RADIATION RESEARCH **155**, 837–846 (2001)
0033-7587/01 $5.00

Measurement of Tumor Oxygenation: *In Vivo* Comparison of a Luminescence Fiber-optic Sensor and a Polarographic Electrode in the P22 Tumor

Beatrice M. Seddon,[a] Davina J. Honess,[b] Borivoj Vojnovic,[b] Gillian M. Tozer[a] and Paul Workman[a,1]

[a] *CRC Centre for Cancer Therapeutics, Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey, SM2 5PT, United Kingdom; and* [b] *Gray Laboratory Cancer Research Trust, P.O. Box 100, Mount Vernon Hospital, Northwood, Middlesex, HA6 2JR, United Kingdom*

Seddon, B. M., Honess, D. J., Vojnovic, B., Tozer, G. M. and Workman, P. Measurement of Tumor Oxygenation: *In Vivo* Comparison of a Luminescence Fiber-optic Sensor and a Polarographic Electrode in the P22 Tumor. *Radiat. Res.* 155, 835–844 (2001).

Hypoxia is important in tumor biology and therapy. This study compared the novel luminescence fiber-optic OxyLite sensor with the Eppendorf polarographic electrode in measuring tumor oxygenation. Using the relatively well-oxygenated P22 tumor, oxygen measurements were made with both instruments in the same individual tumors. In 24 air-breathing animals, pooled electrode pO_2 readings lay in a range over twice that of sensor $pO_{2(5min)}$ values (–3.2 to 80 mmHg and –0.1 to 34.8 mmHg, respectively). However, there was no significant difference between the means ± 2 SE of the median pO_2 values recorded by each instrument (11.0 ± 3.3 and 8.1 ± 1.9 mmHg, for the electrode and sensor respectively, $P = 0.07$). In a group of 12 animals treated with carbon monoxide inhalation to induce tumor hypoxia, there was a small but significant difference between the means ± 2 SE of the median pO_2 values reported by the electrode and sensor (1.7 ± 0.9 and 2.9 ± 0.7 mmHg, respectively, $P = 0.009$). A variable degree of disparity was seen on comparison of pairs of median pO_2 values from individual tumors in both air-breathing and carbon monoxide-breathing animals. Despite the differences between the sets of readings made with each instrument from individual tumors, we have shown that the two instruments provide comparable assessments of tumor oxygenation in groups of tumors, over the range of median pO_2 values of 0.6 to 28.1 mmHg.

INTRODUCTION

Tumor hypoxia is of considerable current interest: It is associated with radiation and drug resistance, and it predicts for poor outcome in clinical studies of tumor treatment. With the growing understanding of its molecular basis, hypoxia is now emerging as a driving force in genetic instability, tumor progression and angiogenesis (*1*). The ability to determine the degree and extent of tumor hypoxia is therefore important prognostically and in the selection of patients for hypoxia-modifying or hypoxia-dependent therapeutic strategies, as well as for novel approaches such as hypoxia-activated gene therapy (*1*).

A widely used and generally accepted method for measuring tumor oxygenation involves a commercially available polarographic electrode. The use of this system has generated a body of evidence indicating that direct pO_2 measurements in human tumors can predict the response to, and outcome of, therapy. Clinical studies in tumor sites including cervix, head and neck, and sarcoma have correlated low tumor oxygenation with a reduction in local control after radiotherapy (*2, 3*), disease-specific survival (*4–6*), and overall survival (*5*). Additionally, tumor hypoxia is related to the presence of metastatic disease at the time of the primary diagnosis (*7*), and predicts for the subsequent development of distant metastases (*2*).

The polarographic electrode consists of a 17-μm gold cathode, encased in a steel needle, and a silver-silver chloride anode. It operates on the principle of electrochemical reduction of oxygen at the cathode, where the current is proportional to the partial pressure of oxygen. The signal-to-noise ratio, and hence the measurement accuracy, is therefore greatest at high pO_2 and falls with decreasing pO_2. After its insertion into the tumor, serial stepping of the electrode through tissue allows multiple sequential oxygen measurements to be made at 1.4-s intervals (*8*). A limitation of the polarographic probe is that the electrochemical reaction occurring at the electrode tip results in oxygen consumption. This may result in an underestimation of the pO_2 measurement, especially at lower oxygen tensions.

A promising new device, the OxyLite fiber-optic oxygen sensor (Oxford Optronix, Oxford, UK) (*9–11*), has recently become available commercially and is now under evaluation. It consists of a ruthenium luminophore incorporated into a silicone rubber polymer at the tip of a fiber-optic probe. After pulsatile excitation from a solid-state blue light

[1] Author to whom correspondence should be addressed at CRC Centre for Cancer Therapeutics, Block E, Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey, SM2 5NG, United Kingdom.

source transmitted by the optical fiber, ruthenium luminescence is detected by a photomultiplier tube. The luminescence lifetime, in the microsecond region, is quenched in the presence of oxygen; it is therefore inversely proportional to the oxygen tension at the probe tip (*11*). Consequently, and in contrast to the polarographic electrode, the signal-to-noise ratio increases with decreasing pO_2. This fact, in addition to the lack of oxygen consumption in the process of the pO_2 measurement, makes the sensor potentially more accurate than the polarographic electrode at low pO_2. It offers further advantages over the polarographic electrode in that it allows oxygen measurements to be made in real time and is compatible with magnetic resonance imaging and spectroscopy systems.

At present, few data are available using the OxyLite system in either animals or humans. Results of one study with the prototype device have been published (*9*); this study compared the fiber-optic sensor with the polarographic electrode in the SaF tumor in mice. The results observed with the two systems were highly comparable, although the fiber-optic sensor gave slightly higher pO_2 values than the polarographic electrode.

Because of the potential utility of the commercially available Oxylite sensor in the clinic, it is important to evaluate it in comparison with the Eppendorf polarographic electrode. We have developed a novel protocol for making a set of pO_2 measurements in a tumor using the fiber-optic sensor, and have compared these measurements to those made by the polarographic electrode as conventionally used by investigators in the field. Using a rodent tumor system, oxygen measurements have been made using both instruments in the same tumor in air-breathing animals. Comparison has also been made in tumors made more hypoxic by inhalation of carbon monoxide to determine whether the two methods of oxygen measurement are equivalent at lower pO_2 values. Measurement in the same tumor is important in view of previous studies showing marked heterogeneity between individual tumors of the same implant (*12, 13*). The P22 carcinosarcoma tumor model has been chosen for this study because it is relatively well oxygenated based on its pO_2 profile, which is close to that reported most frequently for many human tumors; other rodent tumors and human tumor xenografts are considerably more hypoxic (*2, 5, 6, 14*).

MATERIALS AND METHODS

Animals and Tumors

The P22 rat carcinosarcoma arose in the treated site of a male BD9 rat after spinal cord irradiation in the cervical region (*15*). In the present study, the tumor was implanted and grown subcutaneously as a xenograft in the mid or lower back of SCID (severe combined immune deficiency) mice. It was maintained by serial transplantation, using enzymatic disaggregation, for up to 12 passages, followed by a return to definitive frozen stock. Tumors were typically treated at a mean ($\pm$2 SE) diameter of 8.8 $\pm$ 0.2 mm including skin thickness. Animals were restrained unanesthetized within Lucite jigs equipped with nose cones for carbon monoxide inhalation when appropriate. Experimental protocols were approved by the Gray Laboratory Cancer Research Trust Ethical Review Committee and the Institute of Cancer Research Ethics Committee. All mice were housed according to national and local codes of practice for the housing and care of animals used in the scientific procedures, including the UKCCCR guidelines for the welfare of animals in experimental neoplasia (*16*).

Dimensions of the Probe

Photomicrographs of the fiber-optic sensor and polarographic electrode were taken using a conventional video camera (JVC KY-F55B, Japan) equipped with a variable-zoom close-focus lens (Navitar TenX, Rochester, NY, equipped with an epi-illumination adapter). Measurements of the dimensions of the probes were made with reference to a calibrated graticule. The number of cells in immediate contact with the fiber-optic sensor probe tip was calculated using independent measurements of cell dimensions obtained from histological sections using conventional light microscopy. The number of cells ''sensed'' by the polarographic electrode cathode was estimated with reference to a calculation made for an earlier 12-μm version of the polarographic electrode (*17*).

Fiber-optic Sensor Measurements

The OxyLite 4000 four-channel fiber-optic oxygen sensor and OxyData data processing unit (Oxford Optronix, Oxford, UK) were used. The probes were supplied precalibrated by Oxford Optronix. During this calibration, the probes are placed in a temperature-controlled water bath containing physiological saline maintained at 37.0 $\pm$ 0.2°C. N_2 containing 0, 0.5, 1, 2, 5 and 10% O_2 is bubbled through the saline for 2 min at each concentration. Correction is made for atmospheric pressure. The initial calibration is checked at 7 days to assess the stability of the probe, and recalibration is performed within 3 days of shipment. The calibration specification is $\pm$0.7 mmHg or $<\pm$10% of actual pO_2, whichever is greater. Probes measuring oxygen and temperature simultaneously, allowing correction of individual oxygen readings for variations of tumor tissue temperature, were used over a period no longer than 10 weeks (mean 4.2 weeks) after the initial use. Measurements of temperature were made by a fine thermocouple wound loosely around the optic fiber. The pO_2 readings were recorded every 2 s and were displayed by the integral software as a time trace.

Four probes were used simultaneously and were relocated twice to generate 12 traces, each from a different area of the tumor. Placements of the individual probes into the tumor were made through a fresh skin puncture made with a 21-gauge needle. After insertion, the probe tip was retracted by 1 mm, carefully avoiding bending, before it was secured firmly. This minimized the effects of pressure on the probe tip, which can cause spuriously low pO_2 readings (*18*). For each set of 12 traces, the position and depth of the probes were varied to ensure adequate tumor sampling, with some tangential placements to include the tumor periphery. Placement of each probe was recorded pictorially, and the depth of the probe was measured. Each set of four probes was left *in situ* for at least 20 min of uninterrupted recording of pO_2. This procedure was then repeated twice. Acquisition of a complete set of traces took approximately 70 min.

Analysis of Fiber-optic Sensor Data

The fiber-optic sensor produces a pO_2 value by averaging 5 consecutive readings taken over an 8-s period. A new average value is produced every 2 s using a running average algorithm. The resulting time traces were analyzed by taking a mean of the values in 5 min of stable trace ($pO_{2(5min)}$). Each 5-min period therefore comprises 150 individual pO_2 readings. The 5-min period was chosen to be consistent with that used for the *in vivo* measurements with the prototype instrument (*9*). The $pO_{2(5min)}$ values of each tumor were expressed as the median and as the percentage of values $<$2.5, 5 and 10 mmHg.

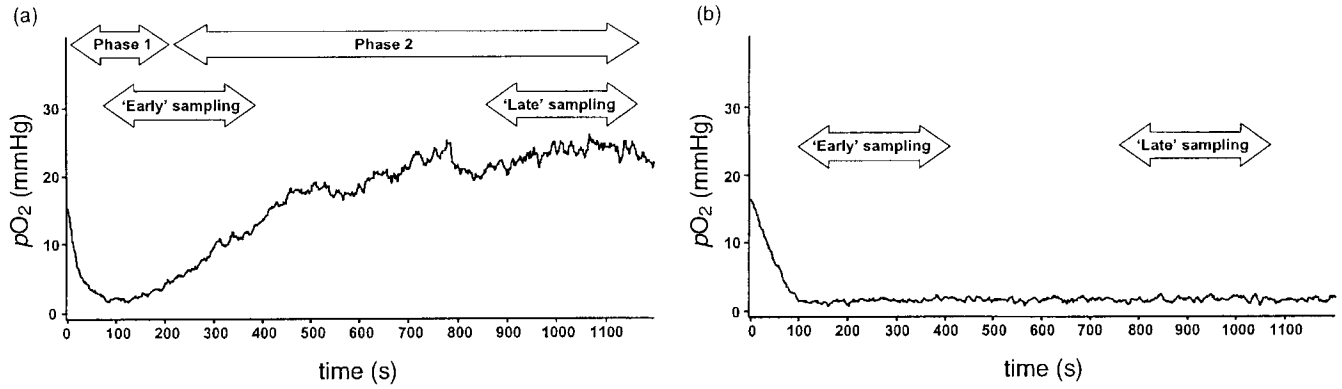

FIG. 1. Fiber-optic sensor pO_2 traces showing (panel a) a rapid fall to a nadir (phase 1) followed by a gradual increase to a second higher pO_2 (phase 2), a two-phase phenomenon, and (panel b) a rapid fall to a nadir which is then maintained. Timing of early and late sampling used to compare pO_2 at the nadir and after at least 10 min of pO_2 recording.

Observed temporal variations in pO_2 prompted an investigation of the time required for the pO_2 to stabilize after placement of the probe. All individual pO_2 traces for the first 12 mice were sampled twice: the first 5 min of trace after a rapid fall to a nadir (''early'' $pO_{2(5min)}$; see Results), and the first 5 min of flat trace encountered after the probes had been *in situ* for at least 10 min (''late'' $pO_{2(5min)}$) (Fig. 1).

Eppendorf Histograph Measurements

The pO_2 measurements were performed using a fine-needle oxygen polarographic electrode (KIMOC-6550, Eppendorf, Hamburg, Germany). Except where stated, the polarographic measurements were made immediately after the fiber-optic measurements. Between six and nine discrete tracks were made in each tumor, with each track at a different angle. For each track, the electrode was inserted to a depth of at least 1 mm, and pO_2 readings were allowed to stabilize; the electrode was then advanced automatically through the tissue in forward (0.9-mm) increments followed by backward (0.3-mm) steps before a pO_2 reading was recorded. Approximately 90 readings (range: 45–105) were taken within each tumor. After final removal of the electrode, a thermocouple was inserted to measure the temperature of the tumor center to permit temperature correction. Calibration of the probe was carried out before and after *in situ* measurements, and was repeated at least every 90 min; this is a two-point linear calibration using oxygen-free N_2 (British Oxygen Company, UK) and air (assumed to be 20.9% O_2), corrected for atmospheric pressure. Any tracks containing more than one pO_2 value calculated as less than -2 mmHg were excluded from analysis because of the possibility of a pressure artifact producing spuriously low pO_2 readings. Results were expressed as the median pO_2 and as the percentage of pO_2 values <2.5, 5 or 10 mmHg, as for the results with the fiber-optic sensor.

Effect of Carbon Monoxide

Carbon monoxide (689–707 ppm in air; British Oxygen Company, UK) was used to modulate tumor oxygenation through the effects of carboxyhemoglobin and perfusion (*19*). The gas was administered by placing the animal in a jig that was flushed at a rate of 1 liter per minute, equivalent to 25 to 30 gas exchanges per minute. The induction and maintenance of hypoxia by carbon monoxide were evaluated in individual tumors using polarographic measurements during gas inhalation for up to 90 min.

Statistical Analysis

Means of median pO_2 values and percentages of values <2.5, 5 and 10 mmHg were compared between two groups of mice using an unpaired two-tailed Student's *t* test, and within the same mouse (comparing the two different methods of pO_2 measurement) using a paired two-tailed Student's *t* test. Means of median pO_2 values and percentages of values <2.5, 5 and 10 mmHg were compared between multiple groups of mice using a standard analysis of variance followed by the Tukey-Kramer honest significance difference test. Correlations were tested by Spearman rank analysis.

RESULTS

Dimensions of the Probes

Figure 2 shows magnified images of the tips of the fiber-optic sensor and the polarographic electrode, with measured shaft diameters of 220 μm and 300 μm, respectively. Previous calculations of the effective volume sensed by a 12-μm electrode estimated this to be 50–100 cells (*17*). The 17-μm electrode currently available senses a roughly 3-fold larger effective volume of 150–300 cells (factor calculated using the volume of a hemisphere, $2/3 \times \pi \times \text{radius}^3$). The number of cells in direct contact with the tip of the fiber-optic sensor probe is approximately 350 in a single layer (calculated using the surface area of a hemisphere, $2 \times \pi \times \text{radius}^2$; measured cell diameter 16.5 μm, cross-sectional area of cell assumed to be $\pi \times \text{radius}^2$). It is estimated that the sensor tip equilibrates with oxygen from at least three cell layers; therefore, the number of cells sensed must be approximately 1350. This gives a ratio for cells sensed by the sensor to the electrode of at least 4.5 to 1.

Optimization of Fiber-optic Sensor Trace Analysis

For the first 12 mice, all 144 pO_2 traces were examined in detail. In 83 cases, the trace initially fell rapidly to a nadir, followed by a slower increase to a second higher pO_2 value (a two-phase phenomenon; Fig. 1a). In 61 cases, the trace fell rapidly to an early nadir that was maintained (Fig. 1b). These two distinct trace types were sometimes observed at different locations in the same tumor. This two-phase phenomenon was investigated by sampling each trace twice, to produce early and late $pO_{2(5min)}$ values (Fig. 1). For each of the 12 animals, the median of the early $pO_{2(5min)}$ values was consistently lower than the median of the late

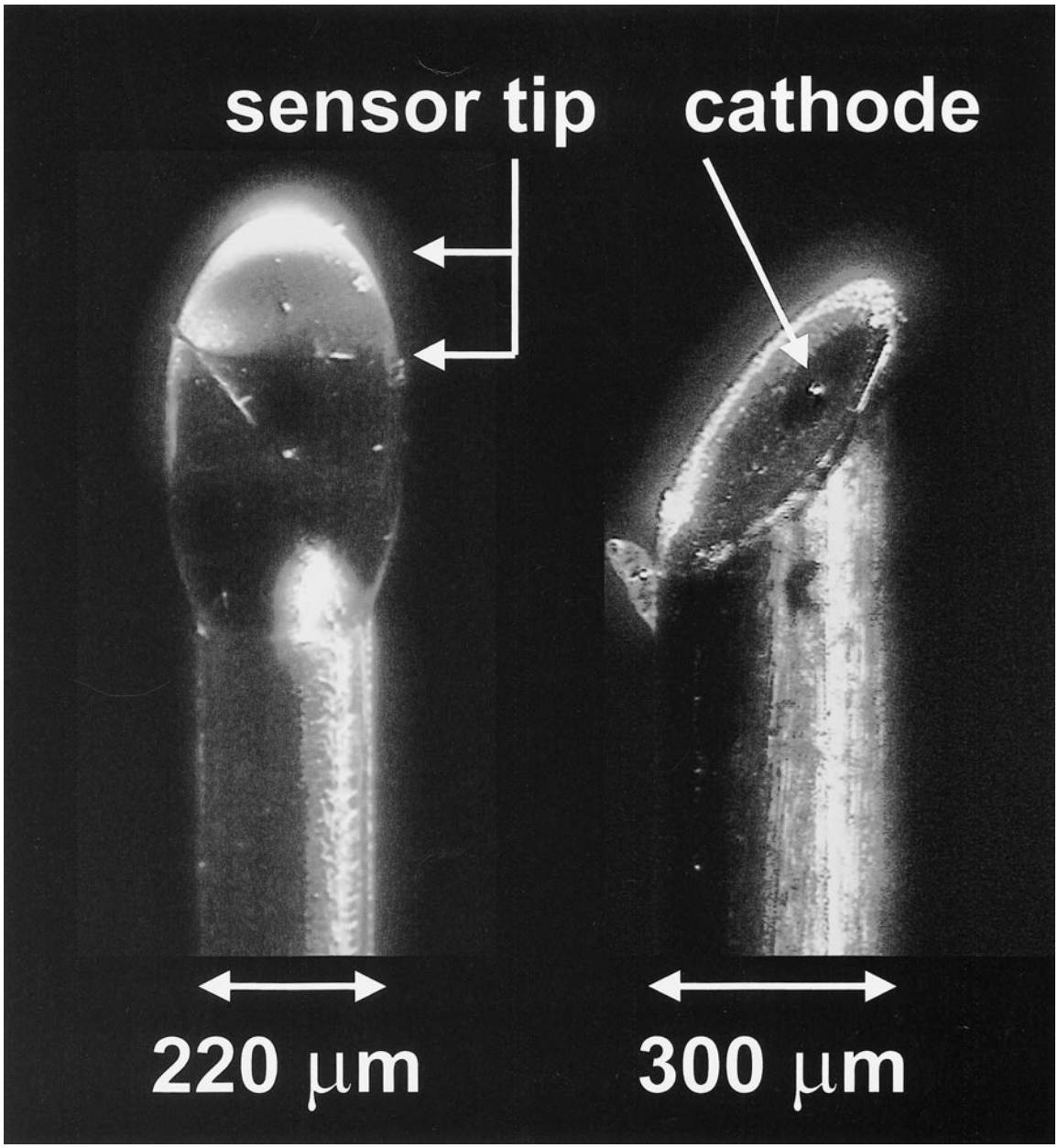

FIG. 2. Comparative magnified images of the fiber-optic sensor and polarographic electrode probe tips.

$pO_{2(5min)}$ values. Moreover, the mean ± 2 SE of the medians of the early values (4.8 ± 1.9 mmHg) was significantly lower than that for the medians of the late values (9.7 ± 3.5 mmHg) ($P = 0.00007$, paired t test). The two-phase phenomenon was uncommon at low $pO_{2(5min)}$ values (0% of traces in which the late $pO_{2(5min)}$ was ≤2.5 mmHg), but was encountered frequently at higher $pO_{2(5min)}$ values (81% of traces in which the late $pO_{2(5min)}$ was >2.5 mmHg).

When two phases were observed in a trace, it was not immediately apparent which value represented the true tumor pO_2 most accurately. The early transient nadir might result from a constrictive tissue reaction to trauma induced by placement of the probe, and it therefore would not reflect the tumor pO_2 status prior to measurement. If so, recovery from this trauma should allow a return to the tumor pO_2 prior to placement of the probe, which would explain the observed rise to a second higher pO_2 level that was then maintained. Thus sampling of the second phase would provide a pO_2 estimate that is potentially more representative, and also more stable and consistent, than that for the first phase. For these reasons, subsequent data analysis was performed using ''late'' sampling.

Dependence of $pO_{2(5min)}$ Values on Probe Depth

The depth of each probe in a tumor was recorded for all measurements made in the first 12 animals, allowing each $pO_{2(5min)}$ value to be related to probe depth. The depths re-

TABLE 1
Comparison of pO_2 Measured by the Fiber-optic Sensor and Polarographic Electrode in the Same Individual Tumors in 24 Air-Breathing Animals

	Mean values			
Probe	Median pO_2 ± 2 SE (mmHg)	Percentage values <2.5 mmHg ± 2 SE	Percentage values <5 mmHg ± 2 SE	Percentage values <10 mmHg ± 2 SE
Polarographic electrode	11.0 ± 3.3	28.5 ± 7.9	39.8 ± 8.0	50.6 ± 7.5
Fiber-optic sensor	8.1 ± 1.9	21.7 ± 8.6	44.0 ± 11.1	57.3 ± 9.3
Paired *t* test	$P = 0.07$	$P = 0.15$	$P = 0.28$	$P = 0.07$

corded were from 1 to 6.5 mm. The majority of readings (63%) were within a range >2 to 4 mm. Ten percent of the readings were made at 1 mm (i.e. peripheral). Individual $pO_{2(5min)}$ values showed no significant correlation with probe depth ($r = -0.14$, $P = 0.12$).

Effect of Measurements with the Fiber-optic Sensor on Tumor Oxygenation

Polarographic measurements were made after approximately 70 min of measurement by the fiber-optic sensor. We investigated whether the use of the fiber-optic sensor prior to the polarographic electrode alters tumor oxygenation significantly. Measurements of pO_2 in the first 12 animals were compared with measurements in an additional group of animals ($n = 10$) bearing size-matched tumors from the same tumor implant that underwent polarographic assessment alone. No significant difference was seen between the two groups, with means of the median ± 2 SE pO_2 values of 9.9 ± 4.2 mmHg (polarographic electrode measurement after sensor measurement) and 12.7 ± 5.0 mmHg (polarographic electrode measurement alone) ($P = 0.38$, unpaired *t* test). Similarly, there were no significant differences between the means of percentages of pO_2 values <2.5, 5 and 10 mmHg ($P = 0.17$, 0.39 and 0.46, respectively). These results indicate that the process of making fiber-optic measurements does not alter tumor oxygenation significantly.

Comparison of the Fiber-optic Sensor and Polarographic Electrode in Unmodulated Tumors

Measurements with the fiber-optic sensor and polarographic electrode were made in a further 12 animals (as controls for the subsequent carbon monoxide modulation experiment). The data for the combined group of 24 animals were analyzed to compare the measurements made by both instruments in the same individual unmodulated tumors. The mean of the median pO_2 values from the fiber-optic sensor (8.1 mmHg) was slightly lower than that from the polarographic electrode (11.0 mmHg), but this difference was not significant ($P = 0.07$). Moreover, no significant differences were seen between the means of the percentages of pO_2 values <2.5, 5 and 10 mmHg produced by each instrument (Table 1). However, considerable tumor-to-tumor heterogeneity in oxygenation was recorded by both instruments (range of median pO_2 values 0.6 to 28.1 mmHg and 2.0 to 19.2 mmHg by electrode and sensor, respectively). Comparison of pairs of the median pO_2 values produced by the two instruments in individual tumors revealed poor correspondence, which was more marked at higher median pO_2 values. To illustrate this point, the pairs were divided into groups in which the median pO_2 for the polarographic electrode was above or below 10 mmHg (Table 2). When the median pO_2 value was <10 mmHg, the corresponding value for the fiber-optic sensor electrode was higher in 10 of 11 (91%) animals; in contrast, when the polarographic value was >10 mmHg, the corresponding value for the fiber-optic sensor was lower in 12 out of 13 (92%) animals.

Examination of the data at the level of individual tumors showed that, for a pool of 2175 polarographic pO_2 readings and 288 sensor $pO_{2(5min)}$ values from the 24 tumors, both instruments recorded pO_2 over a wide range. However, the range of polarographic electrode pO_2 readings (–3.2 to 80 mmHg) was over twice that of the sensor $pO_{2(5min)}$ values (–0.1 to 34.8 mmHg), with the latter recording fewer extreme values, either high or low (Fig. 3).

Effect of Carbon Monoxide on Tumor pO_2 Assessed by Polarographic Electrode

Figure 4 shows the means of median pO_2 values and percentages of values <2.5 mmHg for the control and carbon monoxide treatment groups. These data indicate that the pO_2 was maximally and significantly reduced after 15 min of carbon monoxide inhalation and stably maintained throughout the subsequent 75 min. The means of the percentages of values <5 mmHg and 10 mmHg were consistent with these results.

Comparison of the Fiber-optic Sensor and Polarographic Electrode during Carbon Monoxide Inhalation

Based on the results presented above, fiber-optic sensor measurements were started after 15 min of carbon monoxide inhalation and required up to a total of 90 min of carbon monoxide inhalation for completion. These were followed immediately by measurements with the polarographic electrode, which were completed within the 90 min of carbon monoxide inhalation for which a stable pO_2 profile had been established (Fig. 3). Both instruments measured a statistically significant fall in the means of the me-

TABLE 2
Relationship between Pairs of Median pO_2 Values Produced by Polarographic Electrode and Fiber-optic Sensor in the Same Individual Tumors

Median pO_2 values (mmHg)					
Polarographic median pO_2 value ≤10 mmHg			Polarographic median pO_2 value >10 mmHg		
Polarographic electrode value (mmHg)	Fiber-optic sensor value (mmHg)	Polarographic electrode value lower than fiber-optic sensor value	Polarographic electrode value (mmHg)	Fiber-optic sensor value (mmHg)	Polarographic electrode value lower than fiber-optic sensor value
0.6	6.4	+	10.2	4.8	−
0.6	10.7	+	11.9	2.8	−
1.1	5.6	+	12.6	3.8	−
1.8	2.0	+	14.0	4.2	−
2.2	3.3	+	14.8	11.3	−
2.7	5.5	+	15.3	6.9	−
2.7	5.6	+	16.5	5.5	−
4.1	11.5	+	17.7	12.8	−
8.1	3.4	−	18.5	19.2	+
8.5	13.7	+	19.2	16.0	−
8.6	11.3	+	21.2	9.8	−
			22.5	6.0	−
			28.1	12.3	−

dian tumor pO_2 after carbon monoxide inhalation when compared with the results for air-breathing controls. The electrode recorded 11.1 mmHg, falling to 1.7 mmHg ($P = 8.2 \times 10^{-6}$, unpaired *t* test), and the sensor recorded 8.1 mmHg, falling to 2.9 mmHg ($P = 1.3 \times 10^{-5}$, unpaired *t* test). Comparisons of the results from both instruments in the same individual tumors in carbon monoxide-breathing animals are presented in Table 3. There was a small but significant difference in the means of the median pO_2 values produced by the polarographic electrode and fiber-optic sensor (1.7 mmHg and 2.9 mmHg, respectively, $P = 0.009$, paired *t* test). Comparison of the means of the percentages of pO_2 values <2.5 mmHg (65.9 and 30.3%, respectively, $P = 9 \times 10^{-5}$, paired *t* test) also illustrates that the electrode is reporting pO_2 values slightly lower than those reported by the sensor.

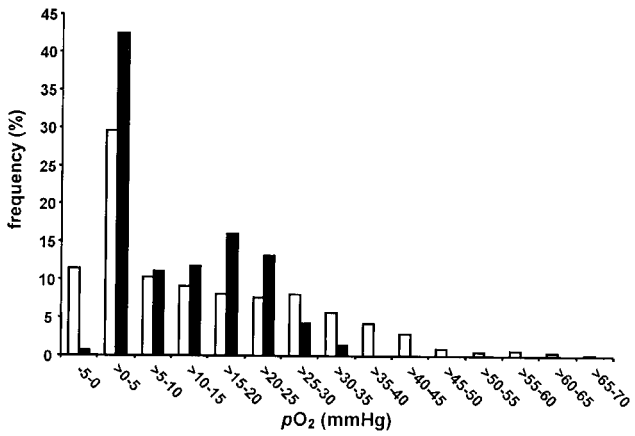

FIG. 3. Frequency distribution of $pO_{2(5min)}$ values measured by the fiber-optic sensor ($n = 288$; solid bars) and pO_2 readings measured by the polarographic electrode ($n = 2175$; open bars) pooled from 24 tumors.

Comparison of pairs of median pO_2 values produced by the two instruments in individual tumors revealed a variable degree of correspondence, similar to that observed in unmodulated tumors in the same pO_2 range. The median pO_2 value for the polarographic electrode was lower than the corresponding value for the fiber-optic sensor in 10 of 12 (83%) cases, consistent with findings in unmodulated tumors in which the median pO_2 for the polarographic electrode was ≤10 mmHg.

Examination of the data at the level of individual tumors after treatment with carbon monoxide showed that, for a pool of 1148 pO_2 readings with the polarographic electrode and 144 $pO_{2(5min)}$ values for the fiber-optic sensor, the range of readings with the polarographic electrode (–2.7 to 40.5 mmHg) was greater than the range of readings for the fiber-optic sensor (–0.3 to 10.6 mmHg). This is consistent with findings for the 24 untreated animals, except that in this case the range of the electrode readings is approximately four times the range of the sensor readings.

The two-phase phenomenon (Fig. 1a) observed in some of the unmodulated animals was noted in only 2 of 144 (1.4%) individual $pO_{2(5min)}$ traces from carbon monoxide-breathing animals; virtually all traces showed a stable pattern at a consistently low pO_2 value (mean of median pO_2 values 2.9 mmHg). This is consistent with the absence of the two-phase phenomenon in traces with a late $pO_{2(5min)}$ value of ≤2.5 mmHg in unmodulated animals.

DISCUSSION

Measurement of hypoxia in human tumors is likely to be valuable for predicting the outcome for a range of treatments. The availability of accurate and robust methods for

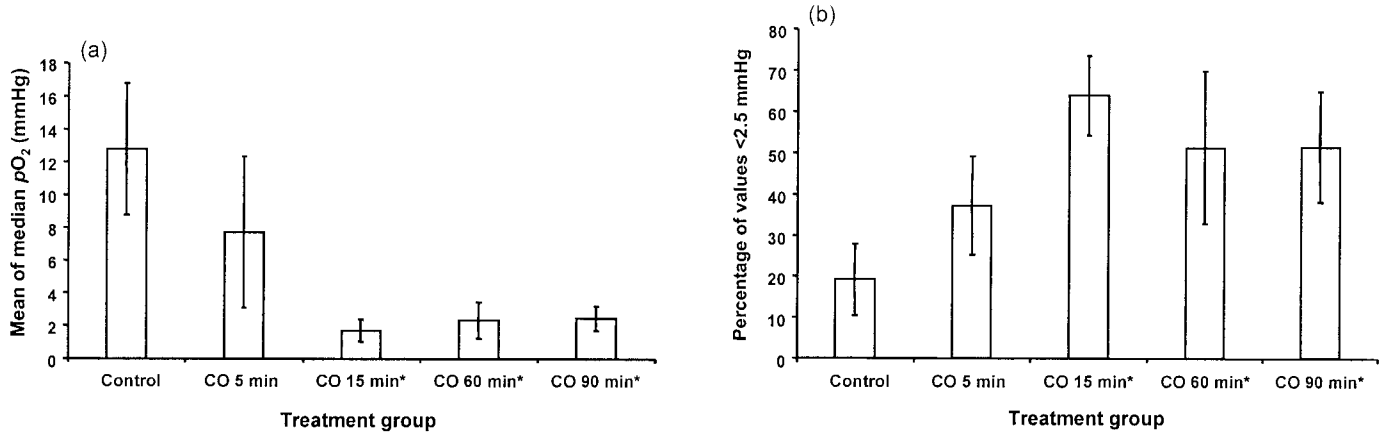

FIG. 4. Determination of the time course of the effect carbon monoxide (CO) on (panel a) the mean of median $pO_2 \pm 2$ SE and (panel b) the mean of the percentage of values <2.5 mmHg ± 2 SE, assessed using the polarographic electrode. A significant difference from the control value is represented by an asterisk (*; $P < 0.05$, analysis of variance and Tukey-Kramer test). Numbers of animals per group: control, 20; carbon monoxide 5 min, 8; carbon monoxide 15 min, 10; carbon monoxide 60 min, 9; carbon monoxide 90 min, 9.

assessing tumor pO_2 is therefore important. In this study, we have developed a protocol for the production of a set of pO_2 measurements by the newly available commercial OxyLite fiber-optic sensor, and have compared these measurements with those made by the widely used and generally accepted Eppendorf polarographic electrode employed in the conventional way. We have specifically chosen to use the P22 tumor because it has an oxygenation profile similar to that in human tumors. We have shown that, when data were compared within groups of air-breathing and carbon monoxide-breathing animals, pO_2 measurements produced by the two instruments were comparable, despite inherent differences in the physical basis upon which each device measures pO_2. However, disparity was seen when data from individual tumors were compared. Furthermore, the range of pO_2 readings produced by the polarographic electrode was wider than that produced by the fiber-optic sensor. These findings have important implications for the context in which each instrument is used.

A specific two-phase pattern of a rapid fall to a nadir (phase 1) followed by a gradual rise to a new, higher pO_2 (phase 2) has been identified in many pO_2 traces after fiber-optic sensor probes have been inserted into a tumor (Fig. 1a). The initial rapid fall of the fiber-optic sensor pO_2 trace to a nadir after placement of the probe probably represents a combination of four effects: (1) the response rate of the probe, quoted as less than 5 s to achieve 90% of the final reading (T_{90}) (manufacturer's data); (2) on introduction of the probe, oxygen present in the probe tip polymer diffuses out into surrounding tissue at a rate that is dependent on the local pO_2 concentration and tissue consumption rate; (3) at the time the probe is inserted, a small volume of oxygen is introduced into the probe track, where it is consumed by the surrounding tissues after sealing of tissue around the probe; (4) placement of the probe may tear blood vessels and exert pressure on nearby capillaries, resulting in a vasoconstrictive tissue reaction.

The latter two effects also occur when the polarographic electrode is inserted. Indeed, a similar fall in recorded pO_2 after electrode placement is well recognized and includes an additional component due to oxygen consumption by the electrochemical reaction at the cathode tip. It is usual practice to ignore this fall and to commence tracking of the electrode through tissue and recording of pO_2 readings when a stable nadir pO_2 is reached, typically after 2–3 min. Hence it is reasonable to consider the initial fall in pO_2 observed in fiber-optic sensor traces as being similarly artifactual.

The nadir observed in fiber-optic sensor pO_2 traces may reflect true low pO_2 in hypoxic locations, or the pO_2 may be spuriously low due to a vasoconstrictive tissue reaction and/or pressure of the probe tip on surrounding tissue.

TABLE 3
Comparison of pO_2 Measured by Fiber-Optic Sensor and Polarographic Electrode in the Same Individual Tumors in 12 Carbon Monoxide-Breathing Animals

	Mean values			
Probe	Median pO_2 ± 2 SE (mmHg)	Percentage values <2.5 mmHg ± 2 SE	Percentage values <5 mmHg ± 2 SE	Percentage values <10 mmHg ± 2 SE
Polarographic electrode	1.7 ± 0.9	65.9 ± 18.5	85.4 ± 7.5	96.3 ± 1.7
Fiber-optic sensor	2.9 ± 0.7	30.3 ± 17.5	94.3 ± 4.5	99.2 ± 1.6
Paired *t* test	$P = 0.009$	$P = 9 \times 10^{-5}$	$P = 0.06$	$P = 3 \times 10^{-5}$

While the vasoconstrictive tissue reaction remains speculative, there is some experimental evidence to support the pO_2-reducing effects of probe tip pressure. A study examining histological changes within muscle tissue surrounding a 350-μm-diameter polarographic needle probe 15–150 s after insertion (*20*) showed compression of surrounding tissue structures, in particular the blood vessels lying within a compression zone of 70 μm around the probe tip, with a reduction in functional capillary lumen volume of almost 50%. Limited passage of erythrocytes through these compressed high-resistance vessels can potentially reduce the oxygen-carrying capacity of blood, with a resultant fall in local tissue oxygenation. An attempt was made in our study to reduce the pressure effect at the sensor tip by pulling it back slightly after placement, but it is probably impossible to guarantee elimination of this effect.

The gradual rise in pO_2 to a new stable level (phase 2) observed in some fiber-optic sensor traces after the phase 1 nadir is probably due to the reversible component of the vasoconstrictive tissue reaction proposed previously, allowing the oxygenation of the tissue to return to a level closer to that prior to placement of the probe. It might be argued that it represents sensor response to a sudden change in pO_2, due for example to a slight movement of the probe tip. However, the rise is too slow for this to be the case. The two-phase phenomenon was present in the majority of traces in which the late $pO_{2(5min)}$ value was >2.5 mmHg, but no traces in which the late $pO_{2(5min)}$ value was ≤ 2.5 mmHg. It is probable that, while tissues that are well oxygenated are able to manifest the pO_2-reducing effects of a vasoconstrictive tissue reaction, in hypoxic locations, any such tissue reaction could have only minimal additional effect on an already low pO_2.

It is important to establish the time during the two-phase phenomenon seen in fiber-optic sensor traces in well-oxygenated tissue with which polarographic electrode readings correspond. On initial placement, the electrode pO_2 is allowed to fall to a stable nadir (as noted above), after which tracking and recording of pO_2 is commenced. However, once the electrode tracks through tissue, each successive dwell position must be considered *de novo.* For each new position (with a dwell time of 1.4 s), the electrode measures pO_2 before sufficient time has elapsed for the occurrence of either substantial oxygen consumption by the cathode or a vasoconstrictive tissue reaction to placement of the electrode. Therefore, the time at which the electrode measures pO_2 corresponds with the time on the fiber-optic sensor trace before the rapid fall in pO_2. Thus the electrode pO_2 readings and sensor $pO_{2(5min)}$ values are effectively measuring the same pO_2 but at different times, before and after the nadir, respectively (Fig. 5). In traces in hypoxic tissue that do not exhibit two phases, the already low pO_2 is unlikely to be decreased further by a vasoconstrictive tissue reaction. Therefore, the initial rapid fall in pO_2 observed as part of fiber-optic sensor traces is due only to tissue consumption of oxygen that has diffused out of the probe tip

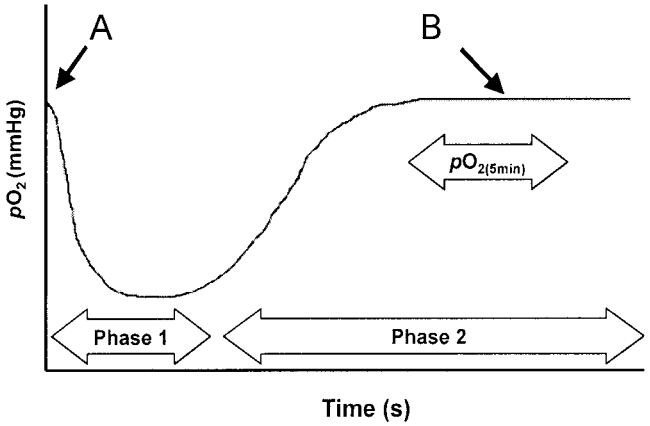

FIG. 5. Diagram of a two-phase fiber-optic sensor trace showing the times at which the polarographic electrode measures corresponding pO_2 at each new dwell position as it tracks through tissue (A), and at which the sensor $pO_{2(5min)}$ is sampled (B).

polymer or has been introduced with probe placement. It then follows that the low pO_2 after the fall represents the true tumor pO_2, and that this corresponds with pO_2 recorded by the electrode tracking through the tumor.

The polarographic electrode and fiber-optic sensor are different in many respects, including the mechanism of pO_2 measurement, the tissue volume sensed by the probe tip, the speed and process of production of pO_2 readings, the mechanism of probe placement and movement through tissue, and the time taken to produce a set of pO_2 measurements. The fiber-optic sensor measures pO_2 most accurately near 0 mmHg, its signal-to-noise ratio becoming progressively lower at higher pO_2 values. At pO_2 values much above 30–50 mmHg, the accuracy and resolution of measurements decrease substantially (*11*). In contrast, the polarographic electrode provides an output that is directly proportional to pO_2; hence the lowest readings are subject to the greatest errors. By choosing to study the relatively well-oxygenated P22 carcinosarcoma, in which 52% of the pO_2 readings with the polarographic electrode are between 5 and 40 mmHg (Fig. 3), and by using a strategy to reduce tumor oxygenation, thereby increasing the scope for comparison at lower pO_2 values, we have been able to compare the two devices over the pO_2 range in which the fiber-optic sensor operates with greatest accuracy. Considering their many differences, it is reassuring that when the two instruments were compared in the same group of tumors in air-breathing animals, no significant difference was seen between the means of median pO_2 values (11.0 and 8.1 mmHg for electrode and sensor, respectively, $P = 0.07$). However, pairs of median pO_2 values produced by the two instruments in the same tumor were rarely the same, reflecting that, by necessity, comparison of a median derived from up to 105 polarographic pO_2 readings with one derived from only 12 fiber-optic $pO_{2(5min)}$ values must be subject to sampling bias. On closer inspection, while pairs of median values do not directly correspond, there is a clear trend be-

tween members of the pairs: In the majority of cases, when the values for the polarographic electrode are ≤10 mmHg, the median pO_2 for the electrode is lower than that for the fiber-optic sensor, whereas when the values for the polarographic electrode are >10 mmHg, the median pO_2 for the electrode is higher. In the former case, this may be because of oxygen consumption by the polarographic electrode; in the latter case, an explanation is less obvious.

Comparison of pooled individual polarographic electrode pO_2 readings and sensor $pO_{2(5min)}$ values shows that the electrode measures pO_2 over a range that is two times that measured by the sensor, extending beyond both the upper and lower limits of the sensor pO_2. This difference in range may be a function of the tissue volume that each probe tip senses. We have estimated that the fiber-optic sensor probe tip senses at least 4.5 more cells than the polarographic electrode. It follows that a single pO_2 reading from this larger volume of cells must involve an element of averaging over space, which may account for the lack of extreme values recorded by the fiber-optic sensor compared to the polarographic electrode. Furthermore, individual polarographic electrode readings, acquired during a dwell time of only 1.4 s, are as a result subject to a poorer signal-to-noise ratio than fiber-optic sensor $pO_{2(5min)}$ values acquired over 5 min; hence larger deviations in pO_2 readings recorded from a given oxygen environment are to be expected. Finally, the fact that the sample size for the pO_2 readings with the polarographic electrode ($n = 2175$) is almost eightfold greater than that of the $pO_{2(5min)}$ readings for the fiber-optic sensor ($n = 288$) increases the probability that the electrode samples will include a wider range of values than the sensor readings.

Results in carbon monoxide-breathing animals were very similar to those in the low pO_2 regions encountered in air-breathing animals. The mean of the median pO_2 determined polarographically was slightly lower than that obtained with the fiber-optic sensor (1.7 and 2.9 mmHg, respectively), with an associated higher percentage of values less than 2.5 mmHg (66 and 30.3%, respectively). This discrepancy may be partially accounted for by polarographic oxygen consumption at the cathode. Moreover, the two instruments would not be expected to produce identical assessments of low pO_2 values, because at such values the sensor operates at its greatest level of accuracy, in contrast to the electrode, which operates at its lowest level of accuracy. While this numerically small difference was statistically significant, its importance in the clinical setting is more debatable. Lack of correspondence of pairs of median pO_2 values from individual tumors, the greater range of pooled individual readings with the polarographic electrode pO_2 compared to sensor $pO_{2(5min)}$ values, and the absence of two-phase traces were also consistent with results from air-breathing animals.

The results for air-breathing and carbon monoxide-breathing animals are consistent with a previous comparative study, which used a prototype fiber-optic sensor using the same oxygen-sensing principle but in a substantially different instrument (*9*). In that study, pooled results from 35 animals undergoing measurements with a polarographic electrode were compared with pooled results from 20 animals undergoing measurements with a fiber-optic sensor in the relatively hypoxic SaF tumor. The median pO_2 for the polarographic electrode was slightly lower than that for the sensor (1.4 mmHg and 2.8 mmHg, respectively), with an associated higher percentage of values <2.5 mmHg (69% and 50%, respectively). These figures are very similar to those obtained from carbon monoxide-breathing animals in our study. We have extended the comparison by use of the commercial instrument, the less hypoxic P22 tumor, and the hypoxia-inducing modulatory agent carbon monoxide, allowing assessment over a wider pO_2 range of greater clinical relevance to human tumors. Furthermore, performance of measurements by both instruments in single tumors has enabled a detailed comparison of pairs of median pO_2 values obtained from the individual tumors.

Currently it is difficult to use the commercially available fiber-optic sensor to produce a pO_2 profile of a tumor in the way achieved by the polarographic electrode. The 12 pO_2 traces per tumor in this study took 70 min to acquire; moreover, it appears from the comparison of median pO_2 pairs in individual tumors that 12 $pO_{2(5min)}$ values may be insufficient as a representative tumor sample. The performance of sufficient measurements to be representative would be time-consuming and laborious, and prohibitively so in humans. The introduction of a stepping mechanism (currently under development), similar to the pilgrim stepping of the polarographic electrode, will enable a greater number of measurements to be made in a shorter time, and may produce a more representative tumor pO_2 profile. However, the presence of the two-phase phenomenon observed in traces in the well-oxygenated areas in this tumor system has implications for the dwell time of the probe tip at each position: It may be that measurements should be made instantaneously prior to the initial fall in pO_2. A further issue to be considered is the pO_2 range in which measurements are to be made: As a result of their different mechanisms of measurement, the fiber-optic sensor operates with greatest accuracy at low pO_2 values, whereas the polarographic electrode operates with the greatest accuracy at high pO_2 values. At present, our view is that, in spite of its limitations, the polarographic electrode remains the instrument of choice for the rapid production of a spatial tumor pO_2 profile, but it cannot provide temporal data. However, the fiber-optic sensor offers two unique and important features: It enables measurement of pO_2 at specific tissue locations over prolonged periods, allowing monitoring of pO_2 fluctuations that either occur spontaneously or are induced by modulatory agents, and it can be used in combination with magnetic resonance imaging and spectroscopy. In summary, it is clear that these two devices are not mutually exclusive, but rather are complementary; used appropriately,

the fiber-optic sensor is a promising new addition to the field of oxygen measurement.

In conclusion, this comparative study of the Eppendorf polarographic electrode and the newly commercially available OxyLite fiber-optic sensor supports and extends the results of a previous comparative study of the polarographic electrode and the prototype device (*9*). We have shown that, despite their very different modes of action, the two instruments produce comparable assessments of tumor oxygenation in groups of animals, over the range of median pO_2 values of 0 to 30 mmHg. In view of the importance of tumor hypoxia and the unique features offered by the OxyLite, our results support the further development and clinical evaluation of this instrument.

ACKNOWLEDGMENTS

We thank Paul Barber and Rosalind Locke for assistance in preparation of photographs for this manuscript; Peter Russell and his staff for care of the animals; and Professor Alan Horwich for his interest and support. This work was supported by the Cancer Research Campaign. PW is a Cancer Research Campaign Life Fellow.

Received: November 6, 2000; accepted: January 25, 2001

REFERENCES

1. J. M. Brown and A. J. Giaccia, The unique physiology of solid tumors: Opportunities (and problems) for cancer therapy. *Cancer Res.* **58**, 1408–1416 (1998).
2. D. M. Brizel, S. P. Scully, J. M. Harrelson, L. J. Layfield, J. M. Bean, L. R. Proznitz and M. W. Dewhirst, Tumor oxygenation predicts for the likelihood of distant metastases in human soft tissue sarcoma. *Cancer Res.* **56**, 941–943 (1996).
3. M. Nordsmark, J. Keller, O. S. Nielsen, O. M. Jensen and J. Overgaard, Hypoxia in human soft tissue sarcoma is associated with poor survival. *Radiother. Oncol.* **48** (Suppl.), S162 (1998). [abstract]
4. A. W. Fyles, M. Milosevic, R. Wong, M. Kavanagh, M. Pintilie, A. Sun, W. Chapman, W. Levin, L. Manchul and R. P. Hill, Oxygenation predicts radiation response and survival in patients with cervix cancer. *Radiother. Oncol.* **48**, 149–156 (1998).
5. D. M. Brizel, G. S. Sibley, L. R. Proznitz, R. L. Scher and M. W. Dewhirst, Tumour hypoxia adversely affects the prognosis of carcinoma of the head and neck. *Int. J. Radiat. Oncol. Biol. Phys.* **38**, 285–289 (1997).
6. M. Höckel, K. Schlenger, B. Aral, M. Mitze, U. Schaffer and P. Vaupel, Association between tumor hypoxia and malignant progression in advanced cancer of the cervix. *Cancer Res.* **56**, 4509–4515 (1996).
7. K. Sundfor, H. Lyng and E. K. Rofstad, Tumour hypoxia and vascular density as predictors of metastasis in squamous cell carcinoma of the uterine cervix. *Br. J. Cancer* **78**, 822–827 (1998).
8. P. Vaupel, K. Schlenger, C. Knoop and M. Hockel, Oxygenation of human tumors: Evaluation of tissue oxygen distribution in breast cancers by computerized O_2 tension measurements. *Cancer Res.* **51**, 3316–3322 (1991).
9. D. R. Collingridge, W. R. Young, B. Vojnovic, P. Wardman, E. M. Lynch, S. A. Hill and D. J. Chaplin, Measurement of tumor oxygenation: A comparison between polarographic needle electrodes and a time-resolved luminescence-based optical sensor. *Radiat. Res.* **147**, 329–334 (1997).
10. J. R. Griffiths and S. P. Robinson, The OxyLite: A fibre-optic oxygen sensor. *Br. J. Radiol.* **72**, 627–630 (1999).
11. W. K. Young, B. Vojnovic and P. Wardman, Measurement of oxygen tension in tumors by time-resolved fluorescence. *Br. J. Cancer* **74** (Suppl. XXVII), S256–S259 (1996).
12. M-C. Kavanagh, A. Sun, Q. Hu and R. P. Hill, Comparing techniques of measuring tumor hypoxia in different murine tumors: Eppendorf pO_2 histograph, [^{3}H]misonidazole binding and paired survival assay. *Radiat. Res.* **145**, 491–500 (1996).
13. M-C. Kavanagh, V. Tsang, S. Chow, C. J. Koch, D. Hedley, S. Minkin and R. P. Hill, A comparison in individual murine tumors of techniques for measuring oxygen levels. *Int. J. Radiat. Oncol. Biol. Phys.* **44**, 1137–1146 (1999).
14. M. Adam, M. J. Dorie and J. M. Brown, Oxygen tension measurements of tumors growing in mice. *Int. J. Radiat. Oncol. Biol. Phys.* **45**, 171–180 (1999).
15. G. M. Tozer and K. M. Shaffi, Modification of tumour blood flow using the hypertensive agent, angiotensin II. *Br. J. Cancer* **67**, 981–988 (1993).
16. P. Workman, P. Twentyman, A. Balkwill, A. Balmain, D. Chaplin, J. Double, J. Embleton, D. Newell, R. Raymond and J. Wallace, United Kingdom Co-ordinating Committee on Cancer Research guidelines for the welfare of animals in experimental neoplasia. 2nd ed. *Br. J. Cancer* **77**, 1–10 (1998).
17. H. B. Stone, J. M. Brown, T. L. Phillips and R. Sutherland, Oxygen in human tumors: Correlations between methods of measurement and response to therapy. Summary of a workshop held November 19–20, 1992, at the National Cancer Institute, Bethesda, Maryland. *Radiat. Res.* **136**, 422–434 (1993).
18. OxyLite User Manual (For OxyLite 2000 and OxyLite 4000 Models). Oxford Optronix Ltd., 1998.
19. C. Grau, A. A. Khalil, M. Nordsmark, M. R. Horsman and J. Overgaard, The relationship between carbon monoxide breathing, tumour oxygenation and local tumour control in the C3H mammary carcinoma *in vivo*. *Br. J. Cancer* **69**, 50–57 (1994).
20. U. Schramm, W. Fleckstein and C. Weber, Morphological assessment of skeletal muscle injury caused by pO_2 measurements with hypodermic needle probes. In *Clinical Oxygen Pressure Measurement II* (A. M. F. W. Ehrly, J. Hauss and R Huck, Eds.), pp. 39–50. Blackwell Ueberrenter Wissenschaft, Berlin, 1990.