AUTORADIOGRAPHIC STUDY OF DNA SYNTHESIS AND THE CELL CYCLE IN SPERMATOGONIA AND SPERMATOCYTES OF MOUSE TESTIS USING TRITIATED THYMIDINE

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ABSTRACT

Mice were injected intraperitoneally with 15 μc of H^3-thymidine. The time course of the labeling in spermatogonia and spermatocytes was studied by using autoradiography on 5 μ sections stained by the periodic acid–Schiff method and hematoxylin over a period of 57 hours after injection. Four generations of type A (called A$_I$, A$_{II}$, A$_{III}$, and A$_{IV}$), one of intermediate, and one of type B spermatogonia occur in one cycle of the seminiferous epithelium. The average life span is about the same in all spermatogonia, *i.e.*, about 27 to 30.5 hours. The average pre-DNA synthetic time, including the mitotic stages from metaphase through telophase and the portion of interphase preceding DNA synthesis, is also not very different, ranging between 7.5 and 10.5 hours. A remarkable difference exists, however, in the duration of DNA synthesis and of the post-DNA synthetic period. The average DNA synthetic time is very long and is highly variable in type B (14.5 hours), a little shorter and less variable in intermediate (12.5 hours) and A$_{IV}$ (13 hours) spermatogonia, and much shorter and very constant in A$_{III}$ (8 hours), A$_{II}$ and A$_I$ (7 to 7.5 hours) spermatogonia. Conversely, the average post-DNA synthetic time, corresponding essentially to the duration of the prophase, is short and very constant in type B (4.5 hours), longer and variable in intermediate (6 hours) and A$_{IV}$ (8 hours) spermatogonia, and much longer and much more variable in A$_{III}$ (11 hours), A$_{II}$ and A$_I$ (14 hours) spermatogonia. The premeiotic synthesis of DNA takes place in primary spermatocytes during the resting phase and terminates just before the visible onset of the meiotic prophase. Its average duration is 14 hours. No further synthesis of DNA takes place in later stages of spermatogenesis.

INTRODUCTION

Labeled thymidine, which is incorporated exclusively into DNA (8, 37), is a powerful tool for studying cell population kinetics and cell turnover. This label is bound firmly within the cells (there is no reduction of labeling in time due to exchange of tritium or turn-over of DNA), the label being subjected only to dilution following cell division (4, 13, 45, 46). Thus the labeling can be followed intact through one or more cell cycles by using autoradiographic techniques. In addition, the pool of labeled precursors is depleted within a very short time after administration, so that the complicating factor of a continuous labeling of the cells is lacking (4, 13, 39, 45). Autoradiographs made using tritium-labeled thymidine, furthermore, have the advantage of a

very high resolving power because of the very low energy and consequently short range of tritium β particles, the average range in tissues being less than 1 μ (7).

Tritiated thymidine has been used in the past few years by a number of workers to study many problems in cell physiology (1, 5, 13, 19, 22, 23, 30, 31, 33, 36, 42, 44–46, 48).

The present research, which utilized these techniques, was undertaken to study the cellular cycle and DNA synthesis in the spermatogonia and spermatocytes of the mouse. These problems have not been investigated as yet in the spermatogonia of mammalian testis. It has previously been shown that the meiotic synthesis of DNA in mammalian testis occurs in resting primary spermatocytes just before the onset of meiotic prophase and that no synthesis of DNA takes place in the course of meiosis (3, 26, 43). Other workers have estimated the duration of the spermatogenesis or its parts (3, 9, 10, 34, 40, 41). It has also been observed that in the mouse testis radiation from tritiated thymidine has no appreciable effect on survival of spermatogonia at the activities that are normally used in tracer experiments using the autoradiographic technique (14).

MATERIAL AND METHODS

(C3H ♂ × 101 ♀)F_1 hybrid male mice, 10 to 13 weeks old, ranging in weight from 21 to 24 gm, were injected intraperitoneally with 15 μc of H^3-thymidine per mouse and sacrificed by cervical dislocation at intervals ranging from 30 minutes to 57 hours after injection. Five mice were used for the 1 hour interval and three mice for each of the other times. Six mice served as non-radioactive controls. The specific activity of the tritiated thymidine (Schwarz BioResearch, Mount Vernon, New York) measured by the supplier was 1.9 curies per millimole.

The testes were removed free of fat, and the tunica cut at one pole. They were then fixed for 24 hours in Orth fluid, washed for 20 hours in running water, embedded in paraffin, sectioned at 5 μ and stained by the periodic acid–Schiff (PAS) technique. After staining autoradiograms were prepared with NTB2 nuclear track liquid emulsion (Kodak, Rochester, New York), and stored in light tight boxes in a refrigerator at 4°C for 21 days. At the end of the exposure period, they were processed in Kodak D-19 at 17°C for 5 minutes, rinsed in tap water, and cleared in Kodak acid fixer for 8 minutes at the same temperature as the developer. Nuclear staining with Ehrlich's acid hematoxylin was carried out through the autoradiographic emulsion after processing.

The seminiferous tubules of the testis were classified according to the description of Leblond and Clermont (18) and Oakberg (28) in twelve successive stages.[1] A fixed number of tubules were scored for each of the 12 stages of the cycle, according to the frequency distribution given by Oakberg (29). Only those tubules cut in cross-section, as judged by their approximation to a circle, were scored.

Counts were made of the number of labeled and unlabeled interphases, early and middle prophases, late prophases, metaphase-anaphases, and telophases in type A, intermediate, and type B spermatogonia. Only cells having more than four grains per nuclei were scored as labeled. The number of labeled and unlabeled resting primary spermatocytes and early leptotene nuclei were also scored at tubule stages VII and VIII. Spermatogonia were classified as necrotic only when degenerative changes were obvious. No attempt was made to count the number of grains lying over the nuclei or to relate this number to the amount of H^3-thymidine incorporated into the

[1] On the basis of the development of the acrosomic system of the head of the spermatids, as revealed by elective staining with the PAS method, the spermiogenesis in mammals can be accurately studied and subdivided into stages (18). Nineteen stages have been described in the rat (18) and sixteen in the mouse (28). Each stage of spermiogenesis is associated with definite types of spermatogonia and spermatocytes, so that the developmental sequences of spermatogonia to spermatocytes can be accurately identified and studied on the basis of the concomitant process of development of spermatids to spermatozoa. The first twelve stages in the mouse correspond to one "cycle" of the seminiferous epithelium, the cycle being defined as the "series of changes occurring between two successive appearances of the same cell association in an area" (18). Four such cycles occur during the entire duration of spermatogenesis in the mouse, from the "dormant" type A spermatogonia to the mature spermatozoa (29). Spermatogonia are divided into three classes, type A, intermediate, and type B. The type A spermatogonia have an ovoid, pale, "dust-like" nucleus with a thin nuclear membrane. The type B spermatogonia have a smaller, spherical nucleus that stains more deeply due to the presence of coarse chromatin masses ("crust-like" spermatogonia) lying mainly against the inner surface of the nuclear membrane. The intermediate type spermatogonia are a transitional form between type A and type B spermatogonia, as shown by a thickening of the nuclear membrane caused by adherent chromatin flakes, a deepening of the nuclear staining and a gradual change in the shape of the nucleus from oval to round (2, 18).

cell, because of the great variability among nuclei in the self-absorption of tritium β^- particles in histological sections.

RESULTS

Spermatogonia

Rate of Labeling with H^3-Thymidine

The percentage of labeled nuclei for each type of spermatogonium (A, intermediate, and B) fluctuates to some extent at the various time intervals after injection of H^3-thymidine, as expected in an asynchronous population of cells. It is relatively low at 30 minutes and increases slightly 1 hour after injection. A slight decrease is then seen at 2 to 4 hours, followed at later times by a further increase up to or above the 1 hour value. The percentage then drops gradually (Table I).

The time course of the change in the percentage of labeled nuclei after injection of H^3-thymidine is interpreted as follows: The 30-minute count is an underestimate of the labeling, since, following intraperitoneal injection, (*a*) about 15 minutes are required for the passage of the thymidine from the peritoneal cavity to the blood stream and (*b*) the amount of H^3-thymidine uptake by the cells during the remaining 15 minutes is, in some cells, insufficient to activate a number of grains above background. The slight drop at 2 to 4 hours is due to the increase of the population by the division of the cells that had completed DNA synthesis at the time of injection and are therefore unlabeled. The subsequent increase is due to the division of the labeled cells (30, 45). The gradual drop in the percentage of labeled cells, which takes place later, is accounted for as being the result of an underestimate of labeled cells owing to the dilution of H^3-thymidine following two cell divisions.

Thirty minutes after injection of H^3-thymidine, 80 to 90 per cent of the labeled spermatogonia are in interphase. As soon as 1 hour after injection, this frequency drops to about 70 per cent and the remaining 30 per cent of labeled cells are in very early prophase. Later, the labeling proceeds through subsequent mitotic stages and finally reappears in the interphase nuclei of the next cell generation (Table II). These data indicate that DNA synthesis in the spermatogonia of mouse testis occurs in interphase and terminates just before the visible onset of prophase.

Cell Generation Sequences

Shortly after administration of H^3-thymidine, labeled spermatogonia are present in six distinct and separate periods of the cycle of the seminiferous epithelium at the following tubule stages: (*a*) late VIII and very early IX, (*b*) late X and very early XI, (*c*) late XII and very early I, (*d*) early II, (*e*) III and very early IV, (*f*) V and very early VI, with very sharp peaks at stages VIII, X, XII, early II, III, and V. The first four peaks correspond to type A, the fifth to intermediate, the sixth to type B spermatogonia (Fig. 1). Very few of the type A spermatogonia present at stages III through VII appear labeled shortly after administration of H^3-thymidine. The average frequency at 1 hour after injection is 1.0 per cent. Resting primary spermatocytes (RPS's) are labeled in very late stage VII and early and middle stage VIII. The peak of labeled RPS's occurs in this latter stage (Table I and Fig. 1). Six distinct successive peaks of spermatogonial mitoses correspond to the six peaks of labeled spermatogonia during the seminiferous cycle. The mitotic peaks, calculated as percentage of spermatogonia in metaphase-anaphase, are localized at tubule stages next to those in which DNA synthesis peaks occur, namely, at stages IX, XI, I, late II, IV, and VI. The first four peaks refer to type A spermatogonia, the fifth and sixth, respectively, to intermediate and type B spermatogonia (Fig. 1).

The type A spermatogonia are present throughout the cycle of the seminiferous epithelium. At the beginning of each cycle, that is, at stage IX, they enter a series of divisions (three in the rat (2), and probably three in the mouse, (28)), thus increasing in number, and most of them transform at the subsequent stage III to intermediate spermatogonia. These divide once at stage IV to form type B spermatogonia which, in turn, divide once at stage VI to give rise to resting primary spermatocytes (RPS's). These are present during stage VII and about the first half of stage VIII, following which they enter meiotic prophase (2, 28). A few type A spermatogonia, however, fail to transform into intermediate spermatogonia, thus becoming "dormant" type A cells that stay undivided until next stage IX and then act as stem cells by initiating a new multiplicative cycle (2, 28). This process accounts for the maintenance of the formation of germ cells while spermatogonia transform into spermatocytes.

These data clearly indicate that four successive generations of type A spermatogonia, one generation of intermediate, and one generation of type B spermatogonia follow one another during a single cycle of the seminiferous epithelium of the mouse. The type A generations are indicated by symbols. A1 is the first spermatogonial generation of the cycle following the "dormant period" (2),

TABLE I

*Percentage of Labeled Spermatogonia (A, Intermediate, and B) and Primary Spermatocytes (RPS's) at Various Time Intervals after Injection of H^3-Thymidine**

Time after Injection	Type A	Intermediate	Type B	RPS Stage VII	RPS and leptotene nuclei stage VIII
hours					
0.5	16.84 ± 1.54	29.13 ± 2.58	30.55 ± 2.01	8.45 ± 0.97	42.75 ± 1.99
1	18.55 ± 0.69	34.97 ± 1.13	36.81 ± 0.86	11.76 ± 0.50	41.31 ± 0.80
2	16.60 ± 0.87	34.58 ± 1.45	27.48 ± 1.02	7.01 ± 0.51	41.49 ± 1.01
4	17.08 ± 1.06	32.38 ± 1.86	27.48 ± 1.32	0.41 ± 0.15	37.43 ± 1.35
7	17.80 ± 1.10	36.10 ± 1.80	37.56 ± 1.31	1.16 ± 0.27	50.44 ± 1.31
10	16.27 ± 0.98	36.08 ± 1.67	30.62 ± 1.24	10.20 ± 0.77	37.06 ± 1.19
13	16.80 ± 1.06	34.42 ± 1.72	31.06 ± 1.27	32.34 ± 1.21	49.40 ± 1.23
16	13.02 ± 0.93	24.96 ± 1.71	47.10 ± 1.36	33.75 ± 1.13	26.94 ± 1.16
19	13.19 ± 1.01	33.85 ± 1.97	38.42 ± 1.46	33.31 ± 1.31	37.15 ± 1.28
22	12.54 ± 1.36				
25	11.66 ± 1.00	29.87 ± 1.95	36.96 ± 1.55	43.16 ± 1.45	13.89 ± 1.06
31	9.61 ± 0.77	28.52 ± 1.61	24.42 ± 1.16	41.01 ± 1.18	18.97 ± 1.00
43	6.94 ± 0.65	14.56 ± 1.25	21.65 ± 1.07	15.20 ± 0.88	15.97 ± 0.86
55	9.05 ± 0.71	15.62 ± 1.18	24.86 ± 1.07	33.67 ± 1.06	25.36 ± 1.01

* Standard deviations are indicated.

TABLE II

Percentage Distribution of Labeled Cells among Mitotic Stages in Spermatogonia after Injection of H^3-Thymidine

Time after injection of H^3-thymidine	Type A					Intermediate					Type B				
	I	EP	LP	MA	T	I	EP	LP	MA	T	I	EP	LP	MA	T
hours															
0.5	90.1	7.9	0.0	0.0	2.0*	81.4	6.8	0.0	0.0	11.8*	80.8	19.2	0.0	0.0	0.0
1	67.8	29.7	0.0	0.0	2.5*	68.4	26.0	0.0	0.0	5.6*	74.7	25.3	0.0	0.0	0.0
4	31.8	68.2	0.0	0.0	0.0	31.9	61.3	6.8	0.0	0.0	23.6	69.6	2.9	2.6	1.3
7	20.0	74.4	5.1	0.5	0.0	8.6	57.5	8.1	9.1	16.7	10.6	76.1	3.4	3.5	6.4
10	5.2	65.7	16.1	5.2	7.8	1.3	64.1	5.7	0.7	28.2	2.8	45.0	7.6	6.4	38.2
13	2.4	50.0	16.7	6.7	24.2	3.0	42.0	9.9	6.8	38.3	27.2	30.4	14.0	6.6	21.8
16	0.0	34.5	25.1	4.7	35.7	22.6	12.0	17.6	11.9	35.9	38.9	15.5	7.6	5.0	33.0
19	2.7	27.7	14.2	6.8	48.6	23.8	14.6	8.6	2.2	50.8	56.2	14.6	5.6	1.7	21.9
22	10.0	20.1	11.2	6.0	52.7										

I, interphase; *EP*, early and middle prophase; *LP*, late prophase; *MA*, metaphase and anaphase; *T*, telophase.

* These labeled telophase figures, seen shortly after injection of H^3-thymidine, represent a very early synthesis of DNA of the following cell generation—namely of intermediate and type B spermatogonia. By withdrawing these labeled telophases from the total number of labeled cells, the percentage distribution of the labeled cells is as follows: about 92 per cent in interphase and 8 per cent in early prophase, at 30 minutes; and about 70 per cent in interphase and the remainder in early prophase at 1 hour after injection.

in which neither DNA synthesis nor division occurs. This period extends from tubule stages III through VII. The type A spermatogonia present throughout this interval are the so-called "dormant" cells (AD), which arise somewhere during the cycle from one of the divisions of type A spermatogonia, remain undivided until next stage VIII, and then function as stem cells by undergoing a series of mitoses leading to the formation of primary spermatocytes in a new cycle (2, 28). The first three of these divisions give rise to the subsequent three generations of type A spermatogonia—AII, AIII, and AIV. AIV is the last type A spermatogonial generation of the cycle. It divides, giving rise to intermediate spermatogonia. The latter undergo one division only. The resulting daughter cells are type B spermatogonia that, in turn, divide once, giving rise to RPS.

Generation Time and Duration of the Various Parts of the Cell Cycle

In an asynchronous and homogeneous population of cells, the labeling of mitotic figures with

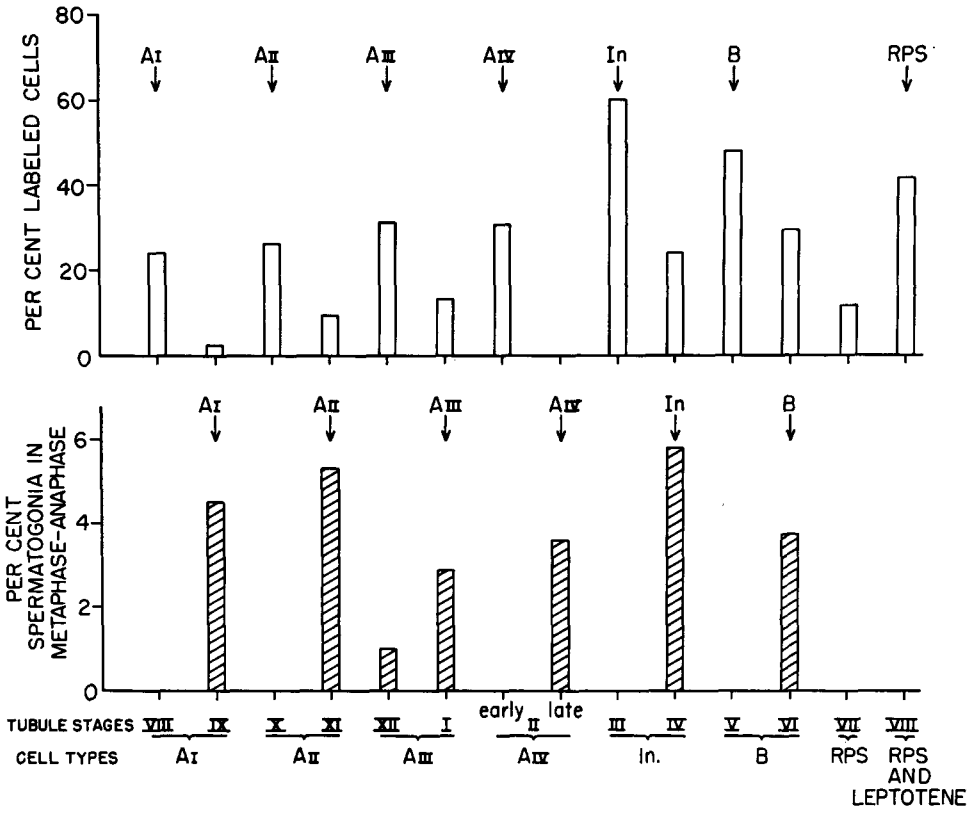

FIGURE 1

Percentage of labeled cells and percentage of spermatogonia in metaphase-anaphase at each stage of the cycle of seminiferous epithelium 1 hour after intraperitoneal injection of H^3-thymidine.

DNA synthesis occurs at seven distinct periods during the cycle, with peaks localized at stages VIII, X, XII, early II, III, V, and VIII. The peaks correspond respectively to four successive generations of type A spermatogonia (indicated as AI, AII, AIII and AIV), to one generation of intermediate spermatogonia (*In*), to one generation of type B spermatogonia (*B*) and to the resting primary spermatocytes (*RPS*). No further synthesis of DNA takes place in later stages of spermatogenesis. Mitoses of spermatogonia occur at six distinct periods during the cycle. The peaks are localized at tubule stages next to those in which DNA synthesis peaks occur, that is, at stages IX, XI, I, late II, IV, and VI. The few intermediate spermatogonia present at very late stage II have been omitted.

respect to time after the administration of the label gives precise information as to the average generation time and position of DNA synthesis in the mitotic cycle. The best information for computing the various time parameters of the mitotic cycle in a population of cells has come from studies performed in tissue cultures (30, 45), or with plant material (48), where the label is made available to the cells for a very short time relative to the total generation time, then withdrawn and substituted with "cold" thymidine. The work by Howard and Pelc (11, 12) and Lajtha, Oliver, and Ellis (16), using P^{32} and adenine-C^{14} labeling, ought to be taken into account in this respect. Evidently only those nuclei that are synthesizing DNA at the time of the addition of the isotope will incorporate the label. The technique consists in following these labeled cells as they pass through two successive mitotic cycles and following the change in the frequency of labeled mitotic figures at various time intervals after the addition of the label. The curve obtained by plotting the frequency of labeled mitotic figures against time would be interpreted as follows.

The time it takes for the first labeled mitotic figures to appear after addition of H^3-thymidine is actually the minimum time between the end of DNA synthesis and the onset of mitosis (minimum post-DNA synthetic time). The time it takes for practically 100 per cent of the mitotic figures to be labeled is the maximum time between the completion of DNA synthesis and the onset of mitosis (maximum post-DNA synthetic time), because at this moment all the cells that had finished DNA synthesis at the time of addition of the label have divided and only those cells that were synthesizing DNA during the labeling move into mitosis. The average post-DNA synthetic time is read from the abscissa at the point where the rising curve crosses the 50 per cent line on the ordinate.

After the maximum post-DNA synthetic time the curve of labeled mitoses forms a plateau, the width of which is greater the longer the duration of DNA synthesis. Thereafter there is a drop of the curve to zero as the cells that were in pre-DNA synthetic period at the moment of labeling move into mitosis.

The time it takes for labeled mitotic figures to disappear is the maximum time between the beginning of DNA synthesis and mitosis. By subtracting from this time the maximum post-DNA synthetic time, one obtains the maximum duration of the DNA synthesis. As the labeled cells go through the next cell cycle, another similar curve of frequency of labeled mitotic figures is obtained. The average generation time is estimated by measuring the interval between two successive peaks of labeled mitotic figures or the interval between the same percentages on two successive rising portions of the curves. Since, in a homogeneous and asynchronous population of cells, the proportion of cells that are labeled after a very short contact with H^3-thymidine is a direct measure of the fraction of the generation time during which DNA is synthesized, the average time for the DNA synthesis can be estimated by multiplying that proportion by the average generation time. By subtracting the average post-DNA synthetic time and the average DNA synthetic time from the average cell generation time, the average pre-DNA synthetic time is estimated. This extends from mitosis to the resumption of DNA synthesis in the next cell cycle.

The slopes of the upward and downward portions of the curves of the percentages of labeled mitotic figures plotted against time after addition of H^3-thymidine are an indication of the variability among cells in the duration of the post-DNA synthetic time and the DNA synthesis, respectively. If the ascending curve rises rapidly, there is little variability in the post-synthetic time; if slowly, cells take different times to proceed from the end of DNA synthesis into mitosis. Accordingly, if the descending curve is symmetrical with the ascending one, there is little or no variability in the time of DNA synthesis; if asymmetrical, cells take different times to complete the synthesis of DNA (30, 48). The rationale for this interpretation is that should all cells take the same time to proceed from the end of DNA synthesis to mitosis, the labeled cells would come to mitosis only when all the unlabeled ones have already gone through division, thus raising rapidly the frequency of labeled mitotic figures from 0 up to 100 per cent. The ascending line would be vertical. Accordingly, the difference between the slope of the downward curve and a vertical line is a direct measure of the combined variabilities of the post-DNA synthetic time and of the DNA synthesis time. If there is no variation in the length of DNA synthetic time, then the ascending and descending slopes would have the

same value and the two portions of the curve would appear symmetrical.

The results on the time course of the labeling of mitotic figures after administration of H^3-thymidine in our experiments are reported in Fig. 2. The data on metaphase and anaphase figures only have been used because the very short duration of these mitotic stages assures a more precise measurement of the various times of the division cycle. Therefore, in the terminology used throughout this paper, the post-DNA synthetic time is the interval between the end of DNA synthesis and metaphase, and, since DNA synthesis terminates just before the visible onset of prophase, this time corresponds essentially to the duration of this part of the mitotic cycle. Accordingly, the pre-DNA synthetic time includes the mitotic stages from metaphase through telophase and the portion of interphase preceding DNA synthesis.

On the supposition that the type A spermatogonia population might possibly be heterogeneous the experimental data were studied individually for each of the four generations of these cells. The distinction between these four cell generations is not based on morphological criteria, since type A spermatogonia are all identical to one another, but on the stage of the cycle of the seminiferous epithelium in which they are present. The testis indeed provides the unique opportunity to differentiate cell lines in a morphologically homogeneous population because of the synchrony between developmental sequences of the spermatogonia and spermiogenesis, as revealed by the development of the PAS-positive acrosomic system of the head of the spermatids. DNA synthesis in the AI generation takes place at tubule stage VIII and division at stage IX. AII generation is present at stages X and XI, DNA synthesis occurring at stage X and division at stage XI. AIII generation is present at stages XII, at which DNA synthesis

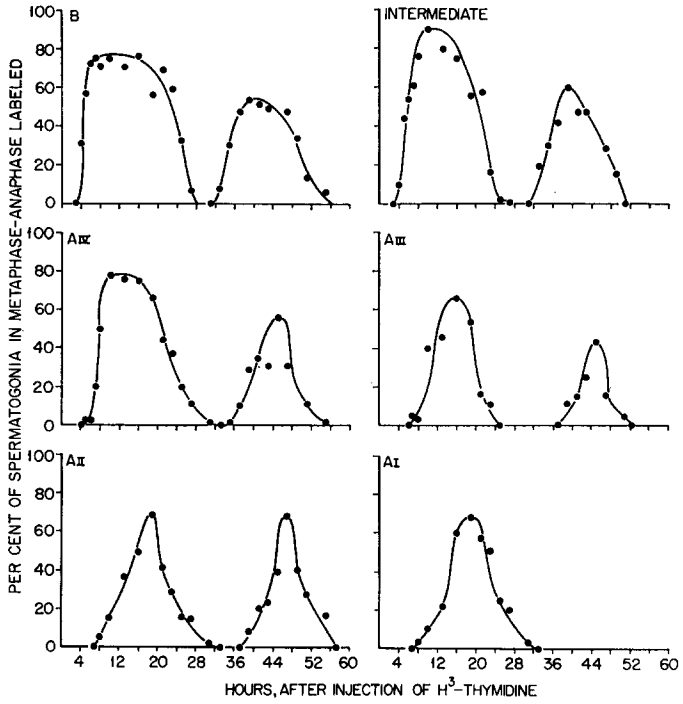

FIGURE 2

Percentage of spermatogonia in metaphase-anaphase labeled at various intervals after intraperitoneal injection of H^3-thymidine.

Each point is the average of the counts performed in three animals. The curves are drawn freehand. See Fig. 1 for abbreviations.

occurs, and I, at which division takes place. The AIV generation is present at stage II in the early and middle parts of which DNA synthesis occurs and, in the late part, division. The generation of intermediate spermatogonia is present in very late stage II and stage III, in which they synthesize their DNA, and in stage IV in which they divide. The generation of type B spermatogonia is present at stages V and VI. In stage V they synthesize DNA and in stage VI divide. Table III lists the frequency of labeled cells of the various generations of spermatogonia at the appropriate tubule stages at 1 hour after injection of H^3-thymidine. The value for the AI generation is missing because there is no way of knowing when the "dormant" period terminates and the interphase of the first type A division begins, since all type A spermatogonia are morphologically identical.

The interpretation of the time course of labeled mitotic figures after administration of H^3-thymidine and of the timing of the various fractions of the cellular cycle requires some explanation.

In experiments using tritiated thymidine *in vivo* and autoradiographs on tissue sections, the experimental conditions are somewhat different from those existing in experiments in tissue culture or with plant material. First, the label remains in the organism for the whole duration of the experiment and the theoretical possibility therefore exists of a continuous labeling of cells. Some evidence shows, however, that this is not the case because the label is completely cleared from the plasma a few minutes after intravenous injection and no further labeling takes place beyond 45 minutes after intravenous injection or beyond 1 hour after intraperitoneal injection. This observation suggests that the pool of labeled precursors for DNA synthesis in the cells becomes depleted at the same time (4, 13, 39, 45). Our data support this conclusion by showing that the percentage of labeled cells reaches a maximum at 1 hour after injection.

A second differential condition in comparison to tissue cultures arises from the thickness of the histological preparations. Owing to the short average range of the beta particles from tritium (less than 1 μ in biological tissues (7)), many labeled nuclei lying at a distance greater than 1 μ from the surface of the sections will not register, thus causing an underestimate of the rate of labeling. That this is the case is proved by the curves of labeled metaphase-anaphases showing that the peaks of the curves in no case reach 100 per cent value (Fig. 2). This fact should not, however, affect the calculation of the generation time because the location of the peaks should not be shifted by this factor. In the second cycle of labeled metaphase and anaphase the peaks are, as expected, even lower, because of the underestimation of labeled cells owing to the dilution of H^3-thymidine following cell division. The difference from 100 per cent of the values observed at the peaks of the curves of labeled metaphases plotted against time after injection is a rough measure of underestimate of labeled cells due to the existence of "falsely negative images." The average difference from 100 per cent, calculated on the total number of labeled metaphase-anaphases at the times of the peaks in the first cycle, is 26.3. This means that 26.3 per cent of actually labeled cells are missed. The true number of labeled cells is, therefore, 35.7 per cent higher than the number of cells scored as labeled. The correction becomes very important for the estimate of the duration of the DNA synthesis based on the frequency of labeled cells 1 hour after injection. Another correction has to be made in this respect for the duration of labeling. After intraperitoneal injection, about 15 minutes are required for the passage of the thymidine from the peritoneal cavity to the blood stream. That leaves 45 minutes for the cells that were in pre-DNA synthetic period at the time of injection to enter DNA synthesis and take up the H^3-thymidine. This time is about 2.5 per cent of the average life span of the spermatogonia (which is about 30 hours, see below). Therefore, 2.5 per cent of the total cell population enters DNA synthesis during the time of labeling and becomes labeled. The correction factor is 0.975. The true frequency of labeled cells at the true zero time from injection is, therefore, to be computed from the number of labeled cells scored at 1 hour after injection $\times$ 1.357 $\times$ 0.975. The corrected frequencies are given in Table III for each generation of spermatogonia. It should also be said that, since the percentage of labeled metaphase figures in no case reaches 100 per cent value in this system (Fig. 2), the average post-DNA synthetic time is to be read from the abscissa not at the point where the rising curve crosses the 50 per cent line on the ordinate, but at the point where a vertical line from the abscissa

crosses the half-way point on the rising curve (Fig. 2).

An additional correction has to be made. Since each cell type studied represents a single cell generation, the second curve of labeled metaphase-anaphase figures given for each cell type, therefore, is represented by cells labeled in the previous cell generation that go through division, develop to the next generation, and again enter mitosis. The time of the second peak of labeled mitotic figures, therefore, will be affected by a possible difference in the length of mitosis between one cell generation and the preceding one. A correction can, however, be made by analyzing for each cell generation the time of reappearance of the first labeled interphases of the next cell generation—after the labeling has moved through the various mitotic stages. This time corresponds to the interval between the end of DNA synthesis and the onset of the interphase of the next generation. A possible difference in the time of this interval between one cell generation and the preceding one is the correction factor for the proper estimate of the time of the second peak of labeled mitotic figures. Table I shows that the first labeled RPS's, after one labeled cycle of type B spermatogonia, reappear in stage VII between 7 and 10 hours. Table II shows that (*a*) the first labeled resting type B spermatogonia, after the labeled nuclei have moved through the mitotic stages of intermediate spermatogonia, reappear in stage V between 10 and 13 hours, (*b*) the first labeled resting intermediate spermatogonia reappear in stages II and III between 13 and 16 hours, and (*c*) the first labeled type A spermatogonia interphases reappear between 19 and 22 hours—at 19 hours AIV labeled interphases and at 22 hours AIII and AII labeled interphases. Therefore, the time of the second peak of labeled metaphase-anaphase figures should be reduced by about 3 hours for type B, intermediate, and AIII spermatogonia and by about 5 hours for AIV spermatogonia. A second cycle of labeled interphases is missing in AI spermatogonia in stage VIII as expected, since these cells are those initiating the spermatogonial cycle after the "dormant" period, in which no DNA synthesis occurs. Accordingly, a second peak of labeled metaphase-anaphases is missing in these cells. It is therefore not possible to compute the life span, and the durations of the DNA synthesis and of the pre-DNA synthetic time in AI generation.

TABLE III

*Frequency of Labeled Cells 1 Hour after Injection of H^3-Thymidine**

Stages of the cycle	Type of cells	No. of cells	No. of labeled cells	Percentage labeled cells		Percentage of spermatogonia in metaphase and anaphase
				Uncorrected	Corrected‡	
X + XI	AII	565	88	15.58 ± 1.53	20.53	3.36 ± 0.77
XII + I	AIII	1045	226	21.63 ± 1.27	28.61	2.01 ± 0.44
II	AIV	752	229	30.45 ± 1.68	40.29	3.59 ± 0.69
II + III + IV	In	1787	625	34.97 ± 1.13	46.28	3.97 ± 0.47
V + VI	B	3151	1160	36.81 ± 0.86	48.71	2.28 ± 0.27
VII + VIII	RPS + leptotene	7915	2039	25.76 ± 0.49	34.09	—

* Standard deviations are indicated. For abbreviations see the text.

‡ Corrected for underscoring of labeled cells (correction factor 1.357) and for the duration of the labeling (correction factor 0.975).

By using the methods and the reasoning outlined previously, the duration of the various parameters of the cell cycle was calculated for the various generations of spermatogonia from the data on Table III and Fig. 2. A statistical analysis of the rate of the slope of the ascending and descending portions of the curves showing the change of the frequency of labeled mitotic figures with respect to time from injection of H^3-thymidine (Fig. 2) was also made in order to have an estimate of the relative variability among cell types in the duration of the post-DNA synthetic time and of the DNA synthesis. The method used and the results obtained are shown in Table IV. The results are in agreement with the rough conclusion drawn by

the visual estimate of the slopes of the curves.

In type B spermatogonia, the average cell life span is 29 to 30 hours and is distributed as follows. DNA synthesis lasts an average of 14.5 hours with very high variability. A few cells take as long as 20 hours to synthesize their DNA (maximum DNA synthetic time). The average pre-DNA synthetic interval is about 10.5 hours. The average post-DNA synthetic time is about 4.5 hours with very little variability. The minimum post-DNA synthetic time is between 3 and 4 hours and the maximum between 6 and 7 hours.

The average life span of intermediate spermatogonia, is about 26 to 28 hours. The average DNA synthetic time is about 12.5 hours, with great variability but to a lesser extent than for type B spermatogonia. The average pre-DNA synthetic time is about 8.5 hours, and the average post-DNA synthetic time about 6 hours, with low variability. The minimum post-DNA synthetic time is between 3 and 4 hours, like in type B spermatogonia, and the maximum between 8 and 10 hours. The fact that some metaphase-anaphase figures are still labeled at 25 hours after injection of the H^3-thymidine (Fig. 2) indicates that a few intermediate spermatogonia take as long as 16 hours to complete DNA synthesis.

In the population of type A spermatogonia the average duration of the cell cycle is about the same for the various cell generations and not different from the duration of intermediate and type B spermatogonia generations. It is about 30 to 31 hours in AIV, 26 to 28 hours in AIII, and 28 to 30 hours in AII generation. Substantial differences exist, however, in the duration of the various parts of the cell cycle. The average DNA synthetic time is 12 hours with great variability in AIV, 7.5 hours in AIII and 6 hours in AII generation, with very low variability. The average post-DNA synthetic time is 8 hours with low variability in AIV, 11 hours in AIII and 14 hours in AII and AI generations, with very high variability. The minimum post-DNA synthetic time

TABLE IV

Variability of the Duration of the Post-DNA Synthetic Period and of DNA Synthesis in the Various Generations of Spermatogonia

Type of Spermatogonia	Rising curve			Descending curve		
	Hours	Slope	95% confidence interval	Hours	Slope	95% confidence interval
AI	7–19	4.2	(3.2; 5.2)	21–31	−3.8	(−2.0; −5.5)
AII	7–19	3.7	(2.8; 4.7)	21–31	−3.0	(−2.0; −3.9)
AIII	6–16	4.8	(2.4; 7.3)	19–25	−6.1	(−1.1; −11.1)
AIV	4–10	10.1	(5.7; 14.5)	19–33	−3.5	(−2.8; −4.1)
In	3–10	9.4	(6.2; 12.6)	13–27	−4.6	(−2.7; −6.4)
B	3–6	17.7	(16.3; 19.1)	19–31	−4.5	(−1.8; −7.1)

The numbers of labeled cells in each sample were assumed to be binomially distributed. The binomial proportions were transformed to angles by the inverse sine transformation, according to the formula θ = arc sine $\sqrt{p}$, where p is the observed proportion. Straight lines were fit through the transformed data by the method of least squares. The slopes of these lines represent the rate of change of the transformed proportions with respect to time since injection of H^3-thymidine.

The slope of the rising curve is very high in type B, lower and with the same value in intermediate and AIV, and much lower in AI, AII, and AIII spermatogonia. This pattern shows that the post-DNA synthetic time has very little variability in type B, higher variability in intermediate and AIV, and much higher variability in AI, AII, and AIII spermatogonia. The slope of the descending portion of the curves is the same as the slope of the ascending one in the first three generations of type A spermatogonia (AI, AII, and AIII), as shown also by the broad overlapping of the 95 per cent confidence intervals. Conversely, the slope of the descending curve is lower than the slope of the ascending curve in AIV and intermediate and mainly in type B spermatogonia; the confidence intervals of the two slopes do not overlap in AIV and in B spermatogonia and overlap barely in intermediate spermatogonia. This pattern shows that the duration of the DNA synthesis is very variable in type B spermatogonia, a little less variable in intermediate and AIV, and very constant in the first three generations of type A spermatogonia.

is 5 to 6 hours in AIV, 7 hours in AIII, and 8 hours in AII and AI generations. The maximum post-DNA synthetic time is between 9 and 10 hours in AIV, between 14 and 16 hours in AIII, and between 17 and 19 hours in AII and AI generations. The average pre-DNA synthetic time is 10.5 hours in AIV, 8.5 hours in AIII, and 9 hours in AII generations.

An approximation of the average duration of the DNA synthesis may also be given from the curves of the time course of the frequency of labeled metaphase-anaphase figures (Fig. 2) as the interval between the half-way points of the ascending and descending portions of the curves. This value must be corrected for the duration of the labeling, namely reduced by about 1 hour. In fact, owing to the cells that enter DNA synthesis and incorporate the label in the interval during which the H^3-thymidine is available to the cells—that is, about 60 minutes (4, 13, 39)—the descending curve is actually shifted by about 60 minutes toward the right. If the labeling were truly instantaneous, the curve should start dropping 1 hour earlier and reach the zero value 1 hour earlier. The estimates of the average duration of DNA synthesis obtained by means of this method are somewhat higher than those computed by multiplying the fraction of cells labeled at zero time after injection by the average cell life span, or 18 hours in type B, 14 hours in intermediate, 13 hours in AIV, 8 hours in AIII, and 7 to 7.5 hours in AII and AI generations.

Spermatocytes

The premeiotic synthesis of DNA, as shown by the cell-labeling shortly after injection of H^3-thymidine, occurs in resting primary spermatocytes at very late VII and early and middle VIII tubule stages and terminates just before the visible onset of the meiotic prophase, because as soon as 2 hours after injection many early leptotene nuclei are also labeled at late stage VIII. Soon after injection of H^3-thymidine, no labeling of nuclei is seen in later stages of spermatogenesis.

One hour after injection of H^3-thymidine, 11.76 per cent of RPS's are labeled at stage VII and the great part of them appear labeled at stage VIII. The figure of 41.31 per cent of labeled cells at stage VIII given in Table I is related to the total number of spermatocytes present at this stage, both labeled resting nuclei and unlabeled early leptotene nuclei. By combining the scoring on tubule stage VII with that on tubule stage VIII, the figure of 25.76 per cent of labeled cells is obtained. The frequency corrected for the underestimate of labeled cells and for the duration of the labeling is 34.09 (Table III). Since, according to the data of Oakberg (29), which were obtained from mice of the same strain and about the same age as those used in the present experiment, the duration of tubule stages VII plus VIII is 41.4 hours, the average DNA synthetic time in RPS's can be estimated by multiplying 41.4 by 0.34, or about 14 hours.

DISCUSSION

The estimate of the average duration of DNA synthesis obtained by multiplying the fraction of cells labeled shortly after administration of H^3-thymidine by the average generation time is based on the concept that this fraction is the measure of the fraction of the cell cycle during which DNA synthesis occurs. This concept is valid only if the cell population is homogeneous and asynchronous and all cells divide. The first two conditions are realized in the present material because single cell generations have been studied individually and because of the random scoring of the various stages of the seminiferous tubules. The third condition needs to be discussed in detail.

If all type A spermatogonia present at the beginning of the cycle of the seminiferous epithelium, at stage VIII, divide four times, the number of intermediate plus "dormant" type A spermatogonia at stage III, namely at the end of the divisions cycle of type A spermatogonia, would be expected to be 16 times as high as the number of "dormant" type A cells present at the beginning of the cycle. Correspondently, if each intermediate and type B spermatogonium divides once, the number of resting type B spermatogonia at stage V and the number of RPS's at stage VII of the cycle would be expected to be twice the number of resting intermediate spermatogonia present at stage III and the number of resting type B spermatogonia present at stage V, respectively. Table V reports the average number of cells per tubule cross-section found at the various stages of the cycle of the seminiferous epithelium. It can be seen that the average number of RPS's per tubule section scored at stage VII (80.00) is almost double the average number of resting type B spermatogonia scored at stage V (42.69)

TABLE V

*Number of Cells per 5 μ Cross-Section at Each Stage of the Cycle of Seminiferous Epithelium**

Stage of the cycle	No. of tubule cross-sections scored	Type of cells	Interphase	Early and middle prophase	Late prophase	Metaphase and anaphase	Telophase	Total cells	Mean no. of cells per tubule cross-section	95% confidence interval	% of spermatogonia in metaphase-anaphase	% of degenerated cells
I	349	AIII	509	1695	321	166	1330	4021	11.52	10.56; 12.48	4.13 ± 0.32	3.29 ± 0.28
II	279	AIV	638	2223	277	182	1029	4349	15.59	14.59; 16.59	4.18 ± 0.31	1.16 ± 0.16
		In	349	0	0	0	0	349	1.25	0.84; 1.66	0.00	
III	133	AD	336	0	0	0	0	336	2.53	2.29; 2.77	0.00	
		In	3045	32	0	0	0	3077	23.14	20.98; 25.30	0.00	0.68 ± 0.15
IV	288	AD	796	0	0	0	0	796	2.76	2.47; 3.05	0.00	
		In	623	3682	508	394	2678	7885	27.38	21.67; 33.09	5.00 ± 0.25	0.30 ± 0.06
V	165	AD	469	0	0	0	0	469	2.84	2.49; 3.20	0.00	
		B	7010	34	0	0	0	7044	42.69	39.13; 46.25	0.00	0.04 ± 0.02
VI	279	AD	789	0	0	0	0	789	2.83	2.53; 3.13	0.00	
		B	1458	7106	666	545	3432	13207	47.34	44.06; 50.62	4.13 ± 0.18	0.10 ± 0.03
VII	319	AD	1235	0	0	0	0	1235	3.87	3.44; 4.30	0.00	
		RPS	25521					25521	80.00	76.12; 83.88		
VIII	319	AI	1085	0	0	0	0	1085	3.40	3.13; 3.67	0.00	1.54 ± 0.37
IX	226	AI	385	328	114	40	240	1107	4.90	4.32; 5.48	3.61 ± 0.57	1.60 ± 0.38
X	186	AII	1088	52	2	1	31	1174	6.31	5.81; 6.81	0.09 ± 0.09	3.53 ± 0.53
XI	328	AII	940	839	147	75	402	2403	7.33	6.82; 7.84	3.12 ± 0.36	2.95 ± 0.35
XII	319	AIII	1378	1370	45	60	135	2988	9.37	8.85; 9.89	2.01 ± 0.26	3.71 ± 0.35
VIII + IX	545	AI	1470	328	114	40	240	2192			1.82 ± 0.29	1.57 ± 0.27
X + XI	514	AII	2028	891	149	76	433	3577			2.12 ± 0.24	3.14 ± 0.29
XII + I	668	AIII	1887	3065	366	226	1465	7009			3.22 ± 0.21	3.47 ± 0.22
II	279	AIV	638	2223	277	182	1029	4349			4.18 ± 0.31	1.16 ± 0.16
II + III + IV	700	In	4017	3714	508	394	2678	11311			3.48 ± 0.18	0.40 ± 0.06
V + VI	444	B	8468	7140	666	545	3432	20251			2.69 ± 0.12	0.08 ± 0.02

* Standard deviations are shown. For abbreviations, see the text.

and this last almost twice the number of resting intermediate spermatogonia scored at stage III (23.14). The observed 6 to 7 per cent deficiency from the expected twofold increase is accounted for by the underestimate of cells caused by crowding in the tubule. The conclusion then is that all intermediate and type B spermatogonia do divide once. The theoretical possibility that some spermatogonia do not divide and some divide more than once seems ruled out by the finding that (*a*) the duration of the stages in which cell division occurs, that is, stage IV for intermediate and stage VI for type B spermatogonia, as given by Oakberg (29), does not allow for more than one division per cell to occur and (*b*) in a given tubule all spermatogonia are in about the same mitotic stage. Conversely, the average number of intermediate plus "dormant" type A (AD) spermatogonia at stage III of the cycle is 25.67, which is only 8.20 times the average number of "dormant" type A spermatogonia at stages III through stage VIII (3.13), instead of the 16-fold increase expected on the basis of four divisions for each "dormant" type A cell.[2] However, as pointed out by Oakberg (28), a correction has to be made for cell degeneration occurring at appropriate tubule stages. The correction reduces the expected number from 50.08 to 41.71. The 38 per cent deficiency of the observed number of 25.67 with respect to the expected number of 41.71 could be accounted for by the concept of Clermont and Leblond (2), concerning the rat, that somewhere during the cycle a few type A spermatogonia fail to undergo the subsequent divisions, thus becoming "dormant" cells that stay undivided until the beginning of next cycle at stage IX when they act as stem cells by entering a new series of divisions leading to the formation of the cells of a new cycle[2]. A difference, however, is likely to exist in this connection between the rat and the mouse. According to Clermont and Leblond (2), the "dormant" type A cells arise in the rat before the last division of type A spermatogonia, by failure of one-fourth of the cells to divide a third time. Since the difference between the expected and the observed value is 38 per cent in the mouse, as compared to 11 per cent for the rat (calculated from (2)), it is likely that the "dormant" type A spermatogonia arise in the mouse much earlier in the cycle than in the rat. The hypothesis that these cells arise after the first division of type A spermatogonia by failure of one out of two cells to undergo the subsequent three divisions fits well the experimental data. On the basis of an average of 3.13 "dormant" type A cells per tubule cross-section at stages III to VIII, one arrives at an expected number of resting type A spermatogonia at stage X of 6.26, at stage XII of 9.39, at early stage II of 15.65, and of resting intermediate plus "dormant" type A spermatogonia at stage III of 28.17. The experimental data are in the order (see Table V): 6.31, 9.37, 13.74 (obtained from the total number of cells at stage II less half of the telophases[3]), 25.67. The small difference between expected and obtained numbers is accounted for by cell degeneration. Such hypothesis is diagrammatically summarized in Fig. 3.

Owing to the existence of a portion of cells escaping cell division in type A population, it seems that a more reliable estimate of the average duration of the DNA synthesis in these cells is that given from the curves of the time course of the percentage of labeled metaphase-anaphase figures (Fig. 2). The estimate is the distance on the abscissa between the half-way points on the ascending and descending portions of the curves, corrected for the duration of labeling. These estimates are indeed a little higher than those computed by multiplying the fraction of cells labeled at the time zero after injection of H^3-thymidine by the average cell life span. The new values are 13 hours for AIV, 8 hours for AIII, and 7 to 7.5 hours for AII and AI generations. The average pre-DNA synthetic times become correspondently shorter, or 9.5 hours for AIV, 8 hours for AIII, and 7.5 hours for AII generation.

Table VI is a summary of the results. It shows that the spermatogonial development from the stem cell to type B spermatogonia is accompanied by a change in the mitotic cycle that is characterized by a gradual lengthening of the duration

[2] The "dormant" period in the cycle of type A spermatogonia actually ends at stage VII, as shown by the high rate of incorporation of H^3-thymidine occurring at the subsequent stage VIII (see Fig. 1), but the increase in number of type A spermatogonia by mitosis begins only at stage IX, with the division of the first type A generation.

[3] The telophase figures were counted as two cells, the anaphase figures as a single cell. The interphases following the fourth cell division are intermediate spermatogonia and were scored as such.

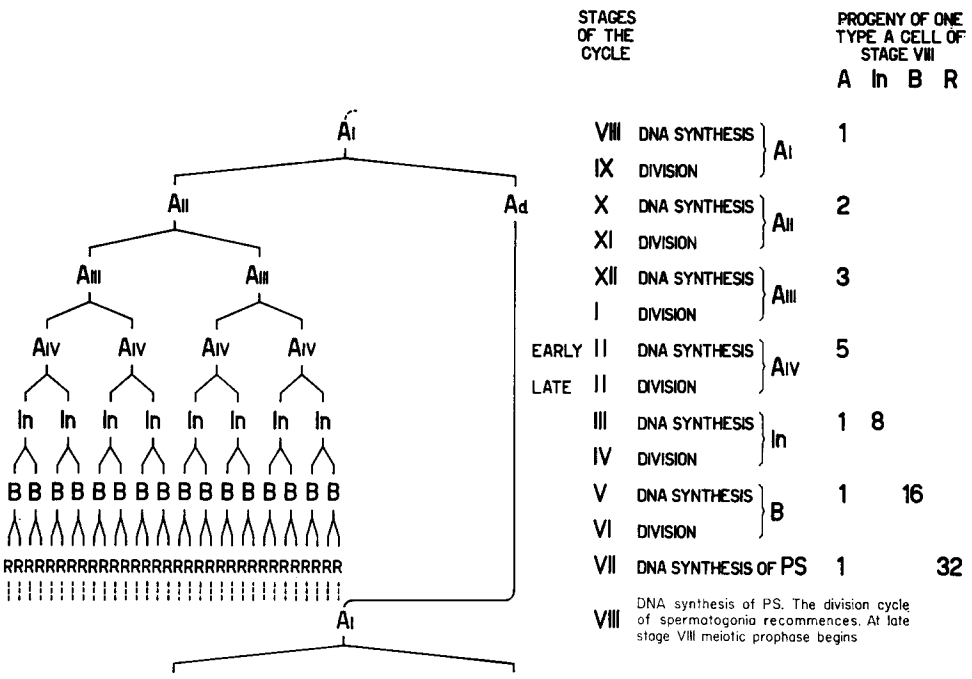

FIGURE 3

Diagrammatic representation of the proposed pattern for the renewal of spermatogonia in the mouse. The method of representation is taken from that used by Clermont and Leblond (2). Abbreviations: AI, AII, AIII, AIV, four successive generations of type A spermatogonia; Ad, "dormant" type *A* spermatogonia; *In*, intermediate type spermatogonia; *B*, type B spermatogonia; *R*, resting primary spermatocytes; *PS*, primary spermatocytes. DNA synthesis for each generation of spermatogonia occurs also at stages IX, XI, I, IV, and VI, but the peaks of the synthesis are localized at stages VIII, X, XII, II, III, and V, as indicated (see Fig. 1).

According to this hypothesis, the daughter cells resulting from the division of each type A cell of stage VIII are functionally different. One is a dividing cell (AII) which undergoes the second of a series of six divisions producing eventually 32 primary spermatocytes. The other daughter cell becomes a dormant type A spermatogonium, which does not divide until stage VIII of the next cycle and then initiates a new multiplicative cycle.

of the DNA synthesis and a parallel shortening of the duration of the post-DNA synthetic time, the duration of the total cellular cycle being constant. It is important to point out that in the type A population the greatest difference exists between the first three type A generations (AI, AII, and AIII) and the last one (AIV), whose daughter cells are intermediate spermatogonia. In the AIV generation the average duration of the DNA synthesis and of the post-DNA synthetic time is not very different from those of intermediate spermatogonia, and quite different from those of AIII, AII, and AI spermatogonia.

An attempt to elucidate the radiation response of mouse spermatogonia on the basis of the findings of the present research has been made (24, 25).

The pre-DNA synthetic period includes, as mentioned above, metaphase, anaphase, telophase, and the resting phase preceding DNA synthesis. The average durations of these stages of the mitotic cycle may be estimated in the following way. The percentage of labeled interphases at 30 minutes after injection of H^3-thymidine, corrected for the underestimate of the labeling and for the labeled cells entering prophase during this time, is a measure of the fraction of the interphase during which DNA synthesis takes place. The corrected frequencies are 0.84 for type B, 0.85 for intermediate, 0.59 for AIV, 0.38 for AIII, and

0.28 for AII spermatogonia. The average duration of the interphase may be estimated by multiplying these frequencies by the average duration of the DNA synthesis. By subtracting from the total duration of the interphase the duration of the DNA synthesis, one obtains the duration of the resting phase preceding the DNA synthesis. The average metaphase-anaphase duration may be estimated by multiplying the fraction of spermatogonia in metaphase-anaphase (Table V) by the average generation time. By subtracting the durations of the metaphase-anaphase and of the resting phase before DNA synthesis from the pre-DNA synthetic time, the average duration of telophase is estimated. The following estimates are obtained for intermediate and type B spermatogonia. In type B spermatogonia: interphase lasts an average of 17.3 hours, the last 14.5 hours being taken for DNA synthesis and the remaining 2.8 hours for the resting phase preceding DNA synthesis; metaphase-anaphase last about 0.8 hour; and telophase 6.9 hours. In intermediate spermatogonia: interphase lasts 14.7 hours, 85 per cent of which (12.5 hours) is taken for DNA synthesis and the first 15 per cent (2.2 hours) for the phase before DNA synthesis; metaphase-anaphase lasts also about 1 hour; and telophase 5 hours. The duration of these parts of the mitotic cycle cannot be reliably estimated in absolute terms in type A spermatogonia on the basis of the frequency of labeled interphases, because of the existence in type A population, throughout the cycle of the seminiferous epithelium, of "dormant" cells not undergoing DNA synthesis and mitosis. It is, however, certain that the duration of the resting phase preceding DNA synthesis is, at least in AIII and AII spermatogonia, much longer than in the other cell types.

The extreme brevity of the resting phase preceding DNA synthesis in type B and in intermediate spermatogonia accounts for the observation that shortly after injection of H^3-thymidine a portion of the telophases of intermediate and AIV spermatogonia are labeled (Table II). Evidently in these cells DNA synthesis starts as early as in the late telophase of the preceding cell generation. This idea is consistent with the observation that a few type B spermatogonia take

TABLE VI

*Timing of the Cellular Cycle and its Parts in Spermatogonia and Duration of DNA Synthesis in Primary Spermatocytes**

		AI	AII	AIII	AIV	In	B	RPS
Average life span (hours)		—	28–30	26–28	30–31	26–28	29–30	
Average DNA synthetic time‡ (hours)		—	6	7.5	12	12.5	14.5	14‖
Average DNA synthetic time§ (hours)		7–7.5	7.5	8	13	14	18	
Variability¶		Low	Low	Low	High	High	Very high	
Post-DNA synthetic** time (hours)	minimum	8	8	7	5–6	3–4	3–4	
	maximum	17–19	17–19	14–16	9–10	8–10	6–7	
	average	14	14	11	8	6	4.5	
Variability¶		High	High	High	Low	Low	Very low	
Average pre-DNA synthetic‡‡ time		—	7.5	8	9.5	8.5	10.5	

* For abbreviations, see the text.

‡ Computed by multiplying the corrected frequency of labeled cells at zero time after injection of H^3-thymidine (see Table III) by the average life span.

§ Estimated from the curves of the time course of the frequency of labeled mitotic figures (see Fig. 2) as the distance on the abscissa between the half-way points on the rising and descending portions of the curves.

‖ Estimated by multiplying the corrected frequency of labeled primary spermatocytes at stage VII and VIII of the cycle at zero time after injection of H^3-thymidine, 0.34 (see Table III), by the average duration of these stages, 41.4 hours as given by Oakberg (29).

¶ Judged by the slope of the ascending and descending portions of the curves of the time course of the frequency of labeled mitotic figures (see Fig. 2 and Table IV).

** From the end of DNA synthesis to metaphase.

‡‡ Including metaphase through telophase and the portion of interphase preceding DNA synthesis.

as long as 20 hours and a few intermediate spermatogonia as long as 16 hours to complete DNA synthesis.

One should take into consideration the possible existence of endogenous radiation effect by intranuclear tritium administered as H^3-thymidine (17, 35, 38), such as was shown to occur by a number of workers (6, 14, 15, 20, 27, 32, 47) and of an interference of the label in the kinetics of thymidine incorporation into DNA. Most of the observations are however consistent with the idea that no appreciable radiation effect by tritium on cell viability and cell physiology is detectable at total activity and time of exposure to the tritium radiation that are normally used in tracer experiments employing autoradiographic technique (13, 14, 21, 48). Specifically, Johnson and Cronkite (14) have shown that the biological radiation damage by doses of H^3-thymidine of about 0.5 μc/gm of body weight, which give excellent autoradiographs, should be of "little experimental concern." This conclusion is consistent with our data, which show that, after exposure of the mice to the tritium radiation from a dose of 0.62 to 0.71 μc/gm of body weight over a period up to 55 hours, the frequency of normal and necrotic cells is not different from that of the controls.

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