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.

1965 #39

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.

transport) are much higher than in mammalian erythrocytes. Cyanide, antimycin-A, and 2,4 dinitrophenol did not alter the active transport of Na^+. Active transport continued, even though cyanide and antimycin-A reduced oxygen consumption by 70%, and 2,4, dinitrophenol, a classic uncoupler of oxidative phosphorylation, stimulated oxygen consumption by 34%. Ouabain, which does not significantly alter O_2 consumption in the dogfish erythrocyte does markedly inhibit Na^+ transport.

It would thus appear that Na^+ transport in this sytem is not dependent upon energy derived from the cytochromechain. Whether anaerobic glycolysis or non-cytochrome mediated oxidative metabolism represents the energy source for cation transport remains to be elucidated.

1965 #40

CHARACTERISTICS OF OXYGEN CONSUMPTION OF THE DOGFISH ERYTHROCYTE

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The metabolic characteristics of the dogfish erythrocyte are of interest because they are nucleated and maintain active metabolic processes at low ambient temperatures. Oxygen consumption of dogfish erythrocytes was studied in a Gilson differential respirometer. The erythrocytes were separated by centrifugation and washed twice with cold dogfish Ringers. Cells from different fish were not mixed since such mixing causes hemolysis. After gassing with O_2, the flasks were isolated and readings taken at 15 minute intervals. Because of the low HCO_3^- in dogfish plasma, a post-gassing technique was developed for adding HCO_3^- in to the Ringers. Cells were studied in oxygen with and without metabolic inhibitors. After 15-30 minutes the vessels stabilized and serial 15 minute readings gave close agreement. The data are summarized in the table.

Table

OXYGEN CONSUMPTION IN μl/hr/gm ERYTHROCYTES

Experimental conditions	30°	12-14°C
Control	49.9 ± 12.0 (22)*	15.4 ± 5.5 (11)
Cyanide, 10^{-3}M	17.1 ± 3.9 (8)	15.5 ___ (2)
DNP, 10^{-5}M	68.9 ± 23.5 (16)	16.8 ± 5.3 (12)
Ouabain, 10^{-4}M	44.3 ± 8.5 (11)	15.0 ± 6.1 (10)
Antimycin-A, 50 pgm/flask	17.8 ± 3.6 (11)	15.9 ___ (2)

*( ) = number of studies performed.

Unlike mammalian erythrocytes there is distinct and measurable O_2 consumption by these erythrocytes. In addition, a cytochrome system is present in dogfish erythrocytes as evidenced by the partial inhibition of O_2 consumption by cyanide and antimycin-A. The increase in O_2 induced by dinitrophenol at 30°C, indicates that oxidative phosphorylation in this system resembles that found in many mammalian systems. Of great interest was the demonstration that both