The Use of NMR Spectroscopy for the Understanding of Disease

GEORGE K. RADDA

Nuclear magnetic resonance spectroscopy can now be used to investigate the biochemical energetics of human tissues and organs noninvasively. The method already has increased our understanding of some muscle diseases, has provided information from muscle metabolism about whole-body functions, control, and hormonal status, has helped in the elucidation of hitherto unrecognized causes of disease, and yielded new ideas about the control of bioenergetics in vivo. Studies on the biochemistry of human brain, liver, heart, and kidney are just beginning. Further investigations of well-selected patients are likely to bring biochemistry and clinical practice closer together.

ALMOST EXACTLY 100 YEARS AGO T. H. HUXLEY, IN HIS anniversary address on 30 November 1885 as President of The Royal Society, talking about the effect of a discovery in one field of investigation on some other remote from it, said: "What an enormous revolution would be made in biology, if physics or chemistry could supply the physiologist with a means of making out the molecular structure of living tissues comparable to that which the spectroscope affords to the inquirer into the nature of the heavenly bodies" (*1, 2*). Physics has provided the basic method by the discovery of nuclear magnetic resonance (NMR) (*3, 4*) and chemistry has given the extensive background for the exploration of chemical structures.

In the study of cellular energetics we are concerned with the reactions involved in the supply and utilization of chemical energy. In skeletal muscle, heart muscle, and brain these reactions are centered around the use of adenosine triphosphate (ATP) for energy supply (Fig. 1). The concentration of ATP is strictly controlled and normally depends on the rate of aerobic ATP production (oxidative phosphorylation in the mitochondrion) in relation to energy demand (ATP hydrolysis). Substrates for oxidative phosphorylation are supplied by the circulating blood (for example, sugars, fats, and amino acids) or are endogenous in the cell (for example, glycogen). Thus, questions about regulation of mitochondrial activity in vivo and of fuel selection become important. Similarly, the mechanism by which anaerobic glycogen breakdown is called into action when aerobic ATP production is inadequate (either because of very high work load or because there is an impaired supply of oxygen) is of importance. Phosphocreatine (PCr) may also act as an energy reserve or may provide an "energy shuttle" between the sites of ATP production and utilization through the special properties of different forms of the enzyme creatine kinase (*5*).

The author is the British Heart Foundation Professor of Molecular Cardiology in the Department of Biochemistry, University of Oxford, Oxford OX1 3QU, United Kingdom, and director of the Medical Research Council, Clinical Magnetic Resonance Group, John Radcliffe Hospital, Oxford.

The concentrations of some of the metabolites shown (Fig. 1), intracellular *p*H (*6*) and lactate production, the fluxes through the major enzyme-catalyzed steps, the flux through glycogenolysis, the way the reactions are controlled, and the way the switch from aerobic to anaerobic processes takes place can now be examined and quantified on the basis of ^{31}P NMR measurements (*7*).

The Phosphorus Nucleus in Biochemistry

Most human biochemical studies by NMR have exploited the phosphorus (^{31}P) NMR signal because phosphate-containing compounds occupy a central role in the bioenergetics of living cells and are present in sufficiently high concentration (>0.5 m*M*) to be detectable by NMR. In addition, the sensitivity of the naturally abundant ^{31}P nucleus is relatively high (one-fifteenth that for ^{1}H) and the chemical shift range is fairly wide (about 30 parts per million for biological phosphates).

In 1974 it was realized that phosphorus-containing metabolites can be detected in intact muscle tissue with the use of ^{31}P NMR (*8*). It is from this work that present-day NMR approaches to human biochemistry have evolved.

In NMR the resonance frequency and the magnetic field are proportional to each other. The constant of proportionality is determined by the magnetic moment of the atomic nucleus of interest. In biology and medicine the most commonly observed signals from the magnetic nuclei are ^{1}H, ^{31}P, ^{13}C, ^{23}Na, and ^{19}F.

NMR is used both for radiological imaging and chemical spectroscopy. NMR imaging is based on the fact that if a magnetic field gradient is applied to an object, the frequency of response of a given type of nucleus will also depend on its position in space. This was first demonstrated experimentally by Lauterbur (*9*), and Mansfield and Grannell reported on a method of NMR diffraction (*10*). A radically different conceptual approach is needed in applying NMR spectroscopy, as opposed to imaging, for the study of human biochemistry. NMR spectroscopy is based on the observation that the NMR frequency of a given nucleus depends to a small extent on the way the electrons shield the nucleus.

The Initial Human ^{31}P NMR Studies: Human Diversity and Control

With the introduction of surface coils (*11*) and the construction of large, horizontal superconducting magnets, it became possible to examine the bioenergetics of human muscle in limbs by ^{31}P NMR (*12, 13*). Spectra can now be recorded in 10 seconds to 1 minute and changes in metabolites and intracellular *p*H can be followed during different kinds of exercise (Fig. 2). During anaerobic (ischemic) contraction only glycogenolysis and PCr can supply ATP, and lactic acid accumulates, leading to a large *p*H decrease. Under aerobic

conditions, particularly at the early stages of dynamic exercise, both oxidative and anaerobic reactions contribute the required energy (*14*). Not surprisingly, there are large variations among different individuals in the way they respond metabolically to the same work (*15*). For example, in individuals with different types of athletic ability the relative importance of oxidative and glycolytic reactions can now be studied noninvasively (*16*). However, the existence of this diversity in normal individuals highlights the need for criteria to identify abnormal (disease) conditions.

From our studies of the forearm muscle (*14*) (flexor digitorum superficialis) and the gastrocnemius muscle of the lower limb, we found that it is the way the reactions in Fig. 1 are controlled that is invariant across the population. For example, the relation between the decrease in PCr and intracellular *p*H during aerobic, dynamic exercise has a characteristic form that does not depend on individual muscle strength or type, or on the way the exercise is performed. This implies that the "metabolic switch" from aerobic to anaerobic energy supply is invariant and can be used to define normal response. Similarly, the controlled rate of oxidative recovery (measured from the PCr resynthesis), the rate of H^+ clearance after exercise (measured from the intracellular *p*H recovery), and the efficiency of coupling between ATP usage and work (*17, 18*) give us characteristic indices for healthy muscle.

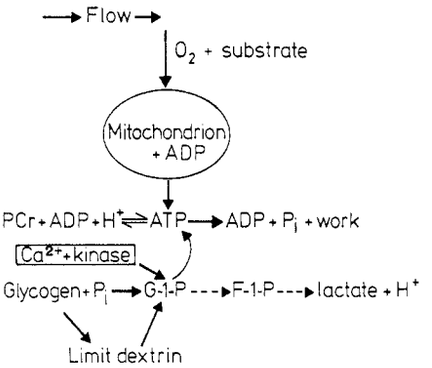

Fig. 1. Biochemical energetics in muscle, heart, and brain. ADP, adenosine diphosphate; ATP, adenosine triphosphate; PCr, phosphocreatine; P_i, inorganic phosphate; G-1-P, glucose 1-phosphate; F-1-P, fructose 1-phosphate.

Biochemistry and Human Muscle Disease

We have devised relatively simple and general tests for the examination of patients with muscle problems and used the criteria outlined above to analyze and compare the data with those from normal controls (*14, 17*). In general, it is the response to stress (exercise in the case of muscle) that has been most informative about abnormal biochemical conditions. Nearly 500 patients have been examined by ^{31}P NMR in Oxford (and at least as many control subjects), many of these for muscle diseases, although a whole body high-field spectrometer has also been used since late 1983 for the study of other organs and disorders (see below) (Tables 1 and 2). Several patients have been examined on many occasions by NMR. These studies have allowed us to learn new biochemistry about known diseases, to elucidate and characterize "new diseases," and to derive new concepts about control of bioenergetics in healthy tissues and subjects. Often the human clinical work has been substantiated by extensive studies on animals and allowed designing of models for the human disease (*18*).

For example, in one recent case detailed NMR studies, together with clinical and biochemical investigations, led to the elucidation of a new muscle disorder. The patient was a 27-year-old male with a 3-year history of muscle pain associated with exercise. On three occasions, exertion resulted in the passage of red urine; one of these episodes was accompanied by an increase in blood creatine kinase activity to 9760 IU/liter (normal maximum <200). Normal electromyogram and inconclusive histology on a muscle biopsy (normal phosphorylase and mitochondria) gave no diagnosis, although a neurological examination suggested an organic myopathy resulting in mild wasting of shoulder girdle musculature. NMR examinations were made on three different occasions. On one occasion ^{31}P NMR measurements [at 1.9 tesla (T)] were made on resting and exercising flexor digitorum superficialis (FDS) (*14*). [Approximately 250 patients had already been examined by an identical protocol, which was evaluated for reproducibility on at least 100 normal control subjects (*18*).] On two occasions the gastrocnemius muscle was studied (also at 1.9 T) at rest and during aerobic dynamic exercise. The comparable protocol has been evaluated in a group of control subjects (n = 10) and in patients suffering from intermittent claudications (*19*). During aerobic dynamic exercise, the change in PCr concentration in gastrocnemius was greater than normal: after 6 minutes it had decreased by 72% (study 1) and 54% (study 2) compared to 25 ± 11% in controls (mean ± SD is used throughout). The *p*H, however, remained in the normal range (6.92 and 7.02 versus 6.94 ± 0.08 in controls). Phosphomonoester was elevated during leg exercise (3.2 and 4.5 m*M* in the two studies compared to control 1.0 ± 0.7 m*M*). During aerobic exercise of FDS, *p*H decreased to 6.82 (close to normal). The recovery of PCr and inorganic phosphate (P_i) concentrations in the patient's muscle was slower than normal and took place at about the same rate, whereas in normals P_i recovery was twice as fast as that of PCr (Table 3) (*14, 18*).

These studies, therefore, showed that increased phosphomonoester was associated with a defect in glycolysis. However, a major deficiency in glycogenolysis or glycolysis—for example, absence of

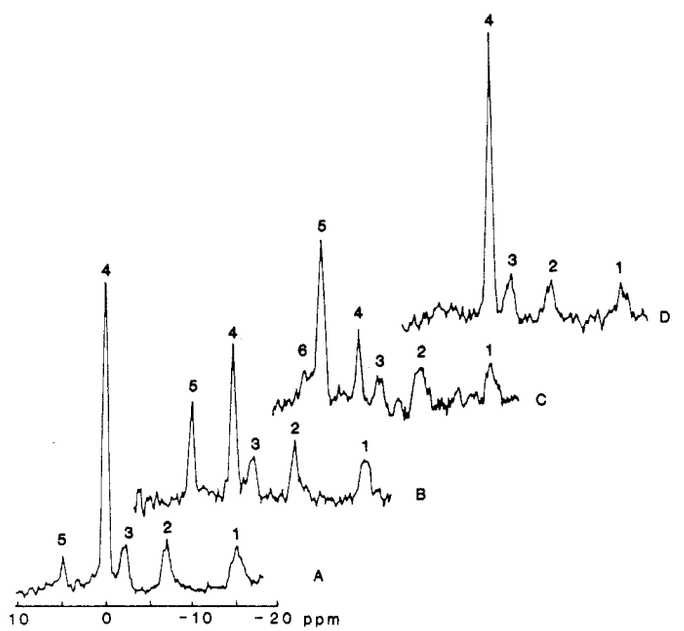

Fig. 2. ^{31}P-NMR spectra of human forearm muscle (flexor digitorum superficialis). (A) Before, (B and C) during, and (D) after exercise. Exercise was carried out for 19 minutes to a pressure of 300 mmHg by repeated squeezing of a bulb. Spectrum B was collected during the first minute of exercise, C during the last minute, and D was obtained 5 to 6 minutes after exercise ceased. Spectrum A was collected over 256 seconds and all others over 64 seconds. Spectra are plotted as signal intensity against parts per million (the fractional frequency shift) and the PCr signal is assigned a value of 0. Peak assignments are: **1**, β-phosphate of ATP; **2**, α-phosphate of ATP plus pyridine nucleotides; **3**, γ-phosphate of ATP; **4**, PCr; **5**, P_i; **6**, phosphomonoesters.

phosphorylase (*12*) or phosphofructokinase activity—is ruled out by the decrease in intracellular *p*H during exercise. Overutilization of PCr during exercise and slow resynthesis in recovery suggested a defect in oxidative phosphorylation. Yet no mitochondrial abnormality was suspected on clinical or histological grounds. The NMR observations are consistent with a defect in a pathway that involves communication between the cytoplasm (glycolysis) and the mitochondrion (oxidative phosphorylation), perhaps at one of the redox shuttles. Since the malate-aspartate shuttle is also associated with mitochondrial transport of P_i, and because of the abnormally slow P_i disappearance during recovery, we concluded that the most likely cause of the muscle disease was an inhibited malate-aspartate shuttle. One prediction of this conclusion, that on exercise the lactate to pyruvate ratio in blood should be elevated in the patient, was confirmed in a 15-minute bicycle ergometer exercise when the lactate-pyruvate ratio increased to 60 (normal <15). The novel diagnosis was finally confirmed by the isolation of muscle mitochondria from a biopsy. The respiratory activities were normal; for example, pyruvate oxidation was 92 nanoatoms of oxygen per minute per milligram of protein (controls, 90 and 72) with a respiratory control ratio of 5.0 (controls, 4.2 and 2.0). The activity of the enzyme-transporter system that links glycolysis to oxidative phosphorylation, the malate-aspartate shuttle, was 20% of control values (20 versus 86 and 91 μmol of reduced nicotine adenine dinucleotide (NADH) per minute per milligram of protein). Thus, on the basis of well-designed NMR experiments, the correct biochemical test could be done to elucidate the nature of this unusual disease (*20*).

Table 1. Forearm muscle studies (n = 614) on patients (n = 327). Some of the results summarized in Tables 1 and 2 are reviewed in (*18*).

Pathology	Subjects (No.)	Remarks
Metabolic myopathies	38	Metabolic diseases often result in major changes in ^{31}P NMR and are of particular interest for providing new biochemical insights (see text)
Mitochondrial		
Glycogen storage		
AMP-deaminase		
Porphyria		
Dystrophy	42	So far ^{31}P NMR has shown major abnormalities in Duchenne and Becker dystrophies only. Preliminary indications are that some manifesting carriers are also abnormal. Disuse atrophy and denervation are being studied in detail for comparisons
Duchenne		
Dystrophia myotonica		
Limb girdle		
Becker		
Others		
Carriers		
Neuropathies	22	Denervation and reinnervation (following surgery) result in change in P_i and phosphorylation potential at rest (mitochondrial activity?)
Brachial plexus lesion		
Motor neuron		
Spinal muscular atrophy		
Others		
Deficient oxygen delivery	16	Pre and post blood-transfusion metabolic responses are being investigated
Low hemoglobin		
Anemia		
Thallasemia		
Scleroderma		
Reduced pulse pressure		
Viral associated weakness	39	See text for an example
Thyroid disease	15	Phosphodiester increases in hypothyroid state are reversed by treatment
Hypothyroid		
Hyperthyroid		No changes have been detected in hyperthyroid
Renal failure	13	Preliminary studies before and after treatment
Diabetes	11	
Heart failure	9	See text
Altered plasma ions	30	Relation between plasma P_i and intracellular P_i has been studied (14 cases). Other ion imbalances only elicit small effects in muscle
Paget's disease		
Osteomalacia		
Hyper- or hypokalemia		
Low Mg^{2+}		
Others		
Alcohol study	6	Indications of impaired glycogenolysis
Other	14	
Myositis		No significant groups yet, only preliminary results
Tumor		
Myasthenia gravis		
Malnutrition		As above
Anorexia nervosa		
Undiagnosed muscle pain or weakness	69	Many "last ditch" referrals. Thirty percent success in detecting abnormal energetics

A second example illustrates a well-known inborn error of metabolism that was the first case examined and studied by ^{31}P NMR. The patient lacked phosphorylase activity (*12*). ^{31}P NMR rapidly showed the lack of acidification of the muscle during ischemic exercise without the buildup of phosphorylated glycolytic intermediates. It was thus concluded that any block in glycolysis must be at the first step (glycogen breakdown). The diagnosis was confirmed by subsequent muscle biopsy and histology. Although the diagnosis could have been made without NMR, during follow-up examinations we have been able to elucidate the biochemical changes associated with the cramp and "second-wind" stages of exercise in these patients (*21, 22*). For this, the patient has been examined by ^{31}P NMR on 29 separate occasions over a period of 3 years. The observations reemphasized and clarified the role of adenosine diphosphate (ADP) in control (see below). Chance and his colleagues had made some interesting observations relating to the role of ADP in the control of mitochondrial activity in a patient with phosphofructokinase deficiency (*23*). My co-workers and I were also able to associate cramp with unusually high ADP levels (see below) and to suggest dietary and exercise therapy for the condition (*22*). Thus, in the examination of established disorders (which by definition can be diagnosed without NMR), repeated noninvasive NMR studies can yield new understanding of the condition and hence provide an objective approach to the evaluation of possible forms of treatment. In this connection we have been investigating in patients with phosphorylase deficiency the metabolic consequences of enzymatic adaptation (*24*) and the role of high protein diet to enhance amino acid utilization as an alternative to glycogenolysis.

The third type of study by NMR involves groups of patients with more common disorders where knowledge of the bioenergetics in muscle or other organs may help in therapy or in understanding the mechanism of the progression of the disease and of the compensatory adaptive changes. For example, the metabolic changes on patients with intermittent claudication as a result of impaired blood flow in the lower limb have been extensively investigated in Oxford (*19*) and Philadelphia (*25*). An interesting group in this category consists of patients with congestive heart failure in whom the severity of exercise intolerance correlates poorly with central hemodynamic abnormalities. This suggests that the pathophysiology may be due in part to alterations in skeletal muscle metabolism or muscle blood flow. We have studied the mechanism of exercise impairment in congestive heart failure by ^{31}P NMR in 11 patients (57 ± 7 years of age) with mean ejection fractions of 16 ± 3% and maximum upright bicycle exercise tolerance ranging from 25 to 125 watts (*26*). Seven age-matched healthy subjects acted as controls. Compared to controls, the patients had similar resting *p*H and PCr values but higher P_i (5.0 ± 1.5 compared to 3.6 ± 4 m*M*, $P < 0.01$) (statistical significance was evaluated with the Student t test). During exercise *p*H decreased more rapidly in patients and remained lower (6.38 ± 0.25 compared to 6.85 ± 0.17 at 100 torr,

$P < 0.001$). PCr also decreased faster in patients. Compared to controls, the pH drop was disproportionate to PCr utilization (*14, 18*) in five subjects, suggesting excessive dependence on glycolysis. Unlike the controls, 5 of the 11 patients developed a split P_i peak during exercise and recovery, indicating two populations of muscle fibers with differing metabolic response to exercise. These findings, together with other measurements, indicate that, in patients with congestive heart failure, skeletal muscle exhibits metabolic abnormalities during exercise that are consistent with impaired oxygen delivery and excessive glycogenolysis. The response of these alterations to treatment of the heart conditions is now being studied.

Abnormal mitochondria are an increasingly recognized cause of neuromuscular disease. We have studied 12 patients with proven mitochondrial myopathies (*27*) and several others subsequently. Of the 12 cases studied in detail, my colleagues and I have seen abnormalities by ^{31}P NMR in 11. In most of the cases (10 of 12) a low phosphorylation potential at rest was observed. About half show impaired rephosphorylation of ADP and fewer show slow PCr resynthesis. This represents the fact that, at the early stages of oxidative phosphorylation, while the ADP levels are still high, more stress is placed on mitochondria (state 3 respiration) and, therefore, the abnormality is more easily detected than when the ADP level decreases and the rate of oxidative phosphorylation reaches a new steady state (expressed by the rate of PCr resynthesis).

The exceptionally high blood lactate during exercise in these patients is consistent with increased glycolytic flux. Yet the fact that the decrease in intracellular pH (pH$_i$) is relatively small and that there is an increased rate of pH$_i$ recovery after exercise suggests that there is an important adaptive mechanism to remove the unusable and potentially damaging lactic acid more rapidly from the muscle cell than in normal subjects.

The dynamic nature of the NMR measurement has uncovered a series of human metabolic abnormalities that previously could not have been detected. For example, several patients suffer from excessive muscular (and often mental) fatigue many months after they have had some viral attack. This condition is not understood and is often regarded as being essentially psychogenic in origin. In one patient with such symptoms the relation between PCr breakdown and pH change is quite abnormal at the early stages of exercise (*28*). This early and rapid acidification has also been seen in more than 14 patients who suffer from postviral fatigue. The biochemical response appears to indicate loss of control and coordination between oxidative and glycolytic mechanisms.

In two patients with undiagnosed and severe muscle pain the opposite effect was observed; that is, on stopping exercise glycogenolysis continued. The possibility that this represents an impaired Ca^{2+} pump remains to be determined.

Examples where some systemic disorder (for example, renal or heart failure, abnormal blood ion concentrations) affects muscle biochemistry are shown in Table 1. Although their discussion is beyond the scope of this article, it should be noted that a detailed study of muscle biochemistry can be used as a window into whole body functions, control, and hormonal status. Since such investigations are simple they might well be added to routine laboratory screening procedures.

Table 2. Patient studies on whole body spectrometer (n = 140).

Organ and condition	Studies (No.)	Remarks
Brain	30	Major changes in tumors and stroke, with unexpected alkalosis. Multiple sclerosis dectectable changes, significance not obvious
Hypoperfusion		
Stroke		
Tumor		
Porphyria		
Multiple sclerosis		See text
Mitochondrial		
Liver	22	
Iron overload		Very useful observation in iron overload. Inflammatory disease: increase in fast relaxing phosphomonoester. Several interesting metabolic defects studied by substrate infusions. ^{31}P components in cyst well resolved
Alcoholic		
Hepatitis		
Metabolic		
Porphyria		
Polycystic		
Malnutrition		
Kidney	3	Preliminary studies on developing appropriate techniques
Transplant		
Heart	2	See text for control studies. Patient investigations only preliminary
Myopathy		
Leg	30	
Claudicants, rest pain, and compartment syndrome	10	See text
Muscle disorders	26	Metabolic as in arm and small changes in phlebitis
Miscellaneous	12	
Others	5	

Table 3. Recovery of phosphocreatine and P_i after exercise ($T_{1/2}$). For controls, n = 10. Error limits are ±SD.

	PCr (sec)		P_i (sec)	
	Patient	Controls	Patient	Controls
Gastrocnemius aerobic exercise				
Study 1	94	33 ± 11	99	17 ± 7
Study 2	91		47	
FDS aerobic exercise	70	57 ± 16	50	27 ± 12
FDS ischemic exercise	108	86 ± 15	106	44 ± 7

We Can Learn New Biochemistry from Human Studies: One Example

The study of altered human biochemistry in disease can help us to understand details of cellular control and energetics, just as the study of bacterial mutations has contributed to the elucidation of specific biochemical mechanisms. For instance, in glycogen phosphorylase deficiency the concentration of ADP rises to about 200 μM during exercise (*22*). In normal individuals the rise in ADP concentration does not exceed 80 to 100 μM and is usually around 40 μM (*15*). These values are obtained because creatine kinase, the enzyme that catalyzes the reaction

$$\text{Phosphocreatine} + \text{ADP} + H^+ \rightleftarrows \text{creatine} + \text{ATP}$$

is close to equilibrium. Thus, when ATP is hydrolyzed the ADP produced is normally kept low by rephosphorylation. In addition, H^+ ions, produced by glycogen breakdown (as lactic acid), also suppress ADP concentration in normal individuals but not in those where glycogenolysis is blocked. If we measure the initial rate of aerobic PCr resynthesis after exercise, it is dependent on ADP concentration in a hyperbolic manner and is faster in patients with phosphorylase deficiency. Thus we can derive the K_m (Michaelis constant) in vivo for the ADP activation of oxidative phosphorylation (27 μM) and the V_{max} for the reaction (43 $mM\ min^{-1}$). We postulated that one of the many roles for creatine kinase is to keep the concentration of free ADP between the resting value of ~1 μM and the value of ~100 μM during exercise that is necessary for

maximal stimulation of oxidative phosphorylation. If the ADP were allowed to rise above this concentration it would block the myosin adenosinetriphosphatase activity ($K_i \sim 200\ \mu M$) and thus lead to muscle failure, as we observed in our patients with phosphorylase deficiency.

These ideas have been tested on heart and skeletal muscle of animals. In some cases my colleagues and I altered creatine kinase function by replacing PCr with a metabolically inert analog (*29, 30*). We were able to show that normal muscle function can be elicited under conditions where the creatine kinase reaction is not faster than ATP turnover, indicating that the enzyme need not act in a PCr shuttle. We also postulated that one reason for "heart muscle" failure may be the high concentrations of ADP rather than the depletion of the high energy phosphates (*31*). These and other NMR studies on the rate of in vivo ATP synthesis (*32, 33*) have resulted in the demonstration that ATP synthetase is effectively irreversible in vivo. A detailed mechanism for this and for how ADP regulates mitochondrial oxidative phosphorylation has been proposed by examining unidirectional P_i-ATP exchange in relation to net ATP synthesis in isolated mitochondria (*34*).

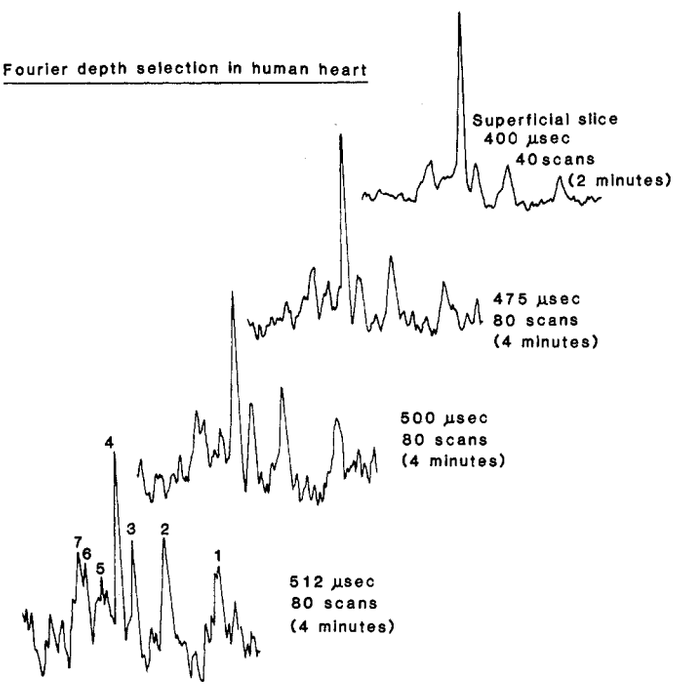

Fig. 3. ^{31}P-NMR spectra of the human heart obtained by Fourier depth selection. Individual spectra represent slices ~1 cm thick (diameter 4 cm) at different distances from the chest wall. Each spectrum required 4 minutes of data accumulation (pulse interval, 3 seconds). Depth selection is carried out with a double concentric surface coil probe (*35*) placed over the chest wall (transmitter, 15-cm diameter, receiver, 6.5-cm diameter). The method of rotating frame imaging has been demonstrated experimentally by observing spectra from the human liver (*35*); the Fourier depth selection is a shortened version of this technique. The heart is more difficult to examine because of the relatively thin tissue mass and proximity to the metabolically similar intercostal muscle. We use depth selection by collecting the signal from the region where the pulse angle is 90°. As the depth of the selected slice is increased, the ratio of PCr to ATP at first falls, then reaches a plateau. The plateau value in ten studies of six normal subjects was 1.7 ± 0.25 (mean ± SE). In three separate studies in one subject the ratio was 1.8 ± 0.12. The corresponding ratio in skeletal muscle is between 3.5 to 4.0. Hence, in the figure shown the superficial slice is from skeletal muscle while the subsequent slices are from heart (calibration of the depth selection was directly compared with echo cardiogram location of the heart). The deepest spectrum shows the double peak from 2,3-diphosphoglycerate, indicating a contribution from blood in the ventricle. The peaks of interest are: **1**, β-phosphate of ATP; **4**, PCr; **5**, phosphodiester; **6**, P_i; **6**, **7**, 2,3-diphosphoglycerate (*42*).

Bioenergetics of Human Organs

The use of different technologies (*35, 36*) has made it possible to record ^{31}P NMR spectra from adult human organs like the brain, liver, heart, and kidney (*22*). Less demanding forms of spatial selection appear to be satisfactory for observing organ metabolism in infants (*37, 38*). In the whole-body system, which operates at 1.9 T, two methods are used for localization in addition to surface coils: static magnetic field profiling (*39*) and rotating frame imaging (*35*) (or a reduced version, the Fourier window technique). For example, spectra from a human heart (Fig. 3) were recorded in 4 minutes per slice, showing that measurements are possible in a clinical setting. However, since patient investigations have many more constraints than the recording of spectra from motivated healthy volunteers of research groups, it will be important to devise meaningful functional tests to elicit metabolic changes. In the case of liver, for example, we can infuse the subject with fructose, and follow the changes in its metabolism (Fig. 4) (*40*). The reactions involve phosphorylation of fructose to fructose 1-phosphate (F-1-P) and its breakdown by aldolase. The synthesis of F-1-P is accompanied by a significant decrease in the concentration of ATP. As F-1-P disappears, there is a large increase in the intracellular P_i concentration. Surprisingly, P_i is only slowly cleared and ATP resynthesis takes several hours. These observations show that phosphate transport in and out of liver is relatively slow and may be the controlling process in some of the metabolic reactions involving phosphate. It also shows that the phosphorylation potential may be substantially reduced in the liver without its leading to cellular damage. This procedure also provides a liver function test for certain metabolic disorders, such as fructose intolerance.

An interesting biochemical question is whether there exists polymorphism and organ specificity of mitochondrial proteins. Skeletal

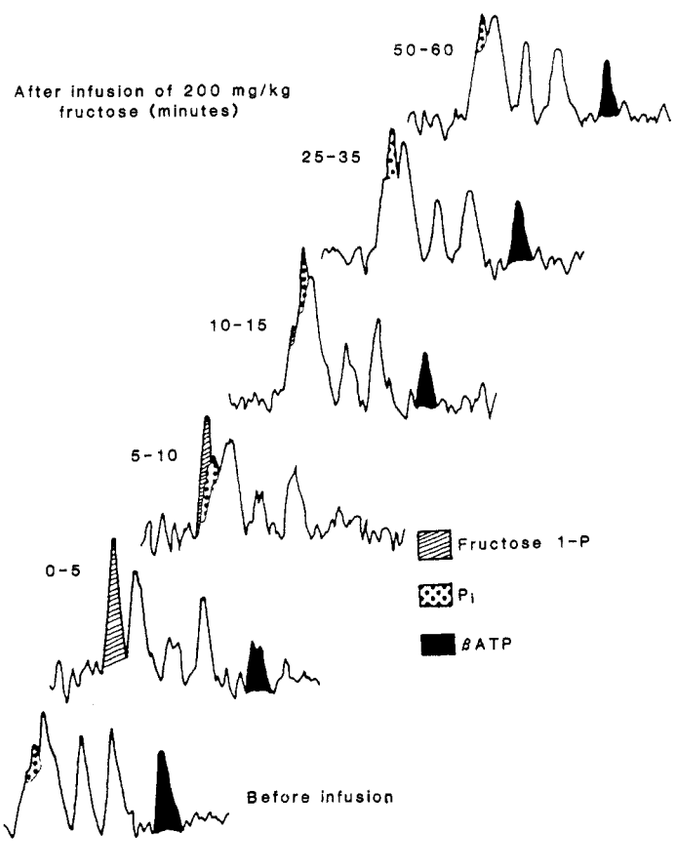

Fig. 4. Time course of metabolic changes in human liver after an intravenous fructose infusion in a healthy subject. [Adapted from (*40*)]

muscle mitochondrial abnormalities are occasionally associated with encephalopathy, cardiomyopathy, or liver disease, for example, as in the case of a 15-year-old girl with muscle and brain involvement (encephalomyopathy) (*41*). Investigations of her skeletal muscle and brain by ^{31}P NMR revealed a low phosphorylation potential in both organs. The defect was further characterized by studies on isolated skeletal muscle mitochondria and found to be within the NADH–coenzyme Q reductase portion of the respiratory chain; thus it is reasonable to assume that the same defect may be being expressed in her brain.

The Direction of Human NMR Spectroscopy

Experience is rapidly being gathered to define the clinical conditions where metabolic studies may be helpful. Abnormalities have been observed in stroke, brain tumors, reduced brain perfusion, iron overload (in liver), glycogen storage diseases, fructose intolerance, and hepatitis; some observations have been reported in infants, such as cardiomyopathy (*37*), liver tumor (*25*), and birth asphyxia (*38*). It is likely that NMR spectroscopy centers will feel financial and clinical pressures to survey large numbers of patients and establish empirical correlations between metabolic patterns, clinical diagnosis, and patient management. There is no doubt that this is a practical and valid approach. However, since diagnosis and understanding of a disease are not synonymous, I hope we also continue to strive toward the elucidation of fundamental biochemical questions in human pathology. This requires selectivity, detailed and repeated investigations of a limited number of patients, parallel research on animal models, and combination of the human study with investigations of cellular and molecular biology. Nuclear magnetic resonance has much to offer as a source of new biochemical information and as a technique that brings clinical practice and biochemical understanding closer together.

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43. This article is based on the first E. C. Slater Plenary Lecture, delivered by the author at the 13th International Congress of Biochemistry, Amsterdam, 1985, and is dedicated to Professor E. C. (Bill) Slater. The work described would not have been possible without the contribution of many colleagues in the past and present and without the financial support of the Medical Research Council, the British Heart Foundation, the Department of Health and Social Security (United Kingdom), and the National Institutes of Health.