Spermatogenesis of the silkworm and its bearing on radiation induced sterility. II

Sado, Toshihiko

https://doi.org/10.5109/22709

出版情報：九州大学大学院農学研究院紀要. 12 (4), pp.387-404, 1963-08. Kyushu University
バージョン：
権利関係：

Journal of the Faculty of Agriculture, Kyushu University, Vol. 12, No. 4.
August 30, 1963

Spermatogenesis of the silkworm and its bearing on radiation induced sterility. II

Toshihiko SADO

DISCUSSION

I. Normal spermatogenesis

Germ cell development in the larva. Although the cytological features of spermatogenesis in the silkworm have already been described in detail by a number of workers (Verson 1889, 1891, 1894, Toyama 1894, 1909, Grüneberg 1903, Katsuki 1918, Oguma 1919, Kawaguchi 1928, Machida 1929, 1935), there has been very little information on the correlation between the stage of spermatogenesis and the developmental phases of the insect. The present study revealed the following facts: (i) as observed by a number of previous workers (Tazima 1947, Takizawa and Tamazawa 1959), the maturation division (metaphase I) rarely takes place before the fifth instar. It becomes prevalent in the middle of this instar, but spermatocytes have already differentiated in the second instar. From the second to the early fifth instar spermatocytes are in the growth stage, or meiotic prophase. (ii) The majority of germ cells in the testis of the early fourth instar are primary spermatocytes in the synaptic and pachytene stages. Spermatogonia are much less numerous than spermatocytes. (iii) During the early fifth instar the testis contains germ cells from the primary spermatogonia to the young spermatids, cells in late meiotic prophase forming the majority. During the growth stage of the spermatocytes, the nuclei enter into meiotic prophase. Thus, spermatocytes in meiotic prophase can also be described as growing spermatocytes.

The correlation between larval stages and germ cell stages is shown schematically in Fig. 1.

Duration of spermatogenesis. Information about the duration of spermatogenesis is required for an accurate interpretation of the results of irradiation experiments. Some conflicting interpretations that have appeared in literature on radiation genetics are doubtless due to lack of accurate knowledge on the duration of spermatogenesis. Bateman's

(1956b) interpretation of his data on radiation-induced dominant lethals in mice and the subsequent discussions by Auerbach (1957), Sirlin and Edwards (1957) and by himself (Bateman 1957) are a case in question. For the mouse reliable data on the timing of cell development in each stage of spermatogenesis are now available (Oakberg 1956, 1957).

A similar situation exists for *Drosophila*. As reviewed in the earlier part of this paper, the period of temporary sterility, or lowest fertility, observed on the 7–9th day after irradiation has been attributed variously to irradiation effects on late spermatogonia and/or youngest spermatocytes (Friesen 1937, Auerbach 1954, Ives 1960), meiotic stages (Demerec and Kaufmann 1941, Bateman 1956), or spermatids (Lüning 1952, Fritz-Niggli 1955). Correspondingly, it has been considered that sperm derived from irradiated spermatogonia can be utilized for fertilization on the 9th day (Friesen 1937, Auerbach 1954), while some others claimed that sperm which were spermatogonia at the time of irradiation may be inseminated first during the 15–19th day after irradiation (Demerec and Kaufmann 1941). Glücksmann (1947) stated that total duration of spermatogenesis in adult *Drosophila* may be about 15 days.

So far as the present author knows, no reliable data on the duration of spermatogenesis have yet been published for the silkworm or other insects.* The answer could readily be obtained if *in vitro* culture of spermatogenic cells were successful, but so far attempts by the author to culture spermatogenic cells of silkworms *in vitro*, have not been successful (Sado 1959a). A rough estimate of the time required for each stage in spermatogenesis has been derived from the time table of the first appearance of germ cells of successive stages in untreated animals. These studies suggest that meiotic prophase extends through about 10 days, while late meiotic stages are fairly short. The time required from the end of the second meiotic division to the completion of spermiogenesis has been estimated as about 6 days. These values are not inconsistent with those obtained from observations on the regenerated germ cells after irradiation. It was noted that irradiation of primary spermatogonia of the silkworm with 1000 r scarcely affects the subsequent development of treated germ cells.

It is interesting to note that in the silkworm, as in the mouse (Oakberg 1957), meiotic prophase and the spermatid stage occupy fairly long periods. This similarity between two widely different species suggests an underlying general feature of spermatogenesis.

Eupyrene and apyrene spermatozoa. As stated in the earlier part

* After the present paper has been sent to the press, Chandley and Bateman (1962) published their data on the timing of spermatogenesis of adult *Drosophila* using H^3-thymidine (Nature, 193: 299–300).

of this paper, two types of spermatozoa, eupyrene and apyrene, were known to occur in the pupal testis of the silkworm. Machida (1929) and Ômura (1936) considered that the former type of spermatozoa is utilized for fertilization while the latter degenerate in the testis. Further, it has been claimed that two kinds of sperm, highly active and weakly motile, are observable in the reproductive tracts of female moths after copulation and that the former is derived from the apyrene spermatozoa and the latter from the eupyrene spermatozoa (Tsukaguchi and Kurotsu 1922, Iriki 1941).

In the present study, it was shown that no apyrene spermatozoa are found in the seminal vesicles of the newly emerged male moths even though the two kinds of sperm were always observable in the bursa copulatrix of females after mating. These two kinds of sperm were also observed in the bursa copulatrix of females mated with sterile males irradiated in the early fifth instar.

This observation leads to the conclusion that the apyrene spermatozoa do not migrate into the extra-testicular organs and, hence, are not ejaculated into female sexual organs. Both active and non-motile sperm observed in the female sexual organs are probably derived from eupyrene spermatozoa.

II. Radiation effects on spermatogenesis

Sensitivity of spermatogenic cells to irradiation. Differential sensitivity of germ cells during various stages of spermatogenesis in animals has been demonstrated in terms of sensitivity of cell-killing, chromosome aberrations, induced mutations and so on.

The results of the histological examination of the irradiated testes of the silkworm were in good agreement with those reported for mice (Hertwig 1938b, Oakberg 1955), grasshoppers (White 1935, Eker 1937, Cocchi and Uggeri 1944) and *Drosophila* (Friesen 1937, Bourgin *et al.* 1955, Welshons and Russell 1957), showing an extreme sensitivity of late spermatogonia to the killing effects of X-rays. According to some earlier investigators the youngest spermatocytes in *Drosophila* and grasshoppers are most readily killed by radiation (Mohr 1919, Friesen 1937); but, judged from the observation of silkworm spermatogenesis, it seems that the youngest spermatocytes are not always easily distinguished from the latest spermatogonia. The present study revealed the following facts: (i) the first sign of degeneration was observed in the late secondary spermatogonia, (ii) the degeneration of secondary spermatogonia became more striking 24 hours or more after irradiation, (iii) secondary spermatogonia were rarely observed 3 days after the exposure, and (iv) degeneration of the youngest spermatocytes was

observed only in very few instances. These results suggest that where necrosis of the youngest spermatocytes was recorded, this may, in fact, have been of cells that were in late secondary spermatogonia at the time of irradiation. This interpretation is supported by evidence presented by Oakberg (1955), who showed that degeneration of spermatogonia of the mouse occurred primarily when damaged cells reached the late interphase or early prophase of their first post-irradiation division; a few cells might undergo one or more division before degeneration. Cocchi and Uggeri (1944) also observed some necrotic early spermatocytes after irradiation, but considered that these cells had been irradiated in the telophase of the last spermatogonial mitoses. However, Kogure and Nakajima (1958) reported in silkworm that "..... until 48 hours after irradiation (of early fifth instar), no apparent distinction (in histocytological sense) in the germ cells of the irradiated and the control testes was found." They further continued in stating that "radiation sensitivity of spermatogonia is much lower than that of prophase spermatocytes." This is quite inconsistent with the present results, in which during 24-48 hours after irradiation highest incidence of necrosis of the secondary spermatogonia was observed in the treated testes. That this disagreement may be due to the differences in radiation quality (X-rays *vs.* γ-rays) or dose-rate (147 r/min. *vs.* 17 r/min.) is quite improbable. Because it has been shown that secondary spermatogonia of this insect are killed after acute as well as chronic γ-irradiations (Sado 1961b). Conceivably, they have missed observing necrosis of late spermatogonia in the irradiated testes.

Spermatocytes have been considered fairly resistant to radiation (Russell 1954), but there have been indications that irradiation of spermatocytes leads to the production of non-functional sperm (Schaefer 1939, Oakberg 1955, Casarett and Casarett 1957a, Bateman 1958, Oakberg and DiMinno 1960). The present experiment revealed that in the silkworm spermatocytes in early meiotic prophase, especially in synapsis and pachytene, are more resistant than those in late meiotic stages. The majority of cells irradiated in the former stages with 2000 r and those treated at the latter stages with 1000 r could not develop into normal functional sperm but degenerated during spermiogenesis or turned into non-functional sperm. It was, however, reported by Kogure and Nakajima (1958) that early prophase spermatocytes are most sensitive to radiation and cells irradiated at this stage later transform into apyrene spermatozoa. This again is not consistent with the present results. There are possible explanations for these apparent discrepancies. First, it seems that they have misidentified spermatocytes at late meiotic prophase as early prophase, because they claimed that spermatocytes at early prophase are the most prevalent cell type in the testis of

early fifth instar. If so, it is expected from the present experiments that in their experiments extremely high sensitivity to the sterilizing effect of radiation occurs late in the fifth instar. However, this was not the case in their experiment. Second, in their report majority of the structurally abnormal eupyrene spermatozoa in the present experiments were described as apyrene spermatozoa. When the present author discussed with Professor Kogure and his colleagues on this point, he was told by them that they classified structurally abnormal spermatozoa, as shown in Plate 12, Fig. 20, into apyrene spermatozoa in their report. Thus, although there are some discrepancies in the descriptions of cytological features of irradiated testes between Kogure's (1958) and the present results, the same conclusion may be reached that irradiation of silkworm larvae at early fifth instar causes the functional oligospermy of the treated individuals as a result of reduction in number of functional eupyrene spermatozoa.

As mentioned before, it has been suggested that spermatocytes carrying gross chromosomal aberrations as a result of irradiation will not give rise to sperm capable of fertilizing eggs (White 1937, Creighton 1941, Ohnuki 1958, Oakberg and DiMinno 1960). Possibly the majority of degenerating spermatids or abnormal spermatozoa reported in this paper were derived from the cells that had gross chromosomal disturbances induced by X-rays, but no cytological proof for this assumption is available.

At any rate, the finding that spermatocytes at early meiotic stages of the silkworm are more resistant to X-rays than those at late meiotic stages is in good agreement with Sparrow's (1951) observations on radiation effects on pollen mother cells of *Trillium*. Quite similar data have recently been published by Oakberg and DiMinno (1960) for mouse primary spermatocytes.

Spermatids and sperm are much more resistant to X-rays than early spermatocytes. Cells irradiated at these later stages of spermatogenesis do not themselves show any detectable changes, but radiation damage may appear in the offspring of the treated cells.

Variations of radiation induced mutation rates during gametogenesis have been reported by a number of workers (Lüning 1952, Auerbach 1954, Bateman 1956a, 1958, Tazima 1958b), and possible reasons for these findings have been discussed. Reviews will be found in the papers of Glass (1956), and Muller (1959) on *Drosophila*, of Hertwig (1957) and Russell, Russell and Oakberg (1959) on the mouse, and of Tazima (1959, 1961) on the silkworm.

Regeneration of spermatogonia. The histological observations on the regeneration of irradiated spermatogonia showed that the fate of regenerated germ cells differed depending on the time at which the

insects had been irradiated. In Table 9, the relation between the stages at which regeneration of irradiated spermatogonia occurs and the type of spermatozoa produced by the regenerated spermatogonia is summarized.

Table 9. Relation of the stages at which regeneration of irradiated spermatogonia occurs to the types of spermatozoa produced by the regenerated spermatogonia.

Stages irradiated	Stages at which regeneration of spermatogonia occurs	Types of spermatozoa developed from the regenerated spermatogonia
Unirradiated	——	Both eupyrene and apyrene spermatozoa
III-1	IV-2	Both eupyrene and apyrene spermatozoa
IV-2	V-3	Apyrene spermatozoa
V-2	V-9	None

It will be seen that spermatogonia regenerated before the IV-2 stage (2nd day of the fourth instar) produced both eupyrene and apyrene spermatozoa as in the unirradiated group. Spermatogonia regenerated at the middle of the fifth instar transform into apyrene spermatozoa but not into eupyrene spermatozoa. Furthermore, spermatogonia regenerated at a very late larval stage or in the pupal stage never transform into either eupyrene or apyrene spermatozoa.

It was noted that when irradiations were given during the second instar the rate of development of germ cells derived from regenerated spermatogonia does not markedly differ from that of unirradiated cells.

It should be remembered that the total duration of spermatogenesis in the silkworm takes about 20 days, of which meiotic prophase occupies more than 10, and that spermatocytes which undergo maturation division in the pupal testes will not differentiate into eupyrene spermatozoa (Machida 1929). In the strain used in the present experiment, it takes about 7 days from the beginning to the end of the fifth instar (maturation of larva) and 14–15 days from maturation to the emergence of moths. 2–3 days before the emergence, eupyrene spermatozoa begin to migrate from the testis into the ductus efferens testis. Thus, in the non-irradiated males, the last formed eupyrene spermatozoa in the pupal testis presumably were spermatogonia in the early fifth instar. It is suggested that under normal conditions spermatogonia present in the late fifth instar larvae or pupae will not be utilized for the production of functional eupyrene spermatozoa.

Stage limit for the production of functional sperm from irradiated spermatogonia. It was confirmed that functional sperm is produced from spermatogonia irradiated during early larval life, i.e., before the third instar. During the early third instar, the testis contains spermatogonia as well as spermatocytes, so that irradiation at this stage results in spermatozoa that might have been derived from either type of treated germ cells. Even after irradiation during the late second instar, a few available spermatozoa might stem from irradiated spermatocytes. However, this is not the case after irradiation with 2000 r or more, because after such high doses most of the primary spermatocytes can not develop into functional sperm. Thus, the fertility curve of males exposed to 2000 r refers only to spermatogonia and provides information on the stage limit for the production of functional sperm from regenerated spermatogonia. From Fig. 2, it will be seen that this limit occurs early in the third instar.

Thus, if one wants to test radiation effects on spermatogonia uncontaminated by other germ cell types, exposure has to be given before the middle of the second instar.

III. Nature of radiation induced sterility of the silkworm males

There are two accepted causes of sterility, in irradiated animals: (a) lack of fertilization due to the destruction of late spermatogonia, and at higher doses—spermatocytes (Hertwig 1938a, b, Russell 1954, Oakberg 1955, Oakberg and DiMinno 1960), (b) embryonal deaths due to dominant lethal mutations, with peak frequency in spermatids (Lüning 1952, Auerbach and Slizynski 1956, Bateman 1958).

It has been known that in the silkworm an extremely sensitive stage to the sterilizing effect of radiation occurs early in the fifth instar of the male larva (Tazima 1958a, Kogure and Nakajima 1958). The present studies show that this excessive sterility can be explained by the fact that cells in the late meiotic prophase are highly sensitive to irradiation and either degenerate during spermiogenesis or form spermatozoa many of which are non-functional, resulting in the functional oligospermy of the treated insects. Thus, in the present studies, sterility of irradiated insects has been attributed to direct damage of irradiated germ cells, and the degree of sterility has been measured in terms of unfertilized eggs. A distinction between fertilized and unfertilized eggs in the silkworm is usually based on their coloration, unfertilized ones being pale yellow, fertilized ones dark as a result of serosal pigmentation. A possible source of error in this procedure is that eggs dying from dominant lethals at a very early stage may be

unpigmented.

In *Habrobracon* and *Drosophila*, it has been shown that more than 50 per cent of the dominant lethal mutations that are induced in mature oöcytes or mature sperm result in death before formation of blastoderm, i.e., at a very early embryonic stage (von Borstel 1958, von Borstel *et al.* 1959). If the same principle holds true also for the silkworm, some of the lethal carrying embryos may die before serosal pigmentation and hence in the present studies may have been classified as unfertilized eggs.*

This question has been discussed very recently by Tazima (1960, 1961). He found that the incidence of unpigmented eggs was not significantly increased when mature sperm were irradiated with up to 5000r and he concluded that early acting dominant lethals are rare in the silkworm. In the present experiment, there was a negative correlation between the amount of spermatozoa observed in the female reproductive organs, particularly in the receptaculum seminis, and the per cent of unpigmented or unfertilized eggs (see Table 8). Thus, death through early acting dominant lethals does not appear to contribute significantly to radiation induced sterility in the silkworm. The results of the present experiment can best be explained by concluding that destruction or damage of germ cells was the main cause of observed sterility.

This agrees with observations on *Drosophila*, where during the period of lowest fertility about 7–9 days after irradiation the reproductive tracts of females mated with irradiated males contained no or very few spermatozoa (Friesen 1937, Auerbach 1954), while the histological examination of the eggs laid during this time revealed no evidence for sperm entrance (Kaplan 1958).

IV. General considerations on radiation-induced sterility in animals

The observations on irradiated silkworm males differ from those obtained on other animals in several ways.

Firstly, a temporary sterile period due to the destruction of type B, or late, spermatogonia has been observed by the successive brood technique both in mice (Schaefer 1939) and *Drosophila* (Friesen 1937, Auerbach 1954, Alexander and Stone 1955, Ives 1960). However, this technique is hardly applicable to the silkworm, because the adult moths are too short-lived for this technique. On the contrary, in mice and

* In the silkworm, blastoderm formation is completed 15–20 hours after egg laying, while the formation of the serosal membrane and its pigmentation occurs at 30 hours and 48–72 hours after the egg laying respectively.

adult *Drosophila* spermatozoa mature and are ejaculated successively for considerable time and a temporary sterile period appears when cells irradiated as late spermatogonia should have matured into functional sperm. After this period, fertility is restored, because by this time sperm has been produced from regenerated spermatogonia.

Contrary to the findings in mice and adult *Drosophila*, sterility due to the destruction of spermatogonia is hardly observable in the silkworm even though spermatogonia are easily killed by radiation. This seeming contradiction may be explained as follows. When X-irradiation is administered before the third instar, most of the secondary spermatogonia are killed but the regenerated spermatogonia have enough time to develop into functional sperm. On the other hand, irradiation with 1000r of older larvae in the third or fourth instar yields functional sperm from irradiated primary spermatocytes but not from regenerated spermatogonia, because the spermatocytes are fairly resistant to radiation while the regenerated spermatogonia have no time to develop into functional sperm. Thus, irradiation before the fourth instar does not markedly affect fertility of emerging male moths.

Secondly, it should be noted that in the silkworm pronounced sterility occurs when germ cells have been irradiated in late meiotic prophase I. This has not been observed in other animals; it may be explained as follows. In mice, Oakberg (1956, 1957) has shown that the early meiotic prophase stages, especially pachytene, are of very long duration, while the late meiotic stages are fairly short. Hence, when adult mice are treated, only a small proportion of late meiotic stages will be exposed among several other cell types of different radiation sensitivity. Among those, spermatocytes in early meiotic prophase I and young spermatids are fairly resistant to radiation. For this reason, the sterility caused by irradiation of cells in late meiotic stages may not be recognized in mice. This may also be true for adult *Drosophila* males. In the silkworm, however, sterility caused by the irradiation of late meiotic stages can be detected clearly because the synchronous development of germ cells results in only a small admixture of other cell types. Observations of Khishin (1955a, b) on *Drosophila* are highly suggestive in this respect. He described that irradiation with 1000r of prepupae, where cells in meiosis are prevalent, results in complete or almost complete sterility of the emerging flies while irradiation of third instar larvae, where growing primary spermatocytes, or auxocytes, are prevalent, affects fertility much less severely. This observation is consistent with the results obtained on the silkworm.

In silkworms as in mice the sterile period is extended by exposure to high doses of radiation, for most of the irradiated primary spermatocytes at early meiotic prophase do not then develop into functional

Fig. 4. Relation between the stages of spermatogenesis and the sterility induced by moderate and high doses of X-rays.

Dose *	Irradiated stage	Spermatogonia		Spermatocytes		Spermatids	Sperm
		Early	Late	Meiotic prophase I	Meta-phase		
Moderate	Sensitivity	Resistant	Highly sensitive (Destroyed)	Fairly resistant	Sensitive to chromosome aberrations (Later death)	Resistant	Resistant
	Fertility	Fertile	Sterile †	Fertile	Sterile ‡	Fertile	Fertile
High	Sensitivity	Resistant	Highly sensitive (Destroyed)	Sensitive to chromosome aberrations (Later death)		Resistant	Resistant
	Fertility	Fertile	Sterile			Fertile	Fertile

* The dose level differs with species.

† Sterility which corresponds to the destruction of late spermatogonia is not identifiable in the silkworm.

‡ Sterility which corresponds to the irradiation of late meiotic stages is not detectable in the mouse.

sperm. In the silkworm, this was clearly shown in the present experiment. In mice, the length of the initial fertile period continues for about 6 weeks after irradiation with 200 r (Schaefer 1939) but only for 2 to 4 weeks after exposure to 600 r and 1000 r (Russell 1954). Bateman (1958) also observed the earlier onset of the sterile period after 500 r as compared with 200 r and suggested that sperm developed from spermatocytes irradiated with 500 r is incapacitated in some subtle way. Cytological data relevant to the explanation of these phenomema are presented by Casarett and Casarett (1957a) for the rat and by Oakberg and DiMinno (1960) for mice.

From the foregoing discussions, the cytological basis of induced sterility observed after irradiation of adult males, where germ cells at various stages of maturation exist together in the testis, corresponds to the interval in which replacement of later germ cell stages has not yet been completed owing to the destruction of spermatogonia. This was shown in mice and adult *Drosophila* but not in the silkworm. On the other hand, when animals are irradiated as larvae in which the male germ cells develop more or less synchronously, an excessive sterility is induced by the failure of spermatocytes irradiated at late meiotic stages to form functional sperm. This was clearly shown for silkworm larvae and possibly for *Drosophila* larvae (Khishin 1955a, b). When animals are irradiated with high doses, the level of which depends on species and other biological factors, the sterilizing effect of radiation is very severe and extends towards the interval corresponding to the irradiation of primary spermatocytes. This is so because after such doses the cells at these stages cannot develop into functional sperm. Fig. 4 summarizes the relation between the stages of spermatogenesis in animals and the sterility induced with moderate and high doses of X-rays.

Summary

The present study consists of two parts. In the first part of this paper, the normal spermatogenesis of the silkworm was described with special reference to the correlation between the developmental stages of the larvae and the germ cell stages and to the duration of the successive stages of spermatogenesis. In the second part, radiation responses of spermatogenic cells were investigated by means of fertility tests and histological and cytological observations on the irradiated testes (or germ cells). The main purposes of this experiment were, first, to investigate the reasons for the seeming contradiction between the findings on mice and adult *Drosophila* on the one hand, and those of the silkworm on the other as to the cytological causes of radiation

induced sterility and, second, to establish the latest larval stage at which irradiation yields spermatozoa that were treated exclusively as spermatogonia. The results may be summarized as follows.

1. During normal spermatogenesis of the silkworm primary spermatocytes differentiate first around the middle of the second instar and increase in number with the development of the larva. In the testis of the early fourth instar larva, primary spermatocytes in synaptic and pachytene stages prevail. The testis of the early fifth instar larva contains germ cells ranging from primary spermatogonia to very early spermatids, with the spermatocytes in late meiotic prophase forming the majority.

2. The duration of each stage in spermatogenesis of the silkworm was estimated from the time table of the first appearance of cells in successive stages. It was found that meiotic prophase takes about 10 days, while late meiotic stages proceed within a short time. It takes about six days from the beginning of the spermiogenesis up to the completion of the eupyrene spermatozoa.

The estimates obtained from a similar study on the development of regenerated spermatogonia after 1000 r irradiation agreed well with those obtained from unirradiated germ cells. This indicates that irradiation of primary spermatogonia with 1000 r does not affect the subsequent development of the treated germ cells.

3. Examination of the seminal vesicles of the newly emerged male moths showed that no apyrene spermatozoa were transferred from the testis to the extra-testicular organs of males. This observation led to the conclusion that both active and non-motile sperm usually observed in the bursa copulatrix of females after copulation are derived from the eupyrene spermatozoa. The cause for the difference in activity among eupyrene spermatozoa remains unexplained.

4. Results of the histological examination of irradiated testes of the silkworm were in good agreement with those of previous workers on other animals. The secondary spermatogonia, especially in their later stages, were shown to be the most sensitive to damage by radiation and to be easily killed even by low X-ray doses. These dead cells degenerate rapidly and are removed from the testis within a short time.

Spermatocytes were shown to be more sensitive to radiation in late meiotic prophase than in the synaptic and pachytene stages. The majority of the cells irradiated with 1000 r of acute X-rays in the former stage or with 2000 r in the latter stages degenerate in the spermatid stage or turned into non-functional sperm.

Thus, two types of cell death could be distinguished by their time of occurrence, namely the immediate death of irradiated spermatogonia

and the later death of irradiated spermatocytes.

Primordial germ cells, primary spermatogonia, spermatids and sperm were shown to be resistant to irradiation.

5. The regeneration of spermatogonia after irradiation was investigated. It was found that the fate of the regenerated spermatogonia differed with the stage of the animal at the time of irradiation. When irradiation was given in the sufficiently early stages of larval life, i.e., before the early third instar, the regenerated spermatogonia could develop into eupyrene and apyrene spermatozoa as in unirradiated insects. When X-rays were given during the fourth or fifth instar, the regenerated spermatogonia failed to differentiate into eupyrene spermatozoa.

The stage limit for the production of functional spermatozoa from regenerated spermatogonia occurs near the early third instar.

6. Since the spermatocytes begin to differentiate around the middle of the second instar, irradiation should be given before this stage in order to obtain sperm samples treated as spermatogonia. When the irradiation is given in the late second instar, sperm utilized for fertilization will be contaminated by spermatozoa that developed from irradiated primary spermatocytes, although their contribution to the total sperm sample probably is very small.

7. In the silkworm, sterility due to the destruction of late spermatogonia is not detectable, even though the cells are easily killed by radiation. Pronounced sterility after irradiation of the early fifth instar is the result of functional oligospermy because the prevalent cells at this stage, i.e., spermatocytes in late meiotic prophase degenerate in the spermatid stage or turn into non-functional sperm.

8. These findings have been discussed in relation to comparable ones on mice and *Drosophila*, and it has been shown that a consistent picture of radiation induced sterility in animals can be obtained.

Acknowledgements

The author would like to express his sincere appreciation to Dr. Y. Tazima, National Institute of Genetics, under whose guidance and suggestions the present work was performed. He is very grateful to Prof. T. Hayashi and Dr. H. Chikushi, Kyushu University, and Prof. M. Kogure, Tokyo University of Agriculture and Technology, for their valuable suggestions and encouragements throughout the course of the work.

Special thanks are due to Dr. C. Auerbach, Edinburgh University, and Dr. S. Kondo, National Institute of Genetics, for critical discussions and correction of the English manuscript.

The author also indebted to Mr. H. Doira and Mr. T. Gamo, Kyushu University, who kindly reproduced for him the copies of papers not available at his Institute and to the entire staffs of the Silkworm Laboratory of the Institute for rearing of silkworms.

This work was aided in part by a Grant from the Rockefeller Foundation to the National Institute of Genetics and in part by a grant-in-aid for the Cooperative Research from the Ministry of Education for which the author gratefully acknowledged.

REFERENCES

Albers-Schönberg, H. E. 1903. Ueber eine bisher unbekannte Wirkung der Röntgenstrahlen auf den Organismus der Tiere. Münch. med. Wochschr. 50: 1859–1860.

Alexander, M. L. 1954. Mutation rates at specific autosomal loci in the mature and immature germ cells of *Drosophila melanogaster*. Genetics 39: 409–428.

——— 1960. Radiosensitivity at specific autosomal loci in mature sperm and spermatogonial cells of *Drosophila melanogaster*. *Ibid.* 45: 1019–1022.

——— and W. S. Stone 1955. Radiation damage in the developing germ cells of *Drosophila virilis*. Proc. Nat. Acad. Sci. 41: 1046–1057.

———, J. Bergendahl and M. Brittain 1959. Biological damage in mature and immature germ cells of *Drosophila virilis* with ionizing radiations. Genetics 44: 979–999.

Auerbach, C. 1954. Sensitivity of the *Drosophila* testis to the mutagenic action of X-rays. Zeits. Vererbungslehre 86: 113–125.

——— 1957. Sensitivity of immature mouse sperm to the mutagenic effects of X-rays. Nature 179: 725–729.

——— and B. M. Slizynski 1956. Sensitivity of the mouse testis to the mutagenic action of X-rays. *Ibid.* 177: 934–935.

Bateman, A. J. 1955a. Mutagenic sensitivity of maturing *Drosophila* sperms I. Dominant lethals. J. Genet. 51: 400–410.

——— 1956b Sensitivity of the mouse testis to the mutagenic action of X rays. Nature 177: 934–935.

——— 1957. Sensitivity of immature mouse sperm to mutagenic effects of X-rays. *Ibid.* 179: 725–729.

——— 1958. Mutagenic sensitivity of maturing germ cells in the male mouse. Heredity 12: 213–232.

Bergonie, J. and L. Tribondeau 1904. Action des rayons X sur le testicule du rat blanc. Compt. rend. Soc. biol. 56, Pt. 2: 400–402.

Bourgin, R. C., R. Krumins and H. Quastler 1956. Radiation induced delay of pupation in *Drosophila*. Rad. Res. 5: 657–673.

Bryan, J. H. and J. W. Gowen 1956. A histological and cytophotometric study of the effects of X-rays on the mouse testis. Biol. Bull. 110: 229–242.

——— and ——— 1958. The effects of 2560 r of X-rays on spermatogenesis in the mouse. *Ibid.* 114: 271–283.

Casarett, A. P. and G. W. Casarett 1957a. Histological investigations of mechanisms of X-ray effects on spermatogenesis in the rat I. Introduction, purpose, methods and preliminary experiments. Univ. Rochester Atomic Energy Project Report, UR-496, 1-119.

and 1957b. *Ditto* II. Studies comparing effects of acute and chronic irradiations. *Ibid.* UR-497, 1-131.

Cocchi, U. and B. Uggeri 1944. Die Wirkung der Röntgenstrahlen auf den Heuschreckenhoden. Strahlentherapie 75: 96-109.

Creighton, M. 1941. The effects of X-rays on mitotic and meiotic chromosomes and cell division in *Chorthippus longicornis* (Orthoptera). J. Exp. Zool. 87: 347-369.

Demerec, M. and B. Kaufmann 1941. Time required for *Drosophila* males to exhaust the supply of mature sperm. Amer. Nat. 75: 366-379.

Eker, R. 1937. Reaktion der Spermatogonienmitosen auf die Röntgenstrahlung bei der Heuschrecke *Tachycines asynamorus*. Arch. exp. Zellforsch. 19: 458-464.

Friesen, H. 1937. Untersuchungen über künstliche Auslösung von Crossing over in der Meiosis und Mitosis. Biol. Zhur. 6: 1055-1136. (In Russian with German Summary)

Fritz-Niggli, H. 1955. Dominant lethal factors and embryonic mortality after irradiation at 180kv and 31 Mev. Proc. Intern. Conf. Peaceful Uses of Atomic Energy 11: 179-183.

Glass, B. 1956. Differences in mutability during different stages of gametogenesis in *Drosophila*. Brookhaven Symp. Biol. No. 8, Mutation: 148-170.

Glücksmann, A. 1947. The effects of radiation on reproductive organs. Brit. J. Radiol. Suppl. 1: 101-109.

Grüneberg, K. 1903. Untersuchungen über die Keim-und Nährzellen in den Hoden und Ovarien der Lepidopteren. Zeits. wiss. Zool. 74: 327-395.

Hayashi, T. 1935. Effects of X-rays on the silkworm (a preliminary note). Zool. Mag. 47: 155-157. (In Japanese)

Hertwig, P. 1938a. Unterscheide in der Entwicklungsfähigkeit von F_1 Mäusen nach Röntgenbestrahlung von Spermatogonien, fertigen und unfertigen Spermatozoen. Biol. Zentbl. 58: 273-301.

1938b. Die Regeneration des Samenepithels der Maus nach Röntgenbestrahlung, unter besonderer Berücksichtigung der Spermatogonien. Arch. exp. Zellforsch. Gewebezücht. 22: 68-73.

1957. Strahlenschäden und Strahlenschutz in zellulären Bereich. Berichte Verhandl. Säch. Akad. Wissensch. Leipzig, Math.-naturwiss. Klasse 102 (6): 1-43.

Iriki, S. 1941. On the function of apyrene spermatozoa of the silkworm. Zool. Mag. 53: 123-124. (In Japanese)

Ives, P. T. 1950. The effects of γ-rays on fecundity and mutagenesis in Oregon-R males of *Drosophila*. Int. J. Rad. Biol. 2: 54-67.

Jones, E. A. 1960. Number of spermatogonia after X-irradiation of the adult rat. *Ibid.* 2: 157-170.

Kaplan, W. D. 1958. The sterility component of X-ray induced dominant lethals in *Drosophila melanogaster*. Proc. 10th Intern. Cong. Genet. Part 2: 142.

Kashiwabara, T. 1957. On the changes of germ cells in the X-ray irradiated monse. Sci. Rept. Fac. Agr. Ibaragi Univ. 5: 133-146.

Katsuki, K. 1918. Cytologische Studien über die Samenzellen von Seidenraupen. Bull. Imp. Sericult. Exp. Stat. 1: 151-202.

1925. The effect of X-rays on the silkworm. *Ibid.* 6: 475-485. (In Japanese)

Kawaguchi, E. 1928. Zytologische Untersuchungen am Seidenspinner und seinen Verwandten I. Gametogenese von *Bombyx mori* L. und *Bombyx mandarina* M. und ihre Bastarde. Zeits. Zellforsch. mikro. Anat. 7: 519-552.

Khishin, A. F. E. 1955a. The response of the immature testis of *Drosophila* to the mutagenic action of X-rays. Zeits. Vererbungslehre 87: 97-112.

1955b. Effect of larval and pupal irradiation on survival and fertility. DIS 29: 128-129.

Kogure, M. and M. Nakajima 1958. Differential radiosensitivity of silkworm testis for the decline of egg hatchability, with special reference to cytological and biochemical evidences. Proc. 2nd U. N. Intern. Confer. Peaceful Uses of Atomic Energy 22: 351-359.

Kaufmann, B. P. 1951. Chromosome aberrations induced in animal cells by ionizing radiations. In 'Radiation Biology' (ed. A. Hollaender) Vol. 1, Part 2, Chap. 9: 627-711.

Lea, D. E. 1954. Actions of radiations on living cells. Cambridge Univ. Press. (2nd ed.)

Lüning, K. G. 1952. X-ray induced dominant lethals in different stages of spermatogenesis in *Drosophila*. Hereditas 38: 91-107.

Machida, J. 1929. Eine experimentelle Untersuchung über die apyrene Spermatozoen des Seidenspinners *Bombyx mori* L. Zeits. Zellforsch. mikro. Anat. 9: 466-510.

1935. Spermatogenesis of the silkworm, *Bombyx mori* L., with special concern to the cytoplasmic materials and spermiogenesis. Zool. Mag. 35: 512-534. (In Japanese)

Miya, K. 1958. Studies on the embryonic development of the gonad in the silkworm, *Bombyx mori* L. Part I. Differentiation of germ cells. Journ. Fac. Agr. Iwate Univ. 3: 436-467.

1959. *Ditto.* Part II. Formation of the gonad. *Ibid.* 4: 126-151.

Mohr, O. L. 1919. Mikroskopische Untersuchungen zu Experimenten über Einfluss der Radiumstrahlen und der Kältewirkung auf die Chromatinreifung und das Heterochromosome bei *Decticus verrucivorum* (♂). Arch. mikro. Anat. 92: 300-368.

Muller, H. J. 1954. The manner of production of mutations by radiation. In 'Radiation Biology' (ed. A. Hollaender) Vol. 1. Part 2, Chap. 8: 475-626.

1958. Radiation mutagenesis through the studies on *Drosophila*. Proc 2nd U. N. Intern. Confer. Peaceful Uses of Atomic Energy 22: 313-321.

Oakberg, E. F. 1955. Sensitivity and time of degeneration of spermatogenic cells irradiated in various stages of maturation in the mouse. Rad. Res. 2: 369-391.

1956. Duration of spermatogenesis in the mouse and timing of stages of the cycle of the seminiferous epithelium. Amer. J. Anat. 99: 507-516.

1957. Duration of spermatogenesis in the mouse. Nature 180: 1137-1139.

1959. Initial depletion and subsequent recovery of spermatogonia of the mouse after 20r of gamma rays and 100, 300, and 600r of X-rays. Rad.

Res. 11: 700–719.

——— and R. L. DiMinno 1950. X-ray sensitivity of primary spermatocytes of the mouse. Intern. J. Rad. Biol. 2: 196–209.

Obata, R. 1934. The effect of Röntgen-rays on the gonads of the silkworm. Sci. Bull. Agr. Coll. Kagoshima, Suppl.: 519–527.

Oguma, K. 1919. On the chromosomes of the silkworm, *Bombyx mori* L. Zool. Mag. No. 363: 8–14; No. 364: 49–54.

Ohnuki, Y. 1958. Phase cinematography studies on the effects of radiation and chemicals on the cell and the chromosomes I. Types of X-ray induced chromosome abnormalities in grasshopper spermatocytes, with a note on the normal course of the first division as control. Jour. Fac. Sci. Hokkaido Univ. Ser. 4, Zool. 14: 83–91.

Ômura, S. 1936. Studies on the reproductive systems of the male of *Bombyx mori*. I. Structure of the testis and the intratesticular behaviour of the spermatozoa. Jour. Fac. Agr. Hokkaido Univ. 38, Pt. 2: 151–181.

——— 1938a. *Ditto*. II. Post-testicular organs and post-testicular behaviour of the spermatozoa. *Ibid.* 40, Pt. 3: 129–170.

——— 1938b. Structure and function of the female genital system of *Bombyx mori* with special reference to the mechanism of fertilization. *Ibid.* 40, Pt. 3: 111–128.

Russell, W. L. 1951. X-ray induced mutations in mice. Cold Spring Harbor Symp. Quant. Biol. 16: 327–336.

——— 1954. Genetic effects of radiation in mammals. In 'Radiation Biology' (ed. A. Hollaender) Vol. 1, Part 2, Chap. 12: 825–859.

———, L. B. Russell and E. M. Kelly 1958. Radiation dose rate and mutation frequency. Science 128: 1546–1550.

———, L. B. Russell and E. F. Oakberg 1958. Radiation genetics of mammals. In 'Radiation Biology and Medicine' (ed. W. D. Claus) Chap. 8: 189–205.

Sado, T. 1959a. Unsuccessful attempt at *in vitro* cultivation of spermatogenic cells of the silkworm. Ann. Rept. Nat. Inst. Genetics, Japan No. 9: 105–106.

——— 1959b. Histological study of radiation sensitivity of spermatogenic cells of the silkworm (a preliminary note). *Ibid.* No. 9: 101–102.

——— 1960a. Duration of spermatogenesis in the silkworm. *Ibid.* No. 10: 120–121.

——— 1960b. Recovery of irradiated spermatogonia of the silkworm and the stage limit for the production of functional sperm. *Ibid.* No. 10: 119.

——— 1960c. Cytological basis of radiation induced sterility of the male silkworm. Jap. J. Genet. 35: 285–286.

——— 1961a. Sensitivity and time of the degeneration of spermatocytes irradiated at different stages of meiosis of the silkworm. Ann. Rept. Nat. Inst. Genetics, Japan, No. 11: 84.

——— 1961b. Differences in response of silkworm gonia to acute and chronic γ-irradiations. Jap. J. Genet. 36: 393 (Abst. in Japanese)

——— 1961c. Spermatogenesis of the silkworm and its bearing on the radiation induced sterility. Jap. J. Genet. 36, Suppl. 1: 136–151.

Schaefer, H. 1939. Die Fertilität von Mäusenmännchen nach Bestrahlung mit 200 r. Zeits. mikr. anat. Forsch. 46: 121–152.

Sirlin, J. L. and K. G. Edwards 1957. Sensitivity of immature mouse sperm to the mutagenic effects of X-rays. Nature 179: 725–729.

Sparrow, A. H. 1951. Radiation sensitivity of cells during mitotic and meiotic

cycle with emphasis on possible cytochemical changes. Ann. New York Acad. Sci. 51: 1508–1540.

Takizawa, Y. and T. Tamazawa 1959. On the time of maturation division of the silkworm. J. Sericult. Sci. Japan 28: 170–171.

Tazima, Y. 1947. Mosaicism of the silkworm. Hokuryukan, Tokyo. (In Japanese)

—— 1956. Differences in sensitivity of germ cells and chromosomes to radiation among some mutant strains of the silkworm. Proc. Intern. Genet. Symp.: 280–286.

—— 1958a. Changes in sensitivity of silkworm germ cells to X-rays with development. Rad. Res. 9: 193.

—— 1958b. Mutational response pattern of silkworm germ cells to X-rays. Proc. 10th Intern. Congr. Genet. Part 2: 291–292.

—— 1959. Radiation physiology of the silkworm with special reference to the effect of radiation on germ cells. In 'Recent Advances in Experimental Morphology' (ed. K. Takewaki *et al.*), Yokendo, Tokyo, Chap. 8: 249–271.

—— 1960. Some observations relevant to the possible cause of radiation induced sterility in the male silkworm. Ann. Rept. Nat. Inst. Genetics, Japan No. 10: 118.

—— —— 1961. Considerations on the changes in observed mutation rates in the silkworm after irradiation of various stages of gametogenesis. Jap. J. Genet. 36, Suppl. 1: 50–64.

——, S. Kondo and T. Sado 1961. Two types of dose-rate dependence of radiation induced mutation rates in spermatogonia and oögonia of the silkworm. Genetics 46: 1335–1345.

Toyama, K. 1894. On the spermatogenesis of the silkworm. Bull. Agr. Coll Tokyo Imp. Univ. 2: 125–157.

—— —— 1902. Contributions to the study of silkworms. I. On the embryology of the silkworms. *Ibid.* 5: 73–118.

—— —— 1909 Lectures on silkworm eggs. Maruyamasha, Tokyo. (In Japanese)

Tichomirow, A. 1898. Zur Anatomie des Insectenhodens. Zool. Anz. 21: 623–630.

Tsukaguchi, R. and T. Kurotsu 1922. On the apyrene spermatozoa of the Lepidoptera. Zool. Mag. 31: 310–317. (In Japanese)

Verson, E. 1889. Zur Spermatogenesis. Zool. Anz. 12: 100–103.

—— —— 1891. Zur Beurteilung der amitotischen Kernteilung. Biol. Zentralbl. 11: 556–558.

—— —— 1894. Zur Spermatogenesis bei der Seidenraupe. Zeits. wiss. Zool. 58: 303–313.

von Borstel, R. C. 1958. Dominant lethal mutations in *Habrobracon* and *Drosophila*. Proc. 10th Intern. Cong. Genet. Part 2: 303.

—— —— and M. L. Rekemyer 1959. Radiation induced and genetically contrived dominant lethality in *Habrobracon* and *Drosophila*. Genetics 44: 1053–1074.

Welshons, W. J. and W. L. Russell 1957. The effects of X-rays on the *Drosophila* testis and a method for obtaining spermatogonial mutation rates. Proc. Nat. Acad. Sci. 43: 608–613.

White, M. J. D. 1935. The effects of X-rays on mitosis in the spermatogonial divisions of *Locusta migratoria* L. Proc. Roy. Soc. B., 119: 61–84.

—— 1937. The effect of X-rays on the first meiotic divisions in three species of Orthoptera. *Ibid.* 124: 183–196.

Yatsu, N. 1913. Notes on the spermatogenesis of the wild and the domesticated silkworms. Annat. Zool. Jap. 8: 215–220.