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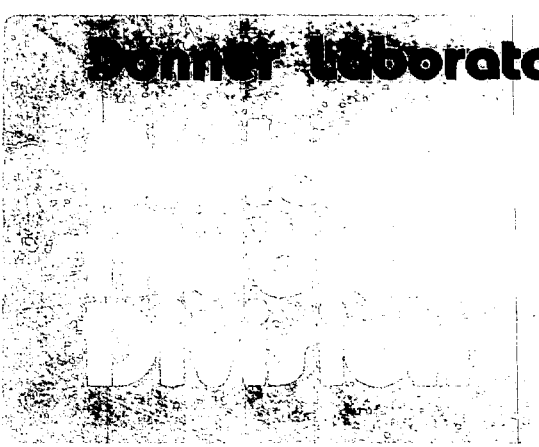

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SPERMATOGONIAL STEM CELL RENEWAL FOLLOWING IRRADIATION

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INTRODUCTION

New concepts of spermatogonial stem-cell renewal in the mouse and rat bear directly on cellular response and cell population kinetics of the seminiferous epithelium and the altered proliferative patterns under stimuli and perturbations of various kinds, particularly low-level of ionizing radiations. Our experiments [1-1] in the mouse, and those of Oakberg [5-8] and Huckins [9-11] in the mouse and rat, lead to a model of spermatogonial stem-cell renewal based on cytological characteristics, quantitative histology, and the techniques of cell population kinetics. In all stages of the seminiferous epithelium, the undifferentiated type A_S stem-cell occurs as single isolated cells; in the mouse it has a long cell cycle time of ∿1-2 weeks (> 8.5 days but < 17 days). Renewal of the type A_S stem-cell occurs by division of some A_S stem-cell spermatogonia to form more isolated A_S stem-cells. Other divisions of type A_S stem-cell spermatogonia result in the formation of A_{pr}-pairs, and this appears to be the initial step in differentiation. Further divisions of the pairs form chains of aligned A_{al} cells, and these transform directly into chains of differentiated type A_1 cells. All type A_1 cells divide, forming A_2, and in turn, types A_3 and A_4 spermatogonia. Sequential division occurs regularly and continuously, and in general, the cell cycle times of the types A_1 to A_4 series are ∿33-35 hr in the mouse [3,4]. There is no evidence of dedifferentiation or return to earlier forms in the maturation sequence. Type A_4 spermatogonia divide to form intermediate (In) cells only, which divide and give rise to type B spermatogonia, and then, by further division to preleptotene spermatocytes.

It is now possible to begin to define the dynamic cell renewal structure of the spermatogonial cell populations in the seminiferous epithelium---where stem cells and their progeny are produced, how transformation and migration take place, and the sequence of differentiation. Further, it is now possible to determine measurements of some time parameters of the spermatogonial cell populations with precision, including cell cycle times, their distribution and proliferating fractions, and the cell birth rates, the compartment residence times and the flow rates through the various subcompartments into which the spermatogonial cell population is divided (Table 1). These new observations provide an insight to the basic questions of tissue homeostasis in the seminiferous epithelium and its response to various perturbing agents, such as ionizing radiations and other mutagenic agents.

CONTINUOUS LOW-LEVEL GAMMA IRRADIATION

Under continuous low-level γ-irradiation at 1.8 rad/day, which will permit the survival of at least 10 generations in the C57BL male mouse, cell renewal during spermatogenesis can achieve a near-steady state of cell population at ~80% of

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control levels for at least 12 cycles of spermatogonial cell renewal. Changes in the patterns of spermatogonial cell population kinetics indicate there is some limited reserve of proliferative capacity. The extent to which these changes may be due to decreasing the cell cycle time of a long-cycling type A_S stem-cell population in the type A cell compartment, rather than the bringing-in of a potentially proliferative type A_S population, is now better understood. These mechanisms are important in maintaining the steady-state of spermatogonial cell renewal under stress. There are delays in the flow of the differentiated types A_{1-4}, In, and B spermatogonia in each proliferative subcompartment through the G_2-period into cell division, associated with a decrease in the duration of DNA synthesis, but with relatively normal cell cycle times.

Under continuous low-level irradiation, there is a differential radiosensitivity among the differentiated types A_{1-4}, In, and B cells affecting proliferation--depopulation is minimal in type B and In cells, less among types A_{1-4} cells and preleptotene spermatocytes, and least in the type A_S stem-cell compartment. In spite of progressive cellular damage associated with increased cell degeneration among spermatogonia in the various tubular stages, there is continued maintenance of some capacity for increased cell proliferation in each cell class, and little change in the cell population structure even under 15 weeks of low-level exposure. Spermatogonial cell production appears to be maintained, to a large extent, by changes in proliferation rates, transit times, and residence times in the type A_S stem-cell precursors. This is important in the regulatory control of homeostasis of the spermatogonial cell renewal system under the stress of continuous low level irradiation.

^{3}HTdR-LABELING INDICES UNDER CONTINUOUS IRRADIATION

Normally, ^{3}HTdR-LI are ~30±4%, ~63±7%, and ~50±6% in type A (A_S + A_{1-4}), In and B spermatogonial cells, respectively (Table 1). Under continuous irradiation, there is increased labeling in all 3 cell classes for up to ~3 wk, and return to normal values by 15 wk. The early changes of increased ^{3}HTdR-labeling indicate increased proliferation rates in type A, In and B cells, and appears to be a response to some cellular depopulation with an attempt to alter the spermatogonial cell cycle kinetics to compensate for cellular radiation death. By 15 wk, the ^{3}HTdR-LI data, together with differential cell type distributions, indicate that the new steady-state of cell proliferation is established and the cell population structure returns to a near-normal pattern.

SPERMATOGONIAL CELL CYCLE KINETICS UNDER CONTINUOUS IRRADIATION

An increase in the ^{3}HTdR-LI may be brought about by increasing the size of the proliferative population, or by decreasing the cell cycle time (T_C) without a change in the duration of DNA synthesis (T_S). Under continuous irradiation, the size of the proliferative population is apparently decreased, since (1) there is an increase in the number of degenerate forms in each cell class, (2) there is no morphological evidence of recruitment of nonproliferating cells into the population, and (3) the proportion of labeled degenerate forms do not change in each spermatogonial subcompartment. We are examining the various cell proliferation kinetic parameters of the different spermatogonial cell cycles to determine how the patterns of cell proliferation change under the perturbation of continuous stress. However, even after continuous low-level γ-irradiation for 15 wk, the curves of the percentage labeled mitoses (PLM) in type A (A_S + $A_{(1-4)}$), In and B spermatogonia are very similar to those of unirradiated animals. The median cell cycle times (T_C) do not change appreciably, and are ~34 hr, ~33 hr, and ~35 hr, respectively. There is lengthening of $T_{(G_2+M/2)}$ in each class to ~5-8 hr, associated with a greater

spread in these values, and with decrease in the number of cells synthesizing DNA in early prophase. This is compensated for by a concomitant decrease in T_S to ∿18, ∿19 and ∿22 hr in type A ($A_S + A_{1-4}$), In, and type B spermatogonia, respectively, by the same duration, ∿3-4 hr, so that no significant change occurs in $\bar{T}_{(G_1+M/2)}$ under irradiation. $\bar{T}_{(G_1+M/2)}$ values are ∿8, ∿7, and ∿8 hr, in the type A ($A_S + A_{1-4}$), In and type B spermatogonia, respectively (Table 2).

While the overall $\bar{T}_C$ does not change appreciably, the effects of continuous low-level irradiation are: (1) a G_2-delay in the flow of cells through G_2 and early prophase; (2) a decrease in the duration of DNA synthesis; and possibly (3) a decrease in the spread of the distribution of cell cycle times. It is of interest that under continuous irradiation, in spite of the shortened S period, ^{3}HTdR nuclear grain counts are lower in all cell types. Decreased grain counts may result from decreased rates of DNA synthesis, or decreased availability or utilization of ^{3}HTdR in proliferating cells, in spite of some radiation-depletion of the cell population.

The extent of the G_2-delay is estimated in part, by the flow-rate of labeled cells from the early into the late prophase subcompartments; delays of up to 8 hr or more occur. Continuous irradiation affects the early prophase cells more than the G_2 cells, and the flow of interphase cells through the $G_1 \rightarrow S \rightarrow G_2$ portion of the cell cycle is least disturbed under the stress of continuous low-level irradiation.

^{3}HTdR REPEATED LABELING INDICES UNDER CONTINUOUS IRRADIATION

An increase in the birth rate in a renewal tissue can be brought about either by (1) an increase in proliferation rate in the already dividing population, or (2) an increase in the size of the dividing population by bringing-in or recruitment of a previously nondividing (or slowly dividing) population. The repeated-labeled mitoses curves to 73 hr rise to ∿95% labeling, but the rate of labeling is slightly slower than in control mice. This reflects a G_2-delay of ∿3-6 hr, and this is seen in the flow-rate of cells into and through the early prophase subcompartment into late prophase. However, whereas in the normal repeated-labeling index curves only ∿70-75% labeling occurs in type A ($A_S + A_{1-4}$) cells by 25 hr and no change in repeated-LI is seen by 73 hr, in the continuously irradiated testis there are much higher repeated-LI in the type A population. Initially the repeated-LI is ∿20% higher, rises promptly to ∿90% labeling by 25 hr, and rises steadily thereafter to ∿95% by 73 hr. Thus, repeated-LI are ∿20-25% higher above normal values in the type A cell compartment at all intervals examined, whereas no change occurs in the In and type B cell repeated-labeling patterns. Further, there is little evidence of unlabeled cells which could be identified as morphologically similar to spermatogonia with oval darkly staining nuclei, or pairs or chains of such cells. Almost all cells label by 25 hr, and no unlabeled type A_S stem-cells are seen at 73 hr. Thus, there is an increased labeling rate, i.e., increased proliferation rate in an already dividing cell population and this could be accounted for by type A_S stem-cell spermatogonia with normally long generation times dividing more frequently. Where previously these cells divide at least once every two cycles of the seminiferous epithelium, i.e., once every ∿8.5-17 days, the evidence suggests that divisions which constantly occur among the A_S stem-cell spermatogonia increase their proliferation rate by decreasing their cell cycle times.

TYPE A SPERMATOGONIAL CELL CYCLE KINETICS

The PLM curves for the four differentiated spermatogonial type A_{1-4} cell cycles demonstrate the types A_2, A_3 and A_4 cell cycle characteristics appear relatively unchanged under continuous irradiation at 1.8 rad/day---the durations of the S and G_2 periods and the cell cycle times (~33-35 hr) remain much the same as in the unirradiated control (Table 3 and Table 4). But this is not the case for the type A_1 spermatogonia. Under continuous low-level exposure, there is a second wave of labeled mitoses, where previously none is seen in the unirradiated controls, and a cell cycle time of ~30 hr, i.e., ~10-15% shorter than the other types A_{2-4} spermatogonia. This is further evidence that the type A_1 compartment probably consists of at least two subpopulations---an A_s stem-cell population comprising the undifferentiated progenitor type A_s stem-cells, and a type A_1 cell population already committed to a differentiated pathway.

Two factors which determine the cellular response under continuous low dose-rate irradiation are (1) the rate of cell injury and cell death, that is, the cellular radiosensitivity, and (2) the capacity of the tissue for compensatory cell proliferation under continuous stress. The evidence for the seminiferous epithelium is that the early differentiated types A_{1-4} spermatogonia are very sensitive to radiation death, that this involves interphase death to a large extent, and that this is likely to be the main reason for the low tolerance of the testis to continuous low dose-rate irradiation. This is not necessarily associated with an absence of any compensatory processes which was formally believed to be the case. It appears that a reserve proliferative capacity exists, associated with repair and regeneration in the proliferative compartment of the undifferentiated type A_s stem-cell population. Thus, the spermatogonial cell renewal system is impaired under continuous irradiation, and the cell populations fall to low levels, due to progressive deterioration of the renewal system. Spermatogonial cell production is, however, maintained at near-normal levels provided the dose-rate is not too high, and this is the result of an increased cell proliferation rate in the type A_s stem-cell compartment. This does not appear to be associated with a greater multiplication factor in the recognizable precursor compartment---from the types A_{2-4} through to intermediate and to type B cells---which could be achieved by shortened cell cycle times along the developmental sequence.

ACCUMULATION OF SUBLETHAL RADIATION INJURY IN THE TYPE A_s STEM-CELL COMPARTMENT

Under continuous low-LET irradiation at very low dose-rates, the spermatogonial cell renewal system in the mouse can establish a steady-state of cell population consistent with function. This can occur provided a sufficient proportion of cells per cell generation escape injury, or can repair injury received, to allow the production of an adequate number of cells to carry out essential functions. This cell number may, in the seminiferous epithelium, be considerably less than normal. The one factor that can prevent the establishment and maintenance of the steady state and lead to continuous deterioration of the spermatogonial cell renewal system would be the accumulation of sublethal radiation damage within the proliferating stem-cell compartment from generation to generation up to a lethal limit. To what extent, if at all, does this occur in the seminiferous epithelium under continuous low dose-rate irradiation, and can we determine the extent to which the spermatogonial type A_s stem-cell population accumulates damage under continuous low-level exposure affecting cell proliferation, and thus the speed and efficiency of regeneration?

We are examining the curvature characteristics to an acute exposure of the type A_s stem-cell population in the mouse testis previously exposed to continuous

low-level irradiation for prolonged periods. After 1.8 rad/day for 105 days, mice are exposed to 400 rad x-irradiation acutely to the testes, and the spermatogonial A_S stem-cell response in continuously-irradiated-acutely-irradiated testis is being compared with control-acutely-irradiated testis. Fractions of control-unirradiated values are determined as a function of days after acute exposure; surviving type A_S stem-cells are determined as experimental/control ratios. In the control-acutely irradiated testes, ratios fall promptly to ~10-15% by 8-12 days, and recovery occurs more gradually by 40 days. In continuously-irradiated-acutely-irradiated mice, cellular depopulation and recovery is the same; repopulation is as rapid, but by 32 days it reaches the control-acutely-irradiated levels, and recovery occurs at about the same time, approximately the duration of one cycle of the seminiferous epithelium. The data suggest that there is little serious accumulation of sublethal damage under continuous low dose-rate exposure affecting the regenerative capacity of the renewal tissue.

In ^{3}HTdR-labeling studies, the LI in type A_S + A_1 populations, determined as a function of days after 400 rad acute irradiation, are low in the control testis, but rise rapidly by 8 days, and by 12 days, ~28% of type A_S + A_1 cells are labeled. There is a decline after 16 days, and normal values are reached after ~36 days, or after one complete cycle of the seminiferous epithelium. In the continuously irradiated mouse testis, the ^{3}HTdR-LI after 1.8 rad/day for 105 days followed by 400 rad of acute irradiation of the testes is similar to controls. Although the initial LI are twice the normal values prior to acute exposure, the LI rise to over 25% by 12 days and decline to pre-acute irradiation levels by ~26 days, again, after about one complete cycle of the seminiferous epithelium. Furthermore, there is increased labeling in the type A_S + A_1 cells during the initial phases of cellular depopulation, indicating that surviving cells are rapidly mobilized into the proliferating pool. In repeated-^{3}HTdR-labeling studies over the 72 hr period of days 11-13 after acute irradiation almost 100% labeling occurs in the type A_S + A_1 cells in Stage I. This is to be compared with the ~70-75% labeling in the type A (A_S + A_{1-4}) population which occurs in unirradiated control mice.

The increased ^{3}HTdR-LI and mitotic indices in type A spermatogonia surviving acute irradiation is associated with improved regenerative capacity of the surviving spermatogonial cells during repopulation following radiation depletion of the epithelium. One might expect that continuous low-level irradiation would impair the proliferative capacity by the accumulation of sublethal damage, but this does not appear to be the case. Thus, in most rapidly proliferating cell renewal systems under continuous exposure, provided the dose-rate is not too high, it appears that there is not a serious accumulation of sublethal damage from generation to generation, presumably because of the selective removal of damaged cells during proliferation [12-16]. The possibility also exists that the seminiferous epithelium may possess a small and temporarily adequate reserve for compensatory cell proliferation, but recruitment of this reserve requires triggering.

SPERMATOGONIAL CELL SURVIVAL AFTER ACUTE IRRADIATION

After 400 rad of acute irradiation to continuously exposed and in unirradiated-control mice a pattern of spermatogonial cell survival emerges in which the type A_S cell is the most radioresistant. At 4 days, cell survival in controls is 52±6% for type A_S, 26±4% for A_1 and only ~1-2% for the remaining types A_{2-4} cells. By 8.5 days, survival falls to 16±3% in the type A_S cells, and very few types A_{1-4} cells are seen. The survival characteristics of the seminiferous epithelium in continuously irradiated mice are very similar, except that cellular depopulation occurs more quickly (35±4% of type A_S cells survive at 4 days), the period of cell depletion is slightly more protracted, and recovery is delayed up to 32 days.

Between 8 and 12 days in both groups the surviving populations of type A spermatogonia are almost all type A_S stem-cells, only a few are type A_1 cells, and practically no types A_{2-4} cells are seen.

In repeated-^{3}HTdR-labeling studies of the surviving type A cells under continuous irradiation, during the period of greatest depopulation following acute exposure, only a small percentage (~10%) of surviving type A cells are labeled after 72 hr, but almost all of those A cells labeled have the cytological characteristics of the type A_S stem cells. It is estimated that ~60% of the A_S stem-cells become labeled during the 72-hr labeling period from days 11-13.

In the absence of labeling in the type A_1 cells and with depopulation of the types A_{2-4} compartments, cellular restitution of the depleted seminiferous epithelium occurs solely through proliferation and differentiation of the surviving type A_S stem-cell spermatogonial population, with subsequent differentiation into more mature forms along the developmental sequence. It, therefore, appears in both control-acutely-irradiated and continuously-irradiated-acutely-irradiated mice, that: (1) the type A_S stem-cells are more resistant to irradiation than other forms of recognizable type A cells; (2) regeneration and restitution of the seminiferous epithelium originates from these surviving A_S stem-cells; and (3) there is little evidence of the accumulation of serious damage from generation to generation in the type A_S stem-cell population under low-level irradiation at 1.8 rad/day affecting the regenerative capacity of the seminiferous epithelium. This latter situation occurs for 105 days, i.e., after 12 cycles of spermatogenesis and after 3 complete cycles of the seminiferous epithelium. These studies in the mouse, and those of Oakberg [5-7,17-19], indicate that whereas formally, the type A spermatogonia were considered the most sensitive to radiation injury, these are now recognized to be the type A_{1-4} spermatogonial cells, and they do not contribute to the stem-cell population. The type A_S stem-cells are the most resistant spermatogonia to radiation and injury, and emerge as the most important spermatogonial cells in the assessment of genetic damage in spermatogonia by ionizing radiation and, in large measure, by other mutagenic agents.

CONCLUSION

The spermatogonial cell renewal system can maintain function and a steady level of cell population for relatively long periods of continuous low-level irradiation indicating that there does not appear to be a serious accumulation, over many generations, of damage affecting proliferation. Provided the dose-rate is quite low, there is an effective selective removal of damaged cells with almost complete repair of cellular nonlethal damage. At dose-rates greater than 2 rad/day, spermatogonia are very sensitive to radiation death, and the main reason for the low tolerance to continuous stress could, in part, be the limited extent of compensatory mechanisms regulating spermatogonial cell production. However, there is some capacity to change the patterns of cellular proliferation while still remaining under homeostatic control, and this capacity appears to reside in the relatively radioresistant A_S stem-cell population.

Little is known about the extent to which the spermatogonial cell population can repair nonlethal cellular radiation damage accumulated under continuous stress affecting the regenerative capacity of the tissue. After acute exposure, a minimum number of surviving type A_S stem-cells is required to repopulate the functional seminiferous epithelium, regeneration proceeds along an ordered cell stage sequence and is dependent on the time required for all stages from type A_S spermatogonia to mature spermatozoa. Under continuous irradiation, provided the dose-rate is not too high, the repopulating ability of the seminiferous epithelium is maintained, in

the presence of injury, due to initial repair and long-term repair of cellular radiation damage. There is evidence for initial repair, since a dose-rate effect exists in type A survival, at low doses. Long-term repair occurs due to differential radiosensitivities of spermatogonia.

Our understanding of spermatogonial stem-cell renewal has considerable importance in the assessment of the genetic effects of radiation and other mutagenic agents. In the past, the entire type A spermatogonial cell population was considered to be the most sensitive, but this no longer obtains for evaluating differential spermatogonial cell survival or cell synchronization in relation to radiation dose and mutation induction. The response of type A_S stem-cell spermatogonia is now central to long-term genetic effects, since these are not only the true stem-cells in the testis, but the most radioresistant spermatogonial cell population. The types A_{1-4}, In, and type B cells do not contribute to the stem-cell pool, and are of little importance in assessing genetic damage in spermatogonia. All long-term genetic effects resulting from low-level ionizing radiations will be based on the cellular response and cell population kinetics of the type A_S stem-cell spermatogonia [5-8].

REFERENCES

1 FABRIKANT, J. I., Radiat. Res. 19:46 (1971)
2 FABRIKANT, J. I., Am. J. Roentgenol., Rad. Therapy and Nuclear Med. 114:792(1972)
3 HSU, T. H. S., FABRIKANT, J. I., Biological and Environmental Effects of Low-Level Radiation, IAEA Publication STI/PUB/409, 1:157, Vienna (1976)
4 FABRIKANT, J. I., Invest. Radiol. (1979) in press
5 OAKBERG, E. F., Mutation Res. 11:1 (1971)
6 OAKBERG, E. F., Anat. Rec. 169:515 (1971)
7 OAKBERG, E. F., HUCKINS, C., Stem Cells of Renewing Cell Populations, Academic Press (1976)
8 HUCKINS, C., OAKBERG, E. F., Anat. Rec., 192:519 (1978)
9 HUCKINS, C., Anat. Rec., 169:533 (1971)
10 HUCKINS, C. Cell Tissue Kinet. 4:313 (1971)
11 HUCKINS, C., Cell Tissue Kinet. 4:335 (1971)
12 LAMERTON, L. F., Recovery and Repair Mechanisms in Radiobiology, Brookhaven Symp. Biol. 20:212 (1968)
13 LAMERTON, L. F., Radiation Research, North-Holland Publishing Co., Amsterdam(1967)
14 LAMERTON, L. F., Radiat. Res. 27:119 (1966)
15 FABRIKANT, J. I., Pathology of Irradiation, Williams and Wilkins, Baltimore (1971)
16 FABRIKANT, J. I., CRC Critical Reviews in Radiological Sciences, CRC Press, Cleveland (1971)
17 OAKBERG, E. F., Japan J. Genetics (Suppl.) 40:119 (1964)
18 OAKBERG, E. F., Handbook of Physiology, Am. Physiological Society, 5 (1971)
19 OAKBERG, E. F., Anat. Rec. 169:515 (1971)

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Table 1
Spermatogonial Cell Kinetic Parameters

Kinetic Parameter	Spermatogonial Cell Type		
	Type A (A_s + A_{1-4})	Intermediate	Type B
^{3}HTdR-LI	30±4%	63±7%	50±6%
$\bar{T}_c$	34 hr	32 hr	34 hr
$\bar{T}_{G_1+M/2}$	9 hr	6 hr	6 hr
$\bar{T}_s$	22 hr	23 hr	25 hr
$\bar{T}_{G_2+M/2}$	3 hr	3 hr	3 hr
Growth Fraction (G.F.)	0.46	0.88	0.68
Cell Birth Rate (α)	20.3 x 10^{-3}/hr	21.7 x 10^{-3}/hr	2[illegible].3 x 10^{-3}/hr
Specific Birth Rate (β)	0.014/hr	0.027/hr	0.020/hr

Table 2
Spermatogonial Cell Kinetic Parameters
Under Continuous Irradiation

Kinetic Parameter	Spermatogonial Cell Type		
	Type A (A_s + A_{1-4})	Intermediate	Type B
^{3}HTdR-LI	41±5%	64±4%	59±6%
$\bar{T}_c$	34 hr	33 hr	35 hr
$\bar{T}_{G_1+M/2}$	8 hr	7 hr	8 hr
$\bar{T}_s$	18 hr	19 hr	22 hr
$\bar{T}_{G_2+M/2}$	8 hr	7 hr	5 hr
Growth Fraction (G.F.)	0.77	1.11	0.94
Cell Birth Rate (α)	20.3 x 10^{-3}/hr	21.0 x 10^{-3}/hr	19.8 x 10^{-3}/hr
Specific Birth Rate (β)	0.023/hr	0.037/hr	0.027/hr

Table 3
Spermatogonial Type A_{1-4} Cell Cycle Parameters

Cell Cycle Parameter (hr)	Spermatogonial Cell Type			
	A_1	A_2	A_3	A4
$\bar{T}_c$	>64	~35	~35	~35
$\bar{T}_{G_1+M/2}$	>37	~7	~6	~6
$\bar{T}_s$	19	20	22	23
$\bar{T}_{G_2+M/2}$	8	8	7	6

Table 4
Spermatogonial Type A_{1-4} Cell Cycle Parameters
Under Continuous Irradiation

Cell Cycle Parameter (hr)	Spermatogonial Cell Type			
	A_1	A_2	A_3	A_4
$\bar{T}_c$	~30	~32	~35	~33
$\bar{T}_{G_1+M/2}$	3	4	7	6
$\bar{T}_s$	19	21	21	21
$\bar{T}_{G_2+M/2}$	8	7	7	6