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% 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 cyanometemoglobin were as beneficial as normal erythrocytes, but cells in which glutathione was partially blocked were significantly less protective. Analogous results were obtained in vitro. 32P-labeled target cells were released to 70% 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 (O2·−), hydrogen peroxide (H2O2), 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 O2·− 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 H2O2, 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...
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 mM. Therapy usually consists of intravenous sodium bicarbonate (NaHCO₃), but resultant mortality is greater than 60 percent. The metabolic and systemic effects of NaHCO₃ 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 NaHCO₃, both arterial pH and bicarbonate concentration decreased by a similar amount in all three groups of dogs. Additional detrimental effects of NaHCO₃ 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 NaHCO₃ in the treatment of hypoxic lactic acidosis.

The administration of sodium bicarbonate (NaHCO₃) 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 NaHCO₃ 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 NaHCO₃ to acidic blood results in a decline of the pH (10).

Earlier studies from our laboratory have shown that, in three different animal models of lactic acidosis, therapy with NaHCO₃ 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 mM (13). We have developed an animal model of hypoxic lactic acidosis (14) that facilitates a controlled study of the metabolic and systemic effects of NaHCO₃ in the treatment of this disorder.

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 P0₂ values of 25 to 30 mmHg. This results in a stable model of lactic acidosis with blood lactate concentrations above 5 mM and bicarbonate concentrations below 15 mM (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 NaHCO₃ 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 pH, PCO₂, PO₂, 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 extrathoracic splanchic (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
Because CO₂ readily penetrates cellular membranes, a further decrease in intracellular pH in the group treated with NaHCO₃ might occur.

Production of carcass lactate did not change significantly during any of the three treatments. However, the rate of lactate production in animals treated with NaCl was significantly less than the rate in animals receiving NaHCO₃ (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 NaHCO₃ 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 NaHCO₃, 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 NaHCO₃ 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 NaHCO₃ 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 Pco₂ at the hepatic portal vein observed in the animals treated with NaHCO₃ could further decrease intracellular pH and generally contribute to the maintenance of worsening of acidemia. That volume expansion with NaCl may be of benefit is 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 Pco₂ 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 Pco₂ ($n = 80$) was 42 mmHg, which is normal for humans. Our results might be different if the Pco₂ used were different, but considering that the Pco₂ 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.
Systemic effects of NaHCO₃ in experimental lactic acidosis in dogs

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ARIEFF, ALLEN I., WILLIAM LEACH, ROBERT PARK, AND VIRGINIA C. LAZAROWITZ. Systemic effects of NaHCO₃ in experimental lactic acidosis in dogs. Am. J. Physiol. 242 (Renal Fluid Electrolyte Physiol. 11): F586-F591, 1982.—Lactic acidosis is characterized by metabolic acidosis due to accumulation of H⁺ ions from lactic acid with blood lactate of at least 5 mM. The standard treatment is intravenous NaHCO₃ with resultant mortality in excess of 80%. Despite the high mortality, the metabolic and systemic effects of NaHCO₃ used in the treatment of lactic acidosis have not been extensively studied. The present experiments in diabetic dogs were designed to address these questions. Dogs with phenformin-induced lactic acidosis (blood lactate above 5 mM, arterial pH below 7.20) were treated with equimolar amounts of either NaCl or NaHCO₃ or received no therapy. Intravenous NaHCO₃ resulted in a decline of cardiac output and intracellular pH (pHi) of liver and erythrocytes, whereas treatment with NaCl did not. With NaHCO₃ but not with NaCl infusion gut lactate production increased almost stoichiometrically, with no change in arterial pH or bicarbonate but with a doubling of lactate. Bicarbonate also resulted in a decrease of hepatic portal vein blood flow. The mean survival time and percent mortality were similar in NaCl- vs. NaHCO₃-treated animals. Although both groups lived longer than did animals receiving no therapy, the differences were not significant. Thus, treatment of experimental lactic acidosis with either NaCl or NaHCO₃ or with no therapy results in no change of blood pH and bicarbonate and in a similar mortality. In terms of systemic effects, however, NaHCO₃ results in significant decrements of liver and erythrocyte pH, hepatic portal vein blood flow, and cardiac output and in significant increments of gut lactate production, whereas NaCl does not. The data suggest that the rationale for therapy of lactic acidosis with NaHCO₃ should probably be reevaluated.

intracellular pH; sodium bicarbonate; phenformin; sodium chloride

LACTIC ACIDOSIS IS A CLINICAL and biochemical syndrome characterized by metabolic acidosis (pH below 7.25) in which the acidosis is primarily due to accumulation of lactic acid to levels of at least 5 mM (1). Credit for the original clinical descriptions of lactic acidosis is usually given to studies published during 1958–1959 (17, 21), although an experimental model of lactic acidosis was described in 1934 (23). In this model (guanidine-induced lactic acidosis in the dog with blood lactate of 13 mM and arterial pH of 7.08), it was observed that treatment with NaHCO₃ resulted in little or no change in blood bicarbonate, increased blood lactate levels, convulsions, and 100% mortality.

Since the original descriptions of lactic acidosis in humans, there have been many attempts to classify lactic acidosis, the most useful of which has been proposed by Cohen and Woods (cited in Ref. 2). In type A lactic acidosis there is clearly tissue hypoxia, and in type B lactic acidosis there is no clear evidence of tissue hypoxia. For all types of lactic acidosis, therapy has usually consisted primarily of intravenous NaHCO₃. Despite such therapy, however, the overall mortality exceeds 85% in patients with type A lactic acidosis and is at least 50% in type B (24). It is well documented in several case reports that NaHCO₃ administration above 1,000 meq/day often results in no change in blood bicarbonate or pH and an increase in blood lactate (12, 13, 33).

Although mortality is apparently not improved by bicarbonate, it nonetheless remains the mainstay of treatment for lactic acidosis. Despite these facts, the metabolic and systemic effects of intravenous bicarbonate in lactic acidosis are not known. The purpose of the present investigation was to evaluate the metabolic and circulatory effects and the effect on mortality of NaHCO₃ therapy in dogs with experimental lactic acidosis. We have previously described the pathogenesis and cardiovascular manifestations of this experimental model of lactic acidosis in dogs (3, 5).

METHODS

Studies were carried out in five groups of diabetic dogs. Lactic acidosis was induced 7–10 days after surgical pancreatectomy with intravenous phenformin. All the dogs had blood glucose levels above 17.5 mM, lactate levels above 5 mM, and bicarbonate levels below 12 meq/liter (5). The experimental groups were as follows: group 1, diabetic controls; group 2, dogs with lactic acidosis in which samples were obtained before therapy; group 3, dogs with lactic acidosis that were treated with intravenous NaCl; and group 5, diabetic dogs with lactic acidosis that received no therapy. The studies in the animal groups consisted of 1) hemodynamic studies, including mean arterial pressure, cardiac output, and hepatic portal venous (HPV) blood flow; 2) arterial, HPV, and hepatic venous (HV) blood pH, PO₂, PCO₂, and concentrations of lactate, bicarbonate, and pyruvate; 3) liver intracellular pH (pHi), extracellular space (ECS), redox state, and concentrations of lactate and pyruvate; 4) muscle and erythrocyte pH; and 5)
LACTIC ACIDOSIS TREATED WITH NaHCO₃

extrahepatic splanchnic lactate production, hepatic lactate extraction, and net splanchnic lactate production.

For all experiments, overnight-fasted dogs were anesthetized with sodium pentobarbital, intubated, and mechanically ventilated (28). The arterial PCO₂ was adjusted to a mean of 33 mmHg by adjustment of tidal volume (28). The dogs had previously been rendered diabetic by surgical pancreatectomy and were maintained on NPH insulin (5), with fasting blood glucose levels of at least 17.5 mM (without insulin).

The dogs in groups 2-5 were infused with phenformin at 13 mg·kg⁻¹·h⁻¹ for a mean of 150 min (5). At this time, all animals had arterial pH < 7.25, lactate > 5 mM, and bicarbonate < 12 meq/liter. The dogs in groups 3, 4, and 5 were then treated with either intravenous 1.0 NaHCO₃ at 1 ml/min (group 3); intravenous 1.0 NaCl at 1 ml/min (group 4); or had no treatment (group 5). The dogs were observed for up to 4 h. Experiments were terminated if mean arterial pressure fell below 60 mmHg or cardiac output fell by more than 60% of the baseline value during 4 h of therapy. Studies were carried out after 90 min of therapy in groups 3 and 4.

Cardiac output was measured by thermodilution (3) using an Instrumentation Laboratory cardiac output computer, HPV flow via a cuff around the hepatic portal vein using a Statham flowmeter (5), and mean arterial blood pressure via a cannula in the femoral artery connected to a Statham transducer attached to a Statham strip-chart recorder (5). Samples of arterial, HPV, and HV blood were obtained as previously described (5). Measurements of HV, HPV, and arterial pH, Pco₂, Po₂, bicarbonate, lactate, and pyruvate were carried out as previously described (5, 28). Liver and muscle samples were frozen in liquid nitrogen and extracted in 0.55 M perchloric acid (28). The extracts were analyzed for lactate, pyruvate, and ¹⁴C-labeled dimethadione (DMO). Intracellular pH was measured in skeletal muscle, erythrocytes, and liver using the distribution of [¹⁴C]dimethadione relative to either arterial blood (for muscle and erythrocytes) or HPV and HV blood (for liver) (28). Erythrocyte extracellular space was measured as (1 - hematocrit); liver ECS was determined as the chloride space (29). The liver cytosolic redox state was determined from measurements in liver of ECS, lactate, pyruvate, and pH (5). Extrahepatic splanchnic lactate production was determined as the product of the lactate concentration difference from aorta to HPV and HPV blood flow (5). Hepatic lactate extraction was calculated from HPV blood flow and the lactate concentration difference in HPV and HV (5). The contribution of lactate from the hepatic artery was not included in this calculation.

In group 3, tissue (muscle, liver, erythrocyte) pH was measured in 6 of 12 dogs. In the six dogs in which pH was determined, the ureters were ligated prior to treatment to prevent loss of isotope (DMO) in the urine. Similarly, ureters were ligated prior to tissue pH measurements in all six group 4 dogs. The arterial pH, bicarbonate, and lactate levels in the six group 3 dogs that had their ureters ligated were not different from the six that did not. The diabetic dogs in group 1 were anesthetized for 180 min at the time samples were obtained.

Statistical methods. Data are expressed as means ± SE. Statistical significance was determined by use of the paired or unpaired t test, as appropriate. In Table 1, the six dogs in group 4 and the six dogs in group 3, all of which both had their ureters ligated and had lactic acidosis before treatment, are pooled and constitute the 12 dogs in group 2. Animals who then received either NaCl or NaHCO₃ are listed separately in Table 1. For statistical comparisons, the dogs in groups 3 and 4, where indicated, served as their own controls and the paired t test was used. In particular, studies were made after 90 min of treatment in groups 3 and 4 of intracellular pH (erythrocytes, liver, muscle), HPV blood flow, liver lactate uptake, and extrahepatic splanchnic lactate production. In group 3, for measurement of pH; only six dogs were compared. Evaluation after 90 min of therapy was also made of mean arterial pressure, cardiac output, and arterial pH, Po₂, Pco₂, lactate, pyruvate, and bicarbonate. In calculating mean survival time, all dogs that survived more than 4 h of treatment were counted as 4 h. Where possible, data in Table 1 from groups 3-5 were obtained after 90 min of therapy. In dogs that died prior to this time, samples were taken just before termination of the experiment.

RESULTS

Controls. The values for control diabetic dogs (group 1) and those with lactic acidosis (group 2) are given in Table 1. The animals with lactic acidosis had received intravenous phenformin for a mean of 150 min. The weight for 35 diabetic dogs in groups 1-5 was 23.4 ± 1.1 kg. Control flow in five normal dogs was 20.4 ± 5.1 in the HPV and 7.5 ± 3.2 ml·kg⁻¹·min⁻¹ in the hepatic artery.

Treatment with NaHCO₃ (group 3). The values for dogs with lactic acidosis that were treated with NaHCO₃ are given in Table 1. A mortality of 83% was observed in 12 animals, two of which survived for 4 h. The mean survival time in the 12 animals was 122 ± 33 min. The values for arterial pH, bicarbonate, and lactate are given in Table 1. After bicarbonate infusion, the values were pH, 7.18 ± 0.03; bicarbonate, 10.2 ± 1.0 meq/liter; and lactate, 13.6 ± 1.5 meq/liter. The values for pH and bicarbonate were not different from those observed before bicarbonate infusion, but blood lactate increased twofold (P < 0.01). During this time pH fell in both liver and erythrocytes (Fig. 1). In 12 dogs with lactic acidosis (group 2), the pH was 6.82 ± 0.02 in liver and 7.20 ± 0.03 in erythrocytes before treatment. After therapy with NaHCO₃ (group 3), in six of these dogs treated with NaHCO₃ the pH in liver had fallen to 6.72 ± 0.05 (P < 0.01) and to 7.12 ± 0.05 in erythrocytes (P < 0.01) (t test for paired data in these six dogs vs. values in the same six dogs before therapy). The change in muscle pH was not significant. The plasma sodium was 149 ± 2 meq/liter (control, 145 ± 1 meq/liter). The reasons for the rise in blood lactate and fall in tissue (liver, erythrocyte, and muscle) pH were then investigated. The cardiac output in 12 dogs with lactic acidosis was 1.26 ± 0.18 liter/min (group 1, control, 2.24 ± 0.25 liter/min, P < 0.01). After 90 min of bicarbonate therapy, it had further declined to 0.88 ± 0.31 liter/min (P < 0.01, t test for paired data). Along with the decrement in cardiac output, HPV blood
### Table 1. Systemic effects of lactic acidosis and its treatment

<table>
<thead>
<tr>
<th></th>
<th>Blood</th>
<th>Cardiac Output, liter/min</th>
<th>pH</th>
<th>HPV Flow, ml·kg⁻¹·min⁻¹</th>
<th>Survival Time, min</th>
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<tr>
<td><strong>Diabetes (group 1, n = 9)</strong></td>
<td>** mean ±SE**</td>
<td></td>
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<td>Arterial pH</td>
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<td>Arterial HCO₃, mM</td>
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<td>7.5 ± 0.1</td>
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<tr>
<td>Arterial lactate, mM</td>
<td>1.04 ± 0.5</td>
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<td>6.82 ± 0.18</td>
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<td>HPV lactate, mM</td>
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<td>HV lactate, mM</td>
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<td>0.04 ± 0.03</td>
<td>0.05 ± 0.05</td>
<td>1.4 ± 0.9</td>
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**Lactic acidosis (group 2, n = 12)**

| Mean ±SE              | 7.15 ± 0.10 | 6.7 ± 0.4 | 7.2 ± 0.7 | 4.0 ± 0.4 | 3.6 ± 0.4 | 7.2 ± 0.4 |

**NaHCO₃ therapy (group 3, n = 12)**

| Mean ±SE              | 7.18 ± 0.10 | 13.5 ± 0.9 | 17.2 ± 0.6 | 16.6 ± 0.9 | 0.88 ± 0.05 | 6.72 ± 0.06 |

**NaCl therapy (group 4, n = 6)**

| Mean ±SE              | 7.13 ± 0.10 | 9.1 ± 0.6 | 11.0 ± 0.5 | 11.2 ± 0.5 | 1.12 ± 0.05 | 6.98 ± 0.06 |

**No therapy (group 5, n = 8)**

| Mean ±SE              | 7.07 ± 0.10 | 8.7 ± 0.5 | 8.5 ± 0.5 | <2.0 ± 0.1 | <2.0 ± 0.1 | 96 ± 37 |

HPV, hepatic portal vein; HV, hepatic vein; pH, intracellular pH.

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**Fig. 1.** Liver intracellular pH (pHₙ) is shown in 4 groups of dogs. In dogs with lactic acidosis, there was a significant decrement (P < 0.01) in liver pH, compared with control diabetic animals. Liver pHₙ was unaffected by NaCl treatment but decreased significantly (P < 0.01 vs. dogs with lactic acidosis before treatment) after therapy with NaHCO₃.

**Fig. 2.** Extraphasic splanchic lactate production and liver lactate uptake are shown in 4 groups of dogs. In dogs with lactic acidosis, there were significant decrements of both lactate production and uptake of lactate by the liver (P < 0.01). Lactate uptake was unaffected by treatment with either NaCl or NaHCO₃. However, treatment with NaHCO₃ resulted in a significantly higher increase in both extraphasic and net splanchic lactate production (P < 0.01), whereas treatment with NaCl did not. Liver lactate uptake does not include contribution of hepatic artery (see text).

Flow also declined. The control HPV blood flow was 19.2 ± 1.4 ml·kg⁻¹·min⁻¹. In dogs with lactic acidosis, HPV flow had fallen to 10.9 ± 0.9 ml·kg⁻¹·min⁻¹ (P < 0.01), and after bicarbonate therapy the value was 8.9 ± 0.9 ml·kg⁻¹·min⁻¹ (P < 0.01 vs. dogs with lactic acidosis). Accordingly, the decline in HPV flow appeared to parallel the decline in cardiac output.

Extraphasic splanchic lactate production was then calculated from the aforementioned values for HPV blood flow and the lactate gradient from aorta to HPV. In dogs with lactic acidosis, the extraphasic splanchic lactate production was 974 ± 79 µmol·kg⁻¹·h⁻¹ before therapy compared with the control value of 679 ± 84 µmol·kg⁻¹·h⁻¹ (P < 0.01). In dogs with lactic acidosis treated with NaHCO₃, lactate production increased to 1,913 ± 117 µmol·kg⁻¹·h⁻¹ (Fig. 2).

Corresponding values were also calculated for hepatic uptake. In dogs with lactic acidosis, the value was 457 ± 15 µmol·kg⁻¹·h⁻¹ (control value, 691 ± 57 µmol·kg⁻¹·h⁻¹). In dogs treated with NaHCO₃, hepatic lactate uptake was 321 ± 42 µmol·kg⁻¹·h⁻¹ (P < 0.01 vs. dogs before treatment). Therefore, in dogs with lactic acidosis treated with NaHCO₃, the net splanchic lactate production (gut production minus hepatic uptake) was 1,592 ± 121 µmol·kg⁻¹·h⁻¹ (42.2 ± 3.2 meq/h, control value = 0). The rate of bicarbonate infusion was 60 meq/h, with no significant alteration in plasma bicarbonate. Thus, most of the infused bicarbonate (70%) was titrated with lactic acid produced by the gut (Fig. 2).

The effects of bicarbonate infusion on hepatocellular cytosolic redox state were also evaluated. The control
diabetic redox state was 591 ± 82. In dogs with lactic acidosis, the value was 551 ± 28 (P < 0.01). After treatment with NaHCO₃, the redox state was unchanged at 365 ± 36. Accordingly, the decreased liver lactate extraction observed after NaHCO₃ administration could not be explained on the basis of an alteration in hepatic cytosolic redox state.

**Treatment with NaCl (group 3).** Animals with lactic acidosis were then treated with intravenous NaCl infused at 1 meq/min. The values for dogs with lactic acidosis are shown in Table 1. After 90 min of treatment with NaCl, the values for blood lactate (9.1 ± 1.2 meq/liter), arterial blood bicarbonate (10.1 ± 1.1 meq/liter), and pH (7.13 ± 0.07) were essentially unaltered. One of six animals survived (mortality = 83%) and the mean survival time in the dogs treated with NaCl was 114 ± 37 min. This was not different from the mortality or survival time in the NaHCO₃-treated dogs.

After treatment with NaCl, the pHₐ was also evaluated in erythrocytes, muscle, and liver. After NaCl therapy, pHₐ was 6.79 ± 0.06 in liver (Fig. 1), 6.86 ± 0.07 in muscle, and 7.16 ± 0.02 in erythrocytes. Only the pHₐ in muscle was significantly different from controls (P < 0.05). The plasma sodium was 148 ± 1 meq/liter, which was not different from that in group 3 dogs.

The cardiac output in dogs with lactic acidosis was 1.26 ± 0.18 liter/min (group 2, Table 1). After 90 min of NaCl therapy, cardiac output was 1.12 ± 0.2 liter/min, a value not different from that observed prior to therapy.

Net splanchinic lactate production was evaluated after therapy with NaCl. The lactate levels in HPV, HV, and aorta are shown in Table 1. Extrahepatic splanchinic lactate production was 1,140 ± 106 μmol·kg⁻¹·h⁻¹ (P < 0.01 vs. value in dogs treated with NaHCO₃). In these same dogs, the calculated hepatic lactate extraction was 238 ± 30 μmol·kg⁻¹·h⁻¹, which was similar to the value in dogs treated with NaHCO₃. The calculated net splanchinic lactate production in dogs treated with NaCl was 800 ± 49 μmol·kg⁻¹·h⁻¹, significantly less (P < 0.01) than the value in dogs treated with NaHCO₃ (1,592 ± 121 μmol·kg⁻¹·h⁻¹) (Fig. 2).

Thus, NaHCO₃ infusion in dogs with lactic acidosis increased extrahepatic splanchinic lactate production, which was significantly greater than with NaCl infusion. The increased lactate production was manifested by both a doubling of blood lactate after NaHCO₃ infusion and an unaltered blood bicarbonate level despite continuous intravenous NaHCO₃.

**No treatment.** The animals in group 5 were given phenformin for 150 min and then received no therapy whatever. They were observed as were the dogs in groups 3 and 4. The mean survival time in these dogs was 96 ± 8 min (n = 8). This time represents the interval from the onset of lactic acidosis to the time that systolic arterial pressure fell below 60 mmHg and/or cardiac output was below 60% of control value. In these dogs, the arterial pH and bicarbonate were 7.07 ± 0.05 and 9.6 ± 0.7 meq/liter, respectively, and lactate was 8.7 ± 0.7 mmol/liter. All of these values were not significantly different from those in dogs treated with NaCl. The values for HPV blood flow and cardiac output were below the range of our instruments. Cardiac output was below 200 ml/min and HPV flow was less than 2 ml·kg⁻¹·min⁻¹. Thus, no therapy resulted in biochemical alterations similar to the results with NaCl therapy. Although mean survival time was less than in those dogs treated with either NaCl or NaHCO₃, the differences were not significant.

**Discussion**

These data show that in dogs with experimental (phenformin-induced) lactic acidosis, survival time is similar in NaHCO₃- vs. NaCl-treated animals. The metabolic parameters evaluated reveal that NaHCO₃ treatment in dogs with lactic acidosis results in a decrement of cardiac output and intracellular pH in liver and erythrocytes, whereas treatment with NaCl does not. Furthermore, despite continuous infusion of NaHCO₃, blood bicarbonate and pH are unaltered. The detrimental effects of NaHCO₃ infusion are not due to either volume expansion or sodium infusion, as they are not duplicated by NaCl infusion at the same volume and sodium concentration.

The observed effects of NaHCO₃ therapy for experimental lactic acidosis parallel some commonly observed clinical observations in patients. Waters et al. (33) observed that infusion of over 1,000 meq/day of NaHCO₃ did not alter blood pH or bicarbonate. Many other investigators reported similar results (7, 12-14). Fraley and associates (13) have recently shown that in a patient with lactic acidosis and cancer, bicarbonate infusion resulted in an increase of lactate production that almost stoichiometrically paralleled the rate of bicarbonate infusion. The increase in lactate production with bicarbonate is not a feature common to all metabolic acidosis, however. Assal and associates (6) found that in nine patients with ketoacidosis, infusion of NaHCO₃ resulted in normalization of blood pH and bicarbonate. However, in some ketoacidotic patients, bicarbonate infusion has resulted in superimposed lactic acidosis (34).

It appears from the literature that in many instances patients with either type A or type B lactic acidosis (2) will respond to supportive care plus amelioration of the apparent underlying cause of the lactic acidosis. For example, lactic acidosis may occur in association with congestive heart failure (15), grand mal seizures (26), strenuous exercise (32), or malignancy (12, 13). In these cases, removal of the underlying cause often resulted in amelioration of the lactic acidosis, but in several bicarbonate therapy was ineffectual (13, 15).

The decrement in pHₐ and increased lactate production with bicarbonate infusion suggests that bicarbonate may somehow cause tissue hypoxia. The delivery of oxygen to tissues is controlled by a number of factors, including extracellular level of 2,3-diphosphoglycerate (2,3-DPG), extracellular pH, PCO₂, and PO₂, erythrocyte pHₐ, and cardiac output. In general, acute increases of extracellular pH decrease tissue oxygen delivery, as do acute decrements of erythrocyte pHₐ (by decreasing erythrocyte 2,3-DPG levels). Bellinger and associates (8) have shown that infusion of NaHCO₃ to acidic individuals acutely shifts the oxygen-hemoglobin dissociation curve to the left, decreasing tissue oxygen delivery (Bohr effect). Increased production of 2,3-DPG could partially compensate for the Bohr effect. However, in the present experi-
ments, NaHCO₃ infusion actually resulted in a fall of erythrocyte pHₐ. If anything, the fall in pH would be expected to decrease erythrocyte 2,3-DPG, but the experiments were probably too short for this to occur. A compensatory increase in cardiac output could also increase tissue oxygen delivery, but in these dogs cardiac output was already low (3) and fell still further after NaHCO₃ infusion (Table 1). Thus, the combination of a fall of erythrocyte pHₐ, a rise of blood pH, and a fall of cardiac output would tend to increase both tissue hypoxia and lactate production.

Most of the increment in lactate production during NaHCO₃ infusion is accounted for by the extrahepatic splanchnic vascular bed (gut). It is recognized that in actuality there is increased production of lactic acid, not lactate, and that the toxicity of lactic acid is related to increased hydrogen ion (H⁺) generation. However, when lactic acid enters the circulation, it reacts with NaHCO₃ to form sodium lactate and carbon dioxide. The standard assays measure lactate rather than lactic acid and virtually all lactic acid that enters the bloodstream is converted to sodium lactate. Thus, it would be technically incorrect to refer to increased concentrations of “lactic acid” in blood. However, it is recognized that, in fact, the toxic manifestations of hyperlactatemia from lactic acidosis are for the most part due to accumulation of H⁺ ion rather than lactate. The gut is a major contributor to the body lactate pool. Previous studies (27) in humans suggest that the intestine produces almost 10% of the total basal lactate of about 1,300 mmol/day in a 70-kg human. However, data in the dog (5) suggest that basal lactate production from gut alone is about 700 mmol·kg⁻¹·h⁻¹ or about 1,300 mmol/day in a 70-kg human. Thus, gut lactate production may be far in excess of that previously reported. This phenomenon may not be readily apparent because measurement of gut lactate production requires cannulation of the HV, which in turn requires laparotomy. Previous studies suggest that about 80% of the glucose metabolized by the gut is converted to lactate (20).

The reasons for the effect of bicarbonate on pHₐ (liver and erythrocyte) are unclear. It may be that alkalosis, by decreasing tissue blood flow, may contribute to tissue ischemia and increase lactate production. In isolated hepatocytes, addition of bicarbonate also lowers pHₐ (22), suggesting a possible direct effect of bicarbonate, per se, on tissue pHₐ. A similar fall in pHₐ with bicarbonate infusion is observed in erythrocytes (11). In normal dogs, however, liver pHₐ (in vivo) is unaffected by bicarbonate infusion (28).

The increased gut lactate production during bicarbonate infusion was an unexpected finding. In vitro studies with isolated rat jejunum demonstrate that gut lactate production may be linked to sodium uptake (19). However, in the present in vivo studies, NaHCO₃ infusion resulted in significantly more lactate production by the gut than did NaCl infusion (Fig. 2). Infusion of NaHCO₃ resulted in a decrement of cardiac output, whereas infusion of NaCl did not (Table 1). The fall in cardiac output may have contributed to increased gut ischemia and a rise in lactate production by lowering HPV flow. The increase in blood lactate observed after infusion of NaHCO₃, but not NaCl, was not due to decreased hepatic lactate extraction. Calculated lactate uptake by the liver was not different in NaCl- vs. NaHCO₃-treated animals (Fig. 2).

Normally, hepatic arterial flow is 29% of total liver blood flow in anesthetized dogs (19). In five normal dogs, we found that HPV flow was 541 ± 134 and hepatic artery flow was 198 ± 65 ml/min, or 27% of total liver blood flow. In this study, we ignored hepatic artery flow when calculating liver lactate extraction. For all groups in which hepatic lactate extraction was measured, the arterial lactate concentration was lower than that in the HV, and the HV lactate was less than that in HPV (Table 1) (groups 2-4). Thus, calculated lactate extraction by the liver using only HPV flow actually represents an upper limit. The actual extraction may be somewhat lower if one considers the lactate delivery to the liver from the hepatic artery. In the current studies (Table 1) and in those previously reported (5) the pathophysiology of lactic acidosis involved a significant decrement in calculated liver lactate extraction. The values were 456, 321, and 238 mmol·kg⁻¹·h⁻¹ of liver lactate extraction in groups 2, 3, and 4, respectively. If lactate from the hepatic artery is included in the calculation, these values become zero. We have shown that the normal liver can extract over 2,300 mmol·kg⁻¹·h⁻¹ of lactate (5). In the present study the control value for liver lactate removal was 691 mmol·kg⁻¹·h⁻¹, and this quantity fell with the various experimental procedures shown in Fig. 2. Since inclusion of lactate contributed by the hepatic artery flow would only result in a still lower value, it was not included in the calculated liver lactate extraction.

It is recognized that data from the present studies may apply only to phenformin-associated lactic acidosis. Phenformin itself may be cardiotoxic (31), although we have shown that there is depression of cardiac output (5) or cardiovascular collapse (18) in other types of experimentl lactic acidosis. In addition, results from previous studies on effects of intravenous NaHCO₃ in the treatment of experimental lactic acidosis are qualitatively similar to ours. Mino and associates (23) induced experimental lactic acidosis in dogs with guanidine infusion. Treatment of these dogs with intravenous NaHCO₃ resulted in a decrement of arterial blood pressure, no change in blood bicarbonate, and subsequent cardiovascular collapse. In rabbits with ketosis associated lactic acidosis, treatment with NaHCO₃ led to a fall in hepatic pH and cardiovascular collapse (18).

There are other potential disadvantages of NaHCO₃ infusion that were not evaluated in the current study. Fatal hypernatremia and hyperosmolality have been frequently reported as a complication of excessive infusion of NaHCO₃ (4). The usual cause of death is intracerebral bleeding, presumably secondary to rapid shrinkage of the brain. Infusion of hypertonic solutions has also been shown to cause lactic acidemia (1). There may also be circulatory congestion or central nervous system depression (9, 10, 30).

These data show that treatment of experimental lactic acidosis with NaHCO₃ results in a mortality similar to that of either NaCl infusion or no therapy. In terms of systemic effects, NaHCO₃ infusion results in decrements of cardiac output and tissue (liver and erythrocytes) pHₐ, whereas NaCl infusion does not. Additionally, NaHCO₃
but not NaCl infusion increases gut production of lactate in a nearly stoichiometric manner, so that arterial bicarbonate and pH are unaltered and lactate is increased. The effects of bicarbonate in dogs with experimental lactic acidosis are similar to the effects observed in some similarly treated patients with lactic acidosis (12, 13, 25, 33). The rationale for therapy of lactic acidosis with bicarbonate infusion should probably be reevaluated.

REFERENCES


We are grateful to the CIBA-Geigy Corporation for supplying the phenformin for intravenous use.

This work was supported by a grant from the Kroc Foundation (Santa Ynez, CA), by Grant AM-18350 from the National Institute of Arthritis, Metabolism, and Digestive Diseases, by the Research Service of the Veterans Administration, and by a generous gift from the CIBA-Geigy Corp. (Ardsley, NY).

R. Puck is a Research Associate of the Veterans Administration. Received 29 June 1981; accepted in final form 14 December 1981.