Acid-Base Changes of the Thoracic Duct Lymph during Hypercapnia and an Estimation of Cellular Contribution to ECF Buffering Capacity

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Abstract During 90 min of spontaneous or controlled CO₂ breathing in splenectomized dogs, the acid-base parameter changes of the thoracic duct lymph were compared with those of the arterial and postcaval venous blood. (1) The lymph reached an apparent steady state in the acid-base parameters at least 10 min after the blood. (2) The lymph bicarbonate concentration gradually increased almost in parallel with the lymph P_{CO_2} elevation, while the raised plasma bicarbonate concentration remained nearly unchanged until the end of CO₂ inhalation. (3) The *in vivo* buffer slope of the lymph increased strikingly within 10 min, reaching three times the *in vitro* slope at 90 min after inception of CO₂ breathing. After 10 min, the lymph value became higher than the venous value, suggesting early cellular contribution to ECF buffering. (4) Considering the lymph values as representative of the interstitial fluid values, cellular contribution to ECF buffering was computed; they were 7.8, 15.6, and 10.7% in the spontaneously breathing group, and 8.9, 10.6, and 12.0% in the controlled ventilation group at 30, 60, and 90 min of CO₂ breathing, respectively.

Acute hypercapnia is followed by a fall in blood pH and a rapid rise in the plasma bicarbonate concentration. The ratio of changes of these parameters, $\Delta[\text{HCO}_3^-]_p/\Delta\text{pH}$, is generally defined as the slope of the *in vivo* CO₂ titration curve. This slope is a composite result of the chemical and physiological buffering processes, which are (1) chemical buffering and bicarbonate redistribution throughout the extracellular fluids (ECF) including the erythrocytes, (2) ion transport from the intracellular to the extracellular fluids or *vice versa*, (3) bone carbonate release, and (4) renal compensation (BROWN and MICHEL, 1968; NICHOLS, 1958).

It has been demonstrated that the slope of the *in vivo* CO₂ titration curve changes with the duration of hypercapnia (WOODBURY, 1974) and that during the early stage of hypercapnia, the *in vivo* slope is considerably lower than the

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in vitro slope (SHAW and MESSER, 1932; BROWN, 1960; COHEN *et al.*, 1964). This decrease is due primarily to redistribution of the blood-produced bicarbonate into the interstitial fluid (ISF) which has a poor buffer capacity (SHAW and MESSER, 1932; COHEN *et al.*, 1964; BROWN and MICHEL, 1968). Thereafter, ion exchange between the intracellular and extracellular fluids (process 2), bone and renal responses (processes 3 and 4) bring about a gradual increase in the *in vivo* blood buffer slope (BROWN and MICHEL, 1968; WOODBURY, 1974).

However, nonextracellular buffering, designated simply as "cellular" buffering in this paper, may contribute to the value of the *in vivo* slope even from the early stage of hypercapnia. To examine this hypothesis, acid-base changes in the ISF and the blood must be determined simultaneously. Since the ISF, in dynamic equilibrium with the intracellular fluid and the blood through the capillary wall, flows into the lymphatics (GUYTON *et al.*, 1975b; YOFFEY and COURTICE, 1956a), we compared the time course of acid-base changes in the thoracic duct lymph with that of the blood during hypercapnia induced in dogs and estimated the cellular contribution to the buffering capacity of the ECF. Since TAKANO and NAKANO (1974) demonstrated a higher *in vivo* buffering capacity in the spontaneously breathing animals than that in the mechanically ventilated ones, we conducted the experiments on dogs with both types of respiration.

METHODS

Twenty mongrel dogs of both sexes, weighing 9.8 to 21.5 kg, were used. They were anesthetized initially by i.v. injection of 25 mg/kg of sodium pentobarbital and an additional dose of 5 mg/kg every 1–2 hr to maintain light anesthesia. Catheters were inserted into the femoral artery and into the intrathoracic portion of the postcava *via* femoral vein under X-ray fluoroscopy. Endotracheal intubation was performed; the airway P_{CO_2} , measured by an infrared CO_2 analyzer (Godart NV, Capnograph 146), was continuously displayed on a pen recorder (Hitachi, QPD-53). The rectal temperature and ECG were monitored throughout the experiments.

The animals were splenectomized to avoid possible distortion of the circulating blood buffering capacity due to a release of highly concentrated blood from the spleen (BILLINGS and BROWN, 1955). The thoracic duct was cannulated without pneumothorax by careful dissection at the juncture of the external jugular and the subclavian vein (MARKOWITZ *et al.*, 1964). To prevent lymph-clotting in the cannula, heparin (200 U/kg) was given intravenously.

Eleven of 20 dogs were then paralyzed by i.v. injection of 4 mg/kg of gallamine triethiodide followed by 2 mg/kg every 0.5–1 hr; they were mechanically ventilated with a respirator so as to keep the end-tidal P_{CO_2} at about 40 Torr. The other 9 dogs were allowed to breathe spontaneously.

Experimental procedures. Control observations were conducted for at least

30 min after the onset of artificial ventilation or for 1 hr after completion of the surgical procedures. Acute hypercapnia was induced by 90-min inhalation of a gas mixture (12% CO₂-30% O₂ in N₂). In the artificially ventilated dogs, the ventilation volume was increased in hypercapnia to the level 1.5 times higher than that during normocapnia. This was done to induce a similar degree of hypercapnia in both animal groups.

Arterial and postcaval venous blood and thoracic duct lymph were simultaneously collected at the end of the air-breathing period ("Control") and repeatedly at 10, 20, 30, 60, and 90 min after the onset of the CO₂-breathing period ("Hypercapnia"). The volume of each blood sample was 3.5 ml; 2 ml were transferred to a glass syringe to measure pH, P_{O_2} , and the hemoglobin concentration; 1.5 ml were transferred to a plastic syringe to determine plasma CO₂ content and the protein concentration. Each lymph sample was 2 ml in volume, except for four experiments in which it was 1 to 1.5 ml due to a lower flow rate. All samples were stored in ice water until determination of the acid-base parameters.

Analysis of acid-base parameters. Within 2 hr of sampling, pH and P_{O_2} of the lymph and the blood were measured at 37°C, using a blood gas analyzer (Radiometer, BGA2D/1-MK2). The results were corrected to the rectal temperature values, using appropriate temperature coefficients (ROSENTHAL, 1948; SEVERINGHAUS, 1965, 1966).

The CO₂ content of lymph and plasma, the latter separated anaerobically in the plastic syringe, was measured by a Van Slyke micromanometer (VAN SLYKE and PLAZIN, 1961). P_{CO_2} and the bicarbonate concentration in the lymph and plasma were computed from the CO₂ content, pH, pK' , and the solubility coefficient for CO₂ (SEVERINGHAUS, 1965) using the Henderson-Hasselbalch equation. The calculation for the lymph was carried out by using the pK' and the solubility coefficient of cerebrospinal fluid (CSF), since these values in the lymph were considered to be close to those of CSF according to the theoretical estimation of SIGGAARD-ANDERSEN (1974a).

The hemoglobin concentration was colorimetrically determined by the cyanomethemoglobin method. The plasma and lymph protein concentration was measured by a refractometer (Hitachi, PRP-B).

Calculation of buffer slopes. *In vitro* CO₂ titration was performed on the blood and lymph collected at the end of the air-breathing period and at 90 min of the CO₂-breathing period. Each sample was equilibrated with two gas mixtures (3.59 and 7.21% CO₂) in a microtonometer (Radiometer, BGA2D/1-MK2) at 37°C. From the pH values and the P_{CO_2} of gases, the bicarbonate concentrations were calculated by using the pK' and the CO₂ solubility coefficient (SEVERINGHAUS, 1965), respectively. The *in vitro* CO₂ titration curve was drawn by connecting the two points on a pH-[HCO₃⁻] diagram, and the slope of the curve was calculated in slykes (sl) (WOODBURY, 1974).

The *in vivo* CO₂ titration curve was drawn by connecting the "Control" and

"Hypercapnia" pH-[HCO₃⁻] points on a pH-[HCO₃⁻] graph and the slope of the curve was calculated.

Estimation of ECF bicarbonate increment and cellular contribution. The general formula used to obtain the ECF bicarbonate increment was:

$$\text{ECF } \Delta\text{HCO}_3^- \text{ content} = \Delta([\text{HCO}_3^-]_p \cdot V_p) + \Delta([\text{HCO}_3^-]_e \cdot V_c) + \Delta([\text{HCO}_3^-]_{\text{ISF}} \cdot V_{\text{ISF}}), \quad (1)$$

in which:

V_p :	plasma volume in liter/kg body weight
V_c :	cell volume in liter/kg body weight
V_{ISF} :	interstitial fluid volume in liter H ₂ O/kg body weight
$[\text{HCO}_3^-]_p$:	bicarbonate concentration in venous plasma in mmol/liter
$[\text{HCO}_3^-]_{\text{ISF}}$:	bicarbonate concentration in interstitial fluid in mmol/liter H ₂ O
$[\text{HCO}_3^-]_e$:	bicarbonate concentration in erythrocytes, in mmol/liter, given by KELMAN's equation (KELMAN, 1967; SIGGAARD-ANDERSEN, 1974b).

$$[\text{HCO}_3^-]_e = \{0.57 - 0.28(\text{pH}_p - 7.40) - 0.082(\text{pH}_p - 7.40)^2\} [\text{HCO}_3^-]_p, \quad (2)$$

where pH_p was pH of venous blood.

If there are no changes in the ECF volumes during acute hypercapnia, Eq. (1) becomes:

$$\text{ECF } \Delta\text{HCO}_3^- \text{ content} = \Delta[\text{HCO}_3^-]_p \cdot V_p + \Delta[\text{HCO}_3^-]_e \cdot V_c + \Delta[\text{HCO}_3^-]_{\text{ISF}} \cdot V_{\text{ISF}}, \quad (3)$$

where V_p , V_c , and V_{ISF} were assumed to be 0.0451 liter/kg, 0.0335 liter/kg, and 0.1720 liter H₂O/kg, respectively, which were obtained from 34 trials on 17 splenectomized and nephrectomized dogs using ferrocyanide and Evans blue (HATA *et al.*, 1977).

(a) Actual bicarbonate increments (indicated as $\Delta[\text{HCO}_3^-]_{\text{ac}}$) were computed from venous pH and venous and lymph bicarbonate concentrations. First, we obtained $[\text{HCO}_3^-]_e$ by inserting venous pH and bicarbonate concentration into Eq. (2), and then $\Delta[\text{HCO}_3^-]_e$, the difference between the values of "Control" and "Hypercapnia." $\Delta[\text{HCO}_3^-]_p$ and $\Delta[\text{HCO}_3^-]_{\text{ISF}}$ were also obtained from the venous plasma and lymph bicarbonate concentrations of "Control" and "Hypercapnia." $\Delta[\text{HCO}_3^-]_{\text{ac}}$ were calculated by inserting these values into Eq. (3). The lymph bicarbonate concentration, in mmol/liter H₂O, was substituted for $[\text{HCO}_3^-]_{\text{ISF}}$.

(b) Theoretical bicarbonate increments (defined as $\Delta[\text{HCO}_3^-]_{\text{th}}$) were computed from the physicochemical buffering capacity of the blood and ISF. For these calculations we first obtained the values of $\Delta[\text{HCO}_3^-]_p$ and $\Delta[\text{HCO}_3^-]_{\text{ISF}}$ according to the following equations, and then inserted these values into Eqs. (2) and (3):

$$\Delta[\text{HCO}_3^-]_p = -\beta_b \cdot \Delta\text{pH}_p \quad \text{and} \quad \Delta[\text{HCO}_3^-]_{\text{ISF}} = -\beta_{\text{ISF}} \cdot \Delta\text{pH}_{\text{ISF}}, \quad (4)$$

where pH_p and pH_{ISF} were pH of venous blood and ISF, respectively; β_{ISF} was *in vitro* slope of ISF. The lymph pH (pH_L) and the buffer capacity *in vitro* (β_L) were also substituted for pH_{ISF} and β_{ISF} . β_b was obtained from the *in vitro* slope of blood (β_{bo}) by multiplying the factor 0.899 which was reported to be the ratio of body hematocrit to venous hematocrit in splenectomized dogs (REEVE *et al.*, 1953).

$$\beta_b = 0.899\beta_{bo} + 0.104[\text{Pr}]_p(1 - 0.899), \quad (5)$$

$[\text{Pr}]_p$: plasma protein concentration in g/liter.

(c) Cellular contribution to the ECF buffering capacity was computed as follows:

$$\% \text{ of cellular contribution} = \frac{\Delta[\text{HCO}_3^-]_{\text{ac}} - \Delta[\text{HCO}_3^-]_{\text{th}}}{\Delta[\text{HCO}_3^-]_{\text{ac}}} \times 100 \quad (6)$$

Statistical treatment. The Student's *t*-test was applied to paired samples in the same animal group and to unpaired samples in two animal groups with different ventilatory patterns. For the estimation of an apparent equilibration time in the acid-base parameter changes, the *F*-test was applied to the differences between the "Control" and the "Hypercapnia" values. When the *F*-test demonstrated significance, the LSD method was used in comparing the adjacent pair of means (SNEDECOR and COCHRAN, 1972).

RESULTS

Changes in lymph and blood acid-base parameters

Table 1 shows the acid-base parameter changes of the lymph and blood of 9 splenectomized dogs breathing CO_2 spontaneously. After inception of CO_2 inhalation, the lymph pH decreased rapidly, reaching an apparent steady state within 20 min. On the other hand, the lymph bicarbonate concentration increased gradually, almost in parallel with the lymph P_{CO_2} elevation, reaching an apparent steady level at between 30 and 60 min (Table 1 and Fig. 1C). The plasma bicarbonate concentration of arterial and venous blood increased rapidly until 10 min; thereafter, it remained almost constant. Apparent steady pH and P_{CO_2} values were attained earlier in the venous blood than in the lymph. Arterial blood pH and P_{CO_2} values rapidly reached an apparent steady level within 10 min (Table 1 and Figs. 1A, B).

Control P_{CO_2} value of lymph was several Torr higher than that of the mixed venous blood, and this difference increased with P_{CO_2} elevation by CO_2 inhalation. These results may suggest that the continuous P_{CO_2} gradient from the tissue cell to the intravascular fluid exists and that the lymph P_{CO_2} is closer to the tissue P_{CO_2} value.

Table 1. Changes in acid-base parameters during spontaneous CO₂ breathing in 9 splenectomized dogs (mean±S.E.).

		Time (min)	pH	P _{CO₂} (Torr)	[HCO ₃] (mEq/liter)	P _{O₂} (Torr)	Hb (%)	Protein (%)
Arterial blood	Control		7.328±0.0123	40.3±1.87	20.29±0.770	84.7±3.01	16.9±0.71	7.0±0.16
		10	7.072±0.0172	83.7±2.38	23.01±0.762	143.5±4.25	17.0±0.72*	7.0±0.15*
	Hypercapnia	20	7.059±0.0153	85.7±2.66	22.85±0.733	149.6±4.90	16.4±0.66	6.8±0.20
		30	7.059±0.0185	84.9±3.44	22.55±0.769	148.4±4.40	16.3±0.64	6.6±0.18
		60	7.048±0.0163	86.8±3.05	22.52±0.732	150.2±6.01	16.1±0.57	6.6±0.19
Postcaval venous blood	Control	90	7.050±0.0173	87.1±2.68	22.74±0.715	151.5±5.05	16.1±0.63	6.6±0.21
			7.305±0.0148	44.9±1.56	21.44±0.699	58.2±2.56		
	Hypercapnia	10	7.081±0.0159**	83.9±2.27**	23.49±0.567	83.0±2.78		
		20	7.055±0.0143	88.7±1.53	23.43±0.640	83.7±2.63		
		30	7.048±0.0160*	89.2±2.08**	23.20±0.747	83.9±3.09		
Thoracic duct lymph	Control	60	7.031±0.0177	93.7±2.43	23.40±0.680	81.9±2.29		
		90	7.031±0.0180	93.1±2.59	23.21±0.719	83.8±3.03		
	Hypercapnia		7.299±0.0178	51.1±1.98	23.12±0.512	77.8±4.60		4.2±0.22
		10	7.142±0.0179**	80.4±2.36**	25.01±0.502**	77.9±5.26		4.3±0.20
		20	7.078±0.0163	97.4±2.41*	26.09±0.515	78.4±5.36		4.3±0.18
	Hypercapnia	30	7.068±0.0144	101.2±1.99**	26.46±0.499*	83.1±5.25		4.2±0.20
		60	7.059±0.0163	106.1±3.69	27.04±0.498	84.8±5.12		4.0±0.25
		90	7.059±0.0164	104.8±3.11	26.74±0.471	90.7±4.37		3.8±0.23

The differences between the "Control" and the "Hypercapnia" values were inspected by the *F*-test. Only when the *F*-test demonstrated significance, the LSD method was used in comparing the adjacent pair of means; *P*<0.05 (or *P*<0.01) for the pair of mean difference marked by * (or **) and the next mean difference.

Fig. 1. Time course of acid-base parameter changes during spontaneous CO₂ breathing in 9 splenectomized dogs. A, arterial blood; B, postcaval venous blood; C, thoracic duct lymph. Vertical bars indicate the mean $\pm$ S.E. Note that during acute hypercapnia, the lymph reached an apparent steady state in the acid-base parameters at least 10 min after the blood.

Similar results were obtained in the 11 splenectomized dogs subjected to controlled ventilation (Table 2). The time course of acid-base parameter changes was strikingly similar in both animal groups. During acute hypercapnia, the

Table 2. Changes in acid-base parameters during controlled CO₂ breathing in 11 splenectomized dogs (mean $\pm$ S.E.).

		Time (min)	pH	P _{CO₂} (Torr)	[HCO ₃ ⁻] (mEq/liter)	P _{O₂} (Torr)	Hb (%)	Protein (%)
Arterial blood	Control		7.350 $\pm$ 0.0139	38.3 $\pm$ 1.60	20.32 $\pm$ 0.619	91.5 $\pm$ 3.38	16.8 $\pm$ 0.43	6.7 $\pm$ 0.17
		10	7.070 $\pm$ 0.0132**	87.8 $\pm$ 1.36**	23.98 $\pm$ 0.530	153.8 $\pm$ 3.33	16.7 $\pm$ 0.37	6.8 $\pm$ 0.15
	Hypercapnia	20	7.041 $\pm$ 0.0160	94.5 $\pm$ 3.60	23.99 $\pm$ 0.572	157.9 $\pm$ 3.44	16.6 $\pm$ 0.39	6.7 $\pm$ 0.16
		30	7.043 $\pm$ 0.0140	93.2 $\pm$ 2.48	23.86 $\pm$ 0.616	158.0 $\pm$ 3.35	16.5 $\pm$ 0.35	6.6 $\pm$ 0.17
Postcaval venous blood	Control	60	7.031 $\pm$ 0.0153	95.7 $\pm$ 2.99	23.80 $\pm$ 0.689	159.6 $\pm$ 2.70	16.5 $\pm$ 0.38	6.5 $\pm$ 0.13
		90	7.024 $\pm$ 0.0173	97.7 $\pm$ 3.54	23.93 $\pm$ 0.660	161.1 $\pm$ 4.04	16.5 $\pm$ 0.40	6.5 $\pm$ 0.13
	Hypercapnia	10	7.310 $\pm$ 0.0126	44.4 $\pm$ 1.49	21.45 $\pm$ 0.607	57.4 $\pm$ 2.89		
		20	7.072 $\pm$ 0.0133**	86.5 $\pm$ 2.25**	23.90 $\pm$ 0.579	86.5 $\pm$ 4.59		
Thoracic duct lymph	Control	30	7.041 $\pm$ 0.0156	95.1 $\pm$ 3.62	24.18 $\pm$ 0.616	85.9 $\pm$ 5.27		
		60	7.026 $\pm$ 0.0156*	98.1 $\pm$ 3.68*	24.06 $\pm$ 0.583	83.7 $\pm$ 4.74		
	Hypercapnia	60	7.005 $\pm$ 0.0181	104.1 $\pm$ 4.73	24.25 $\pm$ 0.578	83.4 $\pm$ 4.60		
		90	6.993 $\pm$ 0.0205	106.7 $\pm$ 5.68	24.39 $\pm$ 0.650	84.8 $\pm$ 4.96		
Thoracic duct lymph	Control		7.298 $\pm$ 0.0171	51.2 $\pm$ 2.57	23.05 $\pm$ 0.554	91.6 $\pm$ 5.15		5.0 $\pm$ 0.25
		10	7.113 $\pm$ 0.0136**	86.0 $\pm$ 2.76**	24.98 $\pm$ 0.487**	101.6 $\pm$ 6.07		5.0 $\pm$ 0.25
	Hypercapnia	20	7.057 $\pm$ 0.0133	102.1 $\pm$ 3.19	26.00 $\pm$ 0.509**	100.6 $\pm$ 6.48		5.0 $\pm$ 0.26
		30	7.039 $\pm$ 0.0149	109.4 $\pm$ 3.60	26.71 $\pm$ 0.536	99.2 $\pm$ 6.98		4.9 $\pm$ 0.23
		60	7.020 $\pm$ 0.0220	116.8 $\pm$ 5.69	27.10 $\pm$ 0.521	101.6 $\pm$ 5.19		4.8 $\pm$ 0.22
		90	7.014 $\pm$ 0.0218	118.7 $\pm$ 5.32	27.31 $\pm$ 0.616	95.4 $\pm$ 5.22		4.8 $\pm$ 0.21

The differences between the "Control" and the "Hypercapnia" values were inspected by the *F*-test. Only when the *F*-test demonstrated significance, the LSD method was used in comparing the adjacent pair of means; *P* < 0.05 (or *P* < 0.01) for the pair of mean difference marked by * (or **) and the next mean difference.

lymph reached apparently steady pH and P_{CO_2} values at least 10 min after the blood. The lymph bicarbonate concentration continued to increase during the first 30 min of hypercapnia, while there were no significant changes in the plasma bicarbonate increments after 10 min. Increments of lymph bicarbonate concentration, corrected for the Donnan effect and water content, were significantly higher than those of venous plasma after 20 and 30 min of hypercapnia in the spontaneous and controlled ventilation groups, respectively ($P < 0.01$). These characteristic changes in the lymph and plasma bicarbonate concentration were reflected in the time course of *in vivo* buffer slope changes.

The concentration of hemoglobin and other proteins, which are the major non-bicarbonate buffers in the ECF, showed no significant changes in the controlled ventilation group. In the spontaneously breathing group, a small decrease in hemoglobin (5%) and plasma protein concentration (6%) was observed after 90 min of hypercapnia, possibly resulting from sampling the blood and lymph (42 and 12 ml in total, respectively) and from flushing the cannula with saline solution.

Table 3 shows the changes of the *in vivo* buffer slope for the lymph and blood in the spontaneous and controlled ventilation groups. The *in vivo* slope of the lymph increased to 12.1 sl at 10 min of spontaneous CO_2 breathing; this value was significantly higher than that of the venous blood (9.1 sl) obtained at the same

Table 3. Changes in the *in vivo* buffer slope during spontaneous and controlled CO_2 breathing in splenectomized dogs (mean $\pm$ S.E.).

		CO_2 breathing (min)	Arterial blood (slyke)	Postcaval venous blood (slyke)	Thoracic duct lymph (slyke)
Spontaneous breathing group (n=9)	<i>in vitro</i> slope		26.6 $\pm$ 1.14	26.6 $\pm$ 1.14	5.7 $\pm$ 0.42
	<i>in vivo</i> slope	10	10.9 $\pm$ 1.25*	9.1 $\pm$ 1.03	12.1 $\pm$ 0.97*
		20	9.6 $\pm$ 0.91	8.0 $\pm$ 0.74	13.4 $\pm$ 1.00*
		30	8.5 $\pm$ 1.11	7.0 $\pm$ 1.09	14.5 $\pm$ 0.56
		60	8.1 $\pm$ 1.04	7.2 $\pm$ 0.67	16.4 $\pm$ 0.86
		90	9.0 $\pm$ 1.01	6.5 $\pm$ 1.01	15.6 $\pm$ 1.50
Controlled ventilation group (n=11)	<i>in vitro</i> slope		27.7 $\pm$ 0.82	27.7 $\pm$ 0.82	5.7 $\pm$ 0.54
	<i>in vivo</i> slope	10	13.1 $\pm$ 0.99	9.8 $\pm$ 1.65	10.2 $\pm$ 1.12*
		20	11.9 $\pm$ 1.06	10.0 $\pm$ 1.65	12.3 $\pm$ 1.75*
		30	11.6 $\pm$ 1.30	9.2 $\pm$ 1.48	14.6 $\pm$ 1.87
		60	11.0 $\pm$ 1.37	9.3 $\pm$ 1.45	15.7 $\pm$ 2.01
		90	11.4 $\pm$ 1.41	9.6 $\pm$ 1.31	16.4 $\pm$ 2.57

The differences between the *in vivo* and *in vitro* slope values were inspected by the *F*-test. Only when the *F*-test demonstrated significance, the LSD method was used in comparing the adjacent pair of means; $P < 0.05$ for the pair of mean difference marked by * and the next mean difference.

time ($P < 0.01$). Thereafter, the lymph value increased gradually, reaching nearly three times the *in vitro* slope at the end of the 90-min period. The lymph value in the spontaneously breathing group seemed to attain an equilibrium within 30 min, since further changes were not statistically significant. The *in vivo* slope of the venous blood exhibited an abrupt fall to about one-third the *in vitro* slope within 10 min; thereafter, an apparent steady state was maintained.

In the controlled ventilation group, the *in vivo* slope values of the lymph were also higher than those of the venous blood after 20 min of CO_2 inhalation ($P < 0.01$). Only at 10 min was the increment of the lymph slope of this group (4.4 sl) lower than that of the spontaneously breathing group (6.4 sl) ($P < 0.01$). Under our experimental conditions, no essential differences were seen in the result between spontaneously and artificially breathing animals.

The above results strongly suggest that cellular contribution to the ECF buffering capacity may appear within 10 min of hypercapnia.

Increase in the ECF bicarbonate content and cellular contribution

The administered CO_2 is buffered by non-bicarbonate buffers present in body fluids to produce bicarbonate. We defined the bicarbonate increment calculated by the physicochemical buffering capacity of the blood and ISF as the theoretical bicarbonate increment in the ECF. By comparing the theoretical and the actually measured bicarbonate increment, the nonextracellular or cellular contribution to the ECF bicarbonate was estimated.

Regarding the theoretical bicarbonate increments, an equilibrium with CO_2 seemed to be nearly attained at about 20 min (broken line in Fig. 2). An apparent steady state in actual bicarbonate increments was reached about 10 min later (heavy solid line in Fig. 2). The observation that, within 20 min of CO_2 inhalation, the actual value became higher than the theoretical value, suggests the addition of cellular bicarbonate to the increased ECF bicarbonate level at an early stage of hypercapnia. The magnitude of cellular contribution to the ECF buffering capacity was computed. In the spontaneously breathing group, it was 7.8, 15.6, and 10.7%, and in the controlled ventilation group, it was 8.9, 10.6, and 12.0% at 30, 60, and 90 min of induced hypercapnia, respectively.

DISCUSSION

Nature of ISF bicarbonate increase and accuracy of its estimation. The actual bicarbonate increments in the ECF (blood plus ISF) progressively increased in parallel with the rise in lymph bicarbonate concentration (heavy solid line in Fig. 2), while the increments in the blood remained nearly unchanged after 10 min of CO_2 inhalation (thin solid line in Fig. 2). The increase in lymph bicarbonate concentration is produced by ISF buffering, by bicarbonate redistribution from the blood and by cellular contribution. The processes of chemical buffering and

Fig. 2. Time course of ECF bicarbonate increments during spontaneous (A; $n=9$) and controlled (B; $n=11$) CO₂ breathing of splenectomized dogs. The heavy solid line indicates the actual bicarbonate increments in the ECF (blood plus ISF); the thin solid line, the actual increments in the blood only. The broken line indicates the theoretical bicarbonate increments, calculated from the buffering of non-bicarbonate buffers in the blood and ISF. For the calculation, see text. Note that within 20 min of CO₂ inhalation, the actual value became higher than the theoretical one. The cellular contribution to the ECF bicarbonate increments was computed after the theoretical line reached an apparently steady level.

Table 4. Differences in estimation of cellular contribution with different ISF buffer values.

	CO ₂ breathing (min)	Cellular contribution in β_{ISF} taken as	
		2.1 slyke (%)	5.7 slyke (%)
Spontaneous breathing group ($n=9$)	30	27.1	7.8
	60	33.1	15.6
	90	29.4	10.7
Controlled ventilation group ($n=11$)	30	28.2	8.9
	60	29.6	10.6
	90	30.2	12.0

bicarbonate redistribution in the lymph seemed to require at least 20 min; after that period, the increase in bicarbonate concentration was greater in the lymph than in the venous plasma (Tables 1 and 2). The delay of 20 min or more may arise not only due to the time (10 to 20 min) required for completion of chemical

buffering and bicarbonate redistribution (BROWN and MICHEL, 1968); the transit time (10 to 15 min) required for the lymph to proceed from the tissue spaces to the thoracic duct (MAYERSON, 1963; YOFFEY and COURTICE, 1956a) may also contribute to this delay. Our results favor the view that the sampled lymph reliably represents the bicarbonate concentration in the ISF.

Theoretical ECF bicarbonate increments are the function of β_b , pH_p , β_L , and pH_L . Therefore, the accuracy of the theoretical values depends on the correct approximation of the lymph and ISF values. The lymph acquired an apparently stable pH at least 10 min later than the venous blood (Tables 1 and 2, Fig. 1B, C); this period coincides with the above-mentioned transit time. When sufficient time is allowed for the occurrence of a new apparent steady state, changes in lymph pH and lymph gas tensions (BERGOFKY *et al.*, 1962; WITTE *et al.*, 1967) provide a reasonable approach in determining ISF alterations. Therefore, after the theoretical line reached an apparent steady level (broken line in Fig. 2), we computed the magnitude of cellular contribution at 30, 60, and 90 min of CO₂ inhalation.

Regarding the *in vitro* slope of the thoracic duct lymph, the value we obtained (5.7 sl) was higher than that of the average ISF commonly admitted (DELL and WINTERS, 1970). The thoracic duct lymph has a higher protein concentration than the lymph from other tissues, because the liver lymph, which has the highest protein concentration, may contribute one-half of the thoracic duct flow (YOFFEY and COURTICE, 1956b; MAYERSON, 1963; GUYTON *et al.*, 1975c). As a high buffer value results in high theoretical bicarbonate increments, the cellular contribution to the ECF bicarbonate content may be underestimated. We computed the possible magnitude of cellular contribution, using 2.1 sl, a buffer value calculated from the average ISF having a protein concentration of 2% (GUYTON *et al.*, 1975a) and compared the results with those that were obtained when 5.7 sl, the buffer value determined in our experiments, was used. As shown in Table 4, when the 2.1 sl buffer value was applied, cellular contribution was around 30% in both animal groups while at 5.7 sl it was around 10%.

In the present experiments, we assumed the ECF space to be the ferrocyanide space. If, however, the chloride space, which has been preferred by DELL and WINTERS (1970) and is greater than the ferrocyanide space, is taken as the ECF space, the cellular contribution will be 20%. On the other hand, when the inulin space, which has been preferred by ROOS and THOMAS (1967) and is smaller than the ferrocyanide space, is taken as the ECF space, the cellular contribution will be 7%. Since we focused our attention on the bicarbonate distribution in the present experiments, the readily available space to bicarbonate may well be intermediate between the chloride and the inulin space. GIEBISCH *et al.* (1955) thought the $^{35}\text{SO}_4^{2-}$ space to be comprised of plasma volume plus interstitial volume being in ready diffusion equilibrium with plasma, and used it in evaluating the buffering capacity of canine body fluids in respiratory acid-base disturbances; this space was similar to the ferrocyanide space (WALSER *et al.*, 1953). Therefore, we feel

that our preference of the ferrocyanide space in the present study is reasonable.

On deriving Eq. (3), we assumed that the ECF volumes were unaltered by CO_2 inhalation. GIEBISCH *et al.* (1955) reported that neither the sucrose space nor $^{35}\text{SO}_4^{2-}$ space changed during hypercapnia in nephrectomized dogs, and that the calculated water shift into red cells was within the limits of error of estimating the ECF volume. We also obtained the same results in splenectomized and nephrectomized dogs using ferrocyanide (HATA *et al.*, 1977). Regarding the changes in plasma volume during hypercapnia, the experimental data are controversial; no significant changes in splenectomized dogs (BILLINGS and BROWN, 1955), or a 7% increase at 30 min in dogs breathing 12% CO_2 (HATA *et al.*, 1977). If the increase in the plasma volume occurs with consequent decrease in the ISF, the cellular contribution to the ECF buffering will decrease.

The cellular contribution to the ECF buffering capacity. The present study demonstrated that cellular contribution to the ECF buffering capacity emerged in an early stage of hypercapnia; it became appreciable within 20 min after the onset of CO_2 breathing (Fig. 2). Furthermore, after 10 min, the *in vivo* slope of the lymph was significantly higher than that of the venous blood (Table 3). These observations strongly suggest that cellular contribution may appear within 10 min of hypercapnia. Our suggestion coincides with that of TAKANO and NAKANO (1974) who compared the predicted ratio of the *in vivo* to the *in vitro* slope for the mixed venous blood with the actual ratio obtained in CO_2 breathing dogs.

In our experiments, the magnitude of cellular contribution was computed in the range of 10–30% of the ECF buffering capacity in splenectomized dogs breathing 12% CO_2 for 30–90 min (Table 4). GIEBISCH *et al.* (1955) reported it to be 51% from the estimated increments of Na and K in the ECF in nephrectomized dogs breathing 20% CO_2 for 2 hr. The difference may, in part, be attributed to their longer duration of hypercapnia.

The cellular contribution, as defined in this study, represents net bicarbonate inflow through composite processes from the tissues (nonextracellular compartments) to the ECF space. Probable processes are $\text{Na}^+\text{-H}^+$ and $\text{Cl}^-\text{-HCO}_3^-$ exchanges between intra- and extracellular fluids, which have been demonstrated in mammalian skeletal muscles (AICKIN and THOMAS, 1977) in glia cells (LOESCHCKE and AHMAD, 1980), in snail neurons (THOMAS, 1976) and in squid giant axons (RUSSELL and BORON, 1976). Through these processes, more bicarbonate is likely to become available in the ECF space. On the other hand, BETTICE *et al.* (1976) and STROME *et al.* (1977) proved bicarbonate influx to the cardiac muscle, causing an increase in the buffer value in the beginning of high CO_2 inhalation. Thus, the cellular contribution to the ECF bicarbonate increments is the result of the overall exchange in all tissues except erythrocytes. It also includes bone and renal responses, though their contribution to the ECF buffering capacity is probably negligible at an early stage of hypercapnia (WOODBURY, 1974; POYART *et al.*, 1975).

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