

# DNA REPLICATION PATTERNS AND CHROMOSOMAL PROTEIN SYNTHESIS IN OPOSSUM LYMPHOCYTES IN VITRO

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## ABSTRACT

DNA replication patterns were determined in the autosomes and sex chromosomes of phytohemagglutinin-stimulated lymphocytes from the opossum (*Didelphis virginiana*) by employing thymidine-<sup>3</sup>H labeling and high-resolution radioautography. Opossum chromosomes are desirable experimental material due to their large size, low number ( $2n = 22$ ), and morphologically distinct sex chromosomes. The autosomes in both sexes began DNA synthesis synchronously and terminated replication asynchronously. One female X chromosome synthesized DNA throughout most of the S phase. Its homologue, however, began replication approximately 3.5 hr later. The two X's terminated DNA synthesis synchronously, slightly later than the autosomes. This form of late replication, in which one X chromosome begins DNA synthesis later than its homologue but completes replication at the same time as its homologue, is apparently unique in the opossum. The male X synthesized DNA throughout S while the Y chromosome exhibited late-replicating characteristics. The two sex chromosomes completed synthesis synchronously, slightly later than the autosomes. Grain counts were performed on all chromosomes to analyze trends in labeling intensity at hourly intervals of S. By analyzing the percent of labeled mitotic figures on radioautographs at various intervals after introduction of arginine-<sup>3</sup>H, chromosomal protein synthesis was found not to be restricted to any portion of interphase but to increase throughout S and into G<sub>2</sub>.

## INTRODUCTION

The use of thymidine-<sup>3</sup>H to study DNA replication patterns in human chromosomes was first discussed by Taylor (28). Using the pulse-labeling technique and colchicine on cultures from human peripheral blood, German (4) and Lima-de-Faria et al. (13) demonstrated that the termination of DNA synthesis was asynchronous among individual chromosomes, that is, certain chromosomes, notably the sex chromosomes, finished replication later than others. Painter (23) observed that DNA replication began almost simul-

taneously in the chromosomes of HeLa S<sub>3</sub> cultures, but that cessation of synthesis was asynchronous, varying as much as 2 hr among different chromosomes of the complement. This was confirmed in part by Stubblefield and Mueller (25) who believed that DNA synthesis in HeLa cell chromosomes was nonrandom and suggested that the reproducible patterns of early and late labeling were due to the association of DNA with different chromosomal constituents.

Particular attention has been paid to one late-

labeling chromosome in cultures of human female somatic diploid cells, categorized in the 6-X-12 group of the complement, and generally accepted as one of the two X chromosomes (5-9, 11, 12, 19, 20). Considerable evidence now exists to support the concept that late-labeling chromosomes represent the heterochromatic chromosomes of interphase nuclei (14, 24). Lyon (16) suggested that the heterochromatic sex chromatin body of female mammalian, somatic interphase nuclei represented an X chromosome which was genetically inactive. It is now generally accepted that the sex-chromatin body, late-labeling X chromosome, and genetically inactive X chromosome are one and the same structure. The following generalizations may be made concerning DNA synthetic patterns in chromosomes of humans and various laboratory animals: (a) most, but not necessarily all, of the autosomes begin DNA replication either simultaneously or within a narrow time limit; (b) the sex chromosomes begin synthesis later than the autosomes; (c) the autosomes finish DNA synthesis asynchronously; (d) the sex chromosomes, one female X and the Y, are usually the last to complete replication.

The opossum was chosen for this investigation because its chromosomes, being large in size and few in number, are ideally suited for a radioautographic analysis of DNA synthesis (see karyograms, Fig. 25). Emphasis was placed on the sex chromosomes since they are morphologically distinct in all hypotonic spread preparations, a condition found in few laboratory animals. A brief discussion of the temporal relationship of chromosomal protein synthesis to the cell cycle of opossum lymphocytes will be presented.

#### METHODS AND MATERIALS

Lymphocytes were obtained from thoracic duct lymph and cardiac blood from male and female opossums. Short-term cultures containing phytohemagglutinin (Difco PHA-P, Difco Laboratories, Detroit, Mich.) were prepared by employing modifications of the technique of Moorhead et al. (18). With standard techniques (15, 22) generation time studies demonstrated an average S phase of 9 hr and an average  $G_2$  of 2 hr.<sup>1</sup>

Termination of DNA synthesis was studied by utilizing continuous labeling with 0.5  $\mu\text{c}/\text{ml}$  of thymidine-<sup>3</sup>H (specific activity 6.05 c/mmole, New England Nuclear Corp., Boston, Mass.).

<sup>1</sup> Schneider, L. K. Unpublished data.

Samples taken 2-8 hr after introduction of isotope were harvested as hypotonic spread preparations. Colchicine was added at a concentration of 0.1  $\mu\text{g}/\text{ml}$  of culture during the final 2 hr of incubation. 65 metaphase plates from five males and 47 metaphase plates from three females were analyzed. To determine whether thymidine-<sup>3</sup>H continued to be available to the lymphocytes over an extended period of continuous labeling, the lymphocytes were centrifuged and discarded from a culture which had been incubated with the isotope for 7.5 hr.  $\frac{1}{10}$  ml ( $10 \times 10^6$  cells) of DNA-synthesizing ascites tumor (A/jax mouse Sarcoma I) was added to 4 ml of culture supernatant fluid and incubated at 37°C for 1 hr. These tumor cells were analyzed for thymidine-<sup>3</sup>H uptake by radioautography (see Results).

The beginning of DNA synthesis was investigated in cultures partially synchronized by addition of 5-fluorodeoxyuridine (FUdR, courtesy of Hoffman-La Roche, Inc., Nutley, N. J.) at a concentration of 0.1  $\mu\text{g}/\text{ml}$  of culture (10). Cultures were pulse-labeled with thymidine-<sup>3</sup>H (5  $\mu\text{c}/\text{ml}$  for 5 min) followed by incubation in 120 times excess nonradioactive thymidine. Samples were harvested at selected intervals as hypotonic spreads. Colchicine was added during the final 2 hr of incubation. 68 metaphase plates from 2 males and 30 metaphase plates from one female were analyzed.

Chromosome spreads were prepared utilizing a technique adapted from Evans (2). This method employed acetic acid to eliminate background cytoplasmic matrix, a procedure which Swift (26) observed was not responsible for removal of histones from *Drosophila* chromosomes. Karyograms were prepared separating the autosomes into sets of three pairs of large submetacentric chromosomes, three pairs of small submetacentrics and four pairs of telocentric chromosomes.

Chromosomal protein synthesis was investigated by pulse-labeling cultures of opossum lymphocytes with 5  $\mu\text{c}/\text{ml}$  of arginine-<sup>3</sup>H (specific activity 143 mc/mmole, Volk Radiochemical Co., Burbank, Calif.) for 30 min. The cells were then placed in fresh culture medium containing hundredfold excess of nonradioactive arginine. The cultures were

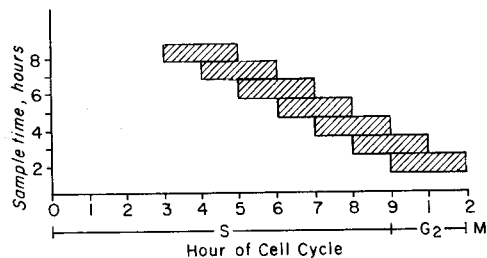


FIGURE 1 Sample intervals for end of S phase

harvested as smears at selected intervals after introduction of isotope.

Slides containing cells labeled with thymidine-<sup>3</sup>H were dipped in liquid Kodak NTB 2 emulsion and exposed for 1 and 3 days. Slides with arginine-<sup>3</sup>H-labeled cells were coated with Kodak NTB 3 emulsion and exposed for 2 wk. All slides were stained with MacNeal's tetrachrome stain (17).

### Data Analysis

DNA synthetic patterns were determined by scoring well-spread metaphase plates on slides from cultures harvested at chosen intervals after addition of thymidine-<sup>3</sup>H. The chromosome complements were photographed with a Leitz camera on Kodak M-plates (4 × 5 inches) at × 1500. The selected

metaphase plates were printed at an enlargement factor of 1.5–2.5 times, and karyograms were prepared. Labeling patterns were investigated by determining the average number of chromosomes in each set which labeled at a given interval, by observing the position of grains over the chromosomes, and by counting grains to determine labeling intensity. Intensity was categorized as light (1–4 gr), moderate (5–9 gr), or heavy (10 gr or more) per chromosome. Background in all preparations was low, averaging approximately 0.1 gr per 100 μ<sup>2</sup>. Since the average area per chromosome ranged from 10 to 20 μ<sup>2</sup>, a single grain over a chromosome represented a degree of labeling 50–100 times above background.

Arginine was chosen to study chromosomal pro-

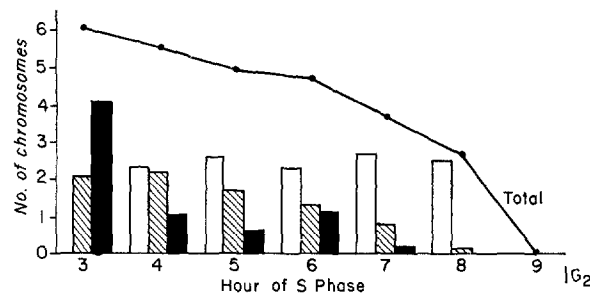


FIGURE 2 Termination of DNA synthesis in large subtelocentric chromosomes. In this graph and similar ones to follow, the line represents the mean total number of chromosomes labeled at a given interval of the S phase. Labeling intensity is indicated by the bars: solid bars denote heavy labeling; cross-hatched bars, moderate labeling; and clear bars, light labeling (see text).

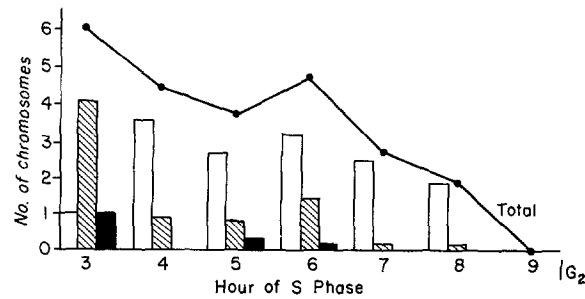


FIGURE 3 Termination of DNA synthesis in small subtelocentric chromosomes.

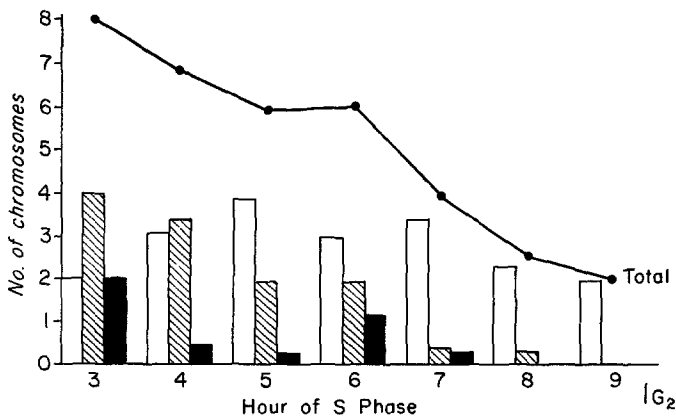


FIGURE 4 Termination of DNA synthesis in telocentric chromosomes.

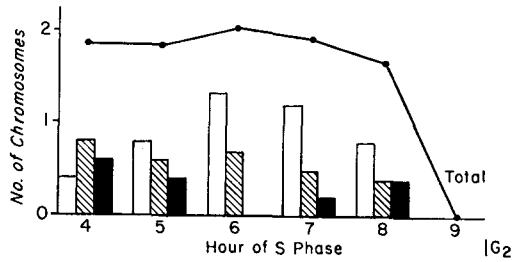


FIGURE 5 Termination of DNA synthesis in female X chromosomes.

tein synthesis because it is a prominent amino acid in histones. Cultures were analyzed by determining the percentage of mitotic figures which were labeled at specific intervals after the addition of arginine-<sup>3</sup>H (see Results). Aside from the isotope used, this

method is the same as that used for determining generation time (15, 22).

## RESULTS

### Termination of DNA Synthesis

Since colchicine was available during the terminal 2 hr of incubation in all cultures, any given interval of G<sub>2</sub> or S represented a 2 hr period. For example, if a culture was sacrificed 4 hr from the end of S, the mitotic figures in it represented the period from 2 to 4 hr from the end of S. Cultures used for the investigation of the terminal phases of DNA synthesis were harvested 2, 3, 4, 5, 6, 7, and 8 hr after initiation of continuous labeling (Fig. 1).

Fig. 2-7 summarize the total labeling (line

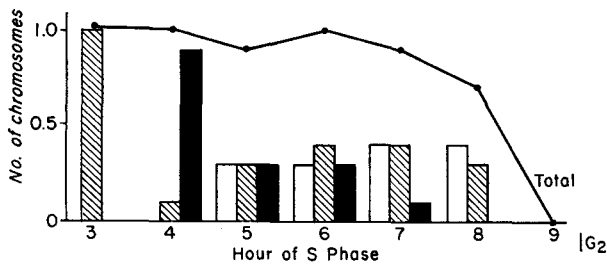


FIGURE 6 Termination of DNA synthesis in male X chromosome.

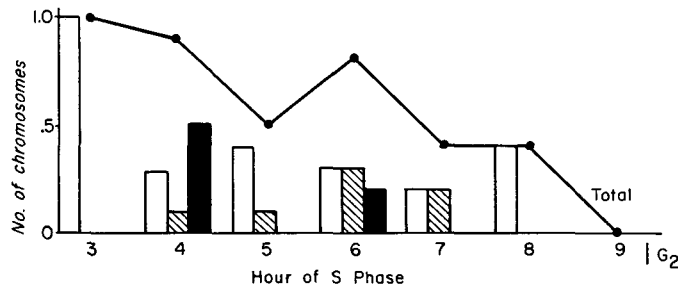


FIGURE 7 Termination of DNA synthesis in Y chromosome.

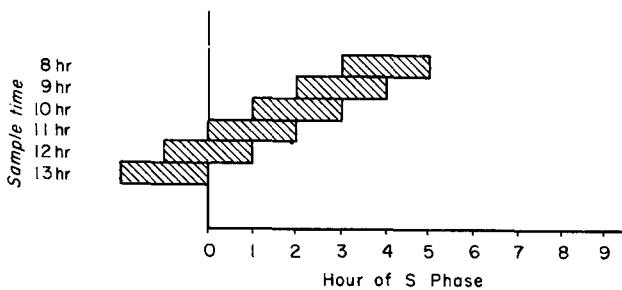


FIGURE 8 Sample intervals for beginning of S phase.

graphs) and the grain count data (bar graphs) for each set of autosomes plus the female X chromosomes, the male X chromosome, and the Y chromosome during the 3rd–the 9th hr of S. The ordinate on each graph represents the number of chromosomes for each set, and the abscissa represents the intervals of the S phase at which labeling of the chromosomes was analyzed. Clear bars represent light labeling, cross-hatched bars indicate moderate labeling, and solid bars represent heavy labeling.

When continuous labeling is employed, any chromosome which is unlabeled when the sample is taken has already completed DNA replication. In Fig. 2, which shows labeling in the large subtelocentric chromosomes, a sample taken 4 hr after introduction of thymidine-<sup>3</sup>H represents the 7th hr of the S phase since the average G<sub>2</sub> is 2 hr and S equals 9 hr. This graph demonstrates an average of 3.6 chromosomes labeled at the 7th hr of S. Since thymidine-<sup>3</sup>H was available for 4 hr, any chromosomes that did not label at this interval must have completed DNA replication prior to the 7th hr of S. Because 3.6 chromosomes labeled during the 7th hr of S, an average of 2.4

of the large subtelocentric chromosomes had completed replication at that time.

Fig. 2 also indicates that the labeling intensity in the large subtelocentric chromosomes proceeded from predominantly moderate and heavy labeling during the earlier stages of S to predominantly light labeling near the end of S. These data indicate that the six chromosomes terminated DNA replication gradually and regularly, and that labeling in the chromosomes became lighter as they proceeded through the S period. All of the large subtelocentrics were labeled at mid-S (3 hr), and all had completed DNA synthesis 1 hr before the end of S (8 hr on the graph).

The six small subtelocentric chromosomes (Fig. 3) followed a pattern similar to the large subtelocentrics: all were labeled at 3 hr of the S period, and all had completed DNA replication 1 hr before the end of S. Similarly, labeling progressed from a moderate-heavy density to a predominantly light grain density near the end of S. The peak in the total labeling curve at 6 hr, i.e. the fact that total labeling is higher at 6 hr than at 5 hr, probably represents variation among cells over the 2 hr sample period.

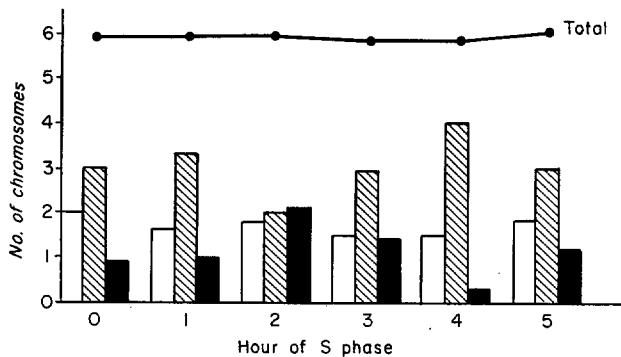


FIGURE 9 Beginning of DNA synthesis in large subtelocentric chromosomes.

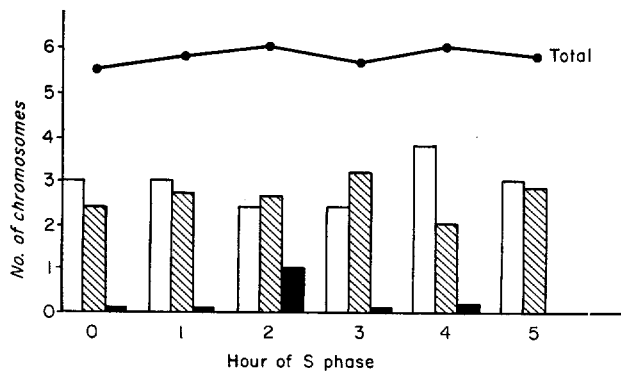


FIGURE 10 Beginning of DNA synthesis in small subtelocentric chromosomes.

The telocentric chromosomes (Fig. 4) repeated the patterns noted in the preceding two sets of chromosomes. Labeling intensity again proceeded from moderate and heavy to light. The chromosomes continued to be labeled into the last hour of S; however, only one sample could be found which labeled at this time, and labeling intensity was very slight. Since over 20 cells were analyzed at the 8th hr of S, the value at this time period was considerably more accurate than at the 9th hr, and the average termination of replication was most likely somewhere between the 8th and 9th hr of the S phase.

The pattern of DNA synthesis in the two female X chromosomes (Fig. 5) was considerably different from that in the preceding autosomal pairs. In general, regions of both chromosomes were labeled throughout the end of the S phase up to the 8th hr of S, at which time labeling dropped sharply to zero. The labeling intensity of the X chromosomes did not proceed from heavy to light. In all stages of S in which labeling was evident (all but the 9th hr), at least one of the X chromosomes exhibited moderate to heavy label-

ing, with the other one generally labeling lightly to moderately. The female X chromosomes finished DNA replication synchronously, i.e., one did not appear to continue synthesis later than did its homologue. In some of the cells sacrificed at the end of the S phase, both of the X chromosomes were labeled, while most of the autosomes had finished DNA replication or were labeled lightly. This was taken as evidence that the X chromosomes in the female replicated their DNA slightly later than did the autosomes (see Fig. 19).

The male X chromosome (Fig. 6) similarly labeled throughout most of the S phase, but labeling began to drop off gradually during the 7th and 8th hr of S. As in the female X chromosomes, heavy and moderate, as well as light, labeling were observed throughout the terminal stages of the DNA synthetic period.

The Y chromosome (Fig. 7) in the 65 male cells investigated demonstrated a cyclic labeling behavior during the termination of S when thymidine-<sup>3</sup>H was continuously available. The chromosome exhibited nearly total labeling during the 3rd and 4th hr of S, dropped to a low level during

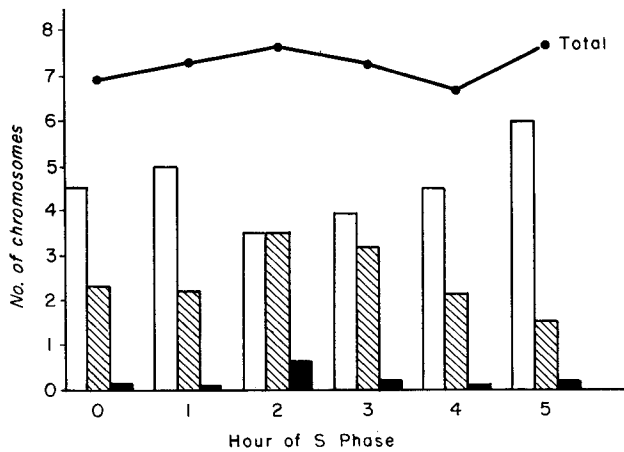


FIGURE 11 Beginning of DNA synthesis in telocentric chromosomes.

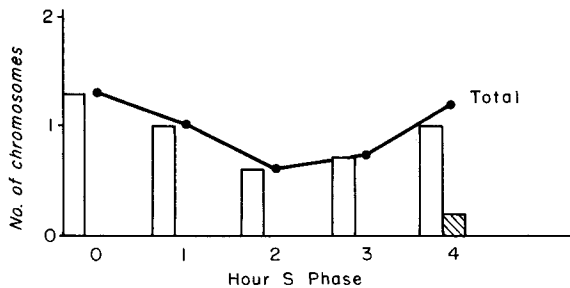


FIGURE 12 Beginning of DNA synthesis in female X chromosomes.

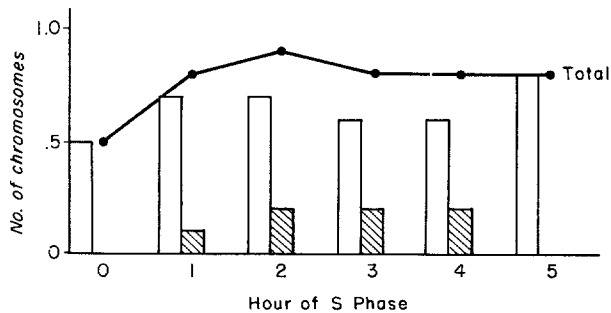


FIGURE 13 Beginning of DNA synthesis in male X chromosome.

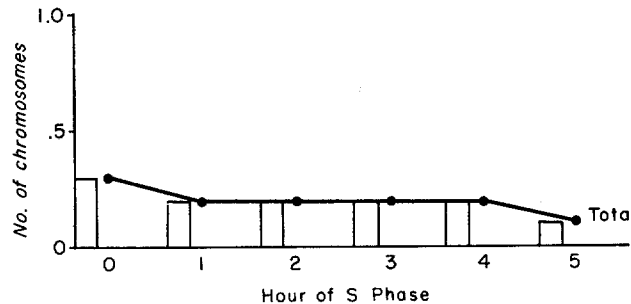


FIGURE 14 Beginning of DNA synthesis in Y chromosome.

the 5th hr, and rose to a high peak during the 6th hr. DNA synthesis was completed at the 8th hr of S. When the data for the end of the S phase and the beginning of the S phase were averaged for the sample intervals which overlapped, the cyclic labeling behavior of the Y disappeared (see Fig. 16). The intensity of labeling was heavy during the 4th hr of S, became predominantly light during the 5th hr, and returned to moderate to heavy during the 6th hr.

As was the case with the female sex chromosomes, the male X and Y completed DNA replication in cadence. Similarly, some cells were observed at the end of the S phase in which both X and Y chromosomes were labeled, but in which the autosomes had, for the most part, completed DNA replication. As in the female, this was not the case in all cells sampled during the same interval. It did, however, indicate that male sex chromosome replication continued slightly later than did autosomal replication (see Fig. 20).

#### Availability of Thymidine-<sup>3</sup>H

Radioautographs were prepared from mouse tumor cells which were incubated for 1 hr in medium freshly removed from cultures of opossum lymphocytes exposed to thymidine-<sup>3</sup>H for 7.5 hr. A count of 500 sarcoma cells showed that approximately 40% had incorporated the isotope (Fig.

22). Control cells incubated with fresh thymidine-<sup>3</sup>H demonstrated a 39% labeling frequency. The degree of labeling was considered as evidence that thymidine-<sup>3</sup>H had been continuously available to DNA-synthesizing lymphocytes during the 7-8 hr in which they had been incubated with the isotope.

#### Beginning of DNA Synthesis

The values for the cell cycle phases and the method of analysis of chromosomal labeling were identical with those values employed in the analysis of the termination of DNA synthesis. That partial synchronization of the cultures did occur with the 5-FUDR-thymidine method (10) was concluded from the presence of approximately two to three times the number of mitotic figures at the initiation of the S phase than at subsequent sample intervals. Cultures were harvested hourly at 8-14 hr after pulse-labeling with thymidine-<sup>3</sup>H. Since colchicine was added to all cultures for the final 2 hr of incubation, each interval sampled represented a 2 hr range.

The 8 hr sample contained cells which had been in the 3rd-5th hr of S at the time of introduction of thymidine-<sup>3</sup>H (Fig. 8). Each of the samples was plotted as though it represented the end of the 2 hr range. Thus, the 8 hr sample represented the 5th hr of S, the 9 hr sample the 4th hr of S, and so

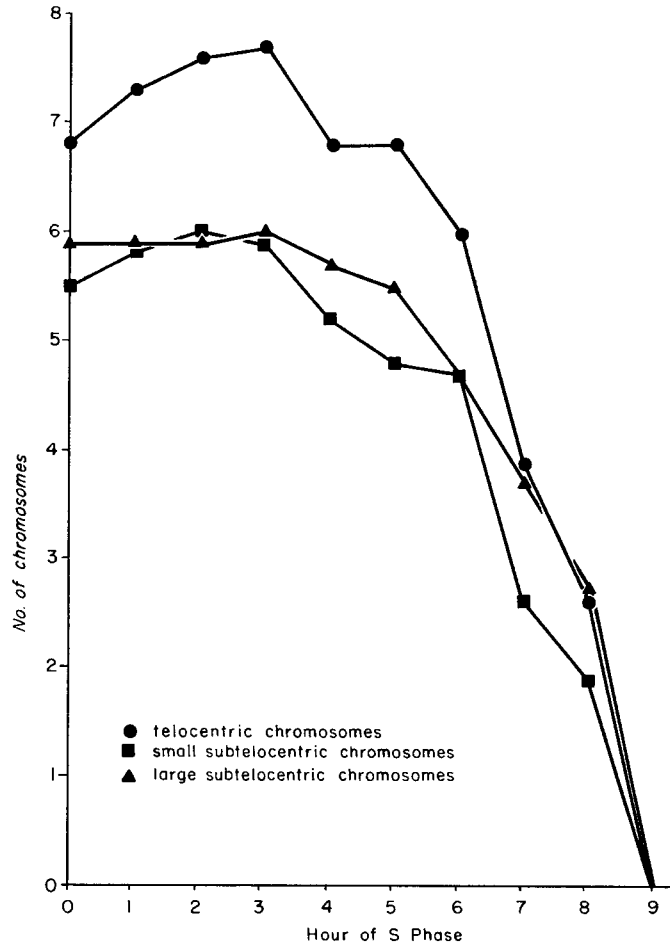


FIGURE 15 Composite labeling patterns in autosomes.

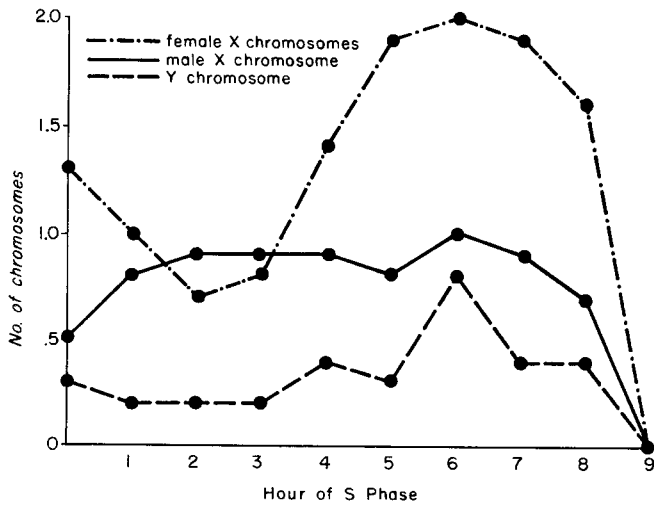


FIGURE 16 Composite labeling patterns in sex chromosomes.



on. The end of the range of the 13 hr sample fell at the zero hour of S, and, therefore, labeled cells in these samples were taken to represent cells at the very beginning of S. Since the 14 hr samples fell outside of the S phase, they were not included in the data analysis. Some of the chromosomes of cells in the 14 hr sample were labeled, but this was interpreted as representing individual variation in the duration of S among cells.

The same type of graph was used as before to demonstrate degree of chromosome labeling (bar graphs) and total labeling (line graphs). Data were plotted at 0, 1, 2, 3, 4, and 5 hrs of S (Figs. 9-14). Composite labeling graphs were constructed for the autosomes and sex chromosomes by averaging the values which overlapped between the termination and beginning of DNA synthesis experiments. These are demonstrated for the autosomes in Fig. 15 and for the sex chromosomes in Fig. 16.

Labeling of the autosomes was strikingly consistent during the first half of S, that is, nearly all of the chromosomes labeled throughout the first 5 hr in all three sets of chromosomes (Figs. 9, 10, and 11). For example, the large submetacentric chromosomes (Fig. 9) produced a horizontal, total-labeling curve with very nearly 100% labeled chromosomes from the initiation through the 5th hr of S. The small submetacentric chromosomes (Fig. 10) and the telocentric chromosomes (Fig. 11) began DNA synthesis with somewhat less than the full complement labeled, but in both cases the total labeling increased during the 1st and 2nd hr of the S phase. In all three sets of autosomes the labeling intensity was predominantly light and moderate although some heavy labeling occurred in the large submetacentric chromosomes. In none of the sets was there evidence of a shift from one intensity to another during S as was observed for the autosomes during the last half of the DNA synthetic period.

Labeling in the female X chromosomes (Fig. 12) was predominantly light during the first half of S, and it is apparent from Fig. 15 that an average of one of the two X's was incorporating thymidine-<sup>3</sup>H during the first 3.5 hr of the DNA synthetic period. The single X chromosome of the male (Fig. 13), however, was labeled in almost all cells at all intervals from the 1st to the 5th hr of the S phase. The labeling intensity was predominantly light as in the single female X which was labeling during the first half of the S phase. The Y chromo-

some (Fig. 14) was for the most part unlabeled during the first half of DNA synthesis, and almost all of the few Y chromosomes that did label (approximately 20%) contained only 1 gr during this period.

Although the average labeling patterns indicated that the sex chromosomes finished DNA synthesis almost simultaneously, one of the X chromosomes in the female was often more heavily labeled than the other (see Fig. 17). Figs. 18 and 19 demonstrate female metaphase plates near the end of the S phase. In Fig. 19 the autosomes, for the most part, have completed replication, while the two X chromosomes are still moderately labeled. Fig. 20 represents a male metaphase plate at the end of S in which the autosomes had completed replication, but in which the sex chromosomes had continued to synthesize DNA. Fig. 21 also shows labeling in a male cell. Fig. 22 demonstrates that isotope was available to lymphocytes during continuous labeling.

#### *Chromosomal Protein Synthesis*

50 mitotic figures were analyzed for chromosomal labeling at each interval (1, 2, 3, 5, 6, 8, 10, 12, 13, 14, 15, and 16 hr) after pulse-labeling cultures with arginine-<sup>3</sup>H. Grains were observed over approximately 80% of all cells in smears, including small lymphocytes, and were found in both the nucleus and cytoplasm although the nuclei contained more grains per unit area than did the cytoplasm. Only mitoses with grains directly over the chromosomes were considered labeled (Fig. 23). Since arginine is a major component of certain histones, it was believed that a portion at least of the protein being synthesized was histone. Fig. 24 demonstrates that when the percent of labeled mitotic figures is plotted against the interval after pulse-labeling with arginine-<sup>3</sup>H, a gradual increase in chromosomal protein synthesis is observed throughout the S phase and into G<sub>2</sub>.

#### DISCUSSION

With certain exceptions, the results reported above generally agree with the reports in the literature regarding DNA synthesis in the chromosomes of humans and animals (21, 23, 28, 29). Elimination of the double-photography technique combined with lower isotope doses and shorter exposure times allowed morphological identification of individual, labeled chromosomes, particularly the sex chromosomes (see Fig. 25 for standard karyo-

grams). Because mean labeling percentages were calculated for a given chromosome or chromosome set from a number of cells, data were often reported as fractions of chromosomes labeled. Each figure, however, represents the average number of chromosomes per set which were labeled at a given interval.

The method of data analysis demonstrated that throughout the S phase the labeling intensity decreased in the autosomes but not in the sex chromosomes. It has, therefore, provided supporting evidence for identifying late-labeling chromosomes; that is, the sex chromosomes in general synthesized their DNA at a higher rate than did the autosomes near the termination of the S phase. It may be possible that chromosomes would present different labeling patterns depending on the quantity of thymidine-<sup>3</sup>H added to cultures, or on the length of exposure. By varying exposure times in these experiments, however, only changes in labeling intensity (in all of the chromosomes) was observed.

The observation of German (7) and Kikuchi and Sandberg (11) that the long autosomes of human leukocytes began DNA synthesis earlier than the short autosomes was noted for opossum lymphocytes since total-labeling peaks were reached later for the short autosomes than for the long ones. In contrast to their findings, however,

the long autosomes from opossum cells did not continue DNA synthesis longer than the short ones, but the three sets appeared to terminate replication in a close relationship.

The sex chromosomes of opossum lymphocytes showed patterns of DNA synthesis at the beginning of S which were similar to those found for the chromosomes of the Chinese hamster (10). In particular agreement was the finding that one X chromosome began DNA synthesis approximately 3.5 hr later in the S phase than did its homologue. The male X chromosome behaved as did its counterpart in the female ("early X"), while the Y showed late-labeling characteristics. If it is assumed that the DNA content in the two X chromosomes of the female is equal, this finding must mean that the late-replicating X synthesized its DNA at a higher rate than its homologue. This supposition is supported by the presence in many cases of one heavily labeled X and one lightly labeled X in lymphocytes from female opossums toward the end of S.

In contrast to the findings of other investigators, who have commonly found that one X in the female and the Y in the male of the various species studied continued synthesizing DNA considerably later than did the other sex chromosomes and autosomes, both female X's and the X and Y in

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FIGURE 17 Female metaphase plate labeled near the end of the S phase. Both X chromosomes are labeled, but the central one more intensely.  $\times 1500$ .

FIGURE 18 Female metaphase plate labeled near the end of the S phase. The autosomes, for the most part, are labeled lightly, but both X chromosomes are heavily labeled.  $\times 1500$ .

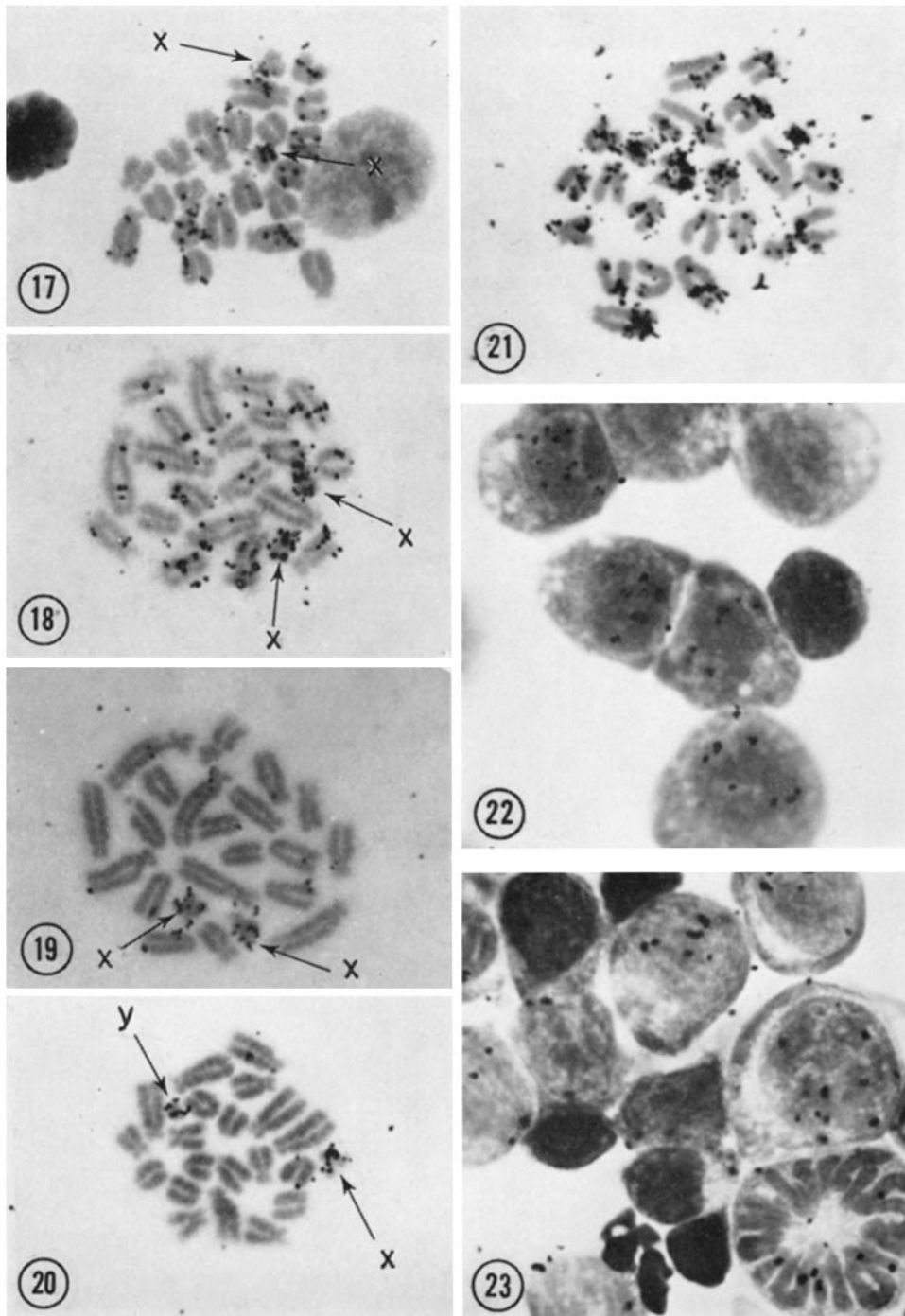
FIGURE 19 Female metaphase plate labeled near the end of the S phase, slightly later than in Fig. 18. The autosomes, for the most part, have completed replication, while both X chromosomes are still labeled.  $\times 1500$ .

FIGURE 20 Male metaphase plate labeled at the termination of the S phase. Note that the X and Y chromosomes are labeled, while the autosomes have completed replication.  $\times 1500$ .

FIGURE 21 DNA synthesis in the autosomes and sex chromosomes of a male opossum during the latter half of the S phase.  $\times 1500$ .

FIGURE 22 Radioautograph of A/jax mouse Sarcoma I ascites tumor cells. The cells were incubated for 1 hr in medium in which thymidine-<sup>3</sup>H had been available to lymphocytes for 7.5 hr. Most of the cells have incorporated the isotope.  $\times 1500$ .

FIGURE 23 Radioautograph of cardiac blood lymphocytes incubated for 2 days with PHA and pulse-labeled with arginine-<sup>3</sup>H. The majority of label is nuclear, and in the mitotic figure the grains are located over the chromosomes.  $\times 1500$ .



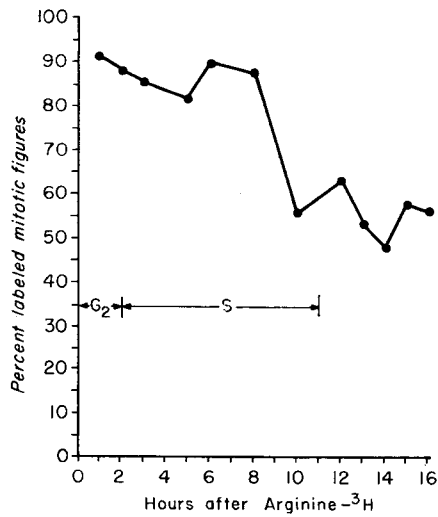


FIGURE 24 Chromosomal protein synthesis in opossum lymphocytes in vitro.

the male opossum terminated DNA synthesis simultaneously. All of the sex chromosomes in the opossum continued replication slightly later than did the autosomes. Termination of DNA synthesis in the sex chromosomes, however, was considerably more abrupt than in the autosomes, that is, the labeling percentages and intensity remained high in the sex chromosomes until the termination of replication. In female bovine cells (3), in which the two X chromosomes began replication simultaneously but terminated asynchronously, the late-replicating X must have synthesized its DNA at a slower rate than its homologue. This assumes, of course, that no gaps exist in the DNA synthetic sequence of the sex chromosomes. The factors controlling the rate of DNA synthesis are unknown.

In comparing this study on the opossum with those on other forms, it appears that at least three means exist by which one sex chromosome of a pair can effect late-replication: (a) it can begin DNA

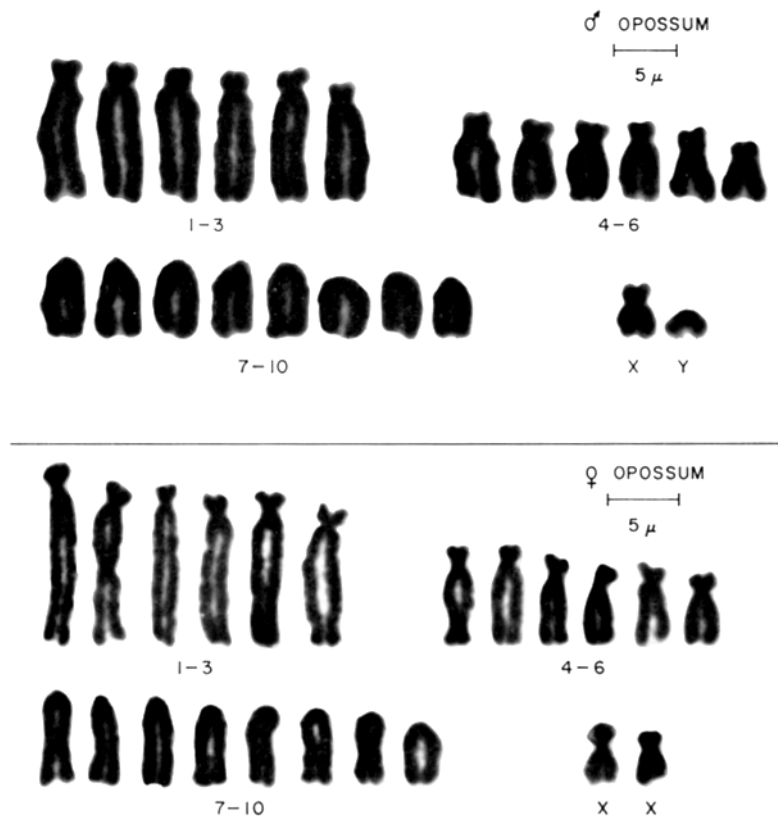


FIGURE 25 Karyograms constructed from metaphase plates of lymphocytes from male and female opossums.  $\times 2000$ .

replication later than its fellow and terminate later than its fellow (Chinese hamster); (b) it can begin DNA replication concomitantly with its fellow and terminate later (cow); and (c) it can begin DNA replication later than its fellow and terminate at approximately the same time (opossum).

The results of chromosomal protein synthesis in opossum chromosomes confirm those of Cave (1) who studied the incorporation of lysine-<sup>3</sup>H into human chromosomes during interphase. Cave demonstrated a lack of functional dependence

between DNA synthesis and protein synthesis, noting that while DNA synthesis was limited to the S phase, chromosomal protein synthesis occurred throughout interphase.

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