OCULAR OXYGEN TOXICITY: THE EFFECT OF HYPERBARIC OXYGEN ON THE IN VITRO ELECTRORETINOGRAM OF SELECTED TELEOSTS, AMPHIBIANS AND MAMMALS

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ABSTRACT

OCULAR OXYGEN TOXICITY: THE EFFECT OF HYPERBARIC OXYGEN ON THE *IN VITRO* ELECTRORETINOGRAM OF SELECTED TELEOSTS, AMPHIBIANS AND MAMMALS

By

John L. Ubels

Electroretinograms were recorded *in vitro* from frog, rat, goldfish, and trout eye cup preparations during exposure to 100% O_2 at 3800 torr in order to study the possible toxic effects of hyperbaric oxygen on the neural elements and supporting tissues of the retina. The frog, rat, and goldfish ERG's were initially enhanced by hyperbaric oxygen.

All components of the frog ERG were significantly attenuated by exposure to hyperbaric oxygen for six hr, as compared to the control ERG recorded under O_2 at 740 torr. The b- and c-waves declined more than the a-wave indicating a toxic effect of O_2 on the neurons lying postsynaptic to the photoreceptors and on the pigmented epithelium, respectively.

John L. Ubels

Hyperbaric oxygen is highly toxic to the rat retina, causing abolishment of the ERG after 90-100 min exposure. The greatest effect is on the a-wave and slow PIII component indicating a toxic effect of O_2 on the photoreceptors. The decline of the b-wave parallels that of the a-wave.

The decline of the ERG in oxygen toxicity may be due to a toxic effect of O_2 on the processes responsible for maintaining transmembrane potentials in the retina.

No significant change in the goldfish ERG was seen during six hr exposure to hyperbaric oxygen. Normal a- and c-waves were also recorded from the trout retina under the same conditions. The trout b-wave was attenuated, however, this decline was not significantly different from a similar decline of the control b-wave. The teleost retina is normally exposed to a P_{O_2} above 400 torr which is generated by the counter-current oxygen multiplier of the choroidal rete. Previous studies have shown the teleost retinal metabolism and enzyme activity are not inhibited by hyperbaric oxygen. The results of the present study give further evidence that the teleost retina is well adapted to high P_{O_2} and that no toxic effect is seen from exposure to P_{O_2} well above that normally encountered. This resistance to oxygen toxicity may involve the presence of protective compounds or specially adapted isozymes.

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By

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DEDICATION

This thesis is dedicated to my parents, Mr. and Mrs. John Ubels, in gratitude for the distinctively Christian education with which they provided me throughout elementary school, high school, and college.

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INTRODUCTION

Oxygen tensions (P_{O_2}) in excess of that found in air are known to have toxic effects on biological systems. One organ which has been shown to be particularly susceptible to oxygen toxicity is the vertebrate eye. Attention was focused on the sensitivity of the eye to high P_{O_2} by the discovery in the early 1950's that the disease retrolental fibroplasia in premature infants was caused by the pure O_2 atmospheres to which these infants were exposed (Patz, Hoeck and De La Cruz, 1952). Since the major effect of oxygen in retrolental fibroplasia is on the retinal blood vessels, most research has centered on the effect of high P_{O_2} on ocular circulation. These studies have shown that high P_{O_2} causes retinal vasoconstriction (Dollery *et al.*, 1964). Few studies have been done on the effect of high oxygen tensions on ocular metabolism, however it has been shown that exposure to hyperbaric oxygen causes a decrease in the metabolic rate of the mammalian retina (Baeyens, Hoffert and Fromm, 1973).

The teleost eye appears to be an exception to general rule of high susceptibility of ocular tissues to oxygen toxicity. In most teleosts a portion of the choriocapillaris is modified into a *rete mirabile* which operates as a counter-current multiplier for oxygen. Ocular oxygen

tensions in excess of 400 torr are generated by this system (Wittenberg and Wittenberg, 1962; Fairbanks, Hoffert and Fromm, 1969). This suggests that the teleost has adapted to high ocular P_{O_2} and is resistant to the toxic effects of oxygen. Baeyens et al. (1973) have shown that retinal metabolism in the trout is in fact enhanced by exposure to hyperbaric oxygen. This apparent resistance makes the teleost an ideal subject for the study of oxygen toxicity in that our understanding of oxygen toxicity may be aided by elucidation of its mechanism of resistance to such toxicity.

Except for the previously mentioned studies on retinal metabolism little work has been done on the direct effect of high oxygen tension on the function of the neural elements and supporting tissues of the retina. The electroretinogram (ERG) is generally accepted as a monitor of the functional integrity of the retina and thus any toxic effect of oxygen on the retina should be evidenced by changed in the ERG. Noell (1962) showed that the in vivo rabbit ERG is severely attenuated by exposure to hyperbaric oxygen. Since this study was done in vivo, however, it is not possible to separate cardiovascular effects of high P_{O_2} from direct toxic effects on the visual cells. The work presented here is a comparative study of the effect of hyperbaric oxygen on the in vitro ERG of an amphibian, a mammal, and two teleosts. Possible cardiovascular effects of increased oxygen tension were eliminated by recording

the ERG from the isolated, non-perfused retina.

The purpose of this study was to answer the following questions:

1. How is the <u>in vitro</u> ERG affected by the exposure of the retina to hyperbaric oxygen?
2. Are there species differences in the response of the ERG to hyperbaric oxygen, especially in view of the fact that teleost ocular tissues are normally exposed to high P_{O_2}?
3. If toxicity of oxygen to the retina is demonstrated, how is each component of the ERG affected and how might this be related to the various cell types in the retina?

LITERATURE REVIEW

The Clinical Uses of Hyperbaric Oxygen

In diseases caused or complicated by hypoxia, treatment by hyperbaric oxygen has often been indicated in order to increase oxygen delivery to the affected tissues. The normal P_{O_2} of mammalian arterial blood is 100 torr. The hemoglobin is 97% saturated and the oxygen in physical solution is 0.3 ml/100 ml. Breathing pure oxygen at normal pressure will increase dissolved oxygen to only 1.5 ml/100 ml, not a substantial increase in terms of oxygen delivery to tissues. However, if through the use of hyperbaric pressures the P_{O_2} is raised to 2000 torr the dissolved oxygen will be 6.0 ml/100 ml. This increase in P_{O_2} will increase the number of O_2 molecules delivered to the tissues and will also increase extravascular penetration of O_2 increasing the volume of tissue supplied with O_2 from a given capillary (Saltzman, 1967).

Studies of the effect of hyperbaric oxygen exposure on cardiac patients have shown the P_{O_2} is in fact increased and that the O_2 can be used since blood lactic acid levels decrease indicating a greater dependence on aerobic metabolism. Hyperbaric oxygen is effective in the relief of pain associated with coronary thrombosis and angina pectoris.

Hyperbaric oxygen has also been used in treatment of carbon monoxide poisoning. In this case oxygen administration prevents hypoxia by increasing plasma dissolved oxygen.

Malignant tumors have been treated by hyperbaric oxygen because oxygen has been found to enhance the effectiveness of X-irradiation. The reason for enhancement is unknown.

The most effective clinical use of hyperbaric oxygen is in the treatment of gas gangrene. The response to treatment is very rapid. Mortality is decreased and limbs are often saved since the development of necrotic tissue is markedly reduced. This is probably due to direct effect of oxygen on the tissue. Elimination of bacterially produced gas bubbles allows increased tissue perfusion by reduction of tissue pressure.

The clinical uses of hyperbaric oxygen are covered in detail in a monograph by Meijne (1970). He stresses the point that there are many unanswered questions concerning the clinical use of hyperbaric oxygen. There is much controversy among clinicians about its value both on theoretical grounds and because of the risk of oxygen toxicity. It appears that in many cases the danger of toxicity outweighs the beneficial effects of hyperbaric oxygen.

In Vivo Oxygen Toxicity

Exposure of animals to oxygen tensions above that in air has profound deleterious effects on the pulmonary, cardiovascular, and central nervous systems. Changes in whole animal metabolism also result from exposure to high oxygen tension.

Since the lung is in intimate contact with the atmosphere it appears to be the organ primarily affected during breathing of pure oxygen. Lesions associated with toxicity include edema, interstitial fibrinization, alveolar cell hypertrophy, congestion and inflammation.

Robinson et al. (1969) exposed several species of sub-human primates to oxygen at 1 atm. Mortality occurred among baboons from days 4-8 while Macaca irus monkeys survived as long as 13 days. The pulmonary lesions listed above were observed in these animals. Several monkeys were killed and examined during the experimental period and these examinations showed that the toxic effects grow worse with longer exposure to oxygen. Humans breathing oxygen at 2 atm complain of substernal pain on inspiration, sore throat and pulmonary congestion (Widdel et al., 1974).

Studies on the effect of oxygen at high pressure on alveolar surfactant show that exposure of cats to O_2 at 3000 torr increases surface tension almost three times. This change takes place before the occurrence of edema or other pulmonary lesions (Beckman and Houlihan, 1973). More recent work has related this increase in surface tension

to increased intra-alveolar cholesterol (Bergren and Beckman, 1975).

The major effect of hyperbaric oxygen on the cardiovascular system is vasoconstriction. Wood et al. (1972) found that exposure of rats to hyperbaric oxygen results in systemic hypertension which produces left vertricular failure. They suggested that much of the pulmonary edema seen in oxygen toxicity is due to cardiac failure. In anesthetized dogs, breathing oxygen at 3 atm causes bradycardia and decreased iliac blood flow. An increase in blood pressure was added to these effects at 5 atm (Hardenbergh et al., 1973). Exposure of humans to hyperbaric oxygen results in decreased cerebral blood flow (Lambertsen et al., 1953; Anderson and Saltzman, 1965). Bridges (1966) observed cardiac arrhythmia and ventricular fibrillation in anesthetized rabbits exposed to hyperbaric oxygen.

High P_{O_2} may also affect capillary permeability. Decreased total plasma protein and haptoglobin in rats exposed to O_2 at 760 torr for seven days was attributed to increased vascular permeability (Grunbaum and Leon, 1975).

The central nervous system is highly susceptible to oxygen toxicity and this may well be the root of the toxic effect of oxygen on other systems. Central nervous system toxicity causes convulsive seizures. These convulsions have been observed in many animals including man. Mice convulse after as little as 30 min under O_2 at 3800 torr

(Hoffert, Baeyens and Fromm, 1975). A similar effect is seen in cats and rats (Beckman and Houlihan, 1973). CNS toxicity is seen in man after only a few minutes of oxygen breathing at 4 atm (Saltzman, 1967). Saltzman states that toxicity can be avoided by limiting exposure to less than 2.5 atm, however more recently Krishnamurtu and Wadhawan (1974) reported seizures in two patients after repeated exposure to oxygen at 2.5 atm for treatment of peripheral arterial disease. They suggest a progressive increase in neuronal irritability by repeated exposure to hyperbaric oxygen.

That CNS effects are not limited to mammals was shown by the work of D'aoust (1969) and Hoffert et al. (1975) who reported losses of equilibrium and muscular control in marine teleosts, rockfish (Sebastodes miniatus) and pilchards (Harengula cupeola) repsectively, exposed to hyperbaric oxygen.

Histological alterations in the rat brain ultrastructure after exposure of the animals to hyperbaric oxygen have been observed (von Schnakenberg and Nolte, 1970). These alterations were seen even when convulsions were suppressed by drugs, indicating a direct effect of O_2 on cellular structure. The toxicity of oxygen to the CNS may well be the most important effect of hyperbaric oxygen since it is probably the cause of lesions associated with high oxygen in other systems. Oxygen toxicity to the CNS results in hyperactivity of the sympathoadrenal system and

this sympathetic activity has been associated with pulmonary lesions (Bean, Zee and Thom, 1966). Indeed, electrical stimulation of the sympathetics causes the same changes in surfactant as seen in oxygen toxicity (Beckman and Houlihan, 1973). Wood et al. (1972) stated that pulmonary effects of high oxygen tension may be secondary to cardiovascular effects, however, since the primary cardiovascular effect of sympathetic stimulation is vasoconstriction it is conceivable that cardiovascular effects of high P_{O_2} are due to CNS toxicity. Some authors have overlooked this point and it is stressed here that in studies like those described above it is important to keep in mind the interactions between organ systems which take place in vivo. Given the present state of knowledge it cannot be stated with certainty which system, if any, can be considered the primary target for oxygen toxicity.

As is evident by the work already described, most oxygen toxicity studies have been done using mammals. Hoffert et al. (1975) have focused attention on oxygen toxicity in lower animals and have chosen an area of study largely ignored by previous investigators: the effect of hyperbaric oxygen on metabolic rate. Oxygen toxicity was defined as a decrease in metabolic rate after exposure to high P_{O_2}. Shrimp (Peneus setriferus), false pilchards (Harengula cupeola), fiddler crabs (Uca pugnax), and mice (Mus musculus) show significant decrease in metabolism after exposure to O_2 at 3800 torr. Metabolic rate was

enhanced in minnows (Pimephales pronmelas) and crayfish (Orconestes propinquus), an interesting difference, indicating that not all animals respond in the same way to high P_{O_2}. These changes in whole animal metabolism are the results of changes at the level of cellular metabolism, a subject to be discussed in the next section of this review.

In Vitro Oxygen Toxicity: Effects on Cellular Metabolism and Enzyme Systems

High oxygen tension has a direct effect on cellular metabolism, causing a decrease in oxygen consumption. Stadie, Riggs and Haugaard (1945) demonstrated a decrease in metabolism of rat brain, liver, kidney, lung, and muscle tissue during six hours exposure to oxygen at 8 atm. More recently it has been shown that oxygen consumption by brain and hepatic tissue of trout, frogs, and dogs is inhibited by exposure to O_2 at 1470 torr for 24 hr (Baeyens et al., 1973).

Numerous studies have shown that the toxic effect of oxygen is at the biochemical level. The greatest effect of high P_{O_2} is on the pathways of carbohydrate metabolism, e.g. the brain, are most susceptible to oxygen toxicity. Oxygen at 760 torr inhibits glucose and pyruvate oxidation (Haugaard, 1968). Glycolysis is inhibited directly by inactivation of the enzyme glyceraldehyde-3-P-dehydrogenase (Horn and Haugaard, 1966; Haugaard, 1968). Another glycolytic enzyme inhibited by high oxygen tension is lactate dehydrogenase (LDH). Baeyens, Hoffert and Fromm (1974)

exposed homogenates of brain and liver tissue from frogs, trout and dogs to hyperbaric oxygen for 24 hr and found that LDH activity was decreased. They hypothesized that the effect of oxygen was on the active and/or allosteric site of the enzyme. Increased Km values indicated a decreased affinity of the enzyme for substrate. Further work by Baeyens (1975) gave similar results for mouse brain.

As far back as 1946 it was hypothesized that oxygen inactivated enzymes by oxidation of essential sulfhydryl groups and formation of disulfide bridges (Haugaard, 1946). Since that time evidence supporting this idea has been presented (Haugaard, 1968). Strong support is offered by the fact that oxygen toxicity can be prevented or reversed by reduced glutathione (Horn and Haugaard, 1966; Baeyens, 1975). Glutathione is a strong reducing agent and may maintain the SH groups of enzymes in their reduced state. The proposed mechanism of oxygen toxicity is:

$$\underset{\text{active}}{2\ \text{En-SH}} \quad \underset{\text{reduction}}{\overset{\text{oxidation}}{\rightleftharpoons}} \quad \underset{\text{inactive}}{\text{EnS-SEn} + 2(\text{H})}$$

(Baeyens, 1972). Addition of reduced glutathione (G) to an enzyme system (En) could reverse the toxic effect of oxygen by the reaction:

$$\text{EnS-S-En} + 2\text{GSH} \rightleftharpoons 2\ \text{EnSH} + \text{GS-SG}$$

Reduced glutathione could protect against oxygen toxicity since it would be more easily oxidized than the enzyme SH

groups. Enzymes other than glycolytic enzymes, such as the SH group containing pyridoxal enzymes and ferredoxins are also inhibited by oxygen. High oxygen can also cause extensive damage by peroxidation of lipids. These subjects have been extensively reviewed by Haugaard (1968).

If carbohydrate metabolism is inhibited one would expect reduced production of high energy compounds. This is in fact the case as shown by Horn and Haugaard (1966) who observed that ATP production is inhibited when rat heart homogenate is incubated with 100% oxygen. ATP concentration in rat heart muscle was decrease 15.7% by exposure to hyperbaric oxygen (Wood et al., 1972).

Another effect of hyperbaric oxygen exposure on cellular metabolism is of interest in view of its convulsive effects. It has been shown that exposure of rats and mice to hyperbaric oxygen causes decreased levels of gamma-amino-butyric acid (GABA) in the brain (Wood, Watson and Murray, 1969; Radomski and Watson, 1973). GABA is an inhibitory neurotransmitter and anti-convulsant (Goodman and Gilman, 1975). The effect of decreasing its level in the brain is obvious. Decreases in GABA are detectable before the onset of convulsions (Radomski and Wood, 1973) which could explain the increased sympathetic activity responsible for other lesions associated with oxygen toxicity, i.e. in the lung. The changes in GABA metabolism in the brain are due either to inactivation by oxygen of glutamic acid decarboxylase which synthesizes GABA or activation

of GABA alpha-oxyglutarate transaminase which breaks it down (Radomski and Watson, 1973). Since the toxic effect of oxygen at the cellular level in most cases is enzyme deactivation the former hypothesis seems more plausible.

Ocular Oxygen Toxicity

The emphasis of the present study is on ocular oxygen toxicity. Therefore descriptions of the effects of high P_{O_2} on ocular tissues have not been included in the previous sections of this review so that attention might be focused on ocular oxygen toxicity at this point.

In the early 1940's a disease which became known as retrolental fibroplasia appeared in premature infants and reached epidemic proportions by the early 1950's. This disease was characterized by irreversible constriction of retinal arteries, cessation of development of new arteries, and proliferation of fibrous tissue in the retina, resulting in blindness. Research efforts using both experimental animals and clinical studies revealed that retrolental fibroplasia was caused by the pure oxygen atmosphere to which premature infants were exposed (Patz et al., 1952; Ashton, Ward and Serpell, 1953; Patz, 1965). Since that time much research on ocular oxygen toxicity has centered on the circulatory and histologic changes in the eye associated with high P_{O_2}. As in other systems high P_{O_2} causes reversible vasoconstriction in the mature retina. Dollery et al. (1964) reported greater constriction of retinal

arteries at 2 atm than at 1 atm during oxygen breathing by humans. The percentage reduction in diameter was greater in smaller vessels than in larger ones. Anderson and Saltzman (1965) concurred with the findings of Dollery et al. (1964) and in addition reported that retinal veins constrict more than retinal arteries during oxygen breathing. Both groups suggest that this vasoconstriction may have protective value. Other workers however disagree with this idea, saying that the vasoconstriction seen with oxygen breathing should not restrict retinal oxygenation (Nichols and Lambertsen, 1969). They also suggest that the retinal vasoconstriction observed in oxygen breathing may at least initially be due to the hypocapnia which accompanies oxygen breathing. A moderate decrease in blood flow to the retina has been observed in monkeys exposed to hyperbaric oxygen (Eperon, Johnson and David, 1975). Exposure to high oxygen tensions may also affect the permeability characteristics of the choriocapillaris. Exposure of dogs to 100% oxygen at 1 atm for 48-50 hr is known to cause retinal and uveal detachment due to leakage of edematous fluid from the choriocapillaris into the subretinal space (Yanoff, Miller and Waldhausen, 1970).

Histological studies have also shown changes in ocular tissue caused by high P_{O_2}. The changes differ from circulatory effects in that they are in most cases irreversible and may take place at relatively low P_{O_2}. Lucas and Trowell (1958) found that rat retinas in tissue culture

became necrotic under 60% oxygen at normal pressure while retinas under room air survived. Histological examinations of retinas from rabbits exposed to 100% O_2 for 48 hr and 80% O_2 for 100 hr at ambient pressure show degeneration of 70% of the visual cell (rods and cones) population (Noell, 1955; 1962). Exposure of the animals to oxygen at 1.66 atm resulted in the death of 100% of the visual cells in 24 hr. Examination of rabbit retinas four days after exposure to 3 atm oxygen for 4 hr shows that the outer nuclear layer is reduced in thickness and that pycnotic nuclei are present. A few days later the visual cells have disappeared by autolysis, however, the ganglion and biopolar cells appear normal (Noell, 1962). Bresnick (1970) essentially repeated Noell's work and showed by electron microscopy that ultrastructural changes appear in the visual cells before the changes observed by Noell.

Corneal and lens tissues are also affected by hyperbaric oxygen. Thinning of the corneal epithelium and darkening of its cytoplasm were observed in guinea pigs after exposure to oxygen at 3 and 5 atm. Pycnosis and loss of lens epithelium nuclei also occurs (Nichols et al., 1972).

At the time of Nichols and Lambertsen's (1969) extensive review of ocular oxygen toxicity little work had been done on the effect of high oxygen tension on ocular metabolism. They state,

> The rates of inactivation of retinal enzymes, and the possible influences of concurrent decreases in nutrient flow and exhaustion of available energy

stores should provide important topics for study of oxygen effects upon vision.

Since that time several studies of oxygen toxicity and retinal metabolism have been done in the laboratory of J. R. Hoffert at Michigan State University. Oxygen at 1470 torr for 24 hr had no effect on the metabolic rate of frog retinas (Baeyens et al., 1973), however, retinal LDH activity was decreased by exposure to O_2 at 740 torr (Baeyens et al., 1974). There is a marked decrease in both metabolic rate and LDH activity of dog retinas exposed to hyperbaric oxygen (Baeyens et al., 1973; 1974). Studies of enzyme kinetics indicate that the toxic effect of oxygen is at the active site of the LDH molecule causing a decreased affinity of enzyme for substrate. In contrast, teleost retinal metabolism and LDH activity were enhanced by hyperbaric oxygen.

The effect of hyperbaric oxygen on the retina may also be studied by observing changes in the electroretinogram (ERG) which is generally accepted as being a good indicator of the functional integrity of the retina. To this author's knowledge only Noell (1955; 1962) and Bridges (1966) have reported studies of this type. Noell (1955) recorded the ERG from rabbits in vivo under normobaric conditions and found that the ERG was significantly attenuated after 20-36 hr under 100% oxygen, and after 50-90 hr under 80% oxygen. He reported that full recovery of the ERG was possible if exposure was not prolonged. At ambient pressure and 100% oxygen ERG attenuation occurred before

histological manifestations of visual cell death (Noell, 1962). Noell (1962) also studied the effect on the ERG of exposure to pure oxygen at 3-7 atm. He gives data only for the b-wave which at 7 atm disappeared within 42 min. Rate of decline changed directly proportionately with P_{O_2}. The latency of the effect was 20 min at 7 atm and 100 min at 3 atm. The change in the ERG was often seen before the systemic effects of hyperbaric oxygen such as convulsions occurred. Bridges (1966) essentially repeated the work of Noell. He used hyperbaric oxygen ranging from 2.5 to 7 atm. Attenuation of the a-wave as well as the b-wave was reported in this study. The time to disappearance of the ERG was an inverse function of pressure with the a-wave lasting longer than the b-wave. Initial enhancement of the ERG was also reported.

As noted earlier these studies were done in vivo and thus direct effects of oxygen on the retinal cells cannot be completely separated from possible circulatory and other systemic effects.

The Resistance of Teleost Ocular Tissues to Oxygen Toxicity

The teleost retina is normally enveloped by oxygen tensions in excess of 400 torr (Wittenberg and Wittenberg, 1962; Fairbanks et al., 1969). Extended exposure to such high oxygen tensions would be toxic to most tissues. These high oxygen tensions are generated by the counter-current oxygen multiplier known as the choroidal rete mirabile. The concentrating potential of the rete may be

appreciated by the fact that the normal P_{O_2} of rainbow trout (Salmo gairdneri) arterial blood is only 21 torr (Fairbanks, 1970). Electroretinographic studies have shown that the trout retina is dependent on these high oxygen tensions for normal function (Fonner, 1973; Fonner, Hoffert and Fromm, 1973).

A mechanism for oxygen concentration has been proposed (Fairbanks et al., 1974) which depends on the enzyme carbonic anhydrase (CA). Erythrocyte CA in the choriocapillaris hydrates CO_2, producing hydrogen ions and releasing O_2 into the plasma by the Bohr and Root effects. In the retina bicarbonate neutralizes lactic acid and CA dehydrates H_2CO_3 forming CO_2 which diffuses into the choriocapillaris again raising the P_{O_2} of the venous blood by the Bohr and Root effects. Oxygen diffuses from the venous side of the rete into the arterial side and is carried to the retina. In the venous side of the rete CA, which is probably located in the walls of the vessels, hydrates CO_2 thereby preventing short circuiting of the system by diffusion of CO_2 into the arterioles. The use of the CA inhibitors, acetazolamide and CL-11,366, showed that this short circuiting does in fact occur when CA is inhibited (Fairbanks et al., 1974; Fonner et al., 1973). Recent histochemical studies have shown that high levels of CA are present in the retial vessels (Eldred, 1975).

The presence of high P_{O_2} at the retina suggests that the ocular tissues of teleosts may be resistant to oxygen

toxicity. Several studies on the effect of hyperbaric oxygen on metabolism of LDH activity in the teleost retina have indicated that this is the case.

Baeyens et al. (1973) showed that trout retinal metabolism is enhanced by exposure to hyperbaric oxygen. Incubation of retinal tissue under 100% O_2 at 154 torr, 400 torr and 1470 torr for 24 hr causes a linear increase in metabolic rate with rising P_{O_2}. Metabolism of lens, cornea and retina of the white grunt (Haemulon plumieri) is enhanced by exposure to O_2 at 3040 torr (Hoffert, Baeyens and Fromm, 1973).

In general the enzyme LDH is inhibited by molecular oxygen. It has been shown however that trout retinal LDH activity is not affected by exposure to oxygen at 1 atm (Baeyens and Hoffert, 1972) indicating that there is a mechanism which prevents oxidation of the enzyme. Trout retina LDH activity is in fact enhanced by exposure to oxygen at 1470 torr for 24 hr (Baeyens et al., 1974).

This evidence indicates that the teleost has developed a mechanism of resistance to ocular oxygen toxicity. The dependence of the teleost retina on high P_{O_2} is probably related to the fact that it has an avascular retina and therefore the whole retina depends on O_2 diffusion from the choroid.

It should be noted that not all teleost tissues and enzymes are resistant to oxygen toxicity (Baeyens et al., 1973; 1974).

The Electroretinogram

The electroretinogram (ERG) has been used as a monitor of retinal function since its discovery by Holmgrem in 1865 (Armington, 1974). The ERG is a complex response of the retina to a photostimulus which can be recorded by an active electrode on the cornea or in the vitreous humor and a reference electrode behind the eye. The response is relatively slow and is made up of a negative deflection known as the a-wave (by convention negative is a downward deflection in electroretinography) a positive b-wave, and in some animals a slow deflection known as the c-wave which may be positive or negative depending on the species.

The ERG is the result of the summation of the responses of the various cell types of the retina to a photostimulus (Brown, 1968). Granit (1933) attributed the ERG to the summation of components which he labeled PI, PII, and PIII based on the order of their disappearance under ether anesthesia. Roughly, PIII corresponds to the a-wave, PII to the b-wave, and PI to the c-wave.

The fact that the ERG is a simultaneous response from several cell types gives it good potential as an experimental tool, however, this same complexity made data analysis difficult until some knowledge of the cellular origins of the ERG was gained. This research was aided by the development of microelectrodes and intraretinal and intracellular recording techniques. The vertebrate receptor cell responds to light by hyperpolarization (Baylor

and Fourtes, 1970; Tomita, 1976). These hyperpolarizations correspond to the a-wave (PIII). Experiments on signal transmission along the rods have provided evidence that the photoreceptor response accounts for a major portion of the a-wave (PIII) (Penn and Hagins, 1969).

Various authors have shown by intraretinal recording that the b-wave originates in the inner-nuclear layer (Brown, 1968). Intracellular recording shows that the only cells which respond to photostimulation with potentials corresponding to the b-wave are the Müller (glial) cells (Miller and Dowling, 1970). The Müller cells are not involved in signal transmission in the retina but act as potassium electrodes responding to increases in K^+ ion concentration in the extracellular fluid. The bipolar cells may make a major contribution to the rise in extracellular potassium (Armington, 1974) however other cells are probably involved also. Tomita (1976) points out that although the Müller cells do make a significant contribution to the b-wave the question of whether it is exclusively of Müller cell origin is still unanswered.

The pigmented epithelium, which lies behind the receptor cells and is not actually part of the retina, has been implicated as the source of the c-wave (PI). Schmidt and Steinberg (1971) using intracellular techniques recorded light induced responses from pigmented epithelium cells which were identical to the c-wave. The responses were shown to be rod dependent.

The ERG has been reviewed extensively by Brown (1968) and Armington (1974). Most recently, Tomita (1976) has published an excellent review of the electrophysiology of the retinal cells.

EXPERIMENTAL RATIONALE

Experiments were designed to study the effect of hyperbaric oxygen on the *in vitro* ERG. Since the ERG is a good monitor of retinal function, any effect of O_2 on the retina should be reflected by changes in the ERG. All experimental preparations were exposed to 100% O_2 at 3800 torr. The control preparations were placed under O_2 at 740 torr because a satisfactory response could not be maintained under room air. Oxygen toxicity was defined as a decrease in the amplitude of ERG recorded from eyes exposed to hyperbaric oxygen as compared to control.

These oxygen toxicity studies were performed using the eyes from teleosts (trout and goldfish), amphibians (frogs), and mammals (rats). Teleosts were chosen because the retina is normally exposed to oxygen tensions in excess of 400 torr and therefore some resistance to oxygen toxicity was expected. The amphibian was chosen because it is a poikilotherm whose eye is not normally exposed to high tensions. The mammal was chosen since it has been shown to be particularly susceptible to oxygen toxicity and also so that a possible relationship between body temperature and susceptibility to oxygen toxicity might be investigated.

MATERIALS AND METHODS

Experimental Animals

Southern grass frogs (Rana pipiens), 15-20 cm long were obtained from Mogul-Ed Corp. (Oshkosh, Wis.) and were kept at room temperature (21 ± 1°C) in a moist aquarium. The frogs were maintained under natural photoperiod.

Goldfish (Carassius auratus) (7-9 cm) were purchased from a local pet dealer. They also were maintained under natural photoperiod at room temperature in dechlorinated tap water in an Instant Ocean aquarium (Aquarium Systems Inc., Wickliffe, Ohio).

Rainbow trout (Salmo gairdneri) (100-200 g) from Midwest Fish Farms (Harrison, Mich.) were maintained in fiberglass tanks at 12 ± 1°C. Dechlorinated tap water flowed continuously through the tanks and was aerated by compressed air filtered through activated charcoal. The photoperiod was 16 hr light, 8 hr dark.

Rats (Long-Evans strain) (200 g) were obtained from Charles River Breeding Laboratories (Wilmington, Mass.). Photoperiod was 12 hr light, 12 hr dark. Long-Evans rats were chosen, rather than the more common albino Sprague-Dawley strain, since their eyes are normally pigmented and it was therefore assumed the ERG recorded from these eyes

would be more comparable to the responses from the other animals used in these experiments.

Tissue Preparation

Frogs were killed by decapitation, fish by cervical section, and the rats by cervical dislocation.

The ERG was recorded from an eye cup which was prepared by the following method: A small incision was made at the junction of the cornea and sclera of an enucleated eye. An iris scissors was inserted into this incision and the cornea and iris were removed by cutting around the circumference of the eye. The lens was then removed with a forceps. In order to reduce the distance for diffusion of oxygen, the aqueous humor and as much of the vitreous humor as possible were removed by blotting with tissue paper. Trout vitreous humor is a semi-gel and therefore it was necessary to remove it by use of a forceps and iris scissors. This process was completed for both eyes within three minutes.

ERG Recording Circuitry

The eye cup preparation was placed in a Lucite recording chamber which was partially filled with non-nutrient Ringer solution (Appendix I). The eye was positioned over a hole in a shelf mounted in this chamber so that the back of the eye made contact with the Ringer solution (Figs. 1 and 2).

Figure 1. *In vitro* ERG recording chamber and electrodes. The plastic chamber on right holds the calomel electrodes and Ringer solution and is connected to the eye chamber on the left by agar bridges. In the background is the hyperbaric chamber.

FIGURE 1

Figure 2. Schematic of *in vitro* ERG recording circuitry. Two conductor shielded cable lead from the pair of calomel electrodes to the P16 preamplifier and was connected to ground. For further details see the text.

In Vitro ERG Recording Circuitry

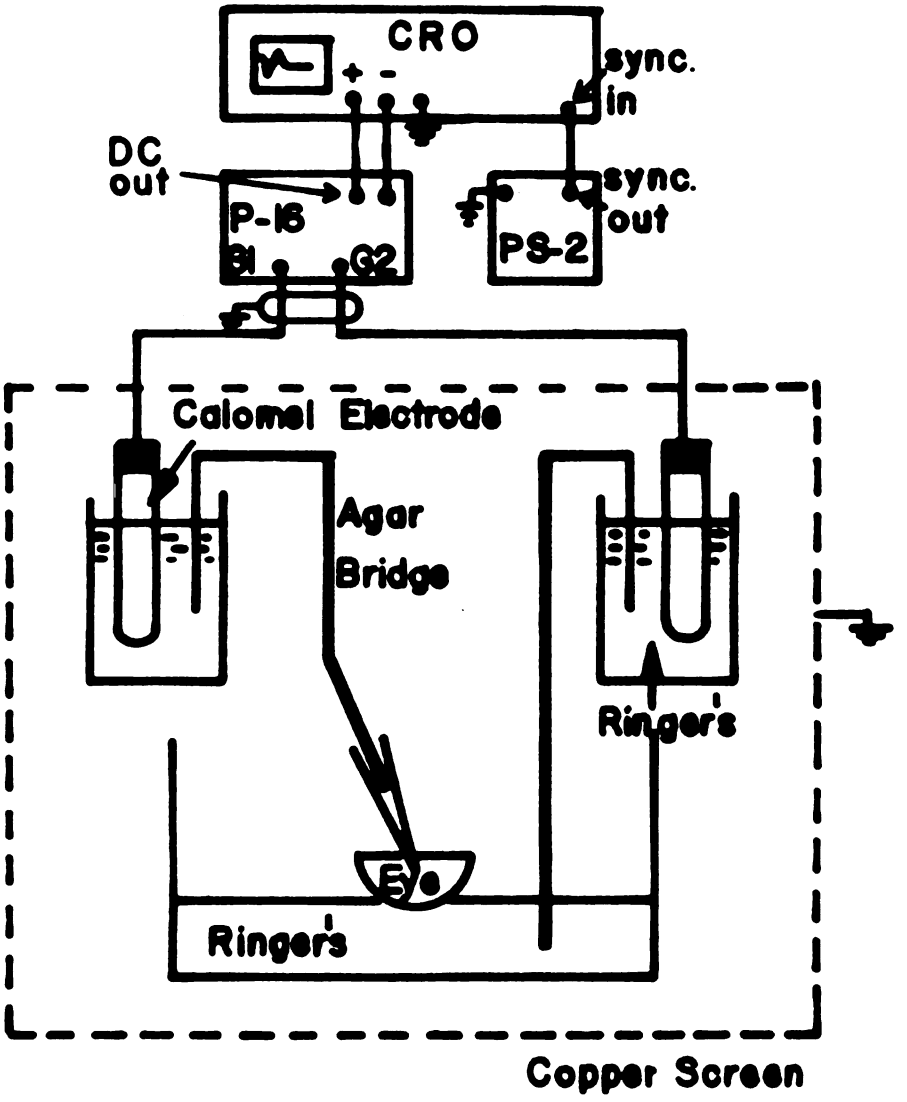


FIGURE 2

The active electrode, which was placed in a holder positioned over the eye cup, was lowered into the remaining vitreous humor. The electrodes used in these experiments were hand drawn from 2 mm I.D. soft glass tubing (0.2 ml Prothrombin Pipette, Dade, Miami, Fla.). The tip diameters of these electrodes were approximately 0.5 mm. The electrodes were filled with 4 g% agar in non-nutrient Ringer solution. These electrodes were connected to agar bridges made from PE 280 tubing also filled with 4 g% agar in Ringer solution. The electrodes and bridges were stored in Ringer's to prevent drying.

The active electrode was connected to Grid 1 (G1) of a Grass P16 AC-DC preamplifier (Grass Instruments, Quincy, Mass.) by an agar bridge and a Ringer's filled calomel electrode (S-30080-17 miniature reference electrode, Sargent-Welch Scientific Co., Detroit, Mich.) (Figs. 1 and 2).

The reference electrode was an agar bridge immersed in the Ringers solution which bathed the back of the eye cup and was connected to Grid 2 (G2) of the preamplifier by a Ringers-calomel electrode. The experimental preparations and electrodes were placed in a light tight electrically shielded cage connected to common ground. This cage was thermally insulated and temperature could be regulated between 15°C and 40°C.

Due to the slow response of the c-wave of the ERG it was necessary to use a direct-coupled output from the P16

preamplifier. The rise time was set at 10 msec (1/2 amp high frequency = 30 Hz). In order to minimize the pick up of extraneous signals the electrodes were wired in the standard push-pull configuration. A double-pole, double-throw switch between the preamplifier and the preparations allowed selective recording from either the control or experimental preparation.

Photostimulation was by means of a Grass PS2 photostimulator (Grass Instruments, Quincy, Mass.). The photostimulator was set at intensity 8 in all experiments. The light source was placed about 70 cm above the experimental preparations. The light was positioned so that both the control and experimental preparations were simultaneously and evenly illuminated. For a discussion of illumination by this instrument see Fonner (1973).

ERG's were displayed on channel 1 of a storage oscilloscope (Tektronix Instruments, Model 5031, Beaverton, Ore.) which was set in the direct-coupled mode. The oscilloscope was synchronized with the PS2 photostimulator and the stimulus was recorded by a photocell and displayed on channel 2 of the oscilloscope.

The instrumentation was calibrated by an internal calibration signal in the P16 preamplifier. Measurements of the amplitudes of the component waves of the ERG were made directly on the oscilloscope screen and selected responses were photographed with a 35 mm camera. The negatives were projected onto paper and traced by hand for reproduction in this dissertation.

Oxygen Toxicity Experiments

The in vitro ERG could not be maintained under room air therefore, it was necessary to place the control preparation in a sealed Lucite box under 100% O_2 at 740 torr.

The experimental preparation was placed in a Bethlehem Environmental Chamber (Model H-70-A, Bethlehem, Pa.) which was purged with pure O_2 and pressurized to 3800 torr (5 atm absolute). The chamber was fitted with a glass window which allowed photostimulation of the preparation. In order to facilitate statistical analysis of the data the right and left eyes of the experimental animal were selected randomly for either the control or experimental preparation and recordings were made alternately from the preparations.

Test flashes were begun after sufficient time for dark adaptation. In earlier experiments on frogs the eyes were stimulated every hour for 6-7 hr. In later experiments on fish stimuli were given every 1/2 hr for 6 hr. The response of the rat retina to hyperbaric oxygen was so rapid that it was necessary to record the ERG every 5 min. Frog and goldfish experiments were run at room temperature (20-22°C). Trout experiments were run at 15°C and rats at 40°C.

Recording of the ERG Using Recirculation Blocks

During this study it was questioned whether or not the eye cup was a satisfactory preparation for recording the trout ERG. Because of the relatively large size of the

eye and because the vitreous humor is difficult to remove it was questionable whether sufficient oxygen could diffuse to the retina.

As an alternative method of recording the ERG a pair of modified Lucite Ussing blocks was used. In preparation for recording, the eye was dark adapted, enucleated, and dissected in a petri dish with Ringer solution under red light. The cornea and lens were removed and a disc of sclera including the optic nerve was removed from the back of the eye. The retina was then teased away from the remaining ring of sclera including the optic nerve was removed from the back of the eye. The retina was then teased away from the remaining ring of sclera. The pigmented epithelium which pulls away from the retina during dark adaptation was peeled off of the retina and as much of the vitreous humor as possible was removed. A millipore filter (Millipore Corp., Bedford, Mass.) was placed on the vitreal side of the retina for support and this preparation was clamped between the reservoirs of the recirculation blocks. The blocks were filled with media and connected by Ringer's-4 g% agar bridges to the calomel cells as previously described.

The recirculation blocks have several advantages for ERG recording: (1) the blocks may be filled with different kinds of media and various substances and drugs may be added to the media. In this study several modifications of Ringer solution were used without success, however, an

ERG could be recorded when the blocks were filled with Tissue Culture Media 199 (Earles Base) (Grand Island Biological Co., Grand Island, N.Y.). (2) Since the media is constantly recirculated and aerated by an air lift siphon the effect of various gases and gas mixtures on the ERG may be studied. (3) It is possible to control the P_{O_2} at the retina.

Although several trout ERG's were recorded using the recirculation blocks, no oxygen toxicity experiments were done due to difficulty with the operation of the air lift siphons in the hyperbaric chamber.

RESULTS

The In Vitro ERG--General Observations

The ERG's recorded in this study were typical of both *in vivo* and *in vitro* examples found in the literature. The a-wave and b-wave were recorded in all cases and a c-wave usually appeared upon dark adaptation. Because of high intra-species variation in wave amplitude, especially in the case of the frog it was necessary to express the graphic data as percent of initial amplitude, i.e. the first recording made after the eye was considered to be dark adapted. Wave amplitudes were measured as shown in Figure 3. An ERG could be recorded from frog and fish eyes for at least six hr if the preparation was under oxygen at 740 torr. Rat eyes could be maintained for only 2-3 hr under oxygen at 740 torr. It was found that if the chamber was purged with air the rat ERG was abolished within three min. If the chamber was again purged with oxygen the ERG recovered within five min.

Actual dark adapted amplitudes of the ERG components are given in Tables 1, 2, 3, and 4. Except in the case of the trout, initial enhancement of the ERG was observed under hyperbaric oxygen.

Figure 3. Schematic of a typical ERG showing how wave amplitudes and latency were measured.

a is a-wave amplitude

b is b-wave amplitude

c is c-wave amplitude

L_b is b-wave latency

⊠ is photostimulus

Schematic Representation of the ERG

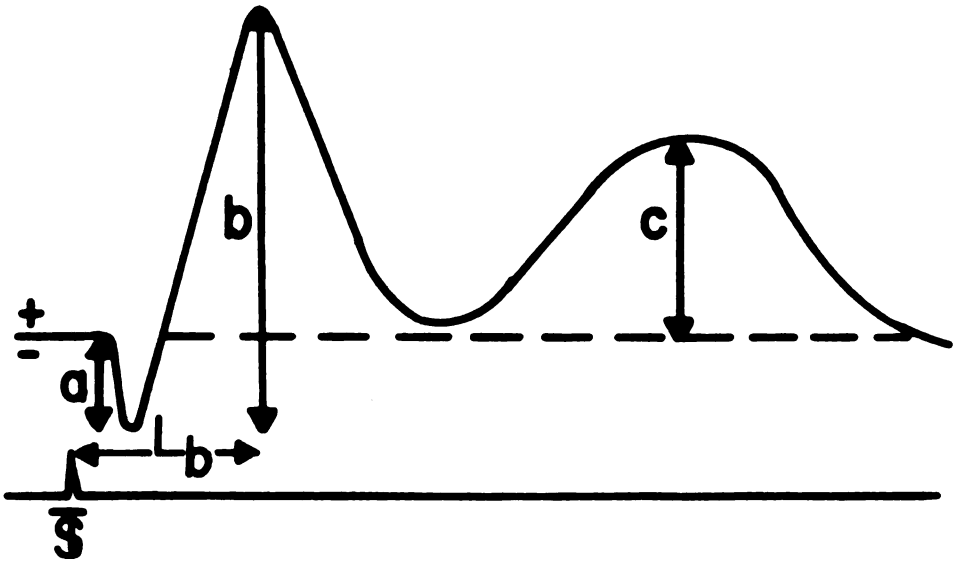


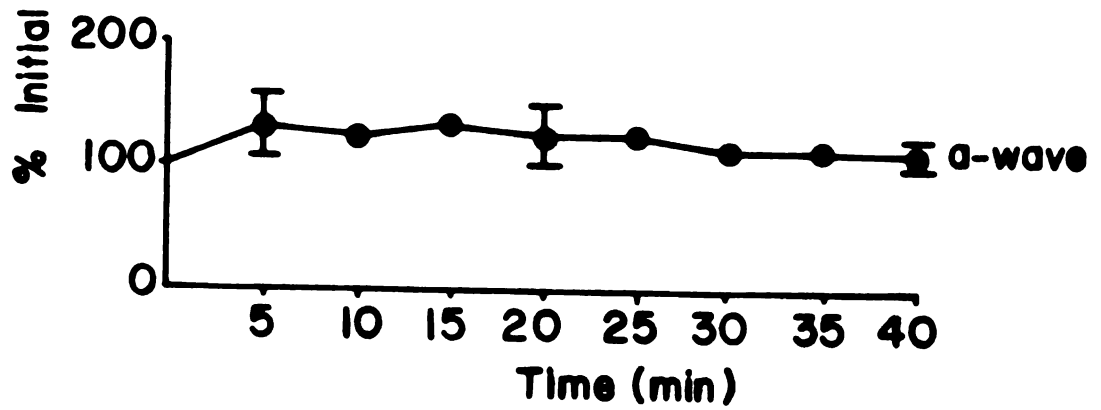
FIGURE 3

Differences in latency and waveform of the ERG from species to species were observed. The frog retina gave the slowest response with a b-wave latency of 500 msec. The b-wave latency was about 200 msec in the other species used. In frog ERG the potential returns nearly to base line before the beginning of the c-wave. This is not the case with the goldfish and trout. No c-wave was recorded from several of the control trout retinas. The c-wave was not present in the rat ERG. The response from the rat eye was unique however, in that a negative wave followed the b-wave. This late negativity is probably part of PIII (Weidner, 1976).

The Effect of Dark Adaptation on the ERG

The ERG is known to change during dark adaptation, therefore for each species used in this study it was necessary to determine the time required for dark adaptation of the isolated retina (Armington, 1974). Eyes were prepared for recording under white light and an ERG was recorded immediately after the experimental chamber was darkened. Subsequent recordings were made every five min until no further change in ERG was seen. The dark adaptation studies on frogs, goldfish and trout are shown in Figure 4, 5, and 6. Based on these results, data collection in oxygen toxicity experiments was begun after sufficient time for dark adaptation so as not to confuse changes in the ERG due to dark adaptation with changes caused by oxygen toxicity.

Figure 4. The effect of dark adaptation on the frog ERG. Amplitudes are expressed as percent initial and means ± SE for 4 experiments are plotted against time ($\bar{m}$in). Initial amplitude at beginning of dark adaptation, a-wave = 21.5 μV, b-wave = 250.0 μV. Recorded under O_2 at 740 torr at 22°C. In subsequent studies dark adaptation was assumed to have occurred by 30 min.


% Initial
200
100
0
5
10
15
20
25
30
35
40
Time (min)
a-wave

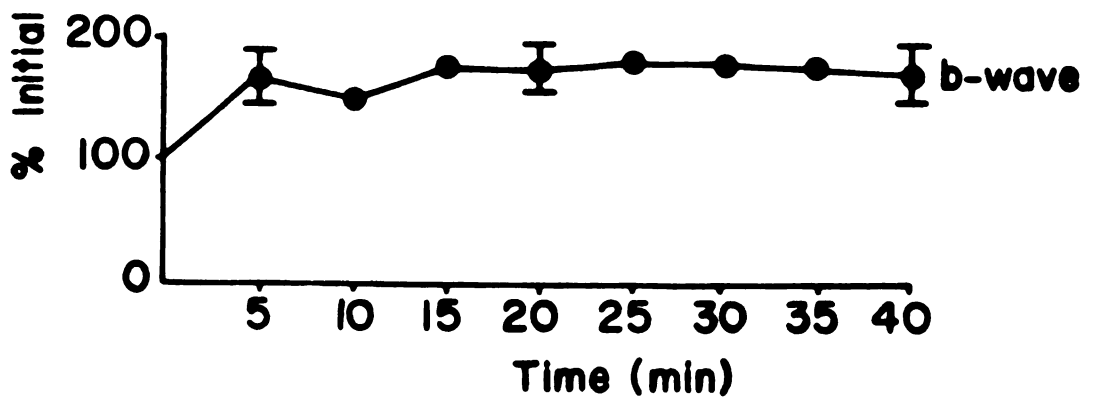
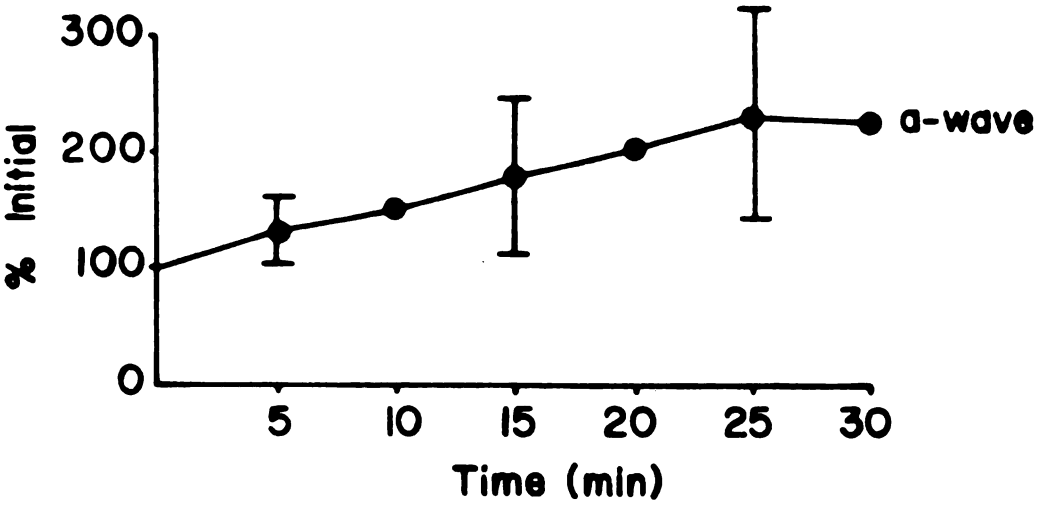

% Initial
200
100
0
5
10
15
20
25
30
35
40
Time (min)
b-wave

FIGURE 4

Figure 5. The effect of dark adaptation on the goldfish ERG. Amplitudes are expressed as percent initial and means ± SE for 4 experiments are plotted against time (min). Initial amplitude at beginning of dark adaptation, a-wave 14.3 μV, b-wave 23.0 μV. Recorded under O_2 at 740 torr at 22°C. In subsequent studies dark adaptation was assumed to have occurred by 30 min.


300
200
100
0
% Initial
a-wave
5
10
15
20
25
30
Time (min)

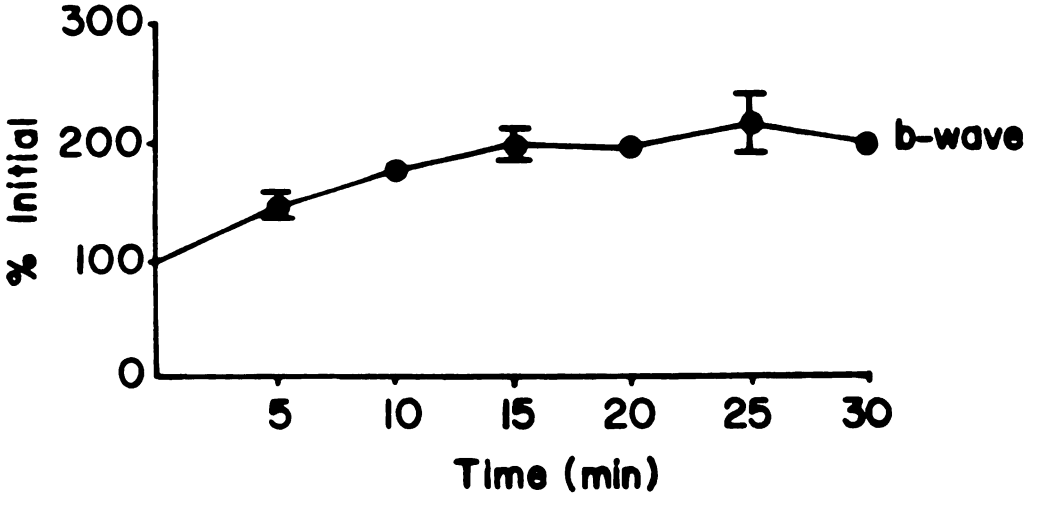

300
200
100
0
% Initial
b-wave
5
10
15
20
25
30
Time (min)

FIGURE 5

Figure 6. The effect of dark adaptation on the trout ERG. Amplitudes are expressed as percent initial and means ± SE for 4 experiments are plotted against time ($\bar{m}$in). Initial amplitude at beginning of dark adaptation, a-wave 14.5 μV, b-wave 16.3 μV. Recorded under O_2 at 740 torr at 22°C. In subsequent studies dark adaptation is assumed to have occurred by 30 min.

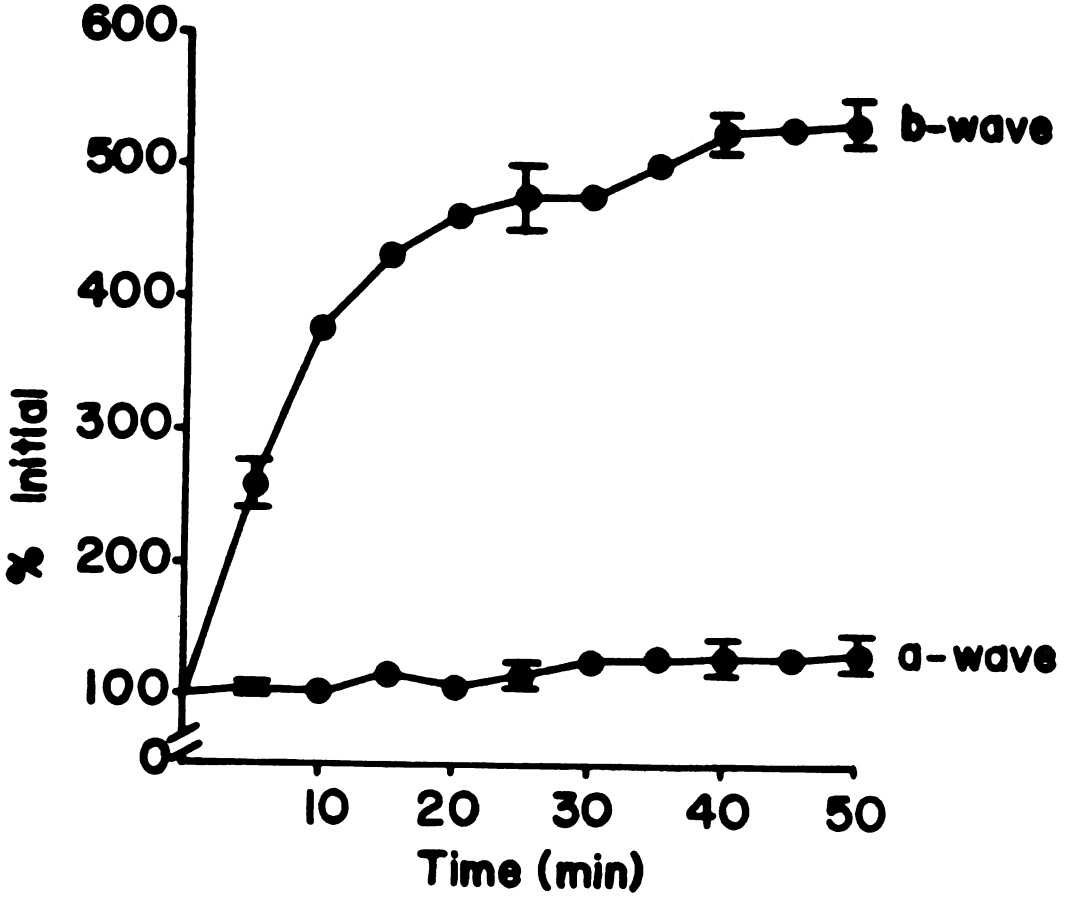

600
500
400
300
200
100
0
% Initial
b-wave
a-wave
10
20
30
40
50
Time (min)

FIGURE 6

Effect of Hyperbaric Oxygen on the Frog ERG

Exposure of the frog retina to oxygen at 3800 torr caused a definite decline of the ERG as compared to control. Wave amplitudes were expressed as percent initial amplitude and the results of all experiments were averaged. Lines were fitted to the data by linear regression (not including initial 100% reading). The reader is cautioned that this method may negate transient changes which take place during the time course of the experiment.

Significant attenuation of the a-wave is seen in both the experimental and control preparations (Table 1). However, the decline of the a-wave under hyperbaric oxygen begins at 2 hr, while the a-wave of the control preparation does not decline until 4 hr (Fig. 7). It should be noted that in both preparations an a-wave could always be recorded throughout the experiment and that the attenuation was not as great as that of the b-wave and c-wave of the retinas exposed to hyperbaric oxygen (Fig. 7; Table 1).

The b- and c-waves recorded from eyes under hyperbaric oxygen were significantly attenuated and sometimes abolished while normal responses could be recorded from the control preparations throughout the experimental period (Fig. 7). Note the sudden drop in b-wave amplitude between 4 and 5 hr. Hyperbaric oxygen causes the retinal cells to respond more uniformly than those under normobaric conditions as indicated by the small standard errors on the experimental data.

Figure 7. Changes in the frog ERG during exposure to O_2 at 740 torr and 3800 torr at 22°C. Points represent means ± SE of 9 experiments with wave amplitudes expressed as percent initial amplitude. Lines were fitted by linear regression.

A. a-wave 740 torr. Slope = -5.63[a], r_{xy} = 0.351[b], n = 57, initial amplitude 30.4 ± 4.2 μV.

B. a-wave 3800 torr. Slope = -9.39[a], r_{xy} = 0.56[b], n = 59, initial amplitude 25.4 ± 2.9 μV. Slopes of plots A and B are not significantly different from each other at 5% level.

C. b-wave 740 torr. Slope = 9.80[a], r_{xy} = 0.332[b], n = 52, initial amplitude 287.0 ± 78.0 μV.

D. b-wave 3800 torr. Slope = -15.38[a], r_{xy} = 0.801[b], n = 55, initial amplitude 575.8 ± 90.1 μV. Slopes of plots C and D are significantly different from each other at 5% level.

E. c-wave 740 torr. Slope = 6.33[c], r_{xy} = 0.192[d], n = 44, initial amplitude 313.4 ± 123.1 μV.

F. c-wave 3800 torr. Slope = -15.08[a], r_{xy} = 0.790[b], n = 44, initial amplitude 860.0 ± 130.9 μV. Slopes of plots E and F are significantly different from each other at 5% level.

a. Slope significantly different from 0 at 5% level.

b. Correlation significantly at 5% level.

c. Slope not significantly different from 0 at 5% level.

d. Correlation not significant at 5% level.

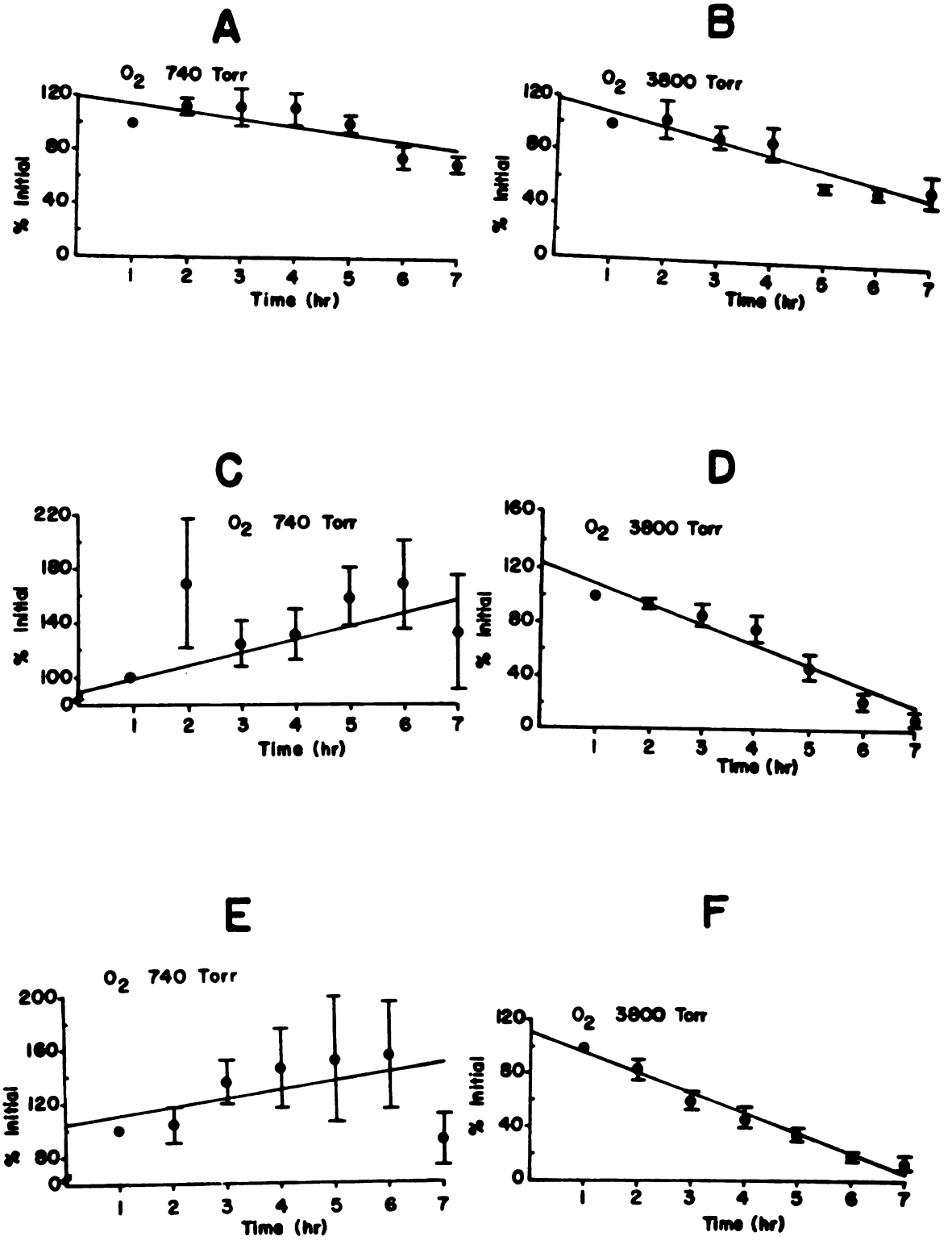

A
O_2 740 Torr
% Initial
Time (hr)
B
O_2 3800 Torr
% Initial
Time (hr)
C
O_2 740 Torr
% Initial
Time (hr)
D
O_2 3800 Torr
% Initial
Time (hr)
E
O_2 740 Torr
% Initial
Time (hr)
F
O_2 3800 Torr
% Initial
Time (hr)

FIGURE 7

Table 1. Changes in the ERG of the isolated frog retina following exposure to pure O_2 at 740 torr and 3800 torr at 22°C.

	1 hr (μV)	6 hr (μV)	d (μV)
a-wave 740	30.4 ± 4.2	20.4 ± 3.1	- 11.1 ± 1.6*
a-wave 3800	25.4 ± 2.9	17.2 ± 3.1	- 9.3 ± 2.6*
b-wave 740	287.0 ± 78.0	260.0 ± 92.4	- 27.0 ± 61.8
b-wave 3800	575.8 ± 90.1	44.8 ± 19.3	-531.1 ± 87.0*
c-wave 740	313.4 ± 123.1	338.0 ± 92.6	+ 24.6 ± 112.8
c-wave 3800	860.0 ± 130.9	128.8 ± 37.8	-731.3 ± 105.5*

Mean ± SE (n = 9)

d = 6 hr - 1 hr

* Significant difference at 5% level.

Figures 8 and 9 and Table 1 give further evidence that the ERG is attenuated by hyperbaric oxygen.

Effect of Hyperbaric Oxygen on the Rat ERG

Changes in the rat ERG took place rapidly compared to the other animals and as pointed out earlier the preparations did not last as long. Therefore recording was begun 15 min after darkening the chamber. Dark adaptation was complete by 35 min (Fig. 10). For this reason that data collected does not lend itself well to the same statistical analyses used for the other animals in this study. Definite differences, however, are seen between the responses of the preparations under normobaric oxygen and hyperbaric oxygen.

While the control a-wave held its dark adapted amplitude for 2 hr the a-wave was greatly attenuated and often abolished after 95-105 min under hyperbaric oxygen (Fig. 10).

Initially the b-wave recorded under hyperbaric oxygen was greatly enhanced over the control b-wave. After dark adaptation, however, both b-waves declined. The waveform of the control ERG (Fig. 11) was like the ERG recorded by Weidner (1976) during hypoxia, indicating that perhaps oxygen at 740 torr is not sufficient to maintain the rat eye cup. The wave-form of the ERG under hyperbaric oxygen has typical a-wave and b-wave components throughout most of the experiment, but the b-wave declines at a faster rate than the control b-wave and is often abolished by

Figure 8. Difference of amplitudes of frog ERG's during O_2 exposure at 740 and 3800 torr at 22°C. Differences in wave amplitudes (control minus experimental) for 9 paired experiments were averaged and plotted against time (hours). Points represent Mean ± SE. Lines were fitted by linear regression.

A. a-wave. Slope = 3.60[a], r_{xy} = 0.130[b], n = 47.

B. b-wave. Slope = 26.43[c], r_{xy} = 0.554[d], n = 43.

C. c-wave. Slope = 18.72[c], r_{xy} = 0.445[d], n = 31.

a. Slope not significantly different from 0 at 5% level.

b. Correlation not significant at 5% level.

c. Slope significantly different from 0 at 5% level.

d. Correlation significant at 5% level.

$\bar{X}$ Dif. Amp. = Mean difference in amplitude (control-experimental)

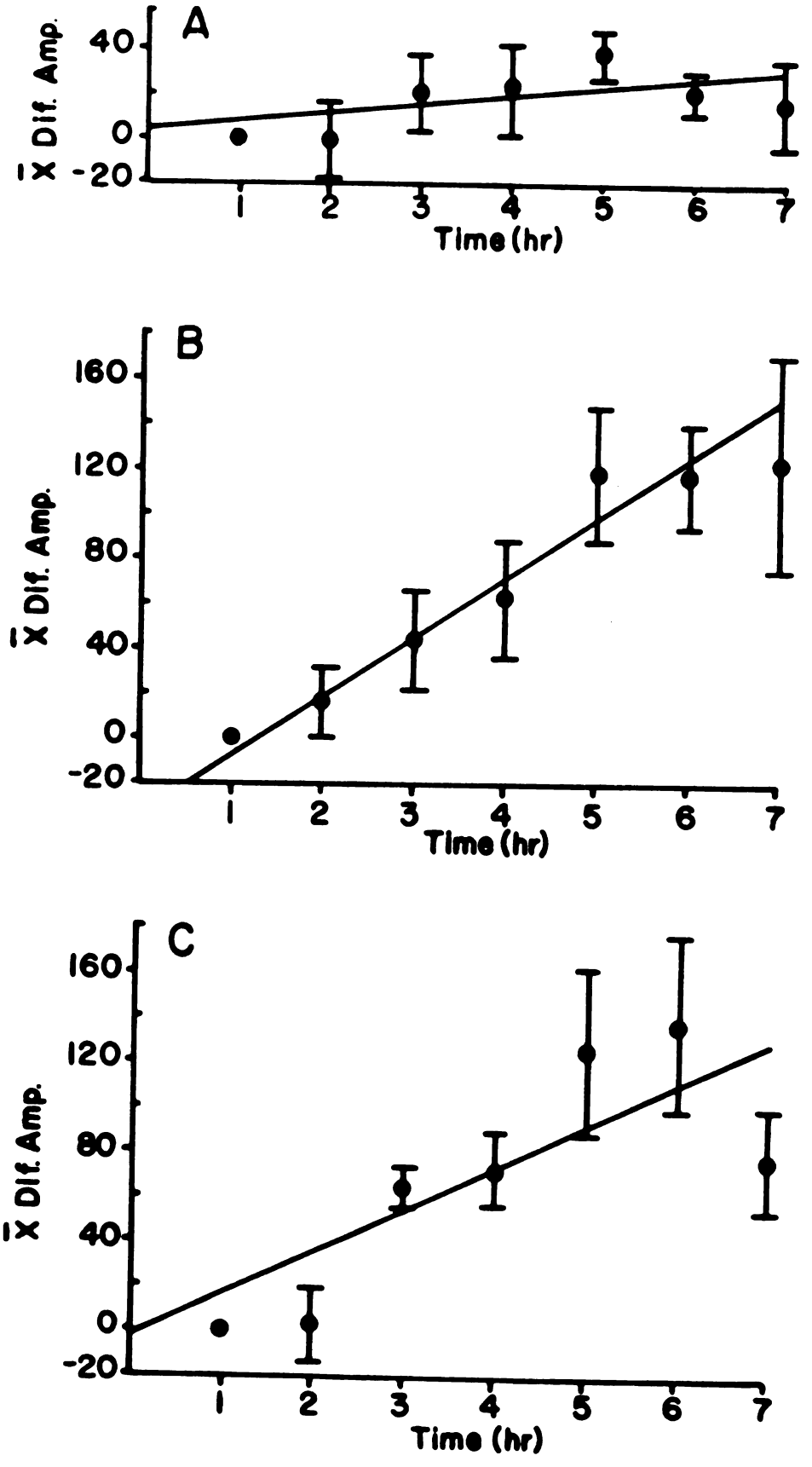

A
40
0
-20
X̄ Dif. Amp.
1
2
3
4
5
6
7
Time (hr)
B
160
120
80
40
0
-20
X̄ Dif. Amp.
1
2
3
4
5
6
7
Time (hr)
C
160
120
80
40
0
-20
X̄ Dif. Amp.
1
2
3
4
5
6
7
Time (hr)

FIGURE 8

Figure 9. Frog ERG's recorded *in vitro* during exposure to oxygen at 3800 torr. Recordings were made over a 6 hr period at 22°C. The response from the control preparation was comparable to those seen in part A and was maintained throughout the 6 hr period. Note change in amplitude calibration in parts C and D.

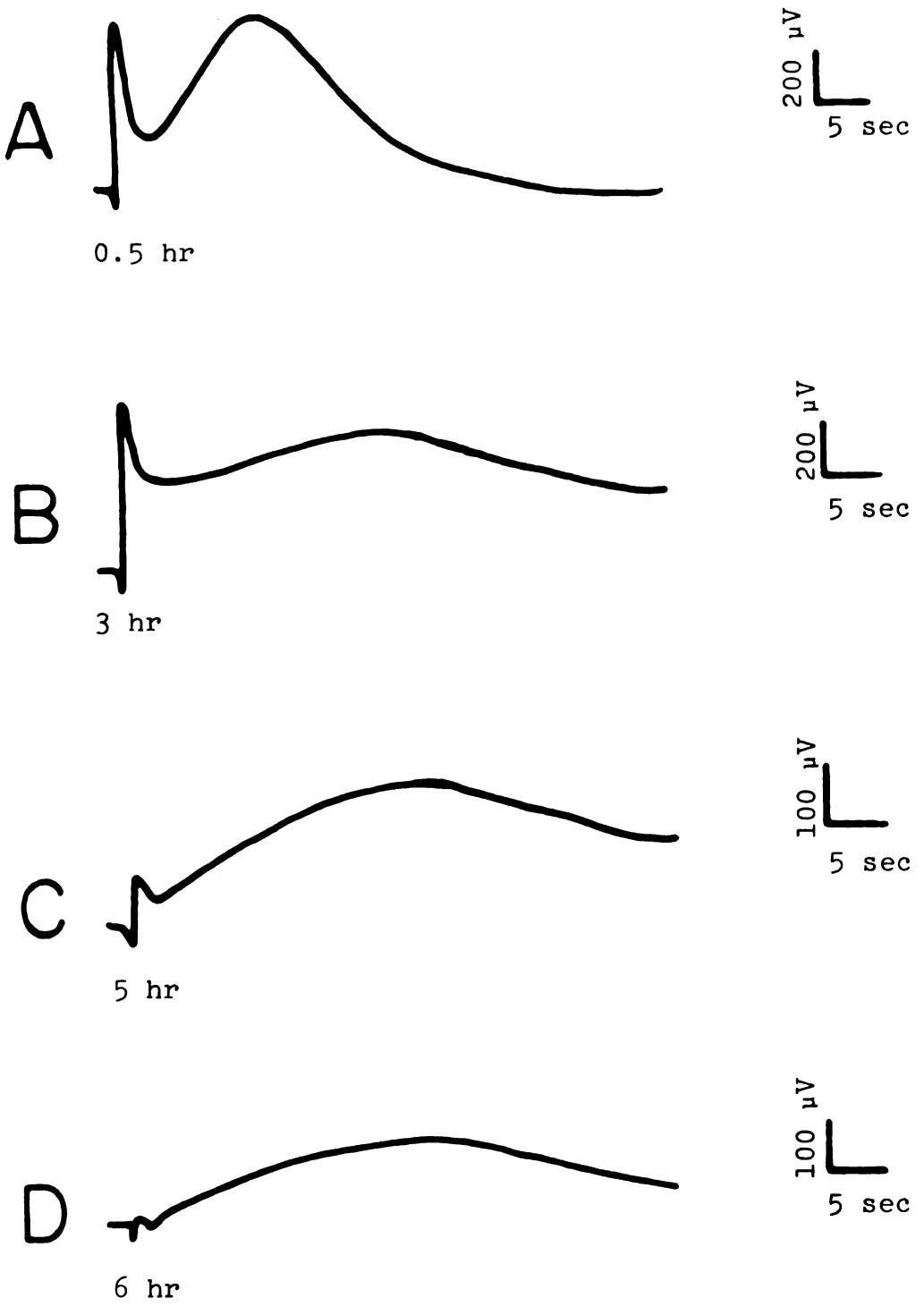

A
0.5 hr
200 µV
5 sec
B
3 hr
200 µV
5 sec
C
5 hr
100 µV
5 sec
D
6 hr
100 µV
5 sec

FIGURE 9

Figure 10. Changes in the rat ERG during exposure to O_2 at 740 torr and 3800 torr at 40°C. Points represent means ± SE for 14 control eyes and 11 experimental eyes. Curves were fitted by hand.

A. a-wave 740 torr.
Initial amplitude 16.1 ± 4.9 μV.

B. a-wave 3800 torr.
Initial amplitude 15.0 ± 1.9 μV.

C. b-wave 740 torr.
Initial amplitude 10.8 ± 2.6 μV.

D. b-wave 3800 torr.
Initial amplitude 32.3 ± 6.6 μV.

E. PIII 740 torr.
Initial amplitude 39.4 ± 9.5 μV.

F. PIII 3800 torr. Slope = -0.91 (significantly different from 0 at 5% level), r_{xy} = 0.520 (significant at 5% level), n = 82, initial amplitude 73.4 ± 10.1 μV.

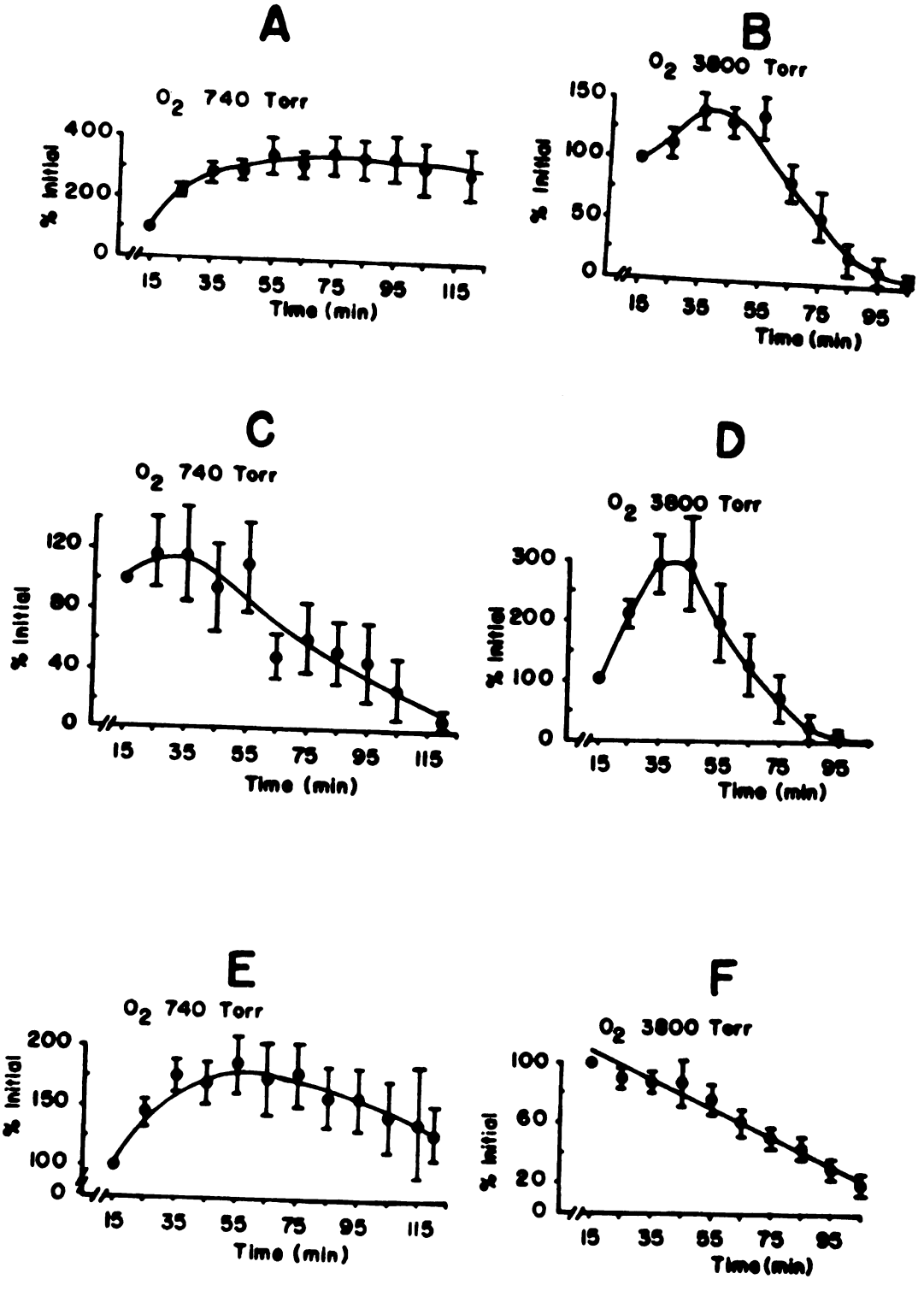

A
O_2 740 Torr
% Initial
400
200
0
15 35 55 75 95 115
Time (min)
B
O_2 3800 Torr
% Initial
150
100
50
0
15 35 55 75 95
Time (min)
C
O_2 740 Torr
% Initial
120
80
40
0
15 35 55 75 95 115
Time (min)
D
O_2 3800 Torr
% Initial
300
200
100
0
15 35 55 75 95
Time (min)
E
O_2 740 Torr
% Initial
200
150
100
0
15 35 55 75 95 115
Time (min)
F
O_2 3800 Torr
% Initial
100
60
20
0
15 35 55 75 95
Time (min)

FIGURE 10

Figure 11. Rat ERG's recorded *in vitro*. A, B, C and D. Recorded during exposure to O_2 at 3800 torr and 40°C. Note change in amplitude calibration.

E and F. Control rat ERG's recorded *in vitro* under O_2 at 740 torr and 40°C. See text for interpretation.

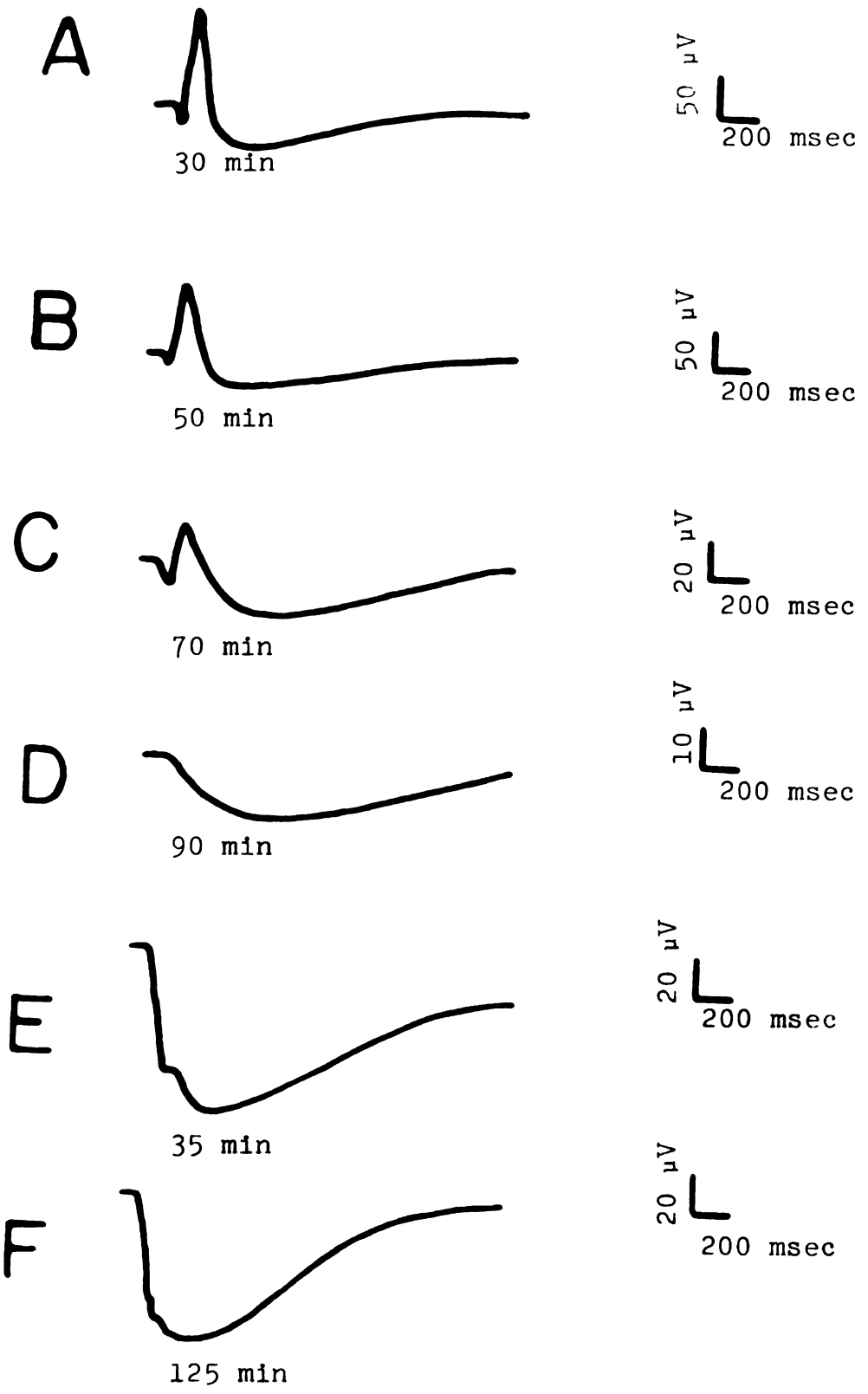

A
30 min
50 µV
200 msec
B
50 min
50 µV
200 msec
C
70 min
20 µV
200 msec
D
90 min
10 µV
200 msec
E
35 min
20 µV
200 msec
F
125 min
20 µV
200 msec

FIGURE 11

85-105 min of oxygen exposure. Note that the decline of the b-wave closely parallels the decline of the a-wave (Fig. 10).

The late negative wave of the in vitro rat ERG makes it possible to study the effect of hyperbaric oxygen on the slow PIII. Initial enhancement of PIII in the control preparation is followed by a decrease in amplitude. The response is well above the initial amplitude at 2 hr (Fig. 10; Table 2). PIII declines continuously during exposure of the eye to hyperbaric oxygen (Figs. 10 and 11; Table 2). Means of differences were not plotted due to a lack of a significant amount of paired data.

Effect of Hyperbaric Oxygen on the Goldfish ERG

The goldfish retina continues to function normally during exposure to hyperbaric oxygen. No significant attenuation of the ERG occurs during the 6 hr experimental period (Figs. 12 and 13; Table 3). Normal a- and b-waves were also maintained in the control preparation, however, the c-wave declined (Fig. 12; Table 3).

Plots of the mean differences in amplitudes (control-experimental) versus time show that there was no significant change in these differences for the a- and b-waves during the experimental period (Fig. 13). ERG recordings are shown in Figure 14.

These results are especially significant when compared to the changes in the frog and rat ERG's under hyperbaric oxygen which both showed attenuation.

Table 2. Changes in the rat ERG during exposure to oxygen at 740 torr and 3800 torr.

	15 min (μV)	35 min (μV)	105 min (μV)	d (μV)
a-wave 740	16.1 ± 4.9	36.2 ± 7.7	32.9 ± 4.6	- 6.7 ± 4.1
a-wave 3800	15.0 ± 1.9	20.8 ± 3.1	1.2 ± 1.2	-19.5 ± 3.0*
b-wave 740	10.8 ± 2.6	11.7 ± 3.1	2.4 ± 1.5	- 9.3 ± 2.4*
b-wave 3800	32.3 ± 6.6	78.7 ± 13.8	0.46 ± 0.46	-78.2 ± 13.6*
PIII 740	39.4 ± 9.5	54.9 ± 11.0	40.3 ± 6.4	-13.9 ± 6.6
PIII 3800	73.4 ± 10.1	62.4 ± 9.3	18.2 ± 6.8	-44.2 ± 7.2*

* Significant difference at 5% level.

d = 105 min - 35 min

n = 14 at 740 torr

n = 11 at 3800 torr

Mean ± SE

Figure 12. The goldfish ERG during exposure to O_2 at 740 torr and 3800 torr at 22°C. Points represent means ± SE for 9 control eyes and 15 experimental eyes. Wave amplitudes are expressed as percent of initial amplitude. Lines were fitted by linear regression.

A. a-wave 740 torr. Slope = 2.07[a], r_{xy} = 0.100[b], n = 88, initial amplitude 10.2 ± 3.0 μV.

B. a-wave 3800 torr. Slope = -1.12[a], r_{xy} = 0.041[b], n = 137, initial amplitude 26.6 ± 3.7 μV. Slopes of plots A and B are not significantly different from each other at 5% level.

C. b-wave 740 torr. Slope = -3.90[a], r_{xy} = 0.100[b], n = 88, initial amplitude 42.9 ± 12.0 μV.

D. b-wave 3800 torr. Slope = 0.62[a], r_{xy} = 0.000[b], n = 137, initial amplitude 128.7 ± 21.6 μV. Slopes of plots C and D are not significantly different from each other at 5% level.

E. c-wave 740 torr. Slope = -11.98[c], r_{xy} = 0.504[d], n = 80, initial amplitude 150.3 ± 17.8 μV.

F. c-wave 3800 torr. Slope = 5.57[a], r_{xy} = 0.148[d], n = 110, initial amplitude 150.3 ± 29.4 μV.

a. Slope not significantly different from 0 at 5% level.

b. Correlation not significant at 5% level.

c. Slope significantly different from 0 at 5% level.

d. Correlation significant at 5% level.

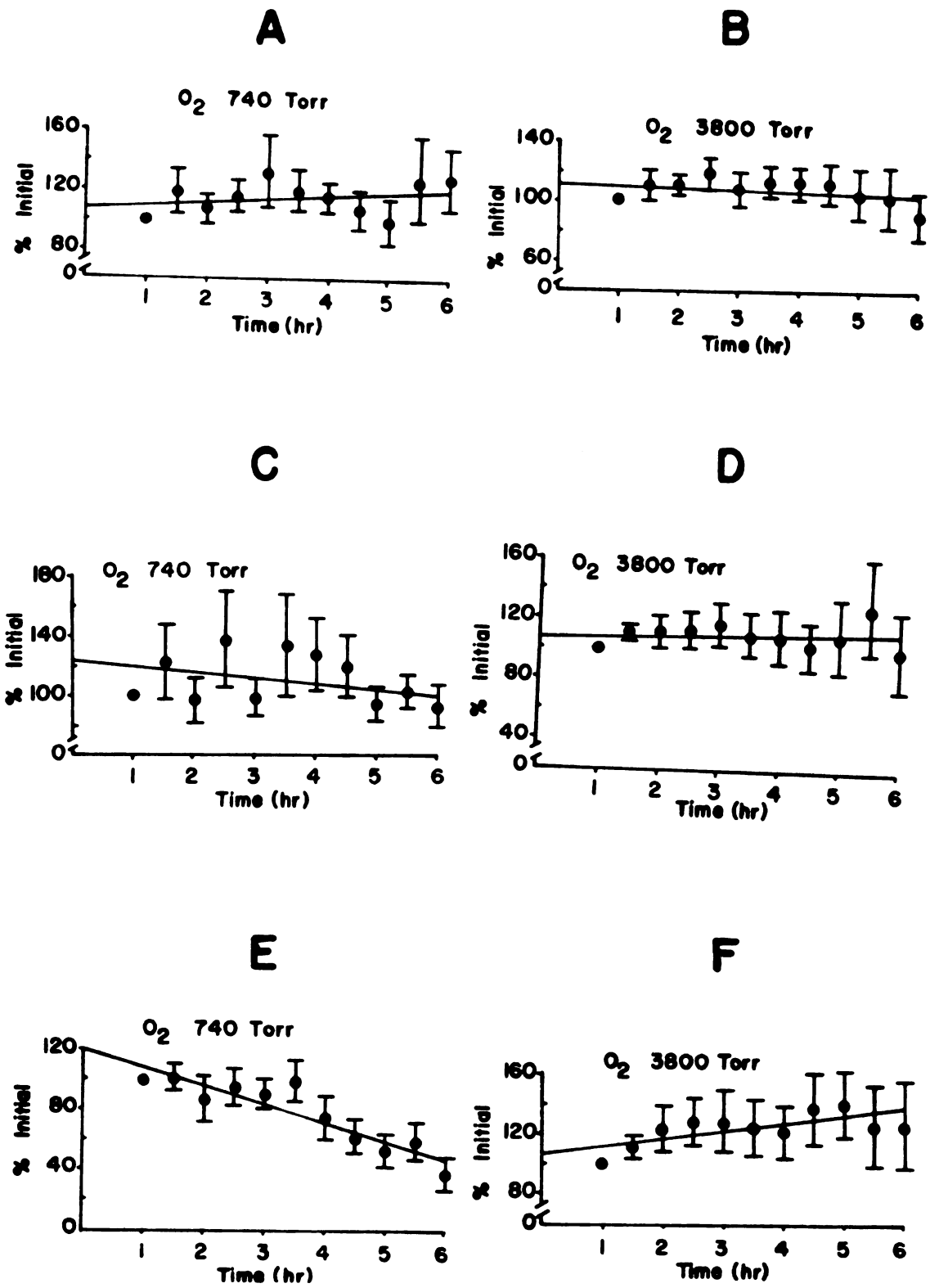

A
O_2 740 Torr
160
120
80
0
% Initial
1 2 3 4 5 6
Time (hr)
B
O_2 3800 Torr
140
100
60
0
% Initial
1 2 3 4 5 6
Time (hr)
C
O_2 740 Torr
160
140
100
0
% Initial
1 2 3 4 5 6
Time (hr)
D
O_2 3800 Torr
160
120
80
40
0
% Initial
1 2 3 4 5 6
Time (hr)
E
O_2 740 Torr
120
80
40
0
% Initial
1 2 3 4 5 6
Time (hr)
F
O_2 3800 Torr
160
120
80
0
% Initial
1 2 3 4 5 6
Time (hr)

FIGURE 12

Figure 13. Differences of amplitudes of goldfish ERG's during exposure to O_2 at 740 torr and 3800 torr at 22°C. Differences in wave amplitudes (control minus experimental) for 9 paired experiments were averaged and plotted against time (hours). Points represent mean ± SE. Lines were fitted by linear regression.

A. a-wave. Slope = 0.45^a, $r_{xy} = 0.037^b$, n = 79.

B. b-wave. Slope = 4.58^a, $r_{xy} = 0.170^c$, n = 72.

C. c-wave. Slope = -15.98^d, $r_{xy} = 0.400^c$, n = 44.

a. Slope not significantly different from 0 at 5% level.

b. Correlation not significant at 5% level.

c. Correlation significant at 5% level.

d. Slope significantly different from 0 at 5% level.

$\bar{X}$ Dif. Amp. = Mean difference in amplitude (control-experimental).

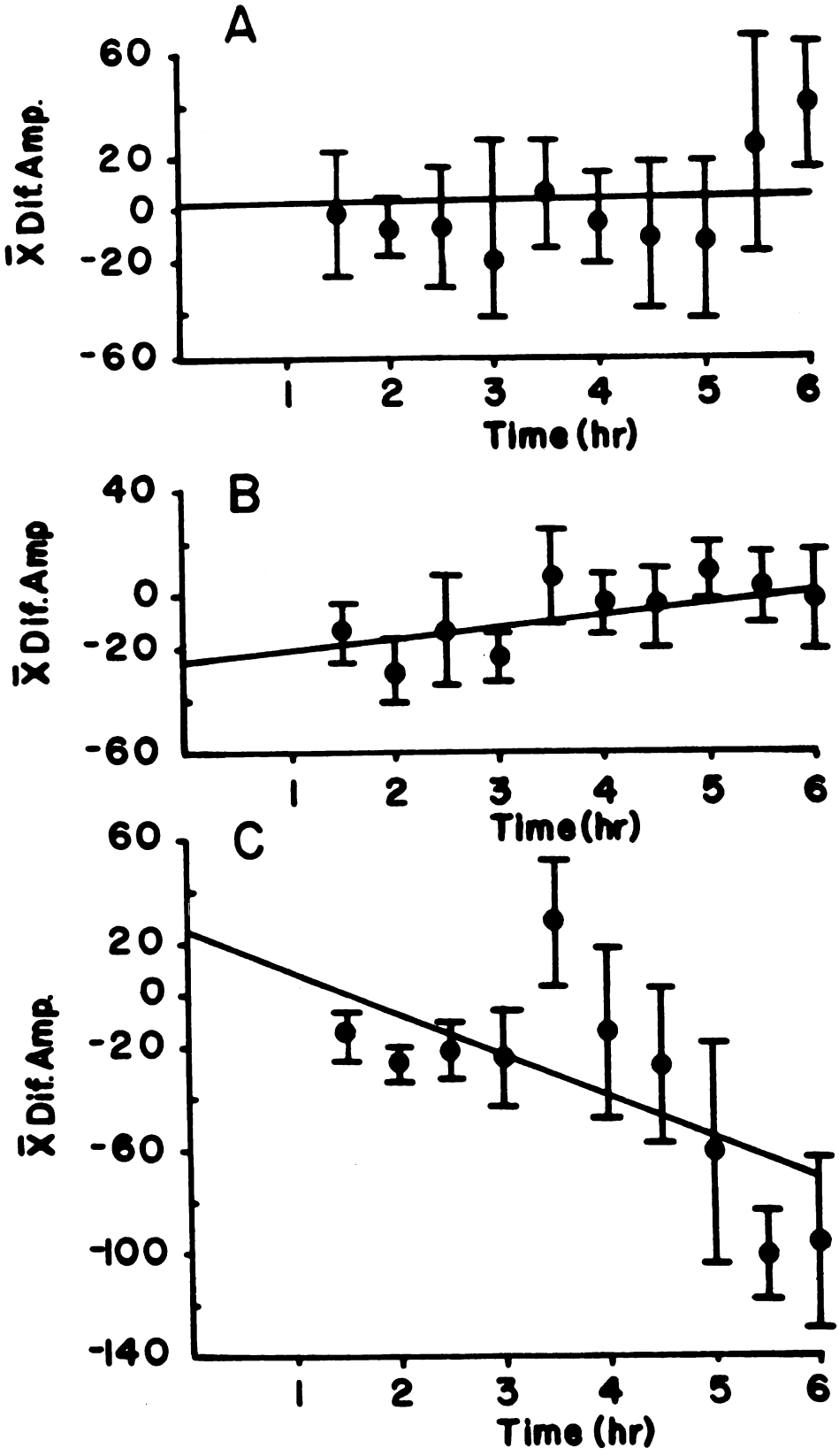

A
60
20
0
-20
-60
X̄ Dif. Amp.
1
2
3
4
5
6
Time (hr)
B
40
0
-20
-60
X̄ Dif. Amp
1
2
3
4
5
6
Time (hr)
C
60
20
0
-20
-60
-100
-140
X̄ Dif. Amp.
1
2
3
4
5
6
Time (hr)

FIGURE 13

Table 3. Effect of exposure to pure O_2 at 740 torr and 3800 torr on the ERG of isolated goldfish retina.

	1 hr (μV)	6 hr (μV)	d (μV)
a-wave 740	10.2 ± 3.0	11.9 ± 3.6	+ 1.1 ± 2.1
a-wave 3800	26.6 ± 3.7	21.2 ± 3.8	- 4.9 ± 2.3
b-wave 740	42.9 ± 12.0	30.9 ± 8.0	-12.0 ± 8.9
b-wave 3800	128.7 ± 21.6	114.3 ± 26.4	-14.3 ± 26.6
c-wave 740	57.9 ± 17.8	25.9 ± 8.4	-32.0 ± 10.9*
c-wave 3800	150.3 ± 29.4	203.4 ± 59.1	+53.1 ± 37.6

Mean ± SE $(n = 9)_{740}$ $(n = 15)_{3800}$

d = 6 hr - 1 hr

* Significant difference at 5% level.

Figure 14. Goldfish ERG's recorded in vitro during exposure to O_2 at 3800 torr. Recordings were made over a 6 hr period at 22°C. The control response was comparable and followed the same course during the experiment.

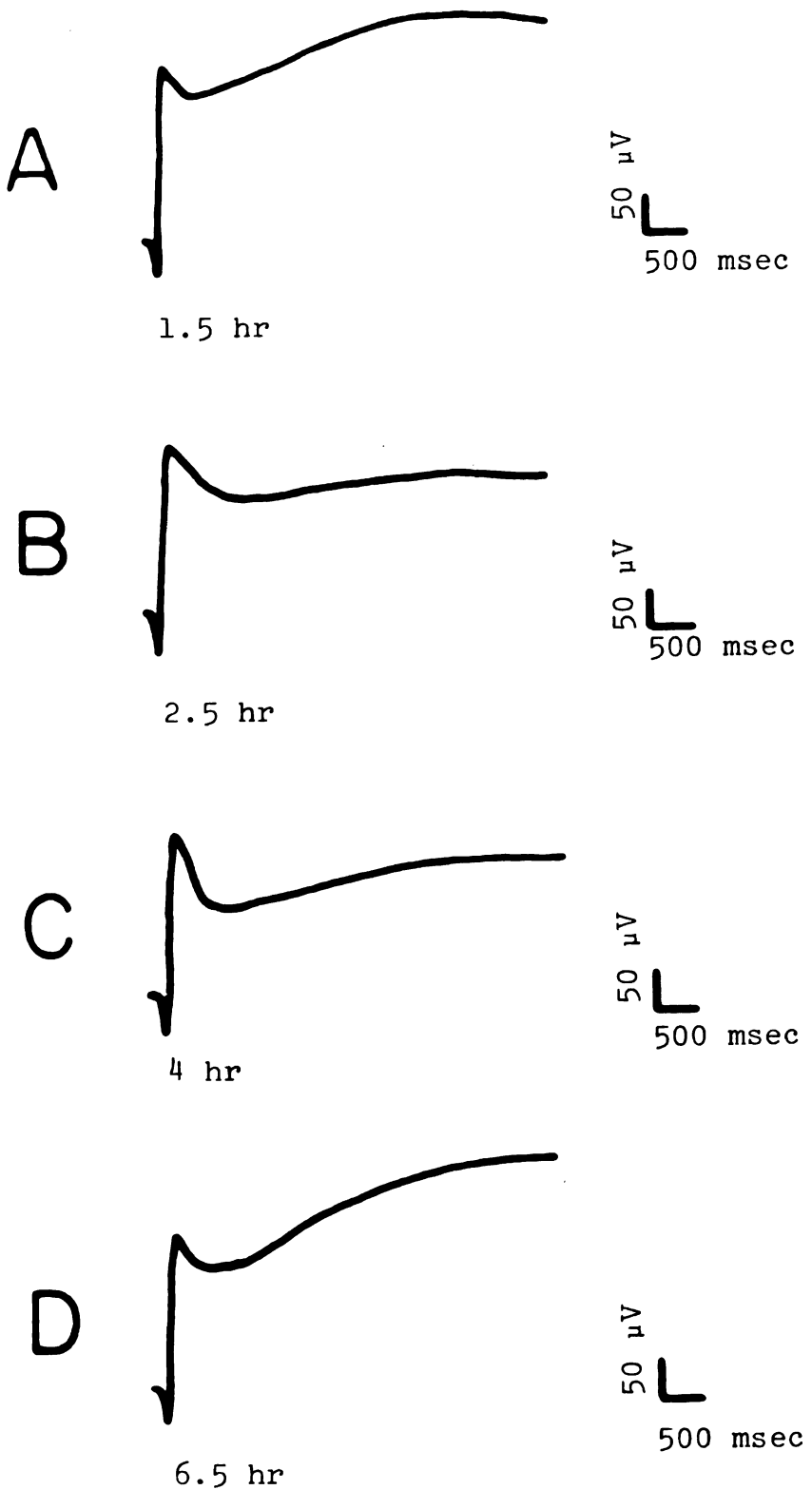

A
50 µV
500 msec
1.5 hr
B
50 µV
500 msec
2.5 hr
C
50 µV
500 msec
4 hr
D
50 µV
500 msec
6.5 hr

FIGURE 14

Effect of Hyperbaric Oxygen on the Trout ERG

ERG's were recorded from the trout retina throughout the 6 hr experimental period under oxygen at 740 torr and 3800 torr at 15°C. The ERG recorded under room air (Fig. 17E) resembled those recorded by Fonner (1973) during hypoxia.

As shown in Figures 15 and 16 and Table 4, there was no significant change in the a-wave during exposure to hyperbaric oxygen and the response was not different from that of the control preparation.

The b-wave of both preparations was significantly attenuated after 6 hr (Fig. 15; Table 4). These responses were not significantly different from each other (Figs. 15 and 16).

The c-wave was a highly variable response and its amplitude fluctuated during the experimental period (Fig. 13). It was not always recorded from the control preparation and a difference of means were not plotted due to a lack of significant number of paired experiments. The c-wave did not reach peak amplitude until 1 hr of dark adaptation and did not change significantly after that time (Fig. 15 and Table 4).

Trout ERG's recorded during hyperbaric oxygen exposure are shown in Figure 17. An ERG recorded using the modified Ussing blocks is also shown. This may be a more satisfactory preparation for study of the in vitro trout ERG in view of the decline of the b-wave recorded from the eye cup.

Figure 15. Changes in the trout ERG during exposure to O_2 at 740 torr and 3800 torr at 15°C. Points represent mean ± SE for 10 experiments with wave amplitudes expressed as percent initial amplitude. Lines were fitted by linear regression.

A. a-wave 740 torr. Slope = -4.76[a], r_{xy} = 0.200[b], n = 102, initial amplitude 33.8 ± 5.6 μV.

B. a-wave 3800 torr. Slope = -3.39[c], r_{xy} = 0.085[d], n = 101, initial amplitude 27.3 ± 3.9 μV. Slopes of plots A and B are not significantly different from each other at 5% level.

C. b-wave 740 torr. Slope = -11.12[a], r_{xy} = 0.539[b], n = 102, initial amplitude 62.5 ± 7.5 μV.

D. b-wave 3800 torr. Slope = -12.52[a], r_{xy} = 0.412[b], n = 101, initial amplitude 60.1 ± 12.7 μV. Slopes of plots C and D are not significantly different from each other at 5% level.

E. c-wave 740 torr. Slope = -2.20[c], r_{xy} = 0.173[d], n = 42, initial amplitude 22.0 ± 3.7 μV.

F. c-wave 3800 torr. Slope = -0.41[c], r_{xy} = 0.00, n = 75, initial amplitude 26.5 ± 6.0 μV. Slopes of plots E and F are not significantly different from each other at 5% level.

a. Slope significantly different from 0 at 5% level.

b. Correlation significant at 5% level.

c. Slope not significantly different from 0 at 5% level.

d. Correlation not significant at 5% level.

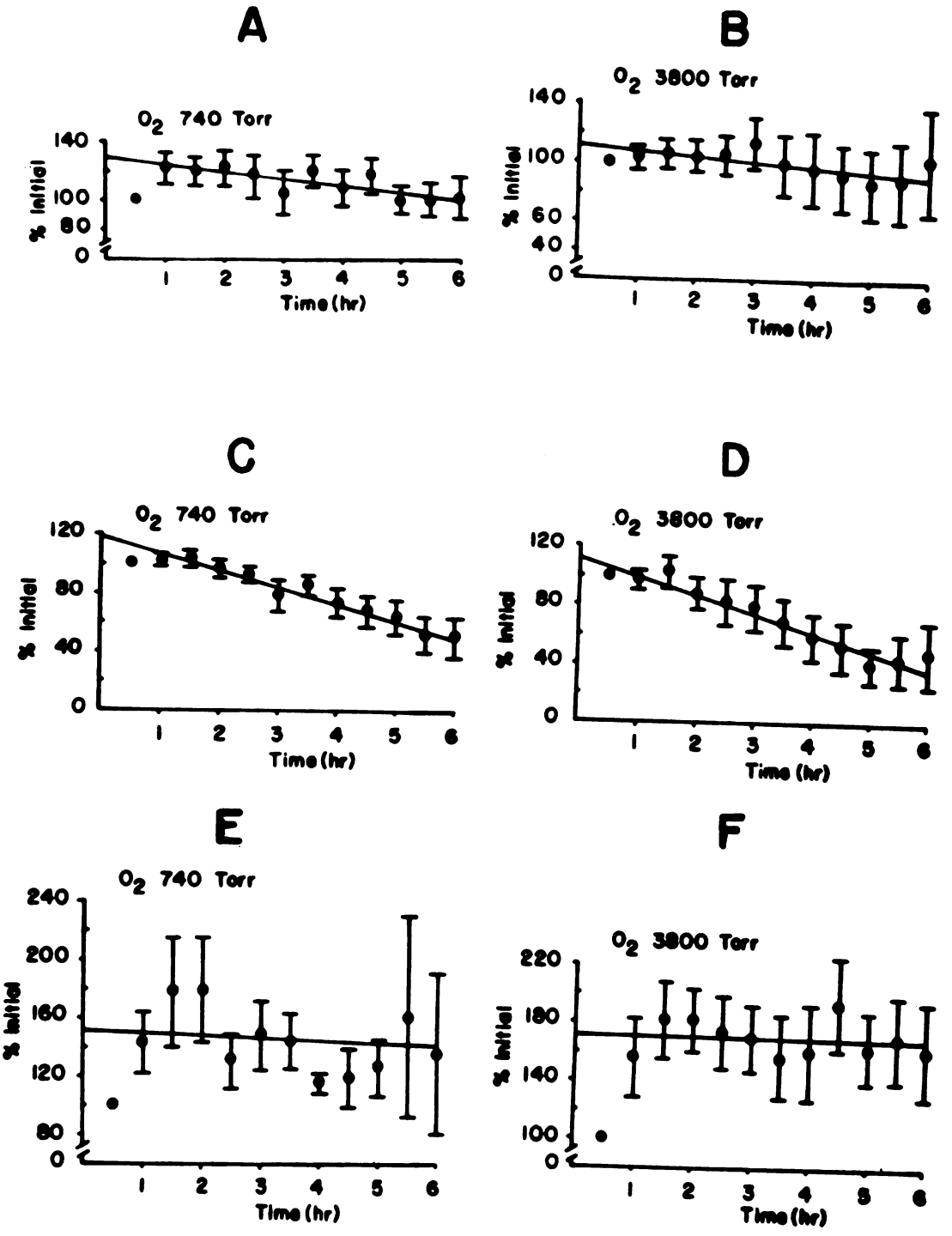

A
O_2 740 Torr
% Initial
140
100
80
0
1 2 3 4 5 6
Time (hr)
B
O_2 3800 Torr
% Initial
140
100
60
40
0
1 2 3 4 5 6
Time (hr)
C
O_2 740 Torr
% Initial
120
80
40
0
1 2 3 4 5 6
Time (hr)
D
O_2 3800 Torr
% Initial
120
80
40
0
1 2 3 4 5 6
Time (hr)
E
O_2 740 Torr
% Initial
240
200
160
120
80
0
1 2 3 4 5 6
Time (hr)
F
O_2 3800 Torr
% Initial
220
180
140
100
0
1 2 3 4 5 6
Time (hr)

FIGURE 15

Figure 16. Differences of amplitudes of trout ERG's recorded during exposure to O_2 at 740 torr and 3800 torr at 15°C. Differences in amplitude (control minus experimental) for 10 paired experiments were averaged and plotted against time. Points represent mean $\pm$ SE. Lines were fitted by linear regression.

A. a-wave. Slope = 0.02^a, r_{xy} = 0.00, n = 101.

B. b-wave. Slope = 0.55^a, r_{xy} = 0.147^b, n = 101.

a. Slope not significantly different from 0 at 5% level.

b. Correlation not significant at 5% level.

$\overline{X}$ Dif. Amp. = Mean difference in amplitude (control-experimental).

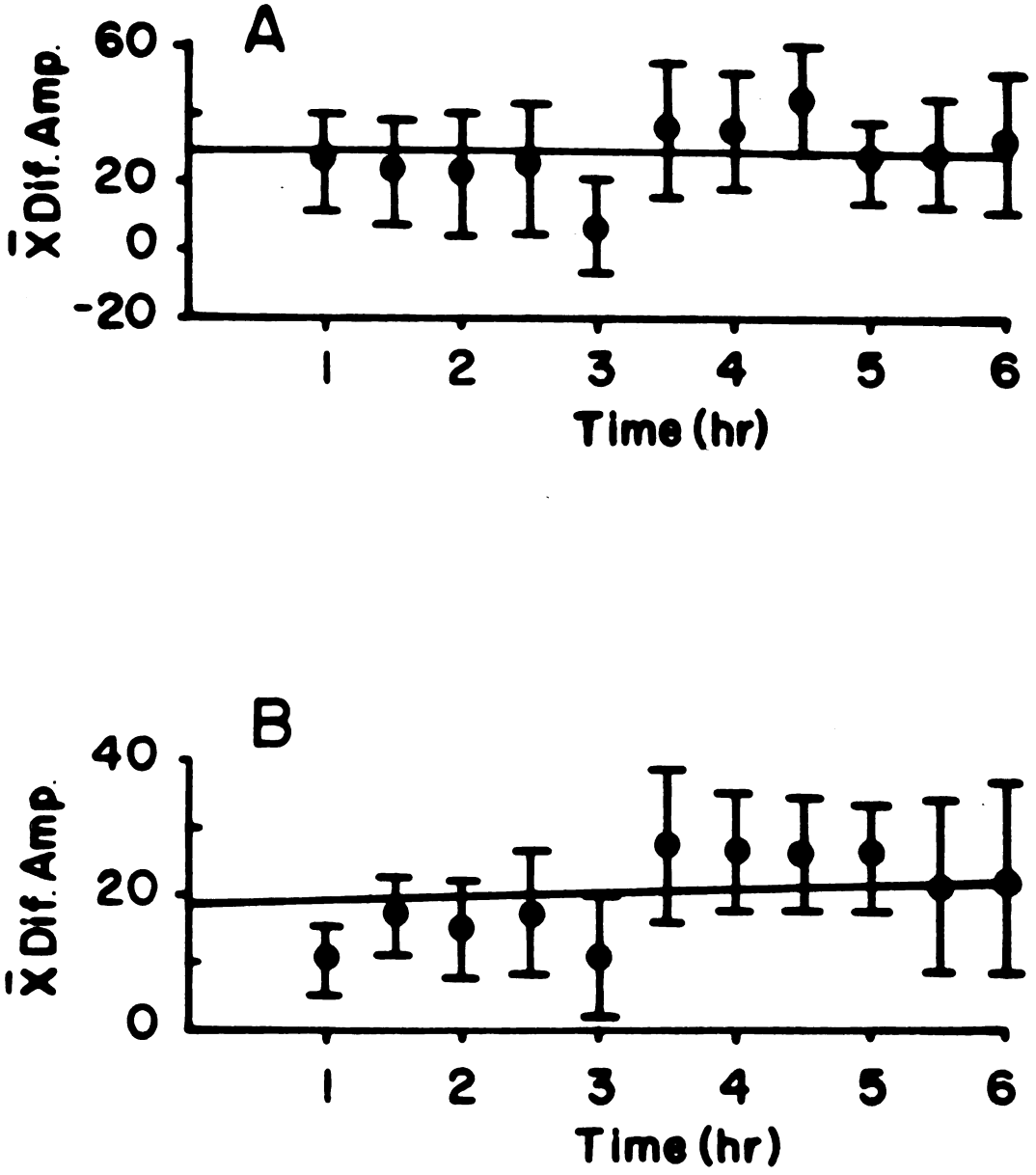

A
60
20
0
-20
X Dif. Amp.
1
2
3
4
5
6
Time (hr)
B
40
20
0
X Dif. Amp.
1
2
3
4
5
6
Time (hr)

FIGURE 16

Table 4. Changes in the trout ERG during exposure to oxygen at 740 torr and 3800 torr.

	1 hr (μV)	6 hr (μV)	d (μV)	n
a-wave 740	27.3 ± 3.9	20.6 ± 1.0	- 6.6 ± 3.3	8
a-wave 3800	33.8 ± 5.6	25.2 ± 5.9	- 8.5 ± 7.4	9
b-wave 740	62.5 ± 7.5	28.0 ± 7.0	-34.5 ± 10.0*	8
b-wave 3800	60.1 ± 12.7	19.7 ± 7.1	-40.4 ± 10.1*	9
c-wave 740	22.0 ± 3.7	16.4 ± 1.3	- 5.6 ± 4.0	5
c-wave 3800	26.5 ± 6.0	32.4 ± 7.9	+ 9.9 ± 10.1	8

Mean ± SE

d = 6 hr - 1 hr

* Significant difference at 0.05.

Figure 17. Trout ERG's recorded *in vitro*. A, B, C, and D. Recorded during 6 hr exposure to O_2 at 3800 torr at 15°C. Control responses were comparable to experimentals.

E. Trout ERG recorded *in vitro* under room air at 15°C.

F. Trout ERG recorded *in vitro* using modified Ussing blocks and Medium 199. Aerated with pure O_2 at 15°C. The pigmented epithelium has been removed.

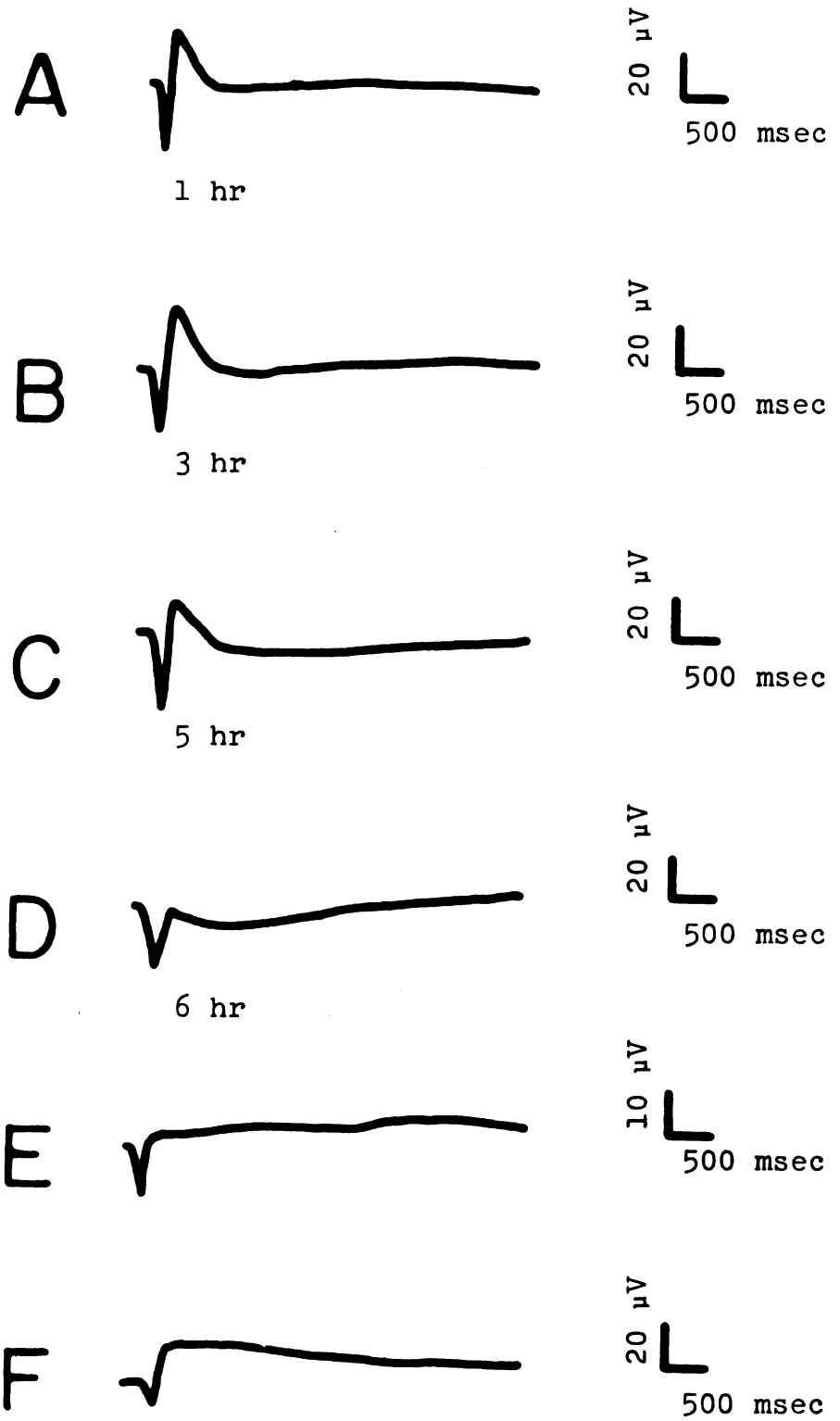

A
1 hr
20 µV
500 msec
B
3 hr
20 µV
500 msec
C
5 hr
20 µV
500 msec
D
6 hr
20 µV
500 msec
E
10 µV
500 msec
F
20 µV
500 msec

FIGURE 17

DISCUSSION

In this study the ERG was used as a monitor of retinal function in investigation of the effect of hyperbaric oxygen on the retinal cells. Attenuation of the ERG amplitude during exposure to hyperbaric oxygen, as compared to control, was considered to be indicative of oxygen toxicity. Maintenance of the ERG, i.e. non-attenuation of amplitude, or a response not different from control was seen as evidence of resistance to oxygen toxicity. The vasculature of the enucleated eye was not perfused, therefore any changes observed in the ERG were due to a direct effect of O_2 on the retina and were not mediated by the cardiovascular, respiratory or nervous systems of the animal.

The data was also interpreted in terms of the effect of O_2 on individual retinal cell types based on our present understanding of the cellular origins of the ERG. Such interpretation should be made with caution in that the ERG is a composite of simultaneous activity in several different parts of the retina. This retinal activity sums algebraically and since the various potentials contributing to the ERG are different in amplitude, polarity, and duration changes in one component may cause apparent changes in another component. These difficulties may be

overcome by surgical removal of the pigmented epithelium, the use of selective inhibitors, or the more tedious technique of intracellular recording.

The ERG's recorded were typical of those recorded both *in vivo* and *in vitro* by other workers. There was no significant difference between the responses recorded from the control chamber and the hyperbaric chamber when both contained O_2 at 740 torr (Appendix II). This indicates that differences in the ERG were not due to differences in the recording geometry, but to the differences in experimental conditions.

The retina was not perfused (perfusion here defined as bathing the retina with a constant flow of oxygenated, nutrient saline) and the question may arise whether or not the eye cup is a good preparation for the type of study in which changes in the ERG over an extended period of time are observed. The simplicity of the preparation is a point in its favor and the fact that a satisfactory response could be recorded from the poikilotherm retinas for at least six hr under control conditions indicates that the eye cup was a satisfactory preparation. Perfusion with a nutrient saline probably would have maintained the rat retina for a longer period of time, since existing stores of nutrients might otherwise be rapidly depleted due to the high metabolic rate of the rat.

Another problem which arises from the use of the eye cup is that of diffusion of oxygen through the vitreous

humor. Preliminary studies indicate that the rate of diffusion is very slow. This was not a problem in the goldfish, frog, and rat studies because almost all of the vitreous body could be removed. In one study the rat ERG was abolished within three min when O_2 at 740 torr was replaced with air. When the chamber was again purged with O_2 the ERG was restored to original amplitude within five min indicating that O_2 was reaching the retina quite rapidly. The vitreous body of the trout eye is very difficult to remove and most of it remained in the eye cup during the recording session. The diffusion path for oxygen is relatively long and it is not known what the P_{O_2} is at the retina in the steady state under O_2 at 3800 torr.

It has been noted that initial enhancement (except in the trout) of the ERG and low variability between preparations was seen under hyperbaric oxygen. The cells responsible for the ERG respond with graded potentials (Armington, 1974). Under control conditions in vitro, some of the retinal cells may be in a slightly anoxic state and respond with lower potentials due to reduced ATP levels. Under conditions of very high P_{O_2} all retinal cells may respond maximally for a given stimulus intensity leading to increased amplitude and a lower variability.

The Toxicity of Oxygen to the Frog and Rat Retinas

The Frog ERG

The attenuation of the frog ERG during exposure to hyperbaric oxygen (Fig. 9) indicates that high P_{O_2} is toxic

to the retinal cells responsible for the generation of the ERG. Although the slopes of the lines fitted to the a-wave data are not significantly different from each other (Fig. 7), examination of the transient changes during the experiment shows that a-wave begins to decline after two hr under hyperbaric O_2 while the control a-wave is maintained for four hr. The decline of the control a-wave is probably due to deterioration of the preparation due to depletion of nutrients since the eye cup is not perfused. Oxygen probably has its toxic effect on processes separate from those causing the decline of the control a-wave. The data for the b-wave and c-waves show a definite toxic effect of oxygen. These waves are a-wave dependent, that is, attenuation of the a-wave should be accompanied by attenuation of the b-wave and c-wave. In this study, the fact that the b- and c-waves decline much more than the a-wave indicates that oxygen must have a direct effect on the cells generating these components of the ERG.

The Rat ERG

The rat retina is highly susceptible to oxygen toxicity. The abolishment of the a-wave (fast PIII) and the attenuation of the late negative wave (slow PIII) indicates that hyperbaric oxygen has a definitive effect on the PIII component of the rat ERG. This effect is much greater than that seen in the frog, a finding which is in keeping with high susceptibility to oxygen toxicity generally seen in

mammals. Baeyens et al. (1973; 1974) also found that the amphibian retina is less susceptible to oxygen toxicity than the mammalian retina. Mammalian metabolic pathways are more highly integrated than those of lower animals. The mammalian enzyme systems operate within narrower limits than those of poikilotherms and there is a greater degree of interaction between these systems so that a given alteration in a pathway will have a greater effect on a mammal than on a lower animal. An example of this is that while cyanide is highly toxic to mammals it will not kill a trout because it can utilize alternative metabolic pathways (Hoffert, personal communication). The same principle appears to hold in this study of oxygen toxicity.

The effect of hyperbaric oxygen on the rat b-wave is not well defined since the control b-wave is also attenuated (Fig. 11). As stated earlier this decline of the control b-wave may be due to hypoxia (Weidner, 1976). The b-wave of the experimental preparation has a greater absolute decline than the control b-wave (Table 2) and since the decline parallels that of the a-wave the explanation of the b-wave attenuation in terms of oxygen toxicity is plausible.

The lack of a c-wave in the rat ERG of this study is puzzling. Weidner (1976) recorded c-waves from Long-Evans rats in vivo, but not from albino rats. In vitro Long-Evans rat eye cup ERG's in this study resemble Weidner's recordings from albino rats in vivo. This problem was not

pursued experimentally but perhaps the large slow PIII component recorded *in vitro* negates the c-wave.

Retinal Cells Affected by Oxygen as Indicated by ERG Changes

In recent years much information has been gained about the origins of the component waves of the ERG. Using this knowledge it is possible to analyze the changes in the ERG during hyperbaric oxygen exposure in terms of the specific retinal cell types upon which oxygen has its toxic effect. As stated earlier this must be approached with caution since the ERG is a summation of the simultaneous activity of several cell types and changes in the activity of one cell group may cause apparent changes in the activity of another group as indicated by the ERG.

The a-wave (fast PIII) is the result of receptor cell activity (Dowling, 1970). Intracellular recordings from mud-puppy rods and cones show hyperpolarizations with latency and waveform of the leading edge which are very similar to the a-wave (Fain and Dowling, 1973). This suggests that the attenuation of the a-wave seen in this study is due to a toxic effect of oxygen on the photoreceptors. The slow component of PIII was also present in our recordings from rat retinas (Fig. 11). Localization of this activity has been difficult, however, Witkovsky, Dudek and Ripps (1975) have suggested that the slow PIII, as well as b-wave, originates in the Müller cells and that it is initiated directly by rod activity through changes in the

extracellular ionic environment. If this is so (it has not yet been confirmed by intracellular recording) the decline of the slow PIII of the rat ERG noted in the present study may indicate a toxic effect of O_2 on the rods.

Intraretinal recording shows that the b-wave originates in the inner nuclear layer of the retina (Armington, 1974) and depolarizations corresponding to the b-wave can be recorded intracellularly from the Müller cells (Miller and Dowling, 1970). The Müller cells act as K^+ electrodes and respond to increasing K^+ in the extracellular fluid as the result of activity of neurons located postsynaptic to the receptors (primarily the bipolar cells) (Miller, 1973). Thus the decline of the b-wave under hyperbaric oxygen which was so evident in the frog data of this study may be due either to a toxic effect of oxygen on the neurons surrounding the Müller cells, or a direct effect on the Müller cells themselves inhibiting their response to K^+ fluxes. As shown above, the slow PIII may also arise in the Müller cells. Since this component could often be recorded from both rat and frog retinas after abolishment of the b-wave the toxic effect of oxygen is probably on the postreceptor neurons rather than on the Müller cells themselves. If slow PIII and the b-wave both arise in the Müller cells we have here a good example of the difficulty in interpretation of the ERG, especially since these potentials are opposite in polarity.

Extracellular recording has confirmed that the pigmented epithelium is the source of the c-wave and that this response is rod dependent (Steinberg, Schmidt and Brown, 1970; Schmidt and Steinberg, 1971). Steinberg and Miller (1973) suggested that the c-wave is a diffusion potential and results from either decreases in K^+ or increases in HCO_3^- around the apices of the epithelial cells. Oakley and Green (1975) have confirmed that the pigmented epithelial cells hyperpolarize in response to decreasing extracellular K^+ as it is reabsorbed by the rods. Conductance of K^+ into a neuron is a slow process (presumably active) which accounts for the slow response of the c-wave.

Attenuation of the c-wave under hyperbaric O_2 could be the result of toxicity to the rods or to the pigmented epithelial cell itself. Since the attenuation of the frog c-wave in this study was greater than the attenuation of the a-wave a direct effect of O_2 on the epithelial cells is suggested. Besides shielding the receptor cells from excess light, a major function of the pigmented epithelium is phagocytosis of spent pigment containing discs which are sloughed off from the receptors (Young, 1970). It is suggested that a toxic effect of O_2 on the pigmented epithelium as indicated by attenuation of the c-wave might also adversely affect these important functions.

Mechanisms of Retinal Oxygen Toxicity

It has been established that oxygen is toxic at the level of cellular metabolism (Haugaard, 1968). Since the retina is neural tissue and often considered to be simply an extension of the brain itself it is possible to speculate on the mechanisms of retinal oxygen toxicity based on what is known about effect of O_2 on brain tissue and on cellular metabolism in general.

Kaplan and Stein (1957) have demonstrated that there is a loss of K^+ and a decrease in active uptake of glutamate by guinea pig brain cortex slices exposed to hyperbaric oxygen. It has further been shown that K^+ and Na^+ gradients continually decrease in the direction of the Donnan equilibrium during exposure of guinea pig cortext to hyperbaric oxygen for 120 min (Joanny, Corriol and Brue, 1970) indicating a possible toxic effect of O_2 on the metabolic pump responsible for maintaining threse gradients. Maintenance of transmembrane potentials in the retina also depends on a metabolic pump which can be inhibited by ouabain (Sillman, Ito and Tomita, 1969) indicating a dependence on Na-K dependent ATPase. Treatment of the retina with ouabain abolishes the ERG (Honda, 1972). Since the maintenance of transmembrane potentials in the retina depends on activity of Na-K ATPase and since a reduction in transmembrane potential is seen during oxygen toxicity to neural tissue it is possible that hyperbaric oxygen causes inhibition of the ATPase enzyme in the retina. It is, of

course, possible that a reduction in transmembrane potential could result simply from changes in the permeability of the retinal cells due to ultrastructural changes in the cell membrane caused by hyperbaric oxygen.

The retina is an obligate glucose user. The enzymes of glycolysis are highly susceptible to oxygen inhibition (Haugaard, 1968; Baeyens et al., 1974) which would stop production of the ATP needed to provide energy for maintenance of membrane potentials. A reduction of ATP in neural tissue exposed to hyperbaric oxygen has been demonstrated (Joanny et al., 1970).

In summary, the attenuation of the ERG during hyperbaric oxygenation could be the result of a reduction in transmembrane potentials due to inhibition of the enzymes of the Na-K pump, inhibition of the enzymes responsible for supplying energy to the pump in the form of ATP, or structural changes in the cell membrane which alter ion permeability.

The Resistance of the Teleost Retina to Oxygen Toxicity

It has previously been shown that O_2 at 1470 torr has no toxic effect on teleost (trout) retinal metabolism and LDH activity (Baeyens et al., 1973; 1974). Fonner (1973) has shown that the teleost retina depends on the high P_{O_2} generated by the choroidal rete for normal function.

In this study no toxic effect was seen on the goldfish ERG during exposure of the retina to hyperbaric oxygen (Fig. 12) and the a-wave and c-wave of the trout ERG

were maintained at their initial amplitudes throughout the experiment indicating a resistance of the teleost retina to oxygen toxicity. This is especially apparent when compared to the data obtained in the frog and rat experiments.

The decline of the trout b-wave (Fig. 15) under hyperbaric oxygen is probably not due to oxygen toxicity since it is not statistically different from the decline of the control b-wave. It might be argued that the O_2 at 760 torr is also toxic to the retina, however, we do not feel that this is the case since in other studies of oxygen toxicity the severity of the toxic effect is seen to increase with increasing P_{O_2}. The decline of the b-wave may be due to deterioration of the preparation caused by problem of oxygen diffusion through the vitreous which was discussed above.

Oxygen Supply to the Teleost Retina from the Choroidal Rete

The teleost retina is avascular. This characteristic is probably an adaptation to the conditions of reduced light under water. The lack of retinal blood vessels allows more light to reach the receptor cells and allows greater visual acuity.

In animals with a vascularized retina oxygen reaches the deep layers of the retina through the retinal arteries. In the teleost all oxygen must diffuse from the choroid. The counter-current multiplier of the choroidal rete generates

oxygen tensions in excess of 400 torr in order to supply adequate oxygen to the whole retina (Fairbanks et al., 1969).

Calculations by Dollery, Bulpitt and Kohner (1969) have shown that theoretically 97% of the human retina between the choroid and deep retinal arteries, a distance of about 100 μ, can be supplied with O_2 when the P_{O_2} in the choroid is 400 torr. At a P_{O_2} of 1430 torr in the choroid the whole retina (260 μ thick) can be supplied with oxygen. Because Dollery made certain assumptions about ocular blood flow and oxygen consumption by the retina which are not applicable to the fish it is not possible to extrapolate his data directly to the trout. It seems clear, however, that trout should have no problem in supplying its whole retina (200 μ thick, unpublished data of Hoffert) with oxygen at a choroidal P_{O_2} of 400 torr, especially when one considers that the metabolic rate of the teleost retina at 15°C is only about one-third that of the mammalian retina at 37°C (calculated from data of Baeyens et al., 1973). Dollery et al. (1969) have also shown that there must be a P_{O_2} gradient in the retina due to O_2 consumption; P_{O_2} decreasing with increasing distance from the choroid. This means that whole teleost retina is not exposed to high P_{O_2}, i.e. the normal level of 400 torr, and raises the question of whether all retinal cells are resistant to oxygen toxicity. (Hoffert et al. (1973) have shown that teleost lens and cornea as well as retina are

resistant to oxygen toxicity). The presence of this gradient also means that the P_{O_2} probably did not reach 3800 torr at the pigmented epithelium in the hyperbaric oxygen experiments, however, at this high pressure it is reasonable to assume that P_{O_2}'s significantly higher than normal existed throughout the retina.

Mechanisms of Resistance to Oxygen Toxicity

There is much evidence that teleost retinal tissue is resistant to oxygen toxicity, although at this time the source of this resistance is unknown. Three mechanisms of protection against oxygen toxicity are proposed here which could serve as a basis for further investigation on this subject.

A mechanism of protection against oxygen toxicity may be the enzyme superoxide dismutase (SOD). This enzyme has been extensively studied by I. Fridovich (1974). During electron transport O_2 may be partially reduced to superoxide radical (O_2^-) by univalent reduction of O_2 or H_2O_2. Univalent pathways are actually favored over mechanisms using electron pairs. Thus in the presence of elevated P_{O_2} high levels of toxic O_2^- may be produced. The function of SOD is to eliminate these poisonous radicals by catalyzing the reaction $2O_2^- + 2H \longrightarrow H_2O_2 + O_2$. The H_2O_2, which is also toxic, is decomposed by the reaction $H_2O_2 \longrightarrow H_2O + 1/2O_2$ catalyzed by catalase. Rats can survive in 100% O_2 (at 1 atm) if acclimatized in 85% O_2

for seven days. Survival is correlated with an increase in SOD in the lungs during the seven day period (Fridovich, 1974) which indicates that SOD can be induced in the presence of high P_{O_2} and may then be produced in relatively large amounts at the teleost retina thereby protecting against oxygen toxicity.

The teleost eye may contain high levels of glutathione. It has been shown that an important function of this compound is to maintain the SH groups of enzymes in their reduced state (Haugaard, 1968). The SH group of glutathione is easily oxidized and may be oxidized more readily than the SH groups of enzymes, or it may reduce SH groups of enzymes which have already been oxidized. Baeyens (1975) has shown that addition of reduced glutathione to mouse brain homogenate prevents inhibition of LDH under hyperbaric oxygen. These proposed high levels of reduced glutathione may originate in the teleost retina or be produced in the pseudobranch, which itself is resistant to oxygen toxicity (Hoffert et al., 1973), and carried to the retina by the afferent pseudobranch artery.

Finally, the enzymes of the metabolic pathways of the teleost retina may in themselves be resistant to oxidation. Possibly these isozymes are specially adapted to high P_{O_2} through structural modifications such as the presence of non-essential SH groups which are more easily oxidized than the SH groups of the active site of the enzyme.

SUMMARY

1. Through the use of isolated, non-perfused eyecups it has been shown that hyperbaric O_2 has a direct effect on the retinal cells irrespective of changes mediated by other systems, e.g. the cardiovascular system.
2. Exposure to hyperbaric oxygen (3800 torr) causes initial enhancement of the goldfish, frog, and rat ERG's as compared to the responses from the control preparations (740 torr).
3. Hyperbaric oxygen is toxic to the frog retina causing attenuation of all components of the ERG. The b-wave and c-wave decline more than the a-wave indicating a direct effect of oxygen on the neurons lying postsynatpic to the receptors and on the pigmented epithelium respectively. The toxicity probably does not involve the Müller cells as evidenced by longer persistence of the slow PIII.
4. Hyperbaric oxygen is highly toxic to the rat retina. The attenuation of the a-wave and slow PIII components of the ERG indicates a direct toxic effect of oxygen on the photoreceptors. The decline of the b-wave under hyperbaric oxygen parallels that of the a-wave reflecting the decline in receptor activity.

5. The teleost ERG is not attenuated by exposure to hyperbaric oxygen. This indicates that the retina is well adapted to the high oxygen tensions generated by the choroidal rete and that no toxic effect is seen from exposure to oxygen tensions well above those normally encountered.

RECOMMENDATIONS FOR FURTHER STUDIES

1. A study similar to the one reported here using microelectrodes, intracellular recording techniques, and perfused retinas in order to study the effect of hyperbaric oxygen on individual cells.
2. The attenuation of the ERG during oxygen toxicity may be due to a reduction in transmembrane potentials. If this is the case the ERG could be restored by perfusing the retina with a high Na^+ solution which initially would restore the transmembrane potential (Sillman et al., 1969).
3. A study of the effect of hyperbaric oxygen on Na-K dependent ATPase.
4. Compare the amount of glutathione and superoxide dismutase in the teleost eye to the levels found in other eyes and tissues.

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APPENDICES

APPENDIX I

Composition of Cold Blooded Ringers

NaCl	6.40 g	111.0 mM
$CaCl_2$	0.20 g	1.8 mM
KCl	0.10 g	1.3 mM
Dist. H_2O	to 1 liter	

APPENDIX II

CHAMBER COMPARISON

The control and hyperbaric chambers (not to be confused with the recording chamber which held the eye cup itself) used in this study were quite different from each other in construction. The control chamber was a clear Lucite box, while the hyperbaric chamber was a steel cylinder painted white on the inside with a glass window. These differences could lead to differences in lighting conditions so it was necessary to compare the ERG's recorded from these chambers under similar atmospheric conditions. This comparison was made by recording ERG's from trout eye cups with both chambers containing O_2 at 740 torr. ERG's were recorded every five min for 70 min following 30 min of dark adaptation. The average wave amplitudes for seven experiments are shown in Table 5.

Table 5. Comparison of trout ERG amplitudes recorded at 15°C from the hyperbaric and control chambers under O_2 at 740 torr.

	a-wave (μV)	b-wave (μV)	c-wave (μV)
Hyperbaric chamber	14.6±2.7(7)	42.7±5.6(7)	23.9±6.3(6)
Control chamber	18.3±3.4(7)	52.6±9.5(7)	27.5±11.6(6)

Mean ± SE(N)
Differences are not significant at 5% level.

These results indicate that there is no significant difference in the ERG amplitudes recorded from the two chambers under similar conditions. The ERG amplitude from the hyperbaric chamber is slightly lower than the response from the control chamber which lends support to the observation that the ERG is initially enhanced by hyperbaric oxygen.