TABLE II. Comparison of Acute Toxicity of Cholinergic Organic Phosphates to Normal and Phenobarbital-Treated Mice.

	Control		Phenobarbital-treated	
Organic phosphate	No. of mice	$LD_{50} \pm SE$ (mg/kg)	No. of mice	$LD_{50} \pm SE$ (mg/kg)
Parathion	57	8.1 ± 0.4	34	14.2 ± 1.1
Methyl parathion	52	9.3 ± 0.8	54	14.3 ± 1.7
EPN	70	23.9 ± 1.1	39	64.4 ± 3.8
Systox	35	3.9 ± 0.1	35	4.8 ± 0.2
Di-Syston	35	6.7 ± 0.6	50	16.3 ± 1.0
Guthion	37	4.0 ± 0.5	48	4.9 ± 0.4
Delnav	75	33.2 ± 2.2	44	87.0 ±10.0
Phosdrin	35	3.2 ± 0.3	30	3.2 ± 0.3
Ethion	36	34.5 ± 5.9	40	40.8 ± 5.5
Trithion	72	27.0 ± 0.8	58	44.0 ± 5.7
OMPA	78	9.6 ± 0.4	70	9.9 ± 0.5
Co-Ral	36	14.7 ± 1.4	36	14.9 ± 1.7
Malathion	48	193.0 ±25.9	42	234.0 ±18.1
Ronnel	38	118.0 ±16.2	40	137.4 ± 7.5
Folex	54	67.7 ± 5.0	60	61.8 ± 5.3

toxicity of 15 cholinergic organic phosphates to normal rats and mice and to animals pretreated with phenobarbital for 5 days. The purpose of the study was to determine whether the hepatic microsomal enzyme induction caused by phenobarbital changed the acute toxicity of the organic phosphates. The study demonstrated that phenobarbital treatment either decreased the toxicity or had no effect on the toxicity of any of the compounds in either species with one exception being an increased toxicity of OMPA to phenobarbital-treated rats.

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Protection of Mice against Radiation Injury by Phenylhydrazine* (33403)

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Phenylhydrazine administration evokes marked erythropoiesis in mammals. The chemical damages erythrocytes, thereby producing an erythropenia which in turn accelerates erythropoiesis. Use of this compound as a means of studying effects of ionizing radiation on hemopoietic tissue was pointed out by Jacobson *et al.* (1), who found less histologic evidence of injury to blood-forming tissue in irradiated rabbits pretreated with phenylhydrazine than in irradiated control animals. Hodgson and co-workers (2, 3) extended this observation and found that rats

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given phenylhydrazine before irradiation displayed an increased radioresistance as measured ferrokinetically. Hodgson *et al.* (4) have also found that phenylhydrazine increases the number of colony-forming cells in hemopoietic tissue, especially in spleen and peripheral blood. As an extension of Hodgson's work it seemed pertinent to determine whether phenylhydrazine protects against acute deaths resulting from whole-body irradiation. Results of the present experiments demonstrate a marked protective effect of this compound.

Materials and Methods. Male (C3H X C57BL)F_1 mice 12–14 weeks old were used. Experimental mice received subcutaneously 1.0 mg freshly prepared PHCl (phenylhydrazine hydrochloride) in 0.1 ml water each day for 3 days. Controls were injected with 0.1 ml of water. In the first series of experiments mice were injected intraperitoneally with 0.1 μCi of ^{59}Fe as citrate on day 1, 3, 4, 5, or 10 after the third PHCl injection. Spleen weight and 24-hr ^{59}Fe uptake was determined for erythrocytes, spleen, and both femurs. In the second series, mice were exposed to 800 R at various intervals after the third PHCl injection and scored for 30- and 60-day survival. Radiation factors were: 250kVp, 15 mA, HVL 0.5 mm of Cu, dose rate in air~160 R/min., TSD of 60 cm. Mice were caged in groups of 5 for all experiments.

Results. Figure 1 shows effect of PHCl on spleen weight and on ^{59}Fe uptake by erythrocytes and spleen. Marked alterations which exemplify accelerated erythropoiesis occurred during the first 5 days. Unexpectedly, a significant reduction in ^{59}Fe uptake by femurs was observed on days 1 through 5; this may reflect PHCl toxicity or a rapid ^{59}Fe utilization and accelerated transit of erythroid elements through the compartment and into the circulation. Mice apparently tolerated PHCl well at the dose given, and no deaths occurred. There was, however, a progressive increase in urine pigment, presumably methemoglobin or its breakdown products. In addition to splenomegaly, the spleen was black and turgid, probably because of congestion with effete erythrocytes and methemoglobin. Although hematocrits were not determined,

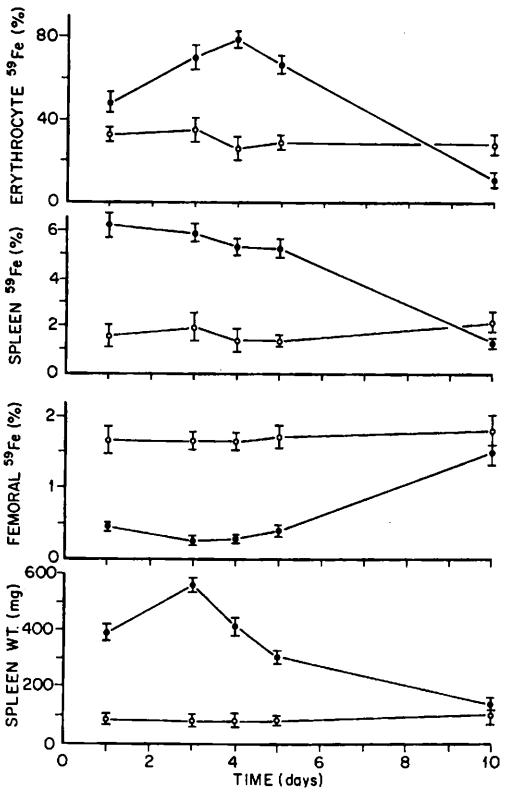

FIG. 1. Effect of phenylhydrazine on spleen weight and on 24-hr ^{59}Fe incorporation (% injected dose) by erythrocytes, spleen, and both femurs of mice. Abscissa is time after the third injection of phenylhydrazine; horizontal bars indicate 95% confidence interval; (●), phenylhydrazine; (○) distilled water; 10 mice/point.

the blood samples clearly indicated that the mice were anemic during the first 5 days after PHCl.

In the survival studies, mean 30-day survival of water-injected controls for all experiments was 10.0% showing that 800 R was about an LD 90/30. Figure 2 shows that PHCl pretreatment substantially improved survival, beginning on day 4 and continuing through day 8 after treatment. Between day 30 and 60, 4 deaths occurred in the PHCl groups.

Discussion. The present results show that PHCl given before irradiation increases radioresistance, thereby confirming the erythropoietic data of Jacobson *et al.* (1) and Hodgson and Eskuche (3). It is reasonable to conclude from results of the 30-day sur-

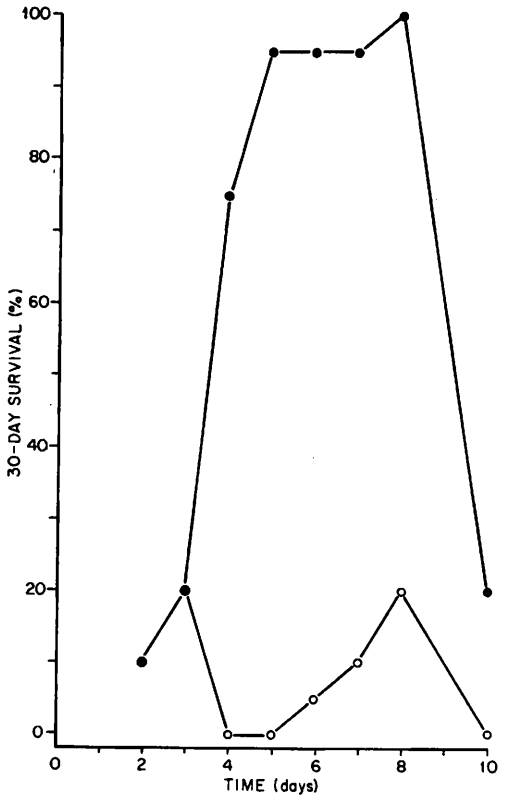

FIG. 2. Effect of phenylhydrazine injected before irradiation (800 R) on 30-day survival of mice. (●), phenylhydrazine; (○), distilled water; 20 mice/point; abscissa is time of irradiation after the third injection of phenylhydrazine.

vival studies that other hemopoietic tissues are also protected by effects of PHCl, presumably since myelopoietic, thrombopoietic, and lymphopoietic compartments must regenerate to prevent acute deaths. The mechanism of protection is not clear, but there are several possibilities to consider. The PHCl or its metabolic breakdown products may afford a true chemical protection like that obtained with the usual protective agents, e.g., cysteine and AET (5). It is not likely, however, that PHCl itself protects chemically, because radioresistance does not increase until day 4 after the third injection. We do not know what the metabolic products of PHCl would be in the mouse, although McIsaac *et al.* (6) have defined products appearing in the urine of rabbits given PHCl orally.

Since erythrocytes, damaged by PHCl, create an acute disposal problem for the animal, hyperactivity of the reticuloendothelial system would be expected to occur (7). Perhaps this hyperactivity enhances radioresistance, by improving the animal's ability to combat infection which results from radiation damage to hemopoietic tissue.

Hodgson and Eskuche (3) suggested that as a consequence of increased erythropoiesis, stem cells are being triggered (8) into cell cycle to meet the demand for erythrocytes, and that these triggered cells may be more radioresistant than nontriggered cells, i.e., cells not in cycle.

Finally, PHCl may effect an increase in the number of hemopoietic stem cells by virtue of compensatory mechanisms brought into play either by direct stem cell damage or as a consequence of erythropoietic stimulation. An increase in stem cell number would improve the animal's chances of survival, because it is known that stem cell killing is an exponential function of X-ray exposure (9–11). Thus the number of stem cells surviving a particular radiation exposure is a function of the number present at the time of irradiation.

Summary. Radioprotection by phenylhydrazine was observed for mice exposed to 800 R of 250 kVp X-rays. Maximum protection, as measured by 30-day survival, was achieved when mice were irradiated on day 5, 6, 7,, or 8 after the third of 3 daily phenylhydrazine injections. We postulate that improved survival resulted from an increase in the number of hemopoietic stem cells at the time of irradiation.

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Transplacental Chemical Induction of Microencephaly in Two Strains of Rats. I. (33404)

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In July 1967, 4 litters of Fischer rats, 14 months old, were sacrificed and unexpectedly found to have various degrees of microencephaly. The assumption was that all mates had been equally and simultaneously exposed to an etiologic agent because the reduction in weight of the brain was strikingly uniform within each litter.

The four litters were parts of a larger study in which the attempt was made to correlate site and frequency of transplacentally induced neoplasms with the stage of fetal development at the time of exposure to methylazoxymethanol (MAM), the aglycone of cycasin and a potent carcinogen. Evidence for transplacental passage of both the glucoside cycasin and its aglycone provided the necessary information to assume a direct chemical interaction between these compounds and embryonic or fetal tissue (1).

The protocols indicated that the respective mother rats had received a dose of MAM equivalent to 20 mg/kg of body weight which had been administered intraperitoneally in three equally divided doses on days 14, 15, and 16 of gestation.

Experiments were started to examine reproducibility, strain dependency, optimal dose and time of fetal development for successful induction, postnatal growth of the brain, effects of mating of microencephalic sisters and brothers on the brain of their progeny, and possible intellectual deficits in microencephalic rats in conjunction with anatomical alterations. It is the purpose of this paper to describe the experimental conditions under which microencephalic litters were produced with regularity in two strains of rats, to report on the growth of the brain during postnatal life and on the result of sister–brother matings of microencephalic rats.

Materials and Methods. Animals. Two strains of rats, the Fischer and the Osborne-Mendel strains, were used. Young males and females were obtained at weanling age from the animal production facilities at the National Institutes of Health. After they had reached maturity, they were bred in our laboratory, and the day on which spermatozoa were identified in the vaginal smear was designated as day 1 of pregnancy. The impregnated females were separated and housed in individual plastic cages. The rats were weighed on day 14 of pregnancy unless stated otherwise and the total dose of MAM was calculated on the basis of 20 mg/kg of body weight. This amount was administered intraperitoneally either in 3 equally divided doses on days 14, 15, and 16 of pregnancy or, in later experiments, as the entire amount on either day 13, 14, 15, or 16 of pregnancy to