Comparison of tumor and normal tissue oxygen tension measurements using OxyLite or microelectrodes in rodents

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The presence of hypoxia in tumors has long been known to adversely affect the sensitivity of tumors to radiation therapy (35). Hypoxia has recently been shown to increase mutation frequency (29), to exert a selective pressure for survival of those cells with lower apoptotic potential (12), to alter gene expression of genes involved in cell cycle regulation and cytokine production (6), and to impact treatment outcome and patient survival (3, 11, 15, 16, 27, 32). Thus there is a continued interest in measurement of oxygen (O2) tension (PO2) in tumors.

Although measurements of PO2 in tumors have been made using optical fluorescent techniques (14) or hypoxia markers (18), most O2 levels in solid tumors have been measured with polarographic electrodes. The electrodes have either been the needle-encased electrode used in the Eppendorf histograph system (19), gold or platinum microelectrodes (22), or recessed-glass polarographic microelectrodes (7). Recently, a new PO2 measurement system has become commercially available. This device is the OxyLite PO2 system (Oxford Optronics; Oxford, UK), which measures PO2 by using a fluorescence quenching technique. The prototype was described by Young et al. (40) in 1996 and was used to measure tumor PO2 and subsequently by Collingridge et al. (4). Briefly, the system uses a ruthenium chloride fluorescent compound immobilized in a polymer at the tip of a fiber-optic probe, which is ~220 μm in diameter. Blue light is emitted from diodes within the unit, and the light is propagated down the fiber to the tip, where it excites the ruthenium chloride.

The lifetime of the resultant fluorescence is inversely proportional to the amount of O2 at the tip.

Although comparisons of tumor PO2 measurements obtained by the OxyLite and Eppendorf systems have been made (4), no detailed analysis of the differences between measurements obtained with small glass O2 microelectrodes and the OxyLite system has been made. Because microelectrodes and the OxyLite are currently being used in laboratories to measure tumor PO2, it is important to know how measurements made with the two probes are similar and how they are different.

In this study, we compared measurements of PO2 made with traditional recessed-tip O2 microelectrodes and with the OxyLite PO2 system. PO2 histograms were measured in rat tumors, mouse spleen, and mouse thymus. To fully compare the two techniques, rat tumor PO2 was also measured continuously at a single location after glucose infusion and 100% O2 breathing.

METHODS

OxyLite PO2 System

The OxyLite PO2 system (Oxford Optronics) measures PO2 by determining the O2-dependent fluorescent lifetime of ruthenium chloride (40). The ruthenium chloride is immobilized at the tip of a 220-μm-diameter fiber-optic probe. Because each probe is calibrated at the manufacturer before shipment, the calibration for each probe was scanned into the computer by using a barcode wand. The probe was then ready...
for measurement of tissue PO₂. The PO₂ signal from the OxyLite probe was a 5-s average value and was recorded to disk with the use of a data-acquisition system (MacLab, ADInstruments; Castle Hill, Australia). For the PO₂ response experiments, the PO₂ values were averaged >10 s and graphed at 10-s intervals.

After the animal was euthanized at the end of the experiment, an in vivo zero value was obtained for each probe. If the PO₂ dropped to a reasonable nonzero value, i.e., 1 or 2 mmHg, it was assumed that there was a slight calibration error and that value was used as the true zero for that probe. All PO₂ measurements made in that experiment were corrected to account for the offset in the zero value.

**O₂ microelectrodes.** Recessed-tip O₂ microelectrodes were produced by using a previously published technique (24). Recess lengths were relatively short, on the order of 30 μm. Several electrodes were used for more than one experiment. The electrodes used to record PO₂ histograms in mice and rats had tip diameters of 8.0 ± 2.2 μm (mean ± SD; n = 15 electrodes). The microelectrodes used in the glucose experiments had tip diameters of 8.0 ± 3.1 μm (n = 11). The O₂ breathing experiments involved microelectrodes with tip diameters of 9.6 ± 3.1 μm (n = 10).

The microelectrodes were polarized at −0.7 V using a commercial polarizing box and picomammeter unit (chemical microsensor model 1201, Diamond General; Ann Arbor, MI). The signal from the microelectrode was digitized at 25 Hz and recorded using data-acquisition software (AT-CODAS; Windaq, DATAQ Instruments; Akron, OH). Electrodes were calibrated before and after each experiment in a saline-filled tonometer alternately bubbled with 0%, 5%, 15%, or 21% O₂ (balance N₂). The saline was warmed to 37°C. An in vivo dead value was also obtained by recording the microelectrode current in the tissue after euthanasia of the rat or mouse with an overdose of pentobarbital sodium. The average sensitivity of the microelectrodes used in the histogram studies was 1.74 ± 0.74 mmHg/pA (mean ± SD; n = 15 electrodes). The average sensitivities of the microelectrodes used for the glucose and O₂ breathing studies were 1.04 ± 0.85 (n = 11) and 1.00 ± 0.27 mmHg/pA (n = 10), respectively.

**PO₂ histograms in mouse spleen and thymus.** Twenty-one female DBA/2 mice (Charles River Laboratories; Raleigh, NC) were used in this portion of the study. At the time of experiment, the mice were ~6–8 wk and weighed 16.7 ± 1.7 g. The mouse was anesthetized with an intraperitoneal injection of 40 mg/kg pentobarbital sodium. Either the spleen or thymus was exposed by an incision in the skin and capsule was then removed to expose the surface of the tumor. This was kept moist by topical application of saline. For the microelectrode experiments, a small incision was then made in the skin. An Ag/AgCl reference electrode was sutured into the subcutis. Body temperature was maintained by placing the rat on a regulated water-heated blanket (K-Module, Baxter Healthcare).

After preparation of the animal and calibration of the electrode, the rat was placed on a water-heated blanket and the left leg was stabilized on a rubber pedestal with tape. Care was taken not to elevate the leg. A micromanipulator (model MO102E, Narishige) was positioned so that a dummy probe could reach the exposed tumor surface, which was covered by a drop of saline. The actual probe or microelectrode was then placed in the micromanipulator and advanced into this saline droplet. The electrode was allowed to polarize for several minutes in the saline before being advanced into the tumor. The OxyLite probe was placed into the mouse and the position on the micromanipulator was noted. The probe was then advanced in 50-μm steps for a total distance of 1,000–2,000 μm. At each location, PO₂ was recorded for 10 s, and an average PO₂ over the interval was calculated later. The probe was then withdrawn and reinserted at a new location. A new penetration was made, and the PO₂ along the second track was recorded. This process was repeated until a total of three to four tracks had been made in the tissue. The total measurements made in each mouse ranged from 50 to 192 points.

At the end of the recording time, an overdose of pentobarbital sodium was injected intravenously. The recordings of all parameters continued for at least 5 min after death. The PO₂ value after death was used in the final microelectrode calibration as a true in vivo zero as described previously (10) or was used to correct for calibration error in the OxyLite probe (see above).

**Spleen PO₂ was measured in 10 DBA/2 mice with a weight of 16.5 ± 1.5 g (mean ± SD). Five histograms were recorded with the OxyLite system, and five were recorded with the microelectrodes. Eleven mice were used to measure thymus PO₂. The mice weighed 16.9 ± 2.0 g. Five or six experiments were performed with the use of each system.**

**Preparation of rats for tumor PO₂ studies.** Forty-six female Fischer 344 rats (Charles River Laboratories) were used in this study. All of the rats received subcutaneous implants of 1- to 2-mm³ pieces of R3320Ac rat mammary adenocarcinoma in the left hindlimb. After the tumor had reached 1 cm in diameter, the tumor-bearing rats were used in the experiments. The rat was anesthetized with an intraperitoneal injection of 50 mg/kg pentobarbital sodium. A small portion (~4–10 mm³) of the skin and tumor capsule was then removed to expose the surface of the tumor. This was kept moist by topical application of saline. For the microelectrode experiments, a small incision was then made in the left forelimb, and an Ag/AgCl reference electrode was sutured into the subcutis. Body temperature was maintained by placing the rat on a regulated water-heated blanket (K-Module, Baxter Healthcare).

After preparation of the animal and calibration of the electrode, the rat was placed on a water-heated blanket and the left leg was stabilized on a rubber pedestal with tape. Care was taken not to elevate the leg. A micromanipulator (model MO102E, Narishige) was positioned so that a dummy probe could reach the exposed tumor surface, which was covered by a drop of saline. The actual probe or microelectrode was then placed in the micromanipulator and advanced into this saline droplet. The electrode was allowed to polarize for several minutes in the saline before being advanced into the tumor. For the OxyLite measurements, a 27-gauge needle was used to pierce the tissue and facilitate penetration of the probe. This step was not necessary for the microelectrode measurements. The OxyLite probe or microelectrode was moved into the tissue and the position on the micromanipulator was noted. The probe was then advanced in 50-μm steps for a total distance of 1,000–2,000 μm. At each location, PO₂ was recorded for 10 s, and an average PO₂ over the interval was calculated later. The probe was then withdrawn and reinserted at a new location. A new penetration was made, and the PO₂ along the second track was recorded. This process was repeated until a total of three to four tracks had been made in the tissue. The total measurements made in each mouse ranged from 50 to 192 points.

At the end of the recording time, an overdose of pentobarbital sodium was injected intravenously. The recordings of in vivo zero PO₂ values were recorded as described above.
Transient PO2 responses in rat R3230Ac tumors: glucose or 100% O2 breathing. After the OxyLite probe or microelectrode had been positioned in the tumor, the probe was moved into the tissue until a clearly nonzero PO2 current was obtained, so that the PO2 had an opportunity to either increase or decrease in response to the perturbation. Because the goal of this study was to look at the response of PO2 to glucose or O2, we did not wish to leave a probe in an area that may have been chronically hypoxic with PO2 readings near zero. Once the probe had been placed, a baseline PO2 was recorded. In the case of the glucose experiments, baseline was recorded for 10 min. A glucose solution (200 mg/ml) was then infused intravenously at a rate of 0.1 ml/min. The infusion lasted from 8 to 10 min. The PO2 was recorded for 90 min after the start of the glucose infusion. The 100% O2 breathing experiments lasted 40 min. A 20-min baseline PO2 was recorded, and 100% O2 was then blown across the snout of the rat via a mask. The PO2 was recorded for 20 min while the animal breathed O2. In both experiments, the probe remained stationary during the entire recording time. After the experiments, the PO2 values were averaged over 10 s and graphed at 10-s intervals. The maximal response was defined as the highest average PO2 change over 1 min after the end of glucose infusion or the start of O2 breathing.

In the glucose experiments, 18 rats weighing 172 ± 6 g were used. Microelectrodes were used in 11 experiments, and OxyLite probes were used in the other seven. Eighteen rats weighing 168 ± 10 g were used in the hyperoxia studies. Microelectrode measurements were made in 10 rats, whereas the OxyLite system was used in the other eight experiments.

Statistics

All of the data were compared using nonparametric analysis. Differences between groups were tested with the use of the Mann-Whitney U-test. The differences in paired data were tested with the use of the Wilcoxon matched-pairs signed-ranks test. Significance was achieved if \( P \leq 0.05 \).

RESULTS

Tissue and Tumor PO2 Histograms

Spleen PO2. Regardless of the probe used for the PO2 measurement, qualitatively the spleen was generally well oxygenated with relatively few PO2 values near zero. The cumulative frequency plot of the histograms measured by the microelectrode showed an almost linear rise from 0 to 1.0 across a PO2 range of 4–31 mmHg (Fig. 1A). In contrast, the OxyLite recorded PO2 values mainly between 10 and 23 mmHg (Fig. 1B). Despite this difference in range, the plots show that the vast majority of the PO2 values measured by either system were between 10 and 25 mmHg. Similarly, the global histograms, including all of the measured points in all mice, revealed that most of the measured PO2 values were between 5 and 30 mmHg (Fig. 1, insets). The striking difference between the two global histograms was at the high end of the distribution. Almost 10% of the values measured by the microelectrode were >40 mmHg, whereas the OxyLite recorded <1% of its values in this range.

The characteristics of the five individual histograms determined by using the two measurement systems are compared in Table 1. None of the parameters was significantly different, although the standard deviation and range approached statistical significance. This could be interpreted to mean that there was a trend for the microelectrode to measure a broader range of PO2 values, which was also in evidence in the cumulative frequency plots and the global histograms (Fig. 1).
Thymus PO₂. In contrast to the distribution of PO₂ in the spleen, the thymus was much more hypoxic, with many values <5 mmHg. This was true whether the microelectrode (Fig. 2A) or the OxyLite system (Fig. 2B) was used for the measurements. The large plot in each panel shows the cumulative frequencies of the individual histograms for each thymus. The cumulative frequency plots of the histograms measured by the microelectrode and the OxyLite both showed a steep rise from 0 to 0.95 across a PO₂ range of 0–17 mmHg. The major qualitative difference is that over 20% of PO₂ values measured by the OxyLite were <1 mmHg. This difference is shown even more dramatically when the global histograms are compared (Fig. 2; insets). Despite this difference at the lowest end of the histogram, the plots still revealed that the vast majority of the PO₂ values measured by either system were between 0 and 17 mmHg.

The characteristics of the individual histograms determined using the two measurement systems are compared in Table 1. None of the parameters were significantly different. Thus in this tissue the two PO₂ measurement systems yielded similar results.

Tumor PO₂. We measured tumor PO₂ with the microelectrode in five rats and with the OxyLite system in another five rats. The discrepancy between the two measurement systems was greatest in this tissue, and the difference in the two techniques is demonstrated best by looking at the cumulative frequency plots (Fig. 3). The microelectrode distribution showed a steady increase in cumulative frequency of PO₂ measurements from 15.7% (median) <1 mmHg up to 80.5% at 5 mmHg and below (Fig. 3A). The OxyLite distribution started at a cumulative frequency of 53.6% <1 mmHg and then steadily rose to 84.3% at 5 mmHg (Fig. 3B). The microelectrode distinguished a more heterogeneous pattern in PO₂ than could be measured by the OxyLite system. With the use of the microelectrodes, <20% of the values were <1 mmHg (Fig. 3A). By using the OxyLite probe, over 50% of the measurements were <1 mmHg in most of the tumors (Fig. 3B). If all of the

Table 1. Characteristics of PO₂ histograms recorded in spleen, thymus, and tumor using oxygen microelectrodes or OxyLite PO₂ system

<table>
<thead>
<tr>
<th>Technique</th>
<th>n</th>
<th>Mean ± SD</th>
<th>Median ± SD</th>
<th>10th %ile ± SD</th>
<th>25th %ile ± SD</th>
<th>% Values ≤ 2.5 mmHg</th>
<th>% Values ≤ 5 mmHg</th>
<th>Skewness ± SD</th>
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<tr>
<td><strong>Mouse spleen</strong></td>
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<td></td>
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<tr>
<td>Microelectrode</td>
<td>5</td>
<td>19.8 ± 3.6</td>
<td>18.8 ± 6.2</td>
<td>9.6 ± 4.7</td>
<td>13.1 ± 4.4</td>
<td>1.3 ± 2.8</td>
<td>4.4 ± 3.7</td>
<td>0.25 ± 0.57</td>
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<tr>
<td>OxyLite</td>
<td>5</td>
<td>16.1 ± 6.7</td>
<td>15.3 ± 6.7</td>
<td>11.1 ± 7.1</td>
<td>12.5 ± 7.5</td>
<td>5.1 ± 11.5</td>
<td>7.7 ± 17.3</td>
<td>0.29 ± 0.67</td>
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<tr>
<td>P value</td>
<td></td>
<td>0.420</td>
<td>0.310</td>
<td>0.690</td>
<td>1.000</td>
<td>0.310</td>
<td>0.420</td>
<td></td>
</tr>
<tr>
<td><strong>Mouse thymus</strong></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microelectrode</td>
<td>5</td>
<td>9.8 ± 3.8</td>
<td>7.6 ± 4.2</td>
<td>4.2 ± 3.0</td>
<td>4.2 ± 3.0</td>
<td>19.4 ± 23.2</td>
<td>33.9 ± 20.2</td>
<td>1.16 ± 1.07</td>
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<tr>
<td>OxyLite</td>
<td>6</td>
<td>9.6 ± 6.5</td>
<td>8.8 ± 7.9</td>
<td>0.3 ± 0.4</td>
<td>2.6 ± 2.5</td>
<td>32.5 ± 14.6</td>
<td>44.8 ± 17.7</td>
<td>0.75 ± 0.64</td>
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<tr>
<td>P value</td>
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<td>0.126</td>
<td>0.428</td>
<td>0.330</td>
<td>0.330</td>
<td>0.428</td>
<td></td>
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<tr>
<td><strong>R3230Ac rat tumor</strong></td>
<td></td>
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<tr>
<td>Microelectrode</td>
<td>5</td>
<td>7.1 ± 4.4</td>
<td>8.4 ± 6.2</td>
<td>0.8 ± 0.7</td>
<td>1.7 ± 1.0</td>
<td>45.3 ± 21.5</td>
<td>69.6 ± 21.4</td>
<td>1.73 ± 0.86</td>
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<tr>
<td>OxyLite</td>
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<td>5.0 ± 5.0</td>
<td>7.3 ± 7.4</td>
<td>0.2 ± 0.5</td>
<td>0.5 ± 0.6</td>
<td>76.3 ± 14.5</td>
<td>83.4 ± 13.9</td>
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<tr>
<td>P value</td>
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<td>0.310</td>
<td>0.016</td>
<td>0.222</td>
<td>0.032</td>
<td>0.420</td>
<td>0.548</td>
<td></td>
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</table>

Values are means ± SD; n, no. of mice in which histograms were recorded. PO₂ values are in mmHg. P < 0.05, statistical significance of 5 histograms.

Fig. 2. Distribution of PO₂ in the thymus of DBA/2 mice measured with microelectrodes (n = 620 points in 5 mice) (A) or the OxyLite PO₂ system (n = 896 points in 6 mice) (B). Figure shows the median cumulative frequency of the given PO₂ ± interquartile range for the set of 5 or 6 mice. Insets: global PO₂ histogram, i.e., all of the PO₂ values measured in all the mice. See METHODS for details.
measurements from the five mice were pooled into one grand histogram, only ~18% of the measurements were in this “near anoxic” range when tumor PO$_2$ was measured with the microelectrodes (Fig. 3A, inset). The grand histogram for the OxyLite measurements showed that >50% of all measurements were in this range (Fig. 3B, inset).

The characteristics of the five individual histograms determined by using the two measurement systems are compared in Table 1. Two parameters were significantly different between the two PO$_2$ measurement devices. The median PO$_2$ measured by the microelectrodes was significantly higher than that measured by the OxyLite probes ($P = 0.016$). The percentage of values <2.5 mmHg was significantly higher for the OxyLite system ($P = 0.032$). None of the other parameters were significantly different, indicating that the major difference between the two histograms occurred at this very low end of the distribution.

**Transient Tumor PO$_2$ Responses**

**Tumor PO$_2$ response to glucose infusion.** The response of tumor PO$_2$ to an intravenous infusion of 1 g/kg glucose is shown in Fig. 4. The PO$_2$ appeared to increase after glucose infusion using both measurement techniques. The microelectrodes detected a mean PO$_2$ increase of ~3 mmHg after the glucose infusion (Fig. 4A), whereas the OxyLite measured a mean PO$_2$ increase of ~6 mmHg (Fig. 4B). Whereas these responses appear similar, none of the changes in PO$_2$
measured by the microelectrodes were statistically significant \((P > 0.05, \text{Wilcoxon ranked-sums test})\), although 8 of the 11 tumors showed some increase in PO2 after glucose infusion. On the other hand, the PO2 measured by the OxyLite was significantly different from baseline PO2 from 8 min (during the infusion) until 41 min (30 min after the end of the infusion) \((P \leq 0.05)\).

Whereas Fig. 4 shows the mean responses and the standard deviations, it is important to note that the variability among the responses is somewhat hidden by this presentation of the data. When the maximal PO2 change is plotted as a function of baseline PO2, it is clear that the response measured by the OxyLite was much more consistent than that measured by the microelectrodes (Fig. 5). When the microelectrode was used to measure tumor PO2, 8 of the 11 measurements showed an increase in PO2 after glucose infusion (Fig. 5A). The other three actually showed a decrease of 2 mmHg or more. The mean maximum change measured with the microelectrode was \(4.2 \pm 7.2 \text{ mmHg (mean } \pm \text{ SD, } n = 11)\). The OxyLite system consistently measured an increase of at least 6 mmHg (Fig. 5B). The mean maximum change measured with the OxyLite probe was \(9.0 \pm 3.2 \text{ mmHg (mean } \pm \text{ SD, } n = 7)\).

Tumor PO2 response to 100% O2 breathing. The response of tumor PO2 to 100% O2 breathing was markedly different when measured with the two techniques. When PO2 was measured with the microelectrode, PO2 did not consistently increase after 100% O2 breathing and the mean magnitude of the change was only \(\sim 10 \text{ mmHg (Fig. 6A)}\). In addition, the increase in PO2 was not immediate, because the PO2 change from baseline PO2 was only significant after 4.3 min. On the other hand, when tumor PO2 was measured with the OxyLite system, tumor PO2 consistently increased immediately after the initiation of O2 breathing and remained elevated for the entire 20 min of gas exposure (Fig. 6B). The mean magnitude of the increase was over 25 mmHg.

Again, to better appreciate the difference between the responses measured by the two measurement techniques, the maximal change in PO2 for each individual experiment was plotted as a function of the baseline PO2 data (Fig. 7). There was no correlation between baseline PO2 and maximal PO2 change for the microelectrode data (Fig. 7A). In 3 of the 10 cases, PO2 did not change at all or even decreased slightly. In four experiments, the maximal PO2 increase was \(<10 \text{ mmHg. The mean maximum change measured with the microelectrode was } 17.9 \pm 25.9 \text{ mmHg (mean } \pm \text{ SD, } n = 10)\). Although the OxyLite data did not show a significant correlation between baseline PO2 and maximal PO2 change, there was a step-like nature to the data (Fig. 7B). One-half of the experiments showed a PO2 increase of at least 37 mmHg. In three of the other four experiments, the PO2 increase was at least 13 mmHg. In the remaining experiment, the baseline air breathing PO2 was near 0 mmHg, and the PO2 increased by \(\sim 3 \text{ mmHg. Thus if the baseline PO2 measured with the OxyLite was } >0\), the O2-induced change in PO2 was at least 13 mmHg. The maximum change in PO2 measured with the OxyLite probe averaged \(27.8 \pm 18.5 \text{ mmHg (mean } \pm \text{ SD, } n = 8)\).

DISCUSSION

In this study PO2 measurements using polarographic O2 microelectrodes and OxyLite optical probes were compared in three tissues and under various conditions. Measurement of PO2 histograms revealed that the results were tissue dependent. In two normal tissues, spleen and thymus, the PO2 distributions measured by the two systems were not statistically different. Differences between the histograms measured by the two techniques were found only in the very hypoxic R3230Ac tumor. In general, the distribution measured by the OxyLite was dominated by lower PO2 values and yielded a higher hypoxic fraction than the histogram.
determined by microelectrodes. Transient responses of tumor Po2 to two different perturbations were also examined. Although both probes showed similar small mean Po2 increases after glucose infusion, the changes measured by the microelectrode were not statistically significant. The responses of the two systems to 100% O2 breathing were drastically different. The OxyLite probe consistently showed a large increase in Po2 immediately after initiation of 100% O2 breathing. The microelectrodes recorded no significant change in Po2, sometimes showing an increase, and sometimes not changing at all. This study is the first to compare these two techniques of measuring Po2 in animal tissues in vivo. The results point to important differences between the two measurement techniques, which need to be incorporated into the interpretation of experimental O2 measurements.

**PO2 Histograms in Normal Tissues**

**Po2 histograms in spleen.** Splenic Po2 has been measured previously in rats and rabbits. Jamieson and van den Brenk (17) measured Po2 in the rat spleen using both 60- and 330-μm-diameter insulated gold wire electrodes. In an extensive study involving 140 rats, they determined a mean splenic Po2 of 17 ± 2 mmHg (means ± SE) and 23 ± 2 mmHg using the smaller and larger electrodes, respectively. In a later report, 3- to 8-μm-diameter gold microelectrodes were used to measure rabbit splenic Po2 (38). In that study, 1,054 measurements were made in 11 rabbits, yielding a grand Po2 histogram with values ranging from 20 to 100 mmHg and a mean Po2 of 63.5 mmHg. In this study, we
determined a mean splenic PO2 of near 20 mmHg using either microelectrodes or the OxyLite (Fig. 1, Table 1). This value is in agreement with the earlier study in rat. A subset of these data are reported in another manuscript, and the possible significance of the O2 levels in the spleen are presented there (C. C. Caldwell et al., unpublished observations).

**PO2 histograms in thymus.** There have been no other measurements of PO2 in the thymus to our knowledge. Regardless of which measurement system was used, the thymus was shown to be a tissue with surprisingly low O2 levels. The mean PO2 was around 10 mmHg with a median near 8 mmHg. Whereas the mean thymic PO2 is lower than might be expected, other organs also have similarly low PO2 distributions. The mean PO2 in the vascularized half of the cat retina (near the vitreous humor) is near 13 mmHg during light adaptation (23). In some studies (25, 37), the myocardium has also been shown to have mean PO2 values as low as 5–10 mmHg. The brain may also have similarly low PO2 levels, although a wide range of PO2 values have been found (37). In a recent study (28), the cat primary visual cortex was shown to have a mean PO2 of 12.8 mmHg with 59.1% of the values <10 mmHg. The retina, myocardium, and brain are all highly metabolic tissues with sufficient blood supplies to maintain oxidative metabolism. It is not known whether the thymus is similar to these tissues or has a modest metabolism and a more limited blood supply. The former may be the case, because the mouse thymus has a dense vascular network with looped capillaries (20). As with the spleen, portions of the thymus PO2 data are reported elsewhere, and the possible significance of these low PO2 values is discussed in detail in that manuscript (C. C. Caldwell et al., unpublished observations).

**Comparison of PO2 histograms in normal tissue: OxyLite vs. microelectrode.** The PO2 histograms measured in normal tissues with the microelectrodes and the OxyLite probes were not statistically different (Table 1). The only parameter that suggested any difference in the distributions was the standard deviation of the histograms measured in the spleen (P = 0.056). The standard deviation of the microelectrode measurements tended to be twice as large as that measured with the OxyLite. This is consistent with the general appearance of the histograms of splenic PO2 (Fig. 1), where the range of measurements made by the microelectrodes is larger than that measured by the OxyLite. Most of the microelectrode measurements fall between 4 and 32 mmHg, with 9% of all measurements >40 mmHg. The OxyLite PO2 measurements lie primarily between 10 and 20–25 mmHg, and <1% of the measurements were >40 mmHg. This slight difference in the histograms can be explained by the fact that the OxyLite averages PO2 over a larger area than the microelectrode. The measuring tip of the OxyLite is 220 μm, whereas microelectrodes have a tip diameter near 10 μm. The effect of averaging on a relatively normal distribution would be a narrowing of the histogram, and this is consistent with the pattern seen in spleen.

**PO2 Histograms in Tumors**

**Features of PO2 histograms in R3230Ac tumors.** There have been many previous measurements of PO2 distributions in both experimental and human tumors. In general, tumors have much lower mean and median PO2 values than normal tissues, and the distributions are severely left shifted, i.e., skewed to the right, with a high percentage of the PO2 values near 0 mmHg (19, 39). The hypoxic fractions (fraction of PO2 values <5 mmHg) can vary widely, depending on such factors as tumor cell line (33) and implantation site (19).

Oxygenation of the R3230Ac tumor has been studied previously by using the Eppendorf PO2 histograph, and it has been found to be very hypoxic. The reported hypoxic fractions (PO2 <5 mmHg) for the R3230Ac range from 49 to 82% (2, 31). The hypoxic fractions measured by the OxyLite (83.4 ± 13.9%) and the microelectrodes (69.6 ± 21.4%) in the present study fall approximately within this range. The median PO2 of the R3230Ac has been reported to be 1–8 mmHg (2) and 3.6 ± 0.3 mmHg (mean ± SE) (31). These values are in agreement with those determined by the OxyLite (1.0 ± 0.7 mmHg) and the O2 microelectrodes (4.0 ± 3.5 mmHg) in the present study.

**Comparison of tumor PO2 histograms: OxyLite vs. microelectrode.** Tumor PO2 distributions measured by the OxyLite system and by microelectrodes were similar in that they both revealed many very low PO2 values. More importantly, however, several statistically significant differences between the distributions were found. As shown in Table 1, the median PO2 determined by microelectrodes was significantly higher than that measured by the OxyLite system. The fraction of PO2 values <2.5 mmHg was also significantly lower in the histogram measured by the microelectrodes. Thus the OxyLite measured many more PO2 values near 0 mmHg.

The first explanation for the difference in low PO2 values might be that the larger OxyLite probe had some physical effect on the tissue, and the extremely low PO2 values are artifactual. The low values could be caused by pressure on the tissue or vascular damage, both of which could reduce tumor blood flow and PO2. Although these explanations are possible, there are two pieces of evidence against this interpretation. First, the effect is only seen at PO2 values near zero. The rest of the histograms measured by the two measurement systems were similar. If the OxyLite probe decreased perfusion, one would expect to see midrange and higher PO2 values affected as well. As shown in the grand histograms (Fig. 3), the OxyLite measured as many tumor PO2 values >10 mmHg as the microelectrodes did. Second, there were no differences in the histograms for spleen and thymus. If the OxyLite probe caused damage in the tumor, it would also be expected to damage the spleen and thymus. There were no significant differences between the descriptive pa-
rameters of the histograms measured by the two different techniques in these normal tissues (Table 1). This second argument may not be valid for the pressure effect of the probe, because this effect would have been more significant in the less compliant tumor than in normal tissues. The issue of the insertion of the probes to increase pressure at the tip was recognized at the start of this study. In an attempt to minimize this effect, both probes were advanced 60 μm and then withdrawn 10 μm. Nevertheless, pressure still could have been high around the tip, and this may have contributed to some of the very low tumor PO2 values measured with the OxyLite probe.

An alternative explanation for the difference in the low end of the histograms involves the sensing volume (measurement volume) of each probe. Although the concept of “measurement volume” can be miscon- strued, it obviously plays a role in determining how PO2 is measured by an O2 sensor. Because both recessed-tip O2 microelectrodes (30) and the OxyLite sensor (40) consume minimal or no O2 and do not disturb the O2 field in front of the probe significantly, the two sensors used in this study essentially measure the PO2 at the tip of the probes. The term “measurement volume” implies that the sensor measures a distinct volume, which is often taken to be hemispherical (15). A more accurate description would be that each sensor measures a region, which is a disk with a circular surface area the size of the cathode or dye tip. If the electrode consumes O2, it will disturb the PO2 field near the tip, and this could cause a hemispherical gradient to be established in the tissue (30). This would alter the true PO2 at the tip of the electrode and change the tissue PO2. With microelectrodes, the use of even a small recess reduces this problem considerably (30). By using this interpretation of the sensing volume, the micro- electrode would measure an area of ~80 μm² or less, and the OxyLite probe would measure an average PO2 in an area of ~38,000 μm². This is a ratio of ~475:1.

To appreciate the effect of this difference in measurement area on the PO2 distribution, a simple conceptual model can be used (Fig. 8). In this model, it is assumed that the microelectrode measures individual points in the tissue and the OxyLite measures an average of 100 points. Thus the ratio here is only 100:1, but it will demonstrate the effect of PO2 averaging. In this example, a hypothetical area of a tumor is presented (Fig. 8A). The tumor section includes a central hypoxic area with small severely hypoxic regions interspersed in the field. The hypothetical distribution of individual PO2 values measured by a microelectrode is shown in Fig. 8B. This field was chosen to yield a distribution similar to that measured in the in vivo study presented earlier (Fig. 3A). When a 100-point average of the PO2 is calculated, a much different distribution results (Fig. 8C). It is characterized by a shift of the lowest PO2 values to the far left or a severe skew to the right. The percentage of PO2 values <1.0 mmHg increases from 22.1% to 44.5%, and the hypoxic fraction (% PO2 values ≤2.5 mmHg) increases from 57.3% to 84.4%. This is the same trend seen in the actual tumor data (Fig. 3, Table 1). Thus the higher hypoxic fraction measured by the OxyLite probe is not necessarily attributable to artifact, but may also be a function of PO2 averaging.

This finding is dependent on the magnitudes of the individual PO2 values and their position in the tissue grid. This can be demonstrated by creating another PO2 field with slightly higher PO2 and a more normally distributed PO2 distribution (Fig. 8D). The hypothetical microelectrode distribution is broad and almost Gaussian in appearance (Fig. 8E). Although the PO2 values are lower, the shape of the distribution is similar to that measured in the spleen (Fig. 1A). The hypothetical OxyLite distribution is narrower and slightly left-shifted or skewed to the right (Fig. 8F). Again, this is reminiscent of what was seen in the spleen, where the OxyLite distribution (Fig. 1B) was slightly skewed to the right, compared with the microelectrode histogram (Fig. 1A).

Although this conceptual model cannot perfectly duplicate the in vivo measurements, it does clearly show that the measurement volumes of the probes can play a key role in the characteristics of the PO2 histograms measured in the tissue, particularly in a heterogeneously hypoxic tissue like tumors.

**Tumor PO2 Responses to Glucose or 100% O2 Breathing**

**Tumor PO2 response to glucose infusion: OxyLite versus microelectrode.** In tissues capable of carrying out glycolysis in the presence of O2, an overabundance of glucose stimulates these tissues to shift metabolism away from oxidative metabolism toward glycolysis (5). The shift results in a decrease in O2 consumption, which would lead to an increase in tissue PO2 if the O2 supply remains constant. This phenomenon is known as the Crabtree effect (5) and has been known to occur in tumors for more than 80 years. Thus it was hypothesized that an increase in tumor PO2 might occur after glucose infusion. A more detailed description of the effects of hyperglycemia on microelectrode PO2 is presented elsewhere (S. A. Snyder et al., unpublished observations).

When using the OxyLite probe, glucose infusion led to a statistically significant increase in tumor PO2 of ~6 mmHg. This glucose-induced increase was not artifactual, because the probe did not respond to glucose in vitro tests (data not shown). When measured with microelectrodes, the mean PO2 increase was near 3 mmHg, but the change was not statistically significant. The difference in the results obtained with the two probes can again most likely be explained by a difference in the measurement area of the probes. The microelectrode measures PO2 in a very local area of the tumor. In 8 of the 11 microelectrode experiments, an increase in PO2 was measured at some point after glucose infusion (Fig. 5A). Half of the time the magnitude of the increase was small (<5 mmHg). If we assume that O2 consumption decreases after glucose, then we would expect the microelectrode PO2 to increase. However, if the drop in consumption were ac-
companied by a local decrease in blood flow (i.e., red blood cell flux), tumor PO2 might decrease (21). Hyperglycemia has been shown to increase red blood cell rigidity, increase blood viscosity, and decrease tumor blood flow (36). Although the 1 g/kg dose of glucose used in this study resulted in no change in tumor blood flow as measured by laser-Doppler flowmetry (S. A. Snyder et al., unpublished observations), there may well have been subtle changes at the microregional level. If the overall effect of this dose of glucose decreases O2 consumption and does not change tumor blood flow, then one would expect a PO2 increase if PO2 is averaged over a large area. This is the case with the OxyLite probe, which samples an area including interstitium and blood vessels. It should be remembered that vascular PO2 in the tumor would be expected to increase after glucose infusion because less O2 would be extracted from the blood as it flows through the tumor parenchyma. Therefore, both the increase in blood and parenchymal PO2 would contribute to the PO2 increase measured by the OxyLite.

**Tumor PO2 response to 100% O2 breathing: OxyLite vs. microelectrode.** Because the presence of O2 is important to the efficacy of radiation therapy, many studies have been performed in an attempt to increase tumor oxygenation. Most of the techniques have involved increasing the O2 supply to the tumor by in-
creasing the O$_2$ content of the blood. One of the simplest methods of increasing blood O$_2$ content is having the animal or patient breathe 100% O$_2$, and this has been studied as a potential means of increasing tumor PO$_2$ levels (34).

Most studies looking at O$_2$ changes in experimental tumors in response to 100% O$_2$ breathing have measured tumor PO$_2$ histograms with the Eppendorf electrode system and have either shown an increase in median tumor PO$_2$ and a decrease in hypoxic fraction (34, 26) or no change in median PO$_2$ or hypoxic fraction (2) during hyperoxia. In one study (34), transient changes at a single point were also measured using a catheter PO$_2$ electrode with an unspecified diameter. Tumor PO$_2$ rose from near 10 mmHg to ~70 mmHg during 100% O$_2$ breathing. That result is similar to the OxyLite results in the current study. The OxyLite probes measured a mean increase in PO$_2$ of over 25 mmHg during 100% O$_2$ breathing (Fig. 6B). In all eight experiments, PO$_2$ increased (Fig. 7B). In contrast to this, microelectrodes measured a hyperoxia-induced increase in PO$_2$ in only 7 of 10 experiments (Fig. 7A), and the mean increase was only ~10 mmHg. Once again, this discrepancy in PO$_2$ response to hyperoxia can most likely be attributed to differences in the sampling characteristics of the two probes. The microelectrode measures PO$_2$ in a small region of the tumor. If the probe is near a blood vessel, there is a chance that the local PO$_2$ will increase in response to hyperoxia. If the microelectrode is in a region at some distance from a vessel or near a vessel far from the arteriolar input, the PO$_2$ might not change. The increased O$_2$ content of the arterial blood may make no difference in that region of the tumor, if all of the O$_2$ has been lost by the time it reaches that location. This extreme longitudinal O$_2$ gradient in tumors due to limited arteriolar input makes improving oxygenation at all locations within some tumors extremely difficult (9). This situation would be exacerbated if tumors increase O$_2$ consumption in response to hyperoxia (8).

In the case of the OxyLite probe, it measures a larger area of tumor, including both vascular and interstitial components. Therefore, at least most of the vascular portion of the PO$_2$ field over which it averages will show an increase during 100% O$_2$ breathing. This would result in an overall increase in PO$_2$ measured by the OxyLite probe. The heterogeneity in tumor response measured by the microelectrode may explain why breathing of high O$_2$ content gases has shown little benefit when used in combination with radiation therapy in this model (1).

Implications of Differences Between Microelectrode and OxyLite PO$_2$ Measurements

The results of this study point out the importance of interpreting O$_2$ data based upon a knowledge of what exactly is being measured by the device. This appears to be particularly true for a tissue with a very heterogeneous PO$_2$ distribution, such as a tumor. The major difference in the two systems is the averaging area of the two probes. Because the OxyLite averages over an area several hundred times larger than the microelectrode, it tends to smooth out some of the heterogeneity in the PO$_2$ distribution. This was particularly evident in the histograms of the spleen (Fig. 1), in which the distribution was narrowed and fewer high PO$_2$ values were recorded by the OxyLite. Despite the averaging, however, it is important to note that there were no significant differences between the distributions obtained by the microelectrode and the OxyLite in normal tissues. The averaging created significant differences only when histograms in the tumor were measured. Because the tumor is known to be hypoxic and have a heterogeneous PO$_2$ distribution, it presents a much different situation than found in normal tissues. The effect of averaging in this tissue was to register many more severely hypoxic PO$_2$ values with the OxyLite than with the microelectrode (Figs. 3 and 8). It is very important to note, however, that there was no difference in the percent of PO$_2$ values ≤5 mmHg (Table 1). Thus in a severely hypoxic tissue like tumor, the OxyLite might tend to give an overestimate of the severely hypoxic fraction.

The effect of the measurement device on interpretation of the data is even more evident in the transient responses, i.e., the response of tumor PO$_2$ to glucose infusion and 100% O$_2$ breathing. When PO$_2$ was evaluated with the microelectrode, both of these experiments showed that the response of tumor PO$_2$ to the systemic changes was heterogeneous within the tumor. In some portions of the tumor, the PO$_2$ increased as predicted, whereas in others there was no change or even a decrease. When the PO$_2$ response was measured with the OxyLite, all of the heterogeneity was lost. There was an increase in tumor PO$_2$ in every case. Although this was consistent with the overall trend seen with the microelectrodes, small pockets within the tumor did not show this same result. Therefore, whereas the OxyLite is extremely useful in measuring overall changes in tumor oxygenation at a regional level, it is important to remember that more subtle changes may be occurring at a microrregional level.

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