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Noninvasive, nondestructive approaches to cell bioenergetics

(^{31}P NMR apparatus/ratio of phosphocreatine to phosphate/optical methods/oxygen delivery to tissue/function of mitochondria)

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ABSTRACT **To demonstrate the feasibility of using NMR spectra of human limbs and larger animals for continuous, noninvasive, nondestructive evaluation of cell bioenergetics, we have constructed a relatively simple and inexpensive ^{31}P NMR apparatus. This apparatus consists of an 18-cm (7-in.) bore superconducting magnet and appropriate transmit-receive components for Fourier transform NMR. The principal signals observed by this instrument in the tissues are due to phosphocreatine and inorganic phosphate. The apparatus can be used to detect tissue normoxia and hypoxia. The large phosphocreatine/phosphate ratio (>10:1), and the low phosphate signal from normoxic tissue (≈10% of the phosphocreatine signal from brain and human skeletal tissue) make an increased phosphate peak a very sensitive indicator of tissue hypoxia. Direct experiments on the human forearm and leg and the brains of dog and rabbit suggest the applicability of ^{31}P NMR to humans and animals. This method and optical methods can both be used for quantitative determination of oxygen delivery to tissue, function of mitochondria, and the coupling of bioenergetic processes to functional activity in skeletal tissue and brain.**

Few methods for the study of cell biochemistry are truly noninvasive. Some that are customarily used examine exhalations and excretions—gases from the lung, urine, and feces. Such gases, liquids, and solids can be used to evaluate the organ chemistry of the lung, blood, and digestive system, but they give only an integrated response characteristic of the average function for the entire body. The data may be difficult to assign to a particular organ, or even to a group of organs. Other methods involve disagreeable and perhaps risky invasion, by which samples of fluids and solid tissues can be excised, frozen rapidly, and analyzed.

Visual inspection of the exposed tissue surface, or that visible through body orifices, provides a direct, superficial signal, and has been extended greatly by catheterization and fiber-optic viewing techniques for the state of many organs. Surface examination of biochemical activities is possible by using exterior optical methods (1) or fiber-optic coupling to interior organs. When these organs are illuminated at appropriate wavelengths, organ-specific absorbance signals of hemoglobin oxygenation (1) and of metabolic function, particularly oxidative metabolism, can be obtained by NADH and flavoprotein fluorometry (2, 3) and cytochrome spectrophotometry (4, 5).

Intraoperative monitoring procedures provide further access to organ surfaces. Optical (ref. 3; unpublished data) as well as surface electrode techniques (6, 7) can be used to evaluate oxygen delivery during a variety of intraoperative procedures, especially those involving brain but also those involving kidney, heart, and lung. At present, only optical techniques permit nondestructive observation of intracellular biochemistry (3, *).

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MATERIALS AND METHODS

^{31}P NMR Signals from Energy-Related Compounds in Tissues. NMR spectra can be used to study the nature and function of atomic nuclei. Phosphorus and carbon, pivotal in cell metabolism, are most useful (8). The spectral (chemical) shift depends on the chemical environment of a particular nucleus. For example, phosphorus atoms attached to a series of energy-related compounds give a radio frequency signal (as a chemical shift) that identifies these compounds in brain, heart, liver, kidney, and skeletal tissue (Fig. 1; ref. 9). Both the high-energy compounds—ATP, the "energy currency" of the body, and phosphocreatine (PCr), the "short-term energy reserve"—and the low-energy forms of these compounds—ADP and inorganic phosphate (P_i) can be identified (10), as can the sugar phosphates derived from glucose metabolism. PCr and P_i are the most prominent signals from skeletal tissue (11–13), heart (14, 15), and brain (16, †). In this paper, we summarize our results and describe an apparatus appropriate to ^{31}P NMR measurements in the human forearm and leg (17–19, ‡).

Experimental Methods for Larger Animals. The use of NMR for tissue study requires wide bore [7.6- to 18-cm (3- to 7-in.)], high-field (1.5 T), superconducting magnets of high homogeneity (1×10^{-7}) over large volumes (2–3 cm^3) (20–22). Because deep penetration into tissue is required, we have constructed and tested a low-frequency transmitting and receiving coil for 24.3 MHz, appropriate to the ^{31}P signal at 1.5 T (19). Superposition of higher order magnetic field gradients onto the main magnetic field gives a uniform central region of adequate homogeneity ($\approx 1 \times 10^{-6}$) for resolving spectra of PCr and P_i (20). Signals from the tissue volume over which the field is homogeneous (≈3 cm^3) are sensitively recorded; signals from other portions of the tissue are "out of focus" and contribute only diffuse background. A sharply defined "focus" may be obtained by using field profiling coils from Oxford Instruments (22).

Fourier transform NMR provides signals simultaneously from all the energy-related phosphorus metabolites. Because signal intensity is determined by the number of nuclei involved, measurement is absolute in the sense that the radio-frequency

Abbreviations: PCr, phosphocreatine; rf, radio frequency.

* Chance, B. (1980) *Proceedings of the International Union of Physiological Science*, Budapest, Hungary, pp. 14–15 (abstr.).

† Jardetsky, O. (1980) *27th International Congress of Physiological Science*, Budapest, Hungary, p. 812 (abstr.).

‡ Chance, B., Eleff, S. & Leigh, J. S., Jr. (1980) *27th International Congress of Physiological Science*, Budapest, Hungary, p. 80 (abstr.).

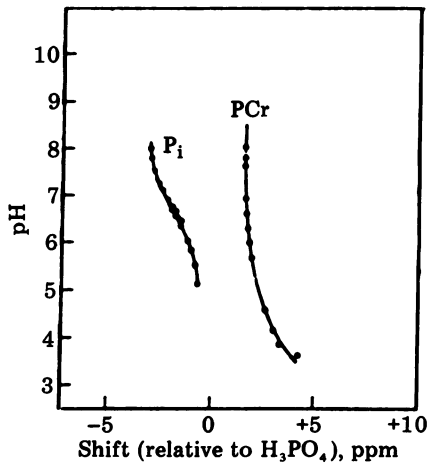

FIG. 1. Chemical shifts of P_i and PCr relative to external 1.0 M H_3PO_4 (taken to be zero).

(rf) signal received is directly proportional to number of nuclei responding to the rf pulse; thus, the amounts of two components can be compared by comparing the peak heights or the area under the traces.

Details of the ^{31}P Tissue NMR Apparatus. We have constructed a simple transmit-receive system and pickup coil, as suggested for proton NMR of human limbs in 1964 (23), except that we use only a few turns of wire. Our equipment has a two-turn, 7.6-cm-diameter coil, an electrostatic shield, and a nonmagnetic field that penetrates a few inches into the tissue (Fig. 2). The coil is tuned to 24.33 MHz and represents a 50-ohm resistance load to the ≈10-W-peak transmitter pulse of 40-μsec duration, an average power that is well below recommended standards for biological studies (24). The faraday shield improves performance of the probe coil and eliminates radio-frequency electric fields in the tissue volume. A low-noise (≈2 dB from theoretical) diode-protected preamplifier and a gated amplifier give 100 dB gain and are coupled to phase-sensitive demodulators and a five-pole filter, and the output signal is coupled to the amplifier-demodulator input to the computer. The computer controls the rf pulse, allows accumulation of repetitive scans, computes their Fourier transform, and plots signal intensity against frequency or chemical shift. Total nuclear spin concentration can be obtained by integration of the x-y signal display or computerized curve-fitting techniques. Often, however, observation of relative amplitude data on the P_i and PCr signals gives a convenient index of tissue metabolic state; a marking point that we have found useful is a level of functional activity that gives equal values to the two.

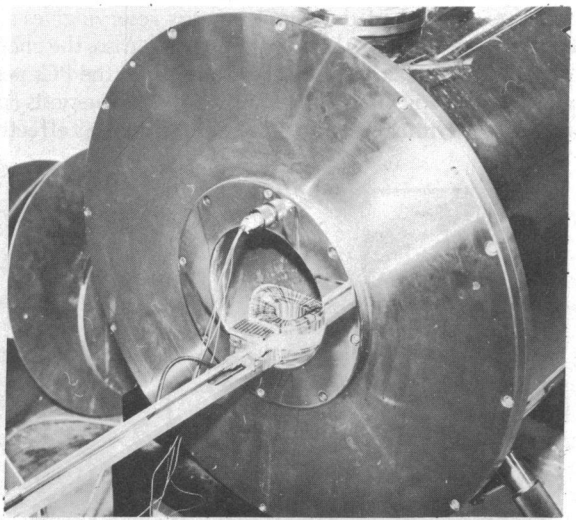

FIG. 2. Superconducting magnet (18-cm diameter) and laboratory-constructed 7.6-cm diameter probe with faraday shield and nonmagnetic tuning components. The sample is inserted into the opposite end of the magnet (see Fig. 4). Typical static and rf magnetic fields are 15 kG and 1 G, respectively (1 G = 1.0×10^{-4} tesla).

Magnets for human-tissue magnetic resonance studies involve special considerations: first, the magnet bore must be large enough to accommodate the organ at a depth within the magnet at which the field is homogeneous. In most cases, that is deep in the interior—usually the midpoint. Recent attempts to achieve adequately homogeneous fields nearer to the end of the magnet have met with some success, but no magnet yet designed allows the human head to reach the homogeneous part of the field without also admitting the shoulders; thus, NMR examination of the head currently requires magnets large enough for the whole body. An overall diameter of 75–90 cm (30–36 in.) has been selected by several designers. Because magnet cost increases as a high power of diameter, we have used a magnet capable of admitting the arm or the leg; a diameter of 18 cm represents a compromise in this respect (see Fig. 2).

Helium holding times of a cryogenic magnet vary from several days to a month, depending on the degree to which thermal losses from the dewar are controlled and whether current leads are disconnected from the superconducting magnet. The 18-cm-diameter magnet loses about 25 ml of He per hr (Oxford Instruments). Optimization of the field homogeneity when the magnet contains a human arm or leg is best done by monitoring the 60-MHz signal from the body water protons.

Current Performance of Wide-Bore ^{31}P NMR. The complete system, the 18-cm magnet, the 7.6-cm coil, and the transmit-receive system, is combined with laboratory-built timing and synchronization circuits and gives signal-to-noise ratios in excess of 10:1 at a 1- to 2-min accumulation time and a resolution of ≈2 ppm. The signals for PCr and P_i, which are appropriate for the detection of hypoxic effects in skeletal tissues and brains, can be adequately resolved at this resolution and a 25-Hz accumulation rate (see below). Sugar phosphates may slightly increase the aerobic signal attributed to P_i in these studies (9). However, P_i is very small under normoxic conditions, and the sugar phosphate level is also very low. In exercise leading to anoxia, P_i increases to a much higher value than sugar phosphate, as shown in small animal studies on brain (16) and skeletal tissue (11, 12).

Effects of Static and rf Magnetic Fields. The 15-kG static magnetic field used in these studies has been tested for its effects on colonies of rats over a several-week period, on a beagle for 2 hr, and on the arm of one of us for 30 min. Examination of the rats, neurological functional tests on the dog, and responses of the human showed no effects attributable to the magnetic field (rats) or to the rf and static magnetic fields (dog and human). These results seem to agree with those of other studies. For example, Purcell (34) states, "Nor, as of this writing, have other biological experiments turned up anything remarkable in the way of magnetic effects on chemical processes. This is not surprising. In its interaction with matter, the magnetic field plays a role utterly different from that of the electric field. Because atoms and molecules are made of slowly moving electric charges, electric forces overwhelmingly dominate the molecular scene." (For a summary, see ref. 25.)

During NMR measurements, the sample is exposed to short pulses of rf magnetic fields at intensities of a few gauss. Exposure to rf electric fields, which cause heating (as in diathermy

treatment), is avoided by the use of the faraday shield, and thus the effects of the 27-MHz rf electric fields (citizen band radio) are not to be expected here.[§]

Problems of Quantitation of ^{31}P NMR Signals. A molecule exhibiting NMR signals may have an *in vivo* bound state unexpected from *in vitro* studies but, because PCr does not have a significant affinity for most intracellular constituents, its concentration can be evaluated by NMR accurately. Similarly, phosphate exists in a highly dissociated state, and the concentration of soluble anion ($HPO_4^{2-} + H_2PO_4^{1-}$) can be assayed with nearly the same precision. However, protein-bound phosphate is not readily available for NMR assay. Thus the PCr/P_i ratios reported here are given simply as ratios of the amplitudes (or areas) of the respective peaks above baseline. The transition between the two forms of phosphate caused by changes of intracellular pH can lead to significant changes of peak position (but not of signal strength), which can be interpreted in terms of the pH and used to determine the degree of lactic acidosis in ischemic, anoxic, fatigued tissues—e.g., compare the chemical shifts of P_i with respect to PCr in Figs. 1 and 3.

The second problem is that of allowing adequate time for relaxation between rf pulses. The relatively low and similar relaxation times of PCr and P_i observed in living tissue vary from organ to organ (26, 27). We have used accumulation rates giving the best signal-to-noise ratio (≈20 Hz); nevertheless, the saturation of the PCr and P_i signals is similar, and thus the accuracy of their ratio is only 10%. However, in animal model systems, excision of tissue examined by cryogenic NMR techniques (16), followed by homogenization and extraction of phosphate compounds by the usual analytical techniques, allows their evaluation for calibration. Thus, the degree to which NMR determines absolute concentrations can be checked in animal cell models or when rapid human organ biopsy is feasible, and such calibrations would be expected to apply to all further studies of that particular organ. P_i is one important exception where NMR data give consistently lower values than those obtained by tissue analysis (27).

RESULTS

The role of energy-related phosphate compounds in tissue was first observed by Otto Meyerhoff and has since been formulated in detail by Fritz Lipmann, who showed their cyclic functions (10). Further work, mainly on isolated mitochondria, cell suspensions, and intact tissue, has identified pathways for synthesis and control of these metabolites in isolated mitochondria, yeast, and tumor cells.

The use of oxygen transported by hemoglobin to peripheral tissues, its diffusion through capillaries to intracellular mitochondria, and their functional response in ATP synthesis and conversion to PCr is a process that can be followed dynamically by optical techniques that show mitochondrial response to both oxygen presence and energy demand. However, the function of mitochondria in producing ATP, and thence PCr, is not determined quantitatively by the optical method. Here, a unique feature of NMR becomes of primary importance—i.e., its capacity to follow skeletal or cortical dissipative functions of physiological or pathological nature that increase tissue P_i above its normal low level.

[§] The American National Standards Institute's limit for continuous exposure is 177 nJ/m^3. A "walkie-talkie" gives more than 200 nJ/m^3 (13) when held next to the head. Such a transceiver has a 1.8-W continuous output; in NMR, the peak power is 10 W but the duty cycle is ≈10^{-4}, so that the average power is only 0.001 W, insignificant compared with the transceiver. Nevertheless, the biological effects of static magnetic and rf electric fields continue to be the topic of scrutiny by us, as well as others (18, 20, 22).

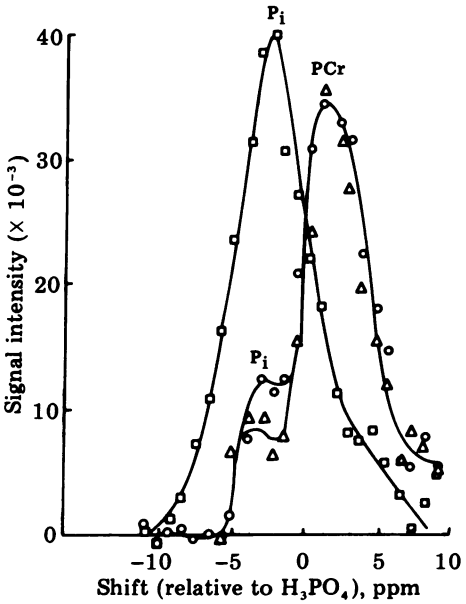

FIG. 3. Effect of strenuous exercise on PCr and P_i levels in the human forearm. ^{31}P NMR scans were for 1.5 min. The forearm was tested before (○), immediately after 1 min of violent exercise (□), and 20 min into the course of recovery (△).

Skeletal Muscles. Skeletal muscles are optimal for NMR study because of their large volume, their homogeneity, and their accessibility. NMR studies of isolated frog skeletal tissues have shown that the fully aerobic tissue has a very high PCr/P_i ratio (28).

Our results on the resting human forearm show a PCr peak (90-sec accumulation) and a PCr/P_i ratio in excess of 10, which is characteristic of animal and human skeletal tissues at rest (Fig. 3). After this measurement, the subject, a trained athlete, exercised his arm for 60 sec in the "Cybex" ergometer at such a rate as to exhaust the forearm muscle. The arm was immediately returned to the NMR; the 90-sec accumulation at this point shows no detectable PCr. Instead, there is a large P_i peak, the muscle ATPase having used ATP to give ADP and P_i. The ADP was rapidly rephosphorylated by creatine kinase to the extent that PCr was available, leaving the accumulated P_i. This process of exhaustion of the muscle energy reserve gives a P_i peak slightly higher than the initial PCr peak. Also, the chemical shift of the P_i peak measured with respect to the PCr peak (compared with Fig. 1) is consistent with lactic acidosis (pH ≈6.4). Thus, a 1-min exercise in the ergometer was effective

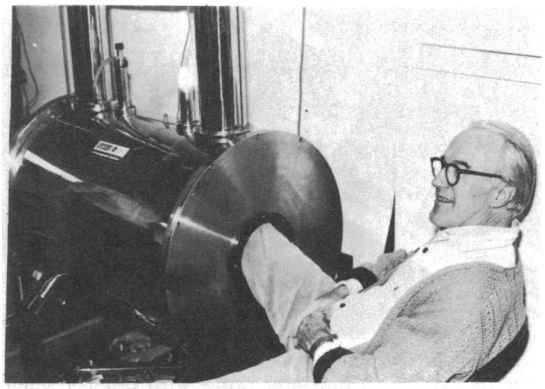

FIG. 4. Insertion of limb into the 18-cm superconducting magnet for detection of PCr and P_i. The calf muscle lies over the probe illustrated in Fig. 2.

in demonstrating the "energy capacity" to do work to the extent available by the energy reserves. (Mitochondrial oxidative phosphorylation and glycolytic activity would contribute only slightly in this brief exercise.) Obviously, steady-state procedures that exercise the muscle at a rate to give PCr/P_i ratios of 1 are also possible and would be more amenable to detailed mathematical analysis. However, both transient and steady-state approaches provide direct noninvasive measures of the coupling between energy expenditure in muscular contraction and bioenergetic efficiency as determined by the muscle energy reserve and resynthesis. Recovery to the resting state can be measured as a function of time during recovery or as completion of recovery.

One case of particular interest is the study of peripheral vascular disease, in which attention is focused on leg muscles. In this disease, insufficient blood flow, and therefore insufficient tissue oxygenation, causes muscle damage that may eventually lead to amputation of the affected limb. Although Doppler flow studies, blood pressure measurements, and arteriograms give indications of the adequacy of blood flow, they do not answer the question of state of tissue oxygenation directly. Stressing the limb with exercise or reactive hyperemia and then measuring the increase and decrease of P_i levels should correlate with clinical observations and lead to a quantitative description of disease progression and a method to compare different treatments.

Use of the 18-cm instrument in the study of human calf muscle metabolism is illustrated in Fig. 4, which shows how the leg can be inserted into the homogeneous portion of the magnetic field. The result of a 2-min scan shows a high PCr level and a low P_i level, as is characteristic of fully aerobic tissue (Fig. 5). Minor movements of the foot for about 3 min cause a decrease in PCr level and an increase in P_i level; for the two cases, the ratios of the amplitude of the PCr peak to that of the P_i peak are >10:1 and 6:1, respectively. More strenuous exercise would have given changes such as those shown in Fig. 3.

Brain. Brain energy metabolism is similar to that of muscle, in that it depends on PCr for its energy storage, but is unlike that of muscle in being in a constant state of high activity. Thus, alterations of blood supply to the brain can be pernicious in either the resting or the active state of the whole body and can lead to symptoms ranging from mild, transient effects to stroke, coma, and death. The bioenergetic state of the brain was evaluated with two living animals, the rabbit and the beagle

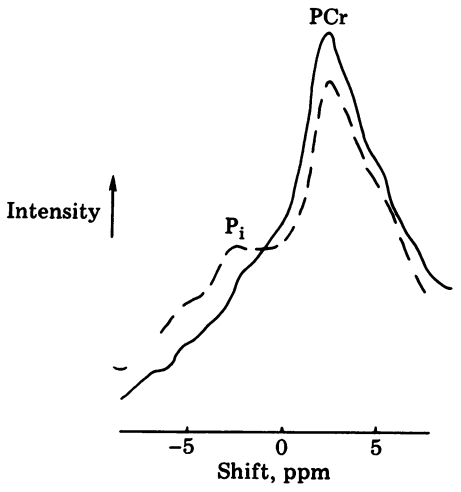

FIG. 5. PCr and P_i levels in resting (—) and mildly exercised (- - -) human limb. The signals were accumulated over intervals of 2 min; the exercise was continued during the second interval of data accumulation.

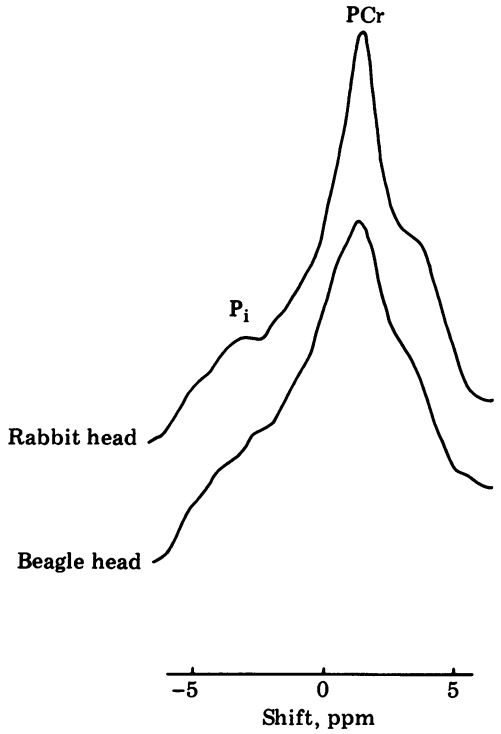

FIG. 6. PCr and P_i signals from anesthetized beagle and rabbit heads (normoxic conditions). Signals were accumulated over 1- and 3-min intervals, respectively.

(Fig. 6). The animal's head was placed on the coil; the NMR spectrum thus contains signals also from skeletal tissues of the head, but controls have shown that these do not prevent evaluation of those due to the brain tissue itself. Similarity of energy-related compounds in brain and skeletal tissue is apparent. In both cases, the resting energy state has a high (>10:1) PCr/P_i ratio. Diminution of oxygen delivery to the brain by any means (such as a simulated head injury) would result in a decreased PCr/P_i ratio (as shown in Fig. 3). This NMR system seems applicable to infant brains and to the adult human head if suitable larger magnets were available.

Future Studies. Many new methods for noninvasive studies of biochemistry in human subjects have emerged from recent developments of optical,* isotopic,¶ chemiluminescent (29), and NMR (16–19, 26–31, †, ‡, ¶, ‖) techniques. Each finds special and appropriate applications—for example, optical detection of highly localized redox states provides an intraoperative procedure for detecting ischemia and anoxia in brain (16) and heart (32). The fluorodeoxyglucose method combined with the position-emission scanner can be used to determine relatively localized glucose metabolism, although the long averaging makes for an indefinite relationship to rapid functional activity.¶ Luminescence, although limited to those organs accessible to fiber optics, is very specific for lipid peroxidation under normal or pathological hyperoxidative process (30). NMR has the ultimate, and as yet unrealized, possibility of imaging human tissue volumes in terms of their specific biochemical constitution (33). We have shown, as a first step toward that goal, that whole organ bioenergetics can be studied in terms of the PCr/P_i ratio.

¶ Reivich, M., Alavi, A., Greenberg, J., Fowler, J., Christman, D., Rosenquist, A., Rentelman, W., Hand, P., McGregor, B. & Wolf, A. (1980) *27th International Congress Physiological Science*, Budapest, Hungary, p. 223 (abstr.).

‖ Chance, B., Eleff, S. & Leigh, J. S. (1980) *27th International Congress Physiological Science*, Budapest, Hungary, p. 80 (abstr.).

Perfused organs are even simpler to study by optical and NMR techniques: the organ to be studied is placed on the surface probe, and perfusates are connected to the main blood vessels through plastic tubes. In this way, heart, liver, kidney, brain, skin flaps, and such have been studied under highly controlled conditions. Typical examples are evaluations of kidney, heart, and liver before transplantation.

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1. Millikan, G. (1944) in *Medical Physics*, ed. Glasser, O. (Yearbook, Chicago), pp. 900–901.
2. Chance, B., Schoener, B. & Schindler, F. (1963) in *Oxygen in the Animal Organism*, eds., Dickens, F. & Neil, E. (Pergamon, London), pp. 367–388.
3. Chance, B., Barlow, C., Nakase, Y., Takeda, H., Mayevsky, A., Fischetti, R., Graham, N. & Sorge, J. (1978) *Am. J. Physiol.* **235**, H809–H820.
4. Chance, B. & Williams, G. (1955) *Nature (London)* **176**, 250–254.
5. Jobsis, F. F. (1977) *Science* **198**, 1264–1267.
6. Davies, P. W. & Brink, F. (1942) *Rev. Sci. Instrum.* **13**, 524–533.
7. Silver, I. A. (1976) in *Advances in Experimental Medicine and Biology*, eds. Reivich, M., Coburn, R., Laihri, S. & Chance, B. (Plenum, New York), pp. 299–312.
8. Navon, G., Ogawa, S., Shulman, R. & Yamane, T. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 888–891.
9. Moon, R. & Richards, J. (1973) *J. Biol. Chem.* **248**, 7276–7278.
10. Lipmann, F. (1946) in *Currents in Biochemical Research*, ed. Green, D. (Interscience, New York), pp. 137–138.
11. Dawson, J., Gadian, D. & Wilkie, D. (1977) *J. Physiol. (London)* **267**, 703–735.
12. Burt, C., Glonek, T. & Barany, M. (1976) *J. Biol. Chem.* **251**, 2584–2591.
13. Banow, K. D., Jamieson, D. & Worton, R. S. (1980) *Eur. J. Biochem.* **103**, 289–297.
14. Gadian, D., Hoult, D., Radda, G., Seeley, P., Chance, B. & Barlow, C. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 4446–4448.
15. Fossel, E., Morgan, H. & Ingwall, J. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3654–3658.
16. Chance, B., Nakase, Y., Bond, M., Leigh, J. S., Jr. & McDonald, G. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 4925–4929.
17. Chance, B., Leigh, J. S., D' Ambrosio, C. & Radda, G. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 2382.
18. Leigh, J. S., Chance, B., Eleff, S., D' Ambrosio, C., Radda, G. K. & Gadian, D. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 2113.
19. Eleff, S., Leigh, J. S. & McDonald, G. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 1807.
20. Hulm, J. K. & Matthias, B. T. (1980) *Science* **208**, 881–887.
21. Hoult, D. (1978) in *Progress in NMR Spectroscopy* (Pergamon, London), pp. 41–77.
22. Oxford Research Systems (1980) *Topical Magnetic Resonance Spectroscopy*, Catalogue No. TMR 10 (Oxford Research Systems, London).
23. Ligon, T. (1964) Dissertation (Oklahoma State Univ., Stillwater, OK).
24. American National Standards Limit (1979) in *An Investigation of Energy Densities in the Vicinity of Vehicles with Mobile Communications Equipment and Near a Hand-Held "Walkie-Talkie"*, ed. Lambdin, D. (Office of Radiation Program, U.S. Environmental Protection Agency, Las Vegas, NV).
25. Tenforde, T. S. (1979) *Magnetic Field Effects on Biological Systems* (Plenum, New York), p. 39.
26. McLaughlin, A., Takeda, H. & Chance, B. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5445–5449.
27. Chance, B., Radda, G., Seeley, P., Silver, I., Nakase, Y., Bond, M. & McDonald, G. (1979) in *NMR and Biochemistry*, eds. Opella, S. & Lu, P. (Dekker, New York), pp. 269–281.
28. Garlick, P., Radda, G., Seeley, P. & Chance, B. (1977) *Biochem. Biophys. Res. Commun.* **74**, 1256–1262.
29. Grove, T., Ackerman, J., Radda, G. & Bore, P. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 299–302.
30. Boveris, A., Cadenas, E., Reiter, R., Filipkowski, M., Nakase, Y. & Chance, B. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 347–351.
31. Ackerman, J., Grove, T., Wong, G., Gadian, D. & Radda, G. (1980) *Nature (London)* **283**, 167–170.
32. Garlick, P., Radda, G. & Seeley, P. (1979) *Biochem. J.* **184**, 547–554.
33. Bendel, P., Lai, C.-M. & Lauterbur, P. C. (1980) *J. Mag. Res.* **38**, 343–356.
34. Purcell, E. M. (1963) *Berkeley Physics* (McGraw Hill, New York), Vol. 2, p. 357.