THE UNIVERSITY OF MANITOBA

Dose Rate Effects of X-Ray-Induced Sex-Linked Lethal Mutations in the Nematode *Panagrellus redivivus*

by

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ABSTRACT

The effect of exposure rate on the induction of sex-linked lethal mutations in the nematode Panagrellus redivivus was investigated for X-ray exposures of approximately 5,700 R. Both male and female mutation rates follow a similar response to dose protraction, decreasing from 28.8×10^{-9} mut/locus/R at an exposure rate of 1158 R/m, to 8.4×10^{-9} mut/locus/R at 57 R/m. In the range of exposure rates studied, the mutation rate increases linearly as a function of the logarithm of the exposure rate.

For prolonged irradiations, the mutation rate decreased in basic agreement with the theory developed by Kellerer and Rossi (Ke72). High exposure rate data, however, failed to conform to the proposed theory. Possible explanations for this failure include a two component system of radiation damage, or an exposure rate dependent repair time.

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List of Pertinent Definitions

Kerma

An acronym for Kinetic Energy Released per unit MAss.

Linear Energy Transfer (LET)

That quantity of energy transferred per unit length of track (keV/micron)

Rad

The unit of absorbed dose of radiation equal to 0.01 joule per kilogram of material (1 gray (Gy) = 100 rads)

Rad Equivalent Therapy (ret)

That quantity defined by units of rads per unit time

Roentgen (R)

The unit of exposure of X- or gamma-rays such that one roentgen produces a charge of $.33 \times 10^{-9}$ coulombs on all the ions of one sign per cubic centimeter of dry air at standard temperature and pressure. It is used only for X- or gamma-rays below 4 MeV. (for X-rays an air exposure of 1 R corresponds to an air kerma of 8.7 mGy)

INTRODUCTION

Ionizing radiation induces biological damage by two separate processes. The "direct action" of radiation results in the ionization and electronic excitation of "target" atoms, ultimately leading to a biological change. Direct action is the primary mechanism for biological damage by high LET (linear energy transfer) radiations. Alternatively, the radiation may interact with "non-target" atoms to produce free radicals (atoms possessing an unpaired electron in the outer shell). These free radicals may in turn react with the critical targets to produce damage. This "indirect action" of radiation is the predominant mode for the induction of biological damage by low LET radiations.

The radiation sensitive targets in the cell are not well defined at the present time, but the mutagenic effect of ionizing radiation, first demonstrated by Muller in 1927 (Mu27), provides conclusive evidence that DNA is at least one of the radiation sensitive targets. The damaging effects of ionizing radiation on DNA can be manifested by base changes, base loss, single or double strand breaks, DNA-DNA crosslinks (intra- or interstrand) as well as DNA-protein crosslinks (Au76).

A wide range of parameters interact to produce variable degrees of radiation-induced damage. The oxygen enhancement

ratio (OER), quantifies the effect of irradiation under oxygenated conditions. Defined as the ratio of hypoxic to aerated doses required to achieve a given biological end point, OER consistently has a value between 2.5 and 3 for sparsely ionizing radiation (Ha78). Oxygen acts at the level of the free radicals, and as expected, the oxygen effect decreases as LET increases. No enhancement is observed for densely ionizing radiations such as alpha-particles.

A vast array of chemicals act as dose modifying agents. Sulfhydryl compounds, such as cysteine, act as radioprotectors. The mechanism by which these compounds act as protectors is not clear, however, the "radical scavenger hypothesis" (Ha78) is favored.

Halogenated pyrimidines, once incorporated into DNA, act as X-ray sensitizers by weakening the DNA chain. Metronidazole and misonidazole are useful hypoxic cell sensitizers. A final class of chemicals add to the effect of radiation, but are not true sensitizers. Actinomycin D, puromycin, methotrexate and 5-fluorouracil act as apparent sensitizers.

The manner in which a given dose of ionizing radiation is administered can also have a drastic effect upon the magnitude of biological damage induced. In general, if a given dose is delivered over an extended period of time,

whether in a number of fractions or a continuous exposure, the biological effect is reduced. The natural explanation for this is the repair of sublethal damage between fractions or during a continuous exposure. (By definition, sublethal damage occurs when only a fraction of the total number of critical targets has been hit).

Fractionation of doses has been fairly well characterized by the Ellis NSD equation (El69):

$$D = (NSD)\ T^{0.11}\ N^{0.24}$$

where:

D = total dose

T = overall treatment time

N = number of fractions
(should be greater than 4)

NSD = nominal standard dose
(biologically effective dose)

The units of NSD are the ret (rad equivalent therapy) including dimensions of dose and time. The effects of a prolonged continuous exposure, however, are less well defined.

Radiotherapeutical or radiobiological studies have employed dose-rates ranging from a few rads per day up to thousands of rads in a fraction of a second. This range may be subdivided, somewhat arbitrarily, into four major regions. An ultra-high dose-rate can be achieved using discharge machines or accelerators to deliver a pulsed

exposure of a fraction of a second. Clinical radiotherapy routinely uses high dose rates (acute exposures of a few minutes) and low dose rates (protracted exposures of many hours or days). The fourth range is an ultra-low dose rate used for continuous exposures lasting weeks, months or even years.

A vast array of biological endpoints have been used to determine the dependency of dose rate upon radiation-induced damage. At the ultra-high dose rate range conflicting results arise. No dose rate effect was observed between 100 and 6,000 rad/min for skin damage in mice (De66), between 80 and 160,000 rad/min for the $LD_{50/30}$ in mice (Li63) or for the life shortening effect in mice (Ha78). More recently, however, Tetsuo (Te80) reports that skin damage in mice increases for an instantaneous dose rate between 10^6 to approximately 10^8 rad/min where a maximum skin reaction was observed. Higher dose rates yet showed a reduction in biological effect. The decrease of biological effectiveness may be due to oxygen depletion or recombination of ionizations and/or radicals (Ha72).

Unfortunately the data concerning cell culture experiments at ultra-high dose rates is also contradictory. Hall (Ha78) reports that no dose rate effect is observed, or even expected, above 100 rad/min since the half-time for

repair of sublethal damage is approximately one hour. Gerwick et al (Ge79), however, found that oxygenated CHO (chinese hamster ovary) cells, are capable of repairing sublethal damage when irradiated at dose rates of the order of 10^{11} rad/sec.

The high and low dose rate regions have best been characterized by using genetic endpoints. Van Buul and Roos (Va77) report that translocation-induction of mouse spermatogonia increases linearly as a function of the logarithm of exposure rate. Lyon et al (Ly72) indicate that specific locus mutations in mouse spermatogonia follow the same general trend if one considers only the data above 0.06 rad/min, but suggest that at lower dose rates there may be an increase in observed mutation frequency. Recent experiments undertaken by Russell and Kelly (Ru82) indicate that the mutation frequency of mouse spermatogonia is independent of dose rates from 0.0007 to 0.8 rad/min.

Evidence for an "inverse" dose rate effect has come from cell culture experiments. Mitchell et al (Mi79) observed more cell killing (S3 HeLa and V79 cells) per unit dose at 37 rather than 74 rad/hr. Yau et al (Ya79) similarly report an inverse split dose effect in a murine lymphoma cell line. Analysis of these results, however, is complicated by the involvement of three factors which influence

radiation dose rate effects. Cell cycle perturbations, repopulation as a result of cell division during protracted exposures, as well as the repair of sublethal damage during irradiation combine to complicate the analysis.

The most clear cut evidence for an inverse dose rate effect comes from the study of membrane damage. Konings et al (Ko79a) found that X-irradiation of model lipid membranes demonstrated an inversely dose rate dependent lipid peroxidation. Biomembranes isolated from liver cells of irradiated mice also showed the inverse dose rate effect (Ko79b). Interphase death of non dividing cell populations could arise from the membrane damage caused by peroxidation of unsaturated lipids (Mi80).

Mathematical attempts to explain the induction of damage by ionizing radiation date back to 1938 (Le38). More recently Kellerer and Rossi have presented the theory of "dual radiation action" (Ke72) based on the arguement proposed by Lea. They derive the general relation:

$$\varepsilon = k\ (\lambda D + D^2) \qquad (1)$$

where:

ε = the yield of elementary lesions, ie, the yield of "impairments taking place at the subnuclear level in the cell"

k = constant

λ = radiation quality constant

D = dose

The linear term of the above equation represents the effect produced by one particle track (intratrack interactions), while the quadratic component reflects interaction of different charged particles (intertrack interactions). Therefore, the linear term is independent of dose rate. The quadratic term, however, is highly dose rate dependent, and in fact may become negligible under chronic exposure conditions.

Given a constant dose rate, it can be shown that (Ke72):

$$q(T) = \frac{2}{T^2} \int_0^T \tau(t)\,(T-t)\,dt \qquad (2)$$

where:

$q(T)$ = ratio of the quadratic effect in the presence of recovery to that in the absence of recovery, ie, to an instantaneous dose

T = time interval of irradiation

$\tau(t)$ = recovery function for sublesions ($\tau(0) = 1$)

If one assumes an exponential recovery function:

$$\tau(t) = e^{-t/t_o} \qquad (3)$$

where:

t = time separation for split dose application

t_o = recovery time

the reduction factor q(t) becomes:

$$q(T) = \frac{2\,t_o}{T} - \frac{2\,t_o^2}{T^2}\left(1-e^{-T/t_o}\right) \qquad (4)$$

from this, one obtains

$$\varepsilon(D) = k\left\{\lambda D + \left[\frac{2\,t_o}{T} - \frac{2\,t_o^2\,(1-e^{-T/t_o})}{T^2}\right]D^2\right\} \qquad (5)$$

If $t_o >> T$, equation (5) reduces to:

$$\varepsilon(D) = k\left[\lambda D + \frac{(1-T)}{3t_o}\,D^2\right] \qquad (6)$$

If, however, $T>>t_o$:

$$\varepsilon(D) = k\left[\lambda D + \frac{2\,t_o}{T}\,D^2\right] \qquad (7)$$

There is substantial experimental support for the theory proposed by Kellerer and Rossi. The basic assumptions have been shown to be valid by Nakamura and Okada (Na81) as their results suggest that there may be two components of gamma-ray-induced mutations in cultured mammalian cells.

According to Nakamura and Okada (Na81);

> One [component] results primarily from repairable damage induced by the indirect action of radiation and shows a clear dose-rate dependency. The other is mainly from non-repairable damage by the direct action of radiation and is only slightly dose-rate dependent.

Henkelman et al (He80) used this theory successfully to explain data for both dose-rate and split dose effects on mouse foot reactions.

The only organism for which extensive dose-rate data has been compiled is the mouse. The accumulation of the mouse data by Lyon et al (Ly72) spanned from 1956 to 1972 and utilized over one million relatively expensive laboratory mice. To reduce both the time and cost of further dose rate studies, an alternative organism, the nematode was chosen.

Nematodes are highly resistant to ionizing radiation. The free living nematode Panagrellus redivivus has an LD_{50} of 750,000 rads gamma-radiation (My60). This resistance is indicative of an extremely efficient repair system. Since a dose-rate effect is dependent upon repair of sublethal damage, Panagrellus redivivus is ideally suited to study the influence of dose rate upon genetic damage.

Three hundred and sixty essential loci are located on the X-chromosome of Panagrellus redivivus. If one examines the frequency of radiation induced X-linked recessive lethal mutations, it is possible to screen 360 loci per trial. This offers a tremendous advantage over the 7 specific-locus mutation test used for mice. Other advantages of the nematode system are:

1) a short life cycle of 4 days

2) a limited developmental repertoire

3) an inexpensive organism

4) easy to manipulate and maintain

This study makes use of the many advantages offered by the nematode, to examine the effect of dose rate on X-ray induced sex-linked lethal recessive mutations.

MATERIALS AND METHODS

The Culture Media

Stocks are maintained on non-nutritive water-agar plates consisting of 15g agar, 1 ml cholesterol solution (5 mg cholesterol/ml ethanol) and enough distilled water to bring the total volume to 1 litre. Limited nutrition was provided by M9-Y (Sa80). This liquid food source was prepared by dissolving 50 mg dried baker's yeast and 1 ml cholesterol solution (5 mg cholesterol/ml ethanol) in one litre of M9 buffer (Br74). M9 buffer is also used for washing animals and consists of 6g Na_2HPO_4, 3g KH_2PO_4, 5g NaCl, 0.25 g $MgSO_4$ and distilled water to make one litre. All of the above solutions were autoclaved at 18 psi for 20 minutes. Agar plates, M9-Y and M9 buffer were stored in the refrigerator (5-10^{o}C) until used.

The strains of Panagrellus redivivus utilized in these experiments were the wild type C15, and two mutant strains b7 and S1. The "wild type" nematode is the standard one typically found in nature. The b7 nematodes have limited movement on agar, and will normally assume a coiled posture in liquid. The b7 mutation at the unc-1 locus is sex-linked recessive and was induced by EMS (ethyl methane sulphonate) (Bu80). The S1 stock, derived from C15, has a wild type phenotype, but contains a putative inversion acting to suppress crossing over the b7-locus.

Sex-Linked Lethal Assay

Virgin b7 females were collected by isolating the individual nematodes before sexual activity begins. Five groups of 30 virgin b7 females were washed and then transferred to water-agar plates in the single droplet of M9 buffer solution. One group served as a control while the other four groups were given a total dose of approximately 5700 R at various exposure rates. A Siemens X-ray machine (250 kV, with a 1 mm Al filter) was used at various source to target distances to achieve the desired dose rates. The dose rate was determined with the Victoreen dosimeter and was monitored throughout the exposure to ensure a constant dose rate. A uniform exposure is ensured by the small target area.

Immediately after irradiation, the treated females were washed and placed on agar plates previously seeded with non-irradiated C15 wild type male in M9-Y liquid growth medium. Eggs are fertilized sequentially and mature independently. The first free-swimming offspring appear at the second juvenile (L2) stage approximately two days after copulation. Male progeny from this cross will carry the b7 allele on the X-chromosome and will thus assume a coiled posture. Heterozygous female offspring will swim normally,

but may possibly carry an X-ray-induced mutation at one of the 360 essential loci located on one of it's two X chromosomes. To identify the females carrying such a mutation, a second cross was required.

The b7 males were removed from the plate and replaced by C15 males. After copulation, gravid F1 females were isolated in depression plates and allowed to drop their offspring. If the F1 female did not carry a mutation, 1/4 of her offspring would express the b7 trait, but if the F1 female carries a mutation, all of her offspring will phenotypically be wild type. The screening method is shown in Figure 1.

The reciprocal experiment was also performed by irradiating S1 males and mating these males to unirradiated b7 virgin females. The F1 were allowed to mate, and again gravid F1 females were isolated and screened for mutations.

Figure 1. Cross of b7 Females by C15 Males.

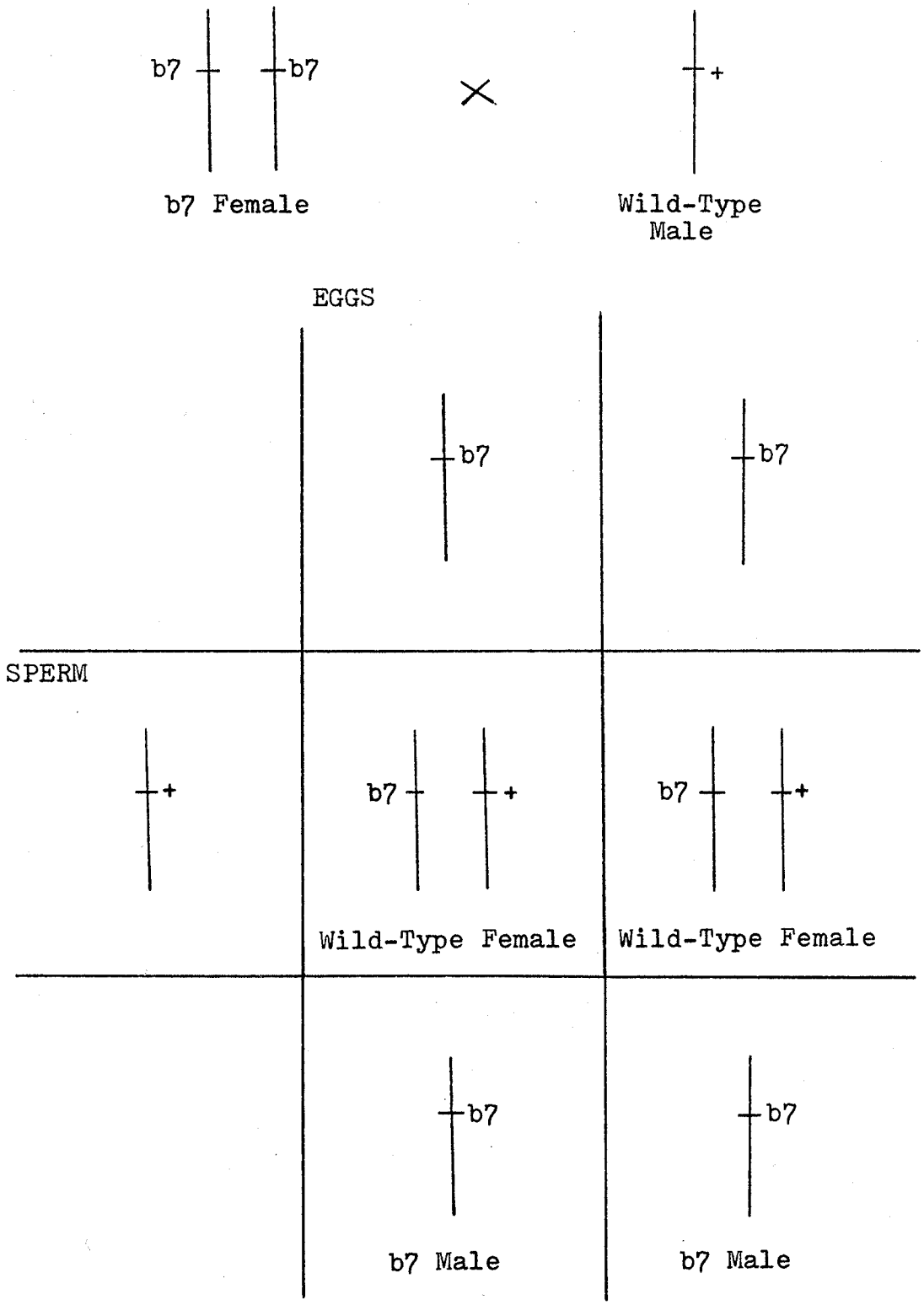
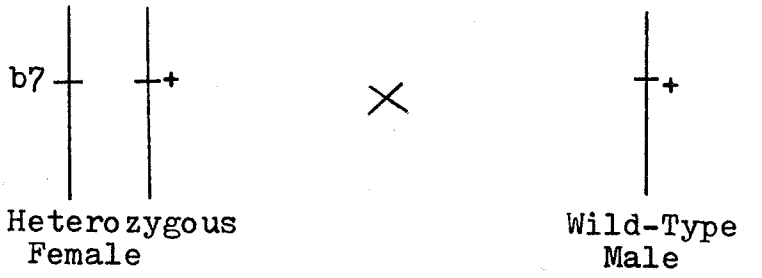
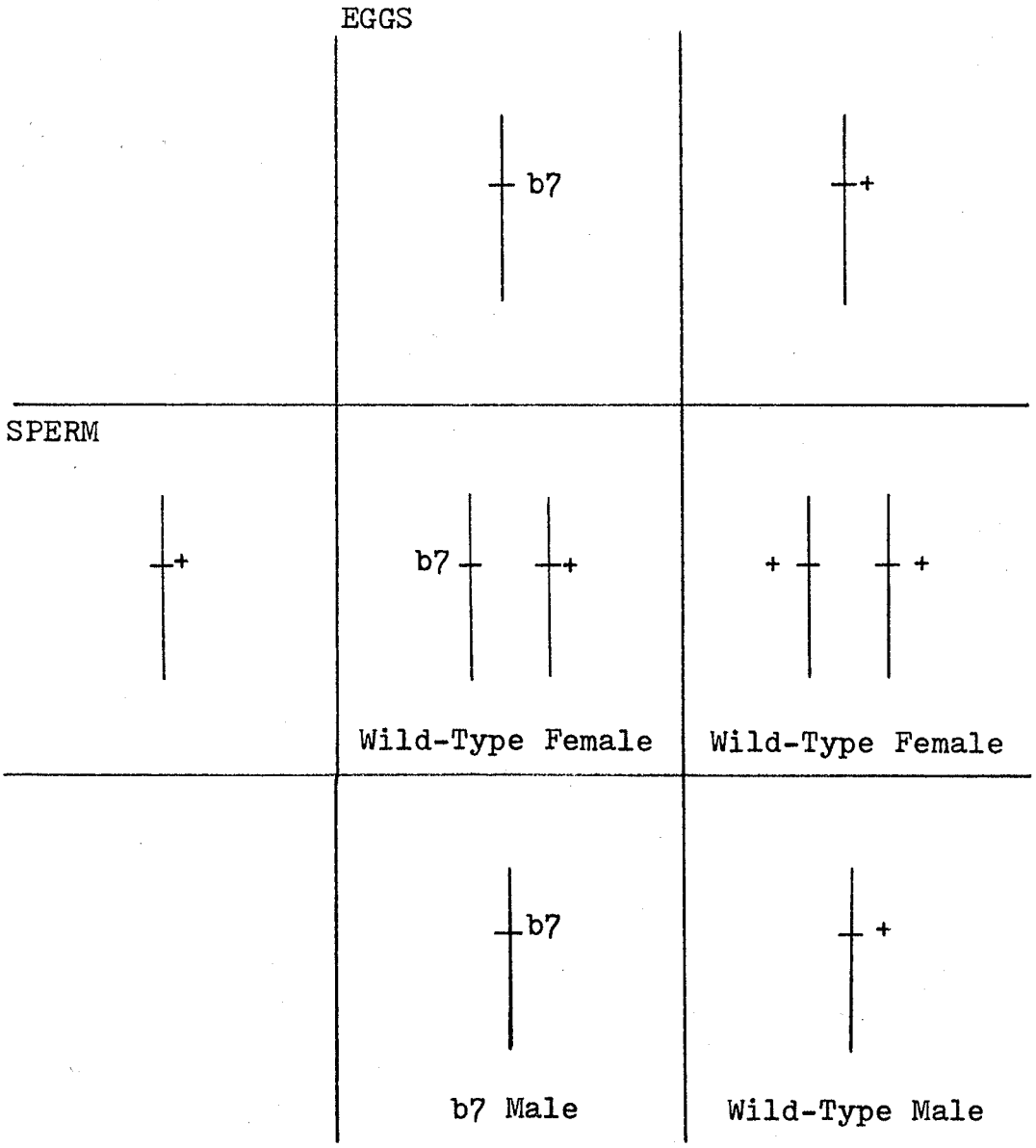

b7
b7
b7 Female
×
+
Wild-Type Male
EGGS
b7
b7
SPERM
+
b7
+
b7
+
Wild-Type Female
Wild-Type Female
b7
b7
b7 Male
b7 Male

Figure 1. Cross of b7 Heterozygous Females by C15 Males. (Part 2)



Heterozygous Female × Wild-Type Male



Wild-Type Female | Wild-Type Female

b7 Male | Wild-Type Male

RESULTS

The results of the sex-linked lethal recessive (SLR) assay are shown in Tables I and II. Mutation rates, expressed as mutations per essential locus per roentgen, mut/locus/R, following X-irradiation are presented in Table III and Figures II and III. Both male and female mutation rates follow a similar response to dose protraction. The mutation rate increases linearly as a function of the logarithm of dose-rate for dose-rates between 56 R/m to 1158 R/m. Within this dose-rate range, the data follows a straight line given by the equation:

$$\text{mutation rate } (\times 10^{9}) = (6.3 \pm .8)\ \ln(\text{dose rate}) - (19 \pm 4)$$

The standard deviation of any point along this curve is 2.4×10^{-9} mut/locus/R.

Table I. Mutation Frequency of Panagrellus redivivus Following X-Irradiation of Females.

Collection time (following exposure) of F1	Induced SLR's/loci screened				
	Control	57 R/m (5750R)	122 R/m (5800R)	225 R/m (5740R)	445 R/m (5740R)
2 days	0/12,600	0/7,560	3/21,960	1/14,760	3/16,920
3 days	1/17,280	0/6,480	0/32,400	1/3,600	2/23,040
4 days	1/14,760	0/20,880	1/15,480	2/19,800	0/5,760
6 days	1/1,890	3/22,680	2/12,600	0/20,160	0/10,440
E. M.*	0/12,960	0/4,680	0/9,360	0/3,600	2/3,600
TOTAL	3/59,400	3/62,280	6/91,800	4/61,920	7/59,760

* Endototia Matracida (offspring developed past the L1 stage inside the Female).

Table II. Mutation Frequency of Panagrellus redivivus Following X-Irradiation of Males.

Collection time (following exposure) of F1	Induced SLR's/loci screened					
	Control	26 R/m (5750R)	56 R/m (5620R)	115 R/m (5630R)	588 R/m (5630R)	1206 R/m (5630R)
2 days	2/59,760	1/7,200	0/8,640	3/21,600	2/16,200	4/12,600
2.5 days	0/12,240	2/27,360	3/21,240	0/13,680	2/20,880	5/21,960
3 days	0/67,320	1/52,920	4/70,920	6/59,040	6/49,680	5/77,760
4 days	1/57,600	3/38,880	0/40,680	2/81,360	0/54,360	1/34,560
5 days	0/24,480	1/24,120		2/34,560	0/30,240	0/28,800
7 days		0/7,560	1/23,040	1/29,520	0/3,600	0/17,280
TOTAL	3/221,400	8/158,040	8/164,520	14/239,760	10/174,960	15/192,960

Table III. Mutation Rates in Panagrellus redivivus Following X-Irradiation of Females.

Dose Rate	Mutation Rate (mut/locus/R)
57 R/m	8.4×10^{-9}
122 R/m	11.3×10^{-9}
225 R/m	11.3×10^{-9}
445 R/m	20.4×10^{-9}

Table IIIb. Mutation Rates in Panagrellus redivivus Following X-Irradiation of Males.

Dose Rate	Mutation Rate (mut/locus/R)
26 R/m	8.8×10^{-9}
56 R/m	8.6×10^{-9}
115 R/m	10.4×10^{-9}
588 R/m	20.3×10^{-9}*
1158 R/m	28.8×10^{-9}*

* Due to an obvious time dependence (see Table II) the mutation rates were calculated as the average of rates obtained on days 2 and 3.

Figure 2. Effect of Dose Protraction on Mutation Rate.

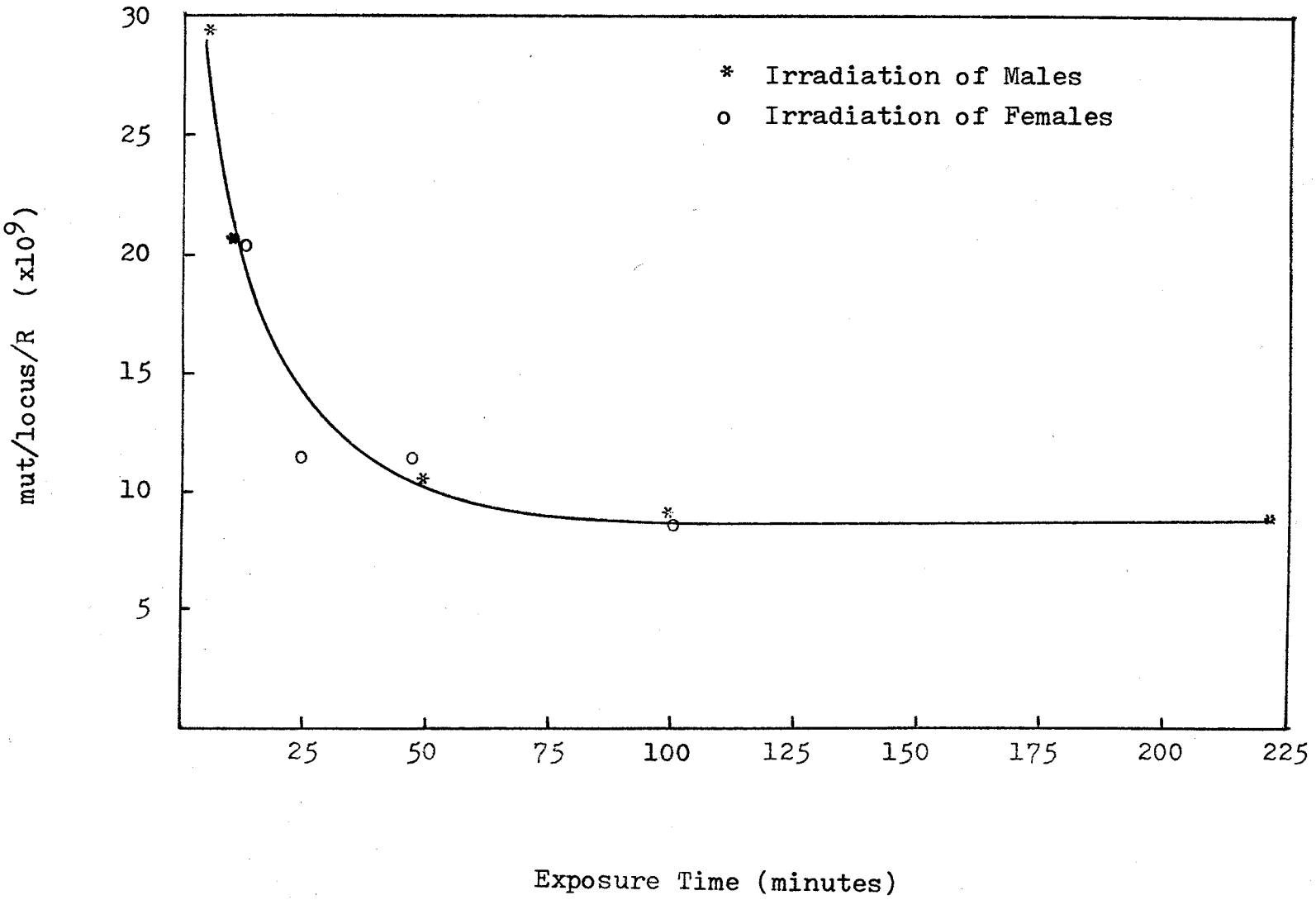
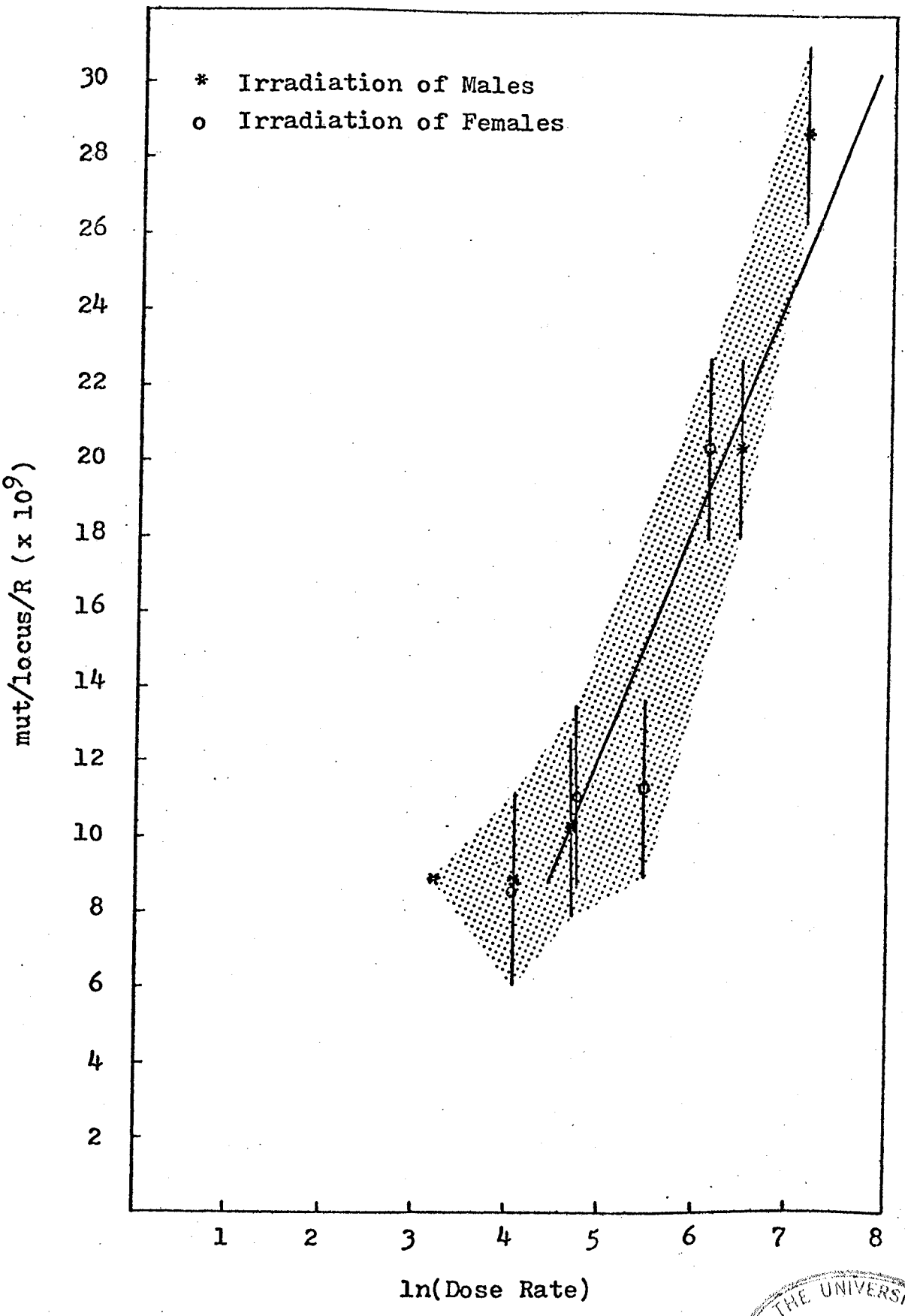

30
25
20
15
10
5
mut/locus/R (x10^9)
* Irradiation of Males
o Irradiation of Females
25
50
75
100
125
150
175
200
225
Exposure Time (minutes)

Figure 3. Mutation Rate as a Function of the Logarithm of Dose Rate.



DISCUSSION

The induction of SLR mutations is used to determine the dependency of dose-rate upon radiation induced damage. The advantages of this biological endpoint are numerous:

1) The frequency of SLR mutations is much greater than that for visible mutations or for dominant lethal mutations
2) SLR mutations occur at a large number of loci (360)
3) SLR mutations can result from several different types of lesions (Au76)
4) Scoring of SLR mutations is highly objective
5) Differential sensitivity of cells (during gametogenesis) is constant from female to female (or male to male) and will not affect the measurement of SLR mutations.

The dose-rate data conclusively demonstrates that Panagrellus redivivus is capable of repairing radiation-induced DNA damage at relatively low dose rates. The absence of a repair capacity would be indicated by a constant mutation rate for a given dose, regardless of the irradiation dose-rate. The decrease of mutation rate with increasing exposure time implies that DNA damage is being repaired, and that the repair is, in fact, dose-rate dependent.

The amount of repair occuring may be quantified in terms of a Recovery Factor such that:

$$\text{Recovery Factor} = \frac{\text{Mutation rate for an acute irradiation}}{\text{Mutation rate for an extended irradiation}} \quad (8)$$

Recovery factors for various exposure times are presented in Table IV and Figure IV. (The acute mutation rate for Panagrellus redivivus was taken as 62×10^{-9} mut/locus/R for a 5850 R, Co^{60} dose delivered at a rate of 4.9 kR/m (Jo81)).

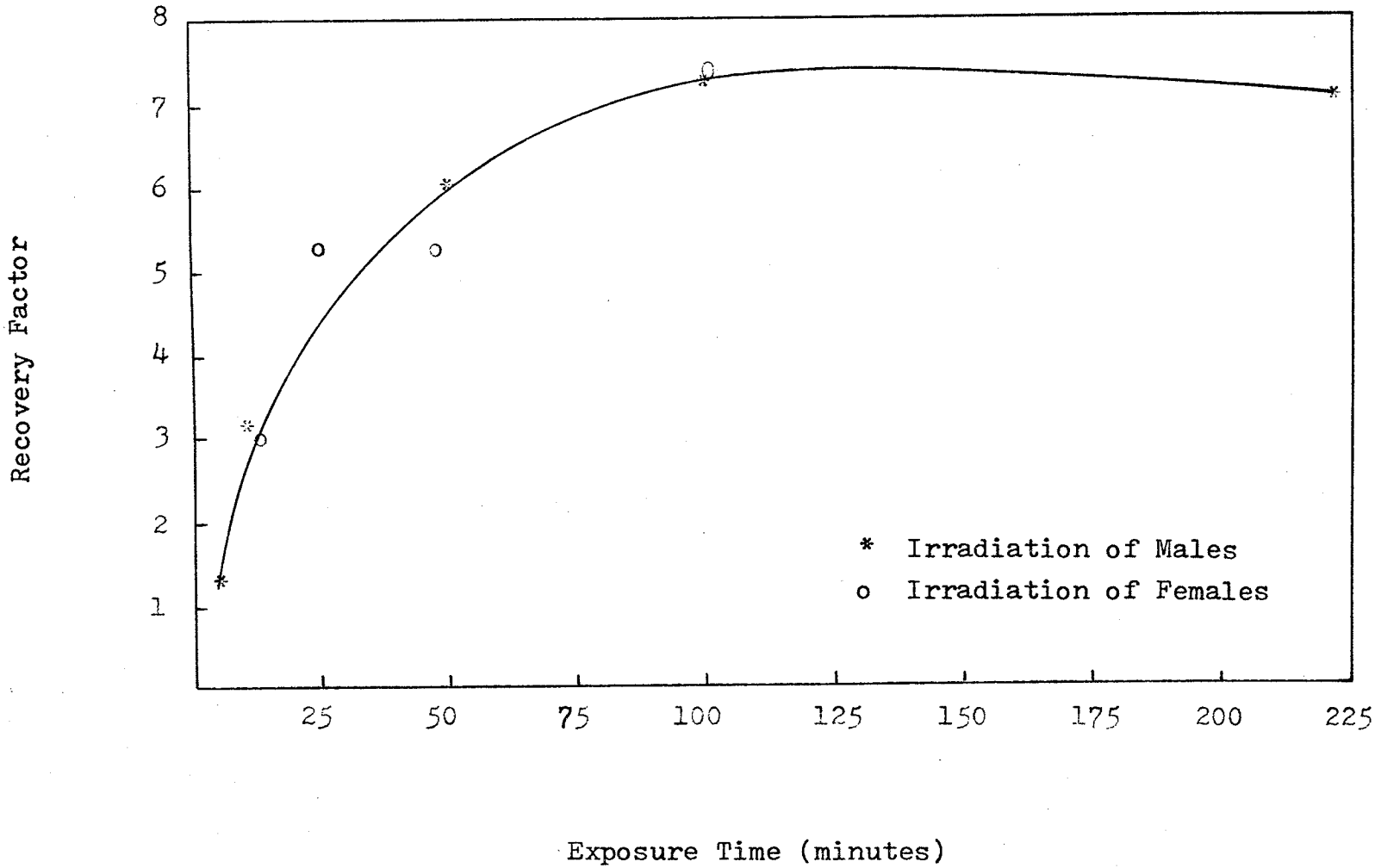
A maximum recovery factor is obtained for an exposure time of approximately 100 minutes, after which the mutation rate remains relatively constant as the exposure time increases (or dose-rate decreases). At this point the repair capacity of the organism is presumed to be saturated, or the damage remaining is of a non-repairable nature.

A more quantitative approach to the data is accomplished by applying the theory developed by Kellerer and Rossi (Ke72). Through the use of equation (4) page 8 , it is possible to calculate a value for the recovery time, t_o, given the exposure time T, and the reduction factor q(T). An accurate estimate of q(T) is not, however, possible unless a mutation rate for an instantaneous exposure is

Table IV. Recovery Factors for Prolonged Exposures.

Exposure Time (min)	Recovery Factor
221	7.0
100.9	7.4
100.4	7.2
49	6.0
47.6	5.5
25.5	5.5
12.9	3.0
9.6	3.1
4.7	2.2

Figure 4. Recovery Factor as a Function of Time.


Recovery Factor
8
7
6
5
4
3
2
1
25
50
75
100
125
150
175
200
225
* Irradiation of Males
o Irradiation of Females
Exposure Time (minutes)

obtained. The question of what exposure time constitutes an instantaneous exposure, ie, one in which no repair takes place, now arises. Assuming a 1.2 minute exposure time is acceptable, values for q(T) can be calculated from data by Jordan (Jo81) and the results are tabulated in Table V. (See Appendix II for calculation procedure).

Excluding the anomalous value obtained for an exposure time of 221 minutes, the average value of t_o calculated is 3 ± 1 minutes. This appears to be significantly lower than the repair time found for most organisms, which is usually on the order of one hour (Ha78). The low value obtained may be due to radiation quality differences between X-rays and gamma-rays, or the possibility that repair is taking place at a dose rate of 4.9 kR/m. A more reliable value for t_o was obtained by using figure IV, and is estimated to be 100 minutes.

Returning to equation (5), it is now possible to determine the constant k. Since the radiation quality constant, λ , can be neglected for X-rays (Ke72), equation (5) reduces to:

$$\varepsilon(D) = k \left[\frac{2\ t_o}{T} - \frac{2\ to^2\ (1-e^{-T/t_o})}{T^2} \right] D^2$$

Table V. Determination of Repair Time t_o.

Exposure Time (m)	Reduction Factor q(T)	$\frac{\text{Exposure Time}}{\text{Repair Time}}$	Repair Time (m)
221	.088	22	10
100.9	.079	24	4.2
100.4	.079	24	4.2
49	.111	17	2.9
47.6	.132	14	3.4
25.5	.129	15	1.7
12.9	.281	6	2.2
9.6	.272	6	1.6
4.7	.412	4	1.2

where $\varepsilon(D)$ is the yield of elemetary lesions, ie, the number of SLR mutations induced. If we let E(D) be the observed mutation rate (mut/locus/R), equation (5) becomes:

$$E(D) = k' \left[\frac{2\ t_o}{T} - \frac{2\ t_o^2\ (1-e^{-T/t_o})}{T^2} \right] D$$

with $k^1 = k/360$. Units of k' are R^{-2}.

Calculated values of k' are listed in Table VI. A possible trend of increasing k' values with increasing dose rate is observed. For exposure times between 25.5 and 221 minutes, however, the constant k' falls within a limited range such that the mean value of k' is $(2.2 \pm .3) \times 10^{-12}\ R^{-2}$.

The anomalous values obtained for high dose rates may indicate the existence of a second component of radiation-induced damage. This component appears to exhibit a dose rate threshold, and is highly dose rate dependent.

The existence of a dose rate threshold could indicate that the damage induced by this second component is two-step in nature. Two step kinetics imply that two hits must occur on one target (two-hit model), or one hit must occur on each of two targets (two-target model) (Ke71). If these two hits must occur within a limited time span (to avoid repair of the damage caused by the first hit) the probability of this two-step damage would be quite low for low dose rates, and would thus exhibit a dose rate threshold. An

Table VI. Determination of Constant k'.

Exposure Time (T) (min)	k' (R^{-2})
221	2.8×10^{-12}
100.9	2.0×10^{-12}
100.4	2.1×10^{-12}
49	2.2×10^{-12}
47.6	2.3×10^{-12}
25.5	2.1×10^{-12}
12.9	3.7×10^{-12}
9.6	3.7×10^{-12}
4.7	5.2×10^{-12}

increase in dose rate (above the threshold dose rate) would increase the probability of the two-hit damage quite dramatically.

An alternate explanation for the anomalous values calculated for k' at high dose rates could be that the repair system is inhibited or damaged in some manner when radiation is administered in a short time span. The net effect of a reduced repair capacity would be an increased repair time, t_o. If t_o is actually larger than the 100 minute value employed in the calculations, the k' values obtained would be erroneously high.

At the present time it is impossible to state conclusively which, if any, of the two proposed theories correctly explains the breakdown of the theory developed by Kellerer and Rossi at high dose rates. Further studies using a higher LET radiation, proton- or neutron-irradiation for example, may provide very useful information. Since an instantaneous exposure would be possible, the repair time, t_o, could be calculated at various dose rates to determine if t_o does actually increase with dose rate.

APPENDIX

I DOSIMETRY

A model 550 III integrating/rate electrometer system with model 550-5 (1.0 MA) integrating probe was used to measure exposure rate. This system is capable of detecting beta, gamma, and X-rays with the following specifications (excluding the probe):

1) Basic electrometer inaccuracy is $\pm$ 0.5% of reading $\pm$ 1 digit (at 22^{o}C)
2) Long term drift is $\pm$ 0.5% per six months
3) Temperature drift is $\pm$ (t^{o}C - 22) (0.03%)
4) Zero drift is $\pm$ 0.05 mR/s after 1 hour
5) Input power changes of $\pm$ 10% result in a change of $\pm$ 0.2% in reading
6) Operating temperature range from 10^{o} - 40^{o}C
7) Exposure rate response time of 1.0 seconds
8) Operating humidity range from 0 - 90% relative humidity

The fully guarded ionization chamber probe is usable in the energy range of 42 keV to 520 keV. The energy response and constant field collection efficiency are outlined in figures A1 and A2. Dose corrections for the parameters used, ie, dose rates less than 20 R/s, at an energy of 80 keV are negligible. Corrections must, however, be considered regarding temperature and pressure fluctuations when using an unsealed cavity type ionization chamber.

Figure A1. Calculated Collection Efficiency to Continuous Radiation.

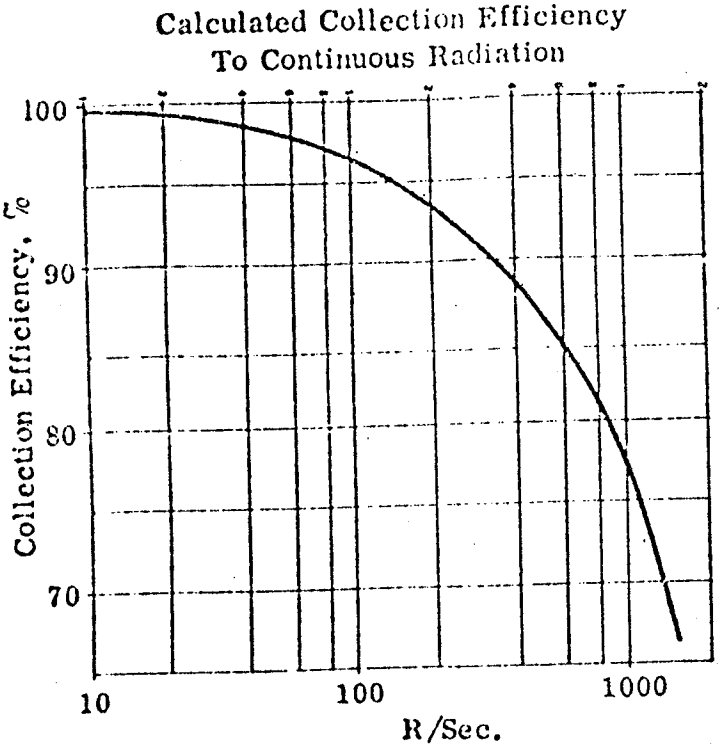
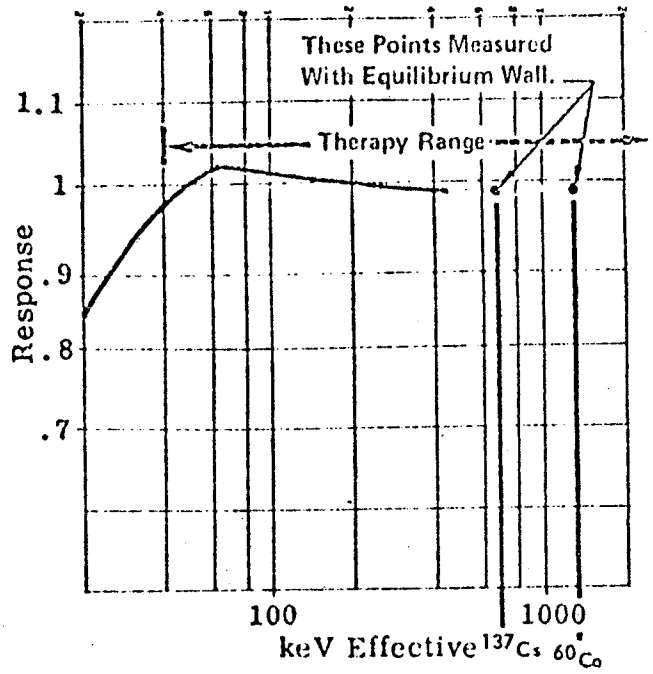

Calculated Collection Efficiency
To Continuous Radiation
100
90
80
70
Collection Efficiency, %
10
100
1000
R/Sec.

Figure A2. Probe Energy Response.


These Points Measured
With Equilibrium Wall.
Therapy Range
1.1
1
.9
.8
.7
Response
100
1000
keV Effective 137Cs 60Co

The exposure is dependent upon the mass of air in a given volume, which in turn, depends upon temperature and pressure. The probe used was calibrated by NBS in International Roentgens at $22^{o}C$ and 760 Torr. For temperatures and pressures other than those specified, a correction factor must be implemented:

$$\text{Correction Factor} = \frac{273 + t^{o}C}{295} \times \frac{760}{p.Torr} \; *$$

The first term of the above expression corrects for the expansion of the gas with an increase in temperature, while the second term corrects for pressure variation. (The loss of charge/current in an unsealed ion chamber due to humidity is negligible and was therefore neglected).

* This equation as well as system specifications are taken from the Operation & Maintenance Instruction Manual for Model 550 Radocon III Integrating/Rate Electrometer System.

APPENDIX

II Determination of Repair Time (t_o)

To determine the repair time, t_o, it is necessary to first calculate the reduction factor q(T):

$$q(T) = \frac{\text{Quadratic effect in presence of repair}}{\text{Quadratic effect in absence of repair}}$$

$$= \frac{\text{MF - SMF for extended exposure}}{\text{MF - SMF for "instantaneous" exposure}}$$

where:

MF = Mutation Frequency (mut/locus) observed at a given dose rate

SMF = Spontaneous Mutation Frequency (mut/locus)

The spontaneous mutation frequency used was found by combining both male and female trials, ie, 6/280,800 or 21×10^{-6} mut/locus. The mutation frequency for an "instantaneous" exposure was taken as 3.05×10^{-4} mut/locus.

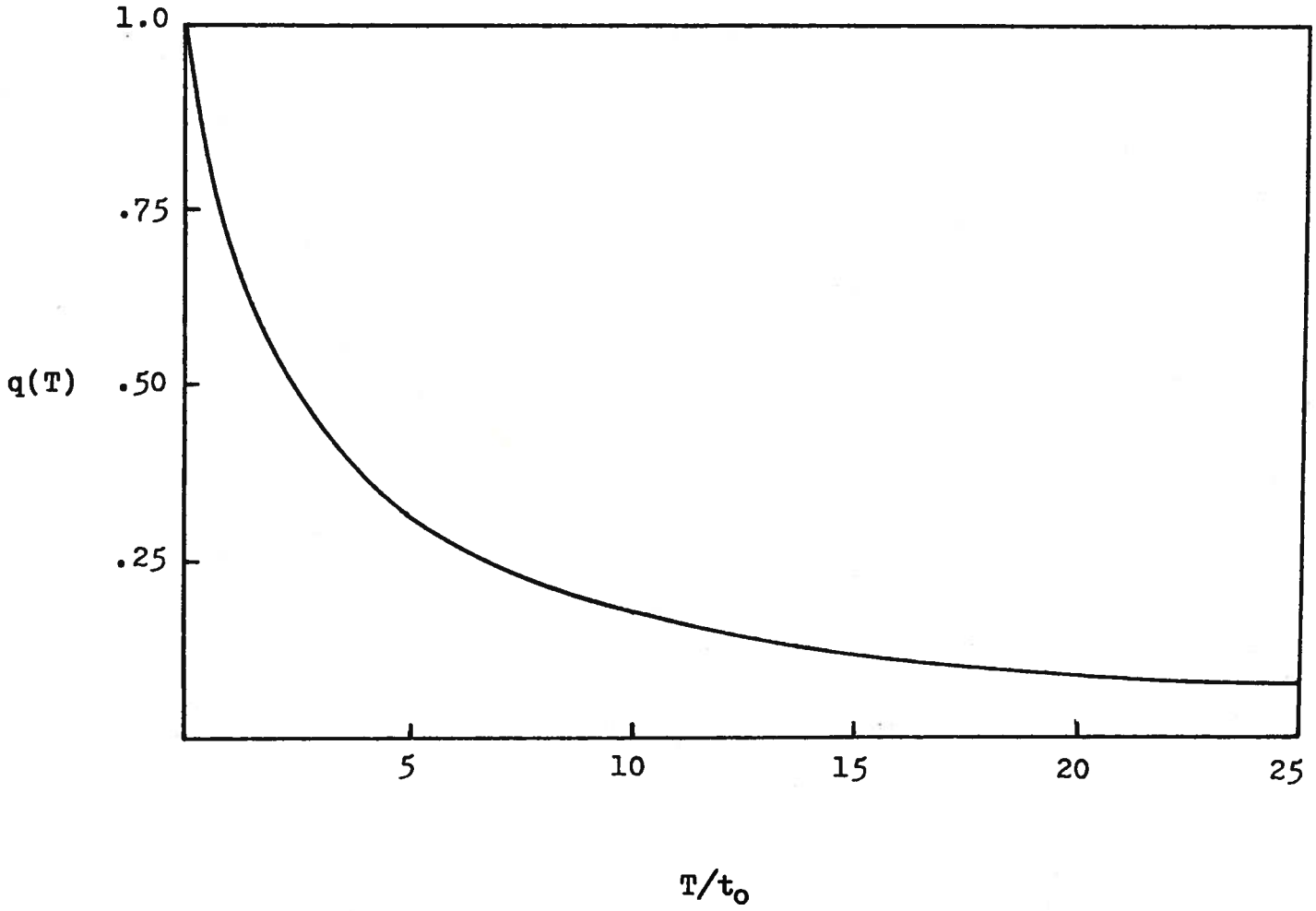
Through the use of equation (4), page 8 :

$$q(T) = \frac{2\ t_o}{T} - \frac{2\ t_o^2}{T^2}\ (1-e^{-T/t_o})$$

it is possible to calculate a value for the recovery time, t_o. Values can be obtained numerically, or more readily from a plot of q(T) versus T/t_o as illustrated in Figure A3.

Figure A3. Reduction Factor, q(T) as a Function of the ratio of irradiation time, T, and recovery time, t_o.

Figure A3. Reduction Factor, q(T) as a Function of the ratio of irradiation time, T, and recovery time, t_o.


1.0
.75
q(T)
.50
.25
5
10
15
20
25
T/t_o

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