LIQUID MEMBRANE PHENOMENA IN DRUG ACTION

Thesis

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DOCTOR OF PHILOSOPHY

By

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"However hard we try to bring
in the new, it comes into
being only in the midst
of clumsy deals."

PETER WEISS.

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE

PILANI, RAJASTHAN.

CERTIFICATE

This is to certify that the thesis entitled "LIQUID MEMBRANE PHENOMENA IN DRUG ACTION" submitted by Mr. S.B. Bhise, ID No. 79RH24010 for the award of the Ph.D. degree of the Institute, embodies original work done by him under my supervision.

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Dated 28.5.82.

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CONTENTS

CHAPTER - 1

INTRODUCTION

1. INTRODUCTION

1.1.1 Membranes and Drug Action:

The barrier properties of membranes have attracted wide attention since life depends on the normal functioning of membranes and membrane transport systems(1). These properties depend on the constituents of the membrane, and an overview of physical properties of the membrane components indicates surface activity to be a common and very significant factor. This is quite understandable because existence of surface activity decides the interfacial location of the molecules constituting the membrane. These generalisations are true of 'biological systems' in general and are also true of 'drug action' in particular. Hence it is logical to expect that 'the drugs acting by altering permeability of cell membranes are likely to be surface active'. It is equally logical to expect that surface active drugs are likely to act by altering the permeability of cell membranes.

1.1.2 Surface Active Drugs:

A wide variety of drugs are known(2-8) to be surface active in nature, their surface-activity being expressed as ability of these drugs to accumulate at interfaces of water with air or any other surfaces. Surprisingly these surface active drugs(2-8) belong to a cross-section of chemical and pharmacological categories.

Thus the co-existence of surface activity and biological activity does not appear to be a fortuitous coincidence. In a number of categories of surface-active drugs a correlation between surface-activity and biological effects has been demonstrated(9-15).

Since the structural requirements for surface-activity are often similar to those for interaction of drugs with receptor sites(16) these correlations appear to be indicative of mechanism of action of surface active drugs. Thus a common feature amongst all surface-active drugs can offer a clue towards the probable common mechanism of action.

1.2.1 Liquid Membrane Phenomena:

Several reports(17-20) indicate ability of surfactants to alter permeability of cell-membranes. In biphasic systems, presence of surfactants has been shown(21-25) to influence mass transfer across the interface. A mechanism to explain such effects on mass-transfer was offered by Kesting(26-28). While investigating reverse osmosis in presence of surfactants like polyvinylmethyl ether, Kesting observed that the surfactants have ability to form a "liquid membrane" at the interface and the supporting membrane, on which the liquid membrane is formed, gets progressively

covered as concentration of the surfactant is increased. This continues upto the critical micelle concentration (CMC) of the surfactant and at concentrations equal to or above CMC of the surfactant the coverage is complete. The 'liquid membrane', thus formed, can influence the mass transfer across it.

An analogous situation exists when a surface-active drug is in contact with the biological cell. Hence it was thought worth investigating whether Kesting's liquid membrane hypothesis has any relevance to the action of surface-active drugs. The possibility appears to be of notable consequence because most of the surface-active drugs are known(4,5,9) to act by altering permeability of cell membranes. The present investigation is an effort to assess validity of this proposition.

1.3.1 Passive Support:

While probing the mechanism of action of surface-active drugs one question that appears to be of paramount importance is: "what factor is really responsible for reduction in permeability of cell membranes in presence of surface active drugs? Is it the specific and active interaction of the drug with constituents of

2) Chlorpromazine, a prototype phenothiazine

3) Reserpine, a rauwolfia alkaloid, and

4) Imipramine, a tricyclic antidepressant.

The first three drugs are antipsychotic drugs. Haloperidol and chlorpromazine are quite extensively used to treat psychotic conditions like mania, schizophrenia. Reserpine is preferred for its antihypertensive rather than neuroleptic action. Imipramine is used to treat endogenous depression.

Haloperidol and chlorpromazine are known(29) to be antagonists of dopamine, a neurotransmitter of central nervous system. Reserpine is known(30) to prevent intraneuronal uptake of catecholamine neurotransmitters and imipramine is known(29) to exert its antidepressant action by reducing uptake of catecholamines from extraneuronal sites. All these drugs are known to be surface-active in nature(6,9,31). It is quite reassuring to note that, while investigating membrane effects of drugs like reserpine, chlorpromazine, imipramine and propranalol, Palm et al(32) have concluded that "irrespective of the chemical structure, the surface activity of these psychotropic drugs mainly determines their potency to affect all kinds of membranes, especially that of catecholamine-storing particles."

1.4.1 Outline of the Thesis:

The thesis is divided in six chapters. After the first introductory chapter, the second chapter presents review of literature upto December 1981 reporting the theme of investigations where surface-activity of the drugs has been considered to be an important factor for their action. Reports of surface activity of drugs, their correlation with clinical potencies or interaction of these drugs with insoluble lipid monolayers has been specially looked into. It also includes a subsection in which biological activity of surface-active agents is presented. A wide spectrum of actions like antimicrobial action, hemolytic action, effects on enzyme activity and other actions, where permeability of cell membranes is considered to be a contributory factor, are reviewed. The effect of surfactants on mass transfer in nonliving systems has also been overviewed.

The third chapter deals with the details of experiments which include determination of hydraulic conductivity coefficient (see Appendix) in presence of various concentrations of surfactant drugs and demonstration of liquid membrane phenomena using mosaic membrane model (see Appendix). It also presents the

transport studies of various permeants viz. neurotransmitters like catecholamines and amino acids, in presence of these liquid membrane forming drugs. The methods of estimation of these permeants are also briefly presented.

In the fourth chapter this experimental data is discussed in the light of the facts known about the effects of these drugs on living cells. The specific orientation of these drugs necessary to reduce transport of these permeants is discussed and its relevance to biological situations is commented. A generalisation regarding orientation of receptors and its logical deduction, complementing our observations is presented.

The fifth chapter gives a summary of the important conclusions of the present investigations and the sixth chapter offers suggestions for further investigations which can be pursued in this area to substantiate and extend the present observations.

CHAPTER - 2

LITERATURE REVIEW

2. LITERATURE REVIEW

A wide variety of drugs are reported to be surface active in nature. The literature reports related to surface activity of these drugs have been classified here according to the pharmacological categories to which they belong.

2.1 Neuroleptic Drugs:

2.1.1 Phenothiazines:

Phenothiazines is a group of compounds in which case surface activity has been reported and investigated extensively to find a clue to the mechanism of their action. Reporting the investigations on a series of antipsychotic neuroleptics, Seeman and Bialy(9) have observed a correlation between average clinical doses of these drugs and the concentrations of these drugs required to reduce surface tension of Ringer solution. They have further commented(9) that 'probably all of the phenothiazines adsorb onto tissue cells and this may be explained by the physical chemistry involved in air-water adsorption'. It is further stated(9) that these drugs form virtually monomolecular films around the cell membrane and reduce transmembrane

permeability to many solutes. Surface activity of promazine, promethazine, and diethazine has been studied(33). Aggregation of phenothiazines has been studied using NMR techniques(34,35)

Effect of Buffers:

In another report(36) while investigating five phenothiazine drugs under a variety of conditions, it was shown that addition of phthalate, citrate and succinate buffers increased surface activity of these drugs.

Effect of Ions:

Presence of short-chain quaternary ammonium and methanesulfonate ions were shown(37) to decrease surface activity of chlorpromazine while ions like bromide, iodide, propanesulfonate, benzenesulfonate and napthalenesulfonate caused increase in surface activity.

Effect of ATP:

In presence of adenosine triphosphate (ATP) surface tension of chlorpromazine solution was found(38) to be markedly reduced. Formation of such a surface active complex has been indicated(38) to be of importance for the action of the drug.

Effect of Substituents:

Phenothiazine derivatives containing alkylated ammonium groups have been investigated(39) for their colloidal properties, association and micelle formation. Micellar weights of phenothiazine derivatives and their dependance on various factors have been reported(40). Structural variation in phenothiazines has been shown(40,41) to influence the surface activity indicating the possibility that variation in the biological activity with structural change may be expressed in the form of altered surface activity. Substitution on the phenothiazine ring was found(41) to enhance surface activity in the order $CF_3 \gg Cl > H$. (all substitutions at position 2). Changes in position of chloro group effected the surface activity in the order 3Cl > 2 Cl > 1 Cl while increase in chain length of alkylamino group at position 10 resulted in increase in surface activity. It is interesting to note that sulfoxide of chlorpromazine, which is a relatively inactive antipsychotic agent, was observed(9) to be less effective in reducing surface tension of an acidic solution.

2.1.2 Tricyclic Antidepressant Drugs:

Surface-activity of tricyclic antidepressants has been investigated(6). In case of amitriptyline, butriptyline, protriptyline, nortryptyline, imipramine, desipramine, clomipramine, dothiepin, dibenzepin, minserin and maprotiline the critical micelle concentration (CMC) have also been reported(6).

In another study, a correlation between surface activity of some of these drugs and their toxicity to Chang liver cultures has been reported(42,43). This observation was also found(44) to be true in case of the cells derived from human liver.

2.1.3 Miscellaneous:

Surface activity of a series of stimulants and depressants has been investigated(45). The drugs included pentylenetetrazole, picrotoxin, amylobarbitone, trimethadione etc. The conclusion is quite interesting. It is concluded(45) that while stimulant drugs tended to populate in the aqueous bulk phase, the depressant drugs accumulated at the air-solution interface. In case of depressant drugs, the surface-activity was observed(45) to be correlated with pharmacological effects of the drugs.

In a study on diazepam, chlorpromazine and haloperidol(46) the drugs have been shown to reduce surface tension of water and blood. At neutral conditions (pH = 7.0) solutions of the drugs exhibited minimum surface tension and maximum surface potential. Colloidal association and dependence of surface activity on chemical structure has been shown(15) in case of phenothiazines, thioxanthene, dibenzocycloheptadiene and dibenzazepine drugs.

Evidence presented, in all these reports supports the observations(47) that physicochemical behaviour of various substituted phenothiazines plays an important role in their activity on the central nervous system. It strengthens the speculation(3) that surface activity of centrally acting drugs is an important feature of their action.

2.1.4 Interaction with Insoluble Monolayers:

Many of the drugs acting on the central nervous system are known to alter permeability of cell membranes. Hence to probe these actions, an appropriate model of cell membrane has been selected and interactions of the membrane-active drugs are investigated. Insoluble monolayers formed by lipids on water surface is one such model.

There are variety of reports indicating interactions of the drugs with lipid monolayers.

Chlorpromazine, chlorpromazine sulfoxide and trifluoperazine have been shown(48,49) to interact with lipid monolayers. Interactions of these drugs were shown(48,49) to correlate with their biological activity and the drugs have been shown to penetrate into lipid film. In case of five phenothiazine drugs it has been shown(36) that ability of these drugs to increase the surface pressure correlated with their nonpolarities. It was further postulated(36) that ion-pair formation may play a role in transport to, and accumulation of these compounds at membrane and other receptor surfaces. Interaction of orphenadrine hydrochloride, chlorpromazine hydrochloride and reserpine with monomolecular films of cholesterol, synthetic phosphoglycerides, sphingomyelin, cerebrosides and gangliosides was investigated(50) indicating gradation in their interaction. Since synaptic vesicles storing acetylcholine are known to be rich in gangliosides, the result(50) appears interesting. Interactions between psychoactive drugs and anionic lipid monolayers pointing importance of coulombic interaction has been reported(51). Interaction of four phenothiazine

derivatives with lecithin and cholesterol monolayers(52) provided evidence that these drugs acted immediately below the lipid monolayers. Effect of UV radiation on interaction of a series of phenothiazines with dipalmitoyl lecithin films indicated(53,54) that ability of these drugs to interact with lecithin monolayer may be a measure of their in-vivo membrane-penetrating and phototoxic properties. It is also indicated(55) that the photoactivated phenothiazines cause photosensitized reactions through formation of new, stable, more specific active compounds.

2.2 Barbiturates:

Butylbarbituric acid has been shown(56) to reduce surface tension of aqueous solutions. Short-acting barbiturates like pentobarbital, quinalbarbital(57) and monoalkyl, dialkyl barbituric acids(58) have also been reported to be surface-active. Linear correlation between protein-binding properties and surface-activity with apparent partition coefficient has been observed(21) in case of barbituric acid derivatives. It has been shown(11) in case of barbital, phenobarbital and pentobarbital that their interaction energies with phospholipid monolayers

expressed as 'the adsorption free energy' correlates with their nerve-blocking potencies. Penetration of barbiturates in membrane lipids resulting in alteration of physical state of lipids is reported(59). Changes in ion-channels and membrane-bound enzymes as a result of the drug-lipid interactions have been indicated to be a mechanism of action of barbiturates(60,61). Barbiturates have been shown to facilitate "melting" or "fluidization" of membrane lipids(61). Studies using fluorescent probes(62) have also supported this observation. Surface charge of synaptic vesicles from cerebral cortex has been shown to be decreased in presence of amobarbital(63).

2.3 Local Anesthetic Drugs:

2.3.1 Surface Activity:

A relation between local anesthetic activity and surface tension in case of 30 diethylaminoethyl esters of substituted carbamic acids has been reported(64). It was interesting to note that inactive derivatives exhibited negligible surface activity.

2.3.2 Interaction with Lipid Monolayers:

Interaction of local anesthetics with lipid monolayers has been extensively investigated(65-68).

Local anesthetics have been shown to increase the surface pressure of lipid monolayers indicating that the drug molecules penetrated the lipid monolayers. The drugs were shown to be expelled out at a high surface pressure. In case of lipids extracted from nerve tissue, penetration of monolayers by the drugs is shown to correlate with their nerve-blocking potency. It has been postulated(69) that local anesthetic drugs block nerve conduction by increasing the lateral pressure within the lipid membrane of nerve cells resulting in blockade of the pores through which ions permeate. The possibility of modification of compositional lipid mosaic by local anesthetics has also been proposed(70) as a mechanism of their action.

2.3.3 Interaction with Veratrum Alkaloids:

Procaine and veratrum alkaloids exhibiting different types of permeability effects on nerves showed(71) correlation between interaction with lipid monolayers and the effects on nerve-fibre membranes. Veratrine, which is known to increase permeability to sodium and potassium ions reduced the area per molecule of a stearic acid monolayer(72). Lipid-alkaloid complex was probably dissolving in the

aqueous subphase, which caused increase in permeability to ions. This effect was shown to be antagonized(71) by procaine. Effect of procaine and veratrine on desorption of monolayer of mono-octadecyl phosphate has also been investigated(73). While procaine prevented desorption apparently by adsorbing at the undersurface, veratrine increased the rate of film desorption. It was suggested(74) that repititive activity observed in case of nerves treated with veratrine alkaloids and the effect on phospholipid monolayers have a common cause.

2.3.4 Other Effects:

In another study(75) interaction of a series of local anesthetic drugs on monolayers of synthetic dipalmitoyl lecithin indicated that minimum blocking concentration of each of these anesthetics lowered the surface tension of lecithin/water interface by approximately same amount.

Effect of local anesthetics on thermal phase transitions of dipalmitoylcholine bilayer membrane has also been reported and it has been shown that this effect is antagonized at high pressures(76).

2.3.5 A Hypothesis:

A recent hypothesis(77) for mechanism of action of local anesthetics indicates that the sodium channel within the cell is surrounded by an annulus of lipids, which is in the crystalline or gel state. Local anesthetics change it to fluid, liquid crystalline phase allowing the sodium channel to close.

2.4 Drugs Related to Acetylcholine:

2.4.1 Cholinomimetic or Cholinolytic Actions:

Surface activity of choline-like compounds is well documented(78-82). Antispasmodic activity which is dependent on antagonism to acetylcholine has also been linked to surface activity. In case of derivatives of papaverine, spasmolytic activity is shown(83) to be proportional to surface activity of the compounds. Curare-like activity of a series of polymethylene-bis-trimethylammonium compound has been shown(84) to be associated with surface-activity. Derivatives of pyrrolidine also exhibit(85) similar behaviour. In this group of compounds, the antispasmodic activity is observed to be related to surface activity of these compounds. Surface activity of a series of synthetic anti-acetylcholine drugs of the category of diphenylmethanes has been reported(8).

2.4.2 Depolarising Agents:

In a subphase containing acetylcholine (1mM) spreading of lecithin monolayers showed(86) a plateau in surface pressure - surface area curves with a larger molecular area for lecithin indicating saturation of the interface. It has been demonstrated(87) that surface potential of lecithin monolayers in presence of acetylcholine is altered either with or without sodium ions. The change in potential was also observed(87) when $\overset{+}{N}H_4$ or $(CH_3)_4\overset{+}{N}$ ions were added to the subphase. It is postulated that depolarising agents can influence the interfacial charge distribution or potential without altering packing of monolayer. The change in phase boundary potentials, thus created might be involved indirectly in the mechanism of action of many substances on natural membranes(87).

Acetylcholine in the concentration of 2mM has been reported(88) to influence the surface potential, but not the surface pressure of egg lecithin films. Higher concentrations of acetylcholine (0.25M) however showed(89) significant increase in the surface pressure of both egg lecithin and dipalmitoyl lecithin.

2.5 Analgesics:

Ability of codeindione and oxycodone to reduce surface tension of water has been reported(90). Surface activity of antipyrine and its alkyl derivatives have also been investigated(91). Analgesics like analgin, amidopyrine, dimedrol, barbamyl have been shown(92) to be surface active. Compression of the adsorption layer of these drugs(92) on trypsin monolayer revealed presende of surfactant admixtures in several preparations. In another study(12) analgesic action of five narcotic compounds was observed to correlate with their surface-activity at the boundry of air and benzene solutions of nerve tissue lipoproteins.

2.6 Psychotomimetics:

In case of a series of psychotomimetic glycolate esters, their interaction with lipid or lipoprotein layer has been well investigated(93-96). Amongst these drugs, N-methyl-3 piperidyl glycolate(94,95) increased the viscosity of stearic acid films possibly because of condensations similar to those produced with calcium indicating similarity in their mode of action. Glycolate esters also showed(96) interaction with adenosine triphosphate (ATP) which was previously interacted with a lipid film.

These studies emphasize lipids as important sites and cell membrane, a important locus for the action of these drugs.

2.7 β-blockers:

Propranalol has been shown(97) to form micelles. Surface-activity has also been reported(98) in case of other β-blockers like sotalol, oxprenolol, labetalol, timolol, metoprolol and acebutolol. Propranalol has been shown(32) to influence nerve-uptake or release of catecholamines. In another investigation(13) in case of nine β-blokers the properties like effect on myocardial conduction velocity, local anesthesia have been shown to correlate with surface-activity and hydrophobicity.

2.8 Histamine and Anti-histamines:

The capillary activity of cetyl phosphate at chloroform-water interface has been shown(99) to be increased by minute concentrations(10^{-8}M) of histamine. Surface activity of a series of antihistamines e.g. diphenhydramine, dimenhydrinate, bromodiphenhydramine, cyclizine, chlorcyclizine, tripelenamine, thenyldiamine and pheniramine have also been reported(7).

Interaction of these antihistamines with L-α-dipalmitoyl lecithin monolayers has been subsequently investigated(100). Penetration of these drugs in the lipid film has been demonstrated. Ability of antihistamines to increase surface pressure was correlated(100) with their surface activity at air-water interface. At high pressures, these drugs were ejected from the monolayer.

A series of compounds containing quaternary ammonium salts, local anesthetics and antihistamines have been shown(101) to prevent loss of intracellular potassium and soluble proteins from liver.

A correlation between the protective activity of these compounds and their interaction at both air/water or lipid/water interface has been demonstrated(101).

2.9 Antibiotics:

2.9.1 Polypeptides:

Surface properties of cyclic decapeptide antibiotics have been well documented(102-104). It is proposed that an electrostatic interaction between amino group of the antibiotics and phosphate group of the bacterial membranes is involved in biological activity of these antibiotics viz. tyrocidin A, gramicidin SA, polymyxin E.

2.9.2 Polyenes:

In case of polyene antibiotics, correlation was observed(105-106) between their interaction with lipid monolayers and ability to produce membrane damage.

Critical micelle concentrations of amphotericin B, mycoheptin, levorin and nystatin have also been recorded and their relation to antimicrobial activity has been shown(107). A strong interaction between polyene antibiotics and cholesterol is well-known as an important factor in membrane damage.

2.9.3 Others:

Increased surface activity of positively charged tetracycline at the solution/membrane interface has been reported(108). Chloromycetin also has been reported(109) to be surface-active. Griseofulvin has been shown(110,111) to increase surface pressure of a stearic acid monolayer at low film pressures, while cholesterol films exhibited loss of surface pressure in presence of griseofulvin suggesting interfacial dissolution.

Surface activity of acridines(112) and antimalarials(113) has also been indicated.

On the basis of interaction of surface-active agents with lipid monomolecular films, a mechanism of lysis has been proposed(114,115). The lysis is either by detergency or by penetration of the lysin into cholesterol monomolecular film. An interesting report(116) investigating surface activity of quaternary ammonium compounds showed that different compounds of this category with equal antimicrobial activity had surface concentrations of the same order of magnitude. This finding is in agreement with another observation(117) that solutions of quaternary ammonium compounds having equal antimicrobial activity have surface tensions of the same order of magnitude.

2.10 Hormones:

2.10.1 Steroids:

Correlation between physiological action of steroid hormones and their packing into a lipoprotein membrane was proposed earlier(118). Progesterone was reported to penetrate monolayers of cholesterol and dipalmitoyl lecithin(119). Interaction of four biologically active steroids with a stearyl alcohol

monomolecular film was investigated(120) indicating influence of these steroids on viscosity of water just below the film. This effect showed specificity towards cations. While desoxycorticosterone and aldosterone reduced viscosity of water more in presence of potassium than of sodium ions, the effects of androsterone and etiocholanolone were not influenced by these ions(120). The monomolecular films of some estrogens and other naturally occuring polycyclic compounds have also been examined(121). Desoxycorticosterone, androsterone and aldosterone have been shown to cause a marked increase in the rate of increase of surface viscosity indicating their ability to accelerate the formation of aggregates(122). Testosterone, testosterone propionate and deoxycorticosterone acetate have been shown(123) to reduce surface tensions of aqueous solutions of salts or proteins. Deoxycorticosterone acetate has further been shown(123) to reduce red cell sedimentation rate and increase rate of diffusion of methylene blue into gelatin.

2.10.2 Polypeptides:

Polypeptide hormones like oxytocin, 8 arginine-vasotocin and l-aspargine-5-valine-angiotensin have ability to interact with lipid monolayers(124) as evidenced by increase in surface pressure. These

hormones are postulated to form "pores" which facilitate movement of water across it.

Insulin in low concentrations is shown(125) to influence interaction of calcium ions with monooctadecyl phosphate monolayer. It inhibited calcium uptake and facilitated release of calcium, previously adsorbed on the monolayer. Insulin analogs e.g. vasopressin, oxytocin, thyrocalcitonin, adrenocorticotropin and adenosine monophosphate (AMP) showed(126) comparatively less effect on uptake of calcium by monolayers of monooctadecyl phosphate. Since calcium ions in the membrane are known to influence permeability of cell membrane, these observations provide a clue to the mechanism of action of these drugs.

2.11 Vitamins:

Fat-soluble vitamins have been proposed(127) to regulate membrane structure and permeability through their ability to act at water/lipid interface. Ability of vitamin A to penetrate lecithin-cholesterol monomolecular films has been demonstrated(128). The hemolytic action of vitamin A is suggested(128) to be because of vitamin A - lecithin interaction. The protection of all-trans-retinol and α-tocophenol against oxidative attack has been indicated(129) to

be because of their interaction with lecithin monolayers, pointing towards a similar biological role for lecithin. Monolayers of rhodopsin at air/water interface have been used(130,131) as a model for structure of rod-sac membrane.

The support for the hypothesis that Schiff base formation may be involved in the mechanism of visual excitation comes from the study(132) of penetration of retinol/retinaldehyde on the phospholipid monolayers. Formation of monolayers in case of biologically important quinones has been demonstrated(133,134). Thiamine(135,136), riboflavine and ascorbic acid are known to influence surface activity of lecithin and lecithin-cholesterol mixtures(137).

2.12 Miscellaneous:

Surface activity of various other types of compounds have also been reported. It has been demonstrated in case of urea(138), ϵ-aminocaproic acid(139), derivatives of lysine(140), biotin(141), iopamidol(142), prostaglandins(143), fatty acid monoesters of ascorbic acid(144), pyrazoles(145), pyrazolones(146), antiarrhythmic drugs(147), heparin(148), betaine(149) and pentagastrin(150).

2.13 Biological Activity of Surfactants:

The role of surface phenomena in biological functions has been thoroughly investigated(151-153). Development of "Bilayer Lipid Membrane" (BLM) as a model to investigate interfacial and electrical properties and permeability characteristics, simulating biological membranes has attracted wide attention in recent years(154,155).

A variety of biological actions are known to be associated with surface-active substances.

2.13.1 Respiratory System:

The functional significance of surfactants present in lungs is widely documented(156). The existence of phospholipids like dipalmitoyl lecithin and their role in uptake of inhaled gases(157), deficiency of phospholipids causing pathological effects on the respiratory system(158) and interactions with biologically active substances like prostaglandins(159) indicate wider implications of such systems to the activity of lungs.

2.13.2 Hemolysis:

Hemolytic activity of surfactants(160) is another such example. The mechanism of hemolytic activity caused by surfactants has attracted some attention(161).

A relation between physicochemical properties of surfactants like critical micelle concentration (CMC), hydrophile-lipophile balance (HLB), relation of surface lytic effects to molecular organization of the membrane and hemolytic activity has been discussed(162). Effect of modification of membrane on hemolysis of erythrocytes has also been reported(163).

2.13.3 Antimicrobial Activity:

Bactericidal property of surface-active agents is yet another area where extensive investigations have been reported(164). The influence of surface-active agents on growth of bacteria(165), the mechanism of bactericidal action of surface-active agents(166), fungicidal action(167,168) and the relation of antimicrobial activity to physicochemical properties of surfactants(169) have received considerable attention.

2.13.4 Effects on Enzyme Activity:

Effect of surfactants on the activity of enzymes is another interesting aspect. The effects of detergents used to isolate membrane components on the activity of variety of enzymes(170) has been documented. Activation of lipase by naturally occuring surfactants like

bile salts is an extensively investigated system. Activation of mammalian brain acetylcholinesterase by non-ionic surfactants has been shown(171) to be by virtue of surface-activity alone. Effect of surface-active agents on the activity of enzymes in brush border of enterocytes has been reported(172). Surfactants like triton-X-100, lubrol have been observed(173) to activate Na-k-ATPase. In one of our investigations(174) micellar inhibition of amylase in the presence of sodium deoxycholate was reported.

2.13.5 Other Effects:

There are several other instances where surface-activity has been considered to be of importance for biological action. On isolated tissues e.g. frog muscle, clam heart etc. the surfactants like sodium tetrapropyl benzenesulfonate have been shown(175) to suppress acetylcholine-induced contractions. Effects like mitotic inhibition(176), inhibition of conjugation and transduction transfer of plasmids(177) have also been observed in presence of surfactants.

Surface-active agents e.g. sodium cholate, Tween-80, ethonium are known(178) to influence regulation of epithelial cell permeability caused by antidiuretic hormone.

In an interesting study(179), dose-response inhibition of water transport in everted hamster jejunal segments with sodium dodecyl sulfate, dioctyl sodium sulfosuccinate, ricinolate was related to CMC of these surfactants.

Effects of surfactants on cell-aggregation have been reported(180). Possible relation of plasma surface activity to thrombosis(181) has been indicated. Surfactants like Brij 58 have been shown to stabilize the platelets(182). Surface-activity of mucoproteins and their adherence to surface phospholipids is indicated(183) to be of some importance for a functional lipoprotein unit. Surface-active agents like digitonin, sodium deoxycholate, triton-X-100, cetyltrimethylammonium bromide are known(184) to interact with lysophosphatidylcholine, which is the cause of effect on platelet aggregation.

Invert soaps have been shown(185) to exhibit curare-like action on frog rectus muscle. Antispasmodic activity of a variety of surfactants on a large number of smooth muscles have been demonstrated(186). Toxicity of surfactants is also well documented(187).

2.13.6 Effects on Permeability:

Effect of surfactants on permeability of epidermis(188), cornea(19), dialysis(189) is reported. Dependance of biological activity on surface properties like CMC, HLB has also been indicated(190).

Diffusion properties of surfactants have been linked(191) to their mechanism of action.

Increase in membrane permeability in presence of surfactants is also a documented observation. Glucose transport has been shown(192) to be facilitated in presence of pluronic F 68. Use of cationic surfactants has been shown(193) to enhance the activity of parenterally administered drugs. Presence of surfactants at liquid/liquid interface influencing mass transfer has been reported(194). Micellar solubilization of intestinal protein increasing transport of substance in everted rat intestinal membrane has been demonstrated(195). Interfacially controlled transport of micelle solubilized sterols across oil/water interface has been investigated(196). Availability of drugs has been shown to be reduced in presence of some surfactants(197).

Effect of surfactants on diffusion of drugs through gels and their dependence on CMC has been investigated(25). Some interesting biological activities of surface-active agents need special mention.

Decrease in surface tension of female urines during menstruation has been shown to be because of a tensio-active substance(198). The damaging effect of surfactants on Ehrlich cancer cells is shown to be function of their surface activity(199). Spermicidal action of surfactants and presence of surface activity for this action has been indicated(200). A relation between surface tension reducing property of nonionic agents and their toxicity against mosquito is shown(201). A relation between acute toxicity of anionic surfactants towards fishes, with surface activity of these compounds has been demonstrated(202). Existence of critical surface tension and its correspondence to the median lethal dose(202) is an important indicator of contribution of surface activity towards lethal action. Surface activity of polypeptide toxins is also documented(203).

2.13.7 Structural Variation and Surface Activity:

Minor structural changes in the compounds are known to alter surface activity and are therefore of

CHAPTER - 3

EXPERIMENTAL

3. EXPERIMENTAL

3.1 Materials:

Various drugs and other materials used in the present investigation are listed below along with their sources.

Haloperidol (B.P., Searle (India) Ltd.)

Chlorpromazine hydrochloride (I.P., May and Baker (India) Ltd.)

Reserpine (B.P. - U.S.P. Roussel UCLAF, Paris)

Imipramine hydrochloride (B.P., Jagson Pal & Co.)

Dopamine chlorhydrate and Adrenaline hydrogen tartrate (Loba Chemie)

L-noradrenaline (Fluka A.G.)

5-Hydroxytryptamine creatinine sulphate and Hydrindantin (Koch Light Laboratories Ltd.)

Histamine acid phosphate, L-glutamic acid, Gamma-amino-butyric acid (GABA), Ninhydrin, Sodium acetate, Acetic acid glacial (B.D.H.)

O-phthalaldehyde (Sigma)

Methyl cellosolve (Riedel-de Haen AG Seelze-Nannover)

Alcohol (B.P., Bengal Chemicals & Pharmaceuticals Ltd.)

Sodium chloride, Potassium chloride, Calcium chloride (AnalaR Grade)

Water, once distilled over potassium permanganate in all pyrex glass still was used.

The all glass transport cell used in the present experiments is diagrammed in Fig. 1 which has been well labelled. It consists of a round bottom flask of about 500 ml capacity to which a glass tube can be attached with help of a standard joint. Placement of a supporting membrane (M) below the tube separates the whole transport cell in two compartments (C and D). A glass capillary (L_1L_2) is attached to compartment D. On each side of the supporting membrane (M) two electrodes (E_1 and E_2) are fixed. A stop-cock is attached to upper part of compartment D to adjust the level of water in capillary (L_1L_2) whenever necessary. Compartment C is connected to a pressure head, the level of which is measured with the help of a cathetometer. During the experiments, the whole transport cell is placed over a magnetic stirrer, whereby the needle in compartment C keeps the solution well stirred. This is done to avoid concentration polarisation of the dissolved substance. The areas of cross-section for the membrane (M) and for the capillary (L_1L_2) were measured before the experiment.

Sartorious cellulose acetate/nitrate millipore filter (Cat. No.11107/11307 of thickness 1×10^{-4}m and area $5.373 \times 10^{-5}m^2$) were used as supports for the

Caption for the Figure

Fig. 1 : The transport cell. M : Supporting membrane. *(cellulose acetate Sartorius millipore filter, Cat. No. 11107), P. bright Platinum electrodes. L_1 L_2: Capillary tube of length 17 cms. and diameter with 1.33×10^{-1}cm. The volume of compartments C and D are 590 ml. and 50 ml. respectively. E_1; E_2; Electrode terminals.

*(cellulose nitrate Sartorium millipore filter, Cat. No. 11307).

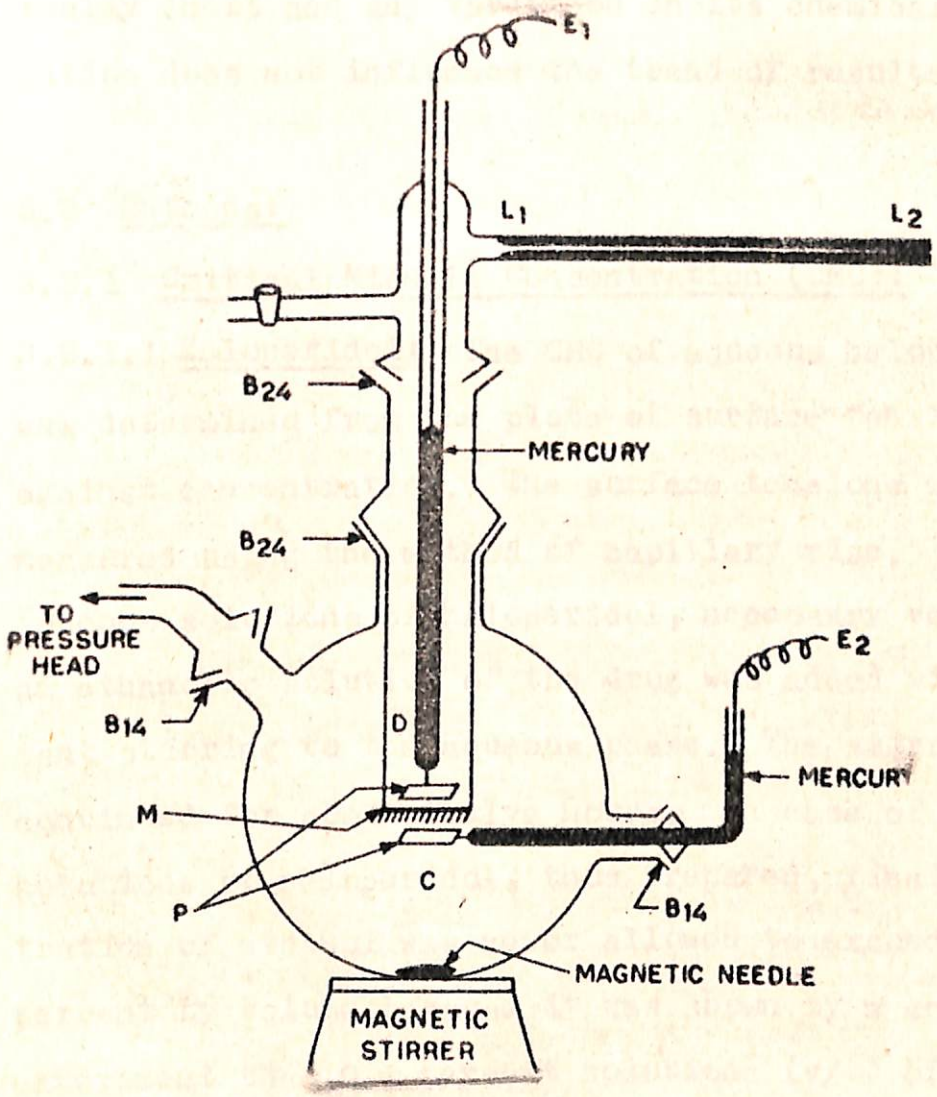
E1
L1
L2
B24
MERCURY
B24
TO
PRESSURE
HEAD
B14
D
E2
MERCURY
M
C
P
B14
MAGNETIC NEEDLE
MAGNETIC
STIRRER

FIG. 1

liquid membrane. Two types of cellulosic supporting membranes were used to ensure that the support is really inert and any variation in its chemical composition does not influence the trend of results.

3.2 Methods:

3.2.1 Critical Micelle Concentration (CMC):

3.2.1.1 Haloperidol: The CMC of aqueous haloperidol was determined from the plots of surface tension against concentration. The surface tensions were measured using the method of capillary rise. To prepare aqueous solutions of haloperidol, necessary volume of an ethanolic solution of the drug was added with constant stirring to the aqueous phase. The stirring was continued for about twelve hours. In case of aqueous solutions of haloperidol, thus prepared, final concentration of ethanol was never allowed to exceed 0.1 percent by volume because it was shown by a control experiment that 0.1 percent solutions (v/v) of ethanol in water did not lower surface tension of water to any measurable extent.

3.2.1.2 Reserpine: Surface tensions were measured by a tensiomat (Fisher Surface Tensiomat Model 21). Reserpine being less soluble in alcohol, the quantity of

alcoholic solution required to obtain maximum reduction in surface tension was around 1.0 percent (v/v). Hence while measuring surface tension values, blanks and other intermediate concentrations were adjusted to contain equivalent (1.0 percent v/v) amount of alcohol.

3.2.1.3 Chlorpromazine Hydrochloride and Imipramine Hydrochloride: For these drugs, water soluble salts were used for the investigation. Surface tensions for varying concentrations were measured using a tensiomat (Fisher Surface Tensiomat Model 21).

3.2.2 Hydraulic Permeability*

For measurements of hydraulic permeability, aqueous solutions of the drugs with following concentration ranges were taken

0 to 1.064×10^{-5}M for Haloperidol
0 to 1.800×10^{-4}M for Chlorpromazine hydrochloride
0 to 6.400×10^{-6}M for Reserpine and
0 to 7.400×10^{-4}M for Imipramine hydrochloride.

These aqueous solutions of the drugs were filled in compartment C of the transport cell (Fig. 1) while compartment D was filled with distilled water. The respective concentration ranges were selected in order

---

* For definition and other details see Appendix.

to get data on both lower and higher sides of CMC of the drugs. Known pressures were applied on compartment C by adjusting the pressure head and the consequent volume flux was measured by noting the rate of advancement of liquid meniscus in the capillary L_1L_2 (Fig. 1) with a cathetometer of least count 0.001 cm and a stopwatch reading upto 0.1 sec. Magnitude of the applied pressures was also measured by noting the position of the pressure head with a cathetometer reading upto 0.001 cm. During the volume flux measurements the solution in compartment C was well stirred and also the electrodes E_1 and E_2 were short circuited so that the electro-osmotic back flow on account of streaming potentials does not interfere with the observations.

From the graph of volume flux against the pressure applied, the value of L_p, the hydraulic conductivity coefficient was calculated. For one set of calculations of L_p, about 8 to 10 observations with prressures, applied in an increasing or decreasing order were recorded. From values of L_p at individual pressures, a mean value of L_p and its mean deviation was calculated. The values of L_p were recorded for a range of concentration of the drugs both below and above its CMC.

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3.2.3.1 Solute Permeability*

Those permeants which are known either to be neurotransmitters or are involved in the transmission of nerve-impulse were used as solutes for the transport study. The drugs and the respective permeants used for trnasport study are listed below.

Haloperidol: adrenaline (acid tartrate), L-noradrenaline, dopamine (chlorhydrate), 5-hydroxytryptamine (creatinine sulphate), histamine (acid phosphate), glutamic acid, G.A.B.A., sodium chloride, potassium chloride and calcium chloride.

Chlorpromazine (hydrochloride): adrenaline (acid tartrate), L-noradrenaline, dopamine (chlorhydrate), 5-hydroxytryptamine (creatinine sulphate), glutamic acid, G.A.B.A.

Reserpine : adrenaline (acid tartrate), L-noradrenaline, dopamine (chlorhydrate), 5-hydroxytryptamine (creatinine sulphate), glutamic acid, G.A.B.A.

Imipramine (hydrochloride): adrenaline (acid tartrate), L-noradrenaline, dopamine (chlorhydrate), 5-hydroxytryptamine (creatinine sulphate) Sodium chloride, potassium chloride and calcium chloride.

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* For definition and other details see Appendix.

The initial concentrations of the permeants were selected to match with their concentrations in the vicinity of nervous tissue.

In case of chlorpromazine hydrochloride, and reserpine, since the estimation of transported biogenic amines was carried out using spectrophotometric absorption, a higher initial concentration was selected so that the transported amount becomes measurable.

For measurement of solute permeability, two sets of experiments were performed. In the first set of experiments, compartment C of the transport cell (Fig. 1) was filled with respective permeants dissolved in aqueous solution of the drug at a concentration above its CMC. The respective concentrations of the drugs were as follows.

4.256×10^{-6}M for Haloperidol (4CMC)

1.800×10^{-4}M for Chlorpromazine hydrochloride (4CMC)

6.400×10^{-6}M for Reserpine (4CMC)

5.920×10^{-4}M for Imipramine hydrochloride (4CMC).

In all these experiments, compartment D was filled with distilled water.

In the second set of experiments, compartment D (Fig. 1) was filled with the concentrations of drugs as indicated above and compartment C was filled with aqueous solutions of known concentrations of the permeants.

For each permeant control experiments without the respective drug were carried out.

The condition of no net volume flux (Jv = 0) was imposed on the system by adjusting the pressure head attached to compartment C of the transport cell (Fig. 1) such that the liquid meniscus in the capillary L_1L_2 remained stationary. After a known period of time ranging from 4 to 12 hours, concentration of the permeant in compartment D was measured. The amount of permeant gained by compartment D divided by the time and area of the membrane gave the value of solute flux (Js). The value of solute permeability (ω) was estimated using the definition(208,209)

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

where $\Delta\pi$ is the osmotic pressure difference. The value of $\Delta\pi$ used in the calculations of ω was average of the values of $\Delta\pi$ at begining of the experiment (t=0)

and at end of the experiment. During the permeability measurements, solution in the compartment C was kept well stirred. All measurements were taken at constant temperature by placing the transport cell (Fig. 1) in a thermostat set at 37 ± 0.1°C.

3.2.3.2 Estimations:

The amounts of various permeants transported to compartment D were estimated as follows.

3.2.3.2. a) Endogenous Amines (in presence of Haloperidol)

Haloperidol did not interfere with the fluorometric estimation of endogenous amines. In case of catecholamines viz. dopamine, noradrenaline and adrenaline, the transported amounts were estimated by measuring intensity of fluorescent light at 325 nm(210). Though the fluorescent intensity of 5-hydroxytryptamine is reported to be maximum at 330 nm(211), since in the present experiments quartz* cuvette was used, the estimations of 5-hydroxytryptamine also were carried out at 325 nm. Histamine was estimated by measuring the fluorescent intensity of its flurophor derived from its reaction with O-phthalaldehyde(210,212) at 450 nm.

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* Quartz, when excited by UV light (<300 nm) itself emits at around 330 nm.

3.2.3.2. b) Biogenic Amines (in the presence of Chlorpromazine, Reserpine or Imipramine)

Chlorpromazine, reserpine and imipramine were observed to quench fluorescence of biogenic amines viz. adrenaline, noradrenaline, dopamine and 5-hydroxytryptamine. Hence their estimations were carried out by measuring absorbance at 282.4 nm (λmax). For this calibration curves were constructed by noting absorbance of the solutions of varying concentrations of biogenic amines prepared in a solution of fixed concentration of the respective drug, which was equal to its concentration in the solute permeability experiments. The calibration curves, thus constructed, were found to be linear in accordance with Beer's law. The amount of transported biogenic amines was estimated with the help of this calibration curve.*

3.2.3.2. c) Amino Acids (in the presence of Haloperidol, Imipramine or Chlorpromazine)

The amounts of transported glutamic acid and G.A.B.A. were estimated from amounts of their reaction

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* A Cary 17-D Spectrophotometer (Varian) with fluorescence attachment was used for the estimation of biogenic/endogenous amines.

products with ninhydrin(213) at 570 nm. These amino acids in 4M acetate buffer (pH 5.5) with a mixture of hydrindantin and ninhydrin in methyl cellosolve produce a blue coloured reaction product with absorption maxima at 570 nm.**

3.2.3.2. d) Cations (in the presence of Haloperidol or Imipramine)

The amounts of sodium, potassium and calcium ions were determined using atomic absorption spectrophotometer (Perkin-Elmer model 306) using emission mode.

The estimations were carried out using following lines.

sodium	589.6 nm.
potassium	766.5 nm.
calcium	422.7 nm.

3.2.3.2. e) Dopamine (in the presence of G.A.B.A. and Haloperidol or Chlorpromazine)

The amount of transported dopamine was estimated in presence of haloperidol and G.A.B.A. or chlorpromazine and G.A.B.A., only in the second set of

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** A Baush & Lomb Spectronic 20 Spectrophotometer was used for estimation of coloured product of amino acids with ninhydrin.

experiments (where the drug and G.A.B.A. were kept in compartment D and the permeant was in compartment C). In case of haloperidol, the estimations were carried out using fluorimetry (325 nm) while in case of chlorpromazine the estimations were carried out using spectrophotometry (282.4 nm).*

---

* Cary 17-D Spectrophotometer (Varian) with fluorescence attachment.

3.3 Representative Calculations

Table 1

3.3.1 Calculations of Hydraulic Conductivity Coefficient

Drug: Reserpine Concentration 1.200×10^{-6}M

A	B	C	D	E	F	G	H	I
1	5.610	8.787	74.024	118.2	0.695	420.4	1.653	0.265
2	6.157	9.799	78.514	57.8	1.630	860.5	1.894	0.024
3	5.812	12.900	82.179	75.8	2.418	1220.0	1.982	0.064
4	6.165	13.234	86.160	55.8	3.276	1610.0	2.034	0.116
5	5.863	14.250	86.160	71.0	3.055	1610.0	1.898	0.020
6	6.420	12.718	82.118	67.5	2.413	1214.0	1.988	0.070
7	6.218	9.975	78.024	59.0	1.647	812.4	2.028	0.110
8	6.335	9.486	74.162	100.5	0.811	433.9	1.869	0.049

Do : Zero setting at 69.734 cm.

Column A : Serial No.

B : Initial capillary reading cm.

C : Final capillary reading cm.

D : Pressure head reading cm.

E : Time in seconds for distance (C-B)

F : Volume flux ($Jv \times 10^5$) ms^{-1}

G : Hydrostatic pressure (ΔP) Nm^{-2}

H : Hydraulic conductivity coefficient (L_p) $m^3s^{-1}N^{-1}$

I : Mean deviation $|L_p - \bar{L_p}|$

where L_p : individual hydraulic conductivity coefficient

$\bar{L_p}$: mean hydraulic conductivity coefficient.

Following are constant parameters of the transport cell (Fig. 1)

r : radius of the capillary (L_1L_2) : 0.0665 cm

$= 6.65 \times 10^{-4}$ m.

R : radius of the membrane (M) : 0.4135 cm

$= 4.135 \times 10^{-2}$ m.

$$Jv = \frac{\pi . r^2 (C-B)}{E} \Big/ \pi . R^2$$

$$= \left(\frac{r}{R}\right)^2 \cdot \frac{C-B}{E}$$

$$= 2.586 \times 10^{-2} \quad \frac{C-B}{E} \qquad \left[\left(\frac{r}{R}\right)^2 = 2.586 \times 10^{-2}\right]$$

For Serial No. 1

$$Jv = \frac{2.586 \times 10^{-2} \ (8.787 - 5.610) \times 10^{-2}}{118.2} \ ms^{-1} \ (C-B \text{ in m})$$

$$= \frac{2.586 \times 3.177 \times 10^{-4}}{118.2} \ ms^{-1}$$

$$= 0.695 \times 10^{-5} \ ms^{-1}$$

$$P = (D - Do) \times 98 \quad Nm^{-2}$$

$$= (74.024 - 69.734) \times 98 \quad Nm^{-2}$$

$$= 4.29 \times 98 \quad Nm^{-2}$$

$$= 420.4 \quad Nm^{-2}$$

$$LP = \frac{Jv}{\Delta P} = \frac{0.695 \times 10^{-5}}{420.4} \quad m^3s^{-1}N^{-1}$$

$$= 1.653 \times 10^{-8} \; m^3s^{-1}N^{-1}$$

3.3.2 Calculations for Mosaic Membrane Model:

Mean L_p value for above 8 readings = 1.918 ± 0.0980

This observation corresponds to 0.75 CMC of reserpine.

L_p for supporting membrane = 2.482 ± 0.086

L_p for supporting membrane fully covered = 1.848 ± 0.057 with the liquid membrane (reserpine conc. = 1 CMC)

Hence expected $L_p = 0.75(1.848 \pm 0.057) + 0.25(2.482 \pm 0.086)$

$= (1.386 \pm 0.043) + (0.620 \pm 0.021)$

$= 2.006 \pm 0.064$

Experimental $L_p = 1.918 \pm 0.090$

3.3.3 Calculations for Solute Permeability(ω):

Permeability of glutamic acid in the presence of 6.4×10^{-6}M reserpine.

$$Js = \frac{c \times v}{\pi . r^2 . t . M}$$

where Js : solute flux per unit area per unit time.

c : concentration of solute in compartment D 77.5 µg/ml.

v : volume of compartment D 7.0 ml.

r : radius of membrane $= 5 \times 10^{-3}$m

t : time = 4 hours = 4 x 3600 seconds.

M : mol. wt. (glutamic acid) = 147.1

$$Js = \frac{77.5 \times 7.0}{\pi (5 \times 10^{-3})^2 \times 4 \times 3600 \times 147.1}$$

$$= 3.262 \times 10^{-6} \text{M m}^{-2}\text{s}^{-1}$$

$$\Pi = C.R.T.$$

where Π : osmotic pressure Nm^{-2}

C : concentration of the solute 500 µg/ml.

R : gas constant 8.31 Nm deg^{-1} mole^{-1}

T : absolute temperature 310°A

$$\text{Initial osmotic pressure} = \frac{500 \times 310 \times 8.31}{147.1} = 8760.4 \text{ Nm}^{-2}$$

*Final osmotic pressure = $8760.4 - \frac{77.5 \times 310 \times 8.31}{147.1}$

$= 8760.4 - 1357.9 = 7402.5 \ Nm^{-2}$

Hence mean osmotic pressure = $\frac{8760.4 + 7402.5}{2}$

$= 8081.5 \ Nm^{-2}$

$$\omega = \left(\frac{Js}{\Delta\pi}\right)_{Jv=0}$$

$$= \frac{3.262 \times 10^{-6}}{8081.5}$$

$$= 4.036 \times 10^{-10} \text{ Moles } s^{-1} N^{-1}$$

---

* Since volume of compartment C is about 10 times larger, the reduction in the amount of solute during the period of experiment is considered to be negligible.

CHAPTER - 4

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

4.1 Hydraulic Permeability:

4.1.1 C.M.C.:

The critical micelle concentrations of the drugs were determined first and they are as follows:

Haloperidol	: 1.064×10^{-6}M
Chlorpromazine hydrochloride	: 4.500×10^{-5}M
Reserpine	: 1.600×10^{-6}M
Imipramine hydrochloride	: 1.480×10^{-4}M.

4.1.2 Hydraulic Conductivity Coefficients(Lp):

From the hydraulic permeability data at various concentrations of these drugs (Fig. 2 - 5), it is obvious that the linear relationship

$$Jv = L_p . \Delta P \qquad \text{(Eq.2)}$$

where Jv represents the volume flux per unit area of the membrane, ΔP the applied pressure difference and L_p, the hydraulic conductivity coefficient, is obeyed in all cases. Complementary to these figures (Fig. 2-5), the values of L_p were calculated individually for each set of applied pressures and the corresponding volume fluxes. Considering about 8 - 10 such observations,

Table 2 : Values of L_p at Various Concentrations of Haloperidol.

Conc.of Haloperidol x 10^7(M)	0	1.064 (0.1CMC)	5.320 (0.5CMC)	10.64 (CMC)	106.4
L_p^* x 10^8 (m^3 s^{-1} N^{-1})	2.804 ±0.4368	2.095 ±0.1273	1.603 ±0.2015	0.7993 ±0.0692	0.7662 ±0.0216
L_p^{**} x 10^8 (m^3 s^{-1} N^{-1})	-	2.6035 ±0.3996	1.8017 ±0.2510	-	-

* Experimental values.

** Calculated values on the basis of mosaic model

Caption for the Figure

Fig. 2 : The hydraulic permeability data. Curves I, II, III, IV and V, are for 0; 1.064×10^{-7}; 5.32×10^{-7}; 1.064×10^{-6}; 1.064×10^{-5} M concentrations of haloperidol respectively.

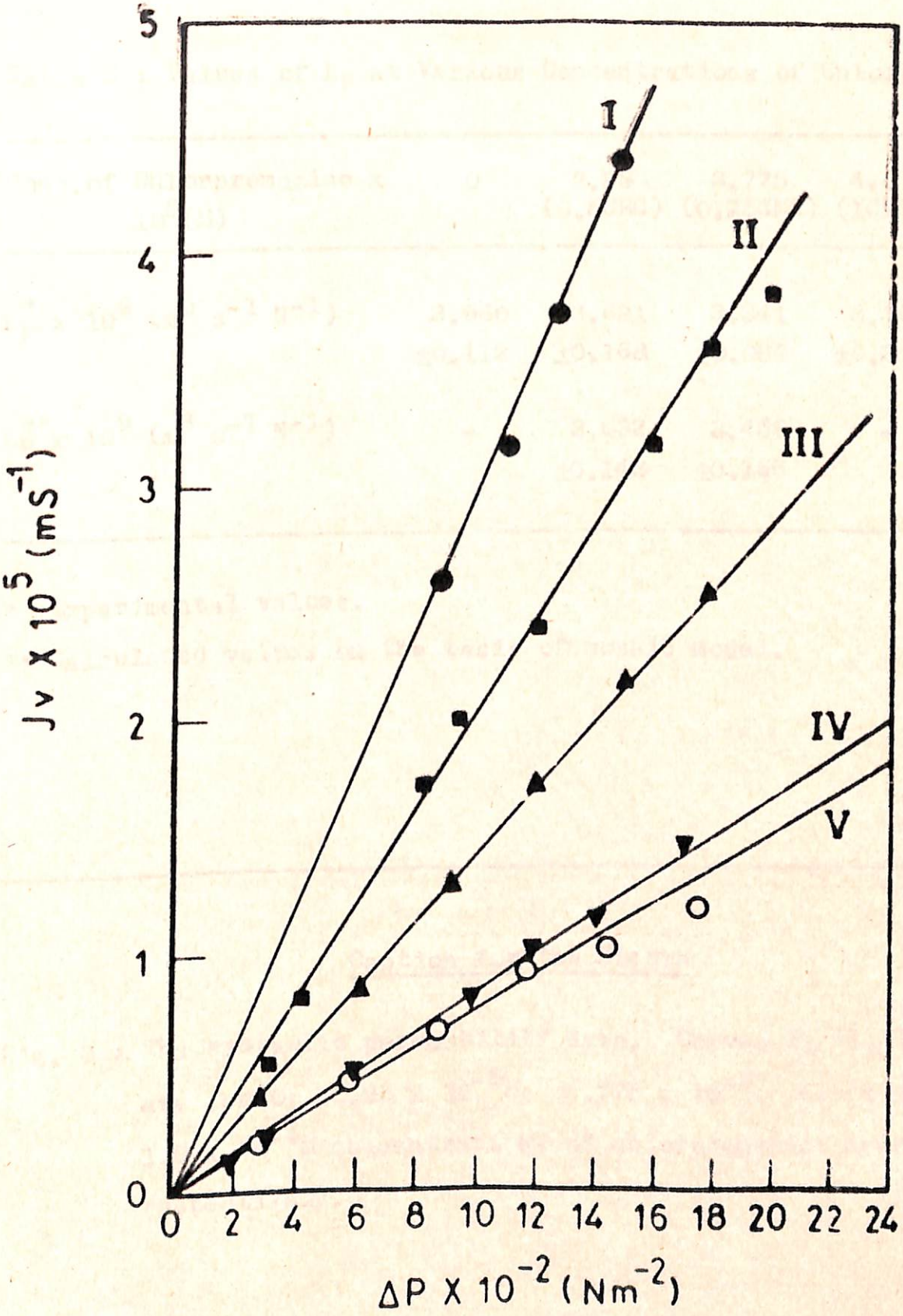

I
II
III
IV
V
0
1
2
3
4
5
0 2 4 6 8 10 12 14 16 18 20 22 24
$J_v \times 10^5 (mS^{-1})$
$\Delta P \times 10^{-2} (Nm^{-2})$

FIG. 2

Table 3 : Values of L_p at Various Concentrations of Chlorpromazine.

Conc.of Chlorpromazine x 10^5(M)	0	2.25 (0.5CMC)	3.775 (0.75CMC)	4.5 (1CMC)	18.0
L_p^* x 10^9 (m^3 s^{-1} N^{-1})	3.960 ±0.112	3.621 ±0.168	3.341 ±0.089	3.102 ±0.286	3.305 ±0.184
L_p^{**} x 10^9 (m^3 s^{-1} N^{-1})	-	3.632 ±0.148	3.468 ±0.166	-	-

* Experimental values.

** Calculated values on the basis of mosaic model.

Caption for the Figure

Fig. 3 : The hydraulic permeability data. Curves I, II, III, and IV are for 0; 2.25×10^{-5}M; 3.775×10^{-5}; 4.5×10^{-5}M and 1.8×10^{-4}M concentrations of chlorpromazine hydrochloride respectively.

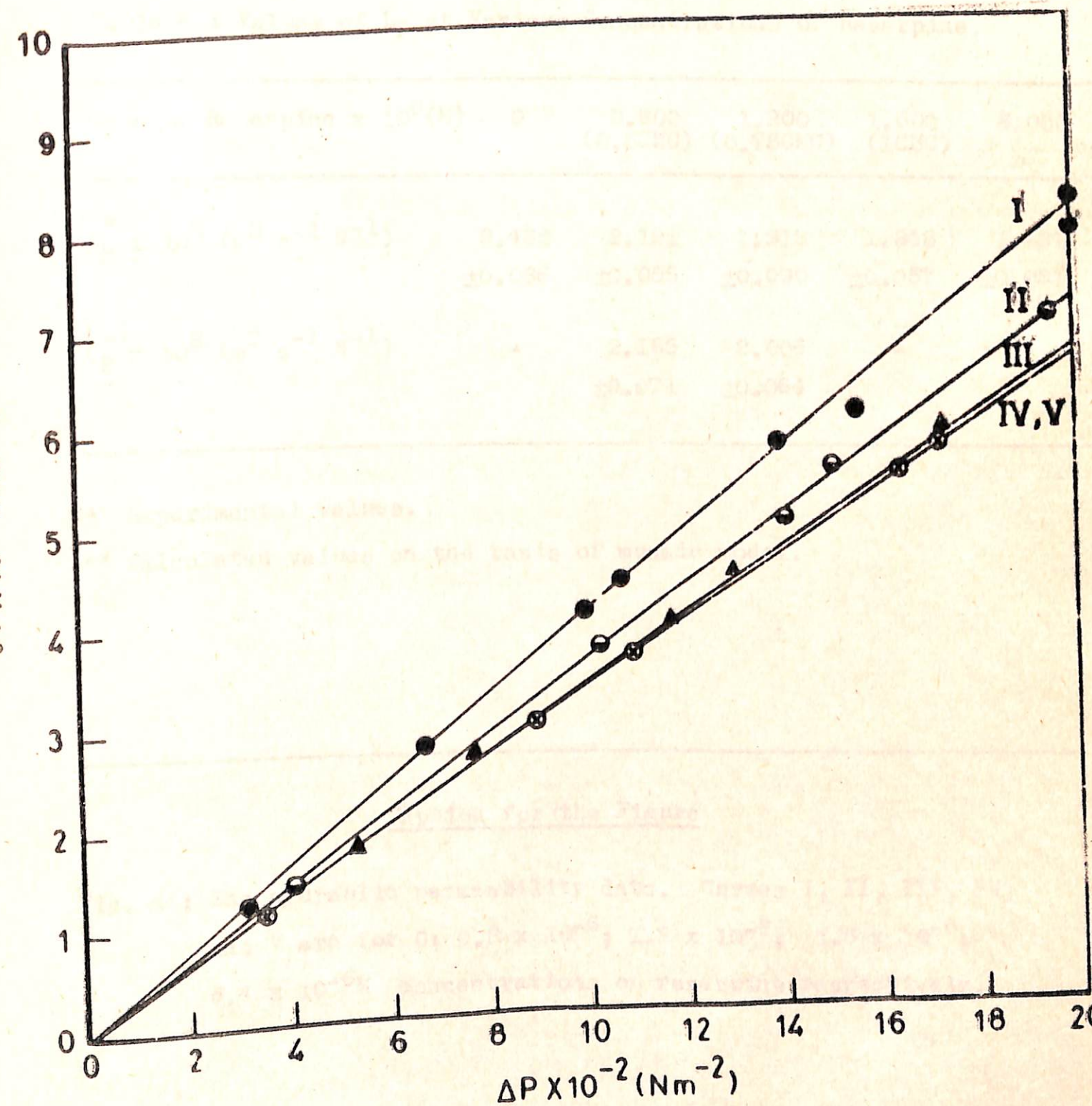

10
9
8
7
6
5
4
3
2
1
0
0
2
4
6
8
10
12
14
16
18
20
I
II
III
IV,V
$J_v \times 10^{-2}$ (mS^{-1})
$\Delta P \times 10^{-2}$ (Nm^{-2})

FIG. 3

Table 4 : Values of L_p at Various Concentrations of Reserpine.

Conc.of Reserpine x 10^6(M)	0	0.800 (0.5CMC)	1.200 (0.75CMC)	1.600 (1CMC)	8.000
L_p^* x 10^8 (m^3 s^{-1} N^{-1})	2.482 ±0.086	2.191 ±0.055	1.918 ±0.090	1.848 ±0.057	1.431 ±0.031
L_p^{**} x 10^8 (m^3 s^{-1} N^{-1})	-	2.165 ±0.071	2.006 ±0.064	-	-

* Experimental values.

** Calculated values on the basis of mosaic model.

Caption for the Figure

Fig. 4 : The hydraulic permeability data. Curves I, II, III, IV, and V are for 0; 0.8 x 10^{-6}; 1.2 x 10^{-6}; 1.6 x 10^{-6}; 6.4 x 10^{-6}M concentrations of reserpine respectively.

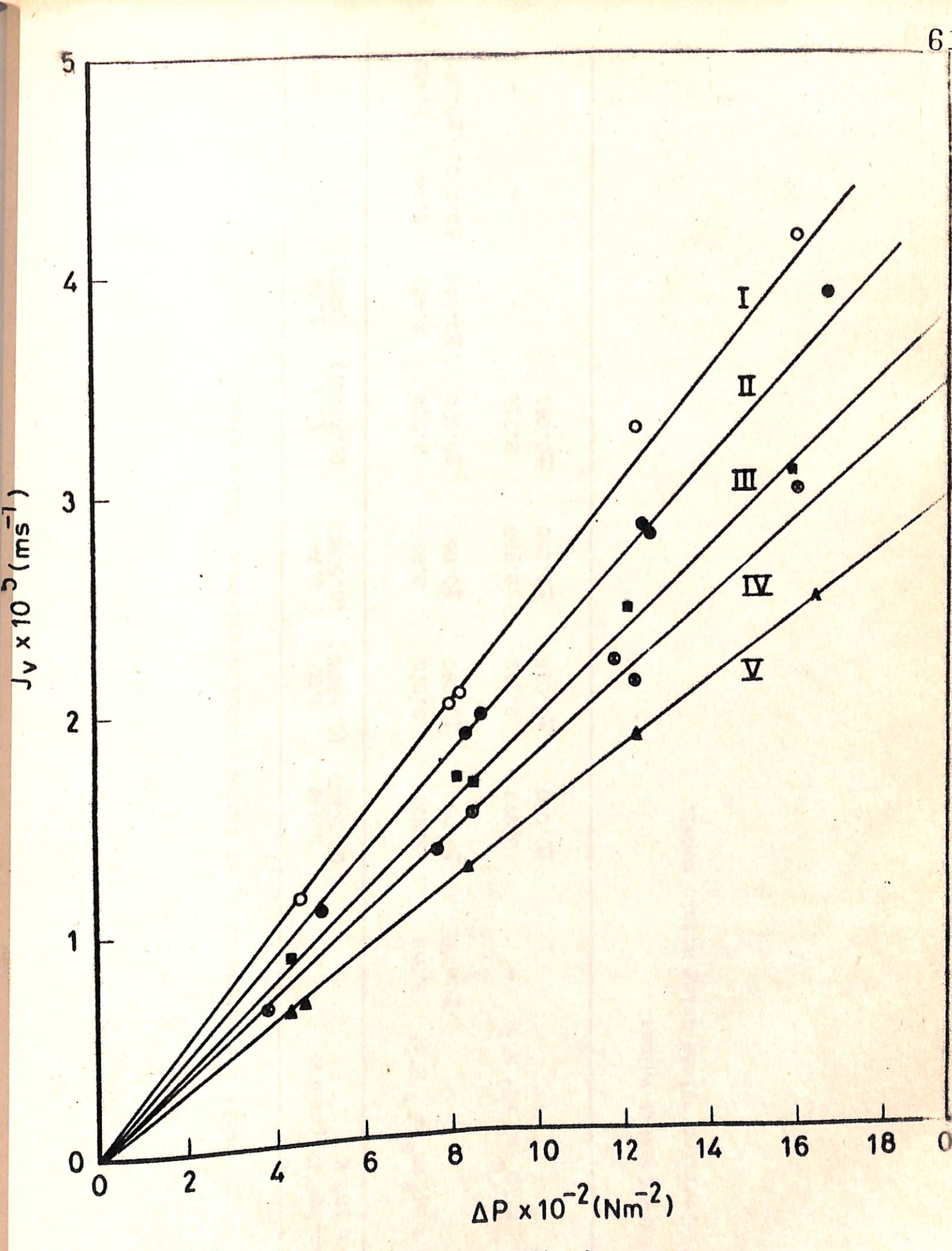

5
4
3
2
1
0
0
2
4
6
8
10
12
14
16
18
I
II
III
IV
V
$J_v \times 10^5 (ms^{-1})$
$\Delta P \times 10^{-2} (Nm^{-2})$

Fig. 4

Table 5 : Values of L_p at Various Concentrations of Imipramine.

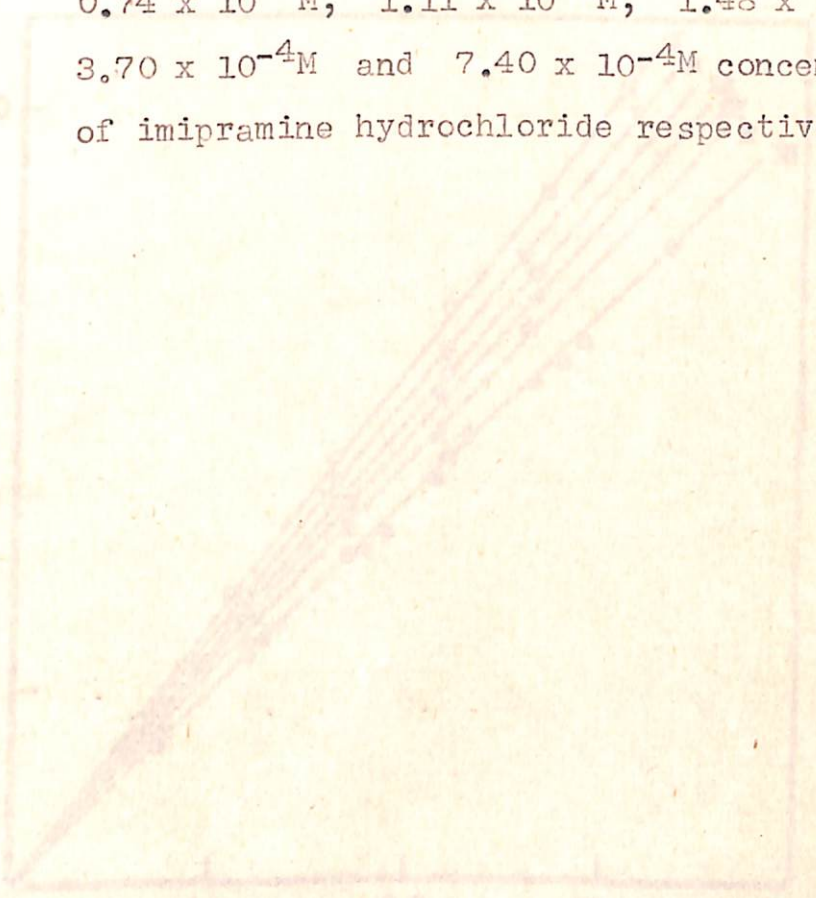
Conc. of imipramine x 10^4 M	0	0.148 (0.1CMC)	0.37 (0.25CMC)	0.74 (0.5CMC)	1.11 (0.75CMC)	1.48 (CMC)	3.70	7.40
L_p^{*} x 10^8(m^3 s^{-1} N^{-1})	3.369 ±0.050	3.233 ±0.090	3.075 ±0.080	2.925 ±0.060	2.702 ±0.070	2.451 ±0.050	2.444 ±0.070	2.442 ±0.080
L_p^{**} x 10^8(m^3 s^{-1} N^{-1})	-	3.277 ±0.050	3.152 ±0.018	2.935 ±0.030	2.718 ±0.041	-	-	-

* Experimental values.

**Calculated values using mosaic model.

Caption for the Figure

Fig. 5 : The hydraulic permeability data. Curves I to VI are for 0, 0.148×10^{-4}M, 0.37×10^{-4}M, 0.74×10^{-4}M, 1.11×10^{-4}M, 1.48×10^{-4}M, 3.70×10^{-4}M and 7.40×10^{-4}M concentrations of imipramine hydrochloride respectively.



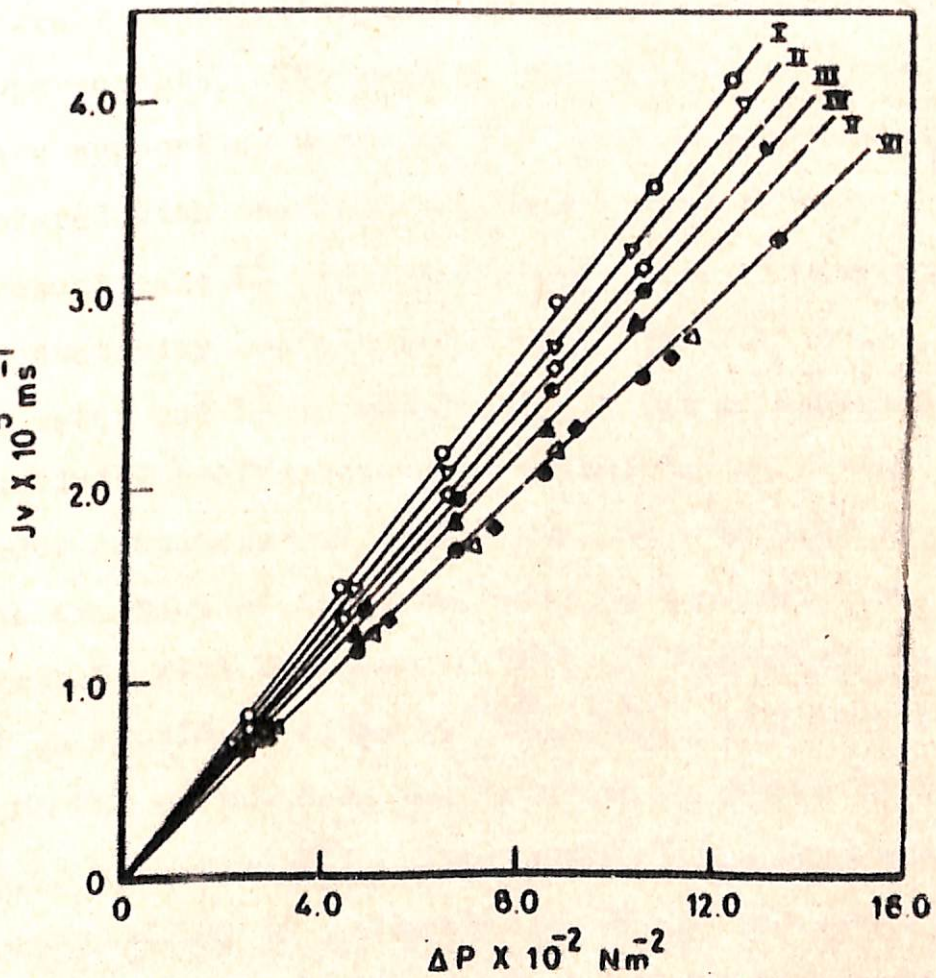


FIG. 5

lower than CMC, the supporting membrane is only partially covered with the liquid membrane, the equation for volume flux of water for such a case can be written as

$$Jv = \left[L_P^c \left(\frac{A^c}{A^c + A^s} \right) + L_P^s \left(\frac{A^c}{A^c + A^s} \right) \right] \Delta P \quad \text{(Eq.3)}$$

where A represents area of the membrane denoted by the superscripts. The superscripts c and s represent the bare supporting membrane and the supporting membrane covered with the liquid membrane respectively. In the present case L_P^c represents the value of hydraulic conductivity coefficient without the drug being added to water and L_P^s represents the value of hydraulic conductivity coefficient when concentration of the drug under consideration equals its CMC. At half the CMC, the fraction of the total area covered with the liquid membrane will be equal to half and therefore the value of L_P should be equal to $\left(\frac{L_P^c + L_P^s}{2} \right)$. Similarly for 0.25 CMC of the drug the value of L_P should be equal to $(0.75\ L_P^c + 0.25\ L_P^s)$. The values of L_P, thus computed, corresponding to a fraction of CMC of the drugs have been presented (Table 2 - 5). For all the drugs investigated, these computed values match well with the experimentally determined values.

4.1.5 Variation in Lp Values:

The absolute values of hydraulic conductivity coefficients for the supporting membranes (Table 2 - 5) show variation. The reason for this variation lies, probably in a vital experimental necessity. The supporting membrane is fixed to the transport cell (Fig.1) with the help of a polymer* solution which solidifies on standing. During this process, there are chances of this solution diffusing inside the membrane area responsible for hydraulic permeability. This, in all probability, will reduce the effective surface area for water permeability reducing the value of L_p as a consequence. This appears to be the case, at least for chlorpromazine, where the absolute value of hydraulic conductivity coefficient is low.

4.2 Solute Permeability in the Presence of Drugs:

4.2.1 Variation in ω Values:

The values of solute permeability (ω) for various permeants in presence of the liquid membrane forming drugs under investigation are recorded (Table 6 - 9). From the definition of ω (see Appendix) it is clear that it is average rate of flow of solute per unit

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* Araldite ..... (Cibatul Ltd.).

Table 6 : Solute Permeability ω of Endogenous Amines, Amino Acids and Cations in Presence of 4.256×10^{-6}m Haloperidol.

	$\omega_1 \times 10^{12}$ moles $s^{-1}N^{-1}$	$\omega_2 \times 10^{12}$ moles $s^{-1}N^{-1}$	$\omega_3 \times 10^{12}$ moles $s^{-1}N^{-1}$	$\omega_4 \times 10^{12}$ moles $s^{-1}N^{-1}$
Dopamine[a]	887.3	680.0	2607.0	274.4
Noradrenaline[b]	75.8	65.9	294.3	
Adrenaline[b]	50.7	undetectable	237.4	
5-Hydroxytryptamine[a]	193.7	94.5	348.1	
Histamine[b]	48.8	109.1	318.8	
Glutamic acid[c]	58.9	47.3	81.0	
G.A.B.A.[d]	119.8	86.6	152.2	
Sodium (chloride)[e]	172.9	53.4	70.7	
Potassium (chloride)[f]	175.5	157.1	101.3	
Calcium (chloride)[g]	119.2	111.7	106.8	

ω_1 : Control value - when no haloperidol was used.

ω_2 : Haloperidol in compartment D of the transport cell.

ω_3 : Haloperidol in compartment C of the transport cell.

ω_4 : In the presence of G.A.B.A. and haloperidol.

a : Initial concentration used 2.0 µg/ml.

b : Initial concentration used 1.0 µg/ml.

c : Initial concentration used 500 µg/ml.

d : Initial concentration used 200 µg/ml.

e : Initial concentration used 5.382 mg/ml.

f : Initial concentration used 10.430 mg/ml.

g : Initial concentration used 0.222 µg/ml.

Table 7 : Solute Permeability ω of Biogenic Amines and Amino Acids in Presence of 1.8×10^{-4}M Chlorpromazine Hydrochloride.

	$\omega_1 \times 10^{12}$ moles $s^{-1}N^{-1}$	$\omega_2 \times 10^{12}$ moles $s^{-1}N^{-1}$	$\omega_3 \times 10^{12}$ moles $s^{-1}N^{-1}$	$\omega_4 \times 10^{12}$ moles $s^{-1}N^{-1}$
* Dopamine	1015.0	344.9	531.5	70.98
* Noradrenaline	778.7	166.3	609.0	
* Adrenaline	2535.0	301.5	2000.0	
* 5-Hydroxytryptamine	842.8	164.1	334.2	
** Glutamic acid	426.0	325.1	366.0	
*** G.A.B.A.	784.7	608.3	624.1	

ω_1 : Control value - when no chlorpromazine was used.

ω_2 : Chlorpromazine in compartment D of the transport cell.

ω_3 : Chlorpromazine in compartment C of the transport cell.

ω_4 : In the presence of chlorpromazine and G.A.B.A.

* : Initial concentration used 10 µg/ml.

** : Initial concentration used 500 µg/ml.

*** : Initial concentration used 200 µg/ml.

Table 8 : Solute Permeability ω of Biogenic Amines and Amino Acids in Presence of 6.4×10^{-6}M Reserpine.

	$\omega_1 \times 10^{12}$ moles $s^{-1}N^{-1}$	$\omega_2 \times 10^{12}$ moles $s^{-1}N^{-1}$	$\omega_3 \times 10^{12}$ moles $s^{-1}N^{-1}$
* Dopamine	1137.0	738.2	883.6
* Noradrenaline	1155.0	67.8	658.3
* Adrenaline	1165.0	567.3	880.2
* 5-Hydroxytryptamine	1063.0	311.6	518.9
**Glutamic acid	403.6	217.5	491.7
***G.A.B.A.	695.1	407.1	1115.0

ω_1 : Control value - when no reserpine was used.

ω_2 : Reserpine in compartment D of the transport cell.

ω_3 : Reserpine in compartment C of the transport cell.

* : Initial concentration used 10 µg/ml.

** : Initial concentration used 500 µg/ml.

*** : Initial concentration used 200 µg/ml.

Table 9 : Solute Permeability ω of Biogenic Amines and Cations in Presence of 5.92×10^{-4}M Imipramine.

	$\omega_1 \times 10^{12}$ moles $s^{-1}N^{-1}$	$\omega_2 \times 10^{12}$ moles $s^{-1}N^{-1}$	$\omega_3 \times 10^{12}$ moles $s^{-1}N^{-1}$
Dopamine[a]	265.70	104.80	433.70
Noradrenaline[b]	98.93	48.20	1210.00
Adrenaline[b]	462.50	176.80	688.70
5-Hydroxytryptamine[b]	227.20	97.30	954.10
Sodium (chloride)[c]	86.20	46.90	71.20
Potassium (chloride)[d]	475.70	138.30	186.10
Calcium (chloride)[e]	56.60	10.20	20.80

ω_1 : Control value when no imipramine was used.

ω_2 : Imipramine in compartment D of the transport cell.

ω_3 : Imipramine in compartment C of the transport cell.

a : Initial concentration used 2.0 µg/ml.

b : Initial concentration used 1.0 µg/ml.

c : Initial concentration used 5.382 mg/ml.

d : Initial concentration used 10.43 mg/ml.

e : Initial concentration used 0.222 µg/ml.

area of the membrane divided by the average osmotic pressure exerted by the solute. Hence it is likely to depend on (a) Initial concentration of the permeants and (b) the total time for the transport of permeants.

Higher the initial concentration of the permeants higher will be the rate of transport. Similarly as more and more time is allowed for the experiment, the concentration gradient across the membrane will reduce gradually, leading to decrease in rate of transport. This will result in lesser average rate. Thus more the time allowed, lesser will be the average rate of solute transported and hence ω value will reduce. In the set of experiments undertaken in the present study, since these factors viz. time and initial concentrations of the permeants have varied for different drugs, the variation in the values of ω in control experiments are understandable. This may also be because of different types of supporting membranes. (Cellulose acetate in case of haloperidol and imipramine and cellulose nitrate in case of reserpine and chlorpromazine).

4.2.2 Concentration of the Drugs:

In all these cases, to ensure that the supporting membrane is completely covered with the liquid membrane

formed by the drug, concentrations of the drugs used for the transport study were always above their CMC values.

4.2.3 Orientation of the Liquid Membranes:

All these drugs being surface-active in nature, their molecules have both hydrophobic and hydrophilic parts in their structure. The orientation of these molecules will, therefore, be significant when they form liquid membranes. The hydrophobic ends of these molecules will be preferentially oriented towards the hydrophobic supporting membrane and the hydrophilic ends will face outwards away from the supporting membrane. When these drugs are in compartment C of the transport cell (first set of experiments) the liquid membranes will preferentially present polar surface to the permeant present in the same compartment. In the second set of experiments, where the drugs are in compartment D (Fig. 1) of the transport cell and the aqueous solutions of the permeants are in compartment C, the liquid membranes will preferentially present hydrophobic surface to the permeants. The orientation of the drug molecules with respect to approaching permeant will thus be different in two sets of experiments.

Since orientation of the drug molecules has influenced transport of the permeants characteristically (Tables 6 - 9), data in case of each drug is discussed individually. The reports on biological cells indicating influence of the drugs on cell permeability are also discussed accordingly.

4.3 Haloperidol:

4.3.1 Effect on Solute Permeability:

The values of ω recorded in table 6 indicate that when hydrophobic surface of the haloperidol liquid membrane preferentially faces the approaching permeant (the second set of experiments), a marked decrease in their permeability is observed. The only exception to this observation is that of histamine acid phosphate in which case, ω was found to be increased indicating increased transport of histamine. In the first set of experiments where haloperidol liquid membrane preferentially presents its hydrophilic ends to the permeants, except for the cations viz. sodium, potassium and calcium permeability of other solute molecules is increased in presence of haloperidol.

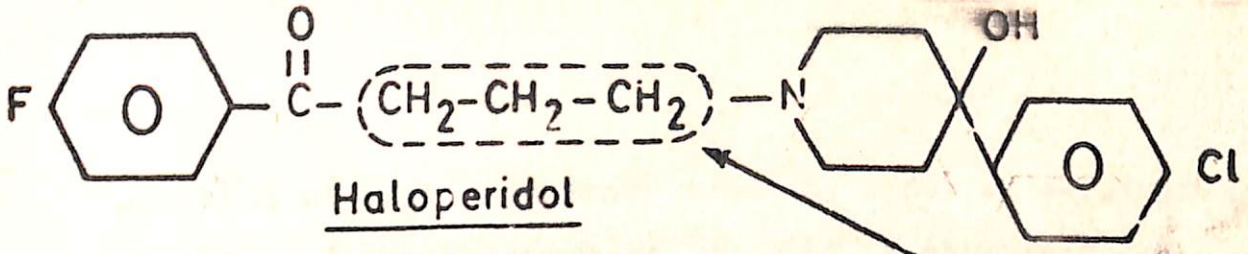
4.3.2 Catecholamines:

Haloperidol liquid membrane, in the second set of experiments offers resistance to transport of catecholamine (Table 6). In biological cells, haloperidol

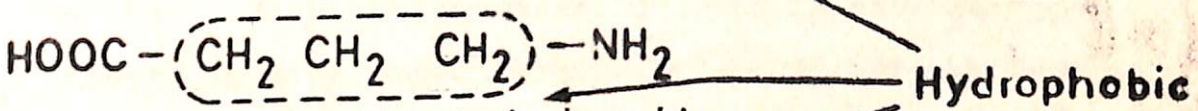
is known(30) to reduce permeability of catecholamines, amongst which reduction in permeability to dopamine is of consequence for the biological effect. The antiemetic and antipsychotic actions of haloperidol are explained(30) on the basis of reduced permeability of dopamine, which is under the influence of GABA-glutamic acid system. To investigate whether similar trend is also observed in case of the present experiments, dopamine transport was measured in presence of G.A.B.A. Quite interestingly, it was observed that presence of G.A.B.A. further increases resistance to flow of dopamine through haloperidol liquid membrane. This can be explained on the basis of strengthening of hydrophobic core of haloperidol liquid membrane by G.A.B.A. which is obvious from the similarity of the hydrophobic components of their structure (Fig. 6). Thus it appears likely that increased passive resistance to the flow of dopamine in the presence of G.A.B.A., coupled with the resistance to the flow of glutamic acid (Table 6) offered by the haloperidol liquid membrane is likely to have a reasonable contribution to the mechanism of action of haloperidol.

4.3.3. Comparison with Chlorpromazine:

Haloperidol is known(217) to be considerably more potent on milligram basis than chlorpromazine in vivo.

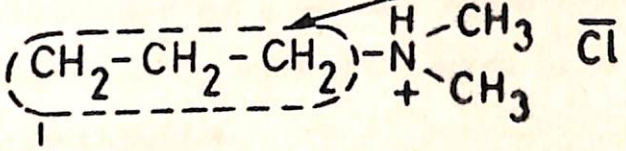


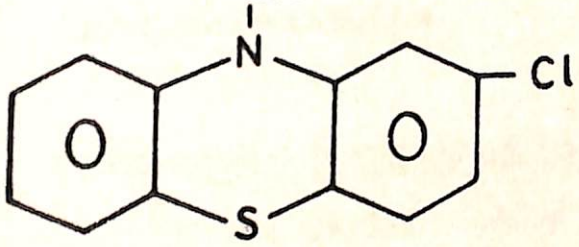
Haloperidol



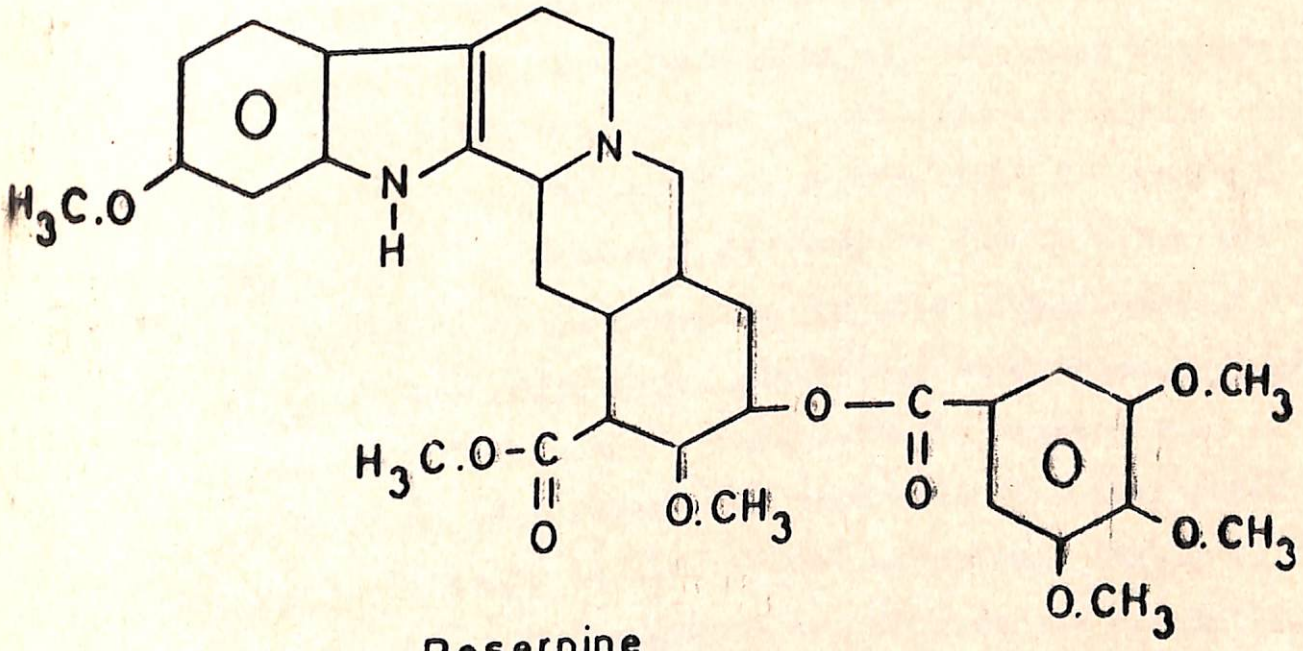
Hydrophobic

gamma-amino butyric acid

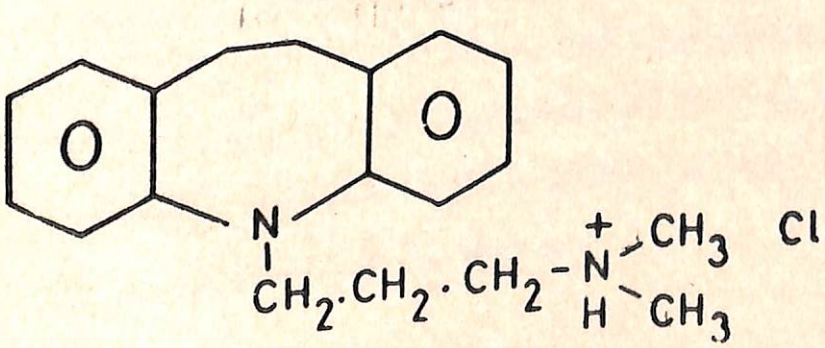




Chlorpromazine hydrochloride



Reserpine



Imipramine hydrochloride

Formation of liquid membrane seems to offer an explanation for this. Since haloperidol exhibits greater surface activity than chlorpromazine(9,30) as is evident from their CMC values (refer 4.1.1) haloperidol will form liquid membranes at a lesser concentration, making it pharmacologically effective even at a comparitively lower concentration.

4.3.4 5-Hydroxytryptamine:

The present study shows that haloperidol, in the second set of experiments reduces permeability of 5-hydroxytryptamine as well (Table 6). This is in agreement with the observation on biological cells(218). The extrapyramidal effects of antipsychotic drugs like haloperidol are reported to be resistant to levodopa therapy(219). This may possibly be due to reduction in transport of levodopa in presence of haloperidol. To test this suggestion, additional experimental proof is necessary. Another possibility to explain this observation is reduction in transport of 5-hydroxytryptamine, as shown in the present experiment (Table 6). This needs special emphasis because reduced concentration of 5-hydroxytryptamine in cerebrospinal fluid has been linked to defect in extrapyramidal function(220,221). Thus reduction in transport of 5-hydroxytryptamine in presence of haloperidol offers a clue to the causations of extrapyramidal symptoms.

4.3.5 Histamine:

The explanation for increased permeability of histamine in presence of haloperidol liquid membrane and its implication, if any, remains to be comprehended.

4.3.6 Cations:

The resistance offered by haloperidol liquid membrane to the flow of cations viz. sodium, potassium and calcium is probably because of hydrophilicity of the ions. The resistance to flow of ions is in both the orientations of haloperidol liquid membrane. This observation may have some biological relevance in relation to nerve conduction.

4.4 Chlorpromazine:

4.4.1 Effect on Solute Permeability:

The chlorpromazine liquid membrane has also been observed to reduce permeability of biogenic amines and amino acids (Table 7). Reduction in permeability is maximum, however, only when hydrophobic surface of the liquid membrane is preferentially oriented towards the approaching permeants.

4.4.2 Biogenic Amines:

Chlorpromazine is known to act by reducing permeability of biogenic amines(222,223) and amino acids.

Effects of chlorpromazine have been noted with membrane containing units like mitochondria(224), nerve-ending particles(225), platelets(226), adrenomedullary particles(227) and muscle fibres. For all these effects, influence of chlorpromazine on the uptake and release of various neurotransmitters(222,223) seems to be of much significance.

4.4.3 Interactions with Monolayers:

In order to investigate the role of accumulation of the drug in biomembranes in the mechanism of its action, studies on interaction of the drug with synthetic monolayers were undertaken by various authors(49,50). However to what extent permeability of the biogenic amines and the amino acids is modified as a result of this interaction has not been reported. The present experiments provide evidence that the liquid membrane of chlorpromazine itself offers resistance to the flow of biogenic amines and the neurotransmitter amino acids.

4.4.4 G.A.B.A.- Glutamic Acid:

Chlorpromazine liquid membrane also reduces permeability to G.A.B.A. and glutamic acid (Table 7). The major factor for antipsychotic action of chlorpromazine is the reduction in permeability to dopamine(229) which

is under control of glutamic acid G.A.B.A. system(31). Similar to the observation in case of haloperidol(Table 6) addition of G.A.B.A. to chlorpromazine liquid membrane further increased the resistance to flow of dopamine (Table 7), which is possibly because of strengthening of hydrophobic core of chlorpromazine liquid membrane by G.A.B.A. (Fig. 6). Thus increased resistance to the flow of dopamine in presence of G.A.B.A. coupled with resistance to the flow of glutamic acid (Table 7) offered by chlorpramazine appears to have a notable contribution to the mechanism of action of chlorpromazine.

4.5 Reserpine:

4.5.1 Effect on Solute Permeability:

The values of solute permeability (ω) for biogenic amines and amino acids (Table 8) indicate that in both the sets of experiments representing two different orientations of reserpine molecules in the liquid membrane, the permeability values for all these solutes are decreased. However maximum reduction in the permeability values is observed only in second set of experiments where hydrophobic surface of the reserpine liquid membrane is preferentially facing the approaching permeants.

4.5.2 Catecholamines:

Reserpine is known to act by inhibiting intraneuronal storage of catecholamines(230). Though active mechanism i.e. inhibition of ATP-Mg^{++} dependent uptake in isolated chromaffin granules has been considered to be a factor governing this fact(231), effect on other subcellular particles is believed to be by a common, unspecific mechanism(32). Results of these experiments indicate that 'liquid membrane phenomena' can be one such mechanism.

4.5.3 Biological Relevance:

While some of the wide ranging actions of reserpine can be explained on the basis of blocking of uptake of catecholamines(230), a common mechanism to reveal other effects appears difficult. Inhibition of experimentally provoked thrombosis formation in rats(232), decreased oxygen utilisation in the brain(233) and liver(234), antitumour effect(235), extrapyramidal symtoms(236) and reduction of thyroid secretion(237) are a few of them. Impairment of release of catecholamines by reserpine has also been reported(238) for which no explanation at a molecular level is available. The liquid membrane phenomena seems to offer a common explanation to all such effects. Alteration in transport of biologically relevant molecules as a result of the liquid membrane could be a plausible explanation.

4.5.4 5-Hydroxytryptamine:

Reserpine is also known to reduce permeability of biological cells to 5-hydroxytryptamine(238), which may have some contribution to its sedative effect. 5-hydroxytryptamine has also been implicated in causation of extrapyramidal symptoms(214,215). The present experiments also show reduction in permeability of 5-hydroxytryptamine as a result of reserpine liquid membrane. This may have some relevance to explain extrapyramidal symptoms caused by reserpine.

4.5.5 G.A.B.A.

Reserpine is also known to lower threshold to electroshock in rats(239), which is related to depletion of G.A.B.A. in brain. Since reserpine liquid membrane reduces permeability of G.A.B.A. (Table 8), the above effect can at least partially be assigned to formation of liquid membrane in situ.

4.6 Imipramine:

4.6.1 Biogenic Amines:

The values of ω (Table 9) in case of imipramine show a similar trend as observed in case of haloperidol. For biogenic amines, when hydrophilic ends of imipramine liquid membrane are preferentially facing the permeants,

permeability of the solutes is increased while in another orientation of imipramine liquid membrane, when its hydrophobic ends are preferentially facing the permeants the biogenic amines experience some resistance.

4.6.2 Relevance to Biological Action:

The antideprassant action of imipramine is known to be caused by reducing uptake of biogenic amines(240). The results of present experiments appear to have biological relevance. In certain tissues imipramine is known to increase outflow of noradrenaline(241,242). Since one orientation of imipramine liquid membrane (Table 9) has shown increase in permeability of biogenic amines, the biological effects may have similar genesis. However more understanding of orientation of imipramine molecules with respect to the relevant biological membrane is necessary.

4.6.3 Cations:

Permeability of sodium, potassium and calcium ions is reduced (Table 9) in both the orientations of imipramine liquid membrane. The reduction is more, however, when hydrophobic ends of imipramine liquid membrane are preferentially facing the cations. This observation may have some relevance to the effect of imipramine on nerve-conduction.

4.7 General Discussion:

Effects of all these drugs, on transport of relevant biomolecules are examined. A few generalisation appear to be quite revealing.

4.7.1 Effect on Permeability:

All these drugs in one specific orientation i.e. when hydrophobic ends of liquid membranes preferentially face the approaching permeants have shown reduction in transport of permeants across the membrane. This clearly indicates that the liquid membrane formed by these drugs, itself, is capable of reducing the transport. This observation is of special importance because on biological cells, when a drug reduces transport of a permeant, the probable causes are either interaction of the drug with membrane components or effect of the drug itself. The present set of experiments indicate that contribution of the liquid membranes towards resistance to the transport of permeants is also notable, and interaction of these drugs with membrane components may not be the sole cause of reduction in permeability.

4.7.2. Effect on Active Transport:

All these observations (Table 6 - 9) showing transport of the permeants being impeded in presence of surface-active drugs is essentially an effect on passive

transfer. However, this is likely to be accompanied by consequent reduction in active transport as well. The reason is quite evident. Formation of a liquid membrane interposed between a permeant and the active carrier located on the membrane is likely to reduce access of the permeant. If the sequence of events leading to transfer of solute molecules across lipid region of biomembranes are seen(243), the above inference appears to be realistic. Adsorption of the hydrated solute at membrane interface is the initial important event necessary for transport(243). Formation of the liquid membrane may reduce the rate of this very event, leading to reduction in facilitated or active transport.

4.7.3 Orientation of Surfactant Molecules:

One generalisation related to orientation of surface-active drugs forming a liquid membrane appears to be true for most of the permeants, so far as resistance to their transport is concerned. The reduction in their transport is maximum when hydrophobic groups of the drug liquid membrane preferentially face the permeants. Since the drugs under investigation are known to act either by reducing the action of respective agonists at their receptors (e.g. haloperidol, chlorpromazine) or by preventing uptake of catecholamines

(e.g. imipramine, reserpine), this specific orientation of surface-active drugs appears necessary even on biological cells.

This inference has many logical corollaries to follow. If in the case of biomembranes, a surface active drug influencing membrane transport is likely to offer more resistance to permeants when its hydrophobic groups are preferentially facing outside, then the hydrophilic ends of the drug are more likely to be associated with biomembrane. Thus the complementary site on biomembrane with which the surface-active drugs are combining should also be hydrophilic in nature. In other words, for a receptor protein located on the surface of biomembrane, its orientation should be such that the hydrophilic sites face exterior of the cell. This inference is in confirmity with various literature reports about this aspect of receptor orientation(244-246)

Such an orientation of receptor proteins can also be rationalized independently. The receptor, being a membrane component is expected to be surface-active and hence should have hydrophilic and hydrophobic moities in its structure. Since the exterior environment of biological cells is aqueous in nature, it is logical to expect that in a natural environment, hydrophobic part of the

receptor protein will be associated with the hydrophobic core of the lipid bilayers and only hydrophilic part will face the exterior. It is interesting to note that such an orientation of receptor proteins in general has been predicted recently(244).

It is suggested(245,146) that the receptor can be considered to be composed of two regions. A specific region to fit with active group in the drug molecule and a nonspecific site where the hydrophobic groups are attached, the latter involving a weak hydrophobic interaction. While commenting on micellar properties of drugs, it has been pointed out(16) that in case of surface-active drugs, possession of a well-defined hydrophobic head provides opportunities for hydrophobic interactions and existence of a charged hydrophilic head group provides hydrogen bonding interactions with receptor molecules.

Thus it appears safe to generalise that for a membrane-active drug, hydrophilic sites are important to decide binding with the receptor protein and increased hydrophobicity improves probability of such drugs reaching to receptor sites. Thus increase in hydrophobicity is likely to increase effectiveness of a drug making it biologically active even at a comparitively lower

concentration than its prototype drug, so long as hydrophilicity of the drug is unaltered. On the other hand, any alteration in hydrophilic sites of a drug is likely to alter its binding site and therefore may influence the spectrum of biological action shown by the drug.

Efforts have been made(247-249) to discuss the nature of hydrophilic and lipophilic groups in the drugs, their size, shape and position in the drug. Influence of these factors on properties such as surface tension reduction, micelle formation, wetting have also been discussed(247-249). However the relationship between surface activity and molecular structure is dealt with in a qualitative or descriptive manner only.

The dependence of drug activity on lipophilicity of the drugs is a thermodynamically predictable inference(246). The dependance is not linear. There is a limit to which increase in lipophilicity can increase effectiveness of the drug. As a result, relation between drug-activity and lipophilicity is "parabolic" in nature. This "parabolic" nature can be predicted theoretically(246). For development of such a model few assumptions are necessary.

One of the necessary assumption is that lipoidal compartment of the biophase has greater affinity for the hydrophobic part in the structure of the drug than the affinity of receptor for it. Thus it predicts that recepter site should be more polar than the general lipoidal biophase.

4.7.4 Limitations of the Generalisation:

The preceeding discussion makes some generalisations about orientation of receptor molecules with respect to the drug. These generalisations may be true only with membrane active drugs and when a drug is likely to get incorporated in the hydrophobic core of lipid bilayer, there may be some departures from these generalisations. In spite of several efforts towards isolation of receptor proteins, the molecular integrity or 'the physiological state of these molecules' remains incompletely understood. Under such circumstances, conclusions drawn from the experiments, as attempted in the thesis, are expected to offer an important clue to orientation of surface-active drug molecules in relation to the receptors. Relevance of these experiments have to be appreciated in this context. So long as they offer a clue to the causation of biological effects, the conclusions continue to be 'logical speculations'.

There is one fact which needs special emphasis. The serum concentrations of some of the investigated drugs(244) are less than their CMC values at air/water interface (refer 4.1.1). Then the question can be raised 'whether liquid membrane phenomena continues to offer partial explanation even at such low concentrations?' These drugs are known to be surface-active and hence are expected to accumulate at the biological membranes. Because of their tendency to accumulate at the interface, their serum concentrations may not be correct indicators for their actual concentrations at the site of action. What matters for their action is their concentration at the site i.e. brain. These drugs are known to be concentrated in the brain(250) and hence concentrations at the respective sites may be considerably higher than the serum concentrations. Moreover CMC of a surfactant is a parameter, which depends on environmental conditions e.g. presence of ions, pH, presence of protein etc. Hence CMC, found at air/water interface and that at the actual biological interface may be different. Hence only after determining CMC values on a biologically simulating conditions, more predictable conclusions can be drawn.

CHAPTER - 5

CONCLUSIONS

5. CONCLUSIONS

The important conclusions of the present investigations are summarised below.

5.1 The surface-active neuroleptic drugs viz. haloperidol, chlorpromazine, reserpine and imipramine form a liquid membrane.

5.2 The liquid membrane formed by these drugs reduces transport of biologically relevant molecules like neurotransmitters, cations, etc.

5.3 For the above mentioned effect (2), specific orientation of the molecules constituting liquid membrane is essential.

5.4 The specific orientation demands that hydrophobic groups of these drugs preferentially face the approaching permeants.

5.5 Since receptors for a drug have structural complementarity, the hydrophilic groups of the surface-active drugs are expected to associate with hydrophilic part of the receptor indicating that membrane located receptors should possess hydrophilic groups projecting towards exterior of the cell.

5.6 Thus the liquid membrane formation by surface-active drugs appears to be one of the notable steps in the mechanism of their action.

CHAPTER - 6

FURTHER INVESTIGATIONS

- A SUGGESTION

6. Further Investigations - A Suggestion:

Listed below are some of the possible offshoots of the present work to confirm and further explore application of these findings.

6.1 The liquid membrane formed by the drugs can be characterized further to seek following information.

a) Thickness of the liquid membrane.

b) The state of organization of individual molecules in the liquid membrane e.g. liquid expanded film, liquid condensed film.

c) Measurement of surface pressure, surface viscosity, compressibility of the liquid membrane in order to characterize it further.

d) By selecting an extremely sensitive method for estimation of transported permeants (e.g. radioactivity), the specificity of the liquid membrane towards different permeants can be studied at a lower concentration.

6.2 The liquid membrane phenomena can be further investigated by extending it to other classes of surface - active drugs.

6.3 It can be further investigated whether surface-active drugs interact with phospholipids of biological importance, and whether this interaction can alter transport properties of the phospholipid liquid membrane.

6.4 Some efforts to simulate facilitated and active transport of cations on lecithin-cholesterol liquid membrane(251) are in progress. If these experiments succeed, then after incorporating enzymes like Na-K-ATPase in the phospholipid liquid membrane, effect of surface-active drugs can be reinvestigated to study the effect on reconstituted membranes.

APPENDIX

Hydraulic Conductivity Coefficient:

It is the volume of water flowing through a membrane per unit area of the membrane per unit pressure applied.

Definition $L_P = \frac{Jv}{\Delta P}$

where L_P : hydraulic conductivity coefficients ($m^3 s^{-1} N^{-1}$)

Jv : volume flux per unit area of membrane ($m.s^{-1}$)

ΔP : pressure difference (Nm^{-2})

Mosaic Membrane Model(214-216):

According to this model, the membrane is considered to be composed of two regions of different transport properties, and the net flux of the permeant can be calculated on the basis of fractional coverage of the area occupied by the respective regions.

Thus according to Kesting's hypothesis(26) if the area covered by the surfactant layer liquid membrane on the supporting membrane is complete at CMC of the surfactant then at any concentration less than CMC of the surfactant a corresponding fraction of

the supporting membrane will be covered by it.

e.g. If A is the area of the supporting membrane and A^c represents the area occupied by the surfactant layer liquid membrane at concentration C, (C < CMC) and A^s represents the remaining area (that of the base supporting membrane), and L_P^c and L_P^s represent corresponding hydraulic conductivity coefficients, then

$$\therefore Jv = L_p . \Delta P$$

$$\therefore Jv = \left[L_P^c \left(\frac{A^c}{A^c + A^s} \right) + L_P^s \left(\frac{A^s}{A^c + A^s} \right) \right] . \Delta P$$

Coefficient of Solute Permeability (ω) (208-209)

It is the rate at which a solute is transferred per unit osmotic pressure, per unit area of the membrane, when the volume flux because of osmosis is reduced to zero.

Definition:

$$\omega = \left(\frac{Js}{\Delta \pi} \right)_{Jv=0}$$

where ω = coefficient of solute permeability (moles $s^{-1}N^{-1}$)

Js = moles of solute transferred per unit area of membrane, per unit time (moles $m^{-2}s^{-1}$)

$\Delta\pi$ = osmotic pressure (Nm^{-2})

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List of Publications

1. Liquid membrane phenomena in haloperidol action, J. Pharm. Sci. (to appear in May 1982 issue).

2. Liquid membrane phenomena in imipramine action, J. Colloid Interface Sci., 87, 56, (1982).

3. Liquid membrane phenomena in reserpine action, J. Pharm. Sci., (accepted).

4. Liquid membrane phenomena in chlorpromazine action, (communicated).