Studies on liquid membrane Phenomenon in Biological action

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CERTIFICATE

This is to certify that the thesis entitled "STUDIES ON LIQUID MEMBRANE PHENOMENON IN BIOLOGICAL ACTION" submitted by Mr. Dontamsetti Basava Raju, I.D. No. 87 PH XF 001, for the award of the Ph.D. degree of The Birla Institute of Technology and Science, Pilani, embodies original work done by him under my supervision.

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PREFACE

Liquid membrane hypothesis for the action of surface active biological agents has been proposed recently (Adv. Colloid Interface Sci., 20, (1984) 131-161). The central idea of the hypothesis lies in the generalisation that surface active biological agents may generate a liquid membrane at the site of action modifying transport of relevant molecules to these sites - this of course is in addition to the concepts like structural complimentarity of the antagonists enabling them to interact with the same receptor sites with which the agonist molecules interact. According to the liquid membrane hypothesis of drug action modification in the transport of relevant molecules due to the liquid membrane barrier may be an important step common to the mechanism of action of all surface active drugs contributing significantly to their biological action. The liquid membrane hypothesis for biological action, when viewed in the light of existing theories, leads to a more rational biophysical explanation of such agents which act by modifying the permeability of cell membrane. Although several studies substantiating the liquid membrane hypothesis have been conducted in past few years, there is a need to investigate many more biological agents for the role of liquid membrane phenomenon in their action. Since the liquid membrane hypothesis of biological actions is of very recent origin, this need is quite pressing.

The studies conducted with this object in view are contained in this thesis. The thesis is divided into eight chapters. Chapter I contains a consolidated account of the

liquid membrane hypothesis of drug action. Chapter II contains a generalised version of the experimental techniques adopted in the present studies. Chapters III to VI respectively contain an account of the investigations carried out on barbiturates, benzodiazepines, prostaglandins and hydrocortisone to explore the role of liquid membrane phenomenon in their biological actions. In Chapter VII multiplicity of biological action of psychotropic drugs has been discussed in the light of the liquid membrane hypothesis of drug action. Chapter VIII is summary (chapterwise) of the contents of the thesis.

DONTAMSETTI BASAVA RAJU

CHAPTER I

CHAPTER I

THE LIQUID MEMBRANE HYPOTHESIS - BIOLOGICAL IMPLICATIONS

The liquid membrane hypothesis[1-3] was originally propounded to account for enhanced salt rejection in reverse osmosis due to addition of surfactants like polyvinylmethyl ether to saline feed. According to the hypothesis, when a surfactant is added to an aqueous phase, a surfactant layer liquid membrane is generated at the interface which modifies mass transfer across the phase boundary. The hypothesis further postulated that as the concentration of the surfactant is increased, the interface gets progressively covered with the surfactant layer liquid membrane. At the critical micelle concentration (CMC), coverage of the interface with the liquid membrane is complete. Experimental evidence from this laboratory[4,5] furnished additional support in favor of the progressive coverage of the interface with the liquid membrane. Since molecules of a surface active nature are crucial to living matter and its organization, biological implications of the liquid membrane hypothesis have been investigated recently. From these investigations, two significant conclusions have been arrived at. These are:

1. Liquid membrane bilayers generated on a hydrophobic supporting membrane by the constituent lipids of the biomembranes are capable of acting as model systems for biological membranes.

2. Liquid membranes generated by surface active drugs at their respective sites of action, make a significant contribution to the biological actions of these drugs. Infact a liquid membrane hypothesis of drug action has been arrived at[5].

In this chapter, a brief but critical account on the liquid membrane hypothesis of drug action which is relevant to the theme of the investigations in this thesis is presented.

The liquid membrane hypothesis for drug action[6]:

The observed biological effects of drugs are a consequence of interaction of drugs with membrane components. The antagonistic drugs, in general, are stated to interact with the membrane components and occupy the sites with which the agonist drugs would have interacted to give the desired biological response. Thus it can be stated that antagonistic drugs act by creating hindrance in the interaction of agonist drugs with receptor sites. How is this hindrance created, is contained in the liquid membrane hypothesis for drug action, which has been substantiated through investigations on a variety of drugs belonging to different pharmacological categories[7-26].

The membranes represent interface. As a corollary any drug which acts by modifying the permeability of cell membranes after interacting with them, of necessity, has to be surface active in nature. Since surface active substances are capable of forming liquid membranes which can influence mass transfer

across the interface (Kesting's Hypothesis) the formation of liquid membrane at the site of action could be an important event in the mechanism of action of surface active drugs. Thus the central concept in the liquid membrane hypothesis for drug action is that surface active drug may generate a liquid membrane at the site of action either by itself or in association with membrane lipids which act as a barrier modifying the transport of relevant molecules to these sites. This is in addition to the concepts like structural complimentarity of the antagonist drugs enabling them to interact with the same receptor sites with which the agonist molecules interact. The liquid membrane generated by the drug itself, acting as barrier modifying access of relevant molecules to the site of action is a new facet of drug action. If this concept is viewed in the light of the "occupancy theory"[27,28] and the "rate theory"[29-31], a more rational biophysical explanation for the action of surface active drugs acting by modifying the permeability of cell membranes, emerges.

It may, however, be clarified that the liquid membrane hypothesis in no way disputes the specific/active interaction between the agonist drugs and their receptors. The liquid membrane formation is an event which precedes the active interaction. The new point of the hypothesis lies in the assertion that the passive transport which has traditionally been considered unimportant for biological action also makes significant contributions-transport through the liquid membranes are indeed passive in nature.

Implications of the Hypothesis:

The liquid membrane hypothesis can provide a clue to their quantitative action. This is because CMC of the drug indicates the concentration at which the intefface will be completely covered by the drug liquid membrane. At this concentration (CMC), therefore, modification in the permeability of biological membrane would be maximum. This implies that at the CMC, magnitude of biological effect would also be maximum. Hence lower the CMC of a drug, lower is the concentration required to alter the membrane transport and as a consequence, more potent would be the drug. Thus CMCs of a series of drugs with the same pharmacological action can be a good indicator of their potency. The investigations of haloperidol[7] and chlorpromazine[10] justify this conjecture. CMC for haloperidol is 1×10^{-6} M while that of chlorpromazine is 4.5×10^{-5} M. Haloperidol is known to be more potent than chlorpromazine on milligram basis[32]. Although there are several examples[14,17,18,21,25,33], substantiating this result, the example of local anaesthetics is most striking: the lower the CMC the more potent the drug. In a series of local anaesthetics, it was found[12] that CMCs and minimum blocking concentrations (MBC) for nerves are identical. This indicates that formation of liquid membrane between cations like sodium, potassium and the nerve membrane appears to be an important step in the mechanism of action of local anaesthetics. It is proposed that interaction of local anaesthetics with the lipid microenvironment of the sodium channel results in its fluidisation causing blockade of sodium transport[34]. Thus a physical mechanism

can provide satisfactory explanation for local anaesthesia. Formation of liquid membranes by these drugs within sodium channels and polar head interaction of the drugs with the liquid microenvironment of the channels can, therefore, explain why nerve blocking concentrations and CMCs are identical.

The liquid membranes generated by surface active drugs are expected to have two types of orientations with respect to the approaching permeants. The drug liquid membrane can present either hydrophilic or hydrophobic ends to the permeants. It is observed that a change in orientation of the drugs can alter transport of permeants. Whichever orientation shows alterations in permeability, similar to those observed in biological cells, is of predictive value. In the majority of drugs investigated so far, it was found that resistance to transport of permeants is maximum when hydrophobic ends of the surface active drugs face the approaching permeants. This implies that the receptors for these drugs are likely to be oriented in such a manner that their hydrophilic moieties are projected outwards to which hydrophilic ends of the drugs get attached. Therefore, the hydrophobic ends of the drugs project outwards to face the permeants. Such an orientation can be rationalised if one examine the nature of receptors, in general, in relation to the lipid bilayers part of the biomembranes.

The receptors, in general, are membrane proteins and hence should be surface active in nature. Hence they should have hydrophilic and hydrophobic moieties in their structures.

Since the exterior environment of biological cells is aqueous in nature, it is logical to expect that hydrophobic part of these membrane proteins will be associated with hydrophobic core of the lipid bilayers and only hydrophilic part will face the exterior. Prediction about similar orientation of receptor proteins and also the membrane proteins, in general, has been made in literature[10]. Thus the studies on liquid membrane generated by drugs are capable of indicating the possible orientation of receptors, responsible for interaction with the drugs.

Since the biological membrane is comprised of different types of lipids and proteins, a drug can alter transport across the membrane by one of the following mechanisms:

(i) the drug itself may form a liquid membrane which can reasonably explain alteration of transport across the membrane;

(ii) the drug lipid interaction may be responsible for the observed biological effect; or

(iii) the drug protein interaction may be the causative action.

In the case where first possibility is ruled out, because an effect similar to that on biological tissues is not mimicked by the drug liquid membrane alone, interaction with the liquid membrane formed by the lipids needs to be studied. In case of diazepam[11], for example, it was found that the biological actions of the drug i.e. facilitating actions of GABA could not be mimicked in either orientations of the drug, but interaction

with lecithin liquid membrane showed increase in permeability towards GABA.

The multiplicity of biological actions exerted by surface-active drugs can be well explained on the basis of liquid membrane hypothesis e.g. antihistamines are also known[36] to have anti-cholinergic and local anaesthetic action. Chlorpromazine and few other low-potency phenothiazines have mild antihistamine[37] and antiserotonin[37] activity. Such actions can be explained as a result of alteration of transport of relevant permeants because of the liquid membrane interposed between the permeant and the biomembrane.

The liquid membrane hypothesis vis-a-vis existing theories of drug action:

The liquid membrane hypothesis for drug action proposes that in a series of structurally related drugs which are congeners of a common chemical moiety and which act by reducing permeability of hydrophilic substances, any structural variation which increases hydrophobicity of the compound will increase resistance towards transport of the hydrophilic permeant. In other words, any deviation in structure leading to increase in hydrophobicity will reduce CMC of the drug, make it more potent and increase resistance towards a hydrophilic permeant. However, this sequence of events will continue so long as the hydrophilic group of the drug responsible for interaction with the biomembrane is

unaltered. Any alteration of the hydrophilic moieties of the drug may alter specificity towards the membrane and therefore may alter the nature of response towards the permeants e.g. after alteration of the hydrophilic structure, the drug may inhibit transport of another permeant more specifically than the earlier permeant. This offers a clue towards the structure activity relationship. An increase in hydrophobicity will alter the drug action quantitatively i.e. it will increase the potency while change in hydrophilicity may alter the nature of action qualitatively i.e. the specificity of the resistance towards different permeants may change. Similar comments have been made by Burger[38] in connection with structure-activity relationship.

According to rate theory[29-31] the dissociation rate constant can be a good indicator of the nature of action shown by a drug. An antagonist is expected to have a low dissociation constant (K_2) as compared to the agonist. Consequently, in a series of antagonists, as the dissociation constant goes on decreasing the potency of the compound as an antagonist increases. In a series of monoquaternary salts, it is indicated that $K_2 = 0.0038 \times 2.65^{12-n}$, where n = number of carbon atoms in the alkyl chain[30]. In other words, K_2 falls by a constant factor of 2.65 for each methylene group added. In case of monoquaternary salts, Paton[29] has commented: "The association of these monoquaternaries is dictated by long range ionic forces, i.e., by the cationic head, but once, the molecule is bound, its dissociation is more or less hampered by Van der Waals binding

of the molecule to the receptor surface. The association rate would then be similar for all the compounds, but the dissociation constant would be sensitive to length of the alkyl chain to a degree comparable with the manner in which surface tension of alkyl carboxylic acids varies with alkyl chain length". These comments can also be understood in terms of the liquid membrane hypothesis. The addition of each methylene group in an antagonist will increase its hydrophobicity resulting in reduction of its CMC. Lowering of CMC may be linked to increase in potency of the compound as discussed earlier. Besides reducing the CMC, an increase in the methylene groups will strengthen the hydrophobic core of the drug liquid membrane and may offer more resistance to the transport of hydrophilic permeants. The CMC of a drug, therefore, appears to provide the same information which dissociation constant provides in case of rate theory[29].

If dose- response curve of an agonist is compared with dose- response curve of a mixture of an agonist and antagonist, it is observed that there is flattening of dose- response curve in the later case[27-30,39]. This leads to a parallel right shift in case of competitive antagonist - the proposition that agonist replaces antagonist is ruled out[29]. This is further substantiated by low dissociation constants in case of antagonist[29]. The observations, related to dose- response curve can also be explained on the basis of the liquid membrane hypothesis. A liquid membrane generated by a surface active 'antagonist' drug is interposed between receptor in the biomembrane and the agonist. As a consequence, transport of agonist is likely to be

reduced resulting in lesser amount of agonist reaching the receptor. Hence to achieve the same quantum of response, higher amount of agonist will be needed. This effect will result in the shifting of dose- response curve to the right. The nature of the liquid membrane and the extent of the resistance offered to the agonist will determine the nature and extent of shift in the dose- response curve.

One experimental observation in relation to dose- response curve of agonist- antagonist mixture has necessitated the hypothesis of "spare- receptors". It is observed[30] that a mixture of an agonist and an antagonist elicits the same maximum response as in the case of agonist alone, but at a comparatively higher concentration. The dilemma is : if the receptors are occluded by the antagonist, how is it possible to obtain parallel dose- response curve with and without antagonist ? or, inspite of a sizable section of receptors being occupied by an antagonist, how can a maximal response be obtained ? The dilemma has been resolved by proposing[40] existance of "spare- receptors"; i.e., those receptors without combining with which the agonist alone was capable of eliciting maximum response. However, there is a criticism about this hypothesis. A direct experimental demonstration of spare-receptor is still awaited[41]. Efforts have also been made to demonstrate[34] experimentally that there are no spare-receptors. Paton has commented[30] that "for occupancy theory, existance of spare receptors merely seems a puzzling extravagance". In the liquid membrane hypothesis for drug action, the existance of spare receptors is not necessary.

The rate of transport of an agonist across the liquid membrane of an antagonist is dependent on the concentration gradient of the agonist across the liquid membrane. As concentration of the agonist is increased, the rate of flow of the agonist across the liquid membrane generated by antagonist will also increase and at a certain higher concentration of the agonist it will elicit the same quantum of response as in the absence of the antagonist. Thus, rather than existance of "hypothetical additional receptors", the resistance offered by the liquid membrane generated by the antagonist to the flow of agonist is likely to decide the strength of the biological response. An indication of this proposition is available in literature. According to the potential - svergiftung theory[42]; the "action of the agonist was related to its flux across the cell membrane, which inturn was related to the driving force". The driving force is the concentration gradient.

While commenting[41] on the rate theory it is mentioned that, in general, for the rate of action of the drug any one of the following four steps may be the rate determining step:

(a) access to the receptor;

(b) conversion of the drug from an inactive to active form;

(c) rate of production of the response; or

(d) rate of combination with the receptor.

Amongst these steps, access to the receptor seems to be the most common rate-limiting step[41]. Hence any event which is

likely to reduce access of the agonist to the receptor should have profound influence on nature and sequence of agonist-receptor interaction and hence the consequent biological response. Generation of a liquid membrane having ability to reduce access of the agonist to the receptor is one such step. As a result, it is likely to affect the agonist-receptor interaction in a notable manner.

To explain kinetics of reversible antagonism in aortic strips, a biophase model was proposed[43,44]. According to this hypothesis, it was suggested that receptors are situated in a biophase separated from the extracellular space by an interfacial barrier through which agonists (but not antagonists), penetrate quickly; penetration of this barrier is considered as the rate-limiting step dictating the kinetics of antagonism. However, the existance of such a barrier in the case of antagonist has been ruled-out experimentally[30]. Another prediction of the biophase hypothesis, i.e., the dose-ratio (the ratio by which the agonist dose must be increased in order to restore a standard response in the presence of antagonist) should rise/fall exponentially when antagonist is added/removed, is also not true[30]. It is occupancy and not the dose-ratio that is observed to change exponentially. The liquid membrane hypothesis resolves this problem. Though there is no barrier for the antagonist to reach the receptor, a liquid membrane generated by an antagonist can act as a barrier to the flow of the agonist.

A general comment regarding the validity of liquid membrane hypothesis for drug action needs special mention. It is known that majority of transport processes in biological system (especially those of neurotransmitters) are 'active' in nature. Hence, only after showing that the active process is also impeded by a drug - liquid membrane, would the role of liquid membrane phenomenon in the action of antagonistic drugs become acceptable. For any process of active transport, rate of access of the permeant to the active site is an important factor. If this itself is impeded, because of resistance to its transport, even the active transport will be reduced. This reduction can result in antagonism. This is especially true in the case of drug-receptor interaction because access to the receptor has been considered[41] to be a rate-limiting process in the whole sequence of drug action.

Thus, the liquid membrane hypothesis for drug action points out towards a new facet of drug action. This aspect of drug action has hitherto gone unnoticed. The hypothesis provides a physical basis for the action of those drugs which act by modifying the permeability of cell membranes and are surface active in nature.

The task in this thesis:

Since the liquid membrane hypothesis for drug action is a recently postulated one, there is a need to carry out investigations on more categories of drugs/biological agents,

for exploring the role of liquid membrane phenomenon in the mechanism of their biological action with a view to further substantiating the hypothesis. In the present thesis, therefore, an account of the studies on Barbiturates, Benzodiazepines, Prostaglandins and Hydrocortisone is presented. In addition, multiplicity of biological actions exerted by psychotropic drugs has been discussed in the light of the liquid membrane hypothesis for drug action. The already published data on haloperidol[7], imipramine[8], reserpine[9] and chlorpromazine[10] have been utilised for this discussion.

Before an account of these studies is taken up in Chapters III to VII, the experimental methods used in these studies are described in a general manner in the next chapter (Chapter II).

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CHAPTER II

CHAPTER II

EXPERIMENTAL - A GENERALISED ACCOUNT

In the present investigations three main experiments have been performed. These are,

1. Determination of critical micelle concentrations (CMCs) of the biological agents/drugs under study.
2. Determination of the liquid membrane formation in series with the supporting membrane by the biological agents/drugs and the incorporation of these biological agents/drugs in the biomembrane phospholipid-cholesterol liquid membrane generated in series with the supporting membrane.
3. Measurements of solute permeabilities of relevant permeants through the liquid membranes generated by the drugs themselves or in association with the phospholipid and cholesterol.

Determination of the CMCs :

The critical micelle concentration (CMC) of aqueous solutions of the drugs was determined from the variation of surface tension with concentration. The surface tensions were measured using a Fisher Tensiomat Model 21. The values

of the CMCs thus determined are recorded in the relevant chapters.

Determination of liquid membrane formation :

For demonstration of the formation of liquid membranes by the various drugs/biological agents, in series with the supporting membrane, the data on hydraulic permeability in the presence of the various concentration of drugs/biological agents were exploited. For obtaining the hydraulic permeability data the all glass cell diagrammed in Fig. 1 was utilised. The diagram of the transport cell (Fig. 1) has been well labelled to make it self explanatory. It essentially consists of two compartments C and D separated by a cellulosic (cellulose acetate/cellulose nitrate) microfiltration membrane (Sartorious cat. no. 11107 or 11307 pore size 0.2 μm) of thickness 1×10^{-4} m and area 2.55×10^{-5} m^2 which, infact, acts as a supporting membrane for the liquid membranes. The stop cock attached to compartment D could be used to adjust the liquid miniscus in the capillary L_1L_2.

To obtain the hydraulic permeability data at various concentrations of the drugs, aqueous solutions of the drugs of varying concentrations were filled in the compartment C of the transport cell and compartment D was filled with distilled water. Known pressures were applied on compartment C by adjusting the pressure head and the resulting volume flux was measured by noting the rate of advancement of the liquid

miniscus in capillary L_1L_2 using a cathetometer reading upto 0.001 cm and a stop watch reading upto 0.1 second. The magnitude of the applied pressure difference was also measured by noting the position of pressure head using a cathetometer reading 0.001 cm. An important precaution in the measurement of volume flux was to allow sufficient time after the application of pressure on compartment C before the measurement of liquid miniscus in the capillary L_1L_2 were recorded. This was done to ensure that the flow in the capillary was steady flow. In fact, the distance moved by the liquid miniscus was ploted against time. If such plots were found to be straight lines passing through the origin, the flow was taken to be steady flow. During volume flow measurements, solution in compartment C was well stirred and the electrodes E_1 and E_2 (Fig. 1) were short circuited so that the electro-osmotic back flow that could develop due to streaming potential did not become a serious disturbing factor. The volume flux J_v at various values of (ΔP), the applied pressure difference, were calculated using the relation (1) where r and R are radii of the capillary L_1L_2

$$J_v = \frac{\pi r^2 \ell}{\pi R^2 t} = \left(\frac{r}{R}\right)^2 \frac{\ell}{t} \qquad .. (1)$$

and the membrane, M (Fig. 1) respectively and ℓ is the distance travelled by the liquid miniscus in the capillary L_1L_2 in time t. The concentration ranges selected were such that hydraulic permeability data were obtained both below and above the CMC of the drugs.

Analysis of the hydraulic permeability data:

To obtain information on the formation of liquid membrane the data on hydraulic permeability was analysed in the following manner. Volume flux J_v was plotted against applied pressure difference ΔP. In all the cases the data were found to be represented by the proportional relationship

$$J_v = L_p \Delta P \qquad .. (2)$$

where L_p is the hydraulic conductivity coefficient. The values of L_p at various concentrations of the drugs/biological agents under study were estimated from the slopes of the J_v versus ΔP plots. The value of L_p in all cases showed a decreasing trend with the increase in the concentration of the drugs/biological agents. The decreasing trend continued upto the CMC of the drugs/biological agents beyond which the values of L_p became more or less constant. This trend was taken to be indication of the formation of the liquid membranes in series with the supporting membrane in accordance with the Kesting's Liquid Membrane Hypothesis[1-3]. According to Kesting's hypothesis (See Chapter I) as concentration of the surfactant is increased the interface gets progressively covered with the surfactant layer liquid membrane and at the CMC it is completely covered.

Additional evidence in favour of liquid membrane formation was obtained using mosaic model[4-6] and the concept of progressive coverage as contained in the Kesting's hypothesis[1-3]. Since, according to liquid membrane hypothesis[1-3], at CMC, the

supporting membrane is fully covered with the surfactant layer liquid membrane, at concentration lower than the CMC it will be partially covered. The situation is pictorially depicted in Fig. 2. The equation for volume flow for such a situation can be written as

$$J_v(A^s + A^c) = J_v^s \cdot A^s + J_v^c \cdot A^c \quad .. (3)$$

where A represents the area of the membrane denoted by the superscripts and the superscripts s and c represent the bare supporting membrane and the supporting membrane covered with the liquid membrane respectively. In view of the linear relationship between J_v and ΔP, the equation (3) can be transformed into

$$J_v = \left[L_p^s\left(\frac{A^s}{A^s + A^c}\right) + L_p^c\left(\frac{A^c}{A^s + A^c}\right)\right] \Delta P \quad .. (4)$$

Functionaly L_p^s and L_p^c represent the value of L_p at '0' and CMC respectively. The concept of progressive coverage in the liquid membrane hypothesis implies that at half of the CMC the fraction of the total area of the supporting membrane covered with the liquid membrane will be half and hence, the slope of J_v versus ΔP plots in view of equation (4) should be equal to $(L_p^s + L_p^c) / 2$. Similarly when concentration of the surface active agent is one forth its CMC, the value of the slope should be equal to $(3/4\ L_p^s + 1/4\ L_p^c)$ and so on. Thus in general terms if the concentration of the surfactant is n times its CMC, n being less than or equal to 1, the value of the slope of J_v versus ΔP plots should be equal to $[(1-n)L_p^s + nL_p^c]$. Values of L_p thus

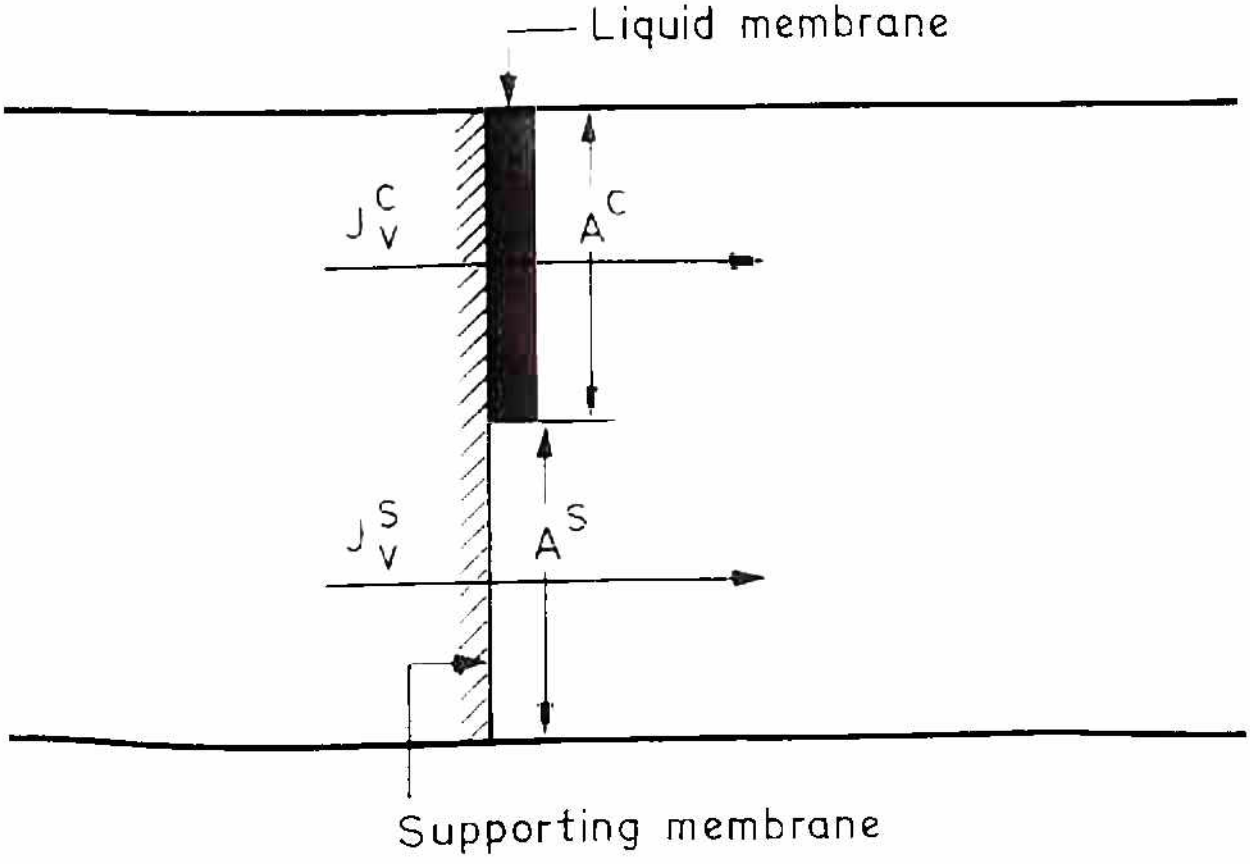

Fig. : The schematic representation of mosaic membrane formed when the concentration of the surfactant is lower than its CMC. J_v^c, J_v^s, A^c and A^s have the same meaning as in equation 3.

computed, at various concentration of the drugs/ biological agents should be in good agreement with the experimentally determined values. This agreement should constitute additional evidence in favour of liquid membrane formation.

Solute permeability measurements:

For measurement of solute permeability , the transport cell (Fig. 1) was used. The compartment C of the transport cell was filled with the solution of desired composition of the liquid membrane generating solution (drug or drug-lipid mixture) along with the solution of permeant of known concentration. The compartment D was filled either with distilled water or the liquid membrane generating solution of the same composition as was filled in the compartment C. In the control experiment no drug or biological agent under study was used.

The values of solute permeability (ω) in presence of the liquid membranes generated by the various drugs were measured using the definition[7,8],

$$\left(\frac{J_s}{\Delta\pi}\right)_{J_v=0} = \omega \qquad \ldots (5)$$

where J_v and J_s are the volume flux and solute flux per unit area of the membrane respectively and $\Delta\pi$ is the osmotic pressure difference. The condition of no net volume flux ($J_v = 0$) during the solute permeability (ω) measurements was attained by adjusting the pressure head attached to the compartment C of the transport cell so that liquid miniscus in the capillary L_1L_2

remains stationary. After a known period of time, which was of the order of several hours, the concentration of the permeant transported to the other compartment - compartment D was measured. The amount of the permeant gained by compartment D divided by the time and the area of the membrane, gave the value of the solute flux J_s for use in the calculation of ω using equation (5), the value of $\Delta\pi$ used in the calculation of ω was the average of the values of π at beginning (t = 0) and at the end of the experiment.

In all these measurements a non-living/non-specific membrane has been deliberately chosen to emphasize the role of passive transport - the transport through liquid membranes is indeed passive in nature.

For the solute permeability measurements the composition of the liquid membrane generating solution was always above the CMC. These compositions were derived from the hydraulic permeability data.

What has been given above is the general description of the experimental procedures. The details will be recorded in the Chapters dealing with the respective studies.

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CHAPTER III

CHAPTER III

LIQUID MEMBRANE PHENOMENON IN THE ACTION OF BARBITURATES*

Barbiturates have been used extensively as sedative-hypnotic drugs. These drugs can produce all degrees of depression of the CNS ranging from mild sedation to general anaesthesia. These drugs reversibly depress the activity of all excitable tissues. Barbiturates are known to be surface active[1-3] and hence should be capable of generating liquid membranes at the interface in accordance with Kesting's liquid membrane hypothesis[4]. There are several instances where the role of surface activity in the biological actions of barbiturates has been indicated[5-7]. In the present studies the formation of liquid membranes in series with a supporting membrane either by barbitures alone or by barbiturates in association with membrane lipids (lecithin and cholesterol), has been demonstrated. For this, data on the hydraulic permeability in the presence of lecithin-cholesterol-barbiturate mixtures, and Kesting's liquid membrane hypothesis[4] have been utilized. Data on modification in the transport of the relevant

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*A paper based on this study has appeared in the Colloids and surfaces., 35, (1989) 17-25.

permeants, namely γ-aminobutyric acid (GABA), glycine, aspartic acid, serotonin and noradrenaline, in the presence of the liquid membranes generated by a lecithin-cholesterol-barbiturate mixture have been obtained and discussed in the light of the reported biological actions of barbiturates.

Sodium phenobarbital and sodium pentobarbital have been chosen for the present study.

MATERIALS AND METHODS

Materials

Lecithin (Patel Chest Institute, CSIR centre for Biochemicals, Delhi), cholesterol (Centron Research Laboratories, Bombay), sodium phenobarbital (Bayer, India), sodium pentobarbital (Abbott, India), glycine, γ-aminobutyric acid (both from BDH, U.K.), serotonin creatinine sulphate (Merck, Darmstadt, F.R.G.), aspartic acid (Loba, Bombay), noradrenaline (Fluka, F.R.G.) and distilled water redistilled in an all-pyrex glass still were used in the present experiments.

Methods

The critical micelle concentrations (c.m.c.) of aqueous sodium phenobarbital (7.5×10^{-5} M) and sodium pentobarbital (5.0×10^{-5} M) were determined from the variation of surface tension with concentration at 37°C. The surface tensions were measured using a Fisher tensiomat, Model 21.

The all-glass cell described in Chapter II (Fig. 1 of Chapter II) was used for obtaining the hydraulic permeability

and solute permeability data. It is essentially a two-compartment cell. The two compartments are separated by a Sartorius cellulose acetate microfiltration membrane (Catalogue No.11107, pore size 0.2 μm) of thickness 1×10^{-4} m and area 2.55×10^{-5} m^2, which acts as a supporting membrane for the liquid membranes. To obtain the hydraulic permeability data, one compartment of the transport cell was filled with aqueous solution of a mixtures of lecithin, cholesterol and a barbiturate of desired composition (Fig. 1 of Chapter II) and the other compartment was filled with distilled water. Details of the method used for the hydraulic permeability measurements were the same as described in Chapter II. The solute permeabilities ω of the relevant permeants in the presence of the liquid membranes generated by the lecithin-cholesterol-barbiturate mixtures were determined using the equation[8]

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

where J_s and J_v are the solute flux and the volume flux per unit area of the membrane, respectively, and $\Delta\pi$ is the osmotic pressure difference. For solute permeability ω measurements, the composition of the lecithin-cholesterol-barbiturate mixture chosen was the one at which the liquid membrane generated by lecithin completely covered the supporting membrane and was saturated with both cholesterol and the barbiturate under investigation. This composition was derived from our earlier studies[9,10] and from the present data on hydraulic permeability in the presence of the varying concentrations of barbiturates

in the mixture of lecithin and cholesterol of fixed composition, i.e. 15.542 ppm with respect to lecithin and 1.175×10^{-6} M with respect to cholesterol. This particular composition of the lecithin-cholesterol mixture was chosen because it was shown in an earlier study[10] that at this composition the liquid membrane generated by lecithin at the interface is completely saturated with cholesterol. For the measurement of solute permeability ω, one compartment of the transport cell was filled with the aqueous solution of the lecithin-cholesterol-barbiturate mixture (Fig. 1 of Chapter II), along with permeant, and the other compartment was filled with distilled water. The condition $J_v = 0$ was imposed on the system and the amount of permeant transported to the compartment filled with distilled water in a known period of time was estimated. Details of the method of measurement of solute permeability ω were the same as described in Chapter II.

All measurements were performed at constant temperature, using a thermostat setting of $37 \pm 0.1^{\circ}$C.

Estimations

The amounts of the various permeants transported to the compartment filled with distilled water were estimated as follows:

Amino acids: The amounts of glycine, aspartic acid and GABA were estimated from the amount of their reaction products with ninhydrin, measured at 570nm[11] using a Bausch and Lomb Spectronic 20 spectrophotometer.

Serotonin and noradrenaline: The amounts of serotonin and noradrenaline were estimated by measurement of absorbance at 281 nm in 0.1N HCl using a Varian Cary 17-D spectrophotometer[12].

RESULTS AND DISCUSSION

The hydraulic permeability data at various concentrations in the case of both barbiturates (sodium phenobarbital and sodium pentobarbital) were found to be in accordance with the proportional relationship

$$J_v = L_p \Delta P \qquad .. (2)$$

where J_v is the volume flux per unit area of the membrane, ΔP is the applied pressure difference across the membrane and L_p is the hydraulic conductivity coefficient. The values of L_p at various concentrations of the drugs, estimated from the J_v versus ΔP plots, are recorded in Table 1. The values of L_p decrease with increasing concentration of the drugs up to their c.m.c., beyond which they become more or less constant. This trend in the values of L_p is consistent with Kesting's liquid membrane hypothesis[4] and is indicative of the formation of liquid membranes by the drugs, in series with the supporting membrane. According to Kesting's hypothesis, which was originally propounded in the context of enhanced salt rejection in reverse osmosis due to the addition of very small amounts of surfactants to saline feed, when a surfactant is added to an aqueous phase, the surfactant layer which forms spontaneously

TABLE 1

Values of the hydraulic conductivity coefficient (L_p)[a] at various concentrations of barbiturates

	Sodium phenobarbital concentration x 10^{-3} (M)						
	0.0	1.875	3.75	5.625	7.5[d]	15.0	30.0
L_p[b] x 10^9 ($m^3 s^{-1} N^{-1}$)	14.360 ± 0.044	12.465 ± 0.147	10.700 ± 0.317	9.110 ±0.029	7.3[illegible] ± [illegible]	7.[illegible] ± [illegible]	7.[illegible] ± [illegible]
L_p[c] x 10^9 ($m^3 s^{-1} N^{-1}$)	-	12.418 ± 0.334	10.679 ± 0.013	9.133 ±0.013	-	-	-
	Sodium pentobarbital concentration x 10^{-3} (M)						
	0.0	1.25	2.5	3.75	[illegible][d]	15.0	15.0
L_p[b] x 10^9 ($m^3 s^{-1} N^{-1}$)	14.360 ± 0.044	12.791 ± 0.147	10.929 ± .179	9.457 ± 0.137	7.767 ± 0.063	7.7[illegible] ± 0.0[illegible]	7.[illegible] ± 0.0[illegible]
L_p[c] x 10^9 ($m^3 s^{-1} N^{-1}$)	-	12.71[illegible] ± 0.[illegible]	11.004 ± 0.0[illegible]	9.41[illegible] ±0.05[illegible]	-	-	-

[a]The values of L_p are reported as arithmetic means of 10 repeats ± S.D.

[b]Experiment values.

[c]Computed values using mosaic model.

[d]Critical micelle concentration.

at the interface acts as a liquid membrane and modifies transport across the interface. As the concentration of surfactant is increased, the interface becomes progressively covered with surfactant layer liquid membrane and at the c.m.c. it is completely covered. Analysis of the values of L_p in the light of the mosaic mode[13-15] lends further support to the formation of the drug liquid membrane in series with the supporting membrane. As has been shown in Chapter II, for a concentration of surfactant n times its c.m.c., $n \leq 1$, the value of L_p should be equal to $[(1-n)L_p^s + nL_p^c]$, where superscripts s and c, respectively represent the values for the bare supporting membrane and the supporting membrane completely covered with surfactant layer liquid membrane. L_p^s and L_p^c represent the values of L_p when the surfactant concentrations are equal to zero and the c.m.c., respectively. The value of L_p thus computed at several concentrations of the drugs below their c.m.c. match with the experimentally determined values (Table 1), lending additional support to the formation of the liquid membranes.

Evidence in favour of the incorporation of barbiturates in the liquid membrane generated by the lecithin-cholesterol mixture can be obtained from the hydraulic permeability data at varying concentrations of these drugs in the lecithin-cholesterol mixtures of fixed composition. The hydraulic permeability data in this case were found to be represented by Eqn. (2). It was observed that as the concentration of drug is increased, the values of L_p first decrease and then become

more or less constant (Table 2). The concentrations of drug beyond which the values of L_p become more or less constant can be taken to be the concentration at which the lecithin liquid membrane at the interface (which is already saturated with cholesterol) is also saturated with the drug (Table 2).

Thus, the concentrations of sodium phenobarbital and sodium pentobarbital required to saturate the lecithin-cholesterol liquid membrane are 6.0×10^{-5} M and 2.0×10^{-5} M respectively. The concentrations of sodium phenobarbital and sodium pentobarbital compare favourably with their reported[16,17] plasma concentrations, at least in order of magnitude. The plasma concentration of sodium phenobarbital is in the range 0.2 - 0.5 mM [16] and that of sodium pentobarbital ranges from 4.2 to 11 μM [17].

In view of these studies, the concentrations of barbiturates in the lecithin-cholesterol mixture of fixed composition used in the solute permeability experiments were either equal to or a little higher than the concentrations required to saturate the lecithin-cholesterol liquid membrane.

Solute permeability data

The solute permeability data recorded in Table 3 appear relevant to the biological actions of the barbiturates.

Electrophysiological studies have indicated that sedative barbiturates inhibit excitatory transmission and enhance inhibitory transmission[18]. This is consistent with the enhanced

TABLE 2

Values of the hydraulic conductivity coefficient $(L_p)^a$ at various concentrations of sodium phenobarbital and sodium pentobarbital in the presence of lecithin-cholesterol mixture of fixed composition.

	Sodium phenobarbital concentration x 10^3 (M)						
	0.0	1.5	3.0	4.5	6.0	7.5	9.0
L_p x 10^7 (m^3 s^{-1} N^{-1})	12.376 ± 0.075	10.036 ± 0.022	8.195 ± 0.207	6.913 ± 0.142	5.158 ± 0.111	5.104 ± [illegible]	5.201 ± [illegible]
	Sodium pentobarbital concentration x 10^3 (M)						
	0.0	1.0	2.0	3.0		4.0	
L_p x 10^9 (m^3 s^{-1} N^{-1})	12.376 ± 0.375	11.436 ± 0.007	8.390 ± 0.087	8.247 ± 0.146		8.417 ± [illegible]	

[a] The values of L_p are reported as arithmetic means of 10 repeats ± S.D.

TABLE 3

Solute permeability (ω)[a] of various permeants in the presence of liquid membranes generated by sodium phenobarbital[b] (ω_1) and sodium pentobarbital[c] (ω_2) in lecithin-cholesterol mixture of fixed composition along with the control values (ω_0) when no barbiturates were used.

Permeants	Initial concentration (10^{-3} mol l^{-1})	$\omega_0 \times 10^5$ (mol s^{-1} M^{-1})	$\omega_1 \times 10^5$ (mol s^{-1} M^{-1})	$\omega_2 \times 10^5$ (mol s^{-1} M^{-1})
γ-Aminobutyric acid (GABA)	1.940	0.284 ±0.014(6.4)	0.710 ±0.038(6.8)	0.855 ±0.076(6.8)
Glycine	1.333	1.077 ±0.021(7.05)	1.401 ±0.011(7.0)	1.074 ±0.017(6.9)
Serotonin creatinine sulphate	0.0847	0.219 ±0.008(6.9)	0.653 ±0.013(6.5)	0.748 ±0.011(6.4)
Aspartic acid	1.127	0.168 ±0.014(4.8)	0.130 ±0.015(4.7)	0.192 ±0.017(4.8)
Norepinephrine	0.059	0.752 ±0.020(7.1)	0.135 ±0.011(6.5)	0.595 ±0.008(6.4)

[a] Values of ω reported as arithmetic means of 10 repeats ± S.D. The figures within parentheses indicate pH of the permeant solution in the lecithin-cholesterol-barbiturate mixture.

[b] Sodium phenobarbital concentration 6.1×10^{-5} M.

[c] Sodium pentobarbital concentration 3.7×10^{-5} M.

permeability of GABA and the reduced permeability of aspartic acid, as observed in the present experiments (Table 3). Electrophysiological evidence has also indicated[14] that GABA has a major role in barbiturate actions. Receptor binding studies have, however, failed to detect any interactions between GABA and barbiturates[19]. On this basis it has been concluded[18] that barbiturates do not affect the post-synaptic binding of GABA, even though GABA mimetic actions have been observed electrophysiologically. The present studies appear to offer an explanation for these observations. The present data on enhancement in the transport of GABA (Table 3) suggest that access to a GABA receptor is likely to be facilitated by the presence of the liquid membrane generated by the barbiturates in association with membrane lipids at the receptor site.

The anticonvulsant activity of phenobarbitone, which has been used in the treatment of epilepsy, is ascribed to its ability to produce an increased concentration of GABA in the brain. Phenobarbitone is reported to be most effective when the brain GABA content has been depleted[20]. The enhancement in the permeability of GABA in the presence of the lecithin-cholesterol-sodium phenobarbital liquid membrane (Table 3) is consistent with these clinical observations.

The two barbitals presently studied are reported to have the following gradation in onset of action[21]: sodium pentobarbital > sodium phenobarbital. This gradation in onset of activity of the barbitals is consistent with the present observation on the

concentration of the barbitals required to saturate the lecithin-cholesterol liquid membrane (Table 2) : sodium phenobarbital > sodium pentobarbital. Thus sodium pentobarbital, which crosses the blood-brain barrier the fastest[21], is required at the lower concentration to saturate the lecithin-cholesterol liquid membrane. Since modification in the permeability of the biological membrane would be maximum when the lipid bilayer is saturated with barbiturate, leading to maximum biological effect, the gradation in the onset of biological action appears to be a consequence of both factors, i.e. how fast it crosses the blood-brain barrier and how small is the concentration of drug required to saturate the lipid bilayer. The gradation in the onset of barbiturate action is also consistent with the conclusion that the c.m.c. is a good indicator of the potency of surface-active drugs - the lower the c.m.c. the more potent is the drug[22]. The c.m.c. of sodium phenobarbital is higher than that of sodium pentobarbital.

Barbiturates are known to disturb the balance of the phases of sleep - the initial effect is that of reducing the proportion of REM (rapid eyeball movement) sleep in comparison to NREM (non-rapid eyeball movement) sleep[23]. This observation can also be explained in terms of the enhanced permeability of serotonin and reduced permeability of noradrenaline in the presence of the liquid membrane generated by the lecithin-cholesterol-barbiturate mixture (Table 3). It is documented that raphe nuclei, which are rich in serotonin, are responsible both for NREM sleep and for the transition to and onset of

REM sleep. When a system of neurons in the pons known as the locus caeruleus (rich in noradrenaline) is destroyed, animals previously deprived of REM sleep fail to take the usual rebound excess of REM sleep when undisturbed[24]. The present data (Table 3) indicate that the liquid membranes likely to be formed in the synaptic cleft by the barbiturates in association with the membrane lipids may enhance the access of serotonin to its site of action in the raphe nuclei and reduce the access of noradrenaline to its site of action in the locus caeruleus, which may also contribute to the causation of imbalance in the phases of sleep by barbiturates.

Barbiturates are known to depress the respiratory drive and to disturb the rhythmic character of respiration[25]. It is also documented that iontophoretically applied GABA and glycine in the bulbar respiratory units have been found to inhibit medullary respiratory neurons[26,27], and glutamic and aspartic acids to excite the ongoing phasic neural activity of both inspiratory and expiratory neurons[27]. Thus the rhythmic character of respiration has been postulated to be a consequence of the actions of inhibitory amino acids like GABA and, excitatory ones like aspartic acid[28]. The present data (Table 3) indicate that enhancement in permeability of GABA and glycine and reduction in the permeability of excitatory neurotransmitters like aspartic acid, due to the liquid membranes formed by barbiturates in association with the membrane lipids in the synaptic cleft of the respective neurons, may also be a factor responsible for disturbance in the rhythmic character of respiration.

Thus the present studies on modification in the permeability of relevant permeants in the presence of the liquid membranes indicate effects which support the liquid membrane hypothesis of drug action and are worthy of further investigation with natural membranes. The pH, which is likely to influence the ionization of the barbiturates and the permeants, is different from its physiological value in the present experiments for solute permeability ω measurements. This fact, however, may not alter the qualitative conclusions because the pH of the experimental solutions are close to the pH of the solutions in the corresponding control experiments, at least in order of magnitude.

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CHAPTER IV

CHAPTER IV

LIQUID MEMBRANE PHENOMENON IN THE BIOLOGICAL ACTIONS OF BENZODIAZEPINES*

In clinical use all the benzodiazepines exert quantitatively different and qualitatively similar effects. In an earlier study[1], the role of liquid membrane phenomenon in the antianxiety action of diazepam has been investigated. Since benzodiazepines in addition to anxiolytic action are also known to exert myorelaxant and anticonvulsant actions[2-4] involving multiplicity of neurotransmitter systems[5] including catecholamines, serotonin, γ-aminobutyric acid (GABA) and glycine, a more detailed study is described in this chapter. The present study has been conducted on two benzodiazepines, namely nitrazepam and chlordiazepoxide. Data on hydraulic permeability have been obtained to demonstrate the formation of liquid membranes by these drugs in series with a supporting membrane and also the incorporation of these drugs into the liquid membranes generated by the lecithin-cholesterol mixtures. Transport of the relevant permeants, viz. glycine, GABA, noradrenaline, dopamine and serotonin, through the liquid membrane generated by the lecithin-

*A paper based on this study has appeared in the Indian J. Biochem. Biophys., 26 (1989) 104-108.

cholesterol-benzodiazepine mixtures has been studied and the data obtained have been utilized to throw light on the role of liquid membrane phenomenon in the biological actions of these drugs. In the present studies, a non-living supporting membrane has been deliberately chosen so that the possibility of active interaction with the constituents of biomembranes is totally ruled out and the role of passive transport through the liquid membranes in the biological actions of the drugs is highlighted.

MATERIALS AND METHODS

Lecithin (Patel Chest Institute, CSIR Centre for Biochemicals, Delhi), cholesterol (Centron Research Laboratories, Bombay), L-noradrenaline (Fluka-AG), serotonin creatinine sulphate (Koch-Light Laboratories), dopamine chlorhydrate (Riedel), glycine (Loba-Chemie), γ-aminobutyric acid (GABA) (BDH England) and water twice distilled in an all pyrex glass still were used in the present experiments.

The critical micelle concentrations (CMCs) of aqueous solutions of nitrazepam and chlordiazepoxide as determined from the variation of surface tensions with concentrations were found to be 8×10^{-4} M and 2.0016×10^{-5} M respectively. The surface tensions were measured using a Fisher Tensiomat Model 21. The aqueous solutions of benzodiazepines, which are sparingly soluble in water, were prepared by adding the requisite volume of ethanolic stock solution of known concentration of the drug to the aqueous phase with constant stirring. In the aqueous solutions of the drugs, thus prepared, the final concentration of ethanol

was never allowed to exceed 0.1% by volume because it was shown by a control experiments that a 0.1% solution of ethanol in water did not affect the surface tension of water to any measurable extent.

The all glass transport cell described in Chapter II (Fig.1 of Chapter II) was also used in the present studies. In the all glass cell, a sartorius cellulose nitrate microfiltration membrane (Cat. no. 11307, average pore size 0.2 μm) of thickness 1×10^{-4} m and area 2.5×10^{-5} m^2 which also acted as a supporting membrane for the liquid membrane divided the cell into two compartments C and D (see Fig. 1 of Chapter II).

The hydraulic permeability data in the presence of various concentrations of the drugs in the lower compartment C of the transport cell were obtained to demonstrate the formation of liquid membranes by the drugs in series with the supporting membrane. The information about the incorporation of the benzodiazepines in the liquid membranes generated by the lecithin-cholesterol mixture was obtained from the data on hydraulic permeability in the presence of varying concentrations of the drugs in the aqueous solution of the lecithin-cholesterol mixture of fixed composition - 15.542 ppm with respect to lecithin and 1.175×10^{-6} M with respect to cholesterol - taken in the lower compartment, C, of the transport cell. This particular composition of the lecithin-cholesterol mixture is derived from our earlier study[5] wherein it has been demonstrated that at this

composition the liquid membrane generated by lecithin completely covers the interface and is saturated with cholesterol. The method used for obtaining the hydraulic permeability data was the same as described in Chapter II.

The values of solute permeability (ω) were estimated using the equation

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

where J_s and J_v are the solute flux and the volume flux per unit area of the membrane and $\Delta\pi$ is the osmotic pressure difference across the membrane. In experiments for determining ω, a solution of desired concentration of the permeant prepared in the aqueous solution of the lecithin-cholesterol-benzodiazepine mixture of known composition was filled in compartment C and water in compartment D of the transport cell. The details of the method were the same as described in Chapter II. The composition of the lecithin-cholesterol-benzodiazepine mixture used in the experiments for ω measurements was derived from the hydraulic permeability data in the presence of varying concentrations of benzodiazepines in the aqueous solution of the fixed composition of the lecithin-cholesterol mixture. The composition of the lecithin-cholesterol-benzodiazepine mixtures used in the solute permeability (ω) measurements were those at which the liquid membrane generated by lecithin completely covers the interface and is saturated with both cholesterol and the benzodiazepine under study.

All measurements were made at constant temperature using a thermostat set at $37^{o} \pm 0.1^{o}C$.

Estimations: The amounts of dopamine, serotonin and noradrenaline transported to compartment D were estimated spectrophotometrically by measuring absorbance at 282.4 nm [7], using a Varian Cary 17-D spectrophotometer.

GABA and glycine were estimated from the amount of their reaction product with ninhydrin measured spectrophotometrically at 570 nm [8], using a Varian Cary 17-D spectrophotometer.

RESULTS AND DISCUSSION

The hydraulic permeability data in all cases were found to be in accordance with the equation

$$J_v = L_p \Delta P \qquad .. (2)$$

where J_v is the volume flow per unit area of the membrane, ΔP is the applied pressure difference and L_p is the hydraulic conductivity coefficient. The values of L_p, as estimated from the J_v versus ΔP plots, in the case of both drugs (Table 1) show trends which are indicative of the formation of the drug liquid membrane in series with the supporting membrane. According to Kesting's hypothesis[9] when a surfactant is added to an aqueous phase the surfactant layer which forms spontaneously at the interface acts as a liquid membrane and modifies transport across the interface. As the concentration of the surfactant is increased, the interface

TABLE 1

Values of the hydraulic conductivity coefficient (L_p)[a] at various concentrations of chloroacetic

	Nitrogen concentration x 10^3 (M)						
	0.00	1.0	4.0	6.0	8.7[d]	10.0	12.0
L_p^o x 10^9 ($m^3 s^{-1} N^{-1}$)	8.061 ±0.142	6.814 ±0.017	5.374 ±0.113	4.128 ±0.081	2.791 ±0.055	2.747 ±0.140	1.765 ±0.071
L_p^c x 10^9 ($m^3 s^{-1} N^{-1}$)	-	6.741 ±0.133	5.415 ±0.096	4.088 ±0.075	-	-	-

	Chloroacetamide concentration x 10^5 (M)					
	0.00	0.0672	1.687	2.0010[d]	2.3052	2.6688
L_p^o x 10^9 ($m^3 s^{-1} N^{-1}$)	8.068 ± .141	6.747 ±0.073	4.209 ± 0.127	2.546 ±0.068	2.532 ±0.074	2.331 ± .011
L_p^c x 10^9 ($m^3 s^{-1} N^{-1}$)	-	6.717 ± 0.115	4.387 ± 0.393	-	-	-

[a]The values are reported as arithmetic means of 10 repeats ± S.D.

[b]Experiment values.

[c]Computed values using mosaic model.

[d]Critical micelle concentration.

gets progressively covered with the surfactant layer liquid membrane and at the CMC it is completely covered. The values of L_p decrease progressively with the increase in the concentrations of the drugs up to their CMCs beyond which they become more or less constant (Table 1). Analysis of the data on L_p values in the light of mosaic model[10-12] further confirms the formation of the drug liquid membrane in series with the supporting membrane. Utilizing the concept of progressive coverage of the supporting membrane by the liquid membrane, as shown in Chapter II, when the concentration of the surfactant is n times its CMC, $n \leq 1$, the values of L_p can be computed using the expression $[(1-n)L_p^s + nL_p^c]$ where L_p^s and L_p^c are the values of L_p when the concentration of the surfactant is equal to zero and CMC respectively. The values of L_p thus computed at different concentrations below the CMC of the drug compare favourably with the corresponding experimentally determined values (Table 1).

The hydraulic permeability data at varying concentrations of the benzodiazepines in aqueous solutions of the lecithin-cholesterol mixture of the fixed composition also obeyed Eq. 2. The values of L_p (Table 2) decrease with the increase in concentration of the drug up to a particular concentration whereafter they become more or less constant. The decreasing trend in the values of L_p indicates the incorporation of the benzodiazepines in the liquid membrane generated at the interface by the lecithin-cholesterol mixture and the concentration whereafter the values of L_p become constant are the concentrations at which the lecithin-

TABLE 2

Values of hydraulic conductivity coefficient (L_p)[a] at various concentrations of nitrazepam in lecithin-cholesterol mixtures[b].

	Nitrazepam concentration x 10^{3} (M)						
	0.00	2.0	4.0	6.0	8.0	10.0	12.0
$L_p^a \times 10^{9}$ (m^{+3} s^{-1} N^{-1})	3.320 ±0.261	1.807 ±0.115	1.320 ±0.079	1.180 ±0.097	1.014 ±0.125	1.050 ± .091	1.007 ± .918
	Chloroacetoxide concentration x 10^{3} (M)						
	0.00	0.5014	1.0028	1.5041	2.0055	2.5069	
$L_p^o \times 10^{9}$ (m^{3} s^{-1} N^{-1})	3.320 ±0.201	2.509 ±0.189	1.840 ±0.139	1.432 ±0.0[illegible]	1.[illegible] ±0.[illegible]	1.030 ±0.0[illegible]	

[a] The values are reported as arithmetic mean of 10 replicate ± S.D.

[b] Lecithin and cholesterol concentrations kept constant at 13.512 μM and 1.175 x 10^{-3} M respectively.

cholesterol liquid membrane at the interface is saturated with the benzodiazepine. It is these particular compositions of the lecithin-cholesterol-benzodiazepine mixtures thst were used in the experiments for solute permeability (ω) determinations.

Solute permeability data and biological actions: Solute permeability data for the various permeants recorded in Table 3 appear relevant to the reported biological actions of the benzodiazepines.

Biochemical and neurophysiological evidences recorded in literature[13-19] have suggested that antianxiety action of benzodiazepines may be exerted by facilitating synaptic action of GABA in the brain. Enhanced permeability of GABA through the lecithin-cholesterol-benzodiazepines composite liquid membrane, as observed in the present study (Table 3), could also facilitate GABA potentiation leading to the antianxiety action of benzodiazepines.

Glycine present in relatively high concentration in the grey matter of the spinal cord is known to cause muscle relaxation by depressing the excessive motor activity[20,21]. The enhanced permeability of glycine through the lecithin-cholesterol-benzodiazepine composite liquid membrane (Table 3) may facilitate its access to the glycine receptor in the central nervous system and thus may also contribute to the reported muscle relaxant action of benzodiazepines.

Use of benzodiazepines in the treatment of epilepsy is documented[20,22]. Electrophysiological and biochemical evidences have linked the actions of benzodiazepines to their ability to

TABLE 3

Solute permeability (ω)[a] of various permeants in presence of lecithin-cholesterol benzodiazepine mixtures[b].

Permeants	Initial concentration (10^{-3} mol l^{-1})	$\omega_0 \times 10^{[illegible]}$ (mol s^{-1} N^{-1})	$\omega_1 \times 10^{[illegible]}$ (mol s^{-1} [illegible])	$\omega_2 \times 10^{[illegible]}$ (mol s^{-1} [illegible])
Glycine	1.330	1.584 ± 0.0[illegible]	2.476 ± 0.071	[illegible].105 ± [illegible]
γ-Aminobutyric acid (GABA)	1.340	0.[illegible]74 ± 0.051	3.151 ± [illegible].116	1.[illegible]17 ± [illegible]
Noradrenaline	0.058	[illegible] ± 0.[illegible]	[illegible].107 ± [illegible].077	[illegible] ± [illegible]
Dopamine	0.0527	0.473 ± [illegible].062	0.342 ± 0.029	[illegible].171 ± [illegible].151
Serotonin	0.3247	0.764 ± 0.016	1.109 ± 0.727	[illegible].657 ± 0.157

[a]Values of ω are reported as arithmetic mean of [illegible] repeats ± S.D.

[b]Lecithin concentration ([illegible] ppm) and cholesterol concentration ([illegible] × $10^{[illegible]}$ M).

ω_0 Control values when no drug was used.

ω_1 [illegible] concentration (7.5 × 10^{-6} M).

ω_2 [illegible] concentration ([illegible] × $10^{[illegible]}$ M).

potentiate the effects of exogeneous GABA or to enhance GABA-mediated presynaptic and post-synaptic inhibitory pathways[23]. The enhanced permeability of GABA, as observed in the present study (Table 3), may contribute to the reported anti-epileptic effects of benzadiazepines.

The benzodiazepines are believed to suppress the ability of the limbic system to active the reticular formation and thus induce sleep in cases of insomnia due to anxiety[24]. This effect appears to be due to the GABA potentiation to which the enhanced permeability of GABA (Table 3) may be a contributing factor.

Nitrazepam, like barbiturates, is known to disturb the balance of the phases of sleep[24]. The initial effect is that of reducing the proportion of REM (rapid eyeball movement) sleep in comparison to NREM (non-rapid eyeball movement) sleep[24]. Raphe nuclei which are rich in serotonin are responsible both for NREM sleep and for the transition to and onset of REM sleep[25]. When the locus caeruleus which is rich in noradrenaline is destroyed, animals previously deprived of REM sleep fail to take the susual rebound excess of REM sleep when undisturbed[25]. The present data (Table 3) indicate that the liquid membrane may be formed by the nitrazepam in association with the membrane lipids in the synaptic cleft and may enhance the access of serotonin to its site of action in the raphe nuclei and reduce the access of noradrenaline to its site of action in the locus caeruleus causing disbalance in the phases of sleep.

It is documented that patients treated with benzodiazepines also show failure of ovulate[26] like those treated with drugs like reserpine and chlorpromazine[27] which impede the transport of dopamine[28]. The present data indicate that impediment in the transport of dopamine (Table 3), due to the liquid membranes of the benzadiazepines in association with membrane lipids, which acts at the level of median eminence[27] to stimulate the release of LH/ FSH-RH, could also be a factor responsible for this side effect of the benzodiazepines.

One side effect of benzodiazepines is reported to be weight gain due to renewed appetite[26,27,29]. Although the pharmacology of eating behaviour is complex and is governed by several factors[29], broadly speaking GABA and noradrenaline acting at the level of hypothalamus are known to act as feeding enhancers and feeding inhibitors respectively[30]. The data recorded in Table 3 on the enhanced permeability of GABA and the reduced permeability of noradrenaline appear consistent with these observations particularly in the case of nitrazepam. The observation that permeability of noradrenaline is enhanced in the case of chlordiazepoxide (Table 3) appears consistant with the report that it is less toxic than nitrazepam[31].

LH/FSH-RH - Luteinizing hormone/ Follicle stimulating hormone - Releasing hormone.

According to the liquid membrane hypothesis of drug action[28] the CMC of the drug is a good indicator of its potency - lower the CMC more potent is the drug. Since the CMC value of nitrazepam is lower than that of chlordiazepoxide it should be more potent than chlordiazepoxide which indeed is the case [-].

Thus it appears that the phenomenon of liquid membrane formation may also contribute to the biological actions of benzodiazepines.

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CHAPTER V

CHAPTER V

ROLE OF LIQUID MEMBRANE PHENOMENON IN THE BIOLOGICAL ACTIONS OF PROSTAGLANDINS: STUDIES ON PROSTAGLANDIN E_1 AND PROSTAGLANDIN $F_{2\alpha}$*

Recently, the liquid membrane phenomenon has been shown to contribute significantly to the biological activity of prostaglandins[1]. Since prostaglandins are ubiquitously distributed in almost every tissue and body fluid and produce a remarkably broad spectrum of biological effects[2], a further discussion on the biological action of prostaglandins in the light of the liquid membrane phenomenon has been attempted in this chapter. The study includes among other actions on the nervous system, both central and peripheral. For this purpose the transport data reported earlier as well as obtained now in the presence of the liquid membranes generated by prostaglandins on a supporting membrane have been utilised. Although the formation of the liquid membranes by prostaglandins in series with a supporting membrane and also the incorporation of the prostaglandins into the liquid membranes generated by the lecithin-cholesterol mixture have been demonstrated earlier[1], it has been done once

*A paper based on this study has been accepted and is in press with the Indian J. Biochem. Biophys. (1989).

again in the case of prostaglandin $F_{2\alpha}$. This was considered desirable because in the present study prostaglandin $F_{2\alpha}$ available to us for experiments was a tris salt from Sigma (Cat. no. P3023) while in the earlier study it was a pure sample obtained from Serva, Germany (Cat. no. 33735).

MATERIALS AND METHODS

Materials :

Prostaglandin E_1 (Sigma Cat. no. P5515), prostaglandin $F_{2\alpha}$ (Sigma Cat. no. P3023), L-noradrenalin (Fluka AG), serotonin creatinine sulphate (Kochlight Laboratories Ltd.), dopamine chlorhydrate (Riedel), lecithin (Patel Chest Institute, CSIR Centre for Biochemicals), cholesterol (Centron Research Laboratories, Bombay) and double distilled water, glass-distilled over potassium permanganate, were used.

Methods :

While critical micelle concentrations (CMC) of prostaglandin E_1, lecithin and cholesterol were same as reported earlier[1], that of the aqueous prostaglandin $F_{2\alpha}$ (tris salt) determined from the variation in surface tension with concentration, using a Fisher tensiomat model 21 was 2.019×10^{-8} M.

The data on the hydraulic permeability demonstrating formation of liquid membrane by prostaglandin E_1 into the liquid membranes generated by the lecithin-cholesterol mixtures of a fixed composition in series with the sartorius cellulose nitrate

supprting membrane were obtained as reported earlier[]. These are reproduced in Tables 1 and 2. Similar hydraulic permeability data for the prostaglandin [] were obtained to demonstrate the formation of liquid membrane by it alone and also its incorporation in the lecithin-cholesterol liquid membrane in series with the supporting membrane. The methods and the ransport cell (Fig. 1 of Chapter II) used for obtaining the hydraulic permeability data were the same as described in Chapter II.

The solute permeability (ω) data for additional permeants, namely serotonin, nordrenaline and dopamine, in the presence of liquid membranes generated by the lecithin-cholesterol-prostaglandin mixtures of desired composition were obtained as described in Chapter 2. The compositions of the lecithin-cholesterol-prostaglandins mixtures were those at which the liquid membrane generated by lecithin in series with the supporting membrane was completely saturated with both cholesterol and the prostaglandin under study. These compositions were derived from our earlier study[] and from the data on hydraulic permeability at varying concentrations of prostaglandins in lecithin-cholesterol mixture of fixed composition (15.542 ppm with respect to lecithin and 1.175×10^{-5} M with respect to cholesterol) obtained in the present work. Since lecithin, cholesterol and the prostaglandins are all surface active in nature it is obvious that in the liquid membranes the hydrophobic tails of these molecules will [] the hydrophobic supporting membrane and the hydrophilic moieties will be drawn outwards away from it.

All measurements were made at constant temperature using a thermostat set at $37 \pm 0.1^{\circ}C$.

The amounts of serotonin, noradrenaline and dopamine in the solute permeability measurements were estimated by measuring absorbance at 282.4 nm[4] using a Varian Cary 17-D spectrophotometer. Calibration curves were constructed by noting the absorbance of the solutions of varying concentrations of biogenic amines, prepared in a solution of lecithin-cholesterol-prostaglandin mixtures of composition equal to the composition used in solute permeability experiments. The calibration curves thus constructed were found to be linear in accordance with Beer's law.

RESULTS AND DISCUSSION

The hydraulic permeability data at various concentrations of prostaglandin $F_{2\alpha}$ were found to be represented by the equation

$$J_v = L_p \Delta P \qquad .. (1)$$

where J_v is the volume flux per unit area of the membrane, ΔP is the applied pressure difference across the membrane and L_p is the hydraulic conductivity coefficient. The value of L_p, as estimated from the J_v versus ΔP plots, showed a decreasing trend with increase in the concentration of prostaglandin $F_{2\alpha}$ up to its CMC beyond which it became more or less constant (Table 1). This trend is in keeping with the Kesting's liquid membrane hypothesis[5] according to which when a surfactant is

TABLE 1

Values of the hydraulic conductivity coefficient (L_p)[a] at various concentrations of [illegible] and prostaglandin $F_{2\alpha}$.

	Prostaglandin E_1* concentration × 10^5 (M)							
	0.0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible][d]	[illegible]	[illegible]
$L_p^c \times 10^7$ ($m^2 s^{-1} H^{-1}$)	[illegible] ±0.085	[illegible] ±0.043	[illegible] ±0.122	[illegible] ±0.054	[illegible] ±0.050	[illegible] ±[illegible]	[illegible] ±[illegible]	[illegible] ±[illegible]
$L_p^e \times 10^7$ ($m^2 s^{-1} H^{-1}$)	-	[illegible] ±0.078	[illegible] ±0.074	[illegible] ±0.063	[illegible] ±[illegible]	-	-	-

	Prostaglandin $F_{2\alpha}$ concentration × 10^5 (M)						
	0.0	[illegible]	[illegible]	[illegible]	[illegible][d]	[illegible]	[illegible]
$L_p^b \times 10^7$ ($m^2 s^{-1} H^{-1}$)	[illegible] ±0.765	[illegible] ±0.155	[illegible] ±0.093	[illegible] ±0.211	[illegible] ±0.083	[illegible] ±[illegible]	[illegible] ±[illegible]
$L_p^e \times 10^7$ ($m^2 s^{-1} H^{-1}$)	-	[illegible] ±0.137	[illegible] ±0.101	[illegible] ±0.115	-	-	-

* The data on prostaglandin E_1 are taken from Ref. 1.

a The values are reported as arithmetic means of 10 repeats ± S.D.

b Experiment values.

c Computed values using mosaic model.

d Critical micelle concentration.

added to an aqueous phase the surfactant layer which forms spontaneously at the interface acts as a liquid membrane and modifies the transport across the interface. The Kesting's hypothesis further states that as concentration of the surfactant is increased the interface gets progressively covered with the surfactant layer liquid membrane and at the CMC it is completely covered. The analysis of the values of L_p in the light of the mosaic model[6-8] furnishes further evidence in favour of the formation of liquid membrane in series with the supporting membrane. As discussed in the Chapter II, if the concentration of the surfactant is n times its CMC, $n \leq 1$ the value of L_p should be equal to $[(1-n)L_p^s + nL_p^c]$ where the superscripts s and c represent the values for the bare supporting membrane and the supporting membrane completely covered with the liquid membrane. L_p^s and L_p^c represent values of L_p at surfactant concentration of zero and CMC respectively. The values of L_p, thus computed at several concentrations of prostaglandin $F_{2\alpha}$ below its CMC, compare favourably with those determined experimentally, thereby lending further support to the formation of the liquid membrane in series with the supporitng membrane.

The hydraulic permeability data at varying concentrations of prostaglandin $F_{2\alpha}$ in the lecithin-cholesterol mixture of fixed composition (15.542 ppm with respect to lecithin and 1.175×10^{-6} M with respect to cholesterol) have been obtained to gain information on the incorporation of prostaglandin $F_{2\alpha}$ in the liquid membranes generated by the lecithin-cholesterol

mixture at the interface. This particular composition of the lecithin-cholesterol mixtures is the one at which, as shown earlier[8], the lecithin liquid membrane completely covers the interface and is fully saturated with cholesterol. The data in this case also were found to be represented by proportional Eqn. (1). The values of L_{lp} recorded in Table 2 reveal a decreasing trend with increase in concentration up to a concentration equal to 7.57×10^{-9} M whereafter they become more or less constant. Thus 7.57×10^{-9} M is the concentration at which the liquid membrane generated at the interface by the lecithin-cholesterol mixture is saturated with prostaglandin $F_{2\alpha}$.

Biological actions: On a macroscopic level inflammation is usually accompanied by the familiar clinical signs of erythema, edema, hyperalgesia and pain[9]. Prostaglandins are always released when cells are damaged and have been detected in increased concentrations in inflammatory exudates[9]. During inflammation chemical mediators like histamine, serotonin etc. are also liberated locally, which stimulate sensory nerve endings and cause pain[10,11]. Prostaglandins by themselves are now known to act directly to stimulate sensory receptors subserving pain[11]. It is documented that histamine or serotonin antagonists have little therapeutic effect in inflammation whereas aspirin-like drugs which have little or no effect upon the release or activity of histamine or serotonin but are well known for their prostaglandin synthetase inhibitory activity are therapeutically important in the treatment of inflammation[9].

TABLE 2

Values of hydraulic conductivity coefficient (L_p)[a] at various concentrations of prostaglandin E_1[*] and prostaglandin $F_{1\alpha}$ in lecithin-cholesterol mixtures[b].

	Prostaglandin E_1[*] concentration x 10^{11} (M)					
	0.0	0.1	0.4	0.8	0.1	1.0
L_p^a x 10^6 (m^3 s^{-1} N^{-1})	1.500 ±0.130	5.158 ±0.190	4.[illegible] ± 0.110	[illegible].529 ±0.100	[illegible].[illegible]60 ±0.002	[illegible].[illegible]00 ± [illegible].[illegible]00

	Prostaglandin $F_{1\alpha}$ concentration x 10^{6} (M)						
	0.00	0.201	0.505	0.757	1.[illegible]10	[illegible].[illegible]00	[illegible].[illegible]00
L_p^a x 10^6 (m^3 s^{-1} N^{-1})	5.550 ±0.136	1.699 ±0.045	1.[illegible]73 ± 0.017	1.[illegible]02 ±0.015	1.[illegible]94 ± [illegible].[illegible]14	1.[illegible]35 ± [illegible].[illegible]07	1.[illegible]91 ± [illegible].[illegible]01

[*] The data on prostaglandin E_1 are taken from ref. 1.

[a] The values are reported as arithmetic mean of 10 repeats ± S.D.

[b] Lecithin and cholesterol concentrations kept constant at [illegible].54[illegible] pm and [illegible].175 x 10^{-6} M respectively.

The present data on the reduced volume flow in the presence of prostaglandins (Tables 1 and 2) indicate that the liquid membranes formed by prostaglandins released in the interstitial fluid offering resistance to the volume flow may be a contributing factor to the causation of edema. Similarly, impediment in the transport of histamine and serotonin in the presence of the liquid membranes generated by the prostaglandins, as observed in the present study (Table 3), may lead to the accumulation of histamine and serotonin in the interstitial region causing hyperalgesia and pain. Thus it appears that the phenomena of liquid membrane formation by the prostaglandins may be a contributing factor to the causation of edema, hyperalgesia and pain in inflammation and its cure by the prostaglandin synthetase inhibiting drugs like aspirin. It may be mentioned that intradermal, intravenous and intra-arterial injections of prostaglandins produce effects strongly reminiscent of inflammation[11].

The suggestion that prostaglandins, particularly prostaglandin E_1, may be implicated in migraine has been made in literature[12,13]. This suggestion has been prompted by the observation that intravenous injection of prostaglandin E_1 in non-migrainous subjects consistently resulted in vascular headache that bore migrainous features. Reduction in the concentration of serotonin at the post-synaptic receptor resulting in defective neurotransmission has also been implicated in migraine[12]. Since serotonin is a prostaglandin

TABLE 3

Solute permeability $(\omega)^{a}$ of various permeants in presence of lecithin - cholesterol prostaglandin E_1 * and prostaglandin $F_{2\alpha}$ mixtures[b].

Permeants	Initial concentration (10^{-}(mg x l^{-1}))	ω_o x $10^{[illegible]}$ (mol s^{-1} N^{-1})	ω_1 x $10^{[illegible]}$ (mol s^{-1} N^{-1})	ω_2 x $10^{[illegible]}$ (mol s^{-1} N^{-1})
Histamine*	10	0.391 ± 0.019	0.244 ± [illegible]	[illegible] ± [illegible]
Glycine*	100	1.517 ± 0.061	[illegible] ± [illegible]	[illegible] ± [illegible]
γ-aminobutyric acid* (GABA)	200	[illegible] ± 0.012	1.27[illegible] ± [illegible]	1.15[illegible] ± [illegible]
Serotonin	10	1.4[illegible] ± [illegible].038	1.17[illegible] ± 0.027	[illegible] ± [illegible]
Dopamine	10	[illegible].362 ± 0.035	0.17[illegible] ± 0.0[illegible]2	[illegible].5[illegible] ± [illegible]
Noradrenaline	10	[illegible].442 ± 0.071	0.328 ± [illegible]	0.711 ± [illegible]

* The data on prostaglandin E_1, histamine, glycine and γ-aminobutyric acid are taken from ref. 1.

[a] Values of ω are reported as arithmetic mean of 10 runs ± S.D.

[b] Lecithin concentration ([illegible].542 mM) and cholesterol concentration (1.17[illegible] x $10^{-[illegible]}$ M).

ω_o Control value when no prostaglandin was used.
ω_1 Prostaglandin E_1 concentration (0.05 x 10^{-8} M). ω_2 prostaglandin $F_{2\alpha}$ concentration (1.019 x $10^{-[illegible]}$ M).

releasing factor, it has been suggested[12] that hypotheses implicating either of these agents are not mutually exclusive. The present data on the reduced permeability of serotonin in the presence of prostaglandin E_1 (Table 3) suggest that the reduced access of serotonin to the postsynaptic receptor due to the prostaglandin liquid membrane formed at the receptor site could also be a contributing factor to the causation of migraine by prostaglandins.

Shock is considered essentially to be an inadequate tissue perfusion that impairs normal organ functions[14]. Elevated levels of circulating prostaglandins have been observed in several shock states[15-17], though the exact significance of prostaglandins in the various shock models remains unclear[14]. Aspirin-like drugs which inhibit prostaglandin synthesis are reported to have beneficial effects in several shock states[14]. The present data on the reduction in volume flow in the presence of prostaglandins (Tables 1 and 2) appear to indicate that the liquid membranes formed by prostaglandins in the blood capillaries offering resistance to the volume flow into the interstitial region resutling in impaired tissue perfusion could be a plausible explanation for these observations.

A similar explanation can be offered in the case of secondary glaucoma due to inflammation. Anterior uveitis particularly iridocyclites results in an increased intraocular pressure because of the swelling and the increased rate of fluid formation including the inflammatory exudate. This

condition is reported to respond to non-steroidal antiinflammatory drugs[18]. The ability of prostaglandins to raise intraocular pressure in rabbit eyes is well known[19]. The outflow of aqueous humour in humans and primates occurs primarily through the conventional drainage pathway, i.e. through the angle of anterior chamber via canal of Schlemm[20]. The present data (Tables 1 and 2) appear to suggest that blockade of drainage pathway by the prostaglandin liquid membranes could be a contributing factor to the increased intraocular pressure in the secondary glaucoma due to inflammation.

It is reported that prostaglandins often modify sympathetic neuroeffector junctions in exceedingly low concentration. For example, prostaglandins of E series inhibit noradrenaline output from adrenergic nerve endings and depress the response of the innervated structures whereas contrary effects leading to increased output of noradrenaline or heightened responsiveness of the effector organ have been noted with prostaglandins of F series. These observations appear consistent with the present findings that the permeability of noradrenaline is reduced in the presence of prostaglandin E_1 and enhanced in the presence of prostaglandin $F_{2\alpha}$ (Table 3).

Prostaglandins of E series are known[21] to relax bronchial smooth muscle and produce bronchodilation in the lungs *in situ*. Bronchoconstrictor responses to histamine, serotonin and other bronchospasmogens are counteracted by prostaglandins of E series[22]. The present data (Table 3)

therefore suggest that the [illegible] membrane formed by prostaglandin offering resistance to the transport of histamine and serotonin to their sites of action could be one of the contributing factors to the observed bronchodilation effects of prostaglandin E_1 and also to the observed counteraction of the bronchoconstrictor response to histamine and serotonin.

Prostaglandins of E series are reported[2] to inhibit water reabsorption induced by antidiuretic hormone in toad bladder and rabbit collecting tubules. The reduced values of L_p, as observed in the present study (Tables 1 and 2), may also contribute to the reported inhibition of water reabsorption in the presence of prostaglandins of E series leading to diuresis.

In epileptic patients marked increase in prostaglandin $F_{2\alpha}$ levels in cerebrospinal fluid has been detected[22]. It is also documented that prostaglandins of E series antagonise convulsions induced by pentylenetetrazol, penicillin and picrotoxins[23]. These observations appear consistent with the trends observed in the solute permeability data for excitatory neurotransmitters, viz. dopamine, serotonin and noradrenaline, and inhibitory neurotransmitters, i.e. glycine and γ-amino-butyric acid (GABA), in the presence of prostaglandin E_1 and prostaglandin $F_{2\alpha}$ (Table 3). Transport of the excitatory neurotransmitters is impeded in the presence of prostaglandin E_1 and enhanced in the presence of prostaglandin $F_{2\alpha}$. Transport of the inhibitory neurotransmitter GABA though enhanced in the case of both prostaglandin E_1 and prostaglandin $F_{2\alpha}$, the

transport of glycine is enhanced in the case of prostaglandin E and impeded in the case of prostaglandin $F_{2\alpha}$ (Table 3).

The nerve cell bodies of the paraventricular and supera-optic nuclei have both cholenergic and noradrenergic nerve endings impinging on them. Thus the activity in the neuro-secretory cells is perhaps also controlled by noradrenaline. It is reported that noradrenaline injected into carotid circulation inhibits the release of antidiuretic hormone[24]. The data on the reduced permeability of noradrenaline in the presence of prostaglandin E_1 (Table 3) appear to indicate that access of noradrenaline to the postsynaptic receptor may be reduced due to the resistance offered by the liquid membranes generated by prostaglandin E_1 in association with the membrane lipids and thereby stimulate antidiuretic hormone release. It is interesting to point out that prostaglandins of E series when injected into common carotid artery or the cerebral ventricles also stimulate the release of antidiuretic hormone[22].

At hypothalmic level, the secretion of prolactin in mammals is controlled by the prolactin release inhibiting hormone (P-RIH) and possibly by a prolactin releasing hormone (P-RH). The role, if any, of P-RH is of secondary importance[23]. The release of P-RIH from neuroendocrine transducer cells in the median eminence is controlled by hypothalamic dopamine and it has been shown that the drugs, like reserpine, chlorpromazine and haloperidol, which reduce the permeability of dopamine[25-27] and thereby lower its concentration in the

hypothalamic region, decrease the release of P-RIH and hence promote prolactin release[24]. Systematic administration of prostaglandins, particularly prostaglandin E_1, has been shown to stimulate prolactin release[28]. This observation is consistent with the decrease permeability of dopamine in the presence of prostaglandin [illegible] observed in the present study (Table 3). It appears that the reduced access of dopamine to its site of action in hypothalamus, which also is reported to be the [illegible] of action of prostaglandins[26], may be a contributing [illegible] prolactin release stimulating action of prostaglandin E_1.

Thus it appears that modification in the transport of relevant permeants to their respective sites of action due to the liquid membrane formed by prostaglandins in association with membrane lipids may also contribute to their biological actions.

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CHAPTER VI

CHAPTER VI

TRANSPORT THROUGH LIQUID MEMBRANE BILAYER GENERATED BY PROSTAGLANDIN E_1 IN THE PRESENCE OF HYDROCORTISONE *

Polyene antibiotics are known[1] to create aqueous pores in thin bilayer lipid membranes. It has been shown that the presence of cholesterol is necessary for the formation of aqueous pores[1]. Recent studies by our group on liquid membrane bilayers have also substantiated these observations. Because of the structural similarity of cholesterol to hydrocortisone and the prostaglandins to polyene antibiotics, the possibility of the formation of aqueous pores by hydrocortisone in the liquid membrane bilayers generated by prostaglandins cannot be ruled out and hence merits exploration. The studies reported in this chapter on the transport through liquid membrane bilayers generated by prostaglandin E_1 in the presence of hydrocortisone were undertaken with this object in view. The data indicate formation of aqueous pores by hydrocortisone in the liquid membrane bilayers generated by prostaglandin E_1, with trends comparable to those reported in the case of polyene antibiotics[1,2].

*A note based on this study has been accepted for publication and is in press with the J. Colloid Interface Science, (1989).

MATERIALS AND METHODS

Materials

Prostaglandin E_1 (Sigma P-5515), hydrocortisone acetate (Sigma H-4126), histamine (Sigma H-7250), serotonin creatinine sulfate (E. Merck, Germany), o-phthalaldehyde (Sigma P-1378), and water distilled twice in an all-Pyrex glass still were used in the present experiments.

Methods

The all-glass cell described in Chapter II was used to obtain data on hydraulic and solute permeability. It consisted essentially of two compartments C and D separated by a Sartorius cellulose acetate microfiltration membrane (Cat. no. 11107, pore size 0.2 μm) 1×10^{-4} m thick with an area of 2.55×10^{-5} m^2 which acted as a supporting membrane for the liquid membranes (Fig. 1 of Chapter II). To obtain the hydraulic permeability data, the two compartments of the transport cell were filled with an aqueous solution of mixtures of prostaglandin E_1 and hydrocortisone acetate of desired composition. Known pressures were applied to the lower compartment C and the consequent volume flow was measured with time in the capillary attached to compartment D of the transport cell. The details of the procedure were the same as those described in Chapter II.

For the solute permeability (ω) measurements compartment C of the transport cell was filled with aqueous solutions of known concentrations of the permeant along with aqueous solutions of mixtures of prostaglandin E_1 and hydrocortisone of desired composition and compartment D was filled only with aqueous solutions of the mixture of prostaglandin E_1 and hydrocortisone of desired composition. The equation[3]

$$\left(\frac{J_s}{\Delta\pi}\right)_{\Delta\pi = 0} = \omega \qquad \ldots (.)$$

where J_s and J_v stand for the solute flux and the volume flux, respectively, per unit area of the membrane and $\Delta\pi$ is the osmotic pressure difference across the membrane, was used for estimating the values of ω. The details of the method are the same as those described in Chapter II.

All measurements were made at a constant temperature using a thermostat set at $37 \pm 0.1^{\circ}C$.

Estimations: The amounts of the various permeants transported to compartment D were estimated as follows:

(i) The amount of histamine was estimated by measuring fluorometrically the fluorophor derivative from its reaction with o-phthalaldehyde[4,5]. A Model 540 photovolt fluorescence meter was used.

(ii) The amount of serotonin transported to the other compartment was estimated using a Cary-17 D spectrophotometer by measuring the absorbance at its absorption maxima[6].

RESULTS AND DISCUSSION

Hydraulic permeability data were obtained in the following sets of experiments:

1. Compartment C of the transport cell was filled with solutions of varying concentrations of hydrocortisone prepared in an aqueous solution of fixed concentration of prostaglandin E_1 equal to 3×10^{-8} M and compartment D was filled with distilled water.

2. Both compartments C and D were filled with aqueous solutions of a mixture of prostaglandin E_1 and hydrocortisone of the same composition as that in compartment C in Set 1.

The particular concentration of prostaglandin E_1 used in these experiments, 3×10^{-8} M, is higher than its critical micelle concentration (CMC) and was derived from earlier [illegible], in which it was shown, using Kesting's hypothesis[5], that when surface-active prostaglandin E_1 is added to an aqueous phase, a surfactant layer liquid membrane which comprises the interface at concentrations equal to or greater than its CMC is generated. The CMC of prostaglandin E_1 was found to be 1×10^{-8} M[7]. It is obvious that in the surfactant layer liquid membrane thus generated by prostaglandin E_1 the hydrophobic portions of the prostaglandin E_1 molecules would be preferentially oriented toward the hydrophobic supporting membrane and the hydrophilic moieties would be drawn outward away from it. In the experiments (Set 1) in which an aqueous solution of prostaglandin E_1 was added only

to compartment C, the liquid membrane would be generated only in compartment C in series with the supporting membrane, whereas in set 2 the supporting membrane would be sandwiched between two layers of liquid membrane generated by prostaglandin E_1.

The hydraulic permeability data in all cases were found to be in agreement with the relationship

$$J_v = L_p \Delta P \qquad .. (2)$$

where J_v is the volume flux per unit area of the membrane, ΔP the applied pressure difference across the membrane, and L_p is the hydraulic conductivity coefficient. The values of L_p for the different sets estimated from J_v versus ΔP plots are recorded in Table 1.

The values of L_p in the experiments in which solutions containing concentrations of hydrocortisone prepared in a solution of the fixed concentration of prostaglandin E_1 were added only to the lower compartment C (Set 1) do not show any increase with the increase in concentration of hydrocortisone; on the contrary there is a decrease (Table 1). The decreasing trend in the values of L_p (Table 1) may be due to incorporation of hydrocortisone, which also is surface active in nature[9], into the already existing prostaglandin E_1 liquid membrane at the interface. The values of L_p in the other set of experiments (Set 2), however, show an increasing trend with increase in the concentrations of hydrocortisone (Table 1). The value of L_p increases up to a certain concentration of hydrocortisone

TABLE 1

Values of L_p at various concentrations of hydrochloric acid in the [illegible] on E_1[a] and myristonitrile.

	Concentration of hydrochloric acid $\times 10^{-4}$ (M)					
	0.0	1.0	3.0	7.0	15	30
$L_p^D \times 10^9$ ($m^3\ s^{-1}\ N^{-1}$)	13.69 ± 0.396	6.767 ± 0.105	3.991 ± 0.091	1.031 ± 0.040	0.054 ± 0.110	−0.04 ± 0.044
$L_p^C \times 10^9$ ($m^3\ s^{-1}\ N^{-1}$)	1.061 ± 0.047	1.271 ± 0.029	1.301 ± 0.096	1.407 ± 0.111	1.458 ± 0.071	1.477 ± 0.000

[a] Prostaglandin E_1 concentration in the compartment was 3×10^{-6} M.

[b] Values obtained when the mixtures of prostaglandin E_1 and myristonitrile were added only to the lower compartment D of the transport cell and compartment C was filled with distilled water (Set 1).

[c] Values obtained when the mixtures of prostaglandin E_1 and myristonitrile were added to both of the upper compartments of the transport cell (Set 2).

beyond which it becomes more or less constant (Table 1). These trends in the values of L_p indicate the formation of aqueous pores in the prostaglandin E_1 liquid membrane bilayer only when hydrocortisone is present on both the sides of the membrane. A perusal of Table 1 reveals that the values of L_p increase up to a hydrocortisone concentration of 2×10^{-6} M, beyond which they become more or less constant. Thus the concentration of 2×10^{-6} M hydrocortisone appears to be the concentration at and beyond which complete aqueous pores are formed in the prostaglandin liquid membrane bilayer. Similar observations have been reported in the case of aqueous pore formation by polyene antibiotics[1,2].

The solute permeability (ω) data for histamine and serotonin were also obtained in two sets of experiments. In the first set a solution of known concentration of the permeant, histamine or serotonin, prepared in an aqueous solution of desired composition of the mixture of prostaglandin E_1 and hydrocortisone, was added to compartment C of the transport cell and compartment D was filled with distilled water. In the second set of experiments for ω measurements, compartment D of the transport cell instead of being filled with distilled water was filled with an aqueous solution of the mixture of prostaglandin E_1 and hydrocortisone of the same composition as that in compartment C. In the control experiments no hydrocortisone was used. The composition of the prostaglandin E_1 and hydrocortisone mixture used in the solute permeability

experiments was that at which the value of L_p showed maximum increase indicating the formation of complete aqueous pores in the prostaglandin E_1 liquid membrane bilayer (Table 1). The normalized values (γ) of solute permeability ($\gamma = \omega/\omega_{control}$) for histamine and serotonin are recorded in Table 2. It is obvious that the solute permeability of both histamine and serotonin is enhanced in the second set of experiments whereas in the first set of experiments the solute permeability of serotonin remains unaltered while that of histamine decreases (Table 2). These observations are consistent with the conclusions drawn from the hydraulic permeability experiments (Table 1) that aqueous pores in the prostaglandin liquid membranes are formed only when hydrocortisone is present on both the sides of the liquid membrane.

These trends in the hydraulic permeability and in the solute permeability appear relevant to therapeutic action of hydrocortisone in the treatment of inflammation. On a microscopic level inflammation is usually accompanied by the familar clinical signs of erythema, edema, hyperalgesia, and pain[10]. Prostaglandins are always released when cells are damaged and have been detected in increased concentrations in inflammatory exudates[10]. During inflammation chemical mediators like histamine and serotonin which stimulate sensory nerve endings and cause pain[10,11], are also liberated locally. Hydrocortisone and its synthetic analogs are known to suppress the inflammatory manifestations. The exact mechanism of these therapeutic

TABLE 2

Normalized values (γ) of solute permeability for histamine and serotonin through the liquid membrane generated by the prostaglandin E_1 - hydrocortisone mixture [a]

Permeant	γ [b]	γ [c]
Histamine	0.678	1.903
Serotonin	1.00	1.470

[a] Concentrations of prostaglandin E_1 and hydrocortisone in the mixture were 3×10^{-8} and 2.5×10^{-6} M, respectively.

[b] Values obtained when the mixtures of prostaglandin E_1 and hydrocortisone were added only to the lower compartment C of the transport cell and compartment D was filled with distilled water.

[c] Values obtained when the mixtures of prostaglandin E_1 and hydrocortisone were added to both lower and upper compartments of the transport cell.

effects of hydrocortisone, however, remains unclear[12]. The present data indicate that enhanced solute permeability of histamine and serotonin leading to their reduced concentration at the inflammation site and enhanced volume flow - increase in the values of L_p - due to the formation of aqueous pores in the prostaglandin liquid membrane could be a plausible explanation for the observed suppression of inflammatory manifestation by hydrocortisone.

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CHAPER VII

CHAPTER VII

LIQUID MEMBRANE PHENOMENA IN THE MULTIPLE ACTIONS OF PSYCHOTROPIC DRUGS

One important implication of the liquid membrane hypothesis for drug action[1] is that it can offer a generalised explanation for the multiplicity of biological actions exerted by surface active drugs (see Chapter I). It has been argued[1] that the multiplicity of biological actions exerted by surface active drugs can be explained on the basis of alteration in the transport of relevant permeants because of the drug liquid membrane interposed between the permeant and the site of action.

In this chapter an attempt has been made to discuss in some detail the multiplicity of biological actions exerted by the psychotropic drugs in the light of the liquid membrane hypothesis for drug action. The drugs chosen for the present discussion are haloperidol, chlorpromazine, reserpine and imipramine. The data obtained in the earlier studies[2-4], on the transport of relevant permeants in the presence of the

liquid membranes generated by these drugs have been utilised for the present discussion.

DISCUSSION

The normalised values of solute permeability for relevant permeants in the presence of the drug liquid membranes in case of each of the four psychotropic drugs viz. haloperidol, chlorpromazine, reserpine and imipramine as obtained in the earlier studies[2-5] are recorded in Tables 1-4. The data in Tables 1-4 are from two sets of model experiments, the one in which the permeants face hydrophilic surface of the drug liquid membrane and the other in which they face the hydrophobic surface.

Clinically chlorpromazine, haloperidol and reserpine are used as antipsychotics whereas imipramine is used as an antidepressant. In addition to antipsychotic and antidepressant effects, these drugs are reported to exert a variety of other biological actions as well (multiplicity of drug action). In what follows we present a rationale for the multiple biological actions of these drugs in terms of liquid membrane hypothesis for drug action.

It is well known that central regulation of the pituitary is mediated by the hypothalamus which in turn is under the influence of neurotransmitters. It is, therefore, logical

Key to Tables 1 to 4

Normalized values (γ) of solute permeability in the presence of liquid membranes generated by the drugs:

$\gamma = \omega / \omega_{control}$, ω being the value of solute permeability as defined by the equation $(-\Delta\pi)_{v=0} = \omega$ where J_s and J_v are the solute flux and the volume flow per unit area of the membrane and $\Delta\pi$ is the osmotic pressure difference across the membrane and $\omega_{control}$ the value from control experiments in which no drug was used.

γ_1 : Permeants facing the hydrophobic surface of the drug liquid membrane.

γ_2 : Permeants facing the hydrophilic surface of the drug liquid membrane.

TABLE 1

Haloperidol (ref. 1,3)

Permeants	γ_1	γ_2
Dopamine	0.766	2.938
Noradrenaline	0.869	3.883
Adrenaline	Undetectable	4.682
Serotonin	0.488	1.797
γ-Aminobutyric acid (GABA)	0.723	1.270

TABLE 2

Chlorpromazine hydrochloride (ref. 1,5)

Permeants	γ_1	γ_2
Dopamine	0.340	0.524
Noradrenaline	0.214	0.783
Adrenaline	0.119	0.789
Serotonin	0.195	0.397
γ-Aminobutyric acid (GABA)	0.775	0.795

TABLE 3

Reserpine (ref. 1,4)

Permeants	γ_1	γ_2
Dopamine	[illegible]	0.777
Noradrenaline	0.059	0.570
Adrenaline	0.487	0.756
Serotonin	0.293	0.488
γ-Aminobutyric acid (GABA)	0.586	1.604

TABLE 4

Imipramine hydrochloride (ref.1,2)

Permeants	γ_1	γ_2
Dopamine	0.394	1.632
Noradrenaline	0.487	1.223
Adrenaline	0.382	1.489
Serotonin	0.428	4.199

to expect that the drugs modifying the permeability of neurotransmitter molecules should display altered pituitary functions resulting from the alteration in the release of adenohypophysins and neurohypophysins.

For the actions of dopamine the hydrophilic portions of dopamine receptors which are located in higher brain centres have been considered to be important [10]. It is therefore expected that in the liquid membranes formed at the site of action by haloperidol, chlorpromazine or reserpine which are known to be dopamine antagonists, the hydrophilic parts of the drugs would be preferentially oriented towards the hydrophilic parts of the receptor and the hydrophobic parts of the drugs would be drawn outwards away from it. Therefore the agonist, dopamine, molecules would face the hydrophobic surface of the drug liquid membranes interposed between the agonist and its site of action. Thus the data (Tables 1-3) on the transport of dopamine in the specific orientation of the drug liquid membranes with its hydrophobic surface facing the permeant, dopamine, appear relevant to its biological actions. Similar considerations apply to adrenaline and noradrenaline and also to serotonin, which act on pre and post synaptic receptors [10].

Neurotransmitters through their action on median eminence promote or inhibit the release of hypophysiotrophic hormones, both release and release inhibiting hormones. These hypophysiotrophic hormones in turn act on adenohypophysis and regulate the release of adenohypophysins[11].

Dopamine, noradrenaline and adrenaline are reported[11-15] to inhibit the release of CRH. This inhibition of the release of CRH inturn inhibits the release of ACTH. The neuroleptic drugs namely haloperidol, chloropromazine and reserpine which impede the transport of dopamine, noradrenaline and adrenaline (Tables 1-3) and thereby reduce the acdess of these neurotransmitters to their site of action in median eminence should enhance the release of CRH and consequently of ACTH. This expectation is in agreement with literature reports that administration of these drugs viz. haloperidol, chlorpromazine and reserpine does enhance the release of ACTH[13,14,16]. The role of serotonin, whose permeability is reduced in the presence of imipramine (Table 4), on the release of ACTH is complicated. Both stimulatory and inhibitory actions have been reported[17,18]. This is consistent with the fact that no significant effect of imipramine, which blocks both pre and post synaptic receptors, on the release of adenohypophysins including ACTH have been reported[19]. GABA is known to have inhibitory effect on the release of CRH[13]. The impediment in the transport of GABA in the presence of the three neuroleptics namely haloperidol, chlorpromazine and reserpine (Tables 1-3) in the specific orientation of the drug liquid membranes with their hydrophobic surface facing the permeants is consistent with the reported enhancement in the release of ACTH by these drugs[13,14,16].

CRH = Cortico-trophin releasing hormone.
ACTH = Adrenocorticotropic hormone.
GABA = γ-Aminobutyric acid.

It is documented that dopamine, and GABA have inhibitory effects on the release of TSH whereas noradrenaline and adrenaline have stimulatory effects[13,14,20-22]. The impediment in the transport of noradrenaline and adrenaline in the presence of the chlorpromazine liquid membrane (Table 2) could be a plausible explanation for the reported inhibition of TSH release by chlorpromazine[14]. It has been reported[13], that chlorpromazine has specific affinity for accumulation in the hypothalamus area.

At hypothalamic level, the secretion of prolactin in mammals is controlled by the inhibitory hormone P-RIH and possibly a prolactin releasing hormone, P-RH, the role if any of P-RH is only of secondary importance[11]. The release of P-RIH from neuro-endocrine transducer cells in the median eminence is controlled primarily by hypothalamic dopamine. Neuroleptic drugs which inhibit the transport of dopamine (Tables 1-3) are therefore expected to enhance the prolactin secretion which indeed is substantiated by literature reports[13,24]. It may be mentioned that there are very strong evidences to suggest that dopamine itself functions as P-RIH[13,25].

The role of GABA in the secretion of prolactin is similar to that of dopamine at the level of median eminence and also at the hypophyseal level[26]. The impediment in the

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TSH = Thyroid stimulating hormone.
P-RIH = Prolactin release inhibiting hormone.
P-RH = Prolactin releasing hormone.

transport of GABA due to the liquid membranes generated by the neuroleptic drugs (Tables 1-3) could also be a factor contributing to the increased prolactin secretion brought about by the neuroleptics. The role of adrenaline, noradrenaline and serotonin at the level of median eminence, in prolactin secretion is stimulatory in nature, the magnitude of the stimulatory effect being much smaller in comparison to the inhibitory effect of dopamine[15,17,28]. The neuroleptic drugs should therefore, bring about a decrease in prolactin release on account of impediment in the transport of serotonin noradrenaline and adrenaline due to the liquid membranes generated by these drugs. In order that this effect becomes observable, one should first block the dopamine receptors and then study the effect of neuroleptics on prolactin release.

At the hypophyseal level the effects of noradrenaline and adrenaline on the release of prolactin are similar to that of dopamine[29,30]. It has been shown that adding extracts of hypothalamus to the cultured anterior pituitories decreased the quantity of prolactin released into the medium[31-33]. It has been shown that hypothalamus obtained from rats treated with phenothiazine derivative, perphenazine, when added to the cultures of pituitaries does not inhibit prolactin secretion[34]. The impediment in the transport of noradrenaline, adrenaline and dopamine due to the liquid membranes of phenothiazine drugs like chlorpromazine (Table 2) could be a plausible explanation for the antagonistic effect of phenothiazine on the normally

operating inhibitory influence of the hypothalamus on prolactin secretion at the level of pituitory.

The fact that dopamine plays a key role at the level of median eminence in stimulating the release of LH/FSH-RH is well established[11]. The release of dopamine at the median eminence is in turn controlled by other neurotransmitters viz. noradrenaline, adrenaline, GABA, serotonin and also dopamine at the higher brain centres[35]. The observation that neuroleptics like chlorpromazine, haloperidol and reserpine block the release of LH/FSH-RH can be explained in terms of the resistance offered to the transport of the neurotransmitters by the neuroleptic drugs (Tables 1-3) which are known to accumulate not only in the median eminence but also in the higher brain centres[14].

Dopamine and noradrenaline have been reported to increase growth hormone release in animals and man[ ]. Neuroleptic drugs like haloperidol, reserpine, chlorpromazine etc. caused reduction in the release of growth hormone[ ]. The impediment in the transport of these neurotransmitters viz. dopamine and noradrenaline (Tables 1-3) due to the liquid membranes generated by the neuroleptic drugs can be utilized to explain this observation - the liquid membranes reduce the access of the neurotransmitter to their action sites.

LH/FSH-RH = Luteinizing hormone/Follicle stimulating hormone-Releasing hormone.

Dopamine which acts not only on the median eminence but also on somatotrophs has a paradoxical inhibitory effect on the release of growth hormone in acromegalics. This is attributed to somototrophic cells themselves[13]. It may be mentioned that treating acromegaly with phenothiazines which inhibit the transport of dopamine (Table) has met with little success[38]. This is not unexpected in view of the paradoxical inhibitory action of dopamine in the release of growth hormone in acromegalic [illegible].

MSHS secretion by the pars intermedia of pituitary gland is inhibited by catecholamines viz. noradrenaline, adrenaline and dopamine[39]. Neuroleptic drugs such as haloperidol, reserpine and [illegible] reported to stimulate MSH secretion[39]. This is an expected observation in view of the reduced permeability of adrenaline, noradrenaline and dopamine in the presence of the [illegible] by the neuroleptic drugs reducing access of the catecholamines to their relevant sites of action.

Neurohypophyseal secretions containing ADH, oxytocin and neurophysins are evoked by different stimuli. Acetylcholine and nicotine injected into carotid circulation cause the release of ADH, oxytocin and neurophysins while noradrenaline inhibits their release[40,41]. It is therefore logical

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MSHS = Melanocyte stimulating hormones.
ADH = Antidiuretic hormone.

to expect that the drugs like chlorpromazine and reserpine which are likely to reduce the access of noradrenaline to the receptor site of action due to its reduced permeability through the liquid membranes generated by these drugs (Tables 2,3) should reduce the inhibitory effect and thereby facilitate the release of neurohypophyseal hormones like ADH, oxytocin etc. This indeed has been found to be the case[14,42,43].

Reduction in the concentration of serotonin at the post synaptic receptor resulting in defective neurotransmission has been implicated in migraine[44]. Imipramine in some cases is known to act beneficially in migraine[44], whereas neuroleptics like reserpine are known to aggravate it[45]. Blockade of reuptake of serotonin due to its reduced permeability through the imipramine liquid membrane (Table 4) likely to be formed at the pre-synaptic receptors resulting in improved neurotransmission could also be a possible explanation for the curative action of imipramine. Similarly blockade of serotonin due to its reduced permeability through the liquid membranes likely to be generated by the neuroleptic drugs (Table) at the post synaptic receptor resulting in poor neurotransmission could be a plausible explanation for the aggravation of migraine by the neuroleptic drugs like reserpine.

Neuroleptic drugs viz. haloperidol, chlorpromazine, and antidepressant drugs like imipramine are reported to cause hypothermia[19,46]. This effect can also be explained on the basis of the modification in the permeability of neurotransmitters

due to liquid [illegible] may be caused by these drugs in the hypothalamic region. The hormones ACTH/MSH whose secretion is inhibited by noradrenaline, adrenaline and dopamine at the level of median eminence[11-15,] have been shown to cause a fall in body temperature[46]. Since the neuroleptic drugs and [illegible] ability of noradrenaline, adrenaline and dopamine (Tables 1-4), the presence of these drugs at the hypothalamic level may reduce the [illegible] of these neurotransmitters to the relevant sites of [illegible] caus[illegible] thereby an increase in the secretion of ACTH/MSH. This inturn may b[illegible] responsible [illegible] the hypothermic [illegible]. The poikilothermic e[illegible] chlorpromazine which is sometimes used to facilitate the induction of surgical hypothermia[19] can also be rationalised in terms of modification in the permeability of neurotransmitters due to the presence of neuroleptic drugs like chlorpromazine (Table 2). Neurotransmitters have also been implicated in central regulation of body temperature in normothermia[16]. While most neurons are temperature insensitive, warm-sensitive neurons and cold sensitive neurons located in the preoptic area and in the anterior hypothalamic area have been implicated in thermoregulation in mammals. The firing rates of warm sensitive neurons increase with warming or decrease with cooling while reverse is the case in cold sensitive neurons. In mammals intracerebroventricular injection of serotonin produces a rise in body temperature[47]. It is therefore logical to guess that serotonin acts on cold sensitive neurons and noradrenaline on warm sensitive neurons. It is documented that cold

sensitive neurons lose their thermosensitivity during synaptic blockade while the warm sensitive neurons do not[48,49]. Chlorpromazine during synaptic blockade would therefore impair the thermosensitivity of cold sensitive neurons and leave the thermosensitivity of warm sensitive neurons unaffected leading to poikilothermia.

Parkinson's disease is known to be due to deficiency of neurotransmitters like dopamine in basal ganglia[50]. Neuroleptic drugs e.g. haloperidol, chlorpromazine and reserpine are known to cause Parkinson's disease[24,50]. Reduced permeability of dopamine (Tables 1-3) in the presence of the liquid membranes likely to be generated by these drugs in the region of basal ganglia could be one of the contributing factors for this side effect i.e. drugs induced Parkinsonism. The extrapyramidal effects of antipsychotic drugs like haloperidol are reported to be resistant to levodopa therapy[51]. Since reduced concentration of serotonin in cerebrospinal fluid has also been linked with a defect of extra-pyramidal function[52,53], the reduced permeability of serotonin in the presence of antipsychotic drugs like holoperidol (Table 1) offers a clue to the causation of extrapyramidal symptoms.

Most neuroleptic drugs have marked protective action against nausea and emesis inducing effects of dopamine agonists which are associated with the central dopaminergic receptors in the chemoreceptor trigger zone of the medulla[19]. This effect can also be explained by the reduction in the permeability of dopamine due to the liquid membranes generated by the neuroleptic drugs (Tables 1-3).

It is reported that neuroleptics and also tricyclic antidepressants e.g. imipramine cause orthostatic hypotension[19]. For example in normal man intravenous administration of chlorpromazine causes orthostatic hypotension due to a combination of central action and peripheral α-adrenergic blockade[54]. Although the actions of these drugs on cardiovascular system are complex because these drugs produce direct effects on the heart and blood vessels and also indirect ones through actions on central nervous system and autonomic reflexes, reduced permeability of noradrenaline in the presence of these drugs (Tables 1-4) could also contribute to the causation of orthostatic hypotension.

Neuroleptic drugs particularly chlorpromazine and reserpine, during coition, are known to impair ejaculation without interfering with erection[19,55]. Attribution of this effect to adrenergic blockade though logical remains unsubstantiated[19]. Reduction in the permeability of noradrenaline due to the liquid membranes generated by the neuroleptic drugs (Tables 1-3) is consistent with the conjecture that impairement of ejaculation may be due to adrenergic blockade.

Thus it appears that modification in the permeability of relevant neurotransmitters due to the liquid membranes generated by the psychotropic drugs may be one of the causal factors for the multiple actions of these drugs.

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CHAPTER VIII

CHAPTER VIII

SUMMARY

The Chapterwise summary of the work recorded in this thesis is given below:

Chapter I

Chapter I gives a brief account of the Kesting's liquid membrane hypothesis and its biological implications. A discussion of the liquid membrane hypothesis of drug action vis-a-vis existing theories of drug action has been presented. At the end of the chapter, the tasks intended to be achieved in the thesis have been brought out.

Chapter II

A general account of the experimental methods used in these investigations have been recorded in this chapter.

Chapter III

The liquid membrane phenomenon in barbiturates has been studied. Formation of liquid membranes, in series with a supporting [illegible] by barbiturates in association with [illegible] has been demonstrated. Data on the [illegible] relevant permeants, viz.

γ-aminobutyric acid, glycine, aspartic acid, serotonin and noradrenaline, in the presence of the liquid membrane generated by barbiturates in association with lecithin and cholesterol have been obtained. The data indicate that modification in the transport of these permeants due to the liquid membrane barrier may have a bearing on the mode of action of barbiturates.

The barbiturates chosen for the present study are sodium phenobarbital and sodium pentobarbital.

Chapter IV

The liquid membrane phenomenon in benzodiazepines has been studied. Transport of glycine, GABA, noradrenaline, dopamine and serotonin in the presence of the liquid membranes generated by the benzodiazepines in association with lecithin and cholesterol has been studied. The data indicate that modification in permeabilities in the presence of the liquid membranes is likely to make a significant contribution to several biological actions of the benzodiazepines.

Chapter V

Modifications in the transport of relevant permeants + their respective sites of action due to the liquid membranes formed by prostaglandins in association with lecithin and cholesterol have been discussed in the light of biological

actions of prostaglandins. It has been shown that the phenomenon of liquid membrane formation is likely to make a significant contribution to their biological actions.

Chapter VI

Transport through liquid membrane bilayers generated by prostaglandin E_1 has been studied in the presence of hydrocortisone. The data indicate the formation of aqueous pores when hydrocortisone is added on both sides of the prostaglandin E_1 liquid membrane bilayer. The phenomenon of aqueous pore formation has been utilized to explain the therapeutic action of hydrocortisone in the treatment of inflammation.

Chapter VII

Multiplicity of biological actions of surface active psychotropic drugs has been discussed in the light of modifications in the permeability of relevant neurotransmitter molecules viz. dopamine, noradrenaline, adrenaline, γ-aminobutyric acid (GABA) and serotonin through the liquid membranes generated by these drugs. It has been shown that modification in the transport of relevant permeants through the drug liquid membranes likely to be generated at various sites of action may provide a generalised explanation for multiple actions of the surface active drugs. The drugs chosen for the present [illegible] are, haloperidol, chlorpromazine, reserpine and [illegible]. The data published earlier on the [illegible] through the liquid membranes [illegible] by these drugs [illegible] for the present discussion.

LIST OF PUBLICATIONS

Based on the work recorded in this thesis:

1. Liquid Membrane Phenomenon in the Action of Barbiturates.
   Colloids and Surfaces, 35 (1989) 17 - 25.

2. Liquid Membrane Phenomenon in the Biological Actions of Benzodiazepines.
   Indian J. Biochem. Biophys., 26(2) (1989) 104-108.

3. Role of Liquid Membrane Phenomenon in the Biological Actions of Prostaglandins: Studies on Prostaglandin E_1 and Prostaglandin $F_{2\alpha}$.
   Indian J. Biochem. Biophys., (In Press), (1989).

4. Transport Through Liquid Membrane Bilayers Generated by Prostaglandin E_1 in the Presence of Hydrocortisone.
   J. Colloid Interface Sci., (In Press, 1989).

5. Liquid Membrane Phenomena in the Multiple Actions of Psychotropic Drugs.
   Adv. Colloid Interface. Sci. (Communicated).

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Liquid Membrane Phenomenon in the Action of Barbiturates

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ABSTRACT

The liquid membrane phenomenon in barbiturates has been studied. Formation of liquid membranes, in series with a supporting membrane, by barbiturates alone and by barbiturates in association with lecithin and cholesterol has been demonstrated. Data on the transport of relevant permeants, viz. γ-aminobutyric acid, glycine, aspartic acid, serotonin and noradrenaline, in the presence of the liquid membrane generated by barbiturates in association with lecithin and cholesterol have been obtained. The data indicate that modification in the transport of these permeants due to the liquid membrane barrier may have a bearing on the mode of action of barbiturates.

The barbiturates chosen for the present study are sodium phenobarbital and sodium pentobarbital.

INTRODUCTION

Extensive studies on a wide variety of surface-active drugs belonging to different pharmacological categories have led to what may be called "liquid membrane hypothesis for drug action". An account of this hypothesis and a discussion of its implications is contained in a recent review by Srivastava et al. [1]. It has been shown that the liquid membrane hypothesis for drug action when viewed in the light of existing theories of drug action, particularly the rate theory [2,3] and the occupancy theory [4,5], leads to a more rational biophysical explanation for the actions of such drugs, which act by altering the permeability of cell membranes [1]. The central idea of the hypothesis lies in the generalization that the liquid membranes generated by the drugs, either by themselves or in association with membrane lipids at their respective sites of action, modify the transport of relevant permeants to these sites. This may be an important step common to the mechanism of action of all surface-active drugs. The hypothesis highlights the role of passive transport in biological action. The transport of the relevant permeants through the drug liquid mem-

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brane, which appears to be one of the factors determining their access to the relevant sites and which precedes interaction with the receptors, is indeed passive in nature.

In the present communication, studies on barbiturates are reported. Barbiturates are known to be surface active [6–8] and hence should be capable of generating liquid membranes at the interface in accordance with Kesting's liquid membrane hypothesis [9]. There are several instances where the role of surface activity in the biological actions of barbiturates has been indicated [10–12]. In the present studies the formation of liquid membranes in series with a supporting membrane, either by barbitures alone or by barbiturates in association with membrane lipids (lecithin and cholesterol), has been demonstrated. For this, data on the hydraulic permeability in the presence of lecithin–cholesterol–barbiturate mixtures, and Kesting's liquid membrane hypothesis [9] have been utilized. Data on modification in the transport of the relevant permeants, namely γ-aminobutyric acid (GABA), glycine, aspartic acid, serotonin and noradrenaline, in the presence of the liquid membranes generated by a lecithin–cholesterol–barbiturate mixture have been obtained and discussed in the light of the reported biological actions of barbiturates.

Sodium phenobarbital and sodium pentobarbital have been chosen for the present study.

MATERIALS AND METHODS

Materials

Lecithin (purity 99%; Patel Chest Institute, CSIR centre for Biochemicals, Delhi), cholesterol (purity 99%; Centron Research Laboratories, Bombay) sodium phenobarbital (purity 98.5%; Bayer, India), sodium pentobarbital (purity 99%; Abbott, India), glycine, γ-aminobutyric acid (both of purity 99%; BDH, U.K.), serotonin creatinine sulphate (purity 99%; Merck, Darmstadt, F.R.G.), aspartic acid (purity 98.5%; Loba, Bombay), noradrenaline (purity 99%, Fluka, F.R.G.) and distilled water redistilled in an all-pyrex glass still were used in the present experiments.

Methods

The critical micelle concentrations (c.m.c.) of aqueous sodium phenobarbital ($7.5 \cdot 10^{-5} M$) and sodium pentobarbital ($5.0 \cdot 10^{-5} M$) were determined from the variation of surface tension with concentration at 37 C. The surface tensions were measured using a Fisher tensiomat, Model 21.

The all-glass cell described earlier [13,14] was used for obtaining the hydraulic permeability and solute permeability data. It is essentially a two compartment cell. The two compartments are separated by a Sartorius cellulose

acetate microfiltration membrane (Catalogue No 11107, pore size 0.2 μm) of thickness $1 \cdot 10^{-4}$ m and area $2.55 \cdot 10^{-3}$ m^2, which acts as a supporting membrane for the liquid membranes. To obtain the hydraulic permeability data, one compartment of the transport cell was filled with aqueous solution of a mixtures of lecithin, cholesterol and a barbiturate of desired composition (Fig. 2 of Ref. [14]) and the other compartment was filled with distilled water. Details of the method used for the hydraulic permeability measurements were the same as used in earlier studies (e.g. Ref. [14]). The solute permeabilities ω of the relevant permeants in the presence of the liquid membranes generated by the lecithin–cholesterol–barbiturate mixtures were determined using the equation [15]

$$J_s = \omega \Delta\Pi \qquad (1)$$

where J_s and J_v are the solute flux and the volume flux per unit area of the membrane, respectively, and $\Delta\Pi$ is the osmotic pressure difference. For solute permeability ω measurements, the composition of the lecithin–cholesterol–barbiturate mixture chosen was the one at which the liquid membrane generated by lecithin completely covered the supporting membrane and was saturated with both cholesterol and the barbiturate under investigation. This composition was derived from our earlier studies [16,17] and from the present data on hydraulic permeability in the presence of the varying concentrations of barbiturates in the mixture of lecithin and cholesterol of fixed composition, i.e. 15.542 ppm with respect to lecithin and $1.175 \cdot 10^{-6} M$ with respect to cholesterol. This particular composition of the lecithin–cholesterol mixture was chosen because it was shown in an earlier study [17] that at this composition the liquid membrane generated by lecithin at the interface is completely saturated with cholesterol. For the measurement of solute permeability ω, one compartment of the transport cell was filled with the aqueous solution of the lecithin–cholesterol–barbiturate mixture (Fig. 2 of Ref. [14]), along with permeant, and the other compartment was filled with distilled water. The condition $J_v = 0$ was imposed on the system and the amount of permeant transported to the compartment filled with distilled water in a known period of time was estimated. Details of the method of measurement of solute permeability ω were the same as described in earlier publications (e.g. Ref. [14]).

All measurements were performed at constant temperature, using a thermostat setting of 37 ± 0.1 °C.

Estimations

The amounts of the various permeants transported to the compartment filled with distilled water were estimated as follows:

Amino acids: the amounts of glycine, aspartic acid and GABA were estimated from the amount of their reaction products with ninhydrin, measured at 570 nm [18] using a Bausch and Lomb Spectronic 20 spectrophotometer.

Serotonin and noradrenaline: the amounts of serotonin and noradrenaline were estimated by measurement of absorbance at 281 nm in 0.1 N HCl using a Varian Cary 1, D spectrophotometer [19].

RESULTS AND DISCUSSION

The hydraulic permeability data at various concentrations in the case of both barbiturates (sodium phenobarbital and sodium pentobarbital) were found to be in accordance with the proportional relationship

$$J_v = L_p \, \Delta P \qquad (1)$$

where J_v is the volume flux per unit area of the membrane, ΔP is the applied pressure difference across the membrane and L_p is the hydraulic conductivity coefficient. The values of L_p at various concentrations of the drugs, estimated from the J_v versus ΔP plots, are recorded in Table 1. The values of L_p decrease with increasing concentration of the drugs up to their c.m.c., beyond which they become more or less constant. This trend in the values of L_p is consistent with Kesting's liquid membrane hypothesis [9] and is indicative of the for-

TABLE 1

Values of the hydraulic conductivity coefficient $(L_p)^a$ at various concentrations of barbiturates

	Sodium phenobarbital concentration $\cdot 10^5$ (M)						
	0.0	1.875	3.75	5.625	7.5[d]	15.0	30.0
$L_p \cdot 10^9$ ($m^3 s^{-1} N^{-1}$)	14.360 ± 0.044	12.465 ± 0.147	10.700 ± 0.217	9.110 ± 0.029	7.391 ± 0.002	7.396 ± 0.037	7.439 ± 0.206
$L_p^t \cdot 10^9$ ($m^3 s^{-1} N^{-1}$)		12.618 ± 0.034	10.876 ± 0.023	9.133 ± 0.013			
	Sodium pentobarbital concentration $\cdot 10^5$ (M)						
	0.0	1.25	[illegible]	[illegible]	[illegible]	10.0	[illegible]
$L_p \cdot 10^9$ ($m^3 s^{-1} N^{-1}$)	14.360 ± 0.044	12.791 ± 0.147	10.929 ± 0.179	9.457 ± 0.127	[illegible]	7.746 ± 0.015	7.810 ± 0.019
$L_p^t \cdot 10^9$ ($m^3 s^{-1} N^{-1}$)		[illegible] ± 0.049	11.064 [illegible]	9.413 [illegible]	± 0.063		

[illegible] The values [illegible] are reported as arithmetric means of 10 repeats [illegible]

[a] Experiment values.

[b] Computed values using mosaic model.

[c] Critical micelle concentration.

progressively covered with surfactant layer liquid membrane and at the c.m.c. it is completely covered. Analysis of the values of L_p in the light of the mosaic model [20–22] lends further support to the formation of the drug liquid membrane in series with the supporting membrane. It has been shown in earlier publications [16,17] that for a concentration of surfactant n times its c.m.c., n < 1, the value of L_p should be equal to $[(1-n)\,L_p^o + n\,L_p^s]$ where superscripts o and s, respectively, represent the values for the bare supporting membrane and the supporting membrane completely covered with surfactant layer liquid membrane. L_p^o and L_p^s represent the values of L_p when the surfactant concentrations are equal to zero and the c.m.c., respectively. The value of L_p thus computed at several concentrations of the drugs below their c.m.c. match with the experimentally determined values (Table 1), lending additional support to the formation of the liquid membranes.

Evidence in favour of the incorporation of barbiturates in the liquid membrane generated by the lecithin–cholesterol mixture can be obtained from the hydraulic permeability data at varying concentrations of these drugs in the lecithin–cholesterol mixtures of fixed composition. The hydraulic permeability data in this case were found to be represented by Eqn (2). It was observed that as the concentration of drug is increased, the values of L_p first decrease and then become more or less constant (Table 2). The concentrations of drug beyond which the values of L_p become more or less constant can be taken to be the concentration at which the lecithin liquid membrane at the interface (which is already saturated with cholesterol) is also saturated with the drug (Table 2).

Thus, the concentrations of sodium phenobarbital and sodium pentobarbital required to saturate the lecithin–cholesterol liquid membrane are $6.0 \cdot 10^{-5} M$ and $2.0 \cdot 10^{-5} M$ respectively. The concentrations of sodium phenobarbital and sodium pentobarbital compare favourably with their reported [23,24] plasma concentrations, at least in order of magnitude. The plasma concentration of sodium phenobarbital is in the range 0.2–0.5 mM [23] and that of sodium pentobarbital ranges from 4.2 to 11 μM [24].

In view of these studies, the concentrations of barbiturates in the lecithin–cholesterol mixture of fixed composition used in the solute permeability experiments were either equal to or a little higher than the concentrations required to saturate the lecithin–cholesterol liquid membrane.

TABLE

Value of the hydraulic conductivity coefficient (L_p) at various concentrations of sodium phenobarbital and sodium pentobarbital in the presence of lecithin cholesterol mixture of fixed composition

	Sodium phenobarbital concentration $\cdot 10^3$ (M)						
	0.0		3.0	4.5	6.0	[illegible]	9.0
[illegible] (m [illegible] N	10.226	10.036	8.195	6.913	5.155	5.194	5.201
	[illegible]	± 0.022	± 0.207	± 0.142	± 0.111	± 0.085	± 0.108

	Sodium pentobarbital concentration $\cdot 10^3$ (M)				
	0.0	1.0	2.0	3.0	4.0
$L_p \cdot 10^7$ (m s^{-1} N^{-1})	10.226	11.436	8.390	8.247	8.417
	± 0.075	± 0.007	± 0.087	± 0.146	± 0.068

The values of L_p are reported as arithmetic means of 10 repeats ± S.D

TABLE 3

Solute permeability (ω)[a] of various permeants in the presence of liquid membranes generated by sodium phenobarbital (ω_1)[b] and sodium pentobarbital[c] (ω_2) in lecithin-cholesterol mixture of fixed composition along with the control values (ω_0) when no barbiturates were used

Permeants	Initial concentration (10^{-3} mol l^{-1})	$\omega_0 \cdot 10^9$ (mol s^{-1} N^{-1})	$\omega_1 \cdot 10^9$ (mol s^{-1} N^{-1})	$\omega_2 \cdot 10^9$ (mol s^{-1} N^{-1})
γ-Aminobutyric acid (GABA)	1.940	0.284 ± 0.014 (6.6)	0.719 ± 0.018 (6.8)	0.855 ± 0.006 (6.8)
Glycine	1.333	1.077 ± 0.021 (7.05)	1.402 ± 0.011 (7.0)	1.674 ± 0.017 (6.9)
Serotonin creatinine sulphate	0.0247	0.219 ± 0.008 (6.9)	0.652 ± 0.013 (6.5)	0.748 ± 0.011 (6.4)
Aspartic acid	1.127	0.269 ± 0.014 (4.2)	0.136 ± 0.015 (4.0)	0.192 ± 0.017 (4.0)
Noradrenaline	0.059	0.752 ± 0.020 (7.1)	0.135 ± 0.011 (6.5)	0.595 ± 0.003 (6.2)

[a]Values of ω reported as arithmetic means of 15 repeats ± S.D. The figures within parentheses indicate pH of the permeant solution in the lecithin-cholesterol-barbiturate mixture.
[b]Sodium phenobarbital concentration $6.0 \cdot 10^{-3}$ M.
[c]Sodium pentobarbital concentration $2.0 \cdot 10^{-3}$ M.

Solute permeability data

The solute permeability data recorded in Table 3 appear relevant to the biological actions of the barbiturates.

Electrophysiological studies have indicated that sedative barbiturates inhibit excitatory transmission and enhance inhibitory transmission [25]. This is consistent with the enhanced permeability of GABA and the reduced permeability of [illegible] observed in the present experiments (Table 3). Electrophysiological [illegible] has also indicated [25] that GABA has a major role [illegible] receptor binding studies have, however, failed to detect [illegible] between GABA and barbiturates [26]. On this basis it has been suggested [illegible] that barbiturates do not affect the post-synaptic binding [illegible] GABA even though GABA mimetic actions have been observed electrophysiologically. The present studies appear to offer an explanation for these observations. The present data on enhancement in the transport of GABA [illegible] suggest that access to a GABA receptor is likely to be facilitated by the presence of the liquid membrane generated by the barbiturates in association with membrane lipids at the receptor site.

The anticonvulsant activity of phenobarbitone, which has been used in the treatment of epilepsy, is ascribed to its ability to produce an increased concentration of GABA in the brain. Phenobarbitone is reported to be most effective when the brain GABA content has been depleted [27]. The enhancement in the permeability of GABA in the presence of the lecithin–cholesterol–sodium phenobarbital liquid membrane (Table 3) is consistent with these clinical observations.

The two barbitals presently studied are reported to have the following gradation in onset of action [28]: sodium pentobarbital > sodium phenobarbital. This gradation in onset of activity of the barbitals is consistent with the present observation on the concentration of the barbitals required to saturate the lecithin–cholesterol liquid membrane (Table 2): sodium phenobarbital > sodium pentobarbital. Thus sodium pentobarbital, which crosses the blood–brain barrier the fastest [28], is required at the lower concentration to saturate the lecithin–cholesterol liquid membrane. Since modification in the permeability of the biological membrane would be maximum when the lipid bilayer is saturated with barbiturate, leading to maximum biological effect, the gradation in the onset of biological action appears to be a consequence of both factors, i.e. how fast it crosses the blood–brain barrier and how small is the concentration of drug required to saturate the lipid bilayer. The gradation in the onset of barbiturate action is also consistent with the conclusion that the c.m.c. is a good indicator of the potency of surface-active drugs — the lower the c.m.c. the more potent is the drug [1]. The c.m.c. of sodium phenobarbital is higher than that of sodium pentobarbital.

Barbiturates are known to disturb the balance of the phases of sleep — the initial effect is that of reducing the proportion of REM (rapid eyeball movement) sleep in comparison to NREM (non-rapid eyeball movement) sleep [29]. This observation can also be explained in terms of the enhanced permeability of serotonin and reduced permeability of noradrenaline in the presence

the liquid membrane generated by the lecithin–cholesterol–barbiturate mixture (Table 2). It is documented that raphe nuclei, which are rich in serotonin, are responsible both for NREM sleep and for the transition to and onset of REM sleep. When a system of neurons in the pons known as the locus caeruleus (rich in noradrenaline) is destroyed, animals previously deprived of REM sleep fail to take the usual rebound excess of REM sleep when undisturbed [30]. The present data (Table 3) indicate that the liquid membranes likely to be formed in the synaptic cleft by the barbiturates in association with the membrane lipids may enhance the access of serotonin to its site of action in the raphe nuclei and reduce the access of noradrenaline to its site of action in the locus caeruleus, which may also contribute to the causation of imbalance in the phases of sleep by barbiturates.

Barbiturates are known to depress the respiratory drive and to disturb the rhythmic character of respiration [31]. It is also documented that iontophoretically applied GABA and glycine in the bulbar respiratory units have been found to inhibit medullary respiratory neurons [32,33], and glutamic and aspartic acids to excite the ongoing phasic neural activity of both inspiratory and expiratory neurons [33]. Thus the rhythmic character of respiration has been postulated to be a consequence of the actions of inhibitory amino acids like GABA and excitatory ones like aspartic acid [34]. The present data (Table 3) indicate that enhancement in permeability of GABA and glycine and reduction in the permeability of excitatory neurotransmitters like aspartic acid, due to the liquid membranes formed by barbiturates in association with the membrane lipids in the synaptic cleft of the respective neurons, may also be a factor responsible for disturbance in the rhythmic character of respiration.

Thus the present studies on modification in the permeability of relevant permeants in the presence of the liquid membranes indicate effects which are worthy of further investigation with natural membranes. The pH, which is likely to influence the ionization of the barbiturates and the permeants, is different from its physiological value in the present experiments for solute permeability ω measurements. This fact, however, may not alter the qualitative conclusions because the pH of the experimental solutions are close to the pH of the solutions in the corresponding control experiments, at least in order of magnitude.

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Liquid membrane phenomenon in the biological actions of benzodiazepines

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The liquid membrane phenomenon in benzodiazepines has been studied. Transport of glycine, GABA, noradrenaline, dopamine and serotonin in the presence of the liquid membranes generated by the benzodiazepines in association with lecithin and cholesterol has been studied. The data indicate that modification in permeabilities in the presence of the liquid membranes is likely to make a significant contribution to several biological actions of the benzodiazepines.

Investigations on a wide variety of surface active drugs belonging to different pharmacological categories have revealed that liquid membranes likely to be generated by the drugs either by themselves or in association with the membrane lipids at their respective sites of action may modify access of relevant permeants to these sites. This modification in the access of relevant permeants to the respective sites of action due to the presence of the liquid membrane barrier, though passive in nature, is likely to make a significant contribution to biological actions of the drugs. A consolidated account of this point of view and its implications substantiated in a good number of cases—some of the recent examples being the studies on prostaglandins[1], vitamin E[2], anticancer drugs[3] and antiarrhythmic drugs[4]—has been presented earlier[5].

In an earlier study[6], the role of liquid membrane phenomenon in the antianxiety action of diazepam has been investigated. Since benzodiazepines in addition to anxiolytic action are also known to exert myorelaxant and anticonvulsant actions[7-9] involving multiplicity of neurotransmitter systems[7] including catecholamines, serotonin, γ-aminobutyric acid (GABA) and glycine, a more detailed study is described in this communication. The present study has been conducted on two benzodiazepines, namely nitrazepam and chlordiazepoxide. Data on hydraulic permeability have been obtained to demonstrate the formation of liquid membranes by these drugs in series with a supporting membrane and also the incorporation of these drugs into the liquid membranes generated by the lecithin-cholesterol mixtures. Transport of the relevant permeants, viz. glycine, GABA, noradrenaline, dopamine and serotonin, through the liquid membrane generated by the lecithin-cholesterol-benzodiazepine mixtures has been studied and the data obtained have been utilized to throw light on the role of liquid membrane phenomenon in the biological actions of these drugs. In the present studies, a non-living supporting membrane has been deliberately chosen so that the possibility of active interaction with the constituents of biomembranes is totally ruled out and the role of passive transport through the liquid membranes in the biological actions of the drugs is highlighted.

Materials and Methods

Lecithin (Patel Chest Institute, CSIR Centre), Biochemicals, Delhi), Cholesterol (Centron Research Laboratories, Bombay), L-noradrenaline (Fluka—AG), serotonin creatinine sulphate (Koch—Light Laboratories), dopamine chlorhydrate (Riedel), glycine (Loba-Chemie), γ-amino butyric acid (GABA) (BDH England) and water twice distilled in an all pyrex glass still were used in the present experiments.

The critical micelle concentrations (CMCs) of aqueous solutions of nitrazepam and chlordiazepoxide as determined from the variation of surface tensions with concentrations were found to be 8×10^{-6} *M* and 2.0016×10^{-5} *M* respectively. The surface tensions were measured using a Fisher Tensiomat Model 21. The aqueous solutions of benzodiazepines, which are sparingly soluble in water were prepared by adding the requisite volume of ethanolic stock solution of known concentration of the drug to the aqueous phase with constant stirring. In the aqueous solutions of the drugs, thus prepared, the final concentration of ethanol was never allowed to exceed 0.1% by volume because it was shown by a control experiments that a 0.1% solution of ethanol in water did not affect the surface tension of water to any measurable extent.

*For correspondence

glass transport cell used in the earlier study[6] on diazepam was also used in the present studies. For description of the transport cell see refs 2 or 3. In the all glass cell, a sartorius cellulose nitrate microfiltration membrane (Cat. no. 11307, average pore [illegible] thickness 1×10^{-4} m and area 2.5×10^{-4} [illegible] which also acted as a supporting membrane for the liquid membrane divided the cell into two compartments C and D (see Fig. 2 of ref. 3).

The hydraulic permeability data in the presence of various concentrations of the drugs in the lower compartment C of the transport cell were obtained to demonstrate the formation of liquid membranes by the drugs in series with the supporting membrane. The information about the incorporation of the benzodiazepines in the liquid membranes generated by the lecithin-cholesterol mixture was obtained from the data on hydraulic permeability in the presence of varying concentrations of the drugs in the aqueous solution of the lecithin-cholesterol mixture of fixed composition—15.542 ppm with respect to lecithin and 1.175×10^{-6} *M* with respect to cholesterol—taken in the lower compartment, C, of the transport cell. This particular composition of the lecithin-cholesterol mixture is derived from our earlier study[6] wherein it has been demonstrated that at this composition the liquid membrane generated by lecithin completely covers the interface and is saturated with cholesterol. The method used for obtaining the hydraulic permeability data was the same as used in the earlier study[6].

The values of solute permeability (ω) were estimated using the equation[11]

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

where J_s and J_v are the solute flux and the volume flux per unit area of the membrane and $\Delta\pi$ is the osmotic pressure difference across the membrane. In experiments for determining ω, a solution of desired concentration of the permeant prepared in the aqueous solution of the lecithin-cholesterol-benzodiazepine mixture of known composition was filled in compartment C and water in compartment D of the transport cell. The details of the method were the same as described earlier[1-4]. The composition of the lecithin-cholesterol-benzodiazepine mixture used in the experiments for ω measurements was derived from the hydraulic permeability data in the presence of varying concentrations of benzodiazepines in the aqueous solution of the fixed composition of the lecithin-cholesterol mixture. The composition of the lecithin-cholesterol-benzodiazepine mixtures used in the solute permeability measurements were those at which the liquid membrane generated by lecithin completely covers the interface and is saturated with both cholesterol and the benzodiazepine under study.

All measurements were made at constant temperature using a thermostat set at 37 ± 0.1 °C.

Estimations—The amounts of dopamine, serotonin and noradrenaline transported to compartment D were estimated spectrophotometrically by measuring absorbance at 282.4 nm[18], using a Varian Cary 17-D spectrophotometer.

GABA and glycine were estimated from the amount of their reaction product with ninhydrin measured spectrophotometrically at 570 nm[19], using a Varian Cary 17-D spectrophotometer.

Results and Discussion

The hydraulic permeability data in all cases were found to be in accordance with the equation

$$J_v = L_p \Delta P \qquad (2)$$

where J_v is the volume flow per unit area of the membrane, ΔP is the applied pressure difference and L_p is the hydraulic conductivity coefficient. The values of L_p, as estimated from the J_v versus ΔP plots, in the case of both drugs (Table 1) show trends which are indicative of the formation of the drug liquid membrane in series with the supporting membrane. According to Kesting's hypothesis[14] when a surfactant is added to an aqueous phase the surfactant layer which forms spontaneously at the interface acts as a liquid membrane and modifies transport across the interface. As the concentration of the surfactant is increased, the interface gets progressively covered with the surfactant layer liquid membrane and at the CMC it is completely covered. The values of L_p decrease progressively with the increase in the concentrations of the drugs up to their CMCs, beyond which they become more or less constant (Table 1). Analysis of the data on L_p values in the light of mosaic model[15-17] further confirms the formation of the drug liquid membrane in series with the supporting membrane. Utilizing the concept of progressive coverage of the supporting membrane by the liquid membrane, it was shown earlier[6] that when the concentration of the surfactant is n times its CMC, $n \leq 1$, the values of L_p can be computed using the expression $[(1-n)L_p^0 + n\ L_p^1]$ where L_p^1 and L_p^0 are the values of L_p when the concentration of the surfactant is equal to zero and the CMC respectively. The values of L_p thus computed at different concentrations below the CMC of the drug compare

Table — alues of hydraulic conductivity coefficient L_p at arying concentrations of benzodiazepines

Concentration $\times 10^5$ M	Nitrazepam						
	0.00	4	6	8	10	30	
$L_p \times 10^9$ m/s ^{1}N	068 ± 0.142	6.814 ± 0.017	5.279 ± 0.112	4.118 ± 0.061	2.761 ± 0.05	2.743 ± 0.146	2.753 ± 0.071
$L_p \times 10^9$ m/s ^{1}N		6.741 ± 0.120	5.415 ± 0.098	4.088 ± 0.075			

Concentration $\times 10^5$ M	0.00		2.0016		2.6688	
$L_p \times 10^9$ m/s ^{1}N						
$L_p \times 10^9$ m/s ^{1}N						

[a]Experimental values
Computed values using mosaic model.

Table 2 — Values of hydraulic conductivity coefficient (L_p)[a] at varying concentrations of benzodiazepines in lecithin-cholesterol mixtures[b]

Concentration $\times 10^5$ M	Nitrazepam						
	0.00	2	4	5	6	8	10
$L_p \times 10$ (m s ^{1}N	3.320 ± 0.261	1.807 ± 0.115	1.322 ± 0.079	1.183 ± 0.017	1.014 ± 0.025	1.026 ± 0.041	1.027 ± 0.01

Concentration $\times 10^5$ M	Chlordiazepoxide					
	0.00	0.5004	1.0008	1.3344	1.668	2.0016
$L_p \times 10^9$ m/s ^{1}N	3.320 ± 0.261	2.509 ± 0.189	1.840 ± 0.139	1.233 ± 0.012	1.231 ± 0.014	1.236 ± 0.012

[a]The values are reported as arithmetic mean of 10 repeats ± S.D.
[b]Lecithin and cholesterol concentrations kept constant at 15.542 ppm and 1.175×10^{-6} M respectively.

favourably with the corresponding experimentally determined values (Table 1).

The hydraulic permeability data at varying concentrations of the benzodiazepines in aqueous solutions of the lecithin-cholesterol mixture of the fixed composition also obeyed Eq. 2. The values of L_p (Table 2) decrease with the increase in concentration of the drug up to a particular concentration whereafter they become more or less constant. The decreasing trend in the values of L_p indicates the incorporation of the benzodiazepines in the liquid membrane generated at the interface by the lecithin-cholesterol mixture and the concentration whereafter the values of L_p become constant are the concentrations at which the lecithin-cholesterol liquid membrane at the interface is saturated with the benzodiazepine. It is these particular compositions of the lecithin-cholesterol-benzodiazepine mixtures that were used in the experiments for solute permeability (ω) determinations.

Solute permeability data and biological actions—Solute permeability data for the various permeants recorded in Table 3 appear relevant to the reported biological actions of the benzodiazepines.

Biochemical and neurophysiological evidences recorded in literature[18-24] have suggested that anti-anxiety action of benzodiazepines may be exerted by facilitating synaptic action of GABA in the brain. Enhanced permeability of GABA through the lecithin-cholesterol-benzodiazepines composite liquid membrane, as observed in the present study (Table 3), could also facilitate GABA potentiation leading to the antianxiety action of benzodiazepines.

Glycine present in relatively high concentration in the grey matter of the spinal cord is known to cause muscle relaxation by depressing the excessive motor activity[25,26]. The enhanced permeability of glycine through the lecithin-cholesterol-benzodiazepine composite liquid membrane (Table 3) may facilitate its access to the glycine receptor in the central nervous system and thus may also contribute to the reported muscle relaxant action of benzodiazepines.

Use of benzodiazepines in the treatment of epilepsy is documented[25,27]. Electrophysiological and

Table 3. Solute permeability of various permeants in pres

Permeants	Initial concentration 10^{-3} mole lit	$\omega \times 10^{-3}$ mole s^{-1} N	$\omega \times 10^{-3}$ mole s^{-1} N	$\omega \times 10^{-3}$ mole s^{-1} N
Glycine	1.333	1.584 ± 0.02	2.4 ± 0.071	1.8 ± 0.10
γ-Aminobutyric acid (GABA)	1.940	0.974 ± 0.051	3.151 ± 0.116	1.7 ± 0.082
Noradrenaline	0.059	0.351 ± 0.039	0.197 ± 0.077	0.516 ± 0.019
Dopamine	0.0527	0.473 ± 0.062	0.342 ± 0.039	0.278 ± 0.051
Serotonin	0.0247	0.764 ± 0.016	1.109 ± 0.027	0.837 ± 0.107

[a]Values of ω are reported as arithmetic mean of 15 repeats ± S.D.
[b]Lecithin concentration (15.542 ppm) and cholesterol concentration (1.175×10^{-6} M
Control values when no drug was used.
ω_1—Nitrazepam concentration (7.5×10^{-6} M).
ω_2—Chlordiazepoxide concentration (1.668×10^{-5} M

biochemical evidences have linked the actions of benzodiazepines to their ability to potentiate the effects of exogeneous GABA or to enhance GABA-mediated presynaptic and post-synaptic inhibitory pathways[27,28]. The enhanced permeability of GABA, as observed in the present study (Table 3), may contribute to the reported anti-epileptic effects of benzodiazepines.

The benzodiazepines are believed to suppress the ability of the limbic system to activate the reticular formation and thus induce sleep in cases of insomnia due to anxiety[29]. This effect appears to be due to the GABA potentiation to which the enhanced permeability of GABA (Table 3) may be a contributing factor.

Nitrazepam, like barbiturates, is known to disturb the balance of the phases of sleep[29]. The initial effect is that of reducing the proportion of REM (rapid eyeball movement) sleep in comparison to NREM (non rapid eyeball movement) sleep[29]. Raphe nuclei which are rich in serotonin are responsible both for NREM sleep and for the transition to and onset of REM sleep[30]. When the locus caeruleus which is rich in noradrenaline is destroyed, animals previously deprived of REM sleep fail to take the usual rebound excess of REM sleep when undisturbed[30]. The present data (Table 3) indicate that the liquid membrane may be formed by the nitrazepam in association with the membrane lipids in the synaptic cleft and may enhance the access of serotonin to its site of action in the raphe nuclei and reduce the access of noradrenaline to its site of action in the locus caeruleus causing disbalance in the phases of sleep.

It is documented that patients treated with benzodiazepines also show failure to ovulate[31] like those treated with drugs like reserpine and chlorpromazine[32] which impede the transport of dopamine. The present data indicate that impediment in the transport of dopamine (Table 3), due to the liquid membranes of the benzadiazepines in association with membrane lipids, which acts at the level of median eminence[32] to stimulate the release of LH/FSH-RH*, could also be a factor responsible for this side effect of the benzodiazepines.

One side effect of benzodiazepines is reported to be weight gain due to renewed appetite[31–33]. Although the pharmacology of eating behaviour is complex and is governed by several factors[33] broadly speaking GABA and noradrenaline acting at the level of hypothalamus are known to act as feeding enhancers and feeding inhibitors respectively[34]. The data recorded in Table 3 on the enhanced permeability of GABA and the reduced permeability of noradrenaline appear consistent with these observations particularly in the case of nitrazepam. The observation that permeability of noradrenaline is enhanced in the case of chloridazepoxide (Table 3) appears consistant with the report that it is less toxic than nitrazepam[35].

According to the liquid membrane hypothesis of drug action[5] the CMC of the drug is a good indicator of its potency—lower the CMC more potent is the drug. Since the CMC value of nitrazepam is lower than that of chlordiazepoxide it should be more potent than chlordiazepoxide which indeed is the case[36].

Thus it appears that the phenomenon of liquid membrane formation may also contribute to the biological actions of benzodiazepines.

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*LH/FSH-RH, Leuteinizing hormone/follicle stimulating hormone-releasing hormone.

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