strains were observed in mobilities of individual serum protein fractions or amount of alpha-2 globulin.

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Chemical Prevention of Radiation-Induced Leukemia in Mice.* (31184)

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Total body exposure of animals to ionizing radiation results in a spectrum of damage which is dose-related(1). The destructive effects have been mitigated by shielding of sensitive organs(2), and by pretreatment of exposed animals with chemical agents (3); 2 of the latter are *Salmonella typhosa* endotoxin(4) and 2-aminoethylisothiuronium bromide (AET)(5).

Leukemia virus persists as an occult infection among many strains of mice, and is very likely transmitted to progeny by congenital routes(6,7). Leukemic disease appears spontaneously in the majority of animals of 2 mouse strains (AKR and C58) when 4 to 12 months of age(8,9). In other mouse strains, development of significant levels of disease depends on early exposure to specific chemical agents or to ionizing radiations(10, 11,12). Total body exposures to a series of small doses of X-rays (150 R) are most effective in inducing leukemia in mice(13). By this regime the disease has been induced in germfree and in conventional mice of 6 strains: they are Swiss-Webster, ICR, CFW, Balb/C, C57 BL, and C3Hf(14). Lesions in the spontaneous and in the radiation-induced forms of leukemia are indistinguishable: enlarged thymus, and less frequently swollen lymph nodes, infiltration of visceral organs with large lymphoid cells, and increased numbers of lymphoid cells in the blood.

The mechanism of progressive leukemogenesis, spontaneous or X-ray induced, is as yet unknown. The homeostatic balance between host and virus is altered to the extent that pathology emerges. It may in some way be related to the immune mechanism of the

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host, since procedures such as X-irradiation, inoculation of chemical carcinogens, and thymectomy influence both the immune status of the host and leukemogenesis.

The damaging effects of X-irradiation in mice have been prevented by shielding specific sensitive organs such as spleen and bone marrow, and this benefit is also reflected in a lowered incidence of leukemia(15,16). Since the damaging effects of X-rays can be prevented or modified by drugs, the action of two such chemical agents was examined for leukemia-sparing effect in mice.

Materials and methods. Groups of C57 BL and Swiss-Webster mice, at 1 month of age, were given whole-body exposures to 4 doses of X-rays, each of 150 R, at 7-day intervals. The X-ray source was a 260-KVP therapy X-ray machine operated at 240 KV and 15 ma with filtration of 1.0 mm Al and 0.25 mm Cu at a rate of 35 to 40 R/minute. Groups of isologous mice were administered either (a) 4 doses of 150 R X-rays, or (b) 4 doses of a drug plus 4 doses of 150 R X-rays after the prescribed interval. Each radiation-sparing drug was administered as follows:

A. Difco endotoxin (bacto-lipopolysaccharide), prepared from *S. typhosa* by the phenolic extraction method of Westphal *et al* (17), was dissolved in physiological saline (40 μg per ml), sealed in glass ampoules, and steam-sterilized. Each mouse was inoculated intraperitoneally (I.P.) with 16 μg endotoxin at 24 hours prior to each dose of X-rays.

B. 2 - aminoethylisothiuronium bromide (AET) was dispensed 200 mg per glass ampoule. Sealed ampoules of AET and of physiological saline were steam-sterilized. Each mouse was inoculated I.P. with 5 mg (250 mg/kg body weight) of freshly-mixed AET in saline at 30 minutes prior to each dose of X-rays.

Groups of Swiss-Webster mice were administered 3 weekly doses of a drug: each of endotoxin (16 μg), or of AET (5 mg) and each inoculum was followed respectively in 24 hours or in 30 minutes by 150 R X-rays. On the fourth week, each mouse was given 16 μg endotoxin or 5 mg AET and after the specified interval 650 R X-rays. Each drug dose was tested in 10 mice for 30 days of protective-effect against whole-body exposure to 650 R X-rays.

TABLE I. Effect of Radiation-Sparing Drugs on X-ray Induced Leukemia.

Mouse strain	150 R x-rays ×4	16 μg endotoxin + 150 R x-rays ×4	5 mg AET + 150 R x-rays ×4
C57 BL	19/32 (59)*	10/36 (27)	2/35 (5.7)
Swiss-W.†	10/19 (52)	13/20 (65)	
"	5/10 (50)	6/10 (60)	

* No. leukemia/No. irradiated (%).
† Swiss-Webster.

The experimental mice and their controls were maintained and observed for at least 6 months following the last dose of X-rays. Animals which appeared sick were killed by ether inhalation, and then examined for gross and microscopic evidence of leukemia. All of the animals which survived 6 months were killed and examined for evidence of leukemia.

Results. Leukemia developed in significant numbers of the mice which received only the X-rays (Table I). Repeated treatments of C57 BL and Swiss-Webster mice with endotoxin prior to each irradiation did not prevent the development of leukemia in them. Actually repeated endotoxin treatments were of such little value that the incidence of leukemia was higher in the endotoxin-treated irradiated Swiss-Webster mice than in the irradiated controls. The incubation periods and the lesions observed in the leukemic mice were not modified by the pretreatment with endotoxin, as compared with the untreated irradiated control mice. One dose of endotoxin did protect mice against the damaging effects of a single dose of 650 R X-rays (Table II).

Pretreatment of irradiated C57 BL mice with AET resulted in 90% reduction of leukemia, compared with that in the untreated irradiated control mice (Table I). The incubation periods and lesions induced in the 2 leukemia cases from the AET-treated group were the same as in the irradiated control group of mice.

Among mice which received 3 doses of drug-150 R X-ray combination plus drug-650 R X-rays, only significant numbers of those

TABLE II. Effects of Repeated Treatments with Endotoxin and with AET on Subsequent Challenge with Lethal Doses of X-rays in Mice.

No. mice	Three treatments*	Final treatments	Survivors†
20	Endotoxin + 150 R	Endotoxin + 650 R	1/20 (5)
10		Endotoxin + 650 R	10/10 (100)
10		650 R	2/10 (20)
20	Endotoxin + 150 R	Endotoxin + 650 R	0/20 (0)
37	AET + 150 R	AET + 650 R	24/37 (65)
13		AET + 650 R	7/13 (53)
15		650 R	2/15 (13)
16	AET + 150 R	AET + 650 R	15/16 (93)
10		AET + 650 R	10/10 (100)
10		650 R	1/10 (10)

* Each treatment at intervals of 7 days—Endotoxin, 16 μg; AET, 5 mg.
† At 30 days: Survivors/No. treated (%).

with AET survived the final X-ray challenge (Table II).

One of 20 mice with the endotoxin treatments survived the 30-day observation period. In a control group of mice, administered one 16 μg dose of endotoxin 24 hours before 650 R X-rays, all of 10 mice survived 30 days. Endotoxin protected 5 of 10 mice against whole-body exposure to 650 R X-rays, and AET protected 7 of 10 mice against the same dose of X-rays. The results are essentially in accord with previously reported tests on radiation-sparing effects of the drugs(4,5). Spontaneous leukemia did not appear among mice of the untreated control colonies from which they were derived.

Discussion. The data here presented extend the radiation and the leukemia-sparing properties of AET(18) and the related mercaptoethylguanidine, as described by Upton *et al*(19), except that the Lobund test animals have not developed myelogenous leukemia. Treatment with AET reduced decisively the leukemogenic effects of subsequently administered X-rays.

Since animals respond to inoculated endotoxin with antibody, conceivably such a mechanism might void the radiation-protective attributes of each subsequent inoculum. This is demonstrated in Table II.

While pre-irradiation treatments with AET reduced the subsequent incidence of leukemia, neither the mechanisms of prevention nor of leukemogenesis are thereby clarified. The radiation-sparing effects of repeated inoculums of AET were not neutralized by the host, and this may be attributed to the nonantigenic nature of AET. AET reduced the biological effects of X-irradiation by reduction of X-ray dose in the animal. It is unlikely that AET affected the leukemia virus in the irradiated animal; by reducing the X-ray dose in the animal it blocked the leukemogenic "trigger" effect of X-rays. Since radiation induced leukemia is dose-related, the radiation-sparing effect of AET can be related to the leukemia-sparing effect.

Summary. Mice which were subjected to repeated treatments of endotoxin plus 150 R X-rays developed leukemia to the same extent as the drug-free irradiated controls. Treatments of mice with AET prior to each exposure to 150 R X-rays reduced the incidence of leukemia in them. Mice which had been inoculated with endotoxin were thereby immunized against the radioprotective and leukemia-sparing benefits of the drug. When AET replaced endotoxin in the above scheme, the radioprotective and leukemia-sparing benefits of AET were sustained.

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Alterations in Enzyme Activities as a Consequence of Exercise (Swimming) in the Rat.* (31185)

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Enzymatic changes in blood and tissues in response to both acute and chronic exercise in trained and untrained individuals and animals have been studied most intensively. However, there appears to be little unanimity in this field. Nikkila *et al*(1) found that the greatest increment in enzyme increase was observed in untrained subjects and the least in trained athletes. As total work output increased, mean serum enzyme levels were correspondingly raised. Malic dehydrogenase activity (MDH) increased moderately, while 2 transaminases manifested very significant rises after all forms of exercise. Gardner *et al* (2) studied the effect of exercise on serum enzyme levels in trained subjects and observed a relationship between degree of exercise (treadmill) and serum enzyme levels; a relationship to duration of exercise was evident also. There was no significant difference in pre-exercise serum enzyme values between trained and untrained subjects. The mean increases in glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), lactic dehydrogenase (LDH) and MDH activities after exercises were observed to be significantly less in trained subjects. Harding, Rosen, and Nichol(3) found that changes in liver GPT activity in rats appeared to reflect changes in metabolism that accompanied an enhanced rate of gluconeogenesis. Critz(4) has recently reported on the effect of swimming exercise on serum glutamic-oxaloacetic transaminase (SGOT). He found that SGOT activity was decreased by a brief swim (1 minute), but was elevated after swimming 5 minutes. An exercise period of 10 or 15 minutes produced no significant change in the SGOT activity but swimming for 30, 60 or 120 minutes caused a progressive rise in activity. It is apparent that conditions and duration of exercise are major factors in determining alterations of enzyme activities.

Several other enzymes in animals and man have been studied using various forms of exercise without any uniform pattern being established. Perhaps the major reason for this lack of unanimity is variation in the duration, type, and environment of exercise. Certain standard conditions were established for the experiments described here and changes in liver and plasma MDH and GPT activities with exposure to acute and repeated exercise (swimming) in the rat were then measured.

Materials and methods. In 2 separate experiments, male Wistar rats weighing 240-270 g were divided randomly into 4 groups: (A) control, (B) acute exercise, (C) trained + exercise, and (D) trained. Prior to experimentation rats were maintained in individual wire cages at an environmental temperature

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