In Vivo Myocardial Cell pH in the Dog

RESPONSE TO ISCHEMIA AND INFUSION OF ALKALI

RICHARD M. EFFROS, BUNYAD HAIDER, PHILIP O. ETTINGER, S. SULTAN AHMED, HENRY A. OLDEWURTEL, KATHLEEN MAROLD, and TIMOTHY J. REGAN

From the Department of Medicine, College of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, New Jersey 07103

ABSTRACT Myocardial cell pH has been measured with 5,5-dimethyl-2,4-oxazolidinedione (DMO) in intact anesthetized dogs by a transient indicator dilution technique. Bolus injections of labeled DMO, vascular, extracellular, and water indicators were made into the anterior descending coronary artery, and blood samples were collected from the great cardiac vein. The steady-state distribution of DMO between cells and plasma was calculated from the indicator mean transit times, and the plasma pH was estimated from arterial and great cardiac vein pH. Myocardial cell pH was determined from the distribution value and plasma pH. Normal myocardial cell pH averaged 6.94. Changes in myocardial cell pH averaged 58% of concomitant changes in plasma pH after infusions of acid or alkali. Myocardial ischemia induced by inflation of a coronary artery balloon resulted in progressive decreases in cellular pH to average values of 6.83 within the initial 15 min and to 6.59 within the interval between 20 and 70 min. Infusions of Na_2CO_3 tended to diminish intracellular acidosis although these infusions had little effect on the difference in pH between the myocardial cell and extracellular fluid.

INTRODUCTION

Reductions in coronary blood flow are rapidly followed by the production of large amounts of lactic acid by the myocardium (1). It has been suggested that the decline in myocardial contractility which is observed after the onset of myocardial ischemia is related to the development of acidosis within the heart cells (2). Although it has been possible to show that lactate concentrations in coronary sinus blood increase as myocardial perfusion is reduced, the development of cellular acidosis in ischemic mammalian hearts has not been documented. It was the purpose of the present study to determine the effect of coronary occlusion upon myocardial cell pH in the dog. In addition, measurements were made of the response of myocardial cell pH [1] to infusions of acid and alkali in normal animals and to infusions of alkali during ischemia.

These studies were accomplished by a modification of an indicator dilution technique which was previously used for the measurement of pulmonary tissue pH (3). The principal advantage of this approach is that measurements of myocardial cell pH may be obtained without insertion of electrodes into the myocardium or excision of tissue from the heart. Direct application to clinical studies may therefore be possible.

METHODS

Mongrel dogs, weighing 20–30 kg, were anesthetized with sodium pentobarbital (50 mg/kg and as needed), anticoagulated (10,000 U, sodium heparin USP every 2 h), intubated, and mechanically ventilated. An "injection" catheter (50 cm, no. 5F coronary artery, with inflatable distal balloon in ischemic studies) was introduced under fluoroscopy into the anterior descending coronary artery by way of the carotid artery. A "collection" catheter (50 cm, no. 8 coronary sinus catheter) was passed from the femoral vein into the great cardiac vein. A short "recirculation" catheter (20 cm, polyethylene no. 160) was placed in the ascending aorta. Additional catheters were placed in the ventricular cavity and femoral artery for pressure measurements and in the femoral vein for i.v. infusions.

Reprint requests should be addressed to Dr. Richard Effros, Respiratory Division, Harbor General Hospital-University of California at Los Angeles, Torrance, Calif. 90509.

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[1] As indicated in the text, data obtained in the present study with an anionic indicator should reflect average intracellular hydroxl ion concentrations rather than pH. However, since values of "cell pH" are generally calculated from indicator distribution data, this practice will be followed here as well.

An injection cocktail was prepared with the following ingredients: 0.1 mCi of [^{125}I]human serum albumin (the vascular indicator, Mallinckrodt Chemical Works, St. Louis, Mo., specific activity 9 μCi/mg), 0.1 mCi of [^{51}Cr]EDTA (the extracelluar indicator, Amersham/Searle Corp., Arlington Heights, Ill., specific activity 1 mCi/mg Cr), 1.0 mCi of tritiated water (THO) [2] (the water indicator, New England Nuclear, Boston, Mass., specific activity 25 mCi/g), and 0.1 mCi of [2-^{14}C]5,5-dimethyl-2,4-oxazolidinedione [^{14}C]DMO, (the pH indicator, New England Nuclear, specific activity 1 mCi/mg) in 7 ml of a suspension of erythrocytes from the dog in 2.5 g/100 ml human serum albumin in 0.9 g/100 ml saline solution at the hematocrit of the dog.

Injections of 1.0 ml of the injection solution were flushed from a 1.0-ml syringe pipette with 5 ml of arterial blood from the same dog through the coronary artery catheter into the heart within a 5-s interval. Because the injection solution entered in advance of the flush, it is likely that most of the tracer materials were introduced within 2 s. The actual injection quantity and interval play a very minor role in the calculation of myocardial pH inasmuch as the calculation is based upon a ratio of mean transit time differences. The mean transit times reported and used are therefore uncorrected for the relatively short injection interval. Myocardial perfusion was determined from the mean transit time of water, as indicated below. The mean transit times of water were relatively long and these approximations should have a minor influence on the calculated tissue perfusion rate (see below).

Blood was pumped with a peristaltic pump at 0.3 ml/s from the great cardiac vein into 42 collection tubes which were changed at 5-s intervals by an automatic sampler. Arterial samples were withdrawn by hand from the "recirculation" catheter at times corresponding to the first three 10-s intervals after beginning of injection. Additional arterial samples were drawn at 1, 2, and 3 min and at the end of the run. All samples were processed for beta and gamma activity as indicated previously (4). The activities of each tracer were then divided by the quantity of indicator in the injection bolus to yield comparable "fractional concentrations" (indicated "w" in units of milliliters^{-1}).

Venous fractional concentrations were then plotted on a logarithmic ordinate against time on a linear plot as indicated in Fig. 1 *a*. The areas (A_u) under the uncorrected venous curves were then approximated by drawing a linear downslope and using eq. 14A of the Appendix.

Aortic samples were obtained from the short, small-bore catheter in the brachial artery. Catheter delay at this site was less than 1 s and both this delay and the difference in arrival times of recirculating indicators to the heart and aorta were assumed negligible. The delay involved in the collection of the venous samples from the collection catheter was significantly greater, mean collection catheter times averaged 8 s. Collection catheter delays were calculated from the catheter volumes divided by the catheter flows. As an approximation, the mean catheter times were added to the actual times at which the aortic samples were obtained. Aortic recirculation curves were constructed by interpolating between observed aortic concentrations on the same coordinates, thereby providing concentrations of recirculating indicators which corresponded to the times at which the venous samples were obtained. The venous curves were then corrected for returning arterial concentrations by a

[2] *Abbreviations used in this paper:* [^{14}C]DMO, [2-^{14}C]-5,5-dimethyl-2, 4-oxazolidinedione; THO, tritiated water.

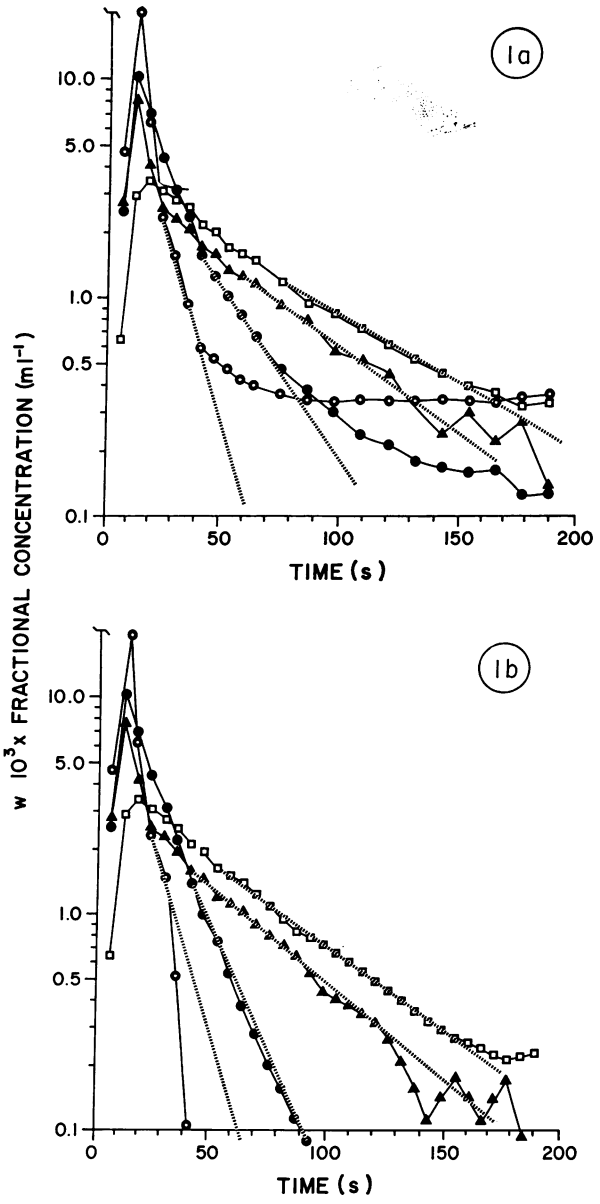

FIGURE 1 Indicator dilution curves in great cardiac vein outflow after injection into anterior descending coronary artery of normal dog heart. Open circles represent [^{125}I]-albumin, closed circles represent [^{51}Cr]EDTA, triangles represent [^{14}C]DMO, and squares represent THO. The curves on the top (*a*) are uncorrected for recirculation, and the dotted lines indicate the extrapolations used to estimate the areas under the curves. The curves on the bottom (*b*) have been corrected for recirculation, and the dotted lines represent the extrapolations used to calculate relative recoveries and mean transit times. The corrected curves tend to have a more monoexponential form. Two points on the [^{125}I]-albumin curve appear to be overcorrected. w represents fractional concentrations.

deconvolution procedure similar to that suggested by Zierler (5):

$$c_i = v_{i-}(\sum_{j=1}^{j=i} a_j v_{i-j+1}/A_u) \quad (1)$$

where v represents the observed venous fractional concentration, a designates arterial concentrations interpolated from observed arterial values as indicated above, and c is the corrected venous concentration. Evidence that this correction for recirculation was adequate is provided in the Discussion section. Corrected venous values are plotted in Fig. 1 *b*. Values for the areas under the corrected indicator curves were calculated with eq. 14A, and indicator mean transit times were calculated with eq. 15A of the Appendix.

The indicator mean transit times were used to calculate the distribution ratio ρ. ρ represents the ratio of DMO concentration within the myocardial cells to that in the plasma which would prevail at infinite time if DMO were infused into the organ at a constant rate. ρ was calculated from the mean transit time difference equation (derived in the Appendix):

$$\rho = (G\bar{t}_Y - H\bar{t}_E - J\bar{t}_R)/(K\bar{t}_W - H\bar{t}_E - N\bar{t}_R). \quad (2)$$

The coefficients G, H, J, K, and N are defined in the Appendix. $\bar{t}_Y$, $\bar{t}_E$, $\bar{t}_R$, and $\bar{t}_W$ designate the mean transit times of [^{14}C]DMO, [^{51}Cr]EDTA, erythrocytes, and tritiated water. Evidence that [^{51}Cr]EDTA is a satisfactory extracellular indicator is provided in the Discussion section. Erythrocyte mean transit times ($\bar{t}_R$) were estimated from the mean transit time of [^{125}I]albumin ($\bar{t}_{^{125}I}$) with the equation

$$\bar{t}_R = 0.868\bar{t}^{125}_{\mathrm{I}} \quad (3)$$

on the basis of eight separate experiments in which the mean transit times of ^{51}Cr-labeled erythrocytes were compared with the mean transit times of [^{125}I]albumin. In these studies, $\bar{t}_R/\bar{t}_{^{125}I} = 0.868 \pm 0.07$[3] ($n = 12$). In ischemic dog hearts, the ratio averaged 0.863±0.08 ($n = 5$). Equations for the calculation of plasma, and extracellular and exchangeable water volumes are provided in the Appendix.

Myocardial cell pH was derived from the cellular hydroxyl concentration $[OH^-]_c$. $[OH^-]_c$ was determined from ρ, the plasma hydroxyl concentration $[OH^-]_p$, and the basic dissociation constant of DMO ($K'_b = 0.1349 \times 10^{-7}$) (6) by solving the equation:

$$\rho = \frac{[OH^-]_c + K'_b}{[OH^-]_p + K'_b}. \quad (4)$$

Plasma pH was estimated from the average of arterial and great cardiac vein pH obtained at the time of each run. The limitations of such an estimate are described below.

Areas under the four corrected indicator dilution curves were averaged in each run. The area under each indicator curve was divided by this average value to obtain relative recoveries. No attempt was made to calculate absolute coronary blood flow because reflux of the injected material into the aorta or collection of blood from other portions of the heart which had not received the injection solution would exaggerate calculated values. Neither reflux nor constant venous dilution should alter indicator mean transit times or calculated values of tissue perfusion, compartmental volumes, or cellular pH.

[3] All means are indicated with SD.

Myocardial perfusion (Q_{THO}, milliliters per minute^{-1} (100 g tissue^{-1})) was determined from the mean transit time of water with the equation (derived in the Appendix):

$$Q_{THO} = 0.78/\{[0.737\ \mathrm{Hct} + 0.956(1 - \mathrm{Hct})]\bar{t}_{THO}\} \quad (5)$$

Perfusion values obtained in this fashion were compared with values obtained within 20 min by the standard ^{85}Kr procedure based on residue detection of the washout of a bolus of this inert gas from the myocardium (7, 8).

Metabolic acidosis was produced with infusions of 0.3 M HCl in 0.9 g/100 ml saline at 4.0 ml/min which were sustained until arterial pH levels had fallen to the desired range. Metabolic alkalosis was generated with infusions of 0.4 M Na_2CO_3 at the same rate of flow. The rationale for the use of Na_2CO_3 rather than $NaHCO_3$ is indicated in the Discussion section. Infusions were continued during the run and lasted from 20 to 40 min. Arterial and great cardiac vein pH values were obtained both before and after the study and averaged (each collection period lasted for 3.5 min; changes in arterial and venous pH over this short interval proved to be less than 0.05 U).

Myocardial ischemia was induced by inflating a balloon near the tip of the coronary artery catheter. Pressure recordings were obtained from the left ventricle or aorta just beyond the site of obstruction. Characteristic signs of S-T elevation were observed in each run (standard lead I).

RESULTS

The observed outflow patterns of [^{125}I]albumin, [^{51}Cr]-EDTA, [^{14}C]DMO, and THO are shown in Fig. 1 *a* and compared with the indicator dilution curves corrected for recirculation in Fig. 1 *b*. Correction for recirculation tended, particularly for [^{125}I]albumin and [^{51}Cr]EDTA, to make the decline of concentrations assume a more monexponential form. Measurements of myocardial perfusion obtained from the tritiated water data appeared to correlate well with the flows determined separately from ^{85}Kr decay (see Fig. 2). The linear regression equation is indicated in Table I. The fact that values obtained from the tritiated water data tended to be slightly greater than those obtained with ^{85}Kr probably reflects the fact that indicator remaining within the organ for long intervals is more easily determined by residue detection than outflow analysis.

The average recoveries of [^{125}I]albumin, [^{51}Cr]EDTA, [^{14}C]DMO, and THO in the control studies were 0.96±0.07, 0.97±0.05, 1.02±0.06, and 1.03±0.06 (SD, $n = 20$). The mean transit time of [^{51}Cr]EDTA averaged 0.373±0.053 of the mean transit time of tritiated water and calculated values of the extracellular volume ($V_{r.p.e}$ including erythrocyte, plasma, and interstitial volumes) averaged 0.298±0.046 of the total water content of the heart. The mean transit time of [^{125}I]-albumin averaged 0.202±0.045 of the mean transit time of water, and the [^{125}I]albumin space averaged 0.130 ±0.035 of the total water content of the heart. As indicated in Table II, both acute and prolonged ischemia appeared to result in a decline in the ratio of the [^{125}I]albumin mean transit time to that of tritiated

water. The corresponding ratio of indicator volumes also diminished during prolonged ischemia. No other significant changes in indicator recoveries or the relative vascular or extracellular volumes were found after infusions of acid or alkali, nor were these relative values altered by ischemia. At control arterial pH (7.39±0.03, $n = 20$), the predicted steady-state ratio (ρ) of DMO within the myocardial cells to DMO in the extracellular space averaged 0.420±0.062, and the calculated value of myocardial cell pH averaged 6.94 ±0.07. Variations in myocardial cell pH between two control runs in each of six dogs were random and averaged 0.08 U. These control runs were obtained at intervals up to 45 min and over periods varying up to 2 h after anesthesia was begun, suggesting that under control conditions myocardial cell pH was relatively constant. Peak values of recirculation varied between 0.02 and 0.20 of the observed venous peak concentrations. Despite variations of as much as fourfold in relative peak recirculation in individual animals, calculated values of myocardial cell pH remained relatively unchanged. These findings suggest that correction for recirculation was adequate.

Infusions of acid tended to increase cellular concentrations of DMO in the heart, reflected by an increase in ρ values, whereas infusions of alkali diminished relative cellular concentrations of DMO with a corresponding decline in ρ. This is illustrated in Fig. 3 *a*. The linear regression relating ρ and average plasma pH is indicated in Table I. With metabolic alterations of plasma pH, changes in cellular pH were only 58%

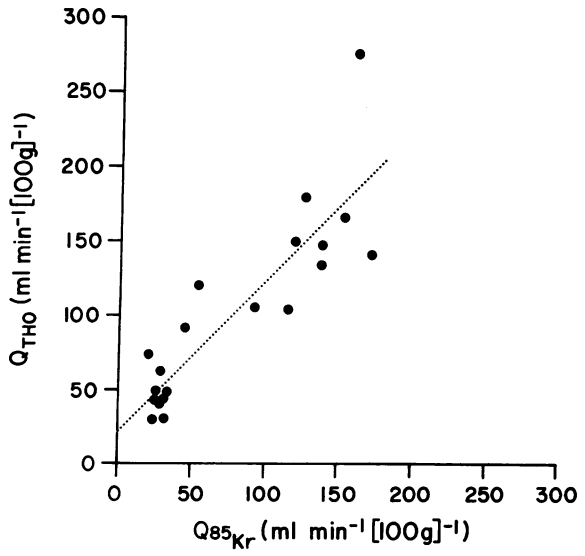

FIGURE 2 Correlation of perfusion determined by ^{85}Kr scanning method ($Q_{^{85}Kr}$) and perfusion determined from mean transit time of THO (Q_{THO}). The regression line (dotted) has a slope close to 1.0 but the y intercept is at 18.65.

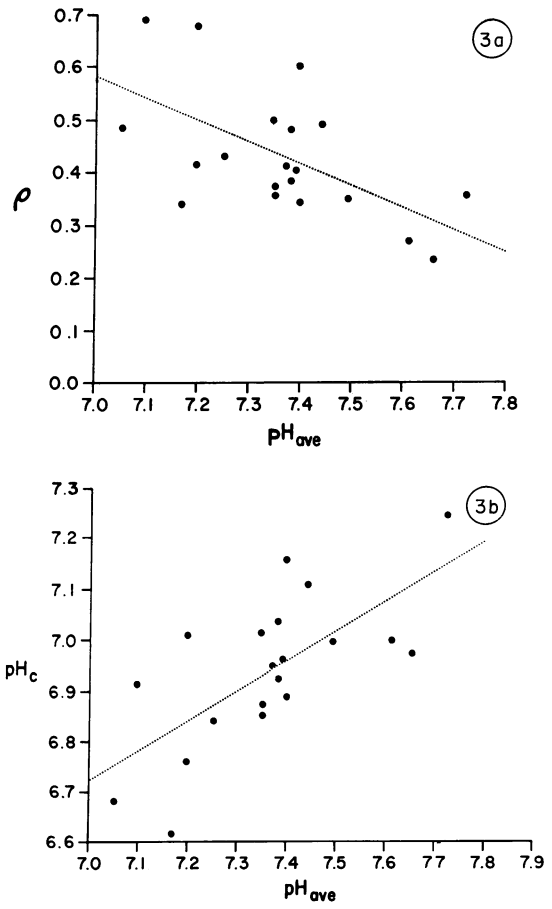

FIGURE 3 Correlation of ρ (*a*) and pH_c (*b*) with the average of arterial and great cardiac vein pH (pH_{ave}). Changes in pH_{ave} were induced with infusions of HCl and Na_2CO_3 solutions (see text).

of changes in concomitant plasma pH (see Fig. 3 *b* and Table I). These infusions had no consistent effect upon blood pressure, heart rate, myocardial perfusion, or the difference between coronary artery and great cardiac vein pH.

Inflation of the balloon on the catheter in the descending coronary artery resulted in the establishment of a hydrostatic pressure difference between the left ventricle and distal artery during systole which averaged 0.75 of the left ventricular systolic pressure (see Fig. 4). Myocardial perfusion declined from an average of 140 ml·min^{-1}·(100 g)$^{-1}$ to an average between 50 and 65 ml·min^{-1}·(100 g)$^{-1}$ (see Table II). At these moderately reduced flow rates, it was difficult to document consistent changes in the difference between coronary sinus pH and coronary artery pH. However, both arterial and venous pH did decline by about 0.03 to 0.04 U during chronic ischemia without a change in P_{CO_2}, suggesting a mild systemic metabolic acidosis. S-T

TABLE I

*Regression Equations and Correlation Coefficients**

Conditions	x	y	n	B	A	r	P
Control and ischemia	Q_{Kr}	Q_{THO}	23	1.034	18.65	0.875	<0.001
Control, HCl and Na_2CO_3 infusions	pH_{ave}	ρ	20	0.417	3.50	0.598	<0.01
	pH_{ave}	pH_e	20	0.586	2.62	0.685	<0.001
Control and ischemia	t	ρ	33	0.00373	0.396	0.709	<0.001
	t	pH_e	33	0.00846	6.91	0.726	<0.001
Control and late ischemia	Q_{THO}	ρ	27	0.00072	0.256	0.436	<0.05
	Q_{THO}	pH_e	27	0.00193	6.56	0.523	<0.01

* The regression equations are of the form: $y = Bx + A$. r designates the correlation coefficient, and P represents the probability that the correlation coefficient is zero. t indicates the duration of ischemia. The remaining symbols are defined in the text.

elevations consistently accompanied the onset of ischemia (Fig. 4), and mean arterial pressures remained unchanged or declined by no more than 20 mm Hg.

Indicator dilution curves obtained after 28 min of ischemia are shown in Figs. 5 *a* and *b*. It will be noted that the downslope of both the uncorrected and corrected curves show at least two exponential components. There is also a tendency for the [^{14}C]DMO to be closer to the [^{51}Cr]EDTA curve and farther from the THO curve than was evident in the control study (Fig. 1). The calculated value for ρ in this study has declined from 0.430 to 0.144, and the calculated cellular pH has fallen from 6.93 to 6.21.

During early ischemia (6–15 min), average values of ρ declined from 0.420 to 0.342 and later (20–70 min) further fell to 0.257 (see Fig. 6 *a* and Table II). This decline in ρ indicates that the myocardial cell contents have become increasingly acid compared to the pH of the myocardial interstitium. Calculated values of myocardial cell pH declined from 6.94 to 6.83 at 6–15 min and to 6.59 at 20–70 min (see Fig. 6 *b* and Table II). Both the decline in ρ and intracellular pH appeared to be better correlated with the duration rather than the magnitude of ischemia (see Table I).

Infusions of Na_2CO_3 were administered to increase arterial pH to an average of 7.57 in dogs with myo-

TABLE II

Effects of Ischemia and Na_2CO_3 Treatment

Condition		Q	pH_{art}	pH_{ven}	Pco_{2art}	Pco_{2ven}	Po_{2art}	Po_{2ven}	$\frac{\tilde{t}_{^{125}I}}{\tilde{t}_{THO}}$	$\frac{\tilde{t}_{[^{51}Cr]EDTA}}{\tilde{t}_{THO}}$	$\frac{V_p}{V_e}$	$\frac{V_{rpi}}{V_e}$	ρ	pH_e
		ml·min^{-1}·(100 g)$^{-1}$			*torr*	*torr*	*torr*	*torr*						
Control	$\bar{x}$	139.8	7.386	7.334	32.9	46.8	94.6	32.4	0.202	0.373	0.130	0.298	0.420	6.94
	SD	51.7	0.031	0.037	4.7	7.5	9.0	7.7	0.045	0.053	0.035	0.046	0.062	0.07
	n	20	20	20	20	20	14	14	20	20	20	20	20	20
Early ischemia, <15 min	$\bar{x}$	53.0	7.387	7.320	32.5	47.1	98.3	30.0	0.128	0.357	0.097	0.270	0.342	6.83
	SD	31.0	0.031	0.044	2.4	5.6	14.1	9.9	0.048	0.078	0.054	0.070	0.054	0.07
	n	8	8	8	8	8	6	6	8	8	8	8	8	8
	*P_{12}	<0.001							<0.001				<0.01	<0.001
Late ischemia, >15 min	$\bar{x}$	55.3	7.344	7.287	33.6	48.9	100.3	29.1	0.148	0.343	0.097	0.268	0.257	6.59
	SD	19.5	0.044	0.042	3.8	6.2	10.9	8.0	0.056	0.086	0.039	0.070	0.085	0.23
	n	10	10	10	10	10	7	7	10	10	10	10	10	10
	*P_{13}	<0.001	<0.01	<0.01					<0.01		<0.05		<0.001	<0.001
	*P_{23}		<0.05										<0.05	<0.02
Ischemia and Na_2CO_3 infusion		64.3	7.572	7.525	34.2	42.9	89.8	24.0	0.177	0.373	0.131	0.316	0.251	6.89
		14.1	0.027	0.029	3.6	3.9			0.046	0.059	0.037	0.045	0.036	0.05
	n	5	5	5	5	5	2	2	5	5	5	5	5	5
	*P_{14}	<0.01	<0.001	<0.001									<0.001	
	*P_{24}		<0.001	<0.001										<0.02

* P_{12}, probability that control and early ischemic value are the same; P_{13}, probability that control and late ischemic value are the same; P_{23}, probability that early ischemic and late ischemic value are the same; P_{14}, probability that control and ischemic and carbonate value are the same; and P_{24}, probability that late ischemic and ischemic and carbonate value are the same.

Only P values less than 0.05 are indicated.

n, number of dogs (if more than one run performed in the same animal under the stated condition, these values were averaged).

cardial ischemia. (These studies are indicated in Figs. 6 *a* and *b* as interrupted lines.) Despite these infusions, ρ values continued to remain low (at 30–90 min, ρ averaged 0.268 with Na_2CO_3 infusions, a value not significantly different than ρ values in the ischemic studies without Na_2CO_3 infusion). This result suggests that the difference between pH_e and pH_p remained large despite the infusion. However, alkalemia did serve to increase pH_e toward control values (see Fig. 6 *b* and Table II). These infusions increased serum osmolality by an average of 18 mosmol/kg.

DISCUSSION

Measurements of cellular pH have been based upon data obtained with microelectrodes or the distribution of pH indicators between cells and surrounding fluid. Considerable technical problems are associated with electrode measurements of cell pH in the contracting heart, and reported values of tissue pH have been obtained with relatively sturdy devices which are much too large to penetrate individual cells without damage (9, 10). Electrodes of this size presumably respond to a mixture of fluids obtained from the plasma, interstium, and ruptured cells.

The chief advantage of the indicator dilution approach is the atraumatic manner in which cellular pH measurements may be obtained. However, these measurements are indirect and are critically related to the behavior of the pH indicator. It is assumed that the ratio of the concentration of indicator within the cell to that in the extracellular fluid is exclusively determined by the difference in hydrogen ion (or hydroxyl ion) activities between these compartments. Since its introduction by Waddell and Butler, DMO has been used extensively for this purpose (6). Although some data seemed to indicate that electrode measurements of skeletal muscle cell pH were considerably lower than

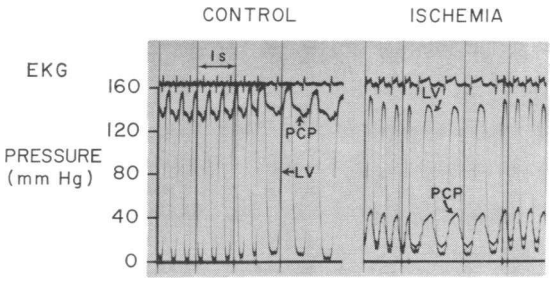

FIGURE 4 Production of myocardial ischemia with balloon inflation. LV is the left ventricular pressure, and PCP is the pressure in coronary artery distal to the site of the balloon. The production of a gradient between systolic pressures in the left ventricle and distal coronary artery with balloon inflation is shown. The appearance of S-T elevations is evident in the tracing at 1 min (standard lead I).

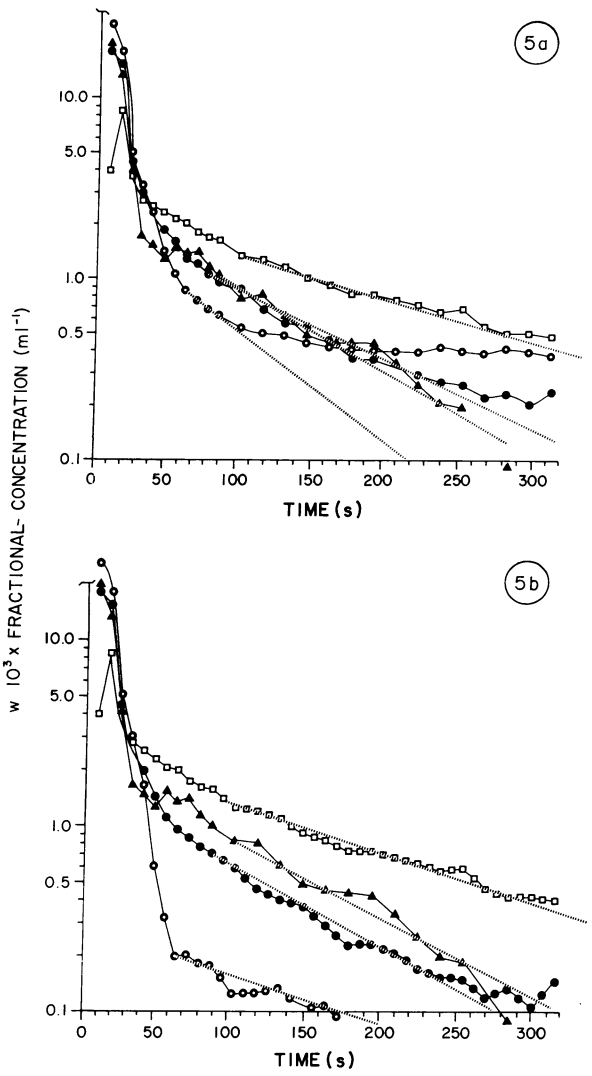

FIGURE 5 Uncorrected (*a*) and corrected (*b*) indicator curves after 28 min of ischemia have elapsed. Open circles represent [^{125}I]albumin, closed circles represent [^{51}Cr]-EDTA, triangles represent [^{14}C]DMO, and squares represent THO. The multiexponential form of these curves reflect slow washout from poorly perfused portions of the heart (compared with the control study in Fig. 1). The [^{14}C]DMO curve lies closer to the [^{51}Cr]EDTA curve in the ischemic study. The calculated value of ρ has fallen from 0.424 to 0.144 with a corresponding decline in pH_e from 6.934 to 6.206. w represents fractional concentrations.

those obtained with DMO (11), more recent evidence suggests that these electrode determinations were technically faulty (12). Justification for use of DMO has been reviewed by Waddell and Bates (13).

Studies with DMO have been generally conducted under steady-state conditions. In some experiments the indicator has been administered systemically with extracellular and water indicators, and tissue samples have

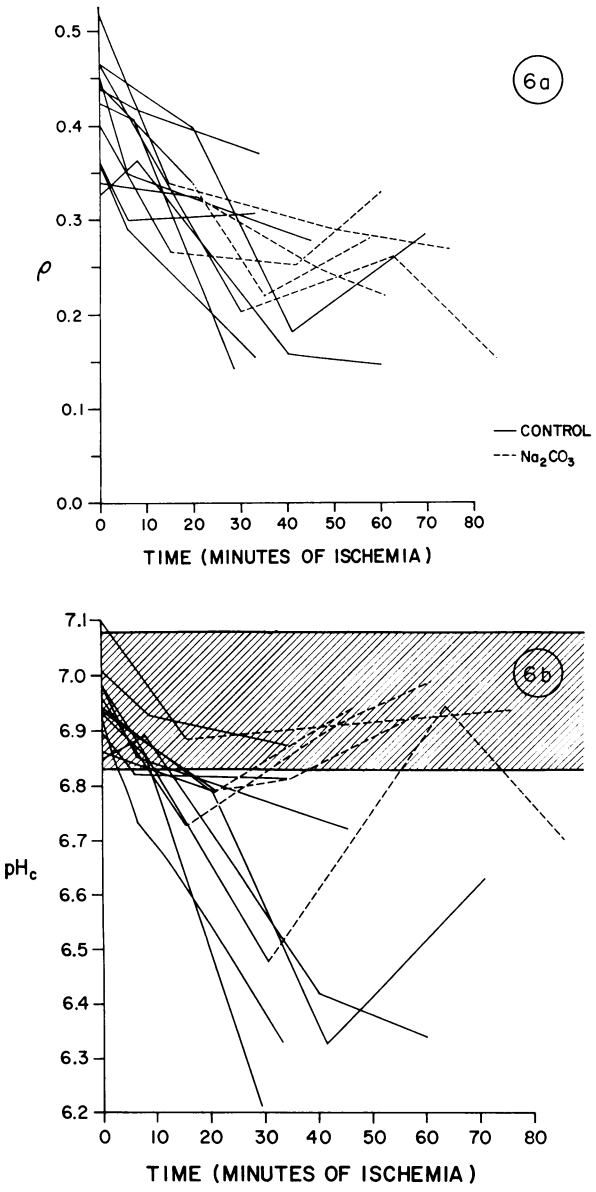

FIGURE 6 Correlation of ρ values with the duration of ischemia. The shaded area represents the normal range of pH_e values (within 2 SD of the mean value). The dotted lines indicate those studies in which infusions of Na_2CO_3 solutions were administered. It is difficult to tell from these studies if these infusions had any effect on the decline in ρ which accompanied ischemia. (*b*) Correlation of myocardial cell pH (pH_e) with the duration of ischemia. Infusions of Na_2CO_3 tended to reverse the myocardial cell acidosis which was produced by ischemia.

been obtained for evaluation of the distribution of DMO between the cellular and extracellular compartments (14–18). Alternatively, the organ may be isolated and perfused and concentrations of these indicators may be determined in the tissues or perfusion fluids (19).

Introduction of a transient method for estimating the distribution of pH indicator between tissues and plasma has made it possible to obtain intracellular pH data for individual organs without the necessity of tissue sampling or organ isolation (4).

In an initial in vivo study of tissue pH, values were obtained for an average pH of the cellular and interstitial volumes of the lung. The failure to find a suitable extracellular indicator made it possible to obtain separate measurements of pH for these compartments. In the present study, [^{51}Cr]EDTA was used as a marker of the extracellular volume of the heart. This indicator has been used previously by Trap-Jensen and Lassen as an extracellular marker (20). The mean transit times of [^{51}Cr]EDTA averaged 0.38 of that of tritiated water, a value very close to that found for sucrose by Ziegler and Goresky (0.40) (21). The calculated ratio of extracellular to total water content of the heart averaged 0.30. This value is considerably smaller than that reported with sucrose by Page and Page (0.40) from observations of isolated perfused hearts (22). In the latter study, the volume of distribution of sucrose increased progressively over a period of 1 h and ultimately represented 60% of the entire organ water. It has been suggested that entry of sucrose into the sarcoplasmic reticulum occurs. It is possible that in the present single circulation studies with [^{51}Cr]EDTA and those of Ziegler and Goresky (21) with [^{14}C]-sucrose, these indicators are primarily confined to the extracellular space. That this may indeed be the case is suggested by the good agreement of these transient data with those of Johnson and Simonds who estimated on histological grounds that the extracellular space represents 0.26 of the total organ volume (23). Since the morphological cellular volume of heart includes more solids than corresponding interstitial volumes, the fraction of cardiac water in the extracellular compartment should be slightly greater than 0.26 and close to the value of 0.30 found in the present study.

The choice of tritiated nicotine rather than [^{14}C]-DMO in the previous study of pulmonary tissue pH was based upon the observation that the outflow pattern of DMO was nearly the same as that of the vascular indicator. Similar observations had been made with a variety of hydrophilic indicators such as Na^+, sucrose, and urea, and measurements of the interstitial volume of the lung have been frustrated by the similarity between the outflow patterns of extracellular and vascular indicators (24). Recent evidence suggests that the low extraction of these indicators, which occurs during a single circulation through the lung, is related to the high rate of blood flow per volume of tissue

which prevails in this organ (25). These high perfusion rates may diminish the extraction of solutes in a predictable fashion (26). When the rate of perfusion is properly considered, the capillary permeability of the lung to Na^+ is similar to that of skeletal muscle.

The perfusion of the heart under control circumstances averaged only 16% of the rate observed in the lungs, and the outflow patterns of both [^{14}C]DMO and [^{51}Cr]EDTA were distinct from that of [^{125}I]albumin. The observed similarity between the distribution of DMO between the heart and plasma calculated from the present transient data and that found in earlier steady-state studies (6) suggests that the mean transit time calculations were not unduly influenced by changes in the indicator dilution curves due to limited permeability.

The decision to use [^{14}C]DMO in the present study was motivated in part by the availability of in vitro cardiac studies with this indicator. In addition it seemed advisable to use an indicator with a relatively shorter mean transit time in the heart in order to minimize recirculation problems and shorten the duration of the study. Since the cellular concentrations of cationic indicators such as tritiated nicotine are greater than plasma concentrations, the mean transit times of these indicators in the heart would presumably exceed those of tritiated water. It would therefore be necessary to prolong the observation interval and thereby make the study more vulnerable to changes in flow or metabolism.

Success of the transient indicator dilution procedure is dependent upon accurate measurements of indicator mean transit times. These mean transit times must be calculated from time concentration curves of cardiac venous blood which may contain considerable amount of recirculated indicator. Correction for recirculation is particularly important when mean transit times are long. The mean transit times of the diffusible indicators may greatly exceed those of the vascular indicators and are further prolonged when local tissue perfusion is diminished. In the present study, returning arterial concentrations were used to derive the first circulation indicator curves. As in all such corrections for recirculation, satisfactory results are dependent upon constant flow and indicator mean transit times. Efforts to collect and divert the entire venous drainage of indicator from the heart in the intact dog as a means of checking correction proved impractical. However, evidence that the recirculation correction was adequate was provided by the observation that control values of myocardial cell pH calculated from the corrected curves were not markedly or systematically altered by variable recirculation. Reasonably good correlation between perfusion rates calculated from the washout of ^{85}Kr (an indicator with relatively little recirculation) and the corrected washout curves of THO in normal and ischemic hearts also suggests that the correction of at least the THO curves was acceptable. The apparent adequacy of the procedure is probably due in part to dilution of recirculating indicator by the cardiac output, in other words, by a factor of more than five.

The average intracellular pH of the heart calculated from the transient method (6.94) was in close agreement with steady-state values reported for both cardiac and skeletal muscle (the majority of reports are between 6.85 and 7.13) (13). Furthermore, the average change in cellular pH obtained from the transient studies amounted to 0.58 of concomitant changes with infusions of acid and alkali in plasma pH, whereas an average change of 0.60 was detected with in vitro studies of rat diaphragm (13) and 0.617 in perfused turtle hearts (19). Scatter is great in both the present and previous data and this apparent agreement may therefore be fortuitous. It has been reported that cell pH in rat diaphragm is relatively more stable to metabolic changes in extracellular pH in the range between 7.2 and 7.5 (27). This observation could not be verified with the present in vivo data of the dog heart.

Measurements of cellular pH in the ischemic heart are associated with several problems. Estimates of extracellular pH within the ischemic area are based upon the pH of blood collected from the great cardiac vein and aorta and are probably greater than local values. The failure to find an increase in the difference between arterial and great cardiac vein pH with ischemia in this study may reflect dilution of the venous samples with blood draining from nonischemic areas. It is therefore likely that the true cellular pH is significantly below the calculated values, which must be considered as upper limit data. It should be noted, however, that the rate of perfusion, the distribution ratio ρ, and the difference between the cellular and extracellular pH are based upon mean transit time data obtained from the ischemic portion of the heart and should not be influenced by a constant flow of blood from nonischemic regions of the heart.

Measurements of cellular pH during ischemia are further complicated by the likelihood that the decline in myocardial perfusion is not uniform. Reductions of coronary flow tend to compromise inner wall perfusion to a greater extent than outer wall perfusion (28), and lactate and pyruvate concentrations within the inner wall appear to exceed outer wall concentrations (29). Differences in regional pH are superimposed upon the heterogeneous distribution of hydrogen ions which normally prevails within the cells. Calculated values of cell pH must always represent an index of the average cellular pH on both a subcellular and regional basis. Values obtained with anionic indicators

such as DMO provide average hydroxyl ion concentrations and tend to represent more alkaline regions. In contrast, cationic indicators such as labeled nicotine, should yield data concerning average hydrogen ion concentrations and are influenced to a greater extent by the more acid portions of the heart (3, 30).

A further consequence of nonuniform flow within the ischemic area is the tendency for outflow concentrations to reflect better perfused areas of the heart which make a more obvious contribution to the outflow curves. The existence of parallel flows through regions of variable perfusion results in the appearance of multiexponential patterns of indicator washout which are more difficult to correct for recirculation and extrapolate for mean transit time calculations.

With these reservations in mind, values were calculated for myocardial cell pH in the ischemic preparation. Considerable variation was found in cellular pH in the presence of ischemia (see Fig. 6 *b*). During the initial 15 min of ischemia, the decline in cellular pH averaged 0.111. Between 20 and 70 min, the cellular pH fell by an average of 0.352 from control values. The severity of cellular acidosis appeared to be more closely correlated with the duration of ischemia than the measured decline in myocardial perfusion.

Inasmuch as systemic infusions of Na_2CO_3 appear to produce increases in myocardial cell pH which average 58% of concomitant increases in plasma pH, the effect of such infusions upon ischemic myocardial cell pH was studied. The choice of Na_2CO_3 rather than $NaHCO_3$ was based upon the fact that smaller volumes of Na_2CO_3 may be used to alkalinize the dog. In addition, Na_2CO_3 tends to diminish CO_2 tensions in blood whereas $NaHCO_3$ increases blood CO_2 tensions and may thereby produce transient "respiratory" acidosis if ventilatory compensation is not complete (31). Small increases in serum osmolality were observed with these infusions (see Results section).

It is difficult to be sure whether such infusions exerted any effect upon the distribution of DMO between the cells and extracellular fluid of the heart and therefore, the increased difference in myocardial cellular and local extracellular pH apparently persists despite these infusions. Nevertheless, the data do suggest that cellular acidosis may have been relieved in part by these infusions. Recent evidence that bicarbonate does have access to both skeletal and myocardial cells has been reported by Heisler and Piiper (32) and Lai, Atterby, and Brown (18).

The potential usefulness of this procedure for measuring myocardial cell pH in a clinical setting remains to be determined. Characterization of tissue pH in regions of the myocardium with severely diminished flow may be impossible by these means because both delivery into the ischemic area and effective washout must be present to ensure successful measurements. However, studies of tissue pH may prove valuable in patients with nonischemic heat disease or those with manifestations of ischemia, in the absence of severely diminished coronary flow.

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APPENDIX

Derivation of volume and distribution equations. The following symbols are used in this derivation:

Y, pH indicator ([^{14}C]DMO);
E, extracellular indicator ([^{51}Cr]EDTA);
W, water indicator (THO);
P, plasma indicator ([^{125}I]albumin);
R, erythrocyte indicator (calculated in these experiments from [^{125}I]albumin data);

$M_{Y,o}$, $M_{E,o}$, $M_{W,o}$, $M_{R,o}$, and $M_{P,o}$, amount (mass) of Y, E, W, R, and P in organ between points of injection and collection during constant infusion (grams);

I_Y, I_E, I_W, I_R, and I_P, flux of Y, E, W, R, and P into the organ during constant infusion (grams/second);

f_r, f_p, fractional volumes of water in erythrocytes and plasma (milliliter water/milliliter erythrocytes or plasma), $f_r = 0.737$, $f_p = 0.956$;

$V_{w,r}$, $V_{w,p}$, $V_{w,pi}$, $V_{w,o}$, $V_{w,rpi}$, and $V_{w,c}$, volumes of water in the following compartments: erythrocytes, plasma, plasma plus interstitium, entire organ, erythrocytes plus plasma plus interstitium, and cells (milliliters);

$\bar{t}_Y$, $\bar{t}_E$, $\bar{t}_W$, $\bar{t}_P$, and $\bar{t}_R$, mean transit times of Y, E, W, P, and R (seconds);

$[Y]_r$, $[Y]_p$, and $[Y]_c$, concentrations of Y in $V_{w,r}$, $V_{w,p}$, and $V_{w,c}$ during constant infusion (grams/milliliter);

F, F_p, flow of blood and plasma through organ (milliliters/second);

$F_{w,r}$, $F_{w,p}$, flow of water in erythrocytes and plasma through organ (milliliters/second).

The mass M_x of any indicator x within the organ which would be present after a sufficiently prolonged infusion at a constant infusion rate is:

$$M_x = I\bar{t}_x, \tag{1A}$$

where $\bar{t}_x$ designates the mean transit time of x. Thus,

$$\begin{aligned} M_{R,o} &= I_R\bar{t}_R, \\ M_{P,o} &= I_P\bar{t}_P, \\ M_{E,o} &= I_E\bar{t}_E, \\ M_{W,o} &= I_W\bar{t}_W, \\ M_{Y,o} &= I_Y\bar{t}_Y. \end{aligned} \tag{2A}$$

Assume that the erythrocyte, extracellular and water indicators become uniformly distributed in the water of their respective compartments during a prolonged infusion. Dividing eq. 2-5 by the steady-state concentration:

$$V_{w,r} = F_{w,r}\bar{t}_R = f_r \text{ Hct } F\bar{t}_R, \tag{3A}$$

$$V_{w,p} = F_{w,p}\bar{t}_p = f_p(1 - \text{Hct})F\bar{t}_P, \tag{4A}$$

$$V_{w,pi} = F_{w,p}\bar{t}_E = f_p(1 - \text{Hct})F\bar{t}_E, \tag{5A}$$

$$V_{w,o} = F_{w,b}\bar{t}_W = [f_r \text{ Hct} + f_p(1 - \text{Hct})]F\bar{t}_W, \tag{6A}$$

and

$$V_{w,rpi} = V_{w,r} + V_{w,pi}. \tag{7A}$$

$$V_{w,c} = V_{w,o} - V_{w,rpi}. \tag{8A}$$

Let us assume that the pH indicator (Y) becomes distributed between three volumes within the heart: (a) the erythrocytes, (b) the plasma and interstitial volume, and (c) the myocardial cells. Moreover, let us assume that during constant infusion, the concentration of Y becomes uniform within each of these volumes though possibly nonuniform between volumes. Then:

$$\begin{aligned} M_Y = I_Y\bar{t}_Y &= M_{Y,r} + M_{r,pi} + M_{Y,c} \\ &= [Y]_rV_{w,r} + [Y]_pV_{w,pi} + [Y]_cV_{w,c}. \end{aligned} \tag{9A}$$

Now

$$I_Y = \{[Y]_rf_r(\text{Hct}) + [Y]_pf_p(1 - \text{Hct})\}F, \tag{10A}$$

and substituting 3A, 5A, 8A, and 10A into 9A dividing by F and solving for $[Y]_c/[Y]_r$ yields:

$$\rho = \frac{[Y]_c}{[Y]_p} = \frac{G\bar{t}_Y - H\bar{t}_E - J\bar{t}_R}{K\bar{t}_W - H\bar{t}_E - N\bar{t}_R}, \tag{11A}$$

where

$$G = \frac{[Y]_r}{[Y]_p}f_r \text{ Hct} + f_p(1 - \text{Hct}) = 0.737\frac{[Y]_r}{[Y]_p}\text{ Hct} + 0.956(1 - \text{Hct}),$$

$$H = f_p(1 - \text{Hct}) = 0.956(1 - \text{Hct}),$$

$$J = \frac{[Y]_r}{[Y]_p}f_r \text{ Hct} = 0.737\frac{[Y]_r}{[Y]_p}\text{ Hct},$$

$$K = f_r \text{ Hct} + f_p(1 - \text{Hct}) = 0.737 \text{ Hct} + 0.956(1 - \text{Hct}),$$

$$N = f_r(\text{Hct}) = 0.737(\text{Hct}).$$

Values for f_r and f_p were derived previously (3). $[Y]_r/[Y]_p$ designates the ratio of DMO concentration within erythrocytes to that in plasma and was calculated (33) from the equation:

$$[Y]_r/[Y]_p = 2.49 - 0.27 \text{ pH}_{ave}, \tag{12A}$$

where pH_{ave} represents average plasma pH (see above).

Calculation of relative recoveries, mean transit times, and organ perfusion. It was assumed that the concentration of each sample represented the average concentration at the midpoint of each collection tube interval. The areas (A) under both the observed indicator dilution curve and that under the curve corrected for recirculation (see Methods section) were calculated from the sample concentrations up to that point at which recirculation appeared (judged in the uncorrected curve by nonlinear decline on the semilogarithmic plot) or at that point at which the data became ragged (in the corrected curves). The downslopes were extrapolated to infinity on the basis of two points along the downslope (collection tubes n_1 and n_2 with indicator fractional concentrations w_1 and w_2) with the equation

$$k = -\ln(w_2/w_1)/[(n_2 - n_1)\tau], \tag{13A}$$

where τ is the collection tube interval. Extrapolation was begun at the end of the n_2 collection interval at which time the fractional concentration is

$$w_f = w_2e^{-k\tau/2}. \tag{14A}$$

The area under the entire curve is

$$A = \sum_0^{n_2} w\tau + w_f/k. \tag{15A}$$

The first term on the right represents the area under the unextrapolated portion of the curve, and the second term represents the extrapolated area.

The mean transit time ($\bar{t}$) of the curve was calculated from the equation:

$$\bar{t} = \left[\left(\sum_0^{n_2} wn - \tfrac{1}{2}\sum_0^{n_2} w\right)\tau^2 + w_f\left(\frac{n_e\tau}{k} + \frac{1}{k^2}\right)\right]\Big/A. \tag{16A}$$

The first term of the numerator designates the sum of products of concentration and time for the unextrapolated portion of the curve, and the second term represents the same product for the extrapolated portion of the curve.

The rate of perfusion (Q) in milliliters/minute per 100 g of tissue was obtained from eq. 6A by assuming that water comprised 0.78 of the wet weight (W) of the heart (34):

$$Q_{\text{THO}} = F/W = 0.78/\{[0.737 \text{ Hct} + 0.956(1 - \text{Hct})]\bar{t}_{\text{THO}}\}. \tag{17A}$$

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