

Effects of Ultraviolet Rays on the Sperm of *Rana japonica*

II. Chromosome Aberrations Induced by UV-irradiation

By

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(With 10 Text-figures)

INTRODUCTION

In Part I of the present study, NISHIOKA, OKUMOTO and KONDO (1981) reported that in the Japanese brown frog, *Rana japonica*, the longer or shorter than 10 seconds the period of UV-irradiation of sperm at 24 erg/mm²/sec, the higher was the survival rate including both normal and abnormal individuals at the hatching stage. When the period of irradiation was increased, normal diploids rapidly decreased in number, while gynogenetic haploids gradually increased. When eggs were inseminated with sperm irradiated for 30, 40 and 60 seconds, 91%, 99% and 100% of the survivals at the hatching stage were haploids, respectively. It is very probable that a small UV dose damaged completely or incompletely the multiplying function of a few chromosomes of some spermatozoa, while a large dose completely destroyed the function of all the haploid chromosomes.

In contrast to such a probability, POGANY (1971) observed in *Rana pipiens* that embryos exposed to a low irradiation dosage were most severely affected in spite of their diploid constitution. Although he (1976) subsequently confirmed that the most impaired survival was accompanied with aneuploid chromosomal conditions, he recognized a relatively long period of time at lower doses during which the exposure to UV was attended by highly abnormal growth without a loss of chromosomes.

It seemed interesting to the present authors to examine the existence of UV doses which would induce abnormal embryonic development without being accompanied by chromosome aberrations. In order to clarify this in the Japanese brown frog, *Rana japonica*, they inseminated eggs with sperm which had been irradiated with low UV doses and observed the developmental capacity and chromosome aberrations of embryos and tadpoles raised from these eggs. The results of these experiments will be reported here.

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MATERIALS AND METHODS

Male and female *Rana japonica* GÜNTHER were collected in the suburbs of Hiroshima. Eggs were obtained from 15 females by pressing their abdomen shortly after natural ovulation. The eggs of each female were divided into several parts and inseminated as soon as possible with irradiated or non-irradiated sperm. Sperm suspension was made from each male by crushing the testes in 5 ml of Cl-free tap-water. Before irradiation, 0.5 ml of sperm suspension was poured into a vial bottle which was 2 cm in both diameter and height and had a flat bottom. Then, the bottle was placed under an ultraviolet lamp (GUL-5-J type, Toshiba KK) operated at 125 mA at a distance of 28.0 cm from the lamp. The UV-ray generated from this lamp was 2537 Å in main wave length and 16 erg/mm²/sec in energy level.

While one part of the eggs of each female was inseminated with non-irradiated sperm as the control, three other parts were inseminated with sperm irradiated for 3, 5 and 8 seconds which had been known from a preliminary examination to cause about 25%, 50% and 75% lethality at the hatching stage, respectively. In addition to these parts, another part was inseminated with sperm irradiated for 10 seconds and four others were inseminated with sperm irradiated for 15, 20, 40 and 60 seconds, respectively. The eggs of one female were always exclusively inseminated with sperm of a single male.

Embryos and young tadpoles were reared in PETRI dishes 18 cm in diameter which contained Cl-free tap-water. Tadpoles at the age of more than 20 days were reared in enameled iron-vessels which were 23 cm × 34 cm × 5 cm in size. Tadpoles were fed on boiled spinach.

Chromosome preparations were made by the squash method with water pre-treatment (MAKINO and NISHIMURA, 1952). Abnormal embryos and tadpoles were put in colchicine solution (200 mg/liter) for 10~15 hours immediately before death and then squashed. The chromosomes of viable tadpoles produced from three females were examined in their tail-tips at TAYLOR and KOLLROS' stage X when they were 30~40 days old. While the viable tadpoles produced from one of the three females were put in colchicine solution before removing their tail-tips, those produced from the other two females were not treated with colchicine. The latter tadpoles were reared until the completion of metamorphosis in order to observe their morphological characteristics.

The developmental stages reported in this paper follow those of *Rana pipiens* established by SHUMWAY (1940) and TAYLOR and KOLLROS (1946) for convenience sake.

OBSERVATION

I. Viability and morphology

When survivals including normal and abnormal individuals raised from eggs of 15 females by inseminating with sperm irradiated for 3~60 seconds were examined at the hatching stage, the proportions of them to the respective total number of eggs were 83%, 80%, 55%, 32%, 17%, 8%, 5%, 69% and 92% in the control and eight experimental series derived from sperm irradiated for 3, 5, 8, 10, 15, 20, 40 and 60 seconds, respectively. It was found that the survival ratio was the lowest by exposure for 15 or 20 seconds and rapidly recovered by exposure for 40 seconds. The HERTWIG effect was evident in this case.

Normal, diploid-shaped individuals distinctly decreased in number with increase in dosage. While 92% of the total individuals was such in the control series, 57%, 32%, 7%, 7% and 2% were those in the series exposed for 3, 5, 8, 10 and 15 seconds, respectively. There were no normally diploid-shaped individuals in the series exposed for 20, 40 and 60 seconds. In contrast to them, abnormal individuals other than haploid-shaped ones rapidly increased in number as the UV dosage was increased, and they attained the maximum by exposure for 10 or 15 seconds. The earliest abnormality was incomplete gastrulation from which exogastrulae or hemi-embryos were produced. At the tail-bud stage, abnormal embryos such as those with spina bifida, acephalia, ascites, warty sur-

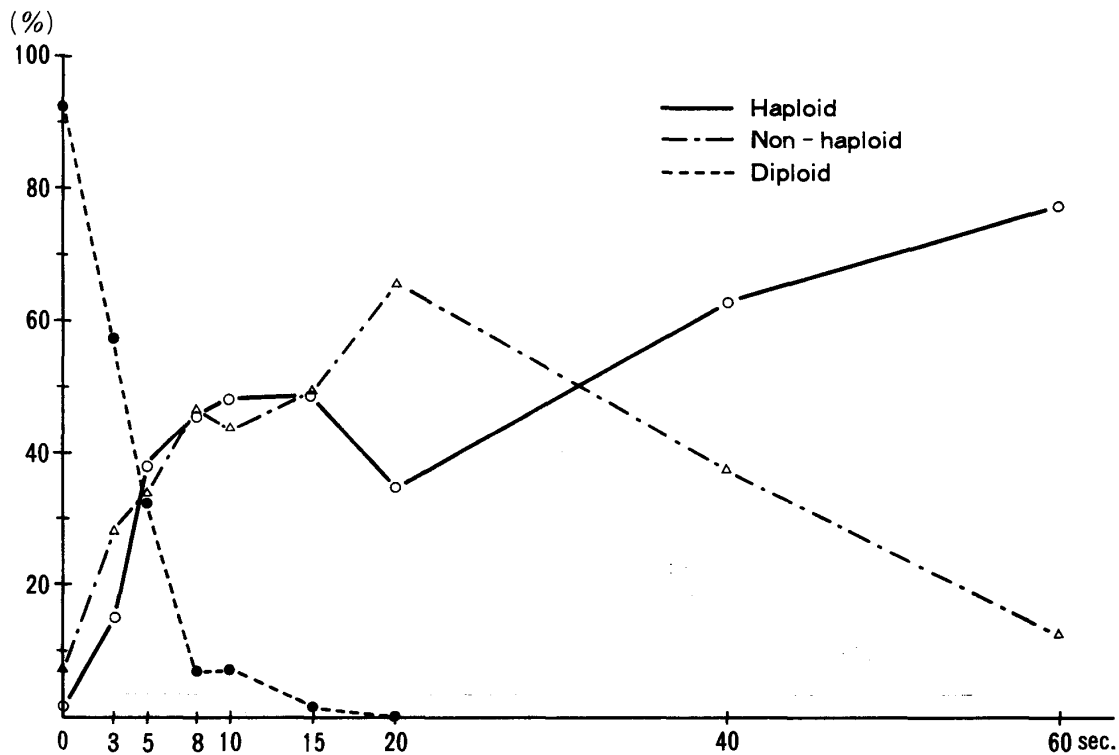


Fig. 1. Distribution curves of various types of embryos and tadpoles.

faces, shortened trunk or shrunk tail appeared abundantly in the series of sperm irradiated for 5~40 seconds. At the feeding tadpole stage, there were some individuals in the series exposed for 3~15 seconds whose body was edematous, underdeveloped or twisted. These abnormal tadpoles were more numerous in the series of sperm irradiated for a period shorter than those in the series of sperm irradiated for a longer period. Embryos of typically haploid shape began to appear in the series of sperm irradiated for 3 seconds and gradually increased in number with increase in UV dosage. In the series of sperm irradiated for 40 and 60 seconds, 63% and 88% of the respective number of normally cleaved eggs were of typically haploid shape (Fig. 1).

II. Chromosomes

Chromosomes were examined in embryos and tadpoles raised from eggs of three females. The number of analyzed mitoses in each individual was usually 4~7. All the individuals were divided into two categories, viable and inviable.

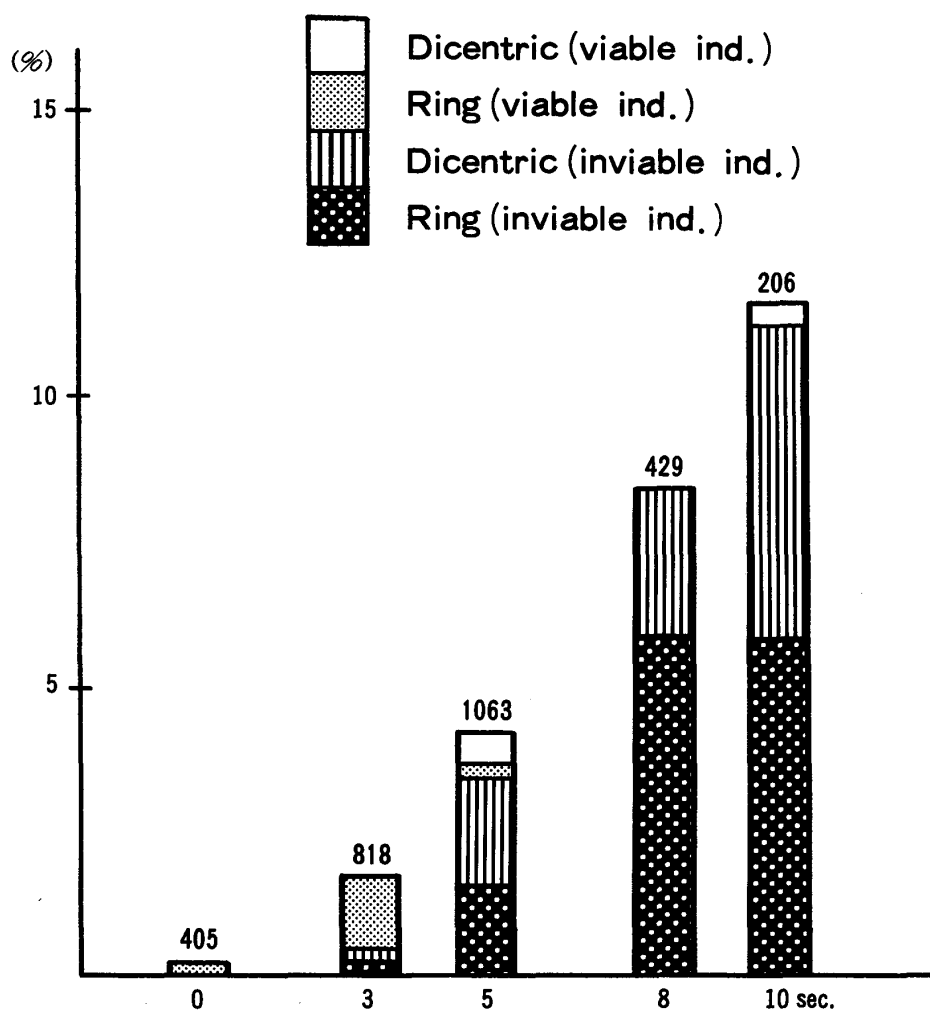


Fig. 2. Frequencies of dicentric and ring chromosomes in viable and inviable individuals raised from UV-irradiated sperm.

The viable individuals were tadpoles which lived for more than 40 days after hatching, while the inviable ones were those which were abnormal at the tail-bud, hatching or earliest feeding tadpole stage. The mitoses of inviable individuals were mostly aneuploid, being intermediate between 13 and 26 in chromosome number. Besides, they frequently contained one or more dicentric or ring chromosomes. Viable individuals often contained hyper- or hypodiploid mitoses in addition to diploid ones. There were also one or more mitoses having a dicentric or ring chromosome in a few viable individuals. When chromosomes were examined in many individuals raised from eggs inseminated with irradiated sperm, a definite interrelation was evident between UV dosage and chromosome aberrations in both viable and inviable individuals (Fig. 2).

1. Inviability individuals

Three groups (1~3) of inviable individuals raised from eggs of three females (Nos. 1~3) were examined in terms of chromosomes. Frequency distributions of various chromosome numbers were pursued in each experimental series of each group. The mean and median values of chromosome numbers in each experimental series were also calculated.

a. Group 1

Eggs of female No. 1 were inseminated with sperm irradiated for 3, 5 or 10 seconds. Frequency distributions of various chromosome numbers in three

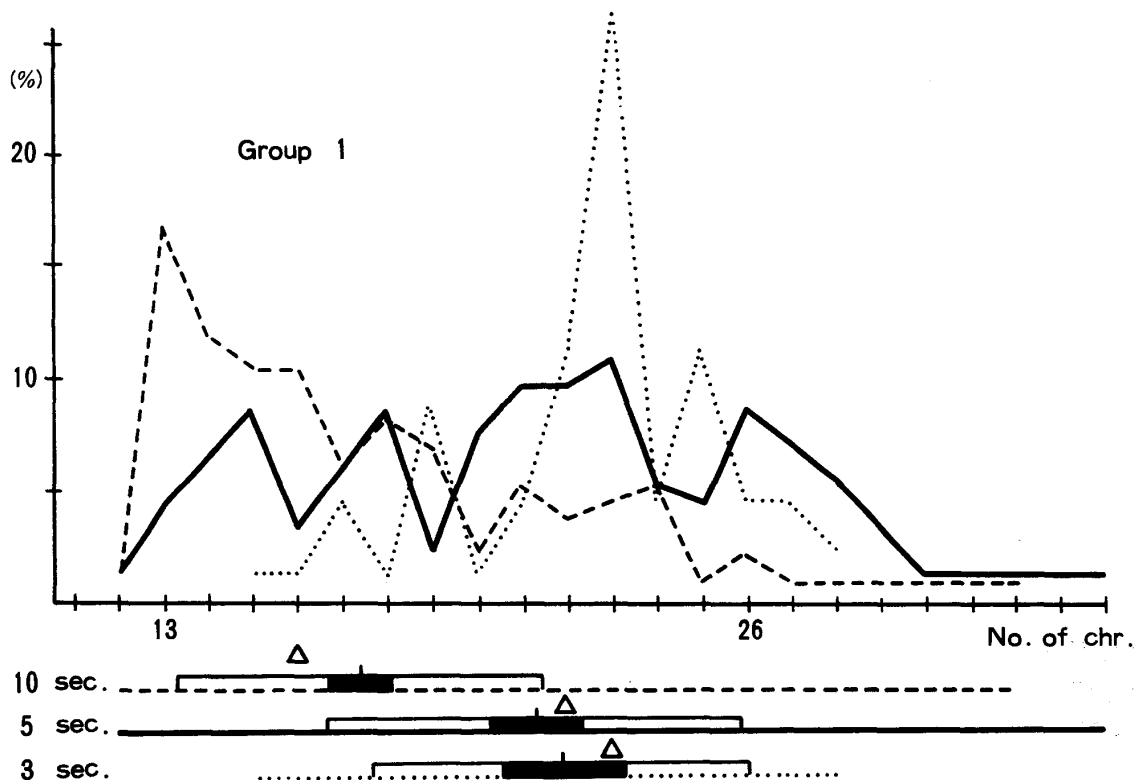


Fig. 3. Frequency distributions of various chromosome numbers in inviable individuals raised from UV-irradiated sperm in group 1 of experimental series.

experimental series are shown in Fig. 3. In the series of sperm exposed to UV for 3 seconds, the chromosome numbers of 45 mitoses from 12 inviable individuals had a very high peak at 23 and were almost bisymmetrically arranged. They were also 23 in median value. In addition to the chromosome numbers shown in Fig. 3, 34 and 50 chromosomes were counted in two mitoses. There were three dicentric and two ring chromosomes in the 45 mitoses. It was remarkable that diploid mitoses were extremely lower in frequency than hypodiploid ones. In the series of sperm exposed for 5 seconds, the chromosome numbers of 93 mitoses from 26 individuals had a peak in the range including 21, 22 and 23 and were almost bisymmetrically arranged. They were 22 in median value. In addition to the chromosome numbers shown in Fig. 3, 36 chromosomes were counted in two mitoses, while 38, 42, 46 and 51 chromosomes were counted in four mitoses. There were five dicentric and five ring chromosomes in the 93 mitoses. In the series of sperm exposed for 10 seconds, the chromosome numbers of 136 mitoses from 43 individuals had a considerably high peak at the haploid number (13) and gradually diminished in frequency toward the larger chromosome numbers. They were 16 in median value and mostly distributed between 12 and 30. However, in addition to the chromosome numbers shown in Fig. 3, 40, 41 and 48 chromosomes were counted in three mitoses. There were 11 dicentric and 12 ring chromosomes in the 136 mitoses. It was noteworthy that haploid mitoses appeared in a high frequency though they were not found in inviable individuals of the series of sperm exposed for 3 seconds.

The mean values of chromosome numbers in the three experimental series of sperm exposed for 3, 5 and 10 seconds were 21.9 ± 1.3 , 21.3 ± 1.0 and 17.4 ± 0.7 , respectively. It is remarkable that the mean value of chromosome numbers became smaller with increase in UV dosage, although there was no significant difference between the series of sperm exposed for 3 seconds and that of sperm exposed for 5 seconds.

b. Group 2

Eggs of female No. 2 were inseminated with sperm irradiated for 3, 5 or 8 seconds. Frequency distributions of various chromosome numbers are shown in Fig. 4. In the series of sperm exposed for 3 seconds, the chromosome numbers of 47 mitoses from nine individuals had a very high peak at 23 and were almost bisymmetrically arranged between 18 and 29. They were also 23 in median value. Diploid mitoses were remarkably low in frequency as compared with those of 23 chromosomes. No haploid mitoses were found in this series. Neither dicentric nor ring chromosome was also found in the 47 mitoses. In the series of sperm exposed for 5 seconds, the chromosome numbers of 273 mitoses from 42 individuals had a peak in the range including 21, 22 and 23 and were almost bisymmetrically arranged. They were 21 in median value. In addition to the chromosome numbers shown in Fig. 4, 35, 36, 37, 38 and 46 chromosomes were counted in five mitoses. There were 13 dicentric and six ring chromosomes in the 273 mitoses. In the series of sperm exposed for 8 seconds, the chromosome

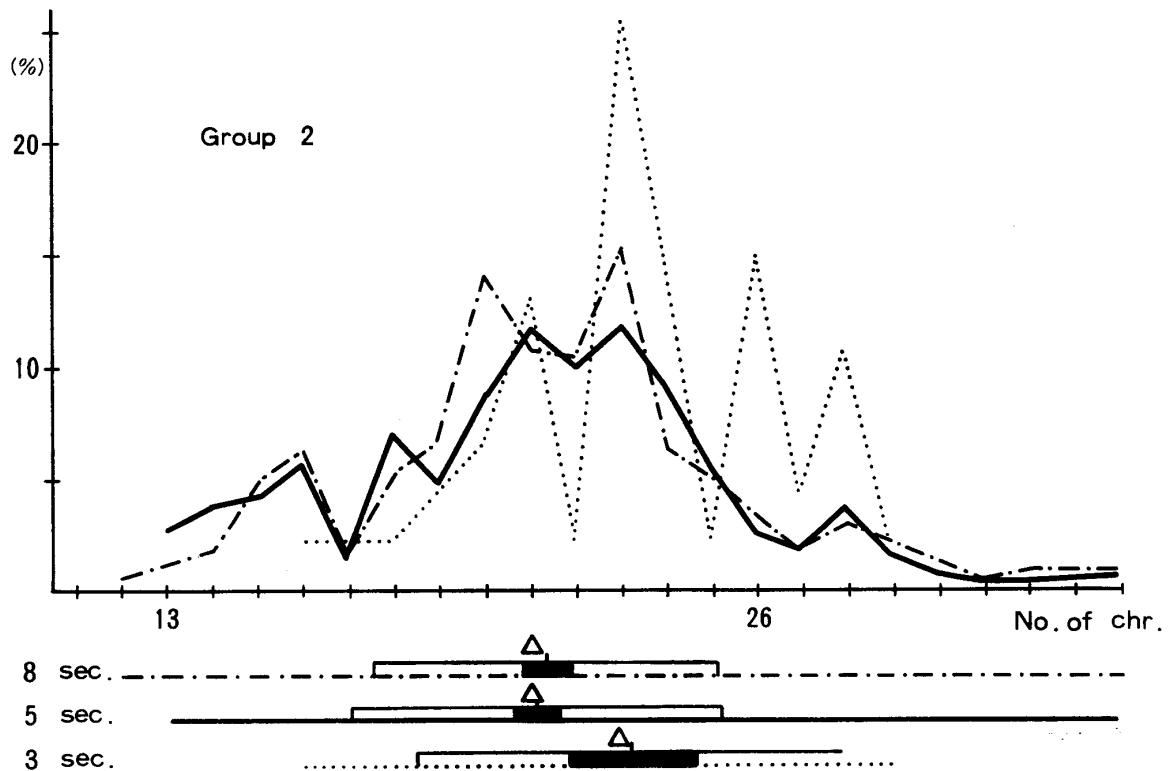


Fig. 4. Frequency distributions of various chromosome numbers in inviable individuals raised from UV-irradiated sperm in group 2 of experimental series.

numbers of 233 mitoses from 47 individuals had a peak in the range including 20, 21, 22 and 23 and were almost bisymmetrically arranged. They were 21 in median value as those in the series of sperm exposed for 5 seconds. In addition to the chromosome numbers shown in Fig. 4, 35, 36, 38, 41 and 45 chromosomes were counted in five mitoses. There were seven dicentric and 18 ring chromosomes in the 233 mitoses.

The mean values of chromosome numbers in the three experimental series of sperm exposed for 3, 5 and 8 seconds were 23.3 ± 1.4 , 21.2 ± 0.5 and 21.4 ± 0.5 , respectively. It was evident that the mean value of chromosome numbers became smaller as the dose of irradiation was increased as observed in group 1 of experimental series, although there was no difference in this respect between the series of sperm exposed for 5 seconds and that of sperm exposed for 8 seconds.

c. Group 3

Eggs of female No. 3 were inseminated with sperm exposed to UV for 3, 5 or 8 seconds. Frequency distributions of various chromosome numbers are shown in Fig. 5. In the series of sperm exposed for 3 seconds, the chromosome numbers of 87 mitoses from 11 individuals had a peak in the range including 22, 23 and 24, were mostly arranged between 17 and 25 and were 22 in median value. In addition to the chromosome numbers shown in Fig. 5, 43 chromosomes were counted in one mitosis. Neither dicentric nor ring chromosomes were found in the 87 mitoses. In the series of sperm exposed for 5 seconds, the chromosome numbers

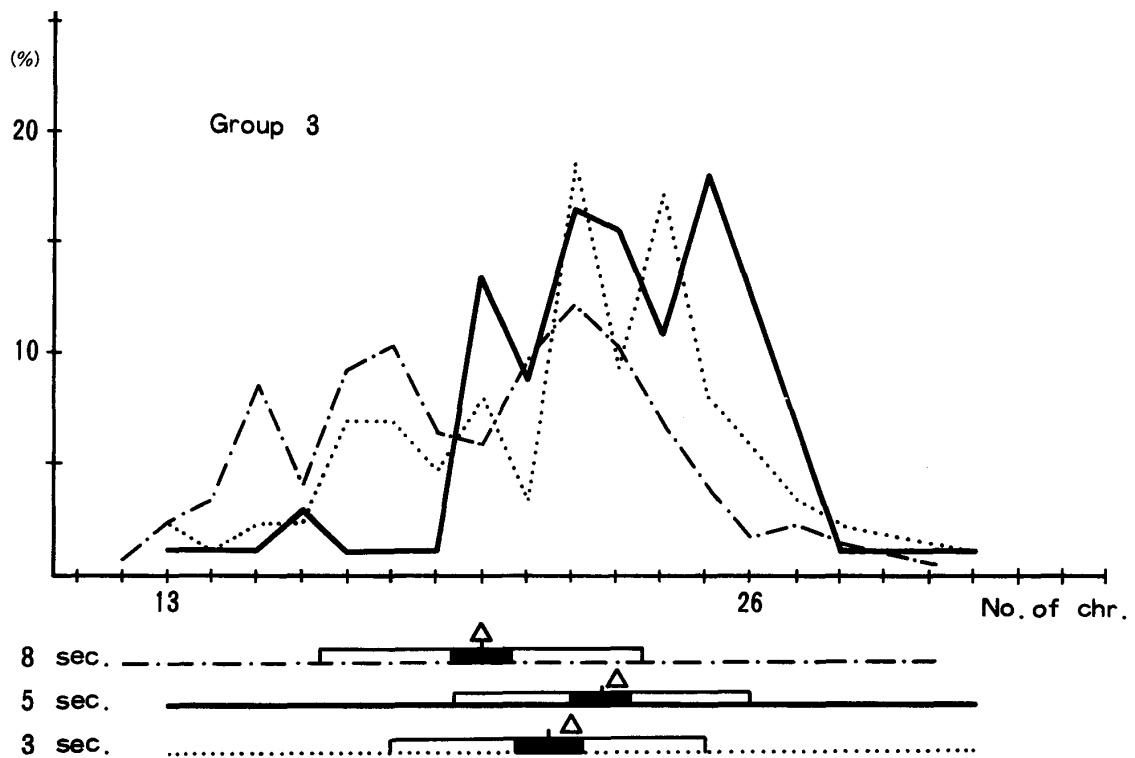


Fig. 5. Frequency distributions of various chromosome numbers in inviable individuals raised from UV-irradiated sperm in group 3 of experimental series.

of 131 mitoses from 22 individuals had a peak in the range including 22, 23, 24 and 25, were mostly arranged between 20 and 26 and were 23 in median value. In addition to the chromosome numbers shown in Fig. 5, 36 and 37 chromosomes were counted in two mitoses. There were two dicentric and five ring chromosomes in the 131 mitoses. In the series of sperm exposed for 8 seconds, the chromosome numbers of 181 mitoses from 26 individuals had a peak at 22, were mostly arranged between 15 and 25 and were 20 in median value. In addition to the chromosome numbers shown in Fig. 5, 46 chromosomes were counted in one mitosis. There were four dicentric and seven ring chromosomes in the 181 mitoses.

The mean values of chromosome numbers in the three experimental series of sperm exposed for 3, 5 and 8 seconds were 21.5 ± 0.8 , 22.7 ± 0.7 and 20.0 ± 0.6 , respectively.

d. Three groups

Frequency distributions of various chromosome numbers observed in the total sum of the inviable individuals belonging to the above three groups are shown in Fig. 6. In the series of sperm exposed for 3 seconds, the chromosome numbers of 180 mitoses from 32 individuals had a high peak in the range including 22, 23 and 24 and were almost bisymmetrically arranged. Their median value was 23. In the series of sperm exposed for 5 seconds, the chromosome numbers of 497 mitoses from 90 individuals had a comparatively low peak in the range including 20~25 and were almost bisymmetrically arranged. The median

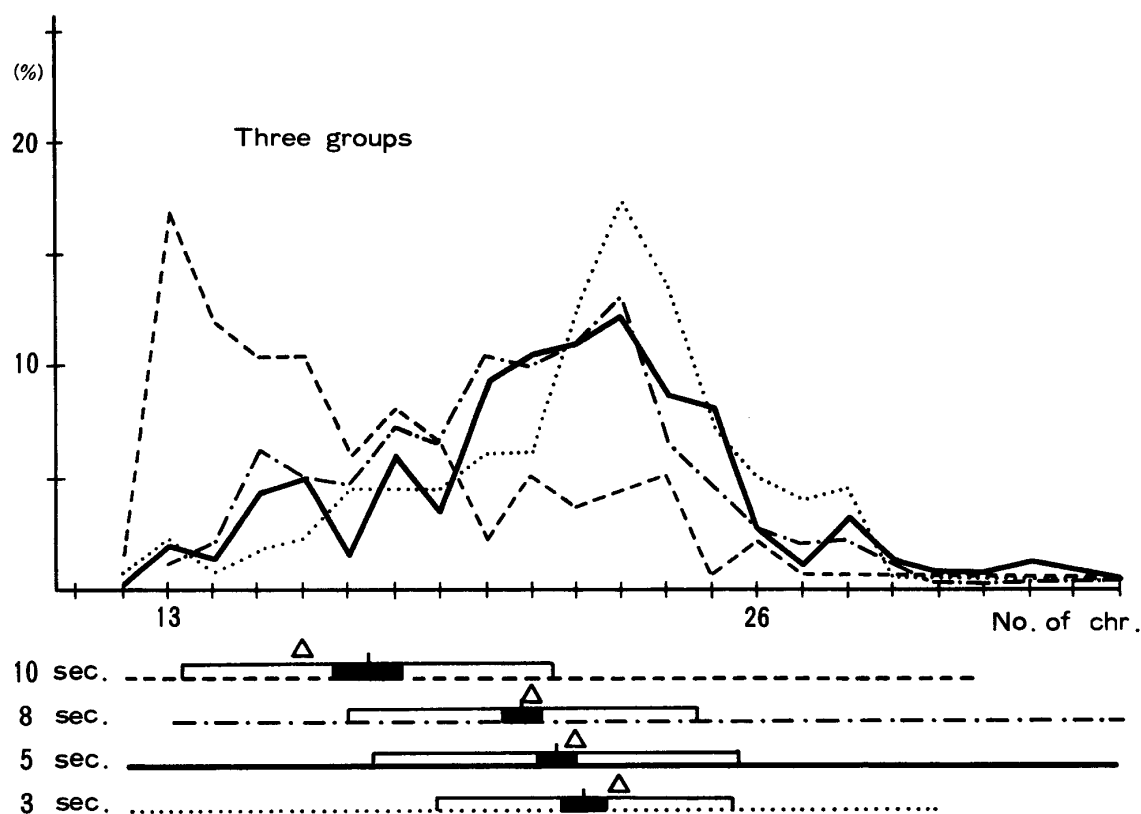


Fig. 6. Frequency distributions of various chromosome numbers in inviable individuals raised from UV-irradiated sperm in 3 groups of experimental series.

value was 22. In the series of sperm exposed for 8 seconds, the chromosome numbers of 414 mitoses from 73 individuals had also a low peak in the range including 18~24 and were almost bisymmetrically arranged. The median value was 21. The chromosome numbers of inviable individuals in the series of sperm exposed for 10 seconds were already described in group 1. They had a peak at 13 and were 16 in median value.

The mean values of chromosome numbers of inviable individuals in the four experimental series of sperm exposed for 3, 5, 8 and 10 seconds were 22.2 ± 0.5 , 21.6 ± 0.4 , 20.8 ± 0.4 and 17.4 ± 0.7 , respectively. There were significant differences in mean values of chromosome numbers between the series of sperm exposed for 3 seconds and that exposed for 8 seconds and between the series of sperm exposed for 8 seconds and that of sperm exposed for 10 seconds. The frequencies of the diploid chromosome number (26) were only 5.0%, 3.2%, 2.7% and 2.2% in the series of sperm exposed for 3, 5, 8 and 10 seconds, respectively. In each of these four series, there were a few mitoses which were 34~51 in chromosome number.

2. Viable individuals

Although tadpoles that lived for more than 40 days after hatching were sorted as viable individuals, a small number of them especially in the series of sperm exposed for 3 or 5 seconds could not grow further. Moreover, a part of the viable

individuals was not always completely normal in external characters. The chromosomes of viable tadpoles raised from eggs inseminated with sperm irradiated for 3, 5, 8 or 10 seconds were examined. These tadpoles were produced from the same three females (Nos. 1, 2 and 3) as those of the inviable individuals described above.

a. Group 1

Eggs of female No. 1 were inseminated with sperm irradiated for 3, 5 or 10 seconds. In contrast to the inviable individuals produced from the same female, the viable tadpoles were mostly 26 in chromosome number, as shown in Fig. 7. However, they had much more numerous aneuploid mitoses than the controls. In the control series, 106 (87.6%) out of 121 mitoses from 29 tadpoles were diploid (26), while 12 others were aneuploids containing 22~30 chromosomes. Another mitosis was haploid and the remaining two were triploid. Neither dicentric nor ring chromosome was found in the 121 mitoses. In the series of sperm exposed for 3 seconds, the chromosome numbers of 122 mitoses from 23 tadpoles were mostly distributed between 21 and 31. Although a peak of the frequency distribution was situated at 26, it was the lowest among those of the three series of sperm exposed for 3, 5 and 10 seconds; only 31 (25.4%) mitoses were diploid, 88 others contained 14~37 chromosomes and the remaining three were haploid. The median value of the chromosome numbers was 25. In the series of sperm exposed for 5 seconds, the chromosome numbers of 326 mitoses from 48 tadpoles were mostly distributed between 21 and 28 and had a very high peak at 26. While 178 (54.6%) were diploid, 139 contained 14~32 chromosomes, six others were haploid and the remaining three were triploid. A single dicentric chromosome was found in one of the 326 mitoses. The median value of the chromosome numbers was 26. In the series of sperm exposed for 10 seconds, the chromosome numbers of 47 mitoses from nine tadpoles were distributed between 20 and 27 except two triploid mitoses. They had a high peak at 26; 17 (36.2%) mitoses were diploid. The median value of chromosome numbers was 25.

The mean values of chromosome numbers in the control series and the three experimental series of sperm exposed for 3, 5 and 10 seconds were 25.9 ± 0.2 , 24.7 ± 0.6 , 25.0 ± 0.3 and 24.2 ± 0.5 , respectively.

b. Group 2

Eggs of female No. 2 were inseminated with sperm irradiated for 3, 5 or 8 seconds. The viable tadpoles raised from these eggs were mostly 26 in chromosome number and had more numerous aneuploid mitoses than those of the controls (Fig. 8). In the control series, 72 (83.7%) out of 86 mitoses from 23 tadpoles were diploid, while the other 14 contained 24, 25, 27 or 28 chromosomes. A single ring chromosome was found in one of the 86 mitoses. The chromosome numbers of tadpoles belonging to each of the three experimental series were also 26 in median value. In the series of sperm exposed for 3 seconds, the chromo-

some numbers of 81 mitoses from 24 tadpoles were mostly distributed between 24 and 28 and had a high peak of their frequency distribution at 26, although this peak was the lowest among those of the three series of sperm exposed for 3, 5 and 8 seconds. Only 31 (38.3%) mitoses were diploid, while 37 others

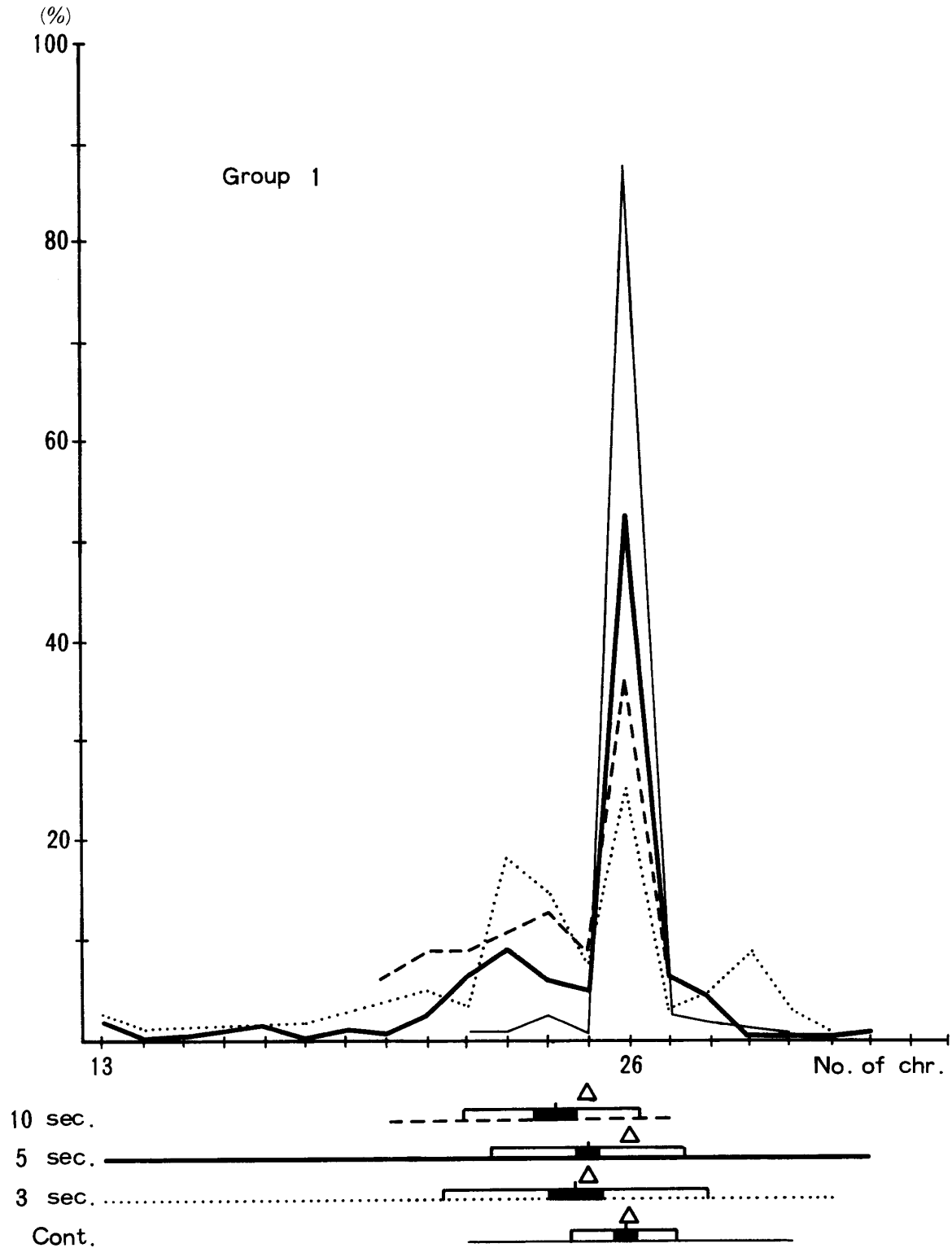


Fig. 7. Frequency distributions of various chromosome numbers in viable individuals raised from UV-irradiated sperm in group 1 of experimental series.

contained 24, 25, 27 or 28 chromosomes and the remaining 13 contained 20~23, 29, 32~34, 39 or 51 chromosomes. The 27 chromosomes were comparatively abundant and were found in 16 (19.8%) mitoses. In the series of sperm exposed for 5 seconds, the chromosome numbers of 52 mitoses from eight tadpoles were mostly distributed between 22 and 29 and had a peak at 26 which was somewhat higher than that of the series of sperm exposed for 3 seconds. Twenty-four (46.1%) mitoses were diploid, while 24 others contained 23~29 chromosomes except 26. The remaining four mitoses contained 17, 18, 19 or 30 chromosomes. In the series of sperm exposed for 8 seconds, the chromosome numbers of 12 mitoses from seven tadpoles were distributed between 23 and 30.

The mean values of chromosome numbers in the control series and the three experimental series of sperm exposed for 3, 5 and 8 seconds were 26.0 ± 0.2 , 26.4 ± 0.5 , 24.7 ± 0.6 and 26.5 ± 1.0 , respectively. Neither dicentric nor ring chromosomes were found in these three experimental series.

c. Group 3

Eggs of female No. 3 were inseminated with sperm irradiated for 3, 5 or 8 seconds. The viable tadpoles raised from these eggs were mostly 26 in chromosome number and had more numerous aneuploid mitoses than those of the controls. The chromosome numbers in each of the three experimental series of sperm exposed for 3, 5 and 8 seconds were 26 in median value (Fig. 9). In the control series, 75 (78.9%) out of 95 mitoses from 27 tadpoles were diploid, while 17 others contained 20~30 chromosomes except 26. The remaining three mitoses contained haploid, triploid and tetraploid chromosomes. In the series of sperm exposed for 3 seconds, the chromosome numbers of 219 mitoses from 40 tadpoles were mostly distributed between 22 and 30. While 106 (48.4%) mitoses were diploid, 46 and 52 contained 22~25 and 27~29 chromosomes, respectively. Six others contained 14, 16 or 21 chromosomes. The remaining nine mitoses contained 30, 31, 39, 41 or 45 chromosomes. Seven ring chromosomes were found among 219 mitoses. In the series of sperm exposed for 5 seconds, the chromosome numbers of 70 mitoses from 16 tadpoles were mostly distributed between 22 and 29. While 20 (28.6%) mitoses were diploid, 3, 5, 8 and 15 mitoses contained 22, 23, 24 and 25 chromosomes, respectively. Fourteen others had 27~29 chromosomes and still two others contained 19 and 34 chromosomes. The remaining three mitoses were haploid. A single ring chromosome was found in one of the 70 mitoses. In the series of sperm exposed for 8 seconds, five out of eight mitoses from one tadpole were diploid, while the other two contained 27 and 24 chromosomes.

The mean values of chromosome numbers in the control series and the three experimental series of sperm exposed for 3, 5 and 8 seconds were 25.9 ± 0.4 , 25.8 ± 0.3 , 25.9 ± 0.1 and 26.0 ± 0.6 , respectively.

d. Three groups

Frequency distributions of various chromosome numbers observed in the total

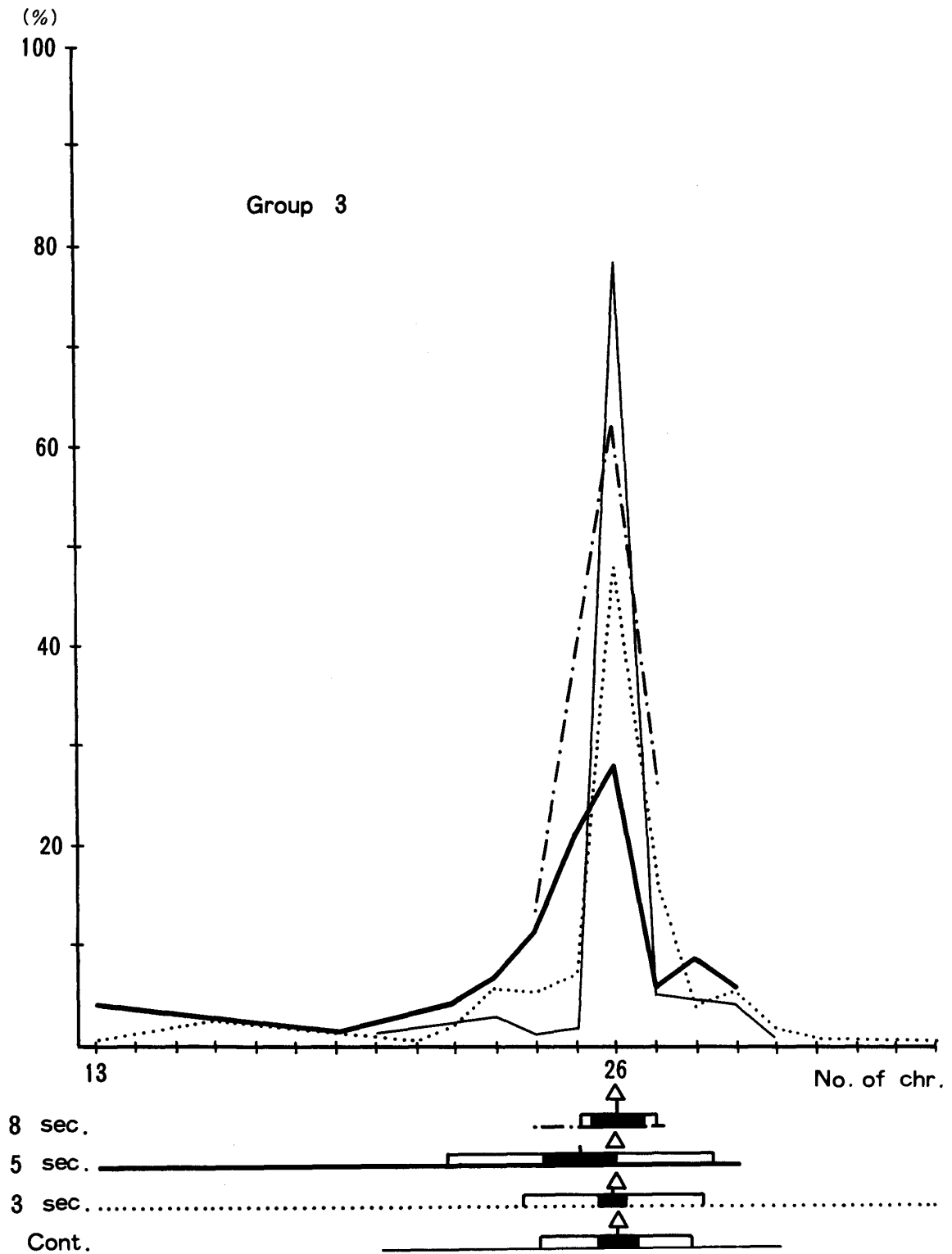


Fig. 9. Frequency distributions of various chromosome numbers in viable individuals raised from UV-irradiated sperm in group 3 of experimental series.

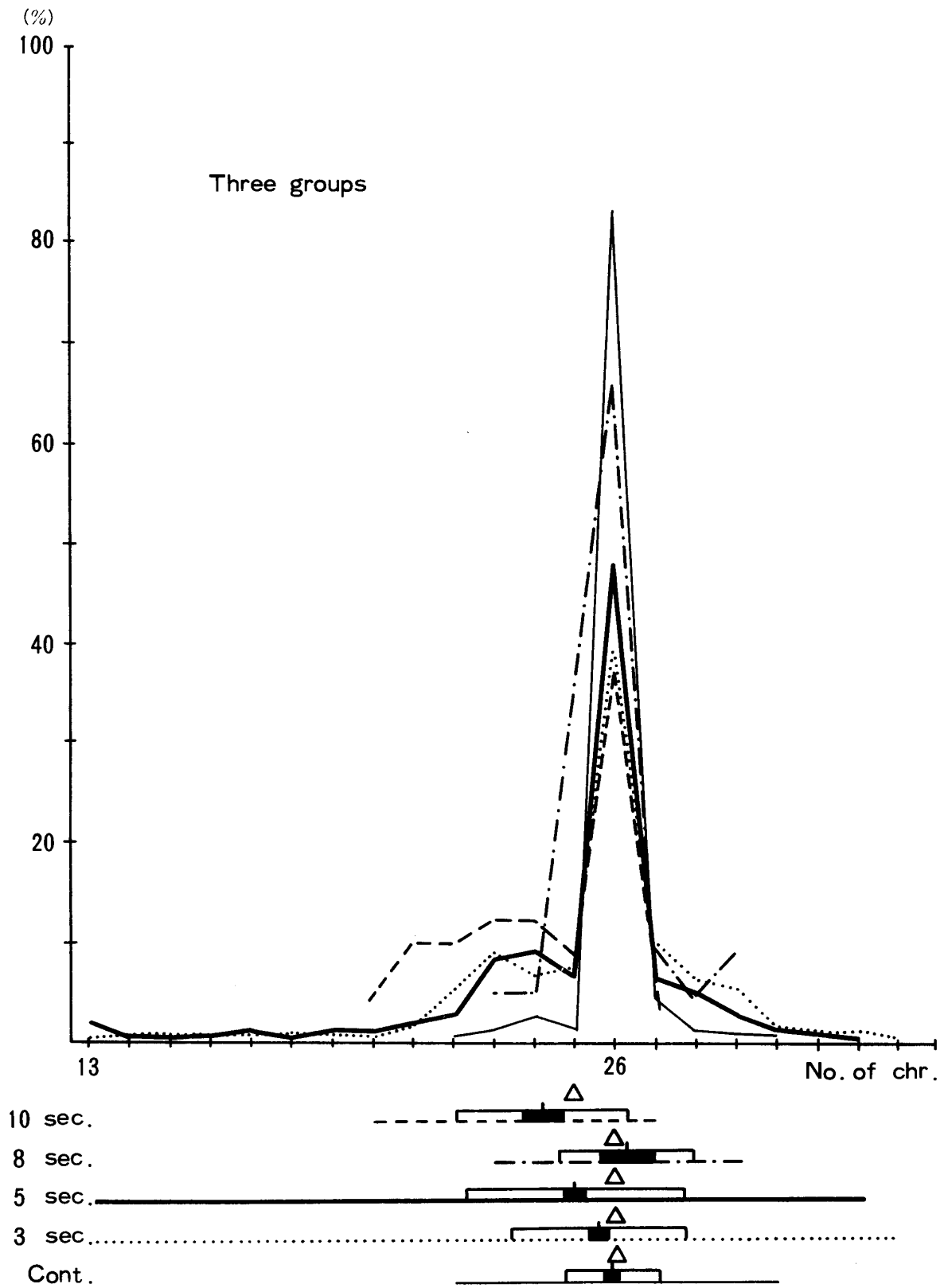


Fig. 10. Frequency distributions of various chromosome numbers in viable individuals raised from UV-irradiated sperm in 3 groups of experimental series.

sum of the viable tadpoles belonging to the above three groups are shown in Fig. 10. While 83.8% of the analyzed mitoses in the control tadpoles had 26 chromosomes, the proportions of mitoses having 26 chromosomes in the four experimental series whose sperm were irradiated for 3, 5, 8 and 10 seconds were 39.4%, 48.2%, 66.2% and 38.0%, respectively. In the series of sperm exposed for 3 seconds, the chromosome numbers of 419 mitoses from 87 tadpoles were distributed between 13 and 33, while those of 298 mitoses from the control tadpoles were distributed between 22 and 30. In the series of sperm exposed for 5 seconds, the chromosome numbers of 458 mitoses from 72 tadpoles were also distributed between 13 and 32. As the other two experimental series of sperm exposed for 8 and 10 seconds were very few in number of mitoses and tadpoles, chromosome numbers were distributed between 23 and 29 and between 20 and 27, respectively. If the series of sperm exposed for 8 seconds was disregarded as the mitoses were especially scanty, it was evident that the mean values of chromosome numbers gradually decreased with increase of UV doses. While the mean value of the chromosome numbers found in the control tadpoles was 25.9 ± 0.2 , those in the tadpoles of the three experimental series whose sperm were exposed for 3, 5 and 10 seconds were 25.6 ± 0.2 , 25.0 ± 0.3 and 24.2 ± 0.5 , respectively. Hypodiploid mitoses were much more abundant in the tadpoles derived from irradiated sperm than those in the controls.

DISCUSSION

Since O. HERTWIG (1911) and G. HERTWIG (1911) disclosed a paradoxical effect of irradiation on gametes in development of *Rana fusca* (= *temporaria*), the so-called HERTWIG effect was confirmed by many investigators in various amphibians (O. HERTWIG, 1913; G. HERTWIG, 1911, 1913; P. HERTWIG, 1913, 1916; SIMON, 1930; DALCQ, 1930; RUGH, 1939; RUGH and EXNER, 1940; HENSHAW, 1943; ROLLASON, 1949; POGANY, 1971, 1973, 1976). POGANY (1971, 1973) reported in *Rana pipiens* that the embryos derived from sperm irradiated with a low UV dose were most severely affected in spite of their diploid constitution. However, the effects of lower UV doses on sperm were not sufficiently analyzed, since the grades in exposure period were very coarse, that is, sperm were irradiated for 5, 15, 30, 60 and 120 seconds at an energy level of 31 erg/mm². Thereafter, POGANY (1976) exposed sperm to UV for 5, 7, 8, 9, 10, 11, 12, 13, 15 and 30 seconds at the same energy level. The results showed that the initial decrease in survival at 5 or 7 second exposure was not accompanied by any chromosomal losses since all cultures were diploids. However, the most impaired survival occurring from 8 second exposure was concomitant with aneuploidy. By 9 second exposure, chromosomal elimination appeared to be complete.

In the present study, sperm were irradiated with UV-rays at an energy level of 16 erg/mm² for 3, 5, 8, 10, 15, 20, 40 and 60 seconds. The proportions of survivals including normal and abnormal embryos at the hatching stage in the series derived from sperm irradiated for these eight kinds of exposure were 80%, 55%,

32%, 17%, 8%, 5%, 69% and 92% of the total number of eggs, respectively. Thus, the survival ratio was the lowest at exposure for 15 or 20 seconds and rapidly recovered at exposure for 40 seconds. Normal, diploid-shaped individuals were 57%, 32%, 7%, 7% and 2% of the total ones in the series of sperm irradiated for 3, 5, 8, 10 and 15 seconds, respectively, while abnormal individuals other than haploid-shaped ones increased rapidly and attained the maximum by exposure for 8 or 10 seconds. These findings showed that the initial decrease in survival occurred in the series of sperm irradiated for 3 and 5 seconds in the present study. While POGANY (1976) observed the phenomenon that the initial decrease in survival was not accompanied by any chromosomal losses, the present authors could not confirm such a phenomenon, as in the following.

Chromosome numbers were counted in both viable and inviable individuals which had been raised from eggs fertilized with irradiated sperm. In viable tadpoles, the proportions of mitoses having 26 chromosomes in two experimental series whose sperm were irradiated for 3 and 5 seconds were 39.4% and 48.2%, respectively, while it was 83.8% in the control series. Hyper- and hypodiploid mitoses were much more abundant in the tadpoles derived from irradiated sperm than those in the controls. In inviable individuals, the frequencies of the diploid chromosome number ($2n=26$) in the two experimental series derived from sperm irradiated for 3 and 5 seconds were 5.0% and 3.2%, respectively. The mean values of chromosome numbers in these series were 22.2 ± 0.5 and 21.6 ± 0.4 , respectively.

On the basis of these results of observation, the initial decrease in survival seems to be attributable to complete or incomplete damage of a part of the chromosomes carried by each spermatozoon. When the multiplying function of a few chromosomes is completely destroyed by UV-rays, a hypodiploid will be produced after inseminating an egg. If the destruction is incomplete, the mitoses in the fertilized egg or those in the embryo raised from this egg will become abnormal and result in producing a chromosomal mosaic. Such an embryo should be an inviable embryo owing to disturbance of balanced morphogenesis. On the other hand, the dicentric and ring chromosomes which were more frequently found in the individuals derived from irradiated sperm than those in the controls seem to indicate the existence of some chromosomes destroyed partially by irradiation.

SUMMARY

1. For the purpose of clarifying the effects of UV-rays on the chromosomes of sperm, eggs of the Japanese brown frog, *Rana japonica*, were inseminated with UV-irradiated sperm of the same species. The UV-ray was 2537 Å in main wave length and 16 erg/mm²/sec in energy level. Sperm suspensions were irradiated for 3, 5, 8, 10, 15, 20, 40 and 60 seconds. The initial decrease in the proportion of survivals including normal and abnormal embryos at the hatching stage occurred in the series of sperm exposed for 3 and 5 seconds. The decrease

in survival attained the maximum by exposure for 15 or 20 seconds and recovered by exposure for 40 seconds.

2. Normal, diploid-shaped individuals distinctly decreased in number with increase in dose, while abnormal individuals other than haploid-shaped ones rapidly increased and attained the maximum by exposure for 8 or 10 seconds. Typically haploid-shaped embryos began to appear in the series of sperm irradiated for 3 seconds and gradually increased in number. In the series of sperm irradiated for 40 or 60 seconds, an overwhelming majority of normally cleaved eggs became typically haploid-shaped embryos.

3. The mean values of chromosome numbers found in inviable individuals were 22.2 ± 0.5 , 21.6 ± 0.4 , 20.8 ± 0.4 and 17.4 ± 0.7 in four experimental series whose sperm were irradiated for 3, 5, 8 and 10 seconds, respectively, while the frequencies of the diploid chromosome number (26) were only 5.0%, 3.2%, 2.7% and 2.2%, respectively. The mean values of chromosome numbers found in viable tadpoles of three experimental series whose sperm were irradiated for 3, 5 and 10 seconds were 25.6 ± 0.2 , 25.0 ± 0.3 and 24.2 ± 0.5 , respectively, while that of chromosome numbers found in the control tadpoles was 25.9 ± 0.2 . Hyper- and hypodiploid mitoses were much more abundant in the tadpoles derived from irradiated sperm than those in the controls. Dicentric and ring chromosomes were also more frequently found in the individuals derived from irradiated sperm than those in the controls.

4. The initial decrease in survival seemed to be attributable to complete or incomplete damage of a part of the chromosomes carried by each spermatozoon.

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LITERATURE

- DALCQ, A. 1930. Interprétation cytologique des effets, sur la gastrulation, de l'irradiation d'un des gamètes, chez *Rana fusca*. C. R. Soc. Biol. (Paris) **104**: 1055-1058.
- HENSHAW, P. S. 1943. Peculiar growth lesions in frogs induced by irradiation of sperm cells with X-rays. J. Nat. Cancer Inst. **3**: 409-417.
- HERTWIG, G. 1911. Radiumbestrahlung unbefruchteter Froscheier und ihre Entwicklung nach Befruchtung mit normalem Samen. Arch. f. mikr. Anat. **77**: 165-209.
- 1913. Parthenogenesis bei Wirbeltieren, hervorgerufen durch artfremden, radiumbestrahlten Samen. Ibid. **81**: 87-127.
- HERTWIG, O. 1911. Die Radiumkrankheit tierischer Keimzellen. Ein Beitrag zur experimentellen Zeugungs- und Vererbungslehre. Ibid. **77**: 1-164.
- 1913. Versuche an Tritoneiern über die Einwirkung bestrahlter Samenfadens auf die tierische Entwicklung. Ibid. **82**: 1-63.
- HERTWIG, P. 1913. Das Verhalten des mit Radium bestrahlten Spermachromatins in Froschei. Ein cytologischer Beweis für die parthenogenetische Entwicklung der Radiumlarven. Ibid. **81**: 173-

182.

- 1916. Durch Radiumbestrahlungs verursachte Entwicklung von halbkerniger Triton- und Fischembryonen. *Ibid.* **87**: 63–122.
- MAKINO, S. and I. NISHIMURA 1952. Water-pretreatment squash technic. A new and simple practical method for the chromosome study of animals. *Stain Technology* **27**: 1–7.
- NISHIOKA, M., H. OKUMOTO and Y. KONDO 1981. Effects of ultraviolet rays on the sperm of *Rana japonica*. I. Production of gynogenetic haploids. *Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ.* **5**: 47–67.
- POGANY, G. C. 1971. Effects of sperm ultraviolet irradiation on the embryonic development of *Rana pipiens*. *Develop. Biol.* **26**: 336–345.
- 1973. Growth inhibition in *Rana pipiens* due to sperm irradiation with ultraviolet rays. *J. Exp. Zool.* **183**: 121–138.
- 1976. Analysis of a HERTWIG effect induced by the irradiation of *Rana pipiens* sperm with ultraviolet. *Develop., Growth and Differ.* **18**: 339–348.
- ROLLASON, G. S. 1949. X-radiation of eggs of *Rana pipiens* at various maturation stages. *Biol. Bull.* **97**: 169–185.
- RUGH, R. 1939. Developmental effects resulting from exposure to X-rays. I. Effect on the embryo of irradiation of frog sperm. *Proc. Am. Philos. Soc.* **81**: 447–471.
- RUGH, R. and F. EXNER 1940. Developmental effects resulting from exposure to X-rays. II. Development of leopard frog eggs activated by bullfrog sperm. *Ibid.* **83**: 607–619.
- SHUMWAY, W. 1940. Stages in the normal development of *Rana pipiens*. I. External form. *Anat. Rec.* **78**: 139–147.
- SIMON, S. 1930. Effets de l'irradiation d'un des gamètes sur la gastrulation chez *Rana fusca*. *C. R. Soc. Biol. (Paris)* **104**: 1025–1055.
- TAYLOR, A. C. and J. J. KOLLROS 1946. Stages in the normal development of *Rana pipiens* larvae. *Anat. Rec.* **94**: 7–24.