INFLUENCE OF X-RAYS ON SOME ELECTRICAL PROPERTIES OF THE BOVINE VITREOUS HUMOUR

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Abstract

In this study, the influence of x-rays on the ionic properties of the vitreous humour of the bovine eyes has been investigated. Each vitreous sample studied was divided into three parts with two parts x-irradiated with a skin dose of 4.43 mGy and 140.15 mGy respectively, while the third part served as a non-irradiated control. Measurements of ionic conductivity, pH and bulk electric potential were made on the irradiated and non-irradiated samples at varying temperatures from 5°C to 45°C in order to study their temperature dependence. It was observed that high radiation dose significantly altered the temperature distribution of the ionic content of the vitreous humour. The possible contributions of the observed physico-chemical changes on the structure and charge distribution in the vitreous body have been discussed.

***Key words**: Bovine, conductivity, vitreous, electrical, liquefaction*

Introduction

The vitreous humour is a clear avascular gelatinous tissue which comprises about 2/3 of the volume and weight of the eye. It contains about 90% of water, the rest being made up of collagen protein and hyaluronic acid. The vitreous humour is known to be a photosensitive tissue. Since the eye constantly interacts in the environment with naturally occurring ionizing radiations, and because the radiations carry energies some of which may be deposited in the materials along their paths, there are therefore increased concerns on the damaging effects of such radiations on the mammalian eye tissues.

Heller and Mayer (1994) reported the effects of ultra-violet (UV) light on the catalase activity of the calf eye vitreous. In that study, a total of 26 calf eyes were irradiated with UV-A light for a period of 3 hours and the catalase activity was investigated and compared with that of un-irradiated eyes. An increase in activity of 33% was reported in the irradiated eyes. In explaining their result, they claimed that the increase in activity was due to the induction of radicals in the vitreous medium by the radiation, coupled with the subsequent intensified catalase formation which enabled the binding of the radicals formed. They therefore recommended that the eye must always be protected from UV radiations. Stepanov et al (1990) also reported photo-damage to the eyes exposed to radiation from an Nd:YAG laser. Their results showed that the radiation induced certain physico-chemical changes affecting the structure and charge distribution in the crystalline lens and the vitreous body. They found a decrease in the density of the vitreous and the lens negative charges resulting from increased liquefaction. They suggested that such effect may be responsible for the shift in the vitreous body or detachment of the retina often noticed after exposure to Nd:YAG lasers, possibly resulting from the collapse of the vitreous gel liquefied components.

Ueno et al (1987) studied the effects of visible light irradiation on the vitreous structure in the presence of a photo-sensitizer. In that work, they investigated the photo-induced changes in calf eyes injected with a photo-sensitizer, riboflavin, prior to irradiation with white light. It was found that charged species of oxygen, i.e. the singlet oxygen, superoxide anion, hydrogen peroxide and the hydroxyl radicals generated by the photo-dynamic action of the radiation in the presence of the sensitizer caused significant liquefaction of the calf vitreous. They related the mechanism of the liquefaction to the formation of vitreous synchysis.

Akiba et al (1994), in order to understand the mechanism of photo-induced vitreous liquefaction, investigated the effects of free radicals on the bovine vitreous collagen proteins and the hyaluronic acid. In that work, they found that free radicals caused an increase in the high molecular weight components and insolubilization of the vitreous collagen protein and also a decrease in the molecular weight of the hyaluronic acid. It was suggested that the change in the molecular properties of the vitreous collagen could be attributed to extensive cross-links of the molecules. They maintained that since riboflavin is present in the vitreous which is irradiated by visible light over a lifetime, both the cross-links of the vitreous collagen and the degradation of the hyaluronic acid may contribute to age-related vitreous liquefaction. The effect of acid degradation in a solution, resulting in higher pH and also affecting the molecular properties of the proteins in the solution has been supported by the work of Laogun (1986) which showed that higher pH caused an aggregation of egg-white lysozyme protein molecules in solution.

Electrical conduction of ions in a solution depends on the ions present and their concentration. Ionic conductivity, therefore is a measure of the flow of ions in the solution. The pH of the solution, which is a measure of the hydrogen ion concentration, $[H^+]$ is given by:

$$pH = -\log [H^+] \qquad (1)$$

The pH is related to the bulk ionic potential, V of the solution by the Nernst diffusion equation (Cole, 1972) given by

$$V = 2.3RT/F + 2.3[RT/F]pH$$

$$= Const + 2.3[RT/F].pH \qquad (2)$$

where F is the Faraday constant and T is the temperature of the sample.

The aim of this study is to investigate the effect of x-rays on the ionic properties of the vitreous humour of the bovine eye. The significance of the work is its relevance to the radiation protection of the eye.

Materials and Method

Research materials and preparation

Twelve bovine eyes from just sacrificed animals were obtained from the government abattoir in Benin City, Nigeria. The vitreous humour was carefully extracted from the eyes by vitreosectomy within one hour of the death of the animals. In order to obtain measurable sample quantity, the vitreous samples from four eye balls were pooled together each time. Two parts of the pooled samples were x-irradiated while the third served as non-irradiated control.

Instrumentation and Measurements

The x-irradiation of the vitreous samples was carried out in the Radiology Department of the University of Benin Teaching Hospital, using a 3 phase diagnostic x-ray machine, model Watson PX 304 (Picker International Ltd, Wembley, U.K.) with a maximum output power of 37.5 kW and an inherent filtration of 3.5 mm aluminum. Two levels of skin dose were chosen for this investigation. The first level is 4.43 mGy, which is within the range being used for skull investigation while the other one is 140.15 mGy, which is well above the normal range. The technical parameters used for the setting of the x-ray machine for the dose levels are as given in table 1 and the equation used for the dose calculation (Edmonds, 1984) is as given below.

Table 1. X-ray machine parameter setting and Skin Dose

kVp	mAs	SSD (cm)	Filtration (cm)	Exposure	Skin Dose (mGy)
75	60	91	3.5	1	4.43
100	100	60	3.5	5	140.15

Skin Dose {mGy) =

$$\frac{836x(kVp)^{1.74}\ x\ mAs}{SSD^2}\ x\left(\frac{1}{T}+0.114\right)x\,10^{-3} \qquad (3)$$

where T is the filtration.

Investigation of the ionic properties of the irradiated and non-irradiated vitreous samples was carried out using the following procedures. Each sample was introduced into a glass tube of diameter 3.1 cm and length 15 cm, and then inserted in a temperature controlled bath [Grant Instruments (Cambridge) Ltd. Cambridge, England] working in conjunction with a refrigerated unit. A Jenway 4010 conductivity / temperature meter [Jenway Ltd, Essex, England.] was employed to measure the ionic conductivity of the sample at different temperatures between 0 and 45°C. A Jenway 3020 pH meter [Jenway Ltd, Essex, England] was also used to monitor the pH and the bulk ionic potential of the sample at different temperatures.

Results

The mean values and standard deviations showing the spread of the ionic conductivity, pH and bulk electric potential at selected temperatures for irradiated and non-irradiated vitreous humour are presented in Table; 2.

Table 2. Spread of conductivity, pH and bulk electric potential at selected temperatures

Temperature (°C)		5	10	20	30	40
Ionic Conductivity (Ms m^{-1})	0 mGy	8.05±0.40	8.44±0.40	8.82±0.40	9.39±0.45	9.84±0.50
	4.43 mGy	8.92±0.40	9.03±0.52	9.25±0.45	9.56±0.50	9.80±0.50
	140.15 mGy	11.00±0.40	11.50±0.40	12.30±0.50	12.84±0.50	13.24±0.60
PH	0 mGy	7.66±0.50	7.45±0.52	7.03±0.50	6.74±0.50	6.57±0.50
	4.43 mGy	6.75±0.40	6.85±0.50	7.04±0.50	7.09±0.51	7.06±0.60
	140.15 mGy	7.16±0.60	7.40±0.55	7.61±0.55	7.67±0.55	7.50±0.60
Bulk electric potential (mV)	0 mGy	-39.0±2.0	-27.0±1.5	-1.0±0.05	14.0±0.5	24.0±1.0
	4.43 mGy	14.0±0.5	8.0±0.5	-3.0±0.5	-7.0±0.3	-4.0±0.4
	140.15 mGy	10.0±0.5	-24.0±1.0	-37.0±2.0	-40.0±2.0	-30.0±2.0

A comparison of the variation of ionic conductivity with temperature for irradiated and non-irradiated bovine vitreous humour samples between 5^0 and 45^{0}C is given in figure 1. The conductivities generally increase with temperature. The conductivity of the sample irradiated with a low x-ray dose of 4.43 mGy appears to be slightly higher than that of the non-irradiated sample only at low temperatures between 5^0 and 25^{0}C after which it exhibits approximately the same values with the non-irradiated control. The sample irradiated with a dose of 140.15 mGy however exhibits significantly higher conductivity values than the others over the temperature range considered in the experiment. This result indicates that higher radiation dose increases the ionic conductivity of the vitreous samples. Figure 2 shows the variation of the pH of the irradiated and non-irradiated vitreous samples from 5°C to 45°C. The pH of the non-irradiated vitreous humour decreases from an alkaline pH of 7.75 at 5^{0}C to an acid pH of around 6.5 at 45^{0}C The variation in pH of the samples irradiated with a low dose of 4.43 mGy is confined largely to the neutral pH region. On the other hand, the pH of the samples irradiated with x-ray dose of 140.15 mGy varies between 7.16 and 7.66 in the alkaline region. This result thus indicates that irradiation of the samples leads to a degradation of the acid in the vitreous samples studied.

A comparison of the bulk ionic potential of the irradiated and non-irradiated bovine vitreous samples is given in figure 3. The ionic potential of the non-irradiated samples increases steadily with temperature from around 40 mV at 5 ^{0}C to about 30 mV at 45 ^{0}C, showing that negative ions predominate in the vitreous samples at low temperatures while positive ions predominate at high temperatures. The ionic potential of the samples irradiated with the low x-ray dose of 4.43 mGy seems to vary within the neutral region. However, the potential of samples treated with the high x-ray dose of 140.15 mGy is confined to the negative region in the temperature range from 5 ^{0}C to 45 ^{0}C, showing also that negative ions predominate in the samples.

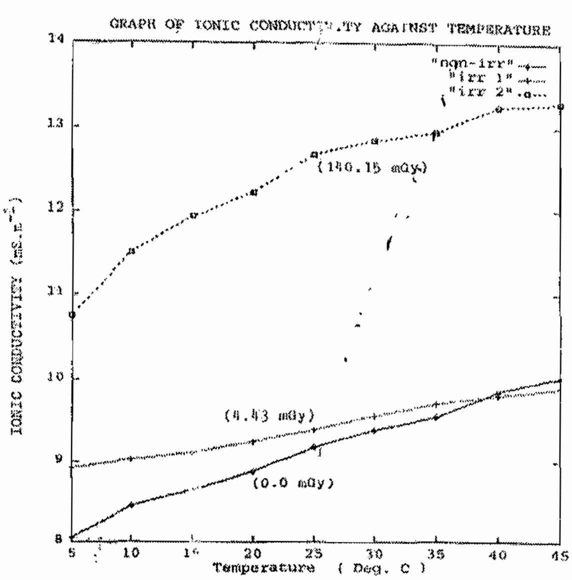

Fig 1 : Comparison of the Variation of the Ionic Conductivi of Irradiated and non-irradiated Bovine Vitreous Humour at Temperatures from 5° to 45°C.

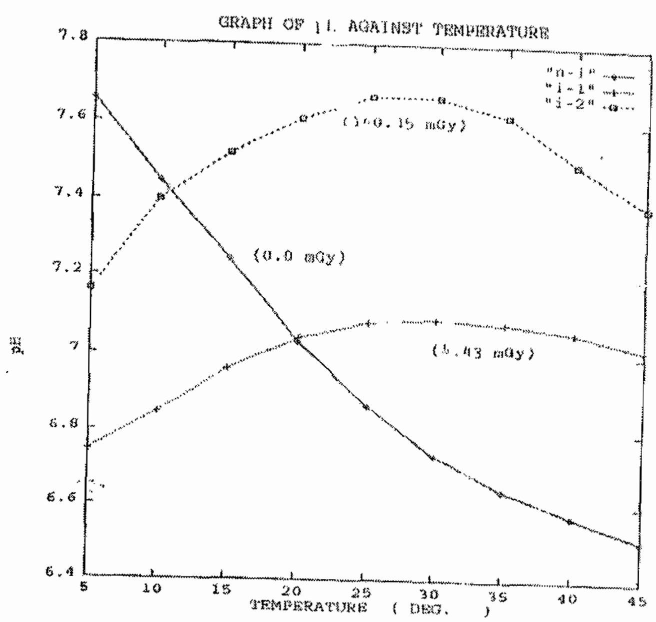

Fig 2 : Comparison of the Variation of the pH of Irradiated and non-Irradiated Bovine Vitreous Humour at temperatures from 5° to 45°C.

Table 3 gives a comparison of ionic conductivities of irradiated and non-irradiated vitreous samples at two standard temperatures, the experimental room temperature of 27 °C and mammalian body temperature of 37 °C. It may be observed from Table 3 that the percentage difference in conductivity of samples irradiated with a low dose of 4.43 mGy is quite low compared to that of the non-irradiated control samples both at the room temperature of 27 °C and body temperature of 37 °C . However, the conductivity of bovine vitreous samples irradiated with a high dose of 140.15 mGy gives a difference of 38% from control at 27 °C and 33% at 37 °C. This result shows that the conductivities of the samples are significantly different from those of the non-irradiated control samples at the two temperatures.

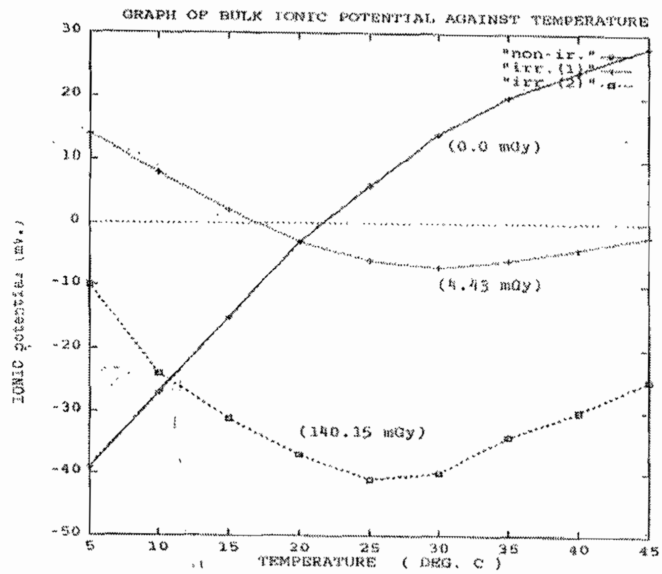

Fig 3 : Comparison of the variation of the ionic potential of Irradiated and non-irradiated bovine Vitreous Humour at Temperatures from 5° to 45°C.

Table 3: Comparison of ionic conductivity of irradiated and non-irradiated bovine vitreous humour at a room temperature of 27 °C and body temperature of 37 °C.

Temp (°C)	Sample	Ionic Conductivity (mS /m)	Percentage difference from control (%)	Remarks
Room Temperature (27 °C)	Non-irradiated control	9.2	0	-
	Irradiated (4.43mGy)]	9.5	0.33	Not significantly different from control
	Irradiated 140.15mGy)]	12.75	38.6	Significantly different from control
Body Temperature (37° C)	Non-irradiated control	9.72	0	-
	Irradiated (4.43 mGy)	9.75	0.3	Not significantly different from control
	Irradiated (140.15mGy)	13.0	33.7	Significantly different from control

The variation of ionic conductivity with temperature can be written as:

$$\sigma = A\exp(-\Delta E_{\sigma}/RT), \quad (4)$$

where ΔE_{σ} is the activation enthalpy, which is the energy required to break the molecular bond to orient the molecule in the applied electric field. R is the molar gas constant and T is the temperature. The Arrheneous plot of the logarithm of conductivity σ against 1/T is presented in figure 4. The activation energy of the vitreous humour was estimated from the graph using the equation:

$$d(\ln\sigma)/d(1/T) = \Delta E_{\sigma}/R. \quad (5)$$

The activation energies of the irradiated and non-irradiated vitreous humour in the temperature range from 5 °C to 45 °C are shown in table 4. The table shows that the activation energy of the non-irradiated control sample is higher than that of the sample irradiated with a low x-ray dose of 4.43 mGy in the temperature range from 5 °C to 45 °C. At the same time, the activation energy of the sample irradiated with a high x-ray dose of 140.15 mGy is higher than that of the non-irradiated control samples at low temperatures from 5 °C to 24.3 °C, while it is lower at high temperatures from 24.3 °C to 45 °C . The activation energy of the samples irradiated with a high xray dose of 140.15 mGy also appears to be slightly higher than those of the samples irradiated with a low dose of 4.43 mGy at high temperatures between 24.3 °C and 45 °C.

Table 4: The activation energies of irradiated and non-irradiated vitreous humour

TEMPERATURE RANGE (°C)	ACTIVATION ENERGY (kJ.mol⁻¹)		
	Non-irradiated (0.0 mGy)	Irradiated (4.43 mGy)	Irradiated (140.15 mGy)
5.0 °C to 24.3 °C	3.92 ± 0.10	2.00 ± 0.06	5.13 ± 0.12
24.3 °C to 45.0 °C	3.92 ± 0.10	2.00 ±0.06	2.11 ± 0.15

Discussion

In this study, interaction of x-rays with bovine vitreous humour has been found to increase the ionic conductivity of the irradiated vitreous samples. The radiation has also been found to affect the pH and the bulk ionic potential of the vitreous samples.

It is believed that the radiation being ionizing may cause a dissociation of the water molecules in the samples (Lea, 1956; Allen, 1961; Parsegian and Clark, 19168), thereby increasing the conductivity. Interaction of the radiation with the vitreous humour samples may also generate hydroxyl radicals and other charged oxygen and hydrogen atoms in the samples, thus increasing the ionic content and the conductivity. In this regard, the high x- ray dose of 140.15 mGy may produce more radicals and ions in the vitreous sample, thus making it more conductive than the samples with a low dose of 4.43 mGy. The radiation-induced radicals and ions may also serve as potential sites for chain initiation, which may result in polymerization. In such polymers formed by ionic and free radical chain mechanisms, cross-linking is usually found to depend on the radiation level and temperature. The Arrheneous plots generally show a break at a specific temperature possibly close to the glass transition temperature T_g of the material, which marks the transition from the low temperature dipole-dipole phase to the high temperature electronic phase. The result obtained in this study for x-irradiation with 140.15 mGy indicates the polymeric nature of the irradiated vitreous sample with estimated T_g equal to 24.3 ^{0}C. The activation enthalpies estimated from the graph in figure 4 gives 5.13 $\pm$ 0.12 kJ per mol. for the low temperature dipole-dipole phase and 2.11 $\pm$ 0.15 kJ per mol. for the electronic phase. It is note-worthy that the non-irradiated samples and those irradiated with a low dose of 4.43 mGy did not show the glass transition temperature phenomenon, possibly because polymerization is low or non-existent in the samples.

Increase in the ion levels in the vitreous has been suggested to cause an imbalance of charge distribution in the crystalline lens Stepanov et al,1990; Bagchi and Emanuel,1991; Rose et al, 1970) and may affect the protein synthesis activities of the lens. High radiation is also known to increase catalase activity by causing a cross-linkage or binding of the hydroxyl ions with other charged atoms in the vitreous (Heller and Mayer, 1994; Allen,1961; Rose et al,1970)1. The non-irradiated vitreous humour contains some amount of hyaluronic acid (Vaughan and Asbury, 1974). The changes in pH observed in the vitreous samples treated with xray doses of 4.43 mGy and 140.15 mGy respectively, therefore indicate a progressive degradation of the acid content. This is also supported by the negative bulk ionic potential exhibited by the samples treated with the high radiation dose.

Increased catalase activity due to radiation is known to cause vitreous liquefaction (Akiba et al, 1994) which has been recognized to have the following effects on the vitreous body and the crystalline lens: reduction of the density of the vitreous humour and weakening of the support to the crystalline lens, changes in the molecular structure of the collagen protein molecules thereby increasing the density and the molecular weight of the protein and also increase in the hydration of the vitreous body. The above effects of vitreous liquefaction may cause injury to the vitreous body leading to the collapse of the vitreous gel and a detachment of the retina (Stepanov et al, 1990; Vaughan and Asbury, 1974).

Conclusion

Because of the damaging effects of ionizing radiations on biological tissues, the eye should always be protected against direct interaction with x-rays and other rays such as gamma, laser and ultraviolet radiations. When x-rays are being used in hospitals especially for examination of the skull, care must be taken to avoid direct exposure of the eye to the radiation. Care must also be taken to always observe the acceptable safety limits of the intensity required when radiation techniques are used in health care (Laogun,1989).

Animal studies often provide useful experimental models for the behaviour of human organs especially where there is a need to excise the organ to be investigated from the body. It is hoped that the information obtained from this study will be relevant in the radiation protection of the human eye especially in hospitals, factories and the environments where people are likely to be exposed to ionizing radiations.

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