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Nuclear Magnetic Resonance Studies of Blood Platelets [and Discussion]

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Phil. Trans. R. Soc. Lond. B 1980 **289**, 413-423
doi: 10.1098/rstb.1980.0058

Phil. Trans. R. Soc. Lond. B **289**, 413–423 (1980) [413]
Printed in Great Britain

Nuclear magnetic resonance studies of blood platelets

By J. L. Costa,† C. M. Dobson,‡ K. L. Kirk,§
F. M. Poulsen,‡ C. R. Valeri‖ and J. J. Vecchione‖

† *Clinical Neuropharmacology Branch, N.I.M.H., Bethesda, Maryland* 20014, *U.S.A.*
‡ *Department of Chemistry, Harvard University, Cambridge, Massachusetts* 02138, *U.S.A.*
§ *Laboratory of Chemistry, N.I.A.M.D.D., Bethesda, Maryland* 20014, *U.S.A.*
‖ *Naval Blood Research Laboratory,* 615 *Albany Street, Boston, Massachusetts* 02118, *U.S.A.*

Blood platelets contain membrane-enclosed granules which have inside them high concentrations of 5-hydroxytryptamine (serotonin) along with adenine nucleotides and divalent metal ions. ^{19}F n.m.r. of fluorinated serotonin incorporated into the granules of both human and pig intact platelets has shown that the motional state of the serotonin is restricted. Comparison with ^{31}P n.m.r. experiments indicates that this restriction of motion is a consequence of high molecular weight aggregates formed by the adenine nucleotides and metal ions, and that it varies with the species from which the platelets are obtained. In the case of human platelet granules, at least, these high molecular weight aggregates are present in the absence as well as in the presence of serotonin. The biological significance of these data is briefly discussed.

Introduction

Blood platelets are cells that play an important role in clotting and coagulation processes (Johnson 1971; Weiss 1975). They are smaller and less numerous than red blood cells, but have the interesting property of storing biogenic amines such as 5-hydroxytryptamine (serotonin), divalent metal ions such as Ca^{2+}, and nucleotides, mainly ADP and ATP (Holmsen & Day 1971; Fukami & Salganicoff 1977). The storage organelle of the cells is a cytoplasmic membrane-enclosed granule, often referred to as a dense body because it is observed to be heavily stained in electron microscopic studies (Tranzer *et al.* 1966). In many of their properties the dense bodies are similar to other storage granules, such as the chromaffin granule (Smith 1968) and synaptic vesicles from neurons (Sneddon 1973), for example, in their uptake of amines and the release of their contents by exocytosis (Poste & Allison 1973). In the cytosol of blood platelets there is a metabolic pool of adenine nucleotides that is believed to serve as the energy source for most platelet functions (Fukami *et al.* 1976; Holmsen *et al.* 1969). We wish to use n.m.r. to provide information about aspects of platelet structure and behaviour that is not readily obtained by other methods. In this paper we are particularly concerned with the structure of the granules and the way in which the serotonin is stored inside them.

N.m.r. has been used in many instances to study cellular systems, and other more or less intact biological systems (Moon & Richards 1974; Dwek *et al.* 1977; Campbell & Dobson 1979). Of most direct relevance to the platelet studies are investigations of chromaffin granules (Daniels *et al.* 1974; Casey *et al.* 1977; Sharp & Richards 1977). As well as using naturally occurring nuclei, such as ^{31}P, we have chosen to study ^{19}F. Fluorine does not normally occur in biological molecules and, therefore, it is possible to use the highly sensitive ^{19}F n.m.r. to observe fluorine analogues of species present in cells in low concentrations, or otherwise not

observable by n.m.r. because their naturally occurring nuclei give rise to broad resonances that are swamped by the signals from other molecules. For these reasons, ^{19}F n.m.r. studies of large proteins (Hull & Sykes 1975), of proteins in membranes (Hagen *et al.* 1978) and of phospholipids in membranes, both isolated and in intact cells (Gent & Ho 1978), have been carried out. As with these earlier studies of proteins and membranes, it is necessary to ensure that the biological behaviour of the fluorine-labelled compound is well understood and that it is compared with that of the naturally occurring molecule. For blood platelets, it has been shown that 4,6-difluoroserotonin is taken up by, and released by, the platelet granules, in a manner that is analogous to that of normal serotonin (Costa *et al.* 1978*a*). The serotonin has been shown, by radioactive labelling methods, to be concentrated virtually exclusively in the granules under the conditions used in the n.m.r. experiments; this permits the use of ^{19}F n.m.r. to study these regions alone in the intact cell. In separate work (Costa *et al.* 1979) we have shown, by incubation of human platelets with 2-fluoroadenosine, that it is possible to observe ^{19}F n.m.r. resonances of labelled adenine nucleotides located exclusively in the cytoplasm. However, in this paper we are concerned entirely with the granules.

Experimental

4,6-Difluoroserotonin was synthesized by procedures described elsewhere (Kirk 1976). Antimycin A, 2-deoxy-D-glucose and serotonin were obtained from Sigma. X537A was a gift of Dr J. Berger of Hoffman-LaRoche. Blood platelets were obtained from human, dog and pig sources as platelet-rich plasma (p.r.p.) and prepared for n.m.r. experiments as described elsewhere (Costa *et al.* 1979). Serotonin or difluoroserotonin were introduced into the platelets in p.r.p. by incubation at 37 °C. Treatment of cells with 2-deoxyglucose and antimycin A or with X537A was carried out in some instances in the n.m.r. suspensions. In other experiments, the cells were treated with these components prior to the preparation of the n.m.r. cell suspensions. Typically, the cells were washed with buffer solution (Costa *et al.* 1977) containing 2 mM ethylene diamine tetra-acetic acid (EDTA), and in this buffer treated, at 37 °C, for 5 min, with 25 mM 2-deoxyglucose and 150 μM antimycin A, or with X537A at 50 μM concentration. Cell extracts were prepared by the addition of perchloric acid followed by addition of EDTA, neutralization and centrifugation.

^{19}F n.m.r. spectra at 254 MHz and ^{31}P n.m.r. spectra at 109.3 MHz were recorded by means of a Bruker spectrometer. ^{19}F n.m.r. spectra at 94 MHz and ^{31}P n.m.r. spectra at 40.5 MHz were recorded by means of a Varian XL-100 spectrometer. Spectra were generally recorded, without ^{1}H decoupling, in the Fourier transform mode. Generally 5000, although in certain cases up to 40 000, transients were accumulated for each spectrum. In the diagrams, frequencies increase to the left in the ^{31}P spectra and to the right in the ^{19}F spectra. Unless otherwise specified, cells were maintained at 0 °C and spectra recorded at 4 °C.

Results

Human blood platelets

At 4 °C, ^{19}F n.m.r. spectra of human blood platelets containing 4,6-difluoroserotonin show no resolvable lines at either 254 MHz or 94 MHz. Release of the granular contents to the extracellular medium, either by total cell destruction with perchloric acid or by stimulation

with the ionophore X537A, results in well resolved peaks (figure 1). The ^{19}F n.m.r. signals of 4,6-difluoroserotonin in the intact cells must, therefore, be very broad under the conditions used here (Costa *et al.* 1979). At higher temperatures very broad resonances, shifted slightly from the extracellular positions, could be detected, but leakage of the 4,6-difluoroserotonin was observed. Further studies are required to establish, with certainty, the origin of these broad resonances, which may arise from the granular compartment or from the cytoplasm.

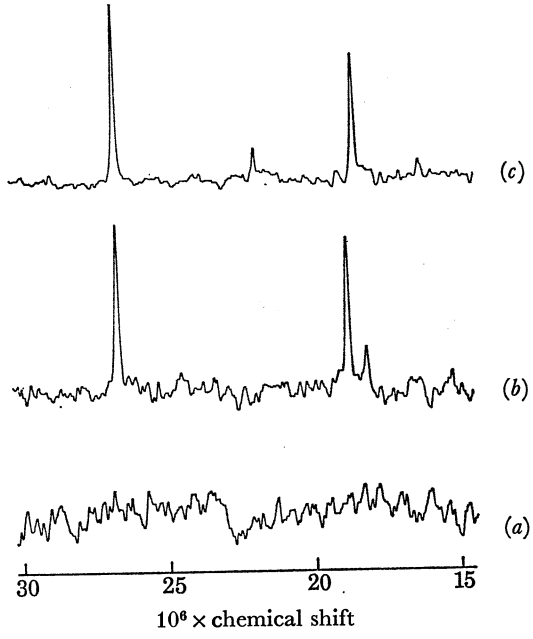

FIGURE 1. 254 MHz ^{19}F n.m.r. spectra at 4 °C of (*a*) intact human blood platelets incubated with difluoroserotonin; (*b*) perchloric acid extract of these cells; (*c*) 1 mM difluoroserotonin in ^2H_2O buffer. The number of accumulations in (*a*) was more than eight times that in (*b*) and (*c*).

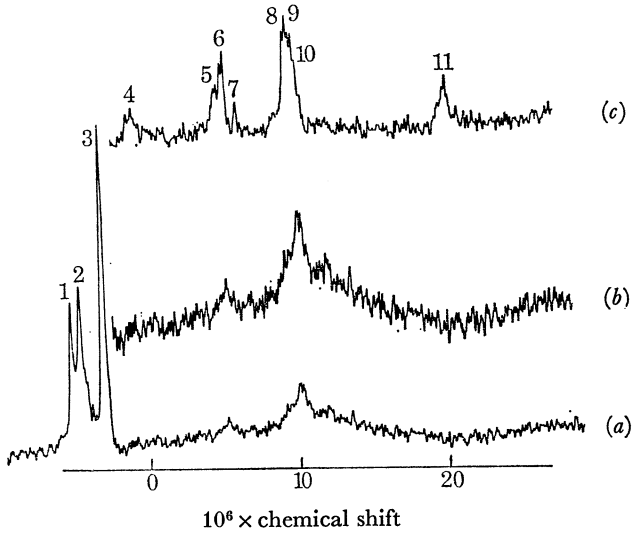

FIGURE 2. 109.3 MHz ^{31}P n.m.r. spectra at 4 °C of (*a*) human blood platelets treated in the n.m.r. suspension for 1 h at 37 °C with antimycin A and 2-deoxyglucose; (*b*) a twofold vertical expansion of part of the same spectrum; (*c*) perchloric acid extract of these cells. Peak assignments: 1 and 2, sugar and nucleotide monophosphates; 3, inorganic phosphate; 4, unassigned; 5, ATP γ-; 6, ADP β-; 7, pyrophosphate; 8, ADP α-; 9, ATP α-; 10, unassigned; 11, ATP β-.

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^{31}P n.m.r. spectra of the intact human platelet cells show signals attributable to ATP and possibly ADP. However, the nucleotides exist in both cytoplasmic and granular compartments and only one set of resonances is observed. Treatment with antimycin A and 2-deoxyglucose causes depletion of the cytoplasmic ATP and ADP (Holmsen *et al.* 1974). A 109.3 MHz ^{31}P n.m.r. spectrum of cells thus treated is shown in figure 2.

Only broad resonances can be observed in this spectrum and no resonance in the position of the β-phosphate of ATP is detectable. In spectra recorded at higher temperatures and at 40.5 MHz no resolved resonances were found that could be attributed to ATP or ADP. The broad resonance in figure 2 arises, in part, from phospholipids of cell membranes. However, an extract of the cells shows that both ADP and ATP were present in the cells. Experiments of this type, and ^{14}C labelling methods, were first able to show that the granular nucleotide resonances were too broad to resolve in the ^{31}P n.m.r. spectrum (Ugurbil *et al.* 1978, 1979).

Normal human platelets do not have their granules saturated with serotonin. However, by treatment of the cells with serotonin it is possible to increase considerably the amount of this substance in the granules (Costa *et al.* 1978*b*). By contrast, careful treatment with the ionophore X537A results in release of all the granular serotonin with only minor reduction in the concentrations of the other granular species (Murer *et al.* 1976; Costa *et al.* 1978*c*). Examination, first at 4 °C and then at higher temperatures, of platelet cells subject to both types of treatment showed no change in ^{31}P n.m.r. spectra, indicating that the nucleotide phosphate peaks are broad whether or not the granules contain serotonin.

Pig blood platelets

The granular composition of blood platelets from different species is known to vary (Pletscher *et al.* 1971; Kinlough-Rathbone *et al.* 1973; Fukami *et al.* 1978), and it is, therefore, of interest to discover whether the n.m.r. spectra differ with species. In fact, in pig platelets, it was found that ^{31}P n.m.r. peaks attributed to granular ATP could be resolved from those of cytoplasmic ATP directly (Johnson *et al.* 1978; Ugurbil *et al.* 1979). We have also observed a similar phenomenon in platelets from dog blood. It was found (Ugurbil *et al.* 1979) that line widths were temperature-dependent and that at low temperatures, even in pig platelet cells, the 145.7 MHz ^{31}P n.m.r. resonances of granular ATP were broad. When recorded at lower magnetic field, the ^{31}P n.m.r. spectra are narrower and, even at 6 °C in pig platelets, two components are apparent in the ATP spectrum (figure 3).

Treatment with antimycin A and 2-deoxyglucose removed one set of resonances, attributed to the cytoplasmic ATP, leaving broad but resolved resonances attributed to granular ATP. Treatment with X537A, by contrast, removed the broad resonances, leaving sharp resonances attributed to cytoplasmic ATP. Poisoning of these cells, or prolonged incubation at 37 °C, resulted in the disappearance of even these resonances. A perchloric acid extract then showed no significant quantity of ATP or ADP remaining in the cell. The ATP resonances of the samples containing only cytoplasmic ATP showed negligible changes in line width with temperature. The ATP resonances of the other sample containing only granular ATP showed a marked and reversible temperature dependence, in accord with the high field results (Ugurbil *et al.* 1979). Prior incubation of cells with serotonin caused no significant change in the ^{31}P resonances of the ATP in the granules.

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Incubation of the pig platelets with difluoroserotonin resulted in no clearly observable ^{19}F n.m.r. resonances at 4 °C at either 254 or 94 MHz, as for human platelets. However, at higher temperature resolved resonances of difluoroserotonin are clearly seen (figure 4).

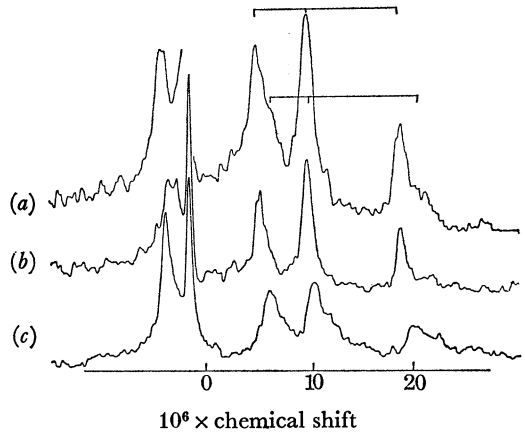

FIGURE 3. 40.5 MHz ^{31}P n.m.r. spectra, at 6 °C, of pig platelets. The n.m.r. samples were prepared after the cells were subjected to the following treatments: (*a*) fresh cells, incubated in buffer with serotonin at 37 °C for 90 min; (*b*) fresh cells incubated at 37 °C in buffer for 90 min, followed by treatment with X537A for 1 min; (*c*) fresh cells incubated as in (*a*) followed by treatment for 5 min with antimycin A and 2-deoxyglucose. The spectrum of fresh cells without treatment is very similar to spectrum (*a*).

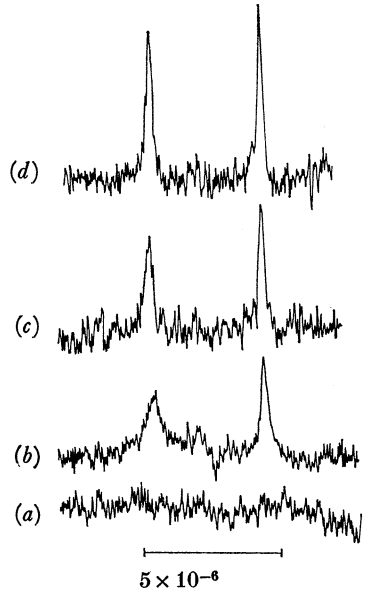

FIGURE 4. 254 MHz ^{19}F n.m.r. spectra of pig blood platelets incubated with difluoroserotonin. The spectra were recorded at (*a*) 4 °C, (*b*) 19 °C, (*c*) 24 °C, (*d*) 29 °C.

The temperature dependence was fully reversible. The line widths at 94 MHz were somewhat narrower than those at 254 MHz and a differential broadening of the resonances of the two fluorines was seen at both frequencies (figure 4). T_1 values, estimated by the inversion-recovery method, were about 0.5 s at 20 °C for the granular difluoroserotonin ^{19}F resonances. Unlike for the human cells, the granular contents leaked extremely slowly even at 37 °C, a

finding confirmed by radioactive labelling experiments. Addition of difluoroserotonin to the extracellular medium, however, showed that chemical shift differences exist between the granular and extracellular difluoroserotonin resonances (figure 5).

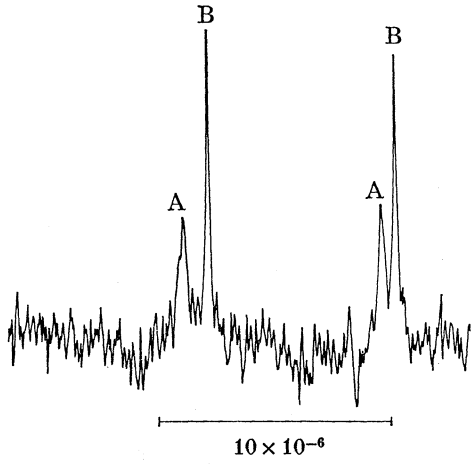

FIGURE 5. 254 MHz ^{19}F n.m.r. spectrum, at 23 °C, of pig blood platelets incubated with a difluoroserotonin and with difluoroserotonin added to the extracellular medium. Peaks A arise from granular, and peaks B from extracellular difluoroserotonin.

DISCUSSION

Mechanism of line broadening

In order to interpret the line widths and line width changes, even qualitatively, it is necessary to understand the relaxation mechanisms involved. In the pig granules, the ^{19}F T_1 values, the relative line widths of the ^{19}F and ^{31}P n.m.r. resonances, and the observed temperature dependences enable us to eliminate paramagnetic ions as a major source of relaxation. In the light of the results with the pig and dog cells it is unlikely that the broadening in the human cells arises from this mechanism. Additionally, the concentrations of paramagnetic ions in the human platelets have been shown (Ugurbil *et al.* 1979) to be insufficient to explain the large line widths. The ^{19}F resonances of the pig platelet granules are somewhat frequency dependent, showing that chemical exchange or chemical shift anisotropy is a relaxation mechanism although not the dominant one here. The ^{1}H–^{19}F dipolar mechanism is therefore likely to be the most important one in this case. The ^{31}P line widths, however, show a marked frequency dependence, as found by comparing spectra recorded at 40.5 MHz with those recorded at 109.3 MHz. This is consistent with chemical shift anisotropy being the dominant relaxation mechanism in this case as, for example, in phospholipid spectra (McLaughlin *et al.* 1975).

The broad line widths in all instances are, therefore, due to restriction of free tumbling in solution. Preliminary calculations show that in pig granules at 10 °C the correlation time for both ATP and difluoroserotonin must be of the same order, about 10^{-7} s.

Storage of serotonin

The extremely broad nature of the ^{31}P n.m.r. spectra of human platelet granules shows that the ATP and ADP have highly restricted motional properties in the intact cells (Ugurbil *et al.*

1978, 1979; Costa *et al.* 1979). Assuming isotropic tumbling, the correlation time for molecular motion cannot be greater than about 10^{-6} s, making reasonable assumptions for chemical shift anisotropy values (Griffin *et al.* 1973). This is also the case for platelets with levels of serotonin either increased or decreased above their natural levels. This immobilization cannot, therefore, be a property due primarily to interaction of these species with serotonin. It is known that divalent metal ions can form aggregates of higher molecular weight with ATP and ADP (Berneis *et al.* 1970; Pletscher *et al.* 1971) and it is, therefore, possible that in the intact human platelet granules such aggregates exist. When the serotonin is taken up by the granules, it too becomes immobilized, as shown by the large ^{19}F line widths of difluoroserotonin, which require a correlation time of less than 10^{-6} s.

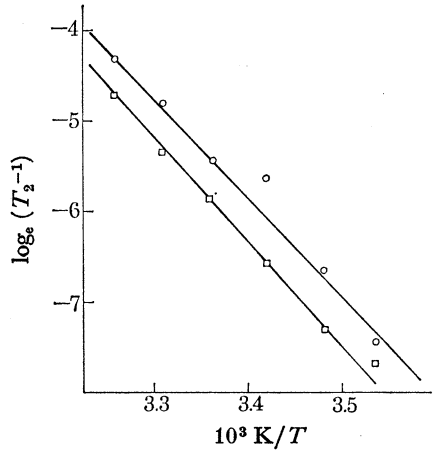

FIGURE 6. Plots of $\log_e(T_2^{-1})$, for the 254 MHz ^{19}F n.m.r. spectrum of difluoroserotonin-incubated pig blood platelets, against the reciprocal of the temperature. The two lines refer to the two ^{19}F resonances from the molecule (see figure 4).

The ATP in pig platelet granules is less immobilized than that in human granules, the apparent correlation time changing dramatically with temperature. This difference from human platelet granules is presumably a result of the change in composition. Pig platelet granules (Kinlough-Rathbone *et al.* 1973) contain predominantly ATP rather than the ADP of human granules, and Mg^{2+} rather than the Ca^{2+} of human granules (Fukami *et al.* 1978). The nature of aggregates of ATP is known to be strongly dependent on the metal ion nature and concentrations, and on temperature (Berneis *et al.* 1970; Pletscher *et al.* 1971; Ugurbil *et al.* 1979). The ^{19}F linewidths of difluoroserotonin in pig platelet granules are well defined over a range of temperature. For slowly tumbling species, $1/T_2$ is directly proportional to the correlation time for molecular tumbling whether relaxation occurs by a dipolar or by a chemical shift anisotropy mechanism (Abragam 1962). T_2 is the spin–spin relaxation time and was estimated from the ^{19}F n.m.r. line widths by subtracting the line widths of extracellular difluoroserotonin to correct for magnetic inhomogeneity in the sample. Plots of log $1/T_2$ against $1/T$ should be linear for simple molecular tumbling, and this is found experimentally as figure 6 shows.

An activation energy of 17 kcal/mol (71 kJ/mol) can be estimated from these data. This activation energy is much higher than expected for an aqueous solution, and suggests that a

gel-like phase with a marked temperature dependence of its motion or state of aggregation exists within the vesicles. The apparent correlation time can be estimated to vary from greater than 10^{-8} s at 37 °C to less than 10^{-6} s at 4 °C.

Conclusions

That immobilization of the contents of platelet granules is associated with complexes involving primarily adenine nucleotides and metal ions is consistent with the observed species variation. It is also consistent with the known relatively unrestricted molecular motion prevailing in chromaffin granules (Daniels *et al.* 1974; Casey *et al.* 1977; Sharp & Richards 1977), which contain ATP and amines but do not contain high concentrations of metal ions. Although the biological significance of the present data will not be fully discussed here, our findings are generally consistent with conclusions drawn from model compound studies (Pletscher *et al.* 1971). It is interesting to note that the apparently more immobilized human granules actually leak their serotonin more rapidly than the more fluid pig granules. Perhaps the serotonin is actually required for the structural integrity of the aggregate existing in the pig platelet, while it appears not be to required in the human platelet.

In certain circumstances, such as that described here, ^{19}F is valuable for studying directly even broad resonances from a defined region of a cell. There is no need to deplete the contents of other compartments, or to isolate specific compartments, to use n.m.r. to provide information about specific molecular structures and dynamics. However, it is necessary to ensure that the biological behaviour of the fluorinated compound is understood.

We should like to thank Professor R. J. P. Williams, F.R.S., for many valuable suggestions, particularly during the early stages of this work. We are also grateful to Dr K. Ugurbil, Dr R. G. Shulman and Dr H. Holmsen for valuable discussions and for providing us with information prior to publication. F.M.P. acknowledges support from the Danish Science Research Council. The high field n.m.r. experiments were performed at the N.M.R. Facility for Biomolecular Research located at the Francis Bitter National Magnet Laboratory, M.I.T. The N.M.R. facility is supported by grant no. RR00995 from the Division of Research Resources of the National Institute of Health, and by the National Science Foundation under Contract C-670. The work was also supported by the U.S. Navy through Naval Medical Research and Development Command Research no. 63706N–M0095 PN001–0040. The opinions and assertions contained herein are those of the authors and are not to be construed to be official or to reflect the views of the Navy Department or Naval Service at large.

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Discussion

S. F. J. Cox (*Rutherford Laboratory, Chilton, Didcot OX*11 0*QX, Oxfordshire*). Sir Rex Richards has asked us, in his opening remarks, to consider the safety of the n.m.r. methods used *in vivo*. The possible risks to health will certainly be discussed tomorrow in the sessions on medical imaging; it seems as important for today's programme to consider whether n.m.r. techniques might in any way perturb or change the metabolic processes or rates under study. There are three main considerations:

1. *Quantum energy*. Unlike X-rays, γ-rays or accelerated particles, the quantum energy of the r.f. radiation presently used, that is of photons up to, say, 600 MHz, is far too low to produce ionization (threshold in far ultraviolet) or point defect molecular damage (threshold in infra-red). So the cumulative low level dose is in no way to be feared as regards single photon events.

2. *Changing magnetic fields*. Since the body is conductive, changing magnetic fields will induce eddy current heating. The obvious danger to avoid is clearly that of 'cooking' the subject with the r.f. field! Irreversible and cumulative effects have been reported even below the thermal threshold of 43–45 °C, however; these are presumably the more direct results of the electric fields and currents induced. In Fourier transform n.m.r., the transmitter power, though instantaneously large, is pulsed with a long duty cycle. The average power density used to monitor an n.m.r. signal is typically very low, much lower, for instance, than that already used in medical treatments such as diathermy. This may not be true, however, of the power levels used continuously in saturation or decoupling experiments, and the risk increases, of

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course, with frequency. Pulsed transmitters for *in vivo* n.m.r. should be failsafe, so that they cannot deliver their maximum output continuously. Safety precautions recommended by the M.R.C. presently relate only to continuous power levels, or power averaged over complete pulsed trains, but claims have been made of hazards associated with the modulation pattern.

Normal rates of introduction of subjects into the 'static' field are quite safe (as indicated, for instance, by the rude health of Bubble Chamber physicists); not so, perhaps, is the rate of collapse of the main field during a 'quench'. Safe limits for pulsed field rise times, particularly as regards the electric fields induced in the heart, nervous system or retina, have yet to be determined.

3. *Static fields.* In any obtainable field, the nuclear polarization, that is the imbalance of the spin up and spin down populations, is exceedingly small, and it is difficult to think of any consequences (other than an enhancement of the n.m.r. signal) even if it were otherwise. This is not true of electronic polarization, that of paramagnetic ions (whose magnetic moment is much larger). Since certain chemical reactions proceed only through a particular spin state, it is possible that they could be forbidden by a sufficiently high field, but, in 10 T, for instance, and at body temperature, paramagnetic polarization is still only at a small percentage. The effects may be measurable, but are unlikely to be dangerous. Other 'electronic' possibilities include semiconductor effects in neurons and changes of electron or proton transfer rates; most worrying, perhaps, is the suggestion that proton tunnelling within the DNA molecule may be so affected as to alter the genetic code.

Then there is the question of physical rearrangement in the field: the distortion of bond angles in paramagnetic molecules, the migration of paramagnetic ions in gradient fields and effects of diffusion, for example. Professor Andrew points out that the main magnetic field can also distort cell membranes across which there is a difference in susceptibility.

The literature is vast and inconclusive. Rate changes in metabolism, as well as irreversible effects, have been demonstrated, but it is likely that they will be small at the fields used for adult whole body imaging, if not for laboratory experiments on small creatures. Western nuclear physics laboratories currently recommend a maximum exposure for short periods (minutes) of 200 mT for the whole body or head and of 2 T for arms and hands, but there is no evidence of serious consequences even where this has been greatly exceeded. Eastern European literature, however, which places a greater emphasis on low level biomagnetism, is generally more cautious in recommending exposure limits. A worrying suggestion is that the effects of r.f. and static fields may not be independent; exposure to one may place an organism in a metastable state rendering it more susceptible to damage by the other.

General reference

S. J. St Lorant 1977 *Biomagnetism.* California: Stanford University.

J. F. Gibson†, R. A. Sheppard†, K. J. Micklem‡ and C. A. Pasternak‡ († *Department of Chemistry, Imperial College, London,* and ‡ *Department of Biochemistry, St George's Hospital Medical School, University of London*). Mn^{2+} as a Ca^{2+} probe: use of water proton spin–lattice relaxation measurements. The movement of Ca^{2+} into and out of Lettree cells is mimicked by Mn^{2+}. When cells are treated with paramyxoviruses such as Sendai virus, the permeability properties of the cell membrane are modified so that more Ca^{2+} (or Mn^{2+}) enters. Water proton spin–lattice relaxation measurements on Lettree cells in the presence of 0.02–2.5 mM Mn^{2+} show

two T_1 values, one from intracellular and one from extracellular water. Cells exposed to virus show three T_1 values at similar Mn^{2+} concentrations; the third T_1 value is from another intracellular water compartment, thus indicating a redistribution of intracellular Mn^{2+}. These results show that virus (i) causes more Mn^{2+} to enter cells and (ii) affects its intracellular distribution.

Such measurements of water proton spin–lattice relaxation times may prove useful for a study of Mn^{2+} (and hence Ca^{2+}) movements in cells treated with hormones, mitogens or neurotransmitters, and provide a useful adjunct to studies of normal and diseased (e.g., virally infected) tissues by n.m.r. imaging (zeugmatography).

A. I. Scott, F.R.S., G. Burton, P. E. Fagerness, P. J. Sidebottom and R. L. Baxter (*Department of Chemistry, Texas A & M University, College Station, Texas* 77843, *U.S.A.*). With use of ^{13}C-enriched substrates, metabolism in the bacteria *Rhodopseudomonas spheroides* and *Propionibacterium shermanii*, grown anaerobically, has been followed by time course n.m.r. at 20 and 75 MHz. In this way, glucose metabolism (to propionate, acetate and succinate) and porphyrinogen biosynthesis from ^{13}C-enriched porphobilinogen has been followed, intra- and extracellularly. The method has been extended to fungi (*penicillia*) in which ^{13}C-enriched acetate is sequentially converted to citrate, gentisyl alcohol and the antibiotic patulin. Extension to many other antibiotic fermentations appears feasible. (See, for example, A. I. Scott, P. E. Fagerness & G. Burton 1979 *J. chem. Soc. chem. Commun.* **202**.)

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