Anesthesiology
47:416–422, 1977

# *Oxygen Uptake of Canine Whole Body and Hind Limb with Hypocapnic Alkalosis*

*Richard A. Theye, M.D.,* Gerald A. Gronert, M.D.,† James J. A. Heffron, Ph.D.‡*

**The effect of hypocapnic alkalosis induced by hyperventilation on whole-body and hind-limb oxygen uptake ($\dot{V}O_2$) was studied in dogs anesthetized with pentobarbital. In the intact dog with a self-perfused hind limb, increasing $pH_a$ from 7.41 to 7.58 increased whole-body $\dot{V}O_2$ 8 per cent and decreased hind-limb $\dot{V}O_2$ 6 per cent. Isolated hind limbs perfused with heparinized whole blood had similar decreases in $\dot{V}O_2$ with increases in arterial blood $pH$ ($pHa$). However, isolated hind limbs perfused with whole blood containing citrate, phosphate, and dextrose (CPD) showed muscle twitches, had larger $\dot{V}O_2$ values at identical $pH_a$'s, and had an increase in $\dot{V}O_2$ with increase in $pH_a$. These changes with a CPD perfusate were associated with low levels of ionized calcium (<0.5 mEq/l), disappeared when calcium ion spontaneously increased to 1.0 mEq/l, and could be prevented or abolished by the addition of calcium chloride, dantrolene, *d*-tubocurarine, or succinylcholine. These results are in accord with the findings of others regarding an increase in whole-body $\dot{V}O_2$ with hypocapnic alkalosis, but do not support a contributory role of skeletal muscle to the overall increase. (Key words: Oxygen uptake; Neuromuscular relaxants, dantrolene; Neuromuscular relaxants, *d*-tubocurarine; Neuromuscular relaxants, succinylcholine; Ions, calcium; Acid–base equilibrium, alkalosis, respiratory.)**

EARLY EFFORTS to quantitate the energy costs of the work of breathing by determining the increase in rate of whole-body $O_2$ uptake ($\dot{V}_{O_2}$) with voluntary hyperventilation led Otis[1] and others to question whether the resultant hypocapnic alkalosis increased oxygen requirements independently of the added mechanical work. Numerous investigators have pursued this question, and it is now generally believed that increasing arterial blood $pH$ ($pH_a$) to more than 7.40 results in an increase in whole-body $\dot{V}_{O_2}$ directly attributable to a decrease in hydrogen ions ($H^+$),[2–5] thereby altering the intracellular energy systems of mitochondria throughout the body.[6] It was, therefore, not surprising when Harken[7] recently reported an increase in isolated canine hind-limb $\dot{V}_{O_2}$ of 10 per cent for each increase of 0.1 in $pH$ during controlled perfusion with a pump oxygenator system primed with blood rendered incoagulable by the addition of a mixture of citric acid, sodium citrate, sodium biphosphate, and dextrose (CPD).

Previously, we had failed to demonstrate an increase in isolated canine gastrocnemius muscle $\dot{V}_{O_2}$ with increase in $pH$ (unpublished observations). However, when we followed Harken's protocol, we obtained results similar to his. Pilot studies suggested that Harken's findings were perhaps due to artifactually low levels of ionized calcium in the CPD perfusate. Thus, the following studies were designed to examine the role of ionized calcium in the increased oxygen uptake of hypocapnic alkalosis.

## Materials and Methods

Three situations were studied: the intact, heparinized dog with a self-perfused hind limb (HL), a sacrificed dog with an isolated HL perfused with heparinized whole blood, and a sacrificed dog with an isolated HL perfused with heparinized whole blood containing CPD solution. As in the studies of Khambatta and Sullivan,[5] all dogs were initially anesthetized with pentobarbital, 30 mg/kg, and, while intact, were maintained with a continuous intravenous infusion of pentobarbital, 0.2 mg/kg/min, in physiologic saline solution, 10 mg/kg/h. The trachea was intubated, and the cuff inflated to provide an airtight fit. Ventilation was controlled with a constant-volume ventilator at a tidal volume of 400 ml, using an analyzed, compressed tank supply of oxygen, 23 per cent, and nitrogen, 77 per cent. The heparin dose was 200 U/kg body weight (intact studies) or 2,000 U/l donor blood (isolated HL studies), and was repeated as needed. As in Harken's studies, the CPD dose was 63 ml of USP solution[8] per liter of heparinized donor blood or perfusate, and the dose was not repeated; glucose was infused into the venous reservoir at a rate of 0.5 g/h. Temperatures of whole body (thermistor, lower third of esophagus) and HL (thermistor in central muscle mass) were maintained at 37.0 ± 0.2 C by heating pads, lamps, and heat exchanger, as needed.

The HL was prepared and isolated by tourniquets, as described by Harken,[7] with femoral venous cannulation and unimpeded femoral arterial supply in the intact studies and venous and arterial cannulations in the perfusion studies. The pump-oxygenator sys-

* Professor of Anesthesiology, Mayo Medical School.
† Associate Professor of Anesthesiology, Mayo Medical School.
‡ Senior Research Fellow, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901.

Received from the Department of Anesthesiology, Mayo Clinic and Mayo Medical School, Rochester, Minnesota 55901. Accepted for publication May 23, 1977. Supported in part by Research Grant GM-21729 from the National Institutes of Health, Public health Service.

Address reprint requests to Dr. Gronert.

tem for the isolated perfused HL had a priming volume of approximately two liters and included a bubble oxygenator (Temptrol) and a pulsatile finger pump (Sigma). Bypass was established with a delay of less than 30 seconds, and the flow rate was constant. The chest was then opened, and the heart was stopped by ligating the superior vena cava, inferior vena cava, and azygos vein.

Measurements and methods were as follows: arterial pressure (strain-gauge); blood $p$H, $P_{O_2}$, and $P_{CO_2}$ (electrodes, 37.0 C); $Hb_{O_2}$ (IL-CO-Oximeter); expired gas volume (open-circuit, calibrated gasometer); inspired and expired $O_2$ and $CO_2$ (Haldane); HL venous flow rate (timed collection, graduated cylinder); total plasma calcium (Ca, Atomic Absorption Spectrophotometer, Perkin-Elmer); ionized whole-blood calcium ($Ca^{++}$, calcium electrode, Orion SS20). Whole-body $\dot{V}_{O_2}$ ($[\dot{V}_I \times F_{I_{O_2}}] - [\dot{V}_E \times F_{E_{O_2}}]$) and HL $\dot{V}_{O_2}$ $[\dot{Q} \times (a\text{-}v)_{O_2}]$ were calculated and expressed relative to respective whole-body and HL mass (tissue distal to tourniquet, as determined at autopsy, which included an estimate of muscle fraction). Each HL $\dot{V}_{O_2}$ was based on six determinations of HL $\dot{Q}$ (collection and reinfusion of femoral venous flow) and $(a\text{-}v)_{O_2}$. The range of these six determinations in the intact animal was within 10 ml/min for $\dot{Q}$ and within 0.40 ml/min for $\dot{V}_{O_2}$. In the isolated perfused HL, $\dot{Q}$ was held constant and the range was within 2 ml/min for $\dot{Q}$ and 0.40 ml/min for $\dot{V}_{O_2}$. In the isolated perfused HL, $\dot{Q}$ was maintained at approximately the same flow rate as measured in the intact animal. Incremental flows to double those in the intact dog did not increase $\dot{V}_{O_2}$.

All tabulated mean values resulted from observations at an original $p$H$_a$, an increased or decreased $p$H$_a$, and a return to the original $p$H$_a$, and are based on the average of all findings at the lower $p$H$_a$ and the higher $p$H$_a$. In intact dogs, two 15-minute normocapnic studies were completed; respiratory frequency was then increased from 8–13 to 21–38 breaths/min and was maintained for an hour with three 10-minute hypocapnic studies in the final 30 minutes; then, frequency was returned to the original setting, and, after 30 minutes, two 15-minute normocapnic studies were completed. In isolated-HL dogs, at least three 20-minute study periods were completed in the sequence of hypocapnia, hypercapnia, and hypocapnia, achieved by adjusting the concentration of $CO_2$ in the oxygen–nitrogen mixture entering the oxygenator. The isolated HL preparation was stable for at least three hours; because of different responses in the CPD dogs, additional individual experiments were performed in these animals to examine the effects of dantrolene, succinylcholine, $d$-tubocurarine, and calcium on the HL $\dot{V}_{O_2}$ and electromyographic response. (These are described below in Results.)

TABLE 1. Whole-body (WB) and Intact Hind-limb (HL) Responses to Increase in $p$H$_a$

| | Normocapnia | | Hypocapnia | |
|---|---|---|---|---|
| | Mean | SE | Mean | SE |
| $p$H$_a$ | 7.41 | 0.01 | 7.58* | 0.02 |
| $Pa_{CO_2}$, torr | 37 | 1 | 22* | 1 |
| $\dot{V}_{O_2}$ (WB), ml/min/kg WB | 5.45 | 0.16 | 5.91* | 0.16 |
| $\dot{V}_{O_2}$ (HL), ml/min/kg HL | 2.40 | 0.14 | 2.25* | 0.16 |
| $\dot{Q}$ (HL), ml/min/kg HL | 112 | 9 | 121 | 10 |
| Arterial pressure (mean), torr | 143 | 4 | 141 | 4 |
| $Pa_{O_2}$, torr | 101 | 2 | 122* | 3 |
| $Pv_{O_2}$ (HL), torr | 51 | 3 | 43 | 6 |
| Plasma Ca (total), mEq/l | 4.52 | 0.05 | 4.53 | 0.06 |
| Whole blood Ca (ionized), mEq/l | 2.45 | 0.04 | 2.36* | 0.04 |

* Significantly different ($P < 0.05$) from normocapnic value by $t$ test for paired data (ten dogs).

While it would have been ideal to carry out in each dog, in sequence, all three sets of studies (intact, perfused-heparin, and perfused CPD), this was not technically feasible. Overall, the results reported involved a total of 16 dogs with weights (kg, mean ± SD) of 20 ± 2 (whole-body), 1.4 ± 0.2 (HL), and 0.70 ± 0.12 (HL muscle). Three dogs had intact whole-body studies with self-perfused hind-limb measurements (intact whole body–hind limb); four had intact whole-body–hind-limb, perfused-HL heparin and perfused-HL CPD studies; two had intact whole-body–hind-limb and perfused-HL CPD studies; one had intact whole-body–hind-limb and perfused-HL heparin studies; two had perfused-HL heparin studies only; one had perfused-HL CPD only; one had both perfused-HL heparin and perfused-HL CPD. In these 14 dogs, then, ten had intact whole-body–hind-limb studies, and eight were used in each of the isolated perfused-HL studies. The additional experiments discussed in the preceding paragraph were begun in the original eight perfused-HL CPD dogs, and completed in two other perfused-HL CPD dogs. Statistical significance was tested within and between situations by t tests for paired data and unpaired data, respectively, with $P < 0.05$ regarded as significant. For each situation, a regression equation relating HL $\dot{V}_{O_2}$ and $p$H$_a$ was calculated by the method of least squares.

## Results

In the intact dog with a self-perfused hind limb, increasing $p$H$_a$ from 7.41 to 7.58 by hyperventilation resulted in an increase in whole-body $\dot{V}_{O_2}$ from 5.45 to 5.91 ml/min/kg body weight (table 1). The average increase in $\dot{V}_{O_2}$ was 5 per cent for each 0.1 increase

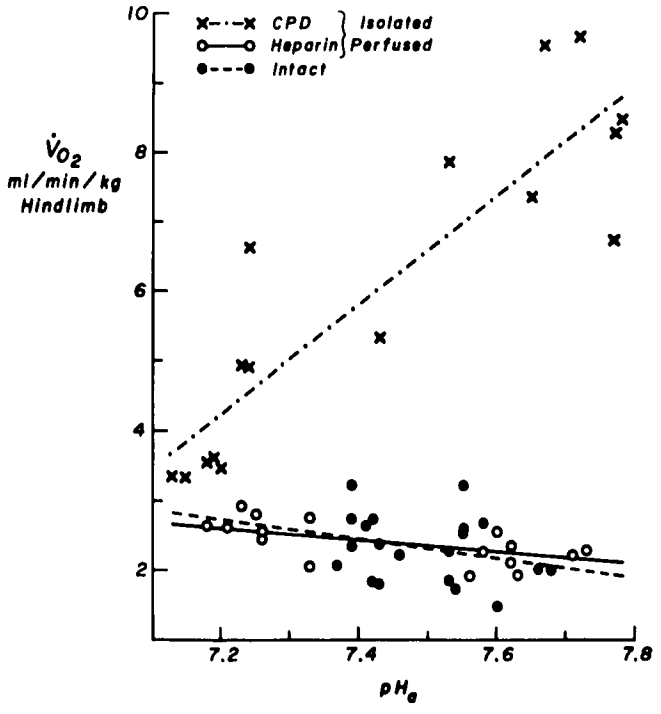


FIG. 1. Relationship between arterial blood $pH$ ($pH_a$) and hindlimb $\dot{V}_{O_2}$ (HL $\dot{V}_{O_2}$) in intact studies (●) and during perfusion with heparinized (○) or CPD (×) whole-blood perfusate. Note that individual values for $\dot{V}_{O_2}$ in the intact HL are similar to those for the heparin group ($\dot{V}_{O_2} = -1.023\ [\pm 0.311]\ pH_a + 10.02$). Note that HL $\dot{V}_{O_2}$ in the CPD group is greater at identical $pH_a$'s and has an opposite slope direction ($\dot{V}_{O_2} = 7.85\ [\pm 1.07]\ pH_a - 52.29$). Correlation coefficient for intact HL $\dot{V}_{O_2}$ (*dotted line*) is not significant.

in $pH$. While whole-body $\dot{V}_{O_2}$ increased as $pH_a$ was increased, HL $\dot{V}_{O_2}$ simultaneously decreased slightly (table 1), although there was not a linear relationship ($r = -0.27$) between $pH_a$ and HL $\dot{V}_{O_2}$ (fig. 1). The lack of a significant correlation is likely to be due to the narrower $pH$ range investigated in the intact–self-perfused-HL group. HL $\dot{V}_{O_2}$ at $pH$ 7.4, calculated from nine individual measurements between $pH$ 7.37 and $pH$ 7.43 (fig. 1), was 2.42 ± 0.16 ml/min/kg HL. The decrease in HL $\dot{V}_{O_2}$ could not be attributed to a decrease in rate of blood flow, oxygen supply, or muscle temperature. The tendency for a decrease in HL venous oxygen tension ($Pv_{O_2}$) to occur during hypocapnia was associated with an increase in oxyhemoglobin as a result of a pronounced leftward shift in the oxyhemoglobin dissociation curve produced by the decrease in hydrogen ions. Plasma values for total calcium were within the range reported as normal for man (4.38 to 5.52 mEq/l)[9] and were not significantly different from each other. Ionized calcium values, determined in whole blood, also were in the normal range (2.02 to 2.52 mEq/l), with the hypocapnic value slightly but significantly less than the normocapnic, as would be expected from the manner in which increased $pH$ increases serum protein binding of calcium, thereby decreasing ionized calcium.[9]

The HL perfused at a constant flow with heparinized whole blood responded the same as the intact HL to increase in $pH_a$ (fig. 1, table 2). The former data cover a wider range in $pH$, and there is now a significant negative linear correlation ($r = -0.66$) between $pH_a$ and HL $\dot{V}_{O_2}$ (fig. 1). The interpolated $\dot{V}_{O_2}$ value (mean ± SE) at $pH_a$ 7.40 was 2.46 ± 0.24 ml/min/kg HL, which was nearly identical to that found in the intact, self-perfused-HL studies. As in the intact studies, there was a tendency towards a lower $Pv_{O_2}$ during hypocapnia. Total calcium, calcium ions, and decreases in calcium ion with increase in $pH_a$ were similar to those of the intact studies.

The response of the HL perfused at a constant flow with CPD blood perfusate to increase in $pH_a$ was entirely opposite to that of either the intact, self-perfused HL or the HL perfused with heparinized blood (fig. 1, table 3). HL $\dot{V}_{O_2}$ increased as $pH_a$ was increased, and there was a significant positive correlation ($r = 0.89$) between $pH_a$ and HL $\dot{V}_{O_2}$ (fig. 1).

TABLE 2. Perfused Hind-limb (HL) Responses to Change in $pH_a$ of Heparinized Blood Perfusate

| | Hypercapnia | | Hypocapnia | |
|---|---|---|---|---|
| | Mean | SE | Mean | SE |
| $pH_a$ | 7.26 | 0.02 | 7.63* | 0.02 |
| $Pa_{CO_2}$, torr | 59 | 3 | 19* | 1 |
| $\dot{V}_{O_2}$ (HL), ml/min/kg HL | 2.61 | 0.09 | 2.21* | 0.08 |
| $\dot{Q}$ (HL), ml/min/kg HL | 107 | 5 | 107 | 5 |
| Arterial pressure (mean), torr | 125 | 6 | 127 | 8 |
| $Pa_{O_2}$, torr | 177 | 5 | 174 | 5 |
| $Pv_{O_2}$ (HL), torr | 71 | 4 | 53* | 6 |
| Plasma Ca (total), mEq/l | 4.22 | 0.07 | 4.22 | 0.07 |
| Whole blood Ca (ionized) mEq/l | 2.20 | 0.02 | 2.06* | 0.02 |

* Significantly different ($P < 0.05$) from hypercapnic value by $t$ test for paired data (eight dogs).

TABLE 3. Perfused Hind-lamb (HL) Responses to Change in $pH_a$ of CPD Blood Perfusate

| | Hypercapnia | | Hypocapnia | |
|---|---|---|---|---|
| | Mean | SE | Mean | SE |
| $pH_a$ | 7.20 | 0.01 | 7.67* | 0.04 |
| $Pa_{CO_2}$, torr | 56 | 2 | 14* | 1 |
| $\dot{V}_{O_2}$ (HL), ml/min/kg HL | 4.22 | 1.18 | 7.90* | 0.51 |
| $\dot{Q}$ (HL), ml/min/kg HL | 106 | 5 | 106 | 5 |
| Arterial pressure (mean), torr | 78 | 3 | 78 | 2 |
| $Pa_{O_2}$, torr | 167 | 2 | 168 | 2 |
| $Pv_{O_2}$ (HL), torr | 63 | 4 | 34* | 3 |
| Plasma Ca (total), mEq/l | 4.39 | 0.10 | 4.41 | 0.09 |
| Whole blood Ca (ionized) mEq/l | 0.37 | 0.05 | 0.35* | 0.05 |

* Significantly different ($P < 0.05$) from hypercapnic value by $t$ test for paired data (eight dogs).

The interpolated $\dot{V}_{O_2}$ value (mean ± SE) at $pH_a$ 7.40 was 5.80 ± 1.08 ml/min/kg HL, which was more than double and significantly greater than values obtained in the other two studies. As expected, ionized blood calcium levels were markedly decreased by the CPD solution. There was no significant correlation between individual ionized blood calcium levels (range 0.12 to 0.53 mEq/l) and individual muscle $\dot{V}_{O_2}$ values at $pH_a$ 7.40 (range 4.72 to 7.70 ml/min/kg HL) or between ionized blood calcium levels and the increment in $\dot{V}_{O_2}$ with increase in $pH$. Occasionally, gross twitches and frequently fine muscle fasciculations were observed in the HL perfused with CPD blood. These were most common and greater in extent at an increased $pH_a$ and were always associated with a large increase in HL $\dot{V}_{O_2}$.

In order to gain some insight into the mechanism involved in the production of muscular twitch and increased HL $\dot{V}_{O_2}$ with a CPD perfusate, the following additional experiments were carried out. While these events were generally stable, they disappeared in two dogs when ionized blood calcium values spontaneously increased (fig. 2). The twitch disappeared initially, and HL $\dot{V}_{O_2}$ then decreased stepwise and leveled off at a value in the range observed in the intact and perfused-HL heparin studies (normal range). In these, as in the tabulated perfused-HL CPD studies, at levels of ionized blood calcium below 1.0 mEq/l, there was no correlation between actual ionized calcium levels and actual $\dot{V}_{O_2}$ values or increments with increase in $pH_a$. In two dogs, the entire phenomenon was eliminated and HL $\dot{V}_{O_2}$ was returned to normal by adding sufficient calcium chloride to the perfusate

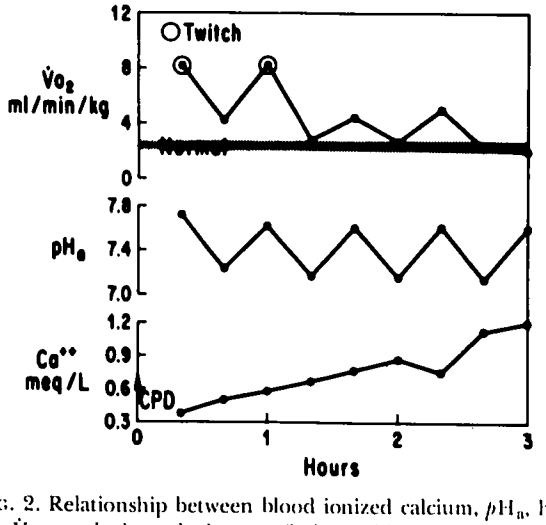


FIG. 2. Relationship between blood ionized calcium, $pH_a$, hindlimb $\dot{V}_{O_2}$, and time during perfusion with CPD whole-blood perfusate. Note the early elevation of $\dot{V}_{O_2}$ and presence of muscular twitching, with gradual return to normal $\dot{V}_{O_2}$ and disappearance of twitches as ionized calcium level spontaneously increased toward normal. Representative values from one dog.

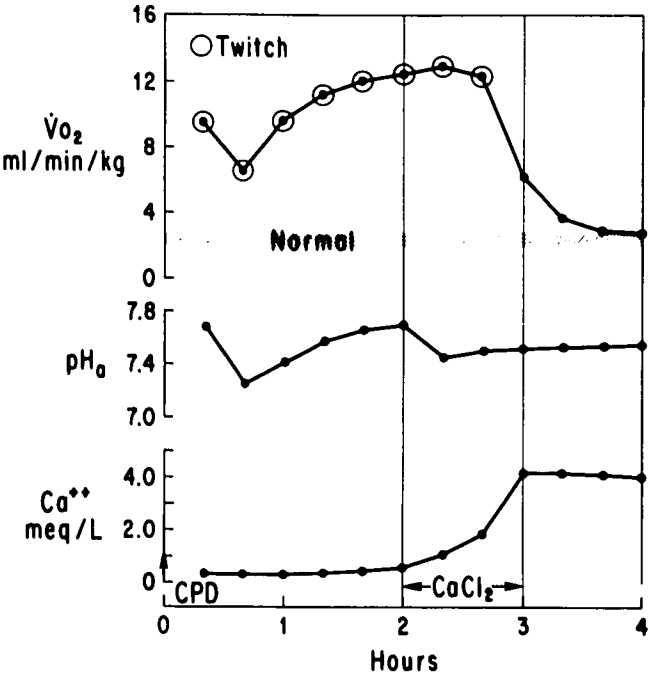


FIG. 3. Relationship between blood ionized calcium, $pH_a$, hindlimb $\dot{V}_{O_2}$, and time during perfusion with CPD perfusate. Note the persistence of an increased $\dot{V}_{O_2}$ and twitch at low ionized calcium levels, with return to normal as ionized calcium was increased by the addition of calcium chloride to the perfusate (0.2 mEq $Ca^{++}$/min for 60 minutes; total dose: 12 mEq $Ca^{++}$). Representative values from one dog.

to restore the ionized calcium values to normal or above (fig. 3). In these studies, ionized calcium, 0.2 mEq/l per minute, was added over a 60-minute period (total dose: 12 mEq $Ca^{++}$). This establishment of a dependence of the phenomenon on abnormally low levels of ionized calcium led to our exploring the response to dantrolene in this situation in two dogs.

Dantrolene is believed to stabilize intracellular ionized calcium levels in muscle and lessen the facility for sarcoplasmic reticulum release of ionized calcium and activation of the contractile process.[10] The amount of dantrolene (mol wt 400) added to the perfusate (22.5 mg dantrolene added to 1.5 liters) was designed to result in a perfusate concentration of $4 \times 10^{-5}$ M. This concentration of dantrolene was totally effective in preventing (fig. 4) and reversing (fig. 5) these events in the continuing presence of low levels of ionized calcium in the perfusate.

Since active muscular contraction was usually found to precede and accompany the increase in $\dot{V}_{O_2}$ induced by the lowering of ionized calcium concentration, spontaneous electromyographic (EMG) activity and the effects of *d*-tubucurarine and succinylcholine on the phenomenon also were examined. Spontaneous EMG activity increased greatly with the addition of CPD to the heparinized blood perfusate. The

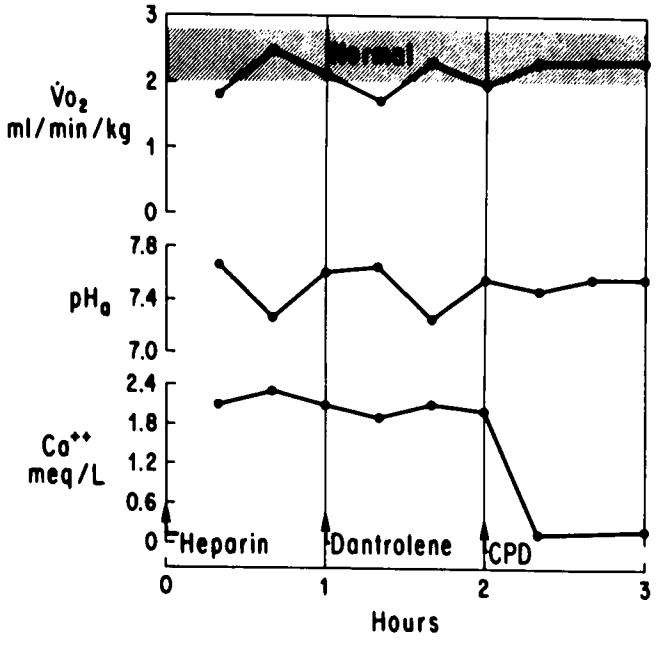


FIG. 4. Effectiveness of dantrolene in preventing an increase in $\dot{V}_{O_2}$ with lowering of the blood ionized calcium level by CPD. Representative values from one dog.

increased EMG activity was accompanied by twitching and an increase in $\dot{V}_{O_2}$. The addition of *d*-tubocurarine chloride to the perfusate in two dogs (10-mg bolus and 1 mg for each 10 minutes thereafter) eliminated the EMG activity and was accompanied by a return of $\dot{V}_{O_2}$ to the normal range (fig. 6). The addition of succinylcholine to the CPD perfusate as a 40-mg bolus and, thereafter, at a rate of 150 mg/h also was totally effective in preventing in one dog (fig. 7) and reversing in one dog (fig. 8) these events in the continuing presence of low levels of ionized calcium in the perfusate.

## Discussion

The results of these studies support the previously reported association of an increase in whole-body $\dot{V}_{O_2}$ with increasing $pH_a$. While our findings were similar in magnitude to those of Huckabee[2] and associates,[3] and Cain,[4] Khambatta and Sullivan[5] observed an increase of 25 per cent with a larger increase in $pH_a$, from 7.43 to 7.72 (8.6 per cent per 0.1 *p*H unit). However, our observation of a significant decrease in hind-limb $\dot{V}_{O_2}$ with increase in $pH_a$ in the absence of CPD does not support the hypothesis that respiratory alkalosis increases the oxygen consumption of all tissues. We agree with Tenney and Lamb[6] that: "little is known of this important chapter in the biochemistry of $CO_2$."

Considerable knowledge has recently accumulated regarding the important role of ionized calcium in the intracellular activity of nearly all cells and, in particular, in the events leading to the contraction of a muscle. It is now established that calcium ions are the link between the nerve-impulse-induced voltage change across the presynaptic motor nerve membrane and the release of acetylcholine at the myoneural junction.[11] Increase in intracellular calcium ions, resulting from either an influx accompanying depolarization of the membrane or a direct intracellular addition, is directly related to the amount of acetylcholine released at the myoneural junction. Excitation–contraction coupling is also now believed to be directly linked to intracellularly released calcium ions. The current view is as follows. The released acetylcholine combines with receptors at the myoneural junction, which gives rise to an action potential that spreads not only along the surface of the muscle fiber membrane but also into the interior of the fiber along the transverse tubules and then, by some as-yet-undetermined mechanism, to the terminal cisternae of the sarcoplasmic reticulum.[12] Depolarization of the latter results in a rapid release of calcium ions, with an increase of sarcoplasmic calcium from $10^{-7}$ M or less, as is required for relaxation, to $10^{-6}$ M, which will result in a contraction. The contraction results from the binding of calcium ions to the thin filaments, thereby allowing formation of cross-bridges between actin and myosin. This activating effect of calcium is mediated by the proteins tropomyosin and troponin, which are part of the thin filaments. Cross-bridge attachment is inhibited by these proteins in the resting state and can be overcome only by binding of calcium ions to troponin. Relaxation is achieved by the sarcoplasmic reticulum, which sequesters calcium ions from the sarcoplasm

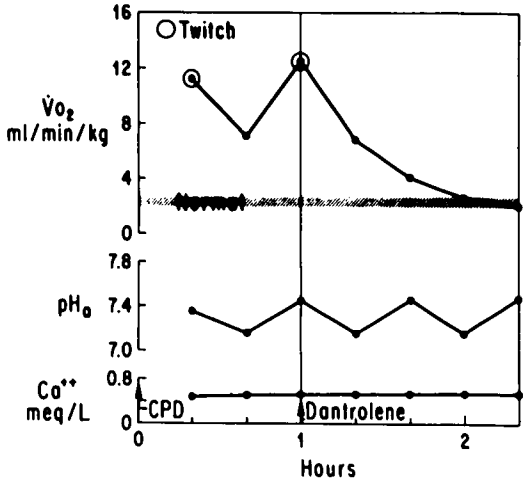


FIG. 5. Effectiveness of dantrolene in eliminating the muscular twitch and increased $\dot{V}_{O_2}$ occasioned by lowering of blood ionized calcium level by CPD. Representative values from one dog.

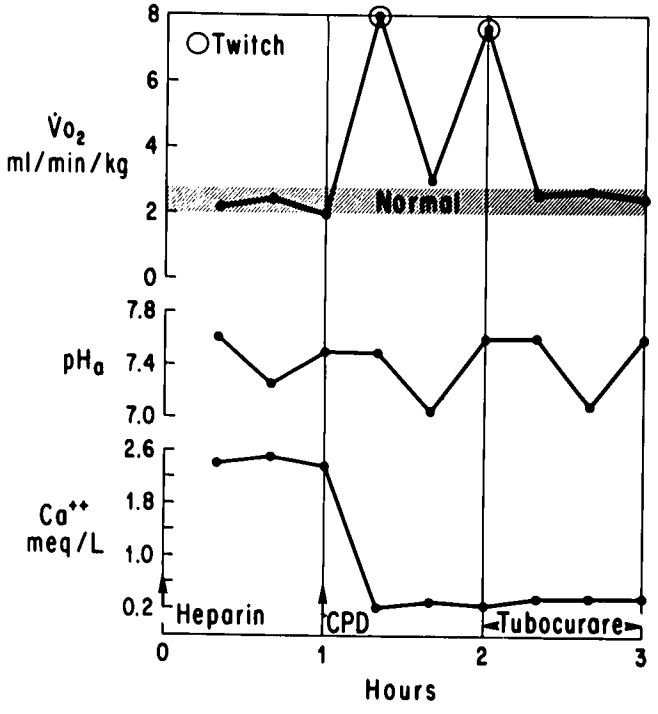


FIG. 6. Effectiveness of *d*-tubocurarine in eliminating the muscular twitch and increased $\dot{V}_{O_2}$ occasioned by lowering of blood ionized calcium by CPD. Representative values from one dog.

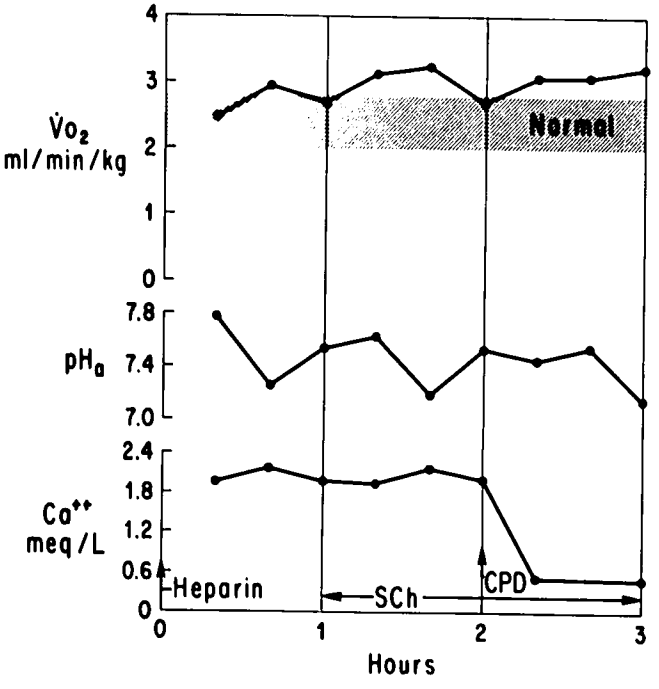


FIG. 7. Effectiveness of succinylcholine in preventing an increase in $\dot{V}_{O_2}$ with lowering of blood ionized calcium by CPD. Representative values from one dog.

until the concentration is decreased to $10^{-7}$ M. This bare outline of a very complex process provides a framework to interpret the results in this study.

In all of this, the stimulus for release of acetylcholine or a muscular contraction is an increase in intracellular level of calcium ion. In our studies, the major increases in electromyographic activity, muscular twitch, and muscle $\dot{V}_{O_2}$ followed an abrupt decrease in extracellular calcium ion level induced by citrate binding of calcium. Bianchi[13] cited similar results from several studies and offered the following paraphrased explanation:

> The removal of extracellular calcium results in a change in membrane structure (from a more crystalline to a more fluid state) that decreases the membrane binding affinity of sites for calcium allowing potassium and magnesium ions to displace calcium into the myoplasm and allows calcium to move across the surface membrane and thereby provokes the contraction.

Guyton[14] cited a similar clinical situation, that of hypocalcemic tetany, and offered the following explanation:

> An extremely important potentiator of excitability is low concentration of calcium ions in the extracellular fluids. Calcium ions normally decrease the permeability of the membrane to sodium. If sufficient calcium ions are not available, however, the permeability becomes increased, and, as a result, the membrane excitability greatly increases—sometimes so greatly that many spontaneous impulses result and cause muscular spasm.

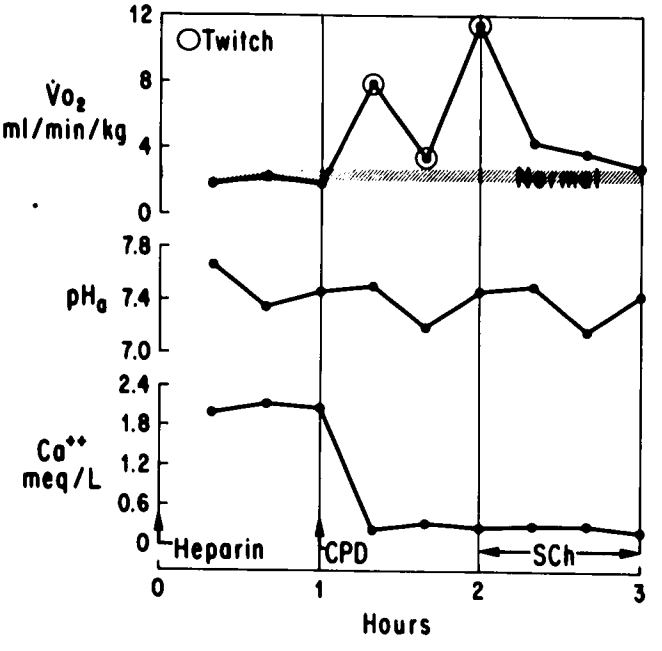


FIG. 8. Effectiveness of succinylcholine in eliminating the muscular twitch and increased $\dot{V}_{O_2}$ occasioned by lowering of blood ionized calcium by CPD. Representative values from one dog.

Although our studies with CPD perfusate did not result in an extracellular fluid free of calcium ions, the concentration of calcium ions was decreased nearly tenfold. Presumably, the disruption and calcium ion influx were greater with the higher *p*H because of the

even lower level of extracellular calcium. Because of the delicate and complicated calcium ion-stabilizing system that operates intracellularly, we conclude that an abrupt decrease in extracellular calcium level of this magnitude can disrupt the system and increase the level of intracellular calcium by the mechanism proposed by Bianchi for the calcium-free state, by the mechanism proposed by Guyton for the hypocalcemic state, or by a combination of these mechanisms.

Whereas *d*-tubocurarine, succinylcholine, and dantrolene were equally effective in eliminating the phenomenon, their sites and modes of action are so dissimilar that they require different explanations and suggest that the phenomenon was initiated at the presynaptic motor nerve ending. *d*-Tubocurarine is commonly regarded as having only postjunctional effects by occupying receptors at the motor end-plate of the sarcolemma and thereby decreasing the opportunity for released acetylcholine to depolarize the receptors in sufficient numbers to initiate an action potential leading to a contraction. Because we observed greatly increased electromyographic activity with the decrease in ionized calcium levels and total elimination of the activity and twitch with *d*-tubocurarine, we must conclude that the event was preceded by the release of actylcholine at the myoneural junction and occasioned by the increase in intracellular calcium level of the presynaptic motor nerve ending that was paradoxically caused by a decrease in extracellular calcium level, which in turn affected membrane permeability to sodium. This view is supported by our observation that succinylcholine also was effective in preventing and eliminating the phenomenon. Succinylcholine is believed initially to provoke an action potential by motor end-plate depolarization and to render the end-plate relatively insensitive to acetylcholine.

Dantrolene, however, is believed to act primarily by stabilizing sarcoplasmic calcium levels and lessening the facility for sarcoplasmic release of calcium and activation of the contractile process.[10] While it has recently been suggested[15] that dantrolene also stabilizes intraneuronal calcium levels in the presynaptic area, this conclusion was based upon the effect of dantrolene in decreasing the frequency, but not the amplitude, of spontaneous miniature end-plate potentials. We cannot apply the latter data directly in interpreting our findings, and therefore cannot be certain of the exact site of action of dantrolene in the present studies.

In summary, our studies confirm the increase in whole-body $\dot{V}_{O_2}$ with increase in $pH_a$ but are incompatible with this being the result of *p*H effects on intracellular events of all cells. In addition, Harken's findings, while valid for the experimental circumstances he described, are not believed relevant to situations with normal levels of ionized calcium.

## References


1. Otis AB: The work of breathing. Physiol Rev 34:449–458, 1954
2. Huckabee WE: Relationships of pyruvate and lactate during anaerobic metabolism. I. Effects of infusion of pyruvate or glucose and of hyperventilation. J Clin Invest 37:244–254, 1958
3. Nahas GG, Ligou JC, Mehlman B: Effects of *p*H changes on $O_2$ uptake and plasma catecholamine levels in the dog. Am J Physiol 198:60–66, 1960
4. Cain SM: Increased oxygen uptake with passive hyperventilation of dogs. J Appl Physiol 28:4–7, 1970
5. Khambatta HJ, Sullivan SF: Effects of respiratory alkalosis on oxygen consumption and oxygenation. ANESTHESIOLOGY 38:53–58, 1973
6. Tenney SM, Lamb TW: Physiological consequences of hypoventilation and hyperventilation, Handbook of Physiology. Section 3: Respiration. Edited by Fenn WO, Rahn H. Washington, D. C., American Physiological Society, 1965, volume 2, pp 979–1010
7. Harken AH: Hydrogen ion concentration and oxygen uptake in an isolated canine hindlimb. J Appl Physiol 40:1–5, 1976
8. The United States Pharmacopeia: Nineteenth Revision: Anticoagulant Citrate Phosphate Dextrose Solution. Rockville, Maryland, The United States Pharmacopeial Convention, Inc., 1974, pp 34–35
9. Moore EW: Ionized calcium in normal serum, ultrafiltrates, and whole blood determined by ion-exchange electrodes. J Clin Invest 49:318–334, 1970
10. Van Winkle WB: Calcium release from skeletal muscle sarcoplasmic reticulum: Site of action of dantrolene sodium? Science 193:1130–1131, 1976
11. Rahamimoff R, Rahamimoff H, Binah O, et al: Control of neurotransmitter release by calcium ions and the role of mitochondria, Calcium Transport in Contraction and Secretion. Edited by Carafoli E, Clementi F, Drabikowski W, et al. Amsterdam, North-Holland Publishing Company, 1975, pp 253–260
12. Taylor SR, Godt RE: Calcium release and contraction in vertebrate skeletal muscle: Calcium in biological systems. Symp Soc Exp Biol 30:361–380, 1976
13. Bianchi CP: Drugs affecting excitation coupling mechanisms, Fundamentals of Cell Pharmacology. Edited by Dikstein S. Springfield, Illinois, Charles C Thomas, 1973, pp 454–468
14. Guyton AC: Textbook of Medical Physiology. Fifth edition. Philadelphia, W. B. Saunders Company, 1976, p 127
15. Statham HE, Duncan CJ: Dantrolene and the neuromuscular junction: Evidence for intracellular calcium stores. Eur J Pharmacol 39:143–152, 1976