Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 334–338, January 1983
Biochemistry

In vivo ^{31}P NMR study of the metabolism of murine mammary 16/C adenocarcinoma and its response to chemotherapy, x-radiation, and hyperthermia

(phosphate metabolism/tumors/cancer therapy/surface coil NMR/breast cancer)

W. T. EVANOCHKO*, T. C. NG*, M. B. LILLY*†, A. J. LAWSON‡, T. H. CORBETT§, J. R. DURANT*†, AND J. D. GLICKSON*¶

*Comprehensive Cancer Center, and Departments of ¶Biochemistry, †Medicine, and ‡Radiation Oncology, University of Alabama, Birmingham, Alabama 35294; and §Chemotherapy Department, Southern Research Institute, Birmingham, Alabama 35255

Communicated by F. A. Bovey, September 22, 1982

ABSTRACT ^{31}P NMR spectroscopy with surface coils has been used to monitor, *in vivo*, the phosphate metabolism of subcutaneously implanted mammary 16/C adenocarcinoma in C3H/He mice. This model tumor was studied during untreated tumor growth and after treatment with adriamycin, hyperthermia, and x-radiation. The mammary 16/C tumor exhibited a Gompertzian growth pattern. Levels of high-energy phosphate metabolites—phosphocreatine and ATP—decreased with increases in tumor mass. There was a concomitant increase in the level of P_i and a decrease in the apparent pH of the tumor. These spectral changes appear to reflect changes in tumor vascularization that accompany tumor growth, the tumor becoming progressively more hypoxic. Partial response of this tumor to chemotherapy with adriamycin was reflected in a small but measurable increase in the phosphocreatine resonance, a decrease in P_i, and a return of the intratumor pH to neutral. Hyperthermia resulted in progressive conversion of the ^{31}P NMR spectrum to that of a dead tumor (high levels of P_i, small levels of residual sugar phosphates and pyridine dinucleotides, and acidic pH). X-irradiation (14.0 Gy) led to disappearance of the phosphocreatine peak within 15 min of treatment. Subsequently, this resonance grew back beyond its pretreatment level. As the tumor receded, its spectrum reflected the characteristics of aerobically metabolizing tissue (high levels of phosphocreatine and ATP and low levels of P_i and sugar phosphates).

Recent progress in the development of high-resolution NMR methods for examining biological tissues *in vivo* (1–10) points to the possible application of this technique to monitoring tumors in experimental animals and in humans. Previous *in vitro* experiments focused on the diagnostic utility of water relaxation rates (11–14) and on ^{31}P NMR spectra of isolated tumor cells (15–19).

Two laboratories have recently reported *in vivo* ^{31}P NMR studies of subcutaneously implanted tumors in rodents. Using a commercial topical NMR spectrometer, Griffiths *et al.* (20) studied glycolysis in Walker 256 carcinosarcoma in the rat during untreated growth and after challenge with 2-deoxyglucose. We recently reported the construction of a surface coil probe that could be adapted to any wide-bore NMR spectrometer and that was suitable for studies of solid tumors in mice (21). Preliminary data from a number of murine tumor models suggested that ^{31}P NMR spectroscopy can monitor changes in the metabolic states of tumors during untreated growth and can detect the response of these tumors to various therapeutic modalities. Similar data were obtained on human breast, colon and lung tumors implanted in athymic mice (unpublished data).

Here we report a more comprehensive ^{31}P NMR study of a single murine tumor line, mammary 16/C adenocarcinoma, which serves as a model for human breast cancer, the most common fatal malignancy in women. We have examined this tumor during various stages of untreated growth and after treatment with adriamycin, x-radiation, and hyperthermia. Our findings provide a basis for the possible clinical application of ^{31}P NMR as a method for predicting and monitoring tumor response to certain therapeutic modalities.

MATERIALS AND METHODS

NMR Spectra. ^{31}P NMR spectra (80.96 MHz) were recorded on a Bruker CXP-200/300 spectrometer with a probe built by us (21). This probe contained a three-turn surface coil (o.d., 2.0 cm; 16-gauge insulated copper wire) 1.2 cm from the surface of a polyethylene cage in which the anesthesized mouse was contained. The subcutaneously implanted tumor protruded through a hole in the side of the cage adjacent to the surface coil. The following spectral parameters were used: 25 μs (65°) pulse, 4,000 data points, 512 scans, 3.2-s recycle time. The resolution was enhanced by the convolution-difference method (line broadenings, 15 Hz and 400 Hz). The sensitivity (mapped with a sample of phosphoric acid in a 2-mm capillary) decreased monotonically from the center of the surface coil and was negligible beyond about 7 mm along an axis perpendicular to the coil and beyond about 8 mm along an axis in the plane of the coil (22). That only tumor resonances were detected was confirmed by the absence of signals when a normal mouse was placed in the probe.

Chemical shifts are reported relative to the phosphocreatine (*P*Cr) resonance (0.00 ppm at pH ≈ 7.0). When no *P*Cr was detectable, an external capillary of methylene diphosphonate placed on the opposite side of the surface coil served as a chemical shift (and intensity) reference (18.7 ppm) (23). All spectra were recorded without an external lock.

Least-squares fitting of the chemical shifts (δ) of a series of phosphate buffers to the Henderson–Hasselbalch equation yielded

$$\delta = 3.22 + 2.51/(1 + 10^{6.803\text{-pH}}). \quad [1]$$

Eq. 1 was used to determine the apparent pH (24) of the tumor from the chemical shift of the P_i resonance.

Mice. Induction, transplantation, and chemotherapy of mammary 16/C adenocarcinoma have been described (25). In these experiments, fragments (30–50 mg) of the tumor (about

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviation: *P*Cr, phosphocreatine.

10^7 cells) were implanted subcutaneously into 8- to 10-week-old male C3H/He mice (supplied through the Division of Cancer Treatment, National Cancer Institute). The size of the tumor was determined from its dimensions (25) measured with calipers. A minimal tumor mass of about 500 mg was required for spectral measurements with this surface coil. Mice were anesthesized with pentobarbital (60 mg/kg, intraperitoneally) before spectral measurements and before x-ray or hyperthermia treatment. At least five mice were used in the ^{31}P NMR studies of each of the therapeutic modalities and of the untreated tumors.

Chemotherapy. Adriamycin in saline (Adria Pharmaceutical, Columbus, OH) was injected (13 mg/kg) into the tail vein.

Radiation. X-radiation was administered to the tumor by means of a Picker Vanguard x-ray unit operated at 280 kV, 20 mA, 1.33 mm Cu (half-value layer), and a target-to-source distance of 47.5 cm. An Ostalloy shield was used to protect normal tissues. Under these conditions, a dose rate to the tumor of 1.10 Gy/min was routinely achieved. Dosimetry was verified with a Victoreen condenser R chamber and meter (model 570).

Hyperthermia. Radiofrequency (1.5 MHz) hyperthermia was applied by procedures analogous to those recently developed for clinical use (26). A rf signal generator (Wavetek model 3002) with variable frequency (330 KHz–35 MHz) was used to drive an amplifier (ENI model A300). Power was transmitted to the anesthesized mouse via a coaxial cable which terminated in two brass electrodes. These electrodes were used to bracket the tumor and were coupled to it with standard electrode jelly. The electrodes themselves were heated or cooled by water from a circulating constant-temperature pump (Lauda, model K2/R). This improved the homogeneity of heating. For temperature measurement, an electronic thermometer (BAT-8, Bailey Instruments, Saddle Brook, NJ) and a Teflon-sheathed copper/constantan thermocouple (Bailey IT-21) were used. The thermocouple was inserted along a tract made with a 25-gauge needle along the longest axis of the tumor. A small amount of rf current was applied and the thermocouple was positioned at the point of maximum temperature. Power was then adjusted to maintain the thermocouple at the target temperature (±0.1°C). Treatment duration was timed from the moment the target temperature was reached.

RESULTS AND DISCUSSION

Untreated Tumor. Fig. 1 displays ^{31}P NMR spectra of an untreated mammary 16/C adenocarcinoma on days 9, 10, and 11 after tumor implantation. The rapid growth of this tumor (2.0, 3.5, and 5.0 g on these successive days) was reflected in changes in the ^{31}P spectrum and in the apparent intratumor pH (7.4, 7.0, and 6.8, respectively).

The indicated spectral assignments were made by comparison with spectra of various normal tissues (1–10). No distinction could be made between the various nucleoside di- and triphosphates, and these peaks have been labeled as ADP and ATP, respectively. Reduced and oxidized pyridine dinucleotides (collectively referred to as NAD) produced a resonance which overlapped with the ATP α- and ADP α-phosphate peaks. The relative intensities of the three high-field resonances clearly indicate that the concentration of nucleoside triphosphates was much greater than that of nucleoside diphosphates and pyridine dinucleotides. A small *P*Cr peak was observed in the day 9 spectrum but was not observed on subsequent days. Similar spectral changes were previously reported for MOPC 104E myeloma (21) and are attributed to the creatine kinase-mediated phosphorylation of ADP:

$$PCr + ADP + H^+ \rightleftarrows Cr + ATP. \quad [2]$$

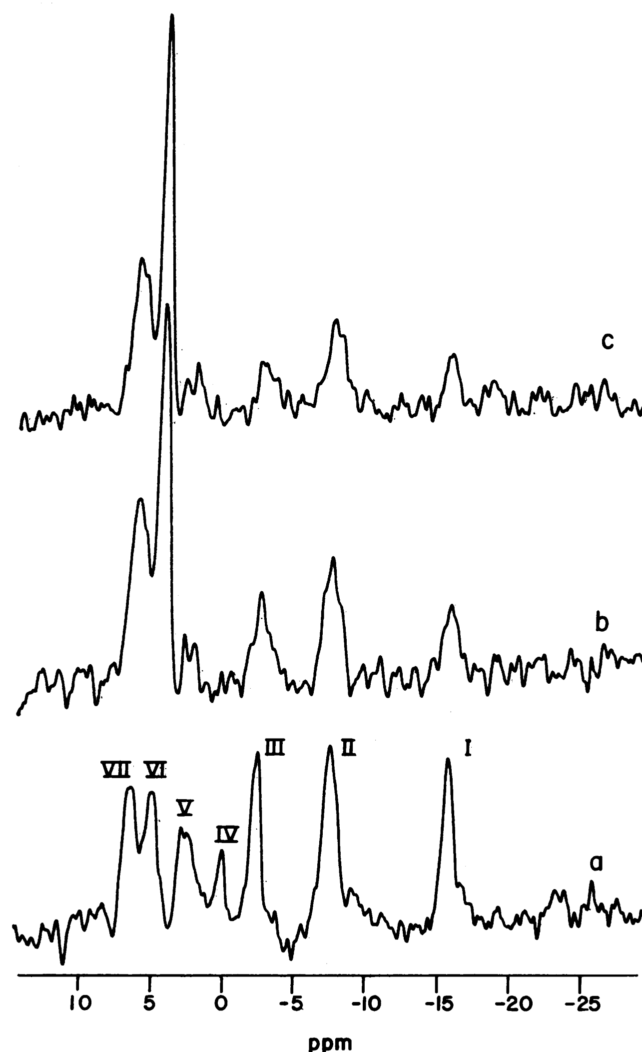

FIG. 1. ^{31}P NMR (80.96 MHz) of a subcutaneously implanted mammary 16/C adenocarcinoma in an anesthesized C3H/He mouse at various times after implantation. Spectra: a, 9 days, 2.0 g, pH = 7.4; b, 10 days, 3.5 g, pH = 7.0; c, 11 days, 5.0 g, pH = 6.8. Peak assignments are: I, ATP_β; II, ATP_α + ADP_α + NAD; III, ATP_γ + ADP_β; IV, *P*Cr; V, unidentified; VI, inorganic phosphate; VII, sugar phosphate.

The initial mammary 16/C spectrum was obtained when the tumor was already hypoxic and the *P*Cr pool was almost depleted. However, although the level of ATP diminished on the subsequent days when *P*Cr was no longer detectable, the ATP concentration of the tumor never decreased below the level of detectability. In fact, even in extremely large tumors (e.g., about 8 g; not shown here) some residual ATP was observed. In contrast, no ATP (or *P*Cr) was detectable in ^{31}P spectra of MOPC 104E myeloma and Dunn osteosarcoma (21) at late stages of tumor growth. We attribute the former observation to the vascularity of the mammary 16/C tumor (25).

The apparent ability of ^{31}P NMR to monitor tumor vascularity noninvasively may prove of clinical significance by predicting responses to specific chemotherapeutic agents, to radiation, and to hyperthermia (27).

Concomitant with the decrease in levels of high-energy phosphates there was an increase in P_i and sugar phosphate. This trend was also observed with the other tumors (21). However, in contrast to MOPC 104E myeloma, the sugar phosphate peak of the mammary 16/C adenocarcinoma was considerably smaller than the P_i peak throughout later stages of tumor growth.

Two unidentified resonances were observed at about 2.0 ppm (Fig. 1). These peaks may originate from phospho*enol*pyruvate, glycerol phosphorylcholine, or other phosphodiesters (28, 29, ‖).

Chemotherapy. Mammary 16/C adenocarcinoma is responsive to adriamycin (25). Small tumors often can be cured by treatment with this agent. However, only a decrease in mass to about 1/3 was induced by adriamycin treatment of the 1.7-g tumor whose spectra appear in Fig. 2.

Two types of spectral changes were observed as the tumor receded. The most obvious change was the expected decrease in signal-to-noise ratio (Fig. 2, compare spectra a and d). A more subtle change in the relative concentrations of certain phosphate metabolites was also detected—PCr increased and P_i decreased. Significantly, these changes were detected within 27 hr of administration of the drug, when there was still no detectable decrease in tumor mass. There was also a significant increase in the pH of the tumor, from 6.8 to 7.4, during this time interval.

These spectral changes are qualitatively similar to but far less dramatic than the changes observed when MOPC 104E myeloma responded to treatment with the alkylating agents cyclophosphamide and 1,3-bis(2-chloroethyl)-1-nitrosourea (21). This may be explained by the fact that adriamycin elicited only a partial response in mammary 16/C adenocarcinoma, whereas treatment of MOPC 104E myeloma with alkylating agents resulted in cures. In the latter study, as in this case, spectral changes were detected before changes in tumor mass. Hence this study of mammary 16/C adenocarcinoma supports our previous conclusions that ^{31}P NMR spectroscopy can detect metabolic changes that precede tumor death and that this technique may serve as an early indicator of tumor response to chemotherapy.

The present study also confirms the previous conclusion that chemotherapy does not result in the accumulation of dead cells in the tumor (21). This would have resulted in an increase in the P_i resonance because dead cells exhibit an intense P_i peak (unpublished data). Rather, it appears that tumor cells are efficiently reabsorbed as they die.

Hyperthermia. Selective susceptibility of certain tumors to this modality has been attributed to the greater heat sensitivity of tumor cells, to the decreased vascularity of tumors (and hence lesser ability to dissipate heat), and to the acidic environment of the tumor (resulting from the accumulation of lactic acid, the end product of anaerobic glycolysis).

Hyperthermia produced the most dramatic spectral changes elicited by any of the therapeutic modalities examined in this study (Fig. 3). Heating this tumor for 15 min at 47°C resulted in only a partial response, which was reflected in a slight decrease in ATP, a slight increase in P_i, and a slight decrease in pH (7.0 to 6.9). A tumor that was heated at the same temperature for 30 min exhibited no residual ATP, a small NAD peak, a very intense P_i resonance, and a small sugar phosphate peak. The pH decreased from 7.2 to 6.8. These are the characteristics of a dead tumor (unpublished data).

We tentatively attribute these spectral changes to the killing of at least 90% of the tumor cells (or perhaps more) which would reduce the number of viable tumor cells below the level of detection by surface coil ^{31}P NMR. In most of the tumors we have examined, reappearance of high-energy phosphates (chiefly ATP), indicative of regrowth of the tumor, did not occur for at least 24 hr after treatment. Fresh tumor growth invariably occurred on the periphery of the tumor where contact with the heating electrodes is expected to have been least efficient. This suggests that, if more efficient methods of selectively heating tumors can be devised, hyperthermia may result in complete destruction of the accessible tumor which could be confirmed by monitoring its ^{31}P spectrum for a prolonged period of time.

‖ Burt, C. T., Pluskal, M., Ribolow, H. & Streter, F. Tenth International Conference on Magnetic Resonance in Biological Systems, Aug. 29–Sept. 3, 1982, Stanford, CA.

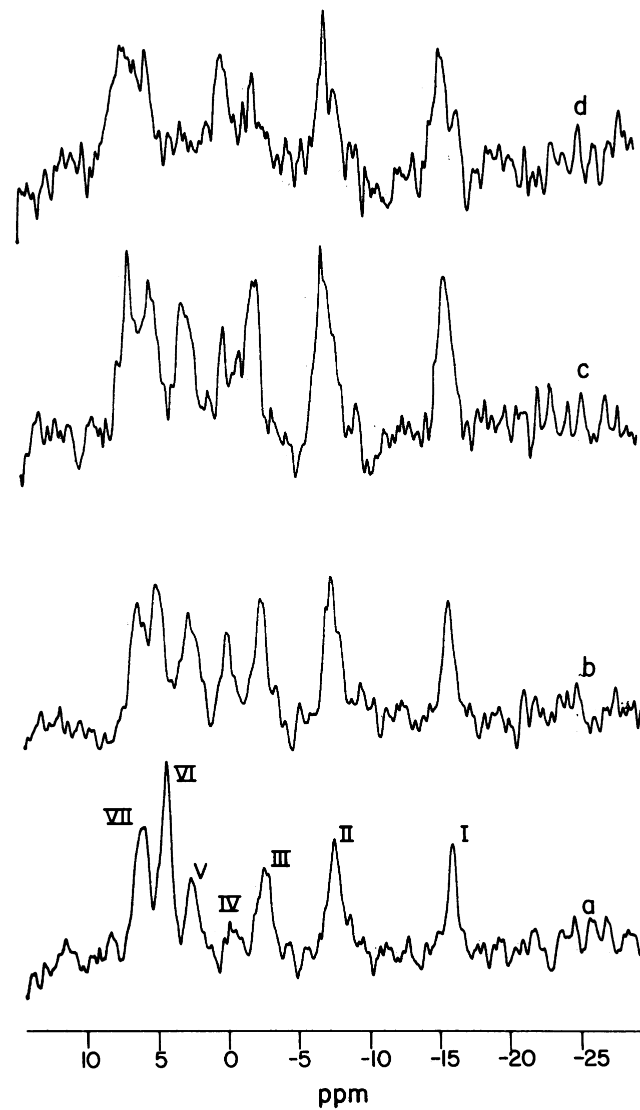

FIG. 2. ^{31}P NMR (80.96 MHz) of a subcutaneously implanted mammary 16/C adenocarcinoma treated with adriamycin (13 mg/kg). Spectra: a, pretreatment, pH = 6.8, 1.7 g; b, 27 hr after treatment, pH = 7.4, 1.7 g; c, 41 hr, pH = 7.4, 1.3 g; d, 72 hr, pH = 7.4, 0.5 g. Spectral assignments are identical to those shown in Fig. 1.

^{31}P NMR appears to be a sensitive noninvasive monitor of tumor response to hyperthermia. This method provides at least a semiquantitative estimate of the extent of tumor response to therapy.

These observations have considerable clinical significance. Administration of hyperthermia therapy is currently performed in an essentially "blind" manner. The patient is treated and sent home, and the extent of response as evident from a decrease in tumor mass is generally evaluated at a subsequent visit, often weeks after therapy. NMR potentially provides a method for assessing the efficacy of hyperthermia treatment immediately after therapy, in time to continue treatment before the patient is sent home.

It is still not clear why hyperthermia, in contrast to chemo-

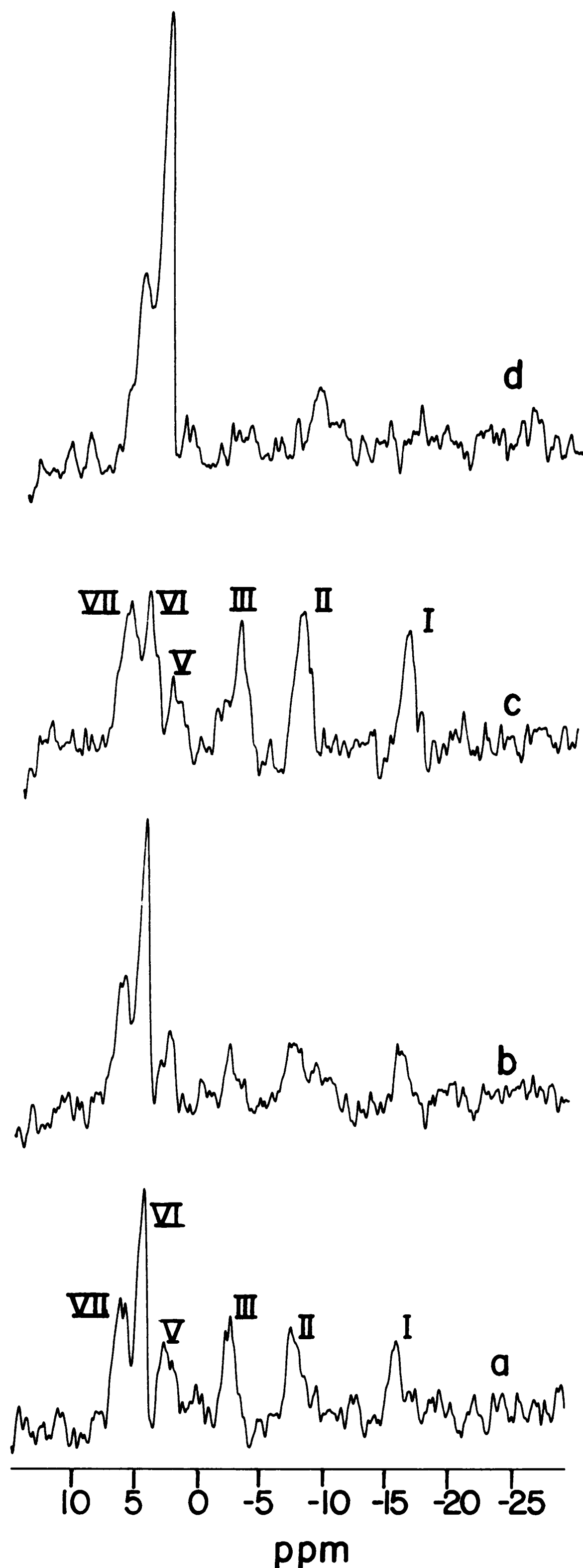

FIG. 3. ^{31}P NMR (80.96 MHz) of a subcutaneously implanted mammary 16/C adenocarcinoma treated with hyperthermia. Spectra: a, pretreatment, pH = 7.0, 1.3 g; b, immediately after treatment (47°C/15 min), pH = 6.9; c, pretreatment, pH = 7.3, 1.1 g; d, immediately after treatment (47°C/30 min), pH = 6.8. Spectral assignments are identical to those shown in Fig. 1.

therapy and x-radiation, results in the accumulation of dead tumor cells. It was previously suggested (21) that this might result from thermal disruption of tumor vascularization. However, heat treatments that are believed to be too mild to produce such an effect (e.g., 40°C for 15 min) produced a detectable increase in the P_i resonance. This suggests that other mechanisms, perhaps involving cellular membranes and tumor vascularization, may also be operative.

X-Radiation. Treatment of mammary 16/C adenocarcinoma with 14.0 Gy resulted in a reduction of the tumor mass to about half the pretreatment level in 1.7 days. Within 15 min of treatment, total disappearance of the *P*Cr resonance was observed (Fig. 4). Nine hours after treatment the *P*Cr resonance was again detected and it eventually grew well beyond its initial relative intensity (to about double the intensity of the ATP peak). As expected, the decrease in tumor mass was accompanied by a decrease in the signal-to-noise ratio of the ^{31}P spectrum (Fig. 2, spectrum e).

Overall, the long-term changes accompanying response of mammary 16/C adenocarcinoma to x-radiation were similar to those exhibited by MOPC 104E myeloma responding to chemotherapy with alkylating agents (21). In both cases the spectral changes appeared to be the inverse of those observed during normal tumor growth—as the tumor receded, its ^{31}P spectrum took on more of the characteristics of tissue performing aerobic metabolism. The tumor *P*Cr resonance disappeared immediately after treatment in each of the five mice that were treated; each of these mice also exhibited the long-term reappearance of this peak, but only in the tumor shown in Fig. 4 did *P*Cr in-

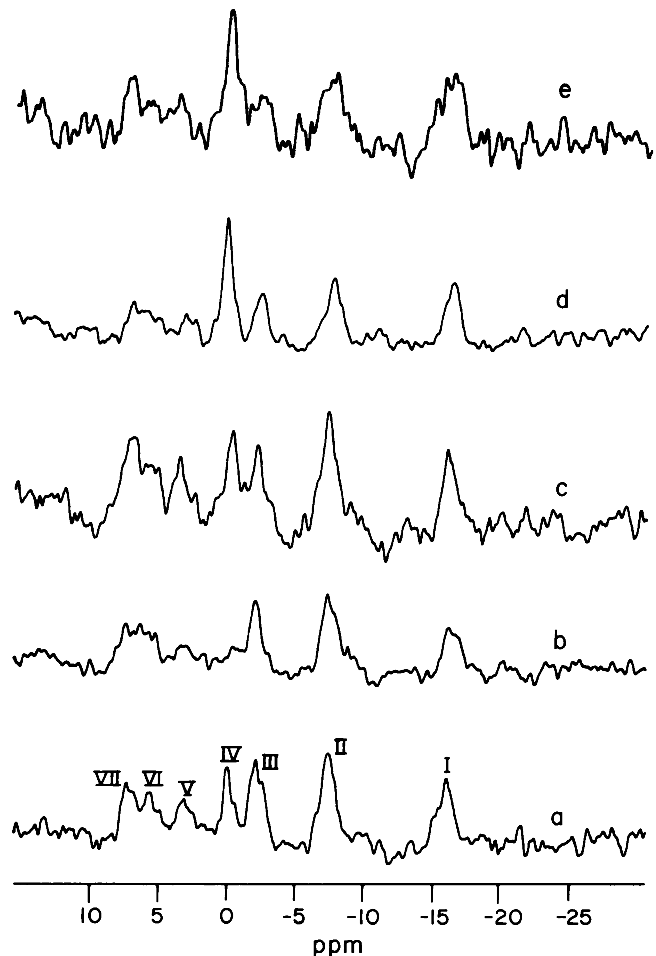

FIG. 4. ^{31}P NMR (80.96 MHz) of a subcutaneously implanted mammary 16/C adenocarcinoma treated with x-radiation (14.0 Gy). Spectra: a, pretreatment; b, 15 min after irradiation; c, 9.0 hr; d, 28.0 hr; e, 40.0 hr. Spectral assignments are identical to those in Fig. 1.

crease beyond the intensity of the ATP resonances.

The initial disappearance of the *P*Cr peak may originate from transient damage to mitochondrial membranes, which would result in a decrease in ATP synthesis. This would be compensated for by the creatine kinase reaction (Eq. 2). Transient damage to blood vessels in the tumor would produce similar effects. The subsequent conversion of the tumor spectrum to one characteristic of a more aerobic state could result from increased availability of oxygen and nutrients to residual tumor cells as the overall tumor mass diminished. Additional experiments are required to test these hypotheses.

CONCLUSIONS

The ^{31}P NMR spectral changes observed during untreated growth of mammary 16/C adenocarcinoma and in response to chemotherapy and hyperthermia are qualitatively similar to those exhibited by other murine tumors (21). Quantitative differences exist between levels of specific metabolites in different tumors before and after treatment with various modalities. These observations on the entire range of spectral characteristics of a tumor before and after therapy with antineoplastic agents, x-radiation, and hyperthermia in a single tumor model, and one that is a model for a prevalent form of human cancer, suggest that NMR (^{31}P as well as other nuclei) may play an important role in the monitoring of human cancer and in the experimental study of this disease.

The greatest potential of this technique appears to be in permitting noninvasive monitoring of the response of tumors to various therapeutic modalities. In the cases studied to date, chemotherapy, x-radiation, and hyperthermia elicit distinct ^{31}P NMR spectral changes that can be detected earlier and more conveniently by this method than by other methods such as biopsy and measurement of tumor mass.

The potential value of this technique in the clinical diagnosis of human cancer is still unclear. It is noteworthy that, in preliminary studies of human adenocarcinoma implanted in athymic mice, the spectral characteristics of this tumor are similar to those of the mammary 16/C model (unpublished data). The metabolic characteristics of tumors, particularly in later stages of growth when the tumor becomes hypoxic, may serve to distinguish neoplastic tissues from surrounding aerobic tissues. However, it remains to be determined if the spectral characteristics of malignant tumors differ from those of benign tumors or from those of nonmalignant abscesses. This will have to be considered for each tumor type and for its benign counterparts. In the case of breast cancer, the relevant control is benign fibrocystic disease, for which there is no suitable animal model. Examination of biopsy samples may be useful if deterioration of the tissue can be minimized. However, the definitive evaluation of the diagnostic utility of NMR for human breast cancer awaits the development of instruments that can directly monitor the spectrum of breast tissue *in situ*.

The assistance of Dr. N. G. Kumar is gratefully acknowledged. This research was supported by Public Health Service Grant CA-13148, Contract NO1-CM-97309, and Fellowship F32 CA-06703 and by Faculty Research Award FRA-162 from the American Cancer Society.

1. Chance, B., Nakase, Y., Bonel, M., Leigh, J. S., Jr., & Radda, G. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 4925–4929.
2. Chance, B., Eleff, S. & Leigh, J. S., Jr. (1980) *Proc. Natl. Acad. Sci. USA* **75**, 7430–7434.
3. Chance, B., Radda, G., Seeley, P. J., Silver, I., Nakase, Y., Bond, M. & McDonald, G. (1979) in *NMR in Biochemistry*, Mildred Cohen Symposium, eds. Opella, S. & Lee, P. (Dekker, New York), pp. 269–281.
4. Ackerman, J. J. H., Grove, T. H., Wong, G. G., Gadian, D. G. & Radda, G. K. (1980) *Nature (London)* **283**, 167–170.
5. Gadian, D. G. & Radda, G. K. (1981) *Annu. Rev. Biochem.* **50**, 69–83.
6. Gadian, D. G., Radda, G. K., Richards, R. E. & Seeley, P. J. (1979) in *Biological Applications of Magnetic Resonance*, ed. Shulman, R. G. (Academic, New York), pp. 463–535.
7. Gadian, D. G. (1982) in *Nuclear Magnetic Resonance and its Applications to Living Systems* (Clarendon, London).
8. Balaban, R. S., Gadian, D. G. & Radda, G. K. (1981) *Kidney Int.* **20**, 575–579.
9. Radda, G. K., Chan, L., Bore, P. B., Gadian, D. G., Ross, B. D., Styles, P. & Taylor, D. (1982) in *NMR Imaging*, eds. Witcofski, R. L., Karstaedt, N. & Partain, C. L. (Bowman Gray School of Medicine, Winston-Salem, NC), pp. 159–169.
10. Grove, T. H., Ackerman, J. J. H., Radda, G. K. & Bore, P. J. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 299–302.
11. Damadian, R. (1971) *Science* **171**, 1151–1153.
12. Hazelwood, C. F., Chang, D. C., Medina, D., Cleveland, G. & Nicols, B. L. (1972) *Proc. Natl. Acad. Sci. USA* **96**, 1478–1480.
13. Weisman, I. D., Bennet, L. H., Maxwell, L. R., Woods, M. W. & Burk, D. (1972) *Science* **178**, 1288–1290.
14. Hollis, D. P., Economou, J. S., Parks, L. C., Eggleston, J. C., Saryon, L. A. & Czeisler, J. L. (1973) *Cancer Res.* **33**, 2156–2160.
15. Evans, F. E. & Kaplan, N. O. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4909–4913.
16. Navon, G., Navon, R., Shulman, R. G. & Yamane, T. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 891–895.
17. Navon, G., Ogawa, S., Shulman, R. G. & Yamane, T. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 87–91.
18. Gupta, R. K. & Yushok, W. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2487–2491.
19. Yushok, W. D. & Gupta, R. K. (1980) *Biochem. Biophys. Res. Commun.* **95**, 73–81.
20. Griffiths, J. R., Stevens, A. N., Iles, R. A., Gordon, R. E. & Shaw, D. (1981) *Biosci. Rep.* **1**, 319–325.
21. Ng, T. C., Evanochko, W. T., Hiramoto, R. N., Ghanta, V. K., Lilly, M. B., Lawson, A. J., Corbett, T. H., Durant, J. R. & Glickson, J. D. (1982) *J. Magn. Reson.* **49**, 271–286.
22. Ng, T. C., Evanochko, W. T., & Glickson, J. D. (1982) *J. Magn. Reson.* **49**, 526–529.
23. Burt, C. T., Glonek, T. & Barany, M. (1976) *J. Biol. Chem.* **251**, 2584–2591.
24. Roberts, J. K. M., Wade-Jardetzky, N. & Jardetzky, O. (1981) *Biochemistry* **20**, 5389–5394.
25. Corbett, T. H., Griswold, D. P., Jr., Roberts, B. J., Peckham, J. C. & Schabel, F. M., Jr. (1978) *Cancer Treat. Rep.* **62**, 1471–1488.
26. Brezovich, I. A., Lilly, M. B., Durant, J. R. & Richards, D. B. (1981) *Int. J. Radiat. Oncol. Biol. Phys.* **7**, 423–430.
27. Teicher, B. A., Lazo, J. S. & Sartorelli, A. C. (1981) *Cancer Res.* **41**, 73–81.
28. Burt, C. T., Cohen, S. M. & Barany, M. (1979) *Annu. Rev. Biophys. Bioeng.* **8**, 1–25.
29. Brady, T. J., Burt, C. T., Goldman, M. R., Pykett, I. L., Buonanno, F. S., Kistler, J. P., Newhouse, J. H., Hinshaw, W. S. & Pohost, G. M. (1982) *NMR Imaging*, eds. Witcofski, R. K., Karstaedt, N. & Partain, C. L. (Bowman Gray School of Medicine, Winston-Salem, NC), pp. 175–180.