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Cell Cycle of Spermatogonia in Mouse Testis

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The mammalian cell cycle consists of four phases: G_1 , S, G_2 , and M. The duration (T) of these phases and the cell cycle time (T_C) can be measured accurately by pulse-labeling with tritiated thymidine ($^3\text{HTdR}$), followed by serial killing, preparation of high resolution autoradiographs, and determination of the percentage of mitoses labeled at frequent intervals between injection and death [1,2]. The present communication measures the spermatogonial cell cycles and their constituent phases of proliferating type A (taken as a whole, including types A_S , A_1 , A_2 , A_3 , and A_4), intermediate (In), and type B cells in the testis of the young mouse, and analyzes the cell cycle and cell population kinetic parameters, such as growth fractions and cell-birth rates from $^3\text{HTdR}$ autoradiographic data.

Young adult (13 weeks) male C57BL/6J mice were used. $^3\text{HTdR}$ (20 μCi , sp. act. 19.2 Ci/mM) were injected intraperitoneally; animals were killed at intervals from 0.5 to 60 hr. Autoradiographs were prepared from wax-embedded tissues, prestained with PAS-Schiff solution, dipped in Kodak NTB-2 liquid nuclear emulsion, exposed for 3 months, processed, and counterstained with Ehrlich's hematoxylin. At least 100 meta-anaphases in each spermatogonial cell type were counted; dividing cells with 4 grains or more on slides with background counts of less than 1 grain per nucleus were scored. The spermatogonial cell types were based on tubular stages and the seminiferous epithelium described by Oakberg [4], and identified in Stages IX, XI, I-II for type A, IV for In, and VI for B mitotic cells, respectively.

Fig. 1 illustrates percentage of mitoses labeled as a function of the interval between injection and death. There are two well-defined waves of labeled mitoses. Mitotic cell labeling of early prophase cells occurs promptly within 1 hr of injection of $^3\text{HTdR}$; because of the labeling of early prophase cells, the kinetic data for analysis of the $\bar{T}_{(G_2+M/2)}$ interval are complicated by the prolonged transitional prophase unique to the spermatogonial cell population. This mitotic stage is unusual, since some 25% of early prophase cells label with $^3\text{HTdR}$ by 1 hr; this indicates that DNA synthesis is occurring immediately prior to the onset of mitosis, and appears to be confined to early prophase [5,6]. $\bar{T}_{(G_2+M/2)}$ in each spermatogonial cell type (A, In, and B) determined at the 50% point on the initial ascending wave of labeled mitoses, is approximately 3 hr, but there was great variation within and among the individual cell populations. The spread in the duration of this period is most noticeable in the type A population. In an ideal renewal system, all cells in DNA synthesis at the time of $^3\text{HTdR}$ injection should be labeled, and should pass through mitosis to give 100% labeling [2,3]; in this study, labeling reached 96% in type A mitoses, and 100% in In and B cells. T_S , determined at the 50% points on the ascending and descending limbs, is 22 hr, 23 hr, and 25 hr in type A, In, and B cells, respectively. The variability in T_S is estimated from differences between slopes of the ascending and descending limbs; since the fall is less steep than the rise, particularly in type A cells, T_S in individual cells is of unequal lengths. T_C is the distance between the 50% points on the rising limbs of the first and second waves; these values are 34 hr, 32 hr, and 34 hr in types A, In, and B cells, respectively. $\bar{T}_{(G_1+M/2)}$, measured by subtraction, is 9 hr, 6 hr, and 6 hr, respectively. The distribution of T_{G_1} in individual cells is great in the type A cell

population, which accounts for a spread of experimental points in the second wave and absence of a well-defined peak for the S phase of the daughter cells. This was not the case in type B cells, and to a much lesser degree, in In spermatogonia.

Estimate of the growth fraction (G.F.) of the proliferating spermatogonial cell populations (type A, In, and B cells) is obtained from 1-hr $^3\text{HTdR}$ -labeling indices (LI) and labeled mitoses [7]. Labeling index of the G.F., $\text{LI}_p = \lambda(T_S/T_C)$, where λ is a constant which depends on the duration and position of S in the cell cycle [3]. In linear growth, λ is ~ 1.0 ; $\text{LI}_p = 46\%$ for type A, 72% for In, and 74% for B spermatogonia. Comparison of observed and calculated LI is a measure of the fraction of cells dividing; $\text{G.F.} = \text{LI}/\text{LI}_p$. For type A spermatogonia, $\text{LI} = 30\%$, and $\text{G.F.} = 0.46$; for In cells, $\text{LI} = 63\%$, and $\text{G.F.} = 0.88$; for B spermatogonia, $\text{LI} = 50\%$, and $\text{G.F.} = 0.68$.

The cell birth rate, α , is the number of cells added to the population by division per unit time; $\alpha = \ln 2/T_C$. Thus, approximately 1.9 type A spermatogonia/hr/1000 type A cells, 2.2 In cells/hr/1000 In cells, and 1.9 type B cells/hr/1000 B cells are available to flow into each subsequent cell compartment along the type A $\rightarrow$ In $\rightarrow$ type B proliferating-differentiating sequence of spermatogonial cell renewal. The specific birth rate, β , is the proportion of cells dividing per unit time; $\beta = \text{LI}/T_S$. These values are 0.14/hr, 0.03/hr, and 0.02/hr for type A, In, and B spermatogonia, respectively. The data are insufficient to calculate extent of cell loss due to ineffective spermatogenesis and physiological attrition.

These observations agree with cell cycle times and phase durations for spermatogonial cell populations in the Wistar rat [8,9], but not with those reported for the F_1 hybrid (101 x C3H) mouse [6]. The data indicate that spermatogonial cell cycle times in the mouse are relatively long, and the

greatest variation occurs in the type A population taken as a whole. This would be due to (1) the heterogeneity of the type A population, which consists of at least 5 types: A_s , and A_{1-4} subpopulations, (2) variation of cell kinetic parameters in each of these type A subpopulations, and (3) the possible existence of at least two type A_s stem cell populations -- one with a relatively short cell cycle time in the range of ~ 42 hr, and one with a fairly long cell cycle time, perhaps 13 days or more [9-12]. The results of this work demonstrate: (1) The cell cycle times of spermatogonial renewal populations are much longer than the rapidly dividing mammalian tissues of the immunohematopoietic system or the intestinal epithelium [1,2]. (2) Spermatogonial cells, as a renewal tissue, are in a dynamic state of cell renewal with precise regulatory control for maintenance of cell population structure in a steady state. The cell birth rate is almost constant in each cell compartment, and this appears to be maintained under homeostatic control through variation in the G.F. and in the specific birth rate in each subcompartment along the proliferation-maturation sequence. For example, the G.F. is low in the type A subcompartment, but this is compensated for by a relatively high specific birth rate; the reverse situation appears to obtain for the In subcompartment. (3) The $\tilde{T}_{(S \rightarrow G_2 \rightarrow M)}$ cell-age sequence is fairly constant in a given renewal tissue in a species, while $\tilde{T}_{G_1}$ varies widely, and is mainly responsible for differences in cell cycle times, both within and between cell populations. (4) The subpopulations of the type A compartment, and particularly the type A_s stem-cell compartment, require further studies for more precise analysis of cell population and cell cycle kinetic parameters. This is presently being done in our laboratory [Work supported by USDOE (USAEC) Contract CH AT (11-1)-3013 and by USPHS training grant No. 39896-03-70].

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Fig. 1. Percentage of labeled mitoses in mouse spermatogonia [type A, intermediate (In), and type B] after injection of $^3\text{HTdR}$; each point represents 1 mouse.

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Figure 1

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