Late DNA Replication in the Sex Chromosomes of *Didelphis virginiana*[1]

The North American opossum *Didelphis virginiana* is a particularly favorable animal for sex chromosome analysis because its X and Y are recognizable at sight in the chromosome complement, the acrocentric Y being approximately one-half the size of the submetacentric X[2]. The present study was undertaken to determine if the sex chromosomes of the opossum demonstrate late DNA synthesis in somatic cells cultured in vitro.

Materials and methods. 4 male and 5 female opossums (*Didelphis virginiana*), age 6–8 weeks, were sacrificed and their femora dissected. Bone marrow cells were cultured in 5 ml of TC 199 and 15% fetal calf serum (Grand Island Biological Supply) at 37 °C for 6 h. Tritiated thymidine (specific activity 1.9 c/mM; Schwarz Bio-Research, Inc., Orangeburg, New York), at a concentration of 0.5 µc/ml of culture medium, was added 30 min after incubation. 2 h prior to harvesting, colchicine (Colcemid, CIBA) 200 µg/ml was added. Chromosome preparations were made utilizing modifications of the technique of KAJII[3] for human material. The slides were stained for 12 h in 2% acetic-lactic orcein, and suitable metaphases were photographed and their locations recorded. Kodak AR-10 stripping film was applied employing the technique of SCHMID[4].

The G2 for opossum bone marrow cells was determined in an adult male opossum employing the above technique. The G2 period extends from the end of the S period to the onset of prophase. Studies were made of cultures of 2, 3, 4, 5, and 6 h duration, with colchicine added in each case during the final hour. Attempts at culturing other tissues were unsuccessful.

Results. The G2 period in the opossum was estimated to be between 3 and 4 h. This estimate was based on the presence of labelled metaphases seen only in cultures containing tritiated thymidine for 4 or more hours, with colchicine added during the final hour. 300 female and 300 male metaphases were counted.

Female. Tritium label was present in 30% of the metaphases analyzed. Of these labelled metaphases, 27.8% (8.3% of total) demonstrated the typical late-replicating pattern of the X chromosome (Figure 1). In 3% of the labelled metaphases (1% of total) one heavily labelled X and a second lightly labelled X were noted.

Male. Of the analyzed metaphases in the male, 23.3% were labelled. Of the labelled metaphases, 28.6% (6.5% of total) demonstrated late labeling sex chromosomes. Both X and Y were late-replicating in 15.7% of the labelled metaphases (3.6% of total); the Y alone was late-replicating in 10% (2.2% of total); the X alone was late-replicating in 2.9% of labelled metaphases (0.7% of total) (Figures 2–4).

Discussion. A unique sexual dimorphism based on sex chromatin is seen in somatic cells of the opossum, *Didelphis virginiana*. Both sexes possess a sex chromatin body with about the same frequency[5,6]. The dimorphism is based on the difference in size of the sex chromatin, it being conspicuously larger in the female. It has been suggested that the small late-labeling Y and even the smaller autosomes are related to the very small regions of dense chromatin seen in the interphase nuclei of some mammals[7,8]. Thus, it is tempting to relate the single late-labeling Y in the opossum to the smaller sex chromatin body seen in the interphase nuclei of male somatic cells.

The present work indicates that sex chromosomes of the bone marrow cells of the opossum synthesize DNA

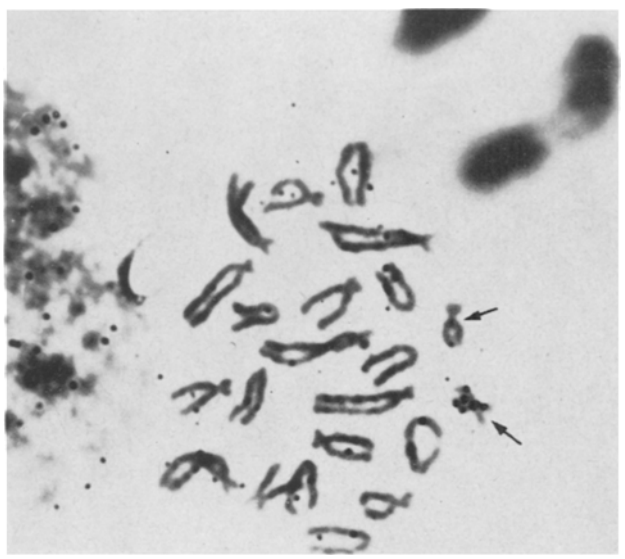

Fig. 1. Metaphase from a female opossum labelled in late S period. Arrows indicate sex chromosomes.

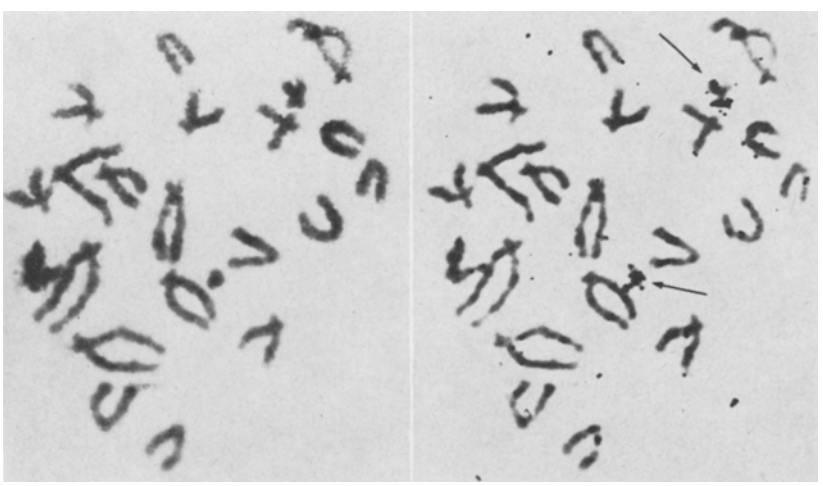

Fig. 2. Metaphase from a male opossum showing both the X and Y chromosome heavily labelled. Arrows indicate sex chromosomes.

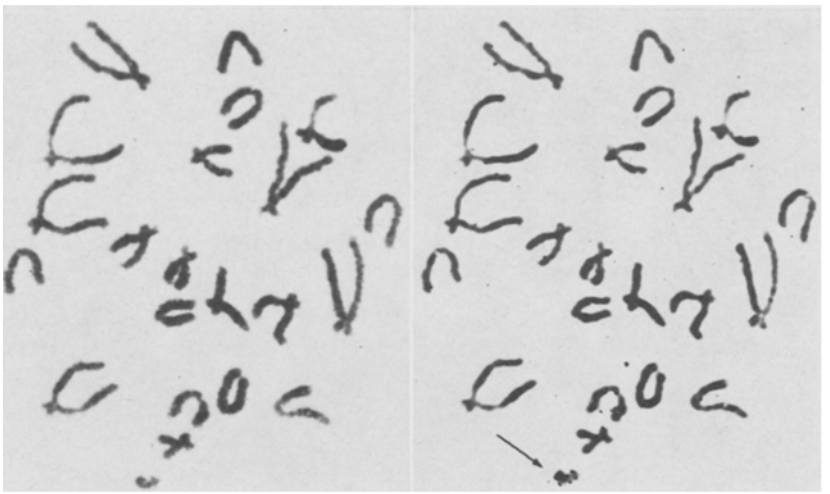

Fig. 3. Metaphase from a male opossum showing a heavily labelled *Y*-chromosome (arrow).

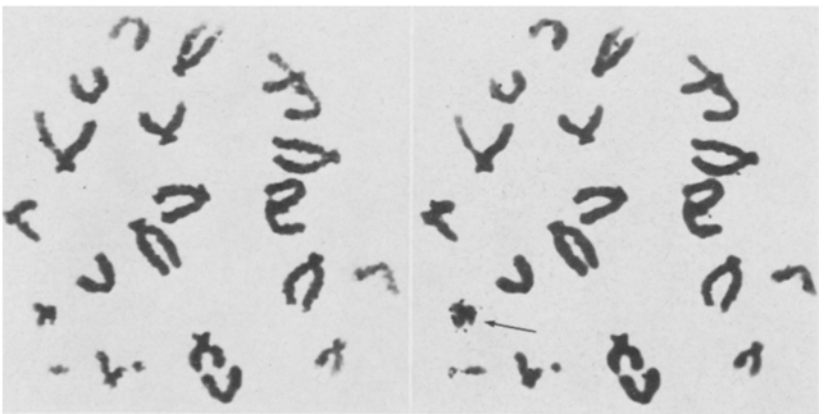

Fig. 4. Metaphase from a male opossum with a heavily labelled *X* chromosome (arrow).

later than do the autosomes. Further, the sex chromosomes of both male and female complete DNA synthesis asynchronously in relation to one another. In a recent study of opossum lymphocytes cultured in vitro, contrary data were reported[9]. These investigators found a simultaneous completion of DNA synthesis of the sex chromosomes of both male and female. This was slightly later than the completion of autosomal replication. The conclusions from the data of another publication by SINHA[10] also differ with those of the present study. SINHA concludes that the autosomes of the opossum complete replication later than the sex chromosomes and therefore that the opossum autosomes contain more heterochromatin than the sex chromosomes. We believe that the differences in our results as contrasted with the two previous investigations can be explained by differences in methodology. Both of the former studies utilized phytohemagglutinin-stimulated lymphocyte cultures of peripheral blood. Our investigation employed instead the short-term culture of bone marrow leucocytes without the presence of a mitotic stimulant. This latter technique would seem to be more likely to reflect the in vivo condition since no mitogenic substance has been added. Whether or not the source of cells used in the studies (bone marrow vs. peripheral blood leucocyte cells) could account for the differences in results must await further study.

Zusammenfassung. Autoradiographisch wurde die späte DNS-Synthese der *X*- und *Y*-Chromosomen von *Didelphis virginiana* (Virginia Opossum) in Knochenmarkzellen untersucht. Die weiblichen Tiere zeigen typische späte Reduplikationsmuster in einem *X*, die männlichen das späte Reduplikationsmuster in *X* und *Y* und in *Y* allein.

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