NMR studies of tumours

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$^{31}$P nuclear magnetic resonance (NMR) spectra have been obtained from animal and human tumours grown in laboratory rodents. The tumour cells are only slightly more acid than the surrounding muscle but they tend to have large $\text{Pi}$ and sugar phosphate peaks, suggesting anoxia, and large but variable phosphodiester peaks. The results indicate that NMR will be an important tool for studying tumours in the laboratory, and for their diagnosis, assessment, and monitoring in clinical practice.

One obvious field for the clinical application of nuclear magnetic resonance (NMR) is oncology. There are several reasons for this: (i) Tumours are normally solid masses of abnormal tissue, easy to detect by $^{31}$P NMR when they exceed 1 cm in diameter. In most cases they do not manifest themselves clinically until they reach this size. (ii) NMR spectra of tumours might provide both diagnostic information (e.g. type of tumour, degree of differentiation, and degree of oxygenation) and an index of the effectiveness of therapy. Diagnosis and the choice of therapy depends heavily on the histological examination of biopsies; but tumours are often inaccessible and some can only be approached surgically, using general anaesthesia (Salvadori, 1980). Even when samples can be obtained one cannot take them repeatedly to follow the course of the disease or the efficacy of therapy. Systems for classifying the stage of a cancer or monitoring its growth or regression depend mainly on measurement of its size (Hayward, 1980). The possibility of making repeated, non-invasive 'biopsies' by NMR is therefore attractive. (iii) NMR studies of tumours in laboratory animals could facilitate the design of new therapeutic methods.

At present, NMR instruments are only large enough to study the distal portion of human limbs, where very few tumours arise. We have therefore studied two types of tumour in laboratory animals: the Walker carcinosarcoma in the rat and human xenografts in mice. Some of our findings have been reported in brief (Griffiths et al., 1981; Stevens et al., 1982).
**Materials and Methods**

NMR spectra of tumours were obtained using surface coils (Ackerman et al., 1980) in an Oxford Research Systems (Oxford, U.K.) TMR-32-200 spectrometer at 40 MHz. Experimental details have been published elsewhere (Griffiths et al., 1981; Stevens et al., 1982).

**Results and Discussion**

*Tumour NMR signals*

What can one expect to see in the signal from a tumour? Any tumour sufficiently large to be examined by NMR is likely to contain >10^8 cells and some of these will be ischaemic and hence anoxic. Thus the signals from sugar phosphates and nucleoside monophosphates, and from Pi are likely to be abnormally large. Figs. 1 and 2 show that this is generally the case. The degree of anoxia in a tumour is an important clinical parameter. Radiotherapy is much less effective in anoxic tissues, so the ability to diagnose its presence and perhaps its degree should assist in the choice of therapeutic measures (e.g. radiotherapy vs. chemotherapy) or in the design of radiotherapy regimes.

Peak C in the spectra shown in Figs. 1 and 2 is in the region of the NMR spectrum where phosphodiester compounds give signals. Glycerolphosphorylcholine and its analogues have been found to give such signals in normal tissues, but no assignments of the signals from tumours have been reported. An unexpected finding concerning these peaks in the phosphodiester region of the spectrum was that they were not constant in magnitude. Fig. 3 shows the variation of such a signal in a fibrosarcoma over a period of 2 days. A similar variation has been found in normal tissue of the animal bearing the fibrosarcoma (Stevens et al., 1982). A signal comparable in magnitude to that of ATP (i.e. ca. 10 mM) was observed in the leg of a mouse which had a tumour implanted in the contralateral flank.

Despite these uncertainties, the triad of elevated sugar phosphates, elevated Pi, and the presence of 'phosphodiesters', i.e. peaks A-C, is common to all the tumour spectra and unusual in normal tissues (Iles et al., 1982). No significant phosphocreatine peak was observed in Walker carcinosarcomas (Fig. 1) when the pulse duration of the NMR pulse was appropriately adjusted. Phosphocreatine signals were observed in the human xenograft tumours in mice (Fig. 2), but they may have arisen from the underlying muscle since these small (ca. 1 cm diameter) tumours are not so easy to distinguish as are the larger (ca. 2 cm diameter) Walker tumours in rats. Unfortunately there is no reliable way at present to define the volume of tissue being studied unless it has clearly different spectral characteristics compared with adjacent tissues. T. H. Koeze (private communication) has observed strong phosphocreatine signals in astrocytoma tumours grown in rabbit brains. The presence of phosphocreatine (and, indeed, of other signals) is likely to depend on the characteristics of the cell type from which the tumour originated and on its degree of differentiation.
Fig. 1. $^{31}$p NMR spectrum of two typical Walker carcinosarcoma tumours in rats. The tumours (2 cm in diameter) were grown subcutaneously in the inguinal region of male Wistar rats for 14 days. The rats were anaesthetized with sodium pentobarbitol, and spectra (320 scans) were obtained at 32.5 MHz in an Oxford Research Systems TMR 32-300 spectrometer using a 2-cm surface coil. Peak A contains signals from sugar phosphates, AMP, and IMP; peak B from inorganic phosphate; peak C from phosphodiesters; peak E from the phosphate moiety of ADP and and the phosphate moiety of ATP; peak F from the phosphate moieties of ATP and ADP and from the two phosphate moieties of NAD; and peak G from the phosphate moiety of ATP.

**Intracellular pH of tumours**

Because tumours are known to be abnormally dependent on aerobic glycolysis for energy metabolism (Warburg, 1931; Racker, 1976) it has long been thought that their intracellular pH would be rendered abnormally acid by the lactate produced. Since intracellular pH can be measured by the chemical shift of the Pi peak (Moon & Richards, 1973), this hypothesis can be tested by $^{31}$P NMR. Table 1 shows that the acidification effect in tumours is very slight, if it exists at all. The Walker carcinosarcoma is a notoriously anoxic tumour, and the examples studied were clearly necrotic in their central regions, yet
were only very slightly more acid than skeletal muscle in the same rats (or in control animals without tumours). Although the difference in pH, namely 0.07, is statistically significant, it must be on the borderline of the precision of the technique. The human tumour xenografts had an almost identical mean intracellular pH to the Walker carcinosarcoma, although it was not significantly different from that of mouse muscle. Many of the earlier studies on tumour pH used glass electrodes to measure the pH of interstitial fluid (Gullino et al., 1965; Meyer, 1974) and it may be that this is depressed by H⁺
Fig. 3. Variation in the 'phosphodiester' peak (C) of a human fibrosarcoma xenograft. (a) Fibrosarcoma; (b) same tumour 9 h later; (c) same tumour next day. Conditions and source as in Fig. 2. Note the reduction in peaks A and B (sugar phosphates and Pi) in the latter two spectra, and the transient increase in peak C. There is also a reduction in peak D (phosphocreatine) in the second spectrum.

secreted from the tumour cells. Later work (Dickson & Calderwood, 1979; T. A. Connors, personal communication), using weak acid indicators, showed little evidence of modification of intracellular pH in tumours.

Studies using 2-deoxyglucose-6-phosphate as an additional probe of intracellular pH have suggested the presence of two pH compartments within the tumour cell (Griffiths et al., 1981). The chemical shift of the 2-deoxyglucose-6-phosphate peak suggests that it is in a compartment of pH 6.3, 0.7 pH unit below the cytosolic pH measured from
Table 1. Intracellular pH of tumours and normal muscle

The intracellular pH of the various tumours shown in Figs. 1 and 2 was measured from the chemical shift of the inorganic phosphate peak (Griffiths et al., 1981; Stevens et al., 1982) and compared with that of skeletal muscle.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>pH$_i$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walker carcinoma in rat</td>
<td>7.14 ± 0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>(13 spectra)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal rat muscle</td>
<td>7.21 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>(13 spectra)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Various human tumours</td>
<td></td>
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<tr>
<td>Xenographs in mice</td>
<td>7.15 ± 0.03</td>
<td>0.13</td>
</tr>
<tr>
<td>(9 spectra)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal mouse muscle</td>
<td>7.22 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>(15 spectra)</td>
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the Pi peak. These data require careful interpretation however. The same effect would be produced if the 2-deoxyglucose-6-phosphate were in rapid exchange between cytosol at pH 7.0 and a much more acid compartment, or if the peak assigned to 2-deoxyglucose-6-phosphate were incorrectly assigned. Further work is in progress to resolve these questions.

Conclusions

Studies of tumours in live animals suggest that $^{31}$P NMR may be useful in clinical (and experimental) oncology. The $^{31}$P spectrum is likely to give information on the tumour type and its degree of differentiation. The degree of anoxia in its cells can also be defined, as can intracellular pH. Serial studies by NMR should make it possible to define tumour response to therapy, both for clinical and research purposes.

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References

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