The In Vivo pH of the Extravascular Space of the Lung

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ABSTRACT The partition of 5,5-dimethyloxazolidine-2,4-dione (DMO) and of 11 amines between the vascular and extravascular spaces of the lung has been determined by the multiple indicator dilution technique. Four amines (nicotine, pentylamine, quinine, and benzylamine) were found to have pH-sensitive tissue to blood concentration ratios. Of these, tritiated nicotine appears to be the most satisfactory indicator of tissue pH and values for the pH of the pulmonary extravascular space (pH_e) have been calculated from the nicotine data. At an arterial pH (pH_{art}) between 7.38 and 7.43 pH_e averaged 6.69 $\pm$0.07.

Changes in pH_e usually paralleled but were consistently less than concomitant changes in pH_{art}. Alterations in P_{CO_2} at constant pH_{art} regularly produced relatively small, parallel changes in extravascular hydrogen ion concentrations. Local alterations in tissue pH due to P_{CO_2} changes are apparently buffered quite rapidly and the pH_e of the lung seems more closely linked to pH_{art} than the cellular pH of other tissues.

DMO, guanidine, methylamine, morphine, and atropine were confined to the vascular volume during the first circulation and could not be used to measure tissue pH. Histamine appeared to be bound to a pH-insensitive site. The extravascular distributions of antipyrine and aniline were unresponsive to alterations in arterial pH, presumably because they are essentially uncharged at pH levels found in the lung.

INTRODUCTION

Measurements of intracellular pH have been obtained directly with microelectrodes (1) and indirectly with a variety of weak acids and bases ("pH indicators") (2). The latter technique avoids cellular damage but requires selection of an indicator which is disbributed between cellular and extracellular water in accordance with its dissociation constant and the concentration gradient of hydrogen ion across the cell membrane. It is assumed that the cell membrane is permeable only to the unionized form of the indicator and that the indicator remains in the aqueous phase, is not bound to protein, and is not actively transported. Provided that an indicator behaves in this fashion, the cellular pH may be calculated from the indicator pK_a', the pH of the surrounding fluid and the ratio of the indicator concentration within the cell to that in the extracellular fluid. Because they appear to satisfy these requirements, carbon dioxide (3) and more recently 5,5-dimethyloxazolidine-2,4-dione (DMO) (4) have been the most frequently used indicators of intracellular pH.

The necessity of measuring indicator concentration ratios between cells and surrounding fluid has limited indirect measurements of pH to whole body determinations or in vitro tissue investigations. In the present study a method for determining tissue pH in vivo is presented and is utilized to measure the pH of the extravascular space of the lung. This method is based upon the sudden injection mutliple indicator dilution technique. Generalization of this approach provides the means of determining steady-state gradients of indicator concentration between tissue compartments in perfused organs (5,6). The distributions of 12 ionizable indicators between blood and the pulmonary parenchyma have been studied in this fashion. Of these, tritiated nicotine appears to be the most suitable for pH measurements in the lung. Accordingly, values for the pH of the pulmonary extravascular space have been calculated from data obtained with this indicator.

Studies in brain (7), skeletal muscle (8, 9), and kidney (10) suggest that alterations in carbon dioxide tension have a more rapid and intense effect on cellular pH than corresponding metabolic changes. The direct contact of pulmonary cells with alveolar gas might therefoer

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expose them to potentially harmful fluctuations in cellular pH. In the present study, evidence has been obtained suggesting that changes of CO_2 tension within the pulmonary tissue are rapidly compensated if a normal arterial pH is maintained.

METHODS

A bolus containing 1–2 ml of a mixture of several indicators is rapidly injected into the jugular vein of a dog. Blood is collected in serial samples from the carotid artery. Included in the injection mixture is a vascular indicator (T-1824), a water indicator (labeled water or antipyrine), and a potential pH indicator (designated "Y"). Indicator concentrations are measured in the collected samples. These values are divided by the quantity of indicator injected and plotted on a logarithmic scale against time on a linear scale. Correction for recirculation is performed by linear extrapolation on these coordinates. The areas under the corrected indicator curves (on linear coordinates) are used to measure blood flow and indicator recoveries. The mean transit time ($\bar{t}$) of each indicator is calculated, permitting evaluation of the vascular and extravascular volumes of the lung.

Conventional extracellular indicators fail to leave the pulmonary capillaries in significant quantities during a single circulation (11–13). It has therefore been impossible to measure the interstitial volume of the lung in these experiments, and the interstitial and cellular spaces are lumped together in the extracellular volume. The symbols $[Y]_e$ and $[Y]_p$ designate the concentrations of a pH indicator Y, which would prevail in the pulmonary extravascular and pulmonary spaces during a prolonged constant infusion of Y.

It is shown below that the steady-state ratio $[Y]_e/[Y]_p$, which would be attained during a constant infusion of Y, is equal to ρ where

$$\rho = \frac{\bar{t}_Y - \bar{t}_{T-1824}}{\bar{t}_{THO} - \bar{t}_{T-1824}}.$$

This estimate does not take into account differences in the concentration of Y within plasma and red cells or mean transit time differences between red cells and plasma labels. The relationship of ρ to arterial pH has been studied for each of the potential pH indicators to determine which indicators have pH-sensitive distributions between blood and tissues.

Data obtained with tritiated nicotine have been used to calculate the pH of the extravascular space of the lung. The steady-state distribution ratio of nicotine between tissues and plasma, $[Nic]_e/[Nic]_p$, is calculated from a modified parameter ρ_c, where

$$\rho_c = \frac{B\bar{t}_{Nic} - C\bar{t}_{T-1824}}{D\bar{t}_W - E\bar{t}_{T-1824}}.$$

The factors B, C, D, and E are defined below. The parameter ρ_c includes corrections for the concentration gradient of nicotine between red cells and plasma (see Appendix), the water content of plasma and red cells, and the more rapid transit of red cells than plasma through the pulmonary capillaries.

Theory

Calculation of the concentration ratio $[Y]_e/[Y]_p$. The theoretical basis for flow and volume measurements by the indicator dilution technique has been comprehensively presented by Zierler (14). Extension of these principles to the measurement of compartmental distribution ratios is discussed in recent publications (5, 6). In the present discussion the following symbols are used (characteristic units are given in parentheses):

Y = pH indicator
W = water indicator (THO or antipyrine-^{14}C)
R = red cell indicator
P = plasma indicator (T-1824)
$M_{Y,o}$, $M_{W,o}$, $M_{R,o}$, $M_{P,o}$ = amount (mass) of Y, W, R, and P in the organ between the points of injection and sampling during constant infusion (grams). $M_{Y,r}$, $M_{Y,p}$, $M_{Y,e}$ refer to the amount of Y in the red cell, plasma, and extravascular compartments during constant infusion.
I_Y, I_W, I_R, I_P = flux of Y, W, R, and P into the organ during constant infusion (grams/second)
f_r, f_p = fractional volumes of water in red cells and plasma (milliliter water/milliliter red cells or plasma)
V_r, V_p = red cell and plasma volumes (milliliter of red cells or plasma)
$V_{w,o}$, $V_{w,r}$, $V_{w,p}$, $V_{w,e}$ = volume of water in entire organ, red cells, plasma, and extravascular space (milliliter water)
$[Y]_r$, $[Y]_p$, $[Y]_e$ = concentrations of Y in $V_{w,r}$, $V_{w,p}$, and $V_{w,e}$ during constant infusion (grams/milliliter water or moles/liter water)
F, F_r, F_p = flow of blood, red cells, and plasma into organ (milliliter/second)
Hct = peripheral hematocrit (millimeter/millimeter)
$\bar{t}$ = mean transit time (second).

Consider an organ with a single vascular inlet and a single vascular outlet which is being perfused at a constant rate with an indicator. (We shall assume that the pulmonary artery represents the inlet and the pulmonary vein, the outlet. The contribution made by the bronchial circulation to pulmonary blood flow will be considered insignificant. Similarly, the error introduced by assuming that injection into the jugular vein is equivalent to injection into the entire flow of blood into the lung is also considered insignificant.) Assume that a steady-state condition is attained in which blood flow is constant and the amount ($M_{X,o}$) of indicator within the organ is also constant. $M_{X,o}$ can be determined directly by clamping the vessels, removing the organ, and measuring the amount of indicator present. Alternatively, $M_{X,o}$ may be determined indirectly with a sudden injection experiment. A bolus of the indicator X is introduced suddenly into the artery and the mean transit time ($\bar{t}_X$) of indicator from the injection point to the collection site is determined. Provided that the indicator dilution curve is not influenced by the dose of indicator injected and does not vary with time, and provided that all of the injected material is recoverable in the venous outlet, the following equation must hold during constant infusion of X:

$$M_{X,o} = I_X\bar{t}_X. \quad (1)$$

Thus

$$M_{R,o} = I_R\bar{t}_R \quad (2)$$

$$M_{P,o} = I_P\bar{t}_P \quad (3)$$

$$M_{W,o} = I_W\bar{t}_W \quad (4)$$

$$M_{Y,o} = I_Y\bar{t}_Y. \quad (5)$$

If the concentration of an indicator X within its distribution volume (V) becomes homogeneous at infinite time during constant infusion, then

$$V = F\bar{t}_X. \quad (6)$$

We shall assume that the densities of red cells, plasma, and water within the organ are constant and that labeling of these compartments by the red cell, plasma, and water indicators

becomes homogeneous during constant infusion of each indicator. Then

$$V_r = F_r i_R = HctF i_R \quad (7)$$

$$V_p = F_p i_P = (1 - Hct)F i_P \quad (8)$$

$$V_{w,o} = F_w i_W = \{f_r(Hct) + f_p(1 - Hct)\}F i_W. \quad (9)$$

It is assumed that f_r and f_p are constant throughout the organ. Thus

$$V_{w,r} = f_r HctF i_R \quad (10)$$

$$V_{w,p} = f_p(1 - Hct)F i_P \quad (11)$$

$$V_{w,e} = V_{w,o} - (V_{w,r} + V_{w,p}) = \{f_r(Hct) + f_p(1 - Hct)\}F i_w - \{f_r(Hct)F i_R + f_p(1 - Hct)F i_p\}. \quad (12)$$

Let us assume that Y is distributed into three aqueous compartments within the organ: red cell, plasma, and extravascular water. Moreover, let us assume that during constant infusion its concentration within each is homogeneous, though possibly heterogeneous between compartments. Then from equation 5

$$M_Y = I_Y i_Y = M_{Y,r} + M_{Y,p} + M_{Y,e} = [Y]_r V_{w,r} + [Y]_p V_{w,p} + [Y]_e V_{w,e}. \quad (13)$$

It is obvious that

$$I_Y = \{[Y]_r f_r(Hct) + [Y]_p f_p(1 - Hct)\}F. \quad (14)$$

Substituting equations 10, 11, 12, and 14 into 13 and dividing by F yields the equation

$$\{[Y]_r f_r(Hct) + [Y]_p f_p(1 - Hct)\} i_Y = [Y]_r f_r(Hct) i_R + [Y]_p f_p(1 - Hct) i_P + [Y]_e\{[f_r(Hct) + f_p(1 - Hct)] i_W - [f_r Hct i_R + f_p(1 - Hct) i_P]\}. \quad (15)$$

Solving for $\frac{[Y]_e}{[Y]_p}$:

$$\frac{[Y]_e}{[Y]_p} = \frac{\left\{\left\{\frac{[Y]_r}{[Y]_p} f_r(Hct) + f_p(1-Hct)\right\} i_Y - \left\{\frac{[Y]_r}{[Y]_p} f_r(Hct) i_r + f_p(1-Hct) i_P\right\}\right\}}{\left\{\{f_r(Hct) + f_p(1-Hct)\} i_W - \{f_r(Hct) i_r + f_p(1-Hct) i_P\}\right\}}. \quad (16)$$

The expression on the right will be designated as ρ_e to indicate that correction has been made for differences in red cell and plasma indicator concentrations and transit times.

If it is assumed that the concentration of the pH indicator is the same in red cells and plasma, and that the mean transit times of red cells and plasma are equal, then equation (16) becomes:

$$\frac{[Y]_e}{[Y]_p} = \frac{i_Y - i_P}{i_W - i_P} = \rho. \quad (17)$$

Values of ρ are plotted against arterial $[H^+]$ for each of the indicators in the present paper and were used in the previously reported calculations of pulmonary extravascular pH with tritiated nicotine (15).

The determination of ρ_e requires information in addition to the indicator mean transit times, viz, f_p, f_r, Hct, the relationship of i_R to i_P, and $[Y]_r/[Y]_p$. Values for f_p and f_r were obtained by drying dog red cells and plasma at 120°C (16) for 24 hr and measuring the fractional loss of weight (0.67 ±0.015 SD, n = 5, for red cells and 0.938 ±0.005 SD, n = 4 for plasma; drying for 18 hr at 90°C yielded similar values). The specific gravity of dog red cells at room temperature averaged 1.0944 ±0.0019 SD (n = 6), and that of plasma 1.0195 ±0.0021 SD (n = 5). Values for the fractional volumes of water in red cells and plasma were calculated from the products of the fractional water weights and the corresponding specific gravities of red cells and plasma: $f_r = 0.737$, $f_p = 0.956$. Alterations of f_r and f_p with changes in pH would play an insignificant role in calculations of tissue pH and have therefore been ignored.

Peripheral microhematocrits obtained at the time of the study were used for Hct. Calculation of organ hematocrit is implicit in equations 7 and 8. Simultaneous sudden injection studies with red cells and plasma indicators by Rapaport, Hiroshi, Haynes, and Deiter (17) and ourselves indicate that the following approximate relationship holds over the range of hematocrit values that obtained in these experiments:

$$i_R = \frac{i_P}{1.06}. \quad (18)$$

A plasma indicator (T-1824) was used exclusively in the present study, and values for i_R were obtained from equation 18. It has been assumed that the excess volume available to plasma indicators is intravascular and that the intravascular plasma volume is given by equation 8. (Recalculation of pH_{art} on the basis of a vascular volume 6% smaller than the T-1824 volume increases the average tissue pH from 6.69 to 6.75 at arterial pH levels between 7.38 and 7.43.) Tritiated nicotine (Nic) was selected as a pH indicator and in vitro studies of $[Nic]_r/[Nic]_p$ are reported in the Appendix. It is shown that the distribution of tritiated nicotine in red cells is correlated with pH gradient between red cells and plasma, and that the ratio $[Nic]_r/(Nic]_p$ may be predicted from the arterial pH with equation 3. Carbon-14-labeled antipyrine (Ant) was used as a water label in the nicotine studies.

The steady-state ratio of the nicotine concentration in the extravascular compartment to that in the vascular compartment may be calculated from the extravascular pH and the mean transit time data with the following equation:

$$\frac{[Nic]_e}{[Nic]_p} = \frac{B i_{Nic} - C i_{T-1824}}{D i_{Ant} - E i_{T-1824}} = \rho_e \quad (19)$$

where

$$B = 0.737\frac{[Nic]_r}{[Nic]_p}(Hct) + 0.956(1 - Hct)$$

$$C = 0.695\frac{[Nic]_r}{[Nic]_p}(Hct) + 0.956(1 - Hct)$$

$$D = 0.956 - 0.219(Hct)$$

$$E = 0.956 - 0.261(Hct).$$

This equation is derived from equation 16 by substituting values for f_r amd f_p and replacing values of i_R with $i_p/1.06$.

The correction introduced by using ρ_e rather than ρ is relatively small for tritiated nicotine. Values of ρ_e were greater than ρ by an average factor of 1.03 ±0.02. As indicated below, this correction resulted in a decline in the calculated average pH_e from 6.71 to 6.69.

Calculation of extravascular hydrogen ion concentration. The following assumptions and approximations are made:

(*a*) The barrier between the vascular and extravascular spaces is freely permeable to the unionized species but is impermeable to the ionized species.

(*b*) The dissociation coefficient of the pH indicator is the same in the vascular and extravascular volumes.

(*c*) The activity coefficients of hydrogen ion and the indicator species are assumed equal to unity. Brackets designate molar concentrations in aqueous solution. ([Y] was defined above in units of mass or moles per volume of water; conver-

sion to moles per liter of solution enables calculation of approximate hydrogen ion molarity. In the present study these units are assumed to be approximately equivalent.) The plasma hydrogen ion concentration is assumed homogeneous in the lung and has been estimated from arterial blood pH values determined with a glass electrode.

(*d*) No active transport or metabolism of the indicator occurs. Lipid solubility and protein binding are not significant.

Granting these assumptions, the distribution of indicator across the capillary membrane can be considered to be determined solely by the hydrogen or hydroxyl ion gradient between the plasma and extravascular compartments (see Orloff and Berliner [18]).

In the case of nicotine:

$$NicH^+ \rightleftharpoons Nic_u + H^+ \quad K_a' = \frac{[Nic_u][H^+]}{[NicH^+]} \tag{20}$$

where $NicH^+$ and Nic_u designate the ionized and unionized forms of nicotine. In the steady-state, constant infusion situation:

$$\frac{[NicH^+]_e}{[NicH^+]_p} = \frac{[H^+]_e}{[H^+]_p}. \tag{21}$$

The extravascular hydrogen ion concentration $[H^+]_e$ may be calculated from ρ_c of nicotine, the K_a' of nicotine, and the plasma concentration, $[H^+]_p$, as follows:

$$\frac{[H^+]_e}{[H^+]_p} = \frac{[Nic]_e}{[Nic]_p} = \frac{[NicH^+]_e + [Nic_u]_e}{[NicH^+]_p + [Nic_u]_p}. \tag{22}$$

It is assumed that the concentrations of the freely unionized form of nicotine are equal in the plasma and extravascular compartments.

$$[Nic_u]_e = [Nic_u]_p. \tag{23}$$

Therefore,

$$\rho_c = \frac{[NicH^+]_e + [Nic_u]_p}{[NicH^+]_p + [Nic_u]_p}$$

$$\frac{[NicH^+]_e}{[Nic_u]_e} = \rho_c\left\{\frac{[NicH^+]_p}{[Nic_u]_p} + 1\right\} - 1$$

$$\frac{[H^+]_e}{K_a'} = \rho_c\left\{\frac{[H^+]_p}{K_a'} + 1\right\} - 1$$

$$[H^+]_e = \rho_c\{[H^+]_p + K_a'\} - K_a' \tag{24}$$

Materials and procedures

The indicators used in the present study are described in Table I. For the sake of simplicity, these indicators are frequently designated by the corresponding unlabeled chemical name. The injection mixture generally contained 45.2 mg of T-1824, 1.0–2.5 mc of a substance labeled with tritium (water or a pH indicator), and 0.1 mc of a substance labeled with carbon-14 label (antipyrine or a pH indicator) in 10 ml of 0.85% saline. A 1.0–2.0 ml volume of this mixture was injected in less than 0.5 sec.

Mongrel dogs were anesthetized with intravenous sodium pentobarbital and paralyzed with intravenous succinylcholine. Ventilation was maintained via an endotracheal tube or tracheotomy tube with a Harvard respiration pump (Harvard Apparatus Co., Inc., Dover, Mass.). The femoral vein was exposed and catheterized for infusions, and the femoral artery was catheterized and connected to a mercury manometer for monitoring blood pressure. The right external jugular vein was exposed, and injections were made directly into this vessel. A catheter was placed in the carotid artery to the level of the aorta and blood was withdrawn at 0.7 ml/sec. 40 samples of blood were collected in heparinized tubes resting in a moving rack collector (23) during a 28 sec period.

TABLE I

Description of Indicators

Indicator	Specific activity	Source	pK_a'*	λ‡§
	mc·mmole^{-1}			
Aniline-^{14}C HCl (UL)	1.3–5.0	NENC	4.58 (19)	—
Antipyrine-N-methyl-^{14}C	2.95	NENC	<2.0‡	0.097
Atropine-^{3}H (UL)	245	NC	9.65 (20)	0.043
Benzylamine-7-^{14}C HCl	1.2–1.5	NRC	9.37 (19)	0.379
5,5-Dimethyloxazolidine-2,4-dione-2-^{14}C	7.5–7.8	NENC	6.13 (37°C) (4)	0.007
Guanidine-^{14}C nitrate	3.29	NENC	13.65 (21)	0.011
Histamine-^{3}H (UL)	5000	NENC	9.80 (22)	0.035
Mescaline-8-^{14}C HCl	9.2–18.4	NENC	10.68‡	0.294
Methylamine-^{14}C HCl	2.0	MN	10.66 (19)	0.072
Morphine-N-methyl-^{14}C HCl	17.6–46.8	NC	8.21 (20)	0.044
Nicotine-^{3}H (UL)	70–259	NC	8.01 (7.77, 37°C)[17]‡	0.616
Pentylamine-1-^{14}C HCl	1.0	MN	10.64 (19)	0.175
Quinine sulfate	Unlabeled	MCB	8.52 (19)	—
T-1824 (Evans Blue)	Unlabeled	EO	—	—
Tritiated water	5500	NENC	—	—

EO, Eastman Organic Chemicals, Rochester, N. Y.; MN, Mallinckrodt Nuclear, Inc., Orlando, Fla; MCB, Matheson, Coleman and Bell, East Rutherford, N. J.; NC, Nuclear-Chicago Corporation, Des Plaines, Ill.; NENC, New England Nuclear Corp. Boston, Mass.; NRC, Nuclear Research Chemicals, Inc., Orlando, Fla.

* pK_a' in water at 25°C unless otherwise stated.

‡ See Methods section.

§ Oil-water partition coefficients.

Analyses were performed on aliquots of whole blood. T-1824 concentrations were determined by diluting 0.2 ml of blood in 2.0 ml of 0.85% saline, centrifuging, and reading the optical density of the supernatant at 620 mμ (Spectronic 20 Bausch & Lomb Incorporated, Rochester, N. Y.). Radioactivity was determined on protein-free supernatants. A volume of 0.9 ml absolute ethanol was added to 0.1 ml of blood, and the mixture in the tube was mixed and centrifuged. A volume of 0.2 ml of the alcohol supernatant was added to 10 ml of a dioxane scintillation mixture described earlier (24), and carbon-14 and tritium counts were obtained in a liquid scintillation spectrometer. Quinine determinations were made fluorometrically: A 0.5 ml volume of the alcohol supernatant was added to 5.0 ml of water and 0.5 ml of 1 N H_2SO_4. Fluorescence was measured in a Turner fluorometer (G. K. Turner, Associates, Palo Alto, Calif.).

Indicator concentrations are divided by the quantity injected yielding fractional concentration w (in milliliters^{-1}). w is plotted on a logarithmic ordinate against time on a linear abscissa, and recirculation is corrected by linear extrapolations on these coordinates.

Blood flow (F) is calculated from the area under the T-1824 curve (on linear coordinates) corrected for recirculation:

$$F = \frac{1}{\Sigma w_c, \text{T-1824}\tau} \tag{25}$$

where τ represents the time interval between samples and the subscript c refers to values of w obtained from the corrected curve.

Mean transit times ($\bar{t}$) are calculated from the equation

$$\bar{t} = \frac{\Sigma n w_c \tau}{\Sigma w_c} - \frac{\tau}{2} - t_{cath} \tag{26}$$

where n designates the tube number and t_{cath}, the catheter delay. t_{cath} is calculated from the catheter volume divided by catheter flow measured immediately before the study and ranged from 1.0 to 1.5 sec. By subtracting $\tau/2$, the time of sampling is adjusted to the middle of the collection interval.

The recovery R_Y in the pulmonary venous blood of the pH indicator is estimated by comparison of the area under the pH indicator curve ($A_Y = \Sigma w_{c,Y}\tau$) with that of an indicator which is assumed fully recoverable during a similar time interval (see Effros, Lowenstsin, Baldwin, and Chinard [24]). Impermeant indicators, Y′, were compared with T-1824 whereas permeant indicators, Y″, were compared with the water indicator (THO or antipyrine).

$$R_{Y'} = \frac{A_{Y'}}{A_{T-1824}} = \frac{\Sigma w_{c,Y'}\tau}{\Sigma w_c, \text{T-1824}\tau} \tag{27}$$

$$R_{Y''} = \frac{A_{Y''}}{A_{T-1824}} = \frac{\Sigma w_{c,Y''}\tau}{\Sigma w_{c,W}\tau} \tag{28}$$

The pK_a' values cited in Table I have been mostly obtained from standard references and refer to measurements obtained in water at 25°C. Morphine, nicotine, and quinine have additional pK_a' values which do not play a significant role at physiological pH and are therefore not shown. The pK_a' of nicotine in water at 37°C is reported to be 7.77 (25). Studies in our laboratory indicate that this parameter is somewhat higher in solutions of physiological ionic strength, and a value of 7.85 was used. These measurements were performed by titrating 0.1 N nicotine in 0.145 M NaCl with 2 N HCl at 37°C, with a glass electrode and a Beckman Research pH meter. The appropriate figure in blood and tissues remains somewhat uncertain. However, an error of 0.08 in the pK_a' of nicotine results in an error of less than 0.06 pH units in the calculated extravascular pH values. The same equipment was used to determine the approximate pK_a's of mescaline and antipyrine at room temperature in water.

The oil-water coefficients of the pH indicators are also shown in Table I. These were obtained in olive oil and pH 7.4 phosphate buffer (80.4 ml M/15 Na_2HPO_4 + 19.6 ml M/15 KH_2PO_4). Indicator, buffer, and oil were incubated with constant mixing for 3 hr and indicator concentrations in each phase were determined in the usual manner. Recoveries were generally greater than 90%.

Indicator dilution volumes were calculated from equations 7, 8, 10, 11, and 12. No correlations with the other tissue and vascular parameters were found and these data are therefore not tabulated.

Arterial pH was determined with a Radiometer pH meter (No. 27, Radiometer Corp., Copenhagen, Denmark). The total carbon dioxide content of 1.0 ml samples of blood was determined with the Van Slyke blood gas apparatus and bicarbonate concentrations and carbon dioxide tensions were calculated in the usual fashion. Arterial CO_2 and tensions were also measured during the study with CO_2 and O_2 electrodes (Radiometer) but are not tabulated here.

"Metabolic" acidosis was induced with infusions of 0.3 N HCl in 0.145 M NaCl at 0.4 ml kg^{-1} min^{-1}. "Metabolic" alkalosis was induced with infusions of 0.4 M Na_2CO_3 or 0.3 N–0.8 N $NaHCO_3$ at 0.4 ml kg^{-1} min^{-1}. Respiratory acidosis was produced with 10.8% CO_2 in air (in study 11-21-67 (3), 8.1% CO_2 in oxygen was used). Respiratory alkalosis was produced with hyperventilation of air. Infusions and respiratory changes were continued until the desired pH was obtained, generally for between 10 and 30 min.

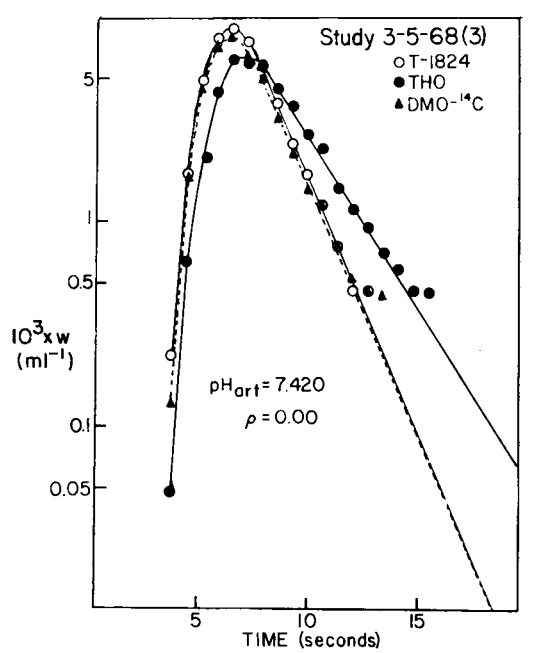

FIGURE 1 Simultaneous indicator dilution curves of DMO-^{14}C, T-1824, and THO. The DMO-^{14}C curve lies close to the T-1824 curve. In this and subsequent illustrations of indicator dilution curves, the value of w found in each tube is assumed to apply to the middle of the collection interval (τ). No correction for catheter delay has been made. The straight lines indicate extrapolations. The curve of the pH indicator is drawn as a dotted line.

RESULTS

DMO (5,5-dimethyloxazolidine-2,4-dione)

Simultaneous indicator dilution curves of DMO-2-^{14}C T-1824, and tritiated water (THO) are shown in Fig. 1. In each study, the DMO-^{14}C curve remained close to that of T-1824, indicating that relatively little DMO-^{14}C leaves the pulmonary capillaries during a single circulation. As indicated in Fig. 2, ρ-DMO remained close to zero at all values of hydrogen ion concentration, averaging 0.06 $\pm$0.04 SD. (Each of the following means is given with the standard deviation.) The recovery of DMO-^{14}C compared with T-1824 averaged 0.99 $\pm$0.04.

Amines with $pK_{a'}$ less than 5.0

Since amines with $pK_{a'}$ less than 5.0 are essentially unionized at pH values that are likely to be present in the lung, changes in arterial pH should exert no effect on the extravascular distribution of these indicators. This proved to be the case for both antipyrine ($pK_{a'} < 2$) and aniline ($pK_{a'} = 4.58$); ρ values for these amines were not influenced by changes in arterial pH (see Fig. 2). Outside of the slightly lower initial values in some of the studies, the antipyrine curves superimposed upon those of water at all values of arterial hydrogen in concentration and at CO_2 tensions of from 36 to 66 mm Hg (see Fig. 3). ρ-antipyrine averaged 1.01 $\pm$0.01 (n = 7) and the recovery of antipyrine compared with THO averaged 1.00 $\pm$0.04 (n = 7). The close approximation of the THO curves by the antipyrine-^{14}C curves provided the basis for the use of antipyrine-^{14}C as a water marker in subsequent studies with tritiated amines (nicotine-^{3}H and atropine-^{3}H).

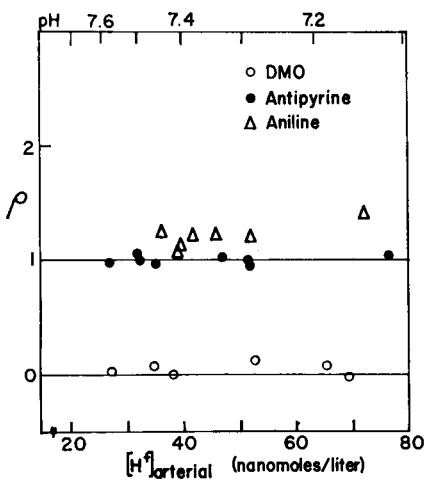

FIGURE 2 The relationship of ρ_{DMO}, $\rho_{antipyrine}$, and $\rho_{aniline}$ to arterial pH. DMO fails to leave the vascular space in significant quantities at any pH whereas $\rho_{antipyrine}$ remains very close to 1.0 and $\rho_{aniline}$ remains somewhat above 1.0. The distribution of each of these indicators is apparently insensitive to changes in pH during the experimental time interval.

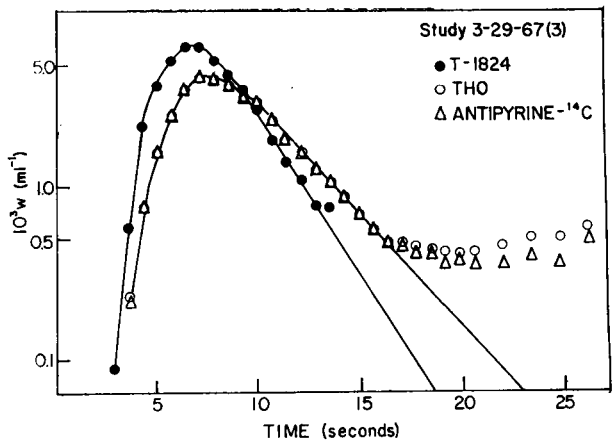

FIGURE 3 Simultaneous indicator dilution curves of T-1824, THO, and antipyrine-^{14}C. The antipyrine-^{14}C curve appears to superimpose upon the THO curve during the first circulation. Divergence during recirculation is evident, indicating that systemic handling of THO and antipyrine differs.

Amines with $pK_{a'}$ greater than 7.0

IMPERMEANT AMINES

Atropine, guanidine, mescaline, methylamine, and morphine remained essentially intravascular and could not be used for tissue pH studies (see Fig. 4). Since guanidine is completely ionized at physiological pH, it

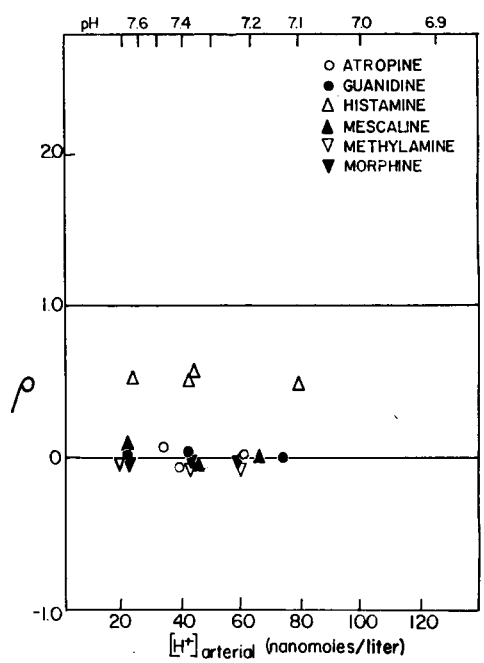

FIGURE 4 The relationship of $[H^+]_{art}$ and ρ values for isotopically labeled atropine, guanidine, histamine, mescaline, methylamine, and morphine. All of these indicators except histamine remained intravascular. The distribution of each indicator was unaltered by changes in $[H^+]_{art}$.

would not be expected to enter the extravascular space during the time intervals available. With the exception of mescaline, the relatively low permeability of the pulmonary barrier to these indicators, as well as to DMO, appeared to correlate well with relatively low oil to water solubility coefficients (See Table I). Histamine appeared to be distributed in a "volume" one-half that of water but was unresponsive to changes in arterial pH. In general, the recoveries of these indicators were greater than 90%.

Permeant Amines

Each of the amines with pK_a' greater than 7.0 which appeared to have access to the entire extravascular water volume proved to have distribution ratios which were responsive to alterations in arterial pH(pH_{art}). These indicators were nicotine, pentylamine, benzylamine, and quinine.

Pentylamine, benzylamine, and quinine. The influence of metabolic acidosis upon the pentylamine-1-^{14}C curve is shown in Fig. 5. From a position after that of the THO curve, the upslope of the pentylamine curve has shifted towards that of T-1824, and lies between the T-1824 and THO curves at the lower arterial pH. ρ-pentylamine has fallen from 1.31 at a pH_{art} of 7.390 to 0.52 at a pH_{art} of 7.155. Alkalosis consistently produced a rise in the extravascular distribution of each of these amines, whereas acidosis resulted in a fall in their extravascular distributions. This is shown in Fig. 6 where ρ values for pentylamine, benzylamine, and quinine are plotted against the arterial hydrogen ion concentration. In these studies, metabolic alterations in pH were employed (see Methods), and the direction of change is indicated by the arrows.

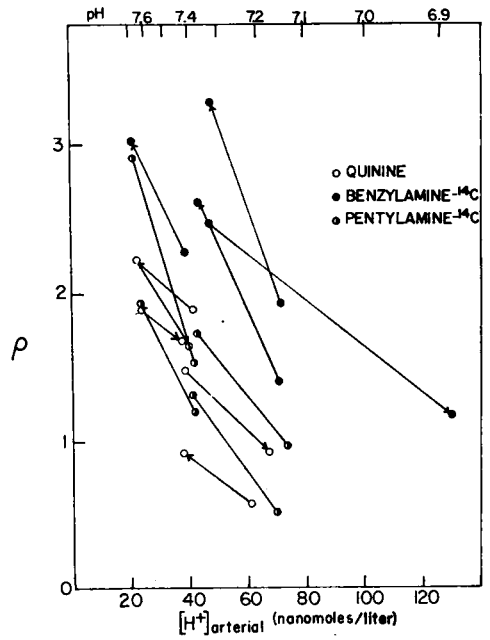

FIGURE 6 The effect of metabolic changes in $[H^+]_{art}$ upon $\rho_{quinine}$, $\rho_{benzylamine\text{-}^{14}C}$, and $\rho_{pentylamine\text{-}^{14}C}$. In each study, the values for ρ fell with acidosis and rose with alkalosis.

The recoveries of pentylamine and benzylamine were incomplete (0.77 ±0.09 [n = 9] and 0.78 ±0.06 [n = 12], respectively), suggesting metabolism or sequestration in a slowly emptying compartment. The true steady-state distribution of these indicators therefore remains in doubt, and they cannot be used for measurement of tissue pH. Although the recovery of quinine is relatively complete (0.88 ±0.06, [n = 11]), evidence has been obtained indicating plasma protein and tissue

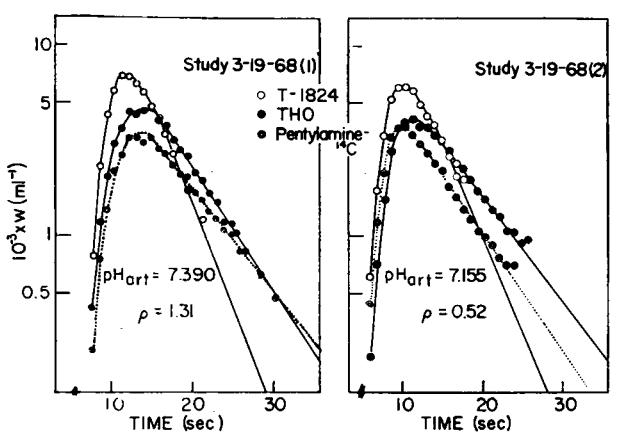

FIGURE 5 Simultaneous indicator dilution curves of T-1824, THO, and pentylamine-^{14}C before and after an infusion of dilute hydrochloric acid. Acidosis has shifted the pentylamine curve towards the T-1824 curve. This is particularly noticeable in the upslope which now preceeds that of tritiated water.

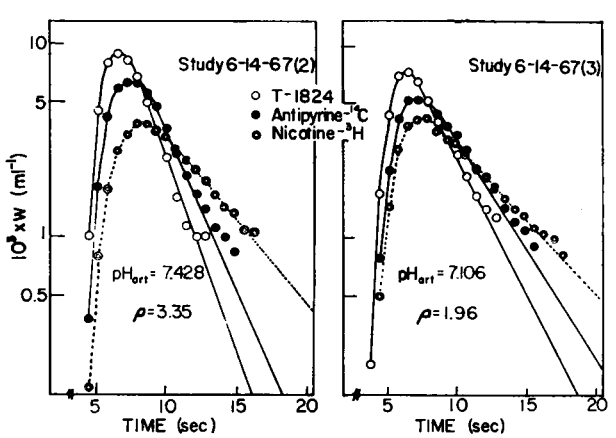

FIGURE 7 Simultaneous indicator dilution curves of T-1824, antipyrine-^{14}C, and nicotine-^{3}H before and after an infusion of dilute acid. Antipyrine-^{14}C has been used as a water marker. After the infusion, the nicotine-^{3}H curve lies close to the antipyrine-^{14}C curve.

binding (26). For this reason, quinine was also rejected as an indicator of tissue pH.

Nicotine. Studies with tritiated nicotine suggested that among the substances investigated, it was the most suitable indicator of pulmonary extravascular pH. The recovery of nicotine compared with antipyrine was relatively complete (0.94 ±0.06, [n = 47]) and, as indicated in the Discussion, protein binding and lipid solubility are probably not significant factors in its distribution between blood and tissue. The recovery of antipyrine compared with T-1824 in these studies was also nearly complete (0.98 ±0.04, [n = 47]), indicating that blood flow remained constant after injection of nicotine.

TABLE II

Results of Experiments with Tritiated Nicotine. Calculated Values of Pulmonary Extravascular pH

Study condition		pH_{art}	$[HCO_3^-]_{art}$	P_{CO_2}	Hct	F	$\bar{t}_{T-1824}$	t_{Ant}	$\bar{t}_{Nic}$	ρ	ρ_0	$[H^+]_e$	pH_e	$\frac{A_{Ant}}{A_{T-1824}}$	$\frac{A_{Nic}}{A_{Ant}}$
			mmoles/liter	*mm Hg*		*ml/sec*	*sec*	*sec*	*sec*			*nmoles/liter*			
pH and P_{CO_2} studies															
4-17-67	(1) N	7.320	24.6	45.1	0.39	38.9	5.55	7.12	11.97	4.09	4.20	246	6.61	1.03	1.13
	(2) 1B	7.551	59.0	67.0	0.25	55.9	3.97	4.93	8.04	4.24	4.39	171	6.77	0.95	0.90
5-9-67	(1) N*	7.354	23.3	43.5	0.49	43.5	4.35	5.21	6.46	2.45	2.55	135	6.87	0.97	0.97
	(2) 2B	7.641	15.5	14.6	0.41	29.7	5.77	6.84	9.09	3.10	3.29	108	6.97	0.98	0.89
	(3) 2B, 1A*	7.442	11.0	16.9	0.37	24.8	6.11	7.58	10.07	2.69	2.81	127	6.90	1.02	0.94
6-14-67	(2) N‡	7.428	20.9	32.5	0.55	26.2	6.59	7.67	10.21	3.35	3.41	162	6.79	0.92	0.89
	(3) 1A	7.106	12.4	40.6	0.54	29.1	6.98	8.68	10.32	1.96	2.01	172	6.77	1.02	0.97
6-27-67	(1) N	7.363	17.9	32.8	0.41	22.1	5.58	6.64	8.22	2.49	2.58	134	6.87	0.94	0.91
	(2) 1A	7.195	11.7	32.0	0.40	15.5	6.58	8.5	10.55	1.98	2.14	153	6.82	0.98	0.97
8-22-67	(1) N	7.285	19.2	41.5	0.42	58.9	5.72	7.01	10.14	3.43	3.48	216	6.67	0.96	1.04
	(2) 1B	7.635	43.0	41.5	0.30	91.6	3.80	4.67	7.50	4.25	4.45	152	6.82	0.98	0.95
8-30-67	(1) 1A	7.095	15.5	52.0	0.43	34.6	3.69	4.71	5.77	2.04	2.08	182	6.74	1.01	1.02
	(2) 1B	7.322	24.0	48.5	0.34	21.6	4.70	5.80	8.09	3.08	3.14	180	6.74	0.92	0.85
	(3) 1B	7.533	45.0	56.0	0.27	29.2	3.24	4.00	6.84	4.74	4.89	199	6.70	0.95	0.92
9-5-67	(1) N*	7.325	22.5	44.0	0.51	52.5	4.96	6.27	9.02	3.10	3.24	185	6.73	0.94	0.92
	(2) 2B	7.570	15.0	16.8	0.47	50.8	4.79	6.12	9.84	3.74	4.07	153	6.81	0.92	0.90
	(3) 2B, 1A*	7.390	9.0	16.0	0.46	43.1	6.14	7.88	11.59	3.13	3.30	167	6.78	1.08	0.87
9-18-67	(1) 2B	7.573	14.0	15.8	0.26	26.7	4.99	7.21	15.26	4.63	4.85	185	6.73	0.90	0.89
	(2) N	7.300	16.1	33.8	0.29	28.1	4.16	6.29	10.27	2.87	2.98	178	6.75	0.92	0.81
	(3) 2A	7.000	22.0	93.0	0.29	22.8	4.79	7.44	11.47	2.52	2.55	278	6.56	0.91	0.98
9-25-67	(1) 2B	7.715	15.2	12.0	0.33	18.3	10.32	13.02	21.30	4.07	4.32	131	6.88	0.96	0.92
	(2) N	7.325	16.8	34.0	0.30	27.2	8.62	10.68	15.81	3.49	3.56	205	6.69	0.95	1.02
	(3) 2A	6.940	21.0	102.0	0.40	32.4	7.72	9.48	12.76	2.86	2.81	349	6.46	0.95	1.16
11-21-67	(1) N*‡	7.385	19.2	33.0	0.32	32.2	7.69	9.67	13.81	3.09	3.18	162	6.79	1.01	0.90
	(3) 2A, 1B*	7.430	45.0	69.5	0.29	36.0	6.50	8.42	13.55	3.67	3.80	181	6.74	1.01	0.88
11-29-67	(1) N*‡	7.390	21.2	36.5	0.46	40.7	6.23	7.85	12.98	4.17	4.33	224	6.65	1.02	0.90
	(2) 2B, 1A*	7.375	14.8	25.5	0.42	38.1	6.03	7.82	12.89	3.83	3.99	211	6.68	0.94	0.92
	(3) 1B, 2A*	7.375	43.0	75.0	0.37	47.1	5.28	6.64	11.66	4.69	4.81	257	6.59	1.00	0.95
12-6-67	(1) N*‡	7.380	24.0	42.0	0.34	31.2	4.53	6.14	11.40	4.27	4.45	235	6.63	0.97	0.95
	(2) 1B	7.650	35.0	33.0	0.27	31.0	4.89	6.40	13.86	5.94	6.23	211	6.67	0.96	0.98
	(3) 1B, 2A*	7.395	56.0	90.0	0.25	33.5	3.93	5.32	10.50	4.60	4.75	245	6.61	0.98	0.95
12-13-67	(1) N*‡	7.410	23.0	38.0	0.42	31.9	4.96	5.88	9.52	4.96	5.01	252	6.60	1.05	0.92
	(2) 1A	7.170	14.3	39.0	0.40	32.0	4.16	5.08	7.25	3.36	3.38	262	6.58	1.01	0.91
	(3) 1A, 2B*	7.405	8.3	14.2	0.33	17.1	6.54	8.68	15.21	4.05	4.20	211	6.68	1.00	0.91
1-8-68	(1) 1A	7.210	15.5	39.0	0.46	55.2	4.40	5.55	9.08	4.07	4.13	299	6.52	0.94	1.08
	(2) 1B‡	7.415	35.5	22.8	0.43	40.7	4.93	6.55	12.30	4.55	4.78	238	6.62	0.94	0.98
1-17-68	(1) 1B	7.622	28.0	28.5	0.39	83.8	2.83	3.48	5.70	4.42	4.64	163	6.79	1.05	0.94
	(2) N‡	7.425	21.0	33.0	0.36	46.1	3.52	4.61	7.55	3.70	3.86	186	6.73	0.99	0.90
Hemorrhage studies															
10-23-67	(1) N	7.398	19.7	33.5	0.47	45.0	4.14	5.24	8.02	3.53	3.69	186	6.73	0.96	0.94
	(2) 3 ‡	7.412	19.0	32.2	0.31	49.3	3.82	4.98	8.04	3.36	3.77	186	6.73	1.01	0.94
	(3) 3	7.380	17.0	30.0	0.20	46.8	3.90	4.96	7.97	3.84	3.92	205	6.69	0.99	0.95
11-6-67	(1) N	7.394	19.5	33.5	0.44	52.5	4.15	5.01	7.00	3.31	3.41	172	6.76	0.97	0.93
	(2) 3 ‡	7.395	19.8	34.0	0.29	66.4	3.43	4.14	5.66	3.14	3.21	161	6.79	0.98	0.89
	(3) 3	7.415	18.5	30.0	0.18	70.8	3.15	3.85	5.44	3.27	3.31	160	6.80	1.00	0.92
11-13-67	(1) N	7.410	20.0	33.0	0.49	48.5	4.98	6.03	9.82	4.61	4.72	237	6.63	0.97	0.96
	(2) 3 ‡	7.408	19.8	32.3	0.27	72.0	3.43	4.32	6.78	3.76	3.86	192	6.72	0.96	0.93
	(3) 3	7.385	18.5	32.5	0.19	69.4	4.07	4.97	7.83	4.18	4.23	214	6.67	1.03	0.94

N = study without infusion, change in P_{CO_2}, or hemorrhage; 1A = infusion 0.3N HCl in 0.85% saline at 0.4 ml/kg per min; 1B = infusion of 0.4M Na_2CO_3 at 0.4 ml/kg per min; 2A = inhalation of 10% CO_2 in air; 2B = hyperventilation with air; 3 = Hemorrhage.

* Data used to calculate average values of $\Delta pH_e/\Delta P_{CO_2}$.

‡ Data used to calculate average values of pH_e, at physiological pH_{art} (7.38–7.43).

Simultaneous indicator dilution curves of nicotine (UL)-^{3}H, T-1824, and antipyrine-N-methyl-^{14}C are shown in Fig. 7. Acidosis produced by an infusion of 0.3 N HCl in normal saline at 0.4 ml min^{-1} Kg^{-1} for 27 min has resulted in a closer approximation of the nicotine and antipyrine curves with a corresponding fall of ρ-nicotine from 3.35 at pH_{art} of 7.428 to 1.96 at pH_{art} of 7.106.

The response of ρ-nicotine to alterations in arterial pH was similar to that of pentylamine, benzylamine, and quinine.

In each of 20 studies, ρ-nicotine rose with arterial alkalosis and fell with arterial acidosis (see Table II and Fig. 8). Metabolic alterations in $[H^+]_{art}$ are indicated by continuous lines, and respiratory alterations by interrupted lines (see Methods). ρ-nicotine remained above one in all instances, suggesting that the extravascular space is relatively acid compared with blood. Alterations in hematocrit or blood flow appeared to have no influence on ρ-nicotine. Values for extravascular pH were calculated from the nicotine studies and are given in Table II. At arterial pH values between 7.38 and 7.43, the extravascular hydrogen ion concentration averaged 203 ±32 nmoles/liter, (n = 10) equivalent to a pH of 6.69 ±0.07.

Alterations in calculated values of pH_e were consistently less than concomitant changes in pH_{art} (see Fig. 9). This indicates that changes in the extravascular hydrogen ion concentration were proportionately smaller than those in plasma and is presumably responsible for the observed alterations in ρ values for each of the pH-sensitive amines (see Discussion). It should be noted, however, that though proportionately smaller, the absolute changes of $[H^+]_e$ were greater than those in $[H^+]_{art}$ in 6 of 12 metabolic studies, and 6 of 8 respiratory studies.

No significant difference was found in the response of extravascular pH to respiratory or metabolic alterations of arterial pH. The ratio of change in extravascular pH to that of arterial pH ($[\Delta pH_e]/[\Delta pH_{art}]$) averaged

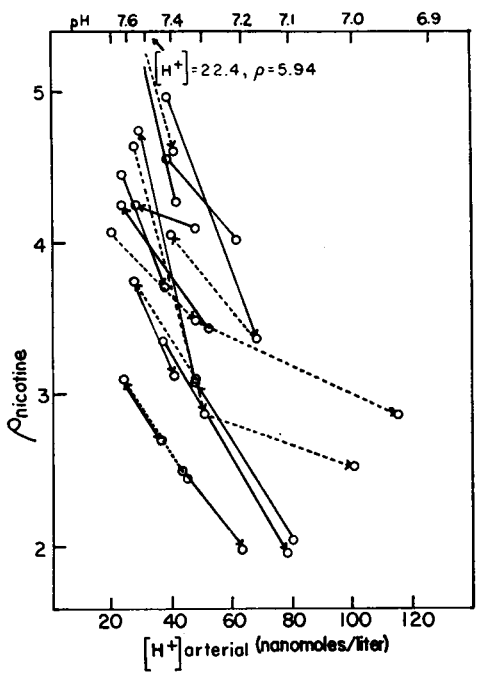

FIGURE 8 The effect of alterations in $[H^+]_{art}$ upon ρ-nicotine, "Metabolic" alterations are denoted with continuous lines, respiratory alterations are denoted by interrupted lines.

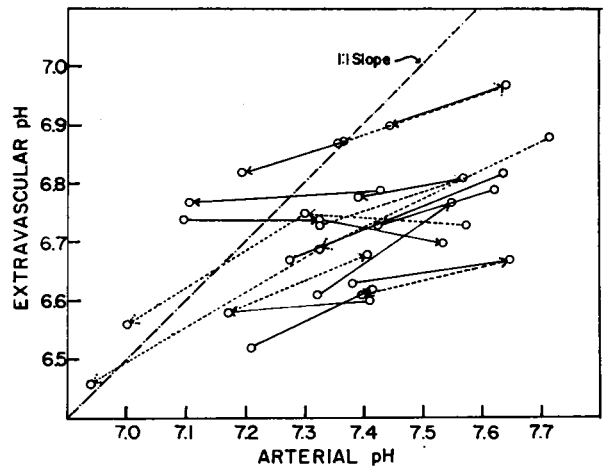

FIGURE 9 The effect of alterations in pH_{art} upon values of pH_e calculated from the nicotine data. "Metabolic" alterations are denoted with continuous lines, respiratory alterations are denoted by interrupted lines.

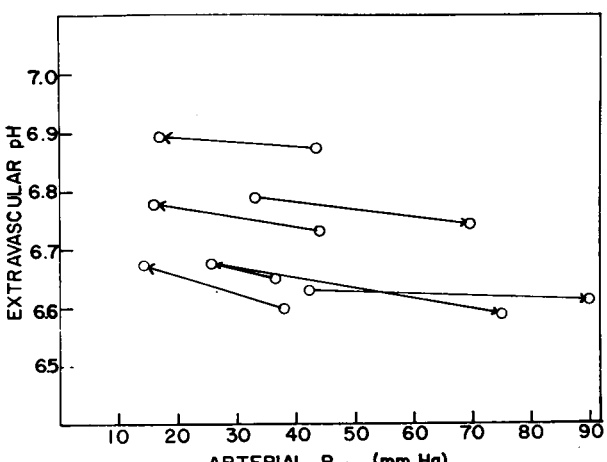

FIGURE 10 The effect of changes in arterial P_{CO_2} at constant arterial pH upon values of pH_e calculated from nicotine-^{3}H data. Hypercapnia induced a modest extravascular acidosis within each study whereas hypocapnia produced a similarly modest extravascular alkalosis.

0.371 ± 0.210 in the respiratory studies, whereas $(\Delta pH_e)/(\Delta pH_{art})$ averaged 0.244 ± 0.230 in the metabolic studies. However, changes in P_{CO_2} at constant arterial pH yielded a relatively minor extravascular acidosis in each of seven runs (see Fig. 10). pH_e fell, on the average, 0.02 U for each rise of 10 mm Hg in arterial P_{CO_2}. Thus increases of P_{CO_2} appear capable of producing a slight tissue acidosis independently of changes of arterial pH. These changes are very small, and it is clear that over the time intervals involved (10–30 min of infusion or respiratory change), tissue pH is more closely linked to arterial pH than to the ambient carbon dioxide tension.

DISCUSSION

Generalization of the multiple indicator dilution approach has made it possible to measure indicator concentration differences between compartments within individual organs in the living and intact animal (5, 6). In the present study this technique has been used to investigate the distribution of ionizable indicators between the extravascular and vascular spaces of the lung and thereby obtain information concerning the pulmonary tissue pH. The conventional intracellular pH indicators, carbon dioxide and DMO, could not be used for this purpose. The former is lost from the alveoli (27, 28), whereas the latter fails to leave the pulmonary capillaries in significant quantities during a single circulation. For this reason a study of the amines was undertaken.

Of the amines with $pK_{a'}$ above 7.0, those which appeared to have access to the extravascular water compartment of the lung proved to have distribution ratios which were sensitive to arterial pH changes. Four pH-sensitive amines were found: nicotine, pentylamine, benzylamine, and quinine. The distribution of cationic amines across a semipermeable membrane reflects the distribution of hydrogen ion across the membrane (see equation 21). Thus, the observation that at normal arterial pH, ρ values for each of these indicators are greater than 1 suggests that the extravascular space is relatively acid compared with the vascular space. Furthermore, the consistent rise of ρ with alkalosis and fall with acidosis provides evidence that the ratio $[H^+]_e/[H^+]_p$ increases with arterial alkalosis and decreases with arterial acidosis. This would be expected if the tissue were relatively acid and changes in tissue hydrogen ion concentration were proportionately less than those in plasma.

Of the four pH-sensitive amines studied, tritiated nicotine appears to be the most suitable indicator of pulmonary tissue pH. Because of incomplete recovery, pentylamine and benzylamine could not be used, and quinine was rejected because of evidence that it is bound to plasma protein and to pulmonary tissue as well (26). Although a minor degree of binding of nicotine to frog muscle has been suggested (29), no binding to plasma protein has been found. Furthermore, though nicotine is readily soluble in lipid, current studies with the higher alcohols suggest that the oil-water partition coefficient of nicotine is too small to appreciably influence its distribution between blood and pulmonary tissue. In vitro metabolism of nicotine by lung is relatively slow and probably inconsequential in vivo during a single circulation (30).

The correlation of nicotine activity with pH was first recorded by Langley and Dickinson in 1889 (31), who found that the ability of nicotine solutions to paralyze the superior cervical ganglion was diminished at acid pH. Richardson and Shepard found that nicotine was more rapidly toxic to mosquitos (32), cockroaches (33), and goldfish (34) at alkaline pH. The ratios of passage of nicotine into urine (35), and through artificial membranes (36), the gastric mucosa (37), and the mucous membranes of the mouth (38) are also pH-dependent. In each of these studies, the rate of entry is probably related to the concentration of the permeant, unionized nicotine molecule which is present in greater abundance at alkaline pH.

Recently, Weiss (29) has presented evidence that the steady-state concentration of nicotine in frog sartorius muscle is largely determined by the pH gradient across the cell membrane (as measured by DMO-^{14}C).

Approximately 10% of the indicator appeared to be reversibly bound to the muscle cell in a pH 7.4 buffer medium. Exposure to relatively high concentrations of nicotine (0.083 mmole/liter) for a full hour produced a rise in the intracellular pH. In the present studies, nicotine concentrations in lung tissue were maintained at much lower levels (below 0.004 mmole/liter) and this exposure lasted no more than a few seconds. A total of 0.125 mg of nicotine was present in the injection bolus. A corresponding dose in man would be less than 2% of the estimated minimum lethal dose (39). Rapid metabolism by the liver should prevent indicator accumulation. No change in cardiac output (as judged by the nearly equal recoveries of T-1824 and water label) was observed during the collection intervals of these studies.

As indicated in the Appendix, the in vitro distribution of nicotine between plasma and red cells also appears to be largely determined by pH gradients across the red cell membrane. A good correlation was found between the plasma pH and the ratio of the nicotine concentration within red cells to that in plasma. It was therefore possible to estimate this ratio from the arterial pH in each experiment and correct calculated values of the gradient of nicotine between tissue and plasma accordingly. This correction proved relatively minor for nicotine in the lung, because the concentration ratio be-

tween tissue and plasma is much greater than that between red cells and plasma, presumably because pulmonary tissue is much more acid than red cells. Values for the average pulmonary extravascular pH based upon ρ averaged 6.71 $\pm$0.09 (see previous abstract [15]), whereas the average obtained using ρ_c was 6.69 $\pm$0.07. The failure to find a correlation between ρ-nicotine and hematocrit in acute bleeding studies also indicates that red cell concentration exerts a relatively minor effect on the distribution of nicotine between plasma and tissues. No information is available concerning the actual rate of nicotine entry into red cells, but in view of its relatively high solubility in lipid and rapid passage through the "tight" pulmonary capillaries, red cell equilibration probably occurs within the pulmonary capillary transit time.

It is assumed that the rate of diffusion of nicotine into the pulmonary extravascular space is very rapid and that alterations in this rate do not alter the indicator dilution curves. The failure of changes in blood flow to influence values of ρ suggests that the time interval required for diffusional equilibration of nicotine between plasma and tissue is very short. Had a long interval been required, errors in extrapolating the tail of the nicotine curve would tend to yield shorter mean transit times at more rapid flows (40). Values calculated for nicotine would consequently be dependent upon blood flow as well as the steady-state concentration ratio of nicotine between tissue and plasma, and tissue pH could not be determined.

The pulmonary extravascular pH obtained in the present study averaged 6.69 $\pm$0.07 at normal pH_{art}. Changes in extravascular pH tended to parallel but were smaller than those occurring in arterial pH, indicating that alterations in tissue pH were proportionately smaller than those occurring in blood. In this respect, the response of lung tissue resembles the in vitro behavior reported for kidney, brain, and muscle. However, the ability of lung tissue to withstand changes in plasma pH would appear to be relatively limited. Changes in tissue hydrogen ion concentration were frequently greater in an absolute sense, though proportionately less, than changes in plasma hydrogen ion concentration.

Although increasing the carbon dioxide tension at constant arterial pH lowered tissue pH and decreasing the carbon dioxide tension raised the tissue pH, these responses were surprisingly modest compared with changes which could be induced by altering arterial pH. Local changes in pulmonary pH secondary to changes in P_{CO_2} are evidently quite well compensated during the 10–30 min exposure to high or low carbon dioxide tensions. Although data has been presented elsewhere that bicarbonate does not equilibrate across the wall during a single circulation, the relatively long intervals of exposure in the present study may be adequate to permit passage of bicarbonate ion across flat alveolar and endothelial cells. Extending the analogy of Kibler, O'Neill, and Robin (7) between the selective permeability of cell membranes and pH electrodes, the pulmonary cell may behave as a "leaky" membrane permitting a slow leakage of bicarbonate ion. Certainly the peculiar configuration of the pulmonary parenchyma would make it more accessible to such a flux than excised muscle or kidney or the large pools of brain and cerebrospinal fluid. Alternatively hydrogen ion may be rapidly transported across pulmonary cell membranes, tending to diminish the hydrogen ion gradient between tissue and blood. In any event, the relative invariance of tissue pH to changes in P_{CO_2} at constant plasma pH observed in these studies is presumably advantageous in the pulmonary parenchyma which is exposed to rapid variations in carbon dioxide tension. The stability of pulmonary tissue pH appears to be linked to blood and thereby whole body acid-base balance, rather than to local buffering mechanisms which would be particularly vulnerable to changes in alveolar ventilation.

Earlier studies (11–13) have shown that the pulmonary capillaries are relatively impermeable to ions and to conventional indicators of extracellular compartments such as sodium, chloride, and sugars, and do not permit their passage during the brief time intervals preceding recirculation. Measurements of the pulmonary interstitial volume were, therefore, not possible, and the hydrogen ion concentration represents an average of the cellular and interstitial values. If one assumes that the pulmonary interstitial pH equals the plasma pH and that the interstitial space represents 32% of the extravascular volume (an estimate made on the basis of the distribution of sucrose in rabbit lungs 1 hr after injection [41]), a value of 6.56 may be calculated for the pulmonary cellular pH at an arterial pH of 7.40. If it is further assumed that the P_{CO_2} of the cell equals the arterial P_{CO_2}, it should be possible to calculate the bicarbonate concentration of the cellular compartment from the cellular pH and arterial P_{CO_2} with the Henderson-Hasselbalch equation. This calculation yields a value of 3.42 mmoles/liter for the cellular bicarbonate concentration (at a P_{CO_2} of 40 mm Hg and a cellular pH of 6.56). If the interstitial bicarbonate may be approximated by the arterial plasma bicarbonate $[HCO_3^-]_{art}$, then the average extravascular bicarbonate $[\overline{HCO_3^-}]_e$ may be calculated from the cellular bicarbonate $[HCO_3]_c$ and the arterial bicarbonate with the equation:

$$[\overline{HCO_3^-}]_e = 0.32\,[HCO_3]_{art} + 0.68\,[HCO_3^-]_c. \quad (29)$$

At $[HCO_3^-]_{art}$ equal to 25 mmoles/liter and $(HCO_3^-]_c$

equal to 3.42 mmoles/liter, $[\overline{HCO_3^-}]_c$ equals 10.33 mmoles/liter.

Estimates of the carbon dioxide content of the extravascular compartment of the lung have been made by a variety of ventilatory procedures. Values of 0.33 ml (42, 43) and 0.35 ml (44) of CO_2/ml of tissue (standard temperature and pressure, dry [STPD], or 14.8 and 15.7 mmoles/liter) have been reported. Hyde, Puy, Raub, and Forster (44) were able to estimate the fraction of the total carbon dioxide content which was in the form of bicarbonate by blocking carbonic anhydrase with a large, intravenous dose of acetazolamide in a single subject. The average bicarbonate concentration of the extravascular space represented 80% of the CO_2 content or 11.87 mmoles/liter.

These authors went on to calculate the extravascular pH, in effect assuming that the bicarbonate concentration of the extravascular space was uniform. They obtained a vaue of 6.97 at a virtual mixed venous P_{CO_2} of 40 mm Hg. Among the explanations that may be offered for the higher pH found by these authors, three are noteworthy: (*a*) If it is again assumed that the bicarbonate concentrations in the interstitium and arterial plasma are the same (25 mmoles/liter), the value of $[HCO_3^-]_c$ calculated from equation 29 will be 5.69 mmoles/liter. At a P_{CO_2} of 40 mm Hg, the cellular pH would be 6.75 and the over-all extravascular pH, 6.90. If the hydrogen ion concentration of the cellular compartment is itself heterogeneous (45), then it would be necessary to further diminish the pH value obtained with an anion such as bicarbonate. (*b*) The subject who received acetazolamide in the ventilatory studies was hyperventilating, and although the mixed venous P_{CO_2} was 40 mm Hg, end-tidal P_{CO_2} was only 14 mm Hg and blood and tissue P_{CO_2} were probably depressed. It is therefore likely that a respiratory alkalosis was present in the end-capillary blood and pulmonary tissues. Although arterial P_{CO_2} and pH values would be helfpul, the administration of acetazolamide probably produces gradients in both P_{CO_2} and plasma pH between the end-capillary and the arterial blood, and neither the P_{CO_2} nor the pH of blood in the pulmonary capillaries may be estimated with confidence under these circumstances. (*c*) It is possible that inspired carbon dioxide in the ventilatory studies has access to compartments which are poorly perfused but well ventilated and, therefore, more alkaline than those which are monitored by the present study in which the indicator is introduced into and collected from the blood.

The failure of the antipyrine and aniline curves to respond to changes in arterial pH was anticipated since they are essentially neutral at the pH of lung tissue. The close similarity of the antipyrine curves to those of tritiated water permitted the use of carbon-labeled antipyrine as a water indicator with tritiated pH indicators (nicotine, atropine, and histamine). With the exception of mescaline, the impermeability of the pulmonary vasculature to DMO and several amines with pK_a' above 7.0 appeared to correlate well with the low oil-to-water coefficients of the indicators. Single-circulation studies do not permit evaluation of the extravascular distribution of indicators which equilibrate slowly between blood and tissues. Although it may be anticipated that DMO would equilibrate in lung tissue during similar constant infusion studies, its relatively slow rate of exchange would make it unreliable as a pH indicator in the presence of abrupt changes in pH. Furthermore, it must be shown that the ionized moiety is significantly less permeant than the unionized moiety; this property has not been well documented for DMO in the past. Individual pH indicators may prove to be applicable to some tissues but not others. The need for cationic pH indicators has recently been emphasized (46) and further studies with labeled nicotine would appear to be indicated.

APPENDIX

The ratio of the concentration of nicotine in red cell water to that in plasma water ($[Nic]_r/[Nic]_p$) was determined in the following manner: Heparinized arterial blood was incubated anaerobically with nicotine-^{3}H and antipyrine-N-methyl-^{14}C and in some instances small amounts of dilute HCl in saline or 0.4 M Na_2CO_3. Incubation was performed at room temperature for approximately ½ hr (up to 3 hr, duration of incubation had no other effects than those attributable to pH changes). The samples were centrifuged at 20,000 rpm for 15 min under mineral oil and the pH, P_{CO_2}, and P_{O_2} of the plasma were determined with the pH, P_{CO_2}, and P_{O_2} electrodes described above. Macrohematocrits were measured on the spun samples and agreed well with standard microhematocrits of the same blood. Blood and plasma were separated and radioactive analyses were performed in the usual fashion. Similar studies with antipyrine and THO indicate that under the experimental conditions and at all values of pH_{art} antipyrine was 10.0 ±2.0% (n = 12) more concentrated in red cell water than in plasma water. Since the antipyrine and tritiated water curves are almost identical in the lung studies, it is likely that their distribution between plasma and red cells is the same during the 1st min and that excessive red cell antipyrine concentration is a consequence of prolonged incubation.

To correct for excessive antipyrine concentration in red cells, the following equation was used

$$\frac{[Nic]_r}{[Nic]_p} = 1.1 \times \frac{\left(\frac{\text{nicotine cpm}}{\text{antipyrine cpm}}\right)}{\left(\frac{\text{nicotine cpm}}{\text{antipyrine cpm}}\right)}. \qquad (30)$$

In Fig. 11, $([Nic]_r)/([Nic]_p)$ is plotted against plasma pH (pH_p) and a regression line is indicated ($P < 0.001$). The equation of this line is

$$\frac{[Nic]_r}{[Nic]_p} = 0.432\ pH_p - 1.768. \qquad (31)$$

This equation was used in the calculation of ρ_c for nicotine (see Methods). Values of red cell hydrogen ion concentration have

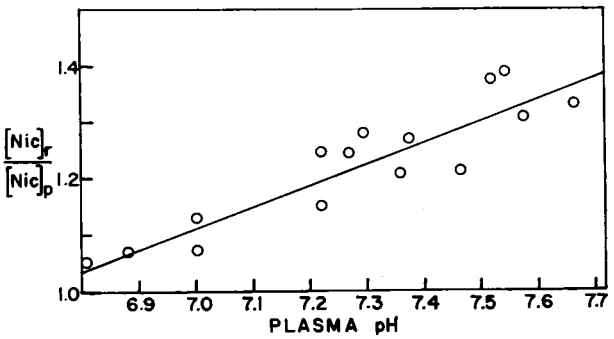

FIGURE 11 Incubation studies: Correlation of the ratio $([Nic]_r)/([Nic]_p)$ of red cell to plasma nicotine concentrations (per ml of water) to plasma pH. $([Nic]_r)/([Nic]_p)$ fell with decreasing values of pH. The equation of the regression line is given in the text.

been calculated using equation 24, substituting $([Nic]_r)/([Nic]_p)$ for ρ_c.

At an average arterial pH of 7.4, the calculated value for red cell pH was 7.28. Extrapolation of the data of Fitzsimmons and Sendroy (47) obtained by electrometic measurements of human hemolysates yielded a value of 7.20 at an arterial pH of 7.4. The ratio of plasma to red cell $[H^+]$ calculated from the nicotine studies declined with decreasing plasma pH in a fashion similar to that observed by others.

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