

# Cellular pH Gradient in Tumor *versus* Normal Tissue: Potential Exploitation for the Treatment of Cancer<sup>1</sup>

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## Abstract

Although limited data exist, electrode-measured pH values of human tumors and adjacent normal tissues, which are concurrently obtained by the same investigator in the same patient, consistently show that the electrode pH (believed to primarily represent tissue extracellular pH) is substantially and consistently lower in tumor than in normal tissue. In contrast, the <sup>31</sup>P-magnetic resonance spectroscopy estimated that intracellular pH is essentially identical or slightly more basic in tumor compared to normal tissue. As a consequence, the cellular pH gradient is substantially reduced or reversed in tumor compared to normal tissue: in normal tissue the extracellular pH is relatively basic, and in tumor tissue the magnitude of the pH gradient is reduced or reversed. This difference provides an exploitable avenue for the treatment of cancer. The extent to which drugs exhibiting weakly acid or basic properties are ionized is strongly dependent on the pH of their milieu. Weakly acidic drugs which are relatively lipid soluble in their nonionized state may diffuse freely across the cell membrane and, upon entering a relatively basic intracellular compartment, become trapped and accumulate within a cell, leading to substantial differences in the intracellular/extracellular drug distribution between tumor and normal tissue for drugs exhibiting appropriate pK<sub>a</sub>s.

## Introduction

Evidence accumulated over the past 50 years and more has shown that electrode-evaluated human tumor pH is on average, lower than the pH of normal tissues (1). Few strategies, however, have been successfully developed to exploit this pH difference for the treatment of cancer. Two factors have hampered the exploitation of this difference. One factor is the overlap of electrode-measured tumor and normal tissue pH values that is observed when values obtained by various investigators are pooled and compared. This overlap appears to be due to largely undefined technical factors associated with the electrode measurement of tissue pH, as well as differences in the physiological and metabolic status of the patients at the time of the analyses. A second fundamental factor is the more recent demonstration using <sup>31</sup>P-MRS<sup>3</sup> procedures that tissue pH is broadly resolvable into two compartments: pH evaluated by electrodes primarily measures interstitial or extracellular tissue pH, whereas pH evaluated by <sup>31</sup>P-MRS primarily reflects the aggregate pHi of tissue. The MRS analyses show that the pHi of tumor and normal tissue are similar, *i.e.*,  $\pm$  approximately 0.1–0.2 pH units.

Most studies designed to exploit the relative acidity of tumor *versus* normal tissue have been based on the electrode pH data showing that

tumors are acidic, with no distinction between the intracellular and extracellular compartment. More recent attempts to exploit tumor acidity involve an enhancement of intracellular acidity (by disruption of the cellular pH-regulating mechanisms), which leads to cell death at sufficiently low pH (2–4). As discussed in this article, the relative acidity of the extracellular/interstitial milieu of tumors compared to normal tissue, along with their invariant pHi, gives rise to a pH gradient difference between these tissues. This gradient difference provides a basis for the selective treatment of cancer.

## Materials and Methods

### pH of Normal and Tumor Tissue

**pHi.** Patient-matched measurements of the extracellular and pHi in both human tumor and normal tissue by the same investigator have not been reported in the literature. As discussed below, this complicates an evaluation of the cellular pH gradient of tissues due to the variability in pH values obtained with pH electrodes. For the measurement of pHi, the majority of values have been obtained using <sup>31</sup>P-MRS. Measurement of pH by MRS is largely standardized, provides accuracy of  $\pm 0.1$  pH units, and is noninvasive (5). Although both the intracellular and extracellular compartments of tissue contain phosphate, because of the relative size of the intracellular compartment and the relative concentration of phosphate in this compartment, pH measured using <sup>31</sup>P-MRS primarily reflects the aggregate pHi of tissue.

A summary of the pHi values in tumors of various histology and three normal tissues is illustrated in Fig. 1. Each of the indicated values is the average for several tumors obtained by one or more investigators (6–15). The results are similar to those obtained in the extensive compilation of tissue pHi values compiled by Vaupel *et al.* (16). The pHi of tissues is relatively constant, ranging from approximately 7.1 to 7.3 for the various tumor types and largely overlap those obtained in three normal tissues, *i.e.*, 7.0–7.2. Values obtained in tumors of the same histology by the same investigator exhibit somewhat more variability ( $\pm 0.1$  pH units) than is obtained in similar studies of the same normal tissue ( $\pm 0.05$  pH units; Refs. 7 and 12). Limited studies indicate that the pHi of tumor tissue is slightly more basic than that obtained in normal tissue (7, 12). In summary, these data indicate that the pHi of tumors and normal tissues is similar and well regulated within  $\pm 0.1$ –0.2 pH units or less.

**pHe of Tumor and Normal Tissue and the Cellular pH Gradient.** As shown in a comprehensive review of the literature by Wike-Hooley *et al.* (1), the electrode-measured pH values in human tumors are on average approximately 0.4 units lower than those observed in normal subcutaneous and muscle tissues. However, substantial heterogeneity and overlap in the reported pH values of these tissues is apparent (1, 17, 18). Of special relevance to the present topic is the range of electrode pH values reported for the same normal tissue. Table 1 shows the mean and SD of measured electrode pH values of subcutaneous tissue by four different investigators. For the same normal tissue, the pH variation between investigators is greater than the pH variation between patients analyzed by the same investigator (19–22). Differences in electrode calibration, electrode stability, local tissue damage at the site of electrode insertion, and the physiological and metabolic status of the patients may all contribute to the observed differences. By considering pH values obtained in both normal and tumor tissue, with the same electrode, at the same time, the interexperimental variation can be eliminated from the calculation of the difference in the pH of tumor and normal tissue. Few studies meet these criteria.

The electrode pH values obtained in 20 patients with glioblastoma is

Received 10/20/95; accepted 1/25/96.

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<sup>1</sup> Supported by National Cancer Institute Grant CA22860.

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<sup>3</sup> The abbreviations used are: MRS, magnetic resonance spectroscopy; pHi, intracellular pH; pHe, extracellular pH.

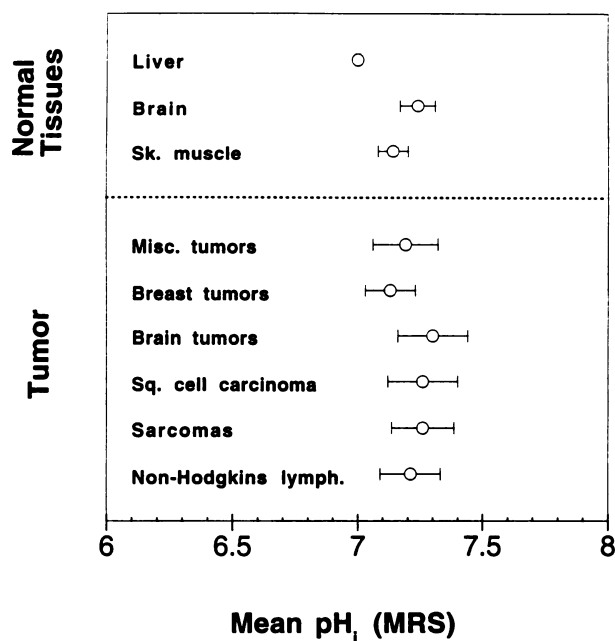


Fig. 1. The  $^{31}\text{P}$ -MRS estimated  $\text{pH}_i$  of various human normal tissues and tumors. Confidence intervals are 1 SD. The data for liver are from Oberhaensli *et al.* (7); for brain from Oberhaensli *et al.* (7) and Hubesch *et al.* (13); for resting skeletal muscle from Sostman *et al.* (13), Semmler *et al.* (9), and Nidecker *et al.* (10); for miscellaneous tumors from Oberhaensli *et al.* (7) and Ng *et al.* (8); for breast tumors from Sijens *et al.* (6) and Oberhaensli *et al.* (7); for brain tumors from Oberhaensli *et al.* (7) and Hubesch *et al.* (14); for squamous cell carcinomas from Ng *et al.* (8); for sarcomas from Sostman *et al.* (12, 13), Dewhirst *et al.* (11), Nidecker *et al.* (10), and Semmler *et al.* (9); and for non-Hodgkin's lymphoma from Ng *et al.* (8) and Smith *et al.* (15).

matched with the pH values obtained in the adjacent normal brain of the same patients (Fig. 2A; Ref. 23). In 18 of 20 cases, the electrode-measured pH values of glioblastomas are equal to or less than those obtained in adjacent normal brain. In addition to these studies, Pampus (23) also measured the pH in 11 patients with astrocytomas and adjacent normal brain (Fig. 2B). In all 11 patients, the tumor pH was equal to or lower than the pH of the normal tissue. Similar results were obtained by Naeslund and Swenson (24), who measured the tissue pH in uterine cancer and normal tissue (Fig. 2C), and Ashby and Cantab (25) in patients with melanoma (Fig. 2D). For the four sets of data shown in Fig. 2, the matched pH values in the tumor were equal to or lower than those obtained in normal tissue in 40 of 42 cases; the mean pH difference being  $0.41 \pm 0.27$  as is observed in comprehensive reviews of unmatched data (1, 17, 19). However, in contrast to these pooled data compilations, matching of the measured pH values for investigator and patient, and time of analysis, markedly reduces the overlap of pH values between tumor and normal tissue. The relatively invariant and similar  $\text{pH}_i$  of tumor and normal tissue and substantially reduced  $\text{pH}_e$  of tumor compared to normal tissue gives rise to a substantially different cellular pH gradient in these tissues. For an average  $\text{pH}_i$  of 7.2 for both tumor and normal tissue and an  $\text{pH}_e$  of 7.4 in normal tissue and 6.8–7.2 in tumor tissue (Fig. 1; Refs. 1, 17, 18, and 25), the average difference between the extracellular and  $\text{pH}_i$  is approximately +0.2 pH units in normal tissue and –0.2 to –0.6 in tumor tissue.

All drugs exhibit neutral, acidic, or basic properties. For drugs which are weak acids (or bases), the extent to which they are ionized is exponentially related to the pH of their milieu. As the presence or absence of charge on a molecule will influence its lipophilicity, slight differences in pH may markedly influence the ability of these drugs to traverse the cell membrane and the intracellular/extracellular equilibrium distribution of the drug.

#### Drug Charge and Drug Transport

The principal barrier to the entry of a drug into an intracellular site of action is the cell membrane. With the exception of the blood-brain barrier, the

vascular wall does not substantially impede the extravasation of biomolecules whose molecular weight is a few thousand or less (26). Similarly, in the absence of binding, drugs of molecular weight of approximately  $M_r$  10,000 or less freely diffuse (similar to water) in the interstitium (27). Entry of a drug into the cell may occur via either carrier or noncarrier-mediated processes (diffusion), and, commonly, membrane transport occurs by both mechanisms. Both inward and outward diffusion may occur simultaneously and independently of carrier-mediated transport, and under certain circumstances, become the predominant mechanism of transport (28).

For noncarrier-mediated molecules (commonly those which are not analogues of naturally occurring biomolecules), diffusion is the sole mechanism of transport. Diffusion across a non-polar lipid barrier is dependent on the lipid solubility or polarity of the diffusing molecule. Ionization substantially decreases lipid solubility and diffusivity. A wide variety of naturally occurring biomolecules (amino acids, proteins, nucleic acids, ATP, etc.) as well as therapeutics are weakly acidic or basic and are therefore charged or uncharged depending on the pH of their microenvironment.

Following drug extravasation across the vessel wall into a relatively acidic extracellular tumor environment, the fraction of a weak acid which is charged decreases, resulting in an increased ability to diffuse across the cell membrane. If the  $\text{pH}_i$  is relatively basic, ionization of the weak acid increases, leading to a decreased membrane permeability and trapping in the relatively basic compartment. Assuming that an undissociated weak acid freely passes between the intracellular and extracellular compartment, and the charged molecule does not, then as shown by Roos and Boron (29), the ratio of the intracellular: extracellular drug concentration of both the charged and uncharged form is:

$$C_i/C_e = (1 + 10^{\text{pH}_i - \text{pK}_a}) / (1 + 10^{\text{pH}_e - \text{pK}_a}) \quad (\text{A})$$

where  $C_i$  and  $C_e$  are the intracellular and extracellular drug concentrations, respectively, and  $\text{pK}_a$  is the negative logarithm of the drug dissociation constant (the pH at which 50% of the drug is dissociated). A similar expression describes the behavior of weak bases. Because of the exponential relationship between the cellular drug concentration and  $\text{pH}_e$ ,  $\text{pH}_i$ , and  $\text{pK}_a$ , small differences in any of the parameters may markedly effect the drug concentration ratio.

#### Results

**pH Gradient,  $\text{pH}_e$ , and Drug Uptake.** As indicated in Table 1 and Fig. 2, literature reported electrode pH values of human s.c. tissue vary significantly. In spite of this variability, the  $\text{pH}_e$  difference between tumor and normal tissues is substantial and relatively invariant when concurrently measured by the same investigator in the same patient (Fig. 2). Over a relevant pH range, the magnitude of the pH gradient across the cell membrane and not the absolute pH values provides the driving force for the selective distribution of weak acids and bases. For example, assuming the  $\text{pH}_e$  of normal tissue ranges from 7.6 to 7.2, with the tumor  $\text{pH}_e$  being 0.4 units lower, the ratio of the intracellular drug concentration in tumor *versus* normal tissue ranges from 2.4 to 2.3 (based on a  $\text{pH}_i$  of 7.2 in both tissues,  $\text{pK}_a = 6.0$ , Equation A). Substantial variation in  $\text{pH}_e$  does not significantly impact the expected preferential uptake of weak acids ( $\text{pK}_a < 6$ ) in tumor compared to normal tissue.

Fig. 3A illustrates the relationship between the calculated intracellular and extracellular drug concentration at variable  $\text{pH}_e$ , assuming the drug is a weak acid with a  $\text{pK}_a$  of 7.0, and the  $\text{pH}_i$  is 7.2. Under

Table 1 Electrode estimated pH of subcutaneous tissue

Measured pH values of human subcutaneous tissue obtained by various investigators. Differences in patients' age, physiology, electrode characteristics, and measurement procedures likely account for the observed differences. From Wike-Hooley *et al.* (1).

Investigator	Sample size	Mean pH $\pm$ SD
van den Berg <i>et al.</i> (19)	26	7.63 $\pm$ 0.17
Harrison and Walker (22)	40	7.54 $\pm$ 0.09
Stamm <i>et al.</i> (20)	10	7.42 $\pm$ 0.05
Vidyasagar <i>et al.</i> (21)	11	7.33 $\pm$ 0.03

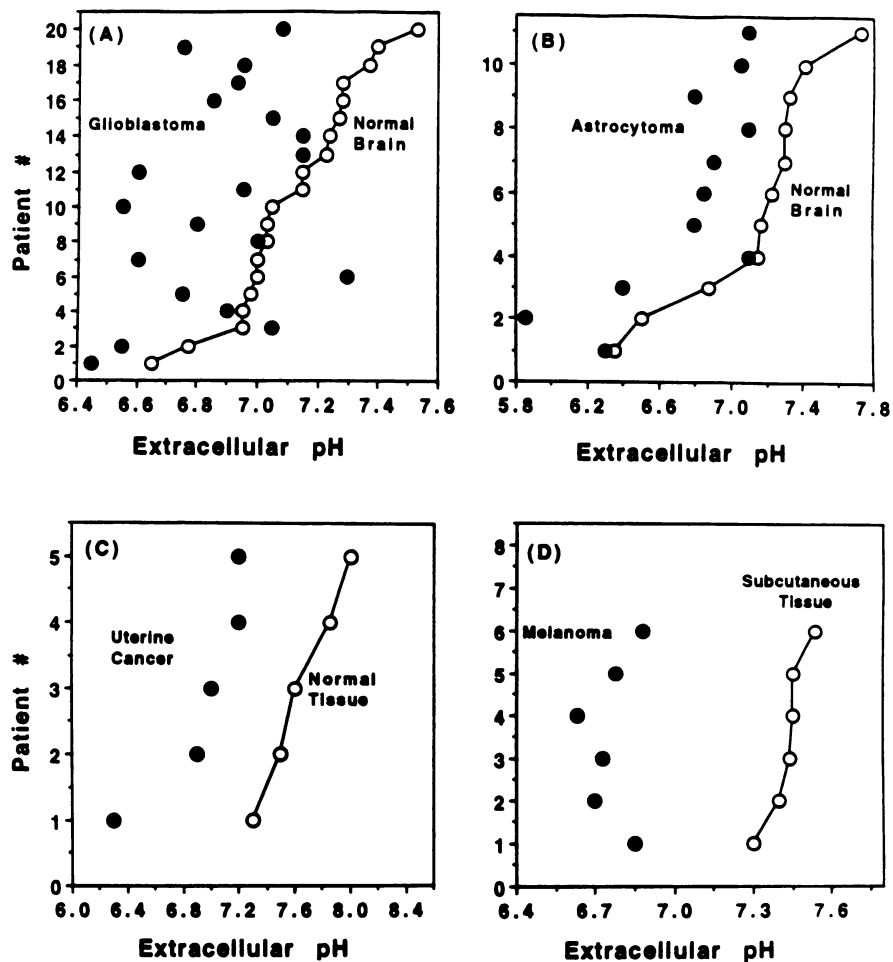


Fig. 2. pH electrode measured pH<sub>e</sub> values concurrently obtained in tumors and normal tissue in the same patient. For glioblastoma (A) and astrocytoma (B), the data are from Pampus (23), for uterine cancer (C) from Naeslund and Swenson (24), and for melanoma (D) from Ashby and Cantab (25).

basic pH<sub>e</sub> conditions, the fraction of the drug that becomes ionized and confined to the extracellular compartment predominates. As the pH<sub>e</sub> approaches the pK<sub>a</sub> of the drug, an increasing fraction of the drug loses its charge, rendering it free to diffuse across the cell membrane. Upon entering the relatively basic intracellular compartment, the drug becomes ionized and trapped, leading to an increased intracellular concentration.

**pK<sub>a</sub> and Drug Uptake.** The influence of pK<sub>a</sub> on the calculated cellular drug distribution ratios is shown in Fig. 3B. Two examples are illustrated. In both cases the pH<sub>i</sub> is assumed to be 7.2: the pH<sub>e</sub> is assumed to be 6.8 in the upper curve and 7.4 in the lower curve. Very weak acids (pK<sub>a</sub> > 9) are essentially nonionized under physiological pH conditions. However, for pK<sub>a</sub>s which are similar to the pH of their milieu, small differences in pH markedly effect the extent of ionization. For a pK<sub>a</sub> of 5 (Fig. 3B, upper curve) ionization is greater at 7.2 than 6.8, and the drug becomes trapped in the compartment in which it is ionized. Similarly, at an pH<sub>e</sub> of 7.4, a greater portion of the weak acid is ionized and confined to the extracellular compartment.

A number of studies have investigated drug partitioning into artificial lipid vesicles and cells as a function of pH and drug pK<sub>a</sub> (30–32). In addition to pH and pK<sub>a</sub>, several additional factors have been shown to affect the predicted intravesicular:extravesicular (or cellular) concentration ratios. pK<sub>a</sub> is influenced by factors such as the solvent in which it is dissolved, ionic strength, and temperature (29, 32). Additionally, the numerical value of the predicted distribution

ratio does not precisely match the observed distribution if the ionized drug is not completely membrane impermeable or is rapidly metabolized or sequestered in the intracellular compartment. Nevertheless, systematic *in vitro* evaluation of cellular drug uptake as a function of drug pK<sub>a</sub> and pH yields results which are substantially consistent with theory. Dennis *et al.* (31) measured the intracellular:extracellular distribution of misonidazole and weak acid and base analogues of misonidazole in V79 cells. Results from their studies are shown in Table 2. In accordance with theory, for the neutral drug misonidazole at equilibrium, the measured intracellular concentration was uninfluenced by pH. Also in accordance with theory, the intracellular concentration of the weak acid azomycin was higher at an pH<sub>e</sub> of 6.6 than at 7.6 (identical extracellular drug concentration). Similarly, as predicted for the weak base Ro 03899, the intracellular concentration was higher at pH 7.6 than 6.6. Although the observed distribution ratios do not match the predicted (calculated) ratios calculated on the basis of the drug pK<sub>a</sub> and the experimentally estimated pH<sub>i</sub>, the impact of the pK<sub>a</sub> on the cellular distribution of these analogues is readily apparent and substantial.

## Discussion

Although the cellular pH gradient differs in tumor and normal tissue, the pH<sub>e</sub> and, therefore, the magnitude of the gradient within a

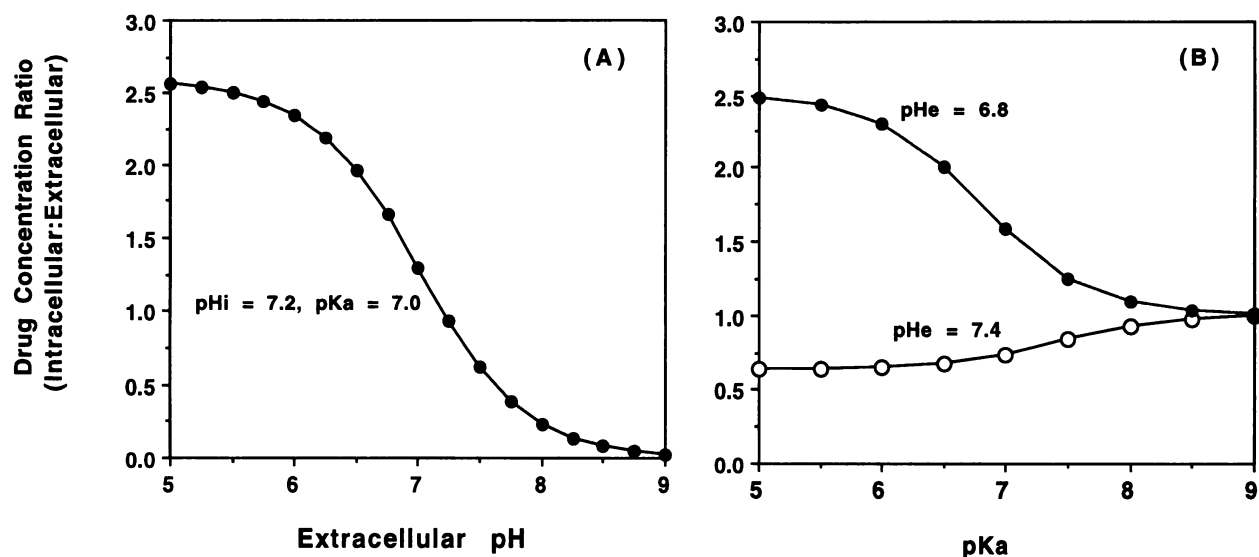


Fig. 3. A, calculated effect of variable  $pH_e$  on the ratio of the intracellular:extracellular distribution of a weak acid. For the example shown, the  $pH_i$  is 7.2, and the drug  $pK_a$  is 7.0. B, calculated effect of variable  $pK_a$  on the ratio of the intracellular:extracellular concentration of a weak acid. For the example shown, the  $pH_i$  is 7.2, and the  $pH_e$  is 6.8 or 7.4.

particular tumor are not uniform. Studies with miniature pH electrodes show that the pH within a tumor may vary from values which are similar to those in normal tissue to substantially more acidic values (1, 33). Most likely, the tumor  $pH_e$  decreases along the length and as a function of the radial distance from the supplying arterial vessel. Substantiation of this possibility is indicated by the studies of Martin and Jain (34), who demonstrated a decrease in pH over a range of  $<50 \mu m$  radially from supplying vessels in a rabbit ear chamber model. As the pH probe employed in these studies was a weak acid, the observed changes in tumor tissue likely underestimated the actual  $pH_e$  decrease radially from the supplying vessel. Nevertheless, these observations are consistent with the expectations that the  $pH_i:pH_e$  gradient may be expected to increase in those cells most distal from the supplying blood vessel. The overall effect would be to enhance drug uptake and killing of cells which are normally exposed to the lowest drug concentration, and especially relevant to radiation therapy, to low concentrations of oxygen.

Although several chemotherapeutics exhibit acidic or basic properties, few exhibit acidic properties with  $pK_a$ s in the range of 4.5–6.5, *i.e.*, the range that would appreciably enhance cellular uptake of the drug in tumor tissue. Not only could weak acids enhance drug uptake in the less accessible and resistant portions of a tumor, but weak bases of the appropriate  $pK_a$ s may be used to enhance the uptake of drugs such as radioprotectors in normal tissues. Other conceptually similar

possibilities for exploiting the pH gradient exist. Using an *in vitro* cell system, Jensen *et al.* (35) showed that the weak base chloroquine, an etoposide antagonist, virtually eliminated etoposide cytotoxicity at an  $pH_e$  of 7.4, but was excluded from cells at an  $pH_e$  of 6.5, resulting in a pronounced etoposide cytotoxicity.

The pH gradient difference between tumor and normal tissue provides a strong rationale for the design and evaluation of the efficacy of drugs as a function of their  $pK_a$ s and the cellular pH gradient. A challenging but appropriate aspect of this evaluation is the development and utilization of experimental tumor models and procedures for the evaluation of tumor and normal tissue toxicity as a function of the tissues' pH gradient and drug  $pK_a$  (36–38).

#### Acknowledgments

We thank Drs. Bruce Chabner, Yves Boucher, Claus Kristensen, and Fan Yuan for their helpful suggestions during the preparation of the manuscript.

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Table 2 Measured and calculated intracellular concentration ratios of misonidazole and acidic or basic analogues

Measured and calculated intracellular concentration ratios of misonidazole and acidic or basic analogues. Values measured in V79 cells under hypoxic conditions at a constant extracellular drug concentration for the various analogues. The calculated intracellular drug concentration is based on the experimentally estimated  $pH_i$ . From Dennis *et al.* (31).

Analogue	$pK_a$	Intracellular concentration ratio	
		$(pH_e = 6.6/pH_e = 7.6)$	
		Observed <sup>a</sup>	Calculated <sup>b</sup>
Misonidazole	Neutral	1.0	
Azomycin (acid)	7.2	2.2	1.5
Ro 03899 (base)	8.9	0.22	0.36

<sup>a</sup> From Dennis *et al.* (31).

<sup>b</sup> Calculated concentration ratios based on the measured  $pH_i$  of approximately 6.87 at  $pH_e = 6.6$ , and  $pH_i$  of 7.45 at  $pH_e = 7.6$ .

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