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## EXAMINATION OF A CASE OF SUSPECTED McARDLE'S SYNDROME BY <sup>31</sup>P NUCLEAR MAGNETIC RESONANCE

BRIAN D. ROSS, D.PHIL., F.R.C.S.,  
GEORGE K. RADDA, M.A., D.PHIL., F.R.S.,  
DAVID G. GADIAN, M.A., D.PHIL.,  
GRAEME ROCKER, M.A., B.M.,  
MARGARET ESIRI, D.M., M.R.C.PATH.,  
AND JAMES FALCONER-SMITH, B.M., M.R.C.P.

McARDLE'S syndrome,<sup>1</sup> an inborn error of metabolism caused by a lack of glycogen phosphorylase activity in skeletal muscle, is a recessive condition of some rarity.<sup>2</sup> Nevertheless, it poses a constant problem in the differential diagnosis of all forms of muscular disorder. Patients usually present with this condition after a long history of inability to sustain exercise; the diagnosis is suggested by the demonstration that ischemic exercise (the forearm-

exercise test<sup>2</sup>) fails to generate lactic acid, and it is confirmed after open-muscle biopsy by the histochemical demonstration of excess glycogen and absent phosphorylase. A conclusive diagnosis and further clarification of various subtypes of the condition come from direct enzymatic assay of glycogen phosphorylases *a* and *b* together with phosphofructokinase.

The biochemical events surrounding this rare condition make it ideal to approach the diagnosis with phosphorus nuclear magnetic resonance (<sup>31</sup>P NMR). This technique permits noninvasive measurements of intracellular pH and of the high-energy, phosphorus-containing metabolites adenosine triphosphate (ATP) and phosphocreatine. In this study, a patient with McArdle's syndrome is shown to differ from normal controls in having no fall in intramuscular pH and an excessive reduction in phosphocreatine in response to exercise. Both these phenomena are predictable results of a lack of ability to break down glycogen and generate lactic acid as the consequence of phosphorylase deficiency.

### <sup>31</sup>P NMR IN BIOLOGY

Since 1974, <sup>31</sup>P NMR has been used extensively to study phosphorus-containing metabolites in isolated, intact tissues and organs such as muscle, heart, and kidney.<sup>3</sup> Recent advances have permitted noninvasive measurement of metabolites from spatially selected regions of living animals, with either surface coils<sup>4</sup> or a field-profiling technique (topical magnetic resonance<sup>5</sup>) or a combination of the two.<sup>6</sup> The construction of large, high-field superconducting magnets (Oxford Research Systems) has now allowed measurements in human beings.

After the demonstration that <sup>31</sup>P NMR spectra can be recorded from human forearm muscle,<sup>7</sup> the biochemical response of muscle to exercise and ischemia was studied.<sup>8</sup> In this paper we report the use of <sup>31</sup>P NMR to support the clinical diagnosis of a patient with McArdle's syndrome.

### What is NMR?

Many atomic nuclei have magnetic properties, and NMR is a way of observing them. When a sample is placed in a uniform magnetic field, such nuclei are "polarized" so that their behavior can be observed with the use of low-energy radio waves. The interaction between the radio frequency and the atomic magnets produces characteristic signals (resonances), because each type of nucleus (e.g., hydrogen, <sup>31</sup>P, or <sup>13</sup>C) acts as a radio transmitter with a given frequency. More important, within the narrow frequency range of each type of atom, the fine tuning of the transmitter is influenced by the chemical properties of the molecule containing that particular atom. We can therefore identify and quantitatively measure specific molecules in a sample. The data are collected in the form of high-resolution NMR spectrums. In such spectrums, the frequency dependence of the absorp-

From the Department of Biochemistry and the Radcliffe Infirmary, University of Oxford, Oxford, United Kingdom. Address reprint requests to Dr. Ross at the Department of Clinical Biochemistry, Radcliffe Infirmary, Oxford OX1 3QU, United Kingdom.

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tion of radio waves is recorded. When the spectrometer is tuned to phosphorus, the  $^{31}\text{P}$  NMR spectrum from muscle, for example, contains signals from nucleotides (adenosine triphosphate, adenosine diphosphate, and nicotinamide-adenine dinucleotide), from phosphocreatine, and from inorganic phosphate. In addition, the inorganic phosphate resonance gives a direct measurement of intracellular cytoplasmic pH in muscle.<sup>9,10</sup> This effect occurs because the chemical environment of the inorganic phosphate nucleus is different in the phosphate monoanion and dianion, and therefore the two species will give signals at different frequencies. In fact, because there is a rapid equilibrium between the two ionic forms of inorganic phosphate, the observed signal is in an average position depending on the relative amounts of the two forms present.

## METHODS

### Clinical History and Investigations

The patient, a 51-year-old man, had a lifelong history of rapid onset of fatigue; his exercise tolerance was limited to walking 100 to 150 yards on a flat surface. He also reported several episodes of passing dark urine after severe exertion, and he had occasional episodes of Raynaud's phenomenon. There was no history of McArdle's syndrome in his parents or two siblings. On clinical examination, there was no evidence of muscle wasting and no obvious abnormality in muscle power apart from the pain and cramps that developed after exercise.

Laboratory investigations showed elevated levels of serum creatine phosphokinase (486 IU) and aldolase (3.81 IU) but no myoglobinuria after exercise. Electromyography was inconclusive, showing only mild abnormalities in action potentials, with short contraction and relaxation times. The electrocardiogram was normal.

On ischemic exercise of the forearm, there was no elevation of lactate, and this suggestion of McArdle's syndrome was supported by histologic examination of a muscle-biopsy specimen from the vastus lateralis. A histochemical assay revealed a moderate excess of glycogen and virtually absent phosphorylase activity. The absence of phosphorylase was confirmed by a direct enzymatic assay<sup>11</sup> after completion of the  $^{31}\text{P}$  NMR study.

After completion of the examinations outlined, the patient was discharged from the hospital pending trials of therapy.

### $^{31}\text{P}$ NMR Examination

The patient received no special preparation. With his forearm placed into a 1.9-T superconducting magnet with a 20-cm bore over a surface coil 4.5 cm in diameter, we examined about 25 ml of tissue within the flexor compartment of the forearm.

The spectrums were recorded at 32.5 MHz on a TMR-32 Fourier Transform spectrometer constructed by Oxford Research Systems, with an approximately spherical region of homogeneous field (radius, 2 cm). This region, together with the localizing characteristics of the surface coil, limit the observed tissue volume to about 25 ml. Before collection of spectrums, the field's homogeneity was adjusted with the  $^1\text{H}$  NMR signal from the tissue water. The resting spectrum was accumulated with 64 radio-frequency pulses applied at intervals of two seconds; all subsequent spectrums were accumulated with 32 pulses, again applied at intervals of two seconds. The very broad components of the spectrums were removed with a computer (apodization), and before Fourier transformation a 6-Hz

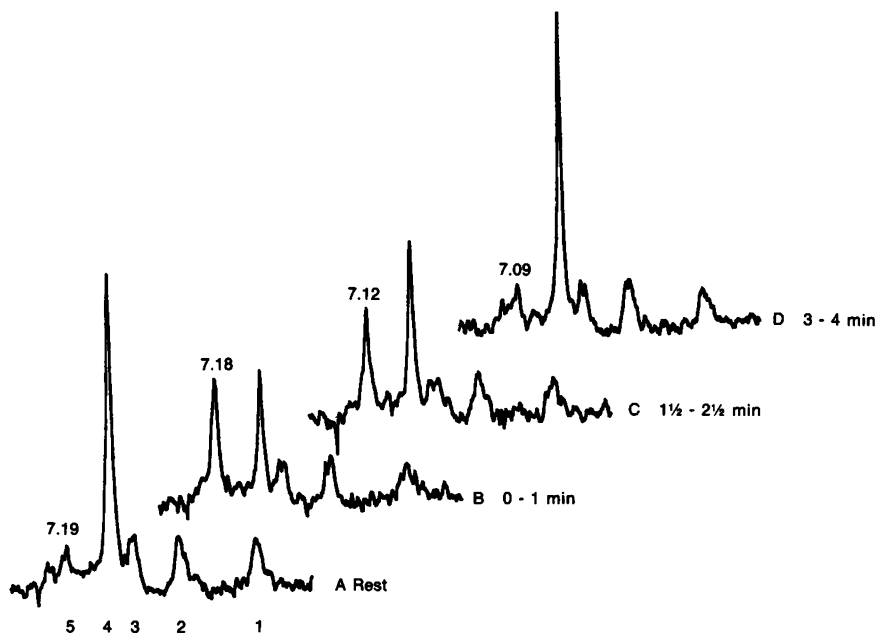


Figure 1. Phosphorus Nuclear Magnetic Resonance ( $^{31}\text{P}$  NMR) Spectrums in the Patient, Showing the Effects of Aerobic Exercise.

The first spectrum (A) was recorded at rest before exercise; subsequent spectrums (B to D) were recorded during the periods shown, in which zero minutes corresponds to the start of exercise. Exercise was maintained during the period from zero to one minute, and aerobic recovery followed. The signals are assigned as follows: 1, 2, and 3, the  $\beta$ ,  $\alpha$ , and  $\gamma$  phosphates of ATP; 4, phosphocreatine; and 5, inorganic phosphate. The pH value given above each inorganic phosphate signal was determined from the frequency separation of the inorganic phosphate and phosphocreatine signals. The resting spectrum was accumulated with 64 radio-frequency pulses applied at intervals of two seconds; all subsequent spectrums were accumulated with 32 pulses, again applied at intervals of two seconds.

line-broadening function was applied. A sweep width of 4 KHz was used (the spectral width displayed in the figures is only 1.1 KHz).

The collection of each spectrum, allowing determinations of pH and of concentrations of phosphate compounds, took one minute. Recordings were made under the following conditions: with the forearm at rest (four minutes of observation); during exercise (flexion of fingers at two-second intervals) with normal blood flow (one minute of observation, after which time the patient was unable to exercise further because of cramp); during recovery (six minutes of observation); and during exercise with complete arterial occlusion (sphygmomanometer cuff pressure, 180 to 200 mm Hg; blood pressure, 130/90). The patient managed to sustain ischemic exercise for only 45 seconds, but arterial occlusion was continued for up to three minutes. Arterial flow was restored, and recovery followed for 5½ minutes. After one hour of rest, spectrums were collected during 10 minutes of arterial occlusion at rest.

The same protocol was carried out in five healthy volunteers 20 to 53 years old. The patient and the control subjects had no ill effects from the examination.

## RESULTS

Spectrums recorded at rest, during aerobic exercise, and during recovery are shown in Figure 1. The relative signal intensities of phosphocreatine, ATP, and inorganic phosphate at rest show the normal pattern seen in control subjects, whereas the patient's intracellular pH of approximately 7.2 was higher than that of the controls. In five control subjects, the muscle pH (mean  $\pm$ S.E.M.) was  $7.02 \pm 0.01$ . (The precision of an individual pH measurement with this technique is approximately  $\pm 0.05$  pH units.) The decrease in phosphocreatine and the increase in inorganic phosphate occurring in the patient during exercise were more extensive than those in the control subjects, in whom relatively small changes were measured even with more prolonged exercise (Fig. 2). The patient's recovery after exercise was not significantly

different from that of the normal subjects. There was no change in ATP in either the patient or the normal subjects.

During ischemic exercise (Fig. 3), phosphocreatine decreased more extensively in the patient than in normal subjects with no measurable decrease, and even an increase, in intracellular pH. This latter observation contrasts markedly with the findings in healthy volunteers, whose intracellular pH always decreased (from  $7.02 \pm 0.01$  to  $6.7 \pm 0.10$  in five subjects, and sometimes to as low as 6.4 to 6.5) (Fig. 4). The recovery of phosphocreatine was again about the same in the patient as in the controls. Again, there was no change in ATP in any subjects. During 10 minutes of arterial occlusion without exercise, no change from the spectrums obtained at rest was detected.

## DISCUSSION

The most striking abnormality in the patient, as compared with the controls, was the relative constancy of intramuscular pH. This value did not fall below 7.0, despite fatigue during ischemic exercise. This observation is entirely consistent with the absence of activation of glycogen phosphorylase. Interesting additional information provided by the  $^{31}\text{P}$  NMR studies was the fall in phosphocreatine during aerobic exercise and the rapid exhaustion of phosphocreatine during minimal ischemic exercise. Both these findings indicate the importance of glycogen metabolism even during aerobic exercise.

Although McArdle's syndrome is exceedingly rare, we have found that the clinical diagnosis can be established by  $^{31}\text{P}$  NMR. We anticipate that observations similar to those described here may be of value

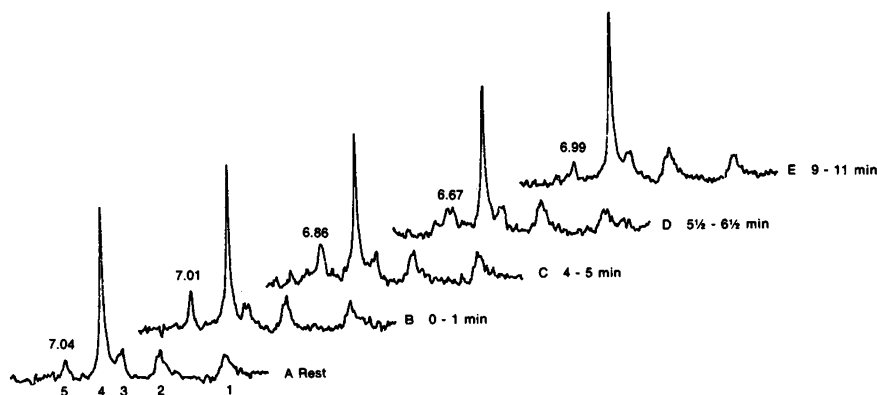


Figure 2. Phosphorus Nuclear Magnetic Resonance ( $^{31}\text{P}$  NMR) Spectrums in a Control Subject, Showing the Effects of Normal Aerobic Exercise.

The peak assignments and spectrometer conditions are as given in the Methods section and the legend to Figure 1, and pH values are given above each inorganic phosphate signal. The first spectrum (A) was recorded at rest before exercise; subsequent spectrums (B to E) were recorded during the periods shown, in which zero minutes corresponds to the time at which exercise was started. Exercise was maintained during the period from zero to five minutes. Spectrums A and E were accumulated with 64 radio-frequency pulses; the other spectrums were accumulated with 32 pulses.

The inorganic phosphate signal in Spectrum D appears to be split into two components. We have observed this type of effect on several occasions, and we interpret it in terms of environments of differing pH (differing in this instance by about 0.4 pH units). The average pH is given above the inorganic phosphate signal.

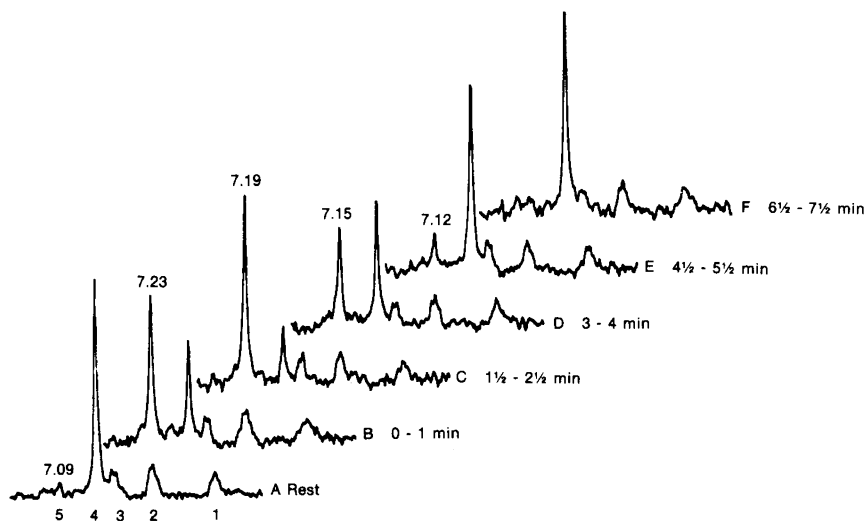


Figure 3. Phosphorus Nuclear Magnetic Resonance (<sup>31</sup>P NMR) Spectrums in the Patient, Showing the Effects of Ischemic Exercise.

Peak assignments and spectrometer conditions are as given in the Methods section and the legend to Figure 1, and pH values are given above each inorganic phosphate signal. The first spectrum (A) was recorded at rest before exercise; subsequent spectrums (B to F) were recorded during the periods shown, in which zero minutes corresponds to the time at which exercise was started. Exercise was maintained during the period from 0 to 3/4 minute, but arterial occlusion was maintained for up to three minutes. Arterial flow was restored after this period.

in monitoring any therapy that may be attempted. Short-term examinations with <sup>31</sup>P NMR should be an improvement over purely subjective criteria applied with the patient at rest.

On the basis of the findings in earlier studies, a number of possible therapies have been proposed to

overcome the muscular weakness and fatigue that are induced by the lack of phosphorylase.<sup>12</sup> These approaches include measures to increase circulating glucose, fructose, and fatty acid levels and to hasten the “second-wind” phenomenon, whereby muscle activity is restored after initial cramp and pain. We pro-

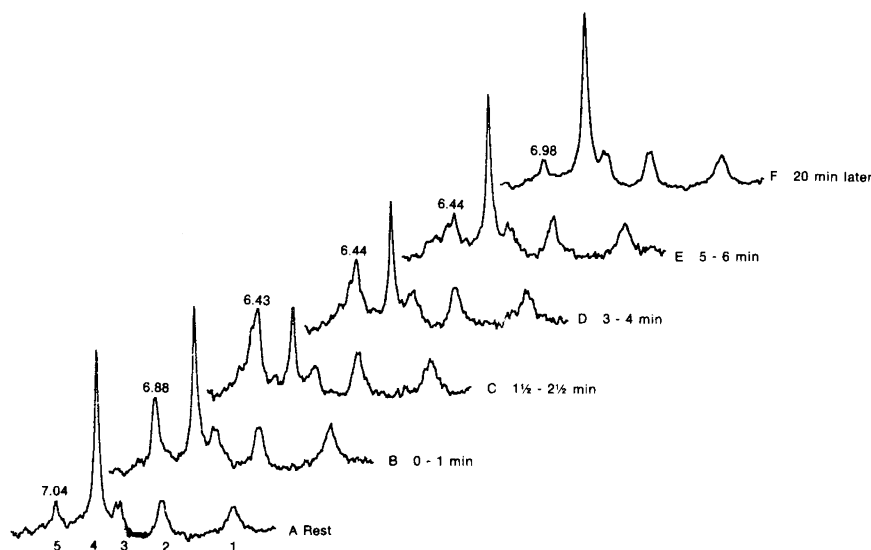


Figure 4. Phosphorus Nuclear Magnetic Resonance (<sup>31</sup>P NMR) Spectrums in a Control Subject, Showing the Effects of Ischemic Exercise.

Peak assignments and spectrometer conditions are as given in the Methods section and the legend to Figure 1, and pH values are given above each inorganic phosphate signal. The first spectrum (A) was recorded at rest before exercise; subsequent spectrums (B to F) were recorded during the periods shown, in which zero minutes corresponds to the time at which exercise was started. Exercise was maintained during the period from zero to 1 1/2 minutes, but arterial occlusion was maintained for up to three minutes. Arterial flow was restored after this period.

pose to use  $^{31}\text{P}$  NMR to test these various possibilities in our patient.

#### Future Applications in Clinical Medicine

The  $^{31}\text{P}$  NMR technique is now at a stage at which the noninvasive (and to our present knowledge, harmless) nature of the measurement make it possible to introduce the method into clinical investigations both as an additional form of diagnosis and as a way to monitor the effectiveness of therapeutic procedures objectively. The space limitations of our magnet restrict the measurements to the lower parts of the limbs and to isolated organs that are used in transplantation surgery. Whole-body magnets should become available in the near future.

Two features of  $^{31}\text{P}$  NMR are likely to be useful in clinical medicine. In the first place, it provides information about intracellular pH — data that until now have been obtainable only with difficulty and uncertainty.<sup>13</sup> A simple noninvasive technique for monitoring tissue pH will considerably extend our understanding of the many clinical conditions in which acid-base disturbances are important. Even in the absence of a whole-body instrument,  $^{31}\text{P}$  NMR studies of muscle in limbs can be important. Secondly, determination of phosphorylated intermediates (in particular, the high-energy compounds ATP and phosphocreatine) will give new information in many tissues apart from muscle. Tissue oxygenation and blood flow influence the energy states of all organs, and we expect that the diagnosis and therapeutic monitoring of ischemic conditions in several organs will be greatly aided by  $^{31}\text{P}$  NMR. It is too early to say which clinical conditions share with ischemia the property of altering the energy state of the cells. The diagnosis of a rare muscle disorder by  $^{31}\text{P}$  NMR thus promises wider clinical applications to more common conditions.

We are indebted to Dr. John Oxbury and Dr. Harvey Sagar for referring this patient to us and to Mr. C. Harman for the assay of phosphorylase.

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#### LAW-MEDICINE NOTES

##### Official Torture and Human Rights: The American Courts and International Law

WILLIAM J. CURRAN, J.D., S.M.HYG.

PHYSICIANS have a particular interest in the prevention of torture and in the effective punishment of barbarous acts of official torture by police and other government agencies. The profession of medicine is based fundamentally on the premise: do no harm. Physicians are often called on to examine the victims of torture. Competent medical evaluation is usually essential to proof of the use of torture on a person. Medical-ethics groups have condemned participation in official torture by the medical profession — or its tacit support — through supplying medical facilities or services to agencies conducting torture. The primary international pronouncement in this field is the World Medical Association's Declaration of Tokyo of 1975.<sup>1</sup>

Physicians have been particularly active in Amnesty International's efforts to seek out and expose official torture in various parts of the world.<sup>2,3</sup> Amnesty International—USA was one of a number of amici curiae in a recent landmark decision in the American federal courts, which is the subject of this Law-Medicine Note.

In *Filartiga vs. Pena-Irala*,<sup>4</sup> the prestigious Second Circuit Court of Appeals in New York, after a long and exhausting series of court reviews, came down with a historic ruling that the deliberate, barbarous torture of a human being perpetrated by government agents under color of law in the home country of the victim at the time was a clear violation of universally accepted principles of the international law of human rights.

The plaintiffs were Dr. Joel Filartiga, a physician, and his daughter, Dolly Filartiga. The case concerned the alleged torture and death of Dr. Filartiga's 17-year-old son, Joelito. The Filartigas were described as longstanding opponents of the government of President Stroessner in Paraguay. Dr. Filartiga claimed that his son had been seized and tortured to death by the Inspector General of Police of Asunción, Paraguay, on or about March 29, 1976. The body had been displayed to Dolly Filartiga and later recovered by Dr. Filartiga. Autopsies had indicated the use of