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LEUKEMOGENESIS BY LOW-LEVEL RADIATION

Shields Warren, M. D. and Olive Gates, M. D.

Cancer Research Institute, New England Deaconess Hospital,

Boston, Massachusetts 02215, USA

A B S T R A C T

Intermittent x-radiation administered in three R doses for ten successive weeks to RAP mice did not increase the incidence of leukemia. A total dose of 100 R (10 R weekly) significantly increased the level. A total dose of 300 R (30 R weekly) increased the incidence of leukemia only slightly but not significantly over that induced by the 100 R total dose. The two leukemogenic doses of x-radiation (100 and 300 R) yielded incidences of leukemia comparable to those following mean doses of perinatal gamma exposure at 700 and 1000 rads. However, continuous lifetime exposure of five successive generations to 30 rads was by far more effective than the higher doses of gamma or x-radiation. In these wide discrepancies between leukemogenic effects of equal low doses of gamma and x-radiation, there are two disparate factors: chronic versus intermittent exposure and the populations at risk; young adult mice versus mice exposed from conception to death.

DISCLAIMER

Evidence of a greater sensitivity of immature as compared with mature mice is not reflected in the leukemic incidence or in the projected lifespan of nonleukemic mice following 1000 rads perinatal gamma exposure.

Aside from the possible greater effectiveness of chronic exposure, the importance of indirect effects is suggested by the greatly extended lifespan of the 30 R x-irradiated mice as compared with those receiving 30 rads gamma.

The force of constitutional factors and/or an inhibitory influence of high doses is apparent in the response of rats (NEDH strain) to whole-body single doses within the range of 700 to 1100 R.

Single rats, or one of a parabiotic pair thus treated, showed only marginal differences in incidence of leukemia compared with control rats, and parabiosis alone yielded a comparable incidence of leukemia.

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LEUKEMOGENESIS BY LOW-LEVEL RADIATION*

Ghields Barron, M. D. and Olive Gates, M. D.

Cancer Research Institute, New England Deaconess Hospital,

Boston, Massachusetts 02219, USA

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The leukemogenic action of low doses of whole-body radiation in mice has been demonstrated for gamma exposures, first by Lorenz et al. [1, 2] and later by ourselves [3]. Intermittent ambient exposures continuous throughout life of LAF_1 mice at the rate of 0.11 R/8 h day to a total dose of 80 rads induced a significant increase of lymphatic leukemia and reticulum cell sarcoma [2]. In our experiments, continuous lifetime exposure to ambient gamma radiation at 0.062 rad/d to an average dose of 30 R yielded a high leukemia incidence in RAP mice but not in AJAX mice [3].

The effect of comparable low doses of x-radiation awaits further study. X-radiation-induced lymphomatosis in mice, first reported by Krebs et al. [4], was later shown to be increased by doses of 200 to 400 R by Barnes & Furth [5, 6].

We present here data on the leukemogenic effect on RAP adult mice of whole-body intermittent x-radiation in doses of 30 to 300 R to be evaluated with the response to gamma ambient radiation of 30 R to adult mice, to perinatal gamma exposures, mean doses of 800 to 1000 rads, and the response of the rat, less prone than the mouse to spontaneous leukemia, to large doses--to 1000 R of x-radiation.

METHODS

X-radiation, provided by a GE Maximar 250 kVp machine, in doses totalling 30, 100 and 300 R respectively, was given in ten weekly intervals to three series of 60-day-old RAP[1] mice of both sexes: 3 R (XR_3, 217 mice), 10 R (XR_{10}, 98 mice), 30 R (XR_{30}, 92 mice).

The gamma radiation previously reported was from ^{60}Co sources applied continuously for differing periods at different stages of development in two ways: ambient from external sources [3] and perinatal from a source in the dam [7].

In a field of ambient radiation from spaced ^{60}Co sources at a rate of 0.062 rad/d averaging a total dose per mouse of 30 rads, five generations of mice were exposed, the first from puberty to death, the four successive generations from conception to death (GA, 147 mice) [3].

A ^{60}Co source placed permanently in the thorax of a female at the time of mating provided continuous exposure to offspring at various doses and dose rates during three periods of development, intrauterine, suckling or both. Mean doses to litter ranged from 147 to 2140 rads (PS, 425 mice) [7].

As controls for these irradiated mice, we have 619 unirradiated RAP mice derived from three sources, stock, offspring of continuously-bred mice and remote inbred descendants of perinatally-irradiated pairs. Since there

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[1]The RAP mouse was penbred by Rockland Farms from strain ICR:hm (Institute for Cancer Research, Fox Chase, Pennsylvania) which had been minimally inbred with continuous selection for large size and large litter size since 1947. It is now designated Roc:(ICR).

was no significant difference in the incidence of leukemia or the mortality rates of the three groups, we have combined the groups.

The mice, about equally divided as to sex, were housed four to six to a cage and provided with Purina Laboratory Chow and water ad libitum. All surviving mice were killed at 600 days, excepting the two series exposed to 100 R (XR_{10}) and 300 R (XR_{30}) which were killed at 600 days.

Rats of the NEDH strain, which have a very low spontaneous incidence of leukemia, were studied in two differently-treated groups. The first group consisted of 227 single rats, the survivors beyond 100 days from a study of LD_{50} levels [8]. These rats had received from 700 to 1100 R of 2.0 kVp x-radiation at about 60 days of age.

The second group consisted of 2172 parabiosed pairs. Littermate rats of the same sex, about 60 days of age, were parabiosed with a common peritoneal cavity [9]. At about 110 days of age the right-hand partner received 1000 R of whole-body 250 kVp x-radiation in a single dose, a lead shield protecting the left-hand partner. Only those pairs surviving over 100 days postradiation are included in this study.

Controls for these two groups consisted of 606 stock single rats and 265 pairs of parabiosed rats. The shielded partners of the irradiated parabiont rats could not be used as controls since leukemic cells pass from one member of a pair to the other.

Male single rats were caged separately or in pairs, females in groups of four. Each parabiont pair was caged separately. Rats were provided Purina Laboratory Chow and water ad libitum. Moribund animals were killed.

All animals were autopsied. Paraffin sections of all but intracranial tissue were stained with hematoxylin and eosin, and when indicated with special stains, and examined histologically to determine the presence or absence of leukemia and the histologic type if present.

RESULTS

In the data herewith presented those relating to males and females are combined. The leukemic rates were substantially the same in each sex barring the single instance of a significantly higher rate of the females than the males following 30 R x-radiation (M_3) (C. R. 2.7)[2] which, however, did not exceed that of control females (Figs. 1 and 2).

The lowest leukemogenic dose was 30 rads ambient gamma (GA) radiation which, in marked contrast to the ineffectual 30 R x-radiation (Fig. 3), yielded an incidence of leukemia higher than that of other gamma or x-radiation doses.

One hundred roentgens x-radiation induced a leukemic incidence significantly above that of control mice and those exposed to 30 R x-radiation (C. R. 2.9) (Fig. 4). The higher incidence following 300 R was not significantly above that following a perinatal (PN) dose of 1000 rads (C. R. 0.7). Perinatal gamma radiation of 200 rads had approximately the same effectiveness as 100 rads (C. R. 0.2) and as 300 R x-radiation (C. R. 0.6).

Within the perinatal group the stage of maturity was without influence on the development of leukemia, with the exception of the higher incidence

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[2] C. R. (critical ratio) 2.6 is significant at the 0.01 level of probability.

in mice exposed to 1000 rads, both in utero and during suckling, as compared with those exposed only in utero (C. R. 2.6).

The types of leukemia varied with dose rather than with other factors of exposure, and significant sex differences did not appear. The relative incidence of lymphatic leukemia, the dominant type in all series, control and irradiated, was significantly elevated following 100 R x-radiation (Table I). However, 100 R did not raise the myelogenous leukemia incidence which was elevated following 300 R (C. R. 2.7) and by higher doses (Fig. 5). Other types of leukemia, consisting of reticulum cell sarcoma, lymphosarcoma, thymoma and unusual forms of leukemia, plasma cell, monocytic and stem cell, were more numerous and diverse following 1000 rads perinatal gamma exposure (Table I).

Inextricably related to radiation-induced leukemia is the effect of radiation on lifespan. The accompanying estimates of longevity are based on projection of the probable age at death of the last surviving mouse in each series (Table II).

Radiation shortens the lifespan of leukemic and to a lesser degree of nonleukemic mice in all series with exception of those exposed to 30 and 100 R x-radiation. The life shortening of leukemic mice is predictably related to the degree of the leukemic incidence rather than to factors of radiation and is most marked following ambient gamma exposure of 30 rads (Table II). There is little difference in lifespan of leukemic mice in the control series and in those mice receiving 300 R x-radiation or 200 rads of gamma radiation.

The projected lifespan of nonleukemic mice in the various experimental series is likewise independent of factors of radiation, viz, the very short lifespan following 200 rads gamma as against the lifespan following 1000 rads and 100 R, both approximately the same as that of control mice (Table II).

The lengthening of life of leukemic mice induced by 30 R x-radiation is correlated with the much lengthened lifespan of the nonleukemic mice of this series (Table II and Fig. 6). That following 100 R is related to a later development of leukemia (Fig. 7). The cumulative mortality of nonleukemic mice in each series is advanced over that of the controls (Fig. 8).

In all series, control as well as irradiated, most leukemic deaths occurred between 200 and 400 days of age (Figs. 7 and 9).

In view of the influence of physiological changes, including the hormonal environment, on leukemic incidence, we investigated the possibility of a correlation with ovarian tumors, the indirect product of radiation through the altered hormonal milieu subsequent to sterilization. Although both ovarian and hematopoietic tissues respond to low doses of radiation, there was no significant coexistence of ovarian tumors and leukemia. However, our data are only suggestive since the long latent period of ovarian tumorigenesis requires lifetime observation for reliable evaluation of dose response relations at low doses. Thirty rads gamma radiation and 30 R intermittent x-radiation had no effect on the ovarian tumor incidence as compared with that of control mice, although in some strains a single dose of 32 R x-radiation is tumorigenic for the ovary [10]. An increase in incidence occurred between 30 and 100 R (C. R. 9.0) and between 100 and 300 R (C. R. 2.8). Perinatal exposure was less effective as the 200 rads yielded

an ovarian tumor incidence only slightly above that of 30 rads ambient gamma exposure (C. R. 2.2), and 1000 rads was slightly but not significantly less effective than 100 R x-radiation (C. R. 2.0). The higher incidence following 100 and 300 R x-radiation is in part due to the longer period of observation.

In contrast to the leukemogenic efficacy of low doses in our series of mice is the marginal effect on rats of high single doses of whole-body x-radiation (700 to 1100 R) under two different experimental conditions. We have data on induction of leukemia in the NEDH rat by doses of x-radiation in the lethal range, as a byproduct of a long-term study of radiation carcinogenesis.

Among survivors from LD_{50} experiments following doses of 700 to 1100 R, the incidence rate of leukemia increased, but only slightly, from 0.8% in control animals to 3.% (Fig. 10).

A single dose of 1000 R whole-body x-radiation to the right-hand partner of a parabiotic pair resulted in a leukemic incidence rate of 2.5%, significantly above that of control single rats. However, parabiosis alone caused a fivefold increase in incidence of leukemia, to 4.5%. The disease also appeared about 200 days earlier than in the control single rats.

DISCUSSION

The discrepancy between the leukemogenic effects of equal low doses of gamma and x-radiation might rest on a greater efficacy of chronic continuous exposure as compared with a short period of intermittent radiation. However, variations in the populations at risk must also be weighed. The XR_3 group (30 R) consisted of young adults. The GA population (30 rads) consisted of

five successive generations. The first was exposed from puberty to death, the following generations from conception to death. The incidence of leukemia from generation to generation varied significantly only with a higher incidence in the fourth generation.

A postulated enhanced susceptibility of immature mice to radiation is inconsistent with our findings: the projected long lifespan (comparable to that of control mice) of nonleukemic mice in the PM series which received 1000 rads of perinatal gamma radiation, and the absence of significant differences in incidence following the four leukemogenic doses (barring the anomalous 30 R ambient exposure) ranging from 100 R x-radiation to adults to 1000 rads gamma perinatal exposure.

The general or indirect effects of radiation as constituting a recognized factor in leukemogenesis are difficult to assess. These, rather than the dose delivered, may have been operative for the differences in leukemogenesis between the GA and XR series reported. This is suggested by the marked differences in the projected lifespan of nonleukemic mice, greatly extended in the XR_3 series and much shortened in the GA series, each having received comparable low doses.

The low incidence rate of spontaneous leukemia in the MEDH rat was but little increased by 1000 R x-radiation in contrast to the striking increase seen in the case of our RAP mice with1100 R perinatal radiation, likewise having a low spontaneous incidence rate. Binhammer et al. [11] did not find leukemia in their parabiotic Holtzman rats following a dose of 700 R. It is to be noted that the leukemogenic response to like doses of radiation of the

single and the parabiosed rats were much the same despite the support from the unirradiated partner.

We have the surprising finding that radiation with a single dose of 1000 R does not increase the incidence of leukemia in parabiotic rats above that found following parabiosis alone and may even decrease it. This suggests either a constitutional factor or an inhibiting mechanism resulting from excessive radiation exposure.

Our findings are congruent with the suggestions made by Mole [12] and numerous authors summarized by Mayncord [13] that with doses above the peak response the leukemogenic effect of radiation may be diminished through the killing of cells made leukemic or susceptible of becoming leukemic.

ACKNOWLEDGMENT

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REFERENCES

[1] LORENZ, E., et al., In "Biological effects of external X and gamma radiation" (ZIRKLE, R.E., Ed), First Ed, Chapters 1-3, McGraw-Hill, New York (1954).

[2] LORENZ, E., et al., J. Nat. Cancer Inst. 15 (1955) 1049.

[3] WARREN, S., GATES, O., Radiat. Res. 47 (1971) 480.

[4] KREBS, C., RASK-NIELSEN, H.C., WAGNER, A., Acta Radiol. Suppl. 10 (1930) 1.

[5] BARNES, W.A., FURTH, J., Proc. Soc. Exptl. Biol. Med. 33 (1935) 386.

[6] FURTH, J., FURTH, O.B., Amer. J. Cancer 28 (1936) 54.

[7] WARREN, S., GATES, O., In "Radiation biology of the fetal and juvenile mammal" (SIKOV, M.R., MAHLUM, D.D., Eds), U. S. Atomic Energy Commission, Division of Technical Information, Oak Ridge, Tennessee (1969) pp. 419-437.

[8] UDUPA, K.B., WARREN, S., CHUTE, R.N., Health Physics 26 (1974) 319.

[9] WARREN, S., HURST, E.E., JR., BROWN, C.E., CHUTE, R.N., Cancer Res. 32 (1972) 983.

[10] UPTON, A.C., et al., Cancer Res. 20, Part 2 (1960) 1.

[11] BINHAMMER, R.T., et al., Radiat. Res. 6 (1957) 339.

[12] MOLE, R.H., In "Proceedings of the Second United Nations International Conference on the Peaceful Uses of Atomic Energy," Vol. 22, "Biological effects of radiation," United Nations, New York (1958) pp. 145-148.

[13] MAYNEORD, W.V., CLARKE, R.H., Brit. J. Radiol. Suppl. 12 (1975) 1.

TABLE I. PERCENT DISTRIBUTION BY TYPE OF LEUKEMIA

	Mean dose	Lymphatic	Myelogenous	Other
Control	0	55	27	18
XR_3	30 R	50	23	27
XR_{10}	100 R	81	9	9
XR_{30}	300 R	45	35	19
PM	200 rads	47	24	29
PM	1000 rads	41	21	38
OA	30 rads	58	24	19

TABLE II. PROBABLE AGE AT DEATH OF LAST SURVIVING MOUSE

	Mean dose	With leukemia	Without leukemia
		days	days
Control	0	890	1250
XR_3	30 R	1040	1640
XR_{10}	100 R	1050	1200
XR_{30}	300 R	880	1070
PN	200 rads	840	910
PN	1000 rads	780	1220
CA	30 rads	680	970

TABLE III. INCIDENCE OF OVARIAN TUMORS

	Mean dose	% ovarian tumors
Control	0	3
XR_3	30 R	3
XR_{10}	100 R	72
XR_{30}	300 R	93
PN	200 rads	22
PN	1000 rads	56
OA	30 rads	7

CAPTIONS

Fig. 1. Incidence rate percent of leukemia following various doses of fractionated x-radiation. Only in the XR_3 series is there significant difference between the sexes.

Fig.2. Incidence rate percent of leukemia following gamma radiation. Note absence of sex differences and the high rate of incidence in the CA series.

Fig.3. Percentage incidence of leukemia. Note the high incidence of leukemia following 30 rads (CA series) and the failure of 30 R (XR_3 series) to show increased incidence above controls.

Fig.4. Comparison of incidence rate percent of leukemia following radiation = x-ray and gamma. Note that 100 R was the lowest leukemogenic dose of x-radiation. Higher doses of x-radiation or of perinatal gamma radiation did not cause significant increase in incidence above that of 100 R. The CA series showed an anomalous increase.

Fig.5. Incidence of types of leukemia at different levels of radiation. Doses above 300 R caused increase in incidence of myelogenous leukemia.

Fig.6. Cumulative percent mortality of nonleukemic mice after various doses of intermittent x-radiation. From this and Figs. 7, 8 and 9, the projected lifespans of the animals given in Table II were calculated using probit-scale graphs. (Most surviving animals had been killed at 500 days.) The projected lifespan of nonleukemic mice was lengthened following 30 R x-radiation.

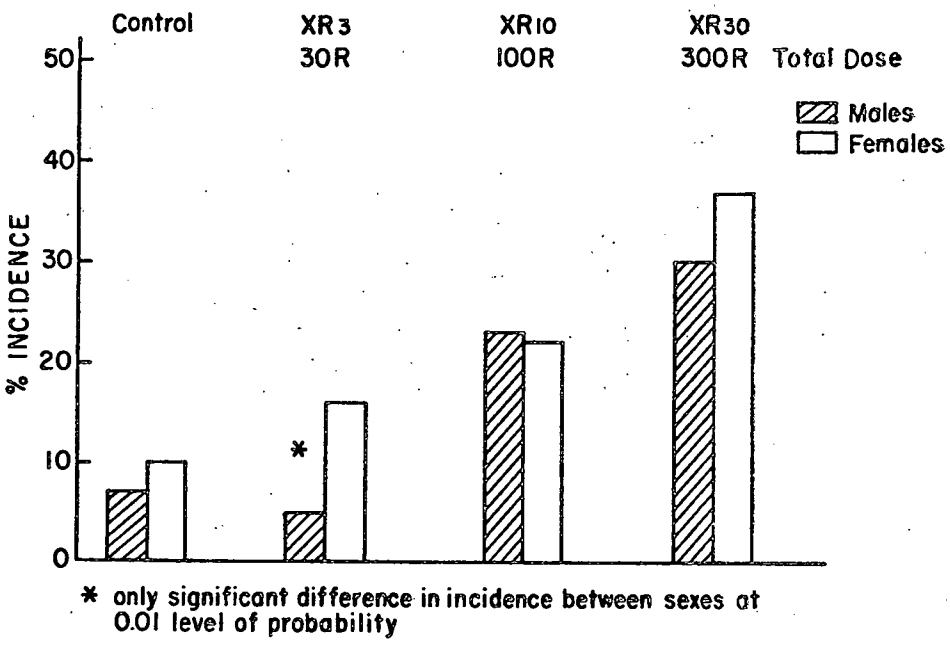
Fig.7. Cumulative percent mortality of mice with leukemia after various doses of x-radiation. Note the generally later occurrence of leukemia in XR_{10} mice and the slow rate of increase in incidence of leukemia.

Fig.8. Cumulative percent mortality of nonleukemic mice after various doses of gamma and x-radiation. Note that all nonleukemic irradiated mice showed greater early mortality than the controls, but that projections of XR_3 and XR_{10} mortality graphs indicate longer survival than the controls.

Fig.9. Cumulative percent mortality of mice with leukemia after various doses of gamma radiation.

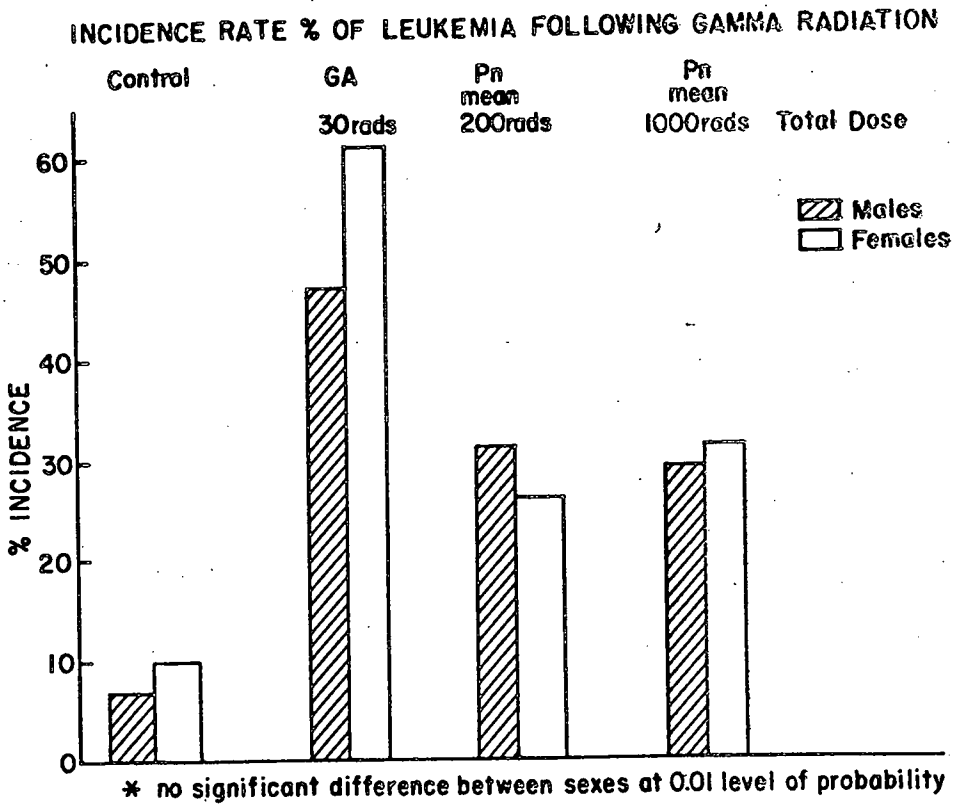
Fig.10. Cumulative percent incidence of leukemia in NEDH rats. Note the difference in incidence of leukemia between control single and control parabiont rats. Radiation did not induce higher levels of leukemia than did parabiosis alone.

Fig. 1



* only significant difference in incidence between sexes at 0.01 level of probability

Fig. 2



* no significant difference between sexes at 0.01 level of probability

Fig. 3

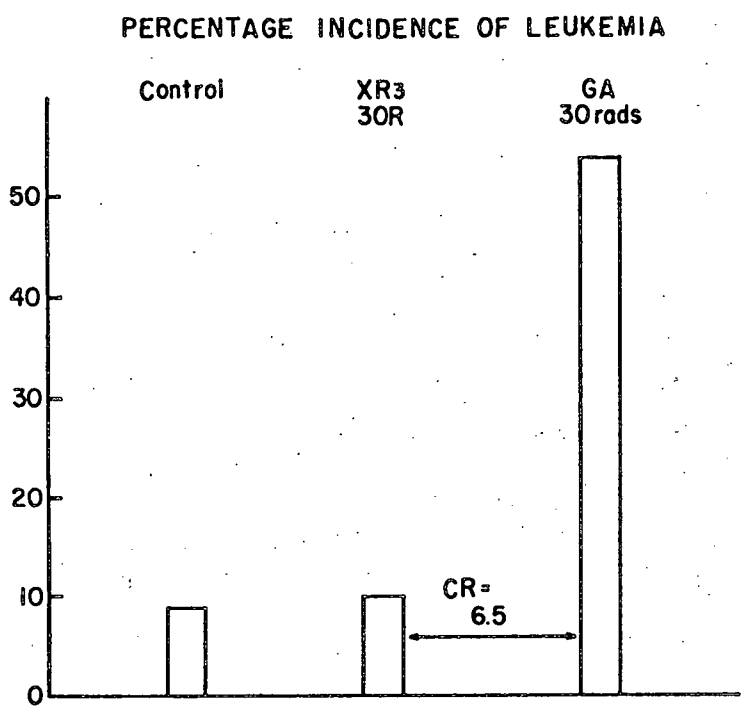
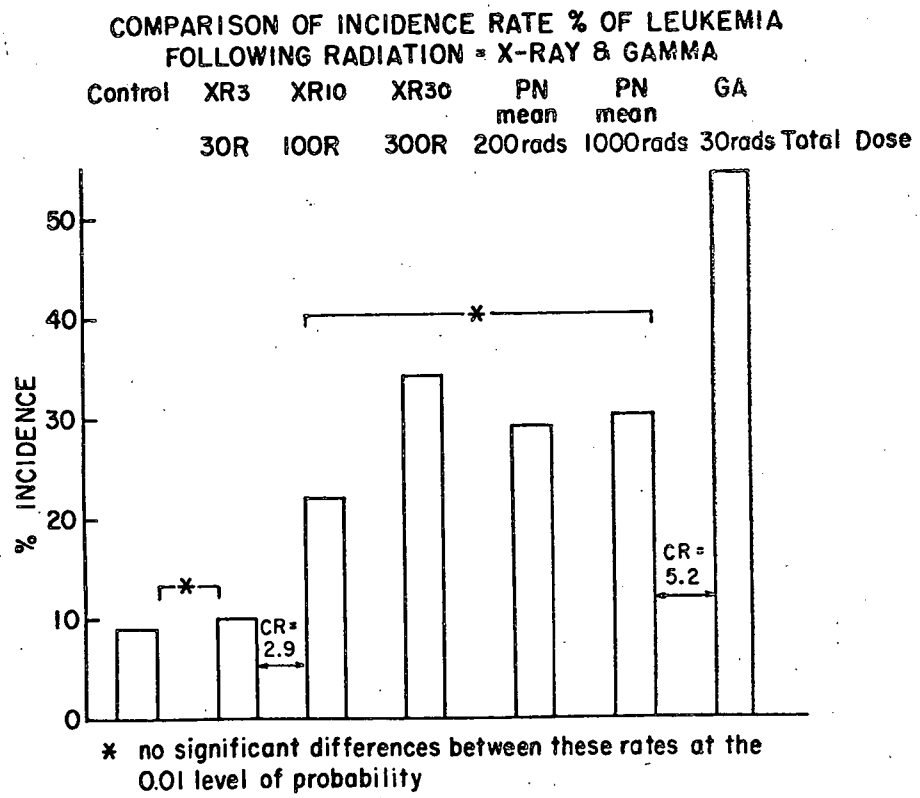


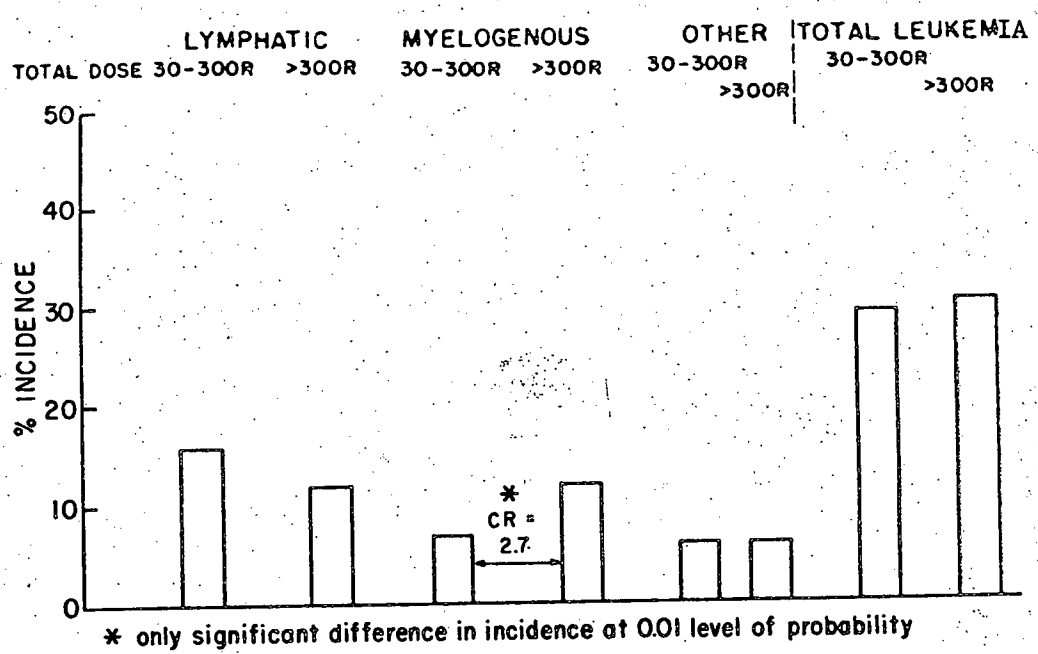
Fig. 4



* no significant differences between these rates at the 0.01 level of probability

Fig. 5

INCIDENCE OF TYPES OF LEUKEMIA
AT DIFFERENT LEVELS OF RADIATION



* only significant difference in incidence at 0.01 level of probability

Fig. 6

CUMULATIVE % MORTALITY OF NON-LEUKEMIC MICE
AFTER VARIOUS DOSES OF INTERMITTENT X-RADIATION

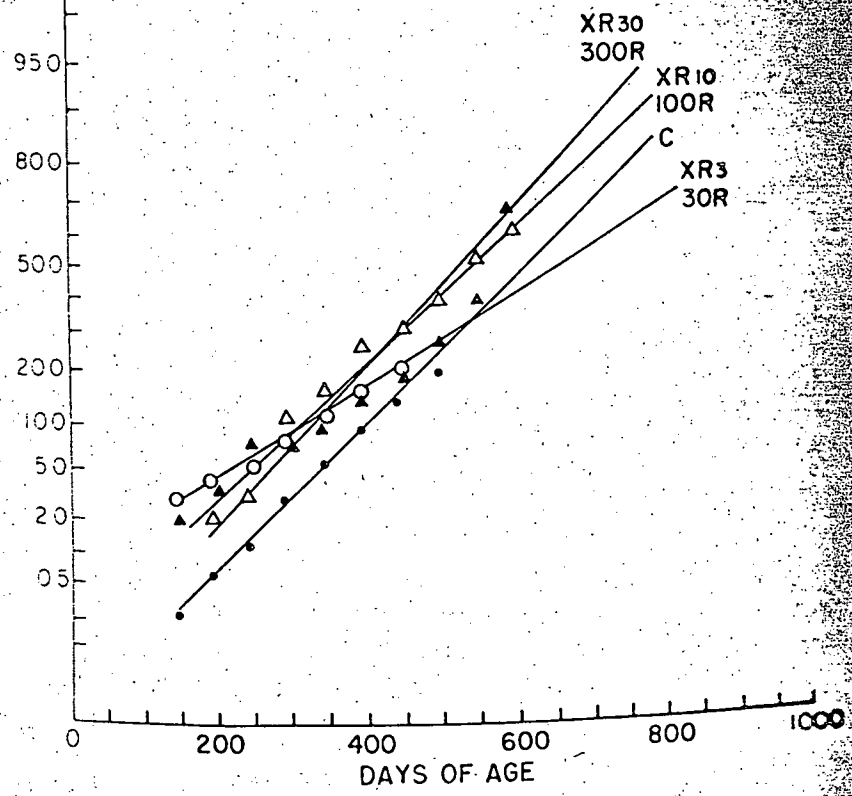
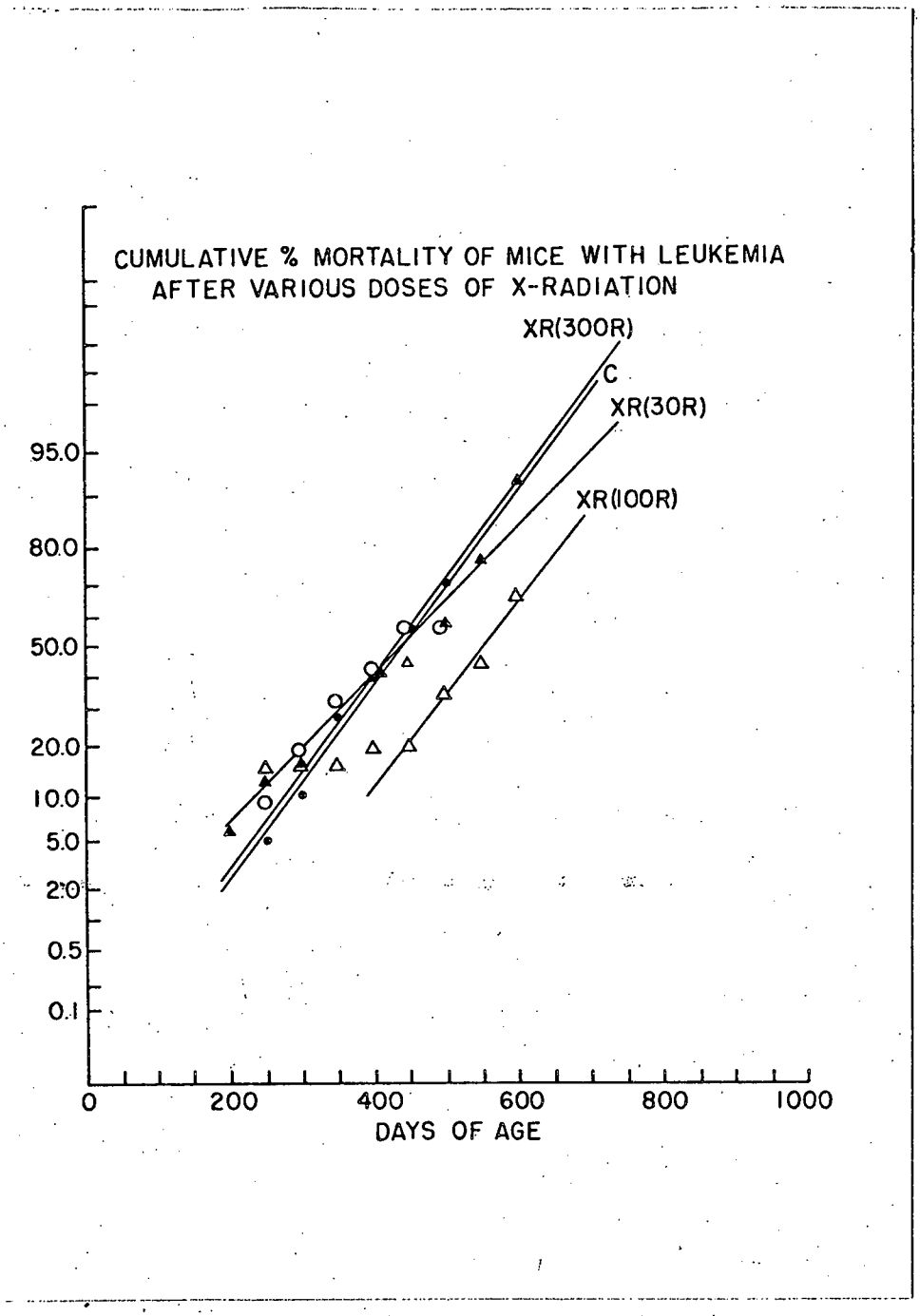


Fig. 7


CUMULATIVE % MORTALITY OF MICE WITH LEUKEMIA
AFTER VARIOUS DOSES OF X-RADIATION
XR(300R)
C
XR(30R)
XR(100R)
95.0
80.0
50.0
20.0
10.0
5.0
2.0
0.5
0.1
0
200
400
600
800
1000
DAYS OF AGE

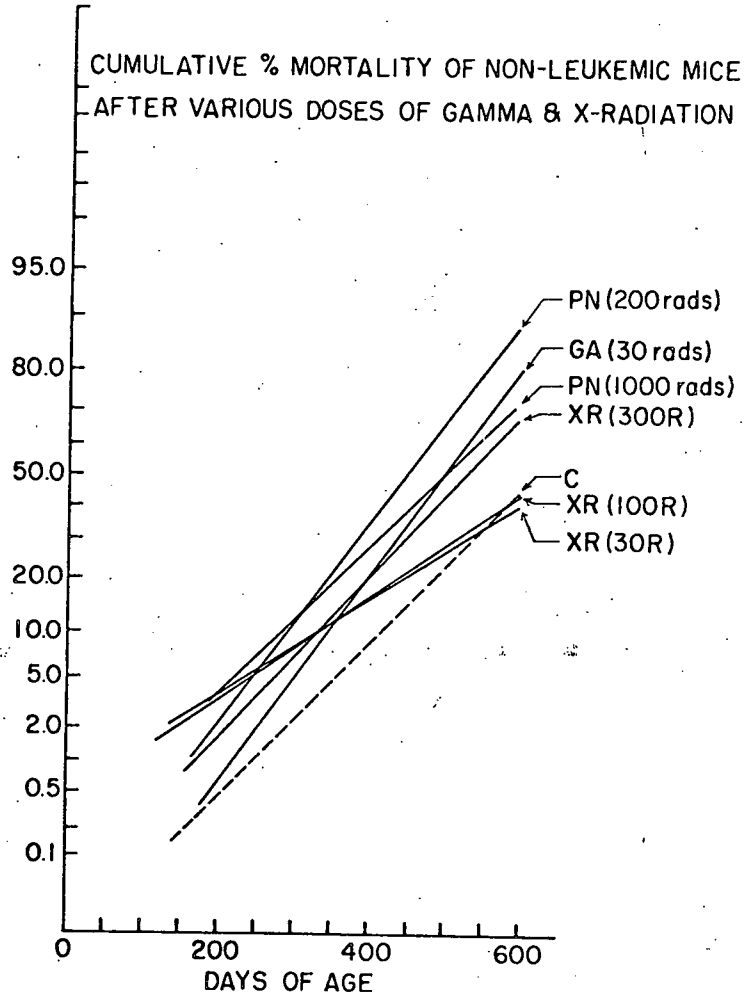
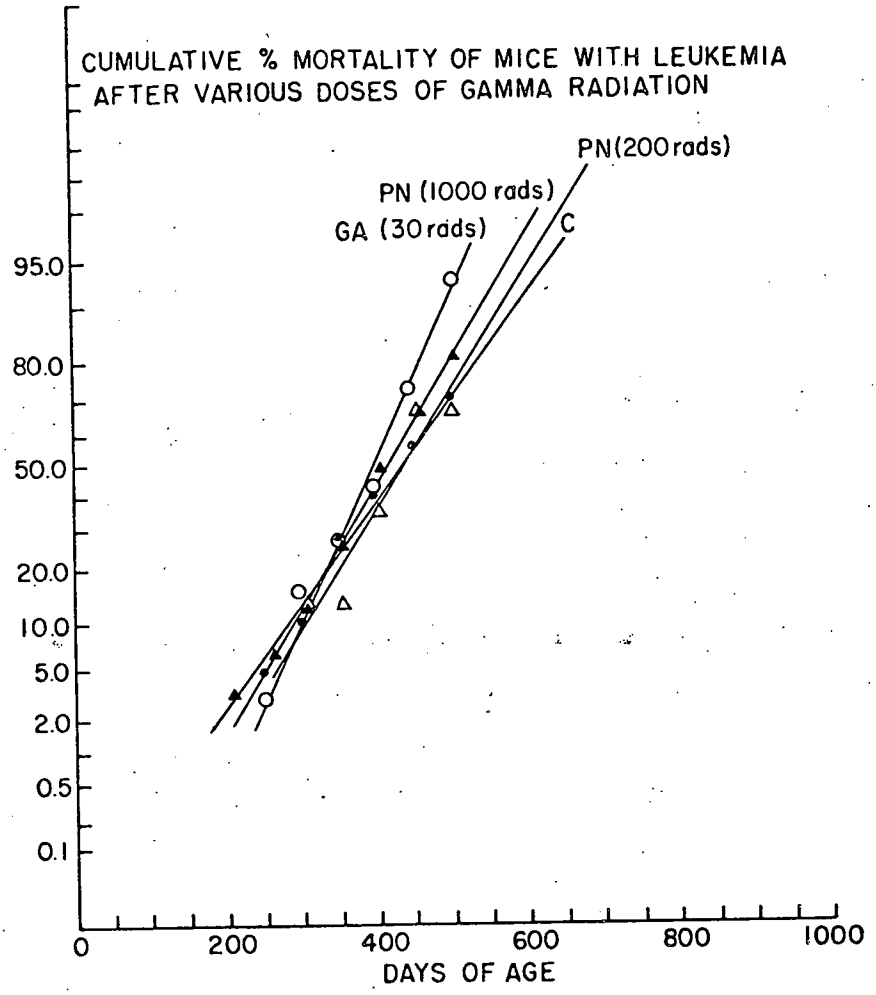
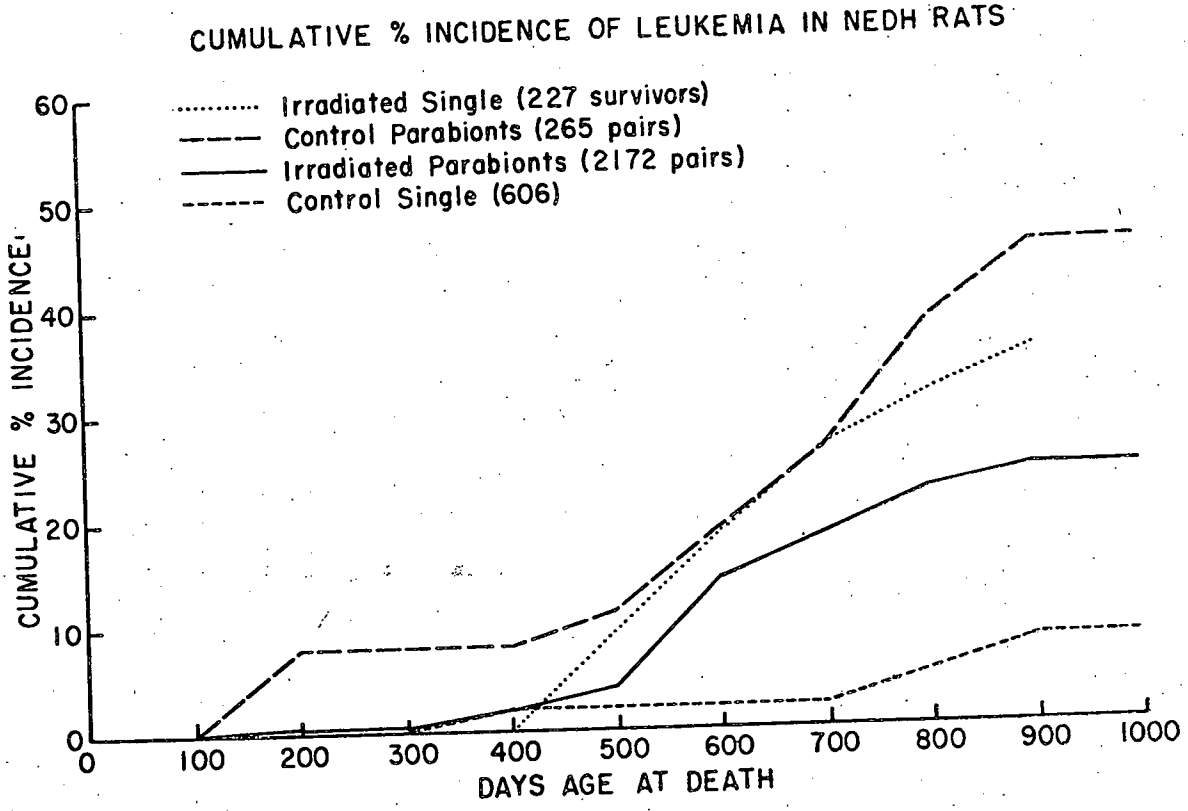

CUMULATIVE % MORTALITY OF NON-LEUKEMIC MICE
AFTER VARIOUS DOSES OF GAMMA & X-RADIATION
95.0
80.0
50.0
20.0
10.0
5.0
2.0
0.5
0.1
0
200
400
600
DAYS OF AGE
PN (200 rads)
GA (30 rads)
PN (1000 rads)
XR (300R)
C
XR (100R)
XR (30R)

Fig. 9


CUMULATIVE % MORTALITY OF MICE WITH LEUKEMIA
AFTER VARIOUS DOSES OF GAMMA RADIATION
PN(200 rads)
PN (1000 rads)
GA (30 rads)
C
95.0
80.0
50.0
20.0
10.0
5.0
2.0
0.5
0.1
0
200
400
600
800
1000
DAYS OF AGE


CUMULATIVE % INCIDENCE OF LEUKEMIA IN NEDH RATS
Irradiated Single (227 survivors)
Control Parabionts (265 pairs)
Irradiated Parabionts (2172 pairs)
Control Single (606)
CUMULATIVE % INCIDENCE
60
50
40
30
20
10
0
0
100
200
300
400
500
600
700
800
900
1000
DAYS AGE AT DEATH