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MEASUREMENT OF H+ EXCRETION BY THE GILL IN RESPONSE TO pCO_2 ELEVATION IN S. acanthias

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The response of S. acanthias to elevated pCO_2 is of considerable interest both on a comparative basis and because it provides insight into special mechanisms of acid-base compensation available to gill breathing animals. Renal titratable acid excretion is relatively constant at about 1 mEq/kg/24 hrs (J. J. Cohen, J. Cell and Comp. Physiol. 53:205, 1959), urine ammonium excretion is low, and acid administration does not alter urinary pH significantly (E. D. Robin, G. P. Rodnan, and M. H. Audrus, Bull. MDIBL 4:65, 1962) so gill mechanisms for acid-base regulation appear to be very important. Rapid elevation of seawater pCO_2 was chosen as a means of studying acute and subacute acid-base alterations and accompanying hemodynamic changes.

Healthy fish were arranged for recording of cardiac output ($\dot{Q}_B$), ventral, and dorsal-aortic pressures, etc. as described earlier (E. C. Peirce, II, et al, Bull. MDIBL 7:40, 1967). Fresh seawater was supplied at about 2200 ml/min by short tubes placed through the spiracles. The water was passed through a small bubble "oxygenator" (Seals Corp.) that could be supplied with a mixture of 2 to 5% CO_2 in air (Figure 1). In a few experiments 3% or 4% CO_2 in oxygen was employed. (The "oxygenator" utilizes a porous ceramic plate to disperse gas in fine bubbles and is an excellent device to use for equilibration of a gas with a fluid. Excess gas is given off through a chimney). Total reaction time at a gas flow of 3L/min was less than 3 seconds. Equilibration

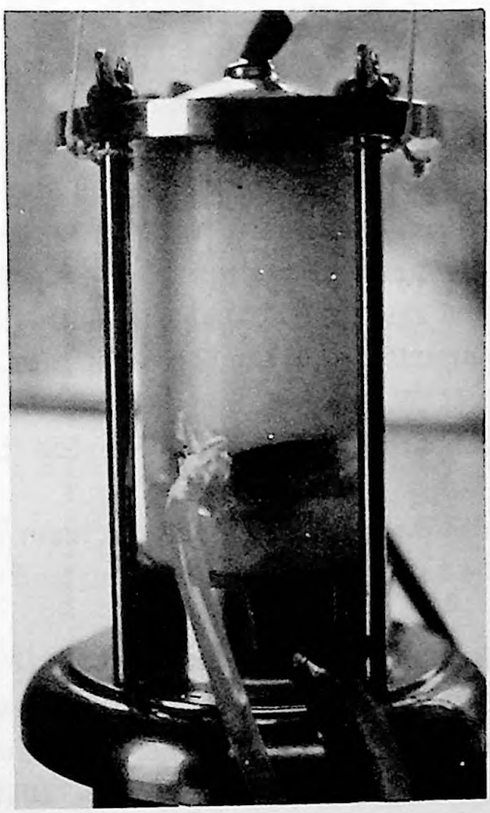


Figure 1

was 67% complete in 20 seconds, providing almost square wave changes of seawater pCO_2 and pH (Figure 2).

The pH of dorsal aortic blood (pH_A) (and of a few paired ventral aortic samples) was measured at 12 to 15°C using an Astrup micro pH electrode (Radiometer), and the values were corrected for the temperature of the fish (0.008 pH units/1°C). A portion of each blood sample was equilibrated with a CO_2 - O_2 mixture of known pCO_2 (between 4.5 and 9.4 mm Hg) at 15°C in an

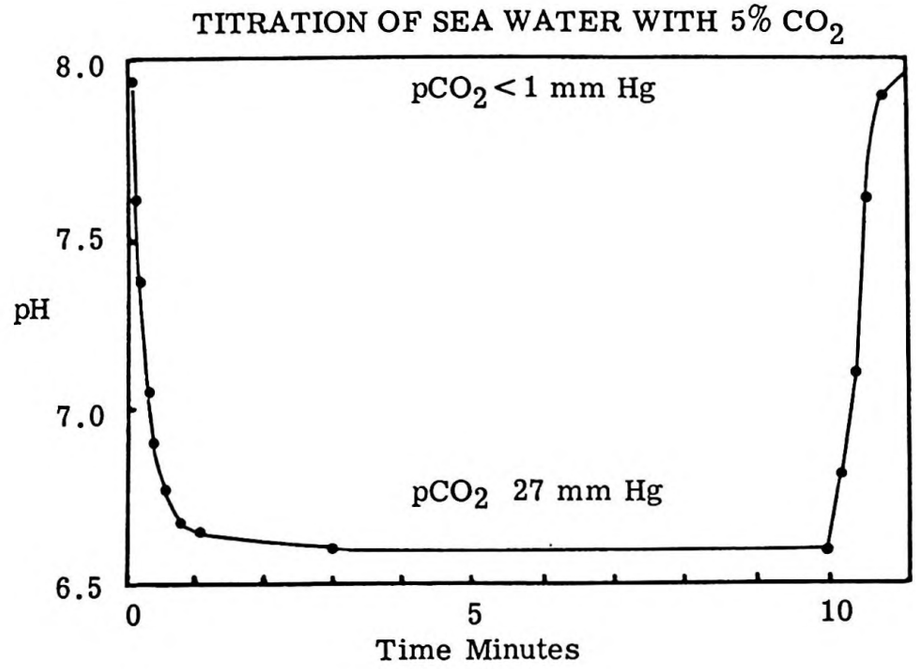


Figure 2

Astrup microtonometer and the pH (pH_E or equilibrated pH) determined. This value does not require temperature correction. All pH measurements were in duplicate, triplicate, or quadruplicate. The pCO_2 and non-carbonic-acid level (NCA) of each sample was estimated from the S. acanthias blood nomogram previously described (E. C. Peirce, II, Bull. MDIBL 7:36, 1967). The hematocrit was measured in duplicate or triplicate by a standard micro method.

The rate of compensation to the acid (pCO_2) load was estimated by two methods.

(a) NCA was assumed to be distributed in the entire extracellular fluid space, which is about 20% of body weight (J. W. Burger, Bull. MDIBL 7-5:5-9, 1967). The change of NCA with time (ΔNCA) could then readily be converted to acid loss in mEq/Kg/hr (Table 1).

(b) The NCA difference across the gill was calculated from the paired ventral and dorsal aortic blood samples. The rate of NCA loss was derived using the simultaneously measured $\dot{Q}_B$ (Table 2).

On CO_2 administration the pH_A fell rapidly and profoundly (Figure 3). The level of depression at 10 min was 0.39 (± 0.018 SEM) pH units for 24 titrations with 2% or 3% gas and 0.64 (± 0.20 SEM) pH units for 22 titrations with 5% CO_2. These changes were associated with marked hemodynamic alterations which are discussed in this bulletin, abstract #20. The pCO_2 of dorsal aortic blood rose approximately to 10 to 15 mm Hg with 2 or 3% CO_2 and to 18 to 28 mm Hg with 5% gas. Initially the pH_E was depressed below the control level indicating a rise in NCA value

Table 1

ESTIMATION OF GILL EXCRETION OF NON-CARBONIC ACID DURING CO_2 ADMINISTRATION BY CHANGE IN ARTERIAL LEVEL WITH TIME (ΔNCA)

Exp. #	CO_2 %	Wt. Kg.	Time* min	ΔNCA		NCA out mEq/Kg/hr
				mEq/L	mEq/Kg	
6	4	3.3	45	6.3	1.26	1.68
15	3	2.8	52	6.9	1.38	1.59
16	3	1.8	60	1.0	0.20	0.20
17	3	1.3	105	12.8	2.56	1.46
19	2	2.9	45	2.4	0.48	0.64
20	5	2.7	110	6.5	1.30	0.71
22	2	1.6	47	2.8	0.56	0.71
41	2	3.9	110	3.0	0.60	0.33
Mean		2.5	72	5.21	1.04	0.92
S.D.				3.53	0.71	0.54
S.E.M.				1.24	0.25	0.19

*Period over which ΔNCA measured.

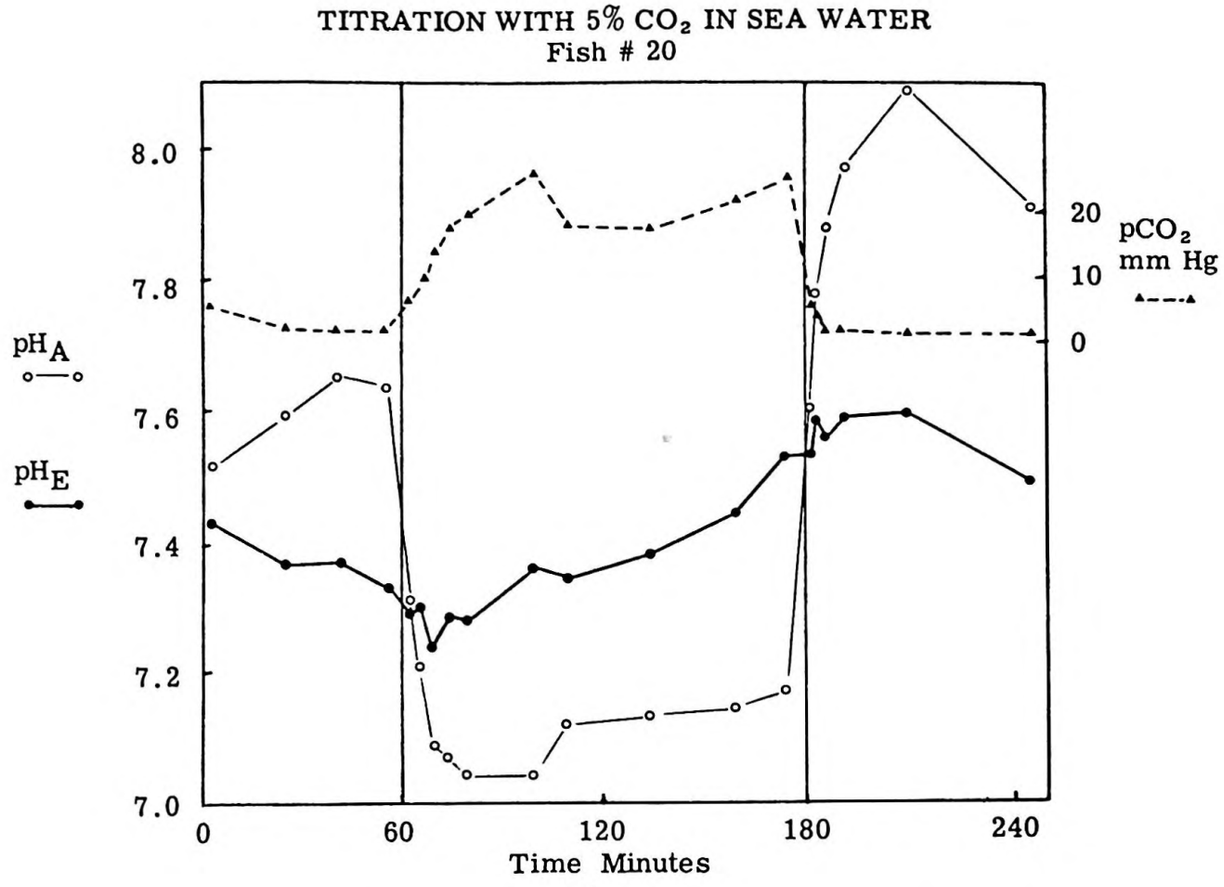
Table 2

ESTIMATION OF GILL EXCRETION OF NON-CARBONIC ACID DURING CO_2 ADMINISTRATION BY MEASUREMENT OF DIFFERENCE ACROSS GILL

Exp. #	CO_2 %	Wt. Kg.	$\dot{Q}_B$*	NCA_V†	NCA_A†	NCA_{V-A}†	NCA out mEq/Kg/hr
20	5	2.7	0.57	6.7	4.3	2.4	1.40
			0.60	2.5	2.5	0.0	0.00
			0.64	2.2	0.5	1.7	1.09
22	2	1.6	1.44	3.2	2.0	1.2	1.73
			1.44	1.7	1.6	0.1	0.14
			1.39	3.4	2.3	1.1	1.53
			1.20	2.6	1.2	1.4	1.68
			1.34	2.3	2.1	0.2	0.27
			1.22	1.8	0.8	1.0	1.22
			1.27	0.9	-0.5	1.4	1.78
41	2	3.9	1.19	4.4	3.4	1.0	1.19
			1.41	4.7	3.6	1.1	1.55
			1.40	4.1	3.7	0.4	0.56
			1.47	4.8	5.1	-0.3	-0.44
			1.29	4.2	3.7	0.5	0.65
			1.51	3.5	1.6	1.9	2.87
			1.38	4.3	3.5	0.8	1.10
17 observations		Mean	1.22	3.37	2.44	0.94	1.08
		S.D.	0.30	1.40	1.44	0.70	0.79
		S.E.M.	0.07	0.33	0.35	0.17	0.19

*L/Kg/hr.

†Non-carbonic acid mEq/L: V, ventral aortic; A, dorsal aortic blood.



pH_A is the pH of the arterial blood. pH_E represents the pH of the same blood sample equilibrated with 1.16% CO_2.

Figure 3

(decrease in buffer concentration). With time, the pH_A and the pH_E rose but did not return to control values in periods of observation as long as 2 hours. These changes represented a decrease in NCA values at a constant pCO_2. On returning to fresh seawater there was a marked overshoot of pH_A, to well beyond the control value, and a further rise in pH_E. Subsequently, in fish observed long enough, both pH_A and pH_E returned toward control levels.

The rate of acid elimination, in 8 fish, calculated by change of NCA during CO_2 administration, was 0.92 (± 0.19 SEM) mEq/Kg/hr (Table 1). The average, in 3 fish, of 17 values derived from the concentration difference across the gill was 1.08 (± 0.19 SEM) mEq/Kg/hr (Table 2).

The initial pattern of response to a CO_2 load in S. acanthias is very similar to that seen in mammals. A given percent change in pCO_2 produces a roughly comparable pH move (E. C. Peirce, II, loc cit). A fourfold rise in pCO_2 in the dog from 40 to 160 mm Hg causes a pH drop of approximately 0.4 pH units which is about what occurs in S. acanthias with a rise from 3 to 12 mm Hg. Since generated HCO_3^- is distributed in the entire extracellular space, there is at first a loss of buffer from the vascular compartment which is manifested by a drop in pH_E. In the mammal only slow renal compensation is available and the limit of compensation is for a pCO_2 of about 65 mm Hg (less than 65% above normal). In the fish there is no such limitation. Free swimming fish with pCO_2 elevations to approximately 12 mm Hg (400% increase), have

pH_A values not significantly different from their controls at 2 hrs (E. D. Robin, personal communication, 1968). Therefore, although our excretory values for acid are high, they are probably less than maximum, the limitation being the conditions of restraint imposed on the fish. The marked overshoot of pH_A and pCO_2 on returning to gill perfusion with fresh seawater suggests either that regulatory chemoreceptors are pre gill (venous) structures or that there is a protracted disturbance of gill perfusion mechanisms as a result of the prolonged high pCO_2.

The values obtained for NCA excretion during high pCO_2 states are close to those found after HCl administration by Robin and Murdaugh (Table 3). Excretion rates, using method (a)

Table 3

ACID-BASE CHANGES IN S. acanthias FOLLOWING ADMINISTRATION OF 3 mEq OF HCl

Time minutes	pH	HCO_3^- mEq/L	pCO_2 mm Hg	NCA mEq/L
Control	7.63	6.59	3	3.0
15	7.09	1.95	4	11.7
30	7.35	3.59	4	7.0
60	7.39	-	-	-
120	7.67	4.42	2	4.2

Data of Robin, E. D., and Murdaugh, H. V., Sharks, Skates and Rays, J. Hopkins Press, 1967, p. 253. Non-carbonic acid (NCA) values added.

above and the estimated NCA values calculated from their data, were 3.76 mEq/Kg/hr for the period from 30 to 120 minutes and 0.43 mEq/Kg/hr for the period from 30 to 120 minutes. For 4 examples cited by the above authors, the average overall rate was 0.86 (± 0.46 SEM) mEq/Kg/hr. The change in concentration of bicarbonate, 0.28 mEq/Kg/hr for the 4 fish, would underestimate the rate of acid excretion. The HCO_3^- is affected by the isohydric shift and the pCO_2 level, neither of which affect the ΔNCA value significantly.

The gill thus can compensate for both a CO_2 load and a fixed acid load by excreting NCA in amounts at least as great as 1 mEq/Kg/hr. Although S. acanthias does not control pH_A within the same narrow range as do mammals, he can accomplish rapidly with his gills what it takes the mammal a long time to do with his kidney. This can certainly explain the relative "disinterest" that the kidney of S. acanthias seems to have in the correction of acidemia.

The mechanism of gill H+ excretion has not been elucidated. Ammonia excretion is inadequate to explain the magnitude of NCA loss. Excretion of trimethylamine and trimethylamine oxide also appear unlikely to explain the large ΔNCA values. The gills are epithelial structures and transport of H+, actively or by ion exchange, would be possible mechanisms (C. A. M. Hogben, personal communication, 1968).

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