Intracellular pH During Metabolic Acidosis of Intracellular and Extracellular Origin.* (32516)

E. B. Brown, Jr., Woo Gyum Kim,† and F. A. Moorhead, Jr.
Department of Physiology, University of Kansas Medical Center, Kansas City, Kan.

It has been demonstrated that intracellular pH may change very little or actually decrease while extracellular pH is increasing during intravenous infusion of sodium bicarbonate solutions(1,2). This is explained by the well-accepted hypothesis that hydrogen ions and bicarbonate ions do not cross cell membranes readily, whereas CO_2 tensions in intracellular and extracellular fluids rapidly reach equilibrium. If this slow distribution of hydrogen ions and/or bicarbonate ions takes place when metabolic acidosis of intracellular origin occurs, extracellular pH might reflect this change very poorly. This raises the following quesion: is there a significant difference in the relationship between intracellular and extracellular pH in acidosis produced experimentally by infusion of a strong mineral acid such as hydrochloric (exogenous source), and that produced by interference with aerobic metabolism as in hemorrhagic shock (endogenous source)? It was the purpose of these experiments to investigate this question.

Procedure. Dogs weighing between 14 and 20 kg were anesthetized with sodium pentobarbital and both kidneys were removed. One gram/kg body weight of sucrose and 0.75 μc/kg body weight of 5,5-dimethyl-2,4-oxazolidinedione 2-C^{14} (DMO) were injected intravenously, and 2 hours were allowed for equilibration and stabilization of arterial and femoral vein pH. During the last one hour of this period a blood sample was drawn every 15 minutes for pH, P_{CO_2}, and sucrose determinations. At the end of the 2-hour period blood and skeletal muscle samples were taken for determination of DMO. From this point on the procedure varied.

Group 1. During the next hour 5 to 8 cc/kg of 0.3 N HCl were infused intravenously and then the infusion was decreased to a rate just sufficient to maintain arterial blood pH constant.

Group 2. These experiments were identical with Group 1 except that 0.3 N gluconic acid was used instead of HCl.

Group 3. In these experiments HCl was infused as in Group 1, but hyperventilation was imposed with a respiration pump with which stroke volume and frequency could be varied. Respiratory ventilation was adjusted to maintain femoral venous blood pH constant.

Group 4. In these experiments .6 N $NaHCO_3$ (approximately 15 mM/kg body weight) was infused while the CO_2 concentration in the inhaled mixture was increased and adjusted so as to maintain a constant venous blood pH.

Group 5. Blood was withdrawn from a carotid artery until blood pressure dropped to a mean of 40 mm Hg at which point it was held constant utilizing a modification of an automatic leveling device and blood reservoir for the control of hypotension as described by Einhaber and Clark(3). The bleeding period lasted approximately one hour.

Following the 1 to 2 hour period of infusion or bleeding, femoral vein pH was fairly stable for the next 1 to 1.5 hours. One ml femoral blood samples were drawn every 5 minutes for pH and C_{CO_2} determination, and at the end of 1 to 1.5 hours of a new stable pH the second muscle sample and blood samples for determination of DMO were taken.

Solutions were infused into the external jugular vein. CO_2 and O_2 mixtures were prepared in a 120 liter spirometer and delivered to the animal with a low resistance, open-circuit system. Arterial blood samples were drawn through a catheter in a carotid artery; venous blood was drawn from a femoral vein by needle puncture. Tissue samples were taken from the gracilis muscle.

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† Fellow of International Atomic Energy Agency. Present address: Dept. of Physiology, Seoul National Univ. Med. School, Seoul, Korea.

Methods. Blood pH was determined with a Sanz electrode system maintained at 37.5°C. Blood pH was corrected to a rectal temperature of the dog by Rosenthal's coefficient (—0.0146 pH/C°). CO_2 tensions were determined with a CO_2 electrode also maintained at 37.5° and corrected for temperature according to the Severinghause nomogram(4). Plasma and intracellular bicarbonate concentrations were calculated from the measured pH and Pco_2 using the Henderson-Hasselbalch equation, with a pK value of 6.10 and CO_2 solubility coefficients of 0.030 and 0.035 for plasma and intracellular fluid, respectively. DMO determinations on blood and tissue were carried out according to the method described by Schloerb and Grantham(5). Sucrose was determined by the method of Clausen(6).

Results. Mean values with their standard errors are presented for each of the groups of experiments in Table I. The results from Groups 1 and 2 in which acid was infused are quite similar with the exception that a more severe extracellular acidosis was produced with gluconic acid, but this did not result in a more severe intracellular acidosis. As a result, whereas the hydrogen ion gradient from inside to outside of the muscle cells changed very little in the case of HCl infusion, it decreased quite measurably with gluconic acid.

With hemorrhagic shock (Group 5), the degree of extracellular acidosis was slightly less than it was for Groups 1 and 2, and the intracellular acidosis was somewhat more severe. With these changes there was a slight increase in the intracellular hydrogen ion (H_i) to extracellular hydrogen ion (H_e) gradient. However this increase was not statistically significant ($P>.05$). In all 3 groups the arterial Pco_2 fell, indicating hyperventilation, however femoral vein blood Pco_2 increased, resulting in an increase in the arterial-venous pH gradient. This was particularly marked in the shock experiments.

In Groups 1 and 5 no significant change in intracellular bicarbonate, $(HCO_3^-)_i$, took place. It appears, therefore, that the rise in Pco_2 produced an increase in $(HCO_3^-)_i$ which was approximately equal to the decrease that resulted from accumulation of hydrogen ions in or efflux of HCO_3^- from the intracellular fluid. With gluconic acid infusion $(HCO_3^-)_i$ decreased in spite of an increase in Pco_2. To evaluate the change in intracellular bicarbonate due to the metabolic acidosis it is necessary to make an estimate of the increase in HCO_3^- which would have been produced by the rise in Pco_2 alone, and to subtract this increase from the value actually obtained. The difference between the control intracellular bicarbonate concentration and the experimental value corrected for change in Pco_2 represents buffering of hydrogen ions transferred from extracellular to intracellular fluid. In previous experiments(7) it was found that intracellular bicarbonate of skeletal muscle increased one mM/l for each 16 mm Hg increase in Pco_2 in a short equilibration period and over the range observed in the present experiments. When this correction is made it would appear that sufficient hydrogen ion entered or remained in the intracellular fluid to decrease intracellular bicarbonate by 1 to 1.5 mEq/l in the case of HCl infusion and hemorrhagic shock, and approximately 3 mEq/l in the experiments in which gluconic acid was infused. Alternatively it could be assumed that this change represented a movement of bicarbonate ions out of the cell rather than of hydrogen ions in.

In Group 3 experiments the fall in venous CO_2 tension from 46 to 27 should have produced, if acting alone, a decrease in intracellular bicarbonate concentration of 1.2 mEq/l; thus the additional decrease of 2.8 mEq/l in intracellular bicarbonate must be accounted for by a transfer of hydrogen ions into the cell. The increase in intracellular pH then represents the predominant influence of the fall in CO_2 tension in the fact of a decreasing bicarbonate concentration. This must be due to hydrogen ions entering the cell or to bicarbonate leaving. In Group 4 the opposite result is apparent. The increase in venous blood CO_2 tension of 71 mm Hg would have been expected to produce a rise in intracellular bicarbonate concentration of approximately 4.5 mEq/l. The

TABLE I. Means and Standard Errors for 5 Groups of Experiments.

	pH_a	pH_e	$(H^+)_e$ nM/l	pH_i	$(H^+)_i$ nM/l	$P_{a}CO_2$ mm Hg	$P_{v}CO_2$ mm Hg	$(H^+)_i - (H^+)_e$	$(H^+)_i / (H^+)_e$	$(HCO_3^-)_e$ mM/l H_2O	$(HCO_3^-)_i$ mM/l H_2O
Group 1. HCl infusion (N=8)											
Control	7.40	7.38	42	6.96	110	37	44	68	2.62	24.9	11.5
S.E.	.01		1.2		8.4	1.6	2.0		.14	.9	.7
HCl	7.15*	7.02*	94*	6.81*	157*	22*	57*	63	1.67*	14.6*	10.9
S.E.	.03		6.2		15	2.4	4.2		.09	1.2	1.1
Group 2. Gluconic acid infusion (N=7)											
Control	7.40	7.36	44	6.98	105	37	52	61	2.39	28.6	13.8
S.E.	.01		1.7		9.7	2.1	4.1		.11	1.8	.9
Gluconic acid	6.96*	6.91*	122*	6.83*	147*	31*	63	25*	1.21*	12.4*	11.8*
S.E.	.01		5.8		4.2	3.2	8.8		.05	.5	1.5
Group 3. HCl and hyperventilation (N=12)											
Control	7.39	7.36	44	6.96	111	36	46	67	2.54	26.5	11.7
S.E.	.01		1.6		5.6	1.2	1.1		.10	1.3	.8
HCl + hypervent.	7.41	7.34	45	7.01*	97*	16*	27*	52*	2.14*	14.8*	7.7*
S.E.	.01		1.3		5.2	1.7	2.1		.12	1.6	.7
Group 4. $NaHCO_3$ and CO_2											
Control	7.41	7.37	43	6.96	110	35	46	67	2.58	25.3	11.6
S.E.	.02		1.9		5.9	.6	2.1		.17	1.3	.7
$NaHCO_3 + CO_2$	7.43	7.37	43	6.87*	135*	99*	117*	92*	3.09*	65.8*	24.2*
S.E.	.02		1.7		12	5.4	7.1		.29	5.1	2.1
Group 5. Hemorrhagic shock (N=8)											
Control	7.38	7.34	46	6.91	126	34	45	80	2.79	23.9	9.9
S.E.	.01		1.8		3.2	1.8	2.9		.12	1.4	1.0
Shock	7.29*	7.06*	87*	6.75*	177*	15*	59*	90	2.03*	16.5*	9.3
S.E.	.02		4.3		11	1.3	4.1		.07	1.1	.9

* Value significantly different ($p > .05$) from the control value.
P values were determined by the t test using paired samples. Interstitial fluid pH, $(pH)_e$, was obtained by adding .02 to venous plasma pH.

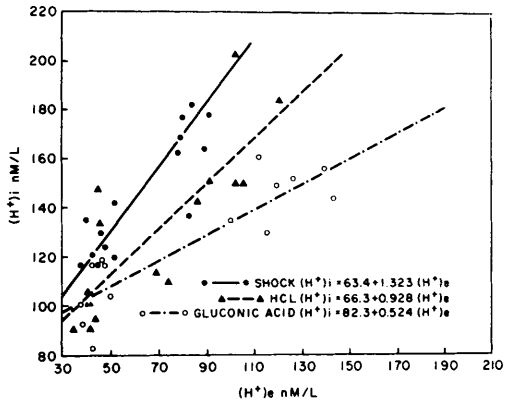

FIG. 1. Regression curves and equations of $(H^+)_e$ against $(H^+)_i$ for 3 groups of experiments.

additional increase of 8.1 mEq/l must have been due to bicarbonate ions crossing the cell membrane with the very high gradient produced by infusion of sodium bicarbonate. The resulting decrease in intracellular pH is considerably less than that usually found with a comparable increase in CO_2 tension.

In Fig. 1 a plot of $(H^+)_i$ against $(H^+)_e$ for the first 3 groups of experiments is presented. Although the regression coefficient for the shock experiments is larger than for HCl, the difference is not statistically significant. The differences between HCl and gluconic acid ($P>.05$), and between shock and gluconic acid ($P>.001$) are significant.

Discussion. It is recognized that the DMO technique for estimation of intracellular pH does not permit evaluation of short transients. At least one hour of steady state must be maintained to allow equilibration of DMO across cell membranes, and this necessarily imposes the time limitation necessary for the experimental procedure plus 1 to 1½ hours of steady state following the imposed intervention before a new value for intracellular pH can be obtained. Given these time limitations however the data in these experiments suggest that for skeletal muscle cells the intracellular-extracellular pH relationship is not markedly different whether the acidosis has been produced by infusion of hydrogen ions into the extracellular compartment or production of hydrogen ions within the intracellular compartment by interference with aerobic metabolism. The suggestion of an increase in shock and decrease with infusion of acids in the $(H^+)_i$-$(H^+)_e$ gradient is in the direction that would be expected if H^+ and HCO_3^- equilibrate slowly across muscle cell membranes. The larger regression coefficient of $(H^+)_i$ against $(H^+)_e$ for shock compared with acid infusion supports this contention. Although the increase in venous blood P_{CO_2} with the decrease in arterial blood P_{CO_2} was not unexpected in the shock experiments, this finding in the acid infusion experiments was somewhat surprising. This may have resulted from vasoconstriction in the leg with a reduction in blood flow to this region. This suggests that the acidosis produced in the skeletal muscles of the leg by infusion of acid in these experiments was partially, at least, identical in mechanism to that of hemorrhagic shock. Both resulted in intracellular acidosis due to reduced tissue perfusion. With this factor operating it is not surprising that the acid infusion and shock results are similar. The results from the two groups in which extracellular bicarbonate varied with constant pH indicate that bicarbonate or hydrogen ions or both cross muscle cell membranes in the equilibration times used in these experiments. At any rate, these findings point out the importance of using venous rather than arterial blood pH in calculation of intracellular pH. In these experiments after infusion of acid and also after institution of shock, arterial blood did not reflect the acid-base condition of interstitial fluid of the leg.

The recent paper by Carter, Rector, Campion, and Seldin(8) indicates that DMO distribution does not measure pH of the bulk phase of skeletal muscle cells. If DMO distribution measures an average pH of cell contents, or if part of the DMO is bound and should not be included in the value used for ionized DMO in calculating intracellular pH, the accumulated data from use of DMO indicates that the value obtained changes with changes in P_{CO_2} as expected and that the changes in pH_i as measured by the DMO technique are probably valid.

Summary. Intracellular pH was determined by distribution of 5,5-dimethyl-2,4-

oxazolidinedione (DMO) in skeletal muscle of dogs 1) infused with .3 N HCl, 2) infused with .3 N gluconic acid, 3) infused with HCl and hyperventilated to maintain extracellular fluid pH constant, 4) infused with .6 N HCO_3 and given CO_2 and O_2 to breathe to maintain extracellular pH constant, and 5) maintained in hemorrhagic shock. After the one to two hours required to reach equilibrium distribution of DMO there was not a striking difference in the intracellular hydrogen ion-extracellular hydrogen ion gradient between animals infused with HCl and those in hemorrhagic shock. Those infused with gluconic acid did show a significant difference from the shock animals. The differences were in the direction that would be expected from slower distribution across cell membranes of H^+ and/or HCO_3^- than CO_2. The experiments in which extracellular pH was maintained constant in the face of infusion of HCl or $NaHCO_3$ also demonstrate the primary dependence of intracellular pH on P_{CO_2} although in these experiments there is additional clear evidence of transfer of H^+ or HCO_3^- between intracellular and extracellular fluid over these short time intervals. With these time intervals of 1 to 3 hours, the intracellular acidosis of hemorrhagic shock is well reflected in the extracellular fluid, and the extracellular acidosis produced by infusion of acid into the blood is reflected in the intracellular fluid.

1. Brown, E. B., Jr., Goott, B., Am. J. Physiol., 1963, v204, 765.

2. Waddell, W. J., Butler, T. C., J. Clin. Invest., 1959, v38, 720.

3. Einheber, A., Clarke, R. W., J. Appl. Physiol., 1957, v11, 493.

4. Bradley, A. F., Stupfel, M., Severinghaus, J. W., ibid., 1956, v9, 201.

5. Schloerb, P. R., Grantham, J. J., J. Lab. & Clin. Med., 1965, v65, 669.

6. Clausen, D. F., Studies on the determination of inulin and Evan's Blue in blood and tissues. (Ph.D. Thesis) Minneapolis, Univ. of Minn., 1955.

7. Clancy, R. L., Brown, E. B., Jr., Am. J. Physiol., 1966, v211, 1309.

8. Carter, N. W., Rector, F. C., Campion, D. S., Seldin, D. W., J. Clin. Invest., 1967, v46, 920.

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Analysis of the Isoimmune Response to Leucocytes: I. Maternal Cytotoxic Response to Fetal Lymphocytes.* (32517)

HAROLD S. GOODMAN AND LILLIAN MASAITIS
(Introduced by Kenneth C. Robbins)
Michael Reese Research Foundation, Chicago, Ill.

Considerable effort is being directed toward the antigenic analysis of human leucocytes and determining their role in histocompatibility(1). If it is established that these antigens are also determinants of histocompatibility, leucocyte typing ultimately will provide a simple means of matching donors and recipients for organ transplantation. A major source of antisera for these studies is found among women sensitized to antigens of fetal leucocytes during pregnancy. Although a number of studies concerned with the incidence of leucocyte isoantibodies among parous women have been carried out(2,3), in these studies leucoagglutination was exclusively used as an index of the antibody response. Our laboratory recently has had the opportunity to re-examine the immune response in parous women, by the now commonly used cytotoxic procedure(4), and to draw certain conclusions concerning the incidence and specificity of the antibodies manifest in this technique.

Materials and methods. Antisera. Blood samples were obtained from maternity patients during their hospital confinement at the time of delivery. The serum was separated and stored at −20°C.

Lymphocyte preparation. Our cell panel was obtained from 10 group O blood donors.

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