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SOME POSSIBILITIES FOR THE USE OF NUCLEAR MAGNETIC RESONANCE SPECTROMETERS IN ORGANIC AND BIOLOGICAL CHEMISTRY

By B. M. Tolbert*

University of California Radiation Laboratory
Berkeley 4, California

October 20, 1955

* The work described in this paper was sponsored by the U. S. Atomic Energy Commission.

The concepts of tracer methodology have been developed with radioactive and stable isotopes and such technical tools as mass spectrometers, falling-drop deuterium water analyses and Geiger counters. Abundant artificial radioactive tracers have greatly increased the value of the method; we are certainly all familiar with the tremendous progress that has been made in the past ten years using pile produced radioactive elements. The discovery of the nuclear magnetic resonance (NMR) phenomenon by Bloch and Purcell[1] (for which they received the Nobel Prize) and its consequent development has made possible a new tracer method. This new tracer method has two important advantages over radioactive tracers and at least one major weakness. It can be applied to all the important elements of biological systems -- carbon, hydrogen, nitrogen and oxygen. It gives important information concerning the nature of the bonding of the isotope, something which neither radioactive analysis nor mass spectrometer analysis does; and its only important weakness is that it is a relatively insensitive analytical method.

I would first like to describe very briefly what a nuclear magnetic resonance spectrometer is and how it works. An atom that has either an odd atomic weight or an odd atomic number, or both, will have a nuclear magnetic moment; that is, the

1 F. Bloch, Phys. Rev., 70, 460 (1946); E. M. Purcell, H. C. Torrey and R. V. Pound, Phys. Rev., 69, 37 (1946).

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nucleus acts as a small magnet. If this nucleus, with a given magnetic moment and a given mass, is put into a large uniform magnetic field of about 10,000 gauss, it will have a resonance frequency of somewhere between 2 to 40 megacycles per second. You will recognize this frequency range as being characteristic of the common short wave bands of our radio system. Our ordinary radio band ends at 1.6 megacycles and is followed thereafter by the various short wave bands. The well-known 20-meter "Ham" band occurs at about 15 megacycles. If we insert two coils of wire parallel to and at right angles to the magnetic field and supply a varying frequency radio-energy to one of these coils and attach the other coil to a sensitive radio frequency analyzer, which is, in effect, a good radio followed by a mechanical display syste, we can scan the radio frequency spectrum. At the frequency at which the magnetic nucleus resonates in the magnetic field, energy will be absorbed by the nucleus and will be re-radiated in all directions. The second coil will pick up this energy and will show a signal at this time (See Fig. 1).

The frequency of this signal is different for the various nuclei and for the different chemical environments of a given nuclear species. The amplitude is proportional to the number of the nuclei present in the field. These three facts are the basis of nuclear magnetic resonance spectrometric technique in chemistry and biology.

In the opening paragraph of this report I mentioned that this technique was applicable to carbon, hydrogen, nitrogen, and oxygen studies. Of these four substances, two are particularly interesting, namely, nitrogen and oxygen, since there are no radioactive isotopes of these elements, and our knowledge of their role in biological systems is extremely meager. Two papers from the University of California Radiation Laboratory, one of them done jointly with Varian Associates in Palo Alto, have laid the groundwork for NMR analysis of oxygen and nitrogen in chemical and biochemical studies. These papers have recently been submitted to the Journal

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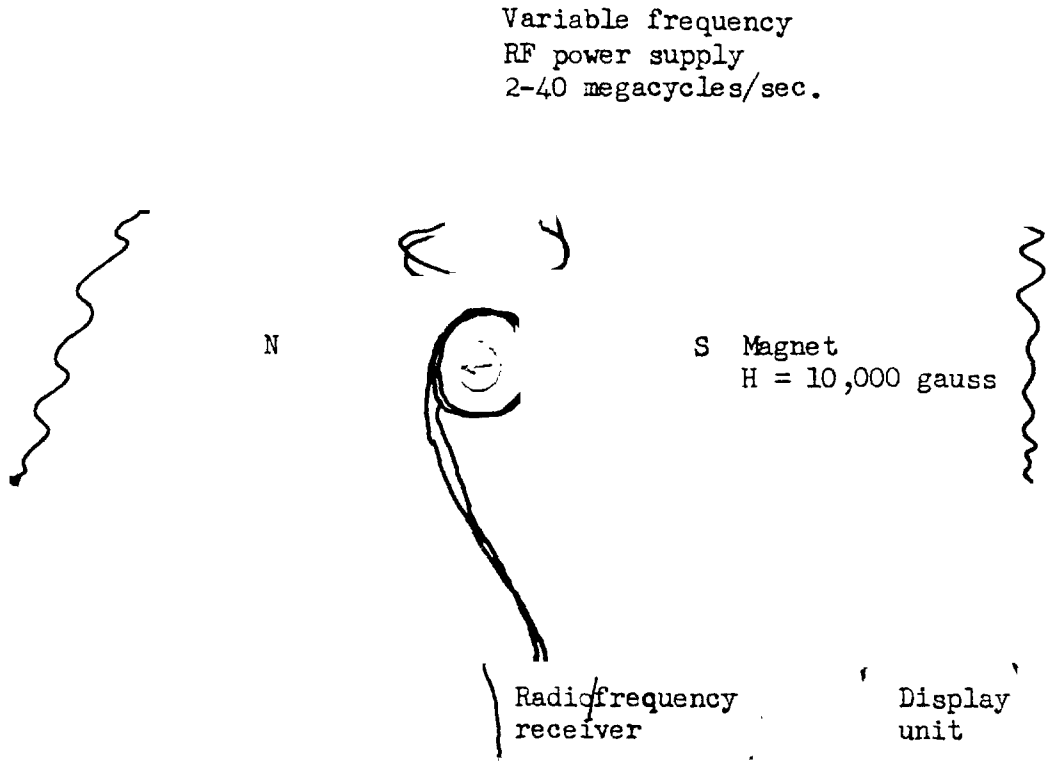

Fig. 1. Simplified schematic diagram of a nuclear magnetic resonance spectrometer. The sample is represented by a magnetic nucleus, ⊖ , and is inside the pick-up coil.

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of Chemical Physics and are entitled, "Chemical Shifts of Nitrogen," by B. E. Holder and M. P. Klein and "Observations of Chemical Shifts of O^{17} Nuclei in Various Chemical Elements," by H. E. Weaver, B. M. Tolbert and R. C. LaForce. In these two papers it was shown that the resonance frequency of the nitrogen and oxygen nucleus varied with the chemical environment and that there were a rather discrete series of resonance frequencies for a number of the common chemical states of oxygen and nitrogen. The difference in frequencies arises from the fact that the magnetic field which the atomic nucleus sees is changed by the circulation of the molecular electrons surrounding it. The effect of the circulation of these electrons is in most cases to decrease the amount of the applied magnetic field and is modified by the nature of the bonding orbital electrons. In the paper by Weaver, et al., O^{17} resonances for the following substances were noted: tetramethyl orthosilicate, butyl nitrite, hydrogen peroxide, acetic acid, formic acid, sodium formate, ethyl nitrate, sodium nitrate, sulfur dioxide, acetone, nitroethane, and sodium nitrite. In the work by Holder and Klein, the following types of nitrogen compounds were studied: ammonium ion, dimethylamine, hydrazine, tetramethyl ammonium bromide, ammonia, urea, hydroxylamine hydrochloride, acetamide, thiocyanate, methyl cyanide, methyl thiocyanate, pyridine, sodium nitrate, nitromethane, nitropropane, and sodium nitrite. The nitrogen and oxygen in these substances showed discrete resonance spectra.

The tracer application of this technique is straightforward. Enhanced concentrations of stable isotopes in various chemical forms can be added to a system. After a reaction has occurred, the new system can be analyzed. Like radioactive isotopes, the technique permits the detection of an enhanced concentration of a given isotope in an unknown without the need for isolating the unknown as a pure substance or even having any idea of its chemical nature. Furthermore these analyses are of a nondestructive nature. These are the great advantages of nuclear magnetic resonance spectrometry over mass spectrometer analysis of stable isotopes. In

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mass spectrometer analysis one must make intelligent guesses as to the nature of the intermediates into which the stable isotope may go, isolate these intermediates and do an analysis on the isolated material.

The other advantages of NMR spectrometry are that at the same time one measures increased isotope concentrations, one also determines something of the nature of the chemical bonds and structure of the molecule. This information is unique to this method and is not obtained from mass spectrometer or radioactive analyses.

The isotopes that are likely to be most important in organic and biological chemistry are carbon-13, deuterium, nitrogen-15 and oxygen-17. Carbon-13, the only stable nucleus of carbon with a magnetic moment, is presently available in limited quantities at a very high price from Eastman Kodak. It costs about $400 per gram of C^{13} in 50% concentration. Both the stable isotopes of hydrogen have nuclear magnetic moments and are readily detected; furthermore, deuterium oxide (heavy water) is available at a fairly reasonable price, $1.00 per gram in small quantities and about 50 cents per gram in very large quantities. Both nitrogen-14 and nitrogen-15 have a magnetic moment and may be detected in an NMR spectrometer. Nitrogen-15 may be used in tracer experiments with nuclear magnetic resonance and is available from Eastman Kodak Co. for about $300 per gram of nitrogen-15 in 60% concentration. Clewett and his research group at the Oak Ridge National Laboratories have made studies on the ammonia-ammonium carbonate system and on the nitric acid-nitric oxide system. They estimate that a plant could prepare 95% N^{15} at the rate of a pound per day for a cost of about $4.21 per gram. This figure would go down by approximately one-fourth for a 5 pound per day plant. There is a great deal of difference between this figure and Eastman's current price for N^{15}.* Oxygen-17 is the only isotope of oxygen with a magnetic moment. This

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* I do not mean to be critical of Eastman Kodak's great contribution to research. They are the only commercial company in America who have undertaken to make these stable isotope concentrations available and have thereby contributed greatly to science and industry. But now more highly enriched and less expensive material is needed.

occurs in natural oxygen to the extent of .037%. Enhanced concentrations of O^{17} are currently available through various distributors from the Weizmann Institute in Israel. The cost has been about $65.00 per gram for material containing 0.7% O^{17}. The advent of this new tracer and chemical structure analysis method will create a great need for more reasonably priced nitrogen-15 and carbon-13 concentrates and for O^{17} in reasonable concentrations at almost any price.

It would not be fair for me to discuss a novel method such as this without discussing something of the limitations of the technique. The primary limitation of the present technique is in the size of the sample that is required to give an adequate signal to noise ratio. For carbon-13, deuterium, nitrogen-15 and oxygen-17 some 10^{16} atoms are the minimum that can be detected theoretically* and 10^{18} - 10^{20} atoms are usually needed to make a measurement. Sizes of samples are currently restricted to about 3 cc., but we are hoping to increase this several-fold in the near future. The fact that so many atoms, relatively speaking, are required by this analytical procedure emphasizes the need of this country for adequate quantities of these critical stable isotopes at reasonable prices.

* The number of atoms required for a 10 to 1 signal to noise ratio in 2-1/2 cc. volume with a Varian** NMR spectrometer is about: C^{13}, 3×10^{18} atoms; D, 5×10^{18} atoms; N^{15}, 5×10^{19} atoms; O^{17}, 2×10^{18} atoms.

** Varian Associates, 611 Hansen Way, Palo Alto, California.