Effect of Methanol intoxication on the Function of Retina of Rabbit

Alaa El-Din A. Gawad and Amal E. Ibrahim

Biophysics and Laser Unit, Research Institute of Ophthalmology

Abstract: Methanol is an ideal candidate to replace fossil fuels. However, alterations in the retinal function are primarily associated with methanol intoxication. In the present work, chronic methanol intoxication was carried out in New Zealand rabbits previously depleted of foliates with methotrexate. We analyze the effect of long-term alcohol consumption on oxidative stress parameters of the rabbit retinas and its correlation to retinal function. We show that methanol has a toxic effect on rabbit retina associated with oxidative stress. Decreases in retina glutathione concentration and increases in catalase activity in whole retina homogenate significantly correlate with ERG a- and b-wave decrease. We show also a marked change in the molecular structure and orientation of rhodopsin in cell membranes of the retina. Chronic methanol consumption induces oxidative stress in rabbit retina associated with an impairment of ERG and molecular changes of membrane proteins.
[Alaa El-Din A. Gawad and Amal E. Ibrahim. **Effect of Methanol intoxication on the Function of Retina of Rabbit.** Journal of American Science 2011;7(6):491-496]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Methanol intoxication, Retina, Rabbit, fossil fuels.

1. Introduction

Integrating methanol into our energy system in the foreseeable future would have numerous economical and environmental benefits. However, considerable toxic effects due to acute or chronic methanol exposure could limit its use.

Exposure of methanol normally occurs through inhalation, skin or eye contact, and ingestion. The response and sensitivity of various species to methanol intoxication is varied (1,2); humans show considerable sensitivity to methanol (*3*). Further, the amount of methanol needed to cause acute toxicity varies widely from person to person (*4*).

In contrast to the acute intoxication, the debate about chronic neurotoxic effects of daily low to moderate methanol exposure is not yet settled. The mammalian metabolism of methanol mainly occurs in the liver. It breaks by alcohol dehydrogenase down into formaldehyde and then to formic acid (*5*). The metabolism of formic acid is mediated through a tetrahydrofolate-dependent pathway (*6*). Clinical findings correlate better with formic acid levels, which cause the profound metabolic acidosis that is a hallmark of methanol poisoning.

Formic acid accumulation gives rise to mitochondrial damage and suppression of oxidative metabolism by inhibiting cytochrome oxidase activity, which may also lead to an increase in H_2O_2 (*7-9*). Interestingly, the formation and stability of reactive oxygen species, for instance OH-like radicals, either by the iron-catalyzed Fenton and Haber-Weiss chemistry is favored in the presence of an acidic pH (*10,11*). Moreover, the metabolic acidosis can trigger iron release (12).

The electroretinogram (ERG) is the measure of action potential produced in the retina by sufficient light. It is a chain of electrical response in the form of graded potential evoked in each layer of the retina, from the photoreceptors to the amacrine cells. The high existence of oxygen and light in the retina even though its essential to vision but it may lead to photo-oxidative damage by means of formation of reactive oxygen species (ROS). Therefore, it is not surprising that it would possess antioxidant system (e.g. GSH, Vitamin E, Lutein and Zeaxanthin) and enzymes (e.g. catalase, glutathione peroxidase, superoxide dismutase) capable of metabolizing such foreign species (*13,14*).

All together, we designed this investigation to explore the effect of chronic methanol poisoning on the molecular biophysical characteristics of proteins in the retinas of rabbits. It has been used FT-IR spectroscopy as a non-invasive tool to detect the molecular changes in protein content.

2. Materials and Methods

Methanol intoxication protocol

Twenty two-month-old New Zealand white rabbits (average body weight 1450 g) fed a standard laboratory chow were divided into two groups for treatment during 7 days. Animals were individually housed in stainless steel cages with free access to food and water. They were maintained on a 12-light/dark cycle. Control and experimental rabbits received water for drinking from the polypropylene bottles.

For treatment, Methyl alcohol (HPLC grade; sigma) was diluted in sterile saline and was administrated as a 20% v/v solution. At the onset of the experiment, rabbits were injected with methotrexate (0.2 mg/kg b.w./day). The treated

animals were given 24-hrs access to a 20 % v/v solution H_2O for one week.

Electroretinogram technique:

The intoxicated rabbits were anaesthetized by 0.1 ml/kg separine, 50 mg/kg ketamine hydrochloride administrated intraprotoneally. ERG was recorded by using three Ag-AgCl electrodes. The active electrode was a disc electrode placed at the corneal periphery. The other two electrodes were placed in the skin of the lower eyelid and in the ear, as a reference and earthed electrodes respectively. A white flash was used in this work with fixed intensity (4 lux) and duration (0.2 sec). The obtained ERG signals were amplified and delivered to a computer system with a wide band (1-100 Hz). Sampling of the ERG records were performed using a custom-made computer system. Details of the technique are found in El-Awadi (15).

Sample preparation

At the ends of experimental periods (1, 3, 5, and 7 days) all the animals were sacrificed. For each experimental period, one animal was used for ERG experiments and the fellow one was dissected. Retinas were removed quickly and placed in iced PBS solution for 1 min, blotted on filter paper, weighed and homogenized (1:9, w/v) in a glass-teflon Potter homogenizer in 0.02 M phosphate buffer pH 7.4. The homogenates were centrifuged at 15,000 x g (4°C) for 30 min to settle the organelles and their membranes. The supernatant received from homogenate and used for subsequent experiments.

Antioxidative defence enzyme activities:

Catalase was measured at 240 nm for 3 min, using the linear decrease of H_2O_2 (10 mM) at pH7.4 in 50 mM potassium phosphate buffer. Activity was calculated using the molar extinction coefficient of 0·0394 $mmol^{-1}$ cm^{-1}, 1, and the results were expressed as nmol H_2O_2/min/mg of protein (16).

GSH concentration was measured in the supernatant. DTNB (5, 5'-dithiobis-2-nitrobenzoic acid) recycling method was used to produce a yellow compound, TMB (5-thio-2-nitrobenzioc acid), which was measured at 412 nm (17).

FTIR spectroscopy:

The absorption measurements were all performed with a Jasco-4100 Fourier-transform-spectrometer. Spectra were recorded in the range 4000 - 400 cm^{-1} using Instrumentation and Services Laboratory, National Research Center. The absorption intensity of the peak was calculated using the base line method.

Protein assay:

Protein contents were quantified with Lowry method (18).

Statistical evaluation

The results were expressed as mean ± SEM. Statistical analysis was performed using Student's t test for unpaired data, and values from to $p < 0.05$ were considered significant.

3. Results:

Figure (1) shows FT-IR spectra of rabbit retinas in the spectral region from 1900 to 400 cm^{-1} for the periods indicated. The major IR bands of the retinal chromophores were assigned to either vibrations of the protein part or the membrane lipids. In Fig (1), the amide I (C=O stretch vibrations) band is centered around 1643 cm^{-1} and amide II (N-H bending vibrations) at 1537 cm^{-1}. This vibration bands are characteristics for α-helical structure of rhodopsin (19). Figure (2) showed the dichoric ratio (amide II_{1537}/amide I_{1643}) decreases with time from 0.954 at 1-day to 0.363 at 7-day. These ratios were employed to calculate the orientation angle of rhodopsin with the membrane normal. Table (1) showed the estimated angles. The exposure of rabbit retinas to methanol for one day has no change in the orientation angle of rhodopsin. The exposure of retinas for longer time (3 and 5 days) showed a remarkable increase in the angle of orientation (31° and 57.5°, respectively). At the seventh day the angle of orientation has been markedly deteriorated.

Catalase, the enzyme that facilitates the breakdown of hydrogen peroxide to oxygen and water. Table (2) shows the activity of catalase in the retinas of methanol-intoxicated rabbits. Catalase activity was increased significantly in a time-dependent manner. The catalase activity in normal rabbit retinas was 4.39±0.93 U/mg protein. With one day of intoxication, the catalase activity increased to 5.12±0.74 U/mg protein. At 3 days post-insult, the catalase activity became 5.48±0.69 U/mg protein. By 5 and 7 days post-intoxication, the catalase activity increased to 6.87± 0.98 and 7.43±1.12 U/mg protein, respectively.

To determine the effect of chronic methyl alcohol administration on antioxidant activity, we measured GSH concentrations per wet weight of retinal cells at the corresponding periods(Table 2). In freshly isolated control retinal cells, the cytosolic GSH were 107.81±6.19 µg/g wet weight. After one day of chronic methanol ingestion, the cytosolic GSH pool was decreased by more than 11% ($P < 0.05$). The methanol ingestion of rabbits for 3, 5, and 7 days caused a significant decrease in the cellular GSH pool ($P<0.05$). By 7 days of methanol intoxication, the drop of GSH concentration reached more than 40% (Table 2).

The traces in figure (3) illustrate a set of control and methanol-intoxicated rabbits ERGs using the flash stimulation technique. In all control animals,

well-defined ERG responses were found. The mean amplitude of a-wave = 0.22± 0.009 mV, and b-wave = 2.29 ± 0.2 mV. It was observed that a- and b-wave amplitudes in the ERGs were significantly reduced with respect to control for all experimental periods (1, 3, 5, and 7 days). The rate of b-wave amplitude change showed a linear decrease (Fig. 4). It was 7.5% after one day whereas this drop was ~ 22% and 27% for 3 and 5 days respectively upon intoxication (Table 3). The rate of change reached 35% on the end of the time course of experiment. On the other hand side, a-wave amplitude showed a sharp increase in the ascending side (Fig. 5). The implicit time of both a-, and b-wave showed a marked changes upon intoxication.

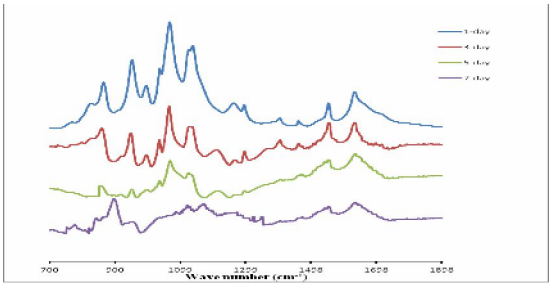

Fig (1): FTIR absorbance spectra of rhodopsin as a function of intoxication time

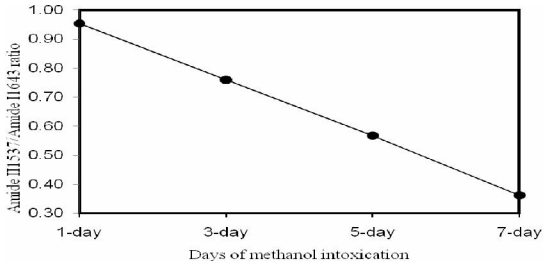

Figure (2):The relationship between dichoric ratio and methanol intoxication time (days)

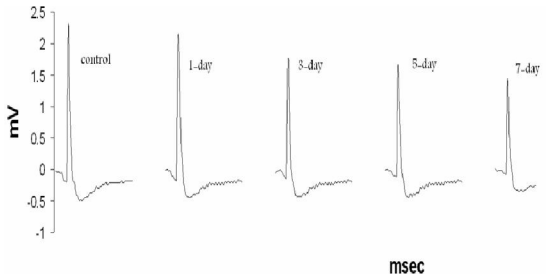

Figure (3): Typical record of ERG of control and methanol-intoxicated rabbits form different periods during dark adaptation

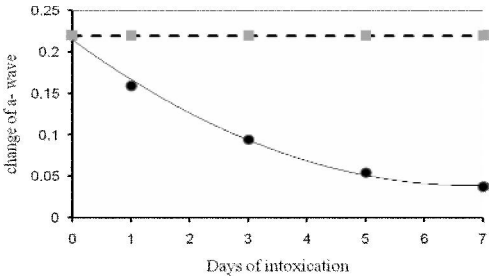

Figure (4):The relationship between of methanol concentration () and the corresponding a-wave (mV) of rabbits during dark adaptation.

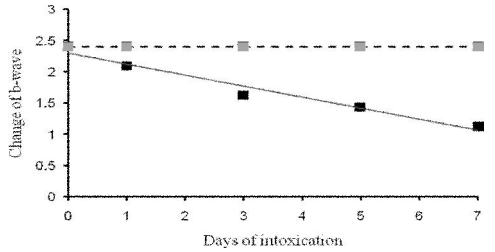

Figure (5):The relationship between of methanol concentration ( ) and the corresponding b-wave (mV) of rabbits during dark adaptation.

Table (1): The angle of the alpha-helix with the membrane normal as a function of the ratio of amide II/amide I

Amide ratio	Orientation angle
0.954	0
0.76	31
0.568	57.5
0.363	ND

Table (2): The effect of methanol poisoning on the reduced glutathione (GSH) and catalase activity.

Item	Catalase activity [a] Mean±SEM	Reduced GSH [b] Mean±SEM	P-value
Control	4.39±0.93	107.81±6.19	
1-day	5.12±0.74	95.32±5.02	<0.05
3-days	5.48±0.69	82.45±3.21	<0.05
5-days	6.87±0.98	75.94±4.67	<0.05
7-days	7.43±1.12	63.49±2.24	<0.05

[a] catalase activity (U/mg protein)
[b] reduced GSH (µg/g wet weight)

Table (3): The rate of change for a-wave and b-waves with the intoxication time course.

Item	a-wave (mV)	b-wave (mV)
Control	0.22 ± 0.01	2.4 ± 0.05
1	0.16 ± 0.02	2.1 ± 0.04
3	0.095 ± 0.002	1.63 ± 0.02
5	0.055 ± 0.0016	1.44 ± 0.03
7	0.038 ± 0.002	1.12 ± 0.04

4. Discussion

Chronic methanol intoxication can severely damage many systems of the human body, especially severe visual dysfunction (20, 21). As in the case of many chronic degenerative diseases, increased productions of reactive oxygen species (ROS) and lipid peroxidation have even been considered to play an important role in the pathogenesis of methanol toxicity (22, 23). In the present study, chronic methanol-intoxicated rabbits have been pretreated with methotrexate, which selectively inhibits formate oxidation and binding dehydrofolate reductase, thereby depleting the animal's folate store (25). Interestingly, the formation and stability of reactive oxygen species either by the iron-catalyzed Fenton and Haber-Weiss chemistry is favored in the presence of an acidic pH (24). Moreover, the metabolic acidosis can trigger iron release (12). Therefore, we analyzed the effect of long-term methyl alcohol consumption on oxidative stress in the retina and its correlation to retinal function by ERG.

The results herein from eyes of methanol-treated rabbits show a significant decrease in the level of endogenous antioxidant (GSH), as well as an increase in catalase activity (Table 3). Methanol induced depletion of glutathione supports the hypothesis that reactive oxygen intermediates generated during the metabolism of methanol lead to glutathione oxidation and lipid peroxidation. These findings agree with previous studies that reported an alteration of oxidative stress metabolites after long term administration of methanol in liver and retina (*22*, *35*). Hyperoxia-induced retinopathy of prematurity and ischemia-reperfusion show a depletion of retinal GSH that may become insufficient to buffer an increased release of free radicals (*24*).

Catalase enzyme serves as a second messenger of oxidative stress. The accumulation of reactive oxygen species giving rise to induction of the expression/activity of catalase enzyme (Table 3). Increase of the catalase activity will lead to a decrease in H_2O_2 concentration within the cell, depriving the Fenton reaction of substrate. Consequently, the formation of hydroxyl radicals through the Fenton reaction (and thus the formation of MDA) will occur at a lower rate. The induction of antioxidant enzymes to varying degrees by H_2O_2 has been reported in other systems, including rat lens (*27*), primary rat hepatocytes (*28*), or the developmentally regulated catalase of E. nidulans (*29*).

A result of free radicals action may be the modification of biologically active proteins, and damage to biological membranes. Our data suggest a quite interesting general inverse relationship between the ratio of Amide II_{1537}/Amide I_{1643} and the time of methanol poisoning ((Fig. 5). The alteration of these dichroic ratios could be used as a monitor to get information about the configuration of main protein component, rhodopsin. The magnitude of changes in these spectral regions indicates significant changes in α-helix configuration of rhodopsin (*30*). In α-helix, the amide I dipole is close to the helix long axis whereas the amide II dipole is roughly perpendicular to it. Though localization of short chain methyl alcohol, in the headgroup region of lipid membrane (*31, 32*) is giving rise to disruption of lipid bilayer packing, i.e. increases membrane fluidity. The enhancement of fluidity seems to make the membrane has less steric constraints (*33*) and may cause changes in the orientation angle between rhodopsin and membrane normal.

ERG analysis in methanol-intoxicated rabbits revealed a significant reduction of the amplitudes of a- and b-waves (Figs. 3, 4). In fact, rhodopsin is a protein anchored in the membranes of discs–flat vesicles that fill the outer segment of rod cells. These membranes contain a high amount of long-chain polyunsaturated fatty acids (*34*), which make them particularly susceptible to oxidative stress. Therefore, hypothesis may directly pertain to the alterations in the ERG is that the function of the retina may be diminished by exposure to methanol and/or the byproducts of methanol metabolism (*35* , *36*). It has also been hypothesized that persistent conditions of oxidative stress due to chronic alcohol exposure can alter the fatty acyl composition of membrane phospholipids (*37, 38*).

Moreover, oxidative stress in the outer rod segment is associated with double bond breaking of 11-*cis*-retinal during *cis*-trans isomerization. In the case of free retinal, this photochemical reaction has been reported to cause the formation of oxygen free radicals. Oxygen radicals can also cause formation of the protein peroxides (*39*). Changes of the primary structure of proteins cause modification in their secondary and tertiary structures.

References

1- Anderson, R. E. and Andrews, L. M. (1982): Biochemistry of photoreceptor membranes in vertebrates and invertebrates. In: *Visual Cells in Evolution*, Westfall J (ed). New York: Raven Press, pp. 1–22.
2- Beers RF and. Sizer IW (1952): a spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem., 195: 130-140.
3- Baker MS and Gebicki JM (1984): The effect of pH on the conversion of superoxide to hydroxyl free radicals. Arch. Biochem. Biophys. 234: 258–264.

4- Chiu C-J, Taylor A (2007): Nutritional antioxidants and age-related cataract and maculopathy. Exp Eye Res., 84: 229-245.
5- Crow JP, Spruell C, Chen J, Gunn C, Ischiropoulos H, Tsal M, Smith CD, Radi R, Koppenol WH, and Beckmann JS (1994): The pH-dependent yield of hydroxyl radical products from peroxynitrite. Free Radic Biol Med., 16: 331–338.
6- Davoli E (1986): serum methanol concentrations in rats and in man after a single dose of aspartame. Food Chem Toxicol 24: 187-189.
7- Dikalova AE, Kadiiska MB, and Mason RP (2001): An *in vivo* ESR spin-trapping in rats from formate intoxication-role of the Fenton reaction. Proc Natl Acad Sci USA, 98: 13549-13553.
8- Eells JT, Black KA, Makar AB, Tedford CE, and Tephly TR (1982): The regulation of one-carbon oxidation in the rat by nitrous oxide and methionine. Arch Biochem Biophys, 219: 316-26.
9- El-Awadi (1999). Effect of radiation on the function and structure of the mammalian eye. PhD Thesis. Biophysics Dept., Faculty of science, Cairo University.
10- Gebicki S and Gebicki J.M. (1993): Formation of peroxides in amino acids proteins exposed to oxygen free radicals. Biochem. J., 289: 743.
11- Kavet R and Nauss KM (1990): The toxicity of inhaled methanol vapors. Crit Rev Toxicol., 21: 21-27.
12- Kawasaki L, Wysong D, Diamond R, Aguirre J. (1997): Two divergent catalase genes are differentially regulated during Aspergillus nidulans development and oxidative stress. J Bacteriol., 179: 3284-3292.
13- Khanna P, Wang L, Ansari NH (1996): Semi-quantitation of mRNA by polymerase chain reaction. Levels of oxidative defense enzymes and aldose reductase in rat lenses cultured in hyperglycemic or oxidative medium..Res Commun Mol Pathl Pharmacol., 92: 3-18.
14- Kuteifan K, Oesterlé, Tajahmady T, *et al.* (1998): Necrosis and haemorrhage of the putamen in methanol poisoning shown on MRI. Neuroradiology, 40: 158–60.
15- Liesivuori J, Savolainen H. (1991): Methanol and formic acid toxicity: biochemical mechanisms. Pharmacol Toxicol., 69: 157-63.
16- Liu JJ, Daya MR, Mann NC (1999): Methanol-related deaths in Ontario. J Toxicol Clin Toxicol., 37: 69–73.
17- Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951): Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193:265-275.
18- Ly H and Longo M (2004): The influence of short-chain alcohols on interfacial tension, mechanical properties, area/molecule, and permeability of fluid lipid bilayers. Biophys. J., 87:1013–1033.
19- Nicholls P. (1976): The effect of formate on cytochrome aa3 and on electron transport in the intact respiratory chain. Biochim Biophys Acta, 430: 13-29.
20- Osborne HB and Nabedryk-viala E. (1977): The hydrophobic heart of rhodopsin revealed by an infrared ^{1}H-^{2}H exchange study. FEBS Lett., 84: 217-220.
21- Patra M, Salonen E, Terama E, Vattulainen I, Faller R, Lee B, Holopainen J, and Karttunen M. (2006): Under the influence of alcohol: the effect of ethanol and methanol on lipid bilayers. Biophys. J., 90: 1121–1135.
22- Paula EM, Mathangi DC, and Namasivayam A. (2003): Free radical changes in methanol toxicity. Indian J Physiol Pharmacol., 47: 207–211.
23- Pawlosky RJ, Flynn BM, Salem N Jr. (1997): The effects of low dietary levels of polyunsaturates on alcohol-induced liver disease in rhesus monkeys. Hepatology, 26: 1386-92.
24- Pawlosky RJ, and Salem N Jr (1999): Alcohol consumption in rhesus monkeys depletes tissues of polyunsaturated fatty acids and alters essential fatty acid metabolism. Alcohol Clin Exp Res., 23: 311-317.
25- Plaziac C, Lachapelle P, and Casanova C. (2003): Effects of methanol on the retinal function of juvenile rats. NeuroToxicol., 24: 255-260.
26- Rajamani R, Muthuvel A, Senthilvelan M, Sheeladevi R. (2006): Oxidative stress induced by methotrexate alone and in the presence of methanol in discrete regions of the rodent brain, retina and optic nerve. Toxicol Lett., 165: 265-273.
27- Rehncrona S, Hauge HN, Siesjö BK (1989): Enhancement of iron-catalyzed free radical formation by acidosis in brain homogenates: difference in effect by lactic acid and CO_2. J Cereb Blood Flow Metab., 9: 65-70.
28- Röe O. (1982): Species differences in methanol poisoning. Crit Rev Toxicol., 10: 275- 86.
29- Röhrdanz E, Kahl R. (1998): Alterations of antioxidant enzyme expression in response to hydrogen peroxide. Free Radic Biol Med., 24: 27-38.

30- Rothschild KJ and Clark NA (1979): Polarized infrared spectroscopy of oriented purple membrane. Biophys J., 25: 473-487.
31- Rowe VK, McCollister SB.(1981): Alcohols. In: Patty's Industrial Hygiene and Toxicology, 3rd ed. Vol. 2C, GD Clayton, FE Clayton, Eds. John Wiley & Sons, New York, pp. 4528-4541
32- Sandhir R, and Kaur K. (2006): Influence of ethanol on methanol-induced oxidative stress and neurobehavioral deficits. J Biochem Mol Toxicol., 20: 247-54.
33- Schalinske KL and Steele RD (1996): Methotrexate alters carbon flow through the hepatic folate-dependent on-carbon pool in rats. Carcinogenesis, 17: 1695-1700.
34- Sedlack J and Lindsay RH (1968). Estimation of total, protein-bound and non-protein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 25: 192-205.
35- Skrzydlewska E, Elas M, Farbiszewski R, and Roszkowska A. (2000): Effect of methanol intoxication on free-radical induced protein oxidation. J Appl Toxicol. 20: 239-43
36- Synder R and Andrews LS (1996): Toxic effects of solvents and vapors. In Casarett and Doulli's toxicology: the basic science of poisons. Klaasen CD (ed.), 5th edition. New York, McGraw Hill. 737-771.
37- Treichel JL, Henry MM, Skumatz CMB, Elles JT, and Burke JM (2004): Antioxidants and ocular cell differences in cytoprotection from formic acid toxicity *in vitro*. Toxicol Sci., 82: 183-192.
38- Van den Brink-van der Laan E, Chupin V, Killian J, and de Kruijff B. (2004): Small alcohols destabilize the KcsA tetramer via their effect on the membrane lateral pressure. Biochemistry. 43:5937–5942.
39- Williams DL (2008): Oxidative stress and the eye. Vet Clin North Am Small Anim Pract., 38(1):179-92.

5/18/2011