

Nuclear Magnetic Resonance Studies of Cancer and Heart Disease

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I. INTRODUCTION

During the last decade, numerous papers have appeared dealing with the application of nuclear magnetic resonance (nmr) techniques to physiology and medicine. Of great interest are the efforts to apply nmr to specific diseases such as cancer and heart disease. The purpose of this review is to describe these efforts and the concepts underlying them and to assess critically the significance and potential of current nmr studies with respect to eventual clinical applications. I have not attempted a thorough compilation of the published literature nor a critical approach to all aspects of the controversial problem of understanding the nmr properties of cells and tissues. Rather, I hope to place the potential medical applications of nmr in perspective and provide the reader with an accurate impression of the current status of this field.

Efforts to apply nmr to biological systems have a long history. In 1952, only six years after Bloch and Purcell first observed nmr in bulk specimens, the first report of the application of nmr to biological specimens appeared (1), and in 1955, nmr results on mammalian tissues were first reported (2). These early studies, although they solved no medical or physiological problems, showed that the nmr properties of biological specimens could be measured and pointed the way toward future approaches to problems related to physiology and medicine.

II. NMR AND CANCER

The proposed applications of nmr to cancer research, detection, and diagnosis are based on the connection

between the nmr parameters of water nuclides (^{17}O , ^1H , ^2H) and other nuclides (^{31}P , ^{23}Na , ^{39}K) and the composition and state of the intracellular water, macromolecules, and ions. This area has been covered recently in an excellent review (3) and will only be summarized here.

A. NMR of Protein Solutions

The presence of dissolved macromolecules enhances the nuclear magnetic relaxation rates of water protons. This suggests an interaction of the water molecules with the protein surface and a rapid exchange between motion-hindered surface and bulk fractions of water. However, surface water is not rigidly immobilized. Kuntz et al (4) have, in fact, shown that the water of hydration of proteins remains in a liquidlike, unfrozen state even at very low temperatures. Quantitative estimates from the nmr spectra of the unfrozen water suggest a single layer of water of hydration covering the surface of the protein. A striking feature of the water nuclides in protein solution is relaxation dispersion, i.e., the dependence of the relaxation rate on the frequency of measurement (5). By measuring the Larmor frequency at the inflection point of the dispersion curves, Koenig and Schillinger (5) determined the correlation time, τ_C , of the water nuclides and found that they were nearly equal to the rotational relaxation time of the protein molecule. Thus, the rate of tumbling of the water molecule depends on how fast the protein molecule is tumbling. The simple and obvious interpretation of these data, namely, that a small fraction of water molecules immobilized on the protein exchange rapidly with bulk, free water molecules does not seem to apply. Calculation of the number of water molecules

firmly bound by the protein and their lifetimes in the bound state gave unreasonable values (6). It appears that no simple hypothesis accommodates all of the nmr data on water nuclides. A combination of exchange between free and immobilized fractions and some degree of ordering of the water molecules with the protein surface may be required. In any case, the nmr relaxation dispersion of protein solutions is still not fully understood although great progress has been made.

B. NMR of Intracellular Fluids

The nmr of water nuclides in erythrocytes was studied by Lindstrom and Koenig (7). The relaxation dispersions induced by hemoglobin in packed erythrocytes, whole blood, and aqueous solutions of hemoglobin were all determined only by the hemoglobin concentration. Inclusion of the hemoglobin within the cell membrane does not significantly affect its molecular motion.

The nmr characteristics of water nuclides in more complex tissues have also been studied. Striated muscles have received the most extensive attention (3). A detailed and convincing study of all three water nuclides by Civan and Shporer (8) indicates that the molecular dynamics of intracellular water are determined only by the molecular composition and not directly by the physiological state of the cell. Changes in physiological state (i.e., malignant transformation, differentiation, or progress through the cell cycle) can, of course, alter the composition of the intracellular fluid and thus alter indirectly the measurable nmr parameters.

Considerable work has also been done on the nmr of intracellular sodium. Early work (9, 10) indicated and subsequent work confirmed beyond doubt (11, 12) that there are at least two components of the transverse relaxation process of intracellular sodium. The results were originally interpreted in terms of a large fraction (> 60%) of immobilized, bound sodium. Later work (13) has indicated that the two-component behavior actually results from quadrupolar effects that do not require the existence of two populations of intracellular sodium. Either a very small immobilized fraction or a uniform free fraction exchanging among domains of electrical charge can account for the results. Although little information is available concerning nmr of intracellular ^{39}K , it appears that it behaves similarly to ^{23}Na (3).

In summary, it appears that the simplest view of the cell as a bag containing a solution of proteins, salts, and other molecules is adequate to describe the known nmr data, at least for erythrocytes. No data exist that indicate long-range organization or special properties of water inside the red cell. More complex, anisotropic cellular systems, such as muscle, affect certain proper-

ties of water in such a way as to alter its nmr properties. Other highly organized cells or tissues in which the macromolecules have long-range order can give similar effects (14). Small differences in motional freedom of water along different axes in space, leading to preferred directions of translation and axes of rotation, can produce noticeable effects on nmr relaxation rates.

C. NMR of Malignant Tissue

Damadian (15) was the first to observe that certain malignant tissues of rats had much longer T_1 values for water protons than normal tissues have and that benign tumor values fell between the two extremes. In these studies the "null" method for T_1 measurements was used. The rat tumors used in Damadian's original study were the Walker sarcoma and the Novikoff hepatoma, two well-characterized malignant solid tumors of the rat. Six normal tissues of the rat were also examined: muscle, kidney, stomach, intestine, brain, and liver. In addition, a single benign tumor, namely a fibroadenoma, was measured. The basic results were that the relaxation times T_1 and T_2 of water protons in the malignant tissues studied were distinctly outside the range of values for the normal tissues. On the basis of these results Damadian suggested using magnetic relaxation methods for rapid discrimination between benign and malignant surgical specimens.

Damadian's original work generated considerable interest in the relationship between the nmr properties and the biological properties of tissues particularly with regard to the possible diagnosis or detection of cancer. These results raised several questions concerning the basis of the observed T_1 and T_2 differences between benign and malignant tissues, as well as the possibilities of their practical application in a clinical setting. Some of these questions are: (a) Are the lengthened T_1 values really characteristic of malignancy? Since Damadian had examined only two malignant tumors, both of them rapidly growing, invasive types, it appeared desirable to study a wider range of experimental malignant tumors differing in characteristics including growth rate, degree of differentiation, and origin. (b) Would it be possible to distinguish malignant tissues from benign lesions, including benign tumors, infections, necrotic tissues, and other abnormal but nonmalignant states? (c) What is the underlying physical basis of the lengthened T_1 and T_2 values of experimental malignant tumors, as compared with normal tissues? (d) Assuming that the elevated relaxation time values were sufficiently characteristic in the case of experimental animal tumors, would the same specificity carry over to the case of human cancers? The experimental animal tumors are generally well-defined,

well-localized lesions, and the nmr measurements can be made on specimens of essentially pure tumor. In the case of human cancers, however, the malignant cells frequently occur in a tissue of a variable and heterogeneous nature. The vascularity of tumors, the amount of supporting stroma, the number of accompanying inflammatory cells, and the amount of hemorrhage and/or necrosis in the tissue could well have significant effects on the relaxation time values. In the seven years since Damadian's original observation much effort in a number of laboratories has been directed toward answering these questions.

Work in several laboratories (16, 17) has substantiated Damadian's observation that experimental malignant tumors in rats and mice generally have longer T_1 values than does any normal tissue. Typical results are shown in Figure 1. It is notable that there is no overlap between any of the experimental tumors and any normal tissue. Later studies, however, used Morris hepatomas, which are a series of chemically induced, transplantable hepatomas of the rat that have different

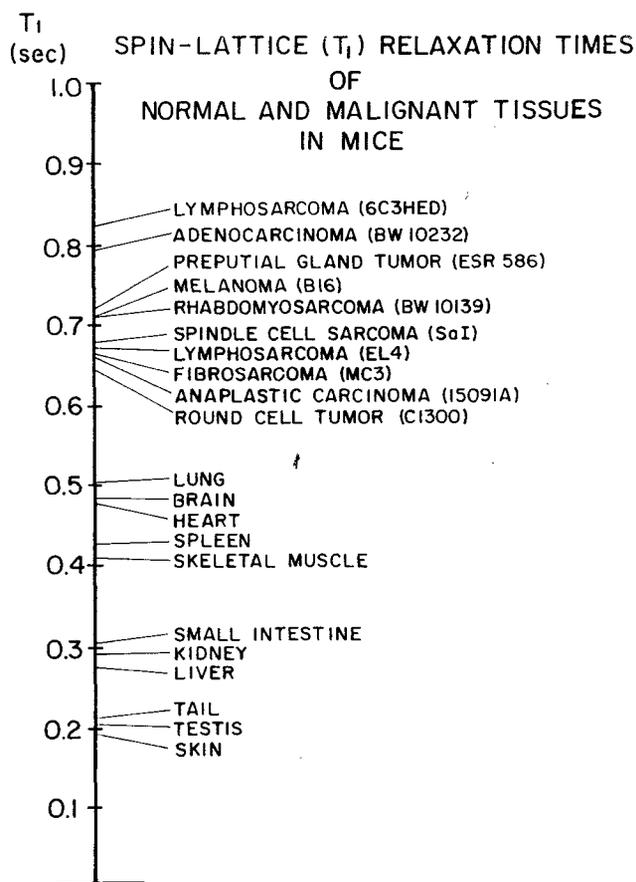


Figure 1. Spin-lattice relaxation times of water protons in normal and malignant tissues of mice. Data obtained at 23 MHz, 27° C.

growth rates and morphological properties and have other characteristics ranging from those of nearly normal liver cells to those of poorly differentiated malignant tumors. These studies revealed a correlation between the degree of differentiation as measured by the growth rate and morphological appearance of the tumor and the T_1 values (18). The Morris hepatomas seem to be truly malignant tumors, at least in the sense that they do metastasize and eventually kill the host animal. It was found in addition that the more slowly growing Morris hepatomas showed T_1 values shorter than those for some normal tissues. These studies also showed that there was a close correlation among the water content, the growth rate, and the T_1 value of the particular tumor being examined. This overlap between the T_1 values of certain normal and malignant tissues unfortunately raised the possibility of confusion between normal and malignant tissues when studied by nmr. Even the most slowly growing hepatomas, however, still had T_1 values considerably longer than normal liver tissue, and the hypothesis that the T_1 values of malignant tumors in experimental animals are longer than those of the tissue of origin still seems to hold.

Figure 2 shows the T_1 values of a selection of normal tissues from rats and mice as compared with a number of malignant tumors. From these results it was suspected that the T_1 value would not be sufficiently characteristic of malignancy to allow reliable distinction between normal and malignant tissues, even in the case of experimental tumors of small animals. The overlap of T_1 values for malignant and normal specimens raises the possibility of confusion between primary tumors adjacent to sites of similar or higher T_1 value or between normal tissue and metastatic tumors having a similar or lower T_1 value. Several groups have shown that the T_1 values of tissue water are closely correlated with the water content, i.e., the degree of hydration of the tissue (19, 20, 21). A change in water content of only a few percent is sufficient to account for the increased proton T_1 values of malignant tissues as compared with normal ones. The ionic and macromolecular composition of the intracellular contents is also expected to affect T_1 (3), and experiments have indicated that such factors do contribute to the relaxation time differences among cells of different types and in different stages of the cell cycle (22, 23). With respect to cancer diagnosis, however, the dependence of T_1 on water content is not a specific property of malignant tissues. The effect of water content on tissue water T_1 is accounted for by a simple two-state model in which a small fraction of the cell water having a short relaxation time is in rapid equilibrium with free water that has a long relaxation time. The extra water entering the cell enters the free-water pool. Since the observed relaxation rate is the

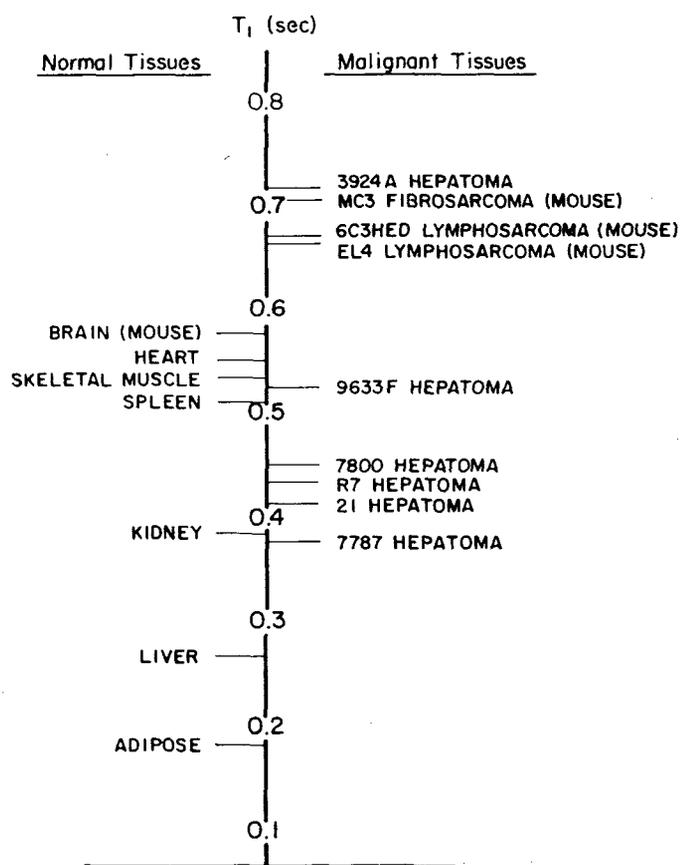


Figure 2. Spin-lattice relaxation times of water protons in normal and malignant tissues of rats and mice. Data obtained at 23 MHz, 27° C. These data differ from those of Figure 1 in that several more slowly growing tumors are included, giving rise to overlap among normal and malignant specimens.

weighted average of the relaxation rates of the two pools, it increases when the water content increases, other factors remaining equal. As mentioned above, changes in intracellular composition can also contribute to T_1 changes independently of the water content.

In summary, there seems to be no theoretical reason to expect nmr relaxation time values to be specific for malignancy, and this is in accord with experimental observations. Efforts to apply nmr relaxation time measurements to distinguish benign abnormal tissues from malignant ones in humans have not been successful (24, 25). As pointed out above, this is not at all surprising because of the variable and heterogeneous nature of human cancers. It should be emphasized that studies designed to distinguish normal from malignant human tissues are of less diagnostic significance, since the distinction between normal and malignant tissues is rarely a clinical problem (24). In view of the

extravagant publicity that has been given to the use of nmr in cancer diagnosis and that has diverted attention from research and from more promising medical uses of nmr, a discussion of the requirements for a useful cancer diagnostic technique seems necessary. Two facts must first be realized: 1) Definitive diagnosis of cancer usually leads immediately to appropriate, vigorous treatment ordinarily involving major discomfort and/or risk to the patient. 2) There is already a very reliable means of diagnosing cancer, namely, microscopic examination of tissue, which appears to diagnose without serious doubt at least 95% of the cases tried (26).

Therefore, the definitive diagnosis of cancer must be soundly based, and any new method suggested must be at least as reliable as the already very reliable method of microscopic examination. The question is whether nmr methods thus far proposed can meet these stern requirements, and the answer seems to be definitely negative. The microscopic examination can be wrong in two ways: 1) It can indicate a cancer where there is none (false-positive), or 2) it can indicate that a lesion is benign when it is in fact malignant (false-negative). How can nmr help in these two cases? To detect a false-positive a decision would have to be made not to treat a patient with a positive histopathological diagnosis of cancer. It is hard to imagine such a decision being made sufficiently often even to provide sufficient data for using nmr to diagnose cancer. To detect a false-negative on the other hand, it would have to be shown that in a large number of cases overt malignancy developed in a region diagnosed as benign by histopathology but malignant by nmr. Since this very seldom happens it would be an immensely time-consuming process to obtain sufficient data to validate the nmr technique, even if it were 100% accurate, which it clearly is not.

Neither nmr nor any other method requiring surgical biopsy is likely to replace or even supplement the standard histopathological techniques with their high reliability based on many years of experience.

In addition to the proposed use of nmr for cancer diagnosis (that is, the definite determination that cancer exists in a particular tissue), another potential application of nmr techniques to cancer is its use as a screening device to detect abnormal conditions, including cancer. Screening identifies individuals with an increased likelihood of having a cancer. A positive result might help the physician in making a definitive diagnosis through surgical biopsy of the questionable area. The requirements for a screening technique are far less rigorous than those for a diagnostic method in that it need only indicate an above-average risk that an abnormality exists. In addition, a screening method must not

carry significant risk or cause much discomfort to the patient. If large numbers of people are involved the screening test must also be fast and inexpensive. As a noninvasive and apparently harmless technique, nmr seems to have potential value as a screening technique. This is especially true in view of the fairly recent development of external imaging techniques based on nmr for use on living tissues (27, 28). Such nmr images owe their contrast to differences in water content and water proton relaxation rates among various tissues. Many types of pathological changes are expected to be reflected in changes in water distribution as well as in associated relaxation times. Spatial resolution of the tissues according to their nmr properties may afford additional useful information to the physician even though the observed changes are not specific for cancer. It might be worth noting that the unwarranted emphasis on nmr and cancer has diverted attention from other suggested medical applications of nmr. For instance, detection of lung edema and coronary infarction are viable and useful possibilities on which significant progress has been made (29, 30, 31).

Needless to say, there are many possible nmr research studies related to the state of water, macromolecules, ions, and membranes in malignant cells and these may eventually lead to clinically useful results perhaps exceeding the direct applications discussed above. This review, however, is limited to discussion of some of the more direct medical applications of nmr.

III. PHOSPHORUS NMR IN CARDIOLOGY

The work of the Oxford group (32) on the phosphorus nmr of living frog skeletal muscle and the demonstration by Moon and Richards (33) that the intracellular pH could be measured by this method opened a new area of biomedical nmr research. The analysis of living tissue by phosphorus-31 nmr has become an active area of research, and a recent review (34) listed more than forty literature references to this subject. More recently much effort has been given to the study of the metabolism of perfused organs including the heart, liver, kidney, brain, and skeletal muscle. All such studies offer excellent potential for basic research in metabolism because the pH, as well as the levels of phosphate metabolites, can readily be measured in a noninvasive manner. This review deals mainly with potential medical applications of nmr, and since the heart studies seem to provide the most promising potential for immediate clinical applications, the remainder of this review focuses on that organ.

A. Magnetic Properties of Phosphorus-31

The phosphorus-31 nuclide is well suited to the study of intact organs, especially heart and skeletal muscle. Phosphorus-31 is the only naturally occurring isotope of phosphorus. Excellent high-resolution phosphorus-31 spectra have been obtained from a variety of biologically significant systems including perfused organs (34). Phosphorus-31 has several favorable properties for the study of heart metabolism. It occurs in high concentrations in only a few compounds. These compounds, however, are crucial to the energy economy of the tissue and can therefore be used to monitor the metabolic state of the tissue. Owing to its 100 percent abundance, phosphorus-31 spectra can be obtained quite rapidly using modern Fourier transform techniques. For example, usable spectra on perfused rabbit hearts have been obtained in as little as 30 seconds (35). Since the nmr technique is noninvasive and nondestructive, serial examinations of a specimen can be made, enabling the determination of kinetic parameters *in vivo*. Furthermore, ^{31}P nmr may be the method of choice for the determination of intracellular pH in intact organs (36).

B. ^{31}P Spectra from Perfused Hearts—General Features

Intact, perfused, beating heart muscle gives especially simple and well-resolved ^{31}P nmr spectra (36). Figure 3 shows a typical ^{31}P spectrum of a perfused rabbit heart. This spectrum was obtained in five minutes on a six-gram heart. Details of the perfusion setup are given elsewhere (35). The assignments of the various signals are indicated. Inorganic phosphate (P_i), phosphocreatine (P-Cr), and the three resonances from ATP are the principal features of the heart spectrum. The unlabelled peak to the left of the inorganic phosphate peak has been assigned to sugar phosphates, while the unlabelled peak to the right of the inorganic phosphate is due to glyceryl phosphoryl choline. Aside from merely indicating the presence of these compounds, the good quality of the spectrum allows us to characterize the signals according to their breadth, relaxation times, intensities, and chemical shifts. The relative intensities of the various peaks are, under appropriate experimental conditions, proportional to the molar quantities of each compound present. Since these relative quantities change as a function of the state of the heart, i.e., oxygen supply, work load, and other factors, it is possible to follow changes in these quantities as a function of time after various in-

terventions (35, 37-40). The chemical shift of the ATP phosphate peaks are significantly downfield from that in simple aqueous solution, while the phosphocreatine and sugar phosphate peaks are at approximately the positions expected for simple aqueous solutions. For skeletal muscle, similar shifts have been interpreted to indicate that the intracellular ATP is complexed with one equivalent of Mg^{2+} (32, 41, 42). The spectrum of Mg-ATP in water accords with this interpretation. Thus, there is good evidence from ^{31}P nmr that virtually all of the ATP in skeletal muscle is complexed with Mg^{2+} . Since the chemical shifts observed for perfused hearts are similar to those of skeletal muscle, it appears that the heart ATP is also completely complexed to ATP, at least in the fully oxygenated heart (35).

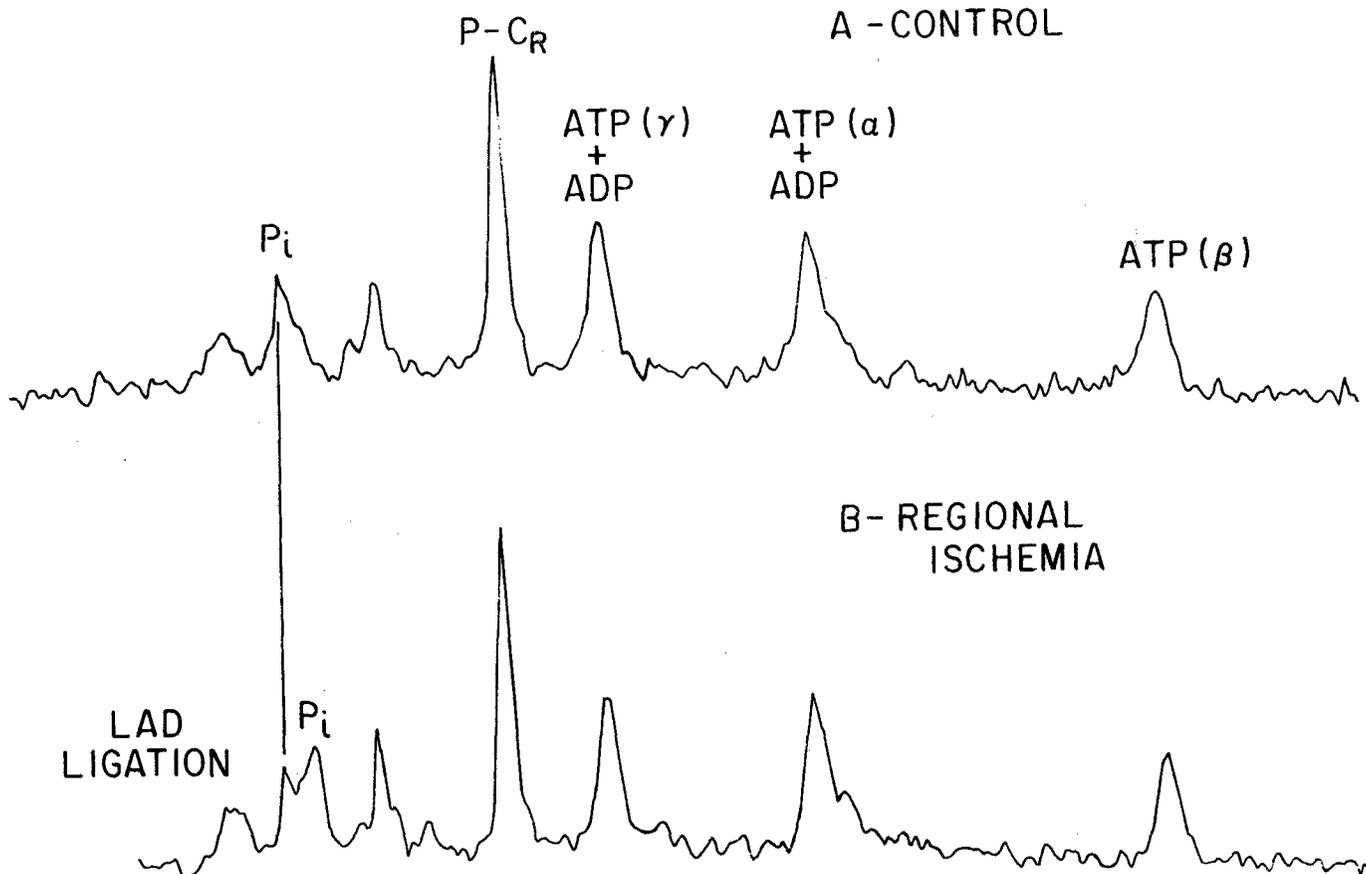
Line widths as well as direct relaxation time measurements can give information concerning possible compartmentation effects in the heart, as demonstrated for skeletal muscle where an overlapping phosphate doublet was probably due to different hydrogen ion concentrations within the muscle (43). In addition, relaxation time measurements may be used in conjunction with intensity measurements to determine steady state reaction rates in the intact heart by the "satura-

tion transfer" technique (44, 45), as demonstrated for *E. coli* by Brown et al (46). Magnetization transfer experiments have also been carried out on intact, perfused hearts in order to study the rate of the creatine kinase reaction *in vivo* (47). Thus, each of the measurable nmr parameters mentioned above can be used to furnish unique information about the intact, beating heart as well as other organs. It is worth pointing out that the heart has a significant advantage over other organs for nmr-metabolic studies in that the function of the heart can be measured in a definite and reproducible manner. Since the function of the heart is that of a pump, the developed pressure can be monitored in a continuous manner (35), and any changes in mechanical performance of the pump can be correlated with the metabolic state of the heart as measured simultaneously by nmr.

C. pH Measurements

A major problem of interest to cardiologists is the role and mechanism by which pH changes influence

Figure 3. ^{31}P nmr spectra of (A) control rabbit heart and (B) the same rabbit heart after ligation of the left anterior descending (LAD) coronary artery. Spectra were obtained at 72 MHz, 37.5° C, and required 5 min accumulation.



the contractility (mechanical performance) of the heart. Poole-Wilson (36) has recently reviewed this area of research. Briefly, non-nmr results suggest strongly that intracellular pH changes play a role in the regulation of cardiac contractility. The isolated, perfused heart is extremely sensitive to alterations in oxygen supply and to changes in flow through the coronary arteries. We refer to a decrease in the oxygen supply during adequate coronary flow as *anoxia*, while a decrease in oxygen supply due to a partial or complete reduction in flow of a fully oxygenated perfusate is called *ischemia*. The response of the heart to total, global ischemia (cross-clamping of the aorta causing a sudden, complete cessation of flow to the heart) is almost immediate. Within a few beats following the onset of total ischemia, a significant reproducible decline in left-ventricular pressure is observed. This decline continues rapidly, and within about one minute the heart has essentially ceased to perform. Measurements by ^{31}P nmr have clearly demonstrated that there is a concomitant decline in the pH during ischemia (35, 37, 48).

The currently most-used techniques for intracellular pH measurements using acid, base, or CO_2 distribution are not applicable to the ischemic heart because they require flow. Present nmr technology allows sequential, time-averaged spectra of the perfused heart to be obtained at intervals as short as 10 seconds (49). This should allow significant new information to be obtained about the biochemical processes occurring during the early stages of ischemia and their relationship to heart function. The ability to measure the pH noninvasively in a no-flow system in the heart as well as in other organs is therefore of considerable significance (36), and it is desirable to validate as thoroughly as possible the accuracy of the nmr spectrometer as a pH meter. It should be noted first that the pH values measured by ^{31}P nmr are reasonable with respect to both absolute value and direction of change during ischemia (35). The best current pH value for the heart with full oxygenation is 7.2 (49). However, factors other than the pH might be expected to affect the inorganic phosphate chemical shift and thus lead to inaccurate results for the absolute pH value. Interactions of the inorganic phosphate with enzymes and ions could in principle contribute to the phosphate chemical shift. Using potassium phosphate buffers at a fixed pH, we measured the effect of mono- and divalent cations on the chemical shift of inorganic phosphate. The data indicated that, within the expected physiological range, alterations in the concentration of K^+ , Na^+ , Mg^{2+} , and Ca^{2+} have insignificant effects on the inorganic phosphate chemical shift (49). To obtain some ideas about possible effects of intracellular enzymes and other macromolecules, titration curves of pH vs inorganic phosphate chemical shift were con-

structed for both aqueous potassium phosphate solutions and homogenized dog heart preparations. In both cases, the data conformed very closely to the theoretical curve based on the Henderson-Hasselbach equation and the fast-exchange case for the phosphate chemical shift of the various phosphate ions present near neutral pH:

$$\text{pH} = \text{pK} - \log \frac{\Delta_o - \Delta_B}{\Delta_A - \Delta_o}$$

The constants were found to be $\text{pK} = 6.90$, $\Delta_A = 3.29$, and $\Delta_B = 5.81$, where Δ_A and Δ_B are the limiting chemical shifts at high and low pH and Δ_o is the observed chemical shift. The agreement of the buffer and homogenate results indicate that phosphate-protein binding interactions have little effect on the observed inorganic phosphate chemical shift. It thus appears that the inorganic phosphate chemical shift provides a valid estimate of average intracellular pH.

By adjusting the intracellular pH of the heart by changing the CO_2 concentration in the perfusate it is possible to demonstrate the strong dependence of heart function on the intracellular pH. Much earlier work had emphasized the role of intracellular pH in the regulation of myocardial contractility (50). Hearts were perfused at 37°C with phosphate from Krebs bicarbonate buffer. The control pH of the heart under these conditions was $7.18 \pm .07$. The left-ventricular developed pressure (LVDP) was stable for more than one hour. The hearts were made more acid (respiratory acidosis) by increasing the concentration of CO_2 in the perfusate (49). By this means, the intracellular pH could be varied from about 7.2 down to 6.9. The striking finding was that an intracellular change of only 0.20 pH units resulted in a 50% decline in LVDP. The results demonstrate a tight coupling between intracellular pH and the depression in heart function under steady-state conditions. Although these results do not prove that the similar early changes in heart function during ischemia are a result of pH changes, they do indicate a method for further study of these early changes. This question is now being explored.

D. Possible Applications

1. Protection of Ischemic Myocardium

Over the past several years the question of how the ischemic heart can be protected against irreversible damage during limited periods of ischemia has become a major area of research. The clinical significance and other aspects of this problem have been thoroughly

discussed in the well-known reviews by Hillis and Braunwald (51, 52). Ischemia is the initial step leading to acute myocardial infarction. Even rather small reductions in irreversible damage during ischemia are believed to have strong positive influence on the final outcome of coronary infarction. In addition, cardiac surgery normally involves a more or less lengthy period of ischemic arrest of the heart, and the desirability of avoiding possible damage to the heart during this period is well recognized. It is not yet clear what biochemical factors lead to the eventual irreversible damage that results from prolonged ischemia, but low pH and/or depleted energy stores, i.e., low ATP and phosphocreatine, are among the factors suspected to contribute to the damage. Phosphorus nmr can potentially play an important role in the understanding of the causes of ischemic damage and in developing methods (drug administration, for example) to minimize ischemic damage to the heart as well as to other organs. This is true because ^{31}P nmr allows measurement both of intracellular pH and the energy status of the heart in terms of the concentration of "high-energy" phosphate compounds, i.e., ATP and phosphocreatine, in the heart (35). During ischemia the pH drops and the ATP and phosphocreatine stores of the heart are severely depleted. The nmr results show that phosphocreatine is depleted within a few minutes and that later the ATP level falls. Reflow leads to a partial recovery of function as well as of pH, ATP, and phosphocreatine values. The extent of recovery depends on several factors including use of cardioplegic (heart arresting) agents such as KCl, the temperature, and the length of time during which ischemia persists.

A promising medical application of nmr is in the development of procedures to afford protection to the heart during ischemia. For example, in an early study we compared the effects of total global ischemia on the biochemical state and the functional recovery of rabbit hearts under conditions of KCl arrest and reduced temperature, with the effects on rabbit hearts at reduced temperatures in the absence of KCl arrest (53). As shown in Figure 4, the pH, the ATP level, and the phosphocreatine level remain higher during ischemia with KCl arrest than during ischemia at low temperature alone, thus demonstrating the efficacy of KCl arrest in protecting the biochemical state of the heart. On reflow following 40 minutes of ischemia, the KCl-arrested heart completely recovers both its function and biochemical profile, but the nonarrested heart recovers function to only a limited extent (~30%). In addition, the latter does not recover its full original ATP concentration. The pH not only drops further without KCl arrest, it also drops faster. We have measured the time course of the pH changes by ^{31}P nmr (53), illustrating the

unique capabilities of this method to monitor noninvasively and sequentially on a single heart the changes in this crucial parameter. Thus, it appears that nmr can be useful in evaluating methods designed to preserve the normal biochemical profile of the heart during ischemia, and we have begun efforts in that direction (54). Most recent experiments (49) have involved an extension of the ischemic period to one hour and an effort to quantitate the results more carefully. In a control group of experiments in which the hearts received no cardioplegic solution (KCl), but were maintained at low temperature (26° – 27°C), intracellular pH fell from a control value of $7.20 \pm .01$ to $6.09 \pm .12$ after 60 minutes of total ischemia. Following reperfusion the pH rose rapidly, reaching or slightly overshooting ($7.30 \pm .05$) control values after 3–4 minutes of reflow. At the end of 45 minutes of reflow, intracellular pH was $7.24 \pm .04$. During this period the heart function returned to $54 \pm 11\%$ of the control value, while coronary flow was $86 \pm 12\%$ of control.

During the period of total global ischemia, intracellular phosphocreatine (P-Cr) fell to $2 \pm 1\%$ of control after 60 minutes of ischemia and then returned to $169 \pm 37\%$ of control after 5 to 10 minutes of reflow. After 45 minutes of reflow the P-Cr level was $108 \pm 11\%$ of control. Thus, despite above-normal levels of P-Cr the hearts were able to function at only about 50 to 55% of control levels. In contrast, ATP levels, which fell to $8 \pm 1\%$ of control values at the end of the 1 hour ischemic period, returned to $47 \pm 14\%$ of control following 45 minutes of reflow. Thus, the return of ATP correlates better with functional recovery than does the return of P-Cr. Inorganic phosphate rose to over 300% of control values during ischemia and remained elevated at about 200% of control after reflow.

Administration of a single dose of KCl cardioplegic solution at the time of initiating ischemia had striking effects on all of these parameters. The KCl slowed the fall of intracellular pH during the ischemic period. At the end of the ischemic period the pH had fallen to only $6.31 \pm .09$ with KCl compared with $6.09 \pm .12$ without. Through the first 45 minutes of ischemia the pH had remained 0.3 to 0.5 pH units higher. There was a comparable improvement in performance with $85 \pm 6\%$ recovery being attained following reflow.

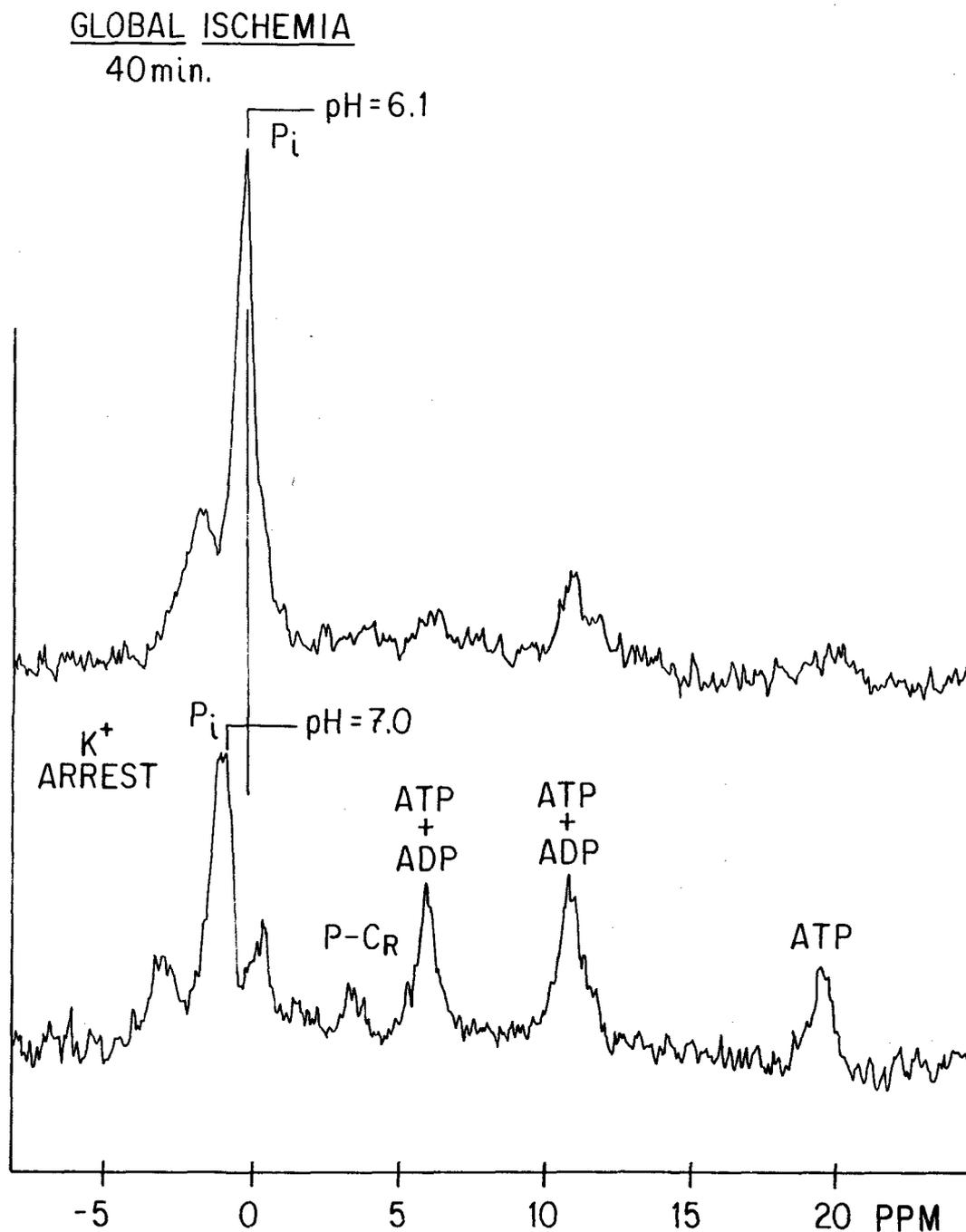
After 60 minutes of arrest with KCl cardioplegia, P-Cr levels fell to $9 \pm 6\%$ of control and then rose to above normal levels ($147 \pm 15\%$ and $136 \pm 15\%$) during the reperfusion period. Again the correlation between function and P-Cr levels was poor while that with ATP levels was good. ATP levels fell to $31 \pm 9\%$ during ischemia, but returned to $85 \pm 8\%$ of control after 45 minutes of reperfusion. Inorganic phosphate rose to 450% of control and remained elevated during the reflow period.

This study illustrates how ^{31}P nmr can be used to correlate the energy status and pH of the heart with functional changes during a particular treatment designed to protect ischemic myocardium. The technique can be applied in trying other protocols involving drug treatment, for example.

Related to the problem of preserving ischemic myocardium is the damage that frequently occurs to

the heart as a direct result of the reflow process. This damage is of clinical significance in cardiac surgery and is believed to be related to calcium influx into the heart cells. A possibly useful nonischemic model of this damage is the so called "calcium paradox" in which perfusion of the heart with a low Ca^{2+} solution followed by perfusion with normal Ca^{2+} concentrations leads to severe, irreversible damage to the heart. ^{31}P nmr studies

Figure 4. ^{31}P nmr spectra of ischemic rabbit hearts. Upper trace: 40 min global ischemia, lower trace: KCl arrest followed by 40 min of global ischemia.



(40) have confirmed that the extreme loss of high-energy phosphate compounds associated with this process occurs only upon reflow and not during the low Ca^{2+} period. ^{31}P spectra are normal during the Ca^{2+} -free period, but are essentially annihilated upon reflow. The results are consistent with the idea that the cell membrane becomes permeable to Ca^{2+} during the Ca^{2+} -free period and that Ca^{2+} influx during reflow produces the resultant cell damage.

^{31}P nmr can also be used to monitor the influx of paramagnetic ions into the heart cell (30). It has been found that the rate of influx of Mn^{2+} into the heart is affected by ischemia, suggesting the possible use of nmr as a probe of the state of the cell membrane as affected by the physiological state of the heart.

2. Regional Myocardial Ischemia

Coronary infarction in man does not involve ischemia of the entire heart. Only a certain region of variable size becomes ischemic due to a partial occlusion of a coronary artery (51, 52). Thus, in seeking to minimize myocardial damage due to coronary infarction, it becomes important to develop methods for detecting, locating, and sizing an ischemic zone in the heart and to distinguish ischemic regions from scars. The possibility that nmr might detect regional tissue metabolism could open new opportunities for investigating the effects of a number of agents proposed to reduce ischemic myocardial necrosis (51, 52).

These would include vasodilators to increase coronary flow, substrates such as glucose plus insulin, and drugs that reduce myocardial oxygen demand. Equally important is the future possibility of observing high-resolution ^{31}P nmr spectra at a specific location within the heart or even of obtaining nmr images of the heart (55). The quantitation of ischemic myocardium is an important problem, and a noninvasive harmless technique would be welcomed. These are the reasons for investigating regionally ischemic hearts by nmr (35, 38).

Figure 3A shows the initial control spectrum of a perfused rabbit heart as described earlier. Figure 3B shows the spectrum of the same heart under identical conditions, immediately after ligating the left anterior descending coronary artery near the septal branch. This produced immediately a dark area covering about 30% of the anterior wall of the left ventricle, the remainder of the heart surface appearing quite normal. The performance of the heart and the coronary flow dropped precipitously as expected, and the ^{31}P nmr spectrum then appeared as in Figure 3B. The crucial point is that the inorganic phosphate resonance has split into two components, one of which remained at the control position corresponding to $\text{pH} = 7.2$, while the other ap-

peared at higher field indicating a pH of 6.2. (These values are somewhat different than those of references (35) and (38) and reflect our best, most recent estimates.) The lower-field (left) peak is assigned to the normally perfused portion of the heart, while the higher-field peak corresponds to the ischemic zone. Although the area of the acidic phosphate peak is related to the volume of the ischemic zone, certain complications prevent direct determination of the ischemic volume. The net amount of tissue phosphate observed as an acidic phosphate peak will depend on the severity and duration of ischemia, the volume of the ischemic zone, and the extent of phosphate washout by collateral flow. Thus, quantitation awaits suitable techniques for spatial resolution of ^{31}P nmr spectra (55). Our results do, however, demonstrate the feasibility of using ^{31}P nmr to detect regional myocardial ischemia in a noninvasive manner, and they suggest the use of ^{31}P nmr for the study of interventions designed to improve coronary flow and metabolism in regionally ischemic tissue.

IV. SUMMARY

This review has dealt with the potential use of nmr in medical applications to two major diseases: cancer and heart disease.

In the case of cancer, it is concluded that it is extremely unlikely that definitive diagnosis of cancer by nmr is possible, since no nmr parameter and no nmr method to date is specific for cancer. In addition, nmr would need to compete with the highly reliable standard method of microscopic examination, and it is unlikely that in cases of disagreement nmr could prevail. The less demanding use of nmr as a cancer-screening technique is somewhat more promising, but its eventual use will require further development of nmr imaging techniques. In addition, nmr has good potential as a research tool for understanding the state of the intracellular fluids.

In the case of heart disease, ^{31}P nmr shows high promise as a method for monitoring the biochemical state of the heart and correlating it with heart function. This has medical significance in that: 1) It can shed light on the biochemical factors affecting heart function, 2) it can provide data for the assessment of protocols designed to preserve ischemic myocardium following coronary infarction and during cardiac surgery, 3) with the development of techniques for spatial resolution of ^{31}P spectra of the heart, sizing and locating ischemic zones, scars, and normal tissue may become feasible. In addition ^{31}P nmr also has great potential in basic studies of living systems including the heart and other organs.

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