EXPERIENTIA

Vol. 29 – Fasc. 5 Pag. 509–644 15. 5. 1973

Magnetic Resonance Methods in Membrane Research

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In a collection of essays by Max Planck, entitled 'Thoughts and Memories', the remark can be found that a scientist must have great belief in the inherent ordering forces of nature. This strength of belief in the ultimate ordered structure in nature is necessary to some degree for each part of our subject. The above title, 'Magnetic Resonance Methods in Membrane Research', includes two quite different problems. On one hand it deals with the magnetic properties of atoms and electrons which are almost the simplest building stones of nature; on the other hand it concerns the examination of biological membranes, which are most complicated structures, formed from many large and small molecules and having a long history of biological evolution.

The first successful experiments to show magnetic resonance phenomena were carried out towards the end of 1945. At that time it would have needed great faith and almost prophetic powers to anticipate the dynamic developments in this field, starting from what would now be regarded as simple experiments on paraffin wax. Today we have very detailed knowledge about almost everything connected with the magnetic properties of the nuclei and electrons. If we consider the definition of KANT that science is only true science in so far as it contains mathematics, then nuclear magnetic resonance (NMR) and electron spin resonance (ESR) can be cited as excellent examples of this concept.

In membrane research, however, we are in a less happy position, although the discovery of the cell membrane goes back to the end of the previous century, namely to the osmotic experiments of W. PFEFFER[1]. Since the cell membrane cannot be seen in the optical microscope – we now know that its average thickness is 60 to 100 Å (100 Å = 10^{-6} cm) – and since it often attaches itself to other structures, it was 30 years after the first propositions of PFEFFER before even the existence of cell membranes was generally accepted. The most important instrument to establish their existence proved to be the electron microscope, which was developed around 1930. Not only did the electron microscope pictures show clearly that the whole cell is enclosed by a thin membrane (the cytoplasmic membrane) but that the various organelles in the cell such as the cell nucleus, the mitochondria, the Golgi apparatus, etc. are also held together by membranes of similar thickness (intracellular membranes). The next steps were the biochemical isolation of such membranes, the determination of their chemical composition, the allocation of specific functions such as ion transport, oxidative phosphorylation, etc. to certain membrane types, and finally the examination of the membrane structure by physical-chemical methods. In all these investigations we are already moving on very uncertain ground. The isolation of a particular membrane in the purest possible form is difficult, and even if this is to some extent successful, there can be regions in this membrane which are chemically, structurally, and functionally distinctly different from one another. The separation of cell membranes into homogenous components has as yet only been achieved in a few cases. Also in relation to the basic chemical units of a membrane, there are gaps in our knowledge. The main elements are the lipids (20 to 80% by weight) and the proteins (80 to 20% by weight).

In the case of lipids we are dealing with substances similar to fats, which are practically insoluble in water. They are ideally suitable as an insulation material to separate the various aqueous regions in a cell from one another. The transport of water and salts can thus be efficiently regulated from one side of the cell membrane to the other, partly by the help of proteins. The impermeability of the lipids is so great that even a bimolecular lipid layer provides a sufficient barrier for biological purposes. This represents a lipid separating wall with a thickness of about 40 to 50 Å, and the basic structure of many biological membranes is therefore such a lipid double layer. The membrane proteins are found partly as insertions in the lipid layer or as surface cover on both sides. This very simple model is illustrated in Figure 1.

The chemical difficulties lie in the fact that, although one now has a reasonable knowledge of the lipid

[1] D. BRANTON and D. W. DEAMER, *Membrane Structure* (Springer Verlag, New York 1972).

composition of a number of membranes, there are as yet only suppositions with regard to the structure and function of the membrane proteins and their interactions with the lipids. Here is a field with many problems for biochemistry. Similarly unsatisfying and incomplete is our physical knowledge about the membrane structure. Electron microscopy and X-ray structural analysis have certainly shown us the approximate arrangement of lipids and proteins, but the pictures are very diffuse and moreover static. If the individual molecules in a membrane are really to be seen and particularly if their movements in the membrane are to be investigated, then we need the help of spectroscopic methods, among which the magnetic resonance method is prominent.

In the following discussion some new developments in magnetic resonance will be indicated and their importance for the determination of membrane structure will be considered. The main emphasis will be on questions of method and not on a description of the present state of membrane research.

1. *High resolution magnetic resonance*[2]

Let us confine ourselves to the proton ^1H. Such a proton has the nuclear spin $I = {}^1/_2\,\hbar$ and has at the same time a nuclear magnetic moment of about $\mu = 2.79\,\mu_k$ ($\mu_k = 5.05 \times 10^{-24}$ erg Gauss^{-1}). If such a proton is put in a homogeneous magnetic field, the magnetic moment orients itself in the magnetic lines of force. In this case only a parallel and an anti-parallel orientation with respect to the external field is possible. These two orientations have slightly different energies. By applying an electromagnetic field with a frequency corresponding exactly to the energy difference between the two orientations, transition between the two energy levels can be induced, i.e. the nuclear magnets will turn in the field. This turning absorbs energy from the applied field and can thus be detected by means of suitable devices as an absorption line in the spectrum.

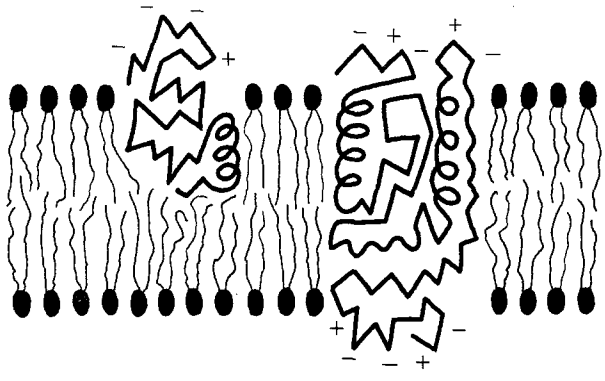


Fig. 1. Simple membrane model: lipid bilayer with proteins [S. J. SINGER and G. L. NICOLSON, Science *75*, 720 (1972)].

The corresponding frequency is called a resonance frequency, and it has been found that an atomic nucleus has slightly different resonance frequencies according to the chemical environment in which it is located. Let us consider for example the molecule decanol and look

$$\underset{1}{HO}\text{–}\underset{2}{CH_2}\text{–}\underset{3}{CH_2}\text{–}\underset{4}{CH_2}\text{–}\underset{5}{CH_2}\text{–}\underset{6}{CH_2}\text{–}\underset{7}{CH_2}\text{–}\underset{8}{CH_2}\text{–}\underset{9}{CH_2}\text{–}\underset{10}{CH_3}$$

at the corresponding ^1H-NMR-spectrum in Figure 2. We recognize essentially 4 absorption lines, which after some experience we can assign to the –OH proton ($\tau \sim 3.2$ ppm), the protons of carbon atom 1 ($\tau \sim 3.6$ ppm), the protons of the methylene groups C-2 to C-9 ($\tau \sim 1.3$ ppm) and to the terminal methyl group ($\tau \sim 0.9$ ppm). (The τ-parameters in brackets are a relative measure of this chemical shift. In this spectrum they refer to the protons of tetramethylsilane, $(CH_3)_4Si$, the chemical shift of which has been arbitrarily set to be 0 ppm. Here we meet the first problem of membrane research by means of nuclear magnetic resonance. This spectrum of decanol shows that the CH_2-protons of groups C-2 to C-9 have such a similar chemical environment that they cannot be separately observed here, i.e. more than 70% of all the protons appear in one single absorption line. For the lipids of a cell membrane, which have hydrocarbon chains almost twice as long, the percentage of unresolved protons would be still more unfavorable. In the last 5 years remarkable progress has been made in the power of NMR to 'see' protons, i.e. the resolving power has been greatly increased.

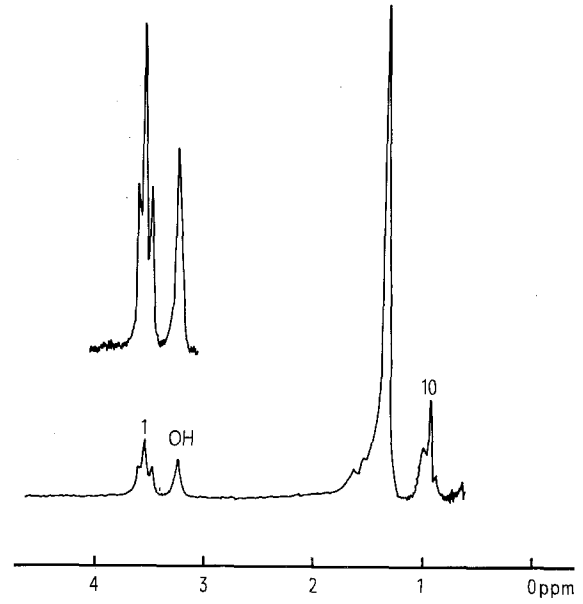
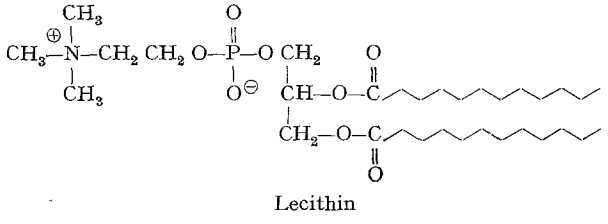


Fig. 2. 100 MHz ^1H NMR-spectrum of n-decanol dissolved in CCl_4 (chemical shifts referred to TMS $\tau = 0$ ppm).

[2] H. STREHLOW, *Magnetische Kernresonanz und chemische Struktur* (Steinkopff Verlag, Darmstadt 1968).

Superconducting magnets

The chemical shift is linearly proportional to the field strength of the external magnetic field, and one possible way to improve the resolution is therefore to increase the field strength. With conventional electromagnets a technical limit is set at about 23.5 KGauss, which corresponds to a resonance frequency of roughly 100 MHz. The spectrum in Figure 2 has been measured using a frequency of 100 MHz, but since the development of superconducting magnets the field strength can be increased several times. Today spectrometers are already in use with a resonance frequency up to 320 MHz and Figure 3 shows again the ^{1}H-NMR-spectrum of decanol, this time measured with a field strength of 63.5 KGauss (at 270 MHz). The protons of the carbon atom 2 are now clearly resolved. Since these methods are quite recent developments, scarcely any results have yet been obtained for biological membranes by this means. Figure 4 shows, however, the 220 MHz spectrum of lecithin, which is an important constituent of many membranes. Although it has a considerably

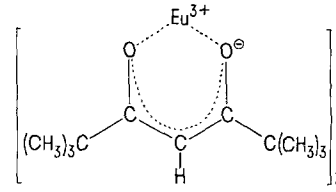


Lecithin

more complicated structure than decanol, good resolution is obtained by means of the larger magnetic field and assignment of many protons is possible.

Shift reagents[3]

Installation and running costs of superconducting magnets are as yet very expensive items. A cheaper (though not always possible) way to spread the spectral lines has been developed since the discovery of the so-called shift reagents in 1969. Here we are dealing with chemical components, which are both paramagnetic (they have thus one or more unpaired electrons) and which also form weakly bound complexes with the substances under investigation. The complexes of the lanthanides have shown themselves specially suitable as shift reagents e.g. *tris* (dipivalomethanato)europium (III), eu(dpm)$_3$:



In this complex, europium has a free coordination site, where a molecule with a functional group like –OH, –CO, or $-NH_2$ can be accommodated. The essential characteristic of the complex is that the unpaired electrons of europium produce a strong magnetic field in the immediate neighbourhood of the metal atom. This microscopic, atomic magnetic field superimposes itself on the external magnetic field of the NMR-spectrometer and can considerably exceed it in effect. Figure 5 shows, as an example, the 100 MHz ^{1}H-NMR-spectra of decanol dissolved in deuterochloroform with varying amounts of eu(dpm)$_3$. With increasing concentration of shift reagent, more and more CH_2-groups are resolved in the spectrum, so that with a high concentration of eu(dpm)$_3$ practically all the ten CH_2-groups can be observed.

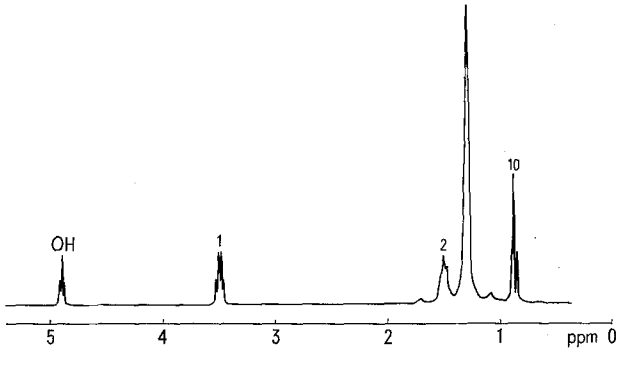


Fig. 3. Superconducting magnet 270 MHz ^{1}H NMR-spectrum of n-decanol.

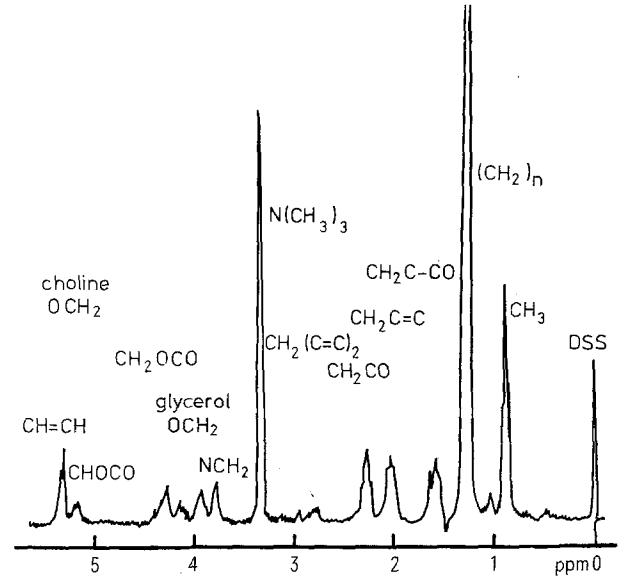


Fig. 4. 220 MHz ^{1}H NMR-spectrum of a natural lipid (egg yolk lecithin) in $CDCl_3$ [E. G. Finer, A.G. Flock and H. Hauser, FEBS Lett. *18*, 331 (1971)].

[3] R. V. Ammon and R. D. Fischer, Angew. Chemie *84*, 732 (1972).

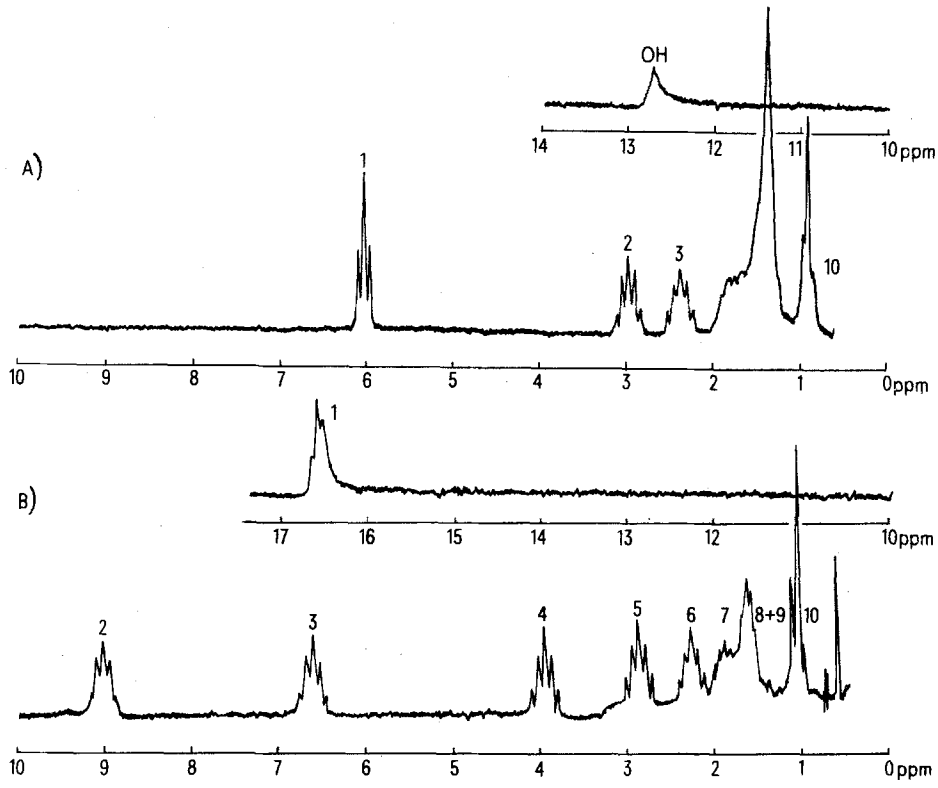


Fig. 5. 100 MHz ^1H NMR-spectrum of n-decanol dissolved in $CDCl_3$ with shift reagent eu(dpm)$_3$. A) $c_{eu}/c_{decanol} = 0.17$; B) $c_{eu}/c_{decanol} = 0.54$ molar ratios.

The use of shift reagents is not confined to proton resonance, as will be shown for the ^{31}P-resonance of phospholipid vesicles[4]. To form such vesicles, lecithin is dispersed in D_2O and the resulting aggregates are broken up by ultrasonification. Spherical structures are then formed – similar to a soap bubble – with a double layer of lipid molecules as an outer membrane (Figure 6). The polar phosphate groups lie on the outer and inner surfaces of this double layer and are in contact with the aqueous phase. The interior of such vesicles is tightly sealed from the outside. Figure 7a shows the normal ^{31}P-NMR-spectrum of such vesicles, which consists of a single sharp line. If a 0.01*M* solution $Pr(NO_3)_3$ is added, then a second equally intense line is observed (Figure 7b). The cause of this new resonance is the adsorption of Pr^{3+} ions on the outer surfaces of the vesicles. The molecular magnetic field of the praseodym only shifts the phosphorus resonance of the outer phospholipids, while the phosphorus resonance of the inner surface, which does not come into contact with the shift reagent, stays unaltered. The use of such shift reagent salts is a very promising method to discover assymetries in natural membranes.

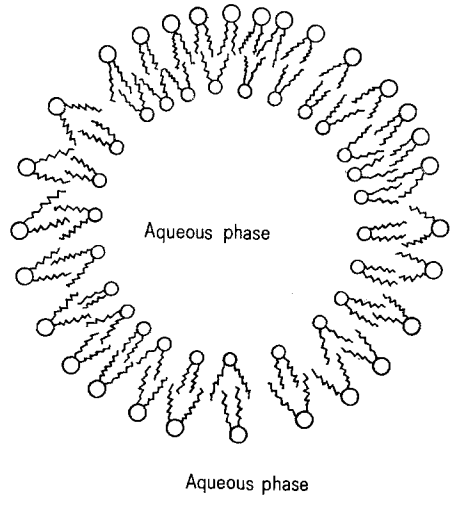


Fig. 6. Schematic picture of a phospholipid vesicle.

Pulse Fourier transform NMR- spectroscopy[5]

Conventional NMR-instruments keep the magnetic field constant, while the frequency of the applied radio waves is continually varied. In this way each absorption frequency is examined and registered in turn (continuous wave method). The recording time for a normal ^1H NMR-spectrum is thus roughly 5 to 10 min. A quite different method is the so-called pulse Fourier

[4] V. F. BYSTROW, Y. E. SHAPIRO, A. V. VIKTOROV, L. K. BARSUKOV and L. D. BERGELSON, FEBS Lett. *25*, 377 (1972).

[5] T. C. FARRAR and D. E. BECKER, *Pulse and Fourier Transform NMR* (Academic Press, New York, N.Y. 1971).

transform spectroscopy, which was first suggested in 1966. By this method all the resonances in the frequency spectrum are excited simultaneously by means of a short and intense high frequency pulse. After the pulse is switched off, the resonances decay slowly, and the various frequencies are superimposed, leading to beats in the radio waves. This interference pattern is recorded. Then, by means of a Fourier transform performed by a fast computing device, the conventional NMR-spectrum is produced. The Fourier technique requires much expensive apparatus, but the observation time for a spectrum is reduced to a few seconds. This enables us to accumulate, for example, 1000 spectra of a substance in a short time and in this way to see signals, which would otherwise, from a single spectrum, be completely hidden in electronic noise. An important advantage of the pulse procedure is therefore the greatly improved sensitivity. Thus atomic nuclei, of which the natural abundancy is small, and which perhaps also have an unfavourable magnetic moment, become accessible to NMR-spectroscopy. In the first place the carbon isotope ^{13}C can be named. Its natural abundance is only 1.1% and its magnetic moment is considerably smaller than that of ^{1}H. (Taking the relative sensitivity of ^{1}H to be 1.00, the sensitivity of ^{13}C is approximately 0.25 for the same number of nuclei and the same frequency.) Since the isotope ^{12}C, which forms 99% of the carbon present, has no magnetic moment, ^{13}C spectroscopy gives the first chance to see directly the molecular frame of the organic compounds. Moreover, compared to ^{1}H NMR-spectroscopy, the ^{13}C measurements have also the advantage of a bigger chemical shift. This is illustrated again in Figure 8 with the example of a lipid molecule, in this case the synthetic dipalmitoyl lecithin[6]. With maximum resolving power, about 22 resonance lines would be expected. Actually only 12 lines are observed, for the absorption lines of the carbon atoms 4–12 of the fatty acid residues coincide. Yet the resolution obtained is remarkable, for at least 5 of the 16 carbon atoms of the palmitic acid residues can be seen separately. An analogous resolution would only be possible with normal ^{1}H NMR-spectroscopy by means of extremely strong superconducting magnets. The pulse method is naturally not confined to ^{13}C. Other nuclei of biological importance, which can now be measured, are ^{2}H, ^{31}P, ^{14}N, ^{23}Na and some others.

A second advantage of the pulse method can only be briefly indicated here. Using suitable sequences of pulses of varying length, the so-called NMR relaxation times can be measured. They are characteristic parameters of the dynamic behavior of the magnetic nucleus and give information indirectly about the molecular movements, namely both about the inner movements of the molecular structural frame and the motion of the whole molecule in relation to its environment. Some preliminary investigations on phospholipid molecules have shown, for example, a distinct

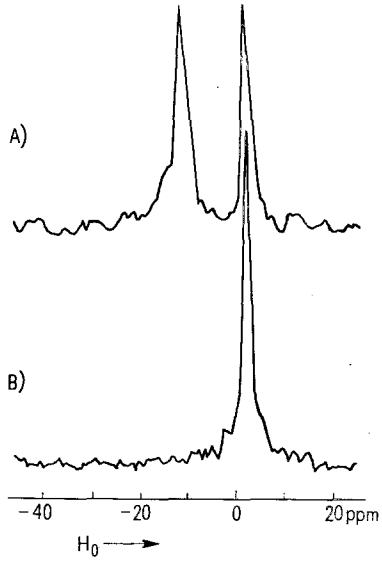


Fig. 7. 36 MHz ^{31}P NMR-spectrum of phospholipid vesicles[4]. A) without shift reagent; B) with 0.01 *N* $Pr(NO_3)_3$.

[6] J. K. Levine, N. J. M. Birdsall, A. G. Lee and J. C. Metcalfe, Biochemistry *11*, 1416 (1972).

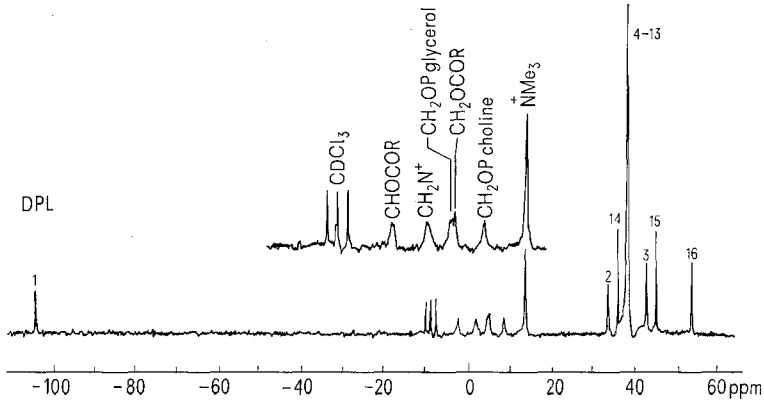


Fig. 8. 25.2 MHz ^{13}C NMR-spectrum[6] of dipalmitoyl-L-α-lecithin in $CDCl_3$ at 52 °C.

increase in the motional freedom towards the methyl ends of the fatty acids. NMR-relaxation-spectrometry could therefore become a valuable addition to the methods of structural analysis, in spite of the fact that the molecular interpretation of the measured relaxation times still demands at the moment a considerable mental effort.

The methods which we have now described have been used mainly for the structural examination of membrane molecules and membrane models such as phospholipid vesicles. If we wish to include biological membranes, there are some practical difficulties, but preliminary results have already been published.

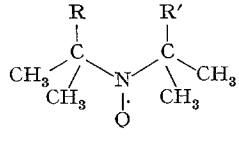
2. *Electron Spin resonance – the spin label method*[7]

Electron spin resonance is concerned with the magnetic properties of electrons and its theoretical basis is very similar to that of NMR-spectroscopy. For its practical application two differences should be noted. Firstly, the magnetic moment of the electron is bigger than that of the nucleus by a factor of about a thousand. This produces a correspondingly higher sensitivity of measurement. Even with comparatively dilute solutions ($\sim 10^{-5}$ molar compared to 10^{-2} molar with NMR-spectroscopy) a good signal is obtained. Secondly. almost all biological systems are diamagnetic, i.e. the electron spins are paired and no ESR signal is observed.

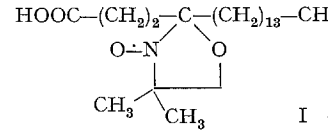
The method of spin labeling consists in attaching an unpaired electron at a specific site in a biological molecule by means of suitable chemical synthesis. The ESR signal can then be assigned unambiguously, since it is the only signal in the spectrum. The quantitative analysis permits conclusions about the fluidity and structure of the matrix, in which the spin label is moving. But before we concern ourselves with these important questions of membrane structure, we must briefly describe the chemical background of the spin label method.

The nitroxide radical[8]

Until a few years ago organic compounds with unpaired electrons (socalled radicals) were regarded as very unstable substances, which were trying to pair their odd electrons by means of chemical reactions. The start of the spin label method first came in 1961 when by chance a radical was discovered, which contradicted all previous chemical experience by its stability. This so-called nitroxide radical has the following chemical structure.



The groups R and R′ indicate that the radical is bound to a larger molecule, for example to a fatty acid.



I (13.2)

This fatty acid spin label I (13.2) is only slightly different in its behavior from a natural fatty acid and can easily be incorporated into a biological membrane. The unpaired electron is localized in the NO-bond. It is shielded by two tertiary carbon atoms and under normal physiological conditions has a practically unlimited lifetime. By means of suitable chemical reactions, the nitroxide group can often be attached at various sites of a biological molecule, as for example at almost every carbon atom of stearic acid. In this way differences in the various parts of the molecule can be observed. The method of spin labeling therefore gives under these circumstances a resolution of 2 to 5 Å, as expressed in the language of microscopy. In this general article it is not possible to discuss in detail the chemistry of the nitroxide radical. It should however be stated that the success of this method is very dependent on the synthesis of suitable spin labels and that the preparative organic chemistry is of decisive importance.

Interpretation of the ESR-spectra – molecular movements in membranes

What kind of information can be gained from ESR-spectra? In aqueous solution or in an organic solvent of low viscosity, the ESR-spectrum of the nitroxide radical looks very simple. As Figure 9a shows, it consists of 3 sharp lines, which are produced by the interaction of the electron with the magnetic moment of the nitrogen nucleus. Depending on the external conditions, quite dramatic spectral changes can be observed. Figure 9b shows, for example, the influence of temperature. A lowering of the temperature corresponds to an increase in the viscosity. The environment of the spin label is more resistant to movement and the absorption lines broaden. At the same time the amplitudes of the outside lines become smaller compared to that of the central line. By calibration with solutions of known viscosity (e.g. glycerin-water mixtures) a definite viscosity can be assigned to each spectrum. If the same spin label is then placed in an unknown environment, for example in a membrane, then the calibration curves allow the viscosity of the

[7] H. M. McCONNELL and B. G. McFARLAND, Q. Rev. Biophys. *3*, 91 (1970).

[8] E. G. ROZANTSEV and V. D. SHOLLE, Synthesis *1971*, 190; Synthesis *1971*, 401.

membrane to be determined immediately. The spin label method therefore gives quantitative results on the speed of molecular movements. A quite different effect is however seen in Figure 9c. The rate of rotation of the spin label is almost as large as in Figure 9a, but the essential difference lies in the fact that the molecule mainly rotates around one single axis. We speak of anisotropic motion in this case. This is generally produced by the special structure of the surroundings and the spin label method therefore offers a possibility to investigate this structure. Figure 9c is a typical spectrum for the movement of a fatty acid in a lipid bilayer.

In the following, some results of the spin label method will be at least qualitatively discussed. With the above-mentioned calibration, it can be shown that many biological membranes, particularly those with a high lipid content, are extremely fluid structures. Their viscosity is comparable with that of light oil. This result is surprising for it is known that the membrane layers are sharply defined in the electron microscope. Before these spin label results had been obtained, i.e. until 4 or 5 years ago, membranes were regarded as relatively crystalline structures. The high fluidity of a membrane on the one hand and the planar nature of the surfaces on the other exclude molecular movements perpendicular to the bilayer. Even very brief jumps of the lipid molecules out of the membrane would produce a diffuse border region. This leads us to the conclusion that the observed viscosity must be due to molecular movements confined to the volume of the membrane. Which molecular movements are possible? Let us take a lipid bilayer, in which here and there proteins are embedded, as the simplest membrane model (Figure 1) and confine ourselves to the movement of the lipid molecules. Then the following elementary processes are conceivable: 1. a rigid body rotation around the long molecular axis, 2. a lateral diffusion, i.e. a diffusion parallel to the membrane surface, 3. a turn through 180° and transition from one side of the membrane to the other and finally 4. the intramolecular movements, i.e. rotations and vibrations of the individual bonds of the molecule. The spin label method has given important details for the understanding of these movements. The rate of transition from the inside to the outside or vice versa can be determined by the following experiment[9]. Phospholipid vesicles are marked with spin labelled lipids. At first there is the same amount of spin label on the inner and outer side of the vesicles. The resonance signals are somewhat different, for the inner membrane surface has a smaller radius of curvature and hence the lipid molecules on the inner surface are more tightly packed. If ascorbic acid is added to this suspension, this reagent reduces the spin labels on the outer half of the vesicles. The spin labels on the inside are not attacked, for the ascorbic acid cannot penetrate the lipid double layer under the chosen experimental conditions (0 °C). Only the inner ESR signal is received. But as time passes, some phospholipids migrate from the inner to the outer surface and the spectrum returns gradually to its initial state. The half value time of this turn around process, which has been determined in this way, is of the order of several hours. We are dealing with a very slow movement.

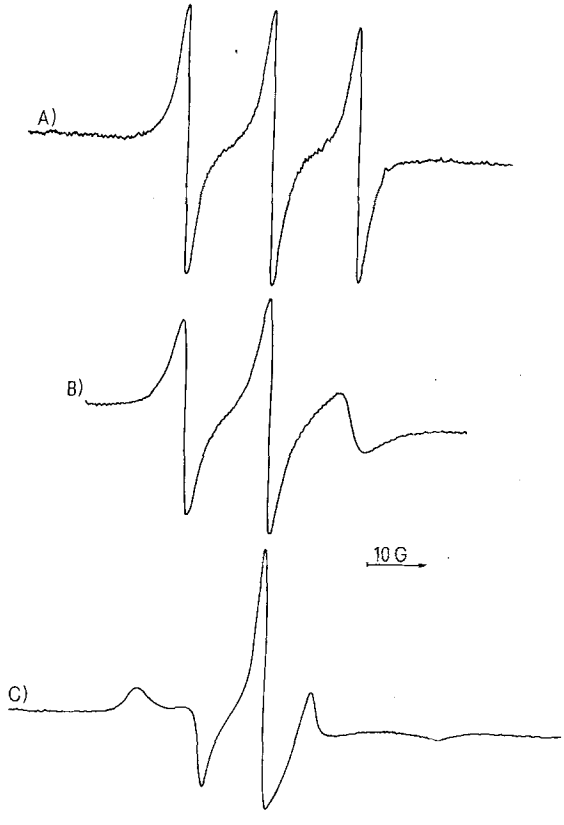


Fig. 9. Electron spin resonance spectrum of I (13.2) in A) methanol at 50 °C, low viscosity; B) methanol at −60 °C, high viscosity; C) phospholipid vesicles at 25 °C, anisotropic rotation.

The experiments for lateral diffusion require a longer theoretical discussion and hence the results only will be given[10]. For phospholipid bilayers and for some biological membranes, diffusion constants of the order of $D \sim 10^{-8}$ cm²/sec have been determined. Although this is slower than the diffusion of a small molecule in water by a factor of a thousand, yet it is still so fast that a phospholipid molecule moves about 10,000 Å (estimated from $x^2 = 2Dt$) in 1 sec. Bacteria, for example, are 10,000 Å or more long and a phospholipid molecule can travel in a few sec from one end of the bacteria to the other. The biological importance of this

[9] R. D. Kornberg and H. M. McConnell, Biochemistry *10*, 1111 (1971).

[10] E. Sackmann and H. Träuble, J. Am. chem. Soc. *94*, 4482, 4492, 4499 (1972). – P. Deveaux and H. M. McConnell, J. Am. chem. Soc. *94*, 4475 (1972).

fluidity could partly lie in the fact that if the cell had only a few centers for synthesis of a certain substance, then the membrane could have the role of a messenger and distributor.

The fastest motions in a lipid membrane are however the rotations of the lipid molecules about their long axes and the internal rotations around carbon-carbon bonds in these molecules. Both motions have a rotational frequency of about 10^{10} Hz. The isomerization of the carbon-carbon bonds also leads to a peculiar structure of the lipid bilayers. The investigation of this specific structure of lipid membranes will be our last example for the usefulness of the spin label method. Infrared- and X-ray-spectroscopic methods have attempted to discover which configuration the fatty acids in a membrane could assume. Two opposing models have been proposed. One view has been that the hydrocarbon chains are stiff and relatively extended, the other that they are flexible and coiled. It has been possible now to attach a spin label to the various carbon atoms of a fatty acid chain and to investigate the motion of each chain segment in the membrane[11]. It has been found that the motional freedom is drastically limited near the polar surfaces. The lipid chains are there arranged almost parallel to each other and rotate mainly around their long axes. The further one goes into the interior of the double layer the more random is the movement. The centre of such a double layer is therefore similar to a liquid. Hence the truth lies between the two extremes mentioned above. At the edge of the membrane the lipid chains are well ordered, whereas in the interior there is a relatively disordered structure. The physical reason for this behavior is not difficult to see. By means of the polar groups the lipids are firmly anchored at the polar region of the bilayer. Each carbon-carbon bond has a certain flexibility, which tends to disturb this ordered structure, and the flexibility increments add up the more one moves into the interior of the double layer. This behavior can also be described quantitatively with suitable models.

This last example has perhaps given a small insight into the ordering principle of a lipid membrane. It should be pointed out, however, that we can at present only understand a very small part of the extremely complicated picture of a biological membrane. We have no answer to the question why there are various polar groups and why there are so many different lipids in the membrane. We know almost nothing about the chemical composition and physical characteristics of membrane proteins and their importance for the membrane structure. Also the connection between membrane function and membrane structure is at present only a broad field for speculation.

If we recall Max Planck's remark made at the beginning, then we must still carry with us for some considerable time our belief in an ordered membrane structure.

Zusammenfassung.

In der kernmagnetischen Resonanz (NMR) und Elektronenspinresonanz (ESR) sind in den letzten Jahren wesentliche methodische Fortschritte erzielt worden. Der Anwendungsbereich dieser Verfahren konnte daher auch auf so komplexe Strukturen wie biologische Membranen erweitert werden. Die Entwicklung supraleitender Magnete und die Entdeckung der Verschiebungsreagentien hatte eine wesentlich verbesserte Auflösung der NMR-Spektren zur Folge. Durch Einführung der Impuls-Fourier-Transformationstechnik wurde ausserdem die Empfindlichkeit der NMR-Spektrometer beträchtlich gesteigert, so dass jetzt neben Protonen auch andere biologisch wichtige Kerne in natürlicher Häufigkeit gemessen werden können. In der biologischen Anwendung der Elektronenspinresonanz kamen wesentliche Anstösse vor allem durch die Methode der Spin-Markierung. Der vorliegende Artikel ist ein Versuch, diese neuartigen spektroskopischen Verfahren möglichst anschaulich darzustellen und ihre Bedeutung für die Membranforschung an einfachen Beispielen zu illustrieren.

[11] J. SEELIG, J. Am. chem. Soc. *92*, 3381 (1970); *93*, 5017 (1971). – J. SEELIG, H. LIMACHER and P. BADER, J. Am. chem. Soc. *94*, 6364 (1972).