in thin lipid membranes, which in the absence of these compounds have a negligible permeability to ions and small polar nonelectrolytes (but high permeability to nonelectrolytes with high partition coefficients). Careful correction for unstirred-layer effects shows that the osmotic water permeability P_{osm} of thin lipid membranes exceeds the diffusional water permeability P_d in the presence of amphotericin and nystatin, as in human erythrocytes, but not in the absence of amphotericin, as in *Valonia*. It has been suggested for amphotericin, and for the related antibiotic gramicidin (Urry, Goodall, Glickson & Mayers, 1971), that several antibiotic molecules combine to form a channel which spans the thickness of a bilayer, presents a hydrocarbon-like exterior, and has many polar groups facing the interior of the channel.

Lieb and Stein (1969, 1971) have argued against attributing the enhanced permeation of small polar nonelectrolytes to a separate pore-like pathway, starting from a suggested analogy between biological membranes and long-chain polymers, in which the inverse dependence of permeability on molecular weight is steep (values of a up to -4). They reanalyze the permeability data for *Chara*, *Nitella*, and rabbit gallbladder and claim to demonstrate that the best fit of the experimental results is to $a=-3.5$, -3.7, and -2.8 in these three preparations, respectively. With these high values of a, σ's or P's for most small polar solutes would fall on the main pattern. However, closer scrutiny of their calculations shows that Lieb and Stein selected for analysis only a fraction of the available solutes in *Chara* and *Nitella*, by an arbitrary criterion that biased the results towards high a; and that they used an incorrect formula to analyze the rabbit gallbladder results. Recalculation of the data in several different ways (Smulders & Wright, 1971; and Appendix of the present paper) yields $a=-2.4$ to -0.2 for *Chara*, -1.8 to -1.2 for *Nitella*, and -0.7 for rabbit gallbladder. A detailed analysis of a and critique of the Lieb and Stein treatment are given in the Appendix.

Relations between Molecular Structure and Permeability

Many principles relating permeability to molecular structure are implicit in the results of Table 2. For instance, σ's for butanol (H_3C–CH_2–CH_2–CH_2–OH), 2,3-butanediol (H_3C–CH(OH)–CH(OH)–CH_3), and erythritol (H_2C(OH)–CH(OH)–CH(OH)–CH_2–OH), respectively, are: -0.05, 0.11, and 0.96 in guinea-pig intestine; 0.00, 0.19, and 0.94 in bullfrog intestine; -0.03, 0.92, and 1.02 in bullfrog gallbladder; -0.22, 0.05, and 1.01 in bullfrog choroid plexus; 0.01, 0.74, and 1.02 in rabbit gallbladder; and 0.10, 0.66, and 1.06 in guinea-pig gallbladder. Thus, introduction of each successive –OH group increases σ or reduces permeability.

Other conclusions following from Table 2 are as follows: (1) If one compares aliphatic solutes with the same number of carbon atoms, permeability is decreased (σ increased) by introduction of the ether link –O–, the hydroxyl group –OH, the amide group $-\overset{\overset{O}{\|}}{C}-NH_2$, and the urea residue $-NH-\overset{\overset{O}{\|}}{C}-NH_2$, in that sequence, –O– having the least effect and $-NH-\overset{\overset{O}{\|}}{C}-NH_2$ the greatest effect. Compared to the effect of –OH, the approximate relative

potencies in reducing permeability are:

$$-\overset{\overset{O}{\|}}{NH-C}-NH_2 \geqq \text{two-OH} \geqq -\overset{\overset{O}{\|}}{C}-NH_2 \quad ; \quad -O- \leqq \text{one-half-OH.}$$

(2) Opportunities for intramolecular hydrogen bonding enhance permeability (decrease σ: compare 2,3-butanediol, $\sigma = 0.19$, and 1,4-butanediol, $\sigma = 0.23$, in bullfrog intestine). (3) As one decreases chain length by removing methylene groups $-CH_2-$, permeability decreases (σ increases), but may increase again when one comes to the lowest members of a homologous series (e.g., acetamide to formamide, methyl urea to urea, propanediol to ethylene glycol), particularly in epithelia with "pores". (4) –Cl has approximately the same effect in increasing permeability as does $-CH_3$. (5) Branched solutes are less permeant than straight-chain homologues.

These rules are the same as those noted previously in rabbit gallbladder and other biological membranes, and arise mainly from solute-water hydrogen bonding and entropy effects in the aqueous phase. A more detailed analysis of their molecular interpretation has been given elsewhere (Diamond & Wright, 1969*a*, 1969*b*).

Preferential Permeability to Basic or Acidic Solutes

Comparisons of different plant cells have suggested variation in preferential permeability to acidic or basic solutes (Collander, 1937). For instance, cells of the plant *Rhoeo discolor* have an unusually low relative permeability to basic solutes, compared to other plants.

The sole convincing instance of this type of variation provided by the available epithelial comparisons is that hexamethylene tetramine has a deviant low σ (high permeability) in bullfrog choroid plexus, as noted by Wright and Prather (1970), but not in rabbit or guinea-pig gallbladders. Hexamethylene tetramine is the most strongly basic solute tested in the present study, because it possesses four tertiary amino nitrogens that act as proton acceptors. For example, the addition of 20% oleic acid to olive-oil increases the olive-oil/water partition coefficients of basic solutes, and the increase for hexamethylene tetramine by a factor of 86 is more than double the increase for any other solute tested, with the average value of the factor being 25 for urea derivatives, 9 for monoamides, and 1.6 for neutral nitrogen-free compounds (Collander & Bärlund, 1933, p. 90 and Table 12). Among those solutes for which partition coefficients are available, the epithelial Collander plots give no indication of systematic

differences between σ's for ureas or amides and other solutes with comparable values of $K_{\text{ether}}M^a$, $K_{\text{oil}}M^a$, or $K_{\text{isobutanol}}M^a$, except for the low σ's of the smallest ureas and amides in epithelia with "pores". Detailed comparison of σ's for ureas and amides with those of nitrogen-free solutes possessing the same number of carbon atoms, restricting comparison to solutes too large to permeate through "pores", also provides no clear examples of differences between epithelia. In bullfrog choroid plexus, substituting $K_{\text{oil+acid}}$ for K_{oil} as the abscissa of the Collander plot overcorrects the deviation for hexamethylene tetramine and creates deviations for all but the smallest ureas and amides, suggesting that choroid plexus membranes are less acidic than olive-oil +20% oleic acid. In the other epithelia studied, Collander plots based on $K_{\text{oil+acid}}$ are considerably worse fits to the experimental σ's.

The molecular interpretation of enhanced permeability to basic or acidic solutes is probably that the membrane contains an excess of proton-donating (acidic) or proton-accepting (basic) groups, by analogy with the effect of oleic acid in increasing K_{oil} of basic substances. For example, since the π electrons of a carbon-carbon double bond can form hydrogen bonds with a proton donor (*cf.* Diamond & Wright, 1969*a*, pp. 305–306), and since biological membrane lipids vary in degree of unsaturation, this variation could be related to biological variation in preferential permeability to acidic solutes.

Discrimination against Branched Solutes

Even in bulk nonpolar solvents such as ether, isobutanol, and olive-oil, branched solutes have lower solvent/water partition coefficients than do their straight-chain analogues, for reasons discussed previously (Diamond & Wright, 1969*a*, p. 312). Biological membranes are also less permeable to branched solutes than to straight-chain analogues, but the effect is even more marked than expected from the bulk-phase partition coefficients. Previous studies by Collander (1959) and Smulders and Wright (1971), and the results of the present study (pp. 114–115), show that different biological membranes differ with respect to the degree of discrimination against branched solutes.

The membrane structural variable that controls discrimination against branched solutes is probably the closeness of packing of fatty-acid hydrocarbon tails in the membrane interior. Within a bilayer, the hydrocarbon tails are more regularly ordered in parallel, more closely packed, less fluid and mobile, with stronger cohesive forces, than in a bulk lipid solvent. Regular close packing should preferentially reduce the permeability of

branched solutes by preferentially reducing both the diffusion coefficient and the partition coefficient: the diffusion coefficient, because branched solutes have a greater cross-sectional area than straight-chain analogues; the partition coefficient, because branched solutes locally disrupt ordered packing and reduce interhydrocarbon cohesive forces more than do straight-chain analogues. Particularly the studies of van Deenen and colleagues have identified some of the compositional differences among membrane lipid constituents which determine the closeness of packing of the membrane interior. Increased cholesterol content in membranes with unsaturated lipids, increased fatty-acid chain length, decreased unsaturation of the fatty-acid chains, and more nearly equal lengths of the two esterified fatty-acid chains in a lecithin molecule all serve to make the interior less fluid, more closely packed (van Deenen *et al.*, 1962). These same four factors correspondingly reduce absolute permeability coefficients to nonelectrolytes (de Gier, Mandersloot & van Deenen, 1968). These factors are similarly expected to increase discrimination against branched solutes, and may underlie the differences observed in this respect among the seven epithelia discussed in the present paper. If so, the relatively fluid membrane interior implied by the relatively low discrimination against branched solutes by the intestine may be of physiological significance, in being associated with high absolute permeability coefficients for nonelectrolytes and hence rapid absorption of lipid-soluble metabolites.

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Appendix

The Mass Dependence of Permeation in Biological Membranes, and a Critique of an Analysis by Lieb and Stein (1969, 1971)

Assume that permeability coefficients P are described by an equation of the form

$$P = P_0 K M^a \tag{A.1}$$

where P_0 and a are constants, M's are molecular weights, and K's are membrane/water partition coefficients. To determine the value of a and the bulk solvent whose partition coefficients are closest to those of the membrane, Lieb and Stein transformed this equation to the form

$$\log P = \log P_0 + s_k \log K + a \log M \tag{A.2}$$

where s_k is a measure of the agreement between membrane/water and bulk-solvent/water partition coefficients. Substituting measured or estimated P's for rabbit gallbladder, *Chara*, and *Nitella* and K's for olive-oil or olive-oil + 20% oleic acid, they calculated by a least-squares double linear regression analysis the values of a and s_k. They concluded that a equalled -2.8, -3.5, and -3.7 for these three membranes, respectively, and that there was no need to postulate a separate permeation pathway ("pores") for the smallest nonelectrolytes. However, these conclusions have been scrutinized and rejected by Smulders and Wright (1971) and by Sha'afi *et al.* (1971). Further examination reveals additional errors.

Rabbit Gallbladder. Lieb and Stein rearranged Eq. (1), after omitting the water-drag term, to yield $P_s=(1-\sigma')\,P_{osm}/v_s$, neglected the difference between σ' (corrected for unstirred layers) and σ (uncorrected for unstirred layers), inserted values of σ measured by Wright and Diamond (1969*b*) to obtain relative values of P_s, used $K_{oil+acid}$ as estimates of membrane K's, and obtained $a=-2.8$ by least mean squares. However, since there is a large unstirred-layer effect on the measured σ values, Eq. (1) is the wrong equation to use (Smulders & Wright, 1971). The correct equation would be Eq. (2), but the value of P_{osm} for rabbit gallbladder in Eq. (2) is unknown, hence P_s cannot be calculated in this indirect way from the measured σ's. More recently, Smulders and Wright (1971) determined values of P_s (corrected for unstirred layers) in rabbit gallbladders directly by means of radioactive tracers, and showed that $a=-0.7$.

Lieb and Stein also observed that most of the small polar solutes which deviated from the Collander plots of rabbit gallbladder are basic (ureas and amides), and they suggested that the deviations were because the gallbladder had an acidic membrane rather than because it had pores. The reason for their observation is that there are only five known, very small ($M \leqq 74$) nonelectrolytes with very low partition coefficients ($K_{ether} \leqq 0.0053$, $K_{oil} \leqq 0.00083$), and four of these are urea, methyl urea, formamide, and acetamide. That the deviations are related to their small size and low K's rather than to their being basic follows from the following facts: (1) The fifth very small nonelectrolyte with low K, ethylene glycol, is also deviant, although it is not basic. Its σ is concentration-independent up to at least 115 mM and thus is unlikely to be carrier-mediated, as Lieb and Stein (1971) postulated to explain away the high permeability of ethylene glycol and urea in human erythrocyte. (2) σ's of the numerous larger basic solutes tested, including several ureas and amides (diethyl urea, butyramide, chloroacetamide, malonamide, isovaleramide, hexamethylenetetramine, piperazine,

antipyrine, succinimide) do not deviate below the main pattern of Collander plots: i.e., their σ's are similar to those of neutral solutes with comparable M and K values. The same reasoning applies to guinea-pig intestine, guinea-pig gallbladder, and bullfrog intestine, where the smallest ureas and amides are deviant but the larger ones are not. (3) σ's of the small ureas and amides co-vary with each other but not with σ's of larger ureas and amides such as butyl urea, butyramide, and propionamide (Table 3). As discussed on p. 126, the only instance of preferential permeability to basic solutes noted in the seven epithelia studied is for the single most basic solute tested in bullfrog choroid plexus.

Finally, Lieb and Stein suggested that the low temperature dependence of σ's for deviant small polar solutes in rabbit gallbladder, compared to other solutes, was not correlated with a distinct permeation pathway, but rather meant that activation energies increased continuously with molecular size in a homogeneous lipid membrane. However, the present study has shown that a given small polar solute exhibits low temperature dependence in epithelia where its σ is markedly deviant from the main pattern, and high temperature dependence in epithelia where its σ falls on the main pattern. Although large solutes do sometimes have higher Q_{10}'s of permeation in biological membranes than small solutes, this is not the general rule that Lieb and Stein (1971) would believe by analogy to diffusion coefficients in artificial polymeric membranes, and the cases of conformity in biological membranes may be at least partly due to low Q_{10}'s in a "pore" pathway (*see* Wright & Diamond, 1969*b*, p. 261, for discussion of these cases). There are at least three groups of examples in which large solutes do not have higher Q_{10}'s than small solutes: (*a*) Based on measurements of permeability coefficients in rabbit gallbladder by means of radioactive tracers, the apparent activation energies for permeation of 1,4-butanediol and antipyrine are both in the range 12 to 15 kcal/mole, although the latter compound has more than twice the molecular weight of the former (Smulders & Wright, 1971). (*b*) Restricting comparison to solutes whose σ's fall on the main pattern, there are several cases in rabbit gallbladder of smaller solutes with more markedly temperature-dependent σ's than larger solutes (Wright & Diamond, 1969*a*, pp. 260–261). (*c*) In lecithin-cholesterol bilayer liposomes, the apparent activation energies for permeation of five dihydroxy alcohols varying in molecular weight from 62 to 104 are approximately the same, and close to the value expected for breaking hydrogen bonds (de Gier *et al.*, 1971). Thus, although Lieb and Stein are probably correct in their theoretical expectation that Q_{10}'s for diffusion coefficients D (as opposed to permeability coefficients) within biological membrane lipids should increase

with size, at least two other factors affect Q_{10}'s of permeability coefficients ($P=KD/d$): the breaking of hydrogen bonds which underlie partition coefficients and interfacial activation energies; and low Q_{10}'s for solutes by-passing lipid and permeating via pores.

Nitella. Collander (1954, Table 3) measured P's for 70 solutes, of which four permeated too rapidly and 14 too slowly to determine P accurately and K_{oil} was not determined for four others. Of the remaining 48 solutes, Lieb and Stein selected only 13 for analysis and calculated $a=-3.7$, using values of K_{oil} as estimates of membrane K's. However, their *Nitella* estimate conflicts with the careful analyses of Collander (1954), Smulders (1970), and Smulders and Wright (1971). Collander concluded that a single empirical relation with $a=-1.5$ held for solutes with molecular weight greater than 70, and that a became increasingly steep for smaller solutes with $M<70$. Smulders (1970) and Smulders and Wright (1971) found that linear regression analysis of all 48 solutes yielded $a=-1.8$, s_k 1.1; analysis of all solutes with $M>70$ yielded $a=-1.2$; and analysis of all solutes with $M<70$ yielded -3.7. Probably $a=-1.2$ applies to permeation through membrane lipid, while a much steeper value applies to permeation of the smallest solutes through pores.

The high a value yielded by the Lieb and Stein subset of 13 solutes is due in part to the fact that it includes a disproportionate number of small solutes (the average M for the subset is 94, compared to an average M of 115 for the whole set of 48 solutes). Since a is steeper for the solutes with $M<70$, over-representation of these solutes in a subset must yield a higher value of a than the value for the whole set. Thus, when Smulders (1970) consciously selected several subsets of 12 solutes to obtain high or low average values of M and calculated a, he obtained: average $M=170$, $a=-1.3$; average $M=102$, $a=-1.7$; average $M=67$, $a=-2.7$; average $M=56$, $a=-3.7$. Evidently, the subset selected by Lieb and Stein is unusual in some additional respect besides its low average M of 95, since the a it yielded (-3.7) is matched only by the Smulders subset with the lowest average M (56), considerably lower than that of the Lieb and Stein subset.

As the stated reason for selecting this particular subset of 13, Lieb and Stein (1969, p. 242) claimed that the least-squares method requires accurate data and hence "only those permeants possessing very accurate values of olive-oil: water distribution coefficients (maximum deviation of any single determination from the mean <10 percent) have been included." While the average coefficient of variation of K_{oil} for the 13 solutes selected by this criterion is only 0.05, the average coefficient of variation for all values

of K_{oil} determined by Collander and Bärlund (1933, Table 10) is still only 0.13, with coefficients for all but five solutes less than 0.20. Since, however, the total range of K_{oil} among the 52 *Nitella* solutes is a factor of 7,100, the gain in accuracy obtained by rejecting K_{oil} values with a coefficient of variation greater than 0.05 is so trivial as to make this criterion irrelevant. Ironically, this criterion resulted in at least two much more serious sources of error, for the resulting subset of 13 solutes is not only skewed with respect to M but also is drawn disproportionately from the solutes with the least accurate P values. The subset contains the two solutes with the most variable P values of the 70 tested in *Nitella* [formamide and acetamide: range of P values a factor of 5 and 13, respectively (Collander, 1954, p. 430)], and also contains diacetin, whose P value Collander reported (1954, pp. 424 and 429) to be time-dependent because of contamination with chemically related solutes. From the ranges of variation given by Collander (1954, pp. 428–431), one may calculate that for all solutes the range from the lowest to the highest individual determination is on the average 44% of the mean value, while for the Lieb and Stein subset the average range is 73%. These ranges are much greater than the ranges of K values which Lieb and Stein used as the basis for rejecting solutes.

Chara. Collander and Bärlund (1933, Table 6) measured P's for 45 solutes, of which five permeated too rapidly and eight too slowly to determine P accurately, and K's were not determined for two. Of the remaining 30 solutes, Lieb and Stein selected only 15 for analysis and calculated $a = -2.9$, using values of K_{oil} as estimates of membrane K's. Excluding the solute for which the data point was most deviant in the direction of low a, they recalculated $a = -3.5$. However, we find that if one uses all 30 solutes, one obtains $a = -2.4$ using K_{oil} as a model, and $a = -1.5$ using $K_{oil+acid}$; and if one uses all solutes with $M > 70$, one obtains $a = -1.8$ using K_{oil} and $a = -0.2$ using $K_{oil+acid}$. The correlation coefficient s_k for $K_{oil+acid}$ is nearer 1.0 than is that for K_{oil} (0.98 *vs.* 1.10), hence the a values based on $K_{oil+acid}$ may be more appropriate. Thus, in *Chara* as in *Nitella*, the mass dependence becomes steeper for the smallest solutes. The reason given by Lieb and Stein for selecting the particular 15 compounds used was the same as in the case of *Nitella*, viz, maximum deviation of any single K_{oil} determination from the mean $<10\%$. Since the total range of K_{oil} among the 30 *Chara* solutes is a factor of 2,400, this criterion again seems irrelevant.

Conclusion

The theoretical discussion of nonelectrolyte permeation by Lieb and Stein contains useful insights. However, their calculations of high mass power

dependence (-2.8 to -3.7) for permeation in biological membranes are invalidated by the use of an incorrect formula in one case and by biased data selection in the two other cases, and their arguments against a separate permeation pathway for small polar nonelectrolytes in some biological membranes do not withstand scrutiny. The mass power dependence of permeation through membrane lipid appears to be approximately -0.7 for rabbit gallbladder, -1.8 to -1.2 in *Nitella*, and somewhere between -2.4 and -0.2 (probably between -1.5 and -0.2) in *Chara*. These values are still higher than the value for diffusion coefficients in bulk water (ca. -0.3 to -0.5). The explanation probably involves both the size dependence of diffusion coefficients, as discussed by Lieb and Stein, and size-dependent partition coefficients. One might expect larger solutes to be preferentially excluded from lipid bilayers because of disrupting the cohesive forces between ordered hydrocarbon tails. Some evidence suggestive of this effect on solute partition has been obtained by Krasne (1971).

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