NMR Researchers Embark on New Enterprise

First medical applications of phosphorus NMR methods suggest that they can produce clinically useful information

All "Star Trek" fans are familiar with the scene. The crewman has collapsed with a baffling malady. Responding to the emergency, Dr. "Bones" McCoy is able to obtain an instant diagnosis simply by passing a space-age gizmo over the patient's body. And in no time flat, thanks to the miracles of starship medicine, the crewman is on his way to recovery.

The phosphorus nuclear magnetic resonance instruments now undergoing their first clinical testing do not approach this level of diagnostic wizardry. By no stretch of the imagination could they be considered portable, for example. But early results suggest that they can produce medically useful information about the biochemistry of internal organs—and do it noninvasively. "First-phase clinical gadgets are going on the air," says Britton Chance of the Johnson Research Foundation of the University of Pennsylvania School of Medicine. "The results speak for themselves. It works."

The first published report of a diagnosis aided by phosphorus NMR recently appeared in the *New England Journal of Medicine*.* Brian Ross, George Radda, and their colleagues at the University of Oxford showed that they could use the technique to detect the biochemical changes in skeletal muscle that are characteristic of a rare genetic condition called McCardle's syndrome. Their achievement illustrates the marked improvement in phosphorus NMR technology during the past few years.

Only since 1974 have researchers been able to obtain NMR signals from the phosphorus compounds of living tissue. Most of these compounds are present in very low concentrations and, even in the best of circumstances, the NMR signals given by phosphorus-31 nuclei are very weak. (Phosphorus-31 is the naturally occurring isotope.) For these reasons, investigators were somewhat surprised to learn that they could use phosphorus NMR spectra to follow changes in the concentrations of such important indicators of metabolic health as adenosine triphosphate (ATP), creatine phosphate, and inorganic phosphate. For example, they could see the concentration changes that occurred when isolated hearts were deprived of oxygen, a finding that immediately suggests that phosphorus NMR methods might play a role in the diagnosis of heart attack or stroke (*Science*, 1 December 1978, p. 958).

As recently as 1978, the small sizes of the magnets available for phosphorus NMR work limited the maximum diameter of the samples, which must be placed inside the magnet bore, to about 2.5 centimeters. Then the Chance group, using a magnet with an 18-centimeter bore that they bought from Oxford Research Systems, built an instrument large enough to examine human arms or legs. At about the same time, Oxford Research Systems developed an instrument with a 20-centimeter magnet, which is what Ross and Radda used to examine the patient with McCardle's syndrome.

The patient simply inserted his forearm into the magnet and the investigators obtained phosphorus NMR spectra from the forearm muscle, for 1 minute each, under four different conditions: when the muscle was at rest, during exercise (flexing the fingers) with normal blood flow, during recovery from the exercise, and during exercise with blood flow to the arm cut off by an inflated blood pressure cuff.

Patients with McCardle's syndrome lack the muscle enzyme (glycogen phosphorylase) that initiates the glycolytic pathway, a series of reactions that produce ATP during periods of oxygen deficiency when the main ATP-synthesizing reactions are shut down. Such periods often occur during exercise when a muscle works so hard that its blood supply becomes temporarily inadequate. With no significant source of ATP under these conditions, the patients can perform little or no exercise.

Using phosphorus NMR methods, Ross and Radda were able to directly detect the biochemical consequences of the enzyme deficiency. They showed that creatine phosphate rapidly disappears from the patient's forearm muscle during exercise. Creatine phosphate, which is the cell's short-term energy storage compound, is broken down to replace the ATP used to drive muscle contraction. At the same time, the concentration of inorganic phosphate, which is released as the ATP is used, increased more rapidly in the patient's forearm than in those of the controls. A third consequence of the enzyme deficiency is that the muscle *p*H fails to fall as it normally does during exercise in response to the production of lactic acid by the glycolytic pathway. (The *p*H value can be derived from phosphorus NMR spectra because the position of the inorganic phosphate peak depends on the degree of ionization of the phosphate group, which in turn depends on the cell *p*H.)

McCardle's syndrome is very rare, but other applications of phosphorus NMR, with much greater potential for clinical use, are also being explored with some success. For example, the Oxford workers recently completed a pilot study to determine whether the method can be used to predict how well kidneys will function immediately after transplantation, a time when many transplants fail for reasons that are not always clear. Radda and his colleagues found that kidneys that appeared to be in good metabolic condition, having high ATP and low inorganic phosphate concentrations, worked better after transplantation into human recipients than did kidneys that appeared not to be in such good condition. In an article to be published in volume 3 of *Organ Preservation* they conclude, "The confirmation that ^{31}P NMR spectroscopy can predict how cadaver donor kidneys function immediately after transplantation is now an urgent task."

Perhaps the greatest potential for the medical application of phosphorus NMR methods is in the diagnosis of conditions such as heart attack and stroke that are caused by oxygen deprivation. When the oxygen supply to a tissue is interrupted, the inorganic phosphate concentration goes up, as those of ATP and creatine phosphate go down. "The phosphate concentration," says Chance, "is a diagnostic indicator of biochemical perform-

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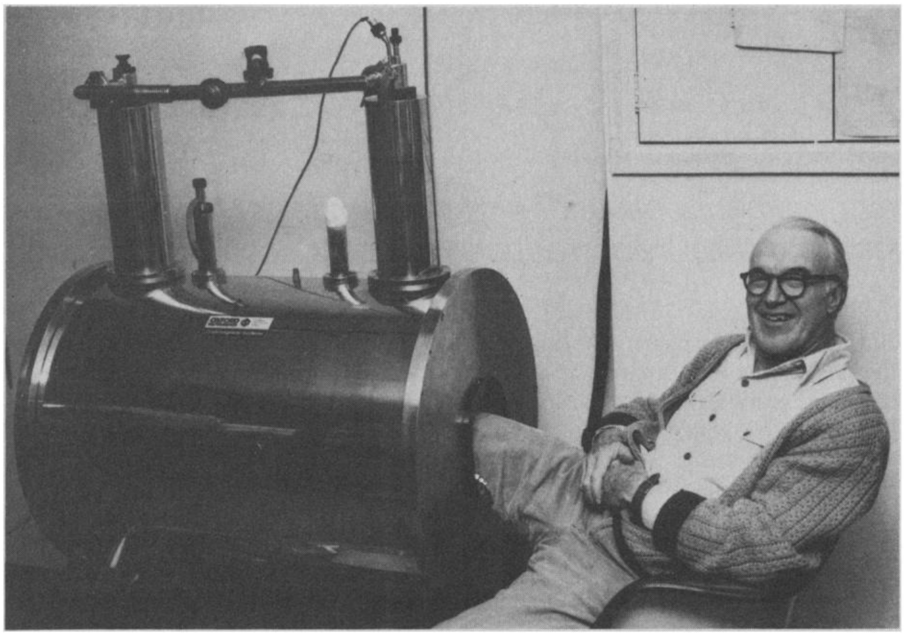

Britton Chance with 18-centimeter bore magnet

Phosphorus NMR spectra can be obtained, apparently without causing discomfort to the subject.

ance. It is like discovering that blue blood means you have a problem with oxygen." Incidentally, one of the discoveries made possible by phosphorus NMR techniques is that the inorganic phosphorus content of normal living tissue is about one half to a third lower than biochemical assays had indicated.

Although phosphorus NMR methods have not yet been used to diagnose human heart attack and stroke patients, experiments with animals have shown that oxygen-deprived brain tissue can be distinguished from normal tissue. In one such study, Radda and his colleagues tied off the right carotid arteries of gerbils. They found the expected changes in ATP, creatine phosphate, and inorganic phosphate concentrations in the right anterior hemisphere of the animals' brains—the region supplied with blood by the right carotid artery. But other areas of the brain showed little change in the concentrations.

Similarly, Ray Nunnally of the University of Texas Health Science Center at Dallas and Paul Bottomley of the General Electric Company Research and Development Center in Schenectady located areas of tissue damage in isolated rabbit hearts after tying off one of the arteries that supply blood to the heart muscle. They also showed that two drugs, verapamil and chlorpromazine, that inhibit calcium ion transport across membranes can protect heart muscle against the deleterious effects of oxygen deprivation. Verapamil in particular is thought to have great promise for treating heart disorders.

Still another application is being investigated by Chance, using one of the 20-centimeter instruments from Oxford Research Systems as well as the slightly smaller instrument constructed by his group. This is the diagnosis of the peripheral blood vessel disease that afflicts many older people, especially those who have diabetes. The blockage of arteries to the limbs, particularly the legs, and consequent oxygen deprivation cause painful muscle cramps. In extreme cases, gangrene may develop. "Phosphorus NMR examination," Chance explains, "can be used for diagnosing the point in the leg where the oxygen supply is insufficient to make the energy supply system work." Usually the arterial blockage is treated by "coring" the blood vessels to open them or by blood vessel transplantation.

Phosphorus NMR methods may have a major advantage over current diagnostic techniques that are invasive or depend on x-rays. Because most investigators consider NMR to be without hazards—although more work will undoubtedly be required on this point—it may be possible to use it repeatedly to follow the effects of therapy as well as to make the initial diagnosis. Nunnally says, "Because it looks like a safe method . . . the prospect of using NMR to follow the efficacy of treatment is one of its most exciting prospects."

At present, there are two major methods for detecting phosphorus NMR signals from discrete portions of whole organs or animals. In gerbil brains and isolated hearts, the investigators detected the NMR signals with surface coils, small flat coils that can be positioned on different areas of the organ to be studied. Coils have an inherent limitation, however. Bottomley points out, "They are sensitive to a depth proportional to their diameter. But you get less localization with larger diameters." In other words, the small coils detect signals from small, well-defined areas close to the surface. Larger coils can pick up signals from deeper within a tissue or animal, as would be required for diagnosis of heart or stroke patients, but may sacrifice resolution.

Even with this possible limitation, surface coils might prove useful for at least one medical application. The liver accumulates manganese ions, which can affect phosphorus NMR signals. Chance, with Alan McLaughlin of the Johnson Research Foundation, found that the signals from phosphorus compounds in liver can be distinguished from those originating in other tissues. He suggests that surface coil methods might detect changes in liver biochemistry, such as those caused by excessive alcohol consumption.

The instruments produced by Oxford Research Systems and used for the human limb studies depend on a different strategy for isolating phosphorus NMR signals from discrete areas within the body. This method, which is called topical magnetic resonance (TMR), allows the selection of signals from well-defined areas by use of very steep magnetic gradients superimposed on the main magnetic field. In this way an area of uniform magnetic density is produced, surrounded by a region where the field strength is rapidly changing. The NMR signals from within the changing field are effectively wiped out, while those from within the homogeneous area are relatively sharply defined.

The principal reason why there have not yet been any phosphorus NMR studies of the hearts or brains of human patients is the lack of large enough magnets. Because small magnets are easier to build then large ones, the next logical step might seem to be construction of magnets large enough to accommodate the human head. In practice, however, this turns out to be impractical. Bottomley explains, "It is important to emphasize the need for highly homogeneous magnetic fields. The chemical shifts for different phosphorus compounds differ by only 1 to 2 parts per million. The homogeneity has to be that good over the whole region you are studying. . . . You can't get a uniform enough signal on the head alone because the shoulders get in the way." To get the head far enough into the magnet so that it is in the homogeneous portion of the field, the shoulders have to go in too. As John Schenck, also of General Electric, says, "If you get a magnet that you could do the head in, you could do the heart."

Currently, Oxford Research Systems has plans for a very large magnet having a 60-centimeter bore and weighing four tons. This will accommodate a moderately sized human thorax. Tests with the instrument will begin in a few months at a number of places, including Oxford, Philadelphia, and Massachusetts General Hospital in Boston.

The cost of the instruments is currently high, but not prohibitive if further clinical trials bear out the promise of the early work. According to Chance, the instrument built by his group cost about a quarter of a million dollars. The smaller Oxford instrument lists at close to a half million dollars and the larger one can be picked up for about three-quarters of a million. This puts their costs in the same range as that of x-ray CAT (computer-assisted tomography) scanners.

Incidentally, phosphorus NMR, which produces biochemical information, could be considered more as a complement to than as a competitor of the x-ray methods, which produce images of body structure. Still, if phosphorus NMR techniques can ever be combined with the imaging methods already developed for proton NMR (*Science*, 17 October 1980, p. 302), it may be possible to obtain two- or three-dimensional images of human tissues and organs that incorporate both biochemical and structural information.—JEAN L. MARX

Staggered Antarctic Ice Formation Supported

If the West Antarctic ice sheet formed as late as 9 million years ago, it may have been the trigger for dramatic ocean changes

Antarctica was not always the continent of ice. According to conventional thinking, little glaciation occurred there until about 16 million years ago. By 13 million years ago, about as much ice had accumulated as is seen today. Oceanographers from the University of Georgia recently found new evidence* for the formation of a relatively small but crucial chunk of ice, the West Antarctic ice sheet, no more than 9 million years ago. Using their more precise dating, they link that glaciation with many of the dramatic chemical and physical changes known to have occurred in the ocean at about the same time.

Paul Ciesielski, Michael Ledbetter, and Brooks Ellwood of the University of Georgia do not base their dating of the West Antarctic glaciation on the usual gauge of the volume of glacial ice—changes in the oxygen isotopic composition of the ocean. They contend that the West Antarctic ice sheet could not have had much effect on the isotopic composition of seawater, in part because of the small volume of ice involved. Instead of isotopic effects, the Georgia group looked for physical effects that glaciation would have caused, especially on the sediments surrounding Antarctica.

Among other effects, extensive West Antarctic ice seems to have cut off a plentiful supply of sediment to the Ross Sea, which flanks West Antarctica proper. Ciesielski and Margaret Savage, also at Georgia, found that prior to 10 million years ago sediment, which was apparently ground off the continent by glaciers, piled up in the Ross Sea at the rate of 100 meters per million years or more. More recently, a typical rate there has been no higher than a few meters per million years. The explanation of the high rates, they say, must be that up until 10 million years ago melting glaciers were dumping their heavy loads of sediment directly into an ice-free Ross Sea. Then, the ice extended to form a floating ice shelf that not only eliminated much of the sediment supply but also, by about 5 to 6 million years ago, scraped away some of the bottom sediments. That erosion can also be seen in the sediments. David Drewry of the University of Cambridge and John Mercer of Ohio State University independently have argued for an ice-free Ross Sea prior to 5 or 6 million years ago. Their hypotheses were based in part on earlier studies with less precisely dated samples.

The date of the formation of the Ross ice shelf is important, Ciesielski says, because the West Antarctic ice sheet could not have formed without that ice shelf. Unlike the lofty East Antarctic ice sheet, much of the ice in the west lies up to 2000 meters below sea level between what would otherwise be islands in a West Antarctic sea. According to some glaciologists, the West Antarctic ice sheet could not have thickened to its 4000-meter depth without ice shelves in the Ross and Weddell seas to buttress the ice sheet and prevent it from flowing laterally under its own weight.

As a second check on the time of ice shelf formation, the Georgia group attempted to more precisely date the appearance of a particular type of sedimentation. They looked for the rock debris carried away from Antarctica by icebergs and dropped on the sea floor as the icebergs melted. Close to the continent at 59°S, ice-rafted debris, which can range from sand to cobble size, first appeared 12 to 13 million years ago, they say. But it did not extend to lower latitudes (about 50°S) until after 10 million years ago, the pebble-sized dropstones not arriving until after 8 million years ago. Because only the large, tabular icebergs calved by ice shelves could survive in those warmer waters, the ice shelves—and thus the West Antarctic ice sheet—could not have formed until after about 9 million years ago, as estimated from the Ross Sea and other cores.

A third date comes from another continent. Although the geological traces of the West Antarctic ice sheet's creation remain buried beneath thousands of meters of ice, researchers can put their hands on the geological record of glacial activity in neighboring South America. Mercer has pointed out that the climate of the southern tip of South America should be closely linked to that of West Antarctica by the prevailing winds and currents. He found that the earliest glacial deposits in South America are between 5.4 and 7.2 million years old. Thus, the Georgia group reasons, the West Antarctic ice sheet must have formed by then.

**Marine Geology*, in press.

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