Concerning the testes of the HCG-treated rats, first signs of histologically visible events, slightly deviating from normal, were observed after a 3 weeks period (Figure 4, a). In spite of 2 months of HCG influence normal-looking interstitium was visible no longer (Figure 4, b).

Along with considerably reduced tubular diameters, the germinal epithelium was in a stage of heavy depopulation. Practically no spermiogenesis and elements no further advanced than primary spermatocytes (Figure 4, b).

Almost identical histological impressions gave slides of the testes which had been 4 and 6 months under the HCG therapy respectively. In fact, there were still areas left containing a few spermatocytes. On the other hand, however, quite a few tubules were completely devoid of normally occurring cellular structures. Instead of this, they were occupied with an abundance of necrotic materials (Figure 4, c). It might be left open to discussion whether the severity of degeneration and necrosis was even further advanced after 6 months as compared to the 4 months pictures (Figure 4, c–d).

Discussion. Although a high dose of TP was given daily up to 6 months, the testicular events retrogressed in the hypophysectomized rat continuously. The longer the replacement therapy, the more a phenomenon became transparent, which is not seen earlier under such an experimental condition[2]. The androgen alone could not sustain spermiogenesis, testicular weight in the adult rat in the long run: almost identical with what was observed after the ablation of the pituitary gland in control rats[7], however, extended over a much longer period of time the well-known sequence of degeneration within the germinal line took place under TP too. However, it must remain open to discussion whether the ultimate stages of atrophy as described earlier in HE rats[8] could also be reached although this steroid is injected.

HCG was much less effective than testosterone, probably, first of all, because of its inability to stimulate androgenic secretion(s) of the interstitial cells beyond a given term. The comparatively high mortality of these HCG-treated rats during the second month of the experiment points to the likelihood of anti-body formation in consequence of the rather high and chronic dose of foreign protein. Under testosterone, such a lack of androgenicity was ruled out: the established parameters for the male hormone, prostate glands and seminal vesicles had been stimulated excessively even after 6 months. These findings support the theory that the dynamics of the seminiferous epithelium and its morphology depend not only on steroidal androgenicity but also on the synergistic action of the FSH[9,10]. At first sight there might arise the somewhat remote objection that the stage of hypophysectomy per se, in other words the absence of the pituitary with its various hormones, could cause this failure of the androgen therapy. However it has been shown extensively that FSH plus endogenous or exogenous androgen gave full replacement for the rat's testis, even after ultimate atrophic conditions such as are seen after extended post-hypophysectomy regression periods[8]. Although the dynamics of spermatogenesis in man and rat are not necessarily comparable, this fact may be of importance in clinical practice.

Zusammenfassung. Langzeitversuche mit hypophysektomierten Ratten ergaben, dass Testosteron oder HCG allein die Spermiogenese, Hodengewicht, nicht aufrechterhalten konnten.

R. VON BERSWORDT-WALLRABE,
M. MEHRING and E. RICHTER-BONACKER

Experimentelle Forschung Pharma Schering AG, Abteilung Endokrinologie, D-1 Berlin 65 (Germany), 6 April 1970.

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RNA Synthesis in the Sex Chromosomes of the Opossum, *Didelphis virginiana*. I. Female

The single active X (Lyon) hypothesis[1] is supported by the following observations: a) female mammalian somatic cells frequently demonstrate sex chromatin bodies[2]; b) these cells contain a late replicating X chromosome[3–7]; c) late replicating chromosomes are heterochromatic in interphase nuclei[8,9]; d) heterochromatin synthesizes RNA at a lower rate than does euchromatin[10–13]; and e) when more than two X chromosomes are present, the number of sex chromatin bodies is one less than the total number of X chromosomes present, and all but one are late replicating[14].

Whereas the majority of evidence favors this hypothesis, certain other findings suggest that both X chromosomes may be genetically active during brief portions of the cell cycle. For example, sex chromatin cannot be identified in all female somatic cells in interphase[15]. Similarly, during periods of rapid growth, both female X chromosomes may fail to undergo heterochromatization in cells with short cycles[16,17]. In addition, all prophase chromosomes of female human lymphocytes incubated briefly with H^3-uridine were found to synthesize RNA immediately prior to mitosis[18,19].

The opossum possesses many unusual cytogenetic characteristics which made it an ideal animal in which to test the single active X hypothesis. Quantitation of RNA synthesis was made possible since the X chromosomes are easily identifiable as the smallest of the complement[20]. In addition, the following observations have been made: a) cultured opossum lymphocytes demonstrate a short cell cycle[21]; b) sex chromatin is present in both sexes[22,23]; c) the amount of heterochromatin appears to vary little between the X chromosomes of lymphocytes from female opossums[24]; and d) there is no typical late replicating X chromosome[25].

Materials and methods. Lymphocytes obtained from cardiac blood of female opossums were cultured according to a previously described method[20]. Following 42–48 h of incubation, the cultures were labeled terminally for

5–20 min with 5 μc/ml of uridine-5-T (specific activity 5 c/m*M*, Amersham/Searle). No colchicine was added. The cells were fixed in 1:3 acetic acid-methanol for 30–60 min to remove the acid soluble labeled nucleotides[26]. Smears and hypotonic spreads were prepared, the latter after 15 min exposure to 50% acetic acid to dissolve background cytoplasm[27]. The slides were coated with Kodak NTB2 liquid emulsion, exposed 3 days, developed and stained[28]. Double photography of either destained or degrained[29] slides was employed to analyze labeling of 89 spreads from 2 opossums. Radioautographs were also prepared from unlabeled cells either destained or unstained to test for possible histochemical or physical reduction of the emulsion. Some labeled preparations were treated with 200 μg/ml of ribonuclease (Mann) in pH 7.0 phosphate buffer for 2 h at 37 °C to test for the possible conversion of uridine-5-T to labeled deoxcytidine and its subsequent incorporation into DNA[30].

Results. Analysis of smears demonstrated that approximately 85% of the newly synthesized RNA remained on prophase chromosomes after 5, 10 or 20 min exposure to uridine-5-T, whereas only 50% or less remained on metaphase chromosomes at the same intervals. For this reason, and because the *X* chromosomes are morphologically distinguishable in prophase as well as in metaphase, emphasis was placed on analysis of RNA synthesis at the earlier stage.

The Figure is a hypotonic spread preparation of a cell in prophase which was labeled for 20 min with uridine-5-T. Both *X* chromosomes are labeled, one more densely than the other. The incidence of RNA synthesis in both *X* chromosomes was higher in prophase (76%) than in metaphase (60%), and increased with the longer exposures to the isotope (from 40% at 5 min to over 84% at 20 min). A total of 63 of the 89 cells, or almost 71% demonstrated incorporation of uridine-5-T into both *X* chromosomes (Tables I and II).

Grain counts were performed on 70 of the 89 spreads which had densities low enough to assure accuracy. The mean number of grains over the total chromosome complement was 90.1. In each spread, the *X* chromosome with the most grains was categorized as the 'active' *X* and that with the fewest grains as the 'inactive' *X*. The mean number of grains per 'active' *X* was 3.1 (3.4% of the total) while that for the 'inactive' *X* was 1.7 (1.9% of the total). Thus on the average, the 'inactive' *X* was 55% as active in RNA synthesis as its homologue. Variation in grain counts between the two *X* chromosomes was slight (0 to 2) in over 84% of the spreads analyzed. Each *X* comprised 2.7% of the area of the total chromosome complement.

Three observations lead to the conclusion that it is RNA and not DNA which is labeled by uridine-5-T: a) in order for uridine to be converted to thymidine, the tritium molecule must be cleaved[31]; b) the cells were labeled a maximum of 20 min before harvest, and the minimum G_2 period for opossum lymphocytes is 90 min[21];

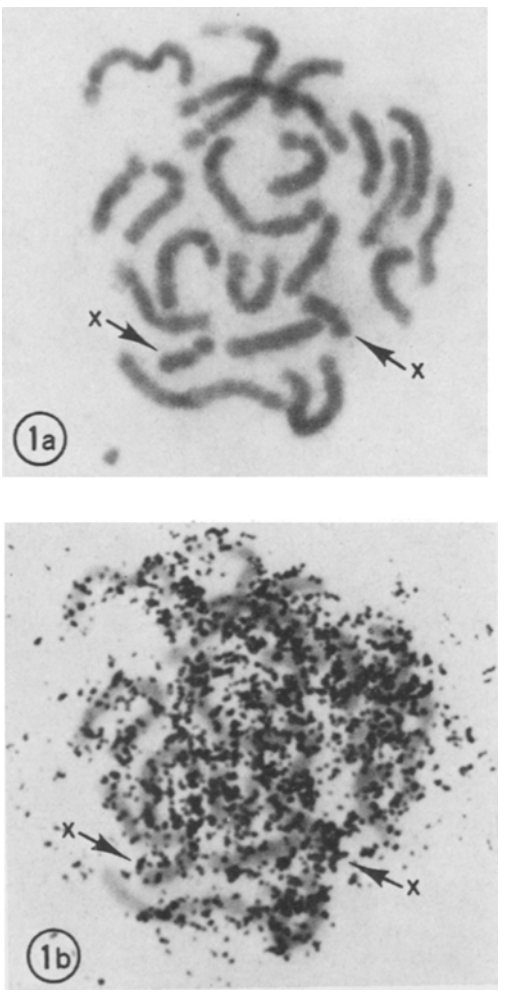

Fig. 1, a and b. Prophase chromosome spread from a culture of female opossum lymphocytes incubated terminally for 20 min with uridine-5-T. Both *X* chromosomes are labeled, one more heavily than the other. × 1375.

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Table I. *X* chromosome labeling at individual mitotic stages

Stage	No. of cells	Neither *X* Labeled		One *X* Labeled		Both *X*'s Labeled	
		No.	(%)	No.	(%)	No.	(%)
Prophase	59	5	8.5	9	15.3	45	76.3
Metaphase	30	1	3.3	11	36.7	18	60.0
Totals	89	6	6.7	20	22.5	63	70.8

Table II. *X* chromosome labeling at various intervals after introduction of uridine-5-T

Time (min)	No. of cells	Neither *X* Labeled		One *X* Labeled		Both *X*'s Labeled	
		No.	(%)	No.	(%)	No.	(%)
5	20	4	20.0	8	40.0	8	40.0
10	18	2	11.1	4	22.2	12	66.7
20	51	0	0.0	8	15.7	43	84.3
Totals	89	6	6.7	20	22.5	63	70.8

and c) treatment with ribonuclease removed over 97% of the grains. Histochemical or physical reduction of the emulsion was judged nonexistent since no significant increase in grains above background was noted in the nonradioactive hypotonic spreads.

Discussion. Due to a variety of cytogenetic peculiarities, the opossum has offered an unusual opportunity to investigate and quantitate *X* chromosomal RNA synthesis. The results demonstrate that both *X* chromosomes of lymphocytes from female opossums synthesize RNA during the brief portion of the cell cycle which was examined. Evidence suggests that RNA synthesized by phytohemagglutinin-stimulated lymphocytes is nonribosomal[32,33]; rather the 'obvious hypothesis' is that it is 'messenger RNA produced in large quantity'[34]. Presumably, therefore, both *X* chromosomes of these cells are genetically active (although one to a lesser degree) just prior to mitosis. It must be kept in mind, however, that differences may exist between cultured cells and those in their natural state.

The *X* chromosomes of opossum lymphocytes appear to be more similar than dissimilar, especially during the interval of the cell cycle just prior to mitosis. Evidence for this similarity stems from the observation that both *X* chromosomes complete DNA synthesis at the same time[25], and that they contain similar amounts of heterochromatin[24]. Opossum lymphocytes also exhibit a short cell cycle[21] which suggests that they may lack sex chromatin (and thus may be genetically active) during periods of rapid growth.

RNA synthesis in the human sex chromatin body has been attributed to juxtaposed euchromatin[11]. This possibility was eliminated in the present study since hypotonic spread preparations allowed for spatial separation of chromosomes. It has also been suggested that labeling in human metaphase chromosomes was the 'result of random, non-specific adherence of labeled RNA'[26]. Release of labeled RNA from prophase chromosomes in the present investigation was slight. Although the possibility of non-specific adherence of some labeled RNA to chromosomes cannot be ignored, it seems improbable that a significant amount of that released in prophase cells would adhere to a single chromosome, specifically the 'inactive' *X*[35].

Résumé. Pour une étude quantitative de la synthèse du RNA, nous avons utilisé des chromosomes sexuels d'Opossum, morphologiquement faciles à distinguer en prophase et possédant des caractères cytogénétiques inusuels. Les résultats suggèrent que les deux chromosomes *X* des lymphocytes femelles sont actifs génétiquement (quoique l'un d'eux le soit moins que l'autre) pendant une courte période du cycle de la cellule.

L. K. SCHNEIDER

Department of Anatomy,
University of Arizona, College of Medicine,
Tucson (Arizona 85721, USA), 9 February 1970.

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