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THE MODE OF GAS TRANSPORT BY ELASMOBRANCH GILL

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Previous work in this laboratory has shown that the mode of gas transport in biological units can be defined by determining the value of 2 parameters.

K_1 = mean expired water - arterial O_2 gradient

K_2 = arterial - mean expired water CO_2 gradient

The precise mechanisms involved in O_2 and CO_2 transport across the elasmobranch gill have not been elucidated. Methods were developed which permit the experimental determinations of these parameters in Squalus acanthias.

Assuming that the amount of O_2 furnished by the gill is equal to the amount of O_2 removed by the gill circulation then

$$K_1 = P_{IO_2} - PaO_2 - \frac{\text{Cardiac output}}{\text{Gill Water Flow}} \times \frac{1}{\alpha O_2} \quad Ca_{O_2} - C_{v_{O_2}}$$

$$K_2 = PaCO_2 - P_{ICO_2} + \frac{\text{Cardiac output}}{\text{Gill Water Flow}} \times \frac{1}{\alpha CO_2} \quad C_{v_{CO_2}} - C_{a_{CO_2}}$$

Cardiac output and gill water flow were determined by dye dilution technique, αO_2 and αCO_2 obtained from standard tables, and the other values measured directly. Nine fish were studied with the following results: K_1 and K_2 were never zero and were never simultaneously negative. Thus, neither simple passive diffusion nor counter-current exchange are the mode of gas exchange in Elasmobranch gill. In animals maintained for relatively long periods in sea water pens there is increasing positivity of K_1 and K_2. This finding suggests that with an unfavorable environment gas transport across the gill becomes increasingly ventilation - perfusion or diffusion limited.

Of great interest was the finding in all animals of a positive K_2 whose magnitude could not be explained on the basis of maldistribution or diffusion limitation in the gill. This suggests that a steady state activity gradient for CO_2 may be maintained between arterial plasma and expired water. Since the isolated positive K_2 was small and the determination of its value is technically difficult, further work will be required to define the significance of this observation.

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UREA SYNTHESIS IN LIVERS OF DOGFISH AND LUNGFISH*

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Elasmobranchs are ureotelic vertebrates. They not only maintain a high concentration of urea in their body fluids but also excrete urea at rates comparable to those of mammals. Lung-

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fish, which are ammonotelic under normal conditions, accumulate urea during estivation. The pathway of urea synthesis has not heretofore been elucidated in these species. In this study the dogfish, Squalus acanthias, and the African lungfish, Protopterus dolloi, were used as representative species for the two groups of fishes. Two possible pathways of urea synthesis were tested. The first pathway, Krebs-ornithine-urea cycle, was examined by following the rate of incorporation of C^{14}-sodium bicarbonate into urea. The second pathway, purein synthesis and degradation, was assayed by determining the rate of incorporation of label from 3-C^{14}-serine and 2-C^{14}-uric acid into urea. For comparative purposes the incorporation of C^{14} from sodium bicarbonate into urea in bullfrog liver slices was measured under the same conditions used for the lungfish experiments.

C^{14}-urea was determined by liberating C^{14}-carbon dioxide with urease in a closed vessel and trapping the gas in a Hyamine-OH-methanol solution. This solution was added to scintillation medium and the level of radioactivity was determined by use of a liquid scintillation counter.

Table 1 shows the rate of incorporation of C^{14} from bicarbonate, serine and urate into urea by dogfish liver slices. The last column of this table indicates that the rate of incorporation of C^{14}-bicarbonate per μmole of total bicarbonate is approximately 20 times greater than the rate of incorporation of 3-C^{14}-serine and 80 times faster than that of 2-C^{14}-uric acid. The incorporation of C^{14}-bicarbonate is linear for one hour. Addition of ornithine to the incubation medium increases the rate of labeling of urea from C^{14}-bicarbonate, and addition of citrulline decreases the rate of labeling. These results support the thesis that labeling of urea by C^{14}-bicarbonate takes place via the Krebs-ornithine-urea cycle.

Table 1

C^{14}-UREA SYNTHESIS FROM C^{14}-BICARBONATE, 3-C^{14}-SERINE AND 2-C^{14}-URIC ACID IN DOGFISH LIVER SLICES*

Experiment No.	Specific activity substrate CPM/μMole			C^{14}-urea CPM/g liver x hr	Urea synthesis μmoles/g liver x hr
	$NaHCO_3$	Serine	Uric acid		
1	2.82×10^4	-	-	18,400	0.65
2	1.03×10^4	-	-	11,500	1.12
3	-	2.0×10^5	-	7,050	0.04
4	-	-	2.2×10^6	11,500	0.01

*Slices were incubated for one hour at 30° in stoppered vessels with 100% oxygen in dogfish plasma.

Two dogfish were injected intravenously, one with 10μC 2-C^{14}-urate and the other with 17.5 μC C^{14}-sodium bicarbonate, and the rate of incorporation of label into urea was followed at timed intervals. Significant labeling of blood urea was observed within 10 minutes after administration of both compounds. At 30 minutes after injection the fish were killed and the C^{14}-urea content of blood, liver and muscle was determined. In both experiments, the level of C^{14}-urea was significantly higher in liver than blood, supporting the view that the liver is a site of urea synthesis in the dogfish. Muscle C^{14}-urea concentration was significantly lower than blood concentration. Minimal rates of urea synthesis were calculated from the rates of C^{14}-urea produc-

tion from either C^{14}-bicarbonate or C^{14}-urate in the whole animals. The minimal rate of urea synthesis from bicarbonate was calculated to be approximately 120 μmoles/kg x hr. This figure is significantly higher than the rate of urea synthesis from uric acid (approximately 15 μmoles/kg x hr.) and may be compared to the rate of urea excretion in vivo—200 μmoles/kg x hr (Dr. John Boylan).

Isolated liver slices from lungfish were incubated for one hour at 25° in a fortified Krebs-Ringer medium in 100% oxygen with substrate. Liver slices from two lungfish which were estivating for about one month produced an average of 0.16 and 0.021 μmoles urea/g liver x hr from bicarbonate, whereas these livers produced 0.89×10^{-3} and 0 μmoles from serine. Some of the urea produced from serine may have been synthesized via carbon dioxide. Slices of a control lungfish liver produced 0.047 μmoles urea/g liver x hr from bicarbonate. Bullfrog liver slices produced 9.4 μmoles urea/g liver x hr from bicarbonate under the same assay conditions.

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METABOLIC PARAMETERS OF SODIUM TRANSPORT BY DOGFISH ERYTHROCYTES

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The relationship of aerobic metabolism to cation transport in the dogfish erythrocytes was studied. The erythrocytes were washed in cold dogfish Ringers, separated by centrifugation, and incubated in Na^{22} labeled dogfish Ringers at 4°C for 6 to 12 hours. The cells were then separated, washed three times with cold non-isotopic dogfish Ringers, and harvested for study. Weighed quantities of erythrocytes were placed in respirometer flasks containing dogfish Ringers, with or without metabolic inhibitors. The flasks were then gassed with 100% O_2 and incubated at 13 or 30°C in a modified Gilson respirometer. Readings of O_2 consumption were obtained at 15 minute intervals. At 0 time, 2 hours and 4 hours the flasks were removed and sampled for measurements of lactate concentrations and for counting. The data are summarized in the table.

Lactate production at 30°C increased in the presence of cyanide or antimycin-A demonstrating a Pasteur effect in this sytem. 2,4 dinitrophenol increased lactate production only slightly.

Absolute rates of total Na^+ transport and of active Na^+ transport (total - ouabain insensitive

Table

	Na^{22} efflux μeg/gm RBC/hr		Lactate production mMoles/gm RBC/hr
	30°C	13°C	30°C
O_2 - Normal	23.3 ± 6.9 (7)*	9.9 ± 3.9 (8)	1.12 ± 0.61 (16)
O_2 + Cyanide	26.3 ± 6.2 (6)		2.04 ± 1.26 (16)
O_2 + DNP	22.9 ± 2.7 (8)	7.8 ± (1.7) (8)	1.63 ± 0.80 (12)
O_2 + Ouabain	9.4 ± 3.1 (8)	1.8 ± 0.7 (8)	.89 ± 0.50 (9)
O_2 + Antimycin-A	21.9 ± 8.7 (4)		3.85 ± 0.89 (9)

*( ) number of studies performed.