AN ABSTRACT OF THE THESIS OF

ROGER DEAN MEYERHOFF for the degree of DOCTOR OF PHILOSOPHY

in FISHERIES presented on May 14, 1980

Title: EXERCISE AND ACID-BASE BALANCE IN RAINBOW TROUT

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Abstract approved: _____

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Acid-base balance in ammoniotelic fish can be accounted for by
the concentrations and net flux rates of four generalized independent
variables. The physicochemical principles of acid-base chemistry
determine the quantitative interaction of acids and bases in an
aqueous solution. In a given medium, acid-base balance is the
instantaneous net result of input and output rates of acids and
bases, as represented by independent variables. Control of acid-base
balance is determined by regulation of input and/or output rates of
at least some independent variables. For the vascular compartment,
the four independent variables of interest are strong ion difference,
total weak acid, total carbon dioxide and total ammonia.

Acid-base balance in the vascular compartment depends on the
input and output rates of all independent variables. Concentrations
of dependent variables, such as H^+ and HCO_3^-, are set by the concen-
trations of independent variables and the laws which govern conser-
vation of mass, electrical neutrality and equilibrium limitations in

dissociation reactions. For the vascular compartment of teleost
fish, input and/or output of independent variables may occur at the
gills, kidney, tissue and gut. Regulation of acid-base balance is
known to occur at the gills and kidney. To regulate acid-base balance
in the vascular compartment, teleost fish rely on their capacity to
control the total weak acid concentration and the strong ion concentra-
tion difference of their blood.

For rainbow trout, fluctuations in exercise levels have the po-
tential to alter the acid/base flux rates and acid-base balance of
the vascular compartment. In order to assess how acid/base load
elimination is amended by rainbow trout to accommodate an increase in
their level of sustained aerobic exercise, elimination of total carbon
dioxide, total ammonia and lactate at the gills was monitored and net
excretion of acid by the kidney was measured. Characteristics of
blood acid-base balance and acid/base load loss were compared.

Total carbon dioxide, total ammonia and net acid efflux rates
rose in Shasta strain rainbow trout that were switched from 20% to
45% of their maximum swimming velocity (U-crit), when compared to
those rates of fish maintained at 20% U-crit. The increased total
carbon dioxide loss rate followed an augmented oxygen consumption
rate and the respiratory coefficient did not change significantly.
The elevated oxygen consumption rate and total carbon dioxide efflux
rate were initially accomplished by a significant but transient
increase in the change of blood $[O_2]$ and total $[CO_2]$ at the gills.

This transient increase was followed by an increased cardiac output rate to maintain the augmented oxygen consumption rate and total carbon dioxide efflux rate. For fish switched from 20% to 45% U-crit (treatment fish), output of titratable acid, inorganic phosphate and net acid carried by the urine significantly increased over the 72 hr experimental period.

The slight respiratory and metabolic acidosis found for treatment fish was followed by a significant increase in blood $[HCO_3^-]$, characteristic of metabolic alkalosis. A rise in blood P_{CO_2} and a significant rise in blood lactate probably accounted for the initial acidosis. Increased loss of NH_4^+ and a decreased loss of Na^+ across the gill may have accounted for the elevated $[HCO_3^-]$ via a change in blood strong ion concentration difference. Re-establishment of normal acid-base status may have relied on increased net influx of Cl^- at the gill due to increased blood $[HCO_3^-]$. Renal acid excretion did not wholly account for any change found in blood acid-base balance.

Exercise and Acid-Base Balance in Rainbow Trout

by

Roger Dean Meyerhoff

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed May 14, 1980

Commencement June 1981

APPROVED:

Redacted for Privacy

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Date thesis is presented _____ May 14, 1980 _____

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ACKNOWLEDGEMENTS

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KEY TO SYMBOLS

[ ]	Concentration
Δ	Change of variable
a	arterial
A^-	Weak acid anion (generalized symbol for sum of all weak acid anions)
b	blood
C	Control fish
°C	Degrees Celsius
Ca^{++}	Calcium
Cl^-	Chloride
cm	Centimeter(s)
CO_2	Carbon dioxide
CO_{2diss}	Dissolved molecular CO_2
$CO_3^=$	Carbonate
EA	Net acid excreted in the urine (TA minus HCO_3^- plus NH_4^+)
F	Function
G	Gauge
G_{sk}	Gut as a sink of independent variables
G_{sr}	Gut as a source of independent variables
GL_{sk}	Gills as a sink of independent variables
GL_{sr}	Gills as a source of independent variables
gm	Gram(s)

gm%	Weight in grams per 100 milliliters of fluid
H^+	Hydrogen ion
HA	Undissociated form of weak acid (generalized symbol for sum of undissociated forms of weak acids)
Hb	Hemoglobin
HCO_3^-	Bicarbonate
H_2CO_3	Carbonic Acid
$HPO_4^=$	Monohydrogen phosphate
$H_2PO_4^-$	Dihydrogen phosphate
hr(s)	Hour(s)
K^+	Potassium
K'_1	Carbonic acid dissociation constant
K_2	Bicarbonate dissociation constant
K_a	Total weak acid dissociation constant
Kg	Kilogram
K_{sk}	Kidney as a sink of independent variables
L	Liter
lac	Lactate
$\dot{M}_{tCO_2}$	Total CO_2 loss rate from the blood at the gills or out through the urine
Mg^{++}	Magnesium
min	Minute(s)
ml	Milliliter(s)
$\dot{M}_{O_2}$	Oxygen consumption rate
MS 222	Tricaine methanesulfonate
$\dot{M}_{(variable)}$	Output rate of variable at the gills or through the urine
n	Number

N	Normality
Na^+	Sodium
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NH_3	Ammonia
NH_4^+	Ammonium
nM	Nanomoles
OH^-	Hydroxyl ion
Pi	Inorganic phosphorus
Pco_2	Partial pressure of CO_2
PE	Polyethelene
pK'	Negative logorithm of a constant that combines the dissociation constant for carbonic acid (pK_1') and bicarbonate (pK_2)
pKa	Negative logorithm of the generalized weak acid dissociation constant
pH	Negative logorithm of the hydrogen ion concentration
RBCs	Red blood cells
Sco_2	Solubility constant of CO_2 in water
SID	Strong ion difference (difference between values of strong electrolyte cations and anions)
$SO_4^=$	Sulfate
tA	Total weak acid: Sum of HA and A^- (generalized symbol for sum of dissociated and undissociated forms of all weak acids)
T	Treatment fish
TA	Titratable acid in urine
tCO_2	Total CO_2: sum of CO_{2diss}, HCO_3^- and $CO_3^=$
T_{EA}	Total acid excreted in urine (TA plus NH_4^+)
tNH_3	Total NH_3 (sum of NH_4^+ and NH_3)

tO_2	Total oxygen (sum of free O_2 and HbO_2)
Torr	A measure of partial pressure (1 Torr = 1 mmHg)
T_{sk}	Tissue as a sink of independent variables
T_{sr}	Tissue as a source of independent variables
u	Urine
U-crit	Maximum (or critical) swimming speed
μl	Microliters
V	Volume
v	Mixed venous blood
$\dot{V}b$	Cardiac output rate
$\dot{V}u$	Urine flow rate

EXERCISE AND ACID-BASE BALANCE IN RAINBOW TROUT

CHAPTER I:

Acid-Base Chemistry, Acid-Base Balance and Control
of Acid-Base Balance in Ammoniotelic Fish

INTRODUCTION

Water breathing fish have very little ability to regulate dissolved molecular $[CO_2]$, or Pco_2, in their blood by ventilation (Janssen and Randall, 1975; Cameron and Randall, 1972; Cameron, 1979; Cameron, 1978; Randall and Cameron, 1973). It follows that these fish have little ability to regulate blood acid-base balance by actively stabilizing blood Pco_2, as do mammals. Instead, it has been suggested that blood $[HCO_3^-]$ and $[H^+]$ is adjusted by direct transfer of HCO_3^- and H^+ (Cameron and Randall, 1972; Randall et al., 1976; Janssen and Randall, 1975) at the gills by ion exchange mechanisms (Evans, 1975; Maetz and Garcia Romeu, 1964; Maetz, 1973; Kormanik and Evans, 1979). But net excretion or uptake of H^+ and HCO_3^- by ion exchange mechanisms at the gills do not generally result in quantitatively corresponding changes in plasma $[H^+]$ or $[HCO_3^-]$. As Stewart (1978) has recently reminded, H^+ and HCO_3^- are dependent variables, the concentrations of which are not regulated directly. At most, changes in plasma $[HCO_3^-]$ and $[H^+]$ result from the interactions that occur when the concentration of one or more independent variable is adjusted. This confusion occurs in the study of ammoniotelic fish when the distinction is lost between acid-base chemistry, acid-base balance, and physiological control of acid-base balance.

With examples from mammals, Stewart (1978) covered physicochemical principles and outlined independent and dependent variables of acid-base control . It is the purpose of this paper to extend the principles covered by Stewart (1978) to physiological values for ammoniotelic fish and to discriminate between acid-base chemistry, acid-base balance, and physiological control of acid-base balance.

DEFINITIONS

Acid-base chemistry

Acid-base chemistry determines the quantitative interactions of acids and bases in aqueous solutions by physicochemical principles.

Acid-base balance

In a given medium, acid-base balance is the instantaneous net result of input and output rates of acids and bases, as represented by independent variables. The concentrations of independent variables, in conjunction with physicochemical principles, determine net protein charge state (Reeves, 1977), acidity of the medium and other dependent variable concentrations (Stewart, 1978).

Control of acid-base balance

Control of acid-base balance rests in the control of input and/or output rates of at least some independent variables to achieve net rates. These net rates establish appropriate steady-state concentrations of independent variables.

Dependent variables

Dependent variables are internal to a system. Their concentrations are set by reaction of the chemical system to externally imposed concentrations of independent variables. Concentrations of dependent variables are determined by the physical properties of the system and by the instantaneous concentrations of the variables inde-

pendent of the system (Stewart, 1978).

Independent variables

The concentrations of independent variables determine the concentrations of the dependent variables. The concentration of an independent variable cannot be altered by any change in the concentration of another independent variable (Stewart, 1978). The concentrations of independent variables are determined by the net result of their input and output rates, which are set outside the medium of interest.

SELECTION OF THE MEDIUM AND INDEPENDENT VARIABLES OF INTEREST

Whole blood in the vascular compartment is the medium in which acid-base chemistry, acid-base balance and control of acid-base balance will be considered. Red blood cells will be assumed to be present, but detailed description of the chemical exchange that occurs between plasma and RBCs will not be attempted. Whole blood is viewed here as a generalized medium. The acid-base chemistry of this medium will be considered to be constantly at equilibrium for purposes of calculating concentrations of dependent variables such as HCO_3^-. The time constants for this assumption to be correct are not realistic, but the assumption reduces the complexity of the chemical description without materially affecting the explanation of the important principles. The acid-base balance of the medium will be assumed to be dynamic.

The generalized independent variables selected must be necessary and sufficient for the description of acid-base chemistry, acid-base balance and physiological control of acid-base balance. The minimum and most general independent variables necessary to determine the acid-base chemistry of plasma are total weak acids (tA) and strong ion difference (SID). These two components are necessary but not sufficient for a description of acid-base balance. Since carbon dioxide is produced and eliminated in large amounts, it is convenient to separate carbon dioxide from the pool of total weak acids. For mammals, Pco_2 is considered to be the independent variable of acid-

base control (Stewart, 1978; Reeves, 1977) that is accounted for
when carbon dioxide is considered separately. Arterial Pco_2 is a
measure of the net result of metabolic total CO_2 input rates and
pulmonary total CO_2 output rates in mammals (Hills, 1973). As has
already been indicated, Pco_2 is not controlled in the blood of fish.
Pco_2 is only an index of the concentration of the dissolved molecular
CO_2 ($[CO_2]_{diss}$) in solution. Pco_2 fails the test for an independent
variable. If blood $[H^+]$ is altered by instantaneously changing
[SID], then Pco_2 and $[CO_2]_{diss}$ change. The Pco_2 component also
fails the test for an independent variable when, over time, carbon
dioxide exchange with another solution is not possible or when that
exchange does not reach equilibrium. Pco_2 is unchanged by alteration
of another independent variable concentration only when the blood is
in continuous exchange equilibrium with another solution. A general
and continuous chemical equilibrium is certainly rare at the gills
of fish. Arterial blood Pco_2 in fish is higher than the Pco_2 of
respired water (Meyerhoff and Weber, unpublished observation; Janssen
and Randall, 1975; Cameron and Randall, 1972). Arterial Pco_2 is
probably set by the re-establishment of chemical equilibrium between
$[CO_2]_{diss}$ and $[HCO_3^-]$ (Cameron, 1978). The values of Pco_2 and $[HCO_3^-]$
in this re-established equilibrium are directly dependent on the
total concentration of carbon dioxide ($[tCO_2]$) in solution. In a
similar manner, the Pco_2 of venous blood is likely dependent on
$[tCO_2]$. Haswell and Randall (1978) suggest that in the closed

compartment of the veins, $[HCO_3^-]$ increases as Pco_2 falls in the re-establishment of equilibrium. For these reasons, $[tCO_2]$ will be considered to be the independent variable that helps determine $[CO_2]_{diss}$, $[HCO_3^-]$ and $[CO_3^=]$ in acid-base chemistry. Blood $[tCO_2]$ is easily measured (Cameron, 1971), whereas direct measurement of blood Pco_2 for cold water fish is fairly inaccurate (Wood, et al., 1977) because low temperatures slow the reaction time of CO_2 electrodes.

In ammoniotelic fish, ammonia contributes to the acid/base load elimination, even though plasma ammonia concentrations are small. The input and output rates of ammonia are probably fairly high compared to those of the remaining compounds of the total weak acid pool. It is convenient, therefore, to separate ammonia from the total weak acid pool for a description of acid-base balance. Arguments similar to those for $[tCO_2]$ establish $[tNH_3]$ as the independent variable of interest which helps account for $[NH_4^+]$ and $[NH_3]$.

The generalized independent variables that will be used here include tA, SID, tCO_2, and tNH_3. For ammoniotelic fish, the concentrations of these generalized independent variables are necessary to adequately determine the concentrations of chemically dependent variables and are appropriate to reflect the dynamics of acid-base balance and control. A complex description of the form in which acids and bases are loaded into the blood is avoided by using tA, tCO_2, and tNH_3 as independent variables. Acids and bases loaded into the blood in their ionic form are accounted for by the simultaneous quantitative changes that occur in the blood [SID]. The independent variables used

here can be measured by standard techniques (Cameron, 1971; Houston and

Smeda, 1979; Hills, 1973; Hillaby and Randall, 1979) except for [tA],

which can be estimated by titration. The value of [SID] is composed of

the abundant cations, such as Na^+, K^+, Ca^{++}, and Mg^{++} and anions, such

as Cl^-, $SO_4^=$, lactate and other strong acids. The value of [tA] is

composed of the many weak acids, but predominantly by imidazole groups

of proteins (Reeves, 1977).

ACID-BASE CHEMISTRY

The laws which determine conservation of mass, electrical neutrality and equilibrium limitations in dissociation reactions govern the quantitative interaction of acids and bases in aqueous solutions. In this section, the components and equations important for understanding acid-base chemistry will be described and pertinent values from the fisheries literature will be used to illustrate the physicochemical relationships between independent variables.

Understanding of acid-base chemistry is facilitated by two concepts: the existence of strong and weak electrolytes and the classification of variables in acid-base chemistry as dependent or independent (Stewart, 1978). In practice, compounds with dissociation constants below about pK 4 or 5 can be considered to be strong electrolytes and to dissociate completely. The net effect of strong electrolytes in acid-base chemistry is described through the law of electrical neutrality and can be represented as the difference between the total concentration of strong acid anions and strong base cations (Stewart, 1978).

The components of the chemical system to be considered here are strong ions, water, H^+, OH^-, HA, A^-, CO_{2diss}, HCO_3^-, $CO_3^=$, NH_3 and NH_4^+. Dissolved CO_2 and H_2CO_3 are considered together here as CO_{2diss}. The dependent variables are functions of the independent variables, which can be expressed as:

$$[H^+] = F_{H^+} ([SID], [tA], [tCO_2], [tNH_3])$$

$$[OH^-] = F_{OH^-} ([SID], [tA], [tCO_2], [tNH_3])$$

$$[A^-] = F_{A^-} ([SID], [tA], [tCO_2], [tNH_3])$$

$$[HA] = F_{HA} ([SID], [tA], [tCO_2], [tNH_3])$$

$$[HCO_3^-] = F_{HCO_3^-} ([SID], [tA], [tCO_2], [tNH_3])$$

$$[CO_3^=] = F_{CO_3^=} ([SID], [tA], [tCO_2], [tNH_3])$$

$$[CO_2]_{diss} = F_{CO_2} ([SID], [tA], [tCO_2], [tNH_3])$$

$$[NH_3] = F_{NH_3} ([SID], [tA], [tCO_2], [tNH_3])$$

$$[NH_4^+] = F_{NH_4^+} ([SID], [tA], [tCO_2], [tNH_3])$$

These functional equations demonstrate that any change in the concentration of a dependent variable only results from a concentration change in one or more independent variable, not from concentration changes in other dependent variables. Each independent variable can affect any dependent variable.

The $[OH^-]/[H^+]$ ratio provides an index of acidity and basicity for physiological solutions (Stewart, 1978). Both $[OH^-]$ and $[H^+]$ are chemically dependent variables that physiologically inter-relate all independent variables. A solution is considered to be neutral and not acidic or basic when $[H^+] = [OH^-]$. The normal arterial blood plasma value of $[OH^-]/[H^+]$ is near 30 for mammals (Stewart, 1978), but may range from about 15 to 45 in poikilotherms at various body temperatures (Rahn and Baumgardner, 1972; Howell, et al., 1970; Reeves, 1977). This fluctuation between different temperatures is in part due to physiological maintenance of the percent dissociation of imidazole groups of proteins (Reeves, 1977).

The laws which determine conservation of mass, electrical neutrality and equilibrium limitations in dissociation reactions allow nine simultaneous equations to be written that quantitatively inter-relate all these components:

Water dissociation equilibrium: $[H^+] \times [OH^-] = K'w$

Total weak acid dissociation equilibrium: $[H^+] \times [A^-] = Ka \times [HA]$

Total weak acid conservation of mass: $[HA] + [A^-] = [tA]$

Carbonic Acid dissociation equilibrium: $[H^+] \times [HCO_3^-] = K_1' \times [CO_2]_{diss}$

Bicarbonate dissociation equilibrium: $[H^+] \times [CO_3^=] = K_2 \times [HCO_3^-]$

Carbon dioxide conservation of mass: $[CO_2]_{diss} + [HCO_3^-] + [CO_3^=] = [tCO_2]$

Ammonia dissociation equilibrium: $[NH_4^+] \times [OH^-] = Kn \times [NH_3]$

Ammonia conservation of mass: $[NH_4^+] + [NH_3] = [tNH_3]$

Electrical neutrality: $[SID] + [NH_4^+] + [H^+] = [OH^-] + [A^-] + [HCO_3^-] + 2[CO_3^=]$

For exact equations, activities, which account for solution ionic strength, would replace concentrations. The rate constants for dissolved CO_2 and carbonic acid are combined as K_1'. The equations describing total weak acid rest on the assumption that this quantity can be adequately represented as a monoprotic acid in the physiological pH range. More noteworthy is the fact that all equilibrium constant values change with the temperature and ionic strength

of the solution. Given the ionic strength and temperature, equilibrium constant values and known concentrations for the independent variables can be combined with the nine equations to compute the concentrations of all nine dependent variables. When the nine equations are solved simultaneously, computer aided techniques are the only practical way to find dependent variable values.

However, we are not restricted to determining values of dependent variables the way it must be done in vivo. The $[OH^-]/[H^+]$ ratio is normally within a given range for physiological solutions. Knowing the temperature of the solution, the dissociation constant of water can be found in references. From this data, $[H^+]$ and $[OH^-]$ can be calculated. The nine equations, $[H^+]$ and $[OH^-]$, the values of the other equilibrium constants and the values of any three independent variables can be used to determine the values of the fourth independent variable and all the remaining dependent variables. This technique allows simultaneous solution of only two or three equations at a time. It also allows combinations of different concentrations of independent variables to be calculated with the $[OH^-]/[H^+]$ ratio in the physiological range. More important for fish, it only requires gross estimates of the values of three independent variables.

Values of $[tCO_2]$, $[tNH_3]$ and [SID] for blood of ammoniotelic fish are available in the literature. In fish not exposed to hypercapnic conditions, values of $[tCO_2]$ range between 4 mM/L and

16 mM/L at various temperatures (Meyerhoff and Weber, unpublished observation; Wood et al., 1977; Neville, 1979; Randall and Cameron, 1973; Cameron and Randall, 1972). An estimate of the anion deficit, a quantity similar to [SID], was determined for rainbow trout and ranged from a mean of about 20 mEq/L to 46 mEq/L for various thermal acclimation regimes (Houston and Smeda, 1979). Under various conditions, total ammonia content of whole blood ranges between 0.080 mM/L and 2.07 mM/L (Hillaby and Randall, 1979; Goldstein et al., 1964; Holmes and Donaldson, 1969). Plasma [tNH_3] ranges between about 20 µM/L and 220 µM/L (Meyerhoff and Weber, unpublished observation).

Estimate of Total Weak Acid

The magnitude of the effective amount of total weak acid in true plasma was estimated using a given set of values for [tCO_2], plasma [tNH_3] plasma [SID], and [OH^-]/[H^+] and with the nine equations and appropriate dissociation constants. In the summer at about 15°C, [tCO_2], plasma [tNH_3], plasma [SID] and plasma [OH^-]/[H^+] in arterial blood of rainbow trout were assumed to be about 8 mM/L, 60 µM/L, 27 to 35 mEq/L and 30, respectively (Meyerhoff and Weber, unpublished observation; Houston and Smeda, 1979). At 15°C, literature values for the equilibrium constants (pK) of water, ammonia, carbonic acid and bicarbonate are 14.346, 4.78, 6.17 and 10.4 (CRC, 1979; Albers, 1970). With the assumption that the predominant weak acid buffering group is imidazole (Reeves, 1977; Randall and Cameron, 1973),

an estimate of the imidazole pKa of 7.14 (Reeves, 1976) was used for the pKa of the total weak acids at 15°C. The water dissociation equilibrium equation and the $[OH^-]/[H^+]$ ratio of 30 were used in conjunction with the pKw value to calculate $[OH^-]$ and $[H^+]$. Next simultaneous solution of the dissociation equilibrium equation and the ammonia conservation of mass equation was completed with known values for $[tNH_3]$, $[OH^-]$ and Kn to yield values for $[NH_3]$ and $[NH_4^+]$. Simultaneous solution of the bicarbonate and carbonic acid dissociation equilibrium equations and the carbon dioxide conservation of mass equation was accomplished with known values of $[tCO_2]$, $[H^+]$, K_1' and K_2 to yield values for $[CO_2]_{diss}$, $[HCO_3^-]$ and $[CO_3^=]$. The concentration of dissociated weak acid, $[A^-]$, was calculated from the electrical neutrality equation by inserting known values of $[SID]$(35mEq/L), $[H^+]$, $[NH_4^+]$, $[OH^-]$, $[HCO_3^-]$ and $[CO_3^=]$. Finally, the concentration of total weak acid, $[tA]$, was calculated using the total weak acid dissociation equation, the total weak acid conservation of mass equation, and known values for $[H^+]$, $[A^-]$ and Ka. For the given conditions, the effective concentration of total weak acid in the true plasma was about 32 mEq/L. When $[SID]$ was 27 mEq/L (Houston and Smeda, 1979), $[tA]$ was about 22 mEq/L. The calculations indicate that the magnitude of $[tA]$ is near the range of 22 mEq/L to 32 mEq/L.

An alternative method for estimating the effective concentration of true plasma total weak acid can be used with data generated by Börjeson (1977). Börjeson titrated whole blood from salmon with HCl. The titration was carried out at 25°C under nitrogen

gas. The salmon were held at 9-12°C. With the assumption that the overwhelming amount of buffering was being done by imidazole groups (Reeves, 1977), the pKa of this blood was estimated to be about 6.96 (Reeves, 1976). Between the pH values of 7.7 (pH_1) and 6.7 (pH_{11}), Börjeson found a blood buffering capacity (β) of about 16 mEq/L for salmon under control conditions. With this information and the equations describing total weak acid equilibrium and conservation of mass, total weak acid concentration can be found with the following equation:

$$[tA] = \frac{\beta}{\dfrac{10^{pH_1 - pKa}}{10^{pH_1 - pKa} + 1} - \dfrac{10^{pH_{11} - pKa}}{10^{pH_{11} - pKa} + 1}}$$

Here the effective concentration of total weak acid in true plasma was calculated as 33 mEq/L.

Combinations of Concentrations of Independent Variables

With the gross estimates of the concentrations of the four independent variables in true plasma, realistic combinations of concentrations of these variables can be calculated which produce given $[OH^-]/[H^+]$ ratios (Figure 1). The figure was constructed from values calculated for conditions of 15°C and plasma osmolality of about 300 mOsm/Kg. The pK values used for water, ammonia, weak acids, bicarbonate and carbonic acid were 14.346, 4.78, 7.14, 10.4 and about 6.2 (CRC, 1979; Reeves, 1976; Albers,

1970). The plasma $[tNH_3]$ for the figure is 60 μM/L. A change in the plasma $[tNH_3]$ in the physiological range has little effect on the position of the lines. The range of $[SID]$, $[tA]$ and $[tCO_2]$ shown in the figure probably encompasses physiologically realistic values for the true plasma of ammoniotelic fish. Although Pco_2 is not shown in the figure, the lowest value of Pco_2 associated with the lines in the figure is about 0.53 Torr and is found at $[OH^-]/[H^+]$ = 45 and $[tCO_2]$ = 2 mM/L. The highest Pco_2 associated with the figure is about 24 Torr and is found at $[OH^-]/[H^+]$ = 2 and $[tCO_2]$ = 18 mM/L. The $[OH^-]/[H^+]$ ratios of 2, 5, 15, 30 and 45 correspond to percent dissociation of imidazole groups of about 60%, 71%, 81%, 86% and 88%, to $[H^+]$ values of 48 nM/L, 30 nM/L, 17nM/L, 12 nM/L and 10 nM/L and to pH values of 7.32, 7.52, 7.76, 7.91 and 8.00.

The utility of the figure is not only in fixing values for the combinations of independent variables, as these values change with temperature, osmolality and better estimates of pK values. Figure 1 is also important because it demonstrates the linear relationships between independent variables and how they must change to account for given $[OH^-]/[H^+]$ values.

A number of phenomena are evident in Figure 1. Given a constant $[tA]$, the slopes of the lines decrease slightly as the $[OH^-]/H^+]$ ratios increase. That is, between any two $[OH^-]/[H^+]$ lines, a slightly larger Δ $[SID]$ is required at high $[tCO_2]$ than is required at low $[tCO_2]$. At any given $[tCO_2]$ and $[tA]$, equal changes

in [SID] produce unequal changes in [H^+] concentration. For example, at [tCO_2] = 10 mM/L and [tA] = 30 mEq/L, changes from [OH^-]/[H^+] = 2 to 5 and then from 5 to 15 result from nearly equal changes in [SID]. Yet the Δ [H^+] corresponding to [OH^-]/[H^+] moving from 5 to 15 is only about 75% of the Δ [H^+] corresponding to [OH^-]/[H^+] moving from 2 to 5. Given a constant [OH^-]/[H^+] value and [tCO_2], changes in [SID] are proportional to equal changes in [tA] and as [tA] increases, so does [SID]. These phenomena are evident in tritration curves and result, in part, from the combined buffering of tCO_2, tNH_3 and tA. The important conclusions from this figure are: 1) quantitative addition of a strong acid certainly does not result in an equivalent change in solution [H^+]; 2) quantitative additions of a strong acid do not even result in [H^+] changes that are a constant proportion of the additions; 3) solution [H^+] is a function of the concentrations of independent variables; 4) any change in solution [H^+] is a function of any change in independent variable concentrations; 5) a change in the concentration of one independent variable must be offset by a proportionate concentration change in another independent variable to maintain a given [H^+].

Figure 1. Combinations of concentrations of independent variables which produce $[OH^-]/[H^+]$ ratios of 2, 5, 15, 30 and 45 in true plasma at 15°C and 300 mOsm/Kg. The plasma $[tNH_3]$ used for this figure was 60 μM/L. The effects of increasing $[tA]$ on the form of the relationship between $[SID]$ and $[tCO_2]$ are demonstrated.
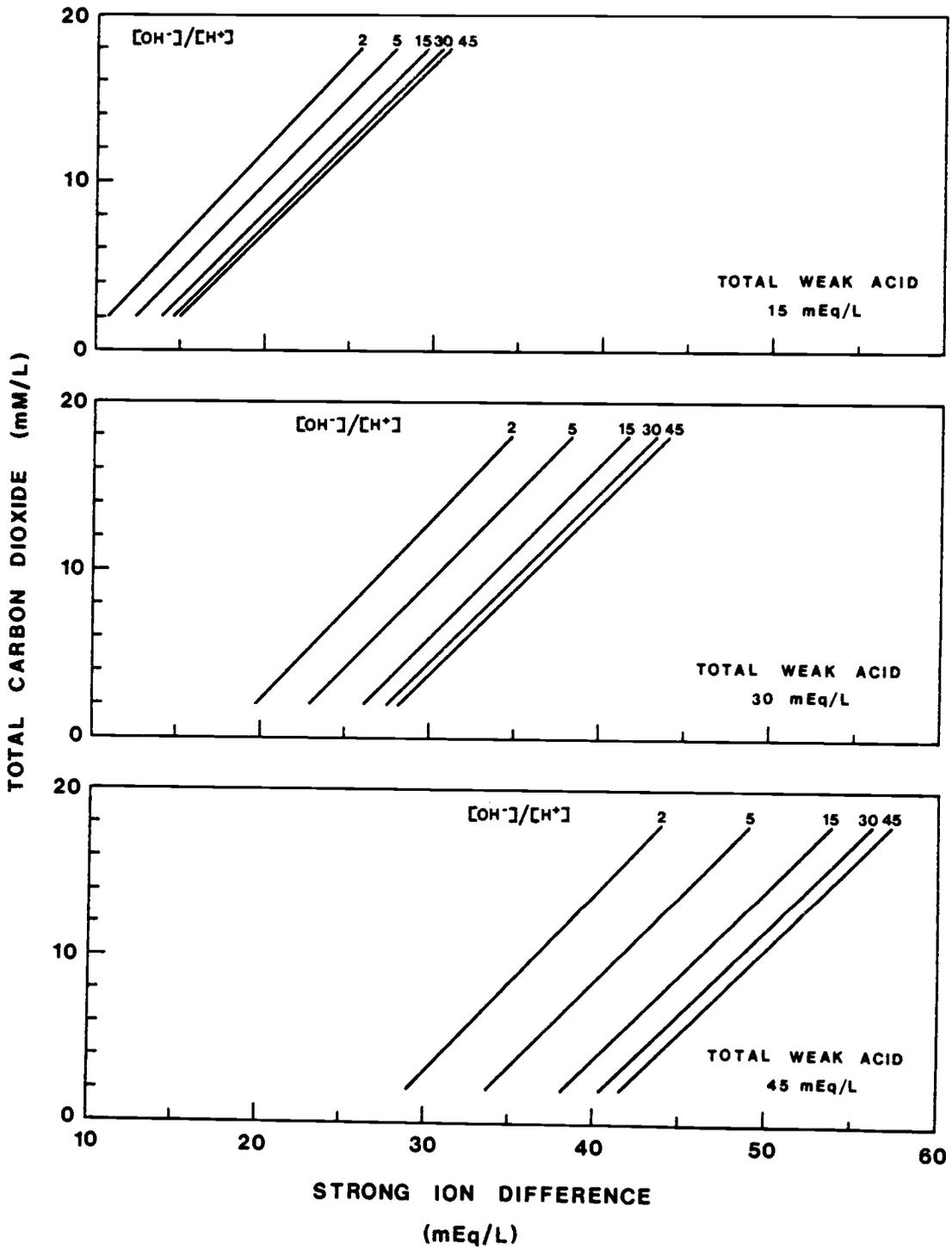
Figure 1

ACID-BASE BALANCE

While acid-base chemistry governs the quantitative behavior between independent and dependent variables, acid-base balance can be described as the net result of the uptake and elimination of acid and base loads in a given physiological compartment (Hills, 1973). These loads are reflected in uptake and elimination of tCO_2, tNH_3, tA and SID. The balance of load uptake and elimination determines $[tCO_2]$, $[tNH_3]$, $[tA]$ and $[SID]$, and consequently, the concentrations of the dependent variables.

Consideration of load uptake and elimination emphasizes the importance of separating $[tCO_2]$ and $[tNH_3]$ from $[tA]$ in the blood. CO_2 is an acid forming molecule (Hills, 1973). It is clear that the uptake of CO_2 must be closely matched by CO_2 elimination to maintain a physiologically normal $[H^+]$ in blood. Because CO_2 uptake and elimination must be so closely matched, they are rarely considered in descriptions of acid-base balance (Hills, 1973). Yet even under basal conditions, excretion of CO_2 in a 1 Kg trout at 15°C is about 3.6 gms/day (Meyerhoff and Weber, unpublished observation). Molecular NH_3 is a base forming compound (Hills, 1973). The loss of tNH_3 at the gills can be estimated from the data of Hillaby and Randall (1979) to be about 0.30 gm/day in a 1 Kg trout. Although this is a considerably smaller output than that for tCO_2, if uptake remained unchecked by elimination, tNH_3 accumulation could significantly alter whole blood $[H^+]$. Both

molecular CO_2 and NH_3 share a common property. They are small and highly membrane permeable. Uptake and elimination of weak acid compounds are probably not as large as they are for tNH_3 and tCO_2. The acids and bases that comprise tA do not easily pass membranes because of their size or charge. These points justify the separate consideration of tNH_3, tCO_2, and tA in the description of acid-base balance.

A simple representation of sources and sinks for independent variables in the vascular compartment is shown in Figure 2. Very little is known about the role of the gut in acid-base balance in fish (Cameron, 1978), although the impact of the gut in acid-base balance could be substantial due to absorption and excretion of ingested acid and base-forming elements (Hills, 1973). Also, little is known about the role of the tissue as a sink, yet it is clear that the tissue probably operates as one at least temporarily (Kobayashi and Wood, 1980). The tissue source of independent variables can be represented as some function of metabolism. The net result of the source and sink action of the gills can be represented as the difference between arterial input and venous output. The role of the kidney as a sink can be represented by the output of acids and bases in the urine. Input and output of independent variables in the vascular compartment are functions of the performance of the gills, gut, tissue and kidney as sources and sinks. This is expressed in the following functional equations.

$$[tCO_2] = F_{tCO_2} (GL_{sr}, GL_{sk}, G_{sr}, G_{sk}, T_{sr}, T_{sk}, K_{sk})$$

$$[SID] = F_{SID} (GL_{sr}, GL_{sk}, G_{sr}, G_{sk}, T_{sr}, T_{sk}, K_{sk})$$

$$[tA] = F_{tA} (GL_{sr}, GL_{sk}, G_{sr}, G_{sk}, T_{sr}, T_{sk}, K_{sk})$$

$$[tNH_3] = F_{tNH_3} (GL_{sr}, GL_{sk}, G_{sr}, G_{sk}, T_{sr}, T_{sk}, K_{sk})$$

Here the symbols represent the gill as a source (GL_{sr}) and sink (GL_{sk}), the gut as a source (G_{sr}) and sink (G_{sk}), the tissue as a source (T_{sr}) and sink (T_{sk}) and the kidney as a sink (K_{sk}).

Figure 2. A representation of sources and sinks for independent

variables in the vascular compartment. Qa and Qv indicate

arterial and venous blood flow rates that carry constitu-

ents to and from the gills. Qu indicates the urine flow

that eliminates acids and bases. MT represents the

metabolic rate times the tissue mass which helps determine

total input of acid and base-forming elements.
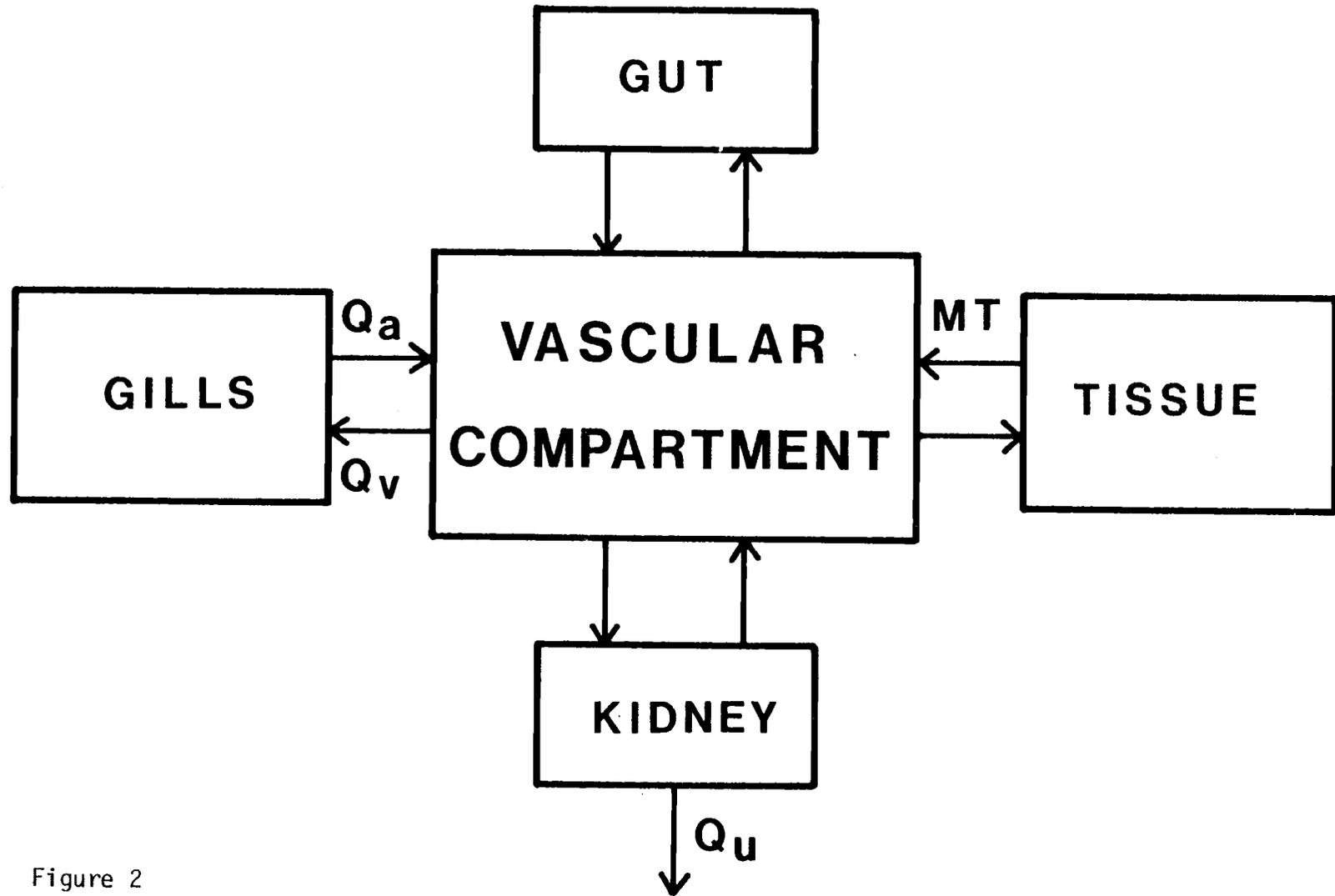
Figure 2

PHYSIOLOGICAL CONTROL OF ACID-BASE BALANCE

The input of an independent variable from its sources and simultaneous output into its sinks occur at various rates. When input and output rates are equal, the concentration of the independent variable in the vascular compartment is constant. When the input and output rates of the independent variable are unequal, that independent variable concentration and all dependent variable concentrations continuously change. As input and output rates of the single independent variable approach equality, the rate of concentration change slows and a new steady state concentration is eventually reached. This new independent variable concentration sets a new steady state for all dependent variable concentrations, including $[H^+]$. If the original $[H^+]$ is to be re-established, equivalent but opposing net rate changes must occur for the original independent variable or another independent variable. Physiological control of acid-base balance comes from regulation of input and output rates of independent variables by the various sources and sinks.

All independent variable input and output rates may not be regulated to achieve acid-base balance. Tissue metabolism and gut absorption of nutrients may not be regulated to produce given input rates of independent variables into the vascular compartment for acid-base balance. The vast proportion of the input and output rates of tCO_2 occurs in the form of CO_{2diss} and is the

result of concentration gradients (Cameron, 1978). It is clear that blood $[CO_2]_{diss}$ is not regulated by gill ventilation rate (Janssen and Randall, 1975). Since $[HCO_3^-]$ is in chemical equilibrium with $[CO_2]_{diss}$ in the blood, any direct regulation of blood $[HCO_3^-]$ by the net result of HCO_3^- input and output rates is precluded. The net result of input and output rates of HCO_3^- is only important when it is indicative of the net result of input and output rates of the strong acid anions or strong base cations. Blood $[tCO_2]$ is an indirect result of $[CO_2]_{diss}$ gradients at exchange membranes and chemical requirements set within the blood by concentrations of SID and tA. Since tNH_3 has a membrane permeable component, the same arguments can be made for its concentration in the blood. Uptake and elimination of tCO_2 and tNH_3 does not normally depend on ion exchange at the gills (Cameron, 1979; Kerstetter, et al., 1970; Hillaby and Randall, 1979; Maetz, 1971), although HCO_3^- and NH_4^+ are involved in ion exchange (Maetz, 1971) and may account for a large proportion of tCO_2 and tNH_3 output (Girard and Payan, 1980; Maetz, 1971).

Physiological control of acid-base balance in ammoniotelic fish then rests with regulation of the input and output rates of total weak acids and of strong acid anions and strong base cations. Cameron (1976) demonstrated that the net flux rate of sodium is adjusted in response to hypercapnic acidosis. Removal of lactate from the strong acid anion pool in the blood can be accomplished by metabolic transformation of lactate to glycogen (Hills, 1973) at many sites, including the gills (Driedzic and Kiceniuk, 1976). After injection of fixed acid, the

kidney responds to regulate acid-base balance with an increased net excretion rate of acid (Wood and Caldwell, 1978). Increased net acid excretion due to exercise is partially accounted for by an increased loss of the weak acid buffer phosphorus in the urine (Meyerhoff and Weber, unpublished observation). In the absence of the rapid physiological control of acid-base balance afforded through ventilatory control of tCO_2 loss rate (Hills, 1973; Davenport, 1974; Reeves, 1977), fish must rely on their capacity to regulate weak acid concentrations and the strong ion concentration difference.

CHAPTER II:

Sustained Aerobic Exercise and Acid-Base Balance in
Rainbow Trout

INTRODUCTION

Acid-base balance in fish is the result of dynamic processes. Redistribution and buffering of an acid or base load are physiological and chemical processes which may temporarily prevent major changes in extracellular free hydrogen ion concentration and in the $[OH^-]/[H^+]$ ratio (Randall, et al., 1976; Eddy, 1976; Wood et al., 1977; Wood and Caldwell, 1978; Kobayashi and Wood, 1980). However, rate changes in the appearance of acids and bases must eventually be countered by equivalent rate changes in elimination to maintain any acid-base balance.

In freshwater teleost fish, regulation of components of blood acid-base balance is known to occur at the gills and the kidney (Cameron, 1978; Evans, 1975; Wood and Caldwell, 1978). Quantitatively, the largest acid and base load reduction occurs at the gills with the loss of carbon dioxide and ammonia, which passively cross the gill membrane down concentration gradients in their unionized form (Cameron, 1978; Cameron, 1976; Cameron, 1979; Kerstetter et al., 1970; Maetz, 1973; de Vooys, 1969; Forster and Goldstein, 1969). Ion exchange mechanisms (Cl^-/HCO_3^- or OH^-; Na^+/H^+ or NH_4^+) in the gill epithelium (Maetz and Garcia Romeu, 1964; Maetz, 1971; Kerstetter and Kirschner, 1972; Kerstetter et al., 1970) have the potential to correct blood acid-base imbalance by changing the

strong ion concentration difference (Stewart, 1978). Correction of
acid-base imbalance following acute exogenous Pco_2 elevation is usually
attributed to net changes in HCO_3^- or H^+ output rates controlled by the
ion exchange mechanisms of the gill (Janssen and Randall, 1975;
Cameron, 1978; Cameron, 1976; Cameron and Randall, 1972). Although
changes in the loading rates of an acid or base with low membrane
permeability can potentially be corrected by ion exchange mechanisms in
the gill, it is not clear that the gill completely assumes that role
with a metabolic acid (Kerstetter and Mize, 1976). The kidney is known
to respond to metabolic disturbances which may affect acid-base balance
(Hickman and Trump, 1969). Fixed acid is excreted by the renal systems
of two Amazon freshwater fish (Cameron and Wood, 1978). A fixed acid
load injected into rainbow trout may be completely compensated for by
renal net acid excretion (Wood and Caldwell, 1978). The renal response
of freshwater rainbow trout to metabolic acidosis is comparable to that
of mammals (Kobayashi and Wood, 1980).

The temporal difference between responses to acid and base
loads may stem from the load type, and from the acute or chronic
appearance of the load. An ephemeral change in the loading rate
only requires temporary buffering and transient increases in load
excretion. Injection of acid or base loads with low membrane
permeability leads to depressed or increased plasma pH and $[HCO_3^-]$
which recover to normal levels within a few hours (Janssen and
Randall, 1975; Hillaby and Randall, 1979; Eddy, 1976). Acutely
anaerobic conditions, induced by exhaustive exercise, result in a

short period of hypercapnic acidosis and a temporally separated metabolic acidosis. Plasma pH returns to near normal within six hours (Wood et al., 1977). During chronic exposure to a new environment with high acid or Pco_2 levels, plasma acid-base imbalance in freshwater teleosts may take days to correct, if corrected at all (Janssen and Randall, 1975; Neville, 1979). Extended exposure to the new environments can result in changes which sustain new levels of hemoglobin and hematocrit (Neville, 1979; Börjeson, 1977), blood buffering capacity (Börjeson, 1977), and net uptake rates of ions across the gills (Cameron, 1976).

Alterations in the level of sustained aerobic exercise have been shown to precipitate changes which may affect how acid-base balance is achieved, even though short term plasma pH changes are small (Kiceniuk and Jones, 1977). Ammonia excretion rate may increase during sustained exercise (Kutty, 1972). White muscle levels of NH_4^+ are heightened when a resting fish is exercised at a sustainable velocity (Dreidzic and Hochachka, 1978). Changes in carbon dioxide excretion rate during increased aerobic exercise are not well understood, although it is clear that production of carbon dioxide should follow elevation in oxygen consumption (Jones and Randall, 1978). Augmented exercise alters the branchial sodium net flux rate (Wood and Randall, 1973a, b) which may affect the strong ion concentration difference. Elevated exercise results in a gain in urine output rate and in a transient adjustment of the net flux rate of body water (Wood and Randall, 1973c;

Hofmann and Butler, 1979). Finally, delivery of acid and base loads to excretory organs may be affected as cardiac output, blood pressure, and glomerular filtration rates change with changes in exercise levels (Kiceniuk and Jones, 1977; Stevens and Randall, 1967a, b; Hofmann and Butler, 1979).

The purpose of this study was to assess how acid/base load elimination is amended by rainbow trout to accommodate an increase in the level of sustained aerobic exercise. Elimination of carbon dioxide, ammonia and lactate at the gills was monitored and net excretion of acid by the kidney was measured. Characteristics of blood acid-base balance and acid/base load loss were compared.

MATERIALS AND METHODS

The two year old Shasta strain rainbow trout used in this study were taken from a laboratory population that shared common environmental conditions. A minimum of three weeks before the experimental procedure began, the fish were placed in a holding facility supplied with 14.5 - 15.5°C well water. All fish were continuously exposed to a slow water current and a natural photoperiod for summer and fall. The fish were deprived of their daily feeding of Abernathy trout diet at least one week before the experimental procedure began to minimize absorption of dietary acids and bases from the gut (Hills, 1973) and to ensure usable urine samples (Wood and Caldwell, 1978). The treatment group consisted of four trout, two of each sex, which were 41.6 ± 0.5 cm long and weighed 935 ± 47 gms ($\bar{x} \pm se$). The control group consisted of four trout, one male and three females, which were 41.1 ± 1.5 cm long and weighed 900 ± 43 gms.

Procedure

Individual fish were transferred from the holding facility to a 41.4 L Blazka respirometer (Blazka, et al., 1960; Hofmann, 1976) to begin the experimental procedure. Each fish was trained to swim in the respirometer by using an electric shocking device at the back of the swim tube. A black plastic curtain over the front of the swim tube provided cover for the fish. Fish not trained within two hours were rejected. One fish was used for each six to seven day experimental procedure.

Following a 24 hr acclimation period with the respirometer set to the minimum swimming speed of each fish, the respirometer was calibrated to the fish. The logarithm of any weight normalized oxygen consumption rate, $\dot{M}o_2$, for trout is linearly related to a percentage of their maximum swimming speed (Kiceniuk and Jones, 1977). Swimming speed in a respirometer is a function of water velocity and drag, which in turn are functions of the cross-sectional area of the fish and the respirometer (Webb, 1971). The rheostat controlling water movement in our respirometer had to be calibrated to percentages of the maximum swimming speed (U-crit) of each fish and corresponding weight normalized oxygen consumption rates. Starting at the minimum swimming speed of each fish, the water velocity was increased every 60 min until U-crit (Brett, 1964) was reached. The time at which U-crit occurred in the final 60 min interval was recorded when the fish failed to swim for 15 continuous seconds. Water velocity values used during the swim test were divided by the water velocity at U-crit to calculate amounts of % U-crit. Each % U-crit value was then paired with the corresponding oxygen consumption rate, which was measured at 40 min into each 60 min swimming speed interval. Since $\dot{M}o_2$ values for low swimming speeds did not significantly change with time or cannula drag, each fish was normalized to similar values of $\dot{M}o_2$ in the subsequent experiment by selecting an appropriate percentage of U-crit.

Twenty-four hours after the calibration swim test, the fish was removed from the respirometer and cannulas and a catheter were implanted so that blood and urine samples could be collected. Surgical anesthesia was induced in the fish by MS 222, dissolved in water at about pH = 7 (Wedemeyer, 1970). Anesthesia was maintained by alternate exposure to oxygenated water with and without 100 mg/L of neutralized MS 222. A Huber pointed 23 G needle, attached to a 60 cm length of PE 50 tubing, was used to cannulate the dorsal aorta (Smith and Bell, 1964). A 23 G needle, bent at a 60° angle and attached to a 60 cm length of PE tubing, was inserted into the ventral aorta (Kiceniuk and Jones, 1977). The cannulas were filled with a 0.9 % NaCl solution, which was adjusted to 290 mOsm/Kg and contained 200 international units/ml of sodium heparin. The slightly flared end of a 120 cm length of PE 90 tubing was used to catheterize the fish bladder (Hofmann and Butler, 1979). Urine was continuously collected by a fraction collector, set for 60 min of sampling, and was withdrawn anaerobically at 0.2 ml/hr into a 5 ml glass syringe. Each fish was allowed to recover from anesthesia in the respirometer for 24-48 hrs at a swimming speed of 20% U-crit.

The experiment and the sampling regime began after the recovery period. Because preliminary observations indicated that cannulated fish exercised between 50% and 60% U-crit would reach exhaustion before 72 hrs, treatment fish were forced from 20% U-crit to a

swimming speed of 45% U-crit. Control fish were allowed to maintain their swimming speed at 20% U-crit for the 72 hr sample period. The 0 hr sampling initiated the experiment and just preceded the immediate increase in the swimming speed of the treatment fish. Other blood samples were taken at 1, 3, 8, 24, 48, and 72 hrs after the experiment began. Blood samples (0.6 ml each), drawn simultaneously and anaerobically into 1 ml glass syringes from the dorsal and ventral aortas, were placed on ice until analyses could be performed (10 min maximum). Oxygen consumption rate was measured 20 min before each blood sample was drawn. An initial 0 hr urine sample, 12 hrs accumulation, was collected before the experiment began. Other urine samples were gathered at 8 hrs, 24 hrs, 48 hrs, and 72 hrs.

Stable water quality conditions were maintained by water flow that provided 95% replacement (Sprague, 1969) of water in the respirometer and its 160 L reservoir in 120 min. Water flow between the respirometer and its reservoir, where the water was vigorously aerated, allowed a 95% replacement of water in the respirometer in 20 min. Water samples were taken at 0, 8, 24, 48, and 72 hrs during each experiment to monitor water quality conditions. Values reflecting water quality conditions in the respirometer throughout all experiments were stable at (mean $\pm$ s.e.): temperature (15.0 $\pm$ 0.0°C); oxygen saturation (97.3 $\pm$ 0.6%); partial pressure of CO_2 (1.10 $\pm$ 0.05 Torr); pH (7.797 $\pm$ 0.012).

Analyses

True plasma pH, total oxygen content, total CO_2 content, hemoglobin, lactate, and plasma total ammonia were measured in each blood sample from the dorsal and ventral aortas. True plasma pH of each blood sample was determined using a Radiometer micro-electrode mounted on a Radiometer BMS3-Mk2 blood micro-system. The electrode was attached to a Radiometer digital output PHM 73 pH/blood gas monitor. The glass micro-electrode was maintained at 15.0°C and was calibrated daily with three standard buffers, pH 6.900, pH 7.445, and pH 8.14. The pH 7.445 buffer was used to check calibration between measurements of sample pH. Total oxygen content was calculated from the Po_2 rise that resulted after 50 µl of whole blood was injected into a 1.445 ml cuvet containing a solution of 0.6 gm% potassium ferricyanide and 0.3 gm% saponin (Tucker, 1967). The Radiometer Po_2 electrode used was fitted with a polypropylene membrane, connected to the digital blood gas monitor, and daily calibrated with gas and liquid at 21% and 0% oxygen content. The Po_2 electrode and cuvet were thermostated to 37°C. Total carbon dioxide content was calculated from the rise in Pco_2 that resulted after 50 µl of whole blood was injected into a 1.440 ml cuvet containing a solution of 0.01 N HCl saturated with octanol (Cameron, 1971). The Radiometer Pco_2 electrode used was fitted with a silicone film membrane and daily calibrated with 5% and 12% CO_2 gas and with 0, 3.6, 7.2, 14.4, and 28.8 mM/L

sodium bicarbonate standards. The scale for Pco_2 was expanded so that the 5% and 12% CO_2 gas read 100 Torr and 240 Torr respectively, on the blood gas monitor before calibration with sodium bicarbonate standards. The digital output was quite stable and repeatable on the expanded scale. The Pco_2 electrode and cuvet were thermostated to 37.0°C. Hemoglobin concentration in 20 µl blood aliquots was measured using the cyanmethemoglobin technique (Sigma Bulletin no. 525; Larson and Snieszko, 1961; Blaxhall and Daisley, 1973). Blood lactate was analyzed enzymatically (Sigma Bulletin no. 826-UV) after 100 µl of blood was diluted in 200 µl of cold 8% (v/v) $HClO_4$. Total ammonia concentrations for 100 µl plasma samples were recorded using an enzymatic reaction to avoid ammonia production by deamination reactions with alkaline reagents. This enzymatic reaction relies on glutamate dehydrogenase, ammonium, and reduced nicotinamide adenine dinucleotide to produce reductive amination of α-ketoglutarate (Sigma Bulletin no. 170-UV). Measurements on repeated subsamples from a common blood pool provided the following coefficients of variation: pH ($\pm$ 0.054%); total oxygen content ($\pm$ 2.1%); total carbon dioxide content ($\pm$ 1.9%); hemoglobin ($\pm$ 1.8%); lactate ($\pm$ 2.5%); ammonia ($\pm$ 14%).

Oxygen consumption rates were calculated by measuring oxygen content in water from the respirometer before and after a 5 min to 10 min period. During this procedure, oxygen content never dropped below 86% saturation. Water oxygen content was measured

with the Winkler method (APHA, 1976) using 0.0250 N phenylarsine oxide as the titrant.

Values of pH, total CO_2, lactate, total ammonia, inorganic phosphorus, and titratable acid were determined for samples of urine. The methods for measuring pH, total CO_2, lactate, and total ammonia were the same as those described for blood samples. Inorganic phosphorus in the urine was measured using the phospho-molybdenum blue complex (Sigma Bulletin no. 670). Titratable acid was determined by titrating urine with either 0.010 N or 0.017 N NaOH to the pH of the corresponding arterial blood sample. The value of titratable acid was adjusted for the calculated amount of hydrogen ions released in bicarbonate formation during the titration (Hills, 1973).

Symbols and Calculations

The symbols used throughout this paper include:

a	Arterial Blood
b	Blood
EA	Net Acid Excreted in Urine
Hb	Hemoglobin
lac	Lactate
$\dot{M}_{variable}$	Output rate of variable at the gills or through the urine
$\dot{M}o_2$	Oxygen consumption rate

Pi	Inorganic Phosphorus
Pco_2	Partial Pressure of CO_2
Sco_2	Solubility constant for CO_2 in water
TA	Titratable Acid in Urine
tCO_2	Total carbon dioxide
T_{EA}	Total Acid Excreted in Urine
tNH_3	Total Ammonia
tO_2	Total oxygen
u	Urine
U-crit	Maximum (or critical) swimming speed
v	Mixed Venous Blood
V	Volume
$\dot{V}b$	Cardiac Output
$\dot{V}u$	Urine Flow Rate
Δ	Change of variable
[ ]	Concentration

Several equations were used to calculate quantities of specific items. Changes in blood $[tO_2]$, $[tCO_2]$, $[HCO_3^-]$, $[tNH_3]$, and $[lac]$ at the gills were derived from $[a] - [v] = \Delta[b]$. Cardiac output was calculated using the Fick Principle: $\dot{M}o_2 \times \Delta[tO_2]^{-1} = \dot{V}b$. The average loss rates of metabolic species across the gills were computed as $\Delta[b] \times \dot{V}b = \dot{M}b$. The urine output rate $\dot{V}u$ was calculated as an average of hourly measurements of urine output over the time interval sampled. Output rate of metabolic species in the urine

was calculated as $[u] \times \dot{V}u = \dot{M}u$. Total excretion of acid in the urine was computed as $TA + NH_4^+ = T_{EA}$. Net excretion of acid in the urine was calculated as $(TA - HCO_3^-) + NH_4^+ = EA$ (Hills, 1973). All inorganic phosphorus in the urine was assumed to exist as $HPO_4^=$ and $H_2PO_4^-$ in equilibrium. With this assumption, urine pH, urine [Pi] and the appropriate pK (Segel, 1976; Hills, 1973), $[HPO_4^=]$ in the urine was calculated. The concentration of $HPO_4^=$ hypothetically filtered from the plasma and available for titration with H^+ in the urine was calculated using the values measured for true plasma pH and urine [Pi]. Urine acidification would account for the change in concentration of filtered $HPO_4^=$ to that of urine $HPO_4^=$. $[HCO_3^-]$ and Pco_2 were calculated using the Henderson-Hasselbach equation, $[tCO_2]$, pH, and values of pK' and Sco_2 (Albers, 1970). Although actual mean $\pm$ se values are given in the results section, values of change between 0 hr and X hr samples were used for statistical analysis unless otherwise indicated. Control and treatment mean values of change, for a given 0 hr to X hr time interval, were analyzed with the t-test (two-tailed). Significance was assigned at $P < 0.05$.

RESULTS

All fish were considered to be from a single population and one relationship between $\dot{M}o_2$ and % U-crit was established (Fig. 3). Separate relationships for treatment and control groups could not be distinguished statistically. The relationship between $\dot{M}o_2$ and % U-crit was fairly stable throughout the experiment (Fig. 4). Oxygen consumption rates of treatment fish were slightly more varied than $\dot{M}o_2$ of control fish.

Within eight hours after the increase in swimming speed, a significant but transient adjustment of oxygen occurred across the gills of treatment fish (Fig. 4). An immediate drop in venous blood $[to_2]$ was responsible for the significant change in the amount of oxygen loaded at the gills of treatment fish. Although the amount of oxygen added at the gills eventually declined below the initial amount for control and treatment fish, loading remained higher for treatment fish than for control fish throughout the experiment. As the experiment progressed, $[to_2]$ in the arterial blood of control and treatment fish changed from 3.60 ± 0.25 $\mu M/ml$ and 3.75 ± 0.50 $\mu M/ml$ at 0 hr to 2.50 ± 0.20 $\mu M/ml$ and 2.75 ± 0.10 $\mu M/ml$ at 72 hrs. The $[to_2]$ in the venous blood of control and treatment fish ranged from 2.00 ± 0.30 $\mu M/ml$ and 1.75 ± 0.15 $\mu M/ml$ at 0 hr to 1.05 ± 0.20 $\mu M/ml$ and 0.95 ± 0.50 $\mu M/ml$ at 72 hrs. The decline in $[to_2]$ followed a drop in $[Hb]$ from 7.6 ± 0.8 gm% to 5.4 ± 0.7 gm% for control fish and from 7.4 ± 0.8 gm% to 5.2 ± 0.7 gm% for treatment fish. The changes in $[Hb]$ occurred as blood samples were taken.

Cardiac output increased in both control and treatment fish (Fig. 5). Although changes in $\dot{V}b$ for control and treatment fish were not statistically different, mean values of $\dot{V}b$ for treatment fish climbed well above those for control fish. The full cardiac output response to the increased swimming speed was not reached until eight hours after the treatment was initiated. Increased $\dot{V}b$ in control fish was roughly concomittant with the drop in [Hb].

Load Elimination

Gills

An initial increase in $\Delta[tCO_2]$ at the gills of treatment fish (Fig. 4) combined with the gradual elevation of cardiac output to maintain an increased $\dot{M}_{tCO_2}$ (Fig. 5). Increased $[tCO_2]$ in the venous blood of treatment fish was responsible for the temporary adjustment of $\Delta[tCO_2]$. Since $[HCO_3^-]$ was about 98% of $[tCO_2]$ in the blood, $\Delta[HCO_3^-]$ at the gills was nearly the same as $\Delta[tCO_2]$. Mean $\dot{M}_{tCO_2}$ for treatment fish peaked at 3 hrs and declined through the 8 hr sample to a fairly stable loss rate at 24 hrs. The $\dot{M}_{tCO_2}$ for treatment fish was most variable at the 8 hr and 24 hr sample periods.

Ammonia was continuously lost from the plasma at the gills of both control and treatment fish. Plasma $\Delta[tNH_3]$ at the gills ranged between 40 ± 10 μM/L and 115 ± 40 μM/L for control fish and between 80 ± 20 μM/L and 150 ± 40 μM/L for treatment fish. When compared to changes (from 0 hr) in control values, a statistically significant increase in the plasma $\Delta[tNH_3]$ for treatment fish was found only at the 1 hr sample period. An increase in venous plasma $[tNH_3]$ of treatment fish partially

accounted for the significantly elevated $\Delta[tNH_3]$. Mean plasma $\Delta[tNH_3]$ values for treatment fish were slightly higher than those for control fish until the 24 hr sample period, when control and treatment mean values stabilized and became nearly the same. The initial increase in plasma $\Delta[tNH_3]$ and later increase in the cardiac output rate combined to keep mean values of plasma $\dot{M}_{tNH_3}$ at the gills of treatment fish slightly higher (about 1.7 $\mu M/Kg/min$ higher) than those for control fish. Mean values of plasma $\dot{M}_{tNH_3}$ ranged between 4.4 and 6.7 $\mu M/Kg/min$ for treatment fish and between 1.6 and 5.7 $\mu M/Kg/min$ for control fish. The largest difference between control and treatment $\dot{M}_{tNH_3}$ occurred at the one hour sample period. Although the change was not statistically different, $\dot{M}_{tNH_3}$ of treatment fish rose to 5.8 $\pm$ 0.9 $\mu M/Kg/min$ and the mean control value declined to 1.6 $\pm$ 0.8 $\mu M/Kg/$ min. Plasma tNH_3 lost at the gills over the entire 72 hr experiment was calculated to be 17.3 $\pm$ 2.8 mM/kg for control fish and 24.5 $\pm$ 4.4 mM/kg for the treatment fish.

Blood lactate concentration was also reduced at the gills of both control and treatment fish. For control fish, 30 $\pm$ 10 $\mu M/L$ and 150 $\pm$ 60 $\mu M/L$ were the high and the low mean values for lactate lost from the blood at the gills over the 72 hr experiment. For treatment fish, high and low mean values over the 72 hr experiment were 50 $\pm$ 10 $\mu M/L$ and 290 $\pm$ 260 $\mu M/L$. Control fish lost lactate over the experiment at mean rates between 0.8 $\pm$ 0.1 $\mu M/Kg/min$ and 8.5 $\pm$ 3.9 $\mu M/Kg/min$. Treatment fish lost lactate from the blood over the 72 hr experiment at mean rates between 3.0 $\pm$ 1.8 $\mu M/Kg/min$ and 14.9 $\pm$ 13.2 $\mu M/Kg/min$. No change in

mean treatment $\Delta[\text{lac}]$ or $\dot{M}_{\text{lac}}$ was statistically significant when compared to changes in mean values for control fish.

Changes in respiratory quotients (ratio of CO_2 excreted to oxygen consumed) and plasma ammonia quotients (ratio of ammonia excreted from the plasma to oxygen consumed) calculated for treatment fish were not statistically significant when compared to control values. Respiratory quotients for control fish declined from 1.05 ± 0.01 at 0 hr to 0.89 ± 0.04 at 72 hrs. In contrast, respiratory quotients for treatment fish increased from 0.84 ± 0.06 at 0 hr to 0.99 ± 0.02 at 3 hrs and subsequently declined to 0.87 ± 0.10 at 72 hrs. Ammonia quotients calculated here only operate as an index of the true ammonia quotients (Kutty, 1978) and are based on tNH_3 lost from the plasma at the gills. tNH_3 loss from RBCs and from deamination of blood amino acids may also contribute to the total $\dot{M}_{tNH_3}$ at the gills. Mean plasma ammonia quotients ranged between 0.042 ± 0.009 and 0.071 ± 0.028 for treatment fish and between 0.027 ± 0.012 and 0.091 ± 0.016 for control fish. Trends in mean values with time were not detected for either group of fish. The overall mean plasma ammonia quotient was 0.057 ± 0.008 for control fish and 0.058 ± 0.004 for treatment fish.

Fig. 3.   The relationship between % U-crit and $\dot{M}o_2$. The forty-
          nine determinations are for fish subsequently exercised
   at 20% U-crit (solid) and 45% U-crit (open).

Figure 3

$$LOG\ Y = 1.7 + 0.0069X$$
$$r^2 = 0.91$$
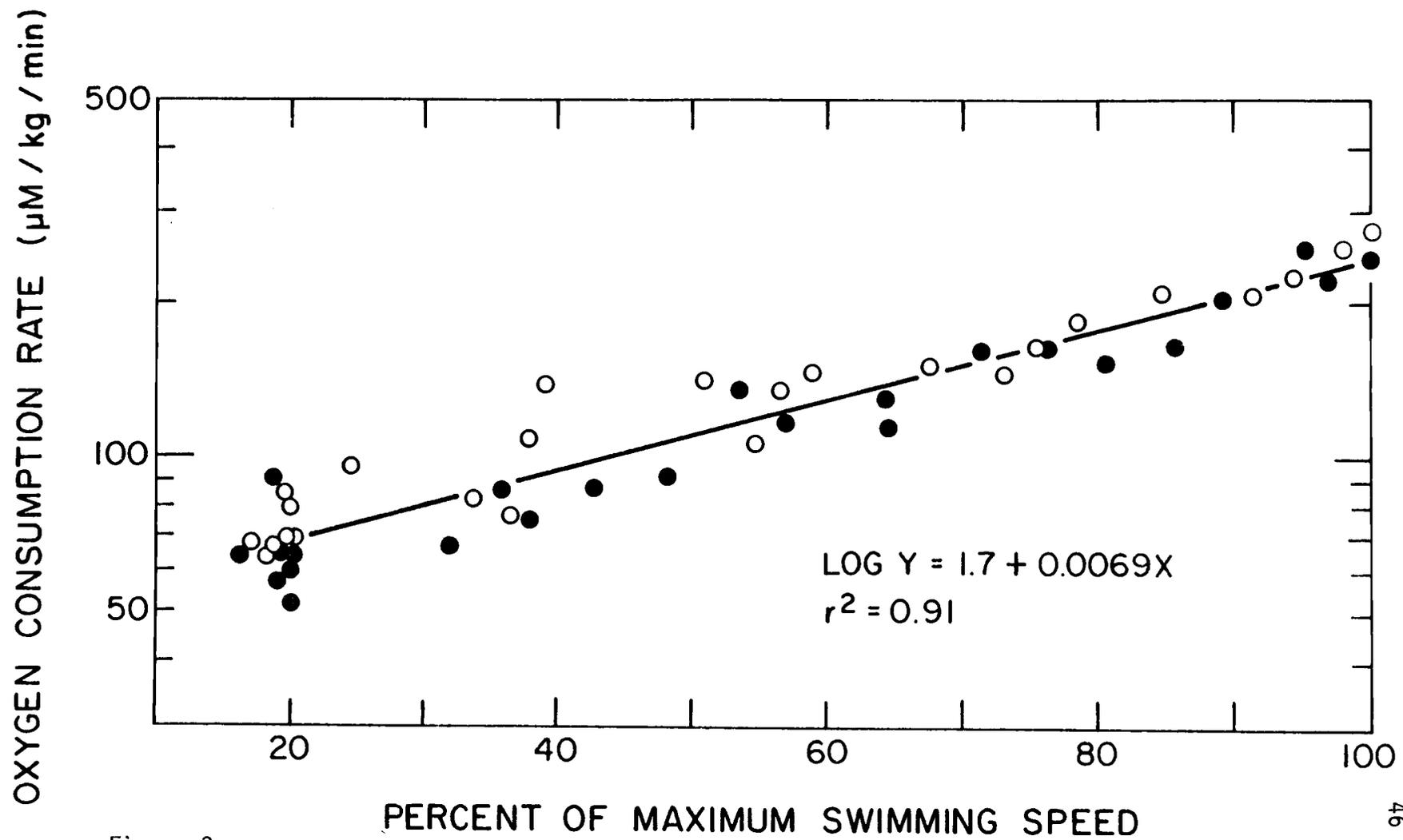
Fig. 4.   Oxygen consumption rates (top) and blood concentration changes oxygen (gain) and total CO_2 (loss) across the gills (bottom). For treatment fish, the 0 hr sample period just preceded the immediate increase in swimming speed from 20% to 45% U-crit. Mean $\pm$ se for fish exercised at 45% U-crit (open) and 20% U-crit (solid). Number of determinations at each point for treatment $(n)_T$ and control $(n)_C$ fish. * indicates mean changes in treatment and control values are significantly different ($P < 0.05$).
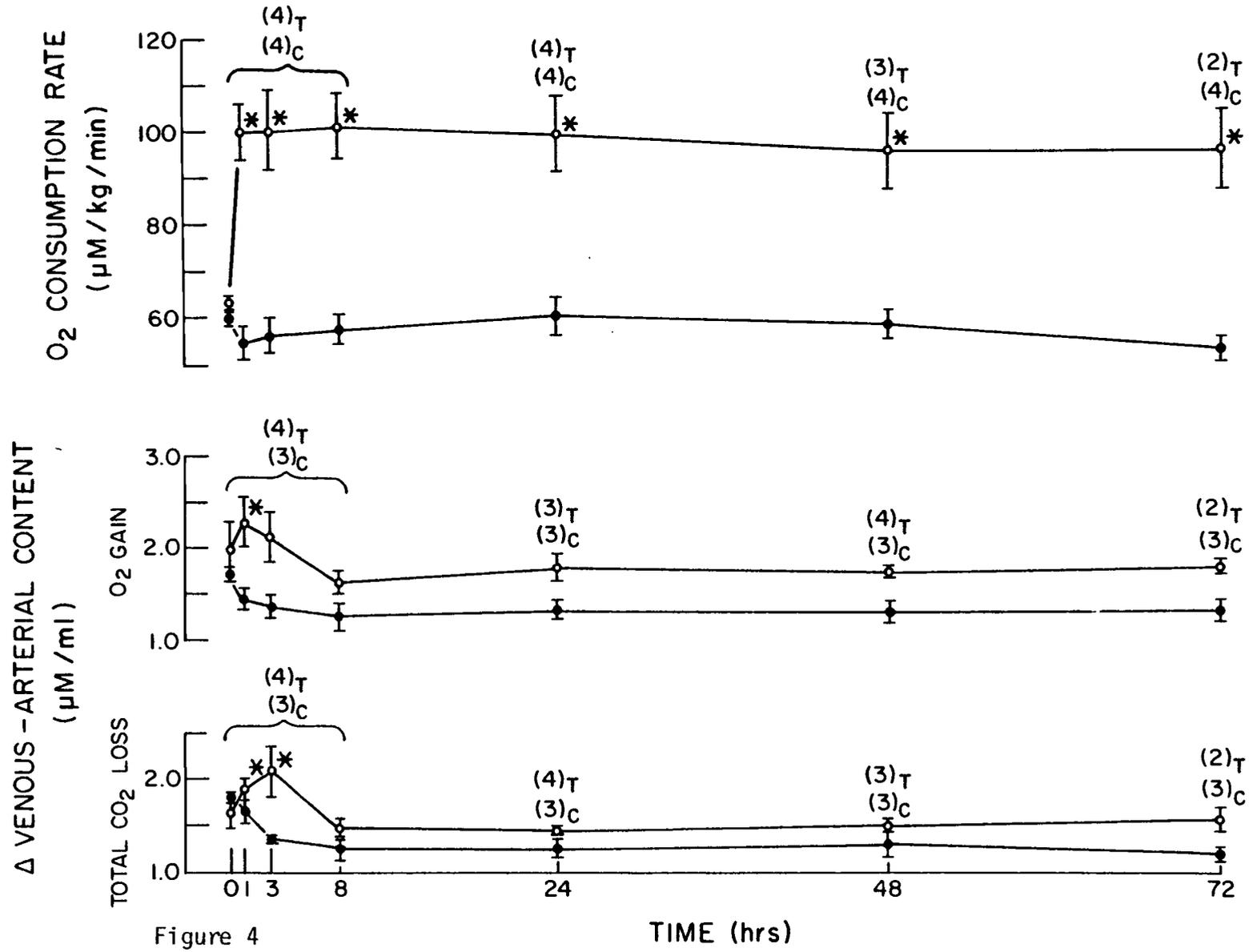
Figure 4

Fig. 5. Cardiac output rates calculated with the Fick Principle (top). Calculated output rates of total CO_2 (bottom). For treatment fish, the 0 hr sample period just preceded the immediate increase in swimming speed from 20% to 45% U-crit. Mean $\pm$ se for fish exercised at 45% U-crit (open) and 20% U-crit (solid). Number of determinations at each point for treatment $(n)_T$ and control $(n)_C$ fish. * indicates mean changes in treatment and control values are significantly different $(P < 0.05)$.

Figure 5

TIME (hrs)
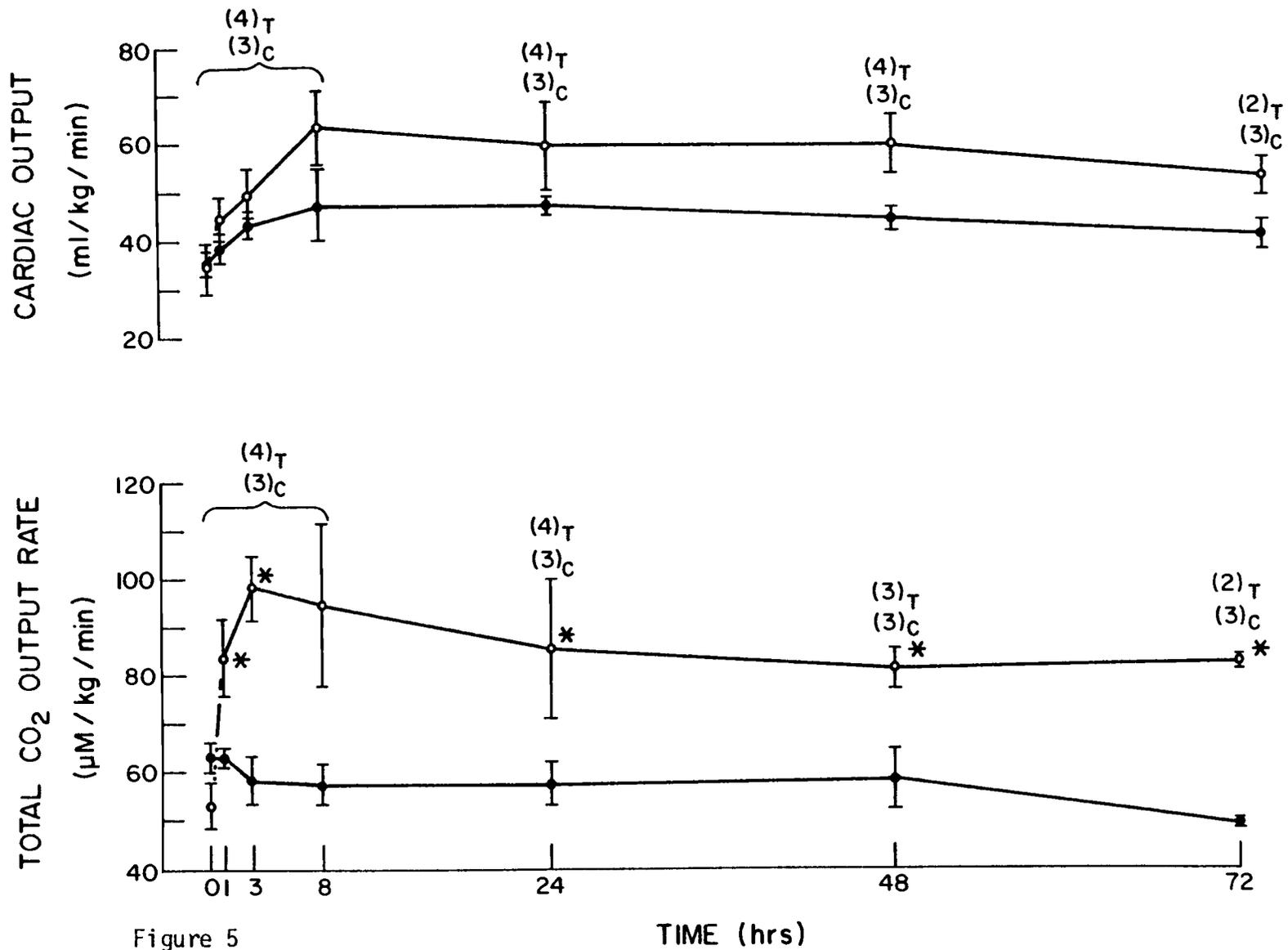
Kidney

Urine [EA] increased significantly for treatment fish in the first sample interval (8 hrs) and was maintained at an elevated level throughout the experiment (Fig. 6D). Urine $[T_{EA}]$ (Fig. 6C) followed the same pattern exhibited by [EA]. The initial increase in $[T_{EA}]$ of treatment fish was due to a significant rise in $[NH_4^+]$ (Fig. 6A). As $[NH_4^+]$ decreased to the control level throughout the remainder of the experiment, [TA] changed to a level much higher than that of control fish (Fig. 6B). The change in [TA] became significant by the last sample interval.

Urine [TA] was related to urine [Pi]. Mean values of [Pi] for control and treatment fish ranged from 1.5 $\mu M/ml$ and 1.5 $\mu M/ml$ before the experiment to 2.0 $\mu M/ml$ and 6.3 $\mu M/ml$ by the end of the experiment. When an estimate of the amount of H^+ titrated by $HPO_4^=$ was paired with corresponding values of [TA], a highly significant relationship was revealed (Fig. 7). The relationship showed that the buffering capacity of $HPO_4^=$ could account for nearly all of [TA], if $HPO_4^=$ was assumed to be filtered from the plasma.

$[H^+]$ and $[tCO_2]$ were measured in urine collected anaerobically and aerobically. Although anaerobically obtained urine contained an average of 120% higher $[tCO_2]$ and 70% higher $[H^+]$ than urine that was exposed to air, absolute differences in concentrations were small. The $[H^+]$ and $[tCO_2]$ data from urine collected anaerobically are reported here. Changes in $[tCO_2]$ for control and treatment

fish urine were not significantly different. Values of $[tCO_2]$ for treatment fish went from 2.7 ± 0.5 $\mu M/ml$ before the experiment to 3.7 ± 0.6 $\mu M/ml$ during the 8 hr to 24 hr sample period and back to 2.2 ± 0.2 $\mu M/ml$ by the end of the experiment. Values of $[tCO_2]$ for control fish went from 2.7 ± 0.4 $\mu M/ml$ at the beginning of the experiment to 3.3 ± 1.1 $\mu M/ml$ by the end of the experiment. Changes in the $[H^+]$ of the urine parallelled changes seen for $[TA]$ and, therefore, were larger for treatment fish than for control fish. Values of $[H^+]$ for control and treatment fish went from 113 ± 37 nM/L and 95 ± 26 nM/L at the beginning of the experiment to 104 ± 8 nM/L and 187 ± 36 nM/L in the 8 hr to 24 hr sample interval. Control and treatment $[H^+]$ values increased to 173 ± 38 nM/L and 303 ± 31 nM/L by the end of the experiment. Overall mean urine pH was 6.85 ± 0.05 for control fish and $6.66 \, {}^{+\ 0.07}_{-\ 0.06}$ for treatment fish. Urine $[H^+]$ was seven to twenty times larger than the $[H^+]$ measured in corresponding blood samples.

Fairly stable $[tCO_2]$ values and increasing $[H^+]$ values for treatment fish produced changing quantities of $[HCO_3^-]$ and Pco_2. Values of $[HCO_3^-]$ in the urine of control and treatment fish ranged from 2.3 ± 0.2 $\mu M/ml$ and 2.3 ± 0.4 $\mu M/ml$ before the experiment to 2.6 ± 1.0 $\mu M/ml$ and 1.4 ± 0.2 $\mu M/ml$ by the last sampling period. $[HCO_3^-]$ accounted for approximately 80% of control and treatment $[tCO_2]$ before the experiment. By the end of the experiment, $[HCO_3^-]$ was still about 80% of $[tCO_2]$ for control fish but dropped to 60% of

[tCO$_2$] for treatment fish. This reflected a rise in urine Pco$_2$
from 6.5 $\pm$ 2.5 Torr to 13.0 $\pm$ 0.9 Torr for treatment fish. Urine
Pco$_2$ for control fish only ranged from 5.8 $\pm$ 1.8 Torr to 8.3 $\pm$
1.5 Torr. Urine Pco$_2$ was larger than Pco$_2$ in the venous blood by
90% to 160% for control fish and by 100% to 280% for treatment
fish.

The [lac] in the urine followed the same pattern as [lac] in
the venous blood. The urine [lac] concentration was generally
less than 50% of the corresponding mean [lac] in whole blood.
Mean [lac] in the urine ranged between 0.10 μM/ml and 0.50 μM/ml.

The combined variability of urine species concentrations and
flow rates eliminated almost all significant changes in species
excretion rates. Mean $\dot{V}u$ values for control and treatment fish
were nearly the same throughout the experiment (Fig. 8A). Mean $\dot{V}u$
did not rise with the increased swimming speed. For all 72 hrs
of the experiment, the overall mean $\dot{M}_{lac}$ values for control and
treatment fish were nearly the same, 0.30 $\pm$ 0.25 μM/Kg/hr and
0.40 $\pm$ 0.30 μM/Kg/hr. The mean urine $\dot{M}_{NH_4^+}$ value for treatment
fish was about three times that for control fish within the first
sample interval (Fig. 8B). Subsequent mean $\dot{M}_{NH_4^+}$ values for control
and treatment fish were nearly the same. Mean $\dot{M}_{TA}$ values for
treatment fish eventually rose above those for control fish and
became significant in the 24 hr to 48 hr sample interval (Fig.
8C). $\dot{M}_{NH_4^+}$ and $\dot{M}_{TA}$ combined to elevate mean treatment values of

$\dot{M}_{TEA}$ from 50% to 100% above mean control values (Fig. 8D). Mean $\dot{M}_{tCO_2}$ for control and treatment fish were similar, decreasing from 8.0 $\pm$ 1.0 μM/Kg/hr and 7.9 $\pm$ 1.5 μM/Kg/hr before the experiment to 5.2 $\pm$ 0.4 μM/Kg/hr and 6.0 $\pm$ 2.0 μM/Kg/hr at the end of the experiment. Mean $\dot{M}_{H}+$ for control and treatment fish went from 0.32 $\pm$ 0.03 nM/Kg/hr and 0.28 $\pm$ 0.07 nM/Kg/hr to 0.38 $\pm$ 0.16 nM/Kg/hr and 0.75 $\pm$ 0.22 nM/Kg/hr. By the end of the experiment, stable $\dot{M}_{tCO_2}$ and increasing $\dot{M}_{H}+$ for treatment fish led to an 85% larger output rate of dissolved carbon dioxide in treatment urine than in urine of control fish. Values of mean $\dot{M}_{HCO_3^-}$ for control and treatment fish went from 6.7 $\pm$ 1.3 μM/Kg/hr and 6.7 $\pm$ 1.3 μM/Kg/hr in the initial urine sample to 3.9 $\pm$ 0.3 μM/Kg/hr and 4.0 $\pm$ 1.5 μM/Kg/hr in the final urine sample. The mean change in $\dot{M}_{EA}$ from 0 hr for treatment fish was 40% to 440% larger than the mean change in $\dot{M}_{EA}$ for control fish (Fig. 8E).

Even though changes in the loss rates of species through the urine of treatment fish were not normally significant, total output of TA, Pi, T_{EA}, and EA over the experimental period was significantly higher for treatment fish than for control fish (Table 1). Mean NH_4^+ output for treatment fish was about 30% more than that for control fish. Output of HCO_3^- was virtually the same for both groups of fish. Before the experiment, output of these urine species by control and treatment fish were statistically equivalent.

Fig. 6. Mean $\pm$ se of concentrations of ammonium (A), titratable acid (B), total acid excreted (C), and net acid excreted (D) in the urine of fish exercised at 20% U-crit (solid) and 45% U-crit (open). For treatment fish, the -12 hr to 0 hr sample period just preceded the immediate increase in swimming speed from 20% to 45% U-crit. The number of determinations for each time interval is indicated in parentheses. * indicates mean changes in treatment and control values are significantly different ($P < 0.05$).
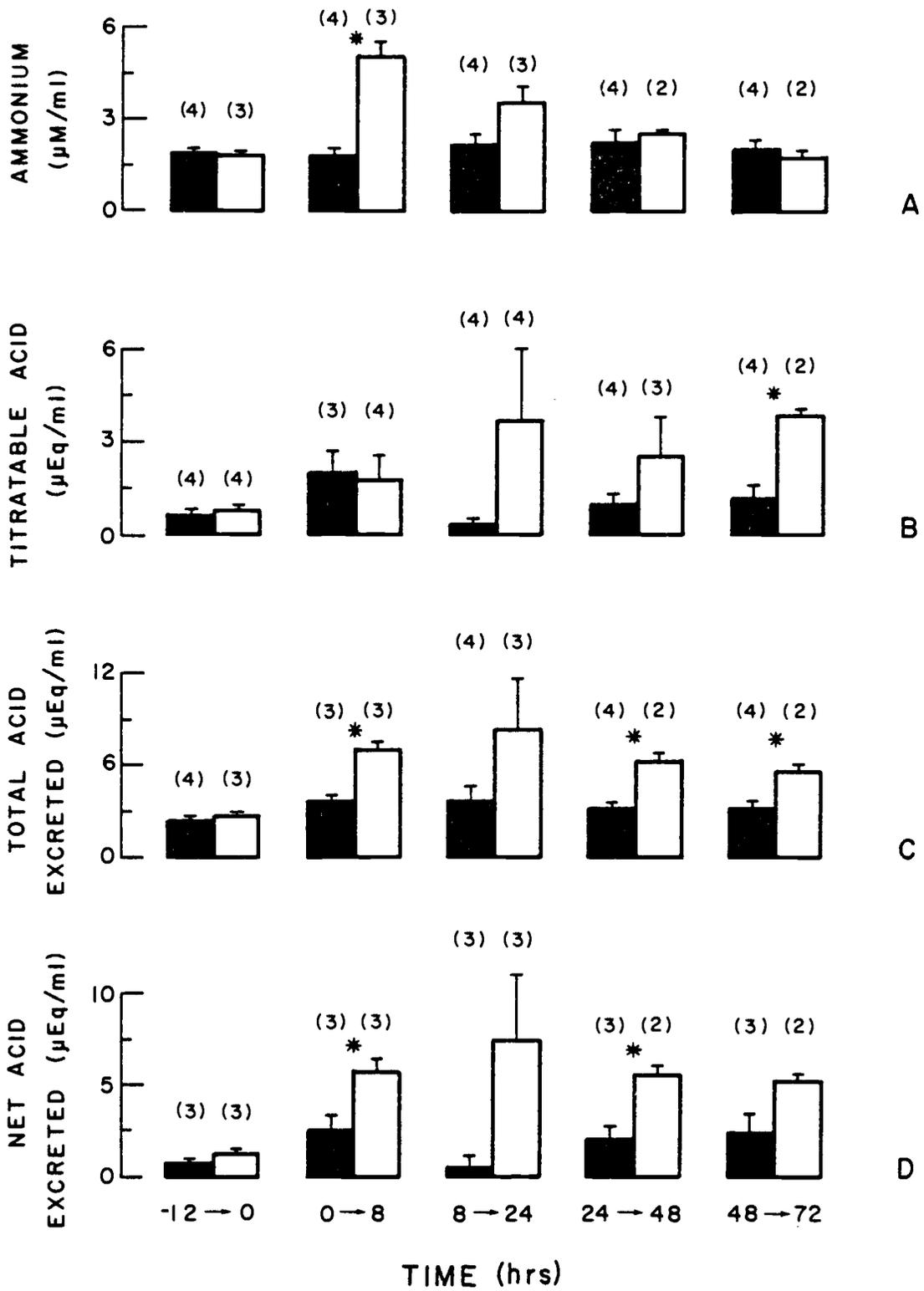
Figure 6

Fig. 7. The relationship between [tA] and [H$^+$] titrated by

HPO$_4^=$. The thirty points are from fish exercised at 20% U-crit

(solid) and 45% U-crit (open).
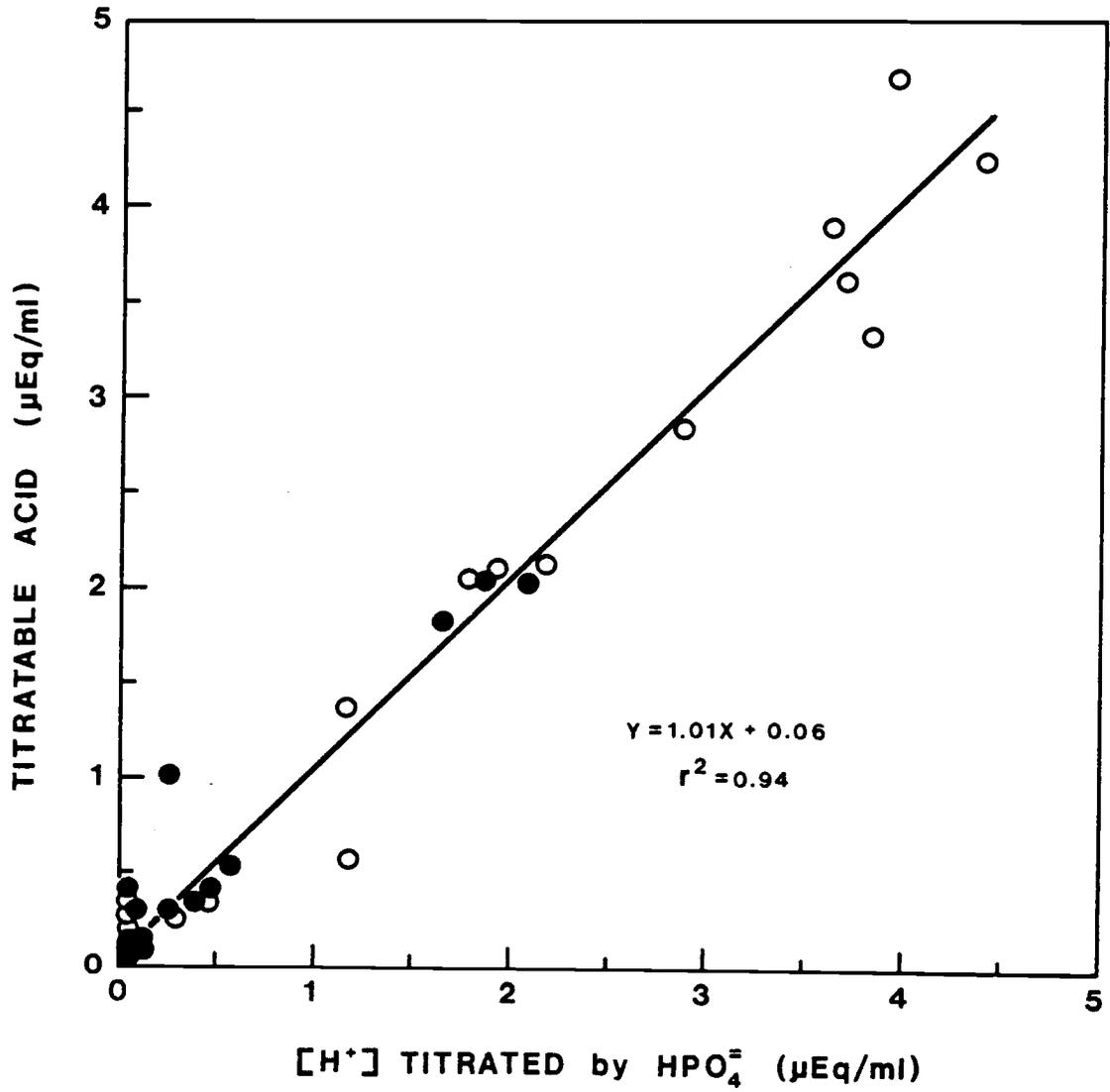
Figure 7

Fig. 8.   Mean $\pm$ se of urine flow (A), ammonium efflux (B), titrat-
able acid efflux (C), total acid efflux (D), and net acid
efflux (E) for fish exercised at 20% (solid) and 45% U-crit
(open). For treatment fish, the -12 hr to 0 hr sample period
just preceded the immediate increase in swimming speed from
20% to 45% U-crit. The number of determinations for each time
interval is indicated in parentheses. * indicates mean changes
in treatment and control values are significantly different (P<
0.05).
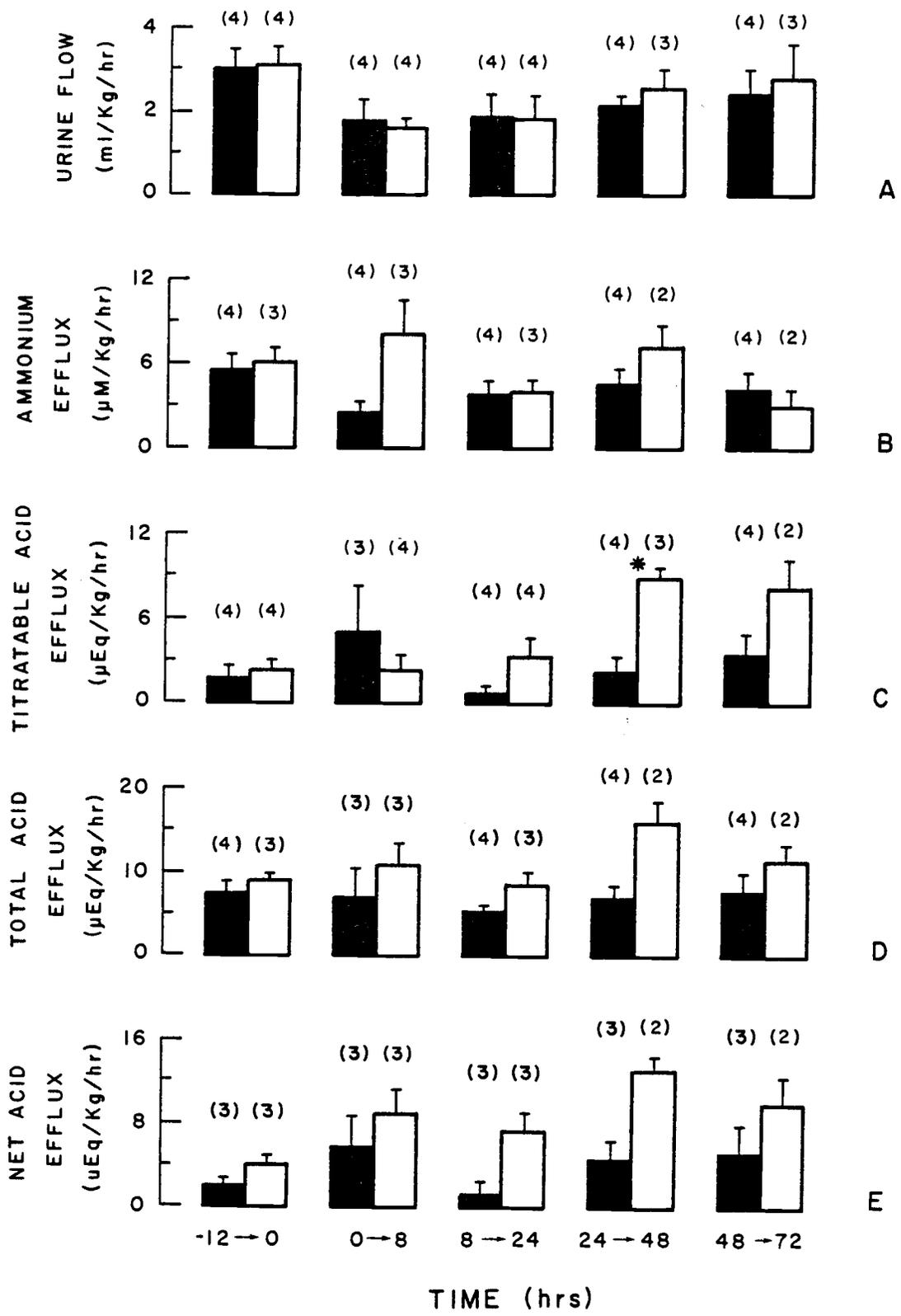
Figure 8

Table 1. Mean $\pm$ s.e. of the total efflux of ammonia, titratable acid, inorganic phosphorus, total acid excreted, bicarbonate, and net acid excreted in the urine. Values are arranged as $\frac{control\ (n)}{treatment\ (n)}$. Control fish were always exercised at 20% U-crit. For treatment fish, 0 hr marked an immediate increase in exercise from 20% to 45% U-crit. For both control and treatment fish, total efflux was measured for a 12 hr interval before 0 hr and for a 72 hr period after 0 hr.

Sample interval	Ammonia (μM/Kg)	Titratable acid (μEq/Kg)	Inorganic phosphorus (μM/Kg)	Total acid excreted (μEq/Kg)	Bicarbonate[a] (μM/Kg)	Net acid excreted (μEq/Kg)
-12 hrs -- 0 hrs	$\frac{67 \pm 12\ (4)}{72 \pm 12\ (3)}$	$\frac{23 \pm 10\ (4)}{28 \pm 9\ (4)}$	$\frac{63 \pm 33\ (4)}{57 \pm 22\ (4)}$	$\frac{90 \pm 21\ (4)}{106 \pm 6\ (3)}$	$\frac{43 \pm 4\ (3)}{64 \pm 17\ (4)}$	$\frac{27 \pm 12\ (3)}{33 \pm 24\ (3)}$
		*	*	*		*
0 hrs -- 72 hrs	$\frac{298 \pm 35\ (4)}{387 \pm 62\ (2)}$	$\frac{174 \pm 80\ (3)}{501 \pm 58\ (2)}$	$\frac{260 \pm 80\ (4)}{910 \pm 60\ (3)}$	$\frac{462 \pm 77\ (3)}{887 \pm 112\ (2)}$	$\frac{162 \pm 22\ (3)}{148 \pm 47\ (3)}$	$\frac{298 \pm 107\ (3)}{737 \pm 70\ (2)}$

a) Values calculated from urine collected aerobically were appropriate here since the urine was titrated aerobically.

* indicates mean treatment and control values are significantly different ($P < 0.05$).

Blood Acid-Base Balance

When compared to changes in control values, subtle but significant elevations (0.35 nM/L to 0.80 nM/L) in mean treatment values of $[H^+]$ were found for arterial and venous blood at 1 hr and for venous blood at 3 hrs (Fig. 9). For both control and treatment fish, mean $[H^+]$ of arterial and venous blood at the 8 hr sample period was significantly lower than the corresponding mean $[H^+]$ value at 0 hr (paired t-test evaluation, $P < 0.05$). Mean $[H^+]$ for all sample intervals ranged between 10.90 ± 0.30 nM/L and 13.20 ± 0.75 nM/L.

At various sample periods for both control and treatment fish, some venous blood samples were slightly more alkaline than arterial blood samples. Values of $\Delta[H^+]$ at the gills ranged throughout the 72 hr experiment between an apparent gain of 0.40 ± 0.05 nM/L and apparent loss of 0.70 ± 0.65 nM/L. No change in $[H^+]$ at the gills was significant when treatment and control values were compared.

Blood $[HCO_3^-]$ (Fig. 9) and $[tCO_2]$ (Fig. 10) were altered after the exercise level for treatment fish was increased. Both arterial and venous $[tCO_2]$ for treatment fish were rising three hours after the experiment began. Arterial and venous $[tCO_2]$ increased significantly by 8 hrs and 24 hrs and returned to near control levels by 72 hrs (Fig. 10). Values of $[HCO_3^-]$ were about 98% of those for $[tCO_2]$ and, therefore, displayed the same changes and levels of

significance shown for [tCO_2]. Although all calculated amounts of
dissolved carbon dioxide were low (0.10 $\mu M/ml$ to 0.32 $\mu M/ml$),
significant increases were recorded for venous blood of treatment
fish at 8 hrs and 24 hrs. Values of arterial and venous P_{CO_2} for
treatment fish increased from 2.6 $\pm$ 0.1 Torr and 3.0 $\pm$ 0.1 Torr at
0 hr to 3.5 $\pm$ 0.3 Torr and 4.3 $\pm$ 0.5 Torr at 24 hrs and then
returned to 2.8 $\pm$ 0.2 Torr and 3.4 $\pm$ 0.2 Torr by 72 hrs. Values
of arterial and venous P_{CO_2} for control fish changed from 2.6 $\pm$
0.2 Torr and 3.2 $\pm$ 0.2 Torr at 0 hr to 3.1 $\pm$ 0.2 Torr and 3.5 $\pm$
0.3 Torr at 24 hrs and then dropped to 2.8 $\pm$ 0.4 and 3.2 $\pm$ 0.5
Torr by 72 hrs.

When compared to control values, arterial blood mean plasma
[tNH_3] for treatment fish increased significantly with exercise
from 43 $\pm$ 7 $\mu M/L$ at 0 hr to 115 $\pm$ 35 $\mu M/L$ at 3 hrs. The mean
value subsequently declined to 61 $\pm$ 7 $\mu M/L$ by 72 hrs. For control
fish, arterial mean plasma [tNH_3] declined from 69 $\pm$ 17 $\mu M/L$ at 0
hr to 37 $\pm$ 20 $\mu M/L$ at 3 hr and then steadily increased to 90 $\pm$ 11
$\mu M/L$ at 72 hrs. Venous plasma [tNH_3] values were always higher
than correspondining arterial values. For treatment fish, mean
values increased from the 0 hr value of 158 $\pm$ 44 $\mu M/L$ by the 1 hr sample
and reached 214 $\pm$ 28 $\mu M/L$ in 3 hrs. Venous plasma [tNH_3] subsequently
declined to 173 $\pm$ 17 $\mu M/L$. Control venous values went from 157 $\pm$ 27
$\mu M/L$ at 0 hr to 123 $\pm$ 30 $\mu M/L$ at 3 hr and then slowly increased to
203 $\pm$ 49 $\mu M/L$ by 72 hrs. Values of [NH_4^+] were about 98% of [tNH_3] for
both control and treatment fish.

All [lac] values were low enough to indicate the dominant form of metabolism was aerobic. For all fish, mean [lac] values ranged over the 72 hr experiment between 0.31 ± 0.09 μM/ml and 0.52 ± 0.08 μM/ml in arterial blood and between 0.36 ± 0.08 μM/ml and 0.79 ± 0.27 μM/ml in the venous blood. When compared to control mean values, slight but significant increases (about 0.25 μM/ml) in treatment mean values of [lac] occurred at 1 hr in both arterial and venous blood.

Fig. 9.   Davenport diagram of mean blood pH and $[HCO_3^-]$ for fish exercised at 20% (solid) and 45% U-crit (open). For treatment fish, the 0 hr sample period just preceded the immediate increase in swimming speed from 20% to 45% U-crit. Arterial blood (top). Venous blood (bottom). S marks the 0 hr samples for control and treatment fish. For treatment fish, arrows indicate the direction of the path from 0 hr samples to samples at 1 hr, 3 hrs, 8 hrs, 24 hrs, 48 hrs and 72 hrs.
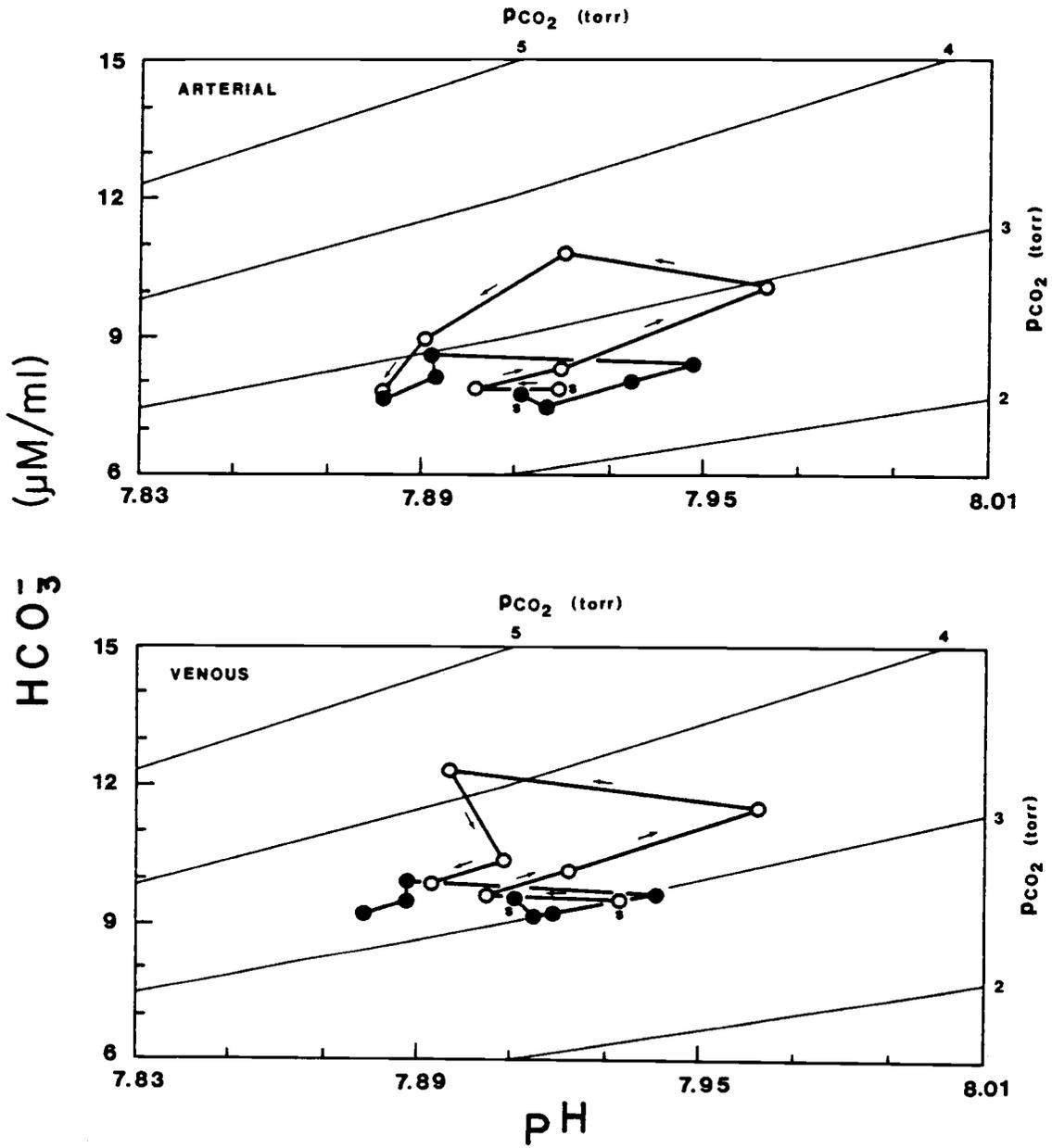
Figure 9

Fig. 10. Total CO_2 concentration in arterial (top) and venous (bottom) blood. For treatment fish, the 0 hr sample period just preceded the immediate increase in swimming speed from 20% to 45% U-crit. Mean $\pm$ se for fish exercised at 45% U-crit (open) and 20% U-crit (solid). Number of determinations at each point for treatment $(n)_T$ and control $(n)_C$ fish. * indicates mean changes in treatment and control values are significantly different ($P < 0.05$).
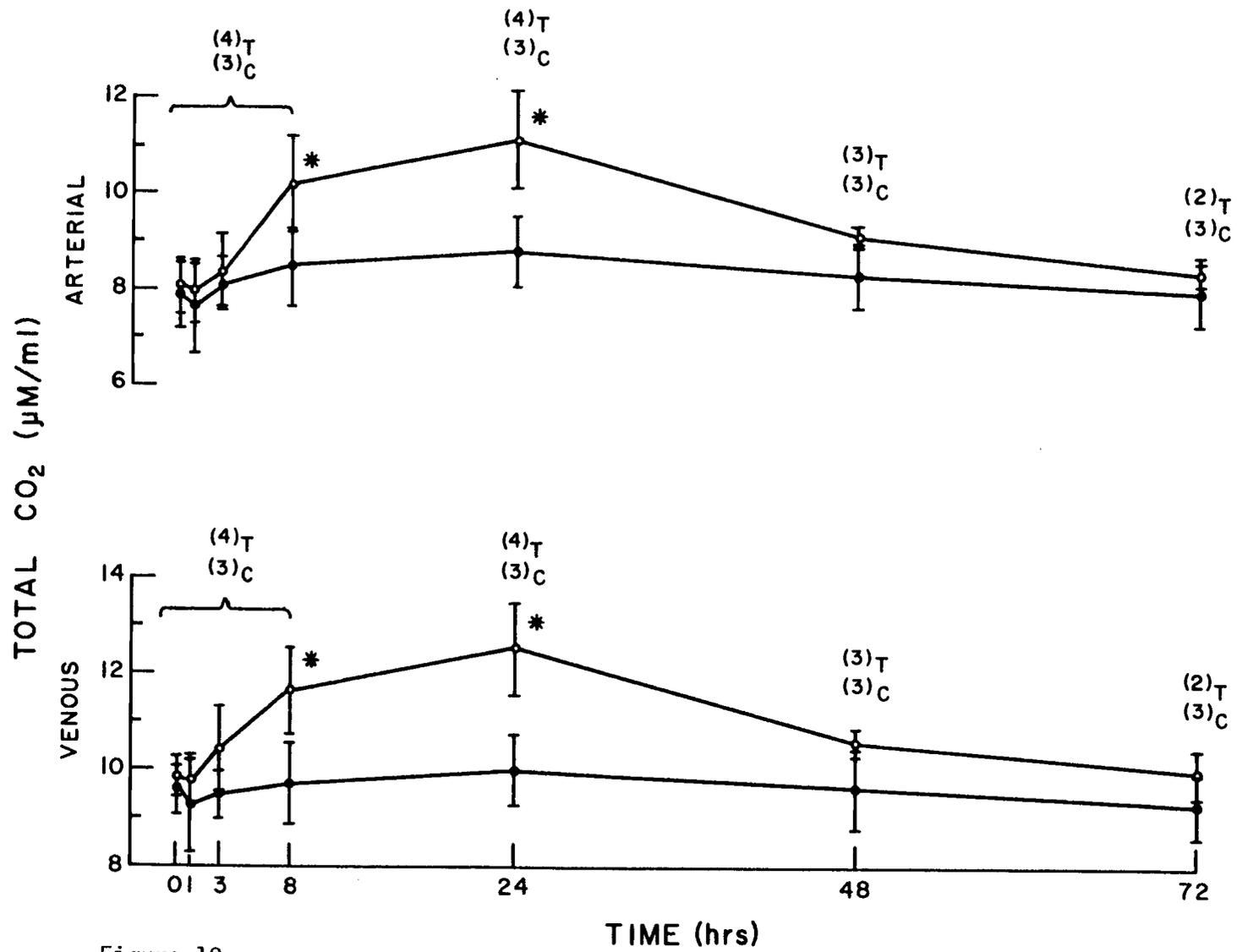
Figure 10

DISCUSSION

An increase in the level of chronic aerobic exercise resulted in changes in blood acid-base balance and in acid/base load elimination. Although blood pH changed very little, Davenport diagrams (Fig. 9) indicated mild respiratory and metabolic acidosis followed by metabolic alkalosis (Davenport, 1974) in the blood of treatment fish. Acid/base load elimination at the gills and kidney increased with exercise.

The increase in load elimination at the gills was characterized by a large and rapid increase in tCO_2 loss. Jones and Randall (1978) pointed out that very little is known about changes in tCO_2 excretion with changes in exercise. In this study, increased tCO_2 loss at the gills was accomplished by increased delivery. Within the first three hours after the increase in exercise, augmented venous $[tCO_2]$ allowed elevated tCO_2 loss. By eight hours, tCO_2 loss per milliliter of blood which crossed the gills had dropped to near control values. Increased respiratory surface area, possibly recruited by a transiently elevated perfusion pressure (Kiceniuk and Jones, 1977), may have been partially responsible for increased $\Delta[tCO_2]$ at the gills. The tCO_2 load elimination rate was maintained by increased delivery as cardiac output increased to a new plateau. It is well established that cardiac output increases with anemia (Cameron and Davis, 1970; Wood, et al., 1979). The slight increase in cardiac output seen for control fish probably resulted from

blood loss and accounted for the decrease in tCO_2 load lost per milliliter of blood at the gills. These patterns of tCO_2 delivery were nearly the same as those for oxygen uptake in control and treatment fish. Mean respiratory quotients were not statistically changed by augmented exercise and this reflected the similarity of $\dot{M}O_2$ and $\dot{M}_{tco_2}$ at the gills.

The burst of activity required to increase the momentum of the rainbow trout to a new sustained velocity may have been substantially anaerobic. An elevation of plasma $[tNH_3]$ and $\dot{M}_{tNH_3}$ lost at the gills and a significant increase in arterial and venous blood lactate concentration was found at the 1 hr sampling period. Anaerobic metabolism is known to lead to production of lactate (Driedzic and Hochachka, 1978) and lactate may be released into the blood (Black et al., 1966). Concentrations of NH_4^+ have been found to increase in the white muscle of carp, Cyprinus carpio, after a short period of activity at a sustainable velocity (Driedzic and Hochachka, 1978) and after the animal was fatigued (Driedzic and Hochachka, 1976). This may have led to the increased plasma $[tNH_3]$ and $\dot{M}_{tNH_3}$ at the gills. Production of ammonia has been found to increase during hypoxia in the mullet, Rhinomugil corsula (Kutty and Mohamed, 1975) and during anoxia in the goldfish, Carassius auratus (van den Thillart and Kesbeke, 1978).

The increase in net acid load efflux by the kidneys of treatment fish was accomplished through an increase in urine net acid concentra-

tion and not by an elevated urine flow rate. Urine flow rate actually dropped for both control and treatment fish, possibly due to blood sampling (Cameron and Wood, 1978).

The increase in total acid excreted in the urine of treatment fish was accomplished by an increase in $[NH_4^+]$ and titratable acid. In acidotic mammals, renal tubular cells excrete increased amounts of unionized ammonia to buffer the urine and increase the acid load of the urine (Hills, 1973). In rainbow trout, urinary NH_4^+ efflux rate increases when the animal is exposed to a mineral acid load (Wood and Caldwell, 1978), but not when exposed to a metabolic acid load (Kobayashi and Wood, 1980). In our experiments, the ammonia lost in the urine was probably excreted by the renal tubular cells. The glomerular filtration rate (GFR) needed to account for $\dot{M}_{tNH_3}$ in the urine was calculated to be between seven and seventeen times the GFR predicted by inulin clearance studies (Hofmann and Butler, 1979). In our study, the titratable acid component of total acid excreted in the urine depended on the buffering of filtered phosphate, as it does in mammals (Hills, 1973). Control of titratable acid and phosphate output in the urine of trout may rest with urine acidification and phosphate recovery by renal tubular cells.

When compared to control values, decreased $[HCO_3^-]$ and increased Pco_2 reflected the elevated acidification of the urine of treatment fish. Since the Pco_2 in the urine of both control and treatment fish was so much higher than the Pco_2 of their blood, urine acidifica-

tion must occur, at least in part, in the distal portion of the nephron. Carbonic anhydrase plays a role in the acidification of tubular fluid (Hills, 1973). Trout have carbonic anhydrase activity in their kidney (Houston and McCarty, 1978) and it has been speculated that this acetazolamide-sensitive esterase resides in the distal segment of the nephron, as it does in mammals (Hickman and Trump, 1969).

Nonvolatile buffers are also responsible for high urine Pco_2 levels. In the presence of nonvolatile buffers, the uncatalyzed dehydration rate of H_2CO_3 in the tubular fluid is outpaced by acidification of bicarbonate (Hills, 1973). Nonvolatile buffers affect tubular fluid $[H_2CO_3]$ and Pco_2 in a concentration dependent manner (Hills, 1973). Inorganic phosphorus concentrations and Pco_2 levels in the urine of the treatment fish were higher than those in the urine of control fish. In the urine of treatment fish, Pco_2 increased with $[Pi]$.

The direction of change in blood acid-base balance found initially for treatment fish was similar to that seen for fish exercised to exhaustion. Wood et al., (1977) found a severe respiratory acidosis followed by a temporally separated metabolic acidosis after exhausting activity in starry flounder, Platichthys stellatus. The two forms of acidosis merged by twenty minutes after exhaustion. A very mild respiratory and metabolic acidosis was found for the blood of treatment fish in our study one hour after the exercise

level for the animal was elevated. These forms of acidosis can be accounted for by a slight increase in blood P_{CO_2} and a small but significant increase in the concentration of blood lactate. Strong ion concentration difference, [SID], probably decreased in the blood (Stewart, 1978). The metabolic acidosis experienced by the starry flounder was larger than could be accounted for by blood lactate increases, so Wood et al. (1977) postulated the presence of another metabolite, such as ammonium. For our treatment fish, we found an increase in plasma [tNH$_3$], which became significant by the three hour sampling period.

The pattern of change in blood acid-base balance and blood pH in treatment and control fish were similar after the one hour sampling period. This was especially true when the P_{CO_2} increased between 8 hrs and 24 hrs in the arterial and venous blood of both control and treatment fish, apparently as a function of blood loss. However, the blood of treatment fish moved significantly further toward metabolic alkalosis by the 8 hr sampling period than did the blood of control fish.

The large [HCO$_3^-$] and [tCO$_2$] found in the shift toward metabolic alkalosis in treatment fish can be accounted for by a change in blood [SID] that is different from that in the blood of control fish. As blood samples were taken, blood [Hb] dropped in a similar manner for treatment and control fish. This presumably reduced the total weak acid buffering power of the blood (Davenport, 1974).

For pH and $[tCO_2]$ to remain as constant as they did in the blood of control fish over the 72 hr experiment, the [SID] of the blood must have decreased in response to decreased [Hb] (Stewart, 1978; Meyerhoff and Weber, unpublished observations). For $[HCO_3^-]$ and $[tCO_2]$ to change as they did in the blood of treatment fish, the decrease in [SID], necessitated by the [Hb] decline, must have been slower than that for the blood of control fish (Fig. 11). This slow drop in blood [SID] in treatment fish blood may stem from elevated urine acidification by the kidney, and the consequent net sparing action on blood cations. The slow drop may also be due to augmented net sodium influx rates or net chloride efflux rates at the gills.

Movement toward metabolic alkalosis cannot be easily explained by reference to net acid efflux in the urine. The elevation in net acid excreted over the entire 72 hr experiment was certainly large enough to rid the vascular compartment of the required amount of acid, but alkalosis occurred within eight hours. When corrected for control values and assuming a 3.5% blood volume (Holmes and Donaldson, 1969) and a total weak acid content of the blood of about 30 mEq/L, urine net acid excretion for treatment fish could only account for 26% of the acid that had to be lost from the vascular compartment to achieve the measured alkalosis. The increase in net acid efflux in the urine may be responsive to changes in whole-body acid-base balance, but it does not completely account for changes in blood acid-base balance. Increased net acid efflux

Figure 11.   Combinations of concentrations of the independent
              variables total CO_2, strong ion difference (SID) and
              total weak acid, represented here by levels of
              hemoglobin, that produce a given blood pH at 15°C.
              The strong ion difference scale has been left off
              because exact values were not known. Normal [Hb]
              and low [Hb] lines were calculated assuming a normal
              [SID] of 30 mEq/L and a low [SID] of 20 mEq/L,
              although these values are not important. The direction
              and relative rate of change of [SID] is important.
              The [tCO_2] and [SID] tracks taken by control (solid)
              and treatment (open) fish between the 1 hr and 72 hr
              sampling periods have been generalized and are shown
              on this graph. The elevated [tCO_2] found in the
              blood of treatment fish at 8 hrs and 24 hrs is
   represented here as a single point.
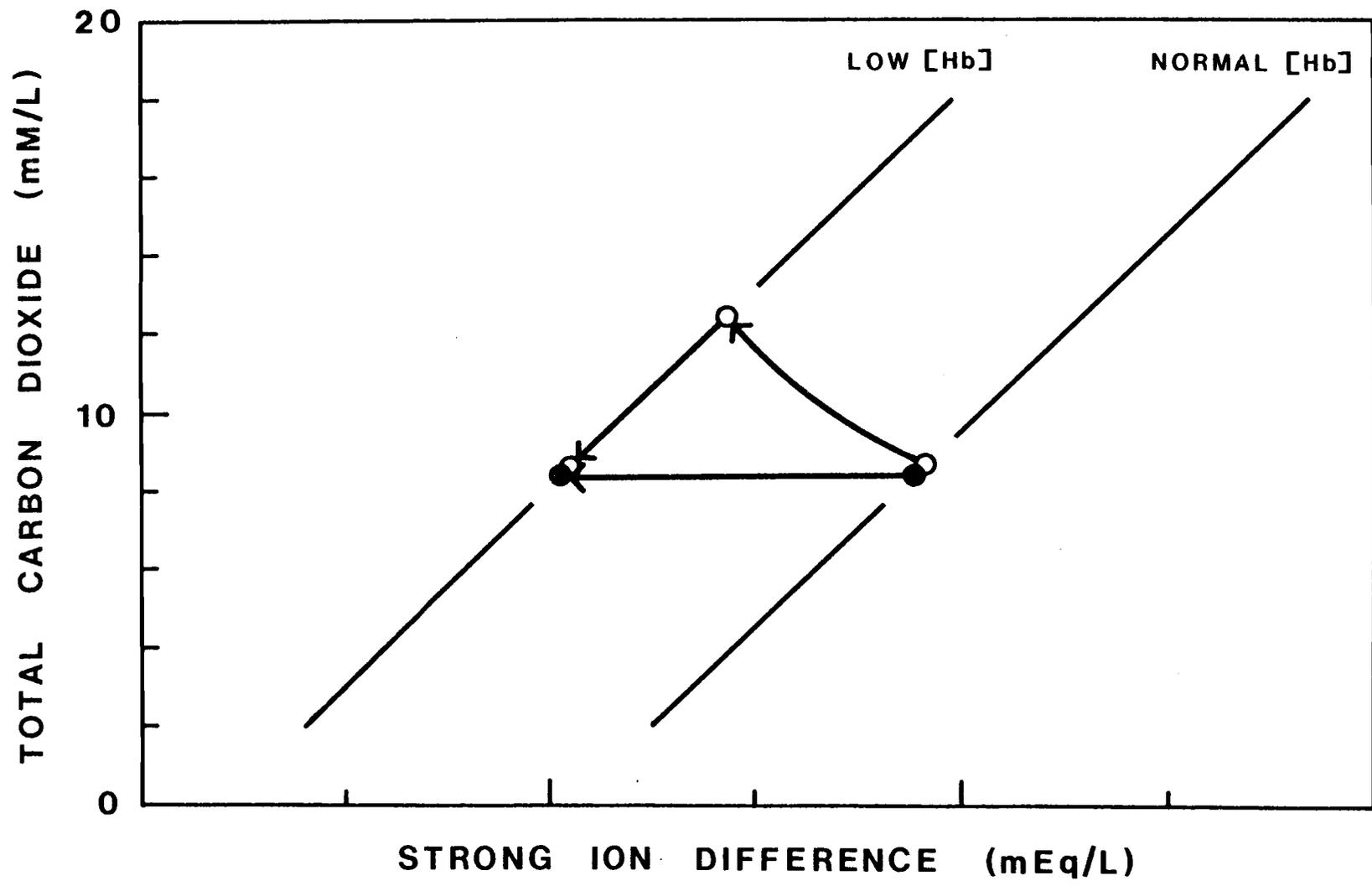
Figure 11

in the urine is maintained by treatment fish through many types of changes in blood acid-base balance. Clearly some other mechanism must be responsible, at least in part, for the trend toward metabolic alkalosis in the blood of treatment fish.

Branchial ion flux changes may have slowed the changes of blood [SID] in treatment fish. Exchange diffusion mechanisms, Na^+/Na^+, Na^+/NH_4^+, Cl^-/Cl^- and Cl^-/HCO_3^-, contribute to ion fluxes at the gills (Girard and Payan, 1980). The slight increase in plasma $\Delta[tNH_3]$ and loss of plasma $\dot{M}_{tNH_3}$ found within the first 8 hrs at the gills of treatment fish may account for the alkalosis. Ammonium efflux and sodium influx have been correlated (Girard and Payan, 1980; Kerstetter and Keeler, 1976; Maetz, 1971). Wood and Randall (1973b) found that after a change in the level of exercise for rainbow trout, Na^+ influx was constant. However, Na^+ efflux decreased after a transient increase. The net sodium influx may have been produced by the sparing action of increased ammonium efflux on sodium efflux. The slight alkalosis produced by the slow rate of change of [SID] in the blood may have resulted from increased NH_4^+ efflux. When corrected for control values and assuming a 3.5% blood volume and total weak acid content of blood of 30 mEq/L, loss of plasma $[tNH_3]$ at the gills of treatment fish was about five times the amount of NH_4^+ efflux necessary to account for the slowed change in blood [SID] and the resulting increase in blood $[HCO_3^-]$. So if a fraction of tNH_3 efflux at the gills was lost as NH_4^+ in

exchange diffusion for Na^+, the trend toward metabolic alkalosis in treatment fish could be explained. Increased blood $[HCO_3^-]$ in treatment fish may have stimulated Cl^-/HCO_3^- exchange and, in conjunction with reduced plasma $\dot{M}_{tNH_3}$ and $[tNH_3]$, blood $[SID]$ and $[HCO_3^-]$ would drop to levels found in control fish.

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