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MAMMALIAN GAMETOGENESIS AND SPECIES COMPARISONS IN RADIATION RESPONSE OF THE GONADS*

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Abstract

MAMMALIAN GAMETOGENESIS AND SPECIES COMPARISONS IN RADIATION RESPONSE OF THE GONADS. Primordial germ cells have an extra-gonadal origin and migrate to the genital ridge early in embryonic development. After a series of mitoses, they transform into oocytes in the female and into type A spermatogonia in the male.

In females, the total supply of germ cells is present either before or shortly after birth; development of the oocyte is arrested in diplodote of meiotic prophase. The nuclear character of the 'arrested' diplodote varies greatly between species, and is an important factor in species differences in ovarian radiation response. In adults of all mammals, the radiation response of the ovary involves a fixed population of primary oocytes. Oogonia are absent; any loss from the oocyte pool cannot be replaced. Type A spermatogonia persist throughout the reproductive life span of the male, and a complete array of gametogenic stages is present in the adult. Radiation resistance of spermatids and sperm results in an initial period of post-irradiation fertility; destruction of spermatogonia and possibly spermatocytes leads to oligospermia with doses of several hundred R; fertility eventually returns owing to repopulation of the seminiferous epithelium from a few surviving type A spermatogonia. The different radiation response of males and females therefore can be attributed to basic differences in gametogenesis and the array of germ cell stages present in the adult gonad. A radiation response typical for all males also occurs in man. However, exact comparison of radiation sensitivity in man and experimental animals is not possible owing to differences in the control of spermatogonial differentiation which results in species differences in both initial depletion and subsequent recovery of the spermatogonial population.

INTRODUCTION

Our understanding of the radiation response of the gonads is dependent on our knowledge of the origin of the germ cells, their development during uterine and pre-pubertal life, and gametogenesis in the adult. Wherever possible, our meager data on man have been included; but for the most part, the formulation of basic principles of normal gametogenesis, and especially of radiation response, has been based on data on experimental animals.

The most striking difference in gametogenesis in mammals is that which occurs between the sexes. In the male, stem cells (spermatogonia) and continued formation of mature gametes occur throughout the reproductive life-span; whereas in the female, the definitive supply of oocytes is formed either before or shortly after birth. Oogonia are not present in the adult ovary. As a result of these fundamental differences in gametogenesis, the radiation response of males differs greatly from that of the female.

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GAMETOGENESIS

Prenatal and neonatal

Migration of primordial germ cells from an extragonadal origin to the germinal ridges was first observed in human embryos [1]. Later, Chiquoine [2] demonstrated selective alkaline phosphate positive staining of the primordial germ cells of the mouse, and by this technique was able to trace their migration from their origin in the region of the caudal end of the primitive streak, root of the allantoic mesoderm, and yolk sac splanchnopleure in the 8-day mouse embryo, to the germinal ridges of the 12-day embryo. The demonstration by Mink [3] that sterility and reduced fertility associated with certain genotypes of the mouse can be correlated with a reduced number of primordial germ cells is conclusive evidence that these are the stem cells of the definitive gametes. Oocyte counts in rat embryos have led to the same conclusion [4]. Mitotic division occurs both during migration of the primordial germ cells and in the germinal ridge. Primordial germ cells transform directly into oocytes in the female [3-6]. In males, they give rise to type A spermatogonia soon after birth in the rat [7], and by 10 years in man [8]. Although Charny et al. [8] did not use the precise system of cell classification given by Clermont [9], their data indicate that the basic developmental pattern observed in other mammals also occurs in man.

Adult male

Our understanding of normal spermatogenesis has advanced markedly in recent years owing to the accurate identification of both cells and developmental sequences made possible by the periodic acid-Schiff technique [9, 10, 11]. In rodents, stages of the cycle of the seminiferous epithelium occupy relatively large areas of the tubule, and a given tubule cross section usually shows a single stage. In man, however, an average of three different stages appears in a single cross section, and while analysis of the cycle of the seminiferous epithelium is therefore very difficult, it has been shown to be fundamentally the same as that observed in other species [12].

Type A spermatogonia are the stem cells of the mammalian testis. By the process of stem cell renewal, the type A population maintains a steady state while giving rise to an unlimited number of differentiated spermatogonial types, which develop into sperm. The following brief description for the mouse is similar to that of Clermont [9] for the rat, and though species differences occur, the same basic process has been observed in guinea pigs, monkeys, and man. Four divisions of type A spermatogonia occur [13]. At the conclusion of the fourth division, most cells transform into intermediate spermatogonia, with morphological characteristics intermediate between those of type A and type B. The intermediate spermatogonia divide to form spermatogonia of type B, which in turn divide to form resting primary spermatocytes. A few type A spermatogonia fail to differentiate, and form the stem cells for the next multiplicative cycle. In the mouse and rat, either four or five cell generations, depending on stage of the cycle of the seminiferous epithelium, occur in a given tubule section. Both the cellular association and number of cells are characteristic for each stage of the cycle; thus quantitation of radiation response of specific cell types is possible. The above model needs to be modified only slightly for man, where two types of type A cells occur, A dark (Ad), and A pale (Ap) [12]. Ad spermatogonia differentiate into Ap, and Ap divide to form spermatogonia B. From there the process is similar, with
spermiogenesis involving changes leading to development of characteristic sperm morphology of each species.

The time required for type A spermatogonia to develop into spermatozoa appears to be a biological constant for each species, and is unaffected by radiation [14] or hormone levels [15, 16] even though both of these procedures can result in severe depletion of the seminiferous epithelium. Estimates of the duration of spermatogenesis are about 35 days in the mouse [17], 49 days in the ram [18], 48 days in the rat [19], and 72-74 days in man [16]. These estimates, together with information on the dynamics of cell populations and relative frequency of stages of the cycle of the seminiferous epithelium, make possible the calculation of the life-span of each cell type. Such calculations, as given for mouse and man in Table I, are invaluable in studying the radiation response of specific cell types, and in comparing the radiation response of different species.

Adult females

The most distinctive feature of oogenesis in mammals is the absence of stem cells (oogonia) from the adult ovary [20]. All oogonia enter meiosis either before or shortly after birth, and usually develop as far as diplophase. Nuclear morphology of the diplophase stage of the "arrested" oocyte, however, varies widely between species; a "typical" diplophase is characteristic of the human, goat, and dog; a synizesis-like diplophase is characteristic of the guinea pig; and a diffuse, interphase-like diplophase (dictyate) is present in mouse and rat [21]. In all species, the chromosomes become diffuse and oxyphilic during growth of the follicle, and probably are in a lambrush stage. The first meiotic division occurs in the ovary; ovulation occurs about 6 h later with the chromosomes in metaphase of the second meiotic division [22, 23].

Since the female has a fixed pool of oocytes established early in life, it would be expected that the number of oocytes would decrease with age owing to follicular atresia and ovulation. This decrease has indeed been observed [24, 25] and constitutes a cogent argument against formation of oocytes in the adult mammalian ovary [24]. Furthermore, in the mouse, DNA synthesis typical of preleptotene occurs only in utero [26, 27], and oocytes labeled in utero persist in the adult ovary [27]. Finally, genetic factors which reduce fertility also reduce the number of primordial germ cells in the embryonic gonad [3]. As a result of the above observations, the direct relationship between primordial and definitive germ cells is now firmly established.

RADIATION RESPONSE

Females

The radiation response of the adult ovary, therefore, will be limited to diplophase and later meiotic stages in the adult; irradiation during embryonic, fetal, and neonatal development will have to be used in study of stages from primordial germ cells through pachytene. Whereas all female germ cells go through a similar developmental process, the correlation of germ cell stage with post-conception age, synchrony of the cell population, and stage at which oocyte development is arrested vary between species; thus, even though specific cells may have a similar radiation response wherever they occur, large species differences in radiation response of the ovary are to be expected.
<table>
<thead>
<tr>
<th>Cell type</th>
<th>Duration (days)</th>
<th>Interval to release of mature sperm from testis (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spermatogonia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>always present</td>
<td>33-36</td>
</tr>
<tr>
<td>Intermediate</td>
<td>1.1</td>
<td>29-30</td>
</tr>
<tr>
<td>B</td>
<td>1.2</td>
<td>28-29</td>
</tr>
<tr>
<td><strong>Primary Spermatocytes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preleptotene</td>
<td>1.3</td>
<td>26-28</td>
</tr>
<tr>
<td>Leptotene</td>
<td>1.3</td>
<td>25-26</td>
</tr>
<tr>
<td>Zygotene</td>
<td>1.6</td>
<td>23-25</td>
</tr>
<tr>
<td>Pachytene</td>
<td>7.3</td>
<td>16-23</td>
</tr>
<tr>
<td>Diplotene</td>
<td>0.9</td>
<td>15-16</td>
</tr>
<tr>
<td>Meiotic divisions</td>
<td>0.4</td>
<td>15</td>
</tr>
<tr>
<td>Spermatocyte II</td>
<td>0.4</td>
<td>14-15</td>
</tr>
<tr>
<td>Spermatids</td>
<td>14.0</td>
<td>0-14</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Duration (days)</th>
<th>Interval to release of mature sperm from testis (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spermatogonia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ad</td>
<td>always present</td>
<td>72-74</td>
</tr>
<tr>
<td>Ap</td>
<td>16.0</td>
<td>73-57</td>
</tr>
<tr>
<td>B</td>
<td>11.2</td>
<td>46-57</td>
</tr>
<tr>
<td><strong>Primary Spermatocytes</strong></td>
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<td></td>
</tr>
<tr>
<td>Preleptotene</td>
<td>1.0</td>
<td>45-46</td>
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<td>Leptotene</td>
<td>3.7</td>
<td>41-45</td>
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<td>Zygotene</td>
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</tr>
<tr>
<td>Pachytene</td>
<td>15.5</td>
<td>23-38</td>
</tr>
<tr>
<td>Diplotene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meiotic divisions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spermatocyte II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spermatids</td>
<td></td>
<td></td>
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</tbody>
</table>

* Computed from data of Heller and Clermont (see ref. 16).
Table II. Mean numbers of oocytes surviving 50 R in the mouse and 200 R in the guinea pig (From Oakberg and Clark; see ref 21)

<table>
<thead>
<tr>
<th>Time after Irradiation (days)</th>
<th>Early follicles (Mice (50 R))</th>
<th>Early follicles (Guinea pigs (200 R))</th>
<th>Late follicles (Mice (50 R))</th>
<th>Late follicles (Guinea pigs (200 R))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. cells</td>
<td>Exptl./Control</td>
<td>No. cells</td>
<td>Exptl./Control</td>
</tr>
<tr>
<td>Control</td>
<td>3,549</td>
<td></td>
<td>33,720**</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>959</td>
<td>0.270</td>
<td>37,780</td>
<td>1.120</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>0.008</td>
<td>21,020</td>
<td>0.623</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>0.010</td>
<td>17,580</td>
<td>0.521</td>
</tr>
<tr>
<td>7</td>
<td>15,420</td>
<td>0.457</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>24</td>
<td>0.007</td>
<td>13,300</td>
<td>0.394</td>
</tr>
<tr>
<td>14</td>
<td>11,560</td>
<td>0.343</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>18,440</td>
<td>0.547</td>
<td></td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>10,500</td>
<td>0.311</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>3</td>
<td>0.001</td>
<td>10</td>
<td>0.217</td>
</tr>
</tbody>
</table>

* All means for mice based on three animals per group.

** Means for guinea pigs based on three controls; data based on one animal for each time after irradiation.
<table>
<thead>
<tr>
<th>Meiotic prophase stage</th>
<th>Survival data</th>
<th>Chromosome breakage D&lt;sub&gt;50&lt;/sub&gt;</th>
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</thead>
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<tr>
<td></td>
<td>Point estimate</td>
<td>95% confidence limits</td>
</tr>
<tr>
<td></td>
<td>LD&lt;sub&gt;50&lt;/sub&gt; (R)</td>
<td>Lower</td>
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<tr>
<td>Preleptotene</td>
<td>205</td>
<td>175</td>
</tr>
<tr>
<td>Leptotene</td>
<td>492</td>
<td>273</td>
</tr>
<tr>
<td>Zygotene</td>
<td>520</td>
<td>413</td>
</tr>
<tr>
<td>Pachytene (early)</td>
<td>404</td>
<td>329</td>
</tr>
<tr>
<td>Pachytene (mid)</td>
<td>382</td>
<td>301</td>
</tr>
<tr>
<td>Pachytene (late)</td>
<td>664</td>
<td>601</td>
</tr>
<tr>
<td>Diplotene</td>
<td>564</td>
<td>365</td>
</tr>
<tr>
<td>Diakinesis – Metaphase I</td>
<td>837</td>
<td>679</td>
</tr>
<tr>
<td>Diakinesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metaphase I</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
MAMMALIAN GAMETOGENESIS

In the mouse, 50 R of x-rays reduces the number of litters to four per female, with permanent sterility thereafter [28]. This can be attributed to killing of the small oocytes, and subsequent failure to replace the older, more resistant stages as they mature and are ovulated [29, 30]. A similar response is observed in the rat [31], but higher doses are required to produce comparable effects. The response in guinea pigs is quite different, however, and a comparison of survival of early and late follicle stages in mice and guinea pigs is given in Table II. In the guinea pig, small oocytes are resistant and growing oocytes sensitive, which is the reverse situation of that in the mouse. As a result, fertility of guinea pigs is unaffected by acute x-ray exposures of several hundred R. Likewise, fertility of dogs is normal after 300 R x-ray exposures [32], rabbits show fertility for a considerable time after irradiation [33], and women have conceived a year or more after exposures of 300-400 R, with presumed normal fertility thereafter [34, 35].

The reasons for species differences in fertility of irradiated females are not well understood, but normal reproductive life-span, normal rate of oocyte depletion, frequency distribution of oocyte stages with different radiation sensitivity, and nuclear characteristics of the "arrested" oocyte all probably are important factors. Differences between species in nuclear cytology of the oocyte have been observed [21], and though we do not understand the basic mechanisms involved, a correlation exists between chromosome appearance and radiation response. High sensitivity to cell-killing is a characteristic of the dictyate nuclei of mouse and rat, which makes these species poor models for generalizations on adult ovarian radiation response in other mammals. However, periods of high sensitivity may occur during prenatal or neonatal development in species where the adult ovary is relatively resistant, and extreme caution should be used in irradiation exposure at this time. The most important fact is that the ovaries of different species show widely divergent radiation response.

Males

Recent advances in our understanding of the normal dynamics of spermatogenesis have made possible the evaluation of the cellular responses in both depletion and regeneration of the seminiferous epithelium. Spermatids show no morphological or gross functional damage (sensitivity to genetic damage is fairly high, however) even after doses of 1000 R or more, complete spermiogenesis, and give rise to the period of initial fertility following doses of several hundred R [36]. After irradiation, the frequency of cells degenerating during meiotic division, and the frequency of bridges and fragments at anaphase are increased. The result is depletion in the number of spermatids formed, and an increase in the proportion of spermatids with various degrees of heteroploidy. A measure of comparative sensitivity of different meiotic prophase stages to degeneration during meiotic division is shown in Table III, and varies from an LD_{50} of 205 R for preleptotene to 837 R for diakinesis-metaphase I. Frequency of abnormal anaphases resulting from chromosome breakage, translocation, and formation of acentric fragments also are given in Table II. The ranking of sensitivities is almost the reverse of that for degeneration, with diakinesis-metaphase I most sensitive and leptotene least sensitive. As a result of degeneration and gross chromosomal abnormality, contribution of spermatocytes to post-irradiation gamete production is dose-dependent.

Since spermatocytes and spermatids have a relatively short duration in comparison to reproductive life-span, spermatogonia are the most important cells in radiation response of the mammalian testis. Killing of differentiating spermatogonia leads to maturation depletion of the testis, azoospermia, and the temporary period...
of infertility characteristic of males given a single radiation exposure. A few type A spermatogonia survive, however, and repopulate the seminiferous epithelium [37]. Depletion and recovery of apparently normal type A spermatogonia in the mouse are shown for a wide range of doses in Fig. 1. The time of maximum depression of cell numbers is dose-dependent owing to initiation of division among surviving cells before the process of cell degeneration has been completed. The interaction of these two processes is characteristic for each dose, and there is no optimum time for comparing the effects of all the doses given. As would be expected, recovery is more rapid after low doses, but a significant increase in the spermatogonial population has occurred by 10 days even after 600 R. Eventually, the entire array of spermatogenic cells is regenerated.

![Graph showing experimental/control ratios for 'normal' type A spermatogonia of the mouse 1 h to 10 days after irradiation.](image)

**Human testis**

In man, the Ad spermatogonia are thought to be the stem cells of the seminiferous epithelium [12, 16]. Radiation response of the Ad spermatogonia to local x-ray exposure of the testis, as measured by number of normal cells, is shown in Fig. 2. (These data are based on slides provided by Dr. Carl G. Helle, Pacific Northwest Research Foundation, Seattle, Washington.) The initial depletion and subsequent recovery observed in experimental animals also occurs in man. There are some important differences, however.

Radiation-induced depletion of the spermatogonial population of the mouse is primarily the result of cell killing [38], whereas both cell killing and differentiation into more mature cell types appear to be important in man. Cell killing results in the initial rapid decrease in the spermatogonial population in man, but the gradual decline which continues until 90 days or more is the result of both cell death and spermatogonial differentiation.

As demonstrated by a comparison of Figs. 1 and 2, repopulation of the seminiferous epithelium also is much slower in man than in the mouse. Comparable data for type A spermatogonia after 300 R in the mouse also are included in Fig. 2 for ease of comparison. The difference is greater than would be expected on the basis of duration of spermatogenesis, for the time required in man is only 72 days compared with 35 days in the mouse. Obviously, other factors are responsible,
and these may concern basic processes in the control of spermatogonial differentiation. In the mouse, the A spermatogonia first increase in number, then differentiate into intermediate and B spermatogonia. In man, however, differentiation into B spermatogonia occurs when the number of Ad cells still is as low as 2 or 3% of control. This represents a "wastage" of cells as far as recovery of the Ad population is concerned, and appears to be a significant factor in the slow repopulation of the radiation depleted human testis.

Comparison of the doses required for comparable depression of spermatogonia in mouse and man suggests that man is the more sensitive by a factor of about 3. However, this estimate must be accepted with cogent reservations. First, the comparison is based on data at 5-7 days after irradiation in the mouse, and on data at 90-200 days in man. Second, the mechanisms of spermatogonial depletion differ in mouse and man. Third, the dynamics of re-establishment of the spermatogonial population in man reveals significant differences in the control of spermatogonial differentiation in mouse and man. Thus, while the general radiation response of the human testis is typical of all mammals, many aspects of the normal process of gametogenesis will have to be more thoroughly understood before radiation sensitivity of experimental animals and man can be adequately compared.

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DISCUSSION

F. H. SOBELS: You mentioned that the period of high genetic radiosensitivity at the end of the dictyate stage of the mouse oocyte seems to coincide with cessation of RNA metabolism. Do you or Dr. Russell know of any other evidence that the apparent repair of premutational damage at earlier stages is associated with RNA metabolism?
E. F. OAKBERG: At present we can only say that RNA metabolism is an indicator of general metabolic activity, which may be connected with repair systems.

L. B. RUSSELL: The low mutation frequency obtained when early follicle stages are sampled may be due to mechanisms other than repair, such as cell selection.

E. F. OAKBERG: A third possibility is that it is connected with a possible difference in intrinsic sensitivity of the genetic material, as W. L. Russell has pointed out (Proc. natn. Acad. Sci. U. S. A. 54 (1965) 1552 - 57).

F. H. SOBELS: What is the mechanism of cell death in the B type spermatogonias?

E. F. OAKBERG: The precise mechanism is unknown, but there is an immediate cell killing which is already detectable 3 - 4 hours after the irradiation in both mouse and man. In man, many of them differentiate into spermatocytes, which is a different response from what we see in the mouse or rat. We really do not understand how the type A spermatogonial population maintains itself. Recently Clermont and his group have suggested there is a so-called A₀ spermatogonium which is a true stem cell forming a reserve population to replenish the seminiferous epithelium. One interpretation of the human results could be that the actual spermatogonial differentiation in man occurs more than 72 days before production of mature sperm, but that we are unable to recognize the initial cell population, so we have the wrong estimate of the duration of human spermatogenesis.

H. M. BEAUMONT: I wonder if you would comment on cell selection in oocytes.

E. F. OAKBERG: We certainly have evidence for increased cell selection through atresia in the irradiated female. It is interesting that the female can ovulate about the normal number of oocytes each oestrus even though she has very few left. But atresia occurs at different times in the control and irradiated females. In the irradiated female degeneration occurs at oocyte stages 1 - 5; however, once a follicle has developed an antrum the oocyte has a better chance of being ovulated in the irradiated than in the control. The increased loss in the irradiated females must be due to selection against those oocytes bearing the more severe types of damage, probably chromosomal.

A. G. SEARLE: Is it known how much the duration of oocyte stages 6 - 8 depends on whether the female is having litters or not?

E. F. OAKBERG: The data on this are not good, because we cannot follow a specific oocyte. There is no difference in the rate of oocyte loss between virgin and continuously mated females: the depletion of the oocyte pools occurred at exactly the same rates. This may result from the fact that most of the loss of oocytes comes through atresia rather than through ovulation.

L. B. RUSSELL: Another result relevant to this point comes from our experiments with 50 R acute X-irradiation given to female mice. When the young were killed at birth, as done in the fertility experiment, an average of four litters was produced. But when you allow them to be raised, as done in the mutation-rate experiments, the average number of litters is smaller because they are more widely spaced. This also points to loss through atresia rather than through ovulation.
A. G. SEARLE: You gave a figure of 46 days for the interval between formation of the primary spermatocyte and of the spermatozoon in man. What is the minimum time between spermatozoal formation and ejaculation?

E. F. OAKBERG: The minimum time between release of the spermatozoa in the tubule and their ejaculation is one day, but the average is probably 10 - 14 days.

A. J. BATEMAN: Is there any evidence for premature death of the spermatocyte before meiotic division?

E. F. OAKBERG: There is no evidence of any loss at this stage. Normal numbers of spermatocytes reach division even with very high exposure, apart perhaps from some at the very early preleptotene stage. Most of the degeneration occurs at metaphase I, so the scoring of abnormal anaphases may be misleading.

A. J. BATEMAN: An irradiated spermatocyte normally forms what is called a restitution cell. Were they scored as survivors or non-survivors?

E. F. OAKBERG: In my opinion a restitution cell is a figment of the imagination. The concept of a restitution cell is erroneous because the spermatocytes which fail in meiosis form a giant spermatid which degenerates. These cells play no role whatsoever in repopulation of the testis. Investigators who have made this claim simply did not look long enough to find the few A type spermatogonia present.

We scored as survivors all those spermatids which appeared to be normal living cells, regardless of nuclear size.

A. J. BATEMAN: If you give a larger dose of about 1000 rads you get very many spermatocytes which have failed to go through meiosis. They accumulate in the testes and as a cell population can legitimately be called restitution cells. 'Restitution' is the classical cytological term for process by which two daughter nuclei fuse, failing to separate, are included within a single nuclear membrane.

E. F. OAKBERG: That is true, but these cells have nothing to do with testes repopulation and the term 'restitution cell' implies that they do.