RADIATION-INDUCED RECESSIVE VISIBLE MUTATIONS OF OOCYTES DURING MEIOSIS IN THE SILKWORM¹

AKIO MURAKAMI

National Institute of Genetics, Yata 1,111, Misima, Sizuoka-ken 411, Japan

Received May 18, 1970

THERE are relatively few publications regarding radiation-induced recessive mutations in female germ cells, although there are numerous papers on mutagenesis in male germ cells which provide an insight into mechanisms of radiation-induced mutational damage to biological systems.

In the female of *Bombyx mori*, TAZIMA (1964) reviewed his data on the changes in sensitivity of the germ cells to radiation at various developmental stages. According to TAZIMA, there were two peaks in mutation rates, one near the time of hatching and the other at about the fifth instar. The stage when the mutation rate is low has been found to be around the second molt. All germ cells from the early larval stage up to the mid-pupal stage have approximately the same mutation rate, and after this stage the sensitivity suddenly increases. The germ cells at late pupal stages correspond cytologically to prophase I oocytes, therefore, it can be said that most of the mutational studies on silkworm females have been on germ cells prior to the meiotic divisions.

In *Drosophila melanogaster* the radiosensitivity of oocytes varies as a function of stage during oogenesis (KING 1970; KOCH, SMITH and KING 1970). WÜRGLE and his associates (PETERMANN 1968; WÜRGLE 1968; WÜRGLE, PETERMANN and GRAF 1968; GRAF, PIATKOWSKA and WÜRGLE 1969) have shown that the radiosensitivity of oviposited eggs remains constant from anaphase I until completion of meiosis. MULLER (1959) reported that immediately after fertilization, eggs show a very low mutation rate, whereas a high frequency was observed in the cleavage division cells. A similar observation has been reported by ULRICH (1958, 1960).

In the silkworm, information available on such problems is meagre. NAKAO (1958, 1959) reported that the frequency of mutations induced by X rays gradually increases with the progress of the meiotic divisions of oocytes in the post-oviposited eggs.

Extension of the investigations on changes in mutational response to radiation for the meiotic division cells of females in this insect seemed worthwhile, and the experiments to be reported here were conducted for this purpose. This paper presents some of the data reported in earlier preliminary communications (MURAKAMI 1965, 1966c).

¹ Contribution No. 797 from the National Institute of Genetics, Misima, Japan.

MATERIALS AND METHODS

Cytology of oocytes in process of meiotic divisions of the silkworm: The cytology of oogenesis in the silkworm has been described by SATO (1926) and by OHTSUKI and MURAKAMI (1968). Oocytes in ovaries of pupae and adults are in the first meiotic prophase. The first meiotic division proceeds when the sperm enters the egg. When the moths are laying on an egg-card, the eggs are in anaphase I and proceed to telophase I within about 10 min. The second meiotic division begins 60–70 min after oviposition with the formation of the second polar bodies. The syngamy of male and female pronuclei occurs 140–150 min after oviposition at 25°C. The cytological observations reported above were carried out with wild type, the bivoltine silkworm strain C108. All experiments were carried out at 25°C.

Experimental techniques for the estimation of radiation-induced mutation frequencies—Detection of mutants: Mutations at both the *pe* and *re* loci were detected by the specific-locus method. Both these recessive genes control egg color. The normal color is black, *pe/pe* eggs are yellow, and *re/re* eggs are red. Eggs homozygous for both *pe* and *re* are also yellow. According to this method, mutant eggs are detectable by the pigmentation in their serosa cells as either whole-body (complete) or fractional-body (mosaic) mutations.* The number of complete and fractional mutants detected was recorded separately for each experimental group and locus, and the overall frequency of mutations was calculated. Spontaneous frequencies for both types of mutations were also checked in unirradiated controls. For each mutation frequency the 95% confidence intervals were calculated according to the method of FISHER and YATES (1953).

Experiments with prophase I oocytes (pre-oviposition eggs): The eggs used in this experiment were laid by wild-type female moths which had been irradiated and mated with marked male moths homozygous for the *pe* and *re* loci. Seven different doses were given: 500, 1000, 1500, 2500, 5000, 10000, and 15000 roentgens (r). The source of the first three doses was X rays at a dose rate of 1000 r/min and that of the remaining four doses was ¹³⁷Cs gamma rays at a dose rate of 60 r/min.

Experiments with cells from the meiotic division stages to the first mitotic division stage (post-oviposition eggs): Two different methods were employed to detect mutations induced by irradiation of oviposited eggs. In the first case, actively laying wild-type moths were used. After allowing oviposition for one hour at 25°C, eggs were collected and subjected to X-ray treatments. The irradiated individuals were allowed to develop into adults and were then crossed with the genetically marked strain. The X-ray doses given were 500, 1000, and 1500 r with a dose rate of 60 r/min. This method was designed to analyze the transmissibility of radiation-induced mutations in post-oviposited eggs. With this method, already discussed by MURAKAMI and TAZIMA (1965), the size of mutant clusters in the same batch was remarkably large. Therefore, it seems that the frequency of mutations cannot always be exactly estimated. The second experiment utilized the following method in order to exclude the effect of mutant clusters.

The second method is essentially the same as that employed for prophase I oocytes in adults. Eggs laid by wild-type females mated with *pe/pe*; *re/re* males were collected every 15 min and allowed to develop to a defined stage at which they were irradiated with 1000 r of X rays. The exposure rate was 1000 r/min.

RESULTS AND DISCUSSION

Mutation induction by radiation of late prophase I oocytes in adults: The experimental results from irradiation of late prophase I oocytes with gamma rays are summarized in Table 1 and presented in Figure 1. The average mutation rate for the two loci varied with the doses used, since the curves of mutation frequency *vs.* radiation dose are not linear. Figure 1 refers only to whole-body

* These terms, whole- and fractional-body mutations, are used in a sense of wholly and partly mutated eggs for either yellow or red color instead of the normal black color.

TABLE 1

The frequency of egg-color mutations after gamma irradiation of oocytes in moths of the silkworm

(Whole-body mutation)									
Dose (R)	Total no. observed	pe locus		re locus		95 % confidence interval for mutation frequency			
		Mut.detd.	Mut.freq.	Mut.detd.	Mut.freq.	pe locus	re locus		
control	195,502	21	10.7×10^{-5}	8	4.1×10^{-5}	6.6-	17.4	1.5-	9.7
2,500	17,180	36	209.5	35	203.7	151.3-	296.9	151.3-	285.2
5,000	61,102	667	1,091.6	521	852.7	1,009.8-	1,175.1	780.7-	929.6
10,000	79,061	2,907	3,676.9	2,000	2,529.7	3,545.4-	3,823.6	2,422.2-	2,620.3
15,000	64,841	5,182	7,991.9	3,957	6,102.6	7,786.7-	8,198.5	5,919.1-	6,287.7
(Fractional-body mutation)									
control	195,502	7	3.6	0	0.0	1.0-	8.7		-2.6
2,500	17,180	0	0.0	1	5.8		-2.6	0.0-	43.2
5,000	61,102	16	26.2	20	32.7	16.4-	44.2	29.5-	44.9
10,000	79,061	71	89.8	56	70.8	70.8-	115.1	64.5-	83.5
15,000	64,841	209	322.3	187	288.4	280.7-	370.1	269.9-	314.6

mutations. The rate lies between 8 and 53×10^{-7} per roentgen per locus. It is remarkable that the frequency of whole-body mutations is much higher than that of fractionals in irradiated prophase I oocytes. These rates seem to be higher than those of oocytes from late pupae (TAZIMA 1961). I will discuss this point in the next section together with the data of post-oviposition eggs. Here I wish to stress the relation between dose and mutation production.

As seen in Figure 1, the dose-response curves are of the multiple-hit type for both whole-body and fractional-body mutation. TAZIMA (1951) has shown that dose-response curves for egg-color mutations at the w_2 (white-2) locus of pupal prophase I oocytes are linear at relatively low doses of X rays. The difference between his results and mine may be due to the different doses used. To investigate this suggestion, one more experiment was carried out in a dose range from 500 r to 1500 r with the same methods as described above, except for the radiation source. The results, summarized in Table 2 and illustrated in Figure 2, suggest that the frequency *vs.* dose relation for whole-body mutations also increases nonlinearly at the lower doses, although the observed mutation rate seems to be somewhat higher than the expected rate based on the data of Table 1. The frequency *vs.* dose curves of fractional-body mutations also seem to be nonlinear, and appear to plateau in the dose range from 500 r up to 1500 r. These observations are in good agreement with the results from X-rayed oocytes in silkworm pupae reported by TAZIMA (1961) and MACHIDA and NAKAO (1969). It can be concluded that the dose-response curve for egg-color mutations induced by lower LET radiations for late prophase I oocytes is nonlinear over the dose range studied from 500 r to 15,000 r. Similar results have been reported for sex-linked recessive lethal mutations in *Drosophila* oocytes (PARKER 1960).

FIGURE 1.—Frequency *vs.* dose curves for mutations induced in prophase I oocytes after irradiation with gamma rays (dose rate: 60 r/min). Solid and broken thick lines indicate whole-body mutations and fractionals at *pe* locus. Solid and broken thin lines denote whole-body mutations and fractionals at *re* locus.

The most remarkable finding in this experiment is that the frequency *vs.* dose relation for whole-body mutations is nonlinear. The simplest explanation is that whole-body mutations are multiple-hit chromosomal changes, as are sex-linked recessive lethals in *Drosophila* oocytes (HERSKOWITZ and ABRAHAMSON 1955; PARKER 1960). In the silkworm, TAZIMA (1964) has demonstrated that the production of whole-body mutations by irradiation of mature sperm in moths follows a linear dose-response relation up to a dose of 15,000 r, while fractionals gave a nonlinear relation. This finding suggested that the whole-body mutations are due to one-hit events, and that fractionals are due to multiple-hit events. Recently TAZIMA and ONIMARU (1969) suggested, on the basis of their own findings and those of others, that radiation-induced whole-body mutations in mature silkworm spermatozoa arise as changes in both strands of the DNA molecule, while fractionals form a mixed type, including changes involving only one strand of DNA and chromosomal aberrations. According to MULLER, VALENCIA and VALENCIA (1950), increasing doses of X rays fail to increase the frequency of mosaic mutants recovered from late prophase I oocytes of *Drosophila*, and none of the sex-linked recessive lethals analyzed resulted from gross chromosomal rearrangements. In agreement with them, MACHIDA and NAKAO (1969) reported that the relation between production of mutations and X-ray dose in prophase I oocytes of the silkworm is nonlinear, while neutrons gave a linear dose-response curve regardless of the type of mutations. They suggested repair mechanisms active in the low dose ranges to interpret the difference in dose-frequency relations between the two radiations.

TABLE 2

The frequency of egg-color mutations after X irradiation of oocytes in moths of the silkworm

(Whole-body mutation)									
Dose (R)	Total no. observed	<u>pe</u> locus		<u>re</u> locus		95 % confidence interval for mutation frequency			
		Mut.detd.	Mut.freq.	Mut.detd.	Mut.freq.	<u>pe</u> locus		<u>re</u> locus	
control	157,728	16	10.1×10^{-5}	7	4.4×10^{-5}	6.3-	17.1	1.3-	10.8
500	82,152	42	51.1	27	32.9	39.0-	69.4	23.1-	48.7
1,000	88,998	92	103.4	79	88.8	84.3-	128.1	71.9-	111.2
1,500	88,998	222	249.4	166	186.5	219.1-	285.4	160.7-	206.7
(Fractional-body mutation)									
control	157,728	0	0.0	0	0.0		-2.6		-2.6
500	82,152	13	15.8	6	7.3	9.7-	28.0	2.4-	19.5
1,000	88,998	20	22.5	8	9.0	14.6-	36.0	3.4-	21.3
1,500	88,998	19	21.4	6	6.7	13.8-	34.8	2.2-	18.0

FIGURE 2.—Frequency vs. dose curves for mutations induced in prophase I oocytes after irradiation with X rays (180 kVp, 25 mA, 1.0 mm Al filter and dose rate at 1000 r/min). Explanations are the same as for Figure 1.

TABLE 3

The frequency of egg-color mutations after X irradiation of inseminated eggs of 5 to 60 min after oviposition in the silkworm

Dose (R)	Total no. observed	<u>pe</u> locus		<u>re</u> locus		95 % confidence interval for mutation frequency			
		Mut.detd.	Mut.freq.	Mut.detd.	Mut.freq.	<u>pe</u> locus		<u>re</u> locus	
control	121,128	15	12.4×10^{-5}	1	0.8×10^{-5}	6.6-	18.2	0.0-	2.5
500	107,888	699	647.9	278	257.7	600.6-	695.2	227.1-	288.3
1,000	57,865	928	1,603.7	599	1,035.2	1,501.8-	1,705.7	952.2-	1,118.1
1,500	32,356	1,862	5,754.7	827	2,555.9	5,492.0-	6,017.4	2,382-	2,729.0

From these considerations, a tentative interpretation for the nonlinear dose-response curve of whole-body mutations may be assumed to involve two components, a repair mechanism in the low dose range and multiple-hit type chromosome changes in the high dose range.

The different kinetics of frequency *vs.* dose for whole-body mutations between

FIGURE 3.—Frequency *vs.* dose curves for whole-body mutations induced by X rays (180 kVp, 25 mA, 1.0 mm Al filter and dose rate at 60 r/min) as the oocytes proceed from anaphase I to the second meiotic division. By employing Method 1, most mutations recovered were whole-body mutations, but fractionals remained at a spontaneous level. Explanations are the same as for Figure 1.

mature oocytes and sperm may be due to some difference in the state of the genetic material in comparable stages of oogenesis and spermatogenesis.

Mutation induction by radiation in cells from the meiotic division stages to the first cleavage division stage:

The results of experiments employing Method 1 are summarized in Table 3 and in Figure 3. The mutation frequency induced by radiation in the oocytes proceeding from anaphase I to the second meiotic division increases more steeply than the first power of the dose over the range studied. This observation does not differ from that on the prophase I oocytes. Almost all mutations recovered by this method were whole-body mutations. The frequency of mutations for this class of oocytes was approximately seven times higher than that found for prophase I oocytes in adults. It was also found that the mutation frequency *vs.* dose relation studied by Method 1 for 3.5 hr eggs (which cytologically correspond to the first cleavage stage) is nonlinear, and the mutation frequency was the same or less than that of oocytes in post-oviposition eggs (MURAKAMI and TAZIMA 1965).

Method 1 was originally designed to determine the transmissibility of radiation-induced mutations in both mature oocytes and early embryos. In this method, as has been described earlier, the size of mutant clusters in the same batch was remarkably large. Hence it appears that the frequency of mutations has been overestimated.

The other experiment used Method 2 in order to get a more reliable estimate of mutation frequency. The oocytes were irradiated with only one dose of 1000r X rays at a dose rate of 1000 r/min. Mutations recovered by this method were of both types: whole-body and fractional-body mutations. Results of the experiment are presented in Table 4. Figure 4, which was constructed from Tables 2 and 4, demonstrates that the total mutation frequencies of whole-body mutations and fractionals stayed constant throughout the meiotic division of oocytes in the pre- and post-oviposition eggs. This finding may be interpreted by the assumption that no DNA synthesis is operating in these oocytes from prophase I to near the end of the second meiotic division. Similar observations were reported in *Drosophila* by WÜRGLE and his associates (PETERMANN 1968; WÜRGLE 1968; WÜRGLE, PETERMANN and GRAF 1968; GRAF, PIATKOWSKA and WÜRGLE 1969). The mutation frequency then increases from the end of the second meiotic division until the first cleavage division, as has been reported in *Drosophila* (MULLER 1959) and in *Dahlbominus fuscipennis* by BALDWIN (1965). In the male germ cells, the spermatid chromosomes are much more mutagenically sensitive to radiation than chromosomes of any other stage of spermatogenesis in both the silkworm (TAZIMA 1958, 1961) and *Drosophila* (MULLER 1959). From these considerations, I speculate it is possible that ootid nuclei immediately after the second meiotic division are mutagenically comparable to spermatids. The high radiosensitivity of this stage may be explained as follows: first, an absence of effective mechanisms to repair genetic damage; and secondly, a rapid progress of the nucleus into mitotic divisions. In other words, the high radiosensitivity may be interpreted as due to a lack of sufficient time to repair premutational damage. A similar thought may be extended to the extremely high muta-

TABLE 4

The frequency of egg-color mutations after irradiation of egg samples collected over 15 min and X irradiated (1000r) at different stages from anaphase of meiosis I until the first cleavage division cell

(Whole-body mutation)		<u>pe</u> locus		<u>re</u> locus		95 % confidence interval for mutation frequency			
Egg age (min)	Total no. observed	Mut.detd.	Mut.freq.	Mut.detd.	Mut.freq.	<u>pe</u> locus		<u>re</u> locus	
control	157,728	16	10.1×10^{-5}	7	0.0×10^{-5}	6.3-	17.1	1.3-	10.8
0-25	47,526	47	98.9	19	40.0	75.7-	132.6	25.2-	65.2
30-45	53,176	39	73.3	14	26.3	54.5-	101.5	16.9-	45.1
65-80	47,871	21	43.9	14	29.2	29.2-	68.9	18.8-	50.1
95-110	48,960	19	38.8	17	34.7	24.5-	63.3	22.5-	57.2
135-150	71,638	23	32.1	14	19.5	22.3-	48.9	12.6-	33.5
165-180	44,820	11	24.5	3	6.7	13.4-	46.9	0.7-	24.5
(Fractional-body mutation)									
control	157,728	0	0.0	0	0.0	-2.6		-2.6	
0-25	47,526	19	40.0	8	16.8	25.2-	65.2	6.3-	40.0
30-45	53,176	20	37.6	9	16.9	24.4-	60.2	5.6-	37.6
65-80	47,871	21	43.9	12	25.1	29.2-	68.9	14.6-	46.0
95-110	48,960	58	118.5	30	61.3	91.9-	155.2	42.9-	90.0
135-150	71,638	143	199.6	124	173.1	170.3-	235.9	142.4-	210.8
165-180	44,820	151	336.9	102	227.6	287.8-	397.1	183.0-	278.9

tion frequency characterizing the first cleavage division cells. As has been reported in the female silkworm, it takes about 60 min from the end of the second meiotic division to the first mitotic division, and it takes 50-60 min for each mitotic cycle thereafter at 25°C (OHTSUKI and MURAKAMI 1968).

As seen in Table 4 and in Figure 4, the cell after fertilization is more sensitive to mutation production by radiation than at any other stage throughout gametogenesis in the female silkworm, as in the female *Drosophila* (ULRICH 1958, 1960; MULLER 1959). In the silkworm, according to NAKAO (1959), the mutability of irradiated meiotic oocytes gradually increases with age of the egg, but mutability of eggs from 120 to 200 min after oviposition varied with the doses applied. It is therefore necessary to carry out more precise investigations in order to determine the period of maximum sensitivity.

The cells in 0-80 min eggs correspond cytologically to the meiotic stages from the later half of the first to the second meiotic division and are roughly comparable to the stages studied by Method 1. The average frequency estimated by Method 2 in these stages is significantly lower than that obtained by Method 1. This difference may result from the cluster effect.

Comparison of spectra of radiation-induced mutations during meiosis in oocytes: I observed a difference in mutational spectra of whole-body vs. fractional-body mutations with the progress of the meiotic divisions (Figure 5). This figure comes from Method 2. It can be deduced from Figure 5 that the ratio of whole-body mutations to fractionals gradually decreases with age of the eggs. The ratio reaches unity in irradiated oocytes at an early second meiotic division stage

FIGURE 4.—The frequency of mutations after irradiation of egg samples collected over 15 min and X irradiated at different stages from anaphase of meiosis I until the first cleavage division. X rays were applied with one dose of 1000r and a dose rate of 1000 r/min. Thick and thin lines denote the total mutation frequencies of whole-body mutations and fractionals at the *pe* and *re* loci, respectively.

and then increases to a ratio similar to that observed in mature sperm (MURAKAMI 1966a; TAZIMA and ONIMARU 1969). In the male silkworm, during the period between the entrance of the sperm and the fusion of the two pronuclei, a sharp increase in the frequency of fractional mutations was observed while the frequency of whole-body mutants remained almost unchanged (MURAKAMI 1966b.) Ito (1967) explained this finding by assuming that gamma rays produce a fairly random distribution of ionizations, and that the swelling of the sperm nucleus separates the paired complementary DNA strands. According to cytological studies, the ootid nucleus gradually increases in size between oviposition and the pronuclear stage, as in other organisms (OHTSUKI and MURAKAMI 1968). It is reasonable to interpret the present finding in oocytes in the same way. A similar idea at the chromatid level was proposed to interpret a differential stage sensitivity for dominant lethal mutations in *Drosophila* oocytes (KOCH, SMITH and KING 1970).

As seen in Figure 5, an extremely high frequency of fractionals as compared to whole-body mutations could be observed in cells around the first cleavage division stage. However, this extremely high sensitivity may result from an increase in the number of targets (nuclei) and from rapid DNA replication during the extremely rapid cleavage divisions. It should be kept in mind, therefore, that the mechanisms operating in this stage are essentially different from that in oocytes.

In addition, there was a marked difference in the radiation-induced mutational spectra detected by Methods 1 and 2. In Method 1, we could not detect fractional-

FIGURE 5.—Changes in the mutation response of meiotic cells in the female silkworm. The egg samples were collected every 15 min and allowed to develop for a definite duration until they were irradiated with the constant dose of 1000r of X rays. Explanations are the same as for Figure 1.

body mutations. On the other hand, two types of mutations, whole-body and fractional-body mutations, could be detected by Method 2. This difference may be explained by assuming that changes in a single strand of DNA in the cells of prereplication stages are converted into changes involving both strands by passing through a number of replication cycles. On the other hand, both types of DNA changes in the cells at postreplication stages would be directly fixed as fractionals and whole-body mutations, respectively. As can be understood from the procedures for the detection of genetic changes, these can be recovered in F_2 and F_1 eggs by Methods 1 and 2, respectively. By employing Method 1, therefore, the cells showing genetic damage should have passed through one round of gametogenesis. Accordingly, whole-body mutants recovered by Method 1 probably arose from both types of DNA changes, whereas whole- and fractional-body mutations detected by Method 2 may have originated from double- and single-stranded DNA changes, respectively.

Some fractional-body mutations detected in oocytes may result from the multiple-hit chromosomal damage, as in spermatozoa (TAZIMA and ONIMARU

1969). If this is the case, part of the multiple-hit type of genetic damage would not be detected by Method 1 because of the elimination of damaged chromosomes passing through gametogenesis. Some contribution from multiple-hit events should not be ruled out, at least in part, in the formation of a few of the fractionals detected by Method 2. We must consider the possibility of a delayed effect of mutation fixation.

The author is grateful to Dr. Y. TAZIMA for his valuable suggestions throughout the course of the present work. The author wishes to acknowledge with thanks his indebtedness to Prof. ROBERT C. KING for his kindness in reading this manuscript and offering his invaluable comments.

SUMMARY

The mutational effects of X rays and ^{137}Cs gamma rays on the germ cells of silkworm females during the meiotic divisions were investigated. The specific-locus method was used, estimating the incidence of mutations at two loci influencing egg color. Nonlinear curves of mutation frequency *vs.* radiation dose were observed for oocytes. No significant difference in mutability was found in oocytes from prophase I to the middle of the second meiotic division but after these periods the frequency of mutations gradually increased with age. Oocytes late in the second meiotic division showed a higher sensitivity to mutation production than did oocytes in any other stage. However, the mutation sensitivity of cells near the first cleavage stage was higher than that of any other cell stage throughout the whole life cycle of this insect. It was also observed that the frequency pattern of fractionals and whole-body mutations induced by radiation changed as the maturation divisions of the oocytes progressed.

LITERATURE CITED

- BALDWIN, W. F., 1965 Radiosensitivity of female germ cell stages of *Dahlbominus*. *Mutation Res.* **2**: 530-533.
- FISHER, R. A. and F. YATES, 1953 *Statistical Tables for Biological, Agricultural, and Medical Research*. Oliver and Boyd, London.
- GRAF, U., B. PIATKOWSKA and F. E. WÜRGLE, 1969 X-ray-induced recessive lethals in newly inseminated eggs of *Drosophila melanogaster*. *Mutation Res.* **7**: 385-392.
- HERSKOWITZ, I. H. and S. ABRAHAMSON, 1955 The effect of X-ray intensity on the dose rate of sex-linked recessive lethal mutations induced by treatment of *Drosophila* oocytes. *Drosophila Inform. Serv.* **29**: 105.
- ITO, T., 1967 Notes on the molecular aspects of mutation events. *Sci. Report Tohoku Univ., Ser. IV (Biol.)* **33**: 265-280.
- KING, R. C., 1970 *Ovarian Development in Drosophila melanogaster*. Academic Press, New York.
- KOCH, E. A., P. A. SMITH and R. C. KING, 1970 Variations in the radiosensitivity of oocyte chromosomes during meiosis in *Drosophila melanogaster*. *Chromosoma* **30**: 98-108.
- MACHIDA, I. and Y. NAKAO, 1969 Comparison of production of mutations by X rays and neutrons in female silkworm pupae. (Abstr.) Records 39th Ann. Meeting Japan. Sericultural Soc. (Tokyo).

- MULLER, H. J., 1959 Advances in radiation mutagenesis through studies on *Drosophila*. pp. 146-160. In: *Progress in Nuclear Energy*, Ser. VI, Vol. 2 (Biol. Sci.). Pergamon Press, London.
- MULLER, H. J., J. I. VALENCIA and R. M. VALENCIA, 1950 The production of mutations at individual loci in *Drosophila* by irradiation of oocytes and oogonia. (Abstr.) *Genetics* **35**: 126.
- MURAKAMI, A., 1965 Radiation-induced mutational responses during meiosis of germ cells in the female silkworm. (Abstr.) *Japan. J. Genet.* **40**: 406. —, 1966a Relative biological effectiveness of 14 MeV neutrons to gamma rays for inducing mutations in mature sperm of the silkworm. *Japan. J. Genet.* **41**: 17-26. —, 1966b Radiation-induced mutational responses of sperm in eggs of the silkworm. (Abstr.) *Japan. J. Genet.* **41**: 473-474. —, 1966c Cell division cycle and radiosensitivity in the early developmental eggs of the silkworm (in Japanese). *Kyoto* **1**: 29-42.
- MURAKAMI, A. and Y. TAZIMA, 1965 Induction of visible recessive mutations by X radiation in silkworm germ cells at the pre-embryonic stage. *Ann. Report Natl. Inst. Genet. (Japan)* **15**: 121-123.
- NAKAO, Y., 1958 X-ray-induced visible mutation rates at three stages immediately after oviposition in the silkworm. (Abstr.) *Radiation Res.* **9**: 158-159. —, 1959 X-ray-induced visible mutation rates at various stages after oviposition in the silkworm. II: (Abstr.) *Japan. J. Genet.* **35**: 280.
- OHTSUKI, Y. and A. MURAKAMI, 1968 Nuclear division in the early embryonic development of the silkworm, *Bombyx mori* L. (in Japanese with English summary). *Zool. Magazine (Tokyo)* **77**: 383-387.
- PARKER, D. R., 1960 The induction of recessive lethals in *Drosophila* oocytes. *Genetics* **45**: 135-138.
- PETERMANN, U. B., 1968 Mutationsraten und Sterblichkeiten nach Röntgenbestrahlung früher Entwicklungsstadien von *Drosophila melanogaster*. *Mutation Res.* **5**: 397-410.
- SATO, H., 1926 On the maturation of the fertilized egg and the behavior of the polar bodies in the silkworm (in Japanese with English summary). *J. Sci. Agric. Soc. Japan* **274**: 232-238.
- TAZIMA, Y., 1951 Radiobiological studies on the silkworm. I. X-ray irradiation during the pupal stage and sensitivity of germ cells (in Japanese with English summary). *Papers Coord. Committee Res. Genet.* **2**: 153-162. —, 1958 Mutational response pattern of silkworm germ cells to X rays. (Abstr.) *Proc. 10th Intern. Congr. Genet.* **2**: 291-292. —, 1961 Considerations on the changes in observed mutation rates in the silkworm after irradiation of various stages of gametogenesis. *Japan. J. Genet.* **36** (Supl.): 50-64. —, 1964 *The Genetics of the Silkworm*. Logos Press, London.
- TAZIMA, Y. and K. ONIMARU, 1969 Frequency pattern of mosaic and whole-body mutants induced by ionizing radiations in postmeiotic cells of the male silkworm. *Mutation Res.* **8**: 177-190.
- ULRICH, H., 1958 The mutagenic action of X rays on uncleaved *Drosophila* eggs and its dependence upon oxygen. (Abstr.) *Proc. 10th Intern. Congr. Genet.* **2**: 298-299. —, 1960 Die Beziehung zwischen Strahlendosis und Mutationsrate bei Röntgenbestrahlung von *Drosophila* Zygoten. *Rev. Suisse Zool.* **67**: 287-295.
- WÜRLER, F. E., 1968 Induced mutations and lethality in *Drosophila* after X irradiation of meiotic and postmeiotic stages of the egg. *Intern. Atomic Energy Agency (Vienna) Publ.* **173**: 43-61.
- WÜRLER, F. E., U. B. PETERMANN and U. GRAF, 1968 X-ray-induced sex-linked recessive lethals in inseminated *Drosophila* eggs. *Records 6th Ann. Meeting European Soc. Radiation Biol. (Interlaken, Switzerland)*.