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Nélida L. DEL MASTRO; Dulcila M.L. BERNARDES and Anna L.C.H. VILLAVICÊNCIO

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DEPARTAMENTO DE APLICAÇÕES NA ENGENHARIA E NA INDÚSTRIA

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RADIOPROTEÇÃO POR DIMETILSULFÓXIDO EM DOIS SISTEMAS BIOLÓGICOS

Nélida L. DEL MASTRO; Dulcila M.L. BERNARDES e Anna L.C.H. VILLAVICÊNCIO

COMISSÃO NACIONAL DE ENERGIA NUCLEAR - SP
INSTITUTO DE PESQUISAS ENERGÉTICAS E NUCLEARES
Caixa Postal 11049 - Pinheiros
05499 - São Paulo - BRASIL

RESUMO

Certos compostos químicos são conhecidos como capazes de oferecer proteção a sistemas "in vivo" ou "in vitro" expostos a radiação gama. O dimetilsulfóxido (DMSO) é conhecido como radioprotetor químico para bactérias e células de mamíferos em cultura. O presente estudo foi conduzido a fim de: a) confirmar dados de outros autores que descrevem capacidade radioprotetora de DMSO para camundongos; b) estabelecer se esse comportamento protetor poderia ser evidenciado num sistema químico "in vitro" que utiliza proteínas do cristalino bovino como alvo. Camundongos fêmeas albinas heterozigotas foram utilizadas para os estudos de sobrevida aos 30 dias da irradiação com 9 Gy de ^{60}Co (taxa de dose: 4,5 Gy/min) injetados 1 h antes com 2000 mg/kg de DMSO intraperitonealmente. Foram também analizadas as curvas de peso corporal durante o mesmo período. Os estudos ao nível molecular foram realizados mediante a adição de DMSO 1M a uma série de soluções protéicas obtidas à partir de cristalinos bovinos e 10 minutos após irradiada com 5 diferentes doses entre 5.000 e 25.000 Gy de ^{60}Co (taxa de dose média: 14 Gy/min). Após a irradiação foram realizadas medidas espectrofotométricas a 600 nm e de grupo tiol livre para avaliar as modificações induzidas pela radiação. O DMSO foi capaz de evitar o aumento de turbidez das soluções, bem como o aumento de grupos sulfidrílicos livres produzidos pela radiação. Os resultados mostraram também que este composto químico fornece uma proteção razoável reduzindo a letalidade em camundongos produzida por exposições à radiação na faixa de dose que afeta a capacidade funcional do sistema hematopoiético e o trato gastrointestinal.

RADIOPROTECTIVE EFFECTS OF DIMETHYL SULFOXIDE IN TWO BIOLOGICAL SYSTEMS*

Nélida L. DEL MASTRO; Dulcila M.L. BERNARDES e Anna L.C.H. VILLAVICÊNCIO

COMISSÃO NACIONAL DE ENERGIA NUCLEAR - SP
INSTITUTO DE PESQUISAS ENERGÉTICAS E NUCLEARES
Caixa Postal 11049 - Pinheiros
05499 - São Paulo - BRASIL

ABSTRACT

Some chemicals are known to offer protection to "in vivo" or "in vitro" systems exposed to gamma radiation. Dimetnyl sulphoxide (DMSO) is a known chemical protector against radiation damage for bacteria as well as mammalian cells grown "in vitro". The present study was conducted: a) to confirm data from others describing a radioprotective capacity of DMSO in mice; b) to establish whether this protective behavior could be evidenced in an "in vitro" chemical system utilizing bovine crystallin protein as target. Heterozigous female albino mice were used for the 30-day-survival studies after 9 Gy ^{60}Co gamma irradiation (dose rate: 4.5 Gy/min) injected 1 h prior with 2000 mg/kg DMSO intraperitoneally. Total body weight curves during the same period were also analysed. For the molecular level studies 1 M DMSO was added to a series of aqueous protein solutions from bovine lens and 10 min later irradiated with 5 different doses from 5,000 to 25,000 Gy ^{60}Co (average dose rate 14 Gy/min). After irradiation, spectrophotometric reading at 600 nm and free thiol group determinations were performed in order to evaluate the radiation-induced modifications. DMSO was able to protect against the increase of turbidity of the solutions by irradiation as well as the radiation-induced augmentation of free sulphydrylic groups. The results shown also that this chemical provided a significant amount of protection reducing lethality in mice following gamma radiation exposures in the range dose expected to inhibit the functional capacity of the hematopoietic system and gastrointestinal tract.

*presented at the 7th Tihany Symposium on Radiation Chemistry, Balatonzeplak, Hungary, 7-14 September 1990.

INTRODUCTION

Radiation damage to cells results from direct and indirect energy depositions in the critical targets. Some chemicals are known to offer protection to "in vivo" or "in vitro" systems exposed to gamma radiation by interfering in the free radical-induced damage through its indirect action on intermediary molecules such as free-radical species resulting from water radiolysis which then interact with and damage cellular molecules (1).

Dimethyl sulfoxide (DMSO) an OH scavenger, is known to act as a radioprotector in different systems (2) (3) (4). It has been used for years as a topical anti-inflammatory agent and as penetrant carrier to enhance absorption (5). Some authors described a radiation protection by topical DMSO application in mice (6) or in intraperitoneally injected female rats with 5 on 7.5 g / kg body weight but a radiosensitization in some animals injected with 10g/kg (7).

The present study was conducted: a) to confirm data from others describing a radioprotective capacity of DMSO in mice; b) to establish whether this protective behavior could be evidenced in an "in vitro" chemical system utilizing bovine crystallin proteins as target.

MATERIALS AND METHODS

The animals used throughout this study were heterozigous female albino mice from our animal house. They were 9 to 12-week-old at the start of the experiment and were kept in plastic cages maintained on usual mouse pellets and water ad libitum. Mice were irradiated with 9.0 Gy of ^{60}Co in a gamma cell 220 Irradiation unit from Atomic Energy of Canada Ltd. at a dose rate of approximately 4.5 Gy/min in a cardboard (9x18) cm cylinder in groups of no more than 3 animals. Animals were injected 1 h prior irradiation with 60 mg DMSO (Merck Darmstadt/0.2 ml saline, being all irradiated in groups composed by 20 animals and injected or normal controls by 10 animals.

The number of survivors after irradiation was recorded during a 30-day period. Total body weight curves during the same period were also analysed.

For the molecular level studies, a protein solution was prepared from bovine crystallins (8). Lens were removed as soon as possible from bovine eyes freshly obtained from the slaughter house and were either used immediately or stored at -18º C. Lens (2 g) was homogenized in 5 ml of water at 0º C with a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 20000 xg for 30 min. The supernatant fluid to which N-ethylmaleimide had been added to a concentration of 10 mM was dialyzed overnight against 0.1 M potassium phosphate buffer (pH 7.4). This concentration of N-ethylmaleimide was the minimum required to mask SH-groups, as determined by the method of Ellman and Lysko (J. Lab. Clin. Med. 70:518, 1967). The dialyzed homogenate was placed in test-tubes and irradiated 10 min after the addition of 1 M DMSO with 5,000 ; 10,000; 15,000; 20,000 and 25,000 Gy ^{60}Co (average dose rate 14 Gy/min). After irradiation spectrophotometric reading at 600nm and free thiol group determinations were performed in order to evaluate the radiation-induced modifications (9). Statistical analysis was performed using the Duncan's test, SAS program, General Linear Models procedure.

RESULTS AND DISCUSSION

Figs. 1,2 and 3 present the 30-day survival and body weight curves after irradiation corresponding to 3 different experiments. The DMSO injected animals showed a higher percentage of survival when compared with controls: average 91.7% (55/60) and 71.7% (43/60) respectively. A minimum in the body weight curves appeared about the 12º day after irradiation being considered due to inhibition of the functional capacity of the hematopoietic system and gastrointestinal tract. All treated and control animals regained weight progressively and almost equally throughout the 30 days observation period. Nevertheless, pre-irradiation DMSO treated animals had a less weight loss and a quicker recovery than only irradiated ones. The results showed that this chemical provided a significant amount of protection preventing lethality

in mice following gamma radiation exposures.

On the other hand, in the experiments using dialyzed bovine lens homogenates exposed to gamma radiation, DMSO prevented augmentation of turbidity (Fig.4) as well as increase of free sulphydrylic groupsproduced by radiation (Tab.I). These results shown a significant 68% of protection of DMSO when compared with the only irradiated samples.

Our results confirm those from others about the increased radiation resistance of animals previously injected with DMSO, even when only one DMSO concentration was assayed. In this connection , the existence of an optimal radioprotective dose : each animal strain and the experimental regime used has to be discussed as mentioned somewhere (6) (7). DMSO presents hypothermic properties achieving the highest result after 60 min when mice are injected ip with 4.5 g/kg. The extension of hypothermia is similar to those produced by other radioprotectors but no direct correlation was found among this effect and the degree of radioresistance induction (4). So, physiological effects like hypoxia and temporary hypothermia must be considered in order to understand the whole biological radiation effect when DMSO is applied in vivo.

Cellular radiation-target environment is an important factor in determining the radiation response of cells as their radiation sensitivity could be made to vary greatly by the addition or removal of substances which were able to react either with free radicals in target molecules or with endogenous hydrogen-donating species (3). Studies on this subject have led to a model for chemical radiosensitization and radioprotection as a generalized version of the oxygen-fixation hypothesis (10). The data presented in this paper are consistent with that model, suggesting that the main role of DMSO be probably that of OH scavenger in a repair-fixation competition for neutral target radicals, showed particulary from our "in vitro" chemical system results. Sevilla and co-workers (11) established from an eletron spin resonance study of the reactions of cysteine and gluthatione thyl radicals with molecular oxygen, the identities of sulfonyl and sulfonyl peroxyl radicals, being

sulfinyl radicals, RSO the final radical species in the reactions of of thyl radicals and oxygen. In that study, sulfonyl peroxyl radicals are predicted to be far more reactive than thiol peroxyl radicals.

The conclusion of those authors may be of significance to interpret our data on the probable interaction of DMSO with biological thiols. Extension of this approach remains to be established.

TAB.I - RELATIVE VALUES OF FREE THIOL GROUP DETERMINATION AFTER ELLMAN'S METHOD. (For details see text).

DOSE (Gy)	CONTROL	PRETREATED WITH DMSO
0	0	0.170 ± 0.01
5,000	0.295 ± 0.01	0.335 ± 0.02
10,000	0.510 ± 0.05	0.495 ± 0.01
15,000	0.955 ± 0.07	0.745 ± 0.04
20,000	1.145 ± 0.01	0.905 ± 0.02
25,000	1.905 ± 0.01	1.035 ± 0.02

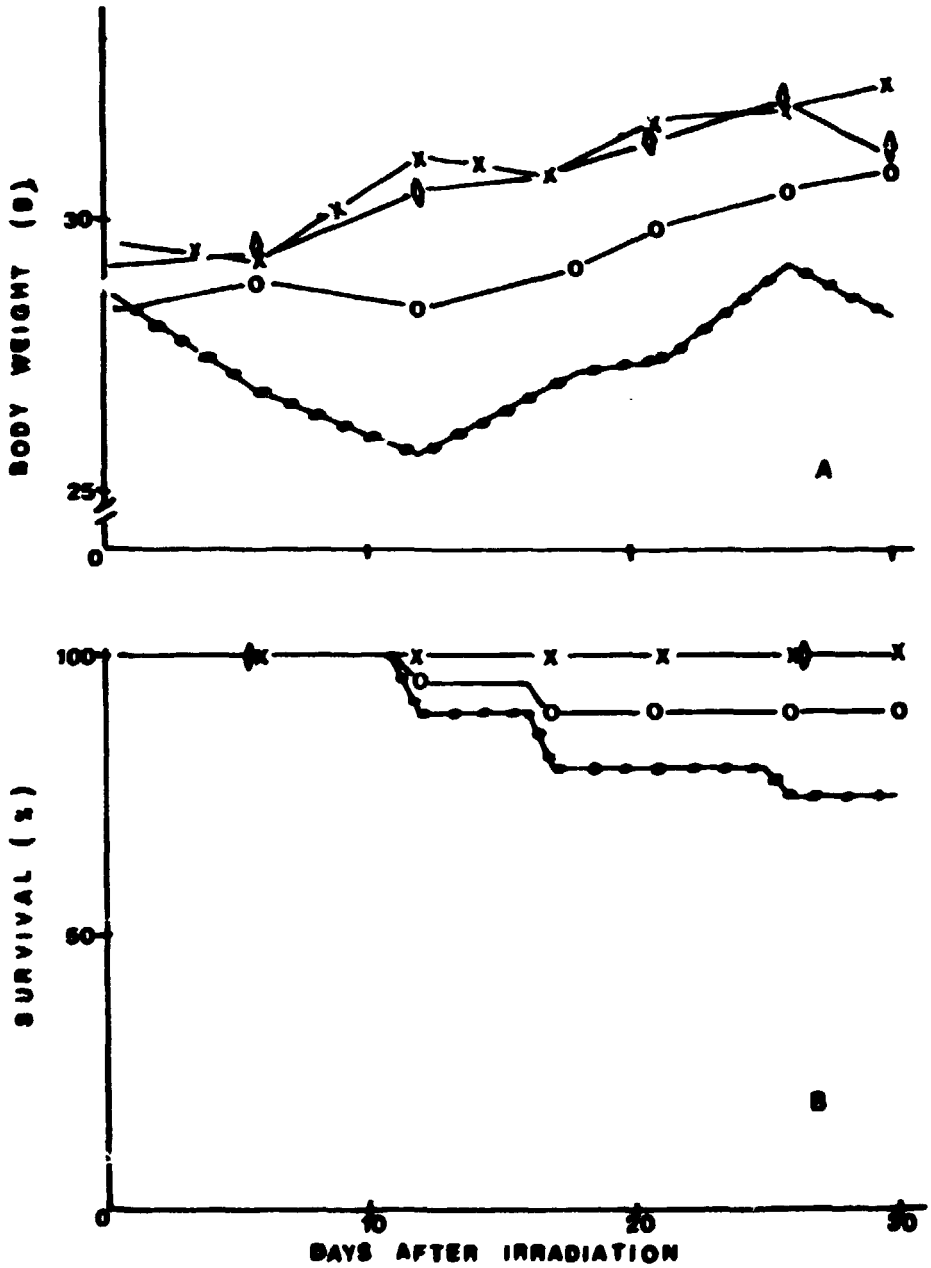

FIG.1 - A - Changes in average body weight of each one of the experimental groups. B - Survival of 11 week-old female mice subjected to 9 Gy ^{60}Co irradiation with or without pre-treatment of 60 mg DMSO/0.2 ml saline. (◆——◆) normal control; (●----●)irradiated; (×——×) DMSO; (○——○) DMSO + irradiated.

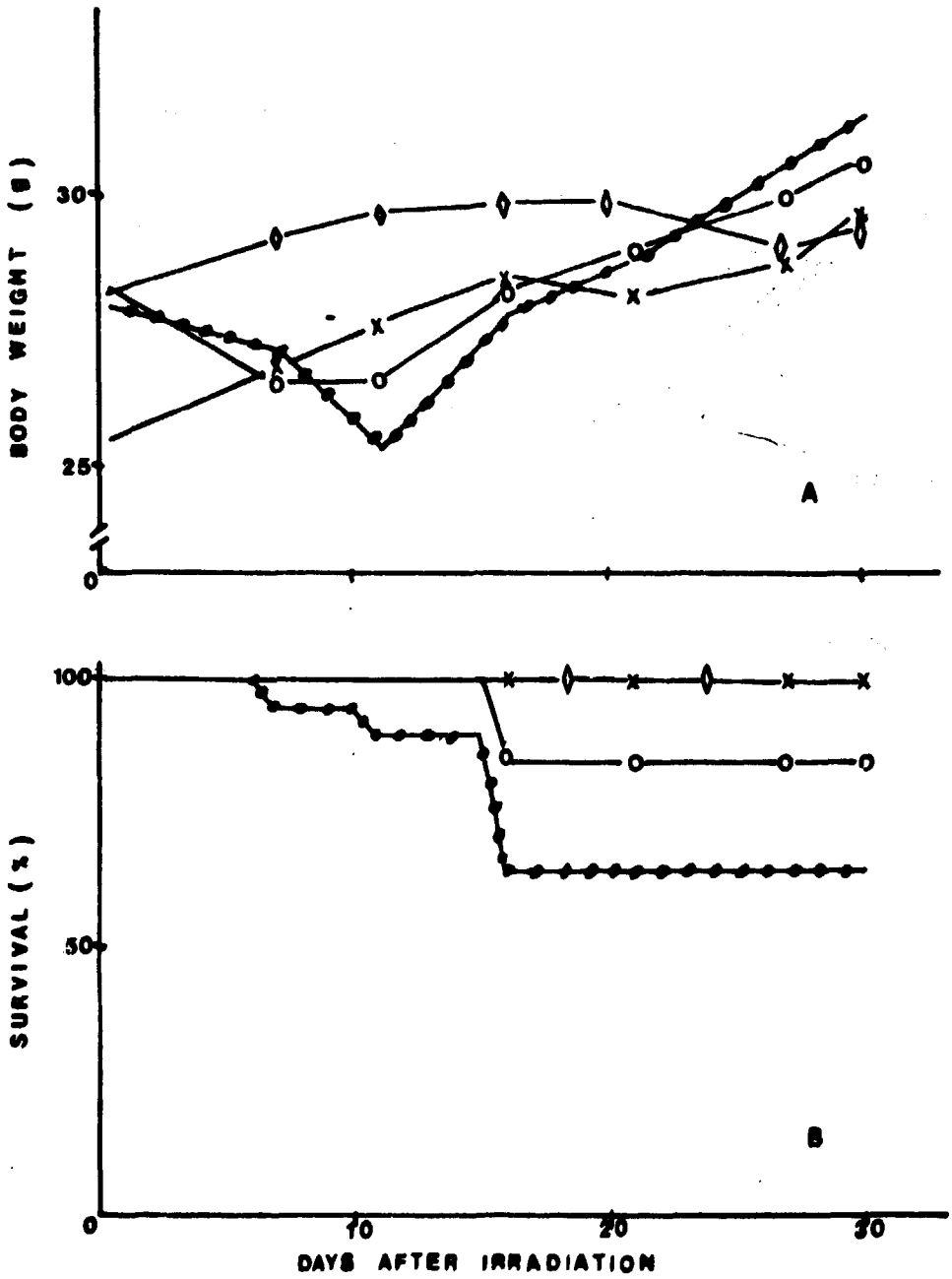

FIG. 3 - A - Changes in average body weight of each one of the experimental groups. B - Survival of 9 week-old female mice subjected to 9 Gy ^{60}Co irradiation with or without pre-treatment of 60 mg DMSO/0.2 ml saline. Legends as Fig. 1.

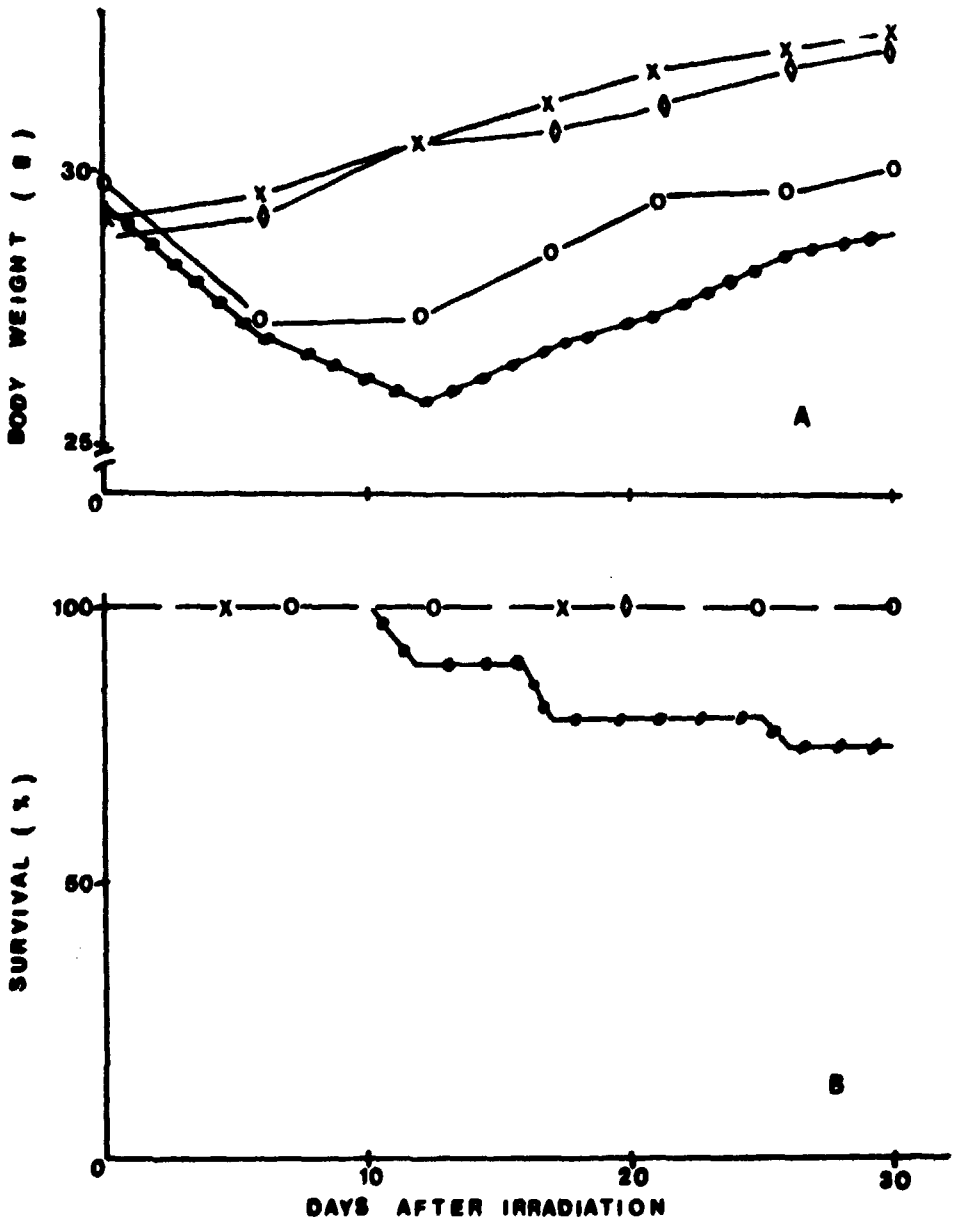

FIG. 2 - A - Changes in average body weight of each one of the experimental groups. B - Survival of 10 week-old female mice subjected to 9 Gy γ irradiation with or without pre-treatment of 60 mg DMSO/0.2 ml saline. Legends as Fig. 1.

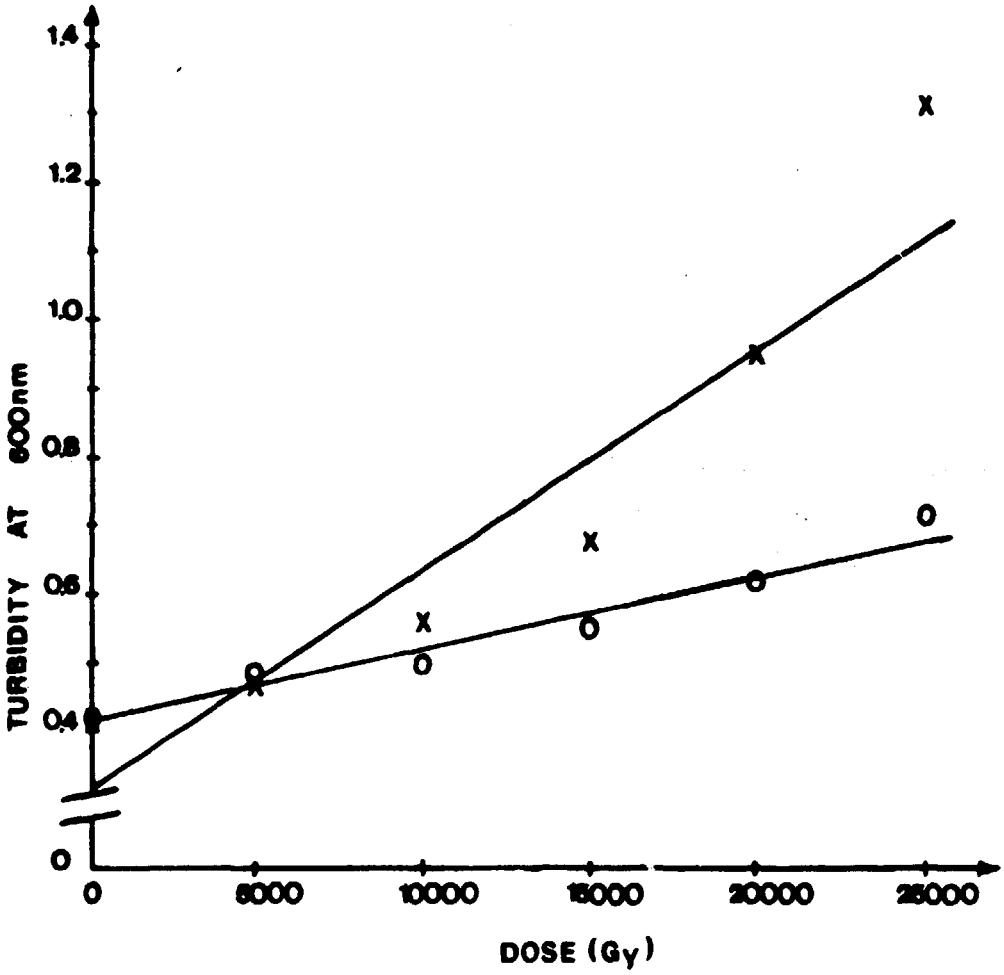

FIG.4 - Average of two turbidity readings of each lens homogenate exposed to ^{60}Co gamma irradiation. (o——o) pre-treated with DMSO; (x——x) control. Statistically significant according to t test $\alpha = 0.05$.

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