DURATION OF PREMEIOTIC DEOXYRIBONUCLEIC ACID SYNTHESIS AND THE STAGES OF PROPHASE I IN RABBIT OOCYTES

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ABSTRACT

To estimate the duration of oocyte DNA synthesis 36, 3-day-old female rabbits received 3, 6, 9, 12, 15, or 18 injections of tritiated thymidine (thy-^{3}H) at hourly intervals. The ovaries, removed at 1, 10, or 20 days after the first injection, were radioautographed. Counts made of the number of silver grains associated with oocyte nuclei in meiotic Prophase I indicate that the duration of DNA synthesis is between 9 and 12 hr. To determine the length of the stages of meiotic Prophase I, a group of 2–3-day-old rabbits was given a single subcutaneous injection of thy-^{3}H, and the ovaries were removed at hourly and/or daily intervals after treatment. The minimum duration of leptotene was 3 hr and the maximum duration probably was less than 8 hr. The maximum durations of zygotene, pachytene, and diplotene were estimated to be 44, 216, and 96 hr, respectively. The interval from the end of oogonial DNA synthesis to the beginning of premeiotic DNA synthesis (G_2 + Mitosis + G_1) appeared to be less than 6 hr.

INTRODUCTION

It has been shown that all oocytes of the rabbit are developed during the neonatal period (3). With the exception of the reports by Peters et al. (8), Teplitz and Ohno (11), and descriptive studies by von Winiwarter (12), the events preceding and including Prophase I of meiosis in female rabbits have not been studied extensively. Monesi (6) estimated that DNA synthesis in mouse primary spermatocytes continued for 14 hr, and Crone et al. (2) estimated that 10.5–12.0 hr was required for mouse oocytes. The age of the animal at which premeiotic DNA synthesis occurred was studied by Lima-de-Faria and Borum (5), but they did not attempt to estimate the duration of the interval.

The duration of the stages of Prophase I was estimated for mouse primary spermatocytes (6), and the over-all length of Prophase I was approximated in rabbit primary spermatocytes (10). The only comparable estimates for oocytes were limited to the leptotene and zygotene stages in the mouse, and were fixed at 3–6 and 12–40 hr, respectively (2). The same authors reported that the minimum duration of pachytene was 60 hr. The purpose of this investigation was to estimate the duration of (*a*) the premeiotic DNA synthesis interval and

(*b*) the stages of Prophase I of meiosis in the neonatal rabbit ovary.

MATERIALS AND METHODS

DNA Synthesis Phase

3-day-old, Dutch-Belted female rabbits were used in two experiments. In Experiment I, 18 rabbits were allocated to six groups and each group received 3, 6, 9, 12, 15, or 18 injections (sc) of 0.2 μc of thymidine-methyl-^{3}H (thy-^{3}H)[1] per gram body weight at hourly intervals. Each animal was one replicate of a 2 × 4 factorial arrangement of treatments with three animals in each group. These treatments consisted of (*a*) unilateral ovariectomy performed 1 or 10 days after the first injection of thy-^{3}H, and (*b*) replicated sections of ovaries prepared for radioautography and exposed for 2.5, 5.0, 10.0, and 20.0 days. The order of excision of left and right ovaries was random. In Experiment II, 18 rabbits were treated similarly in a 2 × 4 × 3 factorial arrangement, except that ovariectomy was accomplished 10 and 20 days after the first thy-^{3}H injection and the radioautographs (ARG's) exposed for 2.5 days were damaged and not tested.

All ovaries were fixed in acetic acid–absolute alcohol (1:3), embedded in paraffin, and 5 μ sections were cut and mounted on acid-cleaned glass slides. Four slides were prepared from each ovary. The slides were stained with the Feulgen technique, countersigned with Fast Green (0.1%), and air-dried. ARG's were prepared as previously described (3).

All grain counts were made at a magnification of ×970. The number of grains were counted in each of 20 randomly selected nuclei that fulfilled the following criteria: (*a*) the center of the nucleus was judged to be included within the section; (*b*) scored nuclei were all in the same stage of meiotic prophase I; (*c*) the meiotic prophase stage selected at any interval was the most advanced stage that was labeled; and (*d*) each nucleus had one or more silver grains associated with it. The labeled oocyte nuclei fulfilling the above criteria in the ovaries removed at 1, 10, and 20 days were in leptotene or zygotene, pachytene, and diplotene phases.

The nuclear areas of five oocytes in each ovary were estimated from two diameter measurements made at right angles to each other so that background corrections could be made. However, this was unnecessary as background counts were low and not significantly correlated (r = 0.26) with uncorrected counts.

Prophase I Stages

The durations of the stages of Prophase I of meiosis were studied in Experiments III and IV in which neonatal rabbits were subcutaneously injected with 1.0 μc of thy-^{3}H[2] per gram of body weight. Experiment III consisted of 14 2-day-old females and Experiment IV of 15 3-day-old females. In Experiment III, an ovary was removed from each female 1 hr after thy-^{3}H injection, and the remaining ovary was removed at 4, 8, 12, 16, 20, or 24 hr, or at 2, 3, 4, 6, 8, 10, 12, or 14 days after injection. In Experiment IV, two females each were sampled as follows: one ovary was removed at 2, 3, 4, 6, 8, 10, or 12 days, and the alternate ovary was removed 1 or 2 days after the first one. The ovaries from the 15th female were removed 14 and 16 days after injection. All ovaries were processed, and ARG's were prepared as described for the DNA synthesis phase.

Each ARG was examined, and the labeled nuclei in the most advanced stage of Prophase I were noted. The nuclear configurations characteristic of each of the Prophase I stages, first described by von Winiwarter (12), are shown in Figs. 1–6.

Sections from one ovary were excluded from the results because each histological section used to identify the most advanced labeled meiotic stage present, also, was required to include unlabeled stages more advanced in development. This minimized the probability of missing the most advanced meiotic stage in a labeled section.

RESULTS

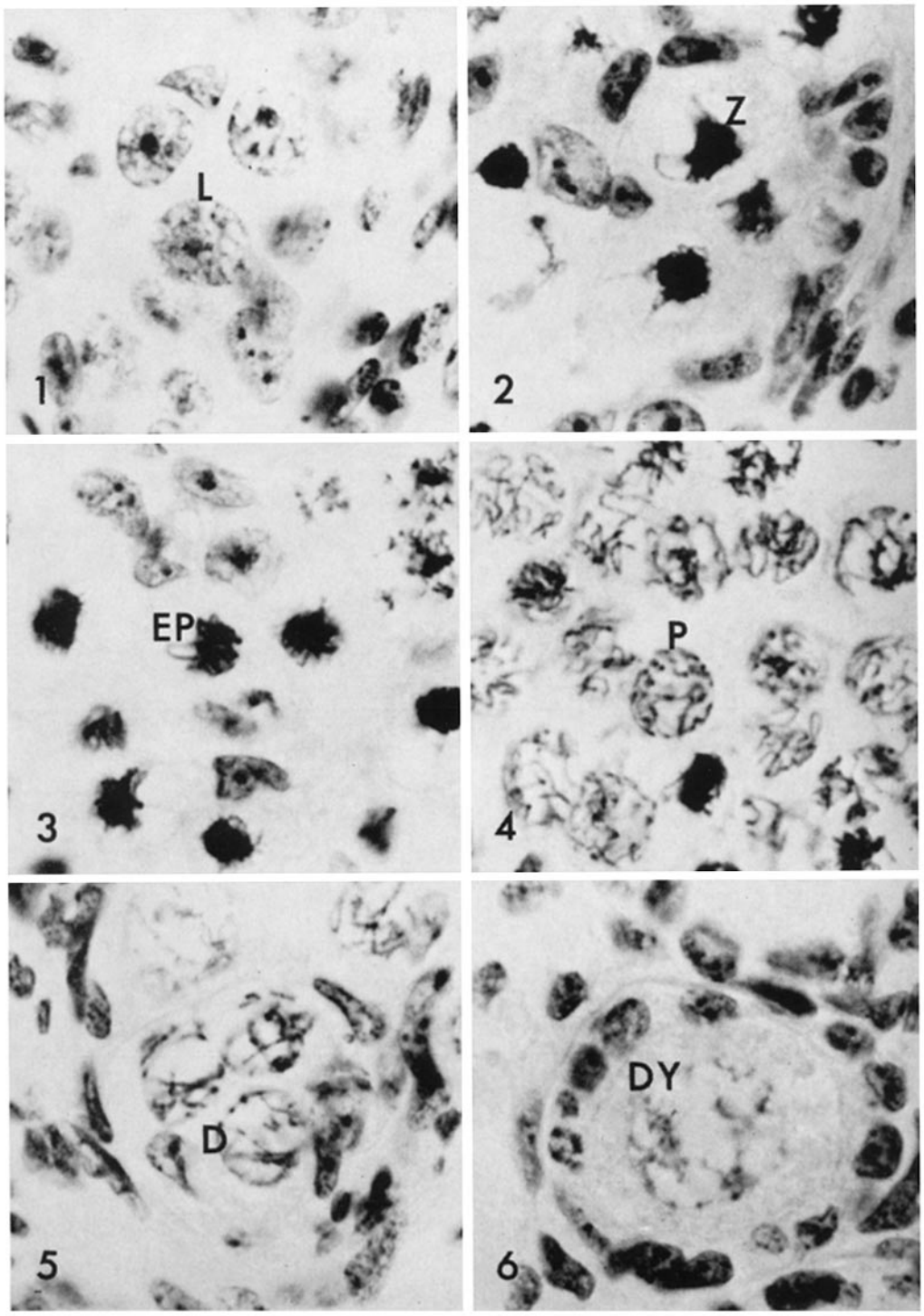
DNA Synthesis Phase

The mean responses for each replicate of the thy-^{3}H injection treatments in Experiment I are shown in Fig. 7. The means for the main effects for Experiments I and II are shown in Table I and selected statistical comparisons are shown in Table II. The grain counts increased linearly from 3 to 9 and from 12 to 18 injections (Fig. 7). The grain counts were consistently higher 1 day after the first thy-^{3}H injection than at 10 days. Also, increasing the exposure time of the ARG's caused a nearly linear increase in the grain count. The mean grain counts after 20 days were lower than at 10 days following thy-^{3}H injection. Since counts were highest after 1 day, the over-all means for Experiment I were higher than for Experiment II. The difference between the two experiments in average counts at 10 days is due primarily to the deletion of the 2.5-day exposure in the second experiment.

The percentage of labeled oocytes observed in 1- and 10-day ovaries for each injection interval in

[1] New England Nuclear Corp., Boston, Mass., sp. ac. = 10.6 curies per millimole.

[2] New England Nuclear Corp., sp. ac. = 6.7 curies per millimole.



FIGURES 1–6 Nuclear configurations of rabbit oocytes in the various stages of meiotic Prophase I. Fig. 1, leptotene (*L*); Fig. 2, zygotene (*Z*); Fig. 3, early pachytene (*EP*); Fig. 4, pachytene (*P*); Fig. 5, diplotene (*D*); and Fig. 6, dictyotene (*DY*).
All figures, × 1375.

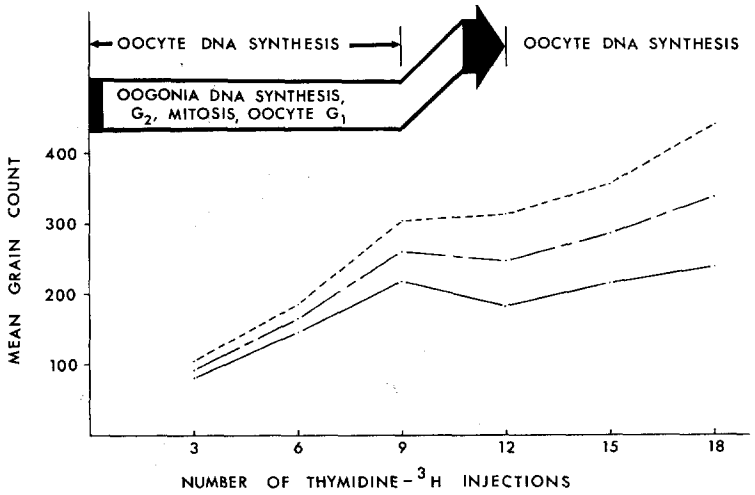


FIGURE 7 Mean nuclear grain count for each thy-^{3}H injection schedule in Experiment I. Each mean is the average of three animals totaled over four exposure times (n = 12). 1 day postinjection (- - - -), 10 days postinjection (·—·), and the mean of 1 and 10 days postinjection (· — - - — ·). Injections were given at hourly intervals, so the number of injections is equivalent to a time scale in hours. The upper diagram illustrates how some of the labeled oocytes may be derived from labeled oogonia following 12 or more injections.

TABLE I

*Mean Grain Counts for Treatments in Experiments I and II**

Experiment	Interval from injection to ovariectomy	No. of thy-^{3}H injections					
		3	6	9	12	15	18
		(Mean grain counts)					
I‡							
	1 day	104	186	305	312	357	441
	10 days	81	146	218	181	215	238
	p:‖	***	**	**	***	***	***
II§							
	10 days	92	132	243	203	273	291
	20 days	62	85	105	135	181	183
	p:‖	*	***	***	**	***	***

* All counts adjusted for background.

‡ Average of three animals totaled over four exposure times (n = 12); 20 nuclei per animal per exposure time.

§ Average of three animals totaled over three exposure times (n = 9); 20 nuclei per animal per exposure time.

‖ * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Experiment I is presented in Table III. The 1-day ovaries showed a marked increase in labeled oocytes after nine injections, whereas the 10-day ovaries showed a sharp increase up to nine injections with only a moderate increase thereafter.

Prophase I Stages

The onset and end of each of the stages in Prophase I in relation to elapsed time from the tritium injections is shown in Fig. 8. The estimated mini-

TABLE II

Orthogonal Comparisons of the Number of Thy-^{3}H Injections and the Statistical Significance of the Effects

Injection schedules compared	Exp I		Exp. II	
	Variance	F-ratio‡	Variance	F-ratio
3 vs. 9	23,982.0	142.84***	9559.6	57.47***
6 vs. mean of 3 & 9	4.3	0	31.1	0.18
12 vs. 18	1,577.3	9.39**	2330.3	14.01**
15 vs. mean of 12 & 18	18.8	0	481.0	2.89
Mean of 3, 6, 9 vs. mean of 12, 15, 18	21,919.2	130.55***	6652.3	39.99***
Error§	167.9		166.3	

‡ ** = $P < .01$.
*** = $P < .001$.
§ Within treatments, among rabbits; d.f. (degrees of freedom) = 12.

TABLE III

*Percentage of Labeled Oocytes**
(Experiment I)

No. of thy-^{3}H injections	Percentage of labeled oocytes	
	1-Day ovaries	10-Day ovaries
3	62.0	50.0
6	68.5	71.0
9	65.0	89.0
12	84.5	91.0
15	78.0	93.5
18	81.0	98.0

* Based on 20-day exposure of ARG's, 200 nuclei per injection, all nuclei with two or more grains scored as labeled.

mum and maximum durations of each stage are shown in Table IV. The maximum duration of leptotene could not be directly determined because these nuclei may have been in this stage for some time when the thy-^{3}H was given. However, it is apparently less than 8 hr. The estimated durations of zygotene, pachytene, and diplotene were 40, 192, and 48 hr, respectively.

DISCUSSION

DNA Synthesis Phase

The linear responses (Fig. 7) during 3–9 and 12–18 hourly injections separated by a plateau are interpreted basically as representing (*a*) oocytes which had incorporated increasing amounts of thy-^{3}H for 9 hr either directly as oocytes or else during the preceding oogonial DNA synthesis, (*b*) a plateau effect at 9–12 hr as the time of injections exceeded oocyte DNA synthesis time, and (*c*) the combined effect of thy-^{3}H incorporation during oocyte DNA synthesis and the preceding oogonial DNA synthesis. The lower mean oocyte grain counts after 10 days, as compared to 1 day after injection, reflect the greater opportunity for any labeled oogonia to become oocytes after the longer time interval.

Based upon these data, Fig. 9 has been constructed. Fig. 9 shows the oocyte DNA synthesis time (S_1) to be 9 hr. It may be slightly longer than this. After 15 hr, the combined effects of two DNA syntheses during the thy-^{3}H injections ($S_0 + S_1$) became evident; thus G_2 + mitosis + G_1 must be less than 6 hr and may be no more than 3 hr. Crone et al. (2) reported that labeled mouse oogonia appear in the oocyte population when the interval between thy-^{3}H injection and necropsy exceeds 6–8 hr. Cameron and Greulich (1) found that the duration of the entire progenitor cycle in mouse embryonic epithelial cells was only slightly longer than the time required for DNA synthesis.

Information is scant on the duration of G_2 and mitosis for mammalian oogonia and of G_1 and G_2 for mammalian oocytes. The G_2 interval of mouse type B spermatogonia, the cell type in the male comparable to the oogonia under question here, ranges between 3 and 7 hr with a mean of 4.5 hr (6). Crone et al. (2) estimated 30 min for the minimum duration of G_2 in mouse oocytes and thought that the total time was short. The results in part II of this study and the report by Crone et al. (2) suggest that the oocyte G_2 interval may be even less than 30 min. The stages in the cell

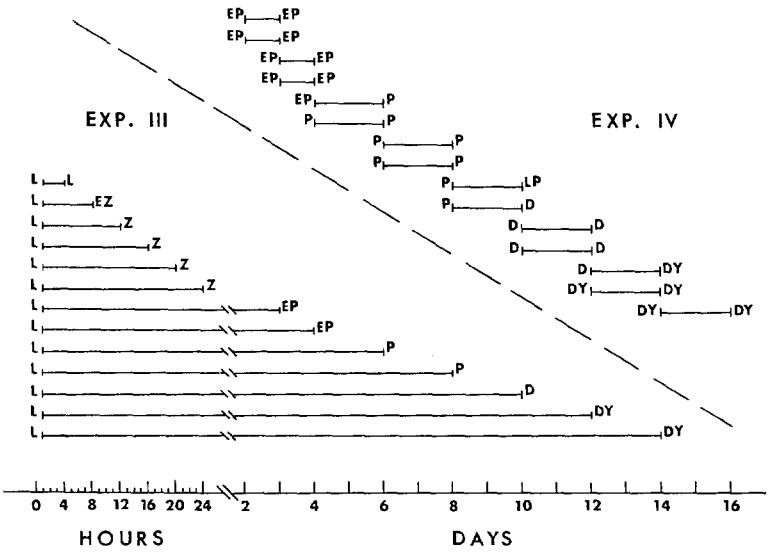


FIGURE 8 The duration of the different stages of meiotic Prophase I in each ovary sampled, as shown by the most advanced labeled meiotic nuclei. Experiment III animals are shown below the diagonal, and Experiment IV animals are above the diagonal. Each line represents one animal and shows (*a*) the time elapsed since thy-^{3}H injection (0 hr) and (*b*) the most advanced meiotic Prophase I stage labeled at that time (*L*, leptotene, *Z*, zygotene; *EP*, early pachytene; *P*, pachytene; *D*, diplotene; and *DY*, dictyotene.

TABLE IV

Duration of the Stages of Prophase I in Rabbit Oocytes

Prophase stage	Duration of stage		
	Minimum	Maximum	Estimated mean
	hr	*hr*	*hr*
Leptotene	3	8	—
Zygotene	16	44	40
Pachytene	144	216	192
Diplotene	48	96	48
Dictyotene	(Age of female minus 2 wk)		—

cycle under study, other than DNA synthesis time, were short.

Indirect evidence that the mean grain counts over oocyte nuclei were affected by the proportion of oocytes which derived some or all of their thy-^{3}H from labeled oogonia is shown in Table III. Following three injections, the percentage of labeled oocytes was reduced between 1 and 10 days, suggesting that oocytes derived from labeled oogonia will go undetected after halving the radioactive DNA at mitosis. The longer exposure of oogonia to thy-^{3}H following six or more injections resulted in the passage of more detectable quantities of radioactive DNA to oocytes after oogonial mitosis, hence the increased percentage of labeled oocytes between 1 and 10 days. Further, the effect of labeled oogonia on the oocyte population following 12–15 injections was minimal in the 1-day ovaries because there was less opportunity for such oogonia to develop into oocytes. The opportunity was greater for the 10-day ovaries, and this resulted in a higher percentage of labeled oocytes.

The decrease in mean grain counts 10 and 20 days postinjection (Table I) is due, in part, to the increasing proportion of oocytes that have formed from cells labeled as oogonia. By 20 days, all oogonia have developed into oocytes (8). In addition, the oocytes that were synthesizing DNA at the time of thy-^{3}H injection are older and they degenerate at a higher rate than younger ones (3, 4), tending to leave a residual population of cells with less thy-^{3}H.

Although grain counts were usually low enough over oocyte nuclei so that coincidence was not considered a serious problem, an effect was observed in some groups with ARG's exposed for 20 days. The mean counts for all groups in Experiment I after 5, 10, and 20 days of exposure,

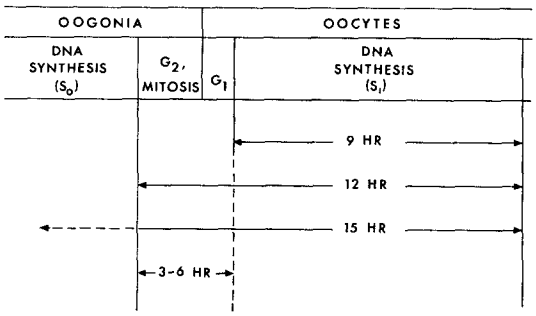


FIGURE 9 A proposed scheme for (*a*) the duration of rabbit oocyte DNA synthesis, and (*b*) the interval between oogonial and oocyte DNA syntheses.

expressed as a percentage of extrapolated counts based on the 2.5-day interval, were 90, 77, and 68, respectively. Except for the group receiving 18 injections, coincidence also increased with an increase in number of thy-^{3}H injections.

Prophase I Stages

The following discussion is based on data illustrated in Fig. 8 and estimates summarized in Table IV. The times are based on the assumption (9) that thy-^{3}H is incorporated into DNA within 1 hr.

LEPTOTENE: The minimum duration of leptotene is fixed at 3 hr and the maximum is probably less than 8 hr. One female whose ovary was removed 4 hr after injection showed no cells labeled beyond leptotene, while another animal's second ovary removed at 8 hr showed labeled nuclei in the early zygotene stage.

ZYGOTENE: The minimum duration of zygotene is estimated to be 16 hr, since zygotene nuclei were the most advanced labeled stage in all ovaries removed between 8 and 24 hr. The maximum duration is probably 44 hr. This estimate is based on the labeled early pachytene nuclei observed in the ovaries of animals in Experiment IV 48 hr after injection, which was 44 hr after the most advanced labeled nuclei in an Experiment III animal were still in leptotene. This agrees quite closely with the 12–40 hr estimate for this stage in mouse oocytes (2).

PACHYTENE: Pachytene nuclei were observed 2–8 days after injection (Experiment IV, Fig. 8). Late pachytene nuclei were labeled in one of four ovaries examined at 10 days, but the labeled oocytes in the three other ovaries at this interval already had developed to the diplotene stage. Thus, minimum duration is equal to 144 hr (6 days). The maximum interval extends from zygotene last observed 24 hr after thy-^{3}H injection to 10 days, or 216 hr. The duration of this stage in mouse primary spermatocytes was estimated to be 175.3 hr (7). The approximate duration of pachytene in rabbit primary spermatocytes was calculated from Swierstra's data (10) to be 9 days, or 216 hr.

DIPLOTENE: Three of four ovaries were in diplotene at 10 and 12 days, which suggests that the minimum interval of 48 hr is reasonable. The maximum duration could be as long as 96 hr. This differs from the 17.6 and 21.4 hr reported as the average duration for mouse primary spermatocytes (2, 8).

DICTYOTENE: Only the onset of dictyotene was recorded, since this condition persists in oocytes until just before ovulation (3). Dictyotene was first recorded 12 days after thy-^{3}H injection, when the rabbits were 2 wk of age, and so onset could be computed in the rabbit as 2 wk less than the age of the female.

Of all the stages of Prophase I of meiosis for rabbit oocytes, leptotene is probably the shortest. The other stages, in order of increasing length, are zygotene, diplotene, pachytene, and dictyotene. Dictyotene first appears when rabbits are 2 wk of age, and some oocytes persist in this condition throughout adult life.

The majority of rabbit oogonia enter Prophase I of meiosis between birth and 10 days of age (8). Multiple injections of thy-^{3}H in 3-day-old rabbits labeled up to 98% of the oocytes. Previous studies (3) have shown that a high proportion of oocytes can be labeled permanently by thy-^{3}H injected shortly after birth.

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