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Relationships between LET and RBE of Ionizing Radiation in the Induction of Somatic Mutations of *Drosophila melanogaster*

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The effects of LET (linear energy transfer) of radiation on the induction of somatic chromosome mutations or gene mutations of *Drosophila melanogaster* were studied. For detecting somatic chromosome mutations and gene mutations, *Drosophila* wing spot system and eye-color spot system were used, respectively. The frequencies of somatic chromosome mutations or gene mutations induced after third instar larval treatment with 23 MeV neutrons, thermal neutrons, X-rays were examined. From these data, the RBE (relative biological effectiveness) values of 23 MeV neutrons relative to X-rays for induction of somatic chromosome mutations or gene mutations were calculated.

The present results suggest that high LET radiations are more efficient than X-ray in producing not only somatic chromosome mutations but also gene mutations.

INTRODUCTION

Studies with Drosophila have helped to answer some basic questions in radiation genetics and have given a understanding of the genetic hazards of radiation exposure of human beings. During the past four decades an impressive number of studies designed to determine the RBE (relative biological effectiveness) of different kinds of radiation have been carried out in *Drosophila melanogaster* (Sankaranarayanan *et al.*, 1976; Muller 1954; King *et al.*, 1958; Sobels *et al.*, 1970). Thus it is now know that the RBE values vary with the dose, dose-rate and the energy spectrum of the neutrons, and may be different for different germ cell stages and for different types of genetic damage.

The idea that cancer cells arise from normal cells through somatic mutation has a long history and is supported by the irreversibility of some cellular changes during carcinogenesis and the stability of most cancer phenotypes. Since the early days of radiation genetics, germ cells of Drosophila have attracted the attention of many workers as a system for studying genetic damages induced after treatment with radiations. By contrast, little is known either about the effects of linear energy transfer (LET) of radiations on the relative

radiosensitivity of the somatic mutation or the manner of its response to high LET radiation. Recently, very sensitive methods for assaying somatic mutation have been developed by Würgler's group (Würgler *et al.*, 1983; Graf *et al.*, 1983, 1984) and Rasmuson's group (Rasmuson *et al.*, 1978). The Drosophila wing spot test system developed by Würgler's group is fast and sensitive for detecting somatic chromosomal mutations. Rasmuson *et al.* (1978) originally observed that a mutationally unstable X-chromosome is more susceptible than a stable X-chromosome to somatic eye-color mutations. The genetic instability of these unstable mutant alleles may be associated with transposable elements inserted in the white locus (Rasmuson *et al.*, 1981, 1984). This eye-color spot system with TE is simple, reliable and sensitive for detecting somatic gene mutations (Ryo *et al.*, 1983).

In the present paper, efficiencies of different LET ionizing radiations in the induction of somatic mutations of *Drosophila melanogaster* were studied. These experiments were carried out by irradiating these assay systems for somatic chromosome mutations and gene mutations with 23 MeV fast neutrons, thermal neutrons and 30 kV X-rays.

MATERIALS AND METHODS

Drosophila stocks: The strain of unstable *zeste* eye color mutant used as assaying system for somatic gene mutation was obtained from Sohei Kondo. The unstable *zeste* strain, with the genotype *sc z' w^+ sn* will be abbreviated the UZ strain. The *zeste* phenotype, the eye color is lemon yellow, in the UZ strain is unstable owing to the insertion of transposable element Dm 225 near the locus w^+ (Rusmuson *et al.*, 1981).

For assaying somatic chromosome mutations, the female stock with *y;mwh j v* and the male stock *y;Dp (1:3) scJ4, flr/TM1, Mé* (from F.E. Würgler) were used. These strains will be abbreviated *mwh* strain and *flr* strain, respectively.

Culture conditions: For assaying somatic gene mutation, female and male flies of the UZ strain were aged separately for 3-4 days, mated for 24 hr at a ratio of 80 females to 80 males in culture bottles containing 40 ml of sugar-agar medium (agar 1.5%, sugar 3%, propionic acid 0.5%), and then transfered to fresh culture bottles containing 40 ml of standard medium to oviposit for 20 hr at 25°C. After the oviposition, parental flies were discarded and resultant eggs were allowed to develop at 25°C.

For assaying chromosome mutations, females of the *mwh* strain and males of the *flr* strain were aged separately for 3-4 days, mated for 24 hr at a ratio of 20 females to 20 males in vial containing 10 ml of agar medium, and then transferred to fresh culture vials containing 10 ml of standard medium to oviposit for 10 hr at 25°C.

Treatment with X-rays and neutrons: X-rays were applied with 6 MeV Mevatron-67 Linac (30 kV and 100 A) at 254 rad/min. Larvae cultured in milk bottle or vial were irradiated and cultured at 25°C until they emerged.

23 MeV neutrons were produced by bombarding a Beryllium target with protons by using cyclotron accelerator (MC-50-AVF type, Scanditronix, Sweden). Larvae were irradiated at a target distance of 150 cm, where the dose rate was 30 rad/min.

Thermal neutrons were produced by using nuclear reactor, TRIGA MARK-III (Korea Advanced Energy Research Institute). The dose rate was 12.5 mrad/min. γ-rays and β-rays emission in this reaction was contaminated.

Assay of somatic gene mutation: Adult males of the UZ strain were surveyed for eye-color mutations: red sectors consisting of 4 or more ommatidia were scored as somatic mutations. The frequency of somatic mutations is defined as the number of red sectors divided by the number of males examined.

Assay of somatic chromosome mutation: The method of Würgler and his group was adopted. From crosses between females of the *mwh* strain and males of the *flr* strain, we obtained larvae transheterozygous for the locus *mwh* (multiple wing hairs, 3-0.0) and the locus *flr* (flare hairs, 3-39.0), which are on the left arm of chromosome 3. Transheterozygous (*mwh/flr*) larvae obtained 72 hr after oviposition, i.e. in the early third instar, were irradiated and mutant clones on the wing blade of adult flies were scored as chromosome mutations. Conventionally, mutant clones in this transheterozygous system are classified into three types: The *mwh* single spots consisting of one or two cells will be called "small" *(mwh)* single spots. These are supposed to be primarily due to terminal deletion of the left arm of chromosome 3 where the wild-type *mwh* allele is located. The "large" *mwh* single spots consisting of three or more cells arise mostly from somatic recombination between the locus *mwh* and the locus *flr* (Yoo *et al.* unpublished data). The twin spots consisting of two types of adjacent cell clones, one showing multiple hairs and the other flare phenotype, arise mostly form somatic recombination between the centromere of the third chromosome and the locus *flr.* In short, detected mutant clones were small single spots consisting of one to two *mwh* cells, large *mwh* single spots and twin spots consisting of *mwh/flr* clones.

Assay of sensitivity to lethal effect of X-irradiation: Eggs obtained using the same method as assaying chromosome mutations were counted and cultured at 25°C. 72±5 hr old larvae were irradiated and cultured at 25°C and the adult flies that emerged were scored. Survival was recorded as the ratio of the number of adults that emerged to the initial number of eggs.

Preparation of wings for assay of chromosome mutations: Non-yellow flies *(mwh+/+flr)* that emerged were preserved in 75% ethanol. Preserved wings were mounted on glass slides in Faure's solution (gum arabic 30 g, glycerol 20 ml, chloral hydrate 50 g, water 285 ml) and clones of cells with mutant hairs were scored under a microscope at 400-fold magnification.

RESULTS AND DISCUSSION

The surviving fractions of larvae irradiated with X-ray dose at 72±5 hr after oviposition are shown in Fig. 1. The LD_{50} (50% lethal dose) value was 3.5 krad. This value agree with those previously reported (Baker *et al.*, 1978; Yoo *et al.*, 1985).

Frequencies of somatic chromosome mutation of the *mwh/flr* system after third instar larval treatment with X-rays, 23 MeV fast neutrons and thermal neutrons are presented in Table 1. The frequency of small *mwh* spots after irradiation of larvae obtained 72±5 hr after oviposition almostly showed the same level as the control group, except at 0.25 rad of thermal neutrons.

In contrast, the frequency of large spots mostly due to chromosomal recombination increased with the dose in all radiations as shown in Table 1.

The size of mutant clones provides important information on the dynamics and the number of cells in the imaginal disc under study (Garcia-Bellido and Merriam, 1971; Becker, 1976; Haynie and Bryant, 1977). The size distributions

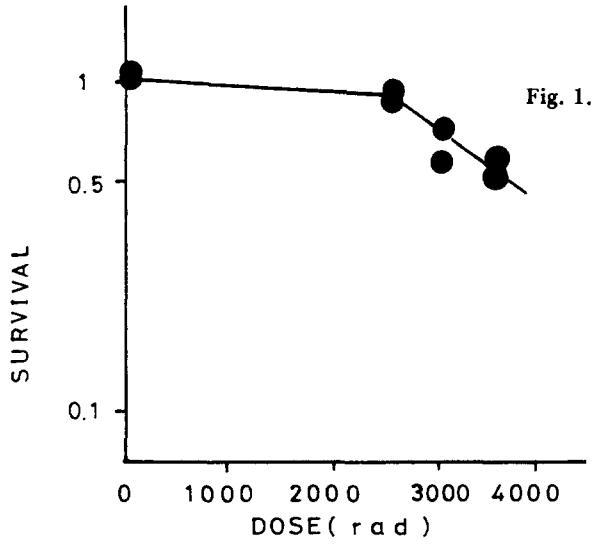

Fig. 1. Effect of X-ray dose on surviving fractions of *Drosophila melanogaster*. Larvae obtained 72 hr after oviposition were irradiated.

Table 1. Frequencies of somatic chromosome mutation in the Drosophila wing spot system by treatment with various LET radiations

Treatment	Dose (rad)	Number of wings scored	Frequency per wing (Number) of			
			Single spots		Twin spots TW	Large spots (LS+TW)
			small SS	large LS		
Control	0	470	0.24(115)	0.01(4)	0.02(8)	0.02(10)
X-rays	500	316	0.29(92)	0.14(43)	0.03(11)	0.17(54)
	1,000	257	0.26(67)	0.29(75)	0.07(19)	0.37(94)
23 MeV neutrons	100	196	0.18(35)	0.09(17)	0.02(2)	0.10(19)
	200	154	0.22(34)	0.17(26)	0.05(8)	0.22(34)
	400	102	0.22(22)	0.33(34)	0.06(6)	0.39(40)
Thermal neutrons	0.125	113	0.34(38)	0.04(5)	0.01(1)	0.05(6)
	0.250	158	0.51(81)	0.09(14)	0.01(2)	0.10(16)

of mutant clones after X-rays or 23 MeV fast neutrons-irradiation of 72±5 hr old larvae are illustrated in Fig. 2. Clone sizes are grouped into integral size classes with limits corresponding to 2^n, where n is a measure for the number of mitotic divisions required for a mutant cells to produce the corresponding clone size (Haynie and Bryant, 1977). The size of mutant clones induced by X-rays decrease with larval age at the time of irradiation from the first to the

third instar. In inverse proportion to the clone size, the frequency of induced mutant clones increase with larval age at the time of irradiation (H. Ryo *et al.*, 1985). As shown in Fig. 2, the large spots induced by X-irradiation had a modal frequency of 65-128 *mwh* cells at 500 rad and 1,000 rad. That induced by 23 MeV neutrons had a modal frequency of 33-64, 65-128, 33-256 *mwh* cells at 100, 200 and 400 rad, respectively. Therefore, large *mwh* clones induced by 23 MeV neutrons had similar pattern of size distribution of mutant clones to those by X-irradiation as can be seen in Fig. 2. This similar pattern of size distribution means that 23 MeV neutrons and X-rays-irradiation were done at the same developmental stage of larvae. Therefore, we can compare efficiencies of 23 MeV neutrons and X-rays in inducing somatic chromosome mutations with the frequency of mutant clones.

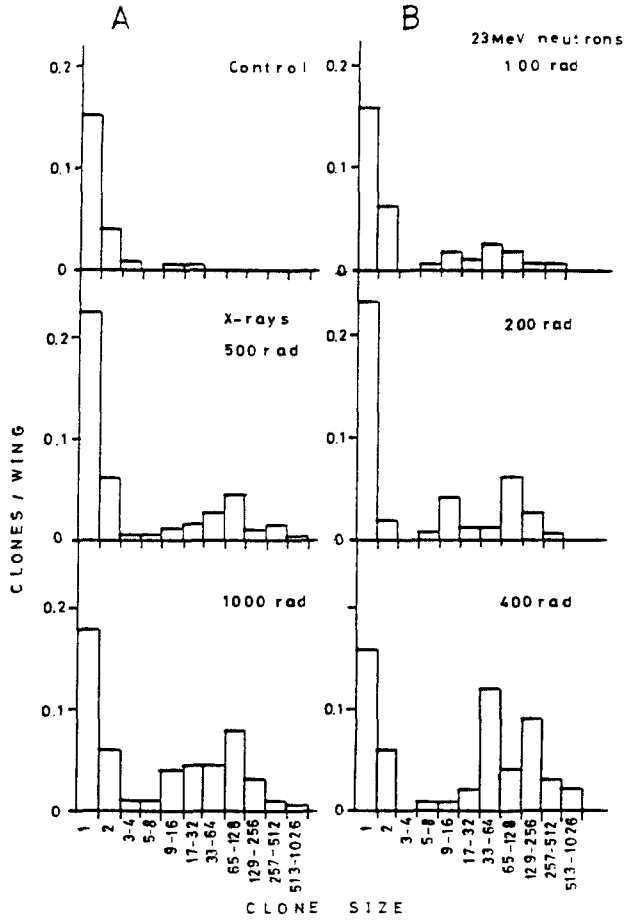

Fig. 2. Distributions of mutant clone sizes after third instar larval treatment with X-rays (A) and 23 MeV neutrons (B). Bars show sums of frequencies of single *mwh* clones and twin *mwh/flr* clones.

The dose-response curves for large spots induced by 23 MeV neutrons and X-rays are presented in Fig. 3. The induced frequencies in Table 1 were fitted to a regression equation of Y=aD (Y=mutant clone per wing, D=dose in rad and a=regression coefficient) by the method of least squares with the reciprocal of variance of the frequencies used as weighting factor. The following regression coefficients were calculated: neutrons-9.4×10^{-4}; X-ray-3.5×10^{-4}. The data on either kind of exceptions did not deviate significantly from the expected linear dose-response relationships. Then, the RBE value was estimated as a ratio of the regression coefficient for neutrons to that for X-rays. The RBE value of 23 MeV neutrons relative to X-rays for induction of somatic chromosome mutations was 2.69.

Frequencies of somatic gene mutation of the UZ strain after third instar larval treatment with 23 MeV fast neutrons and X-rays are presented in Table 2. In the present experiment, X-rays produced lower yields than 0.91% in the UZ strain at 1,100 rad of 180 kV X-rays (H. Ryo *et al.*, 1983) and 0.8% at 1,100 rad of 180 kV X-rays (H. Ryo *et al.*, 1985). The dose-response relationships for somatic gene mutation induced by 23 MeV neutrons and X-rays are shown

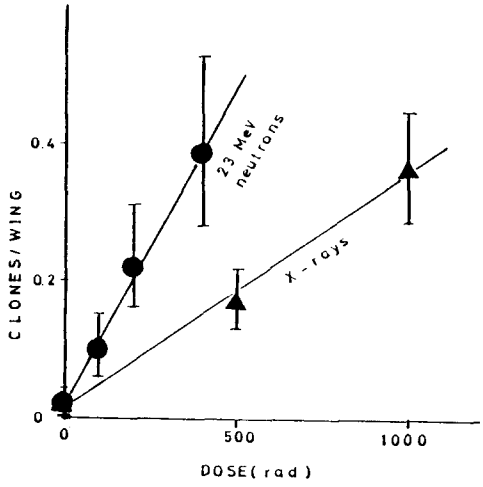

Fig. 3. Dose-response relationships for somatic chromosome mutations in Drosophila wing spot system induced by 23 MeV neutrons and X-rays. Larvae obtained 72 hr after oviposition were irradiated with indicated doses. Vertical lines show 95% confidence intervals.

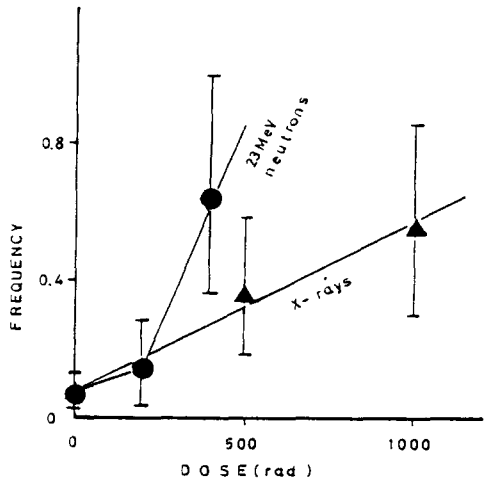

Fig. 4. Dose-response relationships for somatic gene mutations in UZ strain induced by 23 MeV neutrons and X-rays. Larvae obtained 72 hr after oviposition were irradiated with indicated doses. Vertical lines show 95% confidence intervals.

Table 2. Frequencies of somatic gene mutation of the UZ strain by treatment with X-rays and 23 MeV neutrons

Treatment	Dose (rad)	Number of males	Number of red spots	% frequency	(95% confidence interval)
Control	0	10,359	6	0.058	(0.025–0.124)
X-rays	500	3,674	13	0.35	(0.18 –0.58)
	1,000	2,788	15	0.54	(0.29 –0.85)
23 MeV neutrons	200	2,827	4	0.14	(0.03 –0.28)
	400	2,237	14	0.63	(0.36 –1.02)

in Fig. 4. The data obtained for somatic gene mutations, which are presented in Table 2 and Fig. 4, were not suited for evaluating RBE value. This is because the yields of neutrons-induced gene mutations were found to increase faster than linearly with dose (Fig. 4). On the other hand, the data obtained with X-rays did not show a significant deviation from a linear dose-response relationship. As a result of this difference in the dose-response relations, the RBE value for somatic gene mutation depends on dose. It can be seen from Fig.4 that at the yield of 0.14%, the RBE is about 0.85, whereas at the yield of 0.63%, the RBE increases (e.g., about 2.98).

As shown in the present results, it is important to note that 23 MeV neutrons are more efficient than X-rays in producing not only somatic gene mutations but also chromosome mutations.

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초파리의 체세포돌연변이 유발에 대한 방사선의 RBE와 LET와의 관계

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초파리의 체세포 염색체돌연변이와 유전자돌연변이 유발에 대한 방사선의 LET의 영향을 조사하였다. 체세포 염색체돌연변이와 유전자돌연변이 검출을 위해서는 초파리 wing spot system과 eye-color spot system이 각각 사용되었다. 이 두 검출계의 3령기 유충시기에 LET가 다른 23MeV 속중성자, 열중성자, X선을 조사한 후 유발된 염색체돌연변이 빈도와 유전자돌연변이 빈도가 조사되었다. 실험결과로부터 체세포 염색체돌연변이와 유전자돌연변이 유발에 대한 23MeV속중성자의 RBE가 X-ray를 기준으로 계산되었다.

본 연구결과는 high LET방사선이 X선에 비해서 체세포 염색체돌연변이와 유전자돌연변이 유발에 대해 더 효과적임을 시사한다.