concentration (MCHC) and the mean corpuscular hemoglobin (MCH). Further characterization of the hemoglobin change produced in multiple PBC dosed Herring Gulls is in progress. The support of Dome Petroleum Ltd., Imperial Oil Ltd Mobil Oil (Canada) Ltd., and Petro Canada is gratefully acknowledged.

30 THE EFFECT OF HYPERBARIC OXYGEN ON NA+/K+ATPASE AND OXYGEN CONSUMPTION OF DOGFISH, FLOUNDER AND EEL RETINAS

John L. Ubels, Marianne E. Antoine and Henry F. Edelhauser, Departments of Physiology and Ophthalmology, The Medical College of Wisconsin, Milwaukee, Wi.

The choroidal rete mirabile found in the eye of most teleost fish functions as a counter current multiplier for oxygen, generating oxygen tensions at the retina in excess of 400 mmHg (Fairbanks et al., J. Gen. Physiol. 54:203, 1969). Chronic exposure to such high oxygen tensions is toxic to most tissues; however, the rainbow trout (Salmo gairdneri) retina is dependent on these high oxygen tensions for normal function (Fonner et al., Comp. Biochem. Physiol., 46A:559, 1973).

Studies of O_2 consumption (Q_{O_2}), the electroretinogram (ERG) and Na+/K+ATPase have also shown that the rainbow trout retina is resistant to oxygen toxicity at oxygen tensions well above those to which it is normally exposed (Ubels and Hoffert, Exp. Eye Res. 32:77, 1981). In contrast, the above studies show that hyperbaric oxygen (HBO) is toxic to the mammalian retina.

Based on the results of the cited studies, it has been suggested that the resistance of the teleost retina to oxygen toxicity is an adaptation to the high oxygen tensions to which it is chronically exposed. The purpose of the work reported here was to further test this hypothesis by measuring retinal Q_{O_2} and Na+/K+ATPase activity of several marine fish exhibiting a range of rete development and retinal oxygen tensions: the winter flounder (Pseudopleuronectes americanus), the eel (Anguilla rostrata) and spiny dogfish (Squalus acanthias). The flounder has a large rete and the retinal oxygen tension of a closely related species, the fluke (Paralichthys dentatus), has been reported as 255 mmHg. The eel is a teleost without a rete and has arterial oxygen tensions at the retina. The dogfish represents the elasmobranchs which have no rete and low oxygen tensions at the retina (Wittenberg and Wittenberg, Nature 194:106, 1962).

METHODS

Retinas were removed from eyes without the pigmented epithelium and placed in 1 ml of marine teleost or elasmobranch Ringer solution (Forster's media) in 30 mm petri dishes. One eye of each animal was designated experimental and the other control. Eyes to be exposed to hyperbaric oxygen were placed in a hyperbaric chamber which was purged with 100% oxygen and pressurized to 3800 mmHG. Control tissues were held at ambient pressure under 100% O_2 (flounder) or air (other species). Retinas were incubated for 4 hours after which the chamber was depressurized and the retinas removed for metabolic analyses.

Retinal oxygen consumption was measured using a Yellow Springs Instruments Model 53 Biological Oxygen Monitor system. Intact retinas were placed in 3 ml Ringer solution and oxygen consumption was measured over a 10-15 minute period. The retinas were then homogenized in Ringer solution for protein determination.

The Na+/K+ATPase activity of crude homogenates of retinas was determined by a previously reported method (Ubels and Hoffert, Exp. Eye Res. 32:77, 1981). Proteins were measured using the Bio-Rad protein determination reagent and bovine gamma-globulin standard.

RESULTS AND DISCUSSION

Table 1 shows control values for Na+/K+ATPase activity and O_2 consumption of dogfish and flounder retinas immediately upon removal from the animal. As expected, the activity shows temperature dependence.

Table 1

Na+/K+ATPase Activity* and Oxygen Consumption† of Fresh Dogfish and Flounder Retinas

Animal	Temp (°C)			n
Dogfish	15	ATPase	1.19 ± 0.10	4
		Q_{O_2}	2.71 ± 0.29	6
Dogfish	22	ATPase	2.95 ± 0.20	4
		Q_{O_2}	4.40 ± 0.50	4
Flounder	15	Q_{O_2}	6.49 ± 0.79	4
Flounder	22	ATPase	4.48 ± 0.49	4
		Q_{O_2}	7.08 ± 0.64	4

* µmoles Pi/mg protein/hr.

† µL O_2/mg protein/hr.

$\bar{x}$ ± SE.

Comparison of these values with control data from oxygen toxicity experiments shows that the retinas do not deteriorate during a 4-hour incubation.

Exposure of dogfish retinas to HBO resulted in slight toxic effects at 22°C. The Na+/K+ATPase and O_2 consumption activity both decreased; however, the change in O_2 consumption was not statistically significant. No significant changes were seen at 15°C (Tables 2 and 3).

Table 2

Effect of Hyperbaric Oxygen on Retinal Na+/K+ATPase Activity* (PO_2 3800mm Hg for 4 hr)

Animal	Temp (°C)	Control	HBO	n
Dogfish	15	1.03 ± 0.14	1.21 ± 0.19	6
Dogfish	22	4.40 ± 0.68	3.47 ± 0.50†	7
Flounder	22	4.54 ± 0.54	4.67 ± 0.30	8
Eel	15	1.59 ± 0.21	1.46 ± 0.33	5

* µmoles Pi/mg protein/hr.

† Significant difference, $p \leq 0.05$, paired t-test.

$\bar{x}$ ± SE.

The flounder shows marked resistance to oxygen toxicity since no changes in ATPase activity of O_2 consumption were observed at 22°C (Tables 2 and 3). The eel does not fit the hypothetical pattern since, although it has no rete, no effect of hyperbaric oxygen was observed. This is not without precedent since in the studies cited above (Ubels and Hoffert, Exp. Eye Res. 32:77, 1981) no effect of HBO on Q_{O_2} or Na+/K+ATPase of frog

Table 3

Effect of Hyperbaric Oxygen on Retinal Oxygen Consumption* (PO_2 3800mm Hg for 4 hr)

Animal	Temp (°C)	Control	HBO	n
Dogfish	15	2.31 ± 0.17	2.28 ± 0.42	6
Dogfish	22	3.49 ± 0.56	2.35 ± 0.37	11
Flounder	22	6.80 ± 0.65	7.05 ± 0.63	8
Eel	15	6.52 ± 0.84	7.07 ± 1.25	5

* µL O_2/mg protein/hr.

No significant differences, paired t-test.

$\bar{x}$ ± SE.

retina was observed. The frog ERG however was abolished during exposure to HBO. Therefore, further experiments are planned in which ERG's of flounder, eel and dogfish will be recorded under HBO.

31 EFFECTS OF HOMOLOGOUS PITUITARY EXTRACTS ON PLASMA ANDROGEN AND 17β-ESTRADIOL LEVELS IN THE SPINY DOGFISH, SQUALUS ACANTHIAS

Paul Tsang and Ian P. Callard, Department of Biology, Boston University, Boston, Massachusetts

Morphological studies of the elasmobranch pituitary show that it is subdivided into 4 distinct regions - ventral, rostral, median and neurointermediate lobes (Dobson and Dodd, Gen. Comp. Endocrinol. 32:41-52, 1977). Although all four lobes have gonadotrophic activity, the ventral lobe has the most (Sumpter, Jenkins and Dodd, Gen. Comp. Endocrinol. 36:275-285, 1978). In in vivo studies in female oviparous dogfish S. canicula, an annual cycle in pituitary gonadotropin content and plasma 17β-estradiol (E_2) and testosterone (T) levels has been demonstrated (Sumpter and Dodd, J. Fish Biol. 15:687-695, 1979). Further, caudal vein injections of mammalian gonadotropin releasing hormone can induce significant changes in plasma androgen (T and dihydrotestosterone) and E_2 levels (Jenkins and Dodd, J. Endocrinol. 86:171-177, 1980) and ventral lobe extracts increased plasma androgen levels in hypophysectomized dogfish (Sumpter, et al., Op. Cit.).

In the present study, we have begun to investigate the pituitary-gonadal relationship in the ovoviviparous dogfish, Squalus acanthias. The reproductive cycle in the mature female is divided into stages A through D (Hisaw and Albert, Biol. Bull. 92-92, 187-199, 1947). In summer when fish were obtained, they were either in stage A (uterine eggs enclosed in a membranous envelope "Candle Stage") or in stage C, where pups (12CM to 20CM) are maintained free in the uterus and attached to large yolk sacs.

Fresh pituitaries were collected from both stage A and C animals and stored on dry ice until use. An aqueous extract was prepared according to the method of Sumpter et al., (1978). After a single caudal vein injection of extract, blood was collected via the caudal sinus at 1,2,3,4,5,10,24,48,72 hours post injection. Plasma was obtained by centrifugation at 1500 RPM for 20 minutes and frozen until use. After thawing, one ml was taken and extracted with diethyl ether, anesthesia grade and reconstituted in a phosphate-gelatin buffer, pH = 7.4. Duplicate aliquots of each sample were taken and analyzed by radioimmunoassay for T and E_2 content. Since the stage of pregnancy can not be externally detected, this is not determined until autopsy.