

mark resulting in wound size variation. There was, however, no correlation between initial wound size and healing rate within each group. This observation has been reported by Ho et al (Invest. Ophthalmol. 13:804, 1974).

The average healing rates of the sculpin cornea were  $2.54 \text{ mm}^2/\text{hr}$  and  $3.42 \text{ mm}^2/\text{hr}$ , respectively, for 30% and 60% corneal wounds. In shark the corneal wounds healed at  $1.29 \text{ mm}^2/\text{hr}$  (Figure 1). By comparison, the results from this study show that the sculpin corneal healing rate is two- to threefold higher than those reported for many mammalian species (Ubels et al. J. Toxicol.--Cut. & Ocular Toxicol. 1:125, 1982). Following topical treatment of standard corneal wounds in rabbits with epidermal growth factor (EGF), the maximum corneal healing rate has been reported to be  $1.29 \text{ mm}^2/\text{hr}$  (Ho et al., Invest. Ophthalmol. 13:804, 1974), and recently it has been reported that an Eye Derived Growth Factor (EDGF) will increase the corneal healing rate to  $1.45 \text{ mm}^2/\text{hr}$  (Thompson et al., Exp. Eye Res. 34:191, 1982). The value we measured in shark cornea is similar to these stimulated values. It is interesting to note that, in order for these marine animals to survive in their environment, it is essential that vision be preserved, and this study illustrates the high rate of corneal re-epithelialization that these species possess in order to maintain their vision and prevent stromal edema following a corneal laceration. This research was supported in part by NEI Grant EY-00933. Dr. Edelhauser is a 1982 Research to Prevent Blindness, Inc., Olga K. Weiss, Research Scholar.

#### THE EFFECT OF HYPERBARIC OXYGEN ON THE ELECTRORETINOGRAM OF MARINE TELEOSTS AND ELASMOBRANCHS

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**INTRODUCTION**--The mammalian retina is highly susceptible to oxygen toxicity; however, it has been reported that the electroretinogram (ERG) recorded in vitro from the retinas of rainbow trout (Salmo gairdneri) and goldfish (Carassius auratus) is unaffected by exposure of the retina to  $\text{O}_2$  at 3800 mmHg for up to 6 hours (Ubels et al., Comp. Biochem. Physiol. 57A:29, 1977). This resistance to oxygen toxicity is probably an adaptation to the high  $\text{P}_{\text{O}_2}$  ( $> 200 \text{ mmHg}$ ) generated by the counter-current  $\text{O}_2$  multiplier of the choroidal rete mirabile found in most teleost fishes. Since this phenomenon has only been studied in fishes with well-developed retes and elevated retinal  $\text{P}_{\text{O}_2}$ , the present study was designed to test this hypothesis by recording the in vitro ERG from the retinas of several marine fishes with varying degrees of rete development and retinal  $\text{P}_{\text{O}_2}$ , during exposure to hyperbaric oxygen.

**MATERIALS AND METHODS**--Three teleost species were used: the flounder (Pseudopleuronectes americanus) which has a large rete and elevated retinal  $\text{P}_{\text{O}_2}$  ( $> 200 \text{ mmHg}$ ), the goosefish (Lophius americanus) which has a small poorly developed rete and low retinal  $\text{P}_{\text{O}_2}$  ( $< 90 \text{ mmHg}$ ), and the eel (Anguilla rostrata) which has no rete mirabile. ERG's were also recorded from the eyes of two elasmobranchs, the spiny dogfish (Squalus acanthias) and the skate (Raja erinacea) which have no rete and a retinal  $\text{P}_{\text{O}_2}$  reported to be less than 20 mmHg (Wittenberg and Wittenberg, Nature 194:106, 1962). Eyecups were prepared by removing the cornea, iris and lens from enucleated eyes. The ERG was recorded simultaneously from paired retinas under 100%  $\text{O}_2$  at 3800 mmHg and at 760 mmHg (control) as previously described (Ubels et al., Comp. Biochem. Physiol. 57A:29, 1977). The a-wave and b-wave amplitudes were measured directly from the oscilloscope screen, and the ERG's were photographed. All experiments were conducted at  $15^\circ$  to  $17^\circ\text{C}$ .

**RESULTS AND DISCUSSION**--As predicted, based on results of previous studies of trout and goldfish, the flounder ERG was not attenuated by 6-hour exposure to HBO and, in fact, was somewhat enhanced (Table 1; Figure 1A). The goosefish retina, with its poorly developed rete and lower  $\text{P}_{\text{O}_2}$  is susceptible to oxygen toxicity as demonstrated by a 50% reduction in b-wave amplitude after a 6-hour exposure to HBO (Table 1; Figure 1B). The eel does not fit the hypothetical pattern since HBO had no effect on the ERG in spite of the lack of a normally elevated retinal  $\text{P}_{\text{O}_2}$  in this animal. This observation agrees with previous data on the effect of HBO on retinal  $\text{Na}^+/\text{K}^+$  ATPase in the eel (Ubels et al., Bull. MDIBL 21:53, 1981).

Table 1. The effect of  $O_2$  at 3800 mmHg on electroretinogram amplitude (control,  $O_2$  at 760 mmHg).

Species	Percent of initial amplitude*			
	4 hours		6 hours	
	a-wave	b-wave	a-wave	b-wave
Flounder (n = 5)				
Control	79	111	87	87
HBO	133	90	298	180
Eel (n = 3)				
Control	70	117	61	86
HBO	122	111	156	135
Goosefish (n = 4)				
Control	125	133	125	156
HBO	113	109	100	51**
Dogfish (n = 5)				
Control	75	32	70	32
HBO	318†	0**	236†	0**
Skate (n = 4)				
Control	98	94	75	72
HBO	92	60**	126†	21**

\* Initial amplitude = amplitude after 60 min dark adaptation.

† Increase in a-wave amplitude related to b-wave attenuation.

\*\* Attenuation of b-wave attributable to oxygen toxicity.

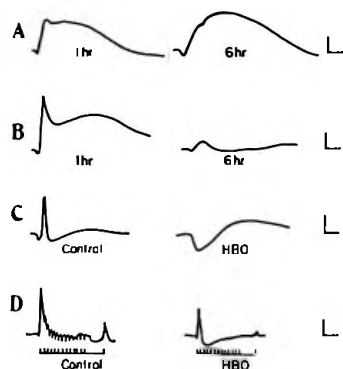


Figure 1a.--Resistance to oxygen toxicity in the flounder retina. 500  $\mu$ V, 500 msec. b. Effect of hyperbaric oxygen on the goosefish ERG. 1 mV, 500 msec. c. Effect of 90-minute exposure to hyperbaric oxygen on the dogfish ERG. 1 mV, 500 msec. d. Effect of 6-hour exposure to hyperbaric oxygen on flicker-fusion in the skate retina. Stimulation at 1/sec. 1 mV, 500 msec.

The b-wave of the dogfish ERG was abolished by between 1.5 and 4-hour exposure to HBO (Figure 1C). The b-wave of the skate ERG was also significantly reduced as compared to control (Table 1). Our data suggest that  $O_2$  is toxic to the cells responsible for b-wave generation or to the process of synaptic transmission between photoreceptors and bipolar cells since the b-wave was abolished without attenuation of the a-wave. The increase in a-wave amplitude during HBO exposure can be explained by the fact that the ERG is the algebraic summation of activity in several retinal cell types. Thus there is an apparent increase in photoreceptor (a-wave) activity as the b-wave is lost. If the oxygen were to affect the photoreceptors first, both a- and b-wave amplitudes would be reduced. The elasmobranch retina is also unresponsive to repetitive stimulation at 1/sec after 6-hour exposure to HBO,

while the control retina responds to each flash (Figure 1D). With the possible exception of the eel data, the results of this study and previous studies (Ubels and Hoffert, Exp. Eye Res. 32:77, 1981; Ubels et al., Bull MDIBL 21:53, 1981) support the hypothesis that resistance to retinal oxygen toxicity is not a general characteristic of all fishes but is directly related to the degree of rete mirabile development and the  $P_{O_2}$  to which the retina is chronically exposed. The teleost retina is therefore a good model for the study of the mechanism of oxygen toxicity and such studies may lead to a better understanding of the effects of high  $P_{O_2}$  on the retinas of premature infants. This research was supported in part by NIH grants EY-00933, EY-05450, EY-04069 and ES-01985.

#### CONTRACTILE ACTIONS OF ISOLATED RENAL TUBULES OF THE WINTER FLOUNDER (*Pseudopleuronectes americanus*)

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**INTRODUCTION**--Radial constrictions of flounder renal tubules have been observed in kidney explants maintained in culture (Townsend and Scott, J. Fish. Res. Bd. Canada 20(1):243, 1963) and in isolated tubular segments (Trump and Bulger, Lab. Invest. 16:453-482, 1967; Beyenbach, Nature 299:54-55, 1982). These systaltic actions are presumably the result of contractions of smooth muscle investments that surround the nephron of flounder species, the English sole (*Parophrys vetulus*) and the plaice (*Pleuronectes platessa*), for which detailed renal ultrastructure has been reported (Bulger and Trump, Am. J. Anat. 123:195-226, 1968; Olsen, Acta Pathol. Microbiol. Scand. 212:81s-96s, 1970). This report provides 1) observations on the constrictive actions of isolated renal tubules of the winter flounder (*Pseudopleuronectes americanus*); 2) confirmation of the presence of smooth muscle cells surrounding the basement membrane of the nephron, and 3) a discussion of the possible role of tubular constrictions to flounder renal function.

**METHODS**--Renal tubules were isolated by microdissection from freshly excised kidney tissue and bathed in flounder Ringer's solution (MDIBL Bull. 21:40-42, 1981). Individual tubular segments were transferred to depression slides and observed under inverted compound light microscopy (400 x). Kidney tissue for transmission electron microscopy (TEM) was prepared as previously described (MDIBL Bull. 21:35-37, 1981).

**RESULTS**--Approximately one fourth of isolated tubular segments examined under light microscopy showed slow, undulatory movements associated in nearly every instance with the presence of sharp radial constrictions as previously described (Beyenbach, 1982). Radial constrictions were observed at one or several (up to 5) sites along a given 200  $\mu$ m portion of the tubular segments and could be sustained or periodic with a frequency of approximately 3-5 constrictions per minute. The constrictions, which in some cases completely occluded the tubular lumen, forced flow of luminal fluid away from the constriction site. Subsequent relaxation allowed luminal fluid to flow back. In one segment a series of constrictions were seen to proceed in a wave-like manner along the tubule. However, multiple constrictions usually appeared independently of each other and true peristalsis was not observed.

Examination of the winter flounder kidney under transmission electron microscopy showed smooth muscle cells attached to the peritubular side of the basement membrane of the nephron (Figure 1). Longitudinal sections of the smooth muscle cells revealed myofilaments and an occasional centrally located nucleus. Single smooth muscle cells imaged in our laboratory to date were associated with proximal tubular segments of the flounder nephron.

**DISCUSSION**--Previously it was not clear if constrictions of renal tubules of the winter flounder were due to the presence of myoepithelial cells or smooth muscle cells (Beyenbach, 1982). Transmission electron microscopy observations confirm the presence of smooth muscle cells adjacent to the proximal segment of the nephron similar in appearance to those described for the English sole (*Parophrys vetulus*) by Bulger and Trump (1968). The presence of individual smooth muscle cells rather than a muscular layer is consistent with the observation of radial constrictions at distinct sites along the tubule.