1968 #20

CARDIOVASCULAR RESPONSES TO CO_2 ADMINISTRATION IN S. acanthias

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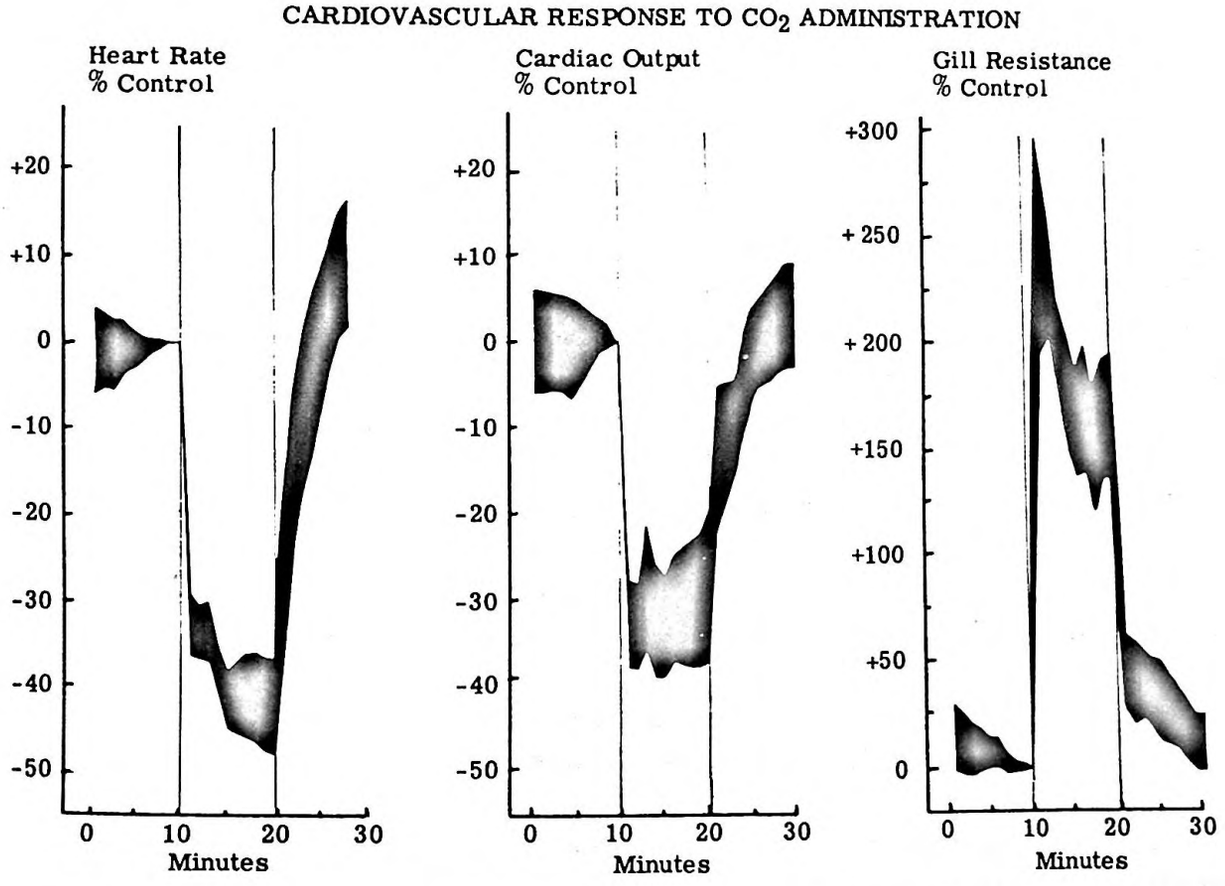
While studying acid-base balance in the dogfish (described in abstract #28) several surprisingly large cardiovascular responses to CO_2 administration were observed. The following study was done to elucidate the nature of these responses.

Twenty fish were placed dorsally in a trough of seawater with their gills continuously perfused with fresh seawater through their spiracles. Cardiac output measurements ($\dot{Q}_B$) were made with an electromagnetic flowmeter as described in Bull. MDIBL 7:40, 1967. In 14 fish ventral aortic pressure (VAP) was recorded from a PE200 polyethylene catheter which was passed through the wall of the apex of the ventricle and threaded into the ventral aorta. Dorsal aortic pressures (DAP) were recorded from an 18 gauge Tuohy needle driven through the ventral midline of the tail into the dorsal aorta. Gill resistance (R_G) was calculated as $R_G = (VAP-DAP)/\dot{Q}_B$. Systemic resistance ($R_S$) was calculated as $R_S = DAP/\dot{Q}_B$, assuming a venous pressure of 0.



The above curves are a composite of results from 12 animals given 2-5% CO_2 in sea water for 10 minute intervals. Values are given as % of control and include the confidence limits.

Figure 1.

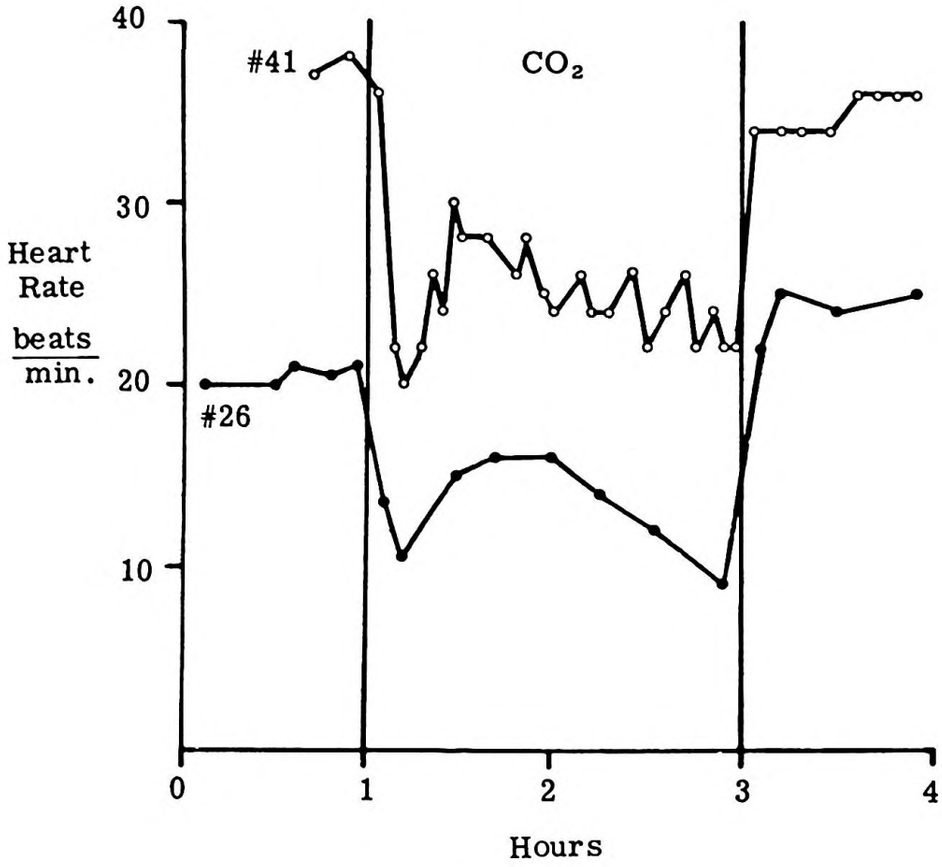


Figure 2

In 6 fish ventricular pressures were recorded from a 20 gauge needle passed into the ventricle through the wall of the apex. The needle was connected to an SF-4 ultra-low volume Statham transducer. The output signal from the transducer was split so that both ventricular pressure and its first derivative were recorded simultaneously. dP/dt was obtained from an RC differentiating circuit with a time constant of 50 msec and linearity between 0.1 and 6 cps. By dividing the peak of the curve of the first derivative of pressure during isovolumetric systole (Max dP/dt) by the simultaneous instantaneous ventricular pressure (IP), an empiric index of contractility ($\frac{\text{Max dP/dt}}{\text{IP}}$) described by Krayenbuhl (Cardiologia 47:95-112, 1965) was calculated.

Complete or partial vagotomies were performed on 3 fish from the ventral aspect utilizing an oro-pharyngeal approach. Removing the epithelial tissue and shaving off underlying cartilage with a high speed air drill between the internal openings of the spiracles gave access to the medulla and the accompanying cranial nerves. The vagi were sectioned at their point of juncture with the medulla. Atropine was given to block the vagi in 6 fish via the needle in the dorsal aorta in doses of 0.8 to 2.0 mg/kg.

CO_2 administration was carried out for 10 minute or 2 hour intervals by perfusing the gills with seawater which had been equilibrated with 2-5% CO_2 in air.

A precipitous fall in dorsal aortic blood pH, averaging 0.51 ± 0.15 SD, on administration of CO_2 was accompanied by dramatic changes in heart rate, cardiac output, and gill resistance as seen in Figure 1. The large increase in gill resistance resulted not only from a lower flow, but also from a 10% elevation in VAP and an 8% decline in DAP. R_S rose 46% during the period of

CO_2 administration. The control values of Max dP/dt/IP (11.34 ± 0.39 SEM) were relatively low in comparison with values generally found in the dog (40 to 60). During 10 minute intervals of CO_2 administration Max dP/dt/IP dropped to 8.87 ± 1.10 SEM or to about 75% of the control.

The heart rate response persisted over a 2 hour period of CO_2 administration (Figure 2). The other cardiovascular responses were also sustained but values did not always return to control levels on cessation of CO_2 administration.

The effects of atropine and vagotomy on the response to CO_2 administration are seen in Table 1. The first column of results are control responses and include the data shown graphically in Figure 1. Lower doses of atropine and partial vagotomy reduced the response. Larger doses of atropine and complete vagotomy abolished cardiovascular changes. There was no measurable decrease in contractility in atropinized fish.

Table 1

EFFECT OF ATROPINE AND VAGOTOMY ON CARDIOVASCULAR RESPONSE TO CO_2 ADMINISTRATION

Atropine*		0.8 mg/Kg	2.0 mg/Kg		
Vagotomy				Partial	Complete
# of Exp.	12	2	4	2	1
H.R.	-38.1 ± 3.6	-20	+6	-17	-8
$\dot{Q}_B$	-30.5 ± 1.6	-13	+1	+20	0
VAP	+10.5 ± 2.0	+25	+6	+26	0
DAP	- 8.4 ± 5.8	-6	+3	+10	0
R_G	+178.5 ± 9.7	+150	-13	+21	+15
R_S	+46.7 ± 2.68	+ 4.5	-3	+ 2	-25

Values are expressed as percentage change from control.

*Average of doses of atropine.

Animals given only CO_2 ± S.E.M.

H.R. = Heart Rate

$\dot{Q}_B$ = Cardiac output

VAP = Ventral aortic pressure

DAP = Dorsal aortic pressure

R_G = Gill resistance

R_S = Systemic resistance

CO_2 administration elicited prompt changes to new steady-state levels in all the cardiovascular parameters studied. Pharmacological or surgical block of the vagus abolished the change; the values of heart rate, contractility and gill resistance remained at control levels indicating little direct influence of elevated CO_2 on the heart and blood vessels.

From the present study it is not possible to conclude whether the circulatory system is re-

sponding to changes in pCO_2 or changes in pH. It is obvious that elevating pCO_2 and circulatory changes are highly correlated and that the vagi are the efferent pathway for the response. There are several possible sources of vagal input. Mammals utilize chemoreceptors to sense acid-base changes; the existence of peripheral chemoreceptors in the dogfish is a possibility. Chemoreceptors could be anywhere in the circulatory system, but the most probable locations would be in the venous circulation or in the gill vasculature. The vagal input could be olfactory. If olfactory receptors were sensitive to pH changes they would be stimulated by the acidic seawater circulating through the nares of the fish. It is also possible that high pCO_2 and low pH are noxious stimuli to the nervous system and elicit a generalized vagal response.

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1968 #21

GLUTAMINE SYNTHETASE IN LOWER VERTEBRATES

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As part of a general study of the comparative biochemistry of nitrogen metabolism the distribution of glutamine synthetase was examined in tissues from a variety of lower vertebrates. The survey was prompted by two contradictory reports in the literature: the finding of an increase in glutamine concentration in blood leaving the liver of the carp (Pequin, Arch. Sci. Physiol. 21:193, 1967) and the reported absence of glutamine synthetase, the only enzyme known to be involved in the de novo synthesis of glutamine, from all tissues except brain of lower vertebrates (Wu, Comp. Biochem. Physiol. 8:335, 1963). In the glutamine synthetase assay hydroxylamine replaces ammonia and the formation of γ-glutamylhydroxamate is measured colorimetrically. Activity was found in all tissues examined (see Table 1). Hepatopancreas and tail mus-

Table 1

GLUTAMINE SYNTHETASE ACTIVITY IN LOWER VERTEBRATES
(μmoles GHA formed/hr x g fresh weight at 37°)

Species	Brain	Liver	Kidney	Skeletal muscle
Myxocephalus scorpius	423 ± 10	40*	121 ± 23	not assayed
Anguilla rostrata	561 ± 118	69† ± 7	106† ± 14	not assayed
Squalus acanthias	423 ± 25	97 ± 12	168 ± 3	not assayed
Xenopus laevis	127 ± 9	65*	98 ± 6	157 ± 46
Rana catesbiana	274 ± 41	97 ± 12	127 ± 11	63 ± 6
Chrysemys picta	525 ± 50	82*	115 ± 6	not assayed
Rat	81 ± 15	249 ± 15	45 ± 6	< 20

*Values uncertain for technical reasons.

†Represent minimal values because of non-linearity of assay.

Values are means ± S.E.M. of four to six animals except for liver values of M. scorpius (1), X. laevis (3) and C. picta (2).