ROLE OF LIQUID MEMBRANE PHENOMENA IN DRUG ACTION

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TO

MY PARENTS

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE

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CERTIFICATE

This is to certify that the thesis entitled "ROLE OF LIQUID MEMBRANE PHENOMENA IN DRUG ACTION" submitted by Mr. C.V.S.Subrahmanyam, ID.No.82P2A7402, for the award of the Ph.D. degree of the Institute, embodies original work done by him under my supervision.

Dated: 24.08.1985.

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CONTENTS

PREFACE

This thesis contains an account of the investigations carried out with a view to exploring the role of liquid membrane phenomena in the action of surface active drugs. The genesis of these investigations is the following. All drugs which act by modifying the permeability of cell membranes, of necessity should be surface active. Not only are a wide variety of drugs really surface active, in a number of cases excellent correlations between surface activity and biological activity have been discovered. This led to the suspicion that there might exist a common mode of action for all surface active drugs. Since, surface active substances are capable of generating liquid membrane at the interface (Kesting's hypothesis) it was suspected that the liquid membranes generated at the site of action of the respective drugs, modifying the transport of relevant permeants, might be an important step common to the mechanism of action of all surface active drugs. Prompted by this conception, a wide variety of structurally dissimilar drugs belonging to different pharmacological categories were investigated. These investigations have led to what may be called a "Liquid Membrane Hypothesis for Drug Action". (see Adv.Colloid Interface Sci., 20, (1984) 131-161).

In this thesis, certain categories of drugs namely antihistamines (H_1-antagonists and H_2-antagonists), histamine release blockers, antiepileptic drugs and antiarrhythmic drugs have been chosen to investigate the role of liquid membrane phenomenon in their action. The data which throw a new light on the mechanism of action of these drugs substantiate the liquid membrane hypothesis for drug action.

The thesis is divided into five Chapters. Chapter I contains a consolidated account of the biological implications of the "Liquid Membrane Phenomena" with special reference to drug action. Chapter II contains a survey of the literature reports on surface activity of the drugs chosen for the present study. Chapter III describes the details of the experiments conducted to demonstrate the formation of liquid membrane and to obtain the data on the transport of relevant permeants through the drug liquid membranes. In Chapter IV, a discussion of the data, high-lighting the role of liquid membrane phenomena in drug action, is presented and in Chapter V a chapterwise summary is presented.

In the present investigations, choice of structurally dissimilar drugs is deliberate to make the role of liquid membranes generated by surface active drugs conspicuous. Similarly the non-living and non-specific membrane like cellulose acetate/ nitrate microfiltration membrane has been deliberately chosen as the supporting membrane for the liquid membranes so that specific and active interaction of the drug molecules with the constituents of bio-membranes as a cause for the modification in the transport of relevant permeants is totally ruled out and the role of passive transport through the drug liquid membranes in the mechanism of their action is highlighted.

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C.V.S. Subrahmanyam

CHAPTER - I

THE LIQUID MEMBRANE HYPOTHESIS AND ITS BIOLOGICAL IMPLICATIONS

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The Liquid Membrane Hypothesis:

Surface active molecules when added to aqueous phase in contact with hydrophobic phase are known to accumulate at the interface in such an orientation that the hydrophobic tails of surfactant molecules are preferentially directed towards the hydrophobic phase and the hydrophilic ends are in the aqueous phase. When concentration of the surfactant exceeds its critical micelle concentration (CMC) the surfactant molecules aggregate themselves as micelles and remain in the bulk of the solution. It has been observed[1] that addition of surfactants modifies material transport across the interface.

Martin discovered[2] that the addition of surfactants like polyvinylmethyl ether (PVME), in very minute quantities say of the order of a few ppm, to saline feed in reverse osmosis dramatically enhanced the salt retention capacity of cellulose acetate membranes with a small decrease in water flux. This phenomenon was explained by Kesting et al [3] on the basis of a liquid membrane hypothesis. According to this hypothesis,

the surfactant layer which forms at the cellulose acetate membrane/saline solution interface acts as a liquid membrane in series with the supporting membrane and is responsible for the enhanced salt rejection. It was also shown that as concentration of the surfactant is increased the interface gets progressively covered by the surfactant layer liquid membrane and at the CMC it is completely covered. Additional experimental evidence in favour of the liquid membrane hypothesis has been furnished by Srivastava and coworkers.[4-6]

BIOLOGICAL IMPLICATIONS

Model Membrane Systems:

Since molecules of surface active nature are crucial to living matter and its organisation, biological implications of the liquid membrane hypothesis have been investigated recently.[7,8] In these studies, bilayers of liquid membranes have been generated from the constituents of biomembranes on a hydrophobic supporting membrane and transport across them has been studied. Not only has the passive transport data on the liquid membrane bilayers thus generated, been

shown to be closer to the corresponding data on biomembranes, some of the biologically relevant transport processes like light induced volume flow through the liquid membrane bilayers (photo-osmosis) generated from chloroplast extract has been mimicked.[9,10] The trends in the data on the photo-osmosis through liquid membrane bilayers generated from chloroplast extract on a hydrophobic supporting membrane have been shown to be consistent with the trends reported in chloroplast BLM.[11] All these investigations have given a strong indication that liquid membrane bilayers generated on a hydrophobic supporting membrane by the constituents of the biomembranes are capable of acting as model systems for biological membranes.

Drug Action:

Formation of biomembranes and the location of receptor proteins in the lipid bilayer part of biomembranes is all a consequence of surface activity. Hence, it is logical to expect that the drugs acting by modifying the permeability of cell membranes after interacting with them are likely to be surface active. A wide variety of drugs are, in fact, known to be surface active in nature.[12,13] This activity does not

appear to be a fortuitous coincidence. In a number of cases, correlations between surface activity and biological effects have been demonstrated.[14-16] While investigating the actions of drugs like reserpine, prenylamine, chlorpromazine, propranolol etc., which inhibit catecholamine transport, it has been concluded[17] that "irrespective of chemical structure, the surface activity of psychotropic drugs mainly determines their potency to affect all kinds of membranes, especially that of catecholamine storing particles". Since the structural requirements for surface activity are often similar to those for interaction of drugs with receptor sites,[18] the correlation between surface activity and biological effects appears to indicate that there might exist a common mode of action for all surface active drugs. In view of the liquid membrane hypothesis, it was suspected that the liquid membranes generated at the site of action of the respective drugs, acting as a barrier to the transport of relevant permeants, might be an important step, common to the mechanism of action of all surface active drugs. Prompted by this conception, a number of investigations as to the role of liquid membrane phenomenon in the mechanism of action

of surface active drugs has been undertaken.[19] For this study, structurally dissimilar drugs of different pharmacological categories were chosen deliberately so as to gain information and highlight the role of liquid membrane phenomena in surface active drugs. Most of the drugs are antagonistic in action and act by altering permeability of relevant permeants to the site of action. The results of these investigations have proved quite revealing. These investigations have, in fact, lead to what can be termed as "Liquid Membrane Hypothesis for Drug Action". A concise account of this hypothesis[19] is given below.

The Liquid Membrane Hypothesis for Drug Action:[19]

The antagonistic drugs, in general, interact with the membrane components, and occupy the same sites with which the agonist drugs would have interacted to elicit the desired response. Thus antagonistic drugs act by creating hindrance in the interaction of agonist drugs with receptor sites. How this hindrance is created, is contained in the liquid membrane hypothesis for drug action,[19] which has been substantiated through investigations on a variety of drugs belonging to different pharmacological categories.

The membranes represent an 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 the 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. The liquid membranes, thus generated by surface active agents, may act as a barrier modifying the transport of relevant molecules to those 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 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"[20,21] and the "rate theory",[22,23] a more rational biophysical explanation for the action of surface active drugs acting by modifying the permeability

of cell membranes emerges. The reason why such a possibility has gone unnoticed so far, appears to be the fact that passive transport processes have traditionally been considered unimportant for biological actions - transport through the liquid membranes are undoubtedly passive in nature. 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 through the liquid membrane also makes a significant contribution to drug action.

Implications of the Hypothesis:

Studies on the liquid membranes generated by surface active drugs can provide a clue to their quantitative action. This is because CMC of a drug indicates the concentration at which interface will be completely covered by the drug liquid membrane. At this concentration (CMC), therefore, modification in the permeability of biological membrane would 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 on haloperidol[24] and chlorpromazine[25] 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.[26] Another example substantiating this is that of local anesthetic drugs[27] - the lower is the CMC, the more potent is the drug. In a series of local anesthetics, it was found[27] that CMCs and minimum blocking concentration (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 anesthetics.

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. The 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 a majority of drugs investigated so far[19] it has been found that the 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, so that hydrophobic ends of the drugs project outwards to face the permeants. Such an orientation can be rationalised if one examines the nature of the receptors, in general, in relation to the lipid bilayer part of the biomembranes.

The receptors, in general, are membrane proteins and hence should be surface active in nature. Hence, they should have both hydrophilic and hydrophobic moieties in their structure. Since, the exterior environment of biological cells is aqueous in nature, it is logical to expect that the hydrophobic part of the membrane proteins will be associated with the hydrophobic core of the lipid bilayer and only the hydrophilic part will face exterior. Prediction about similar

orientation of receptor proteins and also the membrane proteins, in general, has been made in the literature.[28] Thus, the studies on liquid membranes generated by drugs can indicate the possible orientation of receptors responsible for interaction with the drugs.

Since the biological membrane comprises of different types of lipids and proteins, a drug can alter transport across the membrane by any one or more 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 observed biological effect; or

iii) the drug protein interaction may be causative agent.

In the case where first possibility is ruled out, because an effect similar to that on biological tissue 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 it was found[29] 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 of GABA.

The multiplicity of biological actions exerted by surface active drugs can be well explained on the basis of the liquid membrane hypothesis e.g. chlorpromazine and few other low potency phenothiazines have mild antihistamine[30] and antiserotonin activity.[30] Antihistamines are known[31] to have anticholinergic and local anesthetic action. Such actions can be explained as a result of alteration in the transport of relevant permeants because of the liquid membrane interposed between permeant and 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 the permeability of

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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 the permeability of

hydrophilic substances, any structural modification that increases hydrophobicity of the compound will increase resistance to transport of the hydrophilic permeant. In other words, any modification in structure leading to increase in hydrophobicity of a drug will reduce C/C of the drug, make it more potent and increase resistance towards a hydrophilic permeant. However, this sequence of events will continue as long as the hydrophilic group of the drug, responsible for interaction with the biomembrane is unaltered. Any alteration in 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., on 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. Increase in hydrophobicity will alter the drug action quantitatively i.e., it will increase the potency, while change in hydrophilicity may alter the action qualitatively i.e., the specificity of the resistance towards different permeants may change. Similar comments have been made by Burger[32] in connection with structure-activity relationship.

for drug action. The addition of each methylene group in an antagonist will increase its hydrophobicity, resulting in reduction of its CMC. Lowering the CMC may be linked to an increase in potency of the compound as discussed earlier. Besides, reducing the CMC, an increase in 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 the dissociation constant provides in case of rate theory.[22,23]

If the dose-response curve of an agonist is compared with the dose-response curve of a mixture of an agonist and an antagonist, there is a flattening of the dose-response curve in the latter case.[20-23] This change leads to a parallel right shift in the case of competitive antagonists. The proposition that an agonist replaces an antagonist is ruled out. This effect is further substantiated by low dissociation constants in the case of antagonist.[22] These observations related to the dose-response curves can also be explained on the basis of the liquid membrane hypothesis for drug action. A liquid membrane

generated by a surface-active "antagonist" drug is interposed between receptors in the biomembrane and the agonist. As a consequence, transport of agonist is likely to be reduced, resulting in a lesser amount of agonist reaching the receptors. Hence to achieve the same quantum of response, a higher amount of agonist will be needed. This effect will result in the shifting of dose-response curves to the right. The nature of the liquid membrane and extent of the resistance offered to the agonist will determine the nature and extent of the shift in the dose-response curve.

One experimental observation in relation to the dose-response curve of the agonist-antagonist mixture has necessitated the hypothesis of "spare receptors". It is observed[23] that a mixture of an agonist and an antagonist elicit the same maximal response as in the case of an 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 curves with and without an antagonist?

Or inspite of a sizeable section of receptors being occupied by an antagonist, how can a maximal response be obtained? The dilemma has been resolved by proposing[33] the existence of "spare receptors", i.e., those receptors without combining with which the agonist alone was capable of eliciting maximal response. However, there is a criticism of this hypothesis. A direct experimental demonstration for "spare receptors" is still awaited.[34] Efforts have also been made[35] to demonstrate experimentally that there are no 'spare receptors'. Paton has commented[23] that "for occupancy theory, existence of spare receptors merely seems a puzzling extravagance". In the liquid membrane hypothesis for drug action, the existence 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 the concentration of the agonist is increased, the rate of flow of the agonist across the liquid membrane generated by the 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 the existence 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 the literature. According to the "potentialsvergiftung theory"[36] the action of the agonist was related to its flux across the cell membrane, which in turn was related to the driving force. The driving force is the concentration gradient.

While commenting on the rate theory, it is mentioned[34] that, in general, for the rate of action of the drug, any one of the following four steps may be rate determining step:

1) access to the receptors;

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

3) rate of combination with the receptors; or

4) rate of production of response.

Among these steps, access to the receptor seems to be most common rate limiting step.[34] Hence,

any event which is likely to reduce access of the agonist to the receptor should have profound influence on the nature and sequence of the agonist receptor interaction and, hence, on the consequent biological response. Generation of a liquid membrane having the 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 the kinetics of reversible antagonism in aortic strips, a biophasic model was proposed.[37,38] 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 agonist (but not antagonist) penetrate quickly, penetration of this barrier is considered as a rate limiting step dictating the kinetics of antagonism. However, the existence of such a barrier in case of antagonist has been ruled out experimentally.[23] 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 presence of antagonist) should rise/fall exponentially when an antagonist is added/removed, is also not true.[23] It is occupancy and not the dose-ratio that is observed to change exponentially. The liquid membrane hypothesis for drug action 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 the liquid membrane hypothesis for drug action needs special mention. It is known that the majority of transport processes in biological systems (especially those of neurotransmitters) are active in nature. In contrast resistance offered by the liquid membrane generated by the surface active drugs is passive in nature. Hence, only after showing that the active process is also impeded by the drug-liquid membrane, will the role of the liquid membrane phenomenon in the action of antagonistic drugs become acceptable. For any process of active transport, the rate of access of the permeant to the active site is an important factor. If this is impeded, because of

the resistance offered by the liquid membrane to permeants, even the active transport will be reduced. This reduction can result in antagonism. This is especially true in case of drug/receptor interaction because access of the drug to the receptor has been considered[34] to be a rate limiting process in the whole sequence of drug action.

Thus liquid membrane hypothesis for drug action points towards a new facet of drug action. The hypothesis provides a physical basis for the action of antagonistic drugs which are surface active in nature.

Since the liquid membrane hypothesis for drug action is quite recent, there is a definite need to investigate more and more categories of surface active drugs, for the role of the liquid membrane phenomena in the mechanism of their action, to substantiate the hypothesis.

The Present Investigation:

In this thesis, the following categories of drugs have been investigated for the role of liquid membrane phenomenon in the mechanism of their action as postulated in the "Liquid Membrane Hypothesis for Drug Action."

I. Antihistamines:

H_1-antagonists:

Chlorpheniramine maleate,

Diphenhydramine hydrochloride, and

Tripelennamine hydrochloride.

H_2-antagonists:

Cimetidine, and

Ranitidine.

II. Histamine Release Blocker:

Disodium cromoglycate.

III. Antiepileptic Drugs:

Diphenylhydantoin,

Carbamazepine, and

Valproate sodium.

IV. Antiarrhythmic Drugs:

Quinidine hydrochloride,

Disopyramide phosphate,

Procainamide hydrochloride, and

Propranolol hydrochloride.

All the drugs listed above contain hydrophilic and hydrophobic moieties in their structure and hence

are expected to be surface active in nature and capable of generating liquid membranes at the interface.

Before an account of the investigations carried out on these drugs is presented in Chapter III and IV, the literature reports on the surface activity of the drugs listed above are summarised in the next Chapter.

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CHAPTER - II

SURFACE ACTIVITY OF DRUGS

SURFACE ACTIVITY OF DRUGS

In this chapter a quick survey of the literature on surface activity of the drugs experimented with in this thesis is presented.

Antihistamines:

H_1 - antagonists:

Surface activity and micellar aggregation in aqueous solutions[1-4] of diphenhydramine hydrochloride, bromodiphenhydramine hydrochloride, chlorocyclizine hydrochloride, diphenyl-pyriline hydrochloride, phenothiazine derivatives, tripelennamine hydrochloride, mepyramine maleate, brompheniramine maleate have been reported. The effect of electrolytes on the micellar properties of some diphenylmethane derivatives has been investigated. An increase in the aggregation number and decrease in critical micelle concentration (CMC) was observed with increasing concentration of electrolytes.[5]

The interaction of these antihistamines with L-α-dipalmitoyl lecithin monolayers have also been

demonstrated. Ability of antihistamines to increase surface pressure was correlated[6] with their surface activity at air-water interface. At high pressures, these drugs were ejected from the monolayers.

Series of drugs containing quaternary ammonium salts belonging to local anesthetics and antihistamines have been shown to possess[7] the property of preventing liver necrosis. The cause for such a prevention has been attributed to permeability changes, to prevent the loss of intracellular potassium and soluble proteins from liver cells. A correlation between the protective action of these compounds and their interaction at air/water and lipid/water interface has been demonstrated.

H_2 - antagonists:

Physico-chemical properties of a H_2-receptor antagonist, cimetidine,[8] have been investigated. Surface properties of cimetidine, like surface adsorption, surface tension and surface potential were studied. The parameters like hydrophobicity, partition coefficients, which are also related to surface activity are documented[9] in case of cimetidine. However, no definite information on CMC of cimetidine and ranitidine, which are investigated in this thesis,

is available. In general, it appears all the H_2-antagonists may act at membrane water interface.

Antiepileptic Drugs:

Depressant drugs, in general, are reported to populate at the air-water interface.[10] This is a consequence of their surface activity. Antiepileptic drugs are reported[11] to contain both hydrophilic and hydrophobic moieties in their structure. Among the common characteristics of anticonvulsant agents, the highest therapeutic activity is generally seen in agents with relatively complex hydrophobic surface,[11] for example, highly branched carbon atom is found in hydantoins, amides, imides etc. This implies that the hydrophobic interaction of agents with macromolecules of biomembranes may be responsible for their in vivo actions. It has been emphasized[12] that the fundamental molecular mechanisms of action of many CNS depressants can be identical, even though, qualitatively actions differ. This indicates that surface activity and possibly the phenomenon of liquid membrane formation may play an important role in the mechanism of action of antiepileptic drugs just like it does in the case of haloperidol,[13] reserpine,[14] chlorpromazine[15] and diazepam[16].

Antiarrhythmic Drugs:

Antiarrhythmic drugs are known to contain both hydrophilic and hydrophobic moieties in their structure[17] and hence are expected to be surface active in nature. These drugs are observed to cause increase in membrane surface pressure[17] and stabilisation of membranes.[17] The penetration of these drugs in lipid mono and bilayer membranes is documented[18,19] which also is indicative of the possible role of surface activity in the mechanism of their action.

Thus it appears from the survey of literature, that the drugs chosen for the present study are likely to be surface active in nature and hence capable of generating liquid membranes at the interface as per Kosting's hypothesis.[20] The investigations carried out with a view to exploring the role of the liquid membranes generated by these drugs in their action and analyses of the data so obtained in the light of the "Liquid Membrane Hypothesis for Drug Action" summarised in previous chapter are contained in the subsequent chapters - Chapter III and IV. The next chapter, Chapter III, contains an account of the experiments done.

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CHAPTER - III

EXPERIMENTAL

EXPERIMENTAL

This chapter contains an account of the experiments carried out to demonstrate the formation of liquid membranes by the drugs in series with a supporting membrane and to obtain data on the transport of relevant permeants in the presence of the drug liquid membranes thus generated.

Materials:

The drugs and other chemicals used in the present studies are listed in the Table 1.

Preparation of Solutions:

Aqueous solutions of all drugs presently studied except diphenylhydantoin and carbamazepine could be easily prepared. In the case of diphenylhydantoin and carbamazepine whose solubility in water is very low, a different procedure was adopted. To prepare aqueous solutions of the above two drugs, the necessary volume of stock solution of known strength, prepared in acetone, was added to aqueous phase with constant stirring. The final concentration of acetone in the aqueous solutions thus prepared never exceeded 0.025% v/v and

it was shown by control experiments that a 0.025% solution of acetone in water does not lower the surface tension of water to any measurable extent. Aqueous solutions were prepared with triple distilled water, distilled in an all pyrex glass still.

Methods:

Critical Micelle Concentration (CMC) Determination:

The critical micelle concentration (C.M.C) 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 C.M.Cs of the various drugs thus determined are recorded in Tables 4, 6, 8 and 10 in Chapter IV.

Hydraulic Permeability Measurements:

The hydraulic permeability data in presence of various concentrations of drugs, which were exploited to demonstrate existence of the liquid membrane in series with the supporting membrane, were obtained using the all glass transport cell diagrammed in Fig. 1. The diagram of the transport cell (Fig. 1) has been well labelled to make it self explanatory.

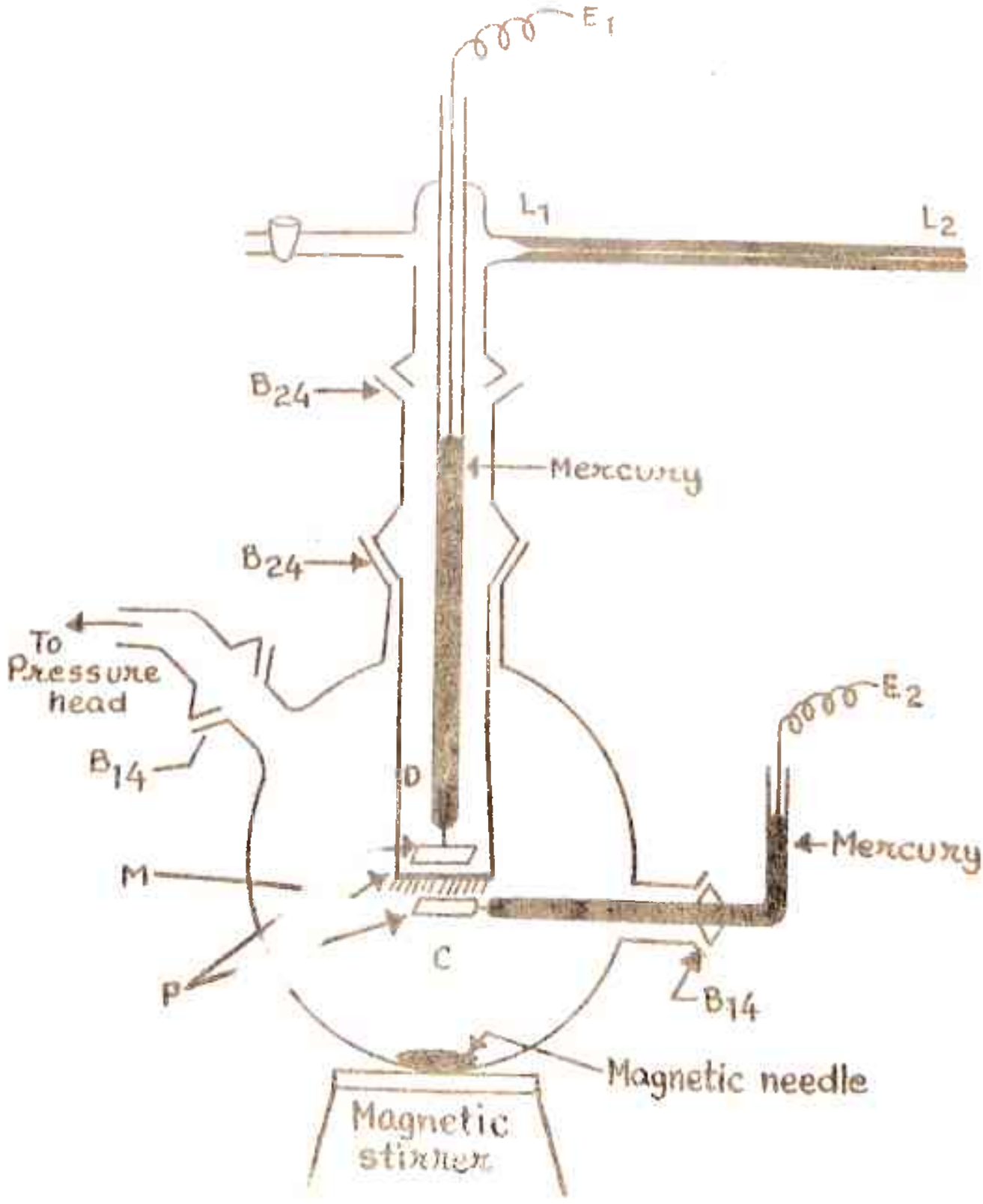

Fig.1 The all-glass transport cell M = supporting membrane (cellulose acetate microfiltration membrane Cat.No.11107 of thickness 1 x 10^{-4}m and area 5.373 x 10^{-5}m^2). P = bright platinum electrodes; E_1 E_2 = electrode terminals; L_1 L_2 = capillary of length 17 cm and diameter 1.16 x 10^{-1}cm.

It essentially consists of two compartments C and D separated by a cellulosic (cellulose acetate/ cellulose nitrate) microfiltration membrane (Sartorius Cat. No. 11107 or 11307, pore size 0.2 μm) of thickness 1×10^{-4}m and area 5.373×10^{-5}m^2 which, in fact, 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_1 L_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_1 L_2 using a cathetometer reading upto 0.001 cm and a stopwatch 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 upto 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_1 L_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 in the capillary was plotted 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, the 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 potentials 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

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

where r and R are radii of the capillary L_1 L_2 and the membrane, M (Fig. 1) respectively and 'l' is the distance travelled by the liquid miniscus in the capillary L_1 L_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.

Solute Permeability Measurements:

For the measurement of solute permeability (ω) of relevant permeants in presence of the liquid membrane generated by the drugs, two sets of experiments were performed. In the first set of experiments, a mixture of aqueous solutions of the relevant permeant and the drug under investigation was filled in compartment C and the compartment D (Fig. 1) was filled with distilled water. In the second set of experiments, the aqueous solution of the relevant permeant was kept in compartment C and compartment D was filled with the aqueous solution of the drug under investigation. In control experiments, however, no drug was used.

The values of solute permeability (ω) in presence of the liquid membranes generated by the various drugs were measured using the definition[1,2]

$$\left(\frac{J_s}{\Delta\pi}\right)_{J_v=0} = \omega \qquad (2)$$

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_1 L_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 2. The value of $\Delta\pi$ used in the calculation of ω was the average of the values of $\Delta\pi$ at beginning ($t = 0$) and at the end of the experiment.

For the solute permeability (ω) measurements, the concentration of the drugs taken were always higher than their CABs. This was done to make sure that the supporting membrane was completely covered with the liquid membrane generated by the drugs - according to Kesting's hypothesis,[3] the supporting

membrane gets completely covered with the liquid membrane when concentration of the surfactant equals or exceeds critical micelle concentration. The initial concentration of the permeants in the solute permeability (ω) experiments was taken to be as far as possible comparable with their in vivo concentrations.

All measurements - solute permeability, hydraulic permeability and the measurement of C_{ss} - were carried out at constant temperature using a thermostat set at $37 \pm 0.1^{\circ}C$.

Estimations:

Histamine:

The amount of histamine transported to compartment D was estimated by measuring fluorimetrically the fluorophor derivative from its reaction with o-phthaldehyde[4,5] A Photovolt fluorescence meter model 540 was used for the estimations.

γ-aminobutyric Acid (GABA):

The amount of GABA transported was estimated by spectrophotometric determination of its reaction product with ninhydrin[6] at 570 nm. The antiepileptic

drugs were found not to interfere with the estimation of GABA. A Spectronic 20 Bausch and Lomb Spectrophotometer was used for estimations.

Cations (Na^+):

The amount of sodium (Na^+) transported was estimated using flame photometer. Elico (India) Ltd., Model CL 22 was used for estimation.

The results obtained and the discussions thereof are contained in the next chapter - Chapter IV.

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CHAPTER - IV

RESULTS AND DISCUSSION

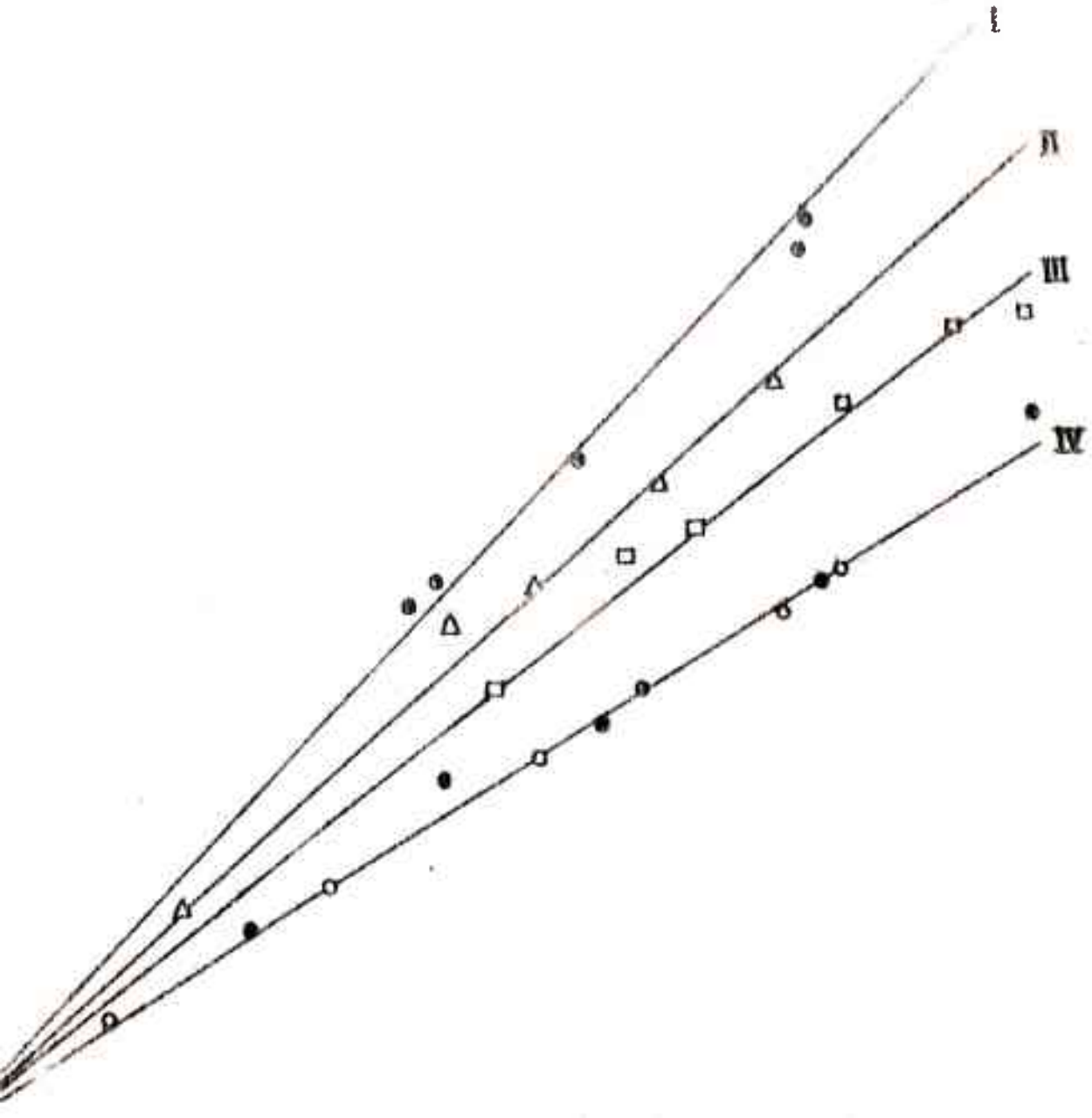
RESULTS AND DISCUSSION

The Liquid Membrane Formation:

The hydraulic permeability data in case of all the drugs were found to obey the linear relationship

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

where J_v represents the volume flux per unit area of the membrane, ΔP is the applied pressure difference and L_p is the hydraulic conductivity coefficient. The typical J_v vs. ΔP plots in a few particular cases - in case of cimetidine, valproate sodium and quinidine hydrochloride are shown in Figs. 1, 2 and 3 respectively. A perusal of the J_v vs. ΔP plots (Figs. 1, 2 and 3) in case of cimetidine, valproate sodium, and quinidine hydrochloride reveals that the value of L_p which measures reciprocal of resistance to volume flow decreases as concentration of the drug increases. The normalised values of L_p i.e., the values of (L_p/L_p^o) where L_p^o represents the value when drug concentration was zero, are plotted against concentration of the drug in case of all drugs and are recorded in Figs.4-7. It can be seen (Fig.4)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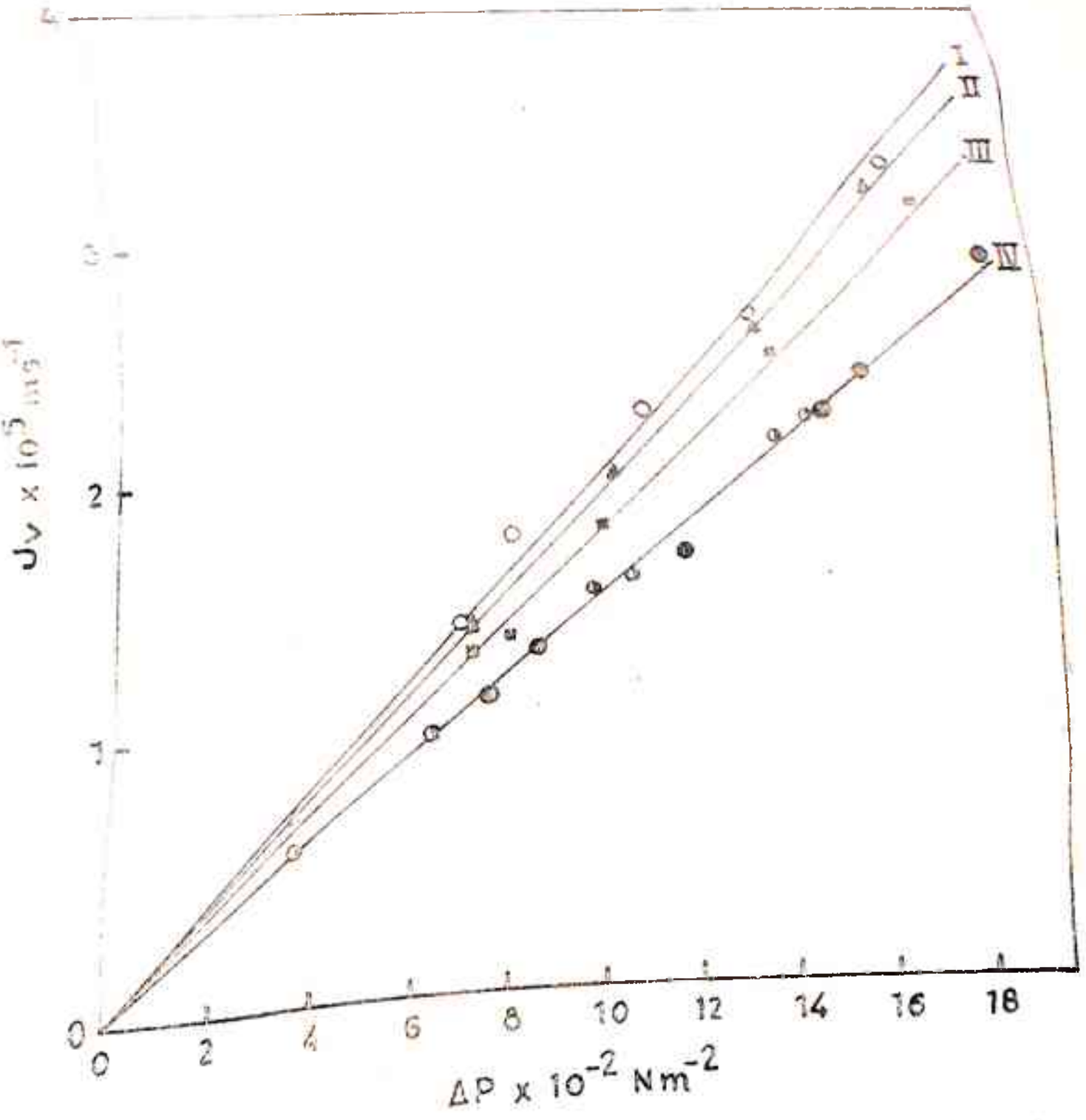
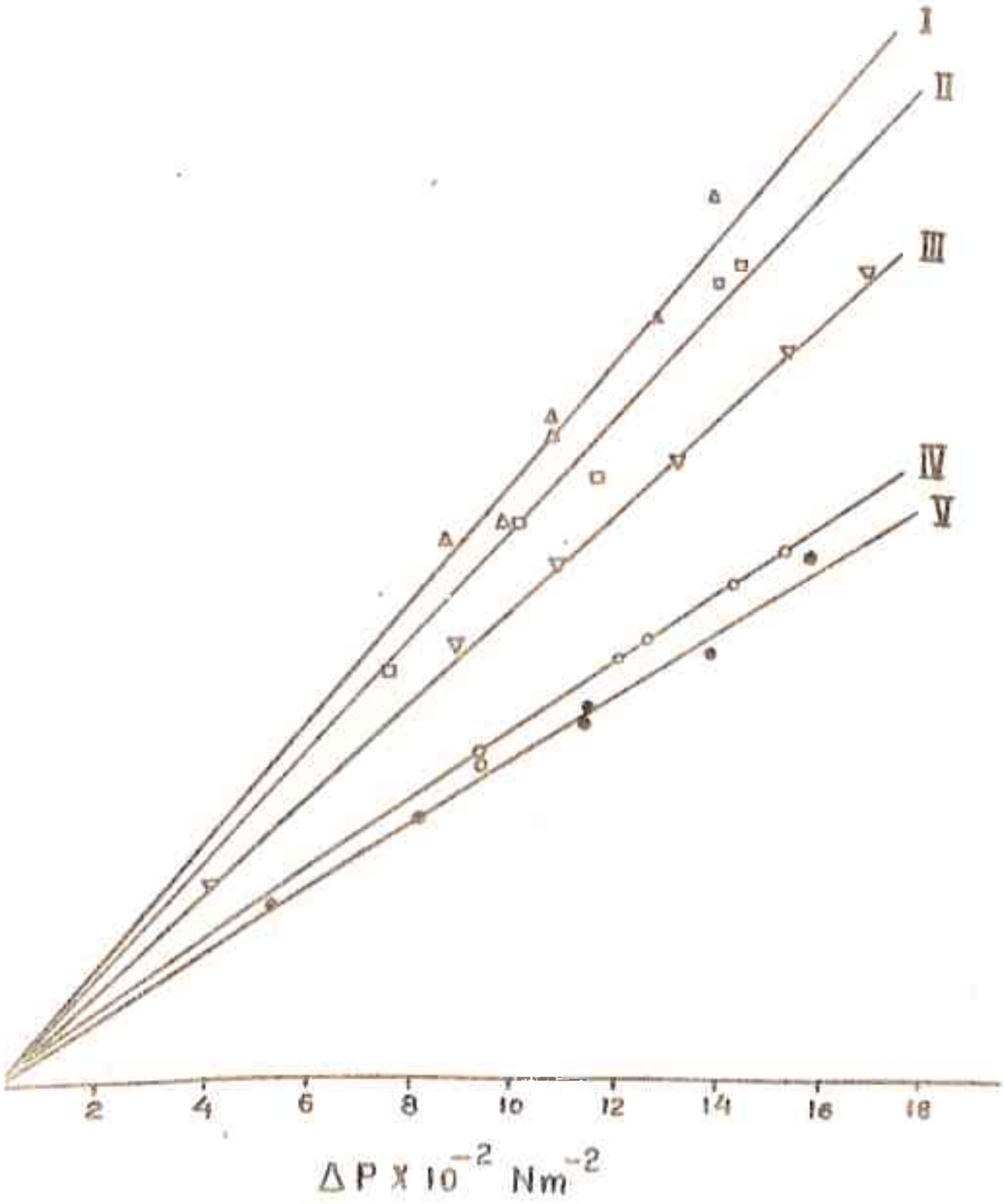
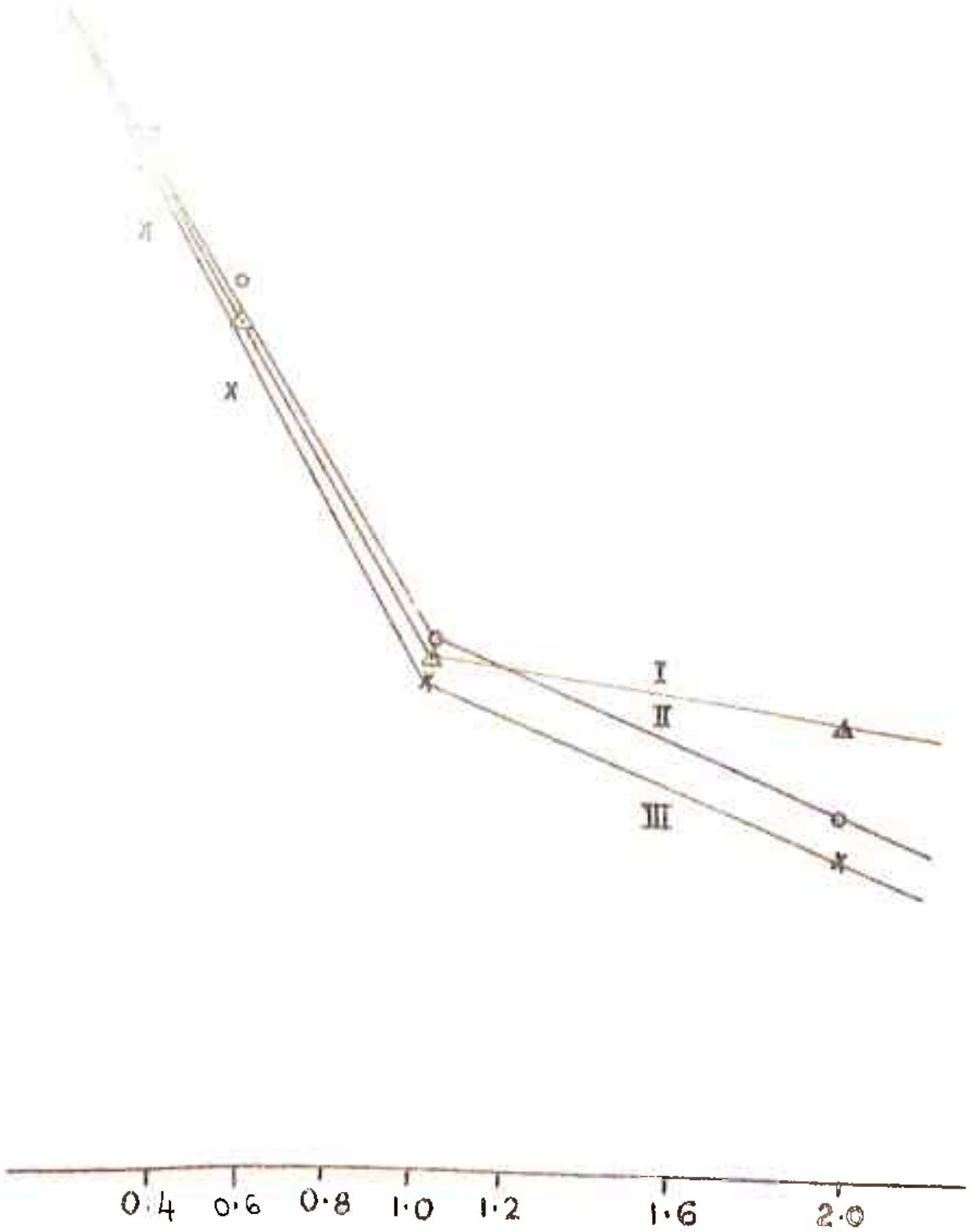


Fig.2 Hydraulic permeability data at various concentration of valproate sodium. Curves, I, II and III are for 0, 1.992×10^{-5}M and 3.984×10^{-4}M concentration of the drug, respectively. Curve IV represents data for concentrations equal to and higher than the CMC of the drug viz., 7.968×10^{-5}M and 15.936×10^{-5}M.



Hydraulic permeability data at various concentrations of quinidine hydrochloride. Curves I, II, III, IV and V are for 0, 0.989×10^{-7}M, 1.978×10^{-7}M, 3.956×10^{-7}M and 7.912×10^{-7}M concentrations of the drug.



Variation of (L_p/L_p^o) with concentration of the drugs. Curves I, II and III represent data in presence of chlorpheniramine maleate, tripelennamine hydrochloride and diphenhydramine respectively.

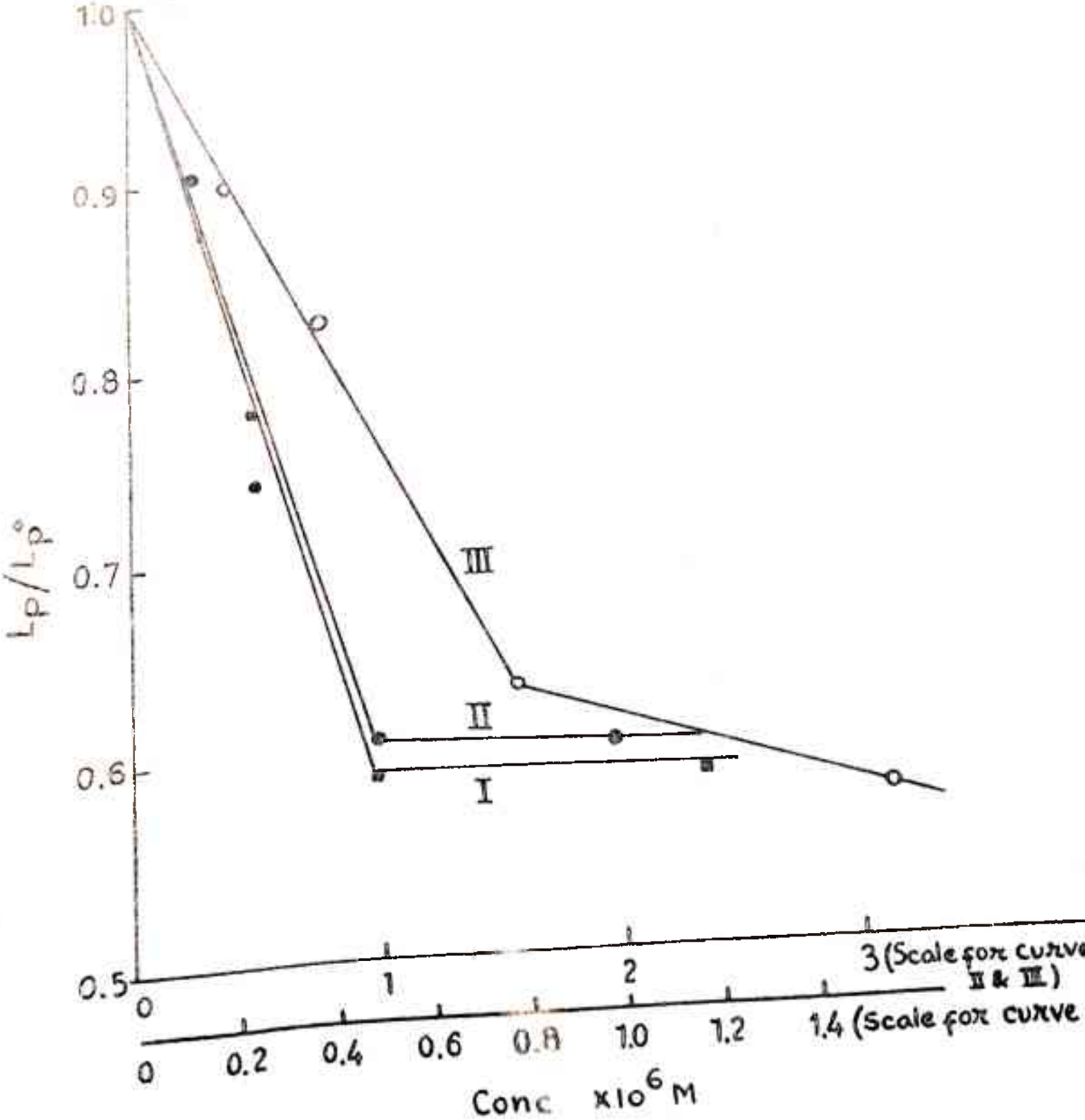
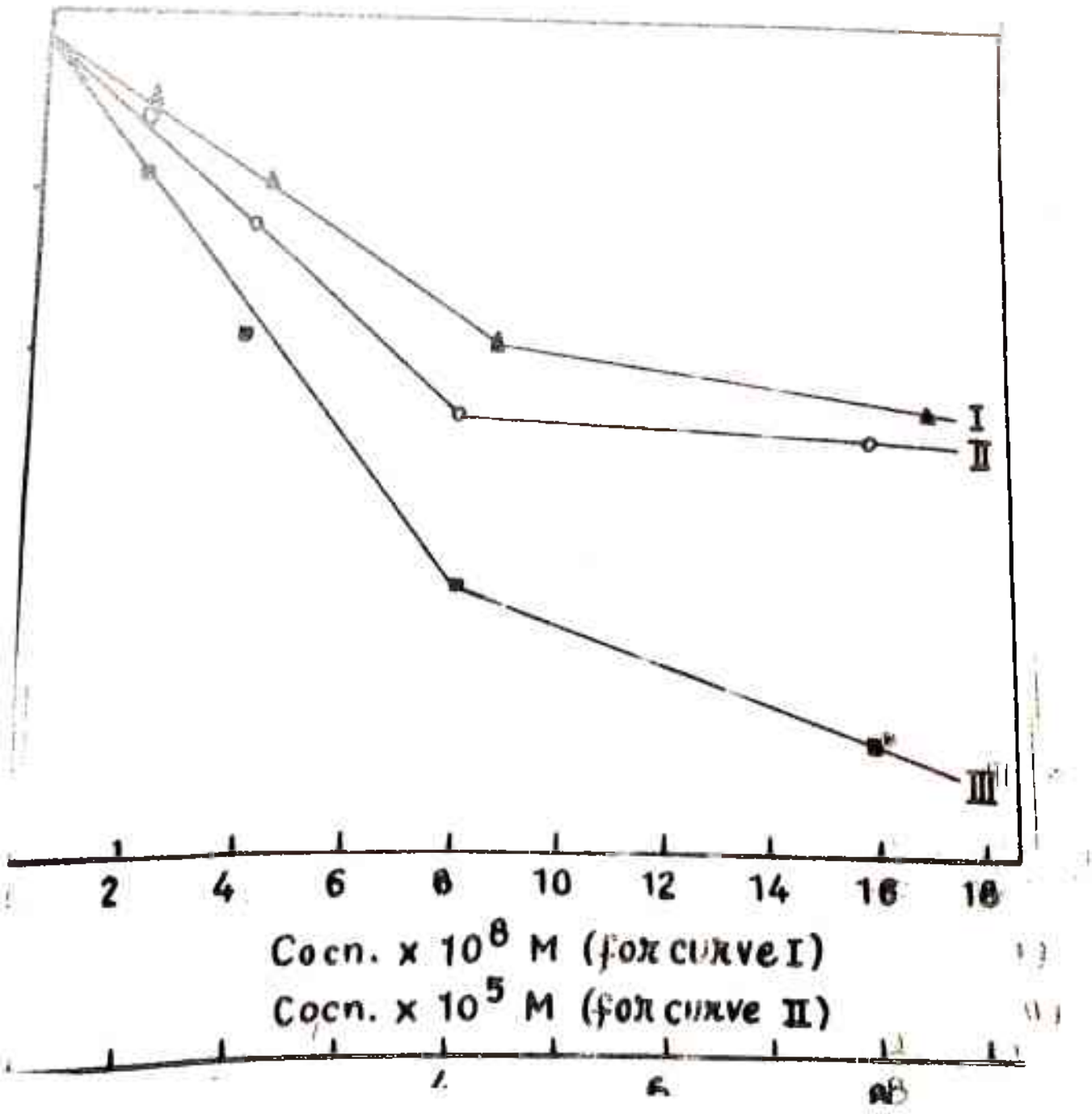


Fig.5 Variation of (L_p/L_p^0) with concentration of the drugs. Curves I, II and III represent data in presence of cimetidine, ranitidine and disodium cromoglycate, respectively.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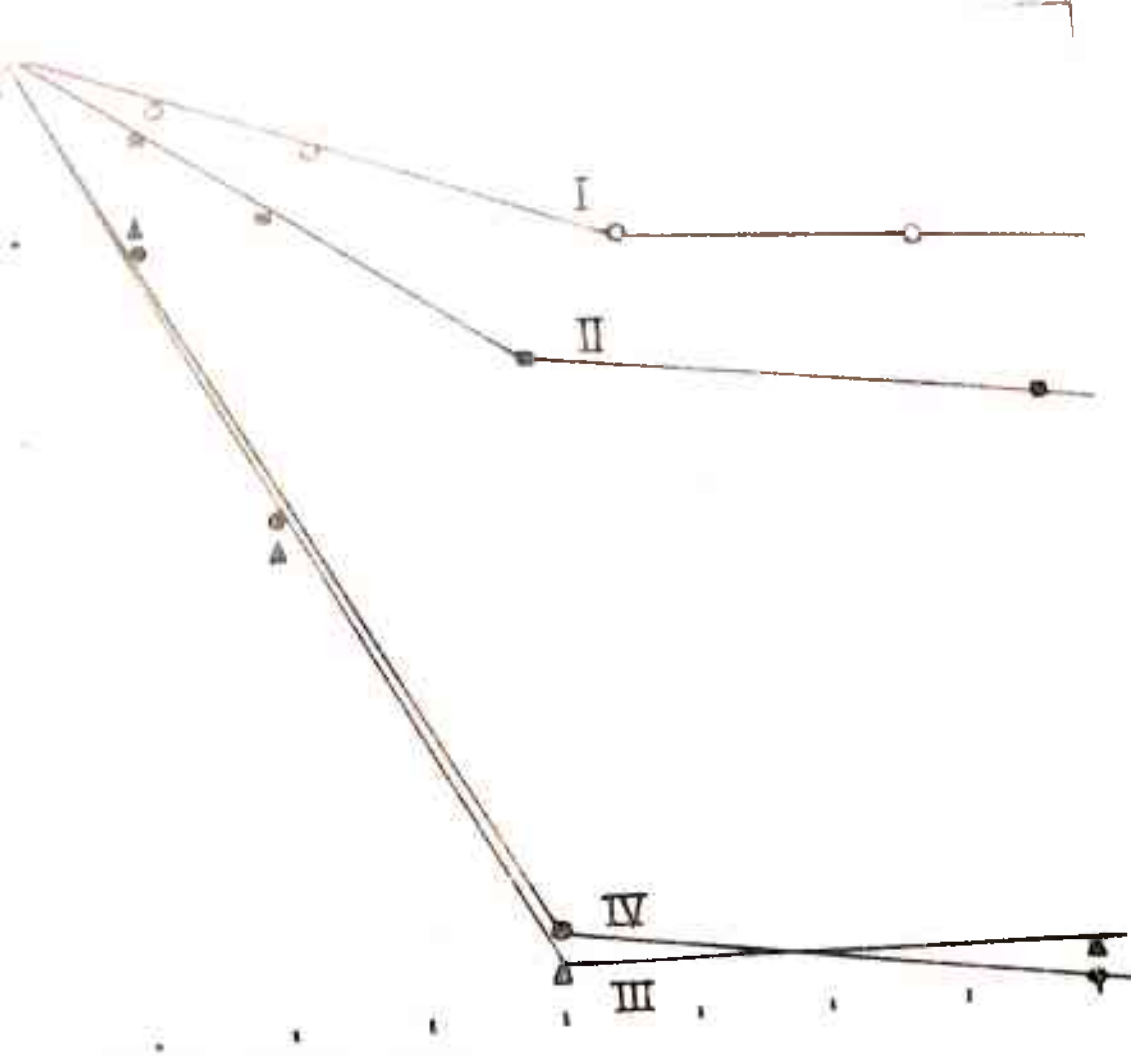
Cocn. x 10^8 M (for curve I)
Cocn. x 10^5 M (for curve II)


I
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that decrease in the value of (L_p/L_p^o) continues upto the critical micelle concentration of the drug, whereafter, it becomes more or less constant. This trend in the variation of hydraulic conductivity coefficient with concentration, which was found to be true in case of all other drugs also presently investigated (Fig. 4 for H_1-antagonists, Fig. 5 for H_2-antagonists and histamine release blocker, Fig. 6 for antiepileptic drugs and Fig. 7 for antiarrhythmic drugs) is inkeeping with Kesting's liquid membrane hypothesis[1]. According to Kesting's hypothesis, as concentration of the surfactant is increased the supporting membrane gets progressively covered with the surfactant layer liquid membrane and at the critical micelle concentration, it is completely covered. The marginal decrease in the normalised values of L_p beyond CMC as observed in a few cases (Fig. 4 for diphenhydramine and tripelennamine, Fig. 5 for sodium cromoglycate, Fig. 6 for diphenylhydantoin and carbamazepine and Fig. 7 for quinidine procainamide and propranolol), may be due to densing of the liquid membrane. Kesting himself has mentioned about such a possibility in the liquid membrane hypothesis.

Analysis of Flow Data:

Analysis of flow data in the light of mosaic membrane model[2,3,4] further supports the existence of the liquid membrane in series with the supporting membrane. Since, according to liquid membrane hypothesis,[1] at CMC, the supporting membrane is fully covered with the surfactant layer liquid membrane, at concentrations lower than the CMC, it will be partially covered. The situation is pictorially depicted in Fig. 8. The equation for volume flow for such a situation can be written as

$$J_v (A^{\wedge} + A^c) = J_v^{\wedge} A^{\wedge} + J_v^c A^c \qquad (2)$$

where A represents the area of the membrane denoted by the superscripts and the superscripts s and c represents 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 (2) can be transformed into

$$J_v = \left[L_p^{\wedge} \left(\frac{A^{\wedge}}{A^{\wedge} + A^c} \right) + L_p^c \left(\frac{A^c}{A^{\wedge} + A^c} \right) \right] \Delta P \qquad (3)$$

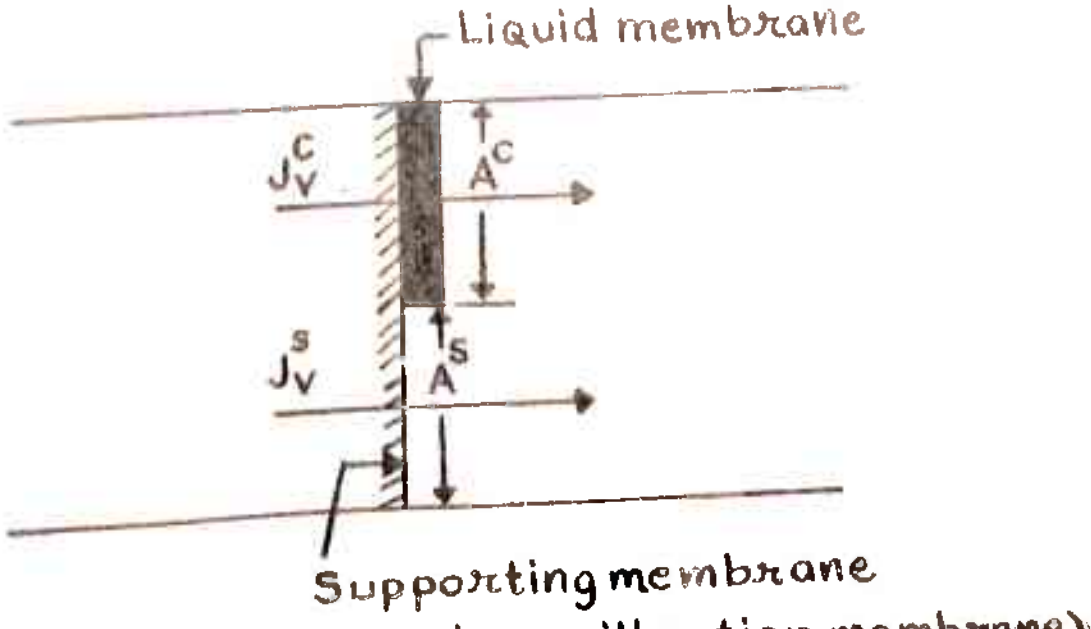

Fig.8 The schematic representation of mosaic membrane formed when the concentration of the surfactant is lower than its CMC. J_V^S, J_V^C, A^S and A^C have the same meaning as in equation 2.

TABLE 1 : Values of L_p at various concentrations of Cimetidine.

	Cimetidine concentration x 10^6 M				
	0	1.276 (0.25 mM)	2.552 (0.5 mM)	5.103	11.923
$L_p^a \times 10^8 (m^3 s^{-1} N^{-1})$	2.0209 ±0.0278	1.7637 ±0.0463	1.5681 ±0.0487	1.1832 ±0.0420	1.1801 ±0.0201
$L_p^b \times 10^8 (m^3 s^{-1} N^{-1})$	-	1.8115 ±0.0314	1.6020 ±0.0349	-	-

	Cimetidine concentration x 10^6/l				
	0	1.276 (0.25 g/l)	2.552 (0.50 g/l)	5.103	11.923
L_p^a x 10^8 ($m^3 s^{-1} N^{-1}$)	2.0209 ±0.0278	1.7637 ±0.0463	1.5681 ±0.0487	1.1832 ±0.0420	1.1801 ±0.0201
L_p^b x 10^8 ($m^3 s^{-1} N^{-1}$)	-	1.8115 ±0.0314	1.6020 ±0.0349	-	-

[a] Experimental values.

[b] Calculated values on the basis of mosaic model. Cellulose acetate microfiltration membrane was used as a supporting membrane.

Table 1 : Values of L_p at various concentrations of Cimetidine.

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	0	1.276 (0.25 GM/L)	2.552 (0.5 GM/L)	5.103	11.923
L_p^a x 10^8 ($m^3 s^{-1} N^{-1}$)	2.0209 ±0.0278	1.7637 ±0.0463	1.5681 ±0.0467	1.1632 ±0.0420	1.1801 ±0.0201
L_p^b x 10^8 ($m^3 s^{-1} N^{-1}$)	-	1.8115 ±0.0314	1.6020 ±0.0349	-	-

a Experimental values.

b Calculated values on the basis of mosaic model. Cellulose acetate microfiltration membrane was used as a supporting membrane.

	Valproate sodium concentration x 10^5 M				
	0	1.992 (0.25 C.E)	3.984 (0.5 C.E)	7.968	15.936
$L_p^a \times 10^8 (m^3 s^{-1} M^{-1})$	2.0899 ±0.0605	1.9312 ±0.0237	1.7878 ±0.0535	1.5494 ±0.0374	1.5189 ±0.0743
$L_p^b \times 10^8 (m^3 s^{-1} M^{-1})$	-	1.9098 ±0.0548	1.7897 ±0.0500	-	-

[a] Experimental values.

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

Cellulose acetate microfiltration membrane was used as a supporting membrane.

	Quinidine hydrochloride concentration x 10^7 M				
	0	0.989 (0.25 C.C)	1.978 (0.50 C.C)	3.956 (C.C)	7.912
L_p^a x 10^8 (m^3 s^{-1} N^{-1})	2.9647 ±0.0902	2.6516 ±0.0648	2.2359 ±0.0250	1.6253 ±0.0337	1.5239 ±0.0311
L_p^b x 10^8 (m^3 s^{-1} N^{-1})	-	2.6299 ±0.0761	2.2950 ±0.0680	-	-

[a] Experimental values.

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

Cellulose nitrate microfiltration membrane was used as a supporting membrane.

Functionally L_p^s and L_p^c represents the value of L_p at 'O' 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 vs. ΔP plot, in view of the equation (3) should be equal to $(L_p^s + L_p^c)/2$. Similarly, when concentration of the surface active agent is one fourth 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 vs. ΔP plots should be equal to $\left[(1 - n)\ L_p^s + n\ L_p^c\right]$. The values of L_p thus computed at various concentrations of the drugs below their CMC were found to be in good agreement with the experimentally determined values in case of all the drugs. The data in case of a few of the drugs is given in Tables 1 - 3, as a sample. This agreement between the computed values of hydraulic conductivity coefficient, L_p, using mosaic model, and the experimentally determined values, furnishes

additional evidence in favour of the formation of liquid membrane by the drugs in series with the supporting membrane. Thus, the present data (Figs. 4 to 7 and Tables 1 - 3) amply indicate that all the drugs investigated in the present study do generate a liquid membrane in series with the supporting membrane in accordance with Kesting's hypothesis.[1]

The Data on Solute Permeability (ω):

For evaluating the values of 'ω' for relevant permeants in presence of the liquid membranes generated

additional evidence in favour of the formation of liquid membrane by the drugs in series with the supporting membrane. Thus, the present data (Figs. 4 to 7 and Tables 1 - 3) amply indicate that all the drugs investigated in the present study do generate a liquid membrane in series with the supporting membrane in accordance with Kesting's hypothesis.[1]

The Data on Solute Permeability (ω):

For evaluating the values of 'ω' for relevant permeants in presence of the liquid membranes generated by the drugs, two sets of experiments have been performed. The details of the procedure are recorded in Chapter III. In the first set of experiments, aqueous solution of the drugs and the permeants were taken in the same compartment - compartment C of the transport cell (Fig. 1, Chapter III) and the compartment D was filled with distilled water. In the second set of experiments, aqueous solutions of the drugs was taken in compartment D of the transport cell (Fig. 1, Chapter III) and the aqueous solution of the permeant, was taken in compartment C. Since, according to Kesting's hypothesis,[1] complete liquid membranes are generated at the interface when concentration of the surfactant

equals or exceeds its CMC, the concentration of the drugs taken in the solute permeability (ω) experiments were always higher than their CMC, to make sure that the supporting membrane is completely covered with the liquid membrane generated by the drug. The data on the solute permeability (ω) for relevant permeants, obtained from both the sets of experiments, for the various drugs investigated are recorded in Tables 5, 7, 9 and 11 along with the values obtained from a control experiment, where no drug was used.

Since, all the drugs investigated are surface active in nature, they have both hydrophilic and hydrophobic moieties in their structure. Hence the orientation of the drug molecules in the liquid membranes with respect to approaching permeant would be different in the two sets of experiments for 'ω' measurements. In view of the surface active nature of the drugs, it is logical to expect that the hydrophobic parts of the drug molecules would be preferentially oriented towards the hydrophobic supporting membrane and the hydrophilic parts would be drawn outwards away from it. Thus in the first set of experiments for ω measurements where the

drug and the permeants are in the same compartment, the approaching permeants would face the hydrophilic surface of the drug liquid membrane whereas in the second set of experiments, the permeants would face the hydrophobic surface of the liquid membrane generated by the drug. The gross picture of the orientation of drug liquid membrane vis-a-vis permeants in the two sets of experiments for ω measurements is depicted in Fig. 9.

In the present studies a non-living/non-specific supporting membrane e.g., cellulose acetate/nitrate microfiltration membrane has been deliberately chosen so that specific/active interaction of the drug with the constituents of biomembranes as a cause for the modification in the permeability of relevant permeants is totally ruled out and the role of passive transport through the drug liquid membranes in the mechanism of their action is highlighted. Further, choosing structurally dissimilar drugs within the same pharmacological category makes the role of passive transport through the drug liquid membranes in the mechanism of their action conspicuous.

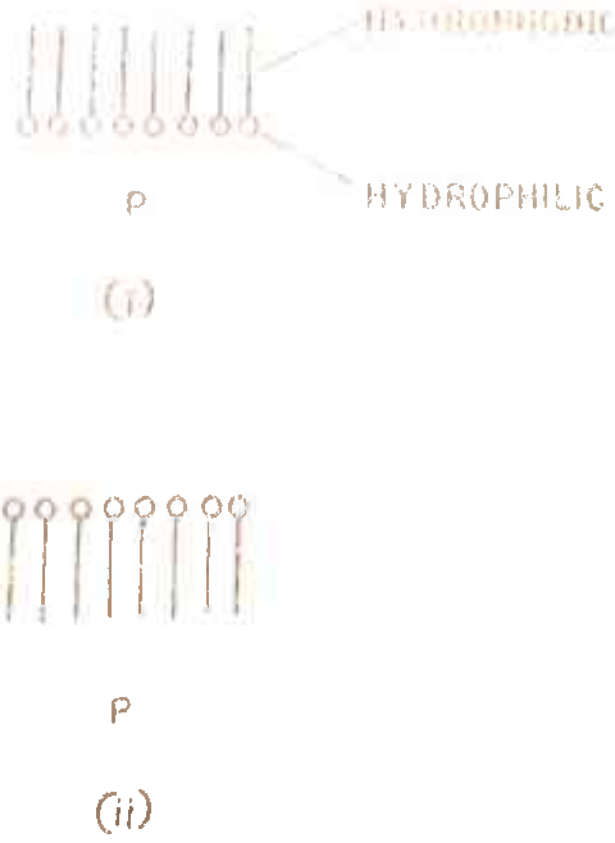

FIG.9. THE GROSS PICTURE OF THE ORIENTATION OF THE DRUG LIQUID MEMBRANE ON THE SUPPORTING MEMBRANE VIS-A-VIS PERMEANTS (P) IN THE TWO SETS OF EXPERIMENTS FOR SOLUTE PERMEABILITY MEASUREMENT — (i) DRUG AND PERMEANT IN THE SAME COMPARTMENT, (ii) DRUG AND PERMEANT IN DIFFERENT COMPARTMENTS.

A discussion of the data highlighting the role of the phenomena of liquid membrane formation in the mechanism of action of the various categories of drugs investigated in the present study is presented below.

ROLE OF LIQUID MEMBRANE PHENOMENA

Antihistamines:

H_1-antagonists:

In this section a discussion of the data on three structurally dissimilar antihistamines (H_1-antagonists), namely, chlorpheniramine maleate, diphenhydramine hydrochloride and tripelennamine hydrochloride is presented to throw light on the role of liquid membrane phenomena in their action. Surface activity of antihistamines is documented in literature.[5]

Literature values of CMCs of antihistamines are also recorded in Table 4 along with the values determined in the present investigation. In case of diphenhydramine hydrochloride, it has been concluded[6] that the drug shows aggregation beyond 0.05M concentration. Hence, the CMC should be above this concentration. The CMC of chlorpheniramine maleate is not documented in the literature. The CMC values determined in the

TABLE 4 : Critical micelle concentration data of antihistamines in aqueous solutions.

	C.C		
	mol.l^{-1}	mol.kg^{-1}	mol.l^{-1}
Chlorphoniramine maleate	$(1 \times 10^{-4})^{*}$	-	-
Diphonhydramine hydrochloride	$(1 \times 10^{-3})^{*}$	0.122**	$\sim$ 0.05***
Tripelennamine hydrochloride	$(1 \times 10^{-3})^{*}$	$\sim$ 0.20**	-

* Present investigation, values are at 37°C.

** Ref. 5, values are at 30°C.

*** Ref. 6, values are at 25°C.

present investigation, though lower than the literature values, were found to be consistent with the hydraulic permeability data (Fig. 4). The resistance to volume flux in the presence of drugs increased with increasing concentration of the drugs upto the C.M.C values - presently determined, beyond which it became more or less constant. This implies that these are the concentrations at which a complete liquid membrane is generated in series with the supporting membrane, in accordance with the liquid membrane hypothesis.[1]

Antihistamines are known[7] to occupy H_1-histamine receptors causing exclusion of histamine from its receptor site. The action is known to be competitive and reversible.[8] The antagonism is considered entirely on account of the specific interaction of antihistamines with the receptors. The present data, however, indicate that the liquid membranes generated by the drugs also contribute to the antihistaminic action.

The data on the solute permeability (ω) of histamine in the presence of the antihistaminic drugs are recorded in Table 5. The values are expressed

TABLE 5 : Permeability of histamine[b] (ω) in presence of Anti-histamines[a].

Drugs	$\omega_1 \times 10^{10}$ (mol.s^{-1}N^{-1})	$\omega_2 \times 10^{10}$ (mol.s^{-1}N^{-1})	$\omega_3 \times 10^{10}$ (mol.s^{-1}N^{-1})
Chlorpheniramine maleate	5.1855 ±0.6379	3.0620 ±0.4604	3.3460 ±0.3398
Diphenhydramine hydrochloride	5.1855 ±0.6379	3.1063 ±0.3251	1.5250 ±0.1420
Tripelennamine hydrochloride	5.1855 ±0.6379	3.1579 ±0.2691	2.6607 ±0.3145

ω_1 = Control value - when no drug was used.

ω_2 = Drug and histamine in the compartment C and water in the compartment D.

ω_3 = Drug in the compartment D and histamine in the compartment C.

a The concentrations of chlorpheniramine maleate, diphenhydramine hydrochloride, tripelennamine hydrochloride were : 2×10^{-4}M, 2×10^{-3}M and 2×10^{-3}M, respectively.

b Initial concentration of histamine 10 µg/ml.

Cellulose acetate microfiltration membrane was used as supporting membrane.

as arithmetic mean ± standard deviation - based on the 15 repeats for each value of ω. The differences between various ω-values in Table 5 were found to be statistically significant. The data in Table 5 clearly indicate that the liquid membranes generated by H_1-antagonists themselves impede the transport of histamine to a notable extent. Chlorpheniramine maleate is known to be most potent[8] amongst all three H_1-antagonists studied. This is consistent with the observation that the CMC of chlorpheniramine maleate is the lowest (Table 4) implying that it forms a complete liquid membrane at a much lesser concentration than the other two drugs. This prima facie indicates that the liquid membrane generated by the antihistamines at the site of action may play a role in the mechanism of their action.

Chlorpheniramine maleate which is known to be most potent of all the three drugs[8] presently studied, impedes the transport of histamine more or less to the same extent in both the orientations - when the permeant faces the hydrophilic or the hydrophobic surface of the drug liquid membrane. The rest of the drugs, however, impede the transport of histamine more when the drug liquid membranes present their hydrophobic surface to

the permeant than when the permeant faces the hydrophilic surface of the drug liquid membranes. Since, in the histamine receptors, existence of both hydrophilic and hydrophobic sites has been indicated,[9] it appears that chlorpheniramine maleate gets attached to both hydrophilic and hydrophobic sites in the formation of liquid membrane, while the other two drugs get attached only to the hydrophilic sites. According to Laud[10] 'potency may imply selectivity'. In other words, the more potent the drug is, the more selective it may be to the receptor. Thus the tendency of chlorpheniramine maleate to attach both hydrophilic and hydrophobic sites implies its selectivity to histamine receptors, which is in keeping with Laud's statement.[10]

It is interesting to note that structure-activity studies of H_1-antagonists have exhibited a relationship with partition characteristics[11,12] and association phenomena[13,14] both of which are related to surface activity.

Although the reduction in the permeability of histamine on account of liquid membranes generated by the antihistamines is passive in nature, it is

likely to be accompanied by a consequent reduction in the active processes as well. This is because the presence of the liquid membrane generated by antihistamines is likely to reduce the access of histamine to its receptors.

The multiple effects[7] associated with H_1-antagonists viz., anticholinergic effects, local anaesthetic effects or sedation may also be explained by modification in the transport of relevant permeants. The liquid membrane generated by H_1-antagonists may offer a varying degree of resistance to the transport of relevant permeants. Further investigations are, however, called for to assess validity of the proposition.

Thus there is enough circumstantial evidence to indicate that the liquid membranes generated by the antihistamines at the site of action may contribute to the mechanism of their action.

H_2-antagonists:

This section contains a discussion of the data on cimetidine and ranitidine in the light of the liquid membrane hypothesis for drug action.

TABLE 6 : Critical micelle concentration data of H_2-antagonists and histamine release blocker.

Drug	CMC
Cimetidine	5.1024×10^{-6} M
Ranitidine	1.0188×10^{-6} M
Cromoglycate disodium	1.5925×10^{-6} M

TABLE 7 : Solute permeability (ω)[a] of Histamine[b] in presence of drugs[c].

Drugs	$\omega_1 \times 10^{10}$ (mol.s^{-1}N^{-1})	$\omega_2 \times 10^{10}$ (mol.s^{-1}N^{-1})	$\omega_3 \times 10^{10}$ (mol.s^{-1}N^{-1})
Cimetidine	5.1855 ±0.6379	3.0379 ±0.3531	3.6516 ±0.2716
Ranitidine	5.1855 ±0.6379	1.6630 ±0.3205	2.6741 ±0.4347
Cromoglycate disodium	5.1855 ±0.6379	1.3139 ±0.1952	2.4207 ±0.2631

ω_1 = Control value - when no drug was used.

ω_2 = Drug and histamine in compartment C and water in compartment D.

ω_3 = Drug in compartment D and histamine in compartment C.

a The values of ω expressed as arithmetic mean ± standard deviation.

b Initial concentration of histamine 10 µg/ml.

c The concentrations of cimetidine, ranitidine and cromoglycate disodium were 2.0410×10^{-5}M, 4.0756×10^{-6}M and 6.3700×10^{-6}M respectively.

Cellulose acetate microfiltration membrane was used as supporting membrane.

Both cimetidine and ranitidine are known[15] to be H_2-antagonists. The data on histamine permeability (ω) in presence of these drugs (Table 7) indicate that the liquid membranes which are likely to be formed at the site of action of the respective drugs may contribute to their biological action. A perusal of Table 7 reveals that the permeability of histamine is reduced to a greater extent in the first set of experiments in which the permeant histamine [illegible]

Ranitidine is known to be a more potent H_2-antagonist than cimetidine.[15] This fact can be rationalised on the basis of CMC values (Table 6) of the two drugs. Since, the CMC of ranitidine is less than that of cimetidine, the former would form the complete liquid membrane offering maximum resistance to the transport of histamine at a lower concentration than cimetidine would require, thus, making

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Histamine Release Blocker:

In case of disodium cromoglycate, a histamine release blocker,[17] the transport of histamine is impeded most when the drug liquid membrane presents its hydrophilic surface to the permeant (Table 7). It appears, therefore, that a similar orientation of the liquid membrane with hydrophilic moieties of disodium cromoglycate molecules facing histamine molecules may be necessary even on mast cells. However, more information on the nature and orientation of the actual site of action on mast cells is called for.

Antiepileptic Drugs:

Antiepileptic drugs are known to stabilise biological membranes[18] after interacting with them. They are known to contain both hydrophilic and hydrophobic moieties in their structure.[19] The antiepileptic drugs, therefore, are expected to be surface active in nature and hence capable of generating liquid membrane at the interface in accordance with Kesting's hypothesis.[1] All the three antiepileptic drugs, namely, diphenylhydantoin, carbamazepine and sodium valproate presently studied have, in fact, been shown to generate liquid membrane in series with the supporting membrane (see Fig. 6 on page 52). Depressant drugs in general are known to populate at the air-solution interface.[20] Since antiepileptic action is determined by the concentration of γ-aminobutyric acid (GABA) in brain, data on the modification of permeability of GABA in presence of the liquid membrane generated by the antiepileptic drugs have been obtained and are recorded in Table 9.

A perusal of Table 9 reveals that in the first set of experiments, where the permeant, GABA, faces the hydrophilic surface of the drug liquid membrane,

TABLE 8 : Critical micelle concentration (CMC) of Antiepileptic drugs.

Drugs	CMC
Diphenylhydantoin	4.00×10^{-4} M
Carbamazepine	8.56×10^{-8} M
Valproate sodium	7.97×10^{-5} M

TABLE 9 : Solute permeability of GABA[a] (ω)* in presence of antiepileptic drugs[b].

Drugs	$\omega_1 \times 10^{10}$ (mol.s^{-1}N^{-1})	$\omega_2 \times 10^{10}$ (mol.s^{-1}N^{-1})	$\omega_3 \times 10^{10}$ (mol.s^{-1}N^{-1})
Diphenylhydantoin	1.1682 ±0.1532	6.8487 ±0.6815	1.1501 ±0.1078
Carbamazepine	1.1682 ±0.1532	4.8175 ±0.4494	1.0680 ±0.1545
Valproate sodium	1.1682 ±0.1532	2.0039 ±0.3782	1.6624 ±0.1534

ω_1 = Control value - when no drug was used.

ω_2 = Drug and GABA in compartment C and water in compartment D.

ω_3 = Drug in compartment D and GABA in compartment C.

a Initial concentration of GABA is 200 µg/ml.

b The concentrations of diphenylhydantoin, carbamazepine and valproate sodium are 8×10^{-7}M, 1.6×10^{-7}M and 1.6×10^{-4}M, respectively.

* Values of ω are reported as arithmetic mean of 10 repeats ± standard deviation.

Cellulose acetate microfiltration membrane was used as supporting membrane.

the permeability of GABA is enhanced considerably in case of all the three drugs. In the second set of experiments, however, there is a distinct reduction in the permeability of GABA, except in case of sodium valproate, where a marginal increase in the permeability is observed (Table 9). Even in the case of sodium valproate, the increase in the permeability of GABA is much more in the first set of experiments than in the second set. The present observations on the increase in the permeability of GABA appear relevant to the antiepileptic action.

The antiepileptic drugs, which, when administered, exert stabilising effect[18] on excitable cell membranes, are known to increase the concentration of GABA in brain. The present experiments appear to indicate that increased permeability of GABA in the presence of the drug liquid membranes which are likely to be formed at the site of action, may be responsible for the increased concentration of GABA in brain. Since enhancement in the permeability of GABA was observed to be maximum in the first set of experiments, it appears that the specific orientation of the drug molecules in the liquid membrane,

with their hydrophilic ends facing the permeant may be necessary even at the actual site of action. To substantiate this conjecture, detailed investigations on the nature of the site of action are called for.

Another indication of the possible role of the liquid membrane phenomenon in antiepileptic action is obtained from the gradation in values of the CMCs of the drugs (Table 8) vis-a-vis the gradation in the concentrations of these drugs in plasma. The CMC values of the three drugs are in the following order

Valproate > diphenylhydantoin > carbamazepine

which also is the gradation in their concentrations in plasma.[21] Concentrations of the drugs in plasma can be taken to be a measure of their concentrations at the site of action. The reported[21] concentration of these drugs are far higher than their CMCs. Hence complete liquid membranes can be generated by the drugs at the site of action. Since modification in the permeability of GABA due to the presence of the drug liquid membranes is responsible for the antiepileptic action, the concentrations of the drugs required to produce maximum biological

response may be related to their CMCs. CMC is the concentration at which the interface is completely covered by the liquid membrane and, therefore, modification in the permeability of biomembranes to GABA will be maximum at this concentration. Hence, agreement between the gradation in the concentration of the drugs in plasma and the gradation in their CMCs is also indicative of the contribution of the liquid membrane generated by these drugs to their antiepileptic action.

Thus, the present study indicates that the formation of liquid membrane at the site of action, by these drugs, modifying the transport of GABA, may be an important step common to the mechanism of action of all the three drugs namely diphenylhydantoin, carbamazepine and valproate sodium. The fact that the three antiepileptic drugs chosen for the present

active in nature. These drugs are known to cause increase in membrane surface pressure[22] and stabilisation of membranes.[22]

The antiarrhythmic action is known to be[23] mainly on account of modification in the permeability of biomembranes to sodium ions. Since antiarrhythmic drugs are expected to be surface active in nature and hence capable of generating liquid membranes at the interface,[1] it is logical to suspect that modification in the permeability of sodium ions may be on account of the liquid membranes generated by them at the respective sites of action. It is of interest to mention that many local anesthetics also show antiarrhythmic action.[24] Since, in local anesthetics, the liquid membranes generated by them have been shown to contribute to the modification in cation permeability,[25] one is encouraged to conjecture that the phenomenon of liquid membrane formation may also be important in the mode of action of antiarrhythmic drugs.

Prompted by this, four structurally dissimilar antiarrhythmic drugs viz., quinidine hydrochloride, disopyramide phosphate, procainamide hydrochloride and propranolol hydrochloride were chosen for investigation. Each of these drugs have been shown to

response may be related to their CMCs. CMC is the concentration at which the interface is completely covered by the liquid membrane and, therefore, modification in the permeability of biomembranes to GABA will be maximum at this concentration. Hence, agreement between the gradation in the concentration of the drugs in plasma and the gradation in their CMCs is also indicative of the contribution of the liquid membrane generated by these drugs to their antiepileptic action.

Thus, the present study indicates that the formation of liquid membrane at the site of action, by these drugs, modifying the transport of GABA, may be an important step common to the mechanism of action of all the three drugs namely diphenylhydantoin, carbamazepine and valproate sodium. The fact that the three antiepileptic drugs chosen for the present study are structurally dissimilar further highlights the role of the liquid membrane phenomenon in their action.

Antiarrhythmic Drugs:

Antiarrhythmic drugs are known to contain both hydrophilic and hydrophobic moieties in their structure[22] and hence are expected to be surface

active in nature. These drugs are known to cause increase in membrane surface pressure[22] and stabilisation of membranes.[22]

The antiarrhythmic action is known to be[23] mainly on account of modification in the permeability of biomembranes to sodium ions. Since antiarrhythmic drugs are expected to be surface active in nature and hence capable of generating liquid membranes at the interface,[1] it is logical to suspect that modification in the permeability of sodium ions may be on account of the liquid membranes generated by them at the respective sites of action. It is of interest to mention that many local anesthetics also show antiarrhythmic action.[24] Since, in local anesthetics, the liquid membranes generated by them have been shown to contribute to the modification in cation permeability,[25] one is encouraged to conjecture that the phenomenon of liquid membrane formation may also be important in the mode of action of antiarrhythmic drugs.

Prompted by this, four structurally dissimilar antiarrhythmic drugs viz., quinidine hydrochloride, disopyramide phosphate, procainamide hydrochloride and propranolol hydrochloride were chosen for investigation. Each of these drugs have been shown to

TABLE 10 : Critical micelle concentration of anti-arrhythmic drugs in water.

Drugs	CMC (M)
Quinidine hydrochloride	3.96×10^{-7}
Disopyramide phosphate	4.00×10^{-7}
Procainamide hydrochloride	4.00×10^{-3}
Propranolol hydrochloride	4.75×10^{-3}

TABLE 11 : Permeability of sodium[a] (Na^+), $(\omega)^*$, in presence of Antiarrhythmic drugs[b].

Drugs	$\omega_1 \times 10^{10}$ ($mol.s^{-1}N^{-1}$)	$\omega_2 \times 10^{10}$ ($mol.s^{-1}N^{-1}$)	$\omega_3 \times 10^{10}$ ($mol.s^{-1}N^{-1}$)

generate liquid membrane in series with the supporting membrane (see Fig. 7 on page 53). The values of the critical micelle concentrations (Table 10) are consistent with the concentrations of the drugs at which the resistance offered to the volume flux is maximum indicating the complete coverage of the supporting membrane with liquid membrane (see Fig. 7 on page 53). Marginal decrease in the values of (L_p/L_p^o) beyond C.M.C, particularly in case of quinidine, procainamide and propranolol may be due to increase in density of the liquid membrane as indicated in the liquid membrane hypothesis itself.[1]

Since antiarrhythmic action is known to be mainly on account of modification in the permeability of biomembranes to sodium ions, data on the permeability of sodium ions in presence of the liquid membranes generated by the antiarrhythmic drugs have been obtained and are recorded in Table 11. A perusal of Table 11 indicates that the liquid membranes generated by the antiarrhythmic drugs, in both the orientations - hydrophilic ends facing the permeant and hydrophobic ends facing the permeant, impede the transport of sodium ions. Antiarrhythmic drugs are known to

TABLE 11 : Permeability of sodium[a] (Na^+), $(\omega)^*$, in presence of Antiarrhythmic drugs[b].

Drugs	$\omega_1 \times 10^{10}$ (mol.$s^{-1}N^{-1}$)	$\omega_2 \times 10^{10}$ (mol.$s^{-1}N^{-1}$)	$\omega_3 \times 10^{10}$ (mol.$s^{-1}N^{-1}$)
Quinidine hydrochloride	5.3255 ±0.3521	2.8934 ±0.1710	3.8757 ±0.1405
Disopyramide phosphate	5.3255 ±0.3521	3.0709 ±0.1731	3.3823 ±0.1540
Procainamide hydrochloride	5.3255 ±0.3521	3.1132 ±0.2108	3.1778 ±0.2475
Propranolol hydrochloride	5.3255 ±0.3521	2.5844 ±0.1293	2.5885 ±0.1542

ω_1 = Control value - when no drug was used.

ω_2 = Drug and sodium ion in compartment C and water in the compartment D.

ω_3 = Drug in compartment D and sodium ion in the compartment C.

a Initial concentration of sodium ion 2117.2 ppm.

* Values of ω are reported as arithmetic mean of 10 repeats ± standard deviation.

b The concentrations of quinidine hydrochloride, disopyramide phosphate, procainamide hydrochloride and propranolol hydrochloride used were 1.6×10^{-6}M, 1.6×10^{-6}M, 8.0×10^{-3}M and 7.315×10^{-5}M respectively. Cellulose nitrate microfiltration membrane was used as a supporting membrane.

stabilise cardiac membrane by a non-specific mechanism.[23] The present study indicates that the liquid membranes generated by antiarrhythmic drugs in series with the cardiac membrane impeding the transport of sodium ion may be such a mechanism. A scrutiny of Table 11 further reveals that the transport of sodium ions is impeded more or less to the same extent in both the orientations of the liquid membranes generated by the drugs which implies that both hydrophilic and hydrophobic moieties in the structure of these drugs may be necessary for antiarrhythmic action. This conjecture is consistent with the literature report[22] that non-specific antiarrhythmic agents interact with both hydrophilic and hydrophobic regions of the biomembranes. Propranolol which is primarily a β-blocker drug is also known[22] to exert a non-specific membrane stabilising action similar to that of quinidine at concentrations higher than those needed for β-blocking action. It is for this reason, that the transport of sodium ions in presence of propranolol was studied. The data on the inhibition of sodium ion transport in presence of propranolol (Table 11) is consistent with its reported antiarrhythmic action.

Concluding Remarks:

1. The present studies strongly indicate that the liquid membranes generated by the drugs at their respective sites of action modifying the transport of relevant permeants might be an important step common to the mechanism of action of all surface active drugs. This concept that liquid membrane generated by the drug itself modify access of relevant permeants to the site of action is the central idea of the "Liquid Membrane Hypothesis for Drug Action" which has been summarised in Chapter I.

2. These studies also throw light on the nature and orientation of receptors. In the solute permeability (ω) experiments, two different orientation of the drug molecules, with respect to permeating species, are possible (see Fig. 9 on page 64) - hydrophilic surface of the drug liquid membrane facing the permeant or hydrophobic surface of the drug liquid membrane facing the permeant. Whichever orientation shows modification in the permeability consistent with the observations reported on biological cell can be of predictive value. Based on this conception, predictions about the nature and orientation

of H_1- and H_2-receptors have been made which have been substantiated by literature reports (see page 74). Similarly, in the case antiarrhythmic drugs, it has been concluded that interaction of the drugs with both hydrophilic and hydrophobic sites is important for antiarrhythmic action, which is in agreement with literature reports (see page 86). In the case of antiepileptic drugs, it has been predicted that interaction of the drugs with hydrophobic sites may be important for their action.

This kind of approach, which is a consequence of the "Liquid Membrane Hypothesis for Drug Action" appears attractive for getting initial information on the nature and orientation of receptors/sites of action.

3. The present studies on the role of liquid membrane phenomena in drug action, indicate that critical micelle concentrations of a series of surface active drugs with the same pharmacological action can be a good indicator of their potency. This inference, which again is a consequence of the "Liquid Membrane Hypothesis for Drug Action", arises because critical micelle concentration of a drug

indicates the concentration at which the interface will be completely covered by the drug liquid membrane. At this concentration (C.M.C), therefore, modification in the permeability of biological membranes would be maximum. This conclusion implies that at the C.M.C, the magnitude of the biological effect would also be maximum. Hence, the lower the C.M.C of a drug, the lower the concentration required to alter the membrane transport and as a consequence, more potent would be the drug. The investigations on H_1-antagonists (see page 69), H_2-antagonists (see page 75) and antiepileptic drugs (see page 80) justify this conjecture. Chlorpheniramine maleate is known to be more potent amongst all the three antihistamines studied. This is consistent with the observation that the C.M.C of chlorpheniramine maleate is the lowest.

4. An explanation for the multiplicity of drug action can also be found in terms of the phenomenon of liquid membrane formation. For example the multiple effects associated with antihistamines, viz. anticholinorgic effects, local anesthetic effects or sedation may be due to the varying degree of resistance which the liquid membrane generated by antihistamines may offer to relevant permeants.

5. Although the data presented in this thesis is all on passive transport through the liquid membranes generated by the surface active drugs, it should not be construed that active processes are unimportant. In fact, the liquid membrane generated by the drug at the site of action modifies the access of the relevant permeants to the active site located on the membrane. Thus if the liquid membrane barrier impedes the transport of the permeant, the active process would also be impeded.

The point that is driven home through these investigations is that the passive transport through the liquid membranes that are likely to be generated at the site of action make a significant contribution to drug action. This aspect of drug action was hitherto considered unimportant.

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CHAPTER - V

SUMMARY

SUMMARY

A concise chapterwise summary is given below:

Chapter - I

This chapter gives an account of the liquid membrane hypothesis and its biological implications. Attempts made on the use of liquid membrane bilayers as model systems for biomembranes and the investigations on the role of the liquid membranes generated by the surface active drugs in the mechanism of their action have been discussed. Special attention has been focussed on the latter aspect - the liquid membrane hypothesis for drug action. The assessment of the "Liquid Membrane Hypothesis for Drug Action" vis-a-vis existing theories of drug action has been presented. Need for investigations on more and more categories of structurally dissimilar drugs to substantiate the liquid membrane hypothesis for drug action has been brought out.

Chapter - II

This chapter presents a quick survey of the relevant literature on surface activity of the drugs

chosen for the present study. An effort has been made to assess how the reports on surface activity of the drugs indicate the possibility of the liquid membranes generated by them contributing to the mechanism of their action.

Chapter - III

This chapter gives the details of the materials and methods used in the present investigation. The transport cell used for obtaining the data on hydraulic permeability and the solute permeability of relevant permeants has been described, along with the procedure adopted for obtaining the data.

Chapter - IV:

This chapter contains a discussion of the data on the hydraulic permeability and the solute permeability in the light of liquid membrane hypothesis for drug action. The following structurally dissimilar drugs belonging to different pharmacological categories have been investigated for the role of the liquid membrane phenomena in their action.

I. Antihistamines:

H_1-antagonists:

Chlorpheniramine maleate,

Diphenhydramine hydrochloride, and

Tripelennamine hydrochloride.

H_2-antagonists:

Cimetidine, and

Ranitidine.

II. Histamine Release Blocker:

Disodium cromoglycate.

III. Antiepileptic Drugs:

Diphenylhydantoin,

Carbamazepine, and

Valproate sodium.

IV. Antiarrhythmic Drugs:

Quinidine hydrochloride,

Disopyramide phosphate,

Procainamide hydrochloride, and

Propranolol hydrochloride.

The choice of the structurally dissimilar drugs is deliberate to make the role of the physical process

like formation of liquid membrane conspicuous in their action.

Each of the drugs listed above has been shown to generate a liquid membrane in series with the supporting membrane. For this, the data on hydraulic permeability in presence of varying concentrations of the drugs has been utilised. In each case, it has been found that the normalised values of the hydraulic conductivity coefficients decrease progressively with increase in concentration of the drugs upto their critical micelle concentrations (CMC). When the concentrations exceed their CMC, the values of the hydraulic conductivity coefficients becomes more or less constant. This trend is consistent with Kesting's liquid membrane hypothesis and is indicative of the fact that as concentration of the surface active drug is increased, the supporting membrane gets progressively covered with the drug liquid membrane and at CMC, it is completely covered. The hydraulic permeability data have been further analysed in the light of mosaic model to obtain additional evidence in favour of the existence of the liquid membrane in series with the supporting membrane. The values of hydraulic conductivity coefficient, L_p computed using mosaic model at various concentrations

lower than critical micelle concentration have been shown to match with the experimentally determined values in case of all the drugs. This agreement lends additional support to the existence of the liquid membrane in series with the supporting membrane.

Solute permeability (ω) data for relevant permeants in presence of the liquid membranes generated by the various drugs have been obtained for both the orientations of the drug molecules in the liquid membrane - the hydrophilic surface of the liquid membrane facing the permeants and the hydrophobic ends of the drug molecules in the liquid membrane facing the permeants. These data amply indicate that the phenomena of the liquid membrane formation makes a definite contribution to the action of these drugs. In these experiments, a non-specific, non-living membrane like cellulose acetate/nitrate microfiltration membrane has been deliberately chosen as supporting membrane for the liquid membrane so that active and specific interaction of the drug molecules with the constituents of biomembranes is totally ruled out and the role of passive transport through the liquid membranes in their action is highlighted. A summary of the significant findings in case of each drug is given below.

I. Antihistamines:

H_1-antagonists:

Of the three antihistaminic drugs studied viz., chlorpheniramine maleate, diphenhydramine hydrochloride and tripelennamine hydrochloride, chlorpheniramine maleate is known to be most potent. This is consistent with the finding that the critical micelle concentration (CMC) of chlorpheniramine maleate is the lowest which in turn is consistent with the liquid membrane hypothesis for drug action. One implication of the liquid membrane hypothesis for drug action is that lower the CMC of the drug, higher is the potency.

The values of solute permeability (ω) for histamine have revealed that chlorpheniramine maleate which is known to be the most potent of the three, gets attached to both, the hydrophilic and hydrophobic sites of the receptors while the other two drugs get attached only to hydrophilic sites making chlorpheniramine more selective to the histamine receptors than the other two drugs. This inference is in keeping with Laud's generalisation that "potency may imply selectivity".

H_2-antagonists:

Of the two H_2-antagonists namely cimetidine and ranitidine, the latter is reported to be more potent than the former. This is consistent with the fact that the C.C of ranitidine is less than that of cimetidine.

The data on the solute permeability (ω) of histamine has also indicated that the orientation of H_2-receptors should be opposite to that of H_1-receptors. This inference on the opposing orientations of the H_1- and H_2-receptors for histamine is in conformity with literature reports.

II. Histamine Release Blocker:

The drug disodium cromoglycate falls in this category. In the case of disodium cromoglycate, it has been found that the transport of histamine is impeded most when the drug liquid membrane presents its hydrophilic surface to the permeant-histamine. This observation predicts that a similar orientation of disodium cromoglycate with its hydrophilic moieties facing histamine may be necessary on mast cells.

III. Antiepileptic Drugs:

Three antiepileptic drugs, namely, diphenylhydantoin, carbamazepine and valproate sodium, have

been investigated. The data indicate that liquid membranes likely to be generated by these drugs at the site of action make significant contribution to their action. It has been found that the transport of γ-aminobutyric acid (GABA) is enhanced when it faces the hydrophilic surface of the liquid membrane generated by the drugs. Since antiepileptic drugs are known to enhance the concentration of GABA in brain, the present studies not only indicate the definite contribution of drug liquid membrane in the antiepileptic action, they also indicate that the specific orientation of the drug molecules with their hydrophilic moieties facing the permeant may be necessary even at the actual site of action.

It has also been found that gradation in the CNS of the three drugs is also the gradation in their concentrations in plasma. This effect also gives the indication of the contribution of the liquid membranes generated by these drugs in the mechanism of their action.

IV. Antiarrhythmic Drugs:

Four structurally dissimilar antiarrhythmic drugs, namely, quinidine, disopyramide, procainamide and propranolol have been investigated. Since antiarrhythmic

action is known to be mainly due to modification in the permeability of cardiac membrane to sodium ions, permeability of sodium ions in presence of the liquid membranes generated by the drugs has been measured. It has been found that the transport of sodium ions is impeded more or less to the same extent in both the orientations of the liquid membrane generated by the drugs - hydrophobic surface of the liquid membrane facing the permeant and hydrophilic surface of the liquid membrane facing the permeant. These findings are consistent with the literature reports that non-specific antiarrhythmic agents interact with both hydrophilic and hydrophobic regions of the cardiac membranes. The data on the transport of sodium ions in presence of propranolol, which is primarily a β-blocker drug, has also been found to be consistent with its reported antiarrhythmic actions.

LIST OF PUBLICATIONS BASED ON THE WORK CONTAINED IN THE THESIS

1 Liquid Membrane Phenomena in Antihistamines, Int. J. Pharmaceutics, 17, 263-272 (1983).

2 Liquid Membrane Phenomena in Cimetidine, Ranitidine and Disodium Cromoglycate, Int. J. Pharmaceutics, 21, 27-33 (1984).

3 Liquid Membrane Phenomenon in Antiepileptic Drugs, Int. J. Pharmaceutics, 24, 297-305 (1985).

4 Liquid Membrane Phenomena in Antiarrhythmic Action, Int. J. Pharmaceutics, (communicated).