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ACTION OF HYDROQUINONE ON THE EFFECTS PRODUCED BY GAMMA RADIATION IN BARLEY SEEDS. EVALUATION OF SEED GERMINATION AND COLEOPTILE AND SEEDLING GROWTH

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RESUMEN

Se ha demostrado que los antioxidantes, en general, tienen acción protectora frente a los efectos biológicos de las radiaciones ionizantes, por ejemplo, al disminuir la fragilidad de los eritrocitos de ratones tratados con rayos x y al aumentar la vida media de ratones y de *Drosophila* irradiados. Se ha descrito, asimismo, que algunos antioxidantes, como las quinonas e hidroquinonas, protegen a los cromosomas de los daños que tienen su origen en los radicales químicos producidos por medios químicos. En este trabajo se demuestra la ineficacia de la hidroquinona como agente radioprotector del crecimiento de los coleóptilos de semillas previamente tratadas con radiación gamma.

ABSTRACT

It has been shown that antioxidants act protectively against ionizing radiations, since they diminsh erythrocyte fargility in mice treated with x rays and, increase the mean life of irradiated mice and *Drosophila*. It has also been shown that some antioxidants, such as quinones and hydroquinones, protect chromosomes from the damage produced by chemically obtained radicals. In this work, however, hydroquinone was shown to be ineffective in protecting the growth of coleoptiles of seeds treated with gamma-radiation.

Introduction

Chemical protection against the biological effects produced by ionizing radiations is attained by using substances which are applied before or at the moment of irradiation.

A knowledge of the primary physico-chemical events which occur during the interaction of radiation with biological material is important, in order to know which substances are indicated, and when they should be applied, in order to correct or to prevent a given effects.

$OH^{\cdot}$ radicals are strong oxidizing agents produced in the radiolysis of water,

and in biological systems, the bases purine and pyrimidine are among their important targets (Schmidt and Borg, 1976). Both radiation damage and the peroxidation of lipids found in membrane structures are attributed to such oxidizing radicals (Tappel, 1972; Vladimirov and Archakow, 1972). When radiolysis is carried out in the presence of oxygen, HO_2 radicals are formed which can react with fatty acid molecules:

$$HO_2^{\cdot} + RH \longrightarrow H_2O_2 + R^{\cdot}$$

With the oxygen present, a new radical ($RO_2^{\cdot}$) is formed: ($R^{\cdot} + O_2 \longrightarrow RO_2$ which can react with another fatty acid molecule: $RO_2^{\cdot} + RH \longrightarrow ROOH + R^{\cdot}$. The radicals obtained can interact among themsleves or react with metallic ions of different valences forming active molecular products. When the radicals react with antioxidants, less active radicals are produced. For example:

$$RO_2^{\cdot} + InH \longrightarrow ROOH + In^{\cdot}$$

where InH is the α-tocopherol molecule. In this case, the new radicals disappear little by little by reacting with other radicals (In · or $RO_2^{\cdot}$) Vladimirov and Archakov, 1972).

Antioxidative action is a general property of a series of phenolic and other biochemical compounds such as adrenaline, serotonine and tyroxine (Pullman and Pullman, 1963). This function is importante because it plays a central role in the prevention of spontaneous oxidation reaction in cellular metabolic processes (Hirsch, 1959).

The oxidation of thiol groups changes the permeability of erythrocyte membranes (Sutherland *et al.*, 1967; Sutherland and Pihl, 1968). This can be avoided by having antioxidants present when irradiation takes place (Kollman *et al.*, 1969). Hoffer and Roy (1975) found that vitamin E diminished the fragility of the erythrocytes of mice that were exposed to x rays, increasing their resistance to spontaneous hemolysis, i. e., vitamin E (alpha-tocopherol) has radioprotective effects.

Hydroquinone is a synthetic antioxidant, more active than adrenaline and less active than alpha-tocopherol in relation to the speed of inhibition of luminescence and electron donation (Petrushevich, 1971). The antioxidant activity of simple phenolic compounds derived from benzene and other complex aromatic hydrocarbons in organic systems is extensively analyzed by means of quantum mechanics. The conclusions derived from these studies may be amplified to include more complicated biochemical antioxidants. These studies start with the empirical observation that the efficiency of the antioxidant goes as the oxidation potential. The efficiency of phenols as antioxidants suggests that the final reaction in the antioxidative chain involves the transfer of a hydrogen atom from the antioxidant to the propagating radical (Pullman and Pullman, 1963).

Greenstock *et al.* (1974) studied the kinetics of radiosensitizing and radioprotective agents on some chemical systems. The sensitizers with an afinity for electrons

react with target radicals, be it DNA or its mononucleotides, by processes that involve the oxidation of the radical or the formation of a sensitizing radical complex. The effectiveness of these processes increases with the affinity for electrons of the radiosensitizers. According to Greenstock *et al* .(1974), nitrofuranes and benzoquinones are very effective sensitizers. Radioprotection implies either the capture of OH or the release of H. Radioprotective agents can compete with sensitizers like nitrofurazone, either by capturing an OH or by repairing target DNA (possibly sugars) by donating an H (SH compounds, amines, ascorbate, etc.) (Greenstock *et al.*, 1974).

The transfer of hydrogen atoms from SH groups to target radicals has been proposed as an explanation for sulfhydrylic protection in bacteriophages, bacteria and cells of irradiated mammals (Howward-Flanders, 1960, Johansen and Howard-Flanders, 1965).

If there exists a quinone-hydroquinone interchange system that manifests itself as oxidized and reduced states, respectively, it is possible to consider that hydroquinone, as the reducer, will donate electrons or H atoms to the target systems from which they have been displaced. Thus, if the quinones have been considered as sensitizers (Kopylov, 1966; Plishevskaya and Solomonova, 1966; Greenstock *et al.*, 1974), hopefully, the hydroquinones will be radioprotectors. It has been shown that the quinones and hydroquinones protect chromosomes from the effects of chemically produced radicals (Dubinin, 1964). However, when x rays were applied, hydroquinone did not protect spring onion chromosomes (Dubinin, 1964). Taking into account these results on hydroquinones a radioprotector, experiments were designed to test its radioprotective action using barley coleoptile as the biological system sensitive to radiation (Palomino *et al.*, 1977).

Material and Method

The barley seeds used (*Hordeum vulgare,* var. común) were obtained from the Escuela Nacional de Agricultura de Chapingo, Edo. de México, from the 1973-1974 harvest. In order to determine its radioprotective qualities, 1.4-benzenediol (hydroquinone), $C_6H_4(OH)_2$, was used whose molecular weight, according to J. T. Baker, is 110.114. The radiation source employed was the Gammabeam 650 (Atomic Energy of Canada, Ltd.) in the Centro de Estudios Nucleares, Universidad Nacional Autónoma de México.

Dismetry of ^{60}Co gamma radiations

For these determinations, the thermoluminescence method with lithium fluroide (Cameron 1961) was employed.

To determine the dose rate, 60 seeds were placed in test tubes where they were mixed with small capsules containing lithium fluoride (30/mg in powder) (J. T. Baker). The test tubes were placed in turn in a holder which was used

for all exposures. This holder was placed in the center of the 12-^{60}Co sources of the Gammabeam 650 which remained open at a diameter of 45 cm, leaving the test tube 30 cm above the base.

The seeds and the capsules containing lithium fluoride were exposed for 1, 2, 3, 4 and 5 minutes. After exposure, small quantities of lithium fluoride (10 mg) were weighed and placed on the high temperature plate of the TLD apparatus (Thermoluminescence Dosimeter, Mod. 2000A. Harshaw Nuclear System). With this equipment, the emission of light brought on by heating the irradiated lithium fluoride is converted into electrical pulses (measured in nano coulombs) and calculated by the following relation:

$$\text{Dose} = 2.415\ I - 0.112\ (Kr)$$

where I is the TLD counter reading. The dose absorbed is expressed in Kilorads (Kr). The average dose rate calculated was 11.7 Kr/min.

The first experiments carried out had a two-fold purpose:

1. To find the adequate concentration of hydroquinone, i. e., the concentration which affects neither germination percentage, coleoptile length nor barley seedling length.
2. To establish the appropiate time of treatment.

Groups of 30 seeds were used which were washed for 2 hours in running water in tubes covered with gauze (Figs. 1 and 2).

Next, the seeds were treated for 1 and 2 hours with hydroquinone at concentrations of 4.5×10^{-5}, 4.5×10^{-4}, 4.5×10^{-3}, 4.5×10^{-2} and 4.5×10^{-1} M, in tubes with air bubbling through them (Fig. 3). These tubes were placed in a constant temperature bath (20° ± 1°C). Afterwards, the seeds were washed in running water for 5 min. to remove excess hydroquinone and then planted in plastic boxes 28 cm wide, 35 cm long and 13 cm deep, which contained a layer of cotton and filter paper to which 450 ml of tap water had been added. All the seeds were planted with the embryo upward, using a system of coordinates to assure easy identification. To maintain the level of humildity desired, the boxes were covered with transparent polyetheylene with small perforations (Fig. 4). The boxes of planted seeds were maintained in a dark room at a controlled temperature of 20° ± 1°C throughout the experiment.

The criterium used for seed germination was the emergence of the coleoptile. The heights reached by the coleoptiles were measured 168 hours after germination began. The heights achieved by the seedling 360 hours after, when the grofth in length had stopped. The results of these preliminary experiments made it possible to select the highest concentration of hydroquinone in the 2 hour treatments which did not affect either germination, coleoptile growth or seedling growth, in order to observe its influence on the effects produced by distinct doses of ^{60}Co γ radiation.

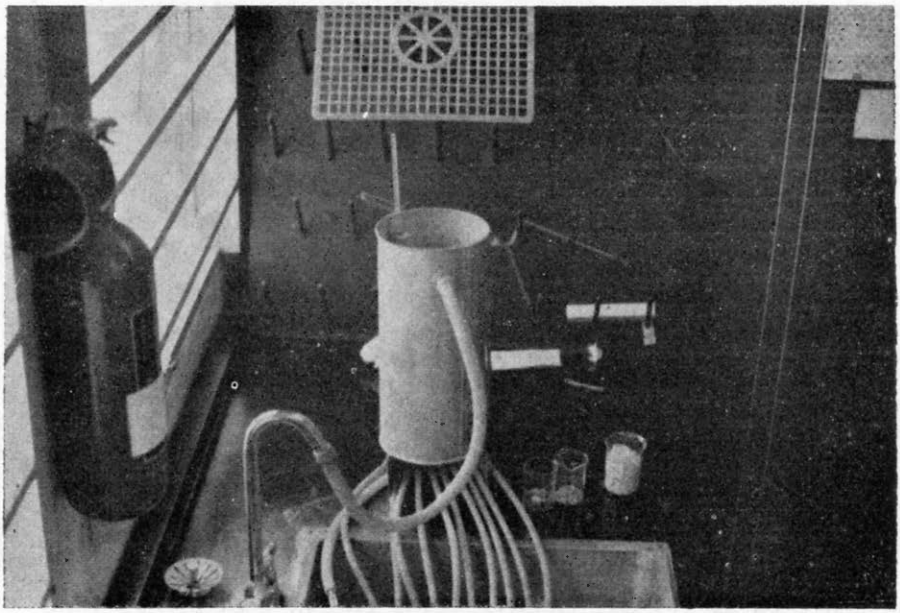

Fig. 1. System used to wash seeds in running water.

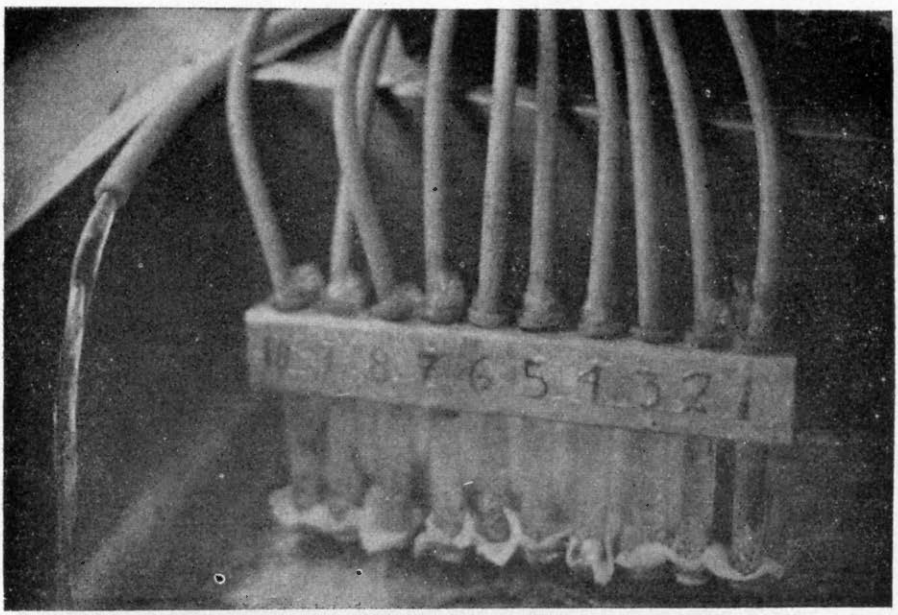

Fig. 2. Tubes with barley seeds (*Hordeum vulgare*, var, común) being washed in running water.

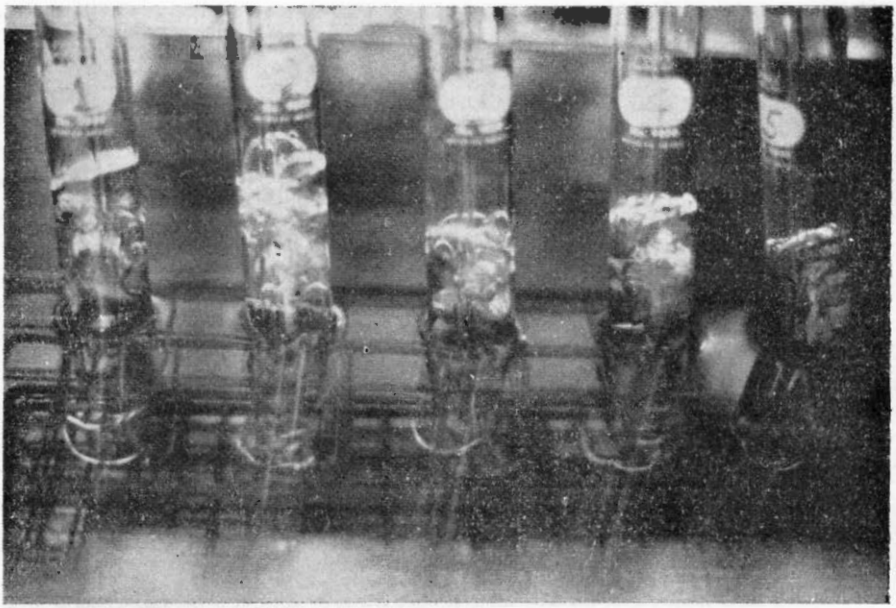

Fig. 3. Seeds being treated with hydroquinone.

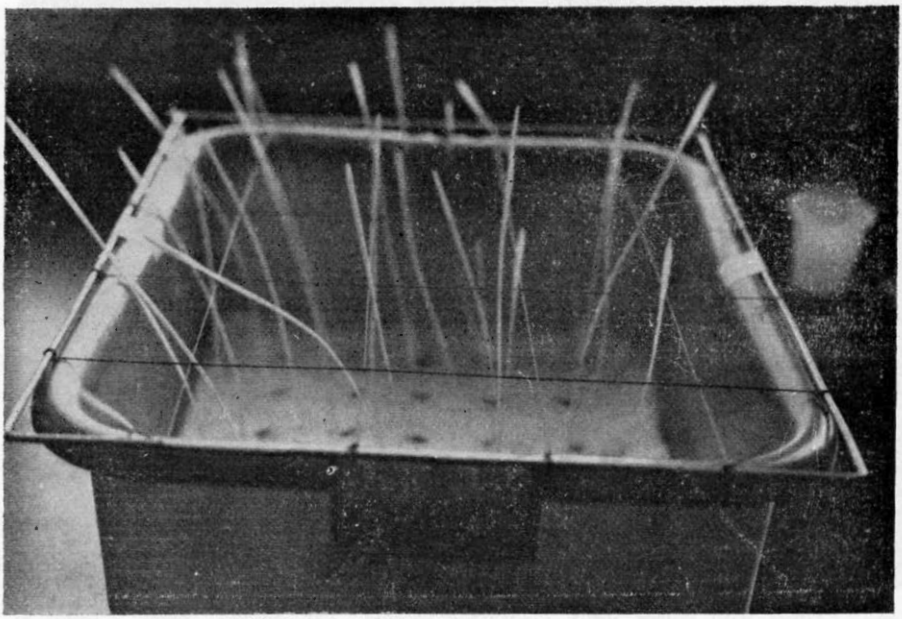

Fig. 4. Barley seedlings growing on cotton and filter paper in a plastic box.

Two groups of 60 seed were exposed to each dose of 10, 20, 30, 40, 50, 100, 125, 150, 175, 200, 300, 400, 500, 600, 700 and 800 of $^{60}Co\gamma$ radiation, applied at an intensity of 11.7 Kr/min. One group was treated with hydroquinone and the other was not. The experimental set-ups used were the same as those employed in the previous experiments. The seeds were placed in Pyrex test tubes to be irradiated. After irradiation they were put to germinate and later the coleoptile and seedling lengths were measured as described above.

Results

1. *Determination of the most adequate hydroquinone dose and treatment time*

The initial experiments were carried out in order to determine the highest dose of hydroquinone which did not affect the response of the batch of seeds with respect to germination and certain variables such as coleoptile length and barley seedling length. The dose selected was 4.5×10^{-3} M.

The times of 1 and 2 hours employed in the treatment did not affect the percentage of germination (Table I, Fig. 5). Smaller doses of hydroquinone stimulated germination. At a concentration of 4.5×10^{-4} M, the percentage of germination with respect to the control was 117.4% for the 1 hour treatment and 121.7% for that of 2 hours. At 4.5×10^{-5} M, the percentages were 121.7% for 1 hour and 126.1% for 2 hours.

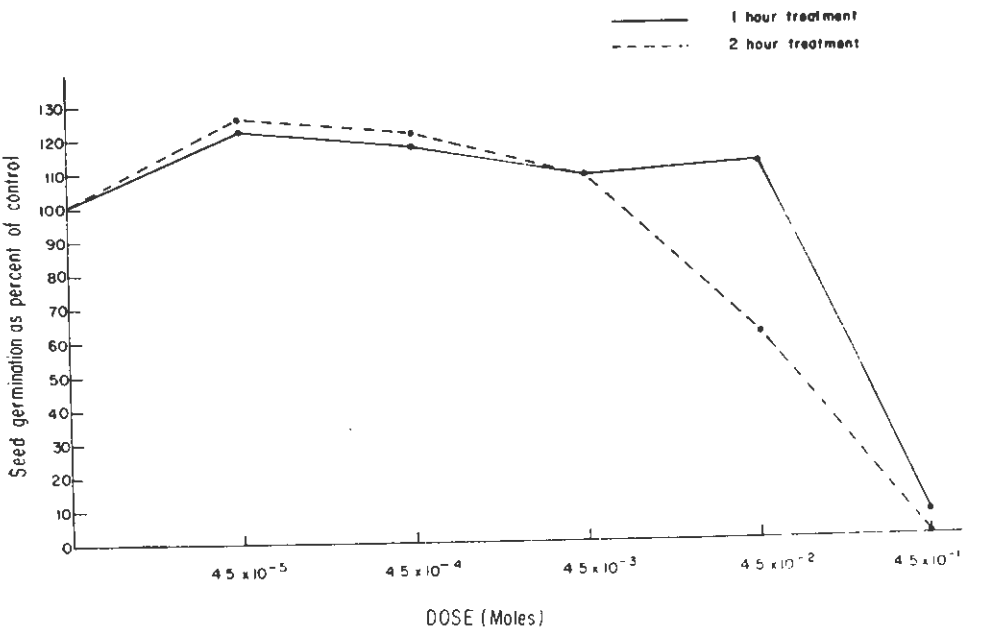

Fig. 5. Effect of hydroquinone on the germination of barley seeds.

On applying higher doses, such as 4.5×10^{-2} M and 4.5×10^{-1} M, after the 2 hour treatment, germination was 61.5% and 0% with respect to the control.

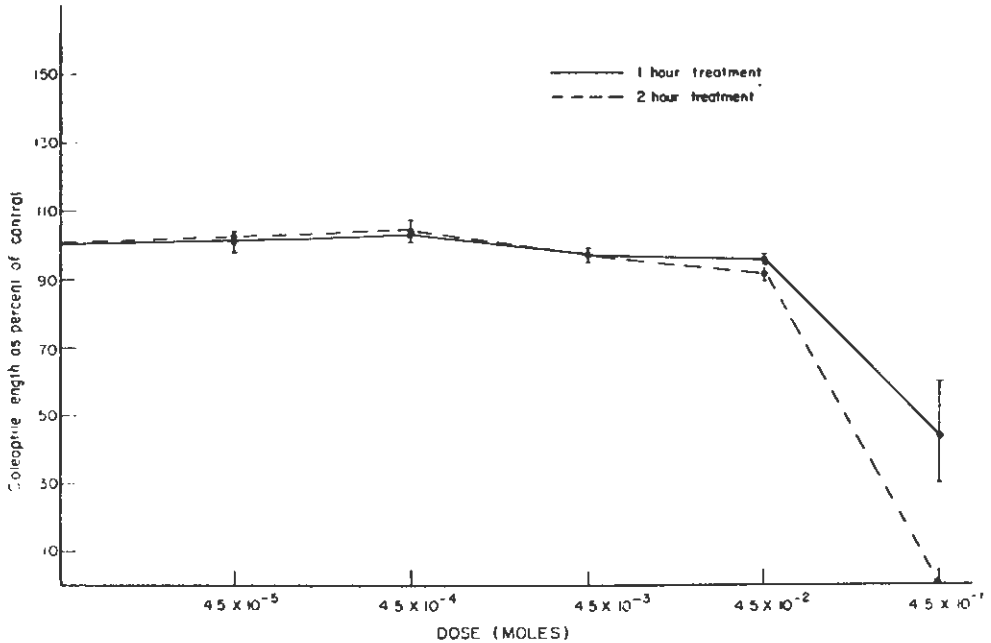

Fig. 6. Final coleoptile length given as a % of the control ± E. E. after treating the seeds with hydroquinone.

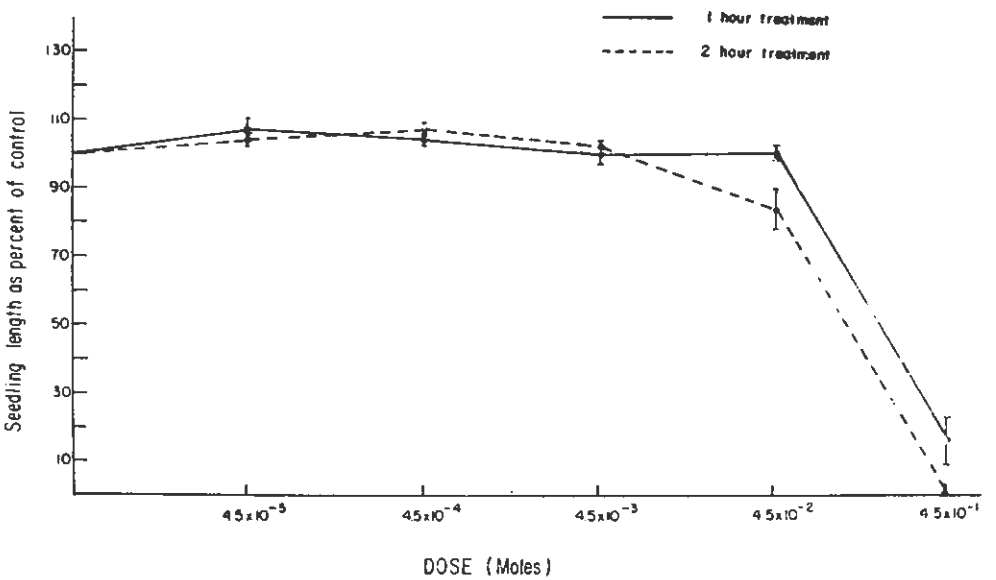

Fig. 7. Average final length ± E. E. achieved by barley seedlings after applying hydroquinone to the seeds.

TABLE I

THE EFFECT OF VARIOUS DOSES OF HYDROQUINONE ON GERMINATION AND AVERAGE FINAL LENGTH OF BARLEY COLEPTILES AND SEEDLINGS

Dose (Moles)	*% germination relative to control*		*Coleoptile length (% control ± E. E.)*		*Seedling length (% control ± E. E.)*	
	1 hour treatment	*2 hour treatment*	*1 hour treatment*	*2 hour treatment*	*1 hour treatment*	*2 hour treatment*
4.5×10^{-5}	121.73	126.08	100.81 ± 2.08	102.51 ± 1.72	107.42 ± 2.69	104.87 ± 1.81
4.5×10^{-4}	117.39	121.73	103.22 ± 2.26	104.37 ± 1.19	103.64 ± 2.19	107.09 ± 1.56
4.5×10^{-3}	108.00	108.00	97.67 ± 1.64	97.69 ± 1.82	100.47 ± 2.69	102.32 ± 2.17
4.5×10^{-2}	111.54	61.54	96.74 ± 1.48	92.01 ± 3.38	101.15 ± 2.40	84.46 ± 6.38
4.5×10^{-1}	7.69	0	44.60 ±16.20	0	16.59 ± 7.07	0

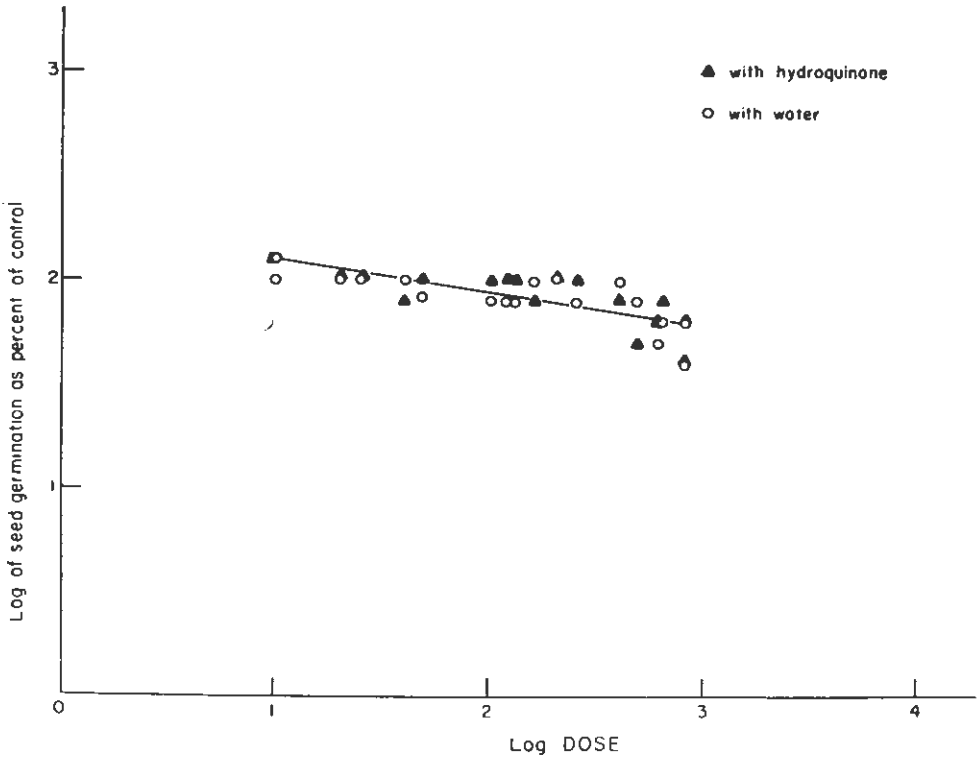

Fig. 8. Influence of hydroquinone (4.5×10^{-3}M) on the effects produced by different doses of ^{60}Co γ –radiation on barley seed germination.

3. *Effects on coleoptile and barley seedling growth*

The seedling appeared only for doses of 10 Kr, and its average length given as a percentage of the control ± standard error was 40.60 ± 3.31 for the group pretreated 2 hours with water, and 33.30 ± 3.26 for the group pretreated for 2 hours with 4.5×10^{-3} M hydroquinone.

Table III contains the coleoptile final lengths after using various doses of ^{60}Co γ-radiation and the two pretreatments (water and 4.5×10^{-3} M hydroquinone).

The differences between the coleoptile average lengths obtained for the two treatments were not significant in the X^2 test ($P > 0.99$) and therefore only one graph that is an average of the two is presented.

The average curve given in Fig. 9 shows the coleoptile growth behavior for seeds pretreated for 2 hours with water and hydroquinone (average result of the 2 pretreatments). Figure 9 also includes results obtained by Palomino *et al.* (1977) with dry seeds treated with ^{60}CO γ radiation and those obtained by Moutschen (1959) with x rays and seeds pretreated for 3 hours with water. Although the behavior is in general similar, some important differences are present.

The dose of hydroquinone mentioned above and treatments of 1 and 2 hours were also used to determine the behavior of other parameters, namley, coleoptile and seedling lengths. Once again the purpose was to find the most adequate dose and time.

No significant differences were found in either case (Table I, Fig. 6 and 7) with respect to the control when doses of 4.5 x 10^{-3} M were employed and the seeds were treated for 1 and 2 hours.

The highest dose of hydroquinone which produced no alterations in the variables selected was 4.5 x 10^{-3} M. Thus, in the experiments with γ -radiations, this dose was applied for 2 hours.

2. *Effects on the percentage of seed germination*

The results are presented in Table II.

TABLE II

THE EFFECT OF ^{60}Co GAMMA RADIATION ON THE GERMINATION OF BARLEY SEEDS WITH AND WITHOUT HYDROQUINONE PRETREATMENT (4.5 x 10^{-3}M)

Dose (Kr)	*% germination relative to control*	
	2 hour pretreatment with water	*2 hour pretreatment with hydroquinone*
10	116	100
20	116	100
30	123	116
40	120	98
50	98	113
100	93	106
125	96	111
150	93	104
175	116	92
200	100	111
300	83	102
400	106	85
500	80	58
600	63	69
700	69	81
800	43	46

Doses less than 500 Kr stimulated seed germination in both groups (those pretreated with water and considered as controls and those pretreated with hydroquinone), reaching values as high as 123% of that of the control.

From 500 Kr on, germination diminished in both cases, being from 43 to 45% of the control at 800 Kr. When the germination values obtained for both groups were plotted on a log-log graph, no significant differences ($P > 0.99$) were found on applying the X^2 test (Fig. 8).

TABLE III

BARLEY COLEOPTILE FINAL LENGTH AFTER APPLYING HYDROQUINONE (4.5×10^{-3}M) AND VARIOUS DOSES OF ^{60}Co GAMMA RADIATION TO THE SEEDS

Dose (Kr)	*Average coleoptile length (% control ± E.E.)* 2 hour pretreatment with water	2 hour pretreatment with hydroquinone
10	41.42 ± 2.12	38.41 ± 2.57
20	25.45 ± 0.82	24.12 ± 0.68
30	26.65 ± 0.80	23.89 ± 0.47
40	26.15 ± 0.67	24.40 ± 0.58
50	28.70 ± 0.59	26.94 ± 0.91
100	35.01 ± 0.66	34.63 ± 1.08
125	32.85 ± 1.07	31.88 ± 0.81
150	45.11 ± 0.81	41.34 ± 1.08
175	38.38 ± 0.85	37.44 ± 1.04
200	42.37 ± 0.67	48.14 ± 0.94
300	49.92 ± 0.95	48.25 ± 0.85
400	33.84 ± 0.87	32.41 ± 1.26
500	34.47 ± 1.35	26.64 ± 1.48
600	33.04 ± 1.10	37.74 ± 1.23
700	25.68 ± 0.90	24.79 ± 0.68
800	25.65 ± 1.07	23.43 ± 0.81

With doses from 10 to 40 Kr, a strong reduction in coleoptile growth is observed, as much as 25% of the control value. At 50 Kr the onset of growth recovery is noted which lasts to 300 Kr where it reaches about 50% of that of the control. From 300 Kr on, coleoptile growth diminishes again, reaching a value of 25% of the control at 800 Kr.

DISCUSSION

Free radicals are produced naturally in the metabolic activity of organisms and their number increases as oxidation and reduction reactions increase (Waters, 1946; Michaelis, 1961). The presence of these natural free radicals has been detected by electron paramagnetic resonance (EPR) studies (Commoner *et al.*, 1954, 1957, Ingram, 1958).

The chain of events starting with the initial absorption of radiation energy up to its final manifestation is a long one that can be modified by several environmental factors. The initial damage caused by exposure to ionizing radiations is modified by factors such as water content, temperature and the proportion of oxygen (Ehrenberg *et al.*, 1952; Bacq and Alexander, 1961).

Houben *et al.* (1964) distinguish 4 types of free radicals induced by radiation in *Pisum sativum*, basing their result on the differential decay of the radicals in different concentrations of water. They confirm other authors observations of the behavior of radiation-induced free radicals in various hydrated materials, noting that the radical decay rate is greater when humidity of the sample is higher.

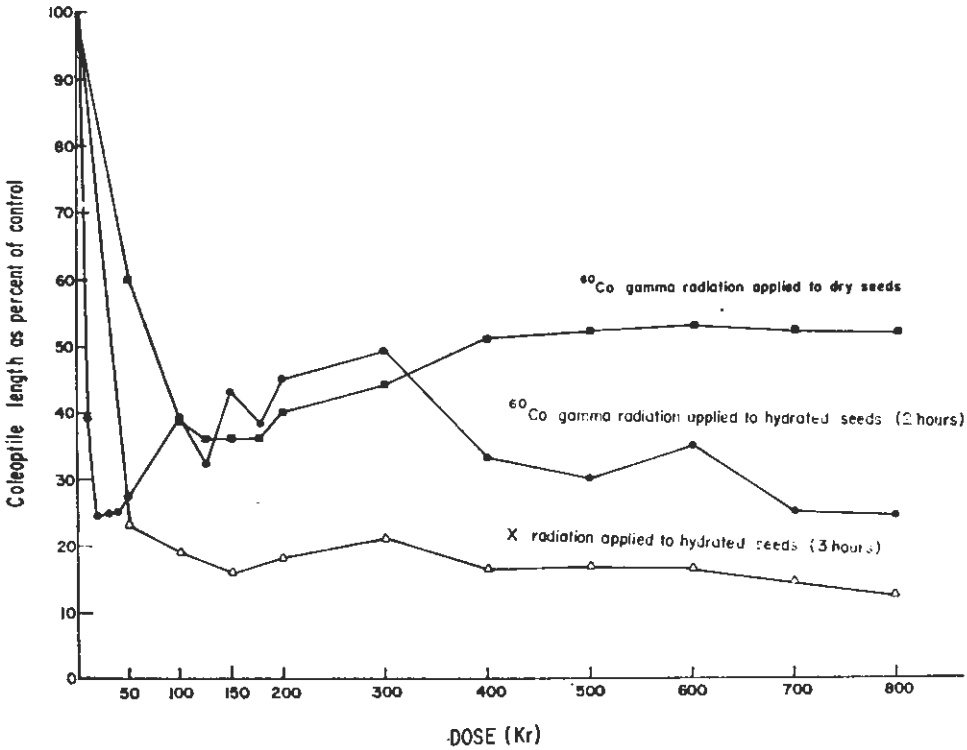

Fig. 9. Effect of treating seeds with low linear energy transfer (LET) radiations on barley coleoptile length. (Δ———Δ Moutshen 1959, ■———■ Palomino *et al.* 1977. ●———● this work).

Kirby and Randolph (1961) consider that the increase in free radical decay in the presence of high humidity levels is probably due to the fact that they recombine rapidly. Conger (1966) relates the effect of the decay of radiation —induced free radicals with radiobiological damage, establishing that the amount of damage is inversely proportional to the number of radicals that remain after they have decayed to a level characteristic of the basal state.

The free radicals produced by ionizing radiations interact with oxygen to form active peroxide radicals, which upon increasing produce more biological damage. When the number of these radicals decreases either by interacting with sulfhydryl compounds, by reacting among themselves or by interacting with antioxidants, they cause less biological damage (Howard-Flanders, 1960; Johansen and Howard-Flanders, 1965).

The antioxidants (butylated hydroxytoluene, proply gallate) that compete with other molecules in scavenging free radicals, increase the mean life of mice (Harman, 1962, 1968, 1969; Harman and Piette, 1966) and of *Drosophila melanogaster* (Félix, 1972), when added to their food. Vitamin E also increases the resistance of mice erythrocytes to the effects of x rays (Hoffer and Roy, 1975).

Studies of the activity of antioxidants in chemical systems where periodizing

reactions of lipids take place, show that there is a relation between the speed of peroxidation and the amount of antioxidant. When the antioxidant reacts with the free radicals, the peroxidation speed diminishes as does the amount of antioxidant (Vladimirov and Archakov, 1972). Tappel (1972) observed that an increase in the peroxidation time increases the absorption of oxygen and the number of toxic peroxidation end products in mice. He also found an inverse correlation between the absorption of oxygen by membranous subcellular organelles and the vitamin E content.

The initial damage due to ionizing radiations is modified by the presence of oxygen and sulfhydryl compounds (Howard-Flanders, 1960; Bacq and Alexander, 1961). The effect of oxygen in inactivating some substances by using x rays could be the result of the competition between molecular oxygen and intra-cellular H donors (such as sulfhydryl compounds) due to the radicals induced by the irradiation of nucleic acid of the bacteriophage T_2 (Howard-Flanders, 1960). Hydrogen, on being donated, occupies the sites left by the hydrogen atoms that have been removed from the DNA by ionization or by the action of radicals (Howard-Flanders, 1960). Studies carried out by Johansen and Howard-Flanders (1965) concerning the protection of bacteria from x-ray effects by means of substances that contain sulfhydryles, revelated the following. On the one hand, the sulfhydryles compete with oxygen in reaction such as: (1) R + O_2 or MSH (mercaptoethanol) giving rise to products that are functional, biologically speaking, and (2) R + O_2 or MSH-inactive products. On the other hand, they may take part in the formation of R by competing with intermediate radicals: DNA + OH (or some other radical) or MSH → R. Therefore, they propose that intermediate radicals with properties similar to the OH radicals are largely responsible for the lethal damage caused by x rays in bacteria subject to aerobic conditions. Studies reported by Greenstock *et al.* (1974) show that radiosensitizers, including oxygen, compete with radioprotectors for the target radicals (R) of the substrate. At high concentrations, the radioprotectors compete mainly with OH radicals. Certain radioprotectors, like SH compounds, amines and ascorbate, repair the damage in DNA and its mononucleotide components, competing with radiosensitizers for the target radicals (R). The effects of some protectors such as the hydrogen donors, for example cysteamine, do not depend on the DNA concentration but rather on the concentration of the sensitizer (Greenstock *et al.*, 1974).

Dubinin (1964) shows that quinones and hydroquinones, the latter being synthetic antioxidants, protect chromosomes from damage caused by OH and HO_2 radicals produced by chemical means. These facts lead to the supposition that hydroquinone is able to diminish the effects produced by radiation on barley coleoptile growth, but the results obtained were different from what was expected. They agree with those reported by Dubinin (1964) where it is shown that hydroquinone did not protect the chromosomes of irradiated onions.

The effect induced by radiation in barley seeds pretreated for 2 hours with water or hydroquinone is expressed in the diminution of coleoptile growth. For doses between 100 and 125 Kr, a slight decrease in growth was observed, whereas,

for doses between 175 and 300 Kr a recovery of growth was noted. In this latter range the growth was 50% of that of the control, in agreement with data from Palomino *et al.* (1977) or irradiated dry seeds, and from Moutschen (1959) on irradiated seeds pretreated with water for three hours. Coleoptile growth decreased again from 300 Kr on, being 25% of that of the control at 800 Kr. This decrease in growth for doses of 300 Kr or higher, agrees with results obtained by Moutschen (1959). Even though the general behavior was similar, there were some differences due to treatment and hydration. The effects produced by radiation on the growth of coleoptiles (Fig. 9) suggest the presence of 4 components, as seen in the dose-response curves. The first is a linear decrease in growth, the second a plateau, the third a region of growth recovery and the last, a drop off that does not reach zero because, before then, germination is inhibited (Palomino *et al.*, 1977). For seeds treated with hydroquinone the dose-response curve appears displaced to the left as compared to the curve obtained by Palomino *et al.* (1977) for dry seeds. The first component of the curve is related to the influence of the radiation on cell division and coincides with the notable reduction in the number of cells as the radiation dose increases. The second component of the curve becomes evident when the reduction in length is interrupted and the plateau appears. Next, the region of recuperation appears as manifested by the increase in size of the coleoptiles as the doses increases. This reversion and recovery of growth produced by high doses was also reported for corn seedlings and was related to a process of cell elongation (Schwartz, 1954) that depends on the interaction of two main factors: chromosome damage and the reduction of the rate of cell division (Schwartz and Bay, 1956). Thus, the diminshing of height was associated to the damage caused to the cell division process, and the recovery was considered as due to a stimulation of cell elongation. At higher doses (higher than 300 Kr) the decrease of cell elongation started, which may be due to the deactivation of the auxins, to some precursor system of the auxins, or to the alteration of the sites where auxins are produced (Gawlik and Shen-Miller, 1974).

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