RELATIVE BIOLOGICAL EFFECTIVENESS OF 14 MeV NEUTRONS FOR INDUCING DOMINANT LETHALS IN MATURE SPERM OF THE SILKWORM: A COMPARISON OF THE RBE FOR DOMINANT LETHALS AND SPECIFIC-LOCUS MUTATIONS¹⁾

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INTRODUCTION

It has been reported in a previous paper (Murakami 1966) that the RBE (relative biological effectiveness) value of 14 MeV neutrons for complete mutations is somewhat higher than for mosaics in mature sperm of the silkworm. Tazima (1964) indicated that the incidence of complete mutations in mature sperm of the silkworm increases linearly with dose throughout the whole dose range observed up to 15 kR, and that the mosaics increase approximately with the square of the dose. Tazima (1964) further proposed that the complete mutations were due to single-hit event or gene mutation, while mosaics were due to two-hit events resulting eventually in deletion or translocation. A similar observation was reported by Inagaki and Nakao (1970) that the radiation-induced mosaics are due to chromosome changes associated with multiple breakage events. In my previous paper (Murakami 1966), the dose-response curves for mosaics were slightly deviated from the linear relation at the doses below 5 kR. Recently, Tazima and Onimaru (1969) have reinterpreted the previous data of Tazima (1964) as indicating that the complete mutations may have occurred due to double-stranded DNA changes, whereas the mosaic mutations may be due to a mixture of single-stranded DNA changes and chromosomal aberrations. The question posed by these considerations is whether the higher RBE of fast neutrons for complete mutations compared with that of mosaic mutations is meaningful.

It is a well-known fact that high LET (linear energy transfer) radiations, when compared with low LET radiations, are highly efficient in producing chromosomal damages such as gross chromosomal aberrations and translocations (e.g., Lea 1955). On the assumption that the origin of the mosaic mutation is a so-called chromosomal event (Tazima 1964; Inagaki and Nakao 1970), we can expect higher RBE values of neutrons for the mosaics than for the complete mutations. The reverse was the case.

It is widely accepted that radiation-induced dominant lethal mutations are attributed to gross chromosomal damages (Pontecorvo 1941; Pontecorvo and Muller 1941; Muller and Pontecorvo 1942). Similar thought has been accepted in the male silkworm (Tazima

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1959). Thus, it is of great interest to compare the RBE value of 14 MeV fast neutrons for the dominant lethals with that for the mosaics. For this reason, the RBE value of the dominant lethals in mature sperm of the silkworm was estimated. In this paper an attempt is also made to interpret the slightly different RBE values between complete and mosaic mutations based on current idea of radiation genetics.

MATERIALS AND METHODS

Experimental details have been given in the previous paper (Murakami 1966), so it will be described here only briefly. The experiment was conducted using mature sperm of the silkworm. Twelve or thirteen days old pupae of the wild type, C108 strain, were exposed to 14 MeV fast neutrons and ^{137}Cs gamma-rays. The irradiated individuals were mated to marker strain homozygous for *pe* and *re* loci. The use of this method permits the detection of wholly expressed complete mutants of either pink or red eggs found among the black eggs of wild phenotype as well as of partly expressed mosaic mutants. The incidence of dominant lethal mutations was estimated for both radiation groups, after the detection of specific-locus mutations, by the percentage of non-hatched eggs per total number of fertilized eggs.

RESULTS

The results obtained for the induction of dominant lethals checked as egg mortalities by neutrons and gamma-irradiation in the silkworm mature sperm are given in Table 1.

Table 1. Rate of dominant lethals by gamma-rays and 14 MeV neutrons in the silkworm sperm

Radiation	Dose in rads	Total no. of observed eggs	No. of hatched eggs	No. of dying eggs	Percent of dying eggs
Control	0	31,607	29,642	1,965	6.2+1.4
	1,940	33,735	31,037	2,698	8.0+1.5
^{137}Cs gamma-rays	3,880	32,352	29,954	2,398	7.1+1.4
	5,820	32,450	29,135	3,315	10.2+1.7
	990	27,503	24,628	2,875	10.5+1.9
	1,880	30,074	25,350	4,724	15.7+2.1
14 MeV neutrons	5,050	31,341	20,257	11,084	35.4+2.7

Although the dose-response kinetics for dominant lethals are essentially multiple-hit types, the kinetics may be either linear or non-linear over the dose-ranges observed in the present experiment. This may be due to few experimental points and relatively low dose-range examined. Obviously, the non-linear relationship of the dose-response curves make difficult to estimate the RBE value. Such being the case, an RBE value for dominant lethals was estimated using gamma-rays as a base line for comparison of the slope of the regression line for 14 MeV neutrons. The weighted linear regression for the percentage of egg-death on dose, as the incidence of dominant lethals, for gamma-

Fig. 1. Regression lines for dose-percentage of egg hatchabilities induced by radiations of mature sperm with 14 MeV fast neutrons and ¹³⁷Cs gamma-rays. Solid and broken lines denote 14 MeV neutrons and ¹³⁷Cs gamma-rays, respectively.

rays ($Y=5.72 \times 10^{-5}D$) and for 14 MeV neutrons ($Y=5.89 \times 10^{-4}D$) are plotted on Figure 1. Thus, the RBE was calculated to be 10.2. This is about 2.1 times higher than that of mosaics. RBE values for different mutational events, including the data in the previous paper (Murakami 1966), are summarized in Table 2.

Table 2. RBE values of 14 MeV fast neutrons to ¹³⁷Cs gamma-rays for specific-locus mutations and dominant lethals in the silkworm sperm

Type of mutation	RBE values		(mean)
	<i>pe</i> locus	<i>re</i> locus	
Complete mutations	5.3	6.7	(6.0)
Mosaics	5.0	4.6	(4.8)
Dominant lethals	10.2		

DISCUSSION

It is a well-known fact that the nature of dominant lethal mutations is characterized by chromosome aberrations (LaChance 1967; Fano and Demerec 1941; Pontecorvo and Muller 1941; Demerec and Fano 1944; Lea and Catcheside 1945). On the basis of ac-

cumulated data on insect sperm, the evidence is overwhelming that the curve relating dominant lethals to radiation dose is a linear relation at low doses and a non-linear relation at high doses (LaChance 1967). It is generally agreed that gross chromosomal changes are induced with high LET radiations more frequently than with low LET (Catcheside 1947; Lea 1955). In the silkworm, it has been reported by Tazima (1959) that the incidence of unhatched embryos in irradiated male gametes is attributable solely to dominant lethals as the chromosomal anomalies.

As can be seen from Table 2, the RBE values of 14 MeV fast neutrons for complete and mosaic mutations in mature sperm of the silkworm are 6.0 and 4.8, respectively. This suggests that the RBE value for mosaics is the same or less than that for complete mutations. The present experimental results indicate that the RBE for dominant lethals is 10.2 and is 1.7 and 2.1 times as high as those of complete and mosaic mutations, respectively. From these comparisons, it can be stated that the nature of genetic damage by radiations for dominant lethals differs significantly from specific-locus mutations. The accumulated data for dominant lethals in *Drosophila* sperm suggest a relatively high figure of RBE between 1.5 and 7.3 (Zimmer 1957; Baker and Von Halle 1954; Mickey 1954; Edington and Randolph 1958; Nakao and Machida 1970), whereas for autosomal and sex-linked recessive lethal mutations the data suggest a relatively low figure of between 0.4 and 3.5 (Ives *et al.* 1954; Mickey 1954; Edington 1956; Zimmer 1957; Edington and Randolph 1958; Dauch *et al.* 1966; Lamb *et al.* 1967; Sobels and Broerse 1970; Nakao and Machida 1970). These findings in *Drosophila* appear to agree, in general, with the present observation in the silkworm.

A slight difference was observed between the two types of specific-locus mutations, i.e., complete and mosaic mutations, suggesting that analogous mechanisms may be involved in these two types of mutations. Tazima and Onimaru (1969) have analyzed the mutational spectra at *pe* and *re* loci of X-irradiated mature sperm in the silkworm and indicated that the complete mutations may have occurred as a result of double-stranded DNA changes of the gene, whereas the mosaics may be due to a mixture of single-stranded DNA changes and chromosomal aberrations. In silkworm sperm, the experiment is still incomplete, preliminary data suggested that almost all radiation-induced mosaic mutations could be transmitted through several DNA replications (Murakami, unpublished data). Thus, it would not seem difficult to conclude that the radiation-induced mosaics in mature sperm, at the doses below 5 kR, are a mixture of predominant DNA strand change mutations and slight chromosomal events, such as a small deficiencies, of the genetic materials. On the other hand, it seems clear that a large RBE value for dominant lethals in the silkworm could be attributed to chromosomal anomalies.

Strictly speaking, little comparable experiments with regard to RBE *versus* LET relations for the induction either of complete mutations or mosaics have been reported in other organisms. Oster (1963) has compared the mutational spectrum of complete and mosaic mutations induced by fast neutrons and by low LET radiations at the *dumpy* (*dp*) locus in *Drosophila* spermatozoa. Based on his data, one can estimate RBE values of fast neutrons for the *dp* locus mutations by assuming that the dose-response curves are linear, regardless of the radiation quality. Thus, based on Oster's data, one can get RBE values of neutrons to X-rays (dose-rate: 300 R/min), which turns out to be 5.2 for

complete mutations and 5.6 for mosaics; whereas those of neutrons to gamma-rays (dose-rate: 3000 R/min) are 2.2 for complete mutations and 2.6 for mosaics. It can be said that there is little difference in the RBE values in *Drosophila* spermatozoa between complete and mosaic mutations. It is of interest to note that these RBE values seem to be the same or less than those of the egg-color-specific-locus mutations in the silkworm sperm. Carlson and Southin (1962) gave evidence that the majority of X-ray induced mutations at *dp* locus of *Drosophila* is gene mutations not associated with multiple breakage events and that mosaics (non-variegated mosaicism) at the same locus usually are free of detectable breakage events.

As can be seen from Table 2, the RBE value for complete mutations of 14 MeV neutrons in mature sperm of the silkworm is about 6. This value is slightly higher than that for mosaics. The RBE value of neutrons for complete mutations in gonial cells is, however, a low figure between 1 and 2 (Murakami and Kondo 1964). In the silkworm, it has generally been accepted that radiation-induced complete or recessive visible mutations are gene mutations (Tazima 1964). Thus, it seems probable that the high RBE values for egg-color-specific-locus mutations cannot always be attributable to gross chromosomal changes. The two different RBE values observed seem to depend on the stage of gametogenesis which indicate different sensitivity of differential stages.

Although numerous data have been reported on sex-linked recessive lethal mutations in *Drosophila*, there are very few comparable experimental data on the RBE for specific-locus mutations. In *Drosophila* sperm treated with fast neutrons from the ORNL cyclotron or from nuclear detonations were approximately four times as efficient in producing specific-locus mutations at the *res* markers as X-rays of 250 kVp (Mickey 1954). Similar figure has been also reported by Ives *et al.* (1954). According to the comment by Zimmer (1957), however, these values should be reduce in half. Even if it was so, it does not change the relative magnitude of RBE values among specific-locus mutations, sex-linked recessive lethal mutations and dominant lethal mutations. It has been widely believed on the basis of accumulated evidence in cytological and genetical analysis of *Drosophila* that the nature of sex-linked recessive lethal mutations are due to deletion and small deficiency (e.g., Lifschytz and Falk 1968). Consequently, even if the RBE value of fast neutrons is a difference between specific-locus and sex-linked recessive mutations, it is probable that a difference of RBE values between these mutations would not be due to the different nature of mutational events. In the latest paper Nakao and Machida (1970) reported, however, that specific-locus mutations of 2.5 MeV neutrons to 200 kVp X-rays is 2.1 and that the RBE for sex-linked recessive lethal mutations is slightly higher than that for specific-locus mutations.

In mice, the RBE value for specific-locus mutations has been estimated to be about 6 in both sperm and spermatogonia (Russell and Kelley 1964; Russell 1965) and the value increases up to 23 or more under chronic irradiations in spermatogonia (Batchelor *et al.* 1966; Searle and Phillips 1964a). By way of precaution, it should be mentioned that there is a consistent picture of RBE values approximately 6 for both dominant lethal and specific-locus mutations in mice post-spermatogonial cells (Russell and Kelley 1964; Russell 1965). As to the nature of specific-locus mutations in mice, Searle and Phillips (1964b) have considered that a part of the radiation-induced mutations was fre-

quently not true point mutations, but really small deficiencies or other chromosomal changes requiring a multiple-hit process. While, Russell (1967) has argued that almost radiation-induced mutations in the specific-locus experiment of post-spermatogonial cells might be double-stranded.

From these connections, it is obvious that the RBE of fast neutrons for specific-locus mutations is different among organisms. Much more data are needed before all aspects of the relationship between RBE value and nature of mutational events are clearly understood.

SUMMARY

Comparisons of RBE values of 14 MeV fast neutrons for the induction of dominant lethals and visible recessive mutations in mature sperm of the silkworm were studied. The main conclusions to be drawn from the results presented are that the RBE of fast neutrons for the dominant lethals (RBE: 10.2) is about 2.1 times that of the mosaics (RBE: 4.8), and the RBE for the mosaics is the same or less than that for the complete mutations (RBE: 6.0).

Considering these findings and others, it seems that the nature of radiation-induced complete and mosaic mutations at the *pe* and *re* loci of the mature sperm of the silkworm, at doses below about 5 kR, could have occurred as a mixture of DNA strand change type mutations and chromosomal type such as deletion and small deficiency, whereas the dominant lethals may correspond to chromosomal changes.

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