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## Effect of Gamma Radiation on the Development and Reproduction of the Brown Planthopper, *Nilaparvata lugens* (STÅL) (Homoptera : Delphacidae)

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*N. lugens* was irradiated at 2.5 to 30 krad in the 5th-instar stage by using  $^{60}\text{Co}$ . The minimum effective doses of gamma radiation to 5th-instar female and male nymphs causing a 100% reduction in egg viability were estimated to be about 2.5 (or somewhat lower) and 6.2 krad, respectively. At 6.2 krad the values of % adult emergence (males and females together), % adult malformation (do.), mean longevity of males (days after irradiation), and LD 50 (do., males and females together) were estimated to be about 100, 19, 17 (29 for the control), and 14 (26 for the control), respectively. Lethal mutation sterility was expected at this dose-level. The internal reproductive organs of adults were anatomically and histologically examined. The spermatozoa were motile in male adults irradiated at 30 krad. The minimum effective dose of gamma radiation to 5th-instar female nymphs required for the interruption of egg-formation was estimated to be between 15 and 20 krad, higher than the minimum effective dose for cessation of egg-laying (10.4 krad).

### INTRODUCTION

Though there are a great number of papers dealing with the effect of gamma and X-ray radiations on various insects, as far as is known, little information concerning the effect of radiation on homopterous insects belonging to the Delphacidae and Cicadellidae has evolved regarding the aspect of sterile-male technique. OSBORN et al. (1966) and SHIPP et al. (1966) showed the possibility of eradicating sugar-cane leafhoppers, *Perkinsiella* spp. (Delphacidae), by repeated releases of sterile insects induced by gamma rays. AMERESEKERE and GEORGHIOU (1971) succeeded in sterilizing the beet leafhopper, *Circulifer tenellus* (BAKER) (Cicadellidae), with gamma radiation. AMERESEKERE et al. (1971) investigated histologically the effect of gamma radiation on the testes and ovaries of the cicadellid. This paper deals with gamma radiation to 5th-instar nymphs and its effect on the subsequent development and reproduction in *N. lugens*, an important pest on rice plants. The results obtained in the present investigation will contribute to a proper understanding of the irradiation effect on *N. lugens*.

### MATERIAL AND METHODS

Nymphs were reared in mass on seedlings of *Toyotama*, a paddy variety of the rice plant, under the condition of continuous illumination by two 20-watt fluorescent tubes at 27°C. Many macropterous and a few brachypterous adults were obtained. These were then employed in the following analysis, unless otherwise described.

Following irradiation newly emerged adults were kept in pair on rice seedlings in test-tubes 1.6 cm in diameter and 16 cm in height. Seedlings were renewed at intervals of several days. Mortality was recorded daily. All the adults which exhibited imperfections such as crumpled wings were termed "malformed". Hatchability was determined by counting all the eggs laid and hatchlings from the eggs. Those eggs in which embryos developed normally and had attained the eye-spot stage by examination under a binocular microscope were classified as hatched ones. This method of classification was employed since many embryos contained eggs yielded by irradiated pairs showed abnormal development which often terminated in death before the eye-spot stage.

Percent reduction in egg viability was calculated by the following formula:

$$\frac{(V_1 - V_2)}{V_1} \times 100$$

in which  $V_1$  is the number of viable eggs/female in the control, and  $V_2$  is the number of viable eggs/female in the test treatment.

The irradiation was conducted with a 200-ci  $^{60}\text{Co}$  source at the Chemical Research Institute, Kyoto University, Uji. The total dose varied between 2.5 and 30 krad at a dose-rate of 1240 rad/minute. Irradiation times ranged between 2 and 24 minutes. Groups of twenty 5th-instar nymphs were irradiated at each dose in small test-tubes stoppered with cotton wool at a temperature of approximately 5 to 7°C. All irradiations mentioned herein are those of 5th-instar nymphs.

For anatomical observation, nymphs and adults were dissected in Ringer solution. For histological observation, specimens were fixed in a F.A.A. solution and embedded in paraplast by the routine method. These were subsequently sectioned at 7  $\mu$  in thickness. Sections were stained with Mayer's acid-haemalaum and eosin.

## RESULTS

### *Adult emergence and malformation*

Emergence of adults irradiated at various doses in the 5th-instar stage is shown in

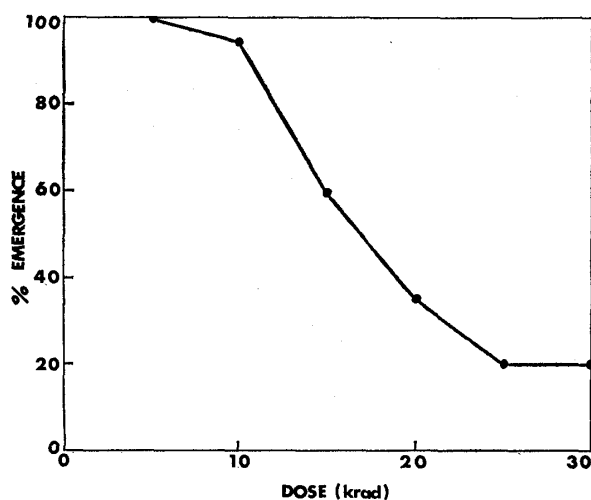


Fig. 1. Emergence of *N. lugens* irradiated in the 5th-instar stage. Males and females were added together. The numbers of insects used were 40 at 0 krad and 20 at each dose other than 0 krad.

Fig. 1. All the 5th-instar nymphs irradiated at 5 krad and lower doses developed to adults. Irradiation at doses above 10 krad resulted in a sharp reduction in adult emergence. The dose at which half of the irradiated 5th-instar nymphs could be expected to emerge into adults was estimated to be about 17 krad.

Some adults which emerged from irradiated nymphs had twisted wings. The number of malformed adults increased in percentage with a rise in doses but displayed an S-shaped curve with a constant value of 75% at 25 and 30 krad (Fig. 2). The dose at which a half of the adults emerged from irradiated nymphs was malformed was about 12 krad.

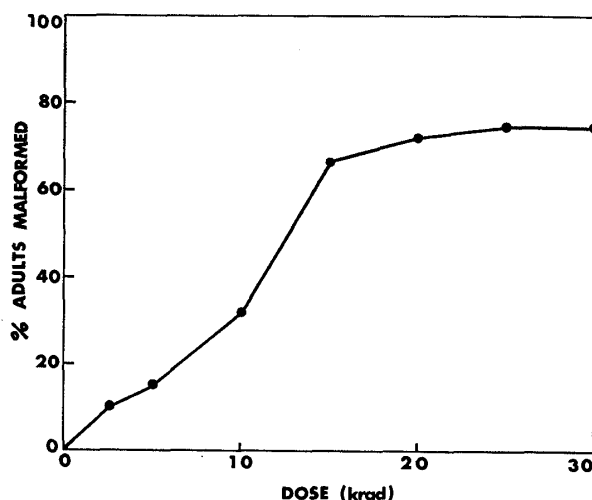


Fig. 2. Adult malformation of *N. lugens* irradiated in the 5th-instar stage. Males and females were added together.

#### *Longevity of combined males and females*

The times required by 5th-instar nymphs irradiated at 2.5 to 30 krad to become adults were not correlated with the doses of irradiation ( $r = -0.129$ , d.f. = 84,  $P > 0.05$ ): The period of the 5th-instar stage following irradiation was an average of 2.0 days. Thus, the longevity expressed here indicates the life-span after irradiation.

The normal life-span was  $28.0 \pm 8.7$  days (mean  $\pm$  S.D.,  $n = 40$ ). Irradiation at doses of 2.5 to 30 krad resulted in a linear reduction in the mean longevity (Fig. 3) expressed by the equation:

$$\log Y = 1.392 - 0.031X \quad (r = -0.955^{**})$$

in which  $X$  is the dose (krad) of irradiation, and  $Y$  is the mean longevity (days). The dose at which the life-span of *N. lugens* irradiated in the 5th-instar stage was reduced to half of that of the control was estimated to be about 7.5 krad.

Survivorship curves for *N. lugens* irradiated are shown in Fig. 4. All the individuals irradiated at 20 and 30 krad died within 10 days after irradiation. About half of the insects irradiated at 10 krad lived longer than 13 days. The shortest and the longest life-span at 10 krad were 5 and 20 days, respectively. Those at 0 krad were 17 and 52 days, respectively. The range in days between the shortest and the longest life-span at each dose of irradiation became broader as gamma radiation dose was reduced. The life-prolongation effect of gamma radiation on *N. lugens* could not

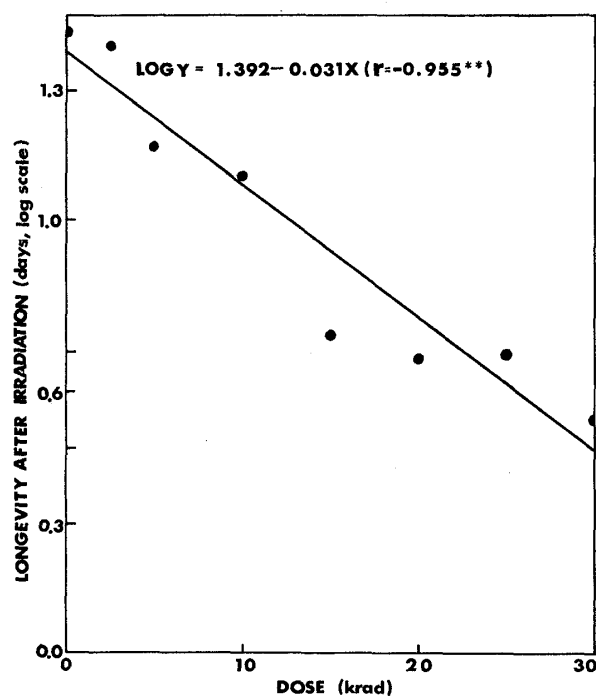


Fig. 3. Mean longevity of *N. lugens* irradiated in the 5th-instar stage. Males and females were added together.

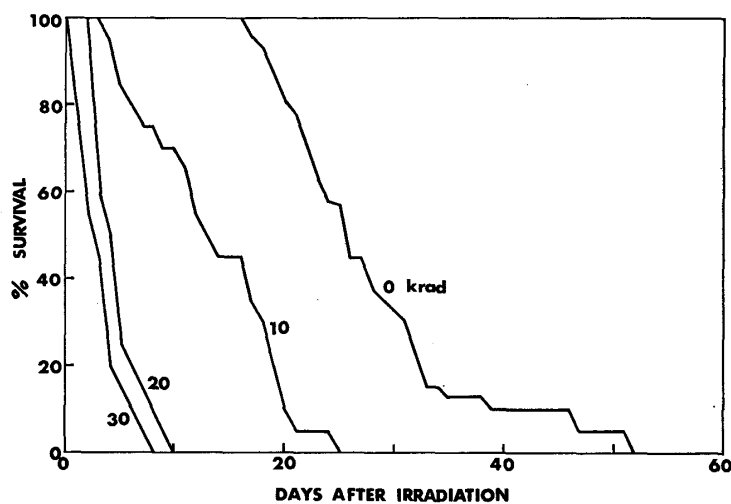


Fig. 4. Survivorship curves for *N. lugens* irradiated in the 5th-instar stage. Males and females were added together.

be observed in the present investigation, contrary to that reported in some insects by O'BRIEN and WOLFE (1964).

#### *LD 50 at different ages*

Percent mortalities at different doses at intervals of 3 or 5 days after irradiation were transformed into probit values to estimate LD 50 at various ages. The L-shaped LD 50

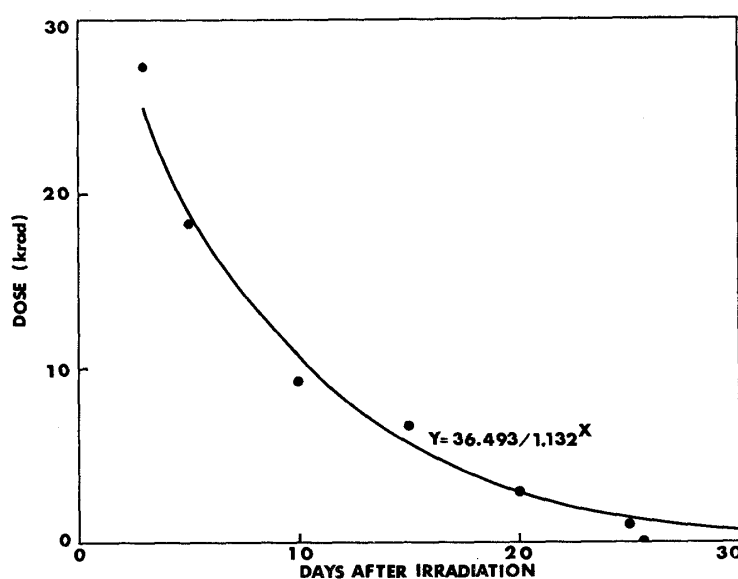


Fig. 5. LD 50 at different ages in *N. lugens*. Males and females were added together.

curve as shown in Fig. 5 can be described by the equation:

$$Y = 36.493 / 1.132^X \text{ or } \log Y = 1.562 - 0.054X \quad (r = -0.994^{**})$$

in which  $X$  is the age after irradiation (days, males and females added together), and  $Y$  is the dose of irradiation (krad). LD 50 values for the 3rd, 5th, 10th and 20th day following irradiation were about 27, 18, 9, and 3 krad, respectively.

#### *Egg-laying and hatchability*

The results are shown in Table 1. In each of the first and second series of crosses in Table 1 (irradiated females  $\times$  irradiated males, and irradiated females  $\times$  normal males), the number of eggs laid/female decreased considerably as the doses of irradiation increased from 0 to 25 krad. In particular irradiation at doses of 15 krad and higher resulted in the absence of egg-laying in all the crosses.

When the first and the second series of crosses were compared with respect to the number of eggs laid/female at each dose of 2.5 to 20 krad, there was no significant difference at each dose. In the final series of crosses (normal females  $\times$  irradiated males), either, normal females showed no difference in the number of eggs laid/female irrespective of pairings with males irradiated at different doses (0 to 5 krad) in the 5th-instar stage. These results indicate that male adults irradiated in the 5th-instar stage had no effect on the number of eggs laid by normal female adults paired with them.

The minimum effective dose of gamma radiation to 5th-instar female nymphs in anticipation of cessation of egg-laying by females irradiated was estimated to be 10.4 krad by the following formula:

$$Y = 144.89 - 13.95X \quad (r = -0.888^{**})$$

in which  $X$  is the dose (krad) of irradiation, and  $Y$  is the number of eggs laid/female (Fig. 6).

When normal females were mated to normal males, the hatchability was 94.6%. When 5th-instar female nymphs were exposed to doses of 2.5 krad and higher, no hatch-

Table 1. EFFECT OF NYMPHAL IRRADIATION ON OVIPOSITION AND HATCHABILITY IN *N. lugens*.

Dose (krad)	Cross <sup>a</sup>	No. pairs examined	No. eggs/female Mean±S. D.	% egg hatch	% decrease in egg viability	No. pairs yielding eggs which hatched	
0.0	♀ × ♂	20	180.0±125.1 <sup>b</sup>	94.6 <sup>b</sup>	0	20	100%
2.5	♀ × ♂	6	101.5±124.5 <sup>bc</sup>	0.0 <sup>c</sup>	100	0	0
5.0	♀ × ♂	4	94.0±113.4 <sup>bc</sup>	0.0 <sup>c</sup>	100	0	0
10.0	♀ × ♂	4	9.5±16.4 <sup>c</sup>	0.0 <sup>c</sup>	100	0	0
15.0	♀ × ♂	3	0.0±0.0 <sup>c</sup>	0.0 <sup>c</sup>	100	0	0
20.0	♀ × ♂	3	0.0±0.0 <sup>c</sup>	0.0 <sup>c</sup>	100	0	0
25.0	♀ × ♂	2	0.0±0.0 <sup>c</sup>	0.0 <sup>c</sup>	100	0	0
0.0	♀ × ♂	20	180.0±125.1 <sup>b</sup>	94.6 <sup>b</sup>	0	20	100
2.5	♀ × ♂	6	56.7±70.8 <sup>cd</sup>	0.0 <sup>c</sup>	100	0	0
5.0	♀ × ♂	8	78.9±86.0 <sup>c</sup>	0.0 <sup>c</sup>	100	0	0
10.0	♀ × ♂	6	5.5±10.4 <sup>d</sup>	0.0 <sup>c</sup>	100	0	0
15.0	♀ × ♂	2	0.0±0.0 <sup>d</sup>	0.0 <sup>c</sup>	100	0	0
20.0	♀ × ♂	3	0.0±0.0 <sup>d</sup>	0.0 <sup>c</sup>	100	0	0
0.0	♀ × ♂	20	180.0±125.1 <sup>b</sup>	94.6 <sup>b</sup>	0	20	100
2.5	♀ × ♂	6	277.0±146.4 <sup>b</sup>	38.4 <sup>c</sup>	37.5	6	100
5.0	♀ × ♂	6	160.0±195.1 <sup>b</sup>	20.3 <sup>d</sup>	80.9	5	83.3

<sup>a</sup> ♀ & ♂ were normal; ♀ & ♂ were treated.

<sup>b-d</sup> Means or percentages followed by different letters are significantly different ( $P < 0.05$ ).

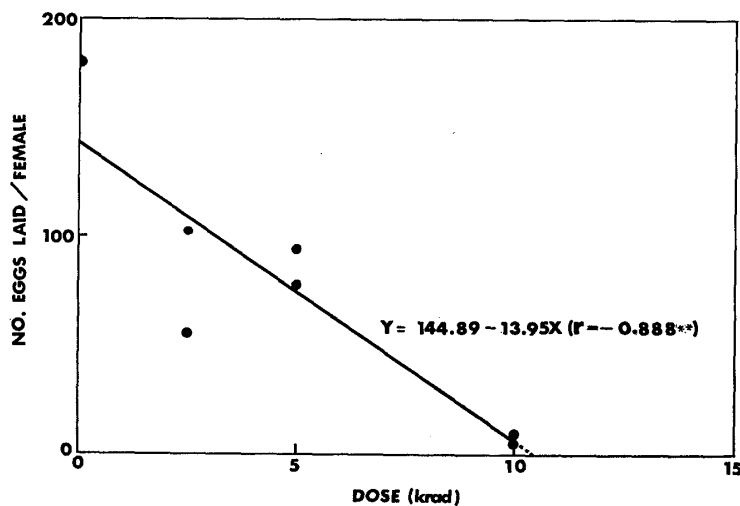


Fig. 6. Estimation of the minimum effective dose of gamma radiation to 5th-instar female nymphs to result in no egg-laying in *N. lugens*. A total of 54 females was examined for egg-laying.

ing appeared from any eggs laid by the female adults from the nymphs irrespective of normal (0 krad) and irradiated (2.5 to 25 krad) males paired with them.

When normal females were mated to males irradiated at doses of 0, 2.5, and 5.0 krad, reductions in egg viability were 0, 37.5, and 80.9%, respectively.

These results indicate that gamma radiation in the 5th-instar stage affected females to a greater degree than males for sterilization of eggs in the subsequent gener-

ation. The minimum effective dose of gamma radiation to 5th-instar female nymphs in order to obtain sterile female adults (or 100% reduction in egg viability in the cross of irradiated females  $\times$  normal males) was estimated to be about 2.5 krad or somewhat lower.

*The minimum effective dose of gamma radiation to obtain sterile male adults and the longevity of the irradiated males*

Fig. 7 shows the method of estimating the minimum effective dose of gamma radiation to 5th-instar male nymphs in the P generation so that the reduction in egg viability in the F<sub>1</sub> generation may become 100% when normal females were paired with irradiated males. The minimum effective dose was 6.24 krad, calculated by a formula as follows:

$$Y = 16.18X - 0.98 \quad (r = 0.999^{**})$$

in which  $X$  is the dose (krad) of irradiation to 5th-instar male nymphs, and  $Y$  is % reduction in egg viability. When reduction in egg viability was 50%, the dose of irradiation was calculated to be 3.15 krad.

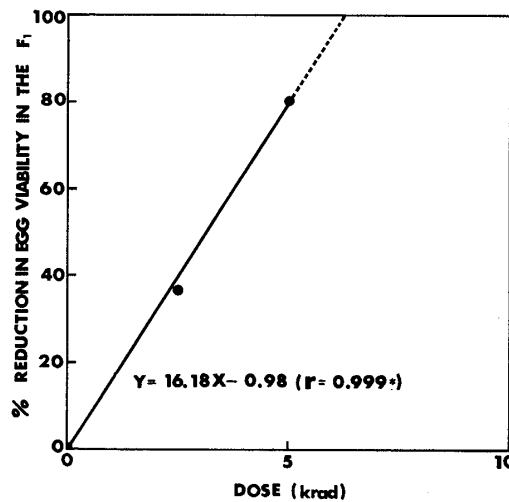


Fig. 7. Estimation of the minimum effective dose of gamma radiation to 5th-instar male nymphs to obtain sterile *N. lugens* male adults.

Table 2. LONGEVITY OF MALES IRRADIATED IN THE 5TH-INSTAR STAGE IN *N. lugens*.

Dose (krad)	No. males examined	Mean $\pm$ S.D. <sup>a</sup> (days)	Relative ratio for the mean of the control
0.0	20	28.95 $\pm$ 8.82	100.0
2.5	4	27.25 $\pm$ 4.49	94.1
5.0	4	18.00 $\pm$ 8.06*	62.2
10.0	4	13.25 $\pm$ 5.40**	45.8
15.0	9	4.78 $\pm$ 1.99**	16.5
20.0	9	4.22 $\pm$ 1.20**	14.6
25.0	14	4.57 $\pm$ 2.10**	15.8
30.0	9	3.00 $\pm$ 1.41**	10.4

<sup>a</sup> Significant differences from the control (0 krad) at 5 and 1% levels are indicated by \* and \*\*, respectively.

The effect of gamma radiation on male longevity, as an important element in consideration of the possibility of sterile-male technique, is shown in Table 2. The mean longevity of males irradiated at 6.2 krad (or the minimum effective dose to obtain sterile male adults) was about 17 days and as short as 59% of that of the control.

*Anatomical observations on the internal reproductive organs*

The organs of males irradiated at 20 krad (Fig. 8 B) were similar in appearance to those of normal ones (Fig. 8 A) soon after emergence. However, the part of the testicular follicles containing spermatozoa (shaded part in Fig. 8) was proportionally somewhat larger in normal males than in males irradiated at 20 krad in later periods (6- to 9-day-old) of the adult age. When male adults irradiated at 30 krad were dissected, the spermatozoa were seen to be motile.

The development of the largest ovarioles and oöcytes of macropterous females irradiated at 0, 5, and 20 krad is illustrated in Fig. 9. In the control females yolk-formation and egg-formation were observed in 1- and 2-day-old females, respectively (Fig. 9 A). In females irradiated at 5 krad those were observed in 2- and 3-day-old females, *i.e.* they were delayed for about one day (Fig. 9 B). In females irradiated at 20 krad, yolk-formation could not be observed even 7 days after emergence. The ovarioles and the younger oöcytes did not show any considerable change in size for several days and

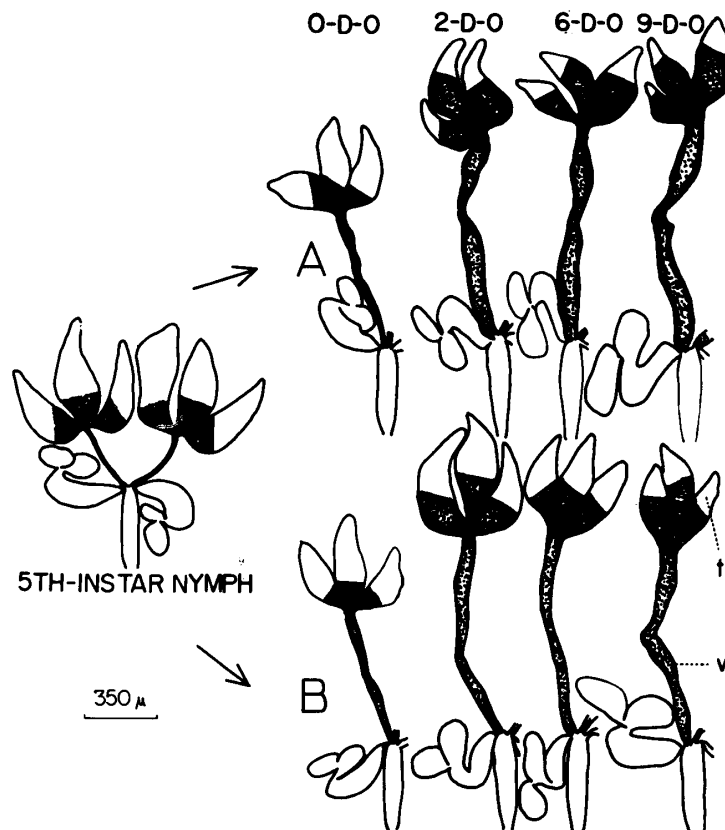


Fig. 8. The internal reproductive organs of *N. lugens* macropterous males irradiated in the 5th-instar stage. A, 0 krad (control); B, 20 krad. 0-D-O, 0-day-old after emergence; t, testicular follicle; v, vas deferens. Shaded parts contain the spermatozoa.



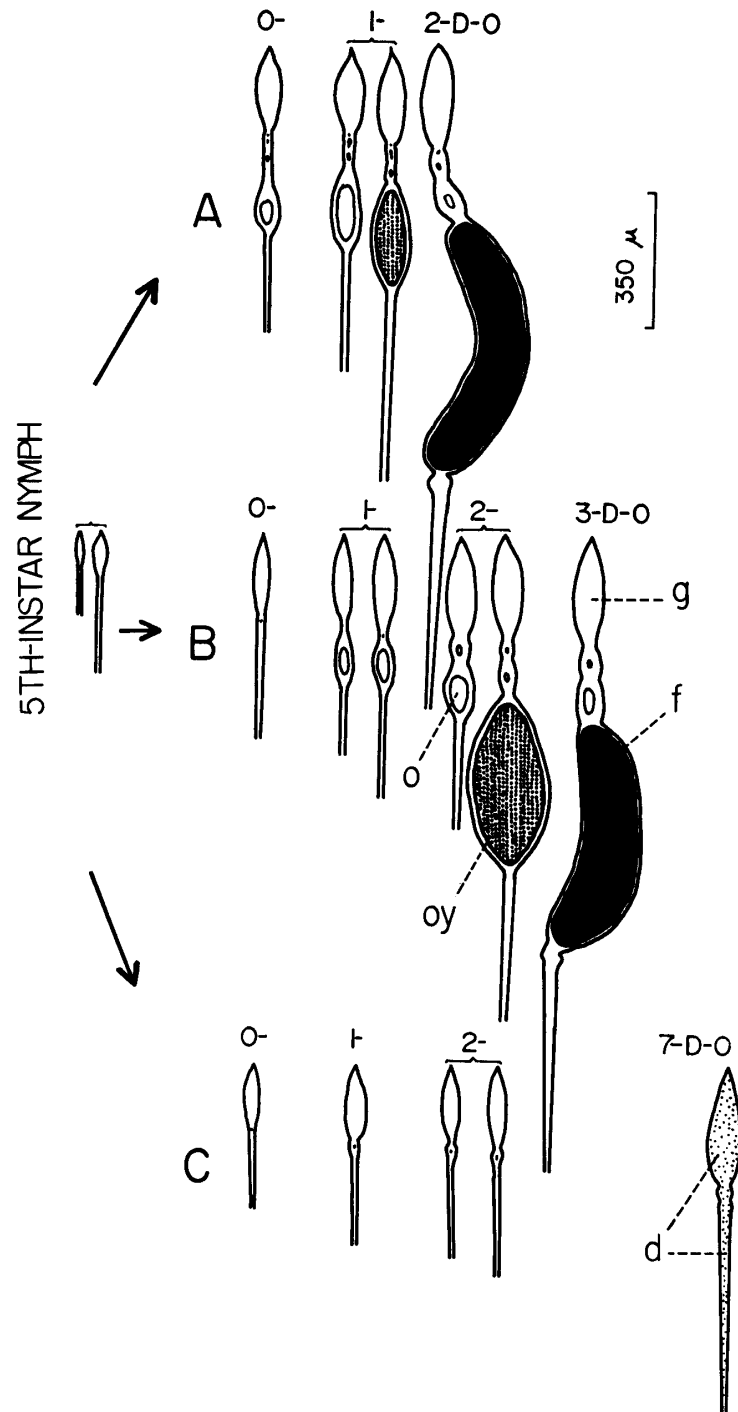


Fig. 9. Development of the largest ovarioles and oocytes within *N. lugens* macropterous females irradiated in the 5th-instar stage. A, 0 krad (control); B, 5 krad; C, 20 krad. 2-D-O, 2-day-old after emergence. d, destroyed cells or tissues; f, fully grown eggs; g, germarium; o, oocyte without yolk; oy, oocyte with yolk. The terminal filaments of the ovarioles are not drawn here.

ceased soon their development. The former contained necrotic cells or tissues within the germariums and vitellariums 7 days after emergence (Fig. 9 C).

Four macropterous adult females, which were irradiated at 15 krad and failed to lay any eggs during their life-span, were dissected. Only one of them possessed some fully grown eggs within her ovaries, but others had no egg.

These results indicate that egg-formation is interrupted between 15 and 20 krad of irradiation.

#### *Anatomical observations on the cuticle and epidermal layer*

The incomplete separation of the cuticle of some irradiated insects has been described (VINSON et al., 1969).

In the present investigation the ratios of irradiated 5th-instar nymphs which died during the final moulting/total irradiated 5th-instar nymphs which failed to emerge as adults were 0 (0/0), 25 (2/8), 31 (4/13), 50 (8/16), and 6 (1/16) % at doses of 0 (control), 15, 20, 25, and 30 krad, respectively. The cuticle of such nymphs were incompletely separated from their epidermal layer at the final moulting, and as a result, they failed to complete the final moulting process. The effect of gamma radiation on the interruption of the normal process of moulting in such nymphs increased proportionally to a rise in the doses of irradiation within a definite range; 0 to 25 krad.

#### *Histological observations on the internal reproductive organs of macropterous adults*

*Normal testes:* Spermatogenesis in *N. lugens* follows the general pattern described for other auchenorrhynchous insects (MOCHIDA, 1970). There is a successive development of germ cells (Fig. 10 A). Spermatocytes showing the maturation division could be seen. The distinct metaphase stage of the first maturation division was frequently observed (Fig. 10 AF).

*Irradiated testes:* No appearance of cytological abnormality was seen in the testes of 0-day-old males irradiated at 5 krad (Fig. 10 C). Though there were many spermatozoa within the testicular follicles, spermatocytes showing maturation division were not seen in males irradiated at 20 krad (Fig. 10 DEG). Spermatogenesis was stopped.

The fact that radiation effect during spermatogenesis is greater in spermatocytes at meiosis in *Drosophila* and *Bombyx* (MULLER, 1958; SADO, 1961, 1963; BORSTEL, 1963) agrees with the results obtained in the present investigation.

*Normal ovaries:* Each ovary consists of about 24 ovarioles. The ovariole is of the telotrophic type. The germarium and vitellarium contain the developing germ cells and their accessory tissues (MOCHIDA, 1970). Soon after emergence yolk was not deposited even in the most developed oöcytes of females. Follicular cells were arranged regularly around younger oöcytes without yolk in the anterior parts of vitellariums (Fig. 11 A). Older females usually contained some fully grown eggs and older oöcytes with yolk within their ovaries (Fig. 11 B). The germariums of older females (Fig. 11 B) were usually larger than those of younger ones (Fig. 11 A), as the germ cells developed and became larger in size within the germariums.

*Irradiated ovaries:* Cellular abnormality could not be found in females irradiated at 5 krad (Fig. 11 CD). In females irradiated at 20 krad (Fig. 11 EF), chromatic clumping and nuclear disintegration of germ cells within germariums were seen, and necrosis and vacuolization were observed in some germ cells and their accessory tissues.

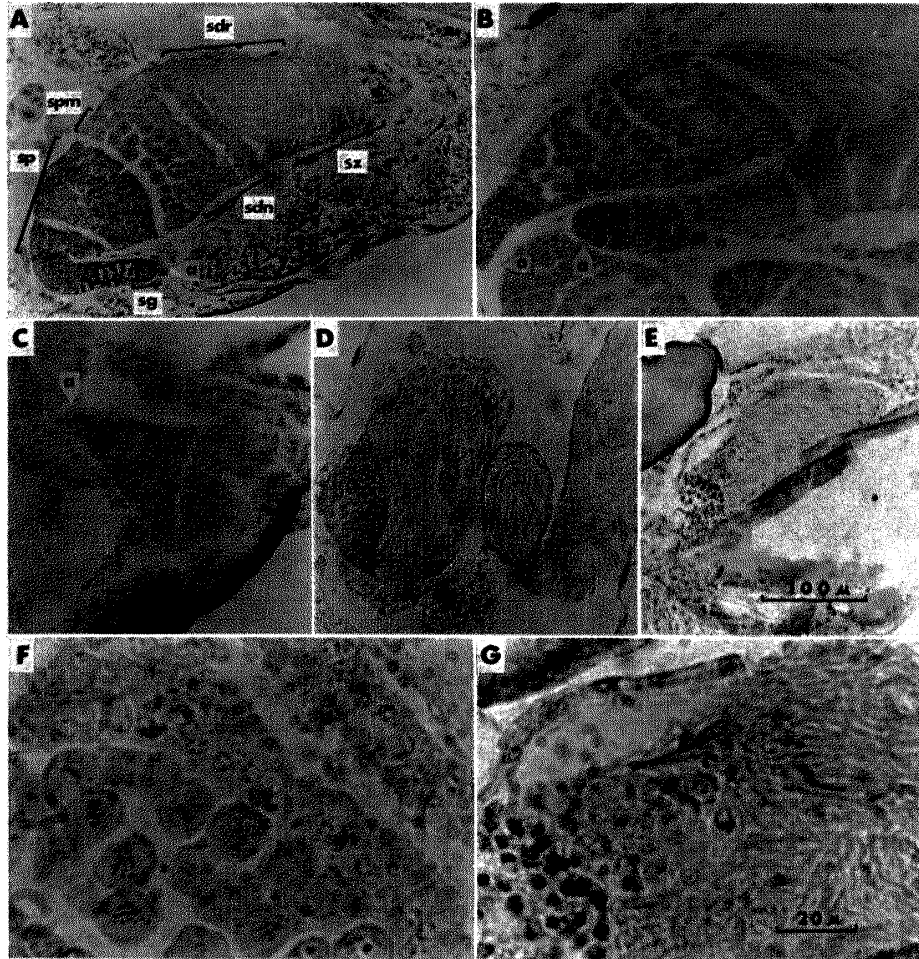


Fig. 10. Longitudinal sections of the testes of normal and irradiated *N. lugens* macropterous adult males. A, Normal 0-day-old after emergence; B, 0-day-old irradiated at 5 krad; C, 6-day-old irradiated at 5 krad; D, 3-day-old irradiated at 20 krad; E, 6-day-old irradiated at 20 krad; F, Part of the testicular follicle of (A); G, Part of the testicular follicle of (E). a, apical part of the testicular follicle; sdn, spermatids with nebenkern; sdr, spermatids with round head; sg, spermatogonia; sp, primary spermatocytes at the prophase (diakinesis) of maturation division; spm, primary spermatocytes at the prophase (diakinesis) or metaphase; sz, spermatozoa. The bar given in (E) is available in (A) to (E), and that in (G) is so in (F) and (G) for magnification.

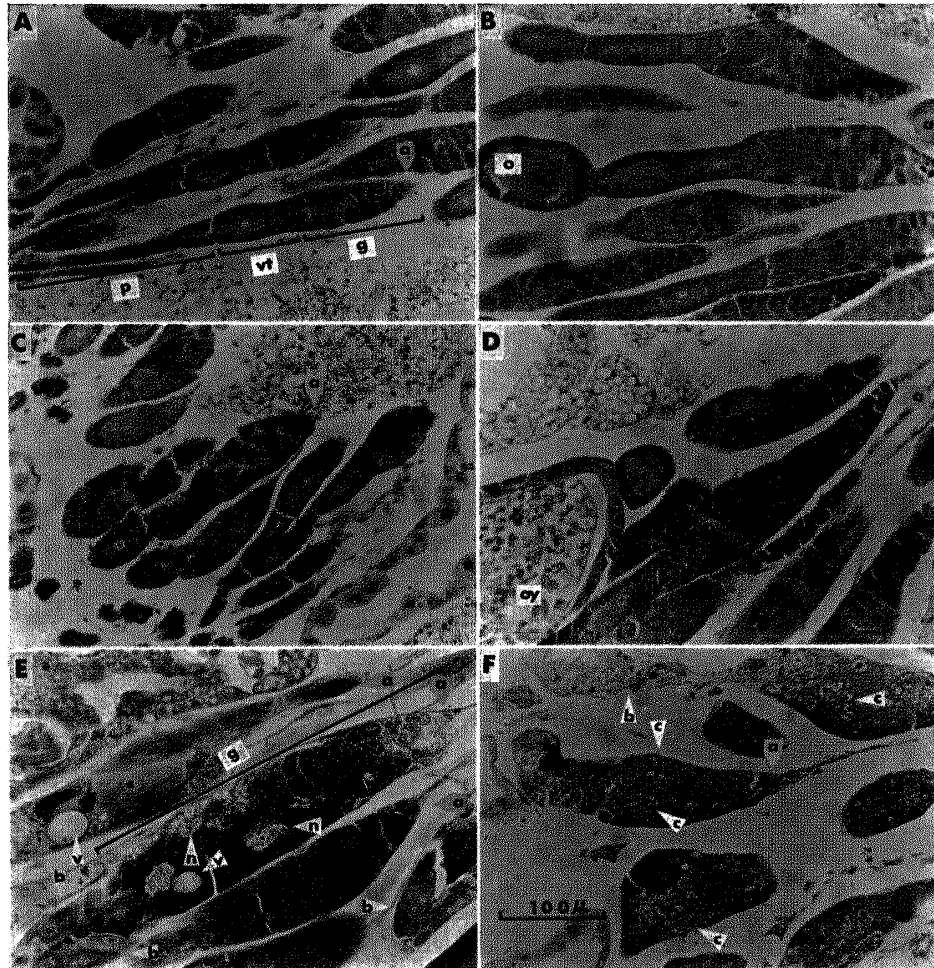


Fig. 11. Longitudinal sections of the ovaries of normal and irradiated *N. lugens* macropterous adult females. A, Normal 0-day-old after emergence; B, Normal 6-day-old; C, 0-day-old irradiated at 5 krad; D, 6-day-old irradiated at 5 krad; E, 9-day-old irradiated at 20 krad; F, Same as (E). a, apical part of the germarium; b, hypertrophic prefollicular or follicular cells; g, germarium; n, necrosis; o, young oocyte without yolk; oy, oocyte with yolk; p, pedicel; v, vacuole; vt, vitellarium. Magnification is all the same from (A) to (F).

Oöcytes disappeared within the anterior parts of vitellariums. Prefollicular or follicular cells around oöcytes showed various degrees of degeneration and hypertrophic or atrophic abnormality. Yolk-formation in normal females is initiated within the oöcytes 350 to 370  $\mu$  in length (MOCHIDA, 1970). Oöcytes no larger than 50  $\mu$  in length could be found in females irradiated at 20 krad. Regular arrangement of follicular cells around oöcytes within the vitellariums was not seen. Oögenesis was completely suppressed. This agrees with the anatomical observations described previously.

*Histological observations on the abdominal fat body cells of macropterous adults*

*Normal male:* The fat body of 0-day-old males consisted of an aggregate of white opaque cells that formed a lobed mass of tissue distributed in the body cavity. Fat body cells were closely adherent, and each cell was polygonal because of compression from neighbouring cells. The cell walls were not easily distinguished from one another. Many fat body cells had round nuclei, and several vacuoles were usually observed in each cell. In 6-day-old males vacuoles increased in size and in number, with concurrent shrinkage of the cytoplasm. As a result, the nuclei were irregularly clumped (Fig. 12).

*Irradiated male:* In males irradiated at 20 krad, the fat body cells possessing round nuclei were distinguishable from one another and filled with cell contents. Few vacuoles could be seen (Fig. 12). The fat body cells in males irradiated at 5 krad (Fig. 12) showed some morphological appearances intermediate between normal and those irradiated at 20 krad.

*Normal female:* The fat body cells of normal females were similar in appearance to those of normal males soon after emergence. Unlike such younger females, many smaller vacuoles appeared in the fat body cells of 6-day-old females. Vacuoles were usually smaller in size in 6-day-old females than in 0-day-old ones. Nuclei were commonly almost round in both younger and older females (Fig. 12).

*Irradiated female:* In both 0- and 6-day-old females vacuolization was intensively seen. The fat body cells were larger in size in females irradiated at 5 krad than in normal females. Those in females irradiated at 20 krad were similar to those of males done at 20 krad: Vacuolization was seldom seen, and round nuclei and cells filled up with cell contents could be observed.

The structural change of fat body cells of the females of manyin sects is usually observed during egg production (WIGGLESWORTH, 1965). In female *N. lugens* irradiated at 20 krad, however, the fact that fat body cells were filled up with cell contents is probably not associated with the cessation of oögenesis, since such appearances of fat body cells were also seen in males as mentioned previously (Fig. 12).

## DISCUSSION

Radiosensitivity of insect testes and ovaries resulting in decline of egg hatchability is known to vary during spermatogenesis and oögenesis. MULLER (1958) suggested on the bases of his and other workers' investigations on *Drosophila* that the sequence of varying susceptibility for the production of recessive lethals may be roughly indicated for the male germ cells as follows: (A) spermatogonia and early spermatocytes 1, (B) meiotic division stages 8, (C) spermatids 12, (D) spermatozoa more than a day

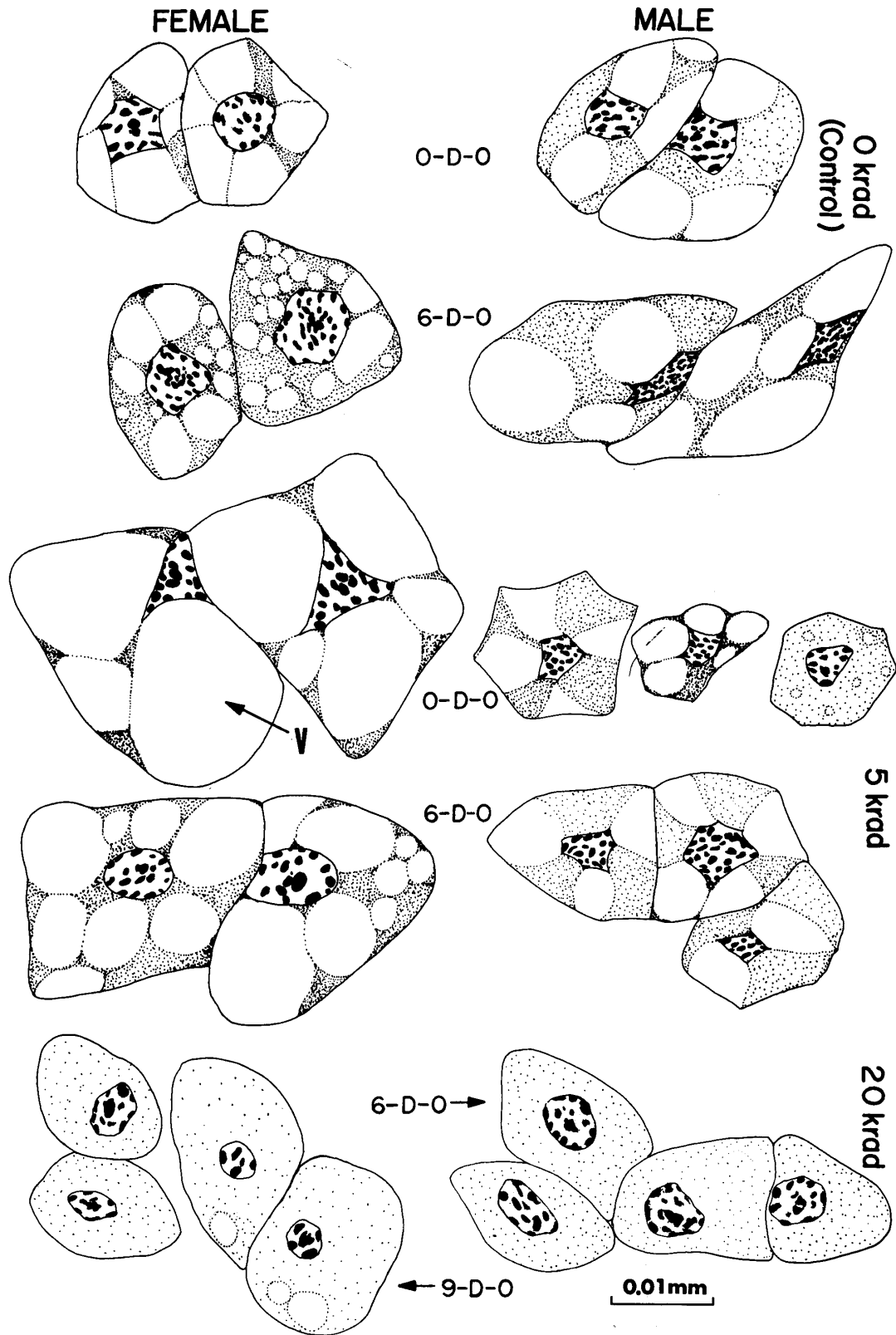


Fig. 12. Abdominal fat body cells of *N. lugens* macropterous adults. 0-D-O, 0-day-old after emergence; V, vacuole.

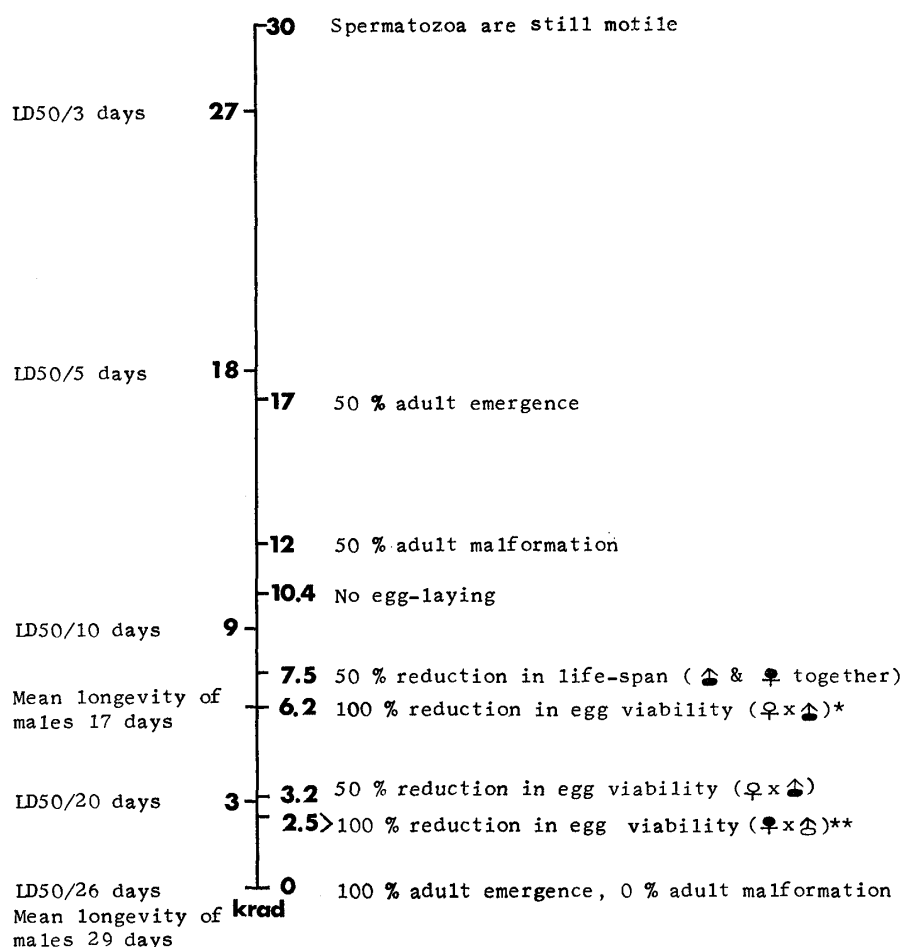


Fig. 13. Effect of gamma radiation on *N. lugens* irradiated in the 5th-instar stage. ♂ ♀, normal; ♂ ♀, irradiated. The values of LD50 are given for males and females added together and in days after irradiation. \* Minimum effective dose to obtain sterile male adults; \*\* Minimum effective dose to obtain sterile female adults.

before their ejaculation 3, (E) spermatozoa within inseminated females 5 to 6, and for the female germ cells: (A) oögonia and early oöcytes 1, (B) late oöcytes 2 to 3. SADO (1961, 1963) showed that an extreme sensitivity of spermatocytes at late meiotic prophase to the sterile effect of X-rays is observed in the silkworm, *Bombyx mori*. In *N. lugens* the majority of the male germ cells are spermatocytes through the 5th-instar stage, and the spermatids are observed in the posterior part of each testicular follicle in the late period of that stage (SUENAGA, 1963). In 5th-instar female nymphs the ovarioles contain younger oöcytes without yolk in the germariums (SUENAGA, 1963). The oöcytes begin to deposit yolk within them and develop to fully grown eggs within several days after emergence (MOCHIDA, 1970). Accordingly, the 5th-instar stage in *N. lugens* is probably suitable for sterilization by gamma radiation.

According to OSBORN et al. (1966) and SHIPP et al. (1966), the 5th-instar nymph of the delphacid, *Perkinsiella saccharicida* (KIRKALDY), is the most suitable stage for irradiation by gamma rays. Sterility is obtained at 3.5 krad to females and 10 krad to males when 5th-instar nymphs were irradiated. At these dose-levels, adult longevi-

ty and mating behaviour are not significantly affected. A dose of 12 krad renders a percentage of the sperm inactive, but adult longevity and mating behaviour are not significantly affected. A dose of 23 krad renders the sperm inactive, shortens adult longevity, and adversely affects wing development.

The results obtained in the present investigation are summarized in Fig. 13. Though experimental conditions were varied, *N. lugens* seems to be more radiosensitive than *P. saccharicida* for adult longevity and adult malformation. The fact that sterility is obtained at lower doses for females than for males in *N. lugens* is in accord with the observations on *P. saccharicida* (OSBORN et al., 1966; SHIPP et al., 1966). A dose of 6.2 krad was calculated as the minimum effective dose of irradiation to 5th-instar male nymphs to obtain sterile male adults or 100% reduction in egg viability in *N. lugens*. At this dose-level the values of % adult emergence (males and females together), % adult malformation (do.), and mean longevity of males (days after irradiation) were estimated to be about 100, 19 (0 for the control), and 17 (about 29 for the control), respectively. Many embryos yielded by normal females × males irradiated at 5 krad developed abnormally and ended in death before the last period of eye-spot stage. Lethal mutation sterility is presumably expected at 6.2 krad to males. The minimum effective dose of gamma radiation to 5th-instar female nymphs for the interruption of egg-formation was estimated to be between 15 and 20 krad, higher than the minimum effective dose for complete prevention of egg-laying.

AMERESKERE et al. (1971) observed histologically the chromatin clumping and necrosis of germ cells in male and female adults of *C. tenellus* irradiated at 15 and 5 krad, respectively.

In the present investigation anatomical observations on the internal reproductive organs indicated the delay of germ cell development in females irradiated at 5 krad and the death of germ cells in both males and females irradiated at 20 krad. These facts were confirmed histologically. The fact that the different stages of spermatogenesis respond with unequal sensitivity to radiation was indicated also in *N. lugens*.

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