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Roy O. Greep

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CHAPTER 11

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Effects of radiation on the testis

E. F. OAKBERG | *Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee*

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RECENT PROGRESS IN understanding the effects of irradiation of the testis on fertility and genetic effects has been made possible by advances in the knowledge of the dynamics and timing of spermatogenesis and of the process of spermatogonial stem-cell renewal. On the basis of periodic acid-Schiff (PAS) staining of the acrosomes of developing spermatids, 14 cellular associations, designated by roman numerals, have been described in the cycle of the seminiferous epithelium in the rat (11, 30). Twelve stages can likewise be described in the mouse (37). This information has made it feasible to observe the response of specific cell types to irradiation; and, at the same time, the radiation response of spermatogonia has provided valuable information on normal spermatogonial differentiation. In mammals, all differentiating germ cells have a relatively short development time in relation to the total reproductive span. Thus the stem spermatogonium, which is radiation resistant, and with a long cycle time, is the most important cell type in reference to both fertility and the genetic effects of radiation (45). Therefore whereas the radiation response of all spermatogenic cells is discussed, particular emphasis is placed on the stem spermatogonia. The following brief review of present concepts of normal stem-cell renewal is given as a basis for discussion of radiation effects on the testis.

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SPERMATOGONIAL STEM-CELL RENEWAL

The problem of stem-cell renewal in germinative tissues was recognized over a century ago, but the process is still not fully understood. Almost every cell in the germinal epithelium of the testis has at some time been designated as the stem cell, and it is only recently that the type A spermatogonium has been clearly demonstrated to fulfill this role (8, 30, 37).

Five classes of type A spermatogonia, recognizable by their nuclear morphology and the stage in which they occur in the cycle of the seminiferous epithelium, can be identified in the rat and mouse (9, 26, 46). The morphological characteristics of these cells in the rat are given by Clermont & Bustos-Obregon (9) and Huckins (26) and by Oakberg (46) for the mouse.

In their most recent model of spermatogonial stem-cell renewal, Clermont & Bustos-Obregon [(9); (Fig. 1*a*)] divide the type A spermatogonia of the rat into two groups. The first constitutes the actively renewing population and comprises the cell types A_1–A_4. According to this model, most A_4 cells divide in stage II in the cycle of the seminiferous epithelium to form intermediate spermatogonia; a few, however, give rise to type A_1 cells, which remain mitotically inactive through stages III–VIII and divide in stage IX to initiate a new cycle. In the model of Clermont & Bustos-Obregon (9) a fifth class of type A spermatogonia, designated A_0, occurs as single or paired cells that normally do not divide and hence do not contribute to the replenishment of A_1 spermatogonia. These cells are considered to be reserve stem cells that become active only if the more mature spermatogonial types are depleted by some noxious agent such as radiation. Two difficulties with this model are readily apparent: *1*) the selective value in evolution of a mechanism that relegates about 25% of the type A spermatogonia of the mouse and about 21% in the rat to an inactive role is not immediately apparent; and *2*) a cellular dedifferentiation is involved in derivation of the A_1 from the A_4 spermatogonia.

However, neither Huckins (26), in her study of the rat, nor Oakberg (46), in an investigation of the mouse,

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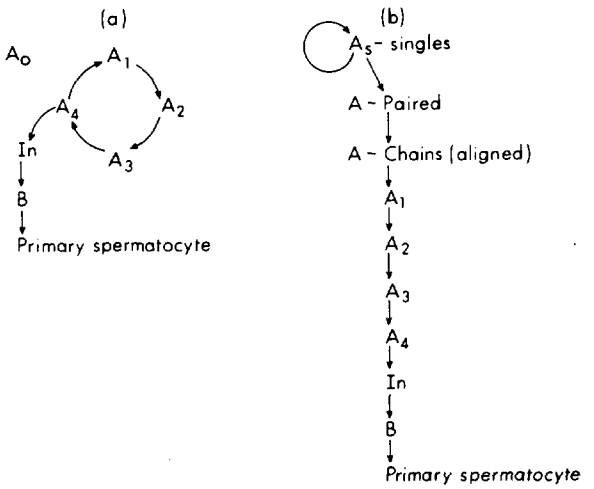

FIG. 1. Diagrammatic representation of spermatogonial stem-cell renewal as proposed by Dym & Clermont (14) for the rat, *a*, and by Huckins (26) for the rat, *b*, and Oakberg (46) for the mouse, *b*. [From Oakberg (45).]

could find a noncycling type A spermatogonium. Instead the isolated A_0 of Clermont & Bustos-Obregon (9) was observed to incorporate ^{3}H-labeled thymidine, to divide, and to generate irregularly aligned chains of cells, which transformed into A_1 spermatogonia (26, 45, 46). In the models of Huckins (26) for the rat and of Oakberg (46) for the mouse, the isolated type A spermatogonia with oval, darkly staining nuclei (Fig. 2) have been designated A_s (A stem) (Fig. 1*b*). These cells have two potentials: they can divide to form more single A_s spermatogonia; or division may result in the formation of a pair of cells with morphology identical both to that of the A_s and to the other member of the pair. Pair formation is considered to be the initial step in an irreversible developmental sequence (Fig. 1). Successive divisions of the pair lead to the formation of a chain of 4, 8, or 16 A spermatogonia, which retain the A_s nuclear morphology; eventually, however, these aligned A spermatogonia stop dividing and transform into the spermatogonia A_1 (46). The A_1 cells divide to form spermatogonia A_2, the A_2 to form A_3, the A_3 to form the A_4, and the A_4 to form intermediate spermatogonia. In agreement with Monesi (34) researchers have found no evidence for formation of anything but intermediate spermatogonia from A_4 cells.

The above description, although applying specifically to the mouse and rat, probably is essentially true for all mammals. The terminology used in different species varies, but a homology of cell stages probably is involved. Thus in the bull, Hochereau-de Reviers (24) has proposed that A_1 spermatogonia are formed in part from the A_0 and in part from A_2 spermatogonia. In man a third type of A spermatogonium, designated A_L, different from both A_{dark} (Ad) and A_{pale} (Ap), appears to be similar to the A_s of mouse and rat (53, 54). A new analysis of the kinetics of stem-cell renewal in the primate testis should be undertaken to settle this point.

The time required for development of spermatozoa from division of type A_1 spermatogonia is about 35 days in the mouse (38) and 52 days in the rat (10). At least one additional cycle (8.6 days in the mouse, 13 days in the rat) is required for development of A_1 from A_s spermatogonia (26, 46), so that duration of spermatogenesis is of the order of at least 43 days in the mouse and 65 days in the rat. A similar correction probably should be made in the 72- to 74-day estimate given by Heller & Clermont (21) for man. Study of the cellular associations after irradiation (38), progression of ^{3}H-labeled thymidine in irradiated testes (16), and endocrinological studies (10, 51) suggest that duration of spermatogenesis measured from beginning of spermatogonial differentiation to spermatozoal release is a constant for each species. There is evidence, however, that the process proceeds more rapidly in the juvenile animal (25). Since the time required for development of all stages derived from the A_s spermatogonium is short in relation to the reproductive life-span in mammals, the A_s spermatogonium is the most important cell in terms of long-term fertility and genetic effects in irradiated males.

PRENATAL AND NEONATAL STAGES

Two periods of high sensitivity to radiation-induced sterility have been observed in the immature rat testis. The first, in the 13.5- to 17.5-day-old embryo, is common to both sexes and is associated with high mitotic activity of the primordial germ cells (4). The second and most sensitive period occurs just before and after birth (19-day-old fetus to 2 days postpartum) in an interval when there is no mitotic activity among the primordial germ cells (25) and when the first type A spermatogonia appear (4). A similar pattern at slightly younger ages occurs in the mouse (55, 56). A rapid increase in resistance parallels the increase of the type A spermatogonia, with a third unexplained period of sensitivity at 17 days

FIG. 2. A_s spermatogonium in control mouse. × 2,500.

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TABLE 1. *Duration of Initial Fertile and Sterile Periods After Irradiation of Males*

Species	Dose, r	Duration, weeks		Reference
		Presterile period	Sterile period	
Mouse	300	6	2	(12)
	500	3	10	(2)
	600	4	4.5	(58)
	800	2.5	10	(61)
	800	3	11	(23)
	800	2	10	(58)
	1,000	2	11	(58)
Rat	300	9	2	(12)
	500	6	12	(12)
Guinea pig	173–864	8	3	(63)
	1,296	4	24	(63)
	1,728	2	24	(63)

From Oakberg (41).

(31). The primary radiation effect in the fetal and neonatal testis can be attributed to killing of germ cells and subsequent deficiency of spermatogonia in the adult (4).

Neither the radiation response nor the time sequences of development (7) in human fetal and prepubertal testes is well documented. Comparable cell stages with sensitivity similar to that observed in mouse and rat would be expected, however, so particular care should be taken in radiation exposure of the fetus and postnatal juvenile stages.

ADULT

The minimum acute radiation dose required to induce a temporary infertile period is 100–300 r, depending on the species and genetic strain. Males are initially fertile because of the continued development of cells irradiated as spermatozoa, spermatids, and possibly spermatocytes. A temporary infertile period, the duration of which is dependent upon dose and species (Table 1), then occurs as a result of spermatogonial killing. With high doses the killing of spermatocytes results in an earlier onset of the sterile period, and a prolongation occurs because of the slow repopulation of the seminiferous epithelium when spermatogonial survival is low. With doses of 800 r and above, the presterile period is further reduced, most probably by the induction of dominant lethals in all spermatids. Provided sufficient type A spermatogonia survive, the seminiferous epithelium is repopulated and fertility returns (36, 40).

SPERMATIDS AND SPERMATOZOA

Morphology and sequence in spermiogenesis of cells irradiated as spermatids are unaffected by doses of 1,500r in the mouse (48); exposure to 32,000 r or more is required to affect the motility of human sperm (19), and the fertilizing capacity of mature rabbit spermatozoa is normal after 65,000 r (6). No viable embryos are formed after such doses because of the extensive chromosome damage. The abnormal spermatids and spermatozoa observed after radiation exposures of 100 r or more, as demonstrated by the wide range in the size of spermatid nuclei in Figure 3, are derived from cells irradiated as spermatocytes (48). Genetic effects ranging from presumed point mutations to deficiencies and chromosome rearrangements are induced with high frequency, especially in early spermatids (57).

SPERMATOCYTES

The sensitivity of the spermatocytes of the mouse to chromosome breakage (Table 2) shows a stage dependence similar to that observed for other species (48). The highest frequency of abnormal anaphases (Fig. 4) was observed for irradiation in diakinesis and metaphase I, and the lowest for leptotene and preleptotene. Pachytene stages were intermediate in sensitivity. In mammals, degeneration in metaphase I (Fig. 5) is relatively common, and a loss of about 13% of the spermatocytes in control mice has been observed (37). Thus it may be argued that certain classes of chromosomal effects were selectively eliminated before scoring in Oakberg & DiMinno's study (48). That this probably is not a valid criticism, however, is indicated by the comparable results obtained in organisms in which this selection does not occur (33, 62). Thus, whereas it may well be that cell death in metaphase I does reduce the frequency of aberrations scored, it apparently does not affect the relative comparisons between stages.

Sensitivity to cell killing, as measured by the number

FIG. 3. Spermatids 5 days after 600 r, demonstrating variable nuclear sizes as a result of chromosome breakage in spermatocytes. × 1,000.

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TABLE 2. *Effect of Meiotic Prophase Stage on Survival and Chromosome Breakage in Primary Spermatocytes of the Mouse*

Meiotic Prophase Stage	Survival Data			Chromosome Breakage D_{50}	
	Point estimate LD_{50}, r	95% Confidence limits		Anaphase I	Anaphase II
		Lower	Upper		
Preleptotene	205	175	233	612 ±93*	
Leptotene	492	273	719	1,443 ±182*	
Zygotene	520	413	637	411	
Pachytene (early)	404	329	470	242	
Pachytene (mid)	382	301	452	265	
Pachytene (late)	664	601	733	321	
Diplotene	564	365	771	275	
Diakinesis-Metaphase I	837	679	1,161		
Diakinesis				204	
Metaphase I					138 ±6*

* Standard errors computed for only those stages giving a linear fit on a semilog plot. [From Oakberg & DiMinno (48).]

FIG. 4. Bridge at anaphase I of meiosis in the mouse, *arrow*, 8.5 days after 150 r. × 2,500.

of spermatids formed, is the reverse of that observed for chromosome breakage (Table 2). Preleptotene is the most sensitive, and diakinesis and metaphase I the most resistant. This had led to the hypothesis that the mechanisms for cell killing and those for chromosome breakage are independent (48).

As a result of the combined effects of the reduction in the number of gametes formed from spermatocytes and the formation of genetically inviable gametes through the process of chromosomal rearrangement or loss, onset of the sterile period occurs earlier with higher doses of radiation. There apparently is a strain effect in this response (2), but it is unknown whether it reflects differences in cell sensitivity, differences in the numbers of germ cells, or differences in the capacity to repair cellular damage.

The response of spermatocytes in man is comparable to that of the mouse (22), but the data do not permit detailed analysis by cell stage. Most observed species' differences are probably more apparent than real because of the failure of the investigator to assess the number of cells in the controls and to correct for differences in the maturation rate of the germ cells. Differences between meiotic prophase stages in the mouse are as great as the differences claimed for species comparisons. Since it requires about 28 days for preleptotene spermatocytes to develop into sperm in the mouse, about 36 days in the rat, and about 46 days in man, it is of extreme importance that the times of observations are adjusted to assure that comparisons are based on strictly comparable cell stages.

SPERMATOGONIA

Definition of the cycle of the seminiferous epithelium and the cellular associations constituting the stages of the cycle made feasible the critical evaluation of the radiation response of the different spermatogonial types. Consequently it was easy to demonstrate that the primary response of spermatogonia is cell death, most of which occurs before cell division [(1, 35, 36); Fig. 6]. Thus the mechanism of chromosome breakage and the resultant aneuploidy appear to be relatively unimportant in the killing of spermatogonia by radiation. Intermediate and late type A (A_2–A_4) spermatogonia of the mouse have a uniformly high sensitivity, with an LD_{50} in the range of 20–24 r of ^{60}Co γ-rays (39), and the effect of any acute

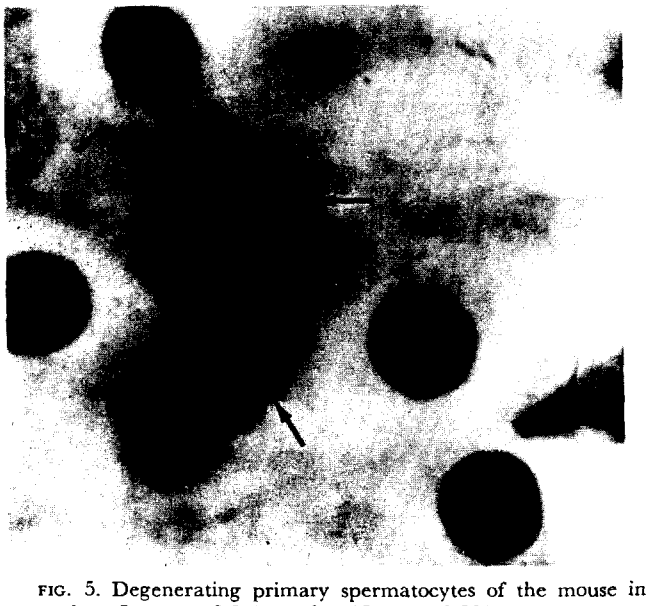

FIG. 5. Degenerating primary spermatocytes of the mouse in metaphase I, *arrows*, 8.5 days after 150 r. × 2,500.

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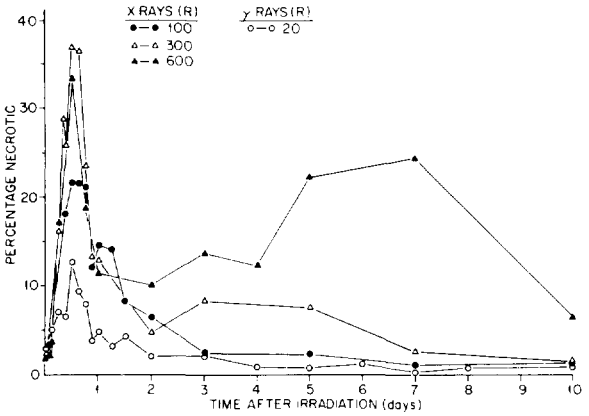

FIG. 6. Frequency of degenerating type A spermatogonia in the mouse at different times after irradiation. [From Oakberg (40).]

dose above 2 rads can be detected (47). Type B spermatogonia have an LD_{50} comparable to that for late type A and intermediate spermatogonia, but respond differently at low doses with a pronounced shoulder followed by a very steep linear decline (35). It was apparent from our early data that the type A spermatogonia were of heterogenous sensitivities and that only a few survivors were required for the regeneration of the seminiferous epithelium (36, 40).

A thorough understanding of the dynamics of spermatogenesis is required for the proper evaluation of the radiation response of spermatogonia. It often is stated that the In and B spermatogonia are the most radiation-sensitive cells of the testis. This misunderstanding has arisen largely because surviving cells continue their differentiation with a resulting difference between the stage irradiated and the stage scored. In the mouse, for example, the effect of irradiating A_4 or In spermatogonia is observed as a depletion of type B spermatogonia 72 hr later, and a high sensitivity of B spermatogonia is claimed. A more accurate interpretation is that the intermediate and B spermatogonia are uniformly sensitive, but that the most sensitive cells occur among the heterogeneous A population. This is demonstrated by the fact that survival of the total A population at doses below 25 r is the same as survival of A_4–In and In–B spermatogonia (39), despite the greater resistance of about 40% of the A population, which has a linear survival curve from 100 to 1,000 r (42). The similar response of the total A and intermediate populations at doses below 25 r has been useful in studies on the effect of low doses and especially in the estimation of the relative biological effectiveness (RBE) of different radiations.

Recent studies of tubule whole mounts of the rat (13) and the mouse (27) and of testis sections of the mouse (45) have suggested that the higher sensitivity of certain A spermatogonia may be related to the presence of intercellular bridges. These results raise fundamental questions previously not considered in mammalian radiobiology and may be germane to differentiating cells other than spermatogonia. In the model of spermatogenesis proposed by Huckins (26) and Oakberg (46), the isolated A_s spermatogonium (Fig. 2) is considered to be the stem cell; formation of a pair of cells constitutes the initial step in differentiation. The pair of cells then embarks on a series of divisions resulting in irregular chains that transform into spermatogonia A_1. The members of these chains appear to have contiguous cytoplasm under the light microscope (27), and, with the electron microscope, intercellular bridges have been observed between A_L spermatogonia in man (53), between spermatogonia of *Macaca* (20) and the rat (15), and between spermatocytes of the rat (18). Figure 7 shows a light-microscopic photograph of a bridge between paired A spermatogonia of the mouse. These bridges are probably an important factor both in maintenance of synchrony of development among members of the chain and in radiation response, where it is observed that the chains respond as a unit. An entire chain either dies or survives (27), with degeneration either affecting the entire chain simultaneously or progressing in a wavelike fashion along the chain. It is unlikely that the death of entire chains of A spermatogonia results from synchrony in a particularly sensitive mitotic stage, for one would not expect lethal events in all members of a chain at a dose of 20 r (i.e., less than the LD_{50}); inactivation of a portion of the chain must result in degeneration of the entire group. In the past we have postulated that the high sensitivity of some spermatogonia (the effect of any acute γ-ray dose above 2 rads can be detected) could be due either to a large, sensitive target or to a multiplicity of targets with inactivation of one being sufficient for cell lethality. The multiple target concept would be fulfilled by the syncytial nature of the aligned spermatogonia. It is interesting that microdosimetric studies (3) have led to the postulate that spermatogonia can be killed by a single particle having traversed the cytoplasm only. Furthermore our observation

FIG. 7. Cytoplasmic bridge between a pair of A spermatogonia in a control mouse, *arrow*. × 2,500.

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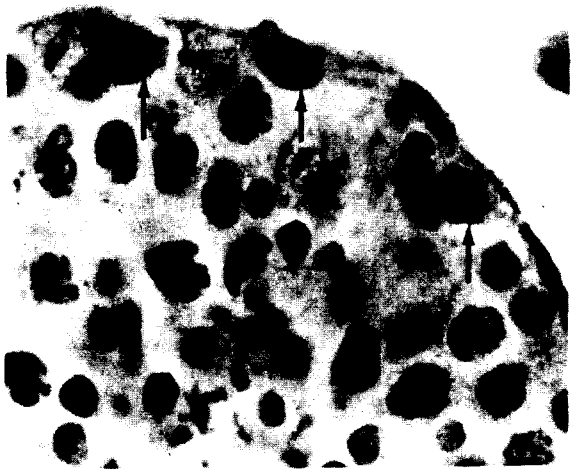

FIG. 8. Three degenerating spermatogonia (probably A_1) at stage IX of the cycle, *arrows*, 5 days after 150 r. × 1,000.

of many years that neither necrotic nor surviving spermatogonia are randomly distributed among cross sections of tubules of any given stage in the cycle of the seminiferous epithelium supports the concept of the cluster response of spermatogonia (Fig. 8).

The A_2–A_4 spermatogonia degenerate rapidly after irradiation, and this results in a peak frequency of necrotic cells 12 hr after irradiation [(40); Fig. 6]. Degeneration of A_1 spermatogonia, however, is delayed until they reach stage IX of the cycle. This difference in response from the A_2 to the A_4 results from the long G_1 of type A_1 cells; A_1 as well as A_2–A_4 spermatogonia degenerate primarily in late interphase to early prophase (35, 36). As a result of this prolonged degeneration, minimum numbers of the total A population are not reached until 120 hr after irradiation. It has been pointed out that repopulation is underway by this time and that the particular manner in which repopulation and degeneration interact to give a minimum value is dose dependent (40).

That A_s spermatogonia are the most resistant of the type A spermatogonia is shown in Figure 9; and Figure 10 is a photomicrograph of an A_s cell 5 days after 150 r. It is identical to those observed normally (Fig. 2). Some paired spermatogonia were observed at 100 and 150 r, but frequency was below control values. At 8.5 days, paired cells were above control values for all doses (Table 3; Fig. 11) and after 150 r this is reflected in higher than normal numbers of A_1 spermatogonia at 14 and 17.25 days, or approximately one cycle of the seminiferous epithelium later. That surviving A_s spermatogonia continue pair formation after irradiation was observed by Huckins in her study of tubule whole mounts after 150 r (27). The data from sections (Table 3) can never be as precise as those from whole mounts at certain intervals, because some members of pairs or chains will be scored as isolated A_s cells. At high doses (Table 3), where cell survival is low, this complication does not arise until newly formed pairs appear. In view of the delayed degeneration of A_1 spermatogonia, and the continuation of pair formation, it is clear that the survivors scored by Dym & Clermont (14) included cells yet to degenerate, repopulating cells, and radioresistant cells. Their conclusion that the survivors were A_0, which were stimulated to relinquish their stem-cell role in repopulation of depleted differentiating spermatogonia, is not compatible with the observation of Oakberg (42, 45) that some of the spermatogonia surviving high radiation doses were labeled when ^{3}H-thymidine was injected prior to irradiation. Thus the survivors were from a cyclic population and not from a noncycling one, as required by the A_0 (reserve-stem-cell) concept. This is conclusive evidence that the stem cells are in continuous, long cycles; and if accelerated division rates do occur after irradiation, it is enhancement of a naturally occurring process. The observation that survivors were randomly distributed among the stages of the spermatogenic cycle (39) and that reestablishment of spermatogenesis occurred in perfect synchrony with resistant spermatocytes and spermatids that survived the irradiation (38) is further proof that the stem cells are continuously cycling; it appeared as if some A spermatogonia already were programmed at the time of irradiation.

The importance of the mechanism of stem-cell renewal cannot be overemphasized in terms of long-term radiation effects. For example if the radiation-sensitive A_4

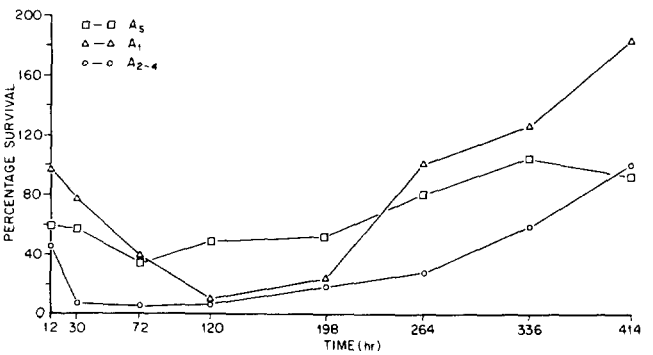

FIG. 9. Frequency of A_s, A_1, and A_2–A_4 spermatogonia in the mouse at different intervals after 150 r.

FIG. 10. Type A_s spermatogonium 5 days after 150 r. × 2,500.

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spermatogonia were able to dedifferentiate and form A_1 spermatogonia as suggested by Clermont & Bustos-Obregon (9), indefinite perpetuation of genetic effects induced in these cells would be possible, making plausible the hypothesis of Oftedal (50) that low doses of radiation are mutagenically more effective owing to the killing of sensitive cells at high doses. The demonstration of the A_s spermatogonia as the stem cells (26, 46) with no reversal in the developmental sequence once pair formation has occurred makes them the most important cell in respect to fertility and genetic damage. All subsequent stages have a relatively short life-span, are rapidly cleared from the reproductive tract, and genetic effects induced in them are of relatively small importance in estimates of the total damage. The A_s spermatogonia, however, with the capacity to replenish their own numbers and to differentiate, persist throughout the reproductive life of the individual. Thus they are the important cell type regardless of total dose, dose rate, or dose fractionation (45).

Mitotic Inhibition

The demonstration of the killing of numerous spermatogonia (Fig. 6) made obsolete the postulate of prolonged mitotic inhibition as an explanation for radiation depletion of the seminiferous epithelium. The problem has become considerably more sophisticated and has implications for radiobiology in general. Monesi (35) observed a delay of about 2 hr in mitosis of spermatogonia after 100 r, a response comparable to that observed in other tissues. It has been demonstrated that the determination of the mitotic rate of surviving spermatogonia is difficult because of the initiation of division in some spermatogonia before the process of cell death had been completed (40). It is now clear that apparent decrease in mitotic rate of surviving spermatogonia may also partly be due to the greater survival of A_s spermatogonia, which normally have a cell cycle much longer than that of the A_1–A_4 types. Thus the normal kinetics of A_s spermatogonia is the proper base line for measuring the effects of any dose of 100 r or more on the mitotic rate; past use of the mitotic rate of the total A population as the base line is incorrect. It is possible that the mitotic rate of these cells may even be increased to replace spermatogonia killed by radiation. This has been claimed by Dym & Clermont (14), but they attribute it to a release of the inhibition of A_0 spermatogonia from a reserve-stem-cell role. It earlier was pointed out, however, that their presumed A_0 population probably was a mixture of cells, some of which were cycling A_s spermatogonia. That the A_0 are not reserve stem cells was demonstrated by Oakberg (42), who observed that ^{3}H-thymidine-labeled cells occur among the survivors when ^{3}H-thymidine is injected before irradiation. Labeled cells can comprise as much as 40% of the surviving population (45). The interpretation of Huckins (26) and Oakberg (46) is that no reserve stem cell exists, but that the slowly dividing A_s spermatogonia become more active during repopulation. This could be a factor in the increased frequency of cells labeled by ^{3}H-thymidine in the irradiated testis (42), but the possibility of an extended S period has not been excluded in these data.

TABLE 3. *Frequency of A Spermatogonia Occurring as Pairs and Chains After X-Irradiation*

Time, days	Control	X-Ray Dose, r			
		100	150	500	1,000
3	0.136	0.079	0.081	0.000	0.000
5		0.025	0.018	0.000	0.000
8.5		0.161	0.175	0.205	0.286

FIG. 11. Newly formed chain of A spermatogonia, *arrows*, 8.5 days after 150 r administered to the mouse testis. × 2,500.

Radiation Quality

The effect of radiations of different types has been studied extensively, and comparisons usually are expressed as the RBE. These comparisons have been largely empirical because it is unknown if different radiations induce damage by the same basic mechanism. Nevertheless the results are of practical use in evaluating potential damage from radioisotopes, use of nuclear energy, and medical procedures. ^{137}Cs γ-rays, X-rays, and 730-Mev protons are about equally effective in killing the spermatogonia of the mouse [(42); Fig. 12]. An RBE of about 2 has been observed for 2.5- and 14.1-Mev neutrons for killing of spermatogonia of the mouse (47), and fission neutrons with an average energy of 1–2 Mev have given an RBE of 6.5 for the 18- to 101-rad dose range and of 4.6 for doses of 175–260 rads (42). These results, in comparison with those for 2.5-Mev neutrons, raise the possibility of very high effectiveness of the low-energy components of the fission neutron spectrum. Thus, of the radiations tested, only neutrons show an increased effectiveness over that observed for X- and γ-rays, and the amount of this

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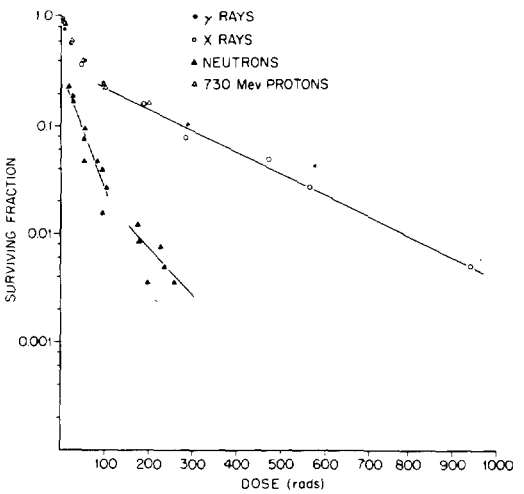

FIG. 12. Relative effectiveness of gamma rays, X-rays, neutrons, and 730-Mev protons on survival of type A spermatogonia in the mouse. [From Oakberg (42).]

increase is dependent both upon neutron energy and dose.

Dose Fractionation

The demonstration of differences among species in the duration of spermatogenesis and the differences in radiation sensitivity of spermatogonial stages explain the conflicting results obtained in attempts to verify the conclusion of Regaud & Nogier (52) that sterility is more readily induced by fractionated than by single exposures. The confusion is cleared up by the knowledge that the effect is a function of the dose of each fraction, the total dose, and the intervals between fractions. The schedules and doses effective in one species may not be applicable to other species because of differences in the rate of gametogenesis. Furthermore proper criteria must be used for evaluation of the response. For example the failure of Kohn & Kallman (28) and Silini et al. (60) to observe a fractionation effect with intervals less than 4–7 days between exposures may be explained by the use of testis weight as the measure of response. Yet the effect on subsequent fertility could be dramatic. This interpretation is supported by Russell's (59) observation that five daily 200 r fractions gave the longest sterile period; two 500 r exposures 24 hr apart, an intermediate period; and a single 1,000-r exposure, the shortest period of infertility. Our observations on cell survival confirm the results of Russell (59), but it is doubtful if these small differences would be detectable by the weight of the testis 28 days after exposure. Thus proper criteria will demonstrate an increased effectiveness of dose fractionation provided proper dose and time intervals are used.

Dose Rate

Amelioration of the radiation effect by reduction of the dose rate is observed in a wide variety of biological materials. Cells apparently have the capacity to either repair or prevent certain types of damage, or both, provided the primary events are widely enough distributed in space and time. The particular response will depend upon the cell, the tissue, the species, and the end effect being studied. No effect of dose rate on spermatogonial killing in the mouse has been observed over the range of 93–0.8 r/min (47). The margin for dose-rate effects appears to be 0.009 r/min, with no effects observed for exposures of 5, 11, and 600 r (i.e., either after very long or short exposure times), but with a much higher survival at 100 and 300 r than after higher dose rates. At 0.001 r/min (about 10 r/week) cells in the seminiferous epithelium reach an equilibrium at about 80% of control and maintain this level for long periods of time (47). These results are in agreement with the repeated observation that fertility of adult male mammals is not impaired by either acute or continuous X-ray or γ-ray exposures until an exposure of more than 2 r/day is given (5, 17, 29).

That the mechanisms resulting in specific locus mutations are different from those involved in cell killing is indicated by the fact that no effect was observed over the 93–0.8 r/min range for cell killing (47) but that the full dose-rate effect on mutation induction is observed with a decrease from 93 to 0.8 r/min (59). These results make unlikely the postulate that cell killing and specific locus mutations are attributable to a common mechanism such as chromosome breakage.

Species Comparisons

The same general pattern of cellular response described here for the mouse has been observed in all species studied, including man (Fig. 13). Within this general framework, however, each species is characterized by the dose required to produce a specific effect, the time and course of initial depletion and recovery, and limitations imposed by reproductive life-span (i.e., in insects, irradiation too late in larval or pupal development may not

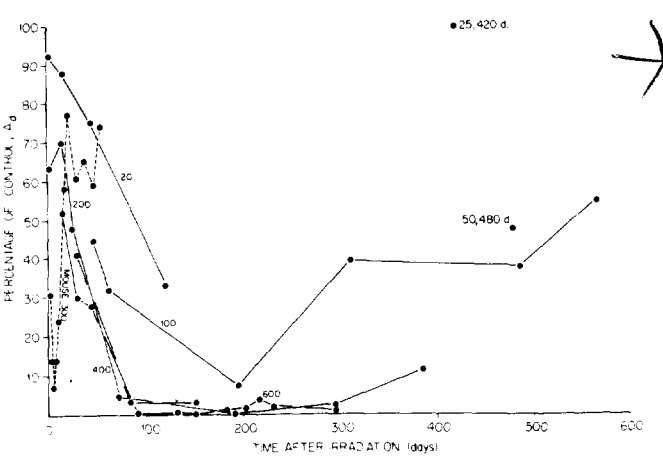

FIG. 13. Frequency of A_d spermatogonia observed at various times after 20–600 r local irradiation of the human testis. [From Oakberg (43).]

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allow sufficient time for surviving spermatogonia to repopulate the testis and form sperm if the imago has a short life-span). Also the relative importance of different spermatogenic stages is a function of the duration of spermatogenesis in relation to the duration of fertility in the adult.

In mammals the dose required to produce aspermia may vary by a factor of 3–5. This dose may be below 100 r in man (22), whereas in mice and other rodents a dose of 300–500 r is required (Table 1). The onset of oligospermia or aspermia (usually resulting in sterility) is a function of normal duration of spermatogenesis and, with the minimal effective dose, represents the time for preleptolene spermatocytes to develop into mature sperm and to traverse the reproductive ducts. This interval is about 35 days in the mouse (48) and 55 days in man (21). In rodents, doses that cause severe killing of spermatogonia (100–300 r) may not result in sterility because of the rapid regeneration of the seminiferous epithelium, whereas in other species, for example man, recovery is slow (32, 49). As a result, depletion of more differentiated cell types exerts its full effect, and a prolonged sterile period may occur.

The reasons for the marked differences among species in the rate of recovery are not well understood, but normal time sequences of spermatogenesis probably are of primary importance. Demonstration of the stem-cell role of the isolated A (A_s) spermatogonium indicates that the total duration of spermatogenesis in the mouse is at least one and possibly two cycles of the seminiferous epithelium longer than previously thought. Demonstration of similar stem-cell kinetics in other species could result in a considerable extension of the duration of spermatogenesis. Since the most primitive stem cells are the most resistant, the full effect on spermatogenesis of the duration of recovery time would be expressed in those species with a long duration of spermatogenesis. Comparison of spermatogonial counts during repopulation in mouse and man suggests that the control of differentiation in them also may be different (43, 44), since in man differentiation occurred when the number of type A spermatogonia was still as low as 2% of control. Our newer concept of stem-cell renewal may change this interpretation, however, since both the stem cell and stem-cell kinetics remain unknown in man. The demonstration of the stem-cell role of the A_s spermatogonium in the mouse and rat and the observation that these also are the most radiation-resistant spermatogonial types have profound significance for both the initial depletion and recovery of spermatogonia after irradiation. The long time (about 100 days) for maximum depletion of spermatogonia in man argues for a system of stem-cell renewal similar to that based on the A_s spermatogonium of mouse and rat. Elucidation of the stem-cell behavior in other species should aid greatly in the interpretation of apparent differences in cell sensitivity and the time required for expression of radiation effects.

In summary the type A_s spermatogonium of the mouse and rat and its counterpart in other species are the most important cells of the mammalian testis in relation to fertility and the genetic effects of radiation in mammals. All long-term effects of dose rate, dose fractionation, total dose, and quality of radiation on these parameters are related to the response of the A_s spermatogonia (45).

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