rating into RNA, since it has been reported to inhibit protein synthesis in mammals by this mechanism (16).

In the past rat studies have been used to develop optimal scheduling programs and predict mechanisms of toxicity of 5-fluorouracil (9, 18). Findings of these investigations are presumed to correlate with clinical observations. In this work, concentrations of 5-fluorouracil in the excreted fluids of rats were used to supplement information derived from the plasma concentration time course. This work has demonstrated that unmetabolized 5-fluorouracil is excreted in detectable amounts in rat bile and parotid saliva. Biliary excretion appears to occur *via* a saturable process. Excretion of 5-fluorouracil in parotid saliva exposes the upper GI tract to this agent, even when administered intravenously. These routes of excretion appear to contribute negligibly to the total drug equivalents eliminated from the body.

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Liquid Membrane Phenomenon in Reserpine Action

S. B. BHISE, P. R. MARWADI, S. S. MATHUR, and R. C. SRIVASTAVA ˣ

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Abstract □ Reserpine was shown to generate a liquid membrane. Transport of adrenaline, noradrenaline, dopamine, 5-hydroxytryptamine, glutamic acid, and γ-aminobutyric acid in the presence of the reserpine liquid membrane was studied. The data indicate that the phenomenon of liquid membrane formation is likely to play a role in the mechanism of reserpine action.

Keyphrases □ Reserpine—liquid membrane phenomenon, biogenic amines, neurotransmitter amino acids, surface activity □ Liquid membrane phenomenon—reserpine action, biogenic amines, neurotransmitter amino acids, surface activity

Surface activity is exhibited by a wide variety of biologically active agents (1). The fact that surface activity may play a role in the mechanism of action of some drugs is evident from the correlations obtained (2) between surface activity and biological effects. Previous researchers (3) have concluded that in the case of psychotropic drugs, surface activity is the primary factor which determines their potency and not the specific chemical structure.

According to a previous hypothesis (4), surface-active agents, when added to water or aqueous solutions, generate liquid membranes which completely cover the interface at concentrations equal to the critical micelle concentration of the surfactant. It is, therefore, logical to expect that the liquid membranes generated by surface-active drugs may play a role in the mechanism of their action. Studies on haloperidol, a surface-active neuroleptic drug, were recently undertaken (5), and it was shown that the liquid membrane generated by haloperidol contributes significantly to the mechanism of its action.

To establish the role of liquid membrane phenomena in the mechanism of action of surface-active drugs, it is necessary to conduct studies, on structurally dissimilar drugs. Reserpine, a drug structurally different from haloperidol, is discussed in the present report. Existence of a liquid membrane generated by reserpine was demonstrated and data on the transport of biogenic amines and relevant neurotransmitter amino acids, through the liquid membrane generated by reserpine, were obtained.

EXPERIMENTAL

Materials—Reserpine[1], dopamine chlorhydrate[2], adrenaline hydrogen tartrate[2], L-noradrenaline[3], 5-hydroxytryptamine creatinine sulfate[4], L-glutamic acid[5], γ-aminobutyric acid[5], and distilled water (glass-distilled once from potassium permanganate) were used.

Methods—The critical micelle concentration (CMC) of aqueous reserpine was determined from the variation of surface tension with concentration. The surface tensions were measured using a tensiometer[6]. To prepare aqueous solutions of reserpine, the necessary volume of an ethanolic solution of known concentration of the drug was added to the aqueous phase with constant stirring. Since the aqueous solution of reserpine always contained some alcohol, in no case >1%, the blanks used also contained the same amount of alcohol in water. The CMC value of aqueous reserpine was found to be 1.6×10^{-6} *M*.

The all-glass cell described earlier (5, 6) was used for transport studies. A cellulose nitrate millipore filter[7], which acted as a support for the liquid

[1] BP-USP Roussel UCLAF, Paris.
[2] Loba Chemie.
[3] Fluka A.G.
[4] Koch-Light Laboratories Ltd.
[5] BDH.
[6] Fisher Surface Tensiomat Model 21.
[7] Sortorious Cat. No. 11307 of thickness 1×10^{-4} m and area 5.373×10^{-5} m^2.

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Table I—Values of L at Various Concentrations of Reserpine

Concentration of Reserpine $\times 10^6$ M	0	0.800 (0.5 CMC)	1.200 (0.75 CMC)	1.600 (1 CMC)	6.400 (4 CMC)
$L^a \times 10^8$ ($m^3 s^{-1} N^{-1}$)	2.482 ± 0.086	2.191 ± 0.055	1.918 ± 0.090	1.848 ± 0.057	1.431 ± 0.031
$L^b \times 10^8$ ($m^3 s^{-1} N^{-1}$)	—	2.165 ± 0.071	2.006 ± 0.064	—	—

[a] Experimental values. [b] Calculated values on the basis of mosaic model.

membrane, separated the transport cell into two compartments, C and D (Fig. 1) (5, 6).

Measurements of hydraulic permeability were carried out using the method described earlier (5, 6) at various concentrations of reserpine, ranging from 0 to 6.4×10^{-6} M. The concentration range was selected to get data from both the lower and the higher sides of the reserpine CMC.

For solute permeability of biogenic amines and amino acids, two sets of experiments were performed. In the first set, compartment C of the transport cell (Fig. 1) (5, 6) was filled with the solution of the respective permeable solutes prepared in 6.4×10^{-6} M aqueous solutions of reserpine, and compartment D was filled with distilled water. In the second set of experiments, compartment C was filled with the aqueous solution of the permeable solutes, and compartment D was filled with the aqueous solution of reserpine of 6.4×10^{-6} M concentration. In the control experiments no reserpine was used. The concentration of reserpine of 6.4×10^{-6} M, well above its CMC, was deliberately chosen for solute permeability measurements to ensure formation of a complete layer of liquid membrane on the supporting membrane. The values of the solute permeabilities (ω) were estimated using (7, 8):

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

where $\Delta\pi$ is the osmotic pressure difference, J_s is the solute flux per unit area of the membrane, and J_v is the volume flux. The method of measurement was described earlier (5).

Since reserpine is known to be photosensitive (9), in all the permeability experiments, the part of the transport cell containing reserpine solution was covered with black paper. All measurements including CMC determinations were carried out at 37 ± 0.1°.

Estimation of Biogenic Amines—Transport of adrenaline, noradrenaline, dopamine, and 5-hydroxytryptamine was estimated in the presence of reserpine. Reserpine was observed to interfere with fluorometric estimation of biogenic amines; therefore, the estimations were carried out using a spectrophotometer[8] by measuring absorbance at 282.4 nm (λ_{max}). Calibration curves were constructed by noting the absorbance of the solutions of varying concentrations of biogenic amines; prepared in a solution of a fixed concentration of reserpine, which was equal to its concentration in solute permeability experiments. The calibration curves thus constructed were found to be linear in accordance with Beer's law.

Estimation of Amino Acids—The amounts of glutamic acid and γ-aminobutyric acid were estimated from the amount of their reaction products with ninhydrin[5], measured spectrophotometrically[9] at 570 nm.

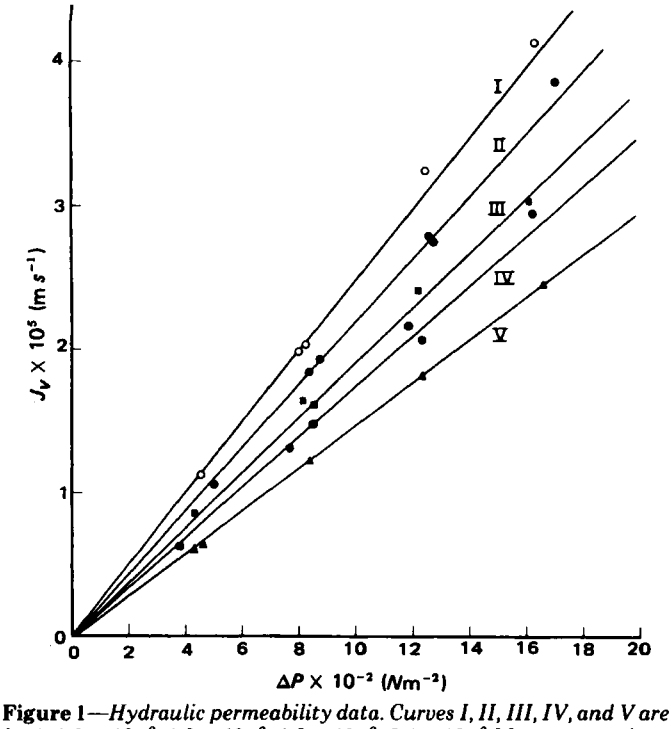

Figure 1—*Hydraulic permeability data. Curves I, II, III, IV, and V are for 0; 0.8×10^{-6}; 1.2×10^{-6}; 1.6×10^{-6}; 6.4×10^{-6} M, concentrations of reserpine, respectively.*

RESULTS AND DISCUSSION

From the hydraulic permeability data at various concentrations of reserpine (Fig. 1), it is observed that the linear relationship:

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

where J_v represents volume flux per unit area of the membrane, ΔP the applied pressure difference, and L the hydraulic conductivity coefficient, holds good in all cases. The values of L (Table I) show a progressive decrease with an increase in concentration of reserpine up to its CMC, beyond which the decrease is only nominal. This trend is indicative of progressive coverage of the supporting membrane with the reserpine liquid membrane in accordance with a previous hypothesis (4). The decrease in the values of L beyond the CMC of reserpine possibly is due to an increase in density of the liquid membrane as postulated previously (4).

Analysis of the flow data (Fig. 1, Table I) in relation to a mosaic membrane model (10, 11) further supports the existence of the liquid membrane in series with the supporting membrane. Following the arguments given earlier (5, 6) it can be shown that if the concentration of the surfactant is n times its CMC, with $n \leq 1$, the values of L would be equal to $[(1-n)L^c + nL^s]$ where L^c and L^s represent the values of L at 0 and CMC of the surfactant, respectively.

The values of L, computed at various concentrations of reserpine, lower than its CMC value, match favorably with the values obtained experimentally (Table I).

The values of ω for the biogenic amines and amino acids recorded in Table II indicate that the maximum reduction in the permeability values is observed in the second set of experiments where compartment D was filled with reserpine and compartment C with permeable solute. When reserpine is present in compartment D (Fig. 1) (5, 6) the liquid membrane generated will present the hydrophobic surface to the permeable solute that is present in compartment C. Since reserpine is known to act by reduction in the uptake of biogenic amines (12), it appears that this specific orientation of reserpine with the hydrophobic ends facing the various solutes may be necessary even in biological cells.

Reserpine is known to inhibit intraneuronal storage of catecholamines (12). Although inhibition of the ATP-Mg^{2+}-dependent uptake mechanism in isolated chromaffin granules has been considered to be a factor governing this mechanism (13), effects on other subcellular particles is also believed to occur by a common, nonspecific mechanism (3). The results of the present experiments indicate that the liquid membrane phenomenon is such a nonspecific mechanism. The phenomenon appears to be of special significance because the liquid membrane is formed at micromolar concentrations of the drug.

While some of the wide-ranging actions of reserpine can be explained on the basis of blocking of catecholamine uptake (12), it is difficult to find a common mechanism for other effects such as inhibition of experimentally provoked thrombus formation in rats (14), decreased oxygen utilization in the brain (15) and liver (16), antitumor effects (17), extrapyramidal symptoms (18), and reduction of thyroid secretion (19). Im-

[8] Varian Cary 17-D Spectrophotometer.
[9] Bausch and Lomb Spectronic—20.

Table II—Solute Permeability (ω) of Biogenic Amines and Amino Acids in The Presence of 6.4×10^{-6} *M* Reserpine

	ω_1[a] $\times 10^{12}$ moles $s^{-1} N^{-1}$	ω_2[b] $\times 10^{12}$ moles $s^{-1} N^{-1}$	ω_3[c] $\times 10^{12}$ moles $s^{-1} N^{-1}$
Dopamine[d]	1137.0	738.2	883.6
Noradrenaline[d]	1155.0	67.8	658.3
Adrenaline[d]	1165.0	567.3	880.2
5-Hydroxy-tryptamine[d]	1063.0	311.6	518.9
Glutamic acid[e]	403.6	217.5	491.7
γ-aminobutyric acid[f]	695.1	407.1	1115.0

[a] Control value, when no reserpine was used. [b] Reserpine in compartment D of the transport cell. [c] Reserpine in compartment C of the transport cell. [d] Initial concentration used, 10 μg/ml. [e] Initial concentration used, 500 μg/ml. [f] Initial concentration used, 200 μg/ml.

pairment of catecholamine release by reserpine has also been reported (20), for which no explanation has been given at a molecular level. The liquid membrane phenomenon seems to offer a common mechanism for all such effects. Alteration in transport of biologically relevant molecules by the reserpine liquid membrane could be a plausible explanation.

Reserpine is also known to reduce the permeability of biological cells to 5-hydroxytryptamine (20), which may contribute to its sedative effect. The present set of experiments also shows reduction in permeability of 5-hydroxytryptamine because of reserpine liquid membrane. Reserpine is known to lower electroshock threshold in rats (21), which is related to depletion of γ-aminobutyric acid in the brain. Since reserpine liquid membrane reduces the permeability of this amino acid (Table II), the above effect can be at least partially assigned to the formation of a liquid membrane by reserpine *in situ*.

Although reduction in the permeabilities of biogenic amines and amino acids (Table II), due to the liquid membrane generated by reserpine, is passive in nature, it is also likely to be accompanied by a subsequent reduction in their active transport. Access of the permeable solutes to the active site located on the biological membrane is likely to be effectively reduced due to the resistance offered by the reserpine liquid membrane.

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