THE EFFECT OF X-RAYS ON THE DROSOPHILA TESTIS AND A METHOD FOR OBTAINING SPERMATOGONIAL MUTATION RATES

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Introduction.—It has long been known that male mice exposed to an acute X-ray dose of a few hundred roentgens remain fertile for a period of from four to six weeks. After a period of sterility, fertility returns and is maintained. The temporary sterility is due to depletion of spermatogonia, which are especially sensitive to killing by radiation.[1] Fertility returns after adequate repopulation of spermatogonia has occurred. Matings made during the presterile period utilize germ cells that were already in postspermatogonial stages at the time of irradiation. Matings made after the sterile period utilize germ cells that were in the spermatogonial stage at the time of irradiation. The genetic effects of irradiation in these two stages, spermatogonial and postspermatogonial, can, therefore, be measured separately by using the sterile period as an indication for the separation. For example, Russell[2] used the sterile period in this way in the determination of X-ray-induced mutation rates in spermatogonia of the mouse.

Do irradiated *Drosophila* males show a similar sterile period which could be used as a reliable sign that the sperm used in matings made after this period were spermatogonia at the time of irradiation? In her determinations of spermatogonial mutation rates in *Drosophila*, Alexander[3] felt that, at the time when her experiments were made, there was no sure way of telling which type of spermatogenic cells were being tested when the male had been irradiated as an adult. To insure that only spermatogonia were being tested, she therefore irradiated larvae at a stage when the testis contained nothing but spermatogonia. The dose had to be limited to 900 r in order to avoid killing the larvae.

Auerbach,[4] on the other hand, concluded that it was possible to determine mutation rates in spermatogonia from irradiated adult *Drosophila*. In experiments designed primarily to study the mutagenic effects of X-rays upon the late stages of spermatogenesis, she found that, at a certain interval after irradiation, *Drosophila* males showed a period of low fertility, and that, after this period, fertility was regained. Other investigators had found the same effect, and one interpretation has been that the low fertility is due to a high incidence of dominant lethality. Some of the earlier part of the period of reduced fertility is undoubtedly due to this factor, but Auerbach examined the reproductive tracts of females that had been mated to irradiated males during the period of lowest fertility of the males, and she found little or no sperm present. She therefore concluded that the low fertility during that period was due to lack of sperm rather than to the loss of embryos from dominant lethality. When the males had regained their fertility, Auerbach obtained some clusters of identical or complementary crossovers. From this observation she concluded that the period of temporary sterility could be used to separate the results of irradiated gonia from those of cells irradiated at a later spermatogenic stage.

As additional evidence for this conclusion, Auerbach called attention to some findings of Friesen[5] which are apparently not well known. Friesen had observed a period of temporary sterility after irradiating adult *Drosophila* males with various doses of X-rays. Even a dose as small as 1,000 r led to a clear-cut period of low fertility. On the basis of experiments on induced crossing over, Friesen concluded that the sperm sampled after the return of fertility were derived from cells which had been irradiated as spermatogonia. He then offered histological evidence that the young primary spermatocytes and some spermatogonia were destroyed by the irradiation, indicating that the period of temporary sterility is due to a deletion of a portion of the spermatogenic cycle.

In contrast to the histological findings of Friesen is the report by Fritz-Niggli[6] that *Drosophila* spermatocytes and spermatogonia are resistant to X-rays. Thus, although the irradiated males observed by Fritz-Niggli showed a period of low fertility comparable to that found by Auerbach and others, Fritz-Niggli does not attribute it to the destruction of young primary spermatocytes and spermatogonia.

It was apparent that further clarification of the problem was needed. The present investigation has attempted to provide this by checking the validity of the conclusions of Auerbach and Friesen. The check was made by a more extensive study of the histology of the irradiated testis at frequent intervals after irradiation with various doses of X-rays.

The present work also explored another point. It was anticipated that if Auerbach and Friesen were correct in supposing that spermatogonial mutations could be obtained by irradiating adults, then there was a possibility that induced mutation rates in spermatogonia could be obtained not only at dosages higher than those possible with larvae, but perhaps at dosages higher than those practicable for the determination of mutation rates in sperm. In the irradiation of sperm, an upper limit is set by the high frequency with which major chromosomal aberrations are induced. Sperm derived from irradiated spermatogonia, however, are essentially free of such aberrations. It was, of course, expected that there would still be an upper practicable limit to the dosage possible, because of the induction of permanent sterility. The present work explored the dose level at which this occurs. Other possible complications arising with the use of high dosages are discussed later.

Methods and Results.—Four experiments were performed, in which males were given 9,867, 8,052, 4,000, or 1,000 r of X-rays. After treatment, the males were mated and kept in cultures maintained at 25° C. A given number of hours after irradiation, a sample of from three to five males was withdrawn from the cultures. The males were placed in a saline solution, and the testes were removed and immediately transferred to a large drop of aceto-orcein stain. They were stained for approximately fifteen minutes. A cover slip was placed over the testes, excess stain was removed, and the cover slip was sealed to the slide with a paraffin-vaseline mixture. These temporary mounts were examined the following day. This is a rapid and easy technique which is patterned after one suggested by Cooper.[7]

When the condition of an irradiated testis is compared with that of an unirradiated one, the damage caused by X-rays is quite apparent at the dosages used here. Although the damage is not easily expressed in quantitative terms, a

roughly quantitative summary of the results was prepared as a basis for discussion. It should be noted that, although there is some variation between time after irradiation and effect, the sequence of changes following a given dose appears to be uniform.

No distinction between secondary spermatogonia and early spermatocytes was made in these preparations. However, obvious damage has been observed in the region where the synchronous divisions of secondary gonia are seen in unirradiated material. Therefore, visible damage in this location is due to the destruction of these gonia, and it is probable that some small young spermatocytes are also killed.

In the following descriptions, only cells in the gonial region (spermatogonia and young spermatocytes), large primary spermatocytes, and cysts of spermatids undergoing spermiogenesis will be used to mark the course of recovery of an irradiated testis. An experiment in which adult *Drosophila* males were given a dose of 4,000 r of X-rays will be described first. The results of experiments at three other dosages will then be compared with the results of the 4,000-r experiment.

Table 1 represents a brief summary of the results of the 4,000-r experiment. Fifty-five hours after the administration of X-rays, the cells in the gonial region were greatly reduced in number, but by day 4 repopulation had occurred. This region then remained normal throughout the rest of the experiment.

TABLE 1

HISTOLOGICAL CONDITION OF *Drosophila* TESTIS AFTER ADMINISTRATION OF 4,000 R OF X-RAYS TO ADULT MALES*

	DAYS AFTER IRRADIATION						
	2¼	3	4	5	6	7	8
Gonial region	+	++	+++	+++	+++	+++	+++
Primary spermatocytes	++	+	0	++	+++	++++	++++
Spermiogenic cysts	+++	+++	+++	+	0	0	0
	9	10	11	12	13	14	15
Gonial region	+++	+++	+++	+++	+++	+++	+++
Primary spermatocytes	+++	+++	+++	+++	+++	+++	+++
Spermiogenic cysts	0	+	+	+	++	++	+++

* The normal condition of a testis is designated by +++.

Cells that are large primary spermatocytes at the time of irradiation are not killed. However, their number gradually decreases, since they proceed through meiosis and are not immediately replaced. By day 4 there are no large primary spermatocytes present. By day 5, new spermatocytes have arisen from the regenerated cells in the gonial region. These new spermatocytes steadily increase in number, until, by days 7 and 8, the irradiated testis actually contains more spermatocytes than the control. By day 9 the number has dropped to the control value, and remains at the control level until the end of the observation period, on day 15. Resumption of meiotic divisions was first observed on day 7.

Spermiogenic cysts are present from the beginning of the experiment until day 4. They decrease in number on day 5, and they are absent from day 6 to day 9.

From day 10 to day 12, one observes them infrequently. Thus, it appears that spermiogenesis can proceed without the formation of these cysts. By day 15 the testis appeared normal in all respects.

Two experiments were performed at higher dosages. The results can be summarized briefly as follows: After the administration of 9,867 r, the cellular elements in the gonial region of the testis had vanished, in mosts cases, by the third day. The large primary spermatocytes followed the course of events outlined above, and by day 4 none was present. There were a few cases of attempted regeneration over the 4–7-day period, followed by a final deterioration of the regenerated elements from day 8 to day 11. In an 8,052-r experiment the results were essentially the same, except that there were a few cases of regeneration which were not followed by deterioration but instead led to complete restoration of the testis. Within the sample observed, this condition never involved both testes of the same male. Viable offspring were obtained from these males.

The results from a 1,000-r experiment were similar to those from the 4,000-r dose except that the damage was not so marked and was more difficult to assess. The number of primary spermatocytes present in the irradiated testis was never reduced to zero, and spermiogenic cysts were absent for only two or three days.

Discussion.—These histological observations are in harmony with the results obtained by Friesen,[5] except that they indicate that the damage to secondary spermatogonia is probably more extensive than Friesen believed. However, Friesen did observe some damage to this cell type after 4,000 r when the observations were made 48 hours after treatment. The present authors' observations indicate that the damage is more obvious at about 55 hours post-treatment. Friesen identified dead young primary spermatocytes 24 hours after irradiation. No such identifications were made in this study, but no observations were made until 55 hours after treatment. Thus it is concluded that X-ray induced degeneration of young primary spermatocytes is best observed 24 hours after irradiation, and that the destruction of secondary spermatogonia does not become apparent until 24 hours later.

It was pointed out earlier that Fritz-Niggli[6] concluded from her experiments that spermatocytes and spermatogonia are resistant to X-rays. The reason for the discrepancy between this conclusion and the present observation is clear. In Fritz-Niggli's work the testis was not examined until three days after administering 3,000 r, at which time the sensitive cells at the tip of the testis must already have been lost and replaced. The conclusion that primary spermatocytes are resistant to the killing effects of X-rays is, however, valid for those that are more mature, for these cells continue through the meiotic divisions even after 9,867 r of X-rays. The observations on spermiogenic cysts become pertinent in this light. It seems that these cysts disappear when the last of the cells irradiated as primary spermatocytes has gone through spermiogenesis. The significance of the observation that spermiogenesis can, for a while following its resumption, take place without the formation of these characteristic cysts remains obscure for the present.

These observations add support to the conclusion of Auerbach[4] that mutations obtained after the return of fertility occurred in cells that were irradiated as spermatogonia. Furthermore, the detailed histology of the effects of radiation is shown to be quite similar to that observed by Oakberg[1] in the mouse. Therefore, sper-

matogonial mutation rates could be obtained in *Drosophila* in the same way that they are obtained in mice.[2]

Since this appears to be a valid method for obtaining spermatogonial mutation rates, it is of interest to compare it with the method of irradiating larvae.[3, 8] There are two reasons why it might be a more convenient method. (1) It is technically easier to irradiate adult males than to irradiate larvae carefully timed for stage in development. (2) The method of using larvae is restricted to doses of not more than about 900 r of X-rays, because larvae cannot survive doses much larger than this. Since adult males live and regain fertility after much greater doses, it seems likely that the determination of spermatogonial mutation rates from adult males could be carried out at dosages higher than those used with larvae. Although an appreciable increase in dosage seems feasible, it is possible that a many-fold increase might introduce the following complication. The number of primary spermatogonia in the testis of the adult *Drosophila* male is not large, and, if there is appreciable killing of these cells at higher dosages, then the number of surviving spermatogonia might be small compared with the number of offspring raised from each male in typical experimental work. Although this factor, which would result in clusters of mutations, complicates the design of experiments, it is of course, of biological interest in itself.

It should be kept in mind that at higher dosages the mutation rate from irradiated gonia may, as in the mouse,[9] depart from a linear relationship with dose. This, again, would be of biological interest.

In presenting this apparently more efficient method for obtaining radiation-induced spermatogonial mutation rates, it is not, of course, implied that induced mutation rates in larvae are no longer worth obtaining. It is quite possible that the rates in larvae and adults would not be identical, and this possibility should be explored.

Summary.—Histological observations were made on the testes of irradiated adult *Drosophila* males given 9,867, 8,052, 4,000, or 1,000 r of X-rays. Contrary to the conclusion of one investigator,[6] these experiments, combined with the earlier work by Friesen,[5] indicate that both secondary spermatogonia and young spermatocytes are sensitive to the killing effects of X-rays. The destruction of these sensitive cells results in a period of temporary sterility following irradiation of adult males. Therefore, this sterile period can be relied upon to separate cells irradiated as spermatogonia from those irradiated at a later spermatogenic stage. It follows that radiation-induced spermatogonial mutations, uncontaminated by mutations at later stages in spermatogenesis, can be obtained from irradiated adult *Drosophila* males in much the same way that they have been obtained from male mice.[2]

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ON A WEDDERBURN-ARTIN STRUCTURE THEORY OF A POTENT SEMIRING*

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1. *Introduction.*—In a recent paper[1] we proved that if S is a potent semiring in which each two-sided ideal contains a minimal right ideal and a minimal left ideal of S, then any right ideal $R \neq (0)$ contains a multiplicative idempotent. The existence of this idempotent now enables us to obtain for a potent semiring a theory analogous to the Wedderburn-Artin structure theory of a semisimple ring with minimum condition.[2]

In this paper we prove that if S is a potent semiring with identity in which each two-sided ideal contains a minimum right ideal and a minimum left ideal and S is a strong direct sum of minimal right ideals, then S is a strong direct sum of semirings isomorphic to matrix semirings over division semirings.

2. *Strong Direct Sum.*—We shall assume that the semiring S possess a *zero* 0, in the sense of Vandiver and Weaver,[3] $0 + s = s + 0 = s$, $0 \cdot s = s \cdot 0 = 0$, for all s in S. For the sake of completeness, we repeat the definition of a *potent semiring* given elsewhere.[1]

Definition 1: A semiring S is said to be potent if it contains no nonzero nilpotent right ideals and no nonzero nilpotent left ideals.

Definition 2: A semiring S is said to be simple if it contains no proper two-sided ideals.

LEMMA 1. *If S is a potent simple semiring and $R \neq (0)$ a minimal right ideal of S, then the mappings $\bar{\rho}(r) = \begin{pmatrix} r \\ \rho r \end{pmatrix}$, $r \in R$, $\rho \in S$, and $\rho R \neq (0)$, form a division semiring.*

Proof: Let Δ be the set of all minimal left ideals $\neq (0)$ of S. Then a former lemma[1] states that $S = \sum_{L \in \Delta} L$ and hence $S\rho = \sum_{L \in \Delta} L$. We note that $L\rho$ is either in Δ or $L\rho = (0)$. If $S\rho R = (0)$, then $S\rho R = SR = (0)$ and $RR = (0)$, which contradicts the potency of S. Therefore, $S\rho R \neq (0)$. Since $S\rho = \sum_{L \in \Delta} L$, there exists an $L \in \Delta$ such that $L\rho R \neq (0)$. We set $L_1 = L\rho$. Since $L_1 R \neq (0)$, then $L_1 \neq (0)$ and $L_1 \in \Delta$. Since $L_1 R \neq (0)$, a former lemma[1] states that $L_1 \cap R \ni e = e^2 \neq (0)$. Hence $eR \neq (0)$ and $eR = R$, $er = r$ for $r \in R$. Since $e \in L_1 \cap R \subseteq L_1 \subseteq S\rho$, then $e = s\rho$, $s \in S$. Therefore, $r = er = s\rho r = \bar{s}\bar{\rho}(r)$, where $\bar{s} = \begin{pmatrix} r \\ sr \end{pmatrix}$ and $\bar{s}\bar{\rho} = \bar{1}$. If $\rho x = \rho y$, for $x, y \in R$, then $s\rho x = s\rho y$, $ex = ey$, and $x = y$. Hence