# Enhancement of Radioresistance in Mice Treated with Diphenylhydantoin

H. LEVAN*, P. GORDON†, and S. STEFANI*

**Abstract** □ Diphenylhydantoin, a pharmaceutical compound of the same chemical class as magnesium pemoline, is found to exhibit significant protection against ionizing radiations. It is suggested that the mechanism of the radioprotective action of diphenylhydantoin is related to its influence on nucleic acid metabolism rather than to its anticonvulsant effects on the brain.

**Keyphrases** □ Diphenylhydantoin—radioresistance enhancement, mice □ Radiation protection—diphenylhydantoin effect, mice □ Irradiation, mice—enhancement of lifespan, diphenylhydantoin effect

Recently, the radioprotective effect of magnesium pemoline, a central nervous system (CNS) stimulant (1–4), has been investigated and reported from these laboratories. These encouraging results led to the studies of other compounds of the same chemical class as magnesium pemoline, among these diphenylhydantoin (DPH). Although pemoline and DPH do not have similar pharmacological actions on the most commonly employed screening system, pemoline is, in fact, 5-phenylpseudohydantoin and has been reported to exert effects similar to DPH and other phenylhydantoins on aspects of learning behavior in the rat and on the enzyme system DNase 1 (5, 6). Because DPH is a relatively nontoxic drug currently in use, while magnesium pemoline still falls in the category of an experimental drug of restricted use, it was felt that the exploration for radioprotective effects exerted by DPH, similar to those of pemoline, might have both theoretical and practical value. In this report, the enhancement of the lifespan of irradiated mice treated with DPH is reported.

## METHOD

$CF_1$ male mice, 50–60 days old, were used. The animals were housed in air-conditioned quarters and had free access to food and water. The experiments started about 10 days after their arrival from the supplier (Carworth Farm). Three hundred mice were divided randomly into two groups. Each group was then divided into 15 cages of 10 animals per cage. A second control group of 60 untreated mice was also used to observe possible pseudomonas infection. The experimental group was treated with 3 mg./kg. of DPH, intraperitoneal route, approximately 15–20 min. before 750 r whole-body X-irradiation; the control group was treated with a 0.3% suspension of tragacanth. Tragacanth was also used to prepare the DPH solution.

Irradiation was carried out with a Maxitron 400 kvp. X-ray machine at 80-cm. target-skin-distance (TSD) and an average exposure rate of 80 r/min. The TSD was determined by measuring the distance between the target and the horizontal level in the cage where the backs of the mice were exposed. The animals were not free to move around in the cage during irradiation. Total exposure was measured by a Victoreen Condenser R-Meter placed in the cage used to irradiate the animals. A turntable rotating at 5 r.p.m. was used to ensure uniform distribution of the radiation field. Less than 3% difference in the total exposure received by the two groups was observed.

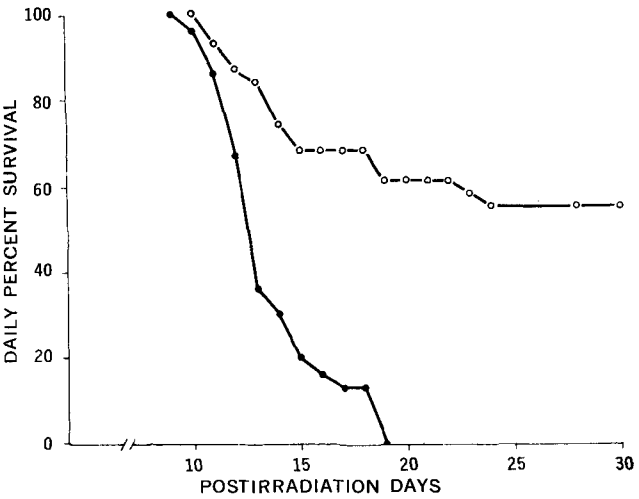


**Figure 1**—*Survival curves for two groups of $CF_1$ mice treated with DPH and tragacanth and exposed to 750 r of X-irradiation. Key:* ●—●, *control (tragacanth); and* ○—○, *experimental (3 mg./kg. DPH).*

The animals spent their postirradiation days in the same cage placed at the same position as before irradiation. Cautious measures were taken to assure minimal changes in the environmental factors between preirradiation and postirradiation periods. The experiment was replicated consecutively three times so that a total of 900 mice was used. Mortality was observed up to 30 days for each cage, and the total results were averaged for each of the two groups from all three experimental runs. Statistical analysis of the three combined experimental results was done according to Mewissen (7). The data plotted were thus based on the mortality of 900 animals.

## RESULTS AND DISCUSSION

No unusual symptoms or sickness were observed in the 60 untreated, nonirradiated control mice. Figure 1 clearly demonstrates a prolongation of survival of mice treated with DPH. All mortalities in the control group occurred within 10 days. By the 19th day after radiation exposure, all animals in this group had died while over 60% of the animals treated with DPH still survived. Over a period of 2 weeks, the survival in the experimental group decreased from 100 to 55%, and no further mortality was observed after the 24th postirradiation day. Most animals in both groups died between the 11th and 14th day after radiation exposure. The highest mortality of the control group was 30%, 13 days after irradiation, *versus* 13% for the DPH-treated group 12 days following radiation exposure.

Although Laird and Fonner (8) have reported a protective effect of DPH in the hyperacute radiation syndrome wherein death is from convulsion, two aspects of the present experimental design required that the authors postulate a mechanism of action for DPH in this experiment employing X-irradiation at 750 r that is distinct from its classical anticonvulsant effect. The exposure to radiation

employed is one where death is delayed for more than 1 week and is ascribable to hematopoietic depression rather than to CNS effects. Mortality in the experiments of Laird and Fonner occurred following exposures in the 10,000-r range 2–3 days postirradiation and was associated with convulsions, which was not the case in this experiment. Furthermore, DPH was given in this experiment in a single injection at a dose level one-fourth the anticonvulsant dose in mice (9) and would certainly not be expected to exert any anticonvulsant activity 10 days following drug administration.

Potentially important nonanticonvulsant actions of the DPH drug group have been identified by Gordon *et al.* and include antileukemic activity (5) and enhancement of the deteriorated learning and memory characteristic of very old animals (10, 11). Furthermore, both DPH and pemoline have been identified by this group as markedly potentiating the activity of the enzyme DNase 1, while DPH has been found by Shafer (12) to increase markedly the turnover of DNA in normal liver. Thus, DPH may exert the radioprotective action reported here by effects on nucleic acid metabolism, which are in no simple way related to its anticonvulsant action on the brain.

Continuing investigations will include exploring the capacity of DPH to exert radioprotective effects when given after radiation because such an effect for DPH has been observed in these laboratories when it is given within the 1st hour. Further, in a series of preliminary experiments, DPH exerted an effect similar to that of pemoline (5-phenylpseudohydantoin) in altering the growth pattern of Ehrlich carcinoma while prolonging the lifespan of tumor-bearing animals (13–15). The fact that DPH and pemoline have a similar chemical structure and exert similar effects on a nucleic acid-metabolizing enzyme suggests the link between biological effects shared by these two compounds.

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# *Erythrina* sp. III: Chemical Constituents of *Erythrina suberosa* Roxb. Seeds


**HARKISHAN SINGH and AMRIK SINGH CHAWLA**



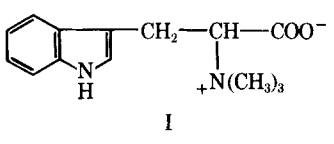
**Abstract** □ A phytochemical investigation of *Erythrina suberosa* seeds has resulted in the isolation of erythraline, erysodine, erysotrine, and hypaphorine. This is the first time that erysotrine has been found to occur naturally, although it is well known as a conversion product of other eryso-alkaloids. The alkaloidal constituents were found to vary in different seed collections. The fatty acid composition of the seed oil was examined, and the sterol part from the unsaponifiable matter was found to be composed of sitosterol, stigmasterol, campesterol, and cholesterol.



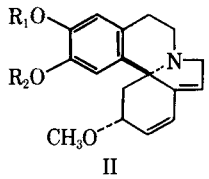
**Keyphrases** □ *Erythrina suberosa* seeds—phytochemistry, chemical constituents □ Mass spectroscopy—identification □ IR spectrophotometry—identification □ TLC—identification


In India, *Erythrina stricta* Roxb., *E. suberosa* Roxb., and *E. variegata* Linn. var. *orientalis* (Linn.) Merrill (syn. *E. indica* Lam.) have been used in the indigenous system of medicine for various ailments (1).

The authors have started a systematic study of *Erythrina* species growing in India. There have been no earlier reports of chemical investigations of *E. suberosa* and *E. stricta*. Hypaphorine (I) has been isolated from the seeds of *E. variegata* var. *orientalis* (2–6), and Folkers and Koniuszy (6) obtained erythraline



I



II

(II; $R_1,R_2 = -CH_2-$) from the seeds. Subbaratnam (5) isolated a neutral entity, $C_{24}H_{50}O_2$, m.p. 82–84°. The fatty acids present in the seeds have been studied (3, 7). The bark of *E. variegata* var. *orientalis* has been investigated by various workers (3, 8, 9), and preliminary studies on the leaves (3, 10) have been made.

Recently, the present authors fractionated the petroleum ether extract of *E. suberosa* bark into wax esters, alcohols, and acids; alkyl ferulates; and stigmasterol, sitosterol, campesterol, and cholesterol (11). In this paper, results from an investigation on the chem-