Topical Magnetic Resonance

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Nuclear Magnetic Resonance Spectroscopy

Certain atomic nuclei possess a nuclear magnetic moment whereby they can interact with magnetic fields. This interaction, previously predicted from optical spectroscopy, was first detected in 1946 by two groups led by Professor F. Bloch at Stanford and Professor E.M. Purcell at Harvard. In 1952 they jointly received the Nobel Prize for their work on demonstrating the Nuclear Magnetic Resonance (NMR) phenomenon.

The basic experiment can be described briefly in the following manner. In the presence of a strong, uniform magnetic field, B_0, magnetic nuclei such as the proton, phosphorus, assume one of two possible orientations with respect to B_0 each with a different corresponding energy as illustrated in Figure 1. At equilibrium the energy levels are not equally populated there being a small excess (1 in 10^6) in the lower level and it is this small population difference which is the key to NMR. The difference in energy ΔE between these levels is proportional to B_0 thus $\Delta E \propto \gamma B_0$ where the constant, γ, is the corresponding nuclear magnetogyric ratio. Expressing this energy ΔE in frequency units (Hz) the basic Larmor equation can be obtained.

$$\nu_0 = \frac{\gamma}{2\pi} B_0$$

Transitions between these levels can be induced by an oscillating magnetic field of frequency ν_0 and as a result resonant absorption is observed. Table 1 gives a list of the resonance frequencies for nuclei of biological significance at a field of 1.39T.

In a sample containing molecules as opposed to isolated nuclei, the field seen by a nucleus is modified by the bonding electrons. This screening, as it is called, results in nuclei from different chemical environments having differing resonance frequencies and produces a spectrum consisting of Lorentzian lines. The areas under these lines are proportional to the relative concentrations of the corresponding nuclei. The differences in resonance frequency are small (in the order of parts per million, ppm) and are termed chemical shifts. Figure 2 illustrates the effect for the three phosphorus nuclei in Adenosine Triphosphate (ATP). These separations are proportional to the applied magnetic field and hence are more easily seen in high field magnets. Narrow resonance lines are generally only found for small molecules in solution.

In addition to the chemical shift, NMR spectra exhibit so called spin-spin coupling which is a manifestation of nuclear/nuclear interactions transmitted via the bonding electrons. If a magnetic nucleus X is connected via one or two chemical bonds to another, chemically distinct nucleus Y then X can sense the two equally probable states of Y, and vice versa. Hence their resonances are split into doublets as illustrated in Figure 2, for the case of ATP.

Two further parameters called the spin-lattice (T_1) and the spin-spin (T_2) relaxation times, can be used to characterise the resonance lines. These relaxation times are a function of the motion of the molecule and describe the time response of the nuclei to a perturbation that disturbs the equilibrium distribution of the nuclei.

The sensitivity of NMR to chemical effects resulted in its development into a major technique in analytical and structural chemistry. With the aid of techniques such as Topical Magnetic Resonance (TMR) these benefits are now becoming of importance to in-vivo biochemistry and clinical research. It is becoming possible to measure in a totally non-invasive way, parameters such as intracellular pH, metabolite concentrations and to perform tracer studies without the use of radio-nuclei or ionising radiation.

Properties of nuclei suitable for use in NMR studies of biological systems

Nucleus	Spin	Frequency* MHZ	Sensitivity**
^{1}H	1/2	80.3	1
^{2}H	1	12.4	1.5×10^{-6}
^{13}C	1/2	20.2	1.8×10^{-4}
^{14}N	1	5.8	1.0×10^{-3}
^{15}N	−1/2	8.1	3.9×10^{-6}
^{17}O	−5/2	10.9	1.1×10^{-5}
^{19}F	1/2	75.5	8.3×10^{-1}
^{23}Na	3/2	21.3	9.3×10^{-2}
^{31}P	1/2	32.5	6.6×10^{-2}

*at 1.9 Tesla **sensitivity for equal number of nuclei at constant field corrected for natural abundance of a given isotope, expressed with respect to proton

FIGURE 1

Basic NMR In the presence of a steady magnetic field, B_o, some nuclei (e.g. ^1H, ^{13}C, ^{31}P) behave like tiny bar magnets and align themselves parallel or anti-parallel to the direction of B_o.

The two orientations, or spin states, have differing energies and transitions between these states can be induced by a radiofrequency magnetic field oscillating at a frequency, ν_0.

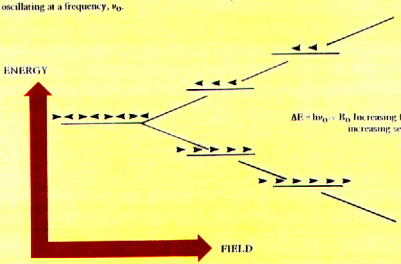
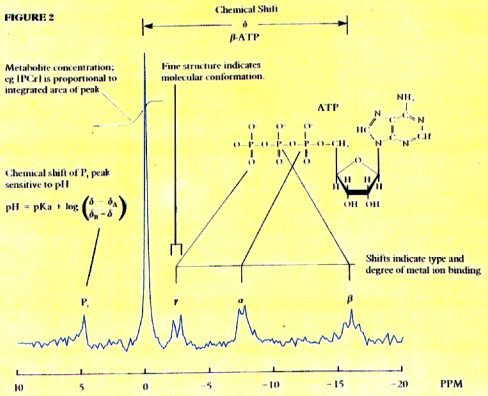


FIGURE 2



The above illustrates the types of information available from a ^{31}P NMR spectrum of a human forearm.

Topical Magnetic Resonance

In order to apply NMR techniques to the study of living bodies, not only is it necessary to have instruments large enough to accommodate them, but it is also necessary to devise ways of obtaining signals from an identifiable part, as opposed to the whole sample, as is the case with conventional spectrometers. Topical Magnetic Resonance (TMR) is a technique invented by Oxford Research Systems Ltd, for obtaining high resolution nuclear magnetic resonance spectra from a selected place in a larger specimen (1,2).

In conventional NMR spectroscopy, the sample is placed within a closely fitting receiver coil and signals are received from all the volume contained within the coil. The magnetic field must be uniform over the whole sample volume.

In TMR spectroscopy a sensitive volume is produced by superimposing high order magnetic field gradients onto the main magnetic field in such a way as to define a central region of uniform field surrounded by rapidly changing fields. The resultant complex magnetic field surface is illustrated on the front page.

A section through this field surface, along the z-axis, is shown in Figure 3 together with a, schematic explanation of the origin of the TMR spectrum. Within the sensitive volume the magnetic field inhomogeneities are less than the typical linewidths and high resolution spectra can be acquired from that part of the sample which lies within this roughly spherical volume. Immediately adjacent to the central volume lies a region where the magnetic field is changing very rapidly with position. Inhomogeneously broadened spectra will be acquired from this region since the range of signal frequencies will still lie within the bandwidth of the spectrometer receiver. In all other regions of the sample the spectral lines will be broadened and shifted so much that the signal frequencies will lie outside the receiver bandwidth and will not be detected.

In Figure 4 spectrum (a) is a typical TMR spectrum. It contains both the narrow, high resolution lines and the inhomogeneously broadened lines which have to be separated out in order to recover the high resolution spectrum of the sensitive volume. TMR uses deconvolution techniques for eliminating the inhomogeneously broadened component and spectra (b) and (c) illustrate the stages in this process. Spectrum (a) is first of all broadened by exponential multiplication to produce spectrum (b). The convoluted spectrum of (a)•(b) then provides the high resolution spectrum (c) of the sensitive volume.

The profiling of the magnetic field is produced by gradient coils which are high strength, modified versions of the conventional shim coils used in wide bore superconducting magnets. The current in the coils can be controlled in a continuous manner to provide a sensitive volume of any diameter within the specified operating range. The main magnetic field contains designed proportions of constant field gradients which are used in conjunction with the variable gradients to adjust the size of the sensitive volume.

The first in-vivo TMR measurements to demonstrate the localising capability of this technique were made on an anaesthetized rat (1). The NMR coil surrounded that part of the abdomen containing the liver so that signals from skeletal muscle, smooth muscle and liver tissue would be collected. Spectrum 5 (a) was acquired from the whole rat in a few minutes. Lines from the ATP, PCr and P_i are clearly visible in addition to a sugar phosphate (SP) line.

Spectrum 5 (b) shows the significant difference obtained by reducing the diameter of the sensitive volume to 2 cm. A marked reduction of PCr is seen which is clear indication that this spectrum is predominantly that of the centrally placed liver, which is known to contain a low level of PCr. The residual PCr line probably comes from the muscle of the diaphragm or gut. Note the low level of inorganic phosphate in healthy tissue.

A further development incorporated in TMR spectrometers is the use of surface coils (3). Unlike conventional coils these do not surround the sample, but as their name suggests, are placed beside the sample and can be used advantageously to obtain signals from a tightly localised region close to the surface of the sample. In combination with TMR, field profiling surface coils can be used to study deeper lying tissues as illustrated by the rabbit heart spectra in Figure 4.

DOCUMENT 20

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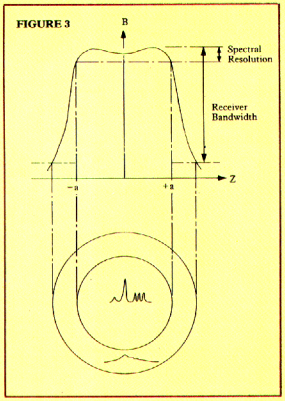
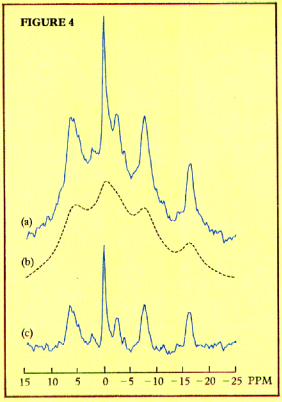
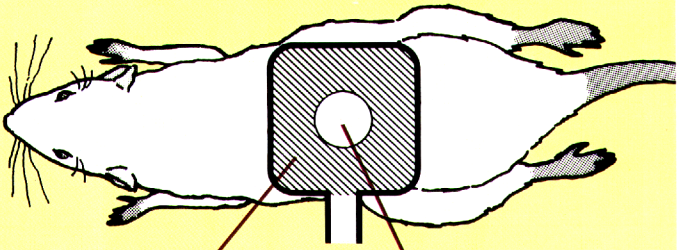
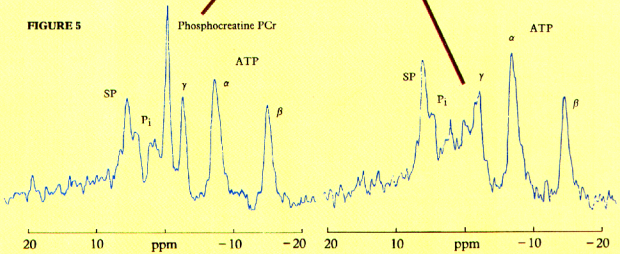








The in-vivo TMR spectrum of ^{31}P obtained from a rat abdomen using a sensitive volume diameter 2a = 5 cm.

The in-vivo TMR spectrum of ^{31}P obtained from a rat abdomen using a sensitive volume diameter 2a = 2 cm.

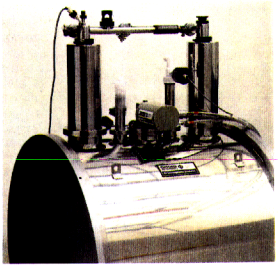
The TMR-32 Spectrometer

A Topical Magnetic Resonance Spectrometer consists of four essential components, the magnet, the probe, the electronics and the data system.

The spectrometer is primarily designed for the observation of biological systems by pulse Fourier Transform NMR. Two versions are available with either 200 or 600 mm clear access diameter. The spectrometer console is the same in both cases but the auxiliary cabinet (which houses the profile coil power supplies) and magnets are different.

The standard TMR 32 system detects ^{31}P resonances at 32.5 MHz and ^{1}H resonances at 80.3 MHz. Accessories are also available for ^{13}C observation with proton noise decoupling and saturation transfer experiments.

Virtually all spectrometer and peripheral functions are under computer control, and can be extended as required to other peripheral devices via the internationally accepted IEEE-488 instrumentation bus.



This magnet with a 200mm bore is equipped with a closed cycle cooler permitting annual helium service.

The Magnet

Both the 200 and 600 mm systems use superconducting magnets operating in persistent mode which are fully protected against quenching. Advanced cryogenic techniques ensure very low cryogen consumption with consequently low running costs and minimal servicing requirements. The shim and field profile coils are resistive and powered from an auxiliary cabinet.

The 200 magnet is suitable for animal model studies and work on human limbs. The sensitive volume is variable from 15 to 40 mm in diameter. The 600 magnet is designed for whole body TMR and has a sensitive volume adjustable from 40 to 100 mm.

The Probe

The TMR-32 system is equipped with probes for each nucleus to be observed, which are capable of accepting a range of easily changeable radiofrequency coils.

The Radiofrequency Electronics

All frequencies used in the instrument are synthesized from a single quartz crystal controlled oscillator. The receiver uses quadrature detection with a "cyclically ordered phase sequence" (CYCLOPS) to eliminate systematic errors. All the major electronic components are housed in separate modules which plug into a common data bus facilitating rapid in-field repair and modification at minimum cost.

Although a highly specialised system, the TMR-32 is extremely flexible and its architecture ensures that it can be adapted for future needs.

The Data System

The data system is designed around general purpose computer architecture which allows the greatest flexibility of use. All spectrometer functions governing the generation and acquisition of the NMR spectrum, e.g. R.F. pulses, amplifier gains etc., are under computer control, providing easy specification of individual experiments. The data is digitised by dual ADCs whose word length can be varied between 2 and 12 bits.

Data is directly acquired with minimal disruption of other computer functions, and accumulation is performed in an extended precision form. Data areas are definable by software, a typical organisation allowing data accumulation into one of the allocated arrays whilst data may be manipulated, plotted etc., in any of the other defined arrays.

The system includes flexible diskettes for both program operation and data storage. High speed data storage devices are available as accessories.

Software

The software system is structured to allow the greatest convenience both for the sophisticated research worker and for routine use. The instructions are arranged in a hierarchical level system so that the operator is presented with a menu of possible options, the choice of one of which will either allow the alteration of a parameter, initiate a spectrometer function or lead to a lower level where another menu is presented. Alternatively, the instructions may be assembled into a program which will run an experiment. Besides the operation of the instrument, the software system will analyse typical spectra. The majority of the software is written in a high-level language so that the user can modify or add to it himself for special purposes.

Spectrometer Control and Data Output

Communication with the spectrometer is principally by means of the alphanumeric keyboard. In addition there are control knobs which can be assigned to specify a variety of functions, e.g. the position of cursors or phase correction angles. The spectrometer communicates with the operator via the visual display unit (VDU) on the left of the console.

The other screen on the left is used to display FIDs and spectra. Cursors can be put onto this display and numerical values of frequency and intensity presented. The information on the display can also be plotted on the X-Y recorder together with calibration scales and additional alphanumeric data.

Additional recording devices, e.g. high speed plotters, can be readily connected via the IEEE-488 instrumentation bus.

Applications

At the present moment three nuclei have been used in TMR studies. The first nucleus to be used was ^{31}P since this was the nucleus on which most fundamental work had been done on model systems. However, recently both ^{1}H and ^{13}C have been shown to be valuable.

^{1}H

Of the naturally occurring nuclei the ^{1}H nucleus, the proton, gives the biggest NMR signal and the isotope is virtually 100% abundant. The very strong water signal obtained from biological samples coupled with the narrow spread of chemical shifts, generally makes it difficult to study the more interesting low concentration ^{1}H-containing molecular species. Consequently very little ^{1}H studies have been carried out in living systems. Recently, however, TMR studies have demonstrated that the ^{1}H NMR spectrum is capable of providing useful in-vivo information. A typical ^{1}H spectrum of human forearm is presented in Figure 6(a) showing signals primarily from H_2O in muscle and the acyl chains of the triglycerides stored in fat tissues. Proton spectra show differences with the physique and sex of the subject.

^{31}P

In 1974 it was shown that ^{31}P spectra could be obtained from intact muscle. The NMR signals were identified as originating from the major phosphate metabolites in the cell, e.g. Adenosine Triphosphate (ATP), phosphocreatine (PCr) and inorganic phosphate (P_i). The presence of adenosine diphosphate (ADP) is generally masked due to its low concentration and overlap with the ATP spectral lines.

In 1941 the work of F. Lipmann demonstrated that these metabolites are involved in many of the energy processes in living systems in a way which is represented diagrammatically in Figure 7.

The ^{31}P NMR spectra can provide detailed information about these biochemical processes. For example, the relative concentrations of the metabolites can be measured from the area of the respective resonance lines and the position of the ATP lines indicates that the ATP is complexed to Mg^{2+} ions. In addition intracellular pH can be measured since the chemical shift of the P_i line is very sensitive to pH in the biological range.

The localised ^{31}P spectra produced by TMR make it possible to identify and study the metabolism of specific tissues within the body. Since different classes of healthy tissue have characteristic amounts of the various phosphate metabolites they will exhibit different characteristic spectra. For example, the primary fuel for skeletal muscle contraction is provided by the breakdown of ATP into ADP and P_i. The ATP level is replenished by PCr. PCr is therefore the energy reservoir of the muscle and as might be expected is stored at a high concentration in the healthy muscle as is shown for the human arm in Figure 6(b). In contrast, an organ such as the liver would not be expected to store PCr and a low level of PCr should be seen in a liver spectrum (see Figure 5). In addition to healthy tissues, diseased tissues can also be studied.

^{31}P NMR is thus a powerful and elegant tool for studying metabolic pathways and energy conversion processes. Topical magnetic resonance enables in-vivo measurements to be carried out over the whole body or in specific organs of live animals.

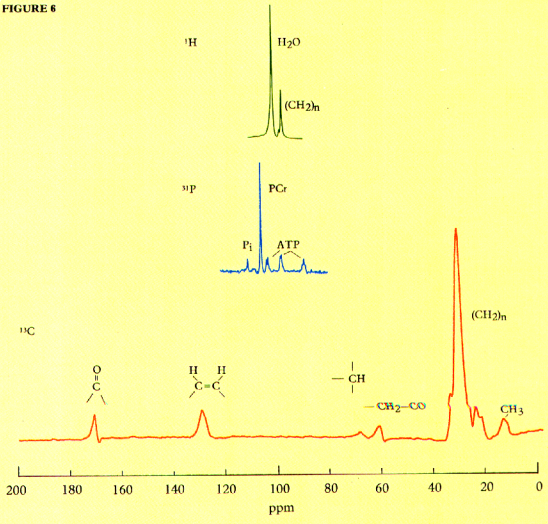
^{13}C

The most recent nucleus to be studied by TMR is ^{13}C. In nature 99% of carbon occurs as the isotope ^{12}C which does not have a magnetic moment and therefore does not produce an NMR signal. The remaining 1% is the isotope ^{13}C which does however give a spectrum rich in chemical information. The ^{1}H-decoupled ^{12}C spectrum of a human forearm is presented in Figure 6(c). Although ^{13}C is relatively insensitive compared to ^{1}H or ^{31}P the wealth of chemical information that is available together with the possibility of using ^{13}C-labelled metabolites compensate for the longer signal acquisition times that have to be employed.

Some of the many in-vivo applications of TMR can be summarised as follows:

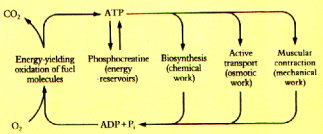
- ☐ **Studies of basic organ metabolism**
- ☐ **Studies of disease in specific organs**
- ☐ **Identification and estimation of:**
  - **extent of ischaemia**
  - **peripheral vascular disease**
  - **myocardial infarction**
  - **cerebral infarction**
- ☐ **Estimation of drug therapy efficacy**
- ☐ **Whole organ transplant viability studies**

FIGURE 6



Proton, phosphorus and carbon spectra of human forearm.

FIGURE 7



Energy processes in living systems.

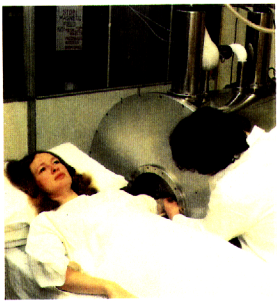
TMR in Medicine

Since their introduction by Oxford Research Systems Ltd in 1980 TMR spectrometers have already been used to provide new information, in a non-invasive manner, about a variety of living systems. In animals, metabolism has been studied in normal and tumour tissues, whilst in humans investigations into muscle function under various conditions of ischaemia and exercise have been carried out. The utility of TMR for such studies has amply justified the expectations based on the previously established work on isolated tissues.

The first clinical use of TMR was announced from the Clinical Magnetic Resonance Group at the Radcliffe Hospital, Oxford. A case of suspected McArdle's syndrome was examined (1). The response of the patient to normal aerobic and ischaemic exercise was observed in a series of quick, simple procedures that measured metabolite concentration and the intracellular pH (see Figure 8). The results conclusively diagnosed McArdle's syndrome. The same group has followed the study with a further clinical investigation of a myopathy.

The foregoing work was based on ^{31}P NMR, but TMR studies are not, however, confined exclusively to this nucleus. ^{1}H and ^{13}C nuclei too have a role to play. The ^{1}H spectrum of the forearm of a normal 13 year old boy is presented in Figure 9(a) (2). Two major peaks are discernible, the H_2O peak from muscle and the $(CH_2)n$ peak arising predominantly from the neutral triglycerides stored in fat tissue. Muscular dystrophy is a debilitating disease characterised by fatty infiltration and gradual replacement of muscle by fat in limbs. The ^{1}H spectrum of the forearm of a dystrophic 14 year old boy is presented in Figure 9(b) and shows a greatly elevated amount of fat compared to the normal spectrum. Such spectra may not be able to elucidate a cure for this disease but should prove useful in the treatment and clinical management of dystrophy.

Despite the low natural abundance of ^{13}C, characteristic ^{13}C spectra can be obtained from various animal and human tissues. The ^{1}H-decoupled ^{13}C spectrum of human forearm is shown in Figure 6(a). The peaks arise from the neutral triglycerides in fat tissue and provide information about nutritional fat deficiency and also fatty infiltration (e.g. in dystrophy). Isolated tissue work has shown that metabolic pathways can be studied in detail by selectively enriching key compounds. The results of one such in-vivo experiment in rat liver are presented in Figure 10. This type of tracer experiment eliminates the hazards inherent in similar radioisotope experiments in current use in clinical practice.



Patient being examined in the Clinical Magnetic Resonance Laboratory, Oxford.

The present TMR spectrometers in use are equipped with 200 mm bore magnets which limits measurements to distal limbs, small animals and isolated organs used in trans-plantation surgery. The availability of whole body magnets is imminent and will open up new possibilities for clinical diagnosis in humans. Preliminary work on rabbit brain (see Figure 11) has demonstrated that ^{31}P spectra can successfully be obtained.

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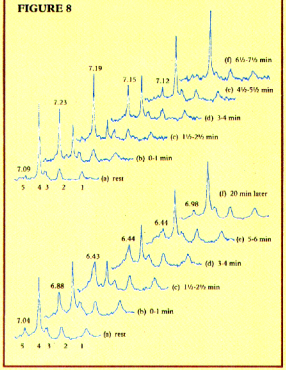
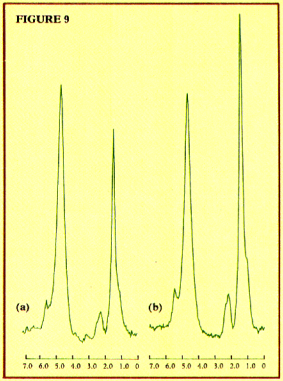
FIGURE 8



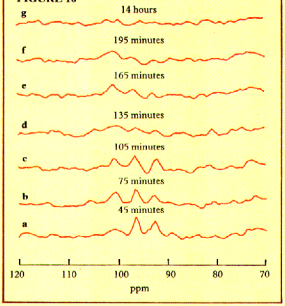
^{31}P spectra of the forearm of a normal person (above) and of a patient with McArdle's syndrome. Spectra (a) were recorded prior to exercise; subsequent spectra (b-f) were recorded during the periods indicated. Ischaemic exercise was maintained for the period 0 to 3 minutes for the normal and 0 to .75 minutes for the McArdle's patient. Arterial occlusion was maintained for 3 minutes after which arterial flow was restored. The pH is reported above the P_i peak (With permission from the New England Journal of Medicine).

FIGURE 9



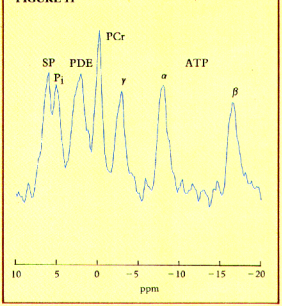
The proton spectra of the forearm of a normal 13 year old boy (a) and that of a boy of similar age suffering from muscular dystrophy (b).
(With permission from *The Lancet*).

FIGURE 10



In-vivo rat abdomen ^{13}C spectra after feeding with D-[1-^{13}C] Glucose. The spectra were collected in half hour blocks. The time between glucose feeding and the spectrum acquisition is given for each spectrum. The signals at 101, 96.8 and 92.3 ppm arise respectively from the C-1 carbons of glycogen and the β and α anomers of D-Glucose. (From the American Association for the Advancement of Science with permission).

FIGURE 11



The ^{31}P spectrum of the brain of a rabbit obtained in 10 minutes. The spectrum shows increased amounts of phosphodiester compared to that obtained in previous in-vivo brain spectra..

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