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Professor Edmond Gabbay died as the experimental work for this paper was being conducted. His creative ideas and experimental work in organic, medicinal, and biochemistry will stand as a memorial to him, and this paper is dedicated to his memory.

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Liquid Membrane Phenomenon in Diuretics

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Abstract □ Surface activity and critical micelle concentrations are reported for two diuretic drugs, furosemide and triamterene. The drugs generate a liquid membrane on a supporting membrane. Transport of chloride, sodium, and potassium ions through the liquid membranes generated by the drugs was studied. The data suggest that the phenomenon of liquid membrane formation may contribute to the diuretic action.

Keyphrases □ Diuretics—liquid membrane phenomenon, critical micelle concentrations, chloride, sodium, potassium ion transport, furosemide, triamterene □ Furosemide—liquid membrane phenomenon, anion/cation transport □ Triamterene—liquid membrane phenomenon, anion/cation transport

Drugs known to act by altering the permeability of cell membranes are mostly surface active. Surface-active agents added to an aqueous phase are known to generate a surfactant-layer liquid membrane at the interface which modifies material transport across it (1). It is therefore likely that the liquid membranes generated by surface-active drugs play a role in the mechanism of drug action. A wide category of surface-active drugs have been investigated in this laboratory (2-7), and results indicated that the liquid membranes contribute to the mechanism of drug action.

Most of the high-ceiling diuretics (8) are known to act by altering the reabsorption of cations (*e.g.*, Na^+) and anions (*e.g.*, Cl^-) in the ascending limb of the loop of Henle (8). Although diuretics act by modifying the membrane permeability, their surface activity is not documented in the literature. In the present study, surface activity of these drugs is demonstrated—critical micelle concentrations (CMC) have been determined—and the existence of liquid membranes generated by them at the interface is confirmed. Transport of relevant cations and anions in the presence of the liquid membranes generated by the drugs has been studied. The data indicate that the liquid membranes generated by the diuretic drugs contribute to the mechanism of their action. A cellulose nitrate microfiltration membrane/aqueous interface was chosen as a site for the formation of the liquid membranes to eliminate the possibility of active and specific interaction of the drugs with the constituents of the biological membranes and to highlight the role of passive transport through the liquid membranes. Two structurally dissimilar diuretic drugs (furosemide and triamterene) were chosen for the present study.

EXPERIMENTAL SECTION

Material—Furosemide[1], triamterene[2], sodium chloride[3], potassium chloride[3], and water (glass distilled over potassium permanganate) were used.

Methods—The critical micelle concentration (CMC) of aqueous furosemide and triamterene solutions were determined from the variation in surface tension with concentration at 37 ± 0.1°C. The surface tensions were measured using a tensiometer[4]. To prepare an aqueous solution of furosemide, the drug

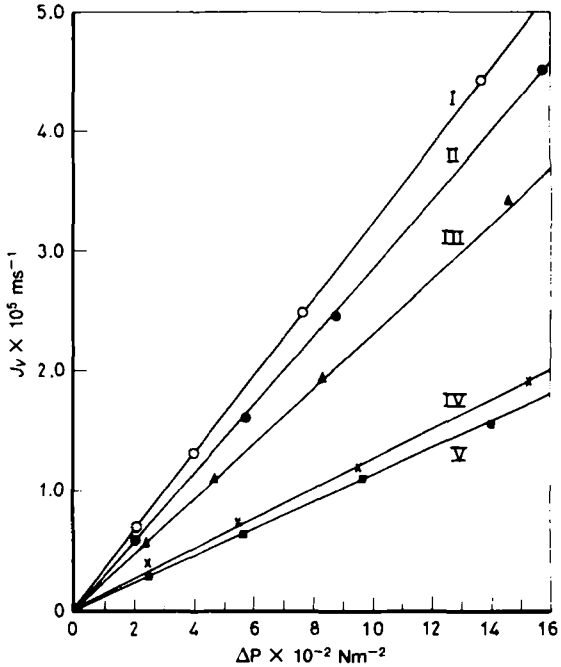

Figure 1—*Hydraulic permeability data. Curves I, II, III, IV, and V are for 0, 2.08 × 10^{-5}, 4.16 × 10^{-5}, 8.3 × 10^{-5}, and 24.9 × 10^{-5} M furosemide concentrations, respectively.*

[1] Hoechst Pharmaceutical, Ltd.
[2] S, K & F Ltd.
[3] Analytical grade.
[4] Model 21 Tensiomat; Fisher.

Table I—Values of the Hydraulic Conductivity Coefficient (L)[a] at Various Concentrations of Furosemide and Triamterene

	Furosemide Concentration, × 10^5 M					Triamterene Concentration, × 10^6 M				
	0	2.08 (0.25 CMC)	4.16 (0.5 CMC)	8.3 (CMC)	24.9	0	2.0 (0.2 CMC)	5.0 (0.5 CMC)	10.0 (CMC)	30.0
$L^b \times 10^8$ ($m^3 \cdot s^{-1} \cdot N^{-1}$)	3.56 ± 0.266	2.73 ± 0.105	2.20 ± 0.416	1.11 ± 0.075	1.26 ± 0.058	3.56 ± 0.090	3.06 ± 0.102	1.95 ± 0.088	0.59 ± 0.072	0.56 ± 0.066
$L^c \times 10^8$ ($m^3 \cdot s^{-1} \cdot N^{-1}$)	—	2.94 ± 0.105	2.33 ± 0.416	—	—	—	2.96 ± 0.102	2.08 ± 0.088	—	—

[a] Expressed as mean ± *SD*. [b] Experimental values. [c] Calculated on the basis of the mosaic model.

was dissolved in acetone and added (stirred constantly) to the aqueous phase. The concentration of acetone in all the furosemide solutions was ≤0.4% (v/v). A corresponding control solution with 0.4% (v/v) acetone was used as a blank. For preparing aqueous solutions of triamterene, a 0.12% (v/v) aqueous solution of dioxane was used as a solvent. A corresponding blank solution, without triamterene, was used for comparison of surface tension values. The CMC values for furosemide and triamterene were 8.3×10^{-5} and 1×10^{-5} M, respectively.

The all-glass transport cell previously desribed (2) was used for the transport studies. A cellulose nitrate microfiltration membrane[5], which acted as support for the liquid membrane, separated the cell into two compartments, C and D. The hydraulic permeability data were obtained at concentrations below and above the CMC values of the drugs, using the method previously described (2).

The solute permeability (ω) of chloride ions was measured in the presence of furosemide; that of sodium and potassium ions was measured in the presence of triamterene using (9, 10):

$$\left(\frac{J_s}{\Delta\pi}\right)_{J_V=0} = \omega \qquad \text{(Eq. 1)}$$

where J_s and J_V are, respectively, the solute flux and the volume flux per unit area of the membrane; $\Delta\pi$ is the osmotic pressure difference across the membrane. The method of measurement has been described (2).

Two sets of experiments were performed for solute permeability measurements. In the first set, aqueous solutions of the drugs and the permeating species were kept in one compartment (compartment C of the transport cell, Fig. 1. of Ref. 2) and the other compartment (D) was filled with distilled water. In the second set of experiments, aqueous solutions of the drugs were taken in compartment D while compartment C contained aqueous solutions of the permeating species. No drug was used in the control experiments. Since hydrophobic ends of the surface-active drugs would be preferentially oriented towards the hydrophobic supporting membrane (the cellulose nitrate microfiltration membrane) in the first set of experiments, the permeating species would face the hydrophilic surface of the drug liquid membrane. In the second set of experiments, however, the permeating species would face the hydrophobic surface of the drug liquid membranes.

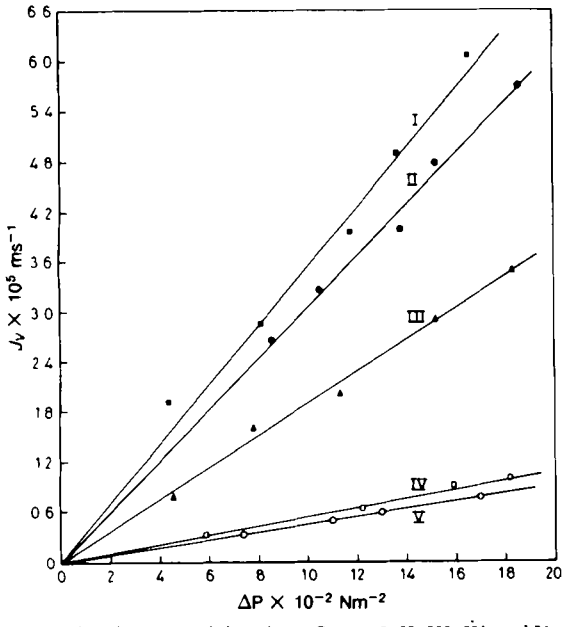

Figure 2—*Hydraulic permeability data. Curves I, II, III, IV, and V are for 0, 2.0×10^{-6}, 5.0×10^{-6}, 1.0×10^{-5}, and 3.0×10^{-5} M triamterene concentrations, respectively.*

[5] Cat. No. 11307; Sartorius (thickness, 1×10^{-4} M; area, 1.794×10^{-5} m^2).

The concentrations of the diuretic drugs used in the solute permeability experiments were always higher than their CMC values to ensure that the supporting membrane was completely covered by the liquid membranes generated by them. The concentration of furosemide was 2.5×10^{-4} M and that of triamterene was 3.0×10^{-5} M. Fifteen replicates were taken for each value of ω. All measurements were made at a constant temperature using a thermostat set at 37 ± 0.1°C.

Assays—The amount of chloride ions transported to the other compartment was measured by the spectrophotometric[6] determination at 540 nm of its product with brucine[3] and potassium persulfate[3] (11). The amounts of sodium and potassium ions transported to the other compartment were measured using a flame photometer[7].

RESULTS AND DISCUSSION

Hydraulic Permeability Data—The hydraulic permeability data at various concentrations of the diuretic drugs, in the case of both furosemide and triamterene, were found to obey the linear relationship (9);

$$J_V = L\Delta P \qquad \text{(Eq. 2)}$$

where J_V represents the volume flux per unit area of the membrane, ΔP is the applied pressure difference, and L is the hydraulic conductivity coefficient. The data for furosemide and triamterene are shown in Figs. 1 and 2, respectively. The values of L, calculated from the slopes of the straight lines in Figs. 1 and 2, show a progressive decrease (Table I) with increase in drug concentration up to the CMC, after which they become more or less constant. This gradation (Table I) is in keeping with the liquid membrane hypothesis (1) and indicates the progressive coverage of the supporting membrane with the liquid membrane with an increase in the concentration of the drug up to its CMC; at this concentration it is completely covered. Analysis of the flow data (Figs. 1 and 2, Table I) in the light of the mosaic membrane model (12–14) furnishes additional support for liquid membrane formation in series with the supporting membrane. The values of L (for both furosemide and triamterene), calculated using the mosaic membrane model at concentrations below the CMC values of the drugs, match the experimentally determined values (Table I) lending support to liquid membrane formation. The method of calculation is described in earlier publications (2–7).

Role of Liquid Membrane Formation in Diuretic Action—The primary action of furosemide is to reduce active absorption of chloride ions (8). The results indicate that the liquid membrane formed by furosemide, even on an inert support, impedes the transport of chloride ions (Table II). Similarly, the liquid membrane generated by triamterene offers resistance to the transport of Na^+ and K^+ ions (Table II). The significance of these observations is enhanced because the concentrations at which the complete liquid membranes are generated in series with the supporting membrane are low (on the order of μM) and compares favorably with the concentrations of these drugs in renal tubules (15, 16).

In the case of triamterene, the data indicate (Table II) that the transport of potassium ions is impeded more than the transport of sodium ions. This agrees with the reported observations on biological cells that triamterene is a potassium-sparing diuretic (17). In spite of the fact that in the present study an inert membrane like cellulose nitrate microfiltration membrane was used as support for the liquid membranes, the trend observed in the permeability of the cations is similar to that expected in biological cells. This strongly indicates that the liquid membranes generated by diuretic drugs, like triamterene, play a significant role in the mechanism of its action.

An examination of Table II reveals that the resistance offered to the transport of chloride ions (in the case of furosemide) and that of potassium ions (in the case of triamterene) is maximal when the liquid membranes generated by these drugs presented a hydrophilic surface to the approaching

[6] Spectronic-20; Bausch & Lomb.

[7] Flame Photometer Type 121; Systronics.

Table II—Solute Permeability (ω) of Ions in the Presence of Furosemide or Triamterene [a]

	ω_1 [b] $\times 10^{12}$ mol · s^{-1} · N^{-1}	ω_2 [c] $\times 10^{12}$ mol · s^{-1} · N^{-1}	ω_3 [d] $\times 10^{12}$ mol · s^{-1} · N^{-1}
	Furosemide [e]		
(Sodium) chloride	250.7 ± 35	189.0 ± 36	419.4 ± 79
	Triamterene [f]		
Potassium (chloride)	168.8 ± 12	91.6 ± 7	359.2 ± 9
Sodium (chloride)	111.2 ± 15	207.4 ± 15	232.5 ± 6

[a] Expressed as mean ± *SD*. [b] The drug in compartment D of the transport cell. [c] The drug in compartment C of the transport cell. [d] Control value; when no drug was used. [e] Concentration, 24.9×10^{-5} M. [f] Concentration, 3.0×10^{-5} M.

permeating species (the first set of experiments). In the light of these observations, it appears likely that the action sites of diuretic drugs like furosemide and triamterene themselves may be hydrophobic so that the hydrophobic ends of these drugs get attached to them leaving the hydrophilic parts to face the permeating species. If the action sites are hydrophobic they should be located within the hydrophobic core of the lipid bilayer of the membranes. To substantiate these conjectures, which appear logical in the light of the trends observed in the present experiments, further investigations are needed.

The permeability of sodium ions is impeded most when the triamterene liquid membrane presents its hydrophobic surface to the cation (Table II). The observation, however, is of limited biological significance because triamterene is known to be a potassium-sparing diuretic (17).

Diuretic drugs are also known to cause reduction in bile flow (18) and to alter ionic fluxes across isolated erythrocytes (19). The phenomenon of liquid membrane formation may be a plausible explanation for these effects. The decrease in reabsorption of water which results in diuresis is considered mainly a consequence of modification in the permeability of ions (8). The present study, however, indicates that the liquid membrane generated by the diuretic drug itself offers resistance to volume flux of water.

Though the observed reduction in permeability of the ions (Table II), due to the liquid membrane generated by the drugs, is passive in nature, it is likely to be accompanied by a consequent decrease in active transport. This would occur because access of the permeating species to the active sites on the biological membrane would be reduced due to the formation of the liquid membranes in series with the biological membrane. Thus, the liquid membranes generated by diuretic drugs may contribute significantly to the mechanism of drug action by impeding transport of ions as well as water.

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