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PERMEABILITY CHARACTERISTICS OF MUSCLE MEMBRANE

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Summary

Unidirectional flux rates of saturated fatty acids, saturated alcohols, and bile acids were measured in an intact rat diaphragm preparation. The logarithm of the permeability coefficients for fatty acids containing from five to ten carbon atoms was a linear function of the number of carbon atoms in the fatty acid chain. Incremental free energies of solution were +336 cal · mol^{-1} for the addition of a hydroxyl group and —258 cal · mol^{-1} for the addition of a methylene group. These incremental free energies were similar to those obtained by other investigators in other animal tissues, and our data suggest a structural similarity between membranes in different tissues and in different species. The muscle membrane exhibited anomalously high permeabilities for fatty acids containing less than five carbon atoms. Since muscle lacks tight junctions, this result suggests that small non-electrolytes traverse polar regions or aqueous pores within the cellular membrane.

Introduction

Permeability characteristics of cellular membranes have been defined by studying permeation patterns of various molecules through these membranes. Numerous experiments have been conducted in various mammalian and non-mammalian epithelial tissues [1—11]. No study, however, has been undertaken to measure the permeability characteristics of the muscle membrane. Studies in muscle tissue would make it possible to compare the permeability characteristics of this tissue with published data from other cell membrane preparations.

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Studies in muscle tissue could also provide insight into the pathways that ions and small non-electrolytes use to enter the cell. Investigators have suggested that these molecules bypass the membrane and traverse 'tight junctions' (zona occludens) which connect epithelial cells [1,2,4,12—14]. Muscle tissue does not have tight junctions and uptake of ions and small non-electrolytes should occur across the membrane itself.

We determined unidirectional flux rates for a homologous series of saturated fatty acids and saturated alcohols and for bile acids with related groupings in intact diaphragm. True permeability coefficients were calculated by correcting flux rates for the unstirred water layer, and incremental free energies ($\delta\Delta F_{w->1}$) were determined for both hydroxyl (-OH) and methylene (-CH_2-) groups using these permeability coefficients. Previously reported permeability coefficients were converted to free energy values and compared with our results.

Materials and Methods

Chemicals and reagents. Radioactively-labeled ([1-^{14}C]-) and unlabeled saturated fatty acids and saturated alcohols were all 99% pure and used as provided from Applied Science Laboratories, Inc., State College, PA. Unlabeled bile acids, obtained from Steraloids, Inc., Pawling, NY, and radiolabeled ([24-^{14}C]-)bile acids, obtained from Mallinckrodt Chemical Works, St. Louis, MO, were purified when necessary by thin-layer chromatography. ^{3}H-labeled dextran (molecular weight 15 000—17 000) was obtained from New England Nuclear Corp., Boston, MA. All chemicals were dissolved in a Ca^{2+}-free Krebs/bicarbonate buffer oxygenated with 5% CO_2/95% O_2 resulting in a pH of 7.4 at 37°C.

Tissue preparation. Male Sprague-Dawley rats (180—220 g) maintained on an ad libitum diet of rat chow were killed by a blow on the head and bled. The intact diaphragm was dissected taking care not to sever any muscle tissue [15]. The sternum was cut horizontally at the level of the fourth to fifth costal cartilage. The abdominal and back muscles were dissected from the lower rib cage and the ribs transected superior to their diaphragmatic insertions. Abdominal and thoracic structures (ligaments, vessels, etc.) transversing the diaphragm or adhering to the central tendon were dissected. The spine was transected above and below the twelfth vertebra freeing the intact diaphragm. All extraneous tissues were removed. The dissection procedure was performed in 3 min with no apparent damage to the diaphragm. Fragments from the dissection were removed by immersing the diaphragm in ice-cold 0.154 M NaCl for 5—10 s. Each diaphragm was secured by tying 3-O silk surgical suture to a portion of protruding rib and placed in 4°C oxygenated buffer where they remained until incubation. No tissue remained in 4°C buffer for longer than 15 min. Prior to incubation each diaphragm was placed in a beaker of Ca^{2+}-free Krebs/bicarbonate buffer oxygenated with 5% CO_2/95% O_2 at 37°C for 5 min (pre-incubation). The only agitation in the pre-incubation or incubation beakers was produced by the stream of oxygenating bubbles.

Flux-rate determinations. Measurements of unidirectional flux rates of saturated fatty acids and bile acids were performed using a modification of the

technique developed in this laboratory [16]. After the 5 min period of preincubation each diaphragm was suspended by the suture in a beaker of Ca^{2+}-free Krebs/bicarbonate buffer at 37° C containing both ^{14}C-labeled test substance (probe) and ^{3}H-labeled dextran, the latter used as a marker to measure adherent mucosal fluid volume, i.e., the fluid adhering to the tissue. After an 8 min incubation period (except during time courses) each diaphragm was washed for several seconds in cold 0.154 M NaCl, blotted, placed on filter paper moistened with 0.154 M NaCl, cut into two portions free of extracellular debris, and transferred to a previously tared counting vial. After drying overnight at 93° C the dry tissue weight was determined by weighing the vial containing dried tissue and subtracting the original weight. The diaphragm was then hydrolyzed with 0.8 ml of 0.75 N NaOH and autoclaved at 110° C for 6 min. After cooling at room temperature, 15.8 ml of an acid/toluene/Triton X-100 scintillation fluid were added to the vial.

Flux-rate measurements of volatile alcohols required a different technique. At the conclusion of the incubation period the diaphragm was bisected. The wet weight of each half of the specimen was determined. One tissue half was transferred to a previously tared counting vial and placed in a drying oven. After drying overnight at 93° C, the dry tissue weight was determined as above. The other tissue half was transferred to a counting vial to which 0.8 ml of 0.75 N NaOH was added. The vial was then capped. The tissue was hydrolyzed in a metabolic shaker at 37° C. After cooling at 4° C for 30 min, 15.8 ml of the acid/toluene/Triton X-100 scintillation fluid were added to the vial. The dry weight of the saponified sample was determined by multiplying the wet weight of the hydrolyzed tissue by the dry weight : wet weight ratio obtained from the half portion dried in the oven.

Radioactivity was measured in a Packard Tri-Carb liquid scintillation counter using an external standard technique to correct for quenching. Flux rates (J_d) are expressed as nmol of the probe absorbed into the muscle per min per 100 mg dry tissue weight (nmol/min per 100 mg). J_d values were normalized to 1 mM concentration after accounting for the mass in the adherent fluid volume measured in microliters of solution per 100 mg dry tissue weight ($\mu l \cdot 100\ mg^{-1}$).

Determination of surface area. Tissue samples (1 cm diameter) obtained by punch biopsy were dried and used for the determination of surface area per 100 mg dry weight ($cm^2 \cdot 100\ mg^{-1}$).

Free diffusion coefficients. The free diffusion coefficients (D) for the fatty acids, bile acids, and alcohols used in this study were obtained as described [8] and are listed in Table I, Column B.

Statistical analysis. Data are expressed as mean net uptake ±1 S.E.

Results

Adherent fluid volume. A period of incubation must be selected in which the entire adherent fluid volume is uniformly labeled by an impermeant molecule. As seen in Fig. 1, [^{3}H]dextran did not achieve equilibrium until after 6 min of incubation. If the time allowed for incubation is less than that required for uniform distribution of the volume marker, a situation will arise where the

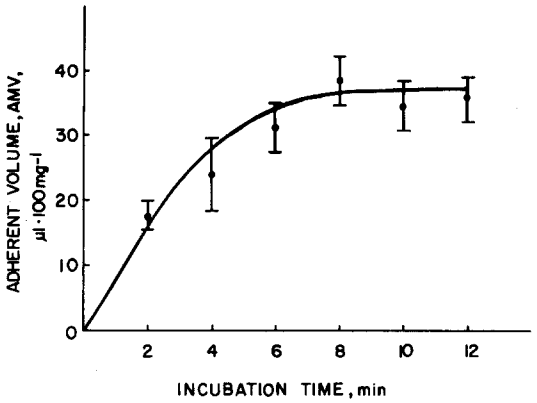

Fig. 1. Characteristics of labeling of the adherent fluid volume in intact rat diaphragm as a function of incubation time. [^{3}H]Dextran was used as the volume marker. Each point represents the mean ±1 S.E. for determinations in six animals.

probe is distributed much more quickly than the marker (i.e., $D_{probe} > D_{marker}$). In such a situation the tissue uptake would be overestimated and the adherent fluid volume underestimated. A time period greater than 6 min was selected for incubation of the diaphragm.

Transport characteristics of tissue uptake. Unidirectional uptake was determined with respect to time and concentration using four saturated fatty acids (hexanoic, heptanoic, octanoic and nonanoic acids) two bile acids (taurocholate and taurodeoxycholate), and one saturated alcohol (decanol) as probe molecules. Competition studies were also performed using these probe molecules.

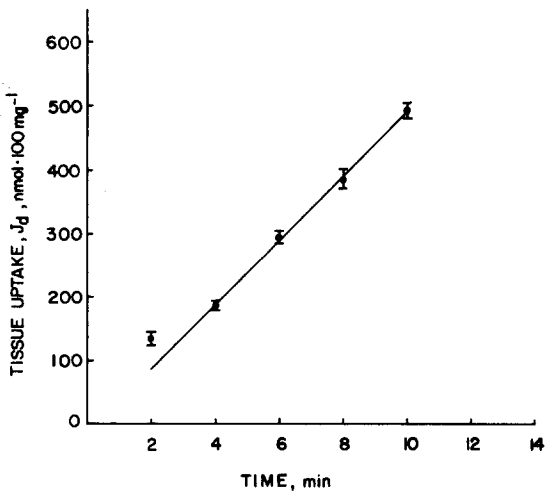

Fig. 2. The uptake, J_d, of octanoic acid into intact rat diaphragms as a function of time. Each specimen was incubated in Ca^{2+}-free Krebs/bicarbonate buffer for 8 min in the concentration course. Each point represents the mean ±1 S.E. for determinations in six animals.

TABLE I

RATES OF UPTAKE OF SATURATED FATTY ACIDS, SATURATED ALCOHOLS AND BILE ACIDS INTO MUSCLE (DIAPHRAGM) TISSUE AND CALCULATION OF PERMEABILITY COEFFICIENTS FOR THESE VARIOUS PROBE MOLECULES

J_d values were determined from solution using concentration of the test molecules of 0.25 to 1.0 mM. All values are expressed as mean uptake ±1 S.E. for at least six animals and were normalized to a concentration of 1.0 mM. To calculate P for various probe molecules, J_d values were corrected for the unstirred water layer as described in the text. D values were obtained as described in Ref. 8. The correction term for the unstirred water layer shown in column D was calculated with a value of 21.3 $cm^2 \cdot 100\ mg^{-1}$ for S_w and 325 μm for d. Two different sets of P values were calculated. The first group (column E) was normalized to tissue dry weight. In the second group (column F) the P values were normalized to the surface area of the diaphragmatic membrane and were calculated by dividing each P value in column E by S_m, estimated to equal 21.3 $cm^2 \cdot 100\ mg^{-1}$.

Test molecule	Uptake rate, J_d (nmol/min per 100 mg) (A)	Free diffusion coefficient, D ($\times 10^6$) ($cm^2 \cdot s^{-1}$) (B)	$10^{-4} \times \frac{J_d}{D}$ (nmol/cm^2 per 100 mg) (C)	$\frac{(J_d)(d)}{(S_w)(D)}$ (mM) (D)	Permeability coefficient, P (nmol/min per 100 mg/mM) (E)	Permeability coefficient, P ($\times 10^6$) ($cm \cdot s^{-1}$). (F)
(A) Saturated fatty acids						
2 : 0	37.4 ± 2.9	16.7	3.7 ± 0.3	0.06	39.8	31.1
4 : 0	23.6 ± 1.7	12.1	3.3 ± 0.2	0.05	24.8	19.4
5 : 0	24.4 ± 2.8	10.9	3,7 ± 0.3	0.06	26.0	20.3
6 : 0	29.2 ± 0.8	10.5	4.6 ± 0.1	0.07	31.4	24.5
7 : 0	33.3 ± 2.2	10.2	5.4 ± 0.4	0.08	36.2	28.3
8 : 0	45.8 ± 0.7	9.5	8.0 ± 0.1	0.12	52.0	40.6
9 : 0	84.4 ± 8.8	8.9	15.8 ± 1.6	0.24	111.1	86.8
10 : 0	98.0 ± 5.2	8.4	19.4 ± 1.0	0.30	140.0	109.4
12 : 0	160.0 ± 11.2	7.6	35.1 ± 2.5	0.54	347.8	271.8
(B) Saturated alcohols						
8 : 0	146.0 ± 12.9	9.9	24.7 ± 2.2	0.38	236.8	185.1
10 : 0	351.2 ± 44.5	8.7	67.3 ± 8.5	1.03	—	—
12 : 0	241.2 ± 23.6	7.7	52.2 ± 5.1	0.80	—	—
(C) Bile acids						
Cholate	32.9 ± 0.9	5.7	9.6 ± 0.3	0.15	38.7	30.2
Taurocholate	3.3 ± 0.4	5.5	1.0 ± 0.1	0.02	3.4	2.7
Deoxycholate	43.8 ± 2.1	5.7	12.8 ± 0.6	0.20	54.8	42.8
Taurodeoxy-cholate	7.2 ± 1.0	5.5	2.2 ± 0.3	0.03	7.4	5.8

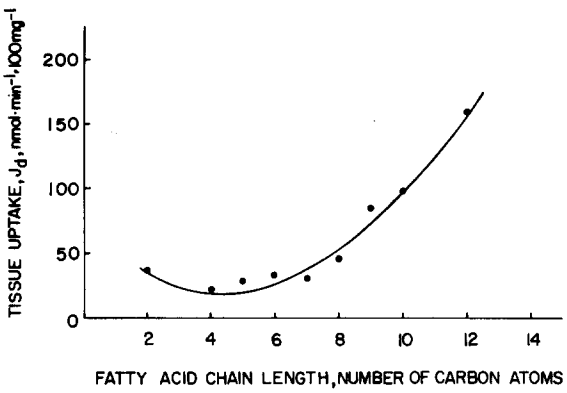

Fig. 3. The relationship between the rates of uptake (J_d) of a homologous series of fatty acids and the number of carbon atoms in each compound. Each specimen was incubated in Ca^{2+}-free Krebs/bicarbonate buffer for 8 min. Each point represents the mean for determinations in six animals.

The rate of tissue uptake was linear with respect to time to 10 min and extrapolated essentially to zero at zero time for all compounds studied (Fig. 2). The finding coupled with the adherent fluid volume data (equilibrium after 6 min), dictated that the standard period of incubation be greater than 6 min and less than 10 min. 8 min was chosen as the experimental incubation period. As would be anticipated, the tissue uptake of all probes tested was artifactually high at 2 min (the probe equilibrated much more quickly than the volume marker).

Tissue uptake of fatty acids was linear with respect to increases in concentration. No data suggestive of saturation kinetics for fatty acids (hexanoic, 0—4 mM; heptanoic, 0—4 mM; octanoic, 0—4 mM; and nonanoic, 0—2 mM) bile salts (taurocholate, 0—20 mM and taurodeoxycholate, 0—20 mM) or an alcohol (decanol, 0—2 mM) were apparent. Tissue uptake was not inhibited by closely related solutes. Octanoate uptake was unaffected by heptanoic or nonanoic acid, and taurocholate uptake was unaffected by taurodeoxycholate. The kinetics exhibited by the diaphragm were those of a passive transport system for those probe molecules studied.

Apparent permeability coefficients of solutes. Flux rates were determined for a homologous series of saturated fatty acids between acetic acid and dodecanoic acid, for saturated monoalcohols between octanol and dodecanol, and for several bile acids (conjugated and unconjugated) as shown in Table I. The pK value for all the fatty acids is significantly lower than pH 7.4 and the population of non-ionized species, the permeable molecule is essentially constant. (The apparent permeability of fatty acids can increase by decreasing the pH from 7.4 to 6.0. This pH shift increases by 50-fold the relative concentration of the permeant non-ionized species.) Flux rates increase in a hyperbolic fashion between pentanoic and dodecanoic acid when the fatty acid chain length is plotted vs. J_d, as seen in Fig. 3. This indicates that the most significant determinant of fatty acid permeability in this preparation is the characteristic of the membrane (see Discussion). The uptakes for acetic and butyric acids are anomalously high.

Discussion

This study was undertaken to define the properties of a mammalian tissue which is functionally and histologically unlike previously examined tissue. Permeability characteristics have been studied in epithelial tissues [1—11], adipocytes [17], red blood cells [18,19], single-celled species [20], and artificial membrane systems [21,22], but never in muscle tissue. Diaphragmatic uptake of various fatty acids, bile acids, and an alcohol is characterized by a process of passive permeation.

We have referred to our permeability coefficients as apparent values because the cell membrane is not the only barrier to a permeating molecule. Adjacent to every biological membrane is water which is more structured and not in equilibrium with the bulk phase solution, the unstirred water layer [23]. There is no instance in which the flux rate of molecular permeation is dictated solely by the cell membrane. A correction must be made for diffusion through the unstirred water layer to determine the true permeability coefficient of the membrane.

The rate of movement, J, of a molecule across the unstirred water layer is defined by the equation:

$$J = D/d\ (C_1 - C_2)\ . \tag{1}$$

where D is the free diffusion coefficient of the solute, d represents the thickness of the unstirred water layer, and C_1 and C_2 equal the concentration of the probe in the bulk phase and at the water/lipid interface, respectively.

Movement of the probe molecules across the cell membrane is defined by the equation:

$$J = P(C_2) \tag{2}$$

where P is the true permeability coefficient for the molecule. To define P the concentration at the cell membrane (C_2) must be known. Solving Eqn. 1 for C_2:

$$C_2 = C_1 - J(d/D) \tag{3}$$

where $J(d/D)$ represents the correction factor for the effect of the unstirred water layer.

The experimental flux, J_d, must be converted into a term which can be substituted in the above equations. J_d is expressed in the units, mass/unit time per 100 mg, and J must be expressed as mass/unit time per unit area. A conversion factor, S_w, representing the effective surface area of the unstirred water layer through which a molecule moves and having the units, area/100 mg, has been derived. It is assumed that S_w is equal to the effective surface area of the cell membrane, S_m, also expressed in terms of area/100 mg and that both surfaces are essentially planar. The assumption is supported by the finding that the rat diaphragm appears flat when examined histologically.

The term, J_d/S_w, can be substituted for J and Eqn. 3 can be rewritten as:

$$C_2 = C_1 - (J_d/S_w)(d/D) \tag{4}$$

and Eqn. 2 becomes:

$$P = J/C_1 - (J_d/S_w)(d/D) \tag{5}$$

When J_d/S_m is substituted for J to correct for the effective surface area of the cell membrane, Eqn. 5 becomes:

$$P = (J_d/S_m)/C_1 - (J_d/S_w)(d/D) \quad (6)$$

S_w and S_m (experimentally determined) were 21.3 cm^2 per 100 mg.

The determination of P depends upon an accurate measurement of d, the only unknown value. Two mathematical methods were used to calculate d:

(1) In the situation where the membrane is the absolute rate-limiting step, i.e., $D/d >>> P$, the unstirred water layer can be calculated from the formula:

$$d = (D \cdot t_{1/2}/0.38)^{1/2} \quad (7)$$

where D is the free coefficient of diffusion for an impermeant molecule and $t_{1/2}$ the half-time required for this molecule to uniformly distribute itself throughout the unstirred water layer [24]. Dextran has been shown in our preparation to require 8 min to uniformly label the unstirred water layer. Solving the equation for dextran with a D value of $1.00 \cdot 10^{-6}$ $cm^2 \cdot s^{-1}$ and a $t_{1/2}$ of 4 min, d equals 251 μm [25].

(2) The thickness of the unstirred water layer can also be calculated by solving Eqn. 4 for d. C_2 may be eliminated (approaches zero) in the special situation where the uptake rate of the probe molecule is limited wholly by the unstirred water layer, i.e., $P >>> D/d$. In this situation:

$$d = (S_w)(D)(C_1)/J_d \quad (8)$$

Molecules which cross the lipid membrane more quickly than the unstirred water layer can be located in Table I by dividing the J_d value of each probe by its respective D value. When a probe molecule's movement into the cell is limited by its rate of diffusion across the unstirred water layer, its J_d/D value should become constant, i.e., J_d in the diffusion-limited solute is directly proportional to D. This relationship develops in the less polar members of any homologous series. J_d/D should increase log-linearly with increasing chain length in molecules of which the uptake is limited by the cell membrane. This relationship exists because the addition of each $-CH_2-$ group to the molecule increases the permeation rate of the cell membrane by a constant factor.

In the homologous series of fatty acids there is no plateau in the values of J_d/D (column 3 of Table I). The flux rates of the fatty acids tested increase by a constant factor when plotted as $\ln(J_d/D)$ vs. chain length, i.e., the cell membrane is the rate-limiting factor (Fig. 4). The $\ln(J_d/D)$ values rise for octanol and decanol. The $\ln(J_d/D)$ value for dodecanol is similar to that of decanol representing a situation where the unstirred layer is rate-limiting, and, therefore, d can be calculated for decanol and dodecanol. The calculated d values for decanol and dodecanol were 317 and 408 μm, respectively. The mean of the calculations by both methods 1 and 2 is 325 μm. This value was used in subsequent calculations.

Using the calculated d value, a correction factor:

$$(J_d/S_w)(d/D) \quad (9)$$

may be determined for any probe molecule (column D of Table I). The value of C_2 may be calculated and the true permeability coefficient may be determined

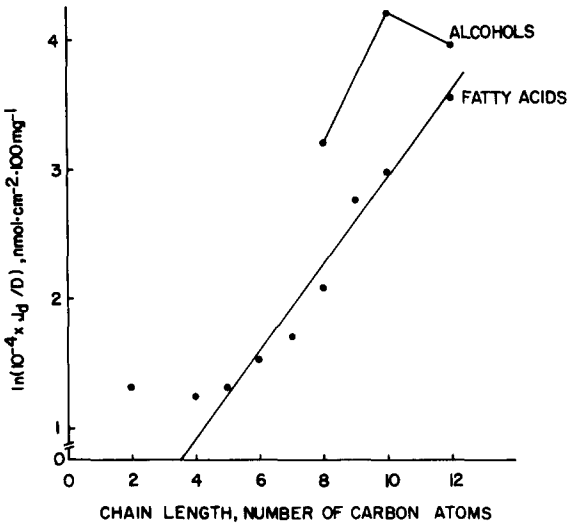

Fig. 4. The relationship between the $\ln(10^{-4} \times J_d/D)$ determined (Table I) and the number of carbon atoms in each compound.

(column F, Table I).

$$P = (J_d/S_m)/C_2 \quad (10)$$

Our data demonstrate the effect of the unstirred water layer on uptake and the error encountered if correction for this barrier is not considered. The data also distinguish between probe molecules of which the movement into the cell is limited by the unstirred water layer and those molecules of which the uptake is dictated by the membrane itself.

In Fig. 5, P values in rat diaphragm are plotted semilogarithmically vs. increasing chain length; comparison is made with rabbit jejunum similarly plotted [11]. Although absolute values vary, the slopes are similar. Both membranes, therefore, appear to have similar permeability characteristics.

The apparent permeability coefficients of bile acid monomers vary with differences in their molecular structure [26]. For any given number of -OH groups, unconjugated bile acids are absorbed more quickly than their conjugated counterparts, and removal of an -OH group increases the permeability coefficient. Differences in molecular weights are insignificant when compared to the magnitude of permeability changes observed. Stein calculated the number of hydrogen bonds bile acids can form in an aqueous solution and has noted that the function $\ln(J_d M^{1/2})$ for a series of bile salts is inversely related to the number (N) of hydrogen bonds that a bile acid can form [26]. When $\ln(J_d M^{1/2})$ of our data is plotted vs. N a linear relationship ($y = 10.662 - 0.526r = 0.94$) exists, i.e., the addition of functional groups which form additional hydrogen bonds decrease $\ln(J_d M^{1/2})$ by a factor of 0.526. When $\ln(J_d M^{1/2})$ was plotted vs. N in rat jejunum, the slopes were -0.386 and -0.607 [9,10]. Rat jejunum and diaphragm have similar slopes and appear to have similar permeability characteristics.

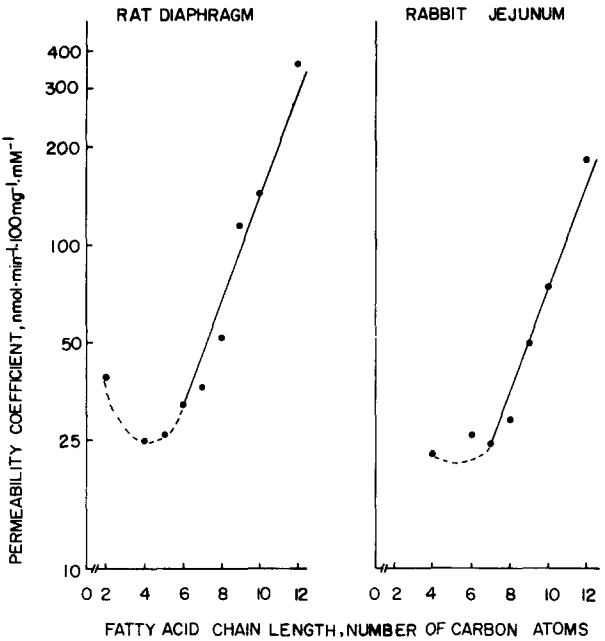

Fig. 5. (Left panel) The relationship between the true permeability coefficients (P) determined for a homologous series of fatty acids (Table I) and the number of carbon atoms in each compound in rat diaphragm. (Right panel) This same relationship in rat jejunum. Data from Ref. 11.

Permeability characteristics of membranes can be described in thermodynamic terminology if accurate values of true permeability coefficients are available. The permeability coefficient of any solute is proportional to K, its partition coefficient (the steady state ratio of a given solute's distribution into two immiscible phases between which the solute can freely migrate). K is proportional to $e^{-\Delta F_{w\rightarrow 1}/RT}$ where $\Delta F_{w\rightarrow 1}$ is the free energy required to transfer 1 mole of a solute from the water to the lipid phase or from an aqueous solution into the lipid membrane, R, the gas constant, and T, the absolute temperature. The ln P is proportional to $\Delta F_{w\rightarrow 1}$.

There is no method of determining absolute free energies of solution but an incremental free energy or $\delta\Delta F_{w\rightarrow 1}$ can be determined by the addition of a functional group:

$$\delta\Delta F_{w\rightarrow 1} = -RT \ln(P^{+}/P^{0}) \tag{11}$$

where P^{+} and P^{0} are the permeability coefficients of the probe molecule with and without the substituent group, respectively [27]. When the logarithm of permeability coefficients is plotted as a function of chain length for the fatty acids from hexanoic to dodecanoic the slopes of the best fit straight lines correspond to an average increase in P by a factor of 1.52 for each -CH_2-group added to the chain. This is equivalent to an incremental free energy change of −258 cal · mol^{-1}. Using the permeability data for the bile acids studied, the addition of an -OH group reduces permeability of the probe molecule by a factor of 0.58 ($\delta\Delta F_{w\rightarrow 1}$, +315 cal · mol^{-1}) and the substitution of a taurine group for a

TABLE II

INCREMENTAL FREE ENERGIES OF SOLUTION, $\delta\Delta F_{w\rightarrow 1}$ AND $\delta\Delta F_1$, ASSOCIATED WITH THE ADDITION OF THE $-CH_2-$ AND —OH SUBSTITUENT GROUPS TO PROBE MOLECULES IN VARIOUS MEMBRANES AND BULK SOLVENTS

$\delta\Delta F_{w\rightarrow 1}$ associated with $-CH_2-$ and —OH groups reported in the literature as determined from both permeability data and partition coefficients. $\delta\Delta F_{w\rightarrow 1}$ was calculated using the equation, $\delta\Delta F_{w\rightarrow 1} = -RT\ln(P+/P^0)$. $P+$ and P^0 represent the permeability coefficients for the probe molecules with and without substituent group, respectively. The values of $\delta\Delta F_1$ were calculated from the relationship, $\delta\Delta F_{w\rightarrow 1} = -\delta\Delta F_w + \delta\Delta F_1$, where $\delta\Delta F_w$ for the $-CH_2-$ and —OH groups was assumed to equal —7000 cal · mol^{-1} and +160 cal · mol^{-1}, respectively.

Membrane or Solvent	Addition of $-CH_2-$		Addition of —OH		References
	$\delta\Delta F_{w\rightarrow 1}$ (cal · mol^{-1}) (A)	$\delta\Delta F_1$ (cal · mol^{-1}) (B)	$\delta\Delta F_{w\rightarrow 1}$ (cal · mol^{-1}) (C)	$\delta\Delta F_1$ (cal · mol^{-1}) (D)	
(A) Non-epithelial tissue and cell *					
(1) Rat diaphragm	—258	—98	+336	—6664	Present study
(2) Rat adipocyte	—547	—387	+1225	—5775	17
(3) Human erythrocyte ghost membranes	—686	—526	—	—	18
(B) Epithelial tissue *					
(1) Rabbit gallbladder	—143	+17	+422	—6561	4
(2) Rabbit jejunum	—258	—98	+564	—6436	11
(3) Rat ileum	—	—	+757	—6243	9
(4) Rat jejunum	—356	—196	+874	—6121	8, 10
(C) Epithelial tissue **					
(1) Frog choroid plexus	—210	—50	+209	—6791	7
(2) Toad urinary bladder	—542	—382	+1195	—5805	7
(D) Plant cells					
(1) Nitella mucronata	—610	—450	+3600	—3400	20
(2) Chara ceratophylla	—	—	+1860	—5140	20
(E) Bulk solvents					
(1) Isobutanol	—530	—370	+1000	—6000	27
(2) Olive oil	—660	—500	+2800	—4200	27
(3) Ether	—670	—510	+2100	—4900	27
(4) Octanol	—690	—530	+1240	—5760	22, 28
(5) Isopentanol	—	—	+1200	—5800	22

* Mamalian.

** Non-mammalian.

carboxyl group reduced permeation by a factor of 0.11 ($\delta\Delta F_{w->1}$, +1360 cal · mol^{-1}). The substitution of an R-COO^- group for an R-H_2COH group reduced permeation by a factor of 0.13 ($\delta\Delta F_{w->1}$, +1252 cal · mol^{-1}).

Table II shows the $\delta\Delta F_{w->1}$ associated with -CH_2- and -OH groups in various membranes and bulk solvents. The $\delta\Delta F_{w->1}$ values of these two substituent groups indicate that: (1) the cell membrane of the muscle tissue is much more polar than bulk solvents; and (2) muscle tissue, rabbit gallbladder, rabbit jejunum, and frog choroid plexus are similar in their permeability characteristics and are the most polar tissues studied to date. The significance of this observation is not clear.

Undirectional flux rates of acetic and butyric acids are higher than would be predicted from a linear extrapolation of the other fatty acids. There are several explanations for this apparently deviant behavior. Firstly, carrier-mediated active or facilitated transport mechanisms might exist within the membrane for low molecule weight polar molecules. Studies in rat intestine [8] and now in rat diaphragm have not revealed kinetics consistent with those of a carrier-mediated system, although kinetic data have only been generated using fatty acids of at least six carbon atoms. In addition, publications from several laboratories reported similar anomalously high permeation rates for different probe molecules that had no chemical or structural similarity. It is unlikely that this behavior can be attributed to the presence of a carrier-mediated transport system operative for all these molecules. Secondly, because the kinetics of low molecular weight polar molecules were initially studied in epithelial tissue, it is possible that these molecules might passively move through relatively low-resistance pathways, i.e., tight junctions [1,2,4,9,13,14]. High permeation rates for these compounds have been demonstrated in isolated adipocytes and red blood cell ghost membranes, thus, this does not appear to be an acceptable explanation [17,19]. Thirdly, existence of enhanced uptake of acetic and butyric acids in our system coupled with recent studies, supports the hypothesis that high rates of permeation must be explained in terms of some inherent property of the cell membrane itself. Muscle tissue unlike epithelial tissue has no tight junctions. Pathways (polar regions or aqueous pores) must exist within the membrane itself [7,17], and low molecular weight polar molecules must use these pathways as their major mode of transmembranal permeation.

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