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AERIAL VERSUS AQUATIC GAS EXCHANGE IN SALAMANDERS IN RESPONSE TO ENVIRONMENTAL HYPERCAPNIA

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THE UNIVERSITY OF OKLAHOMA

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AERIAL VERSUS AQUATIC GAS EXCHANGE IN SALAMANDERS IN RESPONSE TO ENVIRONMENTAL HYPERCAPNIA

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AERIAL VERSUS AQUATIC GAS EXCHANGE IN SALAMANDERS

IN RESPONSE TO ENVIRONMENTAL HYPERCAPNIA

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TABLE OF CONTENTS

LIST OF TABLES

PAPER I

Paper II

LIST OF ILLUSTRATIONS

PAPER I

Figure

PAPER II

Figure

Aerial versus Aquatic Gas Exchange in *Necturus maculosus* in Response to Increasing Aquatic Hypercapnia

This paper was prepared for submission to the journal, *Respiration Physiology*

Abstract

This study documents both aerial and aquatic gas exchange in a predominantly water-breathing salamander in response to five levels of aquatic hypercapnia. Necturus maculosus, acclimated at 20°C and LD 12:12 were subjected to a 3 hr control period of normoxic normocapnia in both media; the aquatic phase was then mixed with a solution having a P_{CO_2} of 20-130 mmHg, and the P_{O_2} of this mixture elevated to that at the beginning of the control period (150 mmHg). While none of the animals registered pulmonary CO_2 release during the control period, 6 out of the 16 did so during hypercapnia. This response occurred only above P_{CO_2} = 40 mmHg. Two animals registered pulmonary O_2 uptake during the control period and both of these plus one other did so during hypercapnia. This contribution of the lungs to overall gas exchange was, however, extremely small; it did not fall into a regular pattern in any animal. Thus, aquatic hypercapnia did not result in a shift in CO_2 release from gills and skin to lungs. Consideration of these results with acid-base balance, respiratory behavior, and ion-exchange in bimodal and other trimodal breathers during short-term and long-term hypercapnia suggests an ion-mediated rather than ventilation-mediated mechanism of acid-base regulation in most amphibians.

KEY WORDS

Necturus maculosus, hypercapnia, gas exchange partitioning, acid-base balance, respiratory control, ion-exchange, amphibians

Introduction

The presence of more than one respiratory organ in amphibians has generated investigation of the physiological roles of each organ in different species; an important consideration is the relative effectiveness of a particular surface in facilitating O_2 acquisition and CO_2 elimination. The distribution of gas exchange under normal temperatures and availability of gases in the respiratory media have addressed this point in studies of the giant salamanders Cryptobranchus alleganiensis, Necturus maculosus, Siren lacertina, and Amphiuma means (Guimond & Hutchison, 1963; Whitford & Sherman, 1968), and the bullfrog Rana catesbeiana (Krogh, 1904). Of the amphibians which spend virtually all their time in water, pulmonary activity is greatest in animals such as Amphiuma and Siren where the lungs are well-developed; aquatic respiration predominates in those with sac-like lungs, regardlesss of whether gills are present (Necturus) or not (Cryptobranchus). The picture which emerges from these studies shows that, in general, trimodal breathers (those with skin, lungs, and gills) use their lungs very little. What then is the function of lungs in these animals?

A second consideration is the possibility that changes in ventilation regulate blood acid-base balance, and is supported by studies on the tiger salamander, Ambystoma tigrinum (Burggren & Wood, 1981), the bullfrog (Jackson & Braun, 1979), the tropical toad, Bufo marinus (Boutilier et al., 1981), the sea turtle, Pseudemys scripta (Jackson & Kagen, 1976), and the alligator, Alligator mississippiensis (Davies, 1975), among others. However, when Crytpobranchus is subjected to an aquatic CO_2 load, increases in pulmonary and buccopharyngeal ventilation do not forestall respiratory acidosis (Boutilier & Toews, 1981).

Thirdly, ecological conditions such as seasonal hypercapnia or changes in ion concentrations are potentially disruptive to an animal's acid-base balance. Aquatic hypercapnia is regularly encountered by sirenids, trimodal breathers with poorly-developed gills. Necturus, with well-developed gills, is rarely exposed to high environmental CO_2.

Finally, ontogenetic factors may be important. When metamorphosing from larval to neotenic adult form, Necturus retains its gills and continues to rely primarily on aquatic respiration. This contrasts with many anurans and ambystomid salamanders, which lose their gills during metamorphosis from a strictly aquatic existence to a fully terrestrial one, thereby experiencing a "physiological hypercapnia" (Stiffler et el., 1983) as they rely primarily on respiration in air, which has a low CO_2 capacitance coefficient.

Thus, the role of the lungs is more obscure in Necturus than it is in other amphibians. Even at high temperatures, pulmonary contribution to total gas exchange is minimal, and as gas exchange is redistributed with increasing temperature, the decrease in the cutaneous component is more than made up for by the increase in branchial respiration (Guimond & Hutchison, 1976). Morphological simplicity and this absence of pulmonary gas exchange suggest that the lung is used in bouyancy control, but lack of direct data renders this explanation speculative.

This study attempts to clarify the role of aerial respiration in Necturus and is the first assessment of the effect of aquatic hypercapnia on the partitioning of gas exchange in a trimodal breather.

Materials and Methods

Necturus maculosus, purchased from commercial suppliers in Wisconsin, were maintained in environmental chambers with an LD 12:12 photoperiod and in tap water at 20°C in open plastic containers for at least 2 weeks prior to experimentation. On the day before an experiment, an individual was transferred to an uncovered 3 L glass chamber partially filled with tap water. An identical chamber filled with 2.75 L of 0.5mM $NaHCO_3^-$ circulated through an external pump was situated in a temperature-controlled water bath and housed the animal during an experiment. Aquatic oxygen consumption was calculated from continuous measurement of P_{O_2} with a Diamond Electro-Tech Clark electrode #731 and Transidyne General Chemical Mircrosensor. Aquatic CO_2 release was determined from continuous titration of the chamber solution to pH 8.00 with 0.1 N NaOH (Gottlieb & Jackson, 1976) with a Fischer Titrimeter II system. The chamber was sealed and the upper portion ventilated at a constant rate from a pump drawing in room air. The outflow from this breathing space was dried with anhydrous $CaSO_4$ (Drierite, W.A. Hammond Company) and passed through a Beckman S-3A Oxygen Analyzer and a Beckman Model 364 Infared Analyzer for measurement of pulmonary oxygen uptake and CO_2 release, respectively. The aerial and aquatic phases of the chamber were separated by mineral oil to prevent gas transfer between air and water.

Aerial and aquatic respiration was monitored under normoxic normocapnia in both respiratory media for at least 3 h. This control period was followed by an experimental period in which the P_{CO_2} of the aquatic phase ($PaqCO_2$) was elevated by mixing the chamber solution with one which had been bubbled with CO_2. Throughout the experimental period, a Radiometer P_{CO_2} electrode was used to measure $PaqCO_2$. Because initial experiments

with low-level hypercapnia (20 mmHg) did not stimulate pulmonary activity, additional tests at P_{CO_2}'s of 30, 40, 60, 80, and 130 mmHg were performed.

The significance of these results was determined with one-way analysis of variance. The arcsin transform was used on percentages. In appropriate cases, Student's t-test was used to determine level of significance. Values of $P<0.05$ were considered significant.

Results

At each level of hypercapnia there was a decrease in O_2 consumption from control to experimental conditions (Table 1). This trend occurred in 9 experiments ($\bar{X}$=31.46± 7.29% of control value in the experimental period), while animals in 5 other experiments increased their total $\dot{V}_{O_2}$ by a mean of 16.50±7.08% over the control value. Although the regression of total $\dot{V}_{O_2}$ against aquatic P_{CO_2} showed a very slight trend towards decreasing oxygen consumption with increasing hypercapnia, this relationship was not significant (lower line, Fig. 1). Similarly, an apparent tendency towards greater reduction of $\dot{V}_{O_2}$ at higher levels of hypercapnia was insignificant when analyzed as the difference between the regression of total $\dot{V}_{O_2}$ against P_{CO_2} for control versus experimental periods (Fig. 1). There was no relationship between body weight and the change in total O_2 consumption from control to experimental periods.

There was no relationship between pulmonary CO_2 release and the level of aquatic hypercapnia. This was true of the absolute level of lung $\dot{V}_{CO_2}$ ($\bar{X}$=5.23±3.00 μlgm^{-1}hr^{-1}) (Fig. 2) and of this value as a percentage of control $\dot{V}_{CO_2}$ ($\bar{X}$=13.32± 6.05%). This activity also bears no relationship to body weight. The lack of pulmonary, and therefore total, CO_2 loss during aquatic hypercapnia means that the respiratory exchange ratio ($R=\dot{V}_{CO_2}/\dot{V}_{O_2}$) was zero, or near zero, during the experimental periods, and decreased greatly from control to experimental periods (Table 1).

Eight individual Necturus were exposed to aquatic hypercapnia in 16 separate experiments. Six animals registered some pulmonary gas exchange. Two animals used lungs for oxygen acquisition, and both did so under both control and experimental conditions; the latter episodes occurred at P_{CO_2}'s of 20 and 80 mmHg for one animal and at 40 and 80 mmHg for the other (Table

1, Np=4). There were no instances in any animal of pulmonary CO_2 release during normoxic normocapnia (Table 1, Np=0). Lung CO_2 loss was measurable in 6 animals (Table 1, Np=6) at experimental P_{CO_2}'s of 40, 60 (2 experiments), 80 (3 experiments), and 130 mmHg. Aerial exchange of both O_2 and CO_2 was sporadic in all cases; in only one experiment was pulmonary activity registered during each 30 min of control and experimental phases. For this reason, all references to total O_2 consumption may be considered equally valid for aquatic O_2 consumption; this equivalence also applies to CO_2 release during the control period. Under hypercapnia the animals were, presumably, prevented from releasing CO_2 into the aquatic medium, and therefore total $\dot{V}_{O_2}$ was the pulmonary value alone.

Discussion

These results demonstrate that even under environmental CO_2 levels far above any the animals would likely encounter in their natural habitat, Necturus remains a 'water-breather'. During hypercapnia, the mean level of lung $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$, expressed as a percentage of the total exchange of each gas (Table 1), was essentially the same as that reported by Guimond & Hutchison (1976) for Necturus at 25°C. In their study, pulmonary exchange peaked at 10% of total O_2 and 12% of total CO_2; these levels apply to animals restrained, and therefore probably stressed, by 2 rubber cuffs encircling the body on either side of the gills. In the experimental period of this study, lungs accounted for a mean 9% of total $\dot{V}_{O_2}$, and 13% of total $\dot{V}_{CO_2}$ measured during the control period. This degree of lung activity does not represent a redistribution of gas exchange from aquatic to pulmonary surfaces in either study, but does constitute a greater role than that measured for unrestrained animals. The animals studied by Guimond and Hutchison (1976) relied completely on skin and gills for exchange of both gases at 5°C and 15°C. The slight recruitment of lungs at 25°C was in accord with control values measured here: 4% of total $\dot{V}_{O_2}$ and 0-1% of total $\dot{V}_{CO_2}$ in both studies. Thus, the pulmonary contribution in unrestrained animals is the same at elevated temperature as it is at elevated P_{CO_2}.

Aquatic surfaces are dominant in respiratory activity under all conditions tested. In this study, cutaneous and branchial exchanges were not measured separately; however, the overall level of aquatic respiration, gauged by absolute values and by comparison of total and aquatic R values, was similar to that previously recorded for restrained animals (Guimond & Hutchison, 1976). We may therefore use their study as a clue to what is

happening at the aquatic surfaces under hypercapnia. As overall respiration increases with increasing temperature, each surface exchanges a greater amount of each gas. The relative contribution of skin versus gills to $\dot{V}_{O_2}$ remains essentially unchanged between 5°C and 25°C (Guimond & Hutchison, 1976). Because the change in aquatic O_2 consumption between control and experimental phases of this study was insignificant and less than that accompanying the changes in temperature (Guimond & Hutchison, 1976), it is reasonable to conclude that hypercapnia did not produce a shift in the relative contribution of gills versus skin to V_{O_2}. However, the animals were subjected to normoxic hypercapnia, and their gills can effectively take up O_2 even at low environmental P_{O_2} (Guimond & Hutchison, 1976). Therefore, given the increased branchial activity, O_2 uptake across the gills may have increased during hypercapnia. If so, gas transfer across the integument must have decreased. This would be consistent with a reduction of cutaneous blood flow to limit CO_2 transfer into the animal. Whatever surfaces mediate O_2 consumption during hypercapnia, the corresponding CO_2 is not eliminated aerially or aquatically. What happens to this CO_2?

At an environmental P_{CO_2} of 22 mmHg, this respiratory CO_2 produces an acidosis in the extracellular compartment, manifested as a decrease in plasma pH accompanying an increase in arterial P_{CO_2} (Stiffler, et al., 1983); this acidosis resulted from 2 hours of hypercapnia and became more pronounced after 24 hours. The 3-hour exposure reported in the present study is, therefore, temporally sufficient to produce an acid-base state which would effect changes in ventilation if an appropriate physiological mechanism existed. The unresponsiveness of pulmonary ventilation to even higher P_{CO_2} is further evidence against the presence of such a mechanism in Necturus. The effects of the extracellular acid-base disturbance on the

intracellular compartment are not documented, but any degree of compensation there cannot be the result of changes in pulmonary ventilation.

Comparison of Necturus' acid-base dynamics with those of other amphibians suggests that significant intracellular compensation does not occur in the mudpuppy. Although dissimilar to Amphiuma and Siren in lung morphology and ecological exposure to CO_2, Necturus is physiologically similar in its extracellular response to aquatic hypercapnia. These species all tolerate significant decreases in arterial pH and increases in plasma P_{CO_2} (Stiffler et al., 1983; Heisler et al., 1982). In Necturus, plasma $[HCO_3^-]$ and electrolytes did not change with short or long-term hypercapnia, while Siren registered a transient decrease in plasma $[HCO_3^-]$ during the first several hours of exposure to a P_{CO_2} of 47 mmHg. During this same period both Amphiuma and Siren exhibited a transient increase in plasma $[Cl^-]$, which suggests a HCO_3^-/Cl^- counterion exchange similar to that in fish gills (Heisler et al., 1982). Siren is the only one of these three species in which intracellular pH has been measured (Heisler et al., 1982), and due to increased tissue $[HCO_3^-]$, is found to be relatively undisturbed by aquatic hypercapnia. For comparison with Siren, data on intracellular compensation in Amphiuma is critical in understanding whether the gills have a role in ion transfer which is relevant to acid-base regulation. The only compensation for respiratory acidosis occurs in the one instance, Siren, where extracellular electrolytes are affected in a manner consistent with a mechanism for compensation.

Larval Ambystoma are like Necturus in being trimodal breathers with poorly-developed lungs. Neither species normally encounters hypercapnia in its natural environment, yet they are quite different in their

physiological responses to elevated P_{CO_2}. In larval Ambystoma, a decrease in plasma [Cl^-] parallels an increase in plasma [HCO_3^-] after long-term (24 h) hypercapnia; these changes did not occur after short-term (2 h) hypercapnia (Stiffler et al., 1983). These ion shifts were associated with a slight but insignificant increase in arterial pH. The overall picture is still consistent with a capacity for compensation, and is also in accord with changes in arterial pH and plasma [HCO_3^-] during similar durations and level of hypercapnia in Cryptobranchus (Boutilier & Toews, 1981) and Typhlonectes compressicauda (Toews & MacIntyre, 1978).

Necturus is the only water-breather in which there is no extracellular compensation for the acidosis resulting from a long-term (several days) exposure to high aquatic P_{CO_2}. Of the 2 gill-less species, both of which have poorly-developed lungs, only Typhlonectes normally encounters hypercapnia in its natural habitat (Toews & MacIntyre, 1978). Neither of the gilled forms usually encounters aquatic hypercapnia; yet one of these, the larval Ambystoma, shows extracellular compensation when experimentally subjected to hypercapnia. Thus, there is no correlation between the presence of gills and acid-base regulation in salamanders. More information on plasma electrolytes and the acid-base state of the intracellular space is required to clarify an association between ion-exchange and acid-base regulation. In addition, it would be helpful to investigate the effect of hypercapnia on the gilled larvae of Typhlonectes.

In amphibians, the focus of inquiry on the effect of environmental hypercapnia has been the potential for ventilatory regulation of acid-base balance, but oxygen acquisition may also direct gas exchange partitioning. In both Necturus and larval Ambystoma, arterial P_{O_2} (PaO_2) increased after 2 h of aquatic hypercapnia, and decreased after 24 h; only the 2 h value in

Necturus was insignificant (Stiffler et al., 1983). These results suggest a short-term hyperventilation followed by a depression of ventilation (Stiffler et al., 1983). Concomitant decreases followed by increases of the difference between arterial and inspired CO_2 tensions ($PaCO_2$-P_ICO_2) in both animals support this suggestion. The results of the present study bear on this point in two ways. First, because aquatic O_2 consumption did not change with hypercapnia, branchial hyperventilation could occur only if cutaneous gas exchange decreased. As noted earlier, the increase in branchial motions during experimental periods occurs under physico-chemical conditions favorable for just such a redistribution of aquatic O_2 uptake. Second, in spite of the behavioral indication of increased pulmonary activity, episodes of pulmonary gas exchange are not markedly increased in Necturus during aquatic hypercapnia, and did not contribute significantly to total exchange of O_2 or CO_2. However, in Necturus, pulmonary gas exchange may not reflect pulmonary ventilation. The pulmonary artery branches directly from the union of the efferent arteries of the second and third gills; unless blood leaving the gills was deficient in oxygen (as a result of aquatic hypoxia or of being shunted away from the respiratory lamellae) little O_2 uptake would take place in the lungs. Thus, the constancy of PaO_2 between control and experimental conditions probably does not constitute evidence against short-term pulmonary hyperventilation in Necturus.

The cardiovascular pattern in larval Ambystoma allows the same inference. However, the increase in PaO_2 in this species may not be due to increased pulmonary ventilation, even if it occurs. The pulmonary artery in Ambystoma can receive blood directly from the efferent artery of the third gill and the bulbous arteriosus of the heart. Due to the presence of a vascular plexus in the latter route and the relative resistances which

usually exist in these vessels, the former path actually supplies the lungs under normal conditions (Malvin, 1983). During hypoxia, circulating catecholamines act to increase total perfusion of both the respiratory lamellae and the pulmonary vascular bed (Malvin, 1983). However, the fraction of cardiac output to the first gill decreases by 80% (Malvin & Heisler, 1984); thus, the increase in the fraction reaching the lungs must be due to an increased blood flow to the third gill. Under these circumstances, elevated PaO_2 could only result from increased pulmonary O_2 uptake. During normoxia, vascular resistance in the gill indicates that the respiratory lamellae receive a small fraction of the cardiac output (Malvin, 1983), and gill ligation does not change O_2 uptake (Heath, 1975). Exposure to CO_2 (at a level comparable to the intermediate ones employed in this study) did not alter vascular resistance in either the respiratory bed or the shunt vessels of isolated Ambystoma gills (Malvin, 1983). While exposure of the respiratory tissues to CO_2 may produce no direct effect on gas exchange, the increased activity of the animal may create a need for oxygen which elicits changes in blood flow similar to those described for the isolated gill in response to hypoxia. If so, increased perfusion of the respiratory lamellae during normoxic hypercapnia could result in an increase in branchial O_2 uptake.

At this point, it is not possible to discern the effect of hypercapnia on the distribution of gas exchange in larval Ambystoma. Comparison of its respiratory and acid-base profiles with those of Necturus suggests that, like the neotenes, the larvae derive no benefit from changes in ventilation in maintaining their acid-base state under short-term hypercapnia. As they decrease their swimming and air-breathing efforts under extended hypercapnia, both species exhibit similar drops in PaO_2. Thus, extracellular compensation in Ambystoma is probably the result of changes

in ion-exchange rather than in respiration. In both species, increased ventilation serves only to elevate PaO_2 in the short-term; due to the cardiovascular patterns, and the lack of pulmonary exchange recorded in this study, aquatic exchange must be the source of this increase. If the animals are experiencing an increased metabolic requirement, and blood flow changes as it did in the isolated Ambystoma gill during hypoxia, then the gills must be responsible for taking up additional O_2. For a full explanation of the response to hypercapnia, these elements must be fashioned into a complete causal chain. The source of increased metabolic demand is needed to complete the scenario suggested thus far. Ion exchange (HCO_3^-/Cl^- and/or HCO_3^-/NH_4^+) is the ready candidate. There is evidence for both renal and extra-renal mechanisms in larval Ambystoma during hypercapnia (Hicks & Stiffler, 1980). In addition, these animals lose their ability for compensation when deprived of external NaCl, and significantly increase active Na^+ transport during hypercapnia (Stiffler, pers. comm.) What implication does the absence of extracellular compensation in Necturus have for this explanation? Because hypercapnia did not produce changes in plasma electrolytes, Necturus need not increase $\dot{V}_{O_2}$ to drive an ion exchange mechanism. Because Necturus does not normally experience hypercapnia in either its ecology or ontogeny, its increased swimming activity during experimental hypercapnia may be a reaction to a stress for which it has no relief mechanism.

The data presented in this study are the only quantification of the consequences of increased respiratory behavior for gas exchange in a trimodal breather subjected to aquatic hypercapnia. There is no indication that respiratory CO_2 exchange plays a significant role in regulation of blood acid-base balance. Neither thermal, physical, (Guimond & Hutchison, 1976) nor physicochemical stress results in a major role for the lungs of

Necturus. The absence of data on lung use for other activities, such as buoyancy control, leaves one explanation for their presence in this species, namely, as auxiliary gas exchangers. However, the circumstances in which this role is important have not been adequately described.

Comparison of cardiovascular patterns and changes in respiratory behavior, ventilation at all gas exchange surfaces, plasma electrolytes, and extracellular acid-base parameters in Necturus maculosus and larval Ambystoma tigrinum leads to the following conclusions: 1) Pulmonary ventilation is not involved in regulation of blood acid-base balance. 2) In contrast to Ambystoma, the absence of extracellular acid-base regulation in Necturus is probably due to the absence of ecological or ontogenetic circumstances which would threaten their normal acid-base state. 3) Increased metabolic demands are met by O_2 uptake across aquatic surfaces. 4) The influence of cardiovascular patterns on gas exchange effectiveness at each surface dictates that increased aquatic O_2 consumption be accomplished by a shift in emphasis from cutaneous to branchial avenues.

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Table Heading

Table 1. Effects of several levels of hypercapnia on gas exchange in Necturus maculosus. Oxygen consumption ($\dot{V}_{O_2}$) and CO_2 production ($\dot{V}_{CO_2}$) are given in $\mu l\ gm^{-1}\ hr^{-1}$. Values in parentheses are percent of total gas exchange for animals with pulmonary activity. C-control period. E-Experimental period. Np-number of animals which registered pulmonary exchange. B-CO_2 release into the aquatic medium was blocked during the experimental periods.

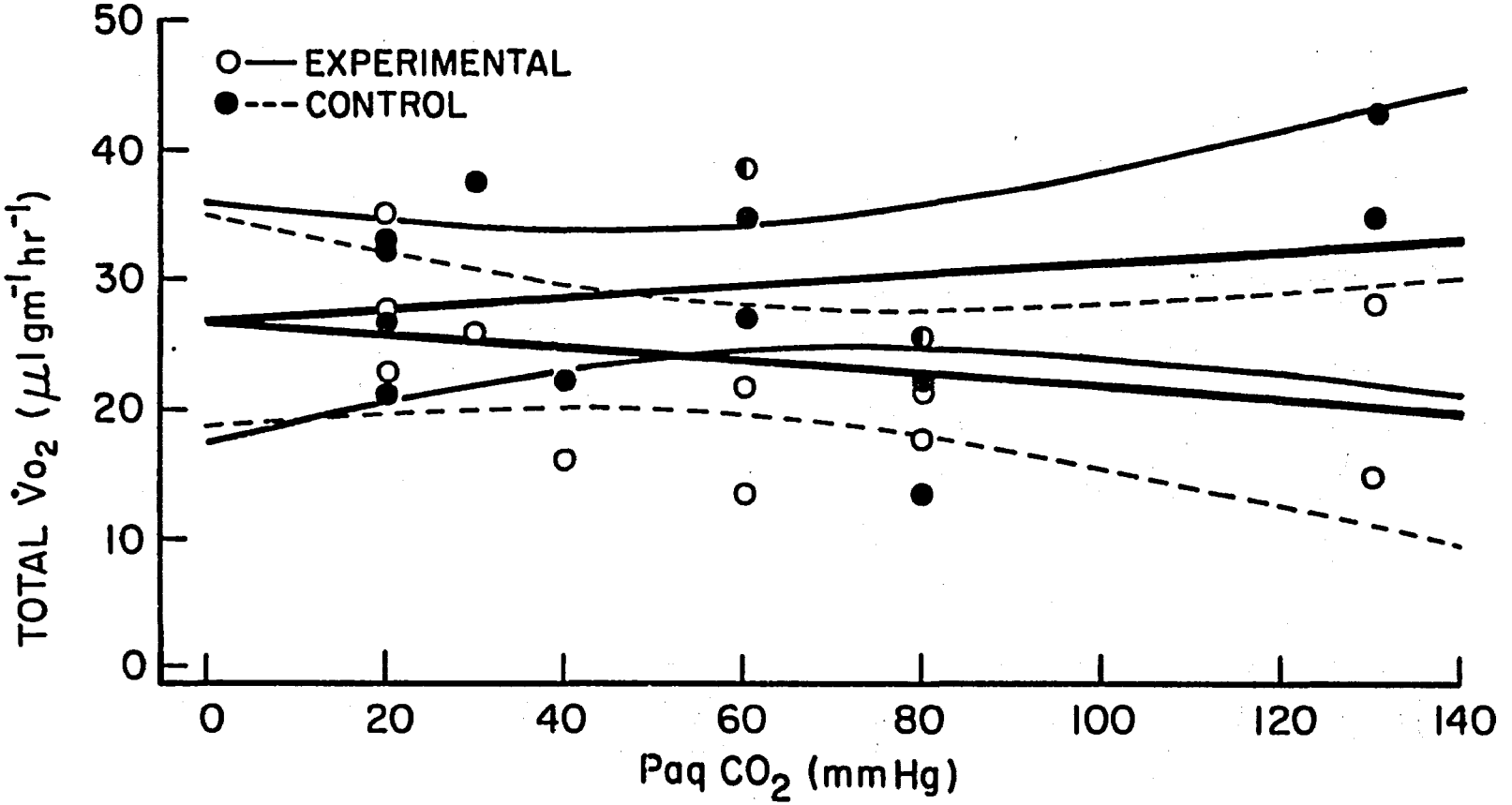
Table 1. Effect of aquatic hypercapnia ($PaqCO_2$) on oxygen consumption ($\dot{V}_{O_2}$) and CO_2 production ($\dot{V}_{CO_2}$) in Necturus maculosus. Values in parentheses are percent of total gas exchange for animals with pulmonary activity. C-Control period. E-Experimental period. B-CO_2 release blocked during the experimental periods.

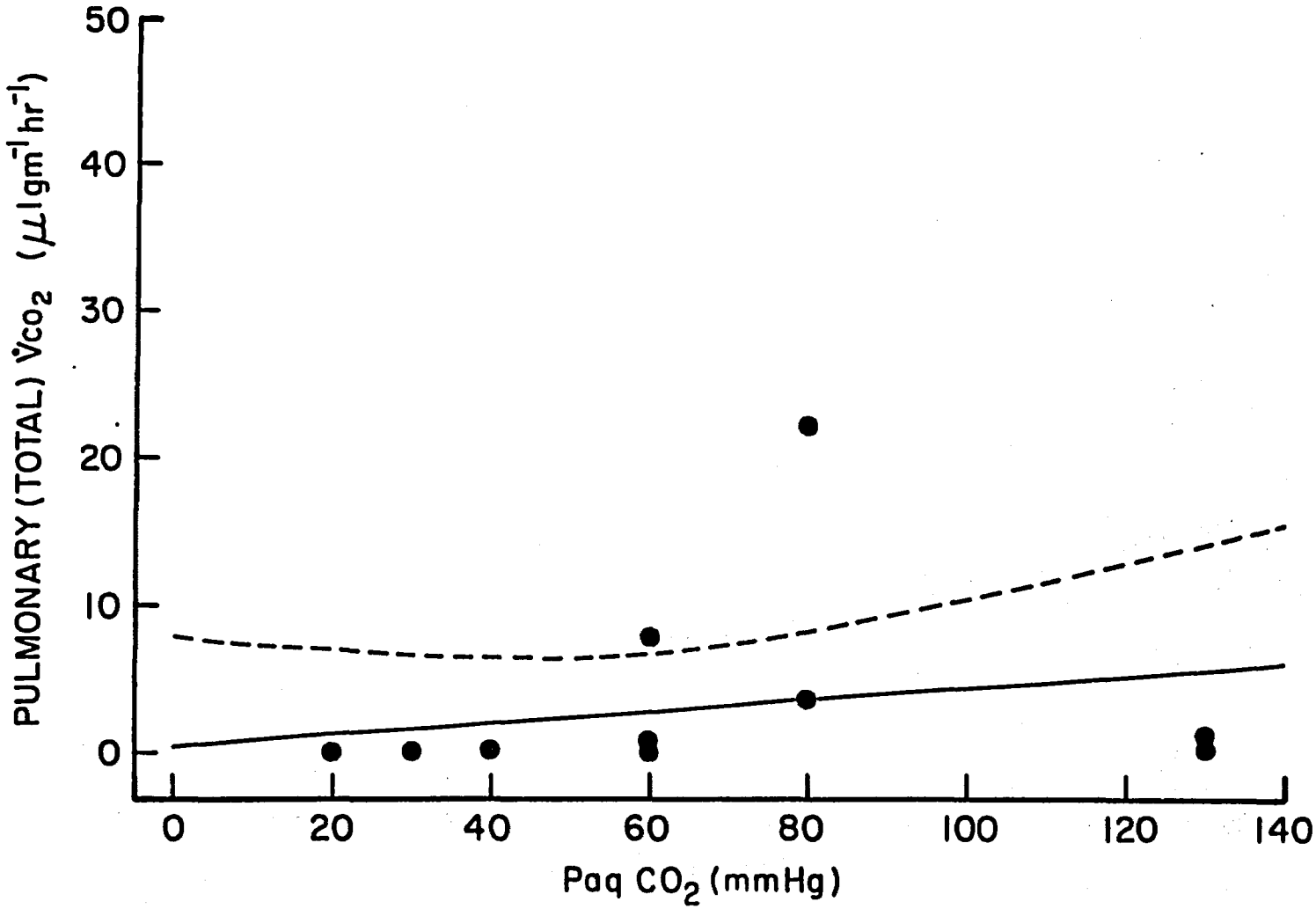
$PaqCO_2$ (mmHg)	BODY WEIGHT (gm)	N		$\dot{V}_{O_2}$ ($\mu lgm^{-1}hr^{-1}$) AQUATIC $\bar{X}\pm SE$	$\dot{V}_{O_2}$ ($\mu lgm^{-1}hr^{-1}$) AERIAL $\bar{X}\pm SE$	Np	$\dot{V}_{CO_2}$ ($\mu lgm^{-1}hr^{-1}$) AQUATIC $\bar{X}\pm SE$	$\dot{V}_{CO_2}$ ($\mu lgm^{-1}hr^{-1}$) AERIAL $\bar{X}\pm SE$	Np	R
20	81.25±6.93	4	C	28.26±2.77 (96.94)	0.66±0.24	1	22.41±3.45	0	0	0.83±0.14
			E	27.70±2.91 (98.72)	0.30±0.25	1	B	0	0	
30	80.00	1	C	37.39	0	0	19.74	0	0	0.53
			E	26.17	0	0	B	0	0	
40	112.00	1	C	21.92	0	0	27.30	0	0	1.24
			E	16.17 (98.65)	0.22±0.22	1	B	0.08±0.08	1	
60	84.00±9.35	3	C	33.21±3.35	0	0	28.35±3.43	0	0	0.89±0.21
			E	24.60±7.39	0	0	B	4.23±3.56	2	
80	93.33±7.00	3	C	20.09±3.63 (95.15)	1.44±0.32	1	49.53±3.67	0	0	2.11±0.01
			E	21.52±2.29 (83.53)	5.88±5.42	2	B	9.10±6.57	2	
130	73.00±7.00	2	C	38.39±3.91	0	0	20.14±8.37	0	0	0.51±0.17
			E	21.48±6.70	0	0	B	0.78±0.21	1	

Figure Captions

Figure 1. Total oxygen consumption ($\dot{V}_{O_2}$) ($\mu l\ gm^{-1}\ hr^{-1}$). Solid symbols are mean values of animals during control periods. Open symbols are mean values of the same group of animals during aquatic hypercapnia ($PaqCO_2$).

Figure 2. Pulmonary CO_2 release ($\dot{V}pCO_2$) as a function of aquatic hypercapnia ($PaqCO_2$). Points are mean values of CO_2 production during each 30 min of experimental period for each animal which registered lung CO_2 loss during hypercapnia.


EXPERIMENTAL
CONTROL
TOTAL $\dot{V}o_2$ (μl gm^{-1} hr^{-1})
50
40
30
20
10
0
0
20
40
60
80
100
120
140
Paq CO_2 (mm Hg)


PULMONARY (TOTAL) $\dot{V}co_2$ (μl gm^{-1} hr^{-1})
50
40
30
20
10
0
0
20
40
60
80
100
120
140
Paq CO_2 (mmHg)

Aerial versus Aquatic Gas Exchange in *Amphiuma tridactylum*, *Siren lacertina*, and *Siren intermedia* in Response to Environmental Hypercapnia

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Abstract

Respiratory surfaces in contact with the aquatic medium are the primary sites of CO_2 loss in the giant salamanders Amphiuma tridactylum, a bimodal breather, and Siren lacertina and Siren intermedia, trimodal breathers, but their relative capacities of aerial and aquatic CO_2 production are unknown. Frequent environmental exposure to partial pressures of CO_2 (P_{CO_2}) up to 60 mmHg may restrict aquatic CO_2 release and and/or produce respiratory acidosis. The presence of well-developed lungs in these animals suggests that a shift to pulmonary CO_2 elimination may be a means of regulating blood acid-base balance. In this study, aerial and aquatic gas exchange was measured in response to aerial (6% CO_2) and aquatic (P_{CO_2} = 40 mmHg) hypercapnia. CO_2 loss increased significantly across lungs and aquatic surface(s) in all 3 species; however, there was no accompanying change in overall CO_2 production ($\dot{V}_{CO_2}$). Concomitant levels of O_2 consumption ($\dot{V}_{O_2}$) indicate that CO_2 loss during experimental hypercapnia is sufficient to maintain the respiratory exchange ratio at control values; in some cases it exceeds this level, thereby including CO_2 from the imposed hypercapnia. Extracellular acidosis resulting from comparable levels of hypercapnia is uncompensated in Amphiuma means and Siren lacertina (Heisler et al., 1982). Thus, the capacity for increased $\dot{V}_{CO_2}$ is apparently not effective for regulating acid-base balance in these species. The presence of gills does not affect the response of gas exchange partitioning to hypercapnia but may influence a role for ion-exchange with the environment in acid-base adjustments. Comparison of these results with those on other amphibians and reptiles suggests that ventilatory mechanisms as well as the ability to readily exchange ions with the environment may underlie the dichotomy between the presence of respiratory acid-base regulation in reptiles and its absence in most amphibians.

KEY WORDS

Amphiuma tridacylum, *Siren lacertina*, *Siren intermedia*, hypercapnia, gas exchange partitioning, acid-base balance, respiratory control, ion-exchange, ventilatory mechanisms, amphibians

Introduction

The mechanisms for physiological control of acid-base balance in ectotherms have been the subject of investigation since the temperature dependence of their blood acid-base state was established (Baumgardner & Rahn, 1967; Howell et al., 1967; Reeves, 1969; Howell et al., 1970). Ventilation and ion-exchange are the two major options for control. In fishes, the limitations of living in a medium of relatively low oxygen availability may not allow enough adjustment of respiration to be effective in acid-base regulation (Randall & Cameron, 1973; Dejours, 1973). Gas exchange appears to be the primary mechanism in air-breathers as diverse as the bullfrog, Rana catesbeiana (Reeves, 1972; Jackson & MacKenzie, 1977), sea turtle, Chelonia mydas (Jackson & Kagen, (1976), tiger salamander, Ambystoma tigrinum (Burggren & Wood, 1981), chuckwalla, Sauromalus obesus (Crawford & Gatz, 1974) and alligator, Alligator mississippiensis (Davies, 1975). An expanding body of work on acid-base balance and ion-regulation between various cellular compartments indicates that changes in ventilation are not a control mechanism in primarily aquatic species. In most amphibians, an increase in temperature causes a shift from an emphasis on aquatic respiration to a greater role for the lungs, particularly in O_2 consumption (Krogh, 1904; Whitford & Hutchison, 1963a,b; Vinegar & Hutchison, 1965; Guimond & Hutchison, 1976). Hypercapnia in one of the respiratory media has been used to investigate ventilatory control in these animals. A largely uncompensated extra-cellular respiratory acidosis occurs in Necturus maculosus, larval Ambystoma tigrinum (Stiffler et al., 1983), Siren lacertina, and Amphiuma means (Heisler et al., 1982) in response to aquatic hypercapnia, and in Typhlonectes compressicauda (Toews & MacIntyre, 1978), Bufo marinus (Boutilier et al., 1979) and the bullfrog (Jackson & Braun, 1979) in response to aerial hypercapnia. In the

bullfrog, aquatic hypercapnia increases pulmonary CO_2 loss, thereby preventing blood acid-base disturbance (Jackson & Braun, 1979). In all but this last case, the significance of the results for respiratory control are implied by the absence of compensation rather than by direct measurement of ventilation.

This study documents the hypercapnic response of aerial versus aquatic respiration in species with different options for gas exchange. Amphiuma tridactylum is a bimodal breather, with cutaneous and pulmonary gas exchangers, while both Siren lacertina and Siren intermedia are trimodal, having gills as well. The lungs of all three species are well-developed for amphibians. All encounter seasonal aquatic hypoxia and hypercapnia, often simultaneously. They are thereby challenged with a reduced oxygen supply as well as respiratory disruption of their acid-base state. Under such conditions, adjustment of gas exchange between aerial and aquatic organs should be useful in understanding metabolic versus physicochemical constraints on respiration.

Materials and Methods

Amphiuma tridactylum, Siren lacertina, and Siren intermedia, purchased from commercial suppliers in Louisiana, were maintained in environmental chambers with an LD 12:12 photoperiod and in tap water at 20°C in open plastic containers for at least 2 weeks prior to experimentation. On the day before an experiment, an individual was transferred to an uncovered 3 L or 6 L (dependent on the size of the animal) glass chamber partially filled with tap water. An identical chamber filled with 2.75 L or 5 L of 0.5mM $NaHCO_3^-$ circulated through an external pump was situated in a temperature-controlled water bath and housed the animal during an experiment. Aquatic oxygen consumption was calculated from periodic measurement of solution P_{O_2} with a Radiometer oxygen electrode, Type E5046, and PHA 927 Gas Monitor. Aquatic CO_2 release was determined from continuous titration of the chamber solution to pH 8.00 with 0.1 N NaOH (Gottlieb & Jackson, 1976) with a Fischer Titrimeter II system. The chamber was sealed and the upper portion ventilated at a constant rate from a pump drawing in room air. The outflow from this breathing space was dried in a tube filled with $CaSO_4$ and passed through a Beckman S-3A Oxygen Analyzer and a Beckman Model 364 Infared Analyzer to measure pulmonary oxygen uptake and CO_2 release, respectively. The aerial and aquatic phases of the chamber were separated by mineral oil to prevent gas transfer between air and water.

Individuals were subjected to two experimental regimens on separate days. Each began with a 3 h control period in which both aerial and aquatic media were normoxic and normocapnic. Then the CO_2 level in the breathing space was raised to 6% or the chamber solution was mixed with a second solution with an elevated P_{CO_2} until the P_{CO_2} in the chamber was at least 40 mmHg.

The significance of these results was determined with one-way analysis of variance on control versus experimental values. The arcsin transform was used on percentages. In appropriate cases, Student's t-test was used to determine level of significance. Values of $P<0.05$ were considered significant.

Results

The three species showed the same patterns of CO_2 loss during both aerial and aquatic hypercapnia. Aerial CO_2 loading, which blocked pulmonary CO_2 release and thereby caused aquatic surfaces to account for 100% of the total CO_2 loss, did not change CO_2 partitioning; aquatic surfaces remained predominant in the percent of total CO_2 eliminated across them (Figs. 1-3, Panel A). The increase in the percent of total CO_2 lost across the aquatic surfaces during aerial hypercapnia was insignificant. Because the pulmonary contribution to CO_2 loss during the control periods was small compared to the aquatic loss, the most striking change in the partitioning of gas exchange occurred when the aquatic surfaces were no longer available for CO_2 release. Under this circumstance, the pattern of CO_2 loss changed completely, from one where nearly all the CO_2 was conveyed across the aquatic surfaces to one where 100% of it was released via the lungs. Thus, aquatic hypercapnia produced an almost complete, and highly significant, shift in CO_2 loss from skin and gills to lungs (Figs. 1-3, Panel B).

The qualitative changes in CO_2 partitioning in response to hypercapnia are a matter of experimental design and are therefore not surprising; but there is a major difference in the effect of an aerial versus an aquatic CO_2 load on O_2 partitioning. An aerial CO_2 load changes the balance between aerial and aquatic O_2 uptake very little, while an aquatic CO_2 load reverses or nearly reverses it. During aerial hypercapnia, the fraction of O_2 taken up across cutaneous and branchial surfaces decreased slightly, but insignificantly, in all 3 species (Figs. 1-3, Panel A). This decrease represented a shift to a dominant role for the lungs only in Amphiuma (Fig. 1, Panel A), where the balance between aquatic and aerial exchange was

close to 50:50. Aquatic surfaces remained predominant in O_2 exchange in the sirenids (Figs. 2 & 3, Panel B). Aquatic CO_2 produced insignificant decreases in the percent of O_2 taken up across the aquatic surfaces (Panel B, Figs. 1-3), but in Amphiuma and Siren intermedia these changes constituted a shift to lungs as the primary site of O_2 uptake.

The large scatter in the data, and the statistical insignificance of the changes in gas exchange partitioning, make it especially imperative to view these changes in the context of absolute quantities of gas exchanged. Aerial CO_2 loading produced a decrease in total $\dot{V}_{CO_2}$ in Amphiuma and an increase in total $\dot{V}_{CO_2}$ in the sirenids (Fig. 4). The opposite trends resulted from aquatic hypercapnia (Fig. 5). Total CO_2 loss increased in Amphiuma but decreased in the sirenids. None of these changes are statistically significant.

In response to aerial CO_2 loading, changes in CO_2 loss were mirrored by the change in total O_2 consumption except in Siren intermedia, where the decrease in total $\dot{V}_{O_2}$ opposes the increase in total $\dot{V}_{CO_2}$ (Fig. 4). The decrease in total $\dot{V}_{O_2}$ exhibited by Siren lacertina during aquatic hypercapnia (Fig. 5) was the only significant change in absolute level of gas exchange ($P<0.025$).

Discussion

These results show that the changes in absolute levels of gas exchange during aquatic hypercapnia render the concomitant redistribution of gas exchange ineffective for acid-base regulation. In Amphiuma and Siren lacertina, absence of compensation for extracellular acidosis resulting from aquatic hypercapnia is in accord with this conclusion (Heisler et al., 1982). In the sirenids, both pulmonary $\dot{V}_{CO_2}$ and its fraction of total $\dot{V}_{CO_2}$ increased with aquatic hypercapnia (Fig.5); however, the concomitant decrease in overall $\dot{V}_{CO_2}$ probably nullifies this pulmonary capacity as an acid-base control mechanism. The decreases in total $\dot{V}_{CO_2}$ match decreases in total $\dot{V}_{O_2}$ (Fig. 5), but because the animals are almost certainly taking in CO_2 across the integument and/or gills (due to reversal of the P_{CO_2} gradient), total $\dot{V}_{CO_2}$ must increase relative to total $\dot{V}_{O_2}$ to maintain acid-base balance. This occurs in Amphiuma, where increases in both fractional and total pulmonary CO_2 loss exceed parallel increases in total $\dot{V}_{O_2}$ (Fig. 5). In this circumstance, increased lung CO_2 loss must alleviate the imposed CO_2 load to some degree; however, blood acid-base parameters show that it is insufficient to relieve extracellular acidosis (Heisler, et al. 1982).

Aerial hypercapnia produces the opposite trends. Both species of Siren exhibit increases in absolute $\dot{V}_{CO_2}$ as well as in the fraction of CO_2 lost across the non-loaded surfaces, i.e. the skin and gills (Fig. 4). In Siren lacertina these changes are slightly greater than the parallel increase in total $\dot{V}_{O_2}$, thereby eliminating some CO_2 from the aerial loading. This is likely the result of an increased transcutaneous and transbranchial P_{CO_2} gradient toward the environment, as blood P_{CO_2} is elevated in the lungs. Siren intermedia must also eliminate some of the

CO_2 taken in from aerial loading, as its increase in total $\dot{V}_{CO_2}$ accompanies a decrease in total $\dot{V}_{O_2}$ (Fig. 4). Although total $\dot{V}_{CO_2}$ actually decreases in Amphiuma during aerial CO_2 loading, this reduction is less than that of total $\dot{V}_{O_2}$, and therefore some of the imposed respiratory CO_2 is being eliminated across the aquatic surfaces. Acid-base parameters have not been measured during aerial CO_2 loading, but the insignificance of the changes in aquatic CO_2 loss make it most unlikely that they achieve any compensation of acidosis. For the same reason, the difference between the decrease in total $\dot{V}_{O_2}$ in Siren intermedia as opposed to the increase in total $\dot{V}_{O_2}$ in Siren lacertina is probably not physiologically meaningful. What is important is that the experimental level of CO_2 production is at least adequate to eliminate the CO_2 generated at the corresponding level of O_2 consumption.

The P_{CO_2} gradient between the animal and its environment is a major determinant of the level of CO_2 production. Therefore, any increase in CO_2 loss during hypercapnia will be influenced by the amount of CO_2 taken in across the gas exchanger(s) in contact with the hypercapnic medium. In Amphiuma, the overall reduction in aerial gas exchange with aerial hypercapnia may minimize the influence of that CO_2 load on blood acid-base balance. Inhibition of aquatic gas exchange with aquatic hypercapnia in the sirenids may have a similar effect. This dichotomy suggests that the different respiratory organs affect the response of bimodal versus trimodal breathers to hypercapnia. Data on chemosensitivity of the various organs would help to clarify this point, but the absence of compensation for hypercapnia-induced extracellular acidosis directs attention to a different issue. Given that the lungs of Amphiuma demonstrate a capacity for increased CO_2 loss, and do so in response to a CO_2 load at or above that which the animals normally encounter in their habitat, what prevents the

exercise of respiratory acid-base control in these animals? The close similiarity of ecology and lung morphology makes this question equally applicable to Siren. Comparison of these 2 species with other air-breathing ectotherms may be helpful in answering this question.

Some compensation of hypercapnia-induced extracellular acidosis has been demonstrated in 5 species of amphibians: Typhlonectes compressicauda (Toews & MacIntyre, 1978), Cryptobranchus alleganiensis (Boutilier & Toews, 1981), larval Ambystoma tigrinum (Stiffler et. al., 1983), Bufo marinus (Boutilier et al., 1979), and the bullfrog, Rana catesbeiana (Jackson & Braun, 1979). The pattern of compensation, as charted on a Davenport diagram, is essentially the same in all but the bullfrog, which was exposed to short-term hypercapnia only. Cryptobranchus and Ambystoma, subjected to aquatic hypercapnia, and Bufo marinus, subjected to aerial hypercapnia, registered some move toward restoration of blood pH to pre-hypercapnic levels only after they were returned to normocapnia. In Typhlonectes, subjected to aerial hypercapnia, elevation of arterial pH began without removal of hypercapnia, but did not achieve the control level until it was removed. In all 4 species, there was no sign of compensation prior to 24 hours after exposure to hypercapnia; pre-hypercapnic levels of plasma bicarbonate $[HCO_3^-]_{pl}$, were not attained.

Respiratory activity (e.g., gill ventilation, swimming to the air-water interface) in all these animals increased during the first several hours of hypercapnia, but was effective in forestalling acid-base disruption only in the bullfrog (Jackson & Braun, 1979). Ventilation may have attenuated the fall in pH and rise in P_{CO_2} in the other species during that initial period, but because compensation began much later, and only with the removal of hypercapnia, respiratory CO_2 loss was not the mechanism for

recovery. Increases in both ventilation ($\dot{V}_E$ in $ml/kg^{-1}min^{-1}$) and the air-convection requirement (V_E/V_{O_2} in ml BTPS/ml STPD) maintain total $[CO_2]$ in the face of temperature dependent $PaCO_2$ changes in a variety of reptiles: the chuckwalla, Sauromalus obesus (Crawford & Gatz, 1974), the turtle, Pseudemys scripta (Jackson & Kagen, 1976), the alligator, Alligator mississippiensis (Davies & Kopetzky, 1976), a monitor lizard, Varanus exanthematicus (Wood et al., 1977), the marine iguana, Amblyrhynchus cristatus (Ackerman & White, 1980), and the desert iguana, Dipsosaurus dorsalis (Bickler, 1981), and one amphibian, the bullfrog, Rana catesbeiana (Jackson & Braun, 1979). In the alligator, this mechanism is also effective in the face of aerial hypercapnia at different temperatures (Davies et al., 1982). This near-dichotomy (the bullfrog being an exception) of the presence of respiratory control in reptiles versus amphibians suggests 2 factors of importance in the regulation of acid-base balance in ectotherms.

One factor is an ability to readily exchange ions with the environment. Amphibians are well-equipped for this task because they possess a well-vascularized integument in direct contact with the aquatic medium, and the ability of their skin to transport ions actively is well-documented (Zerahn, 1956; Snell et al., 1957; Curran et al., 1962,1965; Bricker et al., 1963). Gilled amphibians, including tadpoles (Alvarado et al., 1970; Boonkoom et al., 1971), are doubly well-equipped. Ion-exchange during hypercapnia in Necturus maculosus and larval Ambystoma tigrinum (Stiffler et al., 1983) and Siren lacertina and Amphiuma means (Heisler et al., 1982) supports the hypothesis that ion-exchange with the environment may be involved in regulation of acid-base balance. The latter 3 species do not show significant alteration in ion-exchange nor do they compensate for extracellular acidosis resulting from exposure to aquatic hypercapnia.

Larval Ambystoma tigrinum show both, and their capacity to elevate $[HCO_3^-]$ is impaired when deprived of ions in the external medium (Stiffler, pers. comm.). None of these animals demonstrate an ability to significantly alter respiration during hypercapnia (Braun, 1984). Localization of the ion-exchange is unknown. Ion-exchange mechanisms exist in fish gills (Maetz, 1974), and increased gill ventilation accompanying compensation of extracellular acidodsis during continued aquatic hypercapnia in the dogfish Scyliorhinus stellaris (Randall et al., 1976) encourages investigation of the branchial route. Measurement of GFR in larval Ambystoma during hypercapnia indicates that a renal contribution cannot be ruled out (Stiffler & Hicks, 1980).

The other factor influencing acid-base balance in ectotherms is their mechanism of lung ventilation. Assessment of the utility of various respiratory organs has involved consideration of their morphology. It was therefore thought that Amphiuma and Siren, similar to reptiles in their well-developed (septate and well-vascularized) lungs, would be capable of respiratory acid-base regulation, while other aquatic forms, such as Necturus, larval Ambystoma, and Cryptobranchus, with simple lungs, would not. Lung morphology is certainly a factor, but the effectiveness of filling and emptying the lungs must also be important. Buccal pumping, the mechanism in most amphibians, is considered an inefficient means of gas transfer, and has been cited as the reason why so little CO_2 is eliminated acrosss the lungs of most amphibians during normal circumstances (Gans, 1970). This reasoning has been superseded by measurement of CO_2 loss at different temperatures and during hypercapnia, and by increased appreciation of the advantage of the relative energy-effectiveness of aquatic exchange. However, the ability of the lungs to handle more CO_2 than they do under normal conditions is not an argument for their

effectiveness under any or all circumstances. If acid-base balance can be maintained by ion-exchange with the environment, and this option is available and energy-efficient, pulmonary exchange is an inferior option. Pulse-pumping has been shown to be particularly inefficient in Amphiuma and Siren lacertina where 2 buccal movements are needed for exhalation and several more are required to fill the lungs (Martin & Hutchison, 1979). The discrepancy between the presence of respiratory acid-base control in the bullfrog and its absence in Amphiuma and Siren is probably not attributable to this small difference in their respiratory mechanics. Other factors such as ontogeny and ecology must be considered.

The bullfrog metamorphoses from a completely aquatic and gilled larva to a gill-less animal capable of extended survival on land. This transformation involves exposure to a "physiological hypercapnia" (Stiffler et al., 1983), as the increased reliance on gas-exchange in air, with a CO_2 capacitance much less than that of water, increases blood P_{CO_2} and $[HCO_3^-]$. Amphiuma and Siren possess lungs throughout their life history, and although lungs are critical in meeting oxygen requirements during severe conditions (Guimond & Hutchison, 1976) they are exchanging a gas readily available in the aerial medium. The concentrations of dissolved oxygen and CO_2 vary seasonally and with the presence of the water hyacinth, Eichhornia crassipes, in the habitat of Amphiuma and Siren (Ultsch, 1976). These variations produce distinct mirohabitats delineated by depth, vegetation cover, and temperature (Ultsch, 1976). Fish in the hyacinth community select among these microhabitats; but 3 species of Siren, including the 2 in the present study, avoid the open water, where dissolved gases favor aquatic respiration, and exhibit no preference for microhabitats in the covered areas (Ultsch, 1976). Apparently, other factors, such as predator avoidance, influence the distribution of the sirenids more than do the

levels of dissolved respiratory gases. Thus, the morphological and physiological characteristics of the respiratory organs in animals with more than one gas-exchanger are not the sole determinants of the partitioning of gas exchange.

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Table Heading

Table 1. Effects of aerial and aquatic hypercapnia on aerial and aquatic gas exchange in Amphiuma, Siren lacertina, and Siren intermedia. Oxygen consumption ($\dot{V}_{O_2}$) and CO_2 production ($\dot{V}_{CO_2}$) are given in $\mu l gm^{-1} hr^{-1}$. Figures are mean values of animals under each regimen.
C-Control period. E-experimental period.
B-CO_2 loss blocked during experimental period.

Table 1. Effect of aerial and aquatic hypercapnia on aerial and aquatic gas exchange in Amphiuma tridactylum, Siren lacertina, and Siren intermedia. Oxygen consumption ($\dot{V}_{O_2}$) and CO_2 production ($\dot{V}_{CO_2}$) are given in $\mu l gm^{-1} hr^{-1}$. Figures are mean values of animals under each regimen. C-Control period. E-Experimental period. B-CO_2 release blocked during the experimental periods.

	Aerial CO_2 Loading				Aquatic CO_2 Loading			
	$\dot{V}_{O_2}$		$\dot{V}_{CO_2}$		$\dot{V}_{O_2}$		$\dot{V}_{CO_2}$	
	Aquatic	Pulmonary	Aquatic	Pulmonary	Aquatic	Pulmonary	Aquatic	Pulmonary
				Amphiuma tridactylum				
C	6.39±1.26	6.17±2.04	9.90±1.95	2.22±1.06	4.81±1.05	3.05±0.88	7.44±1.63	0.44±0.14
E	3.24±0.58	4.14±1.23	11.38±1.85	B	2.89±0.71	7.36±3.03	B	15.53±7.65
				Siren lacertina				
C	11.20±4.98	3.85±1.06	15.79±7.01	0.63±0.21	11.18±0.70	3.19±0.47	15.76±0.98	0.46±0.17
E	14.09±6.98	2.34±0.28	20.01±8.50	B	4.87±2.00	2.86±1.64	B	7.86±4.43
				Siren intermedia				
C	8.39±2.88	1.90±0.43	11.84±4.06	0.35±0.11	3.98±1.18	1.49±0.42	5.62±1.76	0.23±0.05
E	6.34±1.49	2.35±0.62	16.15±8.22	B	1.31±0.79	1.78±0.56	B	1.35±0.69

Figure Captions

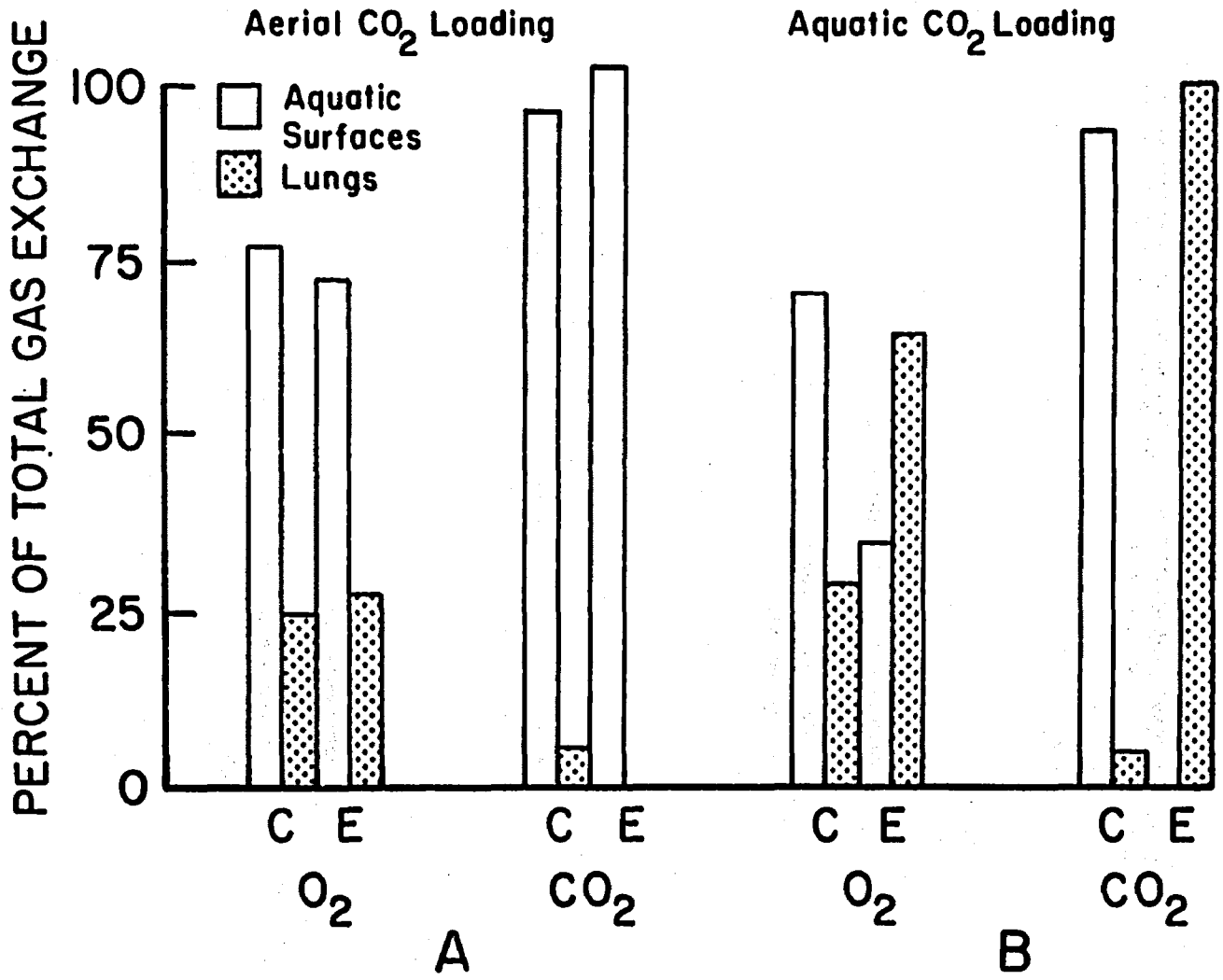
Figure 1. Percentage of total gas exchange across aquatic (cutaneous) and pulmonary surfaces in Amphiuma tridactylum during aerial (Panel A) and aquatic (Panel B) hypercapnia. C-control period. E-experimental period.

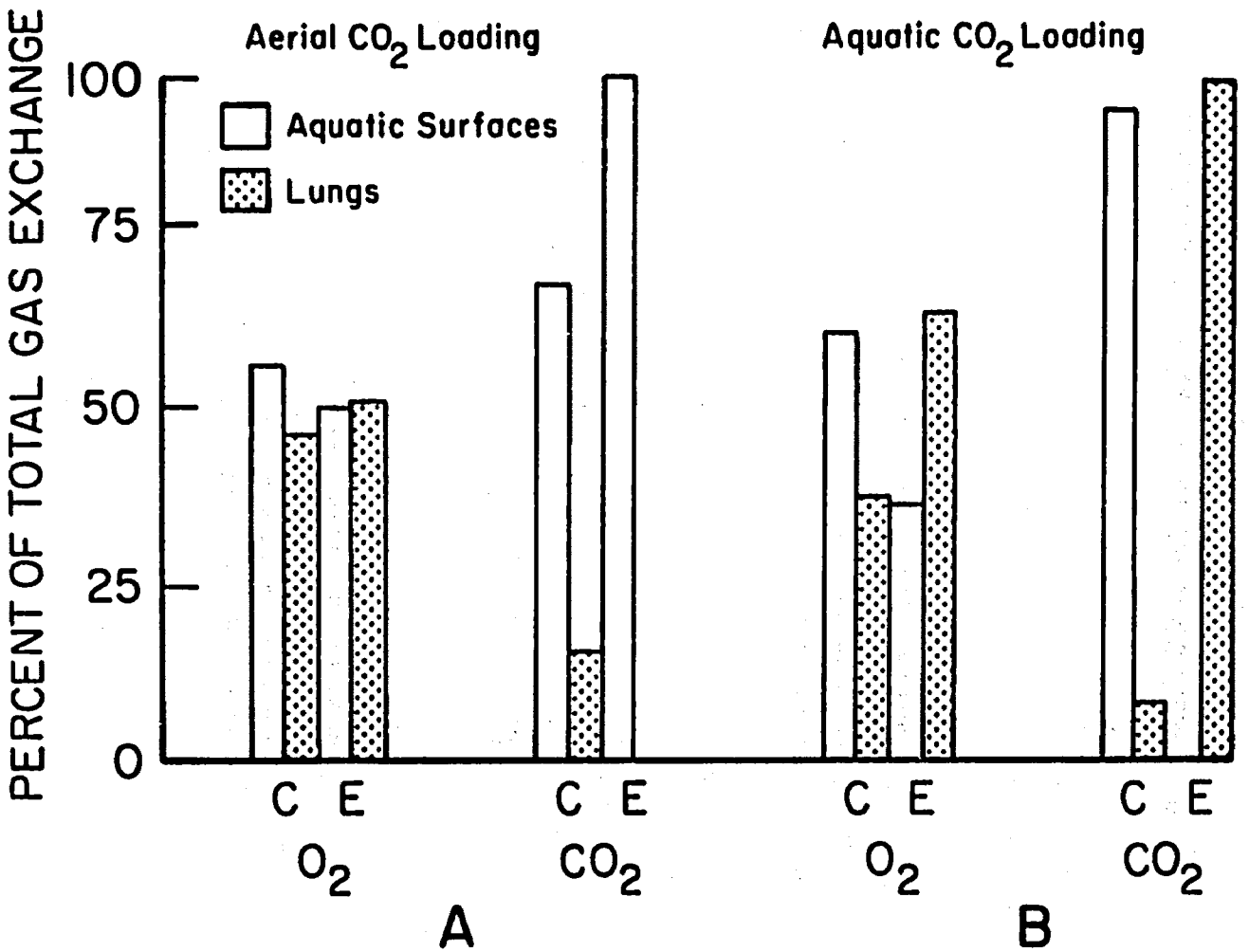
Figure 2. Percentage of total gas exchange across aquatic (cutaneous and branchial) and pulmonary surfaces in Siren lacertina during aerial (Panel A) and aquatic (Panel B) hypercapnia. C-control period. E-experimental period.

Figure 3. Percentage of total gas exchange across aquatic (cutaneous and branchial) and pulmonary surfaces in Siren intermedia during aerial (Panel A) and aquatic (Panel B) hypercapnia. C-control period. E-experimental period.

Figure 4. Oxygen consumption ($\dot{V}_{O_2}$) and CO_2 production ($\dot{V}_{CO_2}$) during aquatic hypercapnia in Amphiuma, Siren lacertina, and Siren intermedia. C-control period. E-experimental period.

Figure 5. Oxygen consumption ($\dot{V}_{O_2}$) and CO_2 production ($\dot{V}_{CO_2}$) during aerial hypercapnia in Amphiuma, Siren lacertina, and Siren intermedia. C-control period. E-experimental period.


Aerial CO_2 Loading
Aquatic CO_2 Loading
PERCENT OF TOTAL GAS EXCHANGE
100
75
50
25
0
Aquatic Surfaces
Lungs
C E
C E
C E
C E
O_2
CO_2
O_2
CO_2
A
B


Aerial CO_2 Loading
Aquatic CO_2 Loading
Aquatic Surfaces
Lungs
PERCENT OF TOTAL GAS EXCHANGE
100
75
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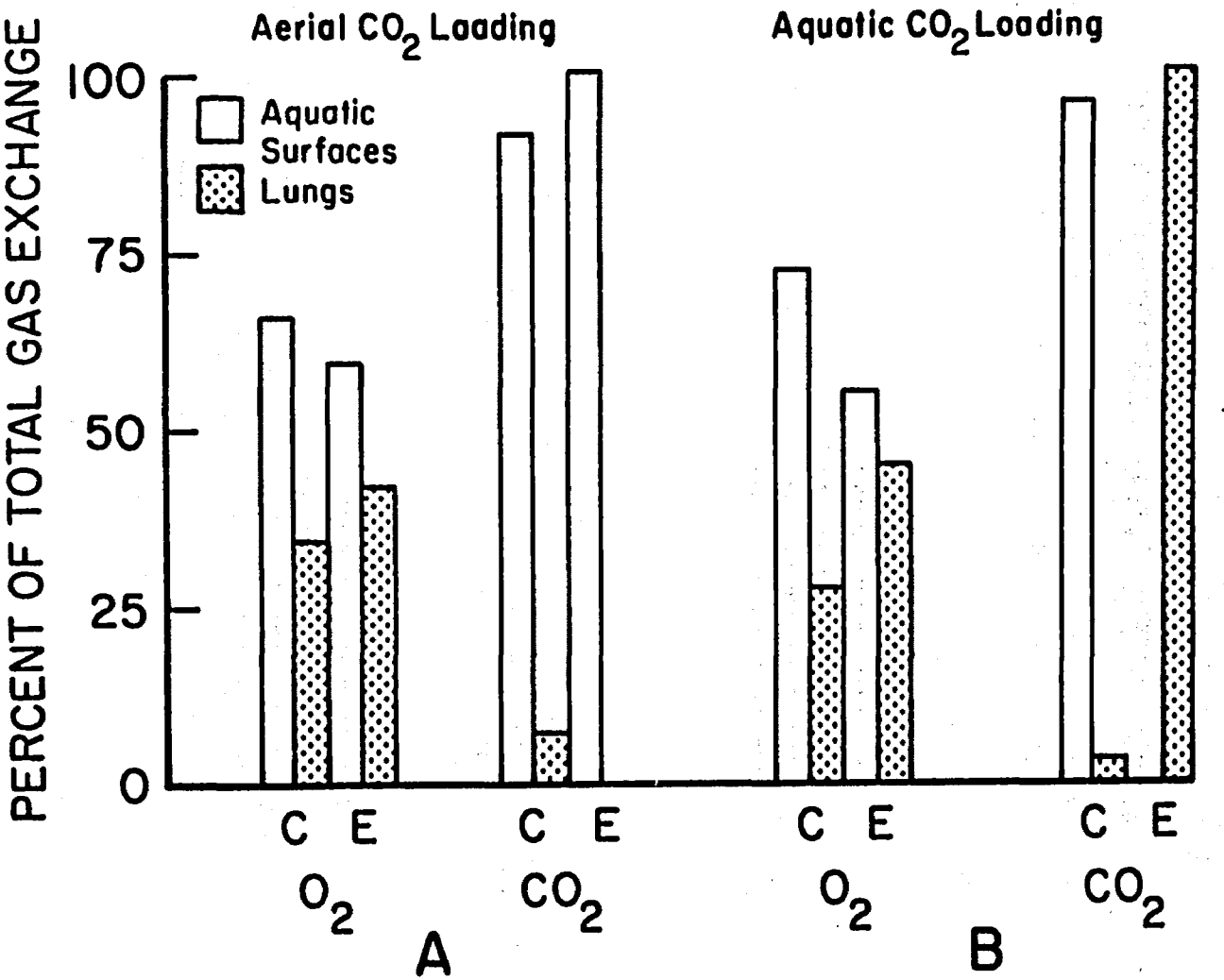
C E
C E
O_2
CO_2
O_2
CO_2
A
B


Aerial CO_2 Loading
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PERCENT OF TOTAL GAS EXCHANGE
100
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Aquatic Surfaces
Lungs
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CO_2
O_2
CO_2
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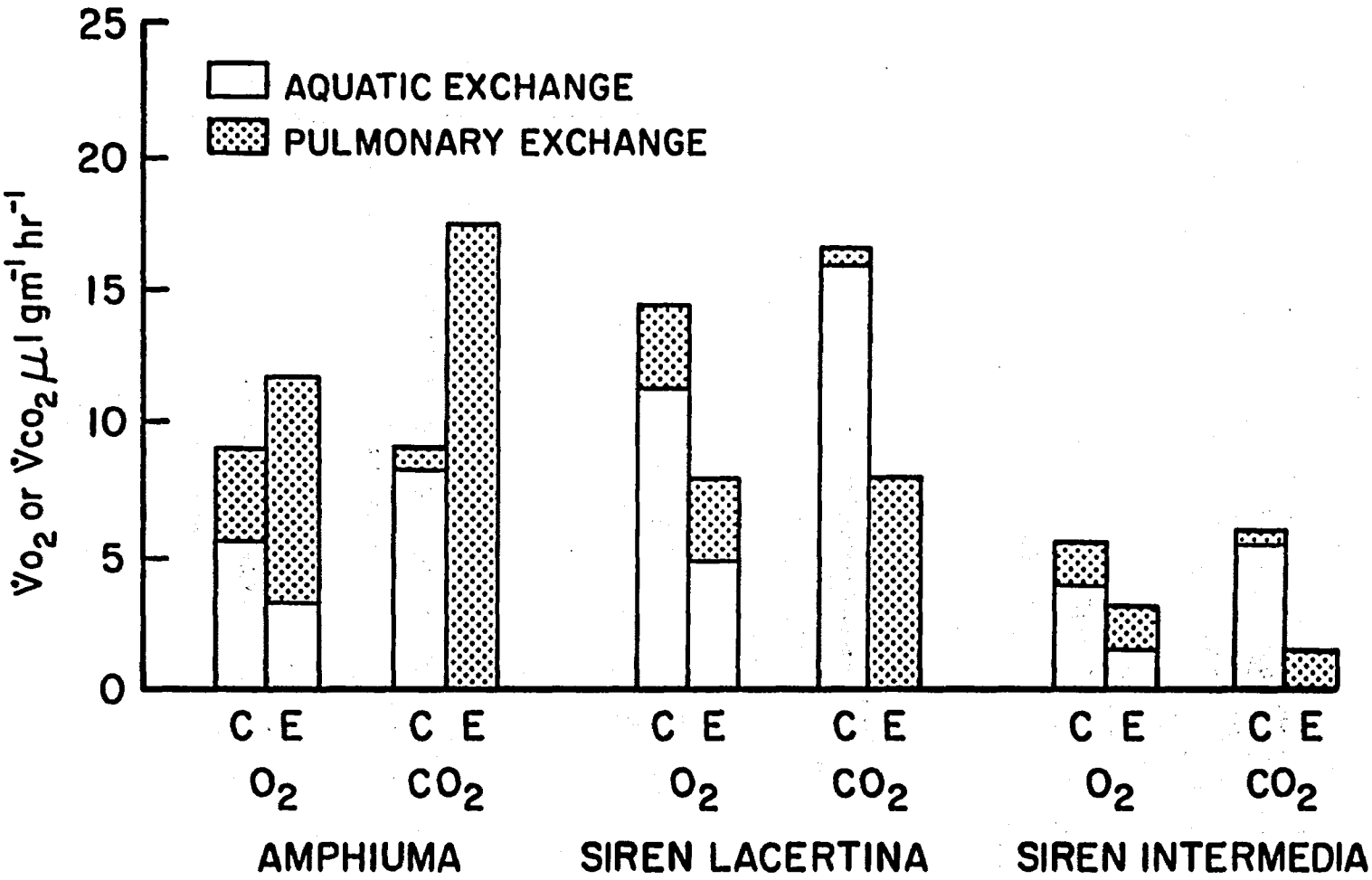
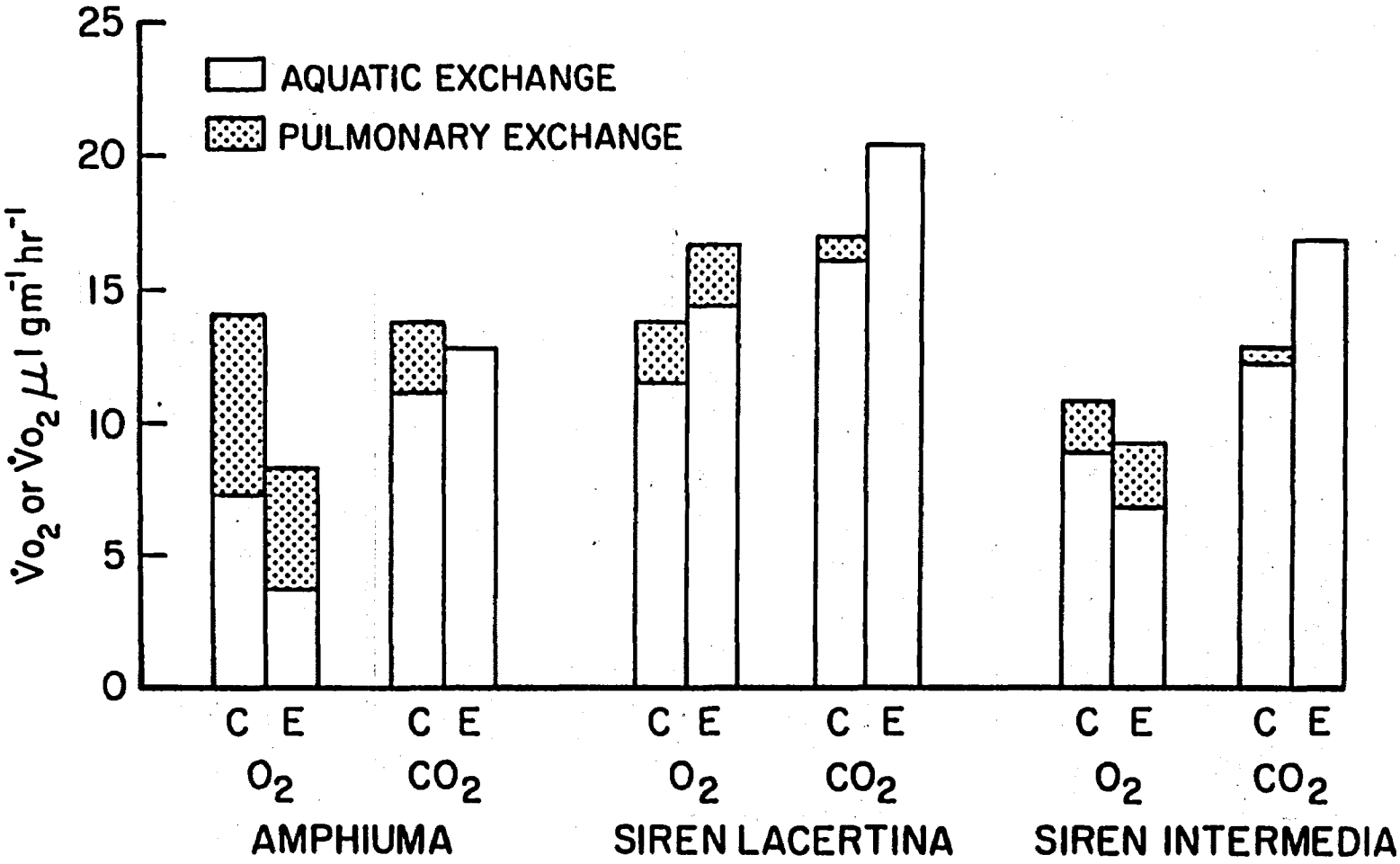
B


25
20
15
10
5
0
AQUATIC EXCHANGE
PULMONARY EXCHANGE
$\dot{V}o_2$ or $\dot{V}co_2$ μl gm^{-1} hr^{-1}
C E
O_2
C E
CO_2
C E
O_2
C E
CO_2
C E
O_2
C E
CO_2
AMPHIUMA
SIREN LACERTINA
SIREN INTERMEDIA


25
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AQUATIC EXCHANGE
PULMONARY EXCHANGE
$\dot{V}o_2$ or $\dot{V}o_2$ μl gm^{-1}hr^{-1}
C E
O_2
C E
CO_2
C E
O_2
C E
CO_2
C E
O_2
C E
CO_2
AMPHIUMA
SIREN LACERTINA
SIREN INTERMEDIA