Further Observations on Late Somatic Effects of Radiomimetic Chemicals and X-Rays in Mice[1]

J. W. Conklin, A. C. Upton, and K. W. Christenberry

(Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee)

SUMMARY

Young adult female RF mice were subjected to four successive midlethal doses of nitrogen mustard (HN2), triethylene melamine (TEM), 1,4-dimethanesulfonoxybutane (myleran), or whole-body x-rays. The agents were given at 14-day intervals, and the survivors were observed for effects on longevity and on the incidence of late occurring diseases. The percentages of mice dying within 30 days after treatment in the various groups were: TEM, 12.5%; HN2, 10%; myleran, 3%; x-rays, 1%; and control, 1%. The life-span of 30-day survivors was shortened in all treated groups, with mean survival times as follows: x-rays, 355 days; TEM, 427 days; HN2, 490 days; myleran, 511 days; and controls, 632 days. Although all the agents were highly oncogenic, the decrease in longevity was not attributable solely to neoplasia, but was correlated with premature mortality in various diseases associated with aging. Thymic lymphomas were induced by all agents, but myeloid leukemia only by x-rays. The incidence of ovarian tumors was increased by TEM, myleran, and x-rays. The incidence of pulmonary adenomas was increased by HN2 and TEM. All agents caused premature development of lens opacities, but only myleran and x-rays induced lens changes significantly more severe than those developing spontaneously in senile controls.

Mice surviving a midlethal dose of the nitrogen mustard methyl-bis(β-chloroethyl)amine (HN2) or of triethylene melamine (TEM) show shortening of the life-span and an increase of tumor incidence, resembling in certain respects the long-term effects of midlethal x-irradiation (4). Comparable effects have been reported for large doses of 1,4-dimethanesulfonoxybutane (myleran) (1). Not all effects of radiation, however, have been reproduced in kind or degree by these chemicals under the conditions tested. It was the purpose of this experiment to study the late somatic effects of HN2, TEM, and myleran in cumulative doses approaching the maximal levels tolerated and to compare them with the effects of comparable doses of x-rays.

MATERIALS AND METHODS

Female RF mice, 10 weeks of age at the start of treatment, were subjected to four successive doses of HN2, TEM, myleran, or x-rays at 14-day intervals (Table 1). HN2 and myleran were injected into a tail vein; TEM was injected intraperitoneally. HN2 and TEM were prepared as 0.1% and 0.03–0.04% solutions, respectively, in sterile 0.9% saline. Myleran was prepared as a warm 0.5% solution in equal volumes of *N*,*N*-dimethylacetamide and water. The x-rays were administered to the whole body, 300 r per exposure, 75–80 r/min., 300 kvP, 0.44 mm Cu HVL, 3 mm Al filtration, and TSD 93.7 cm. The mice were housed in groups of ten per cage, allowed free access to Purina laboratory chow and drinking water, and observed until natural death. All animals were necropsied, and histologic examinations were carried out as necessary for diagnosis. Some of the mice in each treatment group were examined periodically with a slit lamp for lens opacities, as described elsewhere (11).

RESULTS

Survival.—A small percentage of mice given injections of HN2 and TEM died within 2–4 weeks after each dose (Chart 1). TEM, HN2, and myleran caused greater cumulative mortality (12.5%, 10%, and 3%, respectively, within 30 days after the last treatment) than was observed in the x-ray and control groups (1% in each of these groups) (Chart 1). In the HN2 group, the mortality was greatest (5%) after the first dose; whereas in the TEM group, the mortality was of approximately equal intensity following each of the four injections.

The life-span of mice surviving 30 days after the last treatment in each group was shortened in comparison with that of the controls, the x-ray group being most severely affected (Chart 2). The mean survival times (in days) were: x-ray, 355; TEM, 427; HN2, 490; myleran, 511; and control, 632. Analyzed by Seal's method (8),

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TABLE 1

LONGEVITY AND OCCURRENCE OF MAJOR NEOPLASMS IN RELATION TO TREATMENT

Agent	Dose	Original no. of mice	30-day survivors		Mean age at death[a] (days)[b]	THYMIC LYMPHOMA[a]		MYELOID LEUKEMIA[a]		OTHER LEUKEMIAS[a]		LUNG TUMORS[a]		OVARIAN TUMORS[a]	
			No.	(%)		Incidence (%)[b]	Mean age at death (days)[b]	Incidence (%)[b]	Mean age at death (days)[b]	Incidence (%)[b]	Mean age at death (days)[b]	Incidence (%)[b]	Mean age at death (days)[b]	Incidence (%)[b]	Mean age at death (days)[b]
HN2	2.4 mg/kg × 4[c]	115	104	90	490 (±15.7)	21 (±4.0)	358 (28.6)	2 (±1.3)	397 (67.5)	34 (±4.6)	554 (19.7)	68 (±4.6)	566 (13.7)	26 (±4.3)	510 (28.9)
TEM	1.5 mg/kg × 4[c]	113	99	88	427 (±15.5)	33 (±4.7)	367 (22.5)	5 (±2.2)	305 (38.4)	19 (±4.0)	508 (34.4)	53 (±5.0)	523 (17.5)	52 (±5.0)	501 (18.4)
Myleran	12.0 mg/kg × 4[c]	112	109	97	511 (±18.1)	35 (±4.6)	375 (26.4)	5 (±2.0)	362 (58.1)	31 (±4.4)	587 (23.9)	12 (±3.1)	614 (49.6)	45 (±4.8)	621 (21.8)
X-ray	300 r × 4[c]	110	109	99	355 (±9.1)	33 (±4.5)	313 (7.4)	27 (±4.2)	331 (14.6)	20 (±3.8)	414 (20.7)	13 (±3.2)	477 (28.0)	39 (±4.7)	405 (17.6)
Control	—	113	112	99	632 (±12.6)	10 (±2.8)	595 (51.9)	4 (±2.0)	560 (68.4)	37 (±4.6)	654 (17.9)	15 (±3.4)	652 (29.1)	20 (±3.8)	642 (21.6)

[a] 30-day survivors only.
[b] Standard error in parentheses.
[c] Treated at 14-day intervals.

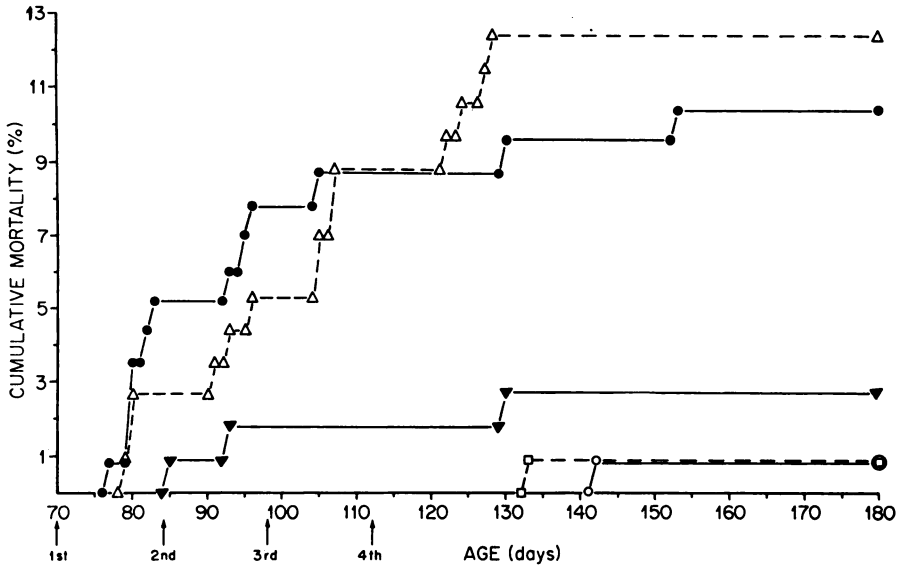

CHART 1.—Influence of four successive doses of HN2, TEM, myleran, and x-rays, respectively, on survival within 30 days after each treatment. *Arrows* indicate ages at treatment. ●HN2; △TEM; ▼myleran; □x-ray; ○control.

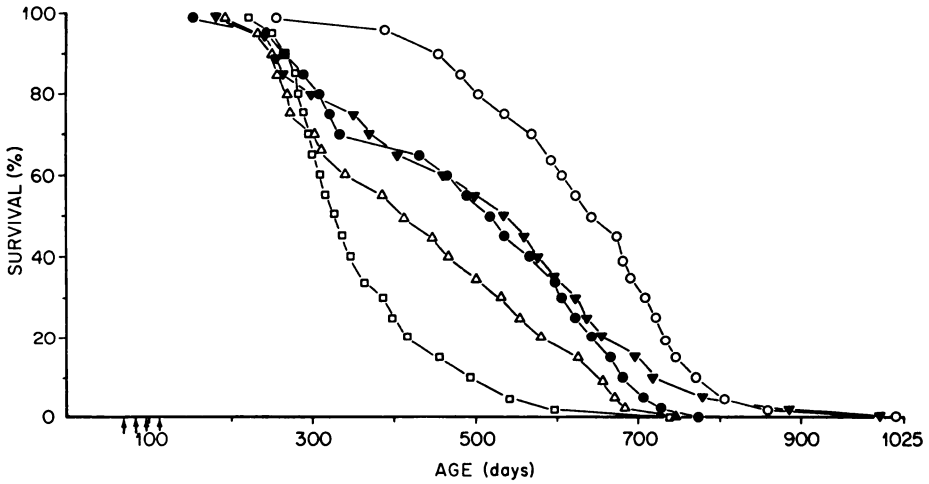

CHART 2.—Influence of HN2, TEM, myleran, and x-rays on life-span of 30-day survivors. *Arrows* indicate ages at treatment. The curves are based on 30-day survivors only. ●HN2; △TEM; ▼myleran; □x-ray; ○control.

the mortality rate curves for all treated groups shifted to the left (Chart 3). This shift was most pronounced early in life, however; the curves for all groups approximated that of the controls late in life and those for the chemically treated mice showed a bimodal character following an early peak. The observed reduction in overall survival was not attributable to neoplasia, since mice without neoplasms showed as much or greater life-shortening than others (Chart 4).

Leukemia.—Thymic lymphoma was increased in frequency by each agent tested (Chart 5). It was also one of the earliest of the major diseases encountered, and its age-distribution was drastically reduced in all treatment groups (Table 1).

Myeloid leukemia, another early disease, increased significantly above the control level only in the x-ray group (Table 1). All the treated groups, however, showed a substantial reduction in the mean age at death of mice with this disease (Table 1).

Other forms of leukemia, predominantly reticulum cell sarcomas and nonthymic lymphomas, appeared earlier in the treated groups than in the controls, although the incidence of these diseases was reduced by all treatments (Table 1). There appeared to be no consistent correlation between age-distribution and incidence, and the mean age at death of mice with these leukemias was greater in each treated group than the mean age at death from all causes (Table 1).

Other neoplasms.—Pulmonary adenomas were increased in frequency and advanced in age-distribution by HN2

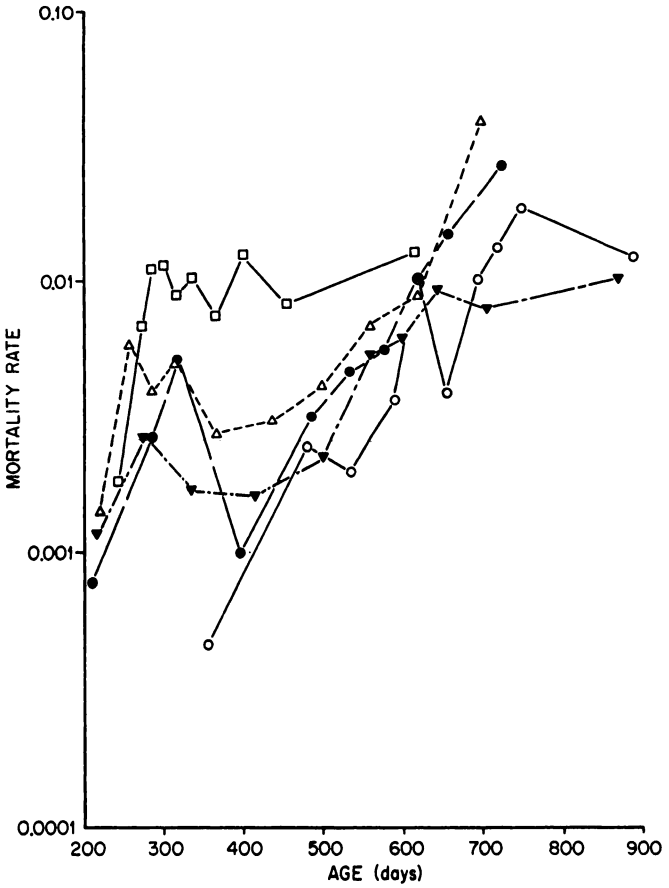

CHART 3.—Influence of HN2, TEM, myleran, and X-rays on age-specific death rate of mice dying from all causes. ● HN2; △ TEM; ▼ myleran; □ x-ray; ○ control.

and TEM (Tables 1 and 2). X-rays advanced their age-distribution even further, but failed to change their frequency. Myleran was without effect on either the age-distribution or incidence of these tumors. The neoplasms were morphologically similar in all groups and resembled those described earlier (11).

Ovarian tumors increased in frequency and appeared early in all treated groups (Table 1, Chart 6). The shift in age-distribution did not, however, parallel the extent of tumor induction—i.e., although the tumors appeared earlier in the x-ray group than in the TEM and myleran groups, they were less frequent in the former than in the latter. Tumors 4 mm in diameter or larger occurred later in life than those of smaller size and were more common in the TEM and myleran groups than in the other groups (Table 3).

Non-neoplastic lesions.—Other diseases encountered in the study are listed in Table 2. None of them appeared to increase in frequency or severity by any of the treatments, although their age-distribution was advanced.

Our data on the incidence of nephrosclerosis are based largely on gross autopsy examinations which underestimate the lesion at its early stages in mice of the RF strain. It is probable that the disease was increased in frequency and severity by the agents studied, especially in view of its marked induction by radiation in other studies (11).

Lens opacities occurred earlier in all the treated groups than in the controls and, in all but the HN2 group, progressed to a significantly greater final severity than in the controls (Chart 7). The opacities induced by the chemicals were qualitatively indistinguishable from those induced by x-rays.

DISCUSSION

Although each of the four successive doses of HN2 and TEM administered was approximately one-half of a midlethal dose (4), it caused some mortality within 2–4 weeks after injection. The acutely lethal effects of successive doses appeared, however, to be nonadditive under the conditions studied. Myleran and x-rays at the dose levels used caused no significant acute mortality.

In animals surviving 30 days after the last of the four

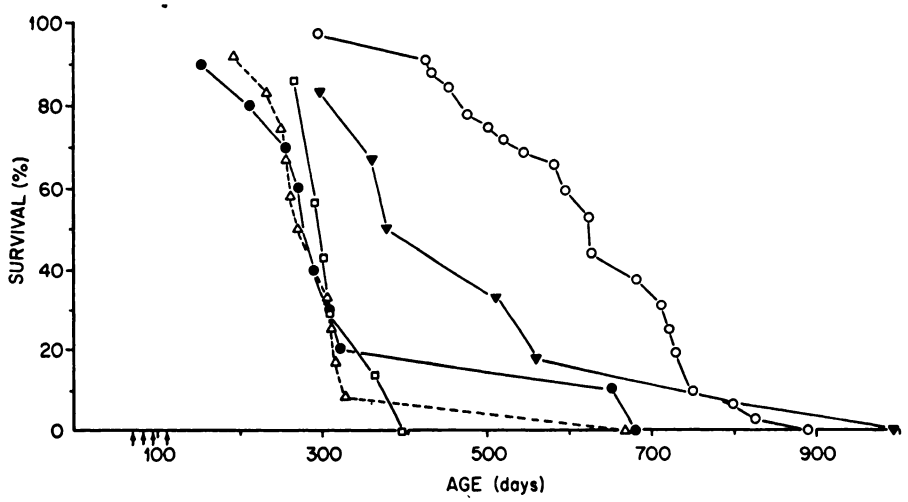

CHART 4.—Influence of HN2, TEM, myleran, and x-rays on long-term survival of mice dying without detectable neoplastic disease. *Arrows* indicate ages at treatment. ●HN2, ten mice; △TEM, twelve mice; ▼myleran, six mice; □x-ray, 7 mice; ○control, 32 mice.

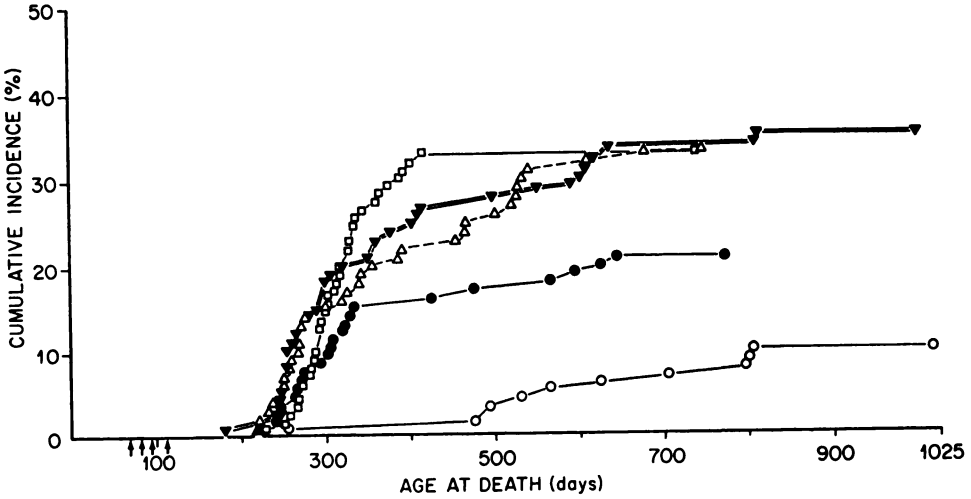

CHART 5.—Influence of HN2, TEM, myleran, and x-rays on cumulative incidence of thymic lymphoma. *Arrows* indicate ages at treatment. ●HN2; △TEM; ▼myleran; □x-ray; ○control.

treatments, the relative life-shortening effectiveness of x-rays, TEM, and HN2 was the same as that observed when the agents were administered in a single dose—i.e., x-rays most effective and HN2 least effective (Chart 8). The reduction of life-span by each agent was greater in this experiment, however, than in the earlier one, owing to the larger total doses given. The extent of life-shortening per unit dose, on the other hand, was less, in this study at least, for x-rays: x-rays, 0.23 days per rad on fractionated exposure *vs.* 0.44 days per rad on single exposure; HN2, 15 days/mg/kg on fractionated dosage *vs.* 19 days/mg/kg on single dosage; TEM, 34 days/mg/kg on fractionated dosage *vs.* 37 days/mg/kg on single dosage. From this it may be inferred that either the successive doses of x-rays failed to be as fully additive in their effects as those of the chemicals, because of partial recovery between treatments, or that the degree of life-shortening is not proportional to the x-ray dose over the dose range studied. Although the former possibility remains to be verified, the latter is strongly indicated by other experiments (A. C. Upton and J. W. Conklin, unpublished data) with γ-rays and fast neutrons.

The life-shortening by myleran confirms earlier observations by Alexander and Connell (1). The decrease in longevity by this agent, as by the others, was not attributable to induction of neoplasia or any one group of effects but appeared to be correlated with premature development of many, if not most, diseases of old age (Table 2).

There was no consistent correlation between the shift in age-distribution of a disease and effects on its incidence (Chart 9); e.g., myeloid leukemia was caused to appear as prematurely by TEM and myleran as by x-rays, but without any increase in incidence. Moreover, ovarian tumors were greatly increased in frequency by myleran without significant shift in age-distribution (Table 1, Chart 6). The relation between induction of a disease and advancement in its time of onset thus clearly requires further study, preferably with serial observations on killed animals, as opposed to those dying naturally.

Although the incidence of neoplasms varied, depending upon the agent and organ in question, all chemicals appeared "radiomimetic" in oncogenic effects on the thymus. The powerful carcinogenic action of HN2 and TEM on the lung has been reported previously (4–6, 9).

The higher, fractionated doses of agents used in this study induced lung tumors at almost the same incidence as the lower doses administered singly (4), but the mean age at death of mice with pulmonary tumors was further reduced, as was the mean age at death from all causes. Myleran resembled radiation in reducing the incidence of lung tumors and increasing the incidence of ovarian tumors. These variations in carcinogenicity among the agents employed illustrates that, although they are "radiomimetic" in the overall sense, they exhibit differences in mode of action or tissue specificity, which cannot be explained without further study.

The observed pattern of lens opacities was similar to that seen with single doses of the same agents (4). The induction of lens opacities by myleran is consistent with earlier observations (10). Although none of the chemicals used in this study was as toxic to the lens as x-rays, the cataractogenic potency of certain alkylating agents may greatly exceed that of sparsely ionizing radiations and approach that of fast neutrons for the same degree of systemic lethality (3).

Exposure to 300 r of x-rays, four times at fortnightly intervals, caused a surprisingly high incidence of myeloid leukemia, in view of the decreasing frequency of this neoplasm with increasing dose when RF mice are given more than 300–400 rad. in a single x-ray exposure (12). Unpublished observations by A. C. Upton and J. W. Conklin indicated that the same total dose (i.e., 1200 r) of x-rays received in 12 exposures at monthly intervals, is almost as highly leukemogenic as the 1200 r of x-rays administered in this experiment, suggesting that the leu-

TABLE 2

MAJOR DISEASES IN RELATION TO TREATMENT

DIAGNOSIS	HN2		TEM		MYLERAN		X-RAY		CONTROL	
	Incidence (%)	Mean age at death (days)	Incidence (%)	Mean age at death (days)	Incidence (%)	Mean age at death (days)	Incidence (%)	Mean age at death (days)	Incidence (%)	Mean age at death (days)
Adrenal										
Cortex, atrophy	—		—		1	808	—	—	—	—
Tumor	—		—		—		—	—	1	526
Colon										
Colitis	3	612	—		1	700	—		2	778
Femur										
Tumor	—		—		1	652	—		—	
Heart										
Abscess	1	320	1	656	—		1	397	1	707
Endocarditis, subacute bacterial	—		—		1	378	—		—	
Left auricle, thrombosis	1	599	3	578	5	609	2	521	4	663
Intestine										
Enterocolitis,										
Ulcerative, acute	—		—		—		—		1	677
Ulcerative, chronic	—		—		1	535	—		1	679
Volvulus	—		1	271	—		—		—	
Kidney										
Abscess	2	541	—		1	700	—		4	724
Hyperplasia	—		1	315	—		—		—	
Hypoplasia	—		—		—		1	367	—	
Nephrosclerosis	7	468	8	535	10	666	7	417	20	673
Pyelonephritis	—		—		2	486	—		2	732
Lung										
Abscess	1	320	—		—		—		—	
Pneumonia	—		1	433	1	744	—		3	561
Tumor	68	566	53	523	12	614	13	477	15	652
Liver										
Abscess	4	503	5	274	4	656	2	365	4	725
Cirrhosis	—		—		1	777	—		—	
Tumor	4	531	2	568	—		1	270	1	437
Mammary gland										
Tumor	2	626	6	595	—		1	320	1	734
Ovary										
Atrophy	—		—		1	494	—		—	
Cystic disease	1	665	1	543	—		—		—	
Tumor	26	510	52	501	45	621	39	405	20	642
Peritoneum										
Abscess	—		2	250	1	707	—		—	
Ascites	7	551	—		5	697	—		5	621
Portal vein										
Anteroposition	1	531	2	356	1	403	3	372	2	509
Spleen										
Abscess	1	320	—		1	319	—		1	748
Tumor	1	467	—		—		—		—	
Stomach										
Abscess	—		—		1	700	—		—	
Thorax										
Hemothorax	—		1	446	1	629	1	300	4	621
Hydrothorax	2	528	—		1	675	1	228	—	
Uterus										
Abscess	2	634	1	636	4	599	1	346	—	
Cystic disease	1	564	—		—		—		—	
Hypertrophy	—		—		1	511	—		1	623
Tumor	—		—		—		—		1	682

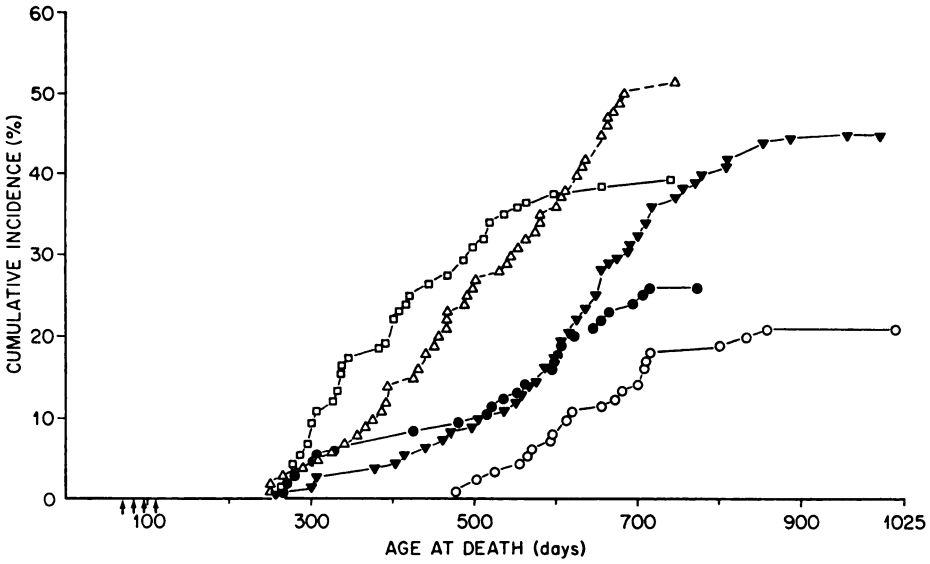

CHART 6.—Influence of HN2, TEM, myleran, and x-rays on cumulative incidence of ovarian tumors. *Arrows* indicate ages at treatment. ●HN2; △TEM; ▼myleran; □x-ray; ○control.

TABLE 3

INCIDENCE OF OVARIAN TUMORS IN RELATION TO SIZE

Agent	Ovarian tumor size: largest diameter (mm)											
	1-2			3			4 or larger			Total[a]		
	Incidence		Mean age at death (days)	Incidence		Mean age at death (days)	Incidence		Mean age at death (days)	Incidence		Mean age at death (days)
	No.	(%)		No.	(%)		No.	(%)		No.	(%)	
HN2	11	11	421	6	6	480	8	8	642	27	26	510
TEM	13	13	419	16	16	488	21	21	569	51	52	501
Myleran	13	12	550	13	12	605	21	19	668	49	45	621
X-ray	20	18	360	13	12	416	7	6	509	43	39	405
Control	9	8	623	5	4	636	8	7	659	23	20	642

[a] Combined total of all size categories, including a small number of tumors of unspecified size.

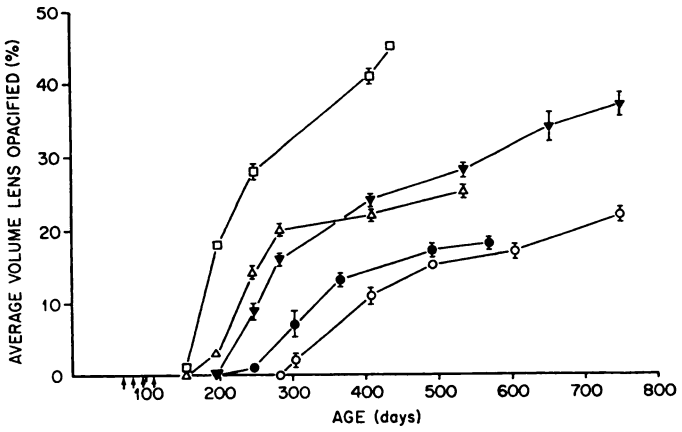

CHART 7.—Influence of HN2, TEM, myleran, and x-rays on development of lens opacities in aging mice. *Arrows* indicate ages at treatment. ● HN2; △ TEM; ▼ myleran; □ x-ray; ○ control.

kemia-inducing action of radiation may be masked on acute irradiation at high-dose levels, because of excessive cytotoxicity or other effects. In this respect, the dose-response data are similar to those observed for the induction of mutations in mouse spermatogonia (7), although this need not imply that leukemogenesis and mutagenesis share common mechanisms. In fact, studies with monofunctional, as compared with bifunctional, alkylating agents have been interpreted to argue against mutagenesis as the basis for oncogenic and other late somatic effects in mice (2).

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REFERENCES

1. ALEXANDER, P., AND CONNELL, D. I. Shortening of the Life Span of Mice by Irradiation with X-rays and Treatment with Radiomimetic Chemicals. Radiation Res., **12**: 38–48, 1960.

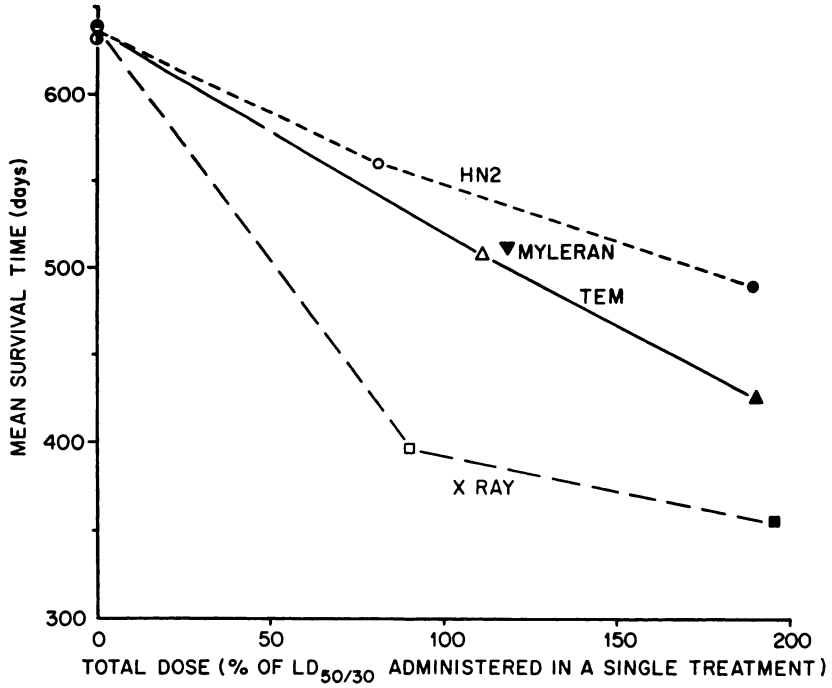

CHART 8.—Shortening of mean survival time in relation to single and fractionated doses of HN2, TEM, myleran, and x-rays. *Solid symbols* represent fractionated doses, given at 14-day intervals, *open symbols* represent single dose. ●HN2, four doses of 2.4 mg/kg each; ▲TEM, four doses of 1.5 mg/kg each; ▼myleran, four doses of 12.0 mg/kg each; ■x-ray, four doses of 300 r each; ◑control. ○HN2, single dose of 3.7–4.5 mg/kg; △TEM, single dose of 3.0–4.0 mg/kg; □x-ray, single dose of 500–600 r; ◓control.

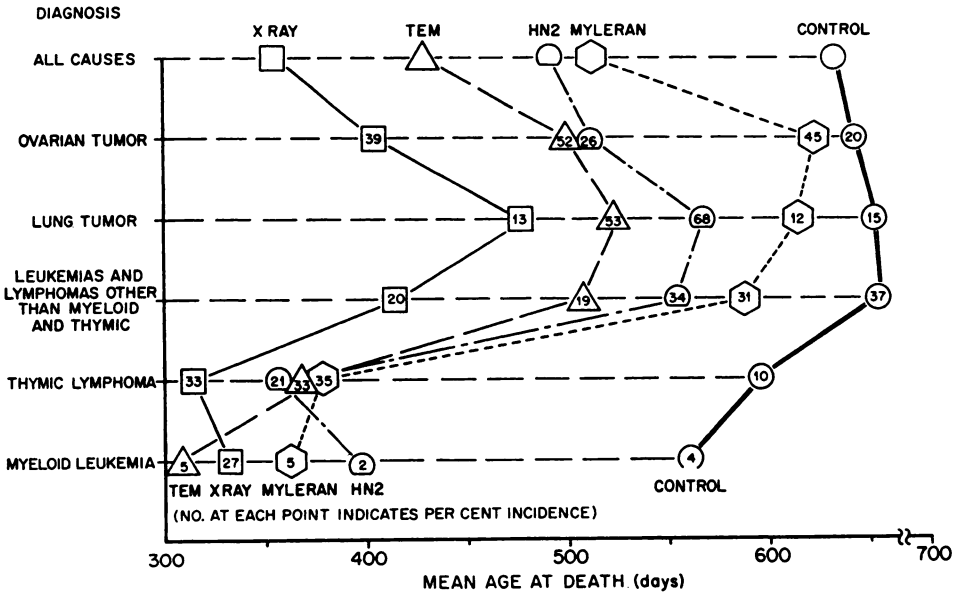

CHART 9.—Incidence of major diseases in relation to mean age at death. □x-ray; △TEM; ⌓HN2; ⬡ myleran; ○control.

2. ———. The Failure of the Potent Mutagenic Chemical, Ethyl Methane Sulphonate, to Shorten the Life-span of Mice. *In:* R. J. C. HARRIS (ed.), Cellular Basis and Aetiology of the Late Effects of Ionizing Radiation, pp. 259–66. New York: Academic Press, Inc., 1963.

3. CHRISTENBERRY, K. W.; CONKLIN, J. W.; UPTON, A. C.; AND COSGROVE, G. E. Induction of Cataracts in Mice by 4-(*p*-Dimethylaminostyryl) Quinoline. Arch. Ophthalmol., **70:** 250–52, 1963.

4. CONKLIN, J. W.; UPTON, A. C.; CHRISTENBERRY, K. W.; AND

McDonald, T. P. Comparative Late Somatic Effects of Some Radiomimetic Agents and X Rays. Radiation Res., **19**: 156–68, 1963.

5. Griffen, A. C.; Brandt, Eugenia L.; and Tatum, E. L. Induction of Tumors with Nitrogen Mustards. Cancer Res., **11**: 253, 1951.
6. Heston, W. E. Induction of Pulmonary Tumors in Strain A Mice with Methyl-bis(β-chlorethyl) Amine Hydrochloride. J. Nat. Cancer Inst., **10**: 125–30, 1949.
7. Russell, W. L. The Effect of Radiation Dose Rate and Fractionation on Mutation in Mice. *In:* F. H. Sobels (ed.), Repair from Genetic Radiation Damage and Differential Radiosensitivity in Germ Cells, pp. 205–17. Oxford: Pergamon Press, 1963.
8. Seal, H. L. The Estimation of Mortality and Other Decremental Probabilities. Skandinau. Actuarietdskr., **37**: 137–62, 1954.
9. Shimkin, M. B. Pulmonary-Tumor Induction in Mice with Chemical Agents Used in the Clinical Management of Lymphomas. Cancer, **7**: 410–13, 1954.
10. Solomon, C.; Light, A. E., and DeBeer, E. J. Cataracts Produced in Rats by 1,4-Dimethane Sulfonoxybutane (Myleran). Arch. Ophthalmol., **54**: 850–52, 1955.
11. Upton, A. C.; Kimball, A. W.; Furth, J.; Christenberry, K. W.; and Benedict, W. H. Some Delayed Effects of Atom-bomb Radiations in Mice. Cancer Res., **20** (part 2): 1–62, 1960.
12. Upton, A. C.; Wolf, F. F.; Furth, J.; and Kimball, A. W. A Comparison of the Induction of Myeloid and Lymphoid Leukemias in X-radiated RF Mice. Cancer Res., **18**: 842–48, 1958.