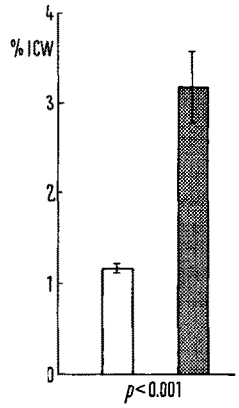
tified as the substance responsible for the antidiuretic effect of plasma by means of the inactivation test with sodium thioglycolate. Student's t-test was used for statistical analyses and p values under 0.05 were considered significant. Means $\pm$ S.E. are shown in the Figure.

Results and discussion. In all dogs of the 1st set of experiments, the osmotic reactivity of hypothalamo-hypophyseal antidiuretic system was higher than that of the thirst mechanism (Figure). Whereas the clear cut increase in plasma ADH level was already observed at cellular dehydration equal to $1.1 \pm 0.01\%$ of intracellular water (ICW), the drinking response was observed at cellular dehydration equal to $3.1 \pm 0.37\%$ of ICW. The difference between these 2 degrees of cellular dehydration was statistically significant ($p < 0.001$). The same results have been obtained in the 2nd group of experiments when different animals were compared. The values of threshold cellular dehydration of 1.4 ± 0.04 and $3.4 \pm 0.17\%$ of ICW were obtained for the antidiuretic system and for the thirst mechanism, respectively. The difference was statistically significant ($p < 0.001$).



Degree of cellular dehydration at which the antidiuretic system (white column) and the thirst mechanism (dashed column) are activated.

The results indicate that there is a difference between the threshold osmotic reactivity of these 2 systems involved in body water conservation. The hypothalamo-hypophyseal antidiuretic system which protects the organism against loss of water and which lowers the thirst threshold is firstly alarmed in conditions of water deficit. As the cellular dehydration increases this action is not fully sufficient and then the thirst mechanism, which controls water intake, is activated.

Résumé. On a examiné la réactivité osmotique du système antidiurétique hypothalamo-hypophysaire et du mécanisme de la soif chez des chiens non narcotisés. Chez tous les animaux examinés, le système antidiurétique s'est montré plus sensible aux stimulus osmotiques que le mécanisme de la soif. On peut supposer que c'est le système antidiurétique qui est activé en premier lieu lors d'un déficit d'eau.

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Random *X*-Chromosome Inactivation in Interspecific Hybrids of *Meriones libycus* (♂) × *Meriones shawi* (♀) (Rodentia:Gerbillinae)

Dosage compensation for *X*-linked genes in mammals is explained by the single active *X*-chromosome theory. This hypothesis, as originally proposed by several authors[1–3] suggests that: 1. One of the *X*-chromosomes in females is inactivated early in embryogenesis; 2. inactivation is a random process with respect to parental origin of the *X*-chromosomes (maternal vs. paternal); and, 3. after inactivation, all subsequent progeny will possess the same inactivated *X*-chromosome (clonal evolution). The association of heterochromatinization, late DNA replication, and gene inactivation has been demonstrated in separate systems, each providing partial proof of the hypothesis[3–10]. Recently, by use of hybrid animals with both suitable chromosomal and enzymatic markers, COHEN and RATTAZZI[11] have shown simultaneous cytological and biochemical evidence of genetic inactivation of one *X*-chromosome in the female mule. This report presents further supportive cytogenetic data for the LYON[1] hypothesis derived from an interspecific hybrid of 2 jird species, *Meriones libycus* Lichtenstein × *M. shawi* Duvernoy.

Materials and methods. Tissue culture. 2 adult hybrid females derived from *M. libycus* (♂) × *M. shawi* (♀) crosses were studied. The animals were sacrificed and skin biopsies were placed in tissue culture. The specimens were minced (approximately 1 mm^3) and the explants placed in 60 mm Petri dishes containing 3 ml of Ham's F-10 nutrient medium with 20% fetal calf serum. The Petri dishes were maintained at 37 °C in an atmosphere of 5% CO_2 in air. When luxuriant fibroblast growth was obvious, the monolayer of cells was trypsinized and replated into 250 ml Falcon tissue culture flasks. The cells were allowed to grow to confluency with medium exchanged at approximately 7-day-intervals.

Cloning. Clones of fibroblasts were established from animal No. 1 by the method of PUCK et al.[12]. Following monolayer trypsinization, 10-fold serial dilutions of the cell suspensions were made. Suspensions of 50 cells/ml to 0.05 cells/ml were then plated into separate Petri dishes. Single cells were located by phase contrast microscopy and surrounded by a glass cylinder ($^1/_4$ inch diameter) made adherent with stopcock grease. When clones became established, they were transferred to Petri dishes and subsequently to Falcon flasks by trypsinization.

Cytogenetic studies. Chromosome preparations were obtained from the mixed population of cells as well as clones derived thereof by a modification of the method of MOORHEAD et al.[13]. When approximately 60–70% confluency was reached, colcemide (0.05 µg/ml) was added for the final 4 h of culture. The cells were then removed from the flasks by gentle scraping with a rubber policeman. After centrifugation, the cells were suspended in 1% sodium citrate for 30 min at 37 °C followed by fixation in 3:1 absolute methanol:glacial acetic acid (at least 2 changes of 10 min each). Several drops of cell suspension were placed on a microscope slide, pre-wet in 70% metha-

nol, and passed through a flame for blaze drying. Staining was achieved by 2% acetic orcein and the cells were examined by means of phase contrast microscopy.

Autoradiographic analysis of late DNA replication patterns of the *X*-chromosomes was achieved by adding ^{3}H-thymidine (final concentration 1.0 μC/ml) for the last 6 h prior to harvest. Colcemide was present for the final 4 h. Harvesting of the cells and slide preparation was performed as described above. The slides were then dipped in Nuclear Track emulsion (Kodak NTB_3) and incubated at 4 °C for 10–14 days. After development of the silver grains, the slides were stained with buffered Giemsa as suggested by SCHMID[4]. Cells with informative labelling patterns were located and photographed. Silver grains were then removed by treatment with 30% potassium ferricyanide (30 min) and several rinses in 24% sodium thiosulphate. Those cells previously studied were located and rephotographed without the overlying silver grains. In this way direct comparisons and definite identification of the labelled chromosomes were possible.

Enzymatic studies. Cellulose acetate gel (Cellogel) electrophoresis was performed on lysates of fibroblasts by the method of RATTAZZI et al.[14]. Particular emphasis was placed on the patterns of glucose-6-phosphate dehydrogenase (G-6-PD) migration, as this enzyme is *X*-linked in most animals.

Results. The karyotype of the *M. libycus* × *M. shawi* hybrid is presented in Figure 1 and agrees with that previously reported by LAY and NADLER[15]. The diploid number is 44 with 20 metacentric, 11 submetacentric and 11 acrocentric autosomes. The sex chromosomes are a large acrocentric (*M. libycus*) and a large metacentric (*M. shawi*). The distinct morphology of each *X*-chromosome allows its immediate identification and assignment as to parental origin. Figure 2 illustrates the late DNA patterns for each of the parental *X*-chromosomes.

The Table shows the results of autoradiographic studies on mixed populations of fibroblasts from the 2 animals examined. In neither individual case was the expected 1:1 ratio obtained ($P < 0.01$ and $P < 0.05$, respectively). Animal No. 1 had 62.6% late replicating *M. libycus* *X*-chromosomes, while animal No. 2 had 63.4% late replicating *M. shawi* *X*-chromosomes. However, when pooling the data from both animals, a random inactivation pattern is obvious ($0.75 > P > 0.50$). 3 clones were successfully established from animal No. 1 and yielded sufficient cells for autoradiographic studies. In clone No. 1, 48 cells were examined, all possessing the late replicating *M. shawi* *X*-chromosome, while in clones 2 and 3, only *M. libycus* *X*-chromosomes were late replicating in 26 and 38 cells respectively.

Unfortunately, efforts to utilize the G-6-PD electrophoresis as an independent biochemical marker failed, since lysates of the mixed populations of cells yielded a single enzyme band. Without individual, separable electrophoretic units, each specific for 1 parental species, biochemical confirmation of the cytologic observations was impossible.

Discussion. The use of interspecific hybrid animals with unique *X*-chromosomes and *X*-linked biochemical markers derived from each parental species has proven valuable in the investigation of mammalian dosage compensation. By using the distinct *X*-chromosomal morphology coupled with parental differences in G-6-PD electrophoretic patterns in the female mule, COHEN and RATTAZZI[11] demonstrated a highly significant correlation between gene inactivation and late *X*-chromosome replication. It was hoped that the interspecific hybrid gerbils studied in these experiments would provide an additional system to demonstrate this phenomenon. Unfortunately, the identical electrophoretic mobilities of G-6-PD in the parental species obviated the biochemical studies. Nonetheless, the cytogenetic investigations provide support for the LYON[1] hypothesis.

It is generally accepted that late *X*-chromosome replication indicates genetic inactivation. Therefore, the data presented in the Table demonstrate that only a single *X*-chromosome is active in a given cell. Although neither animal alone showed the expected 1:1 ratio, it is of

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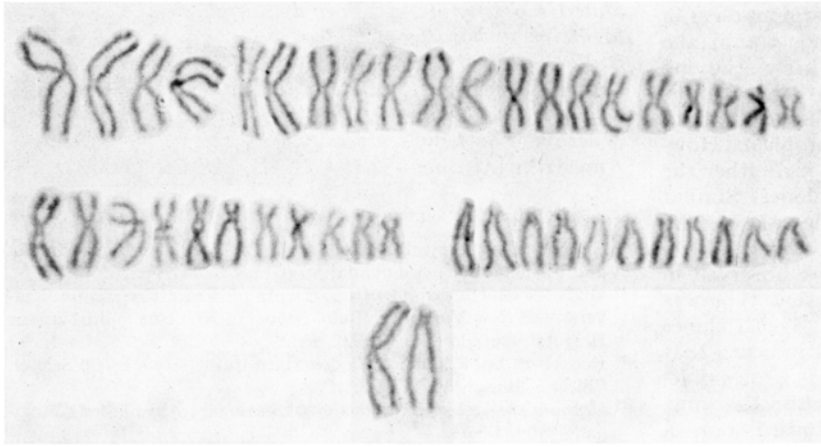

Fig. 1. The karyotype of the female hybrid between *M. libycus* (♂) × *M. shawi* (♀) ($2N$ – 44). Note the distinct *X*-chromosomes in 3rd row (*M. shawi* – metacentric; *M. libycus* – acrocentric).

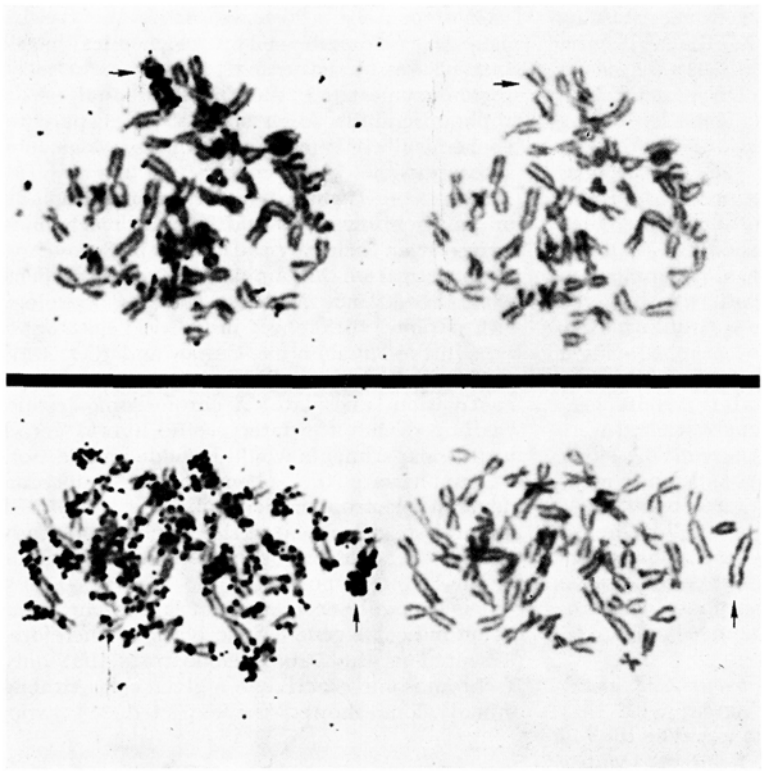

Fig. 2. Autoradiographs of late DNA replication patterns in *M. libycus* (♂) × *M. shawi* (♀). Top, *M. shawi* *X*-chromosome (metacentric) heavily labelled; bottom, *M. libycus* *X*-chromosome (acrocentric) heavily labelled.

Late replication patterns of the *X*-chromosome in female hybrids of *M. libycus* × *M. shawi*

Animal	Late replicating				
	X libycus	*X shawi*	Total cells	χ^2	*P*
No. 1	72	43	115	7.32	$P<0.01$
No. 2	30	52	82	5.90	$P<0.05$
Total	102	95	197	0.12	$0.75>P>0.50$

interest that the deviation from randomness was not the same in both animals. Animal No. 1 showed a preponderance of *M. libycus* (62.6%) *X*-chromosomes late replicating, while animal No. 2 demonstrated an almost identical percentage of *M. shawi* replicating *X*-chromosomes (63.4%). Upon pooling these data, the late replication pattern of the 2 animals was entirely random (*M. lybicus* – 51.8% vs. *M. shawi* – 49.2%). Perhaps larger numbers of cells in the mixed populations are necessary to demonstrate random inactivation patterns within the individual animals.

Biochemical studies utilizing the enzymatic markers in 3 human *X*-linked conditions have demonstrated the clonal concept of the LYON hypothesis[8–10]. By studying females possessing 2 electrophoretic G-6-PD patterns, DAVIDSON et al.[8] demonstrated the expression of both forms in lysates of mixed populations of fibroblasts. However, only single bands of enzymatic activity (either the A or B form) was present in lysates of clones. Similar studies have been undertaken utilizing female heterozygotes with Hurler's[9] and the Lesch-Nyhan syndromes[10]. 2 types of cells, normal and deficient, were observed in mixed populations, but only one or the other type was present in clones derived from such cells. The 3 clones derived from animal No. 1 in our studies indicate that when *X*-chromosome inactivation occurs it is irreversible, and that derived progeny of that cell maintain the same replication pattern. Within each clone studied, only a single *X*-chromosome late replication pattern was observed. However, among the 3 clones, the *X*-chromosome of each parental species was inactivated. These studies, exploiting the cytogenetic uniqueness of the interspecific hybrids, in which each parental species *X*-chromosome can be identified without error, support the basic tenets of the LYON hypothesis[16].

Résumé. Des cultures obtenues à partir d'hybrides femelles entre deux espèces de *Meriones* à 44 chromosomes et différant par l'acrocentrie (*M. shawi*) ou la métacentrie (*M. libycus*) de l'*X* ont permis l'étude de clones cellulaires. C'est alors tantôt l'*X* métacentrique, tantôt l'*X* acrocentrique qui se révèle inactivé («late-replicating»). Bien que la proportion 1/1, significative d'une inactivation due uniquement au hasard, n'ait pas été rigoureusement observée, ces résultats sont nettement en faveur de l'hypothèse de LYON.

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