ple F dissolved when soaked for 24 hours in distilled water. It is interesting that little of the inorganic component of sample F leached out with the soluble fraction. The insoluble residues of both samples were hydrolyzed with sulfuric acid. In both cases roughly one-third of the hydrolyzed samples dissolved in water; the other two-thirds remained insoluble. By the method of Mendel *et al.* (*5*), the fraction soluble after hydrolysis was shown to be almost all carbohydrate.

Although there are some obvious differences in the organic composition of the two samples, especially in the water-soluble fractions, these differences cannot account for all of the differences in combustion properties. In particular, the enhanced glowing combustion of sample F with decreased flaming vulnerability is attributable to the differences in ash content of the two samples.

The direct effect of water in influencing the combustion of vegetation has been recognized for many years. However, live green grass and grass freshly moistened after being allowed to die *in situ* exhibit strikingly different combustion behavior. When exposed to an open flame, a green blade of grass will shrivel, char, and ablate away without igniting; moistened dead grass will burn readily after a brief interval, during which the moisture is driven off. Thus, the difference in combustion properties must be due to something in addition to the difference in moisture content between the living and dead vegetation. Studies of range management have shown that prolonged drought, which so greatly increases the hazards of wildland fires, also influences the composition of forage by decreasing the inorganic constituents of the vegetation, particularly the phosphorous content (*2*). If it can be established that the prevalence of wildland fires during droughts is significantly influenced by the inorganic content as well as the moisture content of the vegetation, methods that would increase the ash content of vegetation could greatly expand the alternatives available to those charged with the responsibilities of fire control.

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DNA Synthesis in Alveolar Cells of the Mammary Gland: Acceleration by Ovarian Hormones

Abstract. *In normal alveolar cells of the mammary gland of C3H/HeJ female mice, DNA synthesis lasts an average of 20.7 hours with a coefficient of variation of 26.6 percent. Treatment of the mice with 1 microgram of 17-β-estradiol and 1 milligram of progesterone per day for 3 to 4 days decreases the average duration to 10.7 hours and the coefficient of variation to 13.8 percent.*

Using a P^{32}-labeled precursor of DNA and radioautography at cellular level, Howard and Pelc (*1*) showed that DNA is synthesized during a discrete part of interphase, the period between two cell divisions. The portion of interphase during which a cell doubles its DNA is called the S phase and is equated to the phase during which cells take up the labeled phosphorus into DNA. The process of DNA synthesis is preceded by a period referred to as G_1 and followed by a G_2 phase. Mitosis (M) follows G_2. These four successive phases form the typical generative or proliferative cycle of cells (*2*).

Progress in the analysis of DNA synthesis in recent years has been based largely on the discovery that thymidine is a highly specific precursor of DNA (*3*) and on the introduction of labeled thymidine in radioautographic studies (*4*). The duration of the process of DNA synthesis can be measured with reasonable ease and accuracy by at least two methods (*5, 6*). This has been done with a number of mammalian cells, and tabulation of data from different sources is available in the literature (*7*). Thus far, duration of DNA synthesis in most of the cell systems investigated appears to vary between 5 and 10 hours, with most of the values clustered around 7 to 8 hours. In contrast, generation time —that is, the duration of the entire generative cycle—can vary widely, from 12 hours to several days (*8*). This suggests that the duration of DNA synthesis in different cell types of a given animal species is fairly constant. As the length of the G_2 phase and of mitosis appear also to be rather constant, it has been further suggested that the whole sequence, DNA synthesis-G_2-mitosis, which is often referred to as the "doubling sequence," is approximately of constant duration and that differences in generation time and thus proliferation rate, both within and between cell populations, depends upon variation of the G_1 phase (*8, 9*).

The above concept is debated by Bullough (*10*), especially on the basis of results which demonstrate that there are noticeable variations in the duration of mitosis in the epidermis of the mouse. The range of variation so far discovered by Bullough and Laurence (*11*) is from 1.5 to 5.3 hours in the epidermis in vitro and in vivo. We found that the duration of mitosis showed diurnal fluctuations and depended upon the adrenalin concentration in the cells. The test of the "constancy" hypothesis of duration of DNA synthesis and in general of the "doubling sequence" was also the subject of a paper by Sherman *et al.* (*12*) on the cell population kinetics of the ear epidermis of the mouse; the cells of this tissue are known to have an extremely long generation time—about 24 days. In these cells the length of DNA synthesis averages about 30 hours, and this appears to disprove the tested hypothesis. As the same authors point out, however, the ear epidermis is under conditions of abnormally low temperature in comparison to most of the tissues of the body. Therefore, the long duration of DNA synthesis can reasonably be interpreted as an aspecific temperature effect on chemical reactions. Reported herein are the results of a more stringent test carried out with an internal

cell system, the mammary gland, whose cell proliferation is known to be under strict hormonal control. If hormones, in this case ovarian hormones, produce their effect on the proliferation rate of this gland only by means of affecting the number of cells in the generative cycle or duration of the G_1 phase (or both), while the "doubling sequence" is constant, then the duration of DNA synthesis should not vary with varying hormonal conditions.

Virgin female C3H/HeJ mice about 6 to 12 months old were used. The duration of DNA synthesis was measured in intact animals and in mice which were ovariectomized and treated with 1 μg of 17-β-estradiol and 1 mg of progesterone per day for 3 to 4 days. The hormones were dissolved in sesame oil and were injected subcutaneously. The duration of DNA synthesis was measured with the double labeling method as described by Wimber and Quastler (*6*).

The mice were injected intraperitoneally with 100 μc of thymidine-H^3; 72 or 90 minutes later they were injected with 50 μc of thymidine-C^{14}. Thirty minutes after the second injection they were killed. Preliminary studies showed that, as in the epithelia of the intestinal tract and mammary tumors (*13*), an intraperitoneal injection of radioactive thymidine produces a radioactive pulse of short duration (30 minutes) in the mammary gland. In every experiment the mice were killed by cervical dislocation, and the integument with the five pairs of mammary glands attached was fixed in cold acidic-alcohol (1 part glacial acetic acid and 3 parts ethanol). The glands were removed from the skin and stained in bulk by the Feulgen method. Alveoli were dissected with fine needles under a stereo microscope and "squashed" between a slide and a cover slip. The squash technique and the radioautographic procedure with a double layer of photographic emulsion are reported elsewhere (*14*). An example of radioautography of a doubly labeled squash preparation of alveoli is shown in Fig. 1.

It should be pointed out that in preliminary experiments not reported here for the sake of brevity, sham ovariectomy and injection of sesame oil did not affect the duration of DNA synthesis in mammary cells; and that ovariectomy produced inhibition of DNA synthesis, which was virtually complete about 3 days after the operation. In the six successive experiments reported here, normal alveolar cells from a total of 19 female mice were studied. Six mice were about 6 months old and intact; another 6 mice about 12 months old were bearing spontaneous mammary tumors and were also studied intact; seven mice were about 6 months old, and were ovariectomized and treated with hormones for 3 to 4 days before the measurements were carried out. The results are shown in Table 1 and allow the following conclusions: (i) No significant difference in duration of DNA synthesis appears to exist between normal alveolar cells from intact mice without tumors and alveolar cells from apparently normal parts of glands in mice with mammary tumors; (ii) in intact mice the average duration of DNA synthesis (20.7 hours) is significantly longer than that of most of the other mammalian cells so far studied; (iii) the variation in the duration of DNA synthesis between animals is remarkable, and a spectrum of values from 12.5 hours to 30.7 hours exists; (iv) the treatment of ovariectomized animals with large doses of hormones drastically reduces both the average duration of DNA synthesis and its variance.

The results show that ovarian hormones accelerate the process of DNA synthesis in mammary cells. At this time a definite mechanism for the hormone effect cannot be given, but two reasonable hypotheses can be presented. (i) The hormones increase the overall rate of the pathway of DNA biosynthesis; this, in turn, could be accomplished in different ways, for example, by some rate-limiting reaction being influenced, or by specific concentrations of substrates or cofactors being affected. (ii) The hormones influence the synchrony of chromosome duplication. Recent research has shown that chromosomes do not duplicate synchronously

Table 1. Duration of DNA synthesis in cells of normal alveoli of the mammary gland of C3H/HeJ mice (intact and injected with ovarian hormones) as measured by the double-labeling method. By this method a cell population is labeled with thymidine-H^3 and then labeled again, after a short period of time, with thymidine-C^{14}. The nuclei of the cells synthesizing DNA at the time of the first injection become labeled with thymidine-H^3. During the interval between the two injections (t_a) a portion of the H^3-labeled cells pass out of the synthetic phase and become labeled with H^3 only. If no labeled cells have divided at the time the mice are killed, then the simple proportion holds: $H/C = t_a/T_s$ where H indicates H^3-labeled cells; C, the cells labeled with C^{14} alone or with both C^{14} and H^3; and T_s, the duration of DNA synthesis. Strictly, this equation holds for a cell population in the steady state only. Since the duration of DNA synthesis is measured over a very short time period, however, the equation can also be used without the introduction of large error in situations where the size of the population varies. It was computed that even in the case of exponential growth, for a t_a of 1.5 hours and a generation time of 30 hours, the error in applying the equation as such for the calculation of T_s is only about 4 percent. In the final calculation of T_s the finite time of pulse-labeling, 0.5 hour, was subtracted.

Expt. No.	Animal No.	*C*	*H*	*C/H*	t_a (hr)	Duration of DNA synthesis (hr) $T_s = \left(\frac{C}{H} \times t_a\right) - 0.5$	Average duration of DNA synthesis ($\bar{x}$) and estimators of variation
				*Intact mice**			
101	1	956	94	10.1	1.5	14.8	
	2	1244	100	12.4	1.5	18.1	
	3	1865	100	18.7	1.5	27.6	$\bar{x} = 20.7$ hours
101	A1	1395	86	16.2	1.2	19.0	sample variance:
	A2	1148	74	15.6	1.2	18.2	$(S^2) = \Sigma x^2/n - 1 = 30.3$ hours
	A3	1286	66	19.5	1.2	22.9	
105	1	2084	100	20.8	1.5	30.7	sample standard deviation:
	2	1221	100	12.2	1.5	17.8	$S = 5.5$ hours
	3	2482	194	12.8	1.5	18.7	coefficient of variation:
114	A1	3378	248	13.6	1.5	19.9	$C = S/\bar{x} = 26.6\%$
	A2	2000	230	8.7	1.5	12.5	
	A3	4964	264	18.8	1.5	27.7	
				Hormone-injected mice†			
102	1	1001	149	6.7	1.5	9.5	$\bar{x} = 10.7$ hours
	2	746	100	7.5	1.5	10.8	sample variance:
	3	1248	150	8.3	1.5	11.9	$S^2 = \Sigma x^2/(n-1) = 2.2$ hours
	4	1102	149	7.4	1.5	10.6	
102	A1	665	102	6.5	1.4	8.6	sample standard deviation:
	A2	950	98	9.7	1.4	13.1	$S = 1.48$ hours
	A3	905	116	7.8	1.4	10.4	coefficient of variation:
							$C = S/\bar{x} = 13.8\%$

* Experiments 101 and 102 were carried out with tumor-free C3H/HeJ female mice aged about 6 months; experiments 105 and 114, with C3H/HeJ females aged about 12 months and bearing spontaneous mammary tumors (in these instances alveoli were dissected from apparently normal parts of the glands). † Three to four days after bilateral ovariectomy the mice were injected subcutaneously with 1 μg of 17-β-estradiol plus 1 mg of progesterone in 0.05 ml of sesame oil for 3 to 4 days before measurement of duration of DNA synthesis was carried out.

during the DNA synthetic phase of the generative cycle, but that a certain spacing in time exists (*15*). The duration of the synthesis of a single chromosome probably corresponds to about one-third or one-half of the total duration of duplication of the genome of the cell (*16*). An increased synchrony would, therefore, result in a reduction of the total time a cell spends in synthesizing DNA.

The ovarian hormones clearly influence the duration of the process of DNA synthesis. Therefore, the simplest interpretation of the variation in the length of this process in mammary epithelium of different intact animals taken at random in the experiment is that this is a consequence of differences in the endocrine titer among mice due to the variable rhythm of the ovarian secretion. As shown in Table 1, the variation in the duration of DNA synthesis is decreased in ovariectomized animals under steady hormonal treatment with 17-β-estradiol and progesterone. This result supports the previous hypothesis.

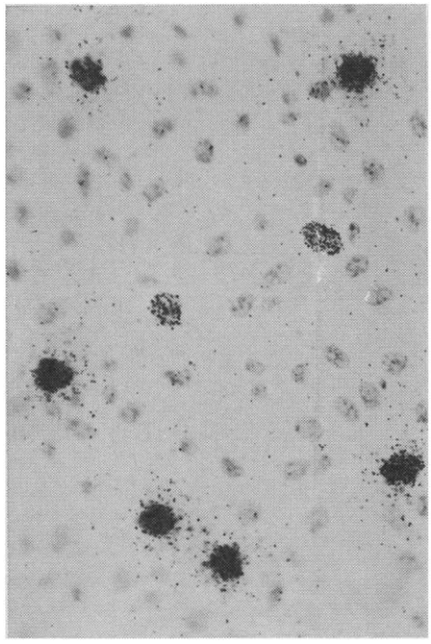

Fig. 1. Radioautography of a "squash" preparation of mammary alveolar cells from a C3H/HeJ female mouse injected first with thymidine-H^3 and then with thymidine-C^{14}. The nuclei containing C^{14} show a spray of β-tracks on several planes (mean energy of C^{14}-β-rays = 49 kev; mean distance traveled, 50 μ), while the nuclei containing only H^3 show photographic grains in their immediate vicinity and on one plane only (mean energy H^3-β-rays = 5.7 kev; mean distance traveled, 1 μ) and thus can be differentiated. Magnification, × 400.

Within the system of the mammary gland, the process of DNA synthesis has proved to be extremely variable in duration. Ovarian hormones are able not only to initiate the process of cell division, but also to accelerate at least a step of this process—that is, the duplication of DNA. The hypothesis of "constancy" of duration of DNA synthesis and of the "doubling sequence" in general, with variation in generation time dependent entirely upon variation of the G_1 phase, appears therefore to be undoubtedly disproved.

The fact remains that most of the cell systems so far studied have a duration of DNA synthesis close to a low of 7 to 8 hours. One might postulate that in these cell systems the duration of DNA synthesis is close to, or has reached, a "minimum" of fairly constant value for different cell types in a given species. This is suggested by the observations that, as shown in Table 1, steady treatment of animals with large doses of hormones decreases variation and clusters the values of duration of DNA synthesis around the low average time of 10.7 hours and that the lowest values of duration of DNA synthesis in cells of the mammary gland are close to those most commonly found in other cell systems of the mouse. A "minimum" duration of DNA synthesis is expected on a theoretical basis. As the quantity of DNA to be synthesized appears to be essentially the same in different cell types of the same species, duplication of DNA at maximum speed should essentially last the same "minimum" time. A certain variance in the duration of this process among different cell types could be expected as a result of difference in synchrony of chromosome duplication. On the other hand, influences or lack of influences on cell metabolism, as for example by hormones, can possibly lengthen the process of DNA duplication without any theoretical expected "maximum." The hypothesis of "minimum" duration appears to give a satisfactory interpretation of all facts known up to now. That is, many cell systems appear to have virtually the same duration of DNA synthesis, while this process is variable in length and can last significantly longer in other cell types in the same animal species.

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Antibody Synthesizing Cells: Appearance after Secondary Antigenic Stimulation in vitro

Abstract. *Suspensions of spleen cells from rabbits immunized with sheep erythrocytes can be stimulated to produce antibody-synthesizing cells in vitro. The cellular response is antigen specific.*

Spleen and lymph node suspensions and slices (*1*) taken from animals that had received in vivo a secondary stimulation by antigen have been shown to synthesize antibody in vitro. After secondary antigenic stimulation in vitro lymph node and spleen fragments synthesize antibody (*1, 2*), and antibody-containing cells appear in lymph node fragments (*3*). We report here on the