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An Account of the Development of Nuclear Magnetic Resonance (NMR) in India

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Abstract

The technique of Nuclear Magnetic Resonance (abbreviated as NMR) was discovered independently, by two groups in USA in 1946. They showed that a nucleus having a spin (I) has 2I+1 nuclear energy levels. The degeneracy between these energy levels is lifted, in an externally applied magnetic field. The system can be excited by applying a suitable radiofrequency field (RF), corresponding to the gap between these energy levels. A resonance signal is obtained by varying the magnetic field such that the frequency matches exactly with the energy gap. A number of parameters dictate the resonance frequency and the observed spectrum shows fine structure. These parameters depend on the chemical structure of the molecule. NMR soon developed as a tool for determination of molecular structures. In early years, spectra were obtained by sweeping the magnetic field. This technique is called continuous wave (CW) NMR. One had to use samples dissolved in a suitable solvent in molar strengths (*low sensitivity*). The dispersion of signals was small, leading to crowded spectra (*low resolution*). This was a major problem, particularly, when dealing with large biological molecules, such as proteins and nucleic acids. Techniques like Fourier transform NMR (FTNMR) invented in 1964, helped to resolve the problem of sensitivity and resolution. The availability of fast computers led to wide spread applications of FTNMR. With the developments in cryogenic and magnet technology, high field magnets became available, which increased both the sensitivity and the dispersion in the spectra. Major developments in electronics also helped in achieving higher sensitivity. With these developments, microgram quantities of samples and large molecules could be studied. This resulted in widespread use of NMR to study molecular structures, research and development in pharmacy, and in the development of medically active compounds. In chemical industries, NMR has become a tool for characterization of products. Nitrogen NMR has been used for the detection of explosives, which often contain nitrogen compounds.

Development of multi-dimensional FTNMR helped in the simultaneous acquisition of spectra of more than one nuclear spin, and allowed study of correlations between two spins. This led to a major thrust in studies of structure, function and dynamics of proteins, nucleic acids and polysaccharides. NMR has also been applied to study animal and human tissues, body fluids and living cells. The technique of Magnetic Resonance Imaging (MRI) was developed, which is used on human and animals. It is also possible to study the biochemistry of tissues *in vivo* by a parallel technique called Magnetic Resonance Spectroscopy (MRS). Functional MRI (f-MRI) enables studies related to cognitive functions like learning and human behaviour. Applications in plant sciences include studies such as development of *trans-genetic* plants.

The development of NMR has been discussed in this article, with particular emphasis of its progress in India. The work on NMR was started in India in the year 1953 by S S Dharmatti at the Tata Institute of Fundamental Research (TIFR), Mumbai. Later, another group with P. Venkteshwarlu started working at the Aligarh Muslim University (AMU), Aligarh. A K Saha and T P Das at the Saha Institute of Nuclear Physics (SINP) wrote one of the earliest books on NMR. In early days, there was an emphasis on building indigenous instruments. However, commercial instruments came into existence and became available after 1955. In India, the first high resolution NMR spectrometers were purchased by TIFR and

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AMU. Today, hundreds of NMR spectrometers are available, for chemical and biological sciences. Medical Institutes and hospitals have MRI, MRS and f-MRI scanners for patient care and research. Using NMR, research is conducted in all branches of sciences such as, physics, chemistry, biophysics, pharmacy, medical sciences, human psychology & behaviour, and plant sciences.

Kew words: History of NMR research in the world, Indian Developments, Industrial Research, NMR in Chemistry, Biology and Medicine, Nobel Prizes in NMR, Nuclear Magnetic Resonance (NMR), Pioneers (Dharmatti, Venketeshwarlu), Safety aspects, Summer Schools and Training Programs in India.

1. Introduction

The technique of Nuclear Magnetic Resonance was discovered in 1946, independently, by groups led by F Bloch at Stanford and E M Purcell at Harvard. The two groups used water and paraffin wax in their study, and observed ^{1}H NMR in these compounds. Results from these two groups were published next to each other in Physical Reviews. The two shared the Nobel award for physics in 1956.

A nucleus having a spin (I) has 2I+1 energy levels. In the absence of a magnetic field, these levels degenerate. This degeneracy is lifted by applying a magnetic field. The gap between these levels and hence the resonance frequency is proportional to the magnetic field at the site of the nucleus. Nuclei are excited by a radiofrequency field (RF), whose frequency corresponds to the energy gap between these levels. Bloch and Purcell observed resonance by varying the magnetic field, keeping the transmitter frequency constant. This is known as continuous wave (CW) technique. The two groups used different names for the newly discovered technique: *nuclear induction* and *nuclear magnetic resonance* (NMR). The second name has become popular.

The resonance frequency of a nuclear spin is directly proportional to the magnetic field at the nuclear site. The signal strength is proportional to $^{3}/_{2}$ power of the field. The typical magnetic field used in NMR ranges from 1 to 18 Tesla and the resonance frequency is in MHz range. For this reason, NMR was also called radiofrequency spectroscopy.

In earlier years, physicists were attracted to NMR because it allowed measurements of properties such as, nuclear magnetic moments and nuclear spins. It allowed precise measurements of magnetic fields. Small changes in earth's magnetic fields were used in search of oil fields. NMR allows studies of metal and alloys through the use of Knight shifts and line-widths in solids. A few years after the discovery of NMR, it was observed that compounds such as NH_4NO_3 gave two signals for ^{14}N. These observations led to the concept of *chemical shifts*. Dharmatti, while working with Bloch, showed that ethyl alcohol gives three lines in the proton (^{1}H) NMR spectrum with intensities in the ratio 3:2:1. These correspond to CH_3, CH_2 and OH protons. This pioneering work gave birth to NMR in organic chemistry. The name *chemical shift* was given to such differences in resonance frequencies, since the effect arises because of chemical bonding. It was later shown that two nuclear spins in a molecule can couple to each other through an interaction called the *coupling constant (J)*, which also depends on chemical structure.

S S Dharmatti returned to India in 1953 and started a research group at TIFR. Another group was started by P Venkateshwarlu at AMU, who later moved to Indian Institute Technology (IIT), Kanpur. In TIFR, ^{59}Co chemical shifts in inorganic complexes were measured in late 1950s. Chemical shifts were used to study hydrogen bonding in liquids. However, support to NMR activities at early stages in India was meager. After 1978, financial support started emerging both from the Government agencies and Industries. Currently facilities in India are considered parallel to some

centres if not best in the world. Research is conducted in all areas of sciences: physics, chemistry, biophysics, biochemistry, pharmacy, medical sciences and human psychology and behaviour. A number of training programs have been organized. International collaborations have been established. Certain facilities also provide services to Universities and Industries. This has resulted in wide use of NMR by scientists.

2. Chemical Sciences

Organic molecules are mainly composed of carbon, hydrogen, oxygen and nitrogen. The main isotopes which are useful in organic chemistry are ^{1}H and ^{13}C. ^{16}O and the more abundant isotope of carbon ^{12}C do not have magnetic moment. Both isotopes of Nitrogen (^{14}N and ^{15}N) have magnetic moments. The more abundant isotope (^{14}N) gives broad lines and therefore, ^{15}N is used. In biological sciences, one also uses ^{31}P. Use has been made of several other spins such as ^{19}F, ^{29}Si and ^{59}Co. NMR would have been a technique of academic interest only but for the discovery of several properties which influences NMR spectral pattern. These parameters are summarized below:

(a) Chemical shifts **(δ_i)**: In molecules, the electronic cloud around the nucleus influences the magnetic field at the nuclear site. Therefore, the magnetic field at the site of the nucleus is different from the one applied. Such an effect depends on the chemical bonding. For the same nucleus more than one line is observed in a molecule. For example, NH_4NO_3 gives two nitrogen signals. Ethyl bromide gives two ^{13}C and two ^{1}H lines. Chemical shifts are measured in Hz. However, these are normally expressed as a dimensionless quantity, called parts per million (ppm), by dividing the frequency differences (in Hz) by the operating frequency of the spectrometer. Typical ranges of chemical shifts are 10 ppm for ^{1}H (which corresponds to 8000 Hz on a 800 MHz spectrometer) and 250 ppm for ^{13}C and ^{15}N. In most compounds of interest, the range of shift for ^{31}P is 15 ppm. Chemical shifts provide information on molecular structures of organic, inorganic and biological compounds.

(b) Spin-spin couplings **(J_{ij}):** Two nuclear spins *i* and *j* in a molecule, can interact with each other, through the bonding electrons. These are called spin-spin couplings and are denoted by the letter J. The values of J depend on the geometry of the molecule and the number of intervening chemical bonds between the two spins. Of particular importance are couplings between nuclei separated by three bonds (^{3}J). These couplings show a dependence on the dihedral angles. Empirical relations between the three bond couplings and dihedral angles have been established for a wide range of couplings and these are often called as *Karplus* type relations. Some examples are:

H-C-C-H, H-C-C-N, H-C-C-N, H-C-N-C, H-C-O-P, H-C-C-F, C-C-O-P, H-C-C-C, H-C-C-F and H-C-O-H

Values of dihedral angles can be estimated from the measured values of ^{3}J, using such relations.

(c) Dipolar Couplings **(D_{ij}):** Nuclear spins can also interact directly in space. Such interactions are called dipolar coupling and are represented by the letter D_{ij}. Dipolar interaction is inversely proportional to the 6^{th} power of the distance (r_{ij}) between the spins *i* and *j*. Distances between atoms can be estimated using dipolar couplings. Through the collective use of such distances, coupled with a computer algorithm, the three dimensional (3-D) structure of a molecule can be established.

(d) Spin-lattice **(*T1*)** ***and spin-spin*** **(*T2*)** ***relaxation times and rates*** **(*R1 and R2*)***:* Once spins have been excited, they return to the ground state. This process is called relaxation. One normally talks about longitudinal (T1) and transverse relaxation (T2) times and their inverse is called as rates, which are denoted by R1 (=1/T1) and R2 (=1/T2),

respectively. Intra- and intermolecular dipolar interactions are involved in relaxation. Because of molecular motions, dipolar interactions lead to fluctuating magnetic fields and help excited spins to return to ground state. Relaxation rates help in the study of *molecular dynamics* in large molecules, while T1 and T2 form the basis of contrast seen between different types of tissues in *magnetic resonance imaging* (MRI).

(e) Nuclear Overhauser Effect* (*NOE*):* Once a spin is excited, its effect is seen by neighbouring spin. There is a change in the intensity of the line due to the second spin. This effect is known as NOE. The main contribution to NOE arises from relaxation processes. Under certain conditions, the value of NOE is inversely proportional to the sixth power of the inter-nuclear distance (r_{ij}), between spins *i* and *j*. NOE thus contains information on inter-atomic distances. Using a suitable computer algorithm, three dimensional structure of a molecule can be obtained.

(f) NMR line shapes and intensities: Line-shape analysis provides information in systems undergoing chemical exchange. It can be used for detecting groups such as N-H and O-H, which undergo chemical exchange with water in aqueous solutions. The intensity of a line is proportional to the number of spins, and is used to find the relative concentrations of compounds in a mixture.

Even though NMR is used in structure determination, study of dynamics of macromolecules, etc., there are several difficulties in its widespread use. These are summarized below:

(a) Sensitivity*:* NMR is a relatively insensitive technique. The energy gap between the ground and excited states of nuclear spins is small. The difference between the populations of spins between the two states is only one in 10^6. This results in weak signals. In the Continuous Wave (CW) technique, the magnetic field is swept slowly to record the entire spectrum. Typical time taken by this technique is few minutes for acquiring a ^{1}H NMR spectrum to several hours for a single ^{13}C NMR spectrum.

(b) Resolution*:* As described earlier. the resonance signals lie in a limited range of frequency (10 ppm for ^{1}H and 250 ppm for ^{13}C), resulting in overlapping lines, especially in proteins, nucleic acids and polysaccharides, which have several similar chemical groups. Because of their large molecular weights, there are large numbers of lines and there is a serious overlapping.

(c) Assignment*:* It is necessary to know as to which resonance line belong to which spin. For small molecules, it is relatively easy to assign spectral peaks. The problem is much more difficult for large molecules. For example, in the peptide backbone, ^{1}H, ^{13}C and ^{15}N are in very similar chemical environments and give highly overlapping resonance peaks.

(d) Solvent Signal*:* Most solvents also give NMR signals. Normally, the molecule of interest (solute) is present in milli-molar (mM) concentrations or less, while the solvent is in molar concentration. The lines of interest are masked by the resonance signals from the solvent. In organic compounds chloroform is generally used as solvent. For biological molecules, water is used as the solvent. Water has two protons and the ^{1}H concentration is 100 M. The strong solvent resonance can be avoided partially, through the use of deuterated solvents ($CDCl_3$ or D_2O). The ^{1}H line intensity due to residual protonated solvent (due to molecules such as HDO) is fairly large. One can use 100% deuterated solvents, but these are expensive.

3. Developments in NMR

Most of the difficulties are surmounted in the developments of technology related to methods of excitation and acquisition of NMR signals, higher field magnets, electronics and computer hardware and software. These are described below:

(i) Fourier transform (FT) technique: In 1964, Richard Ernst while working as a post-doctoral fellow with Varian Associates in USA, came up with a new idea for obtaining NMR. He showed that the entire range of spectral resonances can be excited by using a single RF pulse. The resulting signal (called *free induction decay or* FID) on Fourier transformation provides the CW spectrum. Further, by using two pulses, with frequencies corresponding to different spins, one can simultaneously obtain information on two nuclear spins. Spin-spin correlations such as J_{ij} and D_{ij} can be observed in a single experiment. Ernst returned to Switzerland and started a group on NMR in ETH, Zurich. Kurt Wuthrich collaborated with Ernst to study large biological molecules. Three Indians worked with them as postdoctoral fellows: Anil Kumar, R V Hosur and K V R Chary. All three have returned to India and are working at the Indian Institute of Science (IISc), Bangalore and at TIFR. Ernst received the 1991 Noble prize in Chemistry, while Wuthrich shared the Nobel prize in 2001. Ernst and Wuthrich were elected as honorary Fellows of TIFR, a distinction which has been conferred on very distinguished scientists. They were also elected as Foreign Fellows of the Indian National Science Academy (INSA).

(ii) High field magnets: Electromagnets were used in early days. Such magnets do not provide stable magnetic fields, and were satisfactory for studies only up to 100 MHz frequency for ^{1}H. Higher fields cannot be obtained from such magnets. Super-conducting magnets with high homogeneity were developed around 1970. Use of such magnets resulted in better dispersion of the NMR signals with higher sensitivity. With the availability of cryogenic magnets, higher magnetic fields can be used. Spectrometers are available in India with operating frequency of 800 MHz. In the world 1000 MHz spectrometers are available. These developments resulted in NMR becoming a potential tool for study in biological sciences. A recent addition to these capabilities is the development of dynamic nuclear polarization, through which the sensitivity of less abundant nuclei is increased several fold. Magnets could be designed to suit the requirements of the application. Very high field-magnets are desirable for molecular studies. Magnets for human imaging have lower fields but have a larger pole gap to accommodate the subject under study. Portable magnets are used for botanical studies in the agriculture fields. Desk-top magnets are also available for routine chemical analysis.

(iii) Better electronics and computers: Electronics has improved significantly in recent years. The use of field/frequency lock ensures greater stability. Several spectra can be added coherently, resulting in a better signal to noise (S/N) ratio (time-averaging). Computers help in analysis of multi-dimensional NMR. Spectra can be stored and plotted as and when desired. Special NMR probes kept at low temperatures (cold probes) improve sensitivity.

(iv) Multi-dimensional NMR: Two or more nuclear spins can be excited using multi-dimensional NMR. For example, one can use pulse sequences such that one excites ^{1}H and the other ^{13}C. One obtains a ^{1}H-^{13}C 2D spectrum (so called hetero-nuclear 2D NMR). One can have ^{1}H (homo-nuclear 2D NMR) on both axes. There are two basic 2D techniques. One set of techniques (J-*correlated spectroscopy*), is the earliest technique in this class, known as COSY. J couplings are utilized in these set of pulse sequences to correlate nuclear spins. Advanced techniques under this class include Double Quantum Filter (DQF), COSY and Total Correlation Spectroscopy (TOCSY). Values of J-couplings can be measured using correlated spectroscopy, which help in the estimation of dihedral angles. The other set of 2D pulse sequences utilize nuclear dipolar interactions. Techniques such as NOESY and ROESY belong to this class. Since nuclear dipole-dipole interactions depend on inter-nuclear distances,

such techniques help in the measurements of inter-nuclear distances. One can extend pulsed NMR to more than one dimension. For example, it is possible to obtain a 3D spectrum, with ^{1}H, ^{13}C and ^{15}N, along the three dimensions. Such spectra provide a wealth of information. These developments resulted in higher sensitivity in NMR. The techniques also helped in resolution and assignments of complex spectra. Information can then be obtained on J-couplings from correlated spectroscopy. Inter-nuclear distances can be measured using NOE data. Samples in microgram quantities can be used. Further, new methods for excitation and detection in multi-dimensional NMR have continuously been discovered. There have been parallel software developments for analysis and utilization of NMR data as well.

(v) Other Developments: NMR developed as a tool for macromolecular structure and dynamics mainly through the work of Wuthrich and Ernst. In 1972, Lauterbur published a paper giving the concept of *zeumatography*, which was later called magnetic resonance imaging (MRI). Later, the use of FT method in imaging was developed by Ernst, Mansfield and Edelstein. MRI developed as a clinical tool during 1980-90. Later, *in vivo* magnetic resonance spectroscopy (MRS) methods were developed in conjunction with MRI, which allowed study of metabolism in cells, tissues, organs and whole animals. For their discovery of MRI, Lauterbur and Mansfied won the Noble prize for Physiology and Medicine in 2002. The technique of blood oxygen level detection (BOLD) was developed by S Ogawa and K Ugurbil, which led to a new area called Functional MRI (f-MRI).

4. Early Developments in India

The field of NMR has seen continuous developments since 1946. In early 1970s, in one of the reviews Gutowsky remarked that NMR *is Evergreen*. This holds good even today. It will be desirable to review the developments in India in different periods. We first describe the early work in instrument building and solid state physics. This is followed by developments in other areas. Recent work in India covering fields of chemistry, biology and medicines is discussed later.

(i) Instrument Building: India entered the field of NMR in 1953, with Dharmatti joining TIFR. He started a group named as Nuclear and Electron Magnetism (NEM). Since commercial instruments were not available, he ordered steel pole gaps for two magnets from the Tata Iron and Steel Company (now called Tata Steel). Electric coils were wound around these caps to make electromagnets. One of the magnets was used for NMR and the other to measure the magnetic susceptibility. Electronics for the NMR work was built locally. Because of the lack of homogeneity in the magnetic field in the magnet, NMR lines were broad. However, the instrument was good enough to observe resonances from solids, which anyway give broad lines. The sensitivity was acceptable as one could detect signals from deuterium (^{2}H) and oxygen (^{17}O), which have natural abundances of 0.02% and 0.04%, respectively. ^{17}O NMR was also used for study of double bond character in organic compounds. Mention may be made of two groups, which worked on NMR with meagre facilities. One was at the Department of Crystallography and Biophysics (headed by Prof. R Srinivasan) at the University of Madras. A wide-line NMR spectrometer with a proton frequency of 11.69 MHz was constructed by S Ganpathy. The spectrometer was used to study solids by recording their NMR spectrum and determining the second moments. Dynamics of functional groups in organic compounds and amino acids was studied. Later, N R Jagannathan [now at the All India Institute of Medical Sciences (AIIMS), New Delhi)] upgraded this spectrometer with low level Robinson RF oscillator for higher sensitivity and fabricated a low temperature assembly. At the same time, R Srinivasan and his students built

pulsed NMR spectrometer in mid 1970s for relaxation measurements, at IISc, Bangalore.

(ii) Solid State NMR Studies: The group at TIFR started work in solid state NMR using a home built spectrometer. Attempts were made to search for signals from some previously unexplored nuclei. The approximate values of magnetic moments for such nuclei were known. One had to look for resonances based on these values, so that accurate values of magnetic moments could be calculated. These attempts were unsuccessful. Studies then shifted to study of metals and alloys. TIFR in late 1960's was devoted purely to Physics and Mathematics, and there were no chemists. This posed problems in preparing samples. A scientist (U R K Rao) from chemistry division of Atomic Energy Establishment, Trombay (now called Bhabha Atomic Research Center, BARC) helped in preparation of alloys. Later, the activity shifted to physics of metals and alloys, for which Knight Shift (KS) and line widths were the major source of information. Use was made of ^{23}Na and ^{199}Hg resonances in respective metals and their alloys. Line widths as a function of temperature, non-symmetric quadrupolar effects and other inputs were used to understand the chemical bonds and the phase transitions. Inter-metallic compounds such as $CePt_2$ were studied by NMR and magnetic susceptibility. It was possible to separate out the conduction electron contribution and 4f-contribution to KS by such studies. Deviation from linear relationship between susceptibility and KS at low temperatures was studied in rare earth inter-metallic compounds. Subsequent work on Eu-based systems showed that such anomalies arise from strong 4f-hybridization effects. Conduction electron interactions with nucleus were successfully exploited in explaining the band structure and its modifications in transition metal alloys and rare earth inter-metallic compounds. The non-uniform nature of the conduction electron polarization was established from the observation of two ^{195}Pt lines in RPt_5 (R = rare earth) compounds. This lent strong support to the *Ruderman-Kittel-Kasuya-Yosida* theory. Magnetic susceptibility and ^{195}Pt KS studies were used to explain the effect of crystal field in Sm-based inter-metallic compounds. Studies on $CeSn_3$, a strongly correlated electron system, were one of the earliest such measurements, a heavy fermion compound. Measurements were carried out on systems such as RCu_2Si_2, RRh_3B_2, $EuNi_2P_2$ and $YbCu_4In$. The KS and quadrupole coupling constant of boron, silicon, copper and phosphorus were measured. Magnetic character of a number of Huesler alloys was clarified. Studies were also conducted on Co and Ga to study cluster spin glass behaviour. Issues pertinent to oxidation states were resolved on some materials in the active field of geometrical frustration.

(iii) Development in areas related to NMR: One such area is Electron Resonance Spectroscopy (ESR). Work was started in 1951 at IISc Bangalore. B Venataraman fabricated an ESR machine at TIFR. Other active centres in ESR are at Osmania, Allahabad, IIT Mumbai and Kanpur. In early 1970, S V S Sastry fabricated a 100 KHz field modulated X-band EPR spectrometer in Osmania University, Hyderabad. In late 1970s, K V R Chary indigenously designed and developed a 10 MHz wide-line CW NMR spectrometer for studies on solid compounds in Osmania. The department acted as consultants to the Electronic Corporation of India, Hyderabad. This company collaborated with the Microwave Engineering group of the TIFR in an attempt to market X-band EPR spectrometers, but with a limited success. Another achievement was the construction of a spectrometer at TIFR for the study of nuclear quadrupole resonance (NQR).

(iv) High resolution NMR: Instrument companies soon ventured into the manufacture of NMR instruments. Highly homogeneous magnets were required to achieve good resolution and the companies focussed on such an aspect. In 1955, Varian Associates in San Francisco area, USA started selling such spectrometers. It was possible

to measure the chemical shifts and the coupling constants with such instruments. This development brought two distinct nomenclatures in the field: *wide line* NMR, used for solid state studies by physicists, and *high resolution* NMR used by chemists for structural determination. In later years, techniques were developed for high resolution studies in solid state. TIFR purchased a 30 MHz. high resolution spectrometer (HR30), the third such machine manufactured by Varian. This spectrometer was upgraded in two steps: first to 40 and then to 60 MHz. TIFR also purchased a wide line spectrometer for solids, and a spectrometer to do studies using ESR. However, only one magnet could be purchased. The three groups shared the magnet for 2 days in a week. I joined TIFR in 1959, followed by C L Khetrapal, M M Dhingra and Y P Virmani. A year later, Venkateshwarlu purchased a 40 MHz NMR spectrometer at Aligarh Muslim University. He later moved to IIT, Kanpur and took the spectrometer with him. B D N Rao was his first student and Anil Kumar did his Ph. D. with him. Later Rao left for USA. After their departures, NMR activity at IIT, Kanpur came to a standstill. Anil Kumar returned to India later and joined IISc. Another scientist who had interest in NMR was P T Narasimhan who joined IIT, Kanpur. He was an excellent teacher.

At the low magnetic fields then available, the chemical shifts (in Hz) were comparable to the coupling constants. This gave rise to what were known as *strongly coupled* NMR spectra, a nomenclature developed to denote the spin systems. Spins which were strongly coupled (comparable coupling constants and chemical shifts) were denoted by early letters of alphabet (A, B, C, etc). Weakly coupled spins were denoted by X, Y, Z etc. To extract the values of J couplings and chemical shifts from these spectra, secular determinants had to be solved to determine *eigen-values* and *eigen-vectors*. TIFR had a good computer for solving such secular determinants. The early work, both by the TIFR and IIT groups was on the analysis of strongly coupled spectra and to extract values of the two parameters. This resulted in early publications in International journals from the two groups. My Ph. D. thesis contains analysis of ABC, ABX and A_2B_2X type of spectra from substituted benzenes. The results on chemical shifts in substituted benzenes were correlated with Hametts constants, in terms of inductive and resonance effects. Some other contributions by TIFR scientists may be mentioned. These included, study of hydrogen bonded systems, where mixtures of two liquids were used and studies of substituted coumarins, sesamins and asarinins. These molecules gave overlapping components from AX and ABC type of spin systems. Proton-Fluorine spin couplings in fluoro-benzenes were measured. Solvent effects helped in the detection of self-association in aromatic thiols. Dilution shifts helped in estimation of *aromaticity* in organic molecules. In all, about 25 papers in International Chemistry Journals were published during the period 1959-1965. With this TIFR made an entry into chemical sciences. Prof. T R Sheshadri, from Delhi University, was interested in coumarins. He started getting his compounds analyzed by TIFR. B D Tilak, who was working at the University Department of Chemical Technology, Mumbai and his colleagues got structural analysis of thio-chromans from TIFR. K Venkataramiah from Osmania University entered in a joint collaboration for studies on hydrogen bonding. Apart from these scientists, interest by other chemists in India was almost non-existent. The interest of organic chemists gradually grew because of the services provided by TIFR.

For work on NMR, heavy water and other deuterated solvents were required. There was a ban on exports to India of such compounds by Western countries. Heavy water is used as coolant in nuclear reactors, and the purity is important. The chemical engineering division of AEET (BARC), wanted services of TIFR to get samples of heavy water analyzed, before and after its use

in reactors, to check the deuterium content. The used water also had traces of Tritium. Such analysis was done by TIFR using NMR. In exchange of these services, AEET started providing TIFR with heavy water and deuterated solvents. After the death of Dharmatti in 1965, the NMR activity in TIFR was sub-divided in two newly named groups: Department of Chemical Sciences and Department of Solid State Physics. Today, work at TIFR is done at the National NMR Facility at TIFR. Similar facilities exist at IISc, AIIMS and several other Institutes.

(v) Formal Inauguration of the TIFR facilities by Pandit Jawaharlal Nehru: Till late 1960's, NMR spectrometers in TIFR were housed in military barracks in Holiday Camp (now called Navy Nagar). The construction of the main building of TIFR was completed in 1961 and was formally inaugurated by Pandit Jawaharlal Nehru. Dr. Bhabha made a special point to bring him and other distinguished guests like Shri J R D Tata and the Governor of Maharashtra to show the NMR machine. Dr. Bhabha himself explained the concept of NMR and its applications to Pandit Nehru.

Fig. 1. Inauguration of NMR facilities at TIFR, Mumbai, 1961: Seen in the picture (anticlock wise): J R D Tata, Pandit Nehru (Prime Minister of India), Dr. Homi Bhabha, Prof. SS Dharmatti, Shri Sri Prakasa (Governor of Maharashtra) and the author of this article

(vi) NMR after 1965: The IIT Kanpur group became extinct after Rao and Anil Kumar went abroad. In 1965, Khetrapal, Virmani and the author, went abroad for postdoctoral research. As a post-doc at National Physical Laboratories, UK the author worked on nuclear magnetic double resonance and computer software for NMR and at National Research Council, Canada on hydrogen bonding in gases, and rotational isomerism in substituted ethane. This resulted in some papers on NMR in gaseous state. The main problem with working on gases is that there is a possibility of bursting of the sample tubes in NMR spectrometers, and no one wants to take the risk of spoiling expensive probes. Khetrapal left for Basel, Switzerland. Under the supervision of Peter Diehl, Khetrapal initiated work on structure of organic compounds oriented in liquid crystalline media. During this time, there was very little activity in NMR in India. The work at Kanpur had stopped completely after the departure of Rao. When we returned to India in 1968, TIFR still had the old 30 MHz NMR machine, which had been upgraded to 60 MHz. The capabilities of this machine were very limited and no meaningful work could be done. In view of this, author's research interests were oriented towards quantum chemical calculations of biological molecules, while Khetrapal started working on oriented molecules, in India.

The first spectrometer purchased by a Chemistry Laboratory was a 60 MHz machine by the Central Drug Research Institute (CDRI), Lucknow in 1972. It was used for routine analysis of organic compounds. There was an acute need for modern NMR machines. India had problems of funds and foreign exchange. G N Ramachandran was also interested in getting NMR facility. M G K Menon arranged finances to buy a 270 MHz NMR machine, with joint funding from the Department of Science and Technology (DST) and the Nuffield Foundation of England. The NMR spectrometer was installed at IISc,

Bangalore, as a joint facility for IISc, Raman Research Institute (RRI) and TIFR. From IISc R. Srinivasan was given the overall charge. Khetrapal moved to Raman Research Institute (RRI), Bangalore. The author was supposed to use the RRI spectrometer having almost no facilities to use the machine. At that time, P Balaram joined IISc and started using the NMR spectrometer.

5. Current Activities in Chemical Sciences

The period starting from 1980 may be regarded as modern era of NMR in India. The activities of research in NMR is divided in four sections: Chemical Sciences, Biological Molecules, *in-vivo* and *in-vitro* cellular studies, and Medicine. Over the years, the problems with foreign exchange had become less acute. In addition to the DST and Council of Scientific and Industrial Research (CSIR), a new department namely Department of Biotechnology (DBT) was created under the Ministry of Science and Technology. However, the financial support for NMR continued at a low level. It was limited to providing low field spectrometers under the DST scheme of Sophisticated Instrument Centers (RSIC). After 1985, more liberal funding started coming in all areas of sciences. This brought the NMR culture to a number of Institutes in India. Some of these are: IISc, TIFR, AIIMS, Centre for Cellular and Molecular Biology (CCMB), Hyderabad, Central Drug Research Institute (CDRI), Institute of Nuclear Medicine and Allied Sciences (INMAS), Delhi, Sanjay Gandhi Postgraduate Institute (SGPGI), Lucknow and all the IITs. Unfortunately, funds for less endowed State Universities have still not picked up.

(i) High Resolution NMR: A 500 MHz NMR machine was sanctioned to TIFR by stroke of luck. A new pharmaceutically active compound was discovered by one of the CSIR laboratory. Its structure was established by X-ray crystallography and a patent was taken. Unfortunately, the structure reported in the patent was for the wrong stereoisomer. A company, working on the same molecule, noticed the mistake and took the patent for the correct structure. This led to lot of anguish among top Indian scientists. One scientist from an Industry said that this would not have happened if the structure was determined by NMR. He offered to pay for the entire cost of the instrument to purchase a 500 MHz NMR at TIFR. A meeting was held in Mumbai, and it was agreed that DST will pay for the machine. A spectrometer for the Bose Institute, Kolkata was also sanctioned. Unfortunately, this instrument got damaged in the rain, and did not work.

In recent years, the problems with foreign exchange have become less acute. There are several high resolution NMR spectrometers available in India, ranging in operating frequency from 300 to 800 MHz. Several laboratories have more than one spectrometer. Among major laboratories with good facilities, mention may be made of TIFR, IISc, CDRI, CCMB, Indian Institute of Chemical Technology (IICT), all the IITs, SGPGI, AIIMS, and SINP. A number of Industrial laboratories also have NMR spectrometers.

From the view-point of organic chemistry, there have been two major advances in the NMR spectrometer configuration. One type of NMR spectrometers has automatic sample changer. The samples can be aligned for automatic recording of spectra one after another. The other type of NMR spectrometer is with coupled HPLC and Mass Spectroscopy. Though less popular, such facilities exist in India. With the availability of several high field spectrometers, organic chemists use NMR routinely. Most publications in organic and some in inorganic chemistry, provide NMR data to support the proposed structure. Less endowed Universities generally request to use the facilities set up by DST to get NMR analysis of their samples. Most of such studies are of routine nature and will not be discussed. Studies that deserve mention are listed below.

(ii) NMR of molecules oriented in liquid crystalline media: Khetrapal continued work on molecules oriented in nematic phase of liquid crystals. Later, two other scientists, Kunwar and Ramanathan joined him and have continued collaboration. Solute molecules get partly oriented when dissolved in liquid crystals. Using this technique, dipolar interactions and the ratios of distances can be measured. The method does not provide absolute values of distances. The shapes of a number of several small molecules have been determined, using this technique.

(iii) Quantum computing: Recently, Anil Kumar started working on quantum computing. Though it may sound applied, the work is purely academic. He has published several papers on this subject. A number of his students have started doing similar work in newer academic Institutions like IISER.

(iv) NMR in solid-state: It was realized early by the NMR community that sharp lines can be obtained from solids provided the sample is spun at a very fast rate at an angle (called magic angle of 54.3^0, relative to the applied magnetic field). One problem with such rotors is that they break frequently. The technique of magic angle spinning (MAS) is available at several laboratories. TIFR, IICT, AIIMS, NCL and IISc are the major centres where good solid-state NMR spectrometers are available. In these facilities structure determination of large and small molecules in solid state are being carried out. The techniques have been applied to study: (i) short peptides (ii) surgical samples (tissues), and (iii) peptides and proteins in solid state. At IISc, the work includes development of RF pulse sequences for solid state NMR and study of short peptides, including hair-pin loops in peptides. Investigation of un-conventional *mesogens* has also been carried out using ^{13}C NMR in solid state. Aggregation of the amyloid beta peptide is a likely cause of Alzheimer disease and the beta sheet structures in meta-stable *amyloid oligomers* have been studied, by NMR and single-molecule fluorescence. Dipolar decoupling schemes have been developed. Uniformly ^{13}C labelled solid samples have been used to measure H-C distances. Conducting *perovskites* containing lanthanum ion are useful in battery materials. Such inter-metallic systems have been studied. Applications in biology cover studies on bones and muscles.

(v) NMR in Industrial Research: Industries have also started the use of NMR. Three types of Industries use NMR: (i) those involved in pharmaceuticals, (ii) those involved in cosmetics, and (iii) those working on materials. NMR has also been used to detect explosives. The demands on the configuration for machines for each of these applications are different. Most of this work is protected by patents and very little comes out in published form. Some applications, with which the author was involved are: (i) problem related to Industries in characterizing materials such as paints, glues and other adhesives. The samples are mixtures of several compounds. However, the Industry was not worried as the impure compounds worked. (ii) Compounds from pharmaceutical companies. Here, the chemical structure, stereochemistry and purity of the samples are essential and are highly demanding. The packing of molecules is important as the drug may be used for slow or fast release in patients. (iii) Mention may be made of a court dispute on a facial cream. One company felt that another one has violated their patent by marketing the same molecule under a different trade name. The matter was referred by a court to TIFR. It turned out that the molecules were same. (iv) Among the Industries that have used the NMR facilities at TIFR are: BASF, Biocon, CIPLA, Hindustan Unilever, Sandoz, Lupin Industries, Serum Institute of India and Sun Pharma. TIFR also helped these industries in the interpretation of the NMR spectra.

(vi) Detection of Explosives: Most explosives contain nitrogen compounds. NMR equipments have been installed at airports in USA to detect explosives in checked baggage. The machines use

low-field wide bore magnets such that baggage can pass through. There is no published work on such machines. A report was prepared for the Government of India on the configuration of such machines and the possibilities to introduce the same on Indian airports.

6. Biological Molecules

NMR made inroads in biochemistry and biophysics in 1980, following the wide-spread use of multi-dimensional FTNMR as well as developments in magnet technology, leading to very high-field magnets. With developments in MRI and MRS, its applications has also grown in and the area of medical sciences.

In biological systems, there are two classes of molecules. The first consist of small molecules and metal ions. The most important molecule in living system is adenosine triphosphate (ATP). ATP provides energy for different functions in cells and living system. When body needs energy, ATP is hydrolysed to adenosine diphosphate (ADP). ATP is generated from ADP during the metabolic pathways of living systems such as glycolysis. The balance between ATP and ADP is controlled by living systems. Some of the other important molecules are phospho-creatine and N-acetyl aspartate. Metal ions such as Na and K also play a role and balance between the two is vital for health. Mg is present in chlorophyll. Among the large molecules, the important ones are proteins and nucleic acids. These molecules are present in living systems and control various functions. DNA is the hereditary molecule. By the process of *translation*, it makes another DNA molecule, which is a replica of itself. DNA *transcribes* its information to synthesize RNA. RNA dictates the sequence of amino acids in a protein synthesized by the cellular systems. Proteins act as enzymes and are also involved in transport. Carbohydrates provide food and energy to living systems.

The information transfer in living systems can be sketched as follow:

$$\text{DNA} \rightarrow \text{RNA} \rightarrow \text{Proteins} \rightarrow \text{Cells} \rightarrow \text{Whole organs} \rightarrow \text{Living systems}$$

Cells are unit of living systems. An animal has several types of cells, with its own specialized function. Collectively they perform functions of organs and living systems. Cellular systems have an outer coat called membrane, and inside the cells, there is further compartmentalization. Molecules inside the cells are in highly organized state. Membranes help to separate the intra- and the inter-cellular media, and control the flow of molecules between the intra- and inter-cellular media. Membranes are mainly composed of phospholipids, in the form of lipid-bilayers. Proteins are embedded in lipid bilayers. Lipids are molecules of intermediate size, having molecular weights of around 800 D. Finally, the most complex systems are whole living systems such as animals, human and plants. NMR has been applied to the entire range of biological systems at various level of complexity. This includes: (i) problems dealing with the structure and function of proteins and nucleic acids. Some of these molecules show a dynamic behaviour which is essential for their function. Thus, folding and dynamics are two new themes, which could not be studied by X-ray crystallography. Another theme is the location of active sites in enzymes. (ii) NMR in body fluids, whole cells and tissues. (iii) Whole body NMR leading the studies into two parts: (a) NMR imaging (MRI), and (b) in vivo NMR spectroscopy (MRS). (iv) NMR can help the process of learning and in memory. This field is known as functional NMR (f-MRI). Each subject has been discussed in detail under different sections.

(i) Strategies in structure determination of large molecules: Structural determination of molecules such as DNA and proteins is much more complicated than that of an inorganic or organic molecule. These polymers have a large number of repeating units with spins in very similar chemical environment. Proteins are composed of

a repeating peptide backbone and 21 amino acids, in the side-chains. Nucleic acids are built from 4 bases (G, A, C and T for DNA and (G, A, C and U for RNA). The NMR spectra of such molecules are crowded and problems in resolution and assignment of resonance peaks or spectral features. The use of multi-dimensional NMR has resolved some of these problems. Values of coupling constants and NOE between spin-pairs form the basis for structure determination.

The first level of structural determination is to establish the sequence of amino acid or nucleotide, called the primary structure. There are biochemical techniques to establish the primary structure. The next step is to establish the three dimensional structure. Both the primary and the three dimensional structure is unique for functional proteins and nucleic acids. NMR has been helpful to establish the 3D structure. Structure for a molecule of N atoms, requires knowledge of N-1 inter-atomic distances, N-2 bond angles and N-3 dihedral angles. There are 3N-6 parameters to be determined. The bond lengths and bond angles do not vary much, and can be kept constant. Thus, one has to worry about the values of N-3 dihedral angles.

For each amino acid in a protein, there are about two dihedral angles, phi and psi. For a medium size protein of 100 amino acids, there are thus, 200 parameters to be determined. G N Ramachandran showed that because of steric factors, the possible values of the two dihedral angles, phi and psi lie in a limited range. He gave the allowed ranges in the form of what are known as Ramachandran (phi, psi) plot. Protein structures should satisfy Ramachandran map. With the knowledge of genome of many proteins, these molecules can be synthesized in laboratory and ^{13}C and ^{15}N can be incorporated for better sensitivity.

Nucleic acid structures are more complex. There are five dihedral angles in the backbone (alpha, beta, gamma, delta and epsilon). The pucker of the 5-membered sugar ring (ribose for RNA and deoxy-ribose for DNA) has to be determined. Finally, the intra- and intermolecular hydrogen bonding between the nucleic acid bases has to be established. Thus, unlike proteins, where one has to two dihedral angles per amino-acid, nucleotide structures have five degrees of freedom in the backbone structure. In addition, one has to worry about keeping the hydrogen bonds intact. Similar to Ramachandran's map, stereo-chemically allowed regions for nucleic acids have been constructed using quantum chemical methods. Computer algorithms have been developed for the purpose of determination of 3D structures from the NMR data. The observed data on coupling constants and NOE along with the primary sequence of the molecule under investigation is fed in a computer program. The program calculates possible structures of the molecule, which are consistent with the observed data. For proteins, such software packages can be divided into three categories: (i) metric matrix distance geometry, (ii) variable target function, and (iii) torsion angle approaches. Once the structure has been established, one checks the final structure for its validity. Extensive research in the area of structural determination of proteins and nucleic acids are being carried out at different laboratories.

(ii) NMR of nucleic acids: The usual structure of DNA is the B-form. RNA and DNA-RNA hybrids generally acquire A-form. A model of B-DNA structure was proposed by Watson and Crick from X-ray fibre-diffraction data. It consists of two strands of DNA oriented in anti-parallel orientation, which are held by Watson and Crick base pairs. Nucleic acids can adopt a number of alternative conformations, such as single-stranded hair-pin, triplexes and tetraplexes. Unlike proteins, very little work had been done on nucleic acids. The allowed regions in DNA conformations were investigated using quantum chemical theories. The dependence of the coupling constants on dihedral angles was also established. In collaboration with

Dr. H T Miles at the National Institute of Sciences (NIH), Bethesda, USA structures of several such structures were established. These include: (a) Non-self complementary duplex: GGCAGA GATCCGTA, (b) mismatched base pair such as in GGATGGCCAATCC which has A:A pairs, (c) a strand having hair-pin (d) triple stranded DNA, (e) four stranded DNA, and (f) parallel-stranded DNA. The most interesting work was on a parallel stranded DNA in CCATAATTTACC at acidic pH. Cytosines at the ends, constrain the two strands in parallel orientation. Several drugs bind covalently or non-covalently to DNA. As a result of such binding, cellular processes such as replication or transcription may be affected. The studies are of important for discovering anti-cancerous cells. Studies of drug-DNA binding are being pursued at IIT, Roorkee.

(iii) NMR on Proteins: Protein NMR gradually gained momentum from its initial days especially with the developments in multi-dimensional NMR. It is now one of the most productive fields in NMR. In India, protein structural determination is being done in a number of laboratories. Several new methodologies were developed, which have been used world-wide. Work done in some major laboratories is discussed below. R V Hosur has been working on applications of NMR, both to ordered and random-coil structures. Resonance assignments and structure of several proteins have been carried out. These include, GTPAse, effector domain of dynamin, structural biology and folding of SUMO proteins and their interaction with Ubc9, folding and plasticity of HIV protease and its variant. He has also studied protein aggregation. K V R Chary has solved 3D structures of proteins of Ca binding proteins. Conformational hetereogeneity, dynamics interaction and functional manipulation, using site-directed mutagenesis were also carried out by his group. Methodologies were developed for selective *unlabelling*, for the purpose of residue specific assignments. Stereo-specific assignment of methyl groups Val and Leu residues were carried out. A Arora (CDRI), H S Atreya (IISc), M Deshmukh (CCMB) and N Bhavesh (ICGEB) have all started working on other proteins. Arora's focus has been on important protein drug targets of *Mycobacterium tuberculosis*, for determination of the structure by NMR, and use the structural information in subsequent high-throughput screening for drug development. Atreya has attempted to structurally characterize insulin-like growth factor (IGF) system, implicated in cancer. This protein comprises of the following components: (i) two peptide hormones, IGF-1 and -2, (ii) type 1 and type 2 IGF receptors, (iii) six IGF-binding proteins (IGFBP: numbered 1-6), and (iv) IGFBP proteases. He has also developed new NMR methodologies and has proposed new pulse sequences. Bhavesh's research is on RNA binding proteins, while Deshmukh has focused on RNA binding proteins, RNA interference, RNA metabolism and conformational dynamics. He is currently investigating the structural basis and mechanistic details of the RNAi pathway in C. elegans and Drosophila. They are involved in solving solution structure of RDE-4 (44 kDa) and R2D2 (36 kDa).

(iv) NMR software and pulse sequences: The first pulse sequence developed in India was SUPER-COSY. It was a minor variant of COSY. Hosur developed new NMR pulse sequences such as 3D HNN, 3D HN(C)N, 3D HNN-HNCN, 3D hnCOcaNH, 3D hncoCANH, and 2D (HC)NH for the resonance assignment and structure determination. For high throughput protein structure determination, an algorithm called *Tracked Automated Assignments in Proteins* (TATAPRO) was developed by Chary and co-workers. It is robust to internal sequence homology and unusual chemical shifts for sequence specific resonance assignments. It is one of the most cited methodologies. Methodologies were developed for selective un-labeling of amino acids for residue specific and stereo-specific

assignments. His group also has developed novel methodologies and proposed new NMR pulse sequences which speed up the acquisition of multidimensional NMR data and resolve chemical-shift degeneracy. Information about chemical shifts, pseudo-contact shifts and coupling constants can be obtained. Stereo-specific resonance assignments can also be made.

(v) Comparison of NMR with other methods: Circular dichroism (CD) has been used to study protein structures. However, this method helps only in estimation of the amount of amino acids in helical and sheet structures. The only other technique which helps structure determination at atomic resolution is X-ray crystallography. India has a long tradition of X-ray crystallography with pioneers such as G N Ramachandran and M A Vishwamittra. In modern times, M Vijayan and T P Singh are involved in such studies. The NMR methods and crystallographic methods have some differences. NMR is done in aqueous solutions, the medium of biological systems. Physiological conditions can be generated. The molecules in water are more flexible and are hydrated. Crystallography is done in solid state. During crystallization, some water is present, but the molecules acquire a rigid structure. NMR therefore scores over crystallography in the study of dynamic behavior of macro-molecules. On the other hand, crystallography provides structures with greater resolution. The structures are normally deposited in data-banks. At present the ratio of structures by NMR and X-ray in such data banks are in the ratio 1:3.

6. Cells and Tissues

The next level of biological studies cells, tissues and body fluids. NMR based metabonomics can be used, to address a large range of pharmacological, clinical and related problems. From mammalians fluids like urine and plasma are easily obtained. NMR can also be used to study more complex systems such as seminal fluids, dialysis fluids, biopsy sample and *in-vitro* cells systems. Such studies have widespread applications in diagnosis of metabolic disorders.

(i) Lipid-bilayers: Cells have an outer coat, which separates the ordered interior of the cells from the inter-cellular media. Such a biological membrane is essentially composed of two set of molecules. One set are the lipids that have a hydrophilic head group and two hydrophobic tails. At the ambient temperature of the body, lipid bilayers are in liquid crystalline phase. At lower temperatures, lipid bilayers go in a gel phase. Body selects lipids such that they are in liquid crystalline phase at the body temperature. There are compounds such as cholesterol, which makes the lipid bilayers rigid. The other set of molecules are proteins. Molecules such as ATP help in pumping molecules from outside to the inside of the cells. Study of model membranes was initiated by R Phadke and S Srivastava of TIFR. They prepared lipid bilayers and studied changes in liquid crystalline to gel phase with temperature. The bilayers were incubated with different class of compounds such as anti-oxidants, membrane bound drugs and vitamins. In particular, vitamin E has pharmacological importance. This resulted in a number of useful ideas on what molecules such as cholesterol can do to the fluidity of lipid bilayers. Work was extended to drugs whose pharmacological action depends on their binding with lipids.

(ii) Cellular NMR: The main metabolism in cells consists of burning of carbohydrates to CO_2 and H_2O, which is an exothermic reaction. Cells store energy thus released in the form of ATP. ATP is utilized when energy is required by living system. The metabolism proceeds in several steps and are classified into three main heads: Glycolysis, Citric acid cycle and Oxidative phosphorylation. Work in India on cellular NMR started quite early and we were among the pioneers in studies of animal cells. S Srivastava and R S Phadke started the work on goat spermatozoa in 1980s. These studies are

important from the view point of animal husbandry. Spermatozoa undergo maturation at three levels: *corpus*, *caput* and *cauda*. ^{1}H, ^{13}C and ^{31}P NMR showed dramatic changes as cells maturate. Such studies led to useful information on the metabolic activity in sperms. Cells were fed on ^{13}C labelled glucose and the glycolytic pathway was followed as a function of time. Complete ^{1}H resonance assignments were carried out using TOCSY and DQF COSY spectra of intact cell and cell free extracts. Metabolism was followed in real time. Abnormal or diseased cells could be identified using NMR. To predict for cerebral malaria in mice, H M Sonawat and S Sharma of TIFR have used serum from infected and control mice and measured changes in the lipid to lipoprotein levels, post infection. They have shown that metabolism is differentially regulated in the infected and normal mice. The glucose utilization was followed using labelled glucose and ^{13}C NMR as a function of time. At CBMR, Lucknow, Khetrapal and his group carried out NMR of body fluids obtained from patients like urine, which are helpful in disease diagnosis. In fact, *in-vitro* analysis of samples provides a reliable tool for study of a number of diseases and help in early diagnosis. Jagannathan and his group has carried out the characterization of breast and prostrate tissues with a aim to find out biomarker/s for early detection of cancer and for monitoring therapeutic response of tumors and to study the tissue metabolism.

7. Whole Body NMR

In 1972, the International Society on Magnetic Resonance (ISMAR) held their conference at TIFR. There was an abstract by Paul Lauterbur under the title NMR *Zeugmatography*. The organizers realizing the potential, the poster was upgraded to a plenary lecture. Lauterbur, through images of uncut oranges showed how NMR can be used to look inside a fruit, in a non-invasive manner. This was the beginning of NMR Imaging (called as MRI), which has become today a routine tool for medical diagnostics. Further, it is also possible do NMR of tissue samples or body fluids called *in vitro* spectroscopy. Similarly, spectrum from any organ of the human body can also be obtained which is called as *in vivo* MR spectroscopy, or simply, Magnetic Resonance Spectroscopy (MRS).

(i) Magnetic Resonance Imaging (MRI): MRI utilizes the same principle of NMR to study the distribution and mobility of a particular molecule in a human, plant or animal. Mostly ^{1}H resonance from H_2O is used for this purpose. In some cases ^{31}P has been used to obtain ATP images. Magnetic fields of 0.3 to 3 Tesla are used for routine MRI. The MRI magnets have large pole gaps and are oriented such that a subject can lie in horizontal direction. The subject can then be placed in a *supine* or in a prone position. An important feature of these MRI scanners is that the magnetic field gradients are applied in three perpendicular directions. This results in magnetic field at each volume element (voxel) to be different. Hence, nuclear spins in each voxel have different resonance frequency. At the current level of sensitivity, images from a slice thickness of 0.5 mm or less can easily be obtained. The mobility of the molecules under study as reflected by relaxation times T1 and T2 provides contrast in MR images. By image reconstruction techniques, a 3D picture of an organ can be obtained. An alternative technique for imaging is CT scan that uses X-rays. While NMR provides maps of the soft tissues, CT provides status of hard components of the body such as bones. The two techniques thus complement each other. The first MRI scanner was installed at INMAS, Delhi in 1986. INMAS is a hospital cum research Institute under DRDO organization. The objective was to provide services to army soldiers. This was followed by installation of MRI at the Beach Candy hospital at Mumbai, SGPGI, Lucknow and at AIIMS, New Delhi. Today, there are more than

3000 MRI scanners in India, mostly in hospitals and are used for patient care. Research using MRI and MRS are however being carried out mainly at INMAS, CBMR, CCMB, SGPGI, NIMHANS, Sree Chitra Thirunal Institute of Medical Sciences at Thiruvananthapuram and AIIMS.

An important use of MRI is its ability to image any organ of the human body for evaluating various diseases that affect humans like tumours, abnormalities related to spine, brain injuries, brain damage due to stroke, etc. Radiation induced early brain injury can also be studied. Metabolic alterations due to chronic cold stress have been studied using MRI. Efficacy of Levodopa in Parkinson's disease has been followed using MRI and MRS. The group at INMAS used MRI and MRS techniques on armed force personnel exposed to extreme climatic environment and mental stress. Effect of ageing on brain structure and function has also been studied by researchers. High grade prostatic intra-eptithelial neoplasia is a precursor of prostate cancer. Studies using MRS method on patients showed an increase in choline level with reduced citrate level that may be used as biomarkers of prostate malignancy.

(ii) Magnetic Resonance Spectroscopy (MRS): In MRS, one combines MRI with MR spectroscopy. Most of the applications of *in vivo* MRS have been using ^{1}H and ^{31}P nuclei. . The aim of *in vivo* MRS is to obtain a spectrum that arises exclusively from the volume of interest or region with the best achievable sensitivity and with minimum contribution from other regions. At the current level of sensitivity, volume size of 1 mm^3, give satisfactory MRS signals of ^{1}H. The most important molecules are ATP and phosphocreatine, which provide the energy status of the organ. In general, in-vivo ^{31}P MRS shows signals from ATP (three resonances arising from α, β, and γ phosphates), inorganic phosphate, phospho di-esters (PDE) and phospho mono-esters (PME). The metabolites detected using the ^{1}H *in vivo* MRS from normal brain has three predominant resonances from N-acetylaspartate (NAA at 2.0 ppm), choline (Cho at 3.2 ppm) and creatine (Cr at 3.0 ppm). Early *in-vivo* MRS work was started on neuro-infections by R. K. Gupta of SGPGI. Animal MRI and MRS work was initiated by NR Jagannathan and P Raghunathan at AIIMS. Research using MRS is now being carried out in a number of laboratories. Particular mention may be made of AIIMS, SGPGI, INMAS and NBRC. One of the interesting studies in this area is the work of A Patel and his group at CCMB, Hyderabad. He has carried out *in-vivo metabolic* profile studies using MRI and proton and carbon MRS on regional cerebral energetics in MRTP model of Parkinson's disease. The recovery of cerebral metabolism after treatment was studied. Such work on animal models is important for drug-development. He has also worked on brain energy metabolism in an animal model of alcoholism.

(iii) Functional MRI (f-MRI): Seji Ogawa pioneered the technique of f-MRI. He showed that the processes such as learning can be monitored by NMR. The basis of this technique is that the neural activity is detected by a blood oxygen level dependent (BOLD) signal. In blood, the deoxy-hemoglobin is paramagnetic, while oxy-hemoglobin is dimagnetic. During neural excitation, blood flow increases to the area of excitation. Normally, there is more deoxy-hemoglobin in the area of excitation. This can be monitored by NMR. The technique of f-MRI has a major impact on how the brain functions and store information. The first paper from India on the subject was published from AIIMS by Jagannathan and Raghunathan. Slowly, several groups started to work in this area. At present, there are several groups working on f-MRI. At AIIMS, work is going on in the area of neuro-cognitive changes associated with perception, spatial orientation, language-perception in visually challenged subjects, in epilepsy and stroke and during sleep. In INMAS the fMRI research is being carried on motor system and events related

to sensory motor task and in depression. At NBRC the area of research includes: cortical mapping in Alzheimer disease, multilingual populations, epilepsy, memory performance and analysis. fMRI in cognitive neuroscience is the focus area of research at CBMR, Lucknow. At NIMHANS work related to neuropsychiatric disorder like schizophrenia, movement disorders are being carried out.

(iv) NMR in Plant Sciences: NMR has also been applied to several aspects of plant sciences and had a potential application in development of *transgenic plants*. Not much work has been done in India on plants. PN Tiwari and Gambhir initiated plant NMR by measurements on T1 and T2 relaxation time some plant materials at the Indian Agricultural Research Institute, New Delhi. Recently work has been started at TIFR, to study the acetate metabolism in a unicellular algae *chlamydomonas reinhardtil*. They have studied acetate metabolism during the light and dark phases of growth. Some work has been initiated at AIIMS.

8. Collaborations, Conferences, Schools and Training Programs

India entered into international collaboration in the area of NMR with the National Institutes of Health, USA. The specific work that was identified for collaboration was related to non-standard forms of nucleic acids. The second collaboration was with Italy and was supported under Eurpoean Union. Recently, many Indian laboratories have entered in to research collaboration with France, Germany, and other countries through bi-lateral Indo-French, Indo-Germany programs. In addition, a number of common research and training programs were arranged.

TIFR has a long tradition of organizing summer schools and teaching programs in NMR. The first such school in the field of Magnetic Resonance was held in 1965 at Nainital. Hahn (for NMR) and Stevens (for ESR) were the main speakers. Ironically, Dharmatti died in Mumbai one day before the school was inaugurated. Several physicists attended this conference. The group photograph shows several scientists such as A K Saha, K P Sinha and others. Among the chemists, P T Narasimhan, B Venkatarman and this author attended the school (Fig 2).

The 2nd International Conference of the International Society for Magnetic Resonance (ISMAR) was held in Mumbai in 1972. As stated earlier, Paul Lauterbur delivered a plenary talk on NMR Imaging (MRI). The conference was followed by a workshop. Thereafter, India hosted a number of International Conferences, Schools and workshops on NMR. The XI International Conference of International Society for Magnetic Resonance in Biological Systems (ICMRBS) was held in Goa in 1984. Before this conference, there was a workshop on MRI at IIT, Kanpur hosted by Narasimhan. Preceding the conference, a workshop on bio-molecular NMR was also held. One of the notable participants in the Goa conference was Ovchnikov, the Vice President of USSR Academy of Sciences. At this meeting, Govil, Khetrapal and Anil Kumar were elected to the Council of ICMRBS for 12 years. Later, the author was elected Chairman of the Council for three years. In January 2005, the XXI ICMRBS was held in Hyderabad. This was preceded by a workshop on MRI at AIIMS and after the main event another workshop was also held. Following this conference, Chary, Hosur and Jagannathan were elected to the Council for 12 years. Later, Chary served as President of ICMRBS for three years.

Among the international events, two schools need some special mention. The International Union for Pure and Applied Biophysics (IUPAB) has the tradition of organizing training programs for less developed countries. On author's initiative, two NMR workshops were organized. One was in Japan and

Fig. 2. First NMR School organised by TIFR at Nanital in 1965

the other was in India. The workshop in India was held in Mumbai. In both events, participants from less developed countries in Asia, such as Nepal, Sri Lanka, Bangladesh, and Thailand attended. Two Indo-Italian workshops were also held. One was in Italy and the other at SGPGI, Lucknow.

In addition to above international events, a number of schools and workshops are arranged from time to time to train young students entering the field of NMR. TIFR, Mumbai regularly organizes one school every year. These have been held in Mumbai, Hyderabad and at few other locations. IISc and SGPGI have also held such programs. A partial list of such training programs are: Modern techniques in pulse NMR under Ernst and Wider, at Mumbai (1984); Hardware and software aspects of NMR at Mumbai (1986); Biological Applications of NMR at Mumbai (1988); 2D FT NMR at NEHU, Shillong (1990); NMR in Structural Biology at Mumbai (1995); NMR of solid-state at Mumbai, (2003); Principles of solid state NMR at Mumbai (2010); and Recent Advances in NMR Spectroscopy in 2011 at the Hyderabad campus of TIFR. In addition, some workshops have been organized by the SIF, Bangalore and by SGPGI, Lucknow.

Several years back, an Association of Magnetic Resonance Spectroscopists (AMRS) was established. It started holding meetings at the time of Indian Science Congress. This body stopped functioning after two years. Subsequently in 1995, the National Magnetic Resonance Society (NMRS) was established by scientists interested in NMR, which is now very active and conducts a

three/four day national symposium, each year at different locations in India.

13. Books Published by Indian Authors

As indicated earlier, NMR was the field of interest only for physicists, in early years. A number of physicists got interested in the field. Two physicists, Ajit Kumar Saha and Tara Prasad Das at the SINP were among them. They wrote a book entitled *Theory and Applications of Nuclear Induction*. The book is divided in seven chapters and covers Classical and Quantum theory, experimental techniques, theory of nuclear relaxation times and nuclear quadrupole resonance. Interestingly, the last chapter is devoted to chemical sciences and describes chemical shifts and J-coupling. This 508 page book was among the first one to be published on NMR. A couple of years later, a book written by Pople, Bernstein and Schneider appeared which was devoted entirely to chemistry. In later years, a number of books were written by Indians. It is possible that some books may be missing from the list. Study of structure of molecules by orienting the same in liquid crystals was pioneered by Diehl and Khetrapal. Their early work has been published in a book. Some of the other books are those written by Hosur and Govil, and by Chandrakumar and Subramanian, in 1986 and by Raghunathan in 2007. Chary and Govil's book (2008) possibly gives an exhaustive account of NMR in Biological Systems. A list of edited books is also given in the list.

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