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THE ROLE OF THE ANTIDIURETIC HORMONE DURING WATER DEPRIVATION IN RATS

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It has been suggested that the survival of animals deprived of water is in direct proportion to their ability to concentrate their urine, and that the high concentration of their urine is achieved at the expense of an increased secretion of the antidiuretic hormone. The problem investigated here was to see whether the decrease of the urinary volume observed in laboratory rats deprived of water was the sole factor in the mechanism of water preservation and if so, whether the reduction of urinary secretion was the result of a corresponding increase in secretion of the antidiuretic hormone.

METHODS

White adult male rats of body weight ranging from 250 to 325 g were fed on a standard diet, 100 g of which contained: protein 14 g, fat 4 g, and soluble carbohydrate 49 g; calorific yield 400 cal/100 g. From its composition, it could be calculated that its metabolic water was 52 ml./100 g food: its amount of moisture (i.e. 'preformed water') was 13·0 ml./100 g. The water content of food and of faeces was estimated by drying in an oven at 104° C for 48 hr. The rats were kept in metabolism cages, at a temperature of 21° C; the degree of humidity was not recorded. Urine separators were fixed to the cages and the faeces were collected separately in a vessel with narrow neck so as to diminish water evaporation. Whenever possible fresh faeces were collected for the estimation of their water content. For the estimation of its antidiuretic activity urine was collected in a solution of 3% (v/v) acetic acid (final concentration, 0·2–0·3%). Pituitary glands were removed, dried and extracted as specified in the British Pharmacopoeia (1953); the amount of acetic acid used in their extraction did not exceed 0·2 ml./gland of a 0·25% acetic solution (Bentley & Dicker, 1955). Urine samples were analysed for their Na and K content, using an EEL flame photometer; occasional Cl estimations were performed.

The antidiuretic activity of urine or of dissected neurohypophyseal glands was estimated by intravenous injection in rats under ethanol anaesthesia, with the water load kept constant (Dicker, 1953). The vasopressor activity of the neurohypophysis was estimated on rats anaesthetized with urethane and injected with dibenamine (Dekansky, 1952) and the oxytocic activity on a rat's isolated uterus (Holton, 1948). Each assay of the antidiuretic, vasopressor or oxytocic activity consisted of four doses, two of the standard and two of the unknown, the ratio, high to low dose, being the same for standard and unknown solutions. Results were expressed in terms of the antidiuretic or vasopressor activity of a solution of vasopressin, or in terms of oxytocic activity of a solution of oxytocin.

Drugs. Pitressin (Parke Davis and Co., batch LS 882 H) and Pitocin (Parke Davis and Co., batch E 378212) were used as standard for the estimation of the antidiuretic, vasopressor and oxytocic effects. The antidiuretic and vasopressor activity of the drug Pitressin has been referred to as 'vasopressin', and the oxytocic activity of the drug Pitocin as 'oxytocin'.

RESULTS

Validity of the essays

The errors of the methods for assaying either the antidiuretic or the oxytocic activity of preparations have been defined previously. The present series of assays fell within the limits stated (Dicker & Greenbaum, 1956; Bentley & Dicker, 1955). To estimate the accuracy of the method for assaying the vasopressor activity, known amounts of vasopressin were added to urine collected from well hydrated rats (8·0 ml. water/100 g body weight) kept under ethanol anaesthesia. Control samples had no pressor activity. In experiments where standard and test solutions had the same concentration of vasopressin, and the doses injected were 0·1 and 0·2 ml., the mean recovery was 101·5% ± 0·02, S.E. of six estimations (range: 85–112%) of the stated potency. When, however, the test solutions contained half the amount of vasopressin of the standard solution and the doses of unknown injected were 0·2 and 0·4 ml., the mean amount of activity recovered was 87·5% ± 0·59 (5) only (range: 57–148%) of the stated potency.

Observations on normal rats

Pair weighed rats kept in metabolism cages for up to 5 days were provided with food and water *ad libitum*. The amounts of food and water consumed were recorded as well as those of urine and faeces excreted: the total water intake was calculated as water drunk + preformed water in the food + metabolic water. Observations were made during winter and summer: as no essential differences due to seasonal changes were noted, all results were pooled.

Observations have been made on thirty-four rats. The mean gain of body weight was 2·0 g ± 0·05 (34) in 5 days. Food eaten averaged 10·1 g ± 0·32/100 g body weight/24 hr (34), and the water drunk was 12·1 ml. ± 0·23/100 g/24 hr (34). When expressed in terms of body surface, this represents some 9 ml./100 cm^2, which is of the same order of magnitude as the water drunk by a normal adult man (body surface 1·80 m^2). The urine excretion was 3·5 ml. ± 0·37/100 g body weight/24 hr (34). In two other series of observations on rats on diets of the same calorific value the urine flow was about the same: 3·4 ml. ± 0·22/100 g/24 hr (26) (Dicker, unpublished), and 3·6 ml. ± 0·84/100 g/24 hr (30) (Dicker, 1949). When expressed in terms of body surface, this would be equivalent to a urinary volume of about 270 ml. for adult man. The difference between total water intake (18·5 ml.) and urine excretion (3·5 ml.) represents the insensible water loss, i.e. water lost by evaporation and with faecal matter. The insensible water loss was 15·0 ml./100 g/24 hr, which is of the same order

of magnitude as that calculated by Schmidt-Nielsen & Schmidt-Nielsen (1950) for *ratus norvegicus*. The mean weight of faeces was 2·8 g ± 0·05/100 g/24 hr; the water content was 65 ml. ± 0·3/100 g faeces. From this it could be calculated that the amount of water lost with faecal matter was 1·8 ml./100 g/24 hr, leaving 13·2 ml./100 g/24 hr for evaporation. These figures agree well with those found by Schmidt-Nielsen & Schmidt-Nielsen (1951). There can thus be no doubt that in adult rats, with free access to food and water, the volume of water excreted by the kidneys represents less than 20% of the total water intake and only 25% of the extrarenal water loss (Table 1).

TABLE 1. Water balance of laboratory rat when fed on a standard diet yielding 400 cal/100 g when water is available and when water has been withheld for 96 hr

		Normal rats	After water has been withheld for 4 days
Food intake (g)		10·1 ± 0·32 (34)	0·25 ± 0·08 (30)
Water	From food: preformed (ml.)	1·3	0·03
	From food: metabolic (ml.)	5·3	0·13
	Drunk (ml.)	12·1 ± 0·23 (34)	0
Urine vol. (ml.)		3·5 ± 0·37 (34)	0·5 ± 0·28 (30)
Faeces (wet) (g)		2·8 ± 0·05 (34)	0·2 ± 0·05 (12)
Insensible water loss	By faeces (ml.)	1·8	0·05
	By evaporation (ml.)	13·2	5·76

All results (means and S.E.) are expressed per 100 g body wt./24 hr; in brackets, number or animals.

Day-to-day variations in the ionic urinary excretion were small. The Na excretion was 34·4 mg ± 0·47/100 g/24 hr; that of K was 24·9 mg ± 0·67/100 g/24 hr, with renal clearance values of 9·7 ± 0·36 and 100·7 ± 4·21 mg/100 g/24 hr for Na and K respectively. Ames & van Dyke (1950) found no antidiuretic activity in urine from five stock rats supplied with adequate food and water, though their method could detect an activity as low as 250 μu. With the method used in this investigation, the lowest antidiuretic activity that could be assayed was 5 μu./0·1 ml. urine (Dicker, 1953). As the total urinary volume obtained from a pair of rats was 15–20 ml./24 hr, values of antidiuretic activity below 750–1000 μu. could not be estimated. However, the urine of two pairs of our rats out of the seventeen pairs investigated had some antidiuretic activity: 900 and 1125 μu./24 hr. In nearly all our rats, therefore, the antidiuretic activity in the urine, if present at all, was below 150–200 μu./100 g/24 hr.

Estimations of the vasopressor and oxytocic activities of the excised pituitary glands gave the following values: (*a*) for the vasopressor activity: 150 m-u. ± 10/100 g (28), (*b*) for the oxytocic activity: 140 m-u. ± 9·5/100 g (26). These values were obtained from gland extracts of rats killed by sudden decapitation, and agree with those found by Ames & van Dyke (1950) and by Dicker & Tyler (1953). They are, however, lower than those of some other authors (Simon, 1934; Heller, 1941), who did not mention whether the animals were killed by decapitation or under anaesthesia. To see whether the amounts of

hypophyseal activities would be influenced by the administration of anaesthetics, a series of rats were killed while under the influence of ether, bromethol or urethane and their pituitary glands extracted and assayed. It was found that the vasopressor activity was greater in animals which had been anaesthetized with ether or bromethol than in rats killed by decapitation, though the administration of urethane following that of bromethol appeared to have the opposite effect (Fig. 1).

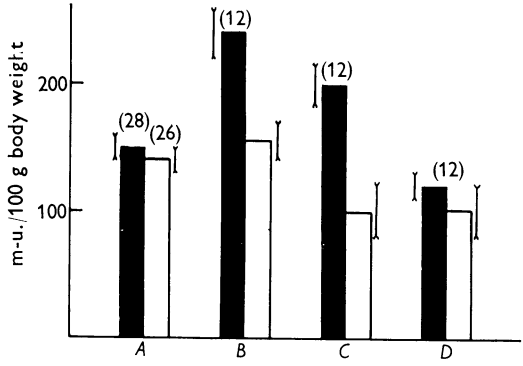

Fig. 1. Effect of anaesthesia on the vasopressor and oxytocic activities of posterior pituitary glands of rats: rats killed, *A*, by sudden decapitation; *B*, after ether anaesthesia (duration 20 min); *C*, after intraperitoneal injection of bromethol; *D*, after administration of bromethol followed by urethane—deep anaesthesia (duration 40–60 min). Black columns, vasopressor activity; white columns, oxytocic activity; results of estimations in m-u./100 g body weight; vertical lines, S.E.; no. of experiments in brackets.

Effects of restricted water supply

As the water balance, in rats on normal diet, seemed to vary within very narrow limits, it was of interest to see (*a*) how much the urinary volume would decline when water intake was restricted, and (*b*) whether a decrease of urinary volume produced by parenteral administration of vasopressin would influence the water intake.

Six series of six rats, kept in pairs in metabolism cages for 5 days, were allowed free food; water, however, was restricted to 7·5, 5·0, 3·0, 2·5, 2·0 and 1·0 ml./100 g/24 hr. Rats allowed amounts of water of 7·5 and 5·0 ml./100 g/24 hr accommodated themselves easily to it. There was a drop in the urinary volume during the first 24 hr, after which the urinary secretion continued without further marked decline at $2{\cdot}7 \pm 0{\cdot}55$ and $1{\cdot}9 \pm 0{\cdot}62$ ml./100 g/24 hr respectively. In rats allowed 7·5 ml. water/100 g/24 hr the Na and K excretion did not differ appreciably from that of rats drinking freely. Moreover, the amount of food eaten was approximately the same as that of control rats. As there was no appreciable loss of body weight, it would seem that the fall of 25 % of urine volume was the main response to the reduction of 25 % in the amount of water

drunk. In animals kept with 5·0 ml./100 g/24 hr, however, there was a loss of body weight particularly marked during the first 48 hr of observation. The food consumption fell from 10 g to about 6 g/100 g/24 hr.

When water supply was further decreased to 3·0 or 2·5 ml./100 g/24 hr, the urine excretion fell sharply at first and continued to decrease during the whole period of observation (Fig. 2). Na and K excretion followed the same trend. The Na secretion decreased from a mean of $24{\cdot}1 \pm 0{\cdot}89$ to $3{\cdot}4 \pm 0{\cdot}67$ mg/100 g/24 hr in 4 days; similarly, that of K fell from a mean of $18{\cdot}4 \pm 0{\cdot}82$ to 0·78 mg/100 g/24 hr, the bigger fall of Na suggesting some depletion of the extracellular fluid phase. The food intake was sharply cut down spontaneously, confirming Adolph's (1947) observations.

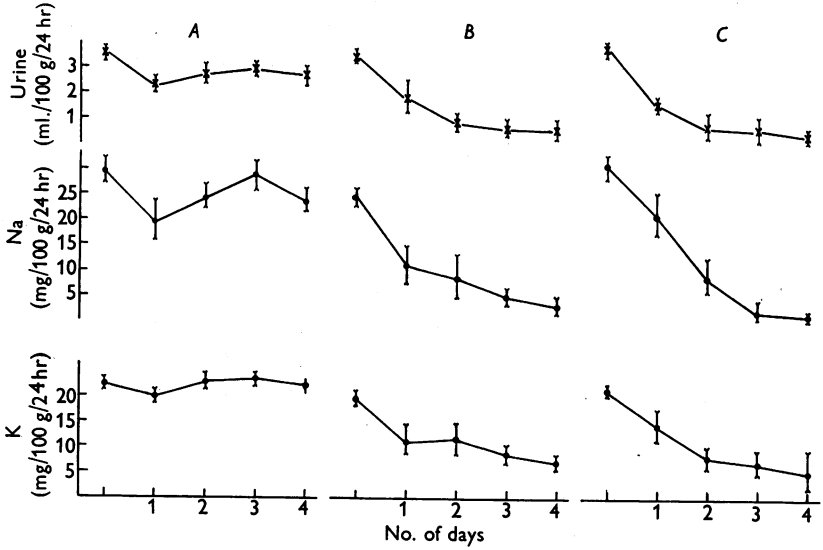

Fig. 2. Urinary secretion in rats kept on a restricted supply of water and in rats deprived of water for 96 hr. *A*, rats kept with 7·5 ml. water/100 g/24 hr; *B*, rats with 2·5 ml. water/100 g/24 hr; *C*, rats deprived of water. The first observation in the three groups represents the mean of four preceding days, when the animals had a free supply of food and water. Upper curve, urine volume; middle curve, Na excretion; bottom curve, K excretion. Results are means and S.E.

To discover whether a reduction of urine excretion produced by administration of vasopressin would help the rats when given a restricted supply of water, 10 m-u. vasopressin-tannate in oil/100 g body weight were injected subcutaneously twice a day into a series of six rats. The animals were either allowed to drink a fixed amount of water, or it was given by mouth one hour after each injection of vasopressin-tannate. Daily amounts of water allowed were 10·0, 5·0, or 3·0 ml./100 g/24 hr.

In rats given two oral doses of 5 ml. water/100 g, vasopressin-tannate had its

usual antidiuretic effect. The decrease of urine volume was accompanied by a decreased excretion of both Na and K. There was no increase of body weight. This was probably due to a spontaneous reduction of food intake of the order of 20%. Cessation of the administration of vasopressin was always followed both by an increased urinary excretion and a decrease of body weight. In contrast with these results, vasopressin-tannate had no antidiuretic effect in rats which were allowed to drink according to their needs a fixed volume of 10 ml. water/100 g/24 hr, i.e. when the rats drank the same volume spread over 24 hr. No explanation of this discrepancy can be offered, beyond the fact that as animals drink mostly at night, the antidiuretic effect of vasopressin-tannate may have been declining.

In rats given 5·0 or 3·0 ml. water/100 g/24 hr, whether given by stomach tube or not, vasopressin-tannate had no antidiuretic effect: rates of water, Na and K excretion were indistinguishable from those observed in non-injected rats allowed similar volumes of water.

Effects of complete withdrawal of water

Some of the observations now reported were made more than seven years ago on a series of rats kept with food but no water, for up to 7 days. As the observations then made agreed with those of the present series of rats, they have been treated together. It must be emphasized that in no case was it possible to observe rats which were deprived of water only: as soon as water was withheld, animals restricted their food intake voluntarily. In Schmidt-Nielsen & Schmidt-Nielsen's (1951) experiments four rats were kept without water for 9 days; the animals were fed on pearl barley, and the amounts ingested increased from 37·7 to 96·8 g. Such an increase never occurred in our experiments. After 48 hr without water, the food consumption had always declined to about one-quarter and after 4 days of water deprivation the food consumption was only one-fortieth of that of control rats. For the last 3–4 days, therefore, our rats were not only dehydrated but also practically starved. As normally 6·5 ml. water/100 g/24 hr was derived from food, self-imposed starvation resulted in an even more acute water shortage; the metabolic + preformed water was only 0·16 ml. water/100 g/24 hr in animals observed for 4 days (Table 1).

As soon as water was withheld, there was a sharp fall of body weight, and both water excreted by urine and water lost by evaporation and faeces decreased. Had the insensible water loss remained at 15 ml. water/100 g/24 hr, as in the control rats, it can be calculated that the loss of body weight would have been of the order of 60% in 5 days, whereas it was only 25%. Some idea of the degree of reduction of extrarenal water loss can be gauged from the following facts: the weight of faeces fell from 2·8 g ± 0·05 in control rats to 0·15 g ± 0·06 (8)/100 g/24 hr. The water content of the faeces decreased similarly from 65 ml. ± 0·3/100 g to 25 ml. ± 10·0/100 g. Thus the water lost through

the faeces had fallen from 1·8 ml. to 0·04 ml./100 g body weight/24 hr, a decrease of 98% in 4 days. The amount of water lost through evaporation, derived from changes of body weight and from the water lost through urine and faeces, followed the same trend. It will be seen from Fig. 3 that after thirsting for 24 hr, the water lost through evaporation had fallen from 13·2 to 7·7 ml./100 g/24 hr; by the end of 4 days it was down to 5·8 ml./100 g/24 hr; i.e. the water lost through evaporation fell by 46% (Fig. 3).

The urine excretion decreased from a mean of 3·5 ml. to 0·5 ml. ± 0·25 (36)/100 g/24 hr, in 3 days, after which it did not change much (Fig. 4 *A*). The initial decrease of urine flow was accompanied by a decrease of ion excretion (Fig. 2 *C*).

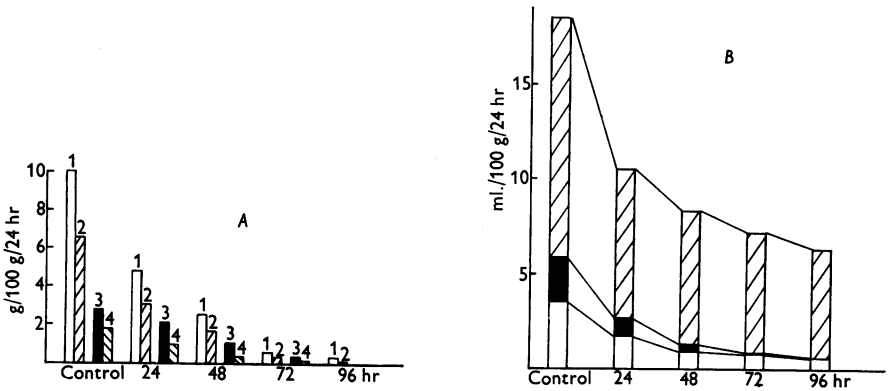

Fig. 3. Decrease of food intake and of insensible water loss related to urine excretion in rats deprived of water. *A*: food and faeces; 1, amount of standard food eaten/100 g body wt./24 hr; 2, total water content of food (moisture + metabolic water) in ml./100 g/24 hr; 3, weight of wet faeces/100 g body wt./24 hr; 4, water content of faeces in ml./100 g/24 hr. *B*: water loss, white column, urine; black column, water from faeces; hatched columns, loss of water by evaporation; all results in ml./100 g body wt./24 hr.

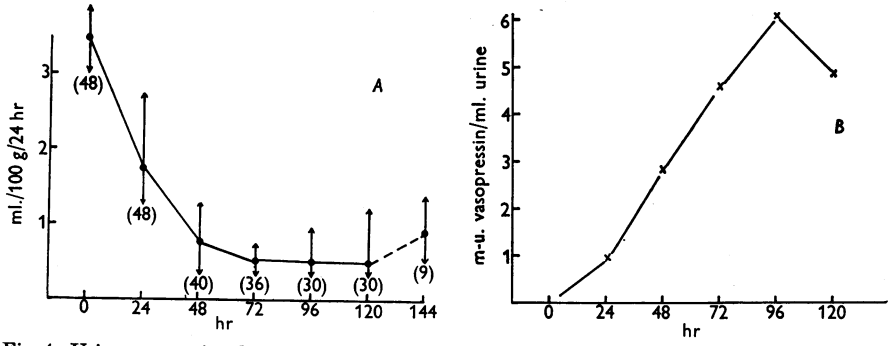

Fig. 4. Urinary secretion during a prolonged period of water deprivation. *A*: urine volume; results are means and range; in brackets, no. of animals; note the increase of urine excretion after the fifth day. *B*: Mean excretion of antidiuretic activity in m-u. vasopressin per ml. urine excreted in forty-eight rats (see fig. 5).

However, from the third to the sixth day of water deprivation, the amounts of Na and K excreted continued to decrease regularly though the urine flow remained more or less unaffected. This may be due partly to the self-imposed restriction of food consumption. In this respect, it will be remembered that normal rats fed on diets free from Na and K excrete a urine devoid of these ions (Elkinton & Danowski, 1955).

It is of interest to note that in about 25 % of the rats there was an increase of the urine flow towards the end of the water deprivation. This was specially evident in animals kept without water for more than 5 days (Fig. 4 *A*). When it occurred there was a fall in the osmolarity of the urine, as measured by depression of freezing point.

To see whether the decreased urine flow followed passively the restriction of water intake or whether it was promoted actively by an enhanced secretion of antidiuretic hormone, the urine of forty-eight rats deprived of water was collected and assayed for its antidiuretic activity. Gilman & Goodman (1937) had found an increase of antidiuretic activity in urine samples from rats kept without water, and claimed that the antidiuretic activity of the urine of rats thirsting 72 hr was equivalent to 100 m-u. vasopressin/ml. urine. Ames & van Dyke (1950), however, showed that the concentration of antidiuretic activity in thirsting rats did not exceed 6·0 m-u. vasopressin/ml. In the present series of experiments it was found that from the first to the fourth day there was a steady increase of the antidiuretic activity in the urine, the maximum concentration of about 6·0 m-u./ml. being reached after 4 days (Fig. 4 *B*). This confirmed Ames & van Dyke's (1950) observations. The total amount of antidiuretic activity excreted by the urine of animals deprived of water for 5 days was of the order of 12 m-u./100 g body weight (Fig. 5). Assuming that (1) the antidiuretic activity of the urine was of neurohypophyseal origin, and (2) that some 10 % of the neural secretion was excreted in the urine (Ginsburg & Heller, 1953; Dicker, 1954), this would represent a release of antidiuretic hormone from the posterior pituitary lobe of about 120 m-u./100 g. To test this assumption, rats were decapitated after 48, 96 and 120 hr of water deprivation and both the oxytocic and vasopressor activities of their neural lobes estimated. It was found, in agreement with Ames & van Dyke's (1950) observations, that up to 48 hr there was an increase of the mean vasopressor activity from 150 to 215 m-u./100 g without a parallel increase of the oxytocic activity. From 48 hr onwards, however, there was a steady decrease of both the oxytocic and the vasopressor activities. At the end of the 5th day, the mean amount of vasopressor activity was 10 m-u. $\pm$ 2·2 (12) and that of the oxytocic activity 5 m-u. $\pm$ 1·7 (12)/100 g body weight, showing that there had been a loss without simultaneous replacement of vasopressor activity of the same order as that forecast by the amount of antidiuretic activity found in the urine (Fig. 6).

In spite of the belief that there is a relation between urine flow and the

presence or absence of antidiuretic activity in the urine, it was clear from these experiments that such a correlation did not exist in rats deprived of water. This absence of causal relation between vasopressin and urine flow, during water deprivation, was further suggested by the following experiments. Rats deprived of water were injected subcutaneously with vasopressin-tannate in oil, alone or enriched with oxytocin. To achieve some form of replacement therapy the daily amounts of vasopressin administered in two doses were equal to 10 times the amounts of urinary antidiuretic activity. When mixed with

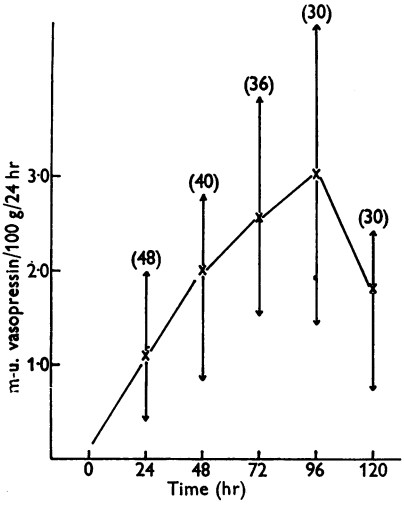

Fig. 5

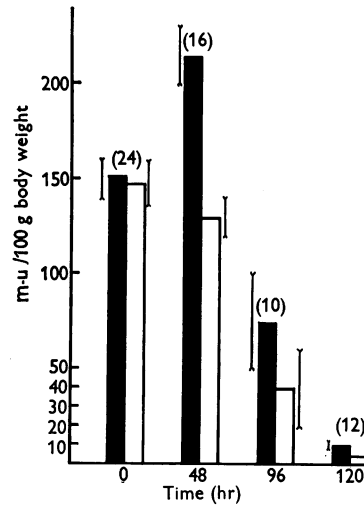

Fig. 6

Fig. 5. Urinary excretion of antidiuretic activity in rats deprived of water for 120 hr; results are means and range; in brackets, number of urine samples assayed.

Fig. 6. Vasopressor and oxytocic activities found in pituitary glands of rats deprived of water for 120 hr. In black, vasopressor-antidiuretic activity; in white, oxytocic activity; in brackets, number of glands extracted (values for 120 hr were obtained by extracting pooled glands); vertical lines beside columns are S.E.

oxytocin, the concentration of oxytocin was 20 times that of vasopressin (Abrahams & Pickford, 1954). Twelve rats were injected with vasopressin-tannate alone, and twelve with vasopressin-tannate and oxytocin. Their urine was measured daily for 5 days; at the end of the experiment, the animals were decapitated and the vasopressor and oxytocic activities of their neurohypophyses were estimated. No difference could be found between the non-injected and the injected rats, whether treated with vasopressin alone or with vasopressin and oxytocin: the volumes of urine secreted and its ionic composition as well as the level of vasopressor and oxytocic activities of the posterior pituitary glands could not be differentiated from those observed in other series of untreated thirsting rats.

DISCUSSION

When fed on diets of the same composition, the water balance of rats is remarkably constant. Tennent (1945) has shown that in the rat the water lost by evaporation is lost through the skin and through the respiratory tract in approximately equal proportions. Heller & Smirk (1932) were the first to emphasize how surprisingly large is the insensible water loss in the rat. They assessed it at 5·67 ml./100 g body weight/3 hr. However, in a later publication (1947) Heller estimated the extra-renal water loss of adult rats, kept at 20/21° C as 0·45 g/100 g/hr, and 2 years later the same author showed that in rats, kept without food or water for 24 hr at 20/21° C, the extrarenal water loss had decreased to 0·23 g/100 g/hr (Heller, 1949). Taking into account the alimentary water (metabolic and preformed), as well as the water drunk, the insensible water loss of the present series of rats has averaged 0·60 g/100 g/hr, a figure which agrees with that given by Schmidt-Nielsen & Schmidt-Nielsen (1950) for rats observed at about 20% relative humidity. It must be stressed that the relative humidity of the air was not estimated in the present experiments, nor in those of Heller (1947, 1949).

The degree to which mammals can concentrate their urine is closely related to that of survival from water depletion. For example, *Dipodomys merriami*, the kangaroo rat, is said to be able to live without any access to water; it can excrete a more concentrated urine than any other mammal. The laboratory rat can concentrate its urine much further than man; thus it survives longer than man when deprived of water. In other words, it would appear that the ability to excrete a highly concentrated urine is an important factor in the process of water conservation. This, however, seems to be only one aspect of the problem. As soon as water has been withheld from rats their food consumption declines; this itself involves a substantial further loss of water intake. Conversely, rats starved but with free access to water reduce their water intake (Dicker, 1949). Had the amount of food eaten by the dehydrated rats remained constant, as in Schmidt-Nielsen & Schmidt-Nielsen's (1951) experiments, there would have been a constant water intake (preformed and metabolic) of some 16 ml., and hence no decrease of urine flow: the water required to excrete a urine with a maximum urea concentration of 14 g/100 ml. would have been of the order of 5·0 ml. per 100 calories of food only, leaving twice that amount available for other metabolic functions. However, according to the present findings, and in agreement with Schmidt-Nielsen & Schmidt-Nielsen's results, the volume of water lost through evaporation and defaecation in the rat is about four times as great as that secreted by the kidneys, whereas in the kangaroo rat the ratio: insensible water loss/urine excretion is only 1·5:1. This is clearly shown in Table 2, which has been computed from Schmidt-Nielsen & Schmidt-Nielsen's (1951) results and from which it will be seen that even if the rat could con-

centrate its urine to the same extent as does the kangaroo rat, its only hope of survival would lie in its ability to cut down its insensible water expenditure. Schmidt-Nielsen & Schmidt-Nielsen (1951) have calculated that this would be achieved if the atmospheric humidity were raised to 20–22 mg. H_2O/litre of air (i.e. close to 100 % relative humidity at 25° C).

In the present investigation the food intake of rats deprived of water was severely reduced. This may have been advantageous to their water economy. As a result of self-imposed starvation, the water content of faeces was reduced to such an extent that it produced an over-all saving of water of about 98 %. Furthermore, there was a marked reduction of water lost through evaporation.

TABLE 2. Comparison of water metabolism of kangaroo rat and white rat when fed on iso-calorific diets but no water

Water loss	Kangaroo rat (ml.)	White rat (ml.)
Urine	3·4	5·1
Evaporation	4·6	19·2
Faeces	0·6	3·4
Total	8·6	27·7

The animals were fed on 25 g of pearl barley (=100 cal) without water to drink; the water yield of 100 cal of pearl barley is 13·4 ml. The data are computed from Schmidt-Nielsen & Schmidt-Nielsen (1951).

Thus the over-all effect of the self-imposed starvation was to reduce the insensible water expenditure by some 40 %, which is roughly equivalent to the amount of water the animal would have saved had it been eating in atmospheric humidity of 100 %. It is of interest to note that this decrease of the insensible water loss is of the same order of magnitude as that achieved normally by desert rodents, such as *Dipodomys merriami*, the kangaroo rat, by *D. spectabilis*, the pocket mouse or by *Perognathus* spp. (Gjönnes & Schmidt-Nielsen, 1952). This reduction, however, was obtained only gradually and its effect was obviously cancelled out by the precarious state of health of our rats.

The remarkable fact that the decline in urine flow is no greater after 5 days than after 3 days of water deprivation requires explanation. There is an increase of the antidiuretic activity of the urine of rats during water deprivation (Gilman & Goodman, 1937; Ames & van Dyke, 1950); there is more antidiuretic activity in the urine of the kangaroo rat than in that of the laboratory rat even after a prolonged water deprivation (Ames & van Dyke, 1950); the posterior pituitary gland of the kangaroo rat contains more antidiuretic hormone than that of the ordinary rat (Ames & van Dyke, 1950). These observations have been explained by supposing that the low urine flow of dehydrated animals is due to the antidiuretic hormone. However, the camel can withstand a much more severe degree of dehydration than most mammals, but the total amount of antidiuretic hormone in the neurohypophysis is about

the same as that found in oxen (Adamsons, Engel, van Dyke, Schmidt-Nielsen & Schmidt-Nielsen, 1956).

The consequences of water deprivation, as described above, involve an initial rise and subsequent decline in the antidiuretic activity of the neurohypophyseal gland, together with progressively increasing amounts of antidiuretic activity in the urine. This might suggest that almost all the neurohypophyseal antidiuretic activity had been expended in an attempt to decrease the loss of water by the urine. But this could only be so if vasopressin has, in fact, an antidiuretic action in hydropenic animals. It is well known, however, that vasopressin has no antidiuretic effect when the urine is concentrated; moreover, it has recently been shown that a desert mammal, *G. gerbillus*, which can concentrate its urine to the same extent as the kangaroo rat, is insensitive to the parenteral administration of vasopressin, whether the animal has been hydrated or not (Burns, 1956). This observation corresponds with that described above on normal and thirsting rats, in which the administration of vasopressin-tannate had no effect on their urinary excretion. This raises the question whether the actual mechanism of renal concentration of urine as observed in desert animals or in hydropenic rats is not entirely independent of the action of the antidiuretic hormone, which may be confined to producing antidiuresis only when the urine is dilute. Evidence in favour of this hypothesis will be given in a following paper.

If, however, there is no causal relationship between the enhanced excretion of antidiuretic activity and the increased urinary concentration observed in thirsting animals, why is it that the neural lobe should be emptied of its hormonal activity? It may be that the antidiuretic hormone has extrarenal effects. Friedman, Friedman & Nakashima (1956) have shown that vasopressin decreases the extracellular space and that this process is iso-osmotic. Another possibility is that the antidiuretic hormone exerts a pressor effect. Considering that the urine of rats deprived of water for 48 hr contains antidiuretic activity of 3–4 m-u., corresponding to a secretion of 30–40 m-u. from the neural lobe, it is not impossible that the neurohypophyseal secretion has had a pressor effect and has contributed thereby indirectly to a reduction in the rate of evaporation.

As for the low urinary secretion observed during hydropenia, it is likely that it is regulated solely by the excretion of solutes in a manner similar to that observed during an osmotic diuresis. The action of the osmotically active load which is increased by the process of catabolism accompanying starvation is very likely restricted by a decrease of the glomerular filtration rate. A true osmotic diuresis may however occur during the final stages of severe and prolonged hydropenia, and account for the terminal increase in urine flow shown in some animals (Fig. 5).

SUMMARY

1. In normal rats the water lost by evaporation and in the faeces is five times as great as that excreted in the urine.

2. When deprived of water, rats reduce their intake of food and thus their absorption both of 'preformed' and metabolic water. The water lost in the faeces as well as by evaporation also declines.

3. The urine flow of rats deprived of water falls sharply during the first 24 hr, but remains more or less stable from the 3rd day onwards. The maximum concentration of the urine is reached after 48 hr. The excretion of ions decreased progressively during the whole period of observation.

4. Parenteral administration of vasopressin-tannate in oil has no effect on the urinary excretion either of normal rats with free access to food and water or of rats kept without water.

5. A steady increase of antidiuretic activity in the urine follows water deprivation, and its maximum concentration is reached in 4 days.

6. In rats deprived of water the vasopressor activity of posterior pituitary glands first increases and subsequently declines.

7. Arguments are presented to show that the decrease of urine secretion during water deprivation is not controlled by the neurohypophyseal hormone.

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