CHAPTER V

SPIN LABEL STUDIES ON THE MECHANISMS OF ACTION OF LOCAL ANAESTHETIC DRUGS ON HUMAN ERYTHROCYTE MEMBRANE

5.1. INTRODUCTION

In recent years, a few drugs in the general class of anaesthetics, analgesics and tranquillizers have been shown to sensitise anoxic bacterial and mammalian cells to ionizing radiation. These drugs have the common property of their primary action on cell membrane and are popularly called membrane specific drugs. The radiosensitising ability of these drugs holds a great promise in radiotherapy of cancer. The mechanism of their action, however, has not been satisfactorily understood to-date.

Various physical techniques have been used to investigate molecular mechanisms of interaction of the drugs with lipids and proteins in model systems. These studies have provided valuable information regarding drug-membrane interactions. For instance, it has been demonstrated that a relation exists between the potency of local anaesthetic drugs and their behavior in model membrane systems (1,2). However, the precise sites of action of these drugs in the membrane have not been understood.
Spin label method of Electron Spin Resonance is a suitable tool to directly observe drug-induced changes in the membrane. The technique is based on the line shape changes in the electron spin resonance spectrum of a nitroxide free radical. The molecular motion of the radical probe provides information on the surrounding environment it faces. The technique is capable of giving information on the polarity of medium as well as molecular properties of the binding sites. This information is not available from other techniques such as radioactive labeling.

The aim of present work was to investigate the effect of local anaesthetic drugs, procaine and tetracaine, on cell membrane. For this purpose, intact erythrocyte as well as ghosts were used. Spin label probes used were those which can covalently bind with the sulfhydryls of proteins such as N-ethylmaleimide spin label and iodoacetamide spin label as well as those which can easily penetrate and localize within the lipid molecules of lipid-bilayer such as phosphatidylcholine spin label (fig. 1b). Effects of local anaesthetic drugs procaine and tetracaine on the ESR spectra of spin labeled membrane were investigated. Results have offered explanations to a number of observations on radiosensitising ability of these drugs. An attempt has also been made to see if any relation exists between effect of drugs on the membrane and their anaesthetic potency.
Fig. 1(a) Chemical structure of local anaesthetics used

Fig. 1(b) Chemical structure of the spin labels used:
1. Phosphatidyl choline
2. Iodoacetamide
3. N-Ethylmaleimide

Fig. 2. Formation of the triplet nitroxide e.s.r. spectrum for a label in solution. The transitions are equidistant and take place between levels of the same nitrogen quantum number. Ref. (15).

Fig. 3. Electron spin resonance spectrum of tempo (label) partitioned between phospholipid vesicles and their aqueous environment; A, spectrum of the bound label; B, spectrum of tempo in aqueous solutions. Ref. (15).
5.2. THEORY OF SPIN LABELS: INFORMATION IN A NITROXIDE SPIN LABEL SPECTRUM

Basic principle of ESR spectroscopy in the study of free radicals has been discussed in Chapter II. In this section effort will be made to briefly discuss various useful parameters of the nitrooxide spin label spectra and relevance of these in the study of problems in membrane research.

The single esr line of unpaired electron on a nitrooxide is split into a triplet due to hyperfine interaction of nitrogen nucleus. The nitrogen nucleus has a spin of 1 and can be polarized with its spin parallel, perpendicular or antiparallel to the static magnetic field. Typical hyperfine Zeeman energy levels of a nitrooxide radical are shown in fig.2.

The hyperfine electron-nuclear interaction, and hence the line splitting, is due to two separate magnetic interactions:

1) Isotropic coupling is due to $\pi$-character of the electronic orbital and is proportional to the probability of overlap of the electron and nucleus in space.

2) Anisotropic magnetic dipolar interactions between the electron and nuclear spins.

All other magnetic interactions i.e. nuclear Zeeman
terms etc. between the unpaired electron and nitrogen nucleus are quite small and can be neglected. This can be formalized in terms of the spin Hamiltonian as follows:

\[ H = \beta H \cdot g \cdot \mathbf{S} + h \mathbf{S} \cdot \mathbf{A} \cdot \mathbf{I} \]  

(5.1)

Zeeman term \hspace{1cm} Hyperfine term
(electron spin- \hspace{0.5cm} (nuclear-electronic
applied field interaction) interaction)

where \( \mathbf{S} \) and \( \mathbf{I} \) are electron spin and nuclear spin operators, 'H' is applied magnetic field vector and 'g' and 'A' are two tensors which describe the interaction of the unpaired electron with the magnetic field and with the nitrogen nuclear magnetic moment.

5.2.1. **THE HYPERFINE COUPLING AND g-TENSOR**

The evaluated values for g and A are anisotropic (3). These tensors are expected to be anisotropic because the molecular orbital containing the unpaired electron is made up in part of a degenerate oxygen \( \pi \)-orbital i.e. 

\( g_{xx} \neq g_{yy} \neq g_{zz} \) and \( A_{xx} \neq A_{yy} \neq A_{zz} \) (3). The hyperfine coupling tensor is however, most often nearly axially symmetric \( A_{zz} \neq A_{xx} = A_{yy} \) because of the occupation by the unpaired electron of a molecular orbital of \( \pi \)-symmetry (4). If the nitroxide free radical is oriented rigidly in a crystalline matrix, the magnetic parameters of g-value and hyperfine coupling can be determined in molecular frame. Three principal values are found, depending on the orientation of
N-O bond relative to the direction of applied magnetic field (5,6). Following values were obtained by doping the nitroxide label on an appropriate host whose crystal characteristics are already known (7).

\[
g_{xx} = 2.0089 \quad A_{xx} = 5.8 \text{ G (16.2 MHz)}
\]
\[
g_{yy} = 2.0058 \quad A_{yy} = 5.8 \text{ G (16.2 MHz)}
\]
\[
g_{zz} = 2.0021 \quad A_{zz} = 30.8 \text{ G (86 MHz)}
\]

The hyperfine coupling values along the principal molecular axis are essentially independent of the molecule to which the free radical is covalently linked.

The conventional axis system for nitroxide is as follows:

\[\begin{array}{c}
\text{Z} \\
\text{X}
\end{array}\]

Above experimental values indicate that the hyperfine coupling is largest and the g-value smallest when the magnetic field is applied in a direction perpendicular to both N-O bond and the ring of the free radical (i.e. in Z-direction). The hyperfine is symmetrical in the
plane of the ring, but the g-value parallel to the N-O bond (X-axis) is larger than the g-value perpendicular to the bond (Y-axis). These parameters can be used to obtain information about the orientation of the label in a rigid matrix. In dilute solutions, however, the isotropic values $A_o = 1/3 (A_{xx} + A_{yy} + A_{zz})$ and $g_c = 1/3 (g_{xx} + g_{yy} + g_{zz})$ are obtained due to averaging i.e. the average of principal values of hyperfine coupling is equal to the isotropic value. Similarly the g-values in solution are average of the three principal values in the molecular frame.

5.2.2. INFORMATION ABOUT THE POLARITY OF ENVIRONMENT

The nitroxide is a resonance hybrid of the structures

\[ \begin{align*}
\text{N} & \quad \text{N}^+ \\
\text{O}^- & \quad \text{O} \\
\end{align*} \]

The charged configuration contributes to the structure to a greater extent when the nitroxide is in a polar environment of high dielectric constant, such as water, than when it is in a nonpolar hydrocarbon environment. Because of the ionic character of nitroxide bond, the values of g- and hyperfine tensor components of a nitroxide will vary with the polarity of the environment. The higher spin density on the nitrogen in a polar environment, leads to the increase in the isotropic
hyperfine coupling with the dielectric constant of the medium from 15.2 Gauss in hexane to 17.1 Gauss in water (8,9). The average g-factor decreases as the polarity of the solvent increases but is less sensitive to the medium polarity as can be seen from the values obtained in water \((g = 2.0056)\) and hexane \((g = 2.0061)\).

The sensitivity of hyperfine coupling and g-factors to the polarity of medium has been used as a criterion for determining the localization of the probe in the membranes or in aqueous environments (10). Provided the exchange between the two environments is slow \((< 10^6 \text{ per sec.})\), two superimposed spectra are observed (fig.3). The partition of the label can be determined from the ratio of the two amplitudes, provided the line widths and shapes are the same in both environments.

5.2.3. INFORMATION FROM MOTION OF SPIN LABELS

The esr spectrum of a nitroxide is extremely sensitive to the nature and rate of the motions the label undergoes. If the nitroxide label tumbles rapidly in solution such that during one oscillation of microwave field it can assume many orientations relative to the static field, then anisotropic dipolar hyperfine coupling is averaged to zero and observed coupling is only due to the isotropic interaction (fig. 4a). The isotropic parameters \(A_o\) and \(g_c\)
are observed giving narrow triplet esr lines of equal width if the nitroxide rotates symmetrically at a rate greater than the frequency corresponding to the largest differences between the principal components of the hyperfine coupling tensor \( (>A_{zz} - A_{yy}) \approx 73 \text{ MHz} \) and g-tensor \( (>g_{xx} - g_{zz}/B_\text{H}^{-1}) \approx 29 \text{ MHz} \) for X-band spectrometer operating at a field of about 3.3 kG.

The anisotropies in the magnetic interactions are averaged out due to fast rotation of the molecule in solution but the electron spin experiences fluctuations in its magnetic environment because during molecular motion, its orientation remains either parallel or antiparallel to the magnetic field. The motional freedom of a molecule is measured in terms of correlation time. The rate of rotation is characterized by rotational correlation time \( (\tau_c) \) which is defined as the inverse rate of the time it takes a molecule to rotate through an angle of 1 radian \( (\tau_c) \). It can be thought of as the time required for the molecules to forget what their previous spatial orientations were. If the rotational correlation times are small \( (10^{-12} - 10^{-9} \text{ sec}) \), the oscillating magnetic fields will be averaged out and the signal will be sharp (fig. 4a). As the rate of molecular rotation is reduced \( (10^{-7} - 10^{-9} \text{ sec}) \), the fluctuations experienced by the electron spin will cause broadening of
Fig. 4. Effect of motion on the e.s.r. spectrum of temga (label 1): (a) $T_c < 10^{-12}$-10^{-10}$ sec; (b) $T_c = 10^{-7}$-10^{-5}$ sec; (c) $T_c > 10^{-7}$ sec; (d) polycrystalline or "rigid-glass" spectrum. Ref. (15).

Fig. 5. Anisotropic spectrum of a fatty acid spin label in phospholipid vesicles. Ref. (15).
esr signals (fig. 4b) but without affecting the averaging of the anisotropic hyperfine coupling.

The broadening of esr lines due to slower rotational motion of molecules is because of two independent processes: (i) The components of fluctuating fields perpendicular to the spin orientation and fluctuating at the resonance frequency will aid the applied oscillating field (incident radiation) and induce further electronic transitions between the energy states i.e. relax the electronic spin. In other words, the effect of oscillating magnetic field would be to shorten the life time of the electron in its energy state which will in turn broaden the esr line. Furthermore, the slower fluctuating fields in a direction parallel to the applied static field will increase the width of energy levels and thus also broaden the signals. The broadening of nitrooxide radical signal depends on the orientation of the nitrogen nucleus in the field. The high field line (the nitrogen spin oriented antiparallel to the magnetic field, $M = -1$) will be broader than the low field line (nitrogen spin parallel to the magnetic field, $M = +1$), while the centre line ($M = 0$) will be least affected for isotropic tumbling (fig. 4c). Expressions have been derived for calculating the rotational correlation times for isotropically tumbling molecules from the line width, provided their rotational correlation times are in the range of $10^{-8} - 10^{-12}$ sec (8,11,12).
ESR SPECTRUM OF 'STRONGLY IMMOBILIZED' LABELS

From fig. 4 it can be seen that the esr spectra of nitroxide radicals are very sensitive to the rate of molecular rotation and cover a range of correlation times from $10^{-10}$ sec (spectrum of three narrow lines of almost equal width) to $10^{-7}$ sec (a strongly immobilized spin label spectrum). The general applicability of the technique to problems of molecular biology lies in this property of spin labels since correlation times of most biological molecules fall in this range.

Incomplete averaging of the anisotropic dipolar hyperfine coupling occur when rotational correlation times becomes higher than $10^{-8}$ sec (fig. 4c). Such a spectrum is said to arise from strongly immobilized labels. In addition, the esr spectrum of a nitroxide completely immobilized and randomly oriented in a rigid glass at liquid nitrogen temperature gives what is called 'rigid glass' or 'polycrystalline' spectra (fig. 4d). This spectrum arises due to envelop of spectra obtained from randomly oriented nitroxide in respect to the applied magnetic field. Polycrystalline spectra are the limit approached by esr spectrum of nitroxide labels which are totally constrained by a large macromolecule of very long correlation time i.e. in this situation the spin labels can
be imagined to stand still with random orientations (in practice however, spin label will often have independent motion relative to that of constraining macromolecule).

Rigid glass spectra are observed not only with 'polycrystalline' samples, but also in systems, where the nitroxide undergoes fast axial rotation while the reorientational rate of the axis relative to the magnetic field is slow ($<<10^{-10}$ sec) as can be seen in fig. 5.

The esr spectrum called strongly immobilized spin labels is most often observed when the motion of a spin label is severely restricted by the structures of the macromolecules to which it is attached. The spectra arising from these anisotropic motions are not suitable to estimate rotational correlation time by line width measurements. However, it has been shown recently that the power saturation behaviour of the esr spectra of strongly immobilized labels can be useful in estimating correlation times (13).

Furthermore, a useful experimental parameter of esr spectra arising in this range of motion is the separation between the two extreme components of the esr spectra. In the limit of the complete immobilization of the label on a macromolecule of a very long correlation time the separation corresponds to $2A_{zz}$. As the site of attachment expands, permitting only restricted rotation in relation to the macromolecule, the
separation decreases. Thus, although the estimation of correlation times from strongly immobilized type of spectra are difficult, the measurement of separation can be used as qualitative estimate of degree of immobilization of spin labels.

It may be noted that here only a qualitative picture of information obtainable from an esr spectra of a nitrooxide molecule has been given but several books and articles have already appeared which deal with the spin label theory in greater details \cite{6,8,14,15} and wherein various applications of spin labels in medical and biological research have been discussed \cite{16}.

5.3. EXPERIMENTAL

Spin labels known to covalently bind with the sulphydryls and used in present experiments are N-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl) iodoacetamide (ISL) and N-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl) maleimide (MSL). Phosphatidyl choline (PC) spin label was used to probe the hydrophobic region of the lipid bilayer. Chemical structures of the drugs and the labels used have been shown in fig. 1a and 1b.

Fresh human red blood cells were washed with 0.9% NaCl containing phosphate buffer until there was no color
observed in the supernatant and the washed blood was used within a day. The ghost membranes were prepared according to Dodge et al., method (17).

Labeling of intact cells or ghosts was carried out by putting in contact with the label solution in PBS at 10:1 ratio for overnight at 4°C under constant stirring condition and the unreacted labels were removed by washing. The labeled ghosts were washed with PBS until no esr signal was observed in the supernatant.

Labeling of intact cells or their ghosts with phosphatidyl choline spin label was carried out by mixing equal volume of washed packed cells and a liposome of the latter and subsequent incubation at 37°C for nearly one hour. The unreacted labels were removed by repeated washing with phosphate buffer saline.

ESR spectra were recorded at room temperature on a ZEOL X-band spectrometer under normal operating conditions. All the solutions were prepared in buffers and for the highest concentrations of drugs studied the pH was never lower than 7.2.

5.4. EFFECT OF PROCAINE AND TETRACAINE ON THE ESR SPECTRA OF ISL AND MSL LABELED MEMBRANE

The esr spectra of ISL and MSL labeled ghost membrane are shown in fig. 6 and fig. 7 respectively. The
Fig. 6. (A) E.s.r. spectra of (a) iodoacetamide spin labeled erythrocyte ghosts (b) the labeled ghosts containing 100 mM procaine.

Fig. 6. (B) E.s.r. spectra of (a) iodoacetamide spin labeled erythrocyte ghosts and (b) the labeled ghosts containing 100 mM tetracaine.
Fig. 7. (A) E.s.r. spectra of (a) N-ethylmaleimide spin-labeled erythrocyte ghosts (b) the labeled ghosts containing 100 mM procaine.

Fig. 7. (B) E.s.r. spectra of (a) N-ethylmaleimide spin-labeled erythrocyte ghosts (b) the labeled ghosts containing 100 mM tetracaine.
site of attachment of these labels on the membrane can be assumed to be the sulfhydryl groups since no binding occurs if the ghosts were pretreated with sulfhydryl binding agent (18). It can be seen from fig. 6 that binding of the labels with the erythrocyte ghosts immobilizes the labels. The major fraction of labels reacted at those sites which impose partial constrain on their motion. It can be seen, however, that a small fraction of labels reacted at those sites which strongly immobilized the labels.

The composite esr spectra obtained from maleimide spin labeled ghosts (fig. 7) arise from partially and strongly immobilized labels. It can be seen that a considerable fraction of labels attach with the sites on membrane which strongly immobilize their motion. Although, maleimide is known to react with sulfhydryl groups (19,20) as well as with imidazole and aminogroups (21,22), the evidence has been presented to show that MSL binds to sulfhydryls on the membrane ghosts (23). Labels used in present experiments also reacted with the sulfhydryl sites of proteins.

The esr spectra of IBL labeled ghosts did not show any significant change on drug treatments. However, both procaine and tetracaine were found to convert the partially hindered labels to the strongly immobilized labels in MSL labeled ghosts (fig. 7). This change varied with the
Fig. 8. Effect of drugs on erythrocyte ghosts. (a) Procaine (1 - 100 mM) (b) Tetracaine (1 - 100 mM).

Nitroxide spectral lines can be taken as a measure of the
concentration of the drugs (0.1 mM - 100 mM) as can be seen in figs. 8a and 8b. The original spectra were, however, obtained once the drugs were washed off from the membrane indicating that drug induced changes in membrane were purely physico-chemical in nature. It can be seen from fig. 9 that ISL and MSL attach to intact erythrocytes at places which give both weakly and strongly immobilized spectra. The effect of drugs on intact cells were not however investigated in the present work but are expected to be similar as those observed in case of their ghosts.

An effort was made to see the effect of metal ions such as Ca$^{++}$ and Mg$^{++}$ on the ISL and MSL labeled membrane. It was found that in the concentration range of these metal ions studied (1-10 mM) no noticeable change occurred in the esr spectra.

5.5. EFFECT OF PROCAINE AND TETRACAINE ON THE ESR SPECTRA OF PHOSPHATIDYL CHOLINE SPIN LABELLED MEMBRANE

The esr spectra of phosphatidyl choline spin labeled intact erythrocytes is shown in fig. 10. The spectral features indicate that the labels are undergoing anisotropic motion and that these are incorporated into the lipid bilayer portion of the membrane. The splitting of nitrooxide spectral lines can be taken as a measure of the
Fig. 9. E.S.R. spectra of (a) N-ethylmaleimide spin labeled intact red blood cells (b) Iodoacetamide spin labeled intact red blood cells.
fluidity of membrane i.e. larger and smaller splittings 
correspond to more rigid and fluid membranes respectively. 
The overall splitting of phosphatidyl choline spin label 
was 52.5 G and the isotropic hyperfine coupling constant 
was 14.7 Gauss. The overall splitting value of PC * labels 
suggests that the labels are present in fluid region of 
membrane. The effect of procaine and tetracaine drugs on 
the PC * labeled cell membranes was examined at non-hemolysing 
as well as at hemolysing concentrations of the drugs.

Tetracaine causes spectral changes at non-hemolysing 
(upto 5 mM) concentration (fig. 10 ) whereas procaine showed 
opt no effect upto these concentrations. It can be further seen 
that some change occurs at 50 mM (hemolysing) concentration 
of tetracaine whereas no significant change was noticed by 
procaine even upto 100 mM concentration.

The esr spectra of phosphatidyl choline spin 
labeled ghosts (fig. 11 ) indicate that the labels were 
present in the fluid region of lipid bilayer and were 
undergoing anisotropic motion. Effect of tetracaine (0.1 - 
50 mM) and procaine (0.1 - 100 mM) were studied and it was 
observed that both the drugs induced changes in the spectra 
of labels but tetracaine appears to be more effective than 
procaine (figs. 11a & 11b).

Metal ions Ca ++ and Mg ++ were found not to show
Fig. 10. Effect of drugs on e.s.r. spectra of phosphatidylcholine spin-labeled intact red blood cells.

(A) Procaine Hydrochloride

(a) Phosphatidylcholine labeled red blood cells.
(b) 10 mM Procaine (c) 50 mM Procaine (d) 100 mM Procaine.

(B) Tetracaine Hydrochloride

(a) Phosphatidylcholine spin-labeled red blood cells (b) 1 mM tetracaine (c) 10 mM tetracaine (d) 25 mM tetracaine.
Effect of drugs on E.S.R. spectra of phosphatidyl choline (PC*) spin labeled erythrocyte ghosts.

(A) Procaine Hydrochloride
- (a) PC* labeled ghost membrane
- (b) 10 mM Procaine
- (c) 50 mM Procaine
- (d) 100 mM Procaine

(B) Tetracaine Hydrochloride
- (a) PC* labeled ghost membrane
- (b) 1 mM Tetracaine
- (c) 50 mM Tetracaine
- (d) 100 mM Tetracaine
any effect on \( R^{*} \) labeled membrane of either intact cells or their ghosts.

5.6. DISCUSSION

The esr spectra of ISL and MSL spin labeled membrane indicate that sulfhydryls of proteins are present at least in two different environments. On one class of sulfhydryls, the motion of the labels on binding was hindered to a smaller extent than the other. These are possibly present at the surface of the membrane facing polar environment. Spectra arising from labels bound to these sites are called 'partially immobilized' (PI) labels. It can be seen from the spectra that ISL mainly attach to these sites in membrane. MSL on the other hand gets attached to the sulfhydryls at the surface as well as to those which imposed stronger restrictions on their motion and are called 'strongly immobilised' (SI) labels. The latter group of sulfhydryls may be deeply buried inside the membrane and/or may be existing in an intimate association with the lipid bilayer.

It is believed that location of sulfhydryls in the membrane decides their role in cell function and the present observations indicate that spin labels can provide information on the changes in environments of these sites.
in membrane induced by external agents including various drugs. These investigations are of direct relevance in structure-function relationship of the cell membrane.

Present results have shown that procaine and tetracaine produce disorganization/fluidisation in the lipid bilayer resulting in changes in the proteins. This can be seen from fig. 8 that the drugs are able to convert the partially immobilized labels to the strongly hindered environment. This effect of the drugs was reversible i.e. the original spectra were obtained on washing off the drugs. These observations provide indications with regard to the radiosensitising effect of these local anaesthetic drugs in bacterial systems. For instance, it has been reported that procaine enhances the killing of *E. coli* B/r cells under anoxic conditions of irradiation (24). The possibility of involvement of a short-lived transient of the drug in sensitisation process was ruled out. It was suggested that a non-radical process may be operative and the effect may be brought about by the interaction of the drug on the cell membrane. Present results have demonstrated that procaine affects the membrane structure and gives rise to changes in environments of proteins. It is thus possible that enhanced radiation cell killing in presence of the drug may be due to interference with the cellular repair machinery.
Present results also explain the observation why enhanced cell killing does not occur once the cells were irradiated after washing off the drug. That the action of drug does not cause any permanent effect on the membrane was established from the fact that drug-induced spectral changes were reversible once the drug was washed off.

Further, it has been found that at the same concentration procaine was more effective than tetracaine in converting the partially hindered labels to strongly immobilized ones (figs. 8a and 8b). This can be explained on the basis of their structural differences (fig. 1a). Procaine contains primary amino group and may not be able to penetrate in the interior hydrophobic region whereas tetracaine has a secondary amino group and thus possesses relatively more hydrophobic character and can penetrate deeper in the central lipid bilayer. This would mean that the effective concentration of procaine may be higher near polar region of bilayer and that of tetracaine may be higher in the central part of bilayer. Thus procaine may cause more perturbation in the vicinity of polar region, possibly reflecting in the change in environment of proteins. The latter may not be affected that efficiently by tetracaine as it will mainly induce disturbances in the centre of bilayer. A similar observation has been made on the
differential fluorescence quenching behaviour of procaine and tetracaine using N-Octa-decyl naphthyl 2-amine 6-sulfonic acid (OCS) fluorescence probe (25). The probe is expected to be present in polar region of bilayer and procaine has been found to quench the fluorescence much efficiently than tetracaine.

Phosphatidyl choline spin labeled intact cells were affected by tetracaine at non-hemolyzing concentration (< 5 mM) whereas procaine showed no effect on the spectra at this concentration. It is seen from spectral changes with the concentration of tetracaine that the drug causes fluidisation of the membrane bilayer. However, it is not clear whether fluidisation of membrane is preconditon or prestage of hemolytic process. A further investigation in this direction is warranted.

In addition, the esr spectra of red blood cell ghost membrane labeled by phosphatidyl choline were found to be affected by tetracaine at higher concentrations. Procaine, however, showed no effect up to 100 mM. These observations can be interpreted in terms of relatively greater hydrophobic character of tetracaine and hence its ability to penetrate into the hydrocarbon interior. The differential effect of tetracaine and procaine on PC* labels in the bilayer can be explained in terms of their effective
concentration. This interpretation is compatible with the efficient quenching of 12-(9-anthroyl) stearic acid (AS) fluorescence by tetracaine than procaine (25). The latter fluorescence probe is supposed to be a probe of lipid bilayer interior. Interpretation of present results finds support from nuclear magnetic resonance data (26). The differential line broadening of proton nmr signals of procaine and tetracaine in artificial membrane has been explained in terms of their relative polar and hydrophobic character (26). These results indicated greater hydrophobic interactions of tetracaine than that of procaine. These observations, similar to spin label results, are consistent with their relative conduction blocking efficiency in frog sciatic nerves (27) and their ability to protect red blood cells against hypotonic hemolysis (28, 29).

In conclusion, present results have demonstrated that both procaine and tetracaine are able to induce changes in lipid bilayer of membrane. The drug effects on membrane are however, reversible in nature. This explained the absence of any residual sensitising effect on cells when the drugs were washed off. Procaine may be localized at higher effective concentration at or near the outer polar layer of membrane than tetracaine. The enhanced killing of cells to radiation in presence of drugs may be due to drug induced alterations in membrane. Tetracaine may localize in the hydrophobic bilayer interior greater than procaine and these
differences can be due to their relative hydrophobic character. The greater effectiveness of tetracaine as compared to procaine on the labels in lipid bilayer is in direct relation with its anaesthetic potency. The drugs, by their action on membrane, potentiate the effect of radiation on cells.

5.7. REFERENCES


