IS THE DURATION OF DNA SYNTHESIS IN SOMATIC CELLS OF MAMMALS AND BIRDS A CONSTANT?

I. L. CAMERON. From the Department of Anatomy, University of California at Los Angeles, California. Dr. Cameron's present address is the Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

The duration and position of the DNA synthetic period (S) in relation to the growth duplication cycle of the cell have now been reported for many cell types. These studies were made possible by the introduction of tritiated thymidine, a labeled specific precursor of DNA, in combination with autoradiographic techniques for detecting radioactive nuclei.

Studies on many mammalian somatic cell types have revealed an S period of relatively constant duration, lasting in most cases from 6 to 8 hours. This rather constant S duration occurs in mammalian cells growing either *in vivo* or *in vitro* and is apparently independent of the cell generation time (5, 9, 16), the species of mammalian cell (1, 3, 5), the number of chromosomes (3, 5), and possibly the ploidy condition of somatic cell type (3, 13).

In this investigation, the duration of the S period was measured in cell populations from embryonic mouse tissues and in several cell types of newly hatched chicken tissues to determine whether the constant S duration holds both (*a*) for conditions of extremely rapid cellular proliferation and (*b*) for the cells of homeothermic organisms other than mammals, *e.g.*, birds.

In the literature covering mammalian cells, a few reports appear of significant deviations from the 6- to 8-hour S period. In the Discussion, an attempt is made to account for these apparent deviations from the 6- to 8-hour interval.

METHODS

Tissues from Newly Hatched Chickens

Twenty-seven cockerels, 2.5 days old and weighing between 38 and 42 gm, were used in this experiment. The animals were maintained at a temperature of 27°C under constant illumination. Each animal was allowed to feed for 18 hours prior to intraperitoneal injection of H^3-thymidine (20 μc in 0.2 ml physiological saline, specific activity 3.0 c/mM). Injections were made at 7:00 A.M. and the animals were killed at intervals from 0.5 to 75 hours after injection. These birds registered cloacal temperatures of about 40.5°C.

Fetal Mouse Tissue

Gravid uteri were removed from a series of 10 pregnant mice. These animals were sacrificed on the 12th day of pregnancy at intervals ranging from 1 to 24 hours after intraperitoneal injection of 10 μc H^3-thymidine in 0.2 ml of physiological saline per animal (specific activity 0.36 c/mM). The pregnancy was timed from the recognition of vaginal plugs. Another series of uteri, treated in the same manner, were taken from 10 8-day pregnant mice. These were sacrificed at intervals from 0.5 hour to 24 hours after injection of thymidine. All mice used in this procedure were maintained at constant temperature (26°C) and under standardized conditions of lighting (13 hours light, 11 hours dark).

Representative portions of tissues and organs were taken from all the animals and fixed immediately in Hollande's modification of Bouin's fluid, embedded in paraffin, and sectioned at 5 or 6 microns. Tissue sections were mounted on slides and autoradiographed according to the dipping method

described by Messier and Leblond (10) using NTB-2 liquid emulsion (Kodak). Preparations were stained either by the Feulgen technique prior to autoradiography or with Harris's hematoxylin and eosin-B after development. Exposure times ranged from 10 days to 3 weeks at 4°C.

RESULTS

Fig. 1 shows the results from an autoradiographic analysis of two newly hatched chicken tissues. The percentage of labeled metaphase figures is plotted against the time between H^3-thymidine administration and sacrifice of the animal. These curves were constructed for all of the other cell populations studied, and Fig. 1 shows representative examples. The time intervals between 50 per cent metaphase labeling on the ascending and descending slopes of these curves were measured and recorded. Table I lists these time intervals for cell types of different chicken tissues. Table II presents similar data from cell types of the embryonic mouse tissue. This time interval is an estimate of the mean duration of DNA synthesis in these cells. The justification for this estimation procedure is well documented (1, 15). As seen from Tables I and II, the mean values for S range from 5 to 6 hours in the chicken tissues and 6 to 8 hours in the embryonic mouse tissues. Table II also records the percentage of nuclei labeled shortly after H^3-thymidine injection. The last column of Table II lists an estimate of the cell generation time based on the percentage of labeled interphase nuclei according to the calculations discussed below.

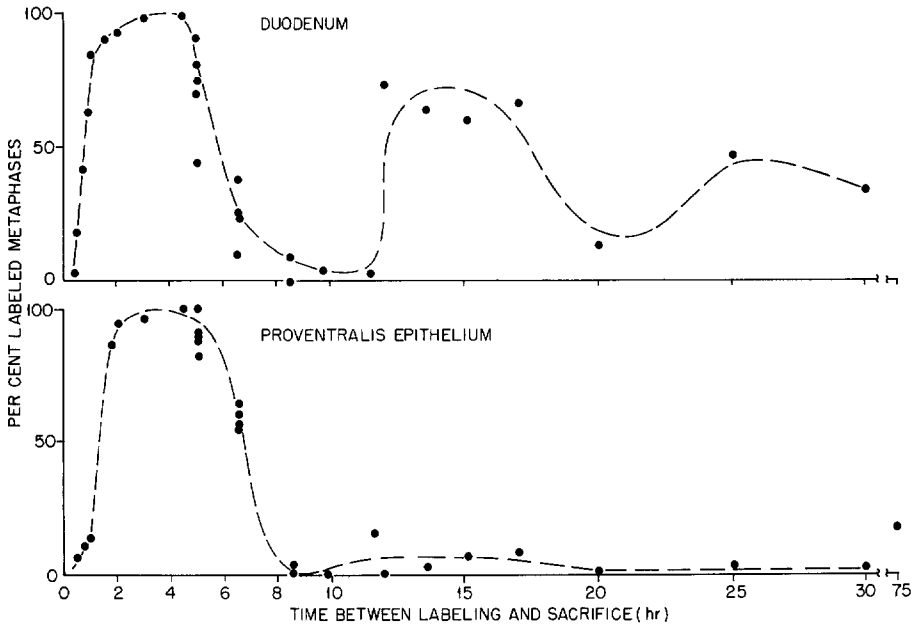

FIGURE 1 Curves showing the percentage of labeled metaphase figures in the duodenal epithelia (above) and proventralis epithelia (below) of chicken, plotted against time between H^3-thymidine injection and sacrifice. Each symbol represents a single animal.

DISCUSSION

Estimation of the mean S period shows that the 6- to 8-hour S value holds for rapidly proliferating placental cell populations (Table I). The chicken cell populations also demonstrate a relatively constant but shorter S period (5 to 6 hours, Table II).

Recently Pilgrim and Maurer (14) have developed a new and different method for measuring S. This new method involves the use of a double labeling procedure. Their method also indicates that the somatic cells from mice and rat tissues have a 6- to 8-hour S period.

The reader is referred to several recent reports that summarize much of the literature on the

6- to 8-hour S period in mammalian cells (1, 3, 5, 13, 14, 16). These references, however, are not to be considered comprehensive, as the number of reports on S periods is rapidly increasing.

Values differing from the 6- to 8-hour S period have been reported, and two of these cases are discussed here: A 30-hour S period has been reported in the epidermal cells of the mouse ear (16). Since the ear temperature is generally less than the animal's core temperature, this variation is most likely a temperature-dependent modification of the normal 6- to 8-hour S period. Mikulicich and Young (11) find a 10.5-hour S period in the lens epithelium of neonatal rat; they also relate this extended S to a lower temperature

TABLE I

Mean Duration of DNA Synthesis in Cells from Newly Hatched Chick Tissue

Type of epithelial tissue	Mean DNA synthetic duration
	hrs.
Proventralis	6.0
Duodenum*	5.0
Proventralis gland	5.0
Liver parenchyma	5.5
Pancreas acini	5.0

* 46 per cent of cells labeled. Calculated growth-duplication cycle duration: 11 hours.

in the anterior chamber of the eye. Recently in our laboratory (Cameron and Cleffmann, submitted to *J. Cell Biol.*) we have measured the duration of S in chickens maintained under experimental circumstances that result in a body temperature of 37°C, 3.5° below the normal. The S is prolonged to 6.9 to 7 hours under these conditions, comparable to the duration of the S period observed at the normal 37°C temperature of mammals. Three independent investigations have demonstrated an extended S period for mammalian spermatogonia (2, 7, 12). As the gonial cells do not fit the 6- to 8-hour S value, a generalized conclusion regarding the constancy of S can relate only to somatic cells under constant temperatures.

Although the evidence for a rather constant S period is not overwhelmingly conclusive, certainly enough data are available to entertain such a concept at this time. Intimations as to the constancy of the S period in mammalian somatic cells have appeared in the literature (1, 4, 9, 16). Variations from this constancy can apparently be explained by temperature considerations, but other variations cannot be eliminated.

Many cell populations of the body are constantly turning over, such that cell loss is balanced by cell birth. This type of cell system is said to be in a steady state or dynamic equilibrium. If, for cell populations in a steady state, one can assume the constancy of S, then it is only necessary to know the "DNA synthetic index" (defined as the percentage of germinal cells labeled shortly after injection of H^3-thymidine before any labeled nuclei have had a chance to divide (1, 6)) in

TABLE II

Mean Duration of DNA Synthesis and Generation Time in Cells from Embryonic Mouse Tissue

Type of tissue	Mean DNA synthetic duration	DNA synthetic index	Estimated generation time
	hrs.		*hrs.*
Yolk sac epithelium 12th day placenta	7.1		
Trophoblast cells 12th day placenta	6.5	43	15
Trophoblast cone cells 8th day placenta	7.0	75	9.4

order to calculate average cell generation times as well as tissue renewal times. Thus, under steady state conditions, average generation time (T) may be computed by T = (mean S duration)/(*DNA* synthetic index) × 100 (15). The generation times listed in Table II were calculated by this method. This formula does not strictly hold for cell populations in a state of continuous growth with a net increase in cell number (*cf.* 8) (*e.g.*, the trophoblastic cells of the embryonic mouse and the cells of the newly hatched chicken and the fetal mouse). In these cases, growth of specialized cell populations is proceeding, while the relative size of the germinal population is shrinking, and the rate of mitosis is becoming progressively slower with time. Consequently, in such populations it is difficult to calculate accurately the duration of the growth-duplication cycle by the proportion method, and the calculations presented in Table II must be considered an

approximation and referable only to the particular fetal age studied.

In mouse somatic cells at 37°C, S is 6 to 8 hours; in chicken somatic cells at the normal body temperature 40.5°C, S is 5 to 6 hours. The data presented in this report support the concept that the S period is of relatively constant duration in somatic cells of mammals, and the data also indicate that somatic cells of birds may have a relatively constant S period of shorter duration.

The skilled technical assistance of Mrs. Zoja Trirogoff is gratefully acknowledged. I also wish to thank Dr. Richard Greulich for his helpful criticisms throughout this study.

This study was supported by Research Grant D-635 to Dr. R. C. Greulich from the National Institutes of Health, Public Health Service.

Received for publication, May 22, 1963.

REFERENCES

1. CAMERON, I. L., and GREULICH, R. C., *J. Cell Biol.*, 1963, **18,** 31.
2. CLERMONT, Y., LEBLOND, C. P., and MESSIER, B., *Arch. anat. mikr. et morphol. exp.*, 1959, **48,** 37.
3. DEFENDI, V., and MANSON, L. A., *Path. et Biol.*, 1961, **9,** 525.
4. DEFENDI, V., and MANSON, L. A., *Proc. Am. Assn. Cancer Research*, 1961, **3,** 218.
5. DEFENDI, V., and MANSON, L. A., *Nature*, 1963, **198,** 359.
6. EDWARDS, J. L., and KLEIN, R. E., *Am. J. Path.*, 1961, **38,** 437.
7. HILSCHER, W., and MAURER, W., *Naturwissensch.*, 1962, **49,** 352.
8. JOHNSON, H. A., *Cytologia*, 1961, **26,** 32.
9. KOBURG, E., and MAURER, W., *Biochim. et Biophysica Acta*, 1962, **61,** 229.
10. MESSIER, B., and LEBLOND, C. P., *Proc. Soc. Exp. Biol. and Med.*, 1957, **96,** 7.
11. MIKULICICH, A. G., AND YOUNG, R. W., Submitted to *Invest. Ophth.*
12. MONESI, V., *J. Cell Biol.*, 1962, **14,** 1.
13. OEHLERT, W., SEESMAYER, N., and LAUF, P., *Beitr. path. Anat.*, 1962, **127,** 63.
14. PILGRIM, C., and MAURER, W., *Naturwissensch.*, 1962, **49,** 544.
15. QUASTLER, H., and SHERMAN, F. G., *Exp. Cell Research*, 1959, **17,** 420.
16. QUASTLER, H., CARLOUGH, M. T., GOOLNICK, B. G., and WOLFSBERG, M. F., *Rad. Research*, 1962, **16,** 561.
17. SHERMAN, F. G., and QUASTLER, H., and WIMBER, D. R., *Exp. Cell Research*, 1961, **25,** 114.