Antidiuretic Hormone in the Urine and Pituitary of the Kangaroo Rat.* (18216)

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The desert rodent *Dipodomys* ordinarily does not have access to water and its survival depends upon an extremely economical use of the water present in food or derived from the oxidation of hydrogen. Its ability to excrete a highly concentrated urine is the important factor in its conservation of water (1-5). This rodent can excrete a more concentrated urine than any other mammal whether terrestrial or marine. According to the Schmidt-Nielsens and their colleagues (2-5), the electrolyte-content of *Dipodomys* urine can reach 1.2 N and urea can be present in a concentration of 3.6 M. The mechanisms by which such unusual concentrations of electrolyte and urea are reached require elucidation. Howell and Gersh (1) concluded that considerable water was removed from the urine in both the collecting tubules and in the bladder. The most important hormonal factor in water conservation by the kidneys is the antidiuretic hormone of the posterior pituitary and it seemed probable that *Dipodomys* requires relatively large quantities of this hormone. A comparison of the urinary excretion of antidiuretic hormone and of the quantity of stored hormone in the pituitary of kangaroo rats *(Dipodomys merriami)* and of stock rats (Long-Evans strain) has been made and will be reported in this communication.

Methods. The kangaroo rats used in this study were obtained from the Arizona desert and were maintained on a diet of dry corn, barley, wheat and rolled oats. Lettuce was offered them 3 times a week but no drinking water was furnished. The animals maintained their weight which was taken to be good evidence of a satisfactory water balance. The comparative studies on stock rats were carried out with the Long-Evans strain of our laboratory colony. The problem of obtaining sufficient urine from the kangaroo rats which usually weighed 35 to 40 g was solved by ligating the neck of the bladder under ether anesthesia. Twenty-four hours later the operated animals were killed by illuminating gas and the accumulated urine was aspirated from the bladder by means of a syringe and fine needle. Approximately 0.5 ml of urine for assay could thus be obtained from each animal. Occasionally the urine was contaminated with blood although there was no apparent reason for hemorrhage into the urinary tract. The best specimens, never containing blood, were aspirated from the bladder at laparotomy before ligation; there was no means of ascertaining the period of collection of these samples. The samples of urine from ligated bladders represented the urine excreted during 24 hours. One-fourth per cent acetic acid was used for the preliminary dilution of the urine. Either the pars neuralis or the whole pituitary was removed under a dissecting microscope and extracted at room temperature by grinding with 0.25% acetic acid in an agate mortar. The diluted fine suspension was kept at 4°C before assay. (Brief extraction at 100°C immediately followed by chilling caused considerable loss of antidiuretic hormone.) Before extraction, the pars neuralis was quickly weighed on a sensitive torsion balance (± 0.02 mg); the whole pituitaries usually were not weighed.

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2. Schmidt-Nielsen, B., Schmidt-Nielson, K., Brokaw, A., and Schneiderman, H., *J. Cell. Comp. Physiol.*, 1948, v32, 331.

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TABLE I. Concentration of Antidiuretic Hormone in the Urine of Kangaroo Rats and of Stock Rats (Long-Evans Strain).

No. of rats	Variety	Bladder ligated	Period of thirst (hr)	Mean and S.D. of antidiuretic hormone in urine as m.u./ml
9	Kangaroo	No		19.3 ± 13.3
11	"	Yes		7.2 ± 4.0
3*	"	"		10.0
5	Stock	"	0	<0.1
4	"	"	48	<0.2 (3) 0.5 (1)
44†	"	No	48-72	1.7 ± 1.5†

* Blood in urine.
† 11 groups of 4 each.

Specimens from the stock rats were also collected in the same manner. In one experiment with 44 stock rats, urine was collected in metabolism cages each containing 4 rats. Antidiuretic hormone was determined in hydrated unanesthetized dogs by the method of Ames, Moore and van Dyke(6). The minimum quantity of hormone which could be detected was 0.25 m.u. (0.00025 U.S.P. unit).

Results. Antidiuretic activity was detected in all urines obtained from the kangaroo rats in concentrations ranging from 1.7 to 50 m.u. per ml (Table I). The average from 9 specimens of kangaroo rat urines before ligation of the bladder was 19.3 ± m.u. per ml. In the 24 hour urine collections from the ligated bladders of 11 rats the average titer was 7.2 ± 4.0 m.u. per ml. Three other specimens from ligated bladders contained gross blood and the average of these was 10.0 m.u. per ml. It was felt that small amounts of blood or serum in these 24-hour collections might account for their lower potency as compared with the urines obtained without ligation of the bladder.

Urine obtained from 5 stock rats on adequate food and water intake contained no antidiuretic activity. When drinking water was withdrawn for 48 hours or more, antidiuretic activity was detected in increasing amounts. There was a small amount of antidiuretic hormone (0.5 m.u. per ml) in only one of 4 rats deprived of water for 48 hours; the amount present in the urine of the other 3 was less than 0.2 m.u. per ml. Urine was collected for the last 24 hours of a 72-hour thirst period from 11 groups of 4 rats in metabolism cages. The average titer of these specimens, each representing 4 rats, was 1.7 ± 1.5 m.u. per ml and the range was 1.0 to 5.9 m.u. per ml. An acid pH was maintained in these urines by the use of collecting cylinders which contained 1% acetic acid. In a control experiment in which pitressin solutions of 2 and 5 m.u. per ml at pH 5.5 were allowed to drip through metabolism cages for a similar period, no loss of activity could be detected.

In Tables II and III, determinations of the amount of antidiuretic hormone in the neural lobe or whole pituitary are summarized. The dissection and weighing of the neural lobes was completed as rapidly as possible. Possibly there was some posterior lobe tissue not removed, especially in the small kangaroo-rat glands, which were found to contain more hormone in the whole gland

TABLE II. Antidiuretic Hormone in Neural Lobe of Kangaroo Rats and of Normal Stock Rats (Long-Evans Strain).

No. of rats	Variety	Avg body wt in g	Avg wt of pars neuralis in mg (fresh)	Antidiuretic hormone in pars neuralis: m.u./μg	Antidiuretic hormone in pars neuralis: m.u./gland S.D. ±
11	Kangaroo	41	0.37	.91	336 ± 110
14	Stock	217	1.07	.26	276 ± 146

TABLE III. Antidiuretic Hormone in Whole Pituitary of Kangaroo Rats and of Normal Stock Rats (Long-Evans Strain).

No. of rats	Variety	Period of thirst (hr)	Avg body wt in g	Mean and S.D. of total m.u. of antidiur. hormone in gland
29	Kangaroo		37	448 ± 128
9	Stock	0	249	275 ± 168
7	"	48	232	320 ± 122
29	"	72	205	490 ± 295

6. Ames. R. G., Moore, D. and van Dyke. H. B., *Endocrinol.*, 1950, v46, 215.

,Table III). Anterior lobe tissue of the kangaroo rat, carefully freed of any neural lobe, contained no antidiuretic hormone. The fresh median eminence of a few rats of each species contained measurable quantities of antidiuretic hormone amounting to 19 m.u. in the kangaroo rat (average of 3 rats) and 11 m.u. in the stock rat (average of 7 rats). From a comparison of the neural lobes of the two species (Table II), it can be concluded that kangaroo rats have as much or more stored antidiuretic hormone than normal stock rats weighing 5 times as much. The observed difference is not statistically significant. The concentration of antidiuretic hormone is more than 3 times as high in the neural lobe of the kangaroo rat than in that of the stock rat.

It was concluded that for complete extraction of the pituitary antidiuretic hormone, whole pituitaries should be used. This plan was followed in the determinations reported in Table III in which an attempt was made to estimate the effect of thirst on the stored hormone in the stock rats. In comparison with normally-hydrated stock rats, kangaroo-rat pituitaries contain significantly more hormone ($P = <0.01$ by Fisher's "t" test). Among the normal and thirsted stock rats, there was found a significantly increased storage of hormone after a thirst of 72 hours ($P = 0.05$).

A few other observations deserve mention. A number of inactivation experiments was completed. In every case, thioglycolate(6) destroyed the antidiuretic activity of urine; under the same conditions posterior-pituitary antidiuretic principle was likewise completely inactivated. Ultracentrifugation of 3 mixtures of kangaroo rat urine was also carried out. In each experiment there was marked sedimentation of the active substance. Before ultracentrifugation the average assay of these urine mixtures was 6.7 m.u. per ml. After ultracentrifugation the top fraction contained 1.2 and the bottom, 18.3 m.u. per ml.

Discussion. Since every urine specimen from the kangaroo rats had a detectable amount of antidiuretic activity it is evident that the secretion of this hormone may be one of the most important factors in the water economy of these desert rodents. It is probable that the kangaroo rat excretes highly concentrated urine in part because of a continuous and high rate of secretion of antidiuretic hormone by the pars neuralis. Our stock rats on an adequate fluid intake did not excrete antidiuretic hormone. However, after they were thirsted from 48 to 72 hours antidiuretic activity could be detected in the urine. The group of 44 rats which were thirsted for 72 hours appeared active and well at the end of this period despite some loss of weight. Increased secretion of antidiuretic hormone evidently induced adequate water conservation. However, if the concentration of antidiuretic hormone in the urine be taken as a measure of the rate of secretion, this rate of secretion is 4 times or more as rapid in the kangaroo rat on its normal diet than in stock rats of the Long-Evans strain thirsted for 48 to 72 hours. In another mammal, the dog, powerful osmotic stimulation is followed by a urinary titer of antidiuretic hormone not exceeding 6 m.u. per ml. The maximum concentration of hormone found in kangaroo-rat urine was 50 m.u. per ml in comparison with 6 m.u. per ml in thirsted stock rats. This high rate of secretion is associated with the storage of a large amount of hormone in the pars neuralis in a much higher concentration than found in the gland of stock rats. Gilman and Goodman(7) using rats for bio-assay injected either the standard posterior-pituitary solution or urine concentrates subcutaneously. They report an experiment in which the urine of laboratory rats thirsted 72 hours was thought to contain 100 m.u. of antidiuretic principle per ml. Urine concentrates contain substances which markedly retard absorption and prolong the action of any accompanying antidiuretic principle when injected subcutaneously. Hence, an assay such as that just cited is subject to gross error(8).

The work of Shannon(9) in the dog sug-

7. Gilman, A. and Goodman, L., *J. Physiol.*, 1937, v90, 113.

8. Gilman, A., personal communication.

9. Shannon, J. A., *J. Exp. Med.*, 1942, v76, 387.

gests that supramaximal amounts of antidiuretic hormone are continuously secreted by kangaroo rats. This may depend upon a different renal response requiring larger amounts, a different rate of destruction of the hormone elsewhere, an excessive rate of secretion or a combination of more than one factor. The amount of antidiuretic hormone in the pituitary of stock rats was found to increase after a thirst of 72 hours. Thus, an increased rate of secretion of the hormone was associated with the storage of an increased amount of hormone in the gland. These results are difficult to compare with those of Simon(10) who found a decrease in pituitary oxytocic and pressor hormones in rats deprived of water for a much longer period (120-168 hours) than our rats (72 hours). He detected no significant change after 72-96 hours in a few rats; however, in view of the variations to be expected, this is not surprising.

Summary. The kangaroo rat *(Dipodomys merriami)* excretes large amounts of antidiuretic hormone in the urine (up to 50 milliunits per ml). The presence of this high concentration of hormone is believed to be related to the ability of this desert rodent to excrete the most concentrated urine of any mammal and to reflect a correspondingly high rate of secretion of antidiuretic hormone by the posterior pituitary. In 2 other mammals, the dog with powerful osmotic stimulation of the cerebrum, and the laboratory rat (Long-Evans strain) deprived of water for 48-72 hours, the maximum concentration of hormone in the urine is about 6 m.u. per ml. The hormone in kangaroo-rat urine undergoes sedimentation in the ultracentrifuge and thus resembles endogenous antidiuretic hormone in canine urine. The pituitary of kangaroo rats contains more antidiuretic hormone than that of normal laboratory rats although the latter are 5 to 6 times larger. After 72 hours of thirst the laboratory rat's pituitary contains an increased amount of hormone. Each microgram of fresh posterior lobe contains about 0.9 m.u. of antidiuretic hormone in kangaroo rats and about 0.3 m.u. in normal laboratory rats.

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Effect of DDT Ingestion on Total Cholesterol Content of Ovaries Of White Rat.* (18217)

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Fitzhugh(1) observed that DDT, up to 600 p.p.m. in the diet of female rats, did not affect survival of young of the first generation. However, in the second generation of feeding DDT poisoned food, there were few living young at birth. Although the high mortality of young in Fitzhugh's DDT feeding tests may have come about through direct toxicity of the chlorinated hydrocarbon, it is possible that a hormonal disturbance may be involved in the whole problem. There is evidence that cholesterol is a precursor of some hormones exerting their influences in the reproductive cycle of the rat(2). Therefore, it seemed promising to investigate ovarian cholesterol in rats subjected to DDT poisoning. In connection with investigations (3) concerned with accumulation of ingested p,p′ DDT in various internal organs of white rats, additional females were exposed to diets

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1. Fitzhugh, O. G., *Ind. and Eng. Chem.*, 1948, v40, 704.

2. Perlman, P. L., and Leonard, S. L., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 24.

3. Tauber, Oscar E., Hughes, Arden B., Tague, R. E., Pappas, Thomas R., and Warns, Thomas F., in preparation.