respective group. Since the size and the extent of the lesions were indistinguishable for the two experiments, the findings in both experiments were pooled, as presented in the Table.

The data show that small bilateral, single-focus lesions (Group 2) produced no elevation in the plasma level of either triglyceride or cholesterol. Triple-focus lesions (Group 3) produced elevations of both triglycerides (36% increase, $p < 0.01$) and cholesterol (13% increase, $p < 0.02$). It is noteworthy that the lipid elevations in the two groups of rats with hypothalamic lesions were not associated with increased food intake. In fact, the plasma cholesterol levels in Group 3 showed a negative correlation with food intake ($r = -0.44$, $p < 0.05$). Body weight, length and obesity index have been included for comparison with previous findings[4-6].

The data indicate that lesions, entirely within the VMN, produced elevations in triglyceride and cholesterol levels and extension outside the VMN was not necessary to obtain an effect. However, as shown in our original study[2], the 'cholesterol area' appears to extend beyond the VMN into the dorsal and lateral areas.

Zusammenfassung. Es wurden ein- und dreipaarige elektrolytische Läsionen in den ventromedialen Kernen von Ratten gesetzt, wobei es zu einer Erhöhung von Triglyceriden und Cholesterin kam. Dieses Hypothalamus-Gebiet enthält Regulationszentren des Serumlipidspiegels.

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The Threshold Osmotic Reactivity of the Hypothalamo-Hypophyseal Antidiuretic System and of the Thirst Mechanism

There is evidence that the hypothalamo-hypophyseal antidiuretic system and the thirst mechanism are both activated by an increase of extracellular fluid osmolality[1-4]. In our previous study[5], we found that a moderate increase of blood ADH level decreases the thirst threshold for osmotic stimuli and thus facilitates water intake. The question arose which of these two systems regulating water balance in the body is first activated due to increasing cellular dehydration. This problem has not yet been examined thoroughly. Wolf[4,6] suggested that osmotic reactivity of the thirst mechanism and of the antidiuretic system is much the same. The purpose of the present study was to find out whether the hypothalamo-hypophyseal antidiuretic system and the thirst mechanism are activated by the same degree of cellular dehydration.

Material and methods. Experiments were carried out on 56 unanaesthetized mongrel dogs. As osmotic reactivity of the thirst mechanism was different in individual dogs, 2 sets of experiments were performed. In the 1st set (10 dogs), the osmotic reactivity of the thirst mechanism and that of the antidiuretic system were compared in the same experiments carried out on the same animals. In the 2nd set, the osmotic reactivity of the thirst mechanism was studied in 25 dogs and the osmotic reactivity of the hypothalamo-hypophyseal antidiuretic system in another group of 21 dogs. The dogs were fasted for 18 h but they had free access to water also during the experiment. The control blood sample was taken and a 5% solution of saline was infused at a rate of 7.5 ml/min into the saphenous vein of a dog. Blood samples were taken every 4 min in the course of the infusion. In the 1st group of experiments, the blood samples were drawn from an external jugular and in the 2nd one from a saphenous vein. When the dog began to drink, the infusion was stopped and a sample of blood was taken instantaneously. If the osmotic reactivity of the antidiuretic system was examined alone, the infusion was stopped after 20 min. The osmotic reactivity was expressed as a threshold value of osmotic stimulus[4]. For the antidiuretic system it was a minimal cellular dehydration causing pronounced increase of plasma ADH concentration; and for the thirst mechanism, it was a minimal cellular dehydration necessary to induce the drinking response. In each dog the extracellular water was measured by using sodium thiocyanate. Total body water was measured by using tritium water in the 1st set of experiments and calculated as the percent of body weight in the 2nd one. The plasma Na concentration was also measured and the total amount of extracellular sodium calculated. As the amount of Na and water in the infusion and in the urine produced during the infusion was measured, hence the shift of water caused by hypertonic infusion and cellular dehydration could be calculated on the basis of the measurements mentioned above. Degree of cellular dehydration was expressed as a percent of initial (control) value of the intracellular water. Plasma antidiuretic activity was measured using a modified method of Czaczkes et al.[7]. ADH was iden-

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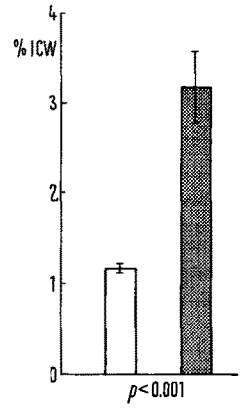
tified as the substance responsible for the antidiuretic effect of plasma by means of the inactivation test with sodium thioglycolate. Student's t-test was used for statistical analyses and p values under 0.05 were considered significant. Means $\pm$ S.E. are shown in the Figure.

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-