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18. We have developed a rapid centrifugation technique that separates viable hepatocytes from nonviable cells and medium (*4*, *15*). In the present study, 0.45 ml of dibutyl phthalate (density, 1.046) was layered over 0.55 ml of 36 percent Percoll (density, 1.06) in a microcentrifuge tube. Rapid separation of cells and medium was accomplished by layering 0.75 ml of isolated hepatocyte suspension over the dibutyl phthalate oil layer and centrifuging for 15 seconds at 13,000*g*. As a result, viable hepatocytes (density, 1.07) were forced through the oil layer and pelleted under the Percoll layer. In contrast, nonviable cells (density 1.02) and medium remain above the oil layer after centrifugation.
19. The measurement of α-tocopherol levels were obtained by adding the internal standard, γ-tocopherol (0.6 nmol in 40 μl of ethanol) to samples of viable hepatocytes. The samples were diluted with 50 percent ethanol (0.4 ml), sonicated, and extracted twice with 0.5 ml of hexane (*20*). Next, the hexane extract was evaporated to dryness with N_2, the residue was dissolved in 0.6 ml of methanol, and 200 μl was analyzed by high-performance liquid chromatography (HPLC). Measurements of α-tocopherol were made on a Spectra-Physics 8700 chromatograph equipped with a Schoeffel 970 fluorescence detector (excitation filter, 205 nm; emission filter, 300 nm (*20*), and a Spherisorb ODS column (20 cm by 4.6 mm; 5-μm particles; Custom LC Inc., Houston). The mobile phase, 70 percent A (80 percent methanol), 30 percent B (100 percent methanol) was run isocratically for 5 minutes and then for 15 minutes at a linear gradient to 1 percent A and 99 percent B at a flow rate of 1.5 ml per minute. The HPLC retention time for γ- and α-tocopherol was approximately 20 and 21 minutes, respectively. Intracellular α-tocopherol concentrations were corrected for the number of viable hepatocytes sampled as determined by DNA content (*4*, *15*) and expressed per 10^6 cells. The lower limit of detection for α-tocopherol was approximately 10 pmol per 10^6 cells.
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* Present address: Department of Pathology, Medical College of Virginia, Richmond 23298.

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Evidence for a Detrimental Effect of Bicarbonate Therapy in Hypoxic Lactic Acidosis

Abstract. *Lactic acidosis, a clinical syndrome caused by the accumulation of lactic acid, is characterized by lactate concentration in blood greater than 5 m*M. *Therapy usually consists of intravenous sodium bicarbonate ($NaHCO_3$), but resultant mortality is greater than 60 percent. The metabolic and systemic effects of $NaHCO_3$ therapy of hypoxic lactic acidosis in dogs were studied and compared to the effects of sodium chloride or no therapy. Sodium bicarbonate elevated blood lactate concentrations to a greater extent than did either sodium chloride or no treatment. Despite the infusion of $NaHCO_3$, both arterial* pH *and bicarbonate concentration decreased by a similar amount in all three groups of dogs. Additional detrimental effects of $NaHCO_3$ were observed on the cardiovascular system, including decreases in cardiac output and blood pressure that were not observed with either sodium chloride or no treatment. Thus there is evidence for a harmful effect of $NaHCO_3$ in the treatment of hypoxic lactic acidosis.*

The administration of sodium bicarbonate ($NaHCO_3$) to patients with metabolic acidosis has become a mainstay of therapy despite limited demonstration of its efficacy in many clinical situations. In cases where metabolic acidosis is largely secondary to actual loss of bicarbonate from the body (such as in renal tubular acidosis), long-term administration of $NaHCO_3$ has been successful (*1*). However, where acidosis is secondary to increased generation of organic acids (in diabetic ketoacidosis and lactic acidosis), the success is less clear (*2*). Both clinical results and those from laboratory studies suggest that bicarbonate may be of no benefit or may actually be harmful under such circumstances (*3–9*). Even in vitro, addition of $NaHCO_3$ to acidotic blood results in a decline of the *p*H (*10*).

Earlier studies from our laboratory have shown that, in three different animal models of lactic acidosis, therapy with $NaHCO_3$ leads to decreased cardiac output, a decline in blood pressure, increased concentrations of lactate in blood, decreased lactate metabolism, and increased subject mortality (*11*, *12*). These studies all dealt with type B (no clinical evidence of hypoxemia) lactic acidosis. A more common clinical problem is type A (hypoxic) lactic acidosis, which can occur with hypoxemia or shock states of various origin, resulting in a patient mortality rate of more than 50 percent when blood lactate concentrations exceed 5 m*M* (*13*). We have developed an animal model of hypoxic lactic acidosis (*14*) that facilitates a controlled study of the metabolic and systemic effects of $NaHCO_3$ in the treatment of this disorder.

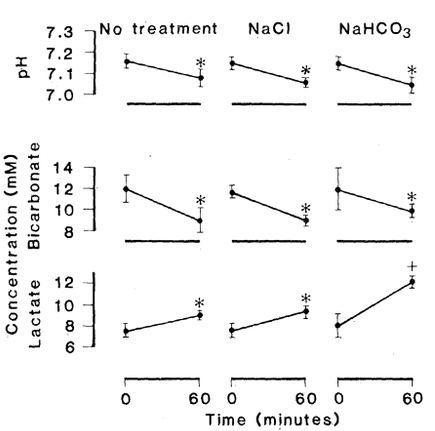

Fig. 1. Blood *p*H and bicarbonate and lactate concentrations in dogs (values are means ± standard error of the mean; $n = 7$ per group) during 60 minutes of therapy of hypoxic lactic acidosis. (*) $P < 0.05$ compared to control at 0 minutes. (+) $P < 0.01$ compared to NaCl as well as animals who received no treatment.

Hypoxic lactic acidosis was induced in intubated anesthetized dogs with controlled ventilation by supplying them, via an anesthesia machine, with a hypoxic gas mixture of approximately 8 percent oxygen and 92 percent nitrogen, producing arterial P_{O_2} values of 25 to 30 mmHg. This results in a stable model of lactic acidosis with blood lactate concentrations above 5 m*M* and bicarbonate concentrations below 15 m*M* (*14*). Three groups of seven dogs each were studied for 60 minutes after the development of hypoxic lactic acidosis: (i) a control group ($n = 7$) receiving no treatment except isotonic fluid infusion equal to estimated fluid losses; (ii) another group ($n = 7$) receiving 1*M* NaCl at a dose of 2.5 meq per kilogram per hour; and (iii) a group ($n = 7$) treated with 1*M* $NaHCO_3$ at the same dose of 2.5 meq per kilogram per hour, which corresponds to that used in clinical settings in the therapy of lactic acidosis (*4*, *6*, *8*). Serial measurement of blood *p*H, P_{CO_2}, P_{O_2}, and concentrations of bicarbonate and lactate were performed as described (*15*). These parameters were monitored in all animals by means of catheters inserted at sites appropriate for measuring production of extrahepatic splanchnic (gut) and skeletal muscle (carcass) lactate and hepatic lactate extraction as described (*15*). Hemodynamic measurements were performed by routine methods (cardiac output was measured by the thermodilution technique, and the mean aortic blood pressure at the femoral artery was measured with a Statham transducer) as described (*11*). For changes within the individual groups, statistical analysis was by the *t*-test for paired data. The statistical analysis for multiple comparisons between the three groups was a one-way analysis of variance with a subsequent

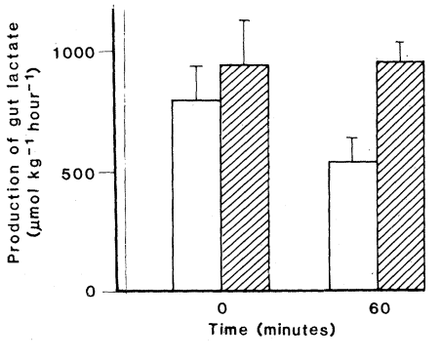

Fig. 2. Production of gut lactate in animals treated with $NaHCO_3$ (□) and NaCl (▨) (values are means ± standard error of the mean; $n = 7$ per group). After 60 minutes of therapy, the rate for animals receiving NaCl was significantly less ($P < 0.001$) than the baseline rate and that for animals treated with $NaHCO_3$. The rate changes in the group receiving no treatment were not significant.

Bonferroni procedure for comparisons of group means.

At baseline (Figs. 1 and 2), dogs in all three groups had similar degrees of metabolic acidosis characterized by arterial blood *p*H of 7.15 ± 0.08 (mean ± standard deviation), bicarbonate concentration of 12.7 ± 2.6 m*M*, P_{CO_2} of 36 ± 6 mmHg, and lactate concentration of 8.1 ± 2.5 m*M*. The major sites of lactate production are gut and carcass, with most of the lactate being produced by the gut. The concomitant depressed capacity of the liver to extract lactate results in lactic acidosis (*14*). The most important hemodynamic alteration at baseline (Fig. 3) was an increased cardiac index of about 130 percent of the value in control animals. The mean aortic pressure was normal, as was the blood flow to the gut and liver.

During the 60 minutes of each treatment there was a slight decrease in both arterial blood *p*H and bicarbonate concentration in all three groups, whereas arterial P_{CO_2} remained unchanged (37 ± 7 mmHg). The animals treated with $NaHCO_3$ showed a greater decrease in blood *p*H and bicarbonate concentration than the animals who received either NaCl or no therapy (Fig. 1). Although blood lactate concentrations increased in all three groups, those in the group receiving $NaHCO_3$ treatment were significantly higher at the end of therapy than those in the other two groups (Fig. 1). Concomitantly, P_{CO_2} at the hepatic portal vein (an expression of gut CO_2 production) was significantly higher after 60 minutes of $NaHCO_3$ therapy (55 ± 16 mmHg) than that observed during NaCl treatment (45 ± 13 mmHg), whereas arterial P_{CO_2} was unaltered in both groups. Because CO_2 readily penetrates cellular membranes, a further decrease in intracellular *p*H in the group treated with $NaHCO_3$ might occur.

Production of carcass lactate did not change significantly during any of the three treatments. However, the rate of gut lactate production in animals treated with NaCl was significantly less than the rate in animals receiving $NaHCO_3$ (Fig. 2). Extraction of lactate was generally unaltered by treatment and was always about 6 percent of the lactate load presented to the liver.

The blood pressure showed no significant changes during NaCl therapy (Fig. 3A). In dogs receiving no treatment, the blood pressure remained constant for the first 30 minutes of hypoxia but then dropped significantly at 60 minutes compared to baseline values ($P < 0.05$) as well as to values for the group treated with NaCl ($P < 0.01$). However, in dogs treated with $NaHCO_3$ there was a significant steady decrease in blood pressure that continued until the end of treatment. The values were always lower for dogs in this group compared to the values for those receiving NaCl and no treatment ($P < 0.001$ and $P < 0.01$, respectively). A similar pattern was found in the changes in cardiac index (Fig. 3B). Whereas the dogs treated with NaCl showed no significant changes in cardiac index after 60 minutes of therapy, dogs receiving no treatment showed a progressive decrease in cardiac index that reached statistical significance compared to NaCl-treated dogs after only 30 minutes ($P < 0.01$). In dogs treated with $NaHCO_3$, the cardiac index after 30 minutes was significantly less than that in dogs receiving NaCl or no treatment ($P < 0.01$). After 60 minutes, there was no significant difference between animals receiving $NaHCO_3$ and no treatment, but both groups had significantly lower values than did the NaCl-treated animals ($P < 0.01$).

These data show that treatment of hypoxic lactic acidosis with $NaHCO_3$ is not only ineffective but has serious adverse effects as well. Infusion of bicarbonate in these animals only resulted in a further increase of blood lactate concentrations that was unexpectedly and substantially greater than that observed with either isosmotic (NaCl) therapy or no treatment. The increased P_{CO_2} at the hepatic portal vein observed in the animals treated with $NaHCO_3$ could further decrease intracellular *p*H and generally contribute to the maintenance or worsening of acidemia. That volume expansion with NaCl may be of benefit is

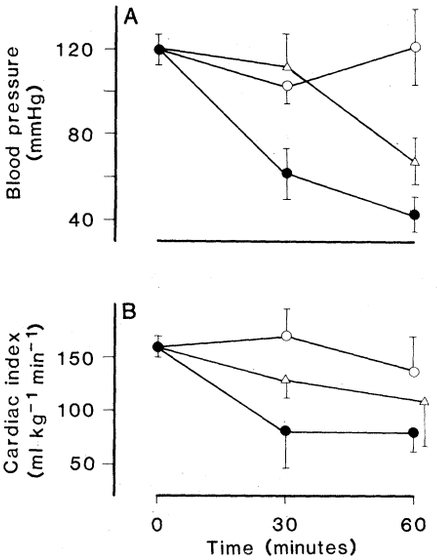

Fig. 3. Hemodynamic changes in dogs receiving NaCl (○), $NaHCO_3$ (●), or no therapy (△) during 60 minutes (values are means ± standard error of the mean; $n = 7$ per group). For significance values see text. Treatment with $NaHCO_3$ resulted in significant decreases in both mean arterial blood pressure (A) and cardiac index (B).

suggested by the decrease in production of gut lactate in NaCl-treated animals. However, aggressive volume expansion might be deleterious in certain patients, particularly those with impaired cardiac or renal function. The same osmotic load, volume, and quantity of sodium was administered to animals receiving NaCl and bicarbonate, but cardiovascular effects and blood chemistries were different (Figs. 1, 2, and 3), thus showing a direct adverse effect of bicarbonate on the production of lactate in tissue and on cardiovascular performance. In this study the arterial P_{CO_2} was set at 36 mmHg, which is normal for the dog (*11*), because in other studies of patients with hypoxic lactic acidosis (*16*) the mean P_{CO_2} ($n = 80$) was 42 mmHg, which is normal for humans. Our results might be different if the P_{CO_2} used were different, but considering that the P_{CO_2} in patients with hypoxic lactic acidosis ranged from 20 to 113 mmHg (*16*), no value could be described as "typical."

The most important goal in treatment of hypoxic lactic acidosis is sustaining tissue perfusion, because any decrease adds a hypoxic stimulus for increased production of tissue lactate. In this respect our data show that administration of sodium bicarbonate is accompanied by a deterioration of cardiovascular performance as evidenced by a fall in cardiac output and blood pressure. The decreases in cardiac index and blood pres-

sure impair tissue perfusion and result in a further increase of blood lactate concentrations. Our results suggest that the basis for use of sodium bicarbonate in the therapy of hypoxic lactic acidosis should be reevaluated.

HELMUT GRAF
WILLIAM LEACH
ALLEN I. ARIEFF*
Nephrology Research, Veterans Administration Medical Center and *University of California, San Francisco, California 94121*

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* To whom correspondence should be addressed at the Veterans Administration, Medical Center (111J), 4150 Clement Street, San Francisco, California 94121.

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Protection Against Lethal Hyperoxia by Tracheal Insufflation of Erythrocytes: Role of Red Cell Glutathione

Abstract. *Intact erythrocytes placed into the tracheobronchial tree of hyperoxic rats dramatically improved their chances for survival. Over 70 percent of the animals so treated survived more than 12 days during continuous exposure to 95 percent oxygen, whereas all of the control animals died within 96 hours. Lungs from erythrocyte-protected rats showed almost none of the morphologic damage suffered by untreated animals. Erythrocytes containing cyanomethemoglobin were as beneficial as normal erythrocytes, but cells in which glutathione was partially blocked were significantly less protective. Analogous results were obtained in vitro: ^{51}Cr-labeled target cells released 70 to 90 percent of their label when exposed briefly to hydrogen peroxide or to toxic oxygen species generated by phorbol ester–stimulated neutrophils. Addition of intact erythrocytes decreased release by approximately 75 percent, but significantly less than this if red blood cell glutathione was partially blocked. These results suggest that insufflated erythrocytes, through their recyclable glutathione, protect rats from toxic oxygen species engendered by hyperoxia.*

A Faustian compact is evident in the treatment of patients with severe respiratory distress who require oxygen at excessive pressures to preserve tissue oxygenation. That is, hyperoxia, itself, provokes pulmonary injury (*1*) by mechanisms not entirely understood. Hyperoxia is directly toxic to explanted lung parenchymal cells (*2*). However, our studies, and those of others (*3*), on the pathogenesis of the adult respiratory distress syndrome have suggested that stimulated inflammatory cells, particularly granulocytes, injure lungs through their production of toxic oxygen species such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (·OH), and the like. We have stressed the paradox that production of these oxidants might be amplified in the hyperoxic environment of lungs ventilated with oxygen at higher than normal pressures.

If toxic oxygen species are the cause of hyperoxic lung injury, scavenging them would seem worthwhile. In fact, levels of pulmonary superoxide dismutase (SOD), a dissipator of O_2^-, can be increased by exposing rats to 85 percent oxygen; such preadapted animals thereafter resist prolonged exposure to hyperoxia (*4*). In addition, prior administration of small doses of endotoxin also protects rats from oxygen toxicity; this again is associated with amplification of lung SOD levels, as well as with increases in other potentially protective moieties, including catalase and glutathione (GSH) (*5*). Glutathione is important in the resistance of diverse tissues to exhibited H_2O_2, organic peroxides, and the oxygen species released from inflammatory cells (*6*).

A more direct approach is intravenous injection of liposomes containing SOD plus catalase. This strategy increases the survival of rats exposed to 100 percent oxygen, whereas SOD and catalase in nonencapsulated form are ineffectual (*7*). Similar data from our laboratory indicate that liposome-encapsulated SOD or catalase when insufflated intratracheally also improves the survival of hyperoxic rats; 95 percent were alive after 72 hours as compared with 22 percent of control animals insufflated with "naked" liposomes (*8*).

We examined the possibility that intact erythrocytes placed in the tracheobronchial tree might act as more physiologic, encapsulated oxygen radical scavengers. Erythrocytes harbor large quantities of catalase, GSH, and SOD. Moreover, we reasoned that their enormous content of oxidizable heme groups might serve to detoxify labile oxygen species. We report that tracheal insufflation of small numbers of intact erythrocytes prevents oxidant lung injury and improves survival in hyperoxic rats, GSH being the beneficial erythrocyte constituent.

The tracheae of ether-anesthetized male Sprague-Dawley rats (275 to 325 g) were cannulated and insufflated with 1 ml of saline or with various red cell suspensions; thereafter, we placed the rats in a 100 by 30 by 30 cm exposure chamber suffused with 100 percent oxygen at 15 liters per minute. The oxygen tension in the chamber was greater than 0.95 atmosphere at all times. The rats were given free access to water and a standard rat diet and were killed after 3 days or 12 days for histologic examination of the lung.

A single insufflated bolus of 0.75 ml of erythrocytes (1 ml of a saline-washed erythrocyte suspension; hematocrit 75 percent) markedly improved the survival of hyperoxic rats: 75 percent of 89 erythrocyte-treated rats survived 96 hours in comparison with none surviving in the control group of 68 rats ($P < 0.001$) (Table 1). Of the animals that survived, some ($n = 24$) were still alive after 12 days of hyperoxia. The beneficial effect of erythrocytes was evidently dose dependent; halving the erythrocyte bolus