11. Morse, E. V., Langham, R. F., *Am. J. Vet. Res.*, 1958, v19, 139.

12. Alston, J. M., Broom, J. C., Leptospirosis in Man and Animals, 1958, E. & S. Livingstone Ltd., Edinburgh & London.

13. West, E. S., Todd, W. R., Textbook of Biochemistry, 1956, Macmillan Co., New York.

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Effect of Gamma Irradiation and AET on Rat Blood Cholinesterase.* (26416)

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Previous work by Sabin(1) has indicated that red blood cell cholinesterase levels of the mouse offer a sensitive biological index to x-irradiation. Williams(2), by a different technic, has shown similar action of gamma irradiation in whole blood cholinesterase of the rat, but the threshold by this index was not determined in this species. In the same study the acute toxicity of acetylcholine was shown to be greater in gamma irradiated mice than in the non-irradiated animals.

With the discovery of Patt, *et al.*(3), that cysteine and glutathion gave radiation protection, further studies by Bach and Herve (4) on radio-protective agents and later work by Shapira, *et al.*(5) led to development of several radio-protective materials. One of these, S, 2-aminoethylisothiouronium bromide, hydrobromide (AET) has been studied rather extensively and was utilized in this investigation.

The purpose of this study was 2-fold: 1) to determine the irradiation threshold for whole blood cholinesterase activity in the rat and to plot a dose response curve of these data; 2) to determine whether AET produces any *in vivo* gamma irradiation protective action insofar as whole blood cholinesterase activity is concerned.

Methods. Rats utilized were irradiated by whole body exposure in lucite boxes to a cobalt-60 source of 820 curies at a source to midline distance of 150 cm. Dosimetry was accomplished utilizing the Victoreen model 70 r-meter and hi-energy chamber model 620. Exposure rate at this distance was 9.37 r per minute.

Dosage levels tested were 0, 75, 150 and 300 r except where otherwise noted. The 10 animals per level utilized were male Wistar strain Charles River rats, weighing 100 to 135 g at beginning of experiment. Cholinesterase determinations were by a micro technique similar to that of Michel(6) and involved the use of one-tenth ml of whole blood. The blood was drawn by tail vein into a one-tenth ml pipette and transferred to a 15 ml centrifuge tube containing 2 ml of distilled water as a hemolyzing agent. The fluid level in the tube was then brought up to 2.5 ml with distilled water and then to 5 ml with a buffer containing 0.82 g sodium diethyl barbiturate, 0.10 g of potassium (monobasic) phosphate and 44.70 g of potassium chloride per liter. The pH of this buffer was adjusted to 8.3 with 0.10 M hydrochloric acid, prior to use. The centrifuge tubes were then placed in a water bath at 37° ± 0.5°C and 0.5 ml of 3 per cent acetylcholine bromide was added.

The initial pH was then read and incubation continued for 2 hours after which the final pH was recorded. The difference between these values was recorded as the delta pH. Non-enzymatic hydrolysis of the acetylcholine was virtually non-existent so no correction was made. Mean initial control whole blood cholinesterase activities, i.e. delta pH, under these conditions were 1.04 pH units per 2-hour incubation period.

All animals used were given Purina Lab Chow and water *ad libitum* and were housed

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TABLE I. Mean Delta pH and Standard Error.

Group	Pre-AET—Pre-irrad.	Days after irradiation 3rd day	7th day	10th day	14th day
Control	.99 ± .028	1.08 ± .01	1.15 ± .003	1.13 ± .003	1.13 ± .02
AET control	1.03 ± .02	1.01 ± .024	1.16 ± .02	1.17 ± .02	1.06 ± .013
75 r	1.05 ± .04	1.13 ± .04	1.11 ± .03	.97 ± .02	1.06 ± .02
150 r	1.00 ± .02	1.06 ± .045	.93 ± .05	.89 ± .05	1.11 ± .07
300 r	1.11 ± .02	1.13 ± .03	.98 ± .02	.86 ± .02	.97 ± .02
AET + 300 r	1.01 ± .01	1.09 ± .02	.89 ± .02	.85 ± .03	.98 ± .004
" + 600 r	1.09 ± .02	1.11 ± .04	.81 ± .01	.82 ± .04	1.02 ± .04

5 to a cage both before and after irradiation. Cholinesterase determinations were made on all animals prior to irradiation, on the 3rd. 7th, 10th and 14th post-irradiation days.

A second series of animals of 10 per level was treated with AET intraperitoneally at 200 mg/kg 15 minutes prior to and 100 mg/kg at time of irradiation according to the method of Hanna(7).

Radiation levels on these animals were 0. 0 plus AET, 300 r plus AET and 600 r plus AET. Cholinesterase determinations were identical with those previously described and occurred before, on the 3rd, 7th, 10th and 14th days after irradiation. All statistical treatment is by students "t" test.

Results. Results obtained in various phases of this study are incorporated into Table I. These values present the mean delta pH and standard error of blood samples taken from beginning groups of 10 animals in each of the treatment groups except control where 20 were utilized.

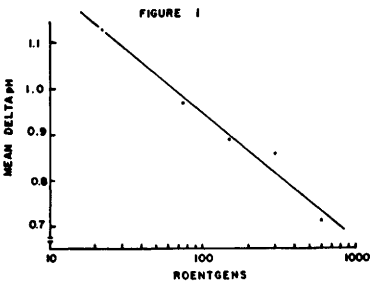
Statistical comparisons of the various groups are found in Table II. It is apparent that peak significant post-irradiation depression of whole blood cholinesterase activity occurs on the tenth day.

TABLE II. Percent Variation in Mean Delta pH from Control.

	Pre-irrad.	Day after irradiation 3rd	7th	10th	14th
300 r	+12.0*	+4.6	−14.8*	−23.8*	−14.2*
150 r	+ 1.0	− 2.0	−19.1*	−21.2*	− 1.8
75 r	+ 6.0	+4.6	− 3.5	−14.1*	− 4.4
AET control	+ 4.0	− 6.4*	+ .8	+3.5	− 4.4*
Percent variation in mean delta pH when 300 r + AET is compared with 300 r alone					
300 r	+9.9*	+3.7	+10.1*	+1.2	−1.0

* P <.05 by Student's "t" test.

It is possible from these data to construct a dose response curve based upon whole blood cholinesterase activity on the tenth day after gamma irradiation (Fig. 1). By adding a 600 r point from our previous study (2) and by extrapolating the line drawn between these points, with the control delta pH on the ordinate, it is possible to estimate the approximate threshold for irradiation which would be necessary to produce just measurable change in whole blood cholinesterase. This extrapolation indicates that the control level falls between 20 and 30 r. Thus, we



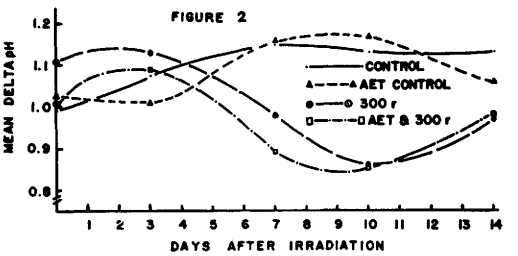


FIG. 1. Radiation levels plotted against the mean delta pH for rat whole blood cholinesterase on tenth day after irradiation.

FIG. 2. Mean whole blood cholinesterase levels (delta pH) at 0, 3, 7, 10 and 14 days after irradiation.

could expect just measurable changes within this range if the phenomenon fits a semi-log plot and if sufficient numbers of animals were used.

Fig. 2 presents data obtained from Table I with specific regard to AET. The upper 2 curves present time-mean delta pH plot of the 2 controls (i.e. with and without AET), while the lower present 300 r, with and without AET (Table II).

Discussion. The finding that apparent threshold levels of gamma irradiation in the rat are in the vicinity of 20 to 30 r for whole blood cholinesterase is in complete agreement with Sabin(1) who found similar activity with x-irradiation down to 25 r in the mouse red cell cholinesterase. Thus the two species are remarkably similar despite differences in type of irradiation and different enzyme entities.

The fact that AET fails to protect whole blood cholinesterase from gamma irradiation is evident from Fig. 2 as the 2 levels are virtually identical, i.e. 1.2% apart, on the tenth day, the time of expected peak depression. AET at the high levels tested did not appreciably affect cholinesterase in the surviving animals, though mild significant depression attributable to AET alone was noted on the third and fourteenth days (Table II).

Summary. 1. Whole body gamma irradiation in the rat produced significant whole blood cholinesterase depression on the tenth day at a dosage level of 75 r. 2. The levels tested when plotted and extrapolated indicated threshold changes in cholinesterase activity would be in the vicinity of 20 to 30 r. 3. AET alone, while producing some mild cholinesterase depression, failed to protect whole blood cholinesterase activity from the effects of gamma irradiation at the levels of agent and irradiation tested.

1. Sabin, J. C., *Am. J. Physiol.,* 1956, v187, 275.
2. Williams, M. W., *ibid.,* 1959, v196, 949.
3. Patt, H. M., Tyree, E. P., Straube, R. L., Smith, D. E., *Science,* 1949, v110, 213.
4. Bacq, Z. M., Herve, A., *Brit. J. Radiol.,* 1951, v24, 617.
5. Shapira, R., Doherty, D. G., Burnett, W. T., Jr., *Radiation Res.,* 1957, v7, 22.
6. Michel, H. O., *J. Lab. Clin. Med.,* 1949, v34, 1564.
7. Hanna, C., O'Brien, J. E., *Fed. Proc.,* 1959, v18, 400.

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Effects of Source of Dietary Carbohydrate on Survival Time of Sublethally X-irradiated Mice.* (26417)

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During an investigation of the effects of diet on response of mice exposed to multiple sublethal doses of total body x-irradiation, it was observed that length of survival of x-irradiated animals was significantly affected by the source of dietary carbohydrate employed. Our findings are reported here.

Procedure. The basal ration employed consisted of carbohydrate, 59%; casein†, 24%; cottonseed oil, 10%; salt mixture‡, 5%; cellulose§, 2%; and the following vitamins per kg of diet: thiamine hydrochloride, 10 mg; riboflavin, 10 mg; pyridoxine hydrochloride, 10 mg; calcium pantothenate, 60 mg; nicotinic acid, 100 mg; ascorbic acid, 200 mg; biotin, 4 mg; folic acid, 10 mg; para-aminobenzoic acid, 400 mg; inositol, 800

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† Vitamin-free Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

‡ Wesson Modification of Osborne-Mendel Salt Mixture, General Biochemicals, Inc., Chagrin Falls, Ohio.

§ Solka Floc, Brown and Co., Boston, Mass.