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THE EFFECTS OF SLOW INFUSIONS OF HYPERTONIC SOLUTIONS ON DRINKING AND DRINKING THRESHOLDS IN RATS

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Wolf (1950) measured the threshold of thirst in man by infusing hypertonic NaCl intravenously and noting the amount which caused the subject to become thirsty. He performed similar experiments on dogs, observing when the animals started to drink. Only NaCl was tested and the possible effect of the rate of infusion on threshold was not studied. Making the assumption that the cells of the body behave as perfect osmometers he concluded that cellular shrinkage of between 1 and 2% of initial cellular size would cause thirst.

In the present infusion experiments on rats several substances were tested, the effect of the rate of infusion noted, and the thresholds of normal and nephrectomized rats compared, because it has been shown previously that normal rats drink less than nephrectomized rats in response to the same stimulus (Fitzsimons, 1961).

A second group of experiments, in which access to drinking water was prevented for up to 24 hr after injection of hypertonic NaCl, was performed in order to obtain information on whether the drinking receptor adapts.

METHODS

Infusion procedure with nephrectomized rats

Albino rats of both sexes, weighing between 150 and 400 g, were prepared for infusion by catheterizing the external jugular vein and by removing both kidneys. These surgical procedures were carried out under open ether anaesthesia and have been described previously (Fitzsimons, 1961).

About 1–1½ hr later the rat was weighed to the nearest 0·1 g and placed in an individual metabolism cage. The jugular catheter was connected by means of a 15 cm length of metal hypodermic tubing, outside diameter 1 mm, to 1–1½ m of polyvinyl chloride tubing, which in turn was connected to a motor-driven syringe. The hypodermic tubing projected out of the metabolism cage and was to frustrate attempts by the rat to chew through the tubing. The polyvinyl chloride tubing was out of reach of the animal but it allowed the animal freedom to move about its cage. The rat remained connected to the tubing throughout the experiment. Water was available from a meter which gave a continuous record of drinking (Fitzsimons, 1958), but food was not available.

When the rat had settled down in its cage, usually 5–15 min after weighing, the infusion

was started at rates of between 3·5 and 14·2 μl./100 g body weight/min and it was continued for between 18 and 245 min. The rate at which solute was administered was regulated by varying the concentration and rate of the infusion. Solutions infused were: 0·154, 0·3, 0·5, 1·0 and 2·0M sodium chloride, 0·1, 0·2, 0·5 and 1·0M sodium sulphate dissolved in 0·154M sodium chloride, 0·5 and 1·0M sucrose dissolved in 0·154M sodium chloride and 2·0M urea dissolved in 0·154M sodium chloride. The hypertonic infusions were generally followed by infusion of 0·154M sodium chloride to wash through the small amount of hypertonic solution remaining in the catheter.

Threshold of drinking was taken to be the point at which the animal started to drink enough water to produce measurable deflexion on the drinking record (about 0·25 ml.). A few rats took sips of water before the onset of sustained drinking, but these sips were too small to be recorded and were regarded as random drinking not caused by the infusion. In experimental rats it was always perfectly obvious when the infusion was the cause of drinking because the animals would refuse to be distracted from drinking. Typical records of drinking are given in Fig. 1. In some experiments the hypertonic infusion was stopped when the animals started to drink, in others it was allowed to continue.

The experiment was terminated with a second weighing of the rat 6 hr after the first, this period allowing the rat at least 100 min after cessation of the infusion to complete any drinking caused by the substance.

The thresholds of drinking are expressed as the percentage increases in initial osmolality at which drinking started, and were calculated for each animal as follows, assuming the initial osmolality of body fluid to be 290 μ-osmole/g H_2O and body water to be 69 g/100 g initial body weight (Fitzsimons, 1961)

$$100\left(\frac{290\times 69+\text{solute load}}{290(69+\text{water load})}-1\right).$$

The solute load just before drinking started was expressed in μ-osmole/100 g initial body weight, using osmotic coefficients of 0·93 for NaCl, 0·82 for Na_2SO_4 and 1·0 for sucrose and urea. The water load at threshold is the difference between the weight of water infused and the evaporative water loss at that time and was expressed in g/100 g initial body weight. The evaporative loss was obtained from the difference between the total amount of water drunk and the change in weight of the whole animal in 6 hr, excluding the weight of the infusion. The rate was assumed to be uniform throughout the experiment.

The standard error of the mean increase in percentage osmolality is due almost entirely to variations in water and solute loads, the standard errors of initial osmolality and initial body weight being very small by comparison. The effects of variations in initial osmolality and body water were not therefore taken into account when calculating individual percentage increases in osmolality.

Procedure with normal rats

The procedure with normal rats was essentially the same as with nephrectomized rats, except that in addition urine was collected within a few minutes of the onset of sustained drinking and also at the end of the experiment, the volumes measured and the Na and K contents determined by flame photometry. The water load at threshold is the difference between the weight of water infused and the weight of water lost as urine and through the lungs and skin at that time. The evaporative loss was calculated from the 6 hr balance figures and the rate was assumed to be uniform throughout the experiment. Solute load at threshold was obtained from the difference between the infused Na and the excreted Na and K. Potassium, the principal intracellular cation, was taken into account because Mudge, Foulks & Gilman (1950) found that potassium excretion can be directly correlated with cellular dehydration, though Wolf (1950) ignores it in his calculations of solute load.

Temporary withholding of drinking water

Experiments similar to those described previously (Fitzsimons, 1961) in which drinking water was temporarily withheld after administration of hypertonic NaCl to nephrectomized rats, were also carried out, but water was withheld for up to 24 hr after injection and the weight changes in 30 hr measured. The animals were not allowed food in this time.

RESULTS

Records of drinking during infusion

Examples of records of drinking in nephrectomized rats after infusion of different concentrations of NaCl or Na_2SO_4 are given in Fig. 1. In three of the experiments (*b*, *c*, *d*) the infusions were stopped when drinking started, in the others (*a*, *e*, *f*) the infusions were allowed to continue after the onset of drinking. The patterns of drinking are typical of all animals infused with hypertonic solutions. Once the rat had started to drink it would continue drinking more or less uninterruptedly until it had taken the bulk of its fluid requirements. During this time if the drinking water were removed and then replaced the rat would start drinking again immediately, and indeed it was generally impossible to prevent the animal from drinking by any other means. The onset of drinking was therefore taken as evidence that the animal was thirsty, and from the water load and amount of solute infused at this time the thirst thresholds were calculated. The results for these animals are given in Table 1 and full results for all animals are given in the next section.

Table 1, column 5, also gives the observed increase in weight (excluding the weight of the infusion) of the six rats at the end of the 6 hr period. The change in weight of the rat is used as a measure of the fluid balance instead of the amount of water drunk, because in 6 hr considerable amounts of water are lost through the lungs and skin and are not therefore available to dilute the solute. The amount of water drunk covers both the gain in fluid by the animal and also the evaporative loss. Column 6 of Table 1 shows the weight of water needed to dilute the excess solute to isotonicity and so is the increase in weight which might be theoretically expected.

The threshold of drinking of normal and nephrectomized rats

The mean increase in osmotic pressure at which ten normal rats infused with 1 M-NaCl started to drink was $1{\cdot}6 \pm 0{\cdot}11$ % (S.E. of the mean) (Fig. 2). Six control rats infused with 0·154 M-NaCl did not drink in 6 hr.

The results for nephrectomized rats were as follows. Hypertonic Na_2SO_4 and NaCl were almost equally effective in causing nephrectomized rats to start drinking. The mean threshold of twenty-two rats infused with Na_2SO_4 was $1{\cdot}97 \pm 0{\cdot}25$ %, and of forty-seven rats infused with NaCl

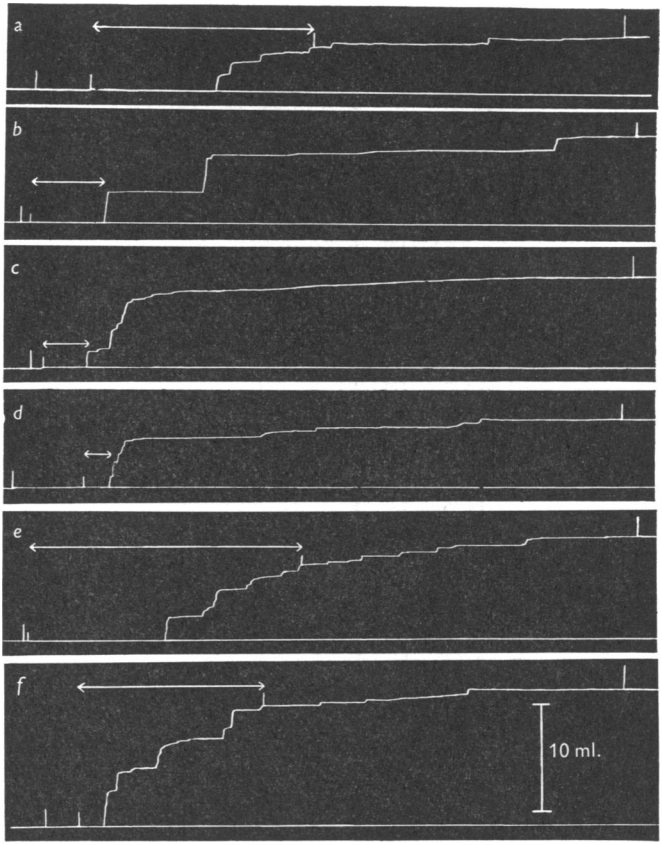

Fig. 1. Records of drinking after infusion of various concentrations of NaCl and Na_2SO_4 into nephrectomized rats. The durations of the infusions are represented by the double-ended arrows. The interval between the first and last marks on each record is 6 hr and the vertical calibration on record *f* applies to all records. The amounts and concentrations of the infusions and other results for these animals are given in Table 1.

TABLE 1

	(1) Initial wt. of rat (g)	(2) Nature and concentration of infusion	(3) Drinking threshold (% increase in osmotic pressure)	(4) Total volume infused (ml.)	(5) Observed increase in wt. at 6 hr (g)	(6) Water needed to dilute excess solute to isotonicity (g)
a	237·8	0·3 M-NaCl	1·95	4·2	2·4	3·9
b	314·2	1·0 M-NaCl	1·87	0·65	4·3	3·5
c	330·3	1·0 M-NaCl	0·93	0·32	4·3	1·7
d	268·4	2·0 M-NaCl	1·89	0·3	2·4	3·5
e	284·5	0·1 M-Na_2SO_4	1·43	4·0	5·4	3·4
f	312·3	0·5 M-Na_2SO_4	0·86	2·0	8·2	8·5

was $1{\cdot}98 \pm 0{\cdot}15\,\%$. Two of the twenty-four rats infused with substantial amounts of Na_2SO_4 only drank a considerable time after the infusion, and three of fifty rats infused with substantial amounts of NaCl did not drink at all. The results from these animals have been excluded from the above mean figures, because it appeared that these animals were unduly upset by the infusions.

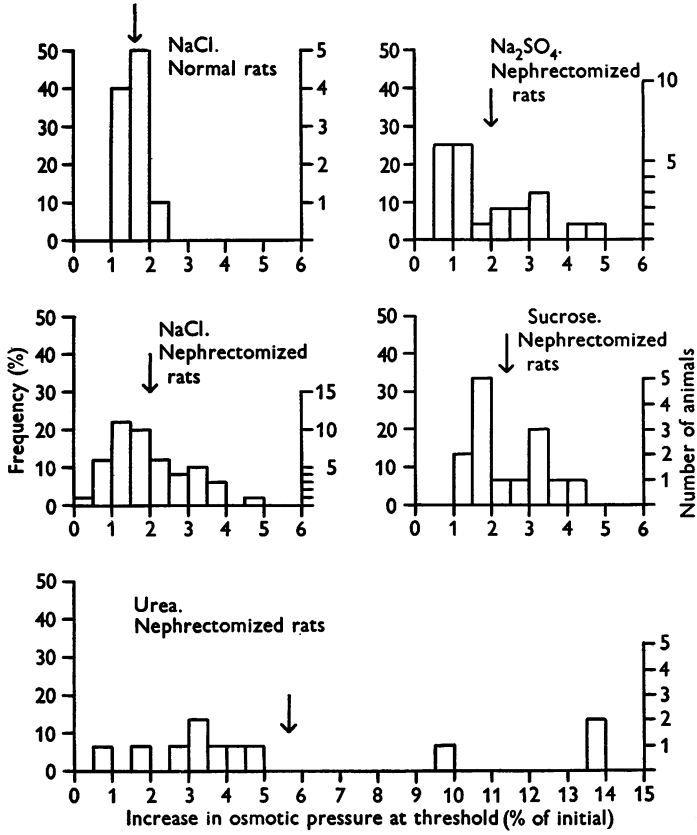

Fig. 2. Histograms of frequency distribution of drinking thresholds in steps of 0·5 % increase in osmotic pressure. The arrows show the mean value for each group.

The sucrose threshold of $2{\cdot}37 \pm 0{\cdot}26\,\%$ was rather higher; one of the fifteen rats infused drank a considerable time after the infusion and the result is therefore not included in the mean value. Finally, the mean threshold of eleven out of fifteen rats which drank during infusion of hypertonic urea was $5{\cdot}65 \pm 1{\cdot}38\,\%$.

On the other hand 0·154 M-NaCl was ineffective in causing nephrectomized rats to drink, since six out of fifteen animals infused did not drink at all in 6 hr and only two drank during the infusions themselves, despite

the fact that the isotonic infusions lasted considerably longer than the hypertonic infusions. Those control animals that did drink took rather small amounts of water so that this group of animals as a whole lost weight (the controls of Fig. 4). Another control group of twelve nephrectomized rats, which was not infused and which was left until all the animals in the group had drunk, lost a mean of 0·9% body weight, representing an increase in osmotic pressure of $1{\cdot}32 \pm 0{\cdot}32$% (S.E. of mean), at the start of drinking. The mean delay before the onset of drinking in this group was just over 5 hr, considerably longer than the mean delays of about 1 hr after infusion of hypertonic Na_2SO_4 or NaCl.

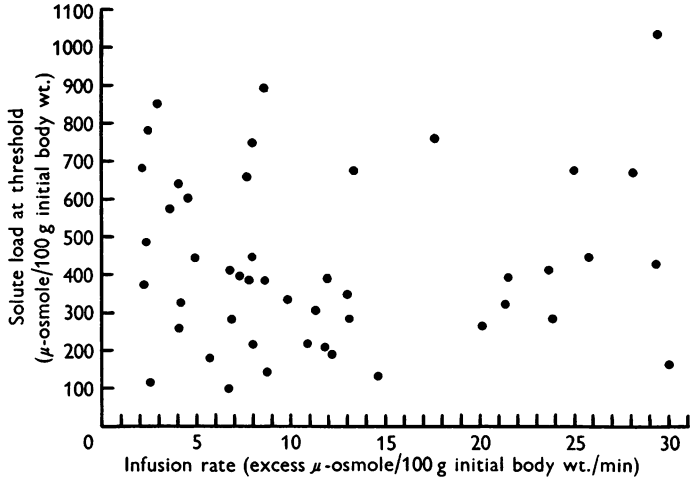

Fig. 3. The relation between the infusion rate of NaCl in nephrectomized rats and the solute load at the threshold of drinking.

With the range of infusion rates used here, the rate of infusion did not appear to be an important factor determining threshold. This is shown in Fig. 3, where the amounts of NaCl infused at the onset of drinking are plotted against the rates of infusion.

Comparison of the effects of rapid injection and slow infusion of hypertonic solutions on the fluid balance of nephrectomized rats

The changes in weight of nephrectomized rats in 6 hr experiments in which they were rapidly injected or slowly infused with hypertonic solutions and allowed to drink are plotted against the amounts of solute given in Fig. 4. The results on rapid injections are from a previous paper Fitzsimons, 1961) with some additional measurements.

The responses to rapid or slow administration of solute were essentially the same for the four substances tested. After Na_2SO_4 and NaCl isotonicity

was restored by drinking, sucrose was less effective and urea least effective. The agreement between the changes in weight of nephrectomized rats rapidly injected or slowly infused with the same amount of solute shows that the infusion procedure was well tolerated by the rats.

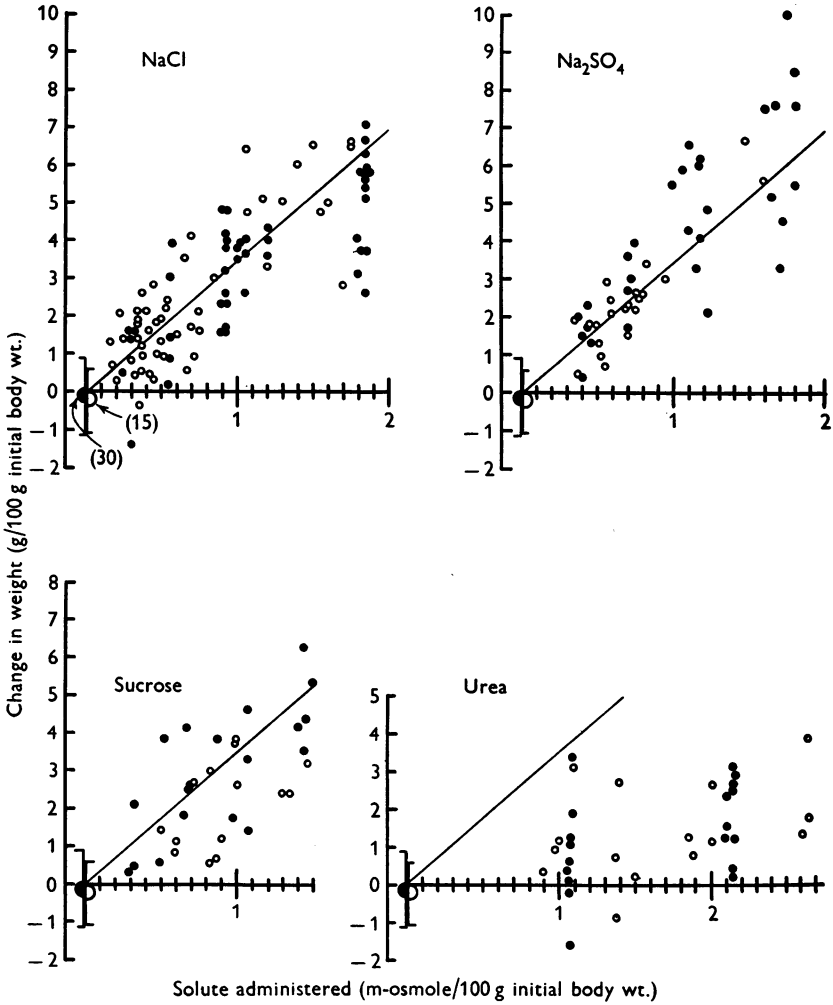

Fig. 4. The relations between the amounts of different solutes administered by rapid injection (●) or by slow infusion (○) and the changes in weight of nephrectomized rats in 6 hr. Each point is one observation except the controls which are mean values ± S.D. with the number of observations in parentheses. The controls were given 0·154 M-NaCl. The solute administered excludes the 0·154 M-NaCl needed to bring the injection volume to isotonicity. The sloping lines delineate the changes in weight required to dilute the solutes to isotonicity.

The effect of withholding drinking water after rapid administration of hypertonic sodium chloride

The withholding of drinking water for 12 and 24 hr after injecting hypertonic NaCl did not alter the amount of water drunk subsequently, compared with the amount drunk when access to drinking water was allowed immediately after the injection. The changes in weight at the end of 30 hr of the immediate and the delayed drinkers were the same (Fig. 5).

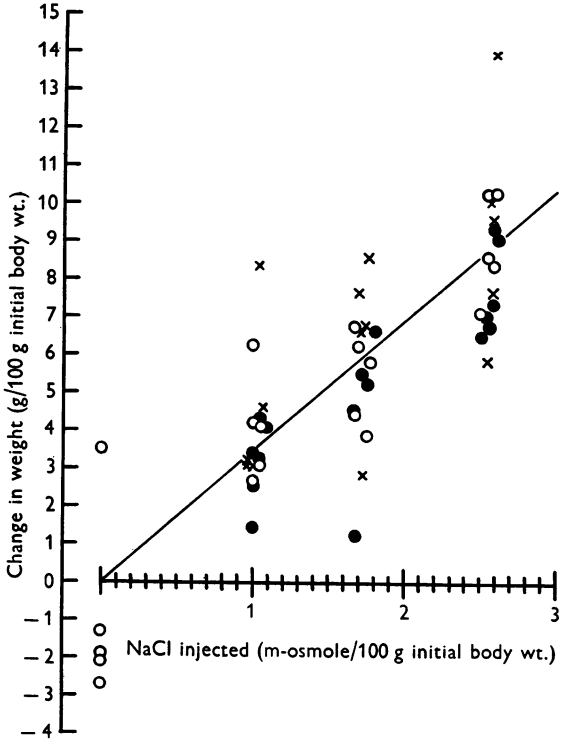

Fig. 5. The changes in weight in 30 hr of nephrectomized rats allowed to drink immediately (○), 12 hr (×) and 24 hr (●) after injection of NaCl. Each point is one observation. The NaCl injected excludes the 0·154 M-NaCl needed to bring the injection volume to isotonicity. The controls were given 0·154 M-NaCl. The sloping line delineates the change in weight required to dilute the excess NaCl to isotonicity.

DISCUSSION

The present results with slowly infused substances demonstrate what has been shown previously for the same substances rapidly injected, namely, that the nephrectomized rat can dilute hypertonic Na_2SO_4 and NaCl to isotonicity by drinking. The result is interesting because the

increase in osmotic pressure produced by slow infusion is similar to the gradual rise that occurs naturally due to a continuing loss of body water.

The measurements of drinking thresholds given here show how sensitive the thirst mechanism is to small solute loads. The idea that increase in effective osmotic pressure is the drinking stimulus is implicit in the manner of expressing thresholds. However, though increase in effective osmotic pressure causes cellular shrinkage, it is simpler to express results as increases in osmotic pressure rather than as Wolf (1950) did, in terms of supposed cellular shrinkage.

The mean NaCl threshold in ten normal rats was 1·6 %. Wolf's (1950) figures for dog and man, recalculated on the same basis as those for rat, though excluding the effect of excreted potassium on solute load for which no figures are given, represent increases in osmotic pressure of 2·8 and 1·2 %, respectively.

The use of nephrectomized rats removed the uncertainty in calculating solute load at the start of drinking because the amount infused is the solute load. Taking water loads into account, the mean increases in osmotic pressure in nephrectomized rats at threshold were about 2 % for Na_2SO_4 and NaCl, 2·4 % for sucrose and 5·6 % for urea. This ranking is the same as that of the generally accepted osmotic properties of the solutes in relation to cells of the body. It is also the same as the effects of these substances on final fluid balance.

The mean threshold of twelve uninfused nephrectomized rats which were left until they drank was 1·3 %, which is a little less than the Na_2SO_4 and NaCl thresholds. The fact that control animals if left long enough do start to drink justifies taking evaporative water loss into account when calculating thresholds. In the animals which received hypertonic infusions the major part of the increase in osmotic pressure was due to infused solute because the intervals between the first weighing and the onset of drinking were rather short compared with the intervals in control animals.

The thresholds of control nephrectomized rats and the thresholds of nephrectomized rats infused with Na_2SO_4, NaCl and sucrose compare well with the NaCl thresholds in normal rats, dogs and men, all values lying between 1·2 and 2·8 % increase in initial osmotic pressure. They also compare well with the few and incomplete data on dehydration thresholds in man and dog. Thus losses of about 2 % body weight in man (Adolph, 1947) and about 0·5 % body weight in dog (Robinson & Adolph, 1943) cause thirst and drinking. Increases in osmotic pressure resulting from such losses cannot be calculated, because no data on the excretion of solutes were given, but the increases were certainly of the same magnitude as in infusion experiments.

The present experiments provide additional evidence that the drinking receptor does not adapt when exposed to slowly changing stimuli, because threshold of drinking was not affected by different infusion rates and the amounts of water subsequently drunk were the same as after rapid injections of the same amount of solute. Furthermore, deprivation of drinking water up to 24 hr after injection of hypertonic NaCl did not affect the amount of water drunk subsequently. On the other hand Adolph (1947) reported that the rate of dehydration alters the thirst threshold in man, though in dog Robinson & Adolph (1943) found no evidence that rate of dehydration influenced threshold, which agrees with the results in Fig. 3. It has also been suggested that the thirst which may occur in hyponatraemia and the occasional absence of thirst in hypernatraemia may be due to accommodation (Wolf, 1958). However, these states are abnormal and for a receptor which normally has to respond to a gradual loss of body water and gradual rise in osmotic pressure adaptation would appear to be a disadvantage.

The similarity between thresholds to osmotically effective solutes and dehydration, the restoration of body fluids to isotonicity after such substances by drinking in nephrectomized rats, and the lack of adaptation to these substances support the theory that increase in effective osmotic pressure of body fluids is a physiological stimulus of drinking.

SUMMARY

1. The amounts of water drunk by nephrectomized rats after slow infusions or rapid injections of varying amounts of hypertonic Na_2SO_4, NaCl, sucrose or urea were the same.

2. The changes in weight produced by drinking were sufficient to dilute infused Na_2SO_4 and NaCl to isotonicity in nephrectomized rats. Sucrose was less effective as a drinking stimulus and urea was least effective.

3. The threshold of drinking of normal rats infused with hypertonic NaCl was 1·6 % increase in osmotic pressure.

4. The threshold of drinking of nephrectomized rats infused with hypertonic Na_2SO_4 and NaCl was about 2 % increase in osmotic pressure. Sucrose threshold was 2·4 % and urea threshold 5·6 %.

5. The threshold of drinking did not depend on the rate of infusion.

6. When drinking water was withheld for up to 24 hr after injection of hypertonic NaCl into nephrectomized rats the change in weight of the animal was the same as when drinking was allowed immediately after injection. Therefore the receptors for thirst do not adapt.

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