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DRINKING BY NEPHRECTOMIZED RATS INJECTED WITH VARIOUS SUBSTANCES

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Gilman (1937), experimenting on normal dogs, observed that hypertonic solutions of sodium chloride, which cause cellular dehydration, gave rise to more drinking than hypertonic solutions of urea, which do not cause loss of cell water. He concluded that thirst is due to cellular dehydration.

Experiments on dogs by Holmes & Gregersen (1950*a*, *b*) in which a variety of substances were given, confirmed that osmotically effective solutes cause most drinking and by implication most thirst, and Adolph, Barker & Hoy (1954) showed that sodium chloride was twice as powerful as urea in causing normal rats to drink. In both species, however, no simple quantitative relationship was found between the amount of substance injected and the amount of water drunk.

In the present work it was thought that the analysis would be simplified if the kidneys were removed, because there could be no renal response to the substance, which could therefore act until neutralized by drinking, and the quantitative relation between the amount of substance injected and water drunk could thus be investigated. It was hoped that the cellular dehydration hypothesis of drinking would be tested in this way.

METHODS

Male albino rats, weighing between 150 and 400 g, were most commonly used in these experiments, but some female rats and a small number of hooded rats and ginger rats of both sexes were used also. Food (Diet 41, Bruce & Parkes, 1949) was available until the beginning of the experiment and water was available before and during the experiment from a drinking meter which enabled the time course of drinking to be recorded (Fitzsimons, 1958).

Experimental procedure

Each animal was anaesthetized with open ether and the external jugular vein catheterized by a soft polyvinyl chloride tube, outside diameter 1 mm. The tip of the catheter was advanced to the right atrium to minimize the complication of dead space when injections were being made. The catheter was brought subcutaneously to the back of the neck and about 2 cm was left projecting through a stab wound in the skin. The catheter, which was filled with 0·9 % sodium chloride solution, was closed with a small plug.

Bilateral nephrectomy was then carried out through a dorsal incision. A clean surgical technique was employed, but no aseptic precautions were taken because the experiments

only lasted for 6 hr. In some early experiments unilateral nephrectomy was performed a few days previously and at the same operation a loose ligature was placed round the pedicle of the remaining kidney and the ends taken out through the skin wound. This ligature was tied just before the start of the experiment with little apparent discomfort to the animal, completing destruction of renal function. The results obtained after the two methods of nephrectomy were the same.

About 1–1½ hr after the operation a known amount of solute in hypertonic solution was injected through the jugular catheter, washing through with 0·9 % sodium chloride solution so that the total injection volume was approximately 1 ml./100 g body weight. After large doses of the less soluble substances this percentage was exceeded. During the injection the rat was not restrained, but allowed to walk around if it wished. If there were signs of discomfort, e.g. head movements, the rate of injection was slowed.

After the injection, the rat was weighed to the nearest 0·1 g and put into an individual metabolism cage. Spontaneous drinking was recorded for the next 6 hr. As will be seen later, 6 hr was usually long enough for the rat to recover from the injection and finish drinking. At the end of 6 hr the rat was weighed, together with any faeces passed; water loss from faeces after voiding was regarded as part of the non-urinary water loss of the animal. The majority of the rats did not in fact defaecate, and those that did passed one or two pellets only.

Control rats were subjected to the same procedures as the experimental animals except that they were given 1 ml./100 g body weight 0·9 % sodium chloride solution intravenously.

In some experiments the inulin space was measured by injecting 1 ml. of 10 % inulin (Kerfoot) intravenously at the time of nephrectomy and estimating the serum concentration at the end of the experiment by a resorcinol method (Bell, 1955).

In some experiments also the serum freezing points were measured thermoelectrically at the end of the 6 hr period.

Solutions

Solutions for injection were prepared from Analar reagents dissolved in 0·9 % sodium chloride solution, except sodium chloride itself which was dissolved in water (Table 1). Sugar and urea solutions were freshly prepared; electrolyte solutions were stored in glass-stoppered bottles. The solutions were not sterilized.

TABLE 1. Concentration (M) of substances in solutions: each substance, except NaCl, was dissolved in isotonic NaCl solution (9 g/l. water)

Electrolytes		Non-electrolytes	
NaCl (in water)	2	Glucose	2
CH_3COONa	2	Methyl glucose	2
Na_2SO_4	2*	Fructose	2
KCl	1	Sucrose	2
CH_3COOK	1	Sorbitol	2
		Mannitol	2*
		Urea	8

* Only dissolves completely when warm.

Expression of results

The weight changes of the whole animal were used as measures of water balance, ignoring respiratory weight changes, metabolic water and water remaining in the alimentary tract. In 6-hr experiments on rats not permitted access to food these amounts are small (Jelleff, Carr & Krantz, 1942), tend to cancel one another, and are presumably approximately the same for control and experimental groups.

The water balance of the animal, i.e. gain or loss of weight, is expressed as a percentage of the initial body water of the animal, which includes the volume of fluid injected. Initial

body water was estimated on 91 rats by subtracting weight changes from final body water measured by evaporating the comminuted carcass to constant weight at 100–105° C. The observed mean of 69 g/100 g initial body weight has been used in all calculations.

The amount of hypertonic solution injected is given in ml./100 g initial body weight, where initial body weight is the post-injection body weight. The amount of solution injected is also expressed in terms of the percentage increase in osmotic pressure of body fluids. To avoid having to restrain and bleed the experimental group, which might affect drinking, the increases in osmotic pressure were not measured directly, but were calculated by using osmotic coefficients appropriate to the ionic strength of the mixture of body fluid (cf. Conway's 1957 data for the rat) and injected substance (Robinson & Stokes, 1955; Bayliss, 1959). The validity of the procedure was confirmed by measuring the freezing points of mixtures of sodium chloride and sodium sulphate. The coefficients of 0·96 for sodium and potassium acetate, 0·93 for sodium chloride and 0·92 for potassium chloride were taken from the International Critical Tables (1928). The sodium sulphate coefficient, which varied between 0·805 and 0·825 depending on the amount of sodium sulphate injected, was taken from the data of Robinson & Stokes (1955). For all non-electrolytes a coefficient of 1 was assumed.

An example of the calculation when sodium sulphate was injected is as follows. A group of rats were given 0·488 ml. 2M sodium sulphate/100 g initial body weight. Assuming that the sodium sulphate remains outside the cells, and that the cells behave as osmometers, the approximate ionic strength of the mixture of extracellular fluid and sodium sulphate is 0·24 and the appropriate osmotic coefficient is 0·81. The amount of sodium sulphate added was therefore $0{\cdot}488 \times 6 \times 0{\cdot}81 = 2{\cdot}372$ m-osmole/100 g initial body weight. The real osmolality of body fluids before the injection was found to be 290 m-osmole/kg H_2O, thus the initial osmotic content of the whole body fluids was $69 \times 290/1000 = 20$ m-osmole/100 g initial body weight. The increase in osmotic pressure was therefore approximately 11·9%. The calculations for the other substances are essentially the same, except that when sodium chloride was given allowance was made for the salt needed to bring the hypertonic volume to isotonicity; other hypertonic solutions were made up in isotonic sodium chloride.

RESULTS

Description of a typical experiment

The behaviour of nephrectomized rats for the first 24 hr after operation was not obviously different from normal. Food and water were still taken though consumption of both fell. Intake of water was sufficient to balance losses for at least the first 24 hr after nephrectomy.

In 30 control rats which had received isotonic sodium chloride solution (Table 2) the average volume of water drunk was 1·82 ml./100 g initial body water and the average weight loss was 0·17 g/100 g initial body water. Change in weight shows the water balance (see under Methods): the controls thus effectively maintained water balance, and the deviations of water balance of the experimental animals from zero may therefore be attributed to the injected solutions.

A kymograph record of drinking by a nephrectomized rat injected with 0·327 ml. 2M sodium sulphate/100 g initial body weight is given in Fig. 1. The increase in osmotic pressure produced by this dose was 8%, the fluid balance after 6 hr was 10·8 g/100 g initial body water and the amount of water drunk was 13·5 ml./100 g initial body water.

The drinking record of Fig. 1 is quite typical of records obtained after injection of a variety of substances, in that most drinking occurred in the first 2 hr. This is further illustrated in Fig. 2, which gives the mean rates of drinking after all the doses of sodium sulphate used. After most doses

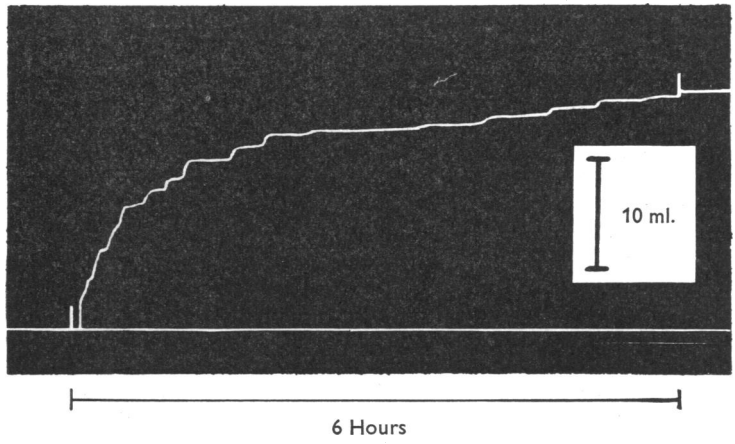

Fig. 1. A kymograph record of drinking by a nephrectomized rat after injection of 0·327 ml. 2M sodium sulphate/100 g body weight.

TABLE 2. The amount of water drunk, fluid balance, inulin space and serum freezing point of nephrectomized rats 6 hr after injection of various doses of sodium chloride

2M NaCl injected (ml./100 g initial body wt.)	Increase in initial osmotic pressure (%)	Water drunk (ml./100 g initial body H_2O)	Fluid balance (g/100 g initial body H_2O)	Inulin space (ml./100 g initial body H_2O)	Difference between control and experimental inulin spaces	Serum freezing point at 6 hr (°C)
Control	0	1·82	−0·17 ± 0·27 (30)	35·52 ± 0·99 (10)	—	−0·566 ± 0·0007 (10)
0·086	1·5	3·5	1·13 ± 0·81 (5)	—	—	—
0·162	2·8	4·47	2·72 ± 0·95 (5)	—	—	—
0·272	4·7	6·36	4·64 ± 0·55 (12)	40·16 ± 1·09 (10)	4·64	−0·562 ± 0·0079 (10)
0·364	6·2	7·17	5·7 ± 0·28 (3)	—	—	—
0·536	9·2	9·17	7·4 ± 0·5 (15)	43·86 ± 1·24 (10)	8·34	—
0·805	13·8	10·98	8·95 ± 0·64 (10)	47·0 ± 1·42 (9)	11·48	−0·585 ± 0·0088 (9)
1·042	17·8	10·09	8·13 ± 1·89 (5)	45·24 ± 2·13 (5)	9·72	−0·636 ± 0·0114 (5)

In this and subsequent tables the mean ± S.E. is given, with the number of observations in parentheses

there was a phase of rapid drinking lasting about 2 hr, followed by a phase in which the rate of drinking was only slightly more than the control rate. After the highest dose the initial rate of drinking was much slowed because of incapacitating tetany that sodium sulphate causes in these doses. It would seem from these results that 6 hr is ample time for a nephrectomized rat to achieve equilibrium after injections which cause drinking.

A comparison of drinking by normal and nephrectomized rats

The amounts of water drunk by normal and nephrectomized rats after various doses of sodium chloride or urea are plotted in Fig. 3. After the smaller doses of both substances the nephrectomized animals drank more water than the normal animals. After the higher doses of urea the position is reversed and the normal animals drank more than the nephrectomized, probably because of the loss of fluid due to the osmotic diuresis. From the trend of the curves in Fig. 3*a*, the same would almost certainly hold after sodium chloride if larger doses had been used.

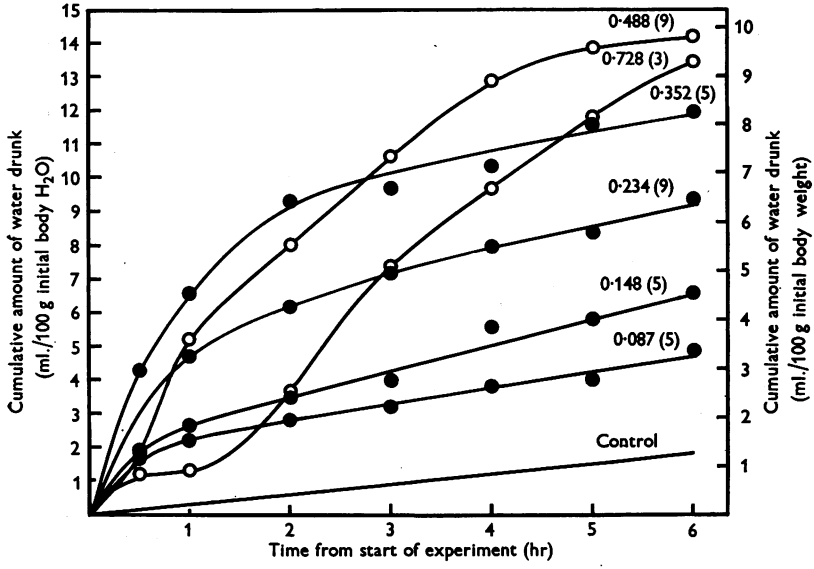

Fig. 2. Cumulative amounts of water drunk after various doses of 2M sodium sulphate. Amount of sodium sulphate injected (ml./100 g body weight) and number of animals used are given on each curve.

Drinking caused by sodium chloride in nephrectomized rats

The amounts of water drunk, the fluid balances, inulin spaces and serum freezing points of nephrectomized rats 6 hr after injection of various doses of sodium chloride are given in Table 2. The doses of sodium chloride are also expressed in terms of increases in initial osmolality. The mean initial serum freezing point of 12 control rats was $-0{\cdot}540 \pm 0{\cdot}0061°$ C (S.E. of mean), giving a real osmolal concentration of *ca.* 290 m-osmole/kg H_2O, and the percentage increases were calculated in the way described above.

When isotonicity is restored by drinking there is numerical equality between the percentage increase in initial osmotic pressure and the fluid balance expressed as a percentage of initial body water. Doses of 2M

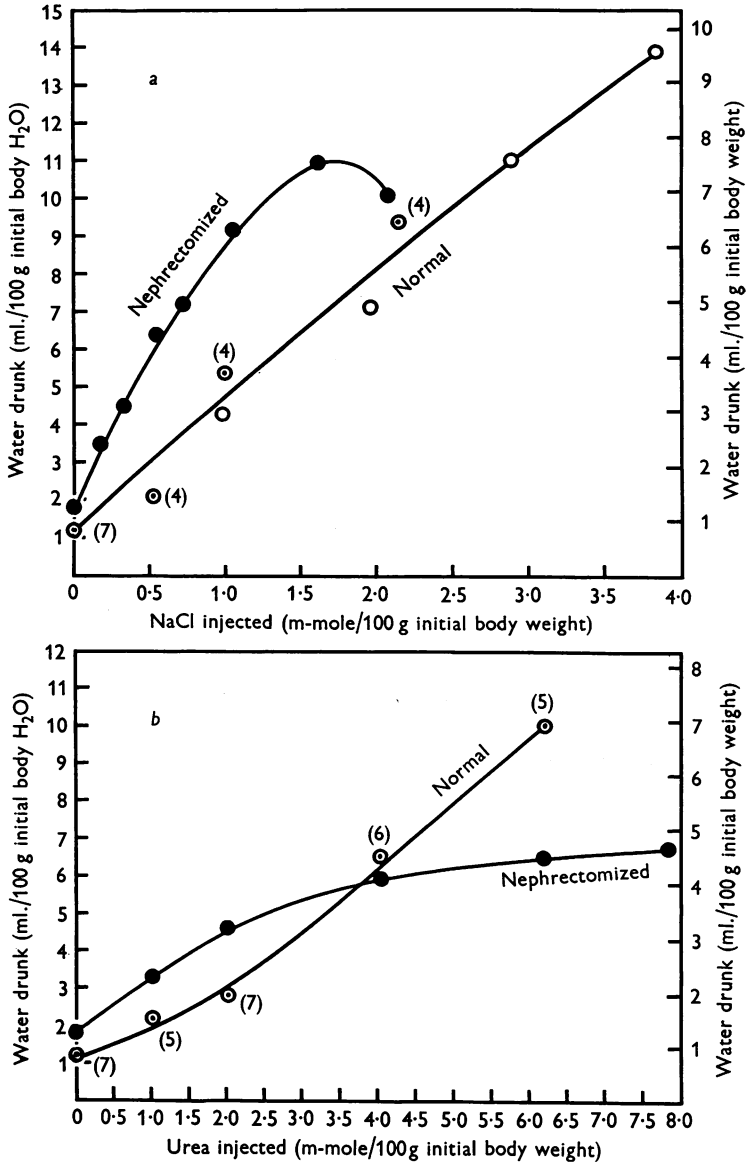

Fig. 3. The amounts of water drunk by normal and nephrectomized rats after various doses of (*a*) sodium chloride, and (*b*) urea. The data for nephrectomized rats plotted as ● are taken from Tables 2 and 7; data for normal rats plotted as ⊙ were obtained in experiments similar to those on nephrectomized animals, described in this paper; those plotted as ○ are calculated from the data of Adolph, Barker & Hoy (1954).

sodium chloride solution up to 0·272 ml./100 g initial body weight, equivalent to a 4·7 % increase in initial osmotic pressure, were diluted to isotonicity by drinking. After higher doses net fluid intake was inadequate to restore osmotic pressure to normal. The serum freezing points confirm this conclusion.

Hypertonic sodium chloride solution produces changes similar to those produced by water deprivation, namely, cellular dehydration and an over-all increase in osmotic pressure of body fluids, without presumably any very marked change in the relative chemical composition of body fluids. One important difference, especially in the nephrectomized rat, is that sodium chloride causes expansion of the extracellular space. This expansion becomes more marked as the nephrectomized animal drinks, and

TABLE 3. The effect of expanding body fluids on drinking induced by hypertonic NaCl in nephrectomized rats

Isotonic NaCl infused (ml./100 g initial body H_2O)	2 M-NaCl injected (ml./100 g post-infusion body wt.)	Increase in initial osmotic pressure (%)	Water drunk (ml./100 g. post-infusion body H_2O)	Fluid balance (g/100 g post-infusion body H_2O)	Fluid balance (g/100 g pre-infusion body H_2O)
0	0·805	13·8	10·98	8·95 ± 0·64 (10)	8·95
3·09	0·819	13·8	10·3	8·98 ± 0·92 (6)	12·8
10·03	0·824	13·6	10·24	8·16 ± 1·33 (9)	20·5

Proportionately larger doses of hypertonic NaCl were given to the infused animals to allow for the greater initial body water.

it may be seen that in the experiments of Table 2 the differences between the control and experimental inulin spaces were approximately equal to the fluid balances, i.e. the net fluid intakes were mostly accommodated in the extracellular spaces.

To determine whether expansion of extracellular space affects drinking, experiments were carried out in which injections of hypertonic sodium chloride solution were immediately preceded by large infusions of isotonic sodium chloride solution, given over a period of about 5 min. It was found that the response to hypertonic sodium chloride, calculated in terms of the augmented initial body water, was the same as for the group which had not been previously infused (Table 3). This occurred despite a fluid balance, including the isotonic infusion, considerably in excess of the largest fluid balance produced by a drinking stimulus alone. Thus this degree of expansion of extracellular fluid is not a factor modifying drinking.

The nephrectomized rat is a suitable preparation to study adaptation of a thirst receptor, because once sodium chloride is injected it must remain in the animal. If drinking is not allowed until some hours after the injection, any adaptation should be revealed by comparing the fluid balance

with the fluid balance when drinking was permitted from the start of the experiment.

It was found (Table 4) that withholding water for 2 hr after injection of hypertonic sodium chloride solution had little effect on the final fluid balance in a 6 hr experiment. When water was withheld for 4 hr, so that the time available for drinking was reduced to 2 hr, net intake of water was a little less, but records of the rate of drinking show that this was because there was less time for drinking rather than adaptation (Fig. 4).

Drinking caused by sodium sulphate, sodium acetate and sucrose in nephrectomized rats

Data for the effects of sodium sulphate, sodium acetate and sucrose in nephrectomized rats are given in Table 5. Sodium sulphate was found to

TABLE 4. The effect of temporarily withholding drinking water after administration of hypertonic NaCl

Duration of water deprivation (min)	2 M-NaCl injected (ml./100 g initial body wt.)	Increase in initial osmotic pressure (%)	Fluid balance (g/100 g initial body H_2O)
0	0·536	9·2	7·4 ± 0·5 (15)
120	0·523	9·0	7·4 ± 1·15 (6)
240	0·541	9·25	6·64 ± 0·73 (6)

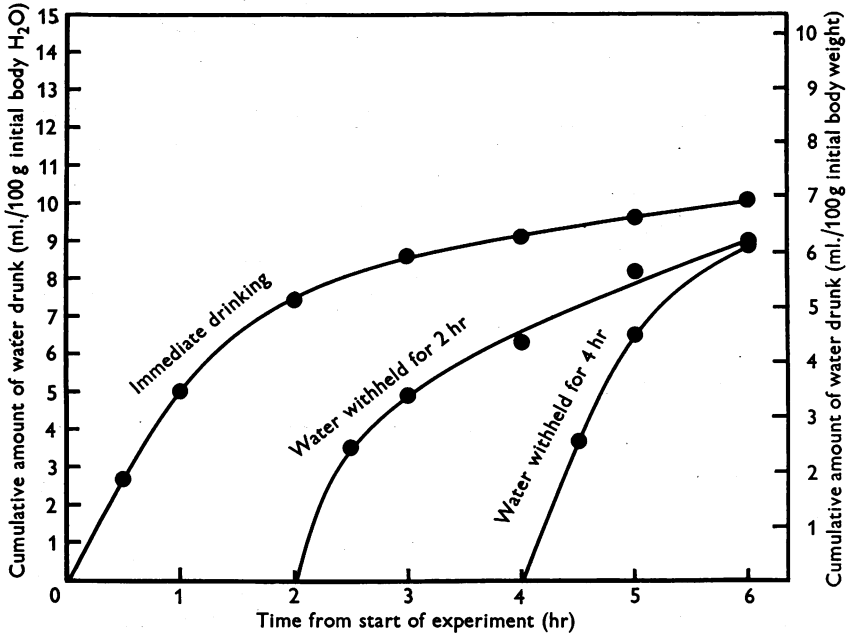

Fig. 4. The effect of temporarily withholding water after injection of hypertonic sodium chloride on the subsequent rates of drinking (see Table 4).

cause the most drinking of all substances tested. Fluid balances were more than sufficient to neutralize up to about 0·49 ml. 2 M sodium sulphate/100 g initial body weight, equivalent to a 12 % increase in initial osmotic pressure. The serum freezing points confirm that the animals tended to drink to hypotonicity after the lower doses of sodium sulphate, and as after sodium chloride the inulin spaces show that the net fluid intake was mostly accommodated in the extracellular space.

TABLE 5. The amount of water drunk, fluid balance, inulin space and serum freezing point of nephrectomized rats 6 hr after injection of various doses of hypertonic sodium sulphate sodium acetate or sucrose

Volume of hypertonic solution injected (ml./100 g initial body wt.)	Increase in initial osmotic pressure (%)	Water drunk (ml./100 g initial body H_2O)	Fluid balance (g/100 g initial body H_2O)	Inulin space (ml./100 g initial body H_2O)	Difference between control and experimental inulin spaces	Serum freezing point at 6 hr (°C)
Control	0	1·82	−0·17 ± 0·27 (30)	35·52 ± 0·99 (10)	—	−0·566 ± 0·0007 (10)
2 M-Na_2SO_4						
0·087	2·2	4·92	2·59 ± 0·25 (5)	36·96 ± 0·39 (5)	1·44	—
0·148	3·7	6·58	4·32 ± 0·56 (5)	—	—	—
0·234	5·8	9·1	6·77 ± 0·78 (9)	44·82 ± 0·37 (7)	9·3	−0·553 ± 0·0078 (8)
0·352	8·6	12·0	9·63 ± 1·02 (9)	—	—	—
0·488	11·9	14·24	12·44 ± 1·34 (9)	51·3 ± 1·81 (9)	15·78	−0·556 ± 0·0044 (9)
0·722	17·5	15·24	12·4 ± 1·68 (6)	48·4 ± 1·82 (6)	12·88	−0·598 ± 0·0043 (6)
2 M-CH_3COONa						
0·149	2·8	4·6	2·61 ± 0·04 (5)	—	—	—
0·243	4·7	5·92	3·99 ± 0·9 (5)	—	—	—
0·496	9·5	8·45	6·67 ± 0·54 (10)	—	—	—
0·745	14·3	10·24	8·34 ± 1·17 (9)	43·92 ± 0·67 (8)	8·4	—
2M Sucrose						
0·159	1·6	2·42	1·36 ± 0·8 (3)	—	—	—
0·335	3·3	5·5	3·29 ± 0·35 (3)	—	—	—
0·536	5·4	6·35	4·8 ± 1·29 (3)	—	—	—
0·738	7·4	7·5	5·8 ± 0·31 (3)	—	—	—
1·055	10·5	8·21	6·59 ± 0·81 (6)	—	—	—
1·58	15·8	10·09	8·35 ± 0·58 (8)	—	—	—

In some animals the larger doses of sodium sulphate caused overt tetany. On two occasions the experiment had to be stopped because the rat was distressed, but usually the rat recovered quite quickly and would start or continue to drink. The mean delays before the onset of drinking after the highest doses of sodium chloride and sodium sulphate show the effect of tetany (Table 6), as do the curves of the rates of drinking in Fig. 2.

The response to sodium acetate (Table 5) was almost the same as to sodium chloride, perhaps surprisingly in view of the fact that acetate is metabolized. However, the presence of sodium ions in the extracellular space requires anions for electrical neutrality, presumably bicarbonate, which subserve the same osmotic function as acetate. It has been shown

that sodium acetate causes an alkalosis in no way different from a bicarbonate alkalosis (Lipsky, Alper, Rubin, Van Eck & Gordon, 1954). Sucrose (Table 5) was as effective as sodium chloride and sodium acetate in causing drinking. The close agreement between the fluid balances after doses of equal osmotic strength is striking.

TABLE 6. Intervals before the onset of drinking after the higher doses of sodium chloride and sodium sulphate

NaCl			Na_2SO_4		
2 M-NaCl injected (ml./100 g initial body wt.)	Increase in initial osmotic pressure (%)	Mean latency (min)	Mean latency (min)	Increase in initial osmotic pressure (%)	2 M-Na_2SO_4 injected (ml./100 g initial body wt.)
0·805	13·8	7·3 (10)	40 (9)	11·9	0·488
1·042	17·8	10 (5)	107 (6)	17·5	0·722

TABLE 7. The amount of water drunk and fluid balance of nephrectomized rats 6 hr after injection of substances which did not cause much drinking, and the effect of temporarily withholding drinking water after injection of urea

Volume of hypertonic solution injected (ml./100 g initial body wt.)	Increase in initial osmotic pressure (%)	Water drunk (ml./100 g initial body H_2O)	Fluid balance (g/100 g initial body H_2O)
Control	0	1·82	$-0·17 \pm 0·27$ (30)
2 M Sorbitol			
0·532	5·3	3·5	$1·98 \pm 0·38$ (6)
1·04	10·4	4·9	$2·89 \pm 0·42$ (5)
1·55	15·5	5·83	$3·2 \pm 0·88$ (7)
2M Mannitol			
1·52	15·2	3·5	$2·01 \pm 1·36$ (5)
2M Glucose			
1·575	15·75	2·4	$0·27 \pm 0·43$ (15)
2M Methyl glucose			
1·59	15·9	2·47	$0·79 \pm 0·66$ (5)
2M Fructose			
1·573	15·7	4·13	$2·09 \pm 0·62$ (10)
8M Urea (immediate access to drinking water)			
0·127	5·1	3·3	$0·98 \pm 0·62$ (10)
0·253	10·1	4·62	$2·34 \pm 0·49$ (8)
0·505	20·2	5·86	$3·44 \pm 0·65$ (8)
0·775	31·0	6·49	$4·25 \pm 0·38$ (9)
0·98	39·2	6·75	$4·59 \pm 0·96$ (5)
8M Urea (drinking water withheld for 165–230 min after injection)			
0·752	30·1	5·6	$3·7 \pm 0·61$ (8)

Substances which did not cause much drinking

The effects of sorbitol, mannitol, glucose, methyl glucose, fructose and urea on the water intake of nephrectomized rats are given in Table 7. In this class, sorbitol, mannitol, fructose and urea were the most effective and glucose and methyl glucose the least effective. Fluid balances after glucose and methyl glucose were only slightly greater than the control

balance. Urea had a definite effect on drinking, but even after the very large dose of 0·98 ml. 8M urea/100 g initial body weight, giving an increase in osmotic pressure of about 39%, the positive fluid balance was only 4·6 g/100 g initial body water. Delayed access to drinking water after urea administration did not markedly alter the amount of water drunk subsequently.

TABLE 8. The amount of water drunk and fluid balance of nephrectomized rats 6 hr after injection of potassium salts, and the effect of temporarily withholding drinking water after injection of potassium chloride

Volume of hypertonic solution injected (ml./100 g initial body wt.)	Increase in initial osmotic pressure (%)	Water drunk (ml./100 g initial body H_2O)	Fluid balance (g/100 g initial body H_2O)
Control	0	1·82	$-0·17 \pm 0·27$ (30)
1M-CH_3COOK			
0·344	3·3	3·13	$1·94 \pm 0·19$ (5)
1M-KCl (immediate access to drinking water			
0·25	2·3	3·5	$1·5 \pm 0·54$ (5)
0·413	3·8	4·5	$2·31 \pm 0·86$ (5)
0·621	5·7	5·84	$4·07 \pm 1·09$ (5)
1M-KCl (drinking water withheld for 130–230 min after injection)			
0·618	5·7	5·12	$3·14 \pm 0·67$ (10)

Drinking caused by potassium salts in nephrectomized rats

Potassium salts were unexpectedly effective in causing nephrectomized rats to drink, even when immediate access to drinking water was prevented (Table 8). Potassium salts, particularly in the large doses used here, are very toxic. Injections were given slowly in divided doses and part of the dose was often given subcutaneously, but despite these precautions toxic manifestations were the rule. These consisted of irregular movements of muscle including the respiratory muscles, temporary paralysis and irregular action of the heart. There was apparently complete recovery 5–10 min later and the rats would start to drink.

DISCUSSION

A number of substances cause nephrectomized rats to drink, casting doubt on the assertion that the sensation of thirst does not occur in the nephrectomized rat (Linazasoro, Jiménez Diáz & Castro Mendoza, 1954). Sodium chloride, sodium sulphate, sodium acetate and sucrose were the most effective substances in causing drinking and were equally effective in isosmotic doses when the doses were small. It is therefore unlikely that drinking was due to specific chemical properties of the substances, but rather it was due to a common or colligative property such as osmotic pressure. Furthermore, these are substances which are known to dehydrate most of the cells in the body. In doses producing increases of up to 5% in

osmotic pressure, comparable with the increases met in normal life, these substances caused enough drinking to restore the osmotic pressure of body fluids to normal, as is shown in Fig. 5 where the line $0A$ represents restoration of isotonicity. However, restoration of body fluids to isotonicity is quite incidental as is shown by the relative failure of substances such as urea and glucose, which penetrate cells, to cause drinking. In the case of

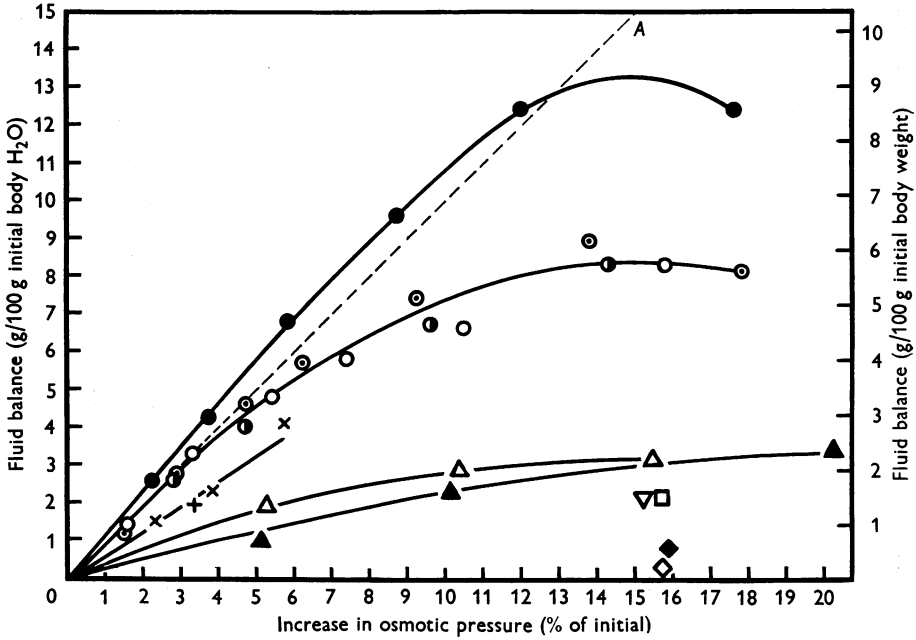

Fig. 5. The relationship between increase in initial osmotic pressure produced by injection of various substances and the net fluid intake of nephrectomized rats. The data are taken from Tables 2, 5, 7 and 8. The line $0A$ delineates the theoretical relationship predicted for an osmotically effective substance by a simple cellular dehydration hypothesis of drinking, indicating restoration of cell size. ● Na_2SO_4, ○ sucrose, ⊙ NaCl, ◑ CH_3COONa, × KCl, + CH_3COOK, △ sorbitol, ▲ urea, ▽ mannitol, □ fructose, ◇ glucose, ◆ methyl glucose.

injected substances which are confined to the extracellular space, sufficient drinking to dilute these substances to isotonicity also restores the cells to their initial size, provided there is no net movement of normal body fluid constituents across the cell membranes. If it be assumed that the cells in a thirst receptor zone behave in the same way as the majority of cells in the body, then as a first approximation these results are in accordance with a hypothesis that thirst and drinking are due to dehydration of cells in a receptor zone.

Further support for the hypothesis is provided by the inulin spaces,

which show the lack of appreciable cellular dehydration after the higher and unneutralized doses of sodium chloride, sodium sulphate and sodium acetate when the animals had voluntarily stopped drinking. The animals had stopped drinking when presumably the receptor cells had been restored to their normal size, even though the injected substances had not been diluted to isotonicity.

Holmes & Gregersen (1950*a*) and Adolph *et al.* (1954) emphasized that in intact animals the amount of water drunk in response to an osmotic stimulus is not simply related to the amount of water required to restore the animal to isotonicity, and that if drinking were not permitted during the first few hours after the injection, the amount of water subsequently drunk was the same as when drinking was allowed immediately after the injection, even though the injected substance had been mostly excreted in this time.

One reason for their failure to find a simple relationship between the amount of substance injected and drinking is that the experiments were carried out on animals with functioning kidneys. Adolph and his colleagues in fact questioned the view that drinking may be modified by a renal response to the injected substance, saying, 'we found no good evidence that drinking was stimulated as an alternative to the performance of osmotic work in the kidneys.' The present experiments provide such evidence, because it was found that nephrectomized animals drank more water than normal animals after identical injections. It seems reasonable to conclude that the extra amount of water was drunk because the injected substances were not removed by renal excretion.

Another complication is that Adolph *et al.* (1954) used oral and intraperitoneal routes for administration of hypertonic urea or sodium chloride. The intraperitoneal injection of hypertonic solutions appears to be painful, and moreover, there may be temporary pooling of fluid in the peritoneal cavity, so that any effect on drinking may be secondary. These authors in fact found different responses to the same amount of solute given by the two routes. The advantages of intravenous injections are that they cause least apparent discomfort to the animals, they avoid the complication of pooling and they allow rapid distribution of the injected substance.

A third reason is that all previous workers measured the amount of water drunk rather than the net fluid intake. In a rapid drinker like the dog this may be reasonable, though there is the complication of temporary gastro-pharyngeal satiation (Montgomery & Holmes, 1955). In a slow drinker such as the rat, however, non-urinary loss of water during the drinking period will appreciably alter the amount of water available for diluting the injected substance.

Though after lower doses of sodium sulphate, sodium chloride, sodium acetate and sucrose, fluid balances were close to the values expected on

a cellular dehydration hypothesis of drinking, after higher doses the response curves fell away from the predicted relationship (Fig. 5). Expansion of extracellular fluid does not appear to be a factor, because in the experiments in Table 3 infusion of isotonic sodium chloride did not modify the drinking caused by subsequent injection of hypertonic sodium chloride. The total fluid balance of these animals, including the infusion, was considerably greater than the largest fluid balance achieved by drinking alone. Nor does adaptation of the receptor appear to be important, because a 4 hr delay had little effect on the response to sodium chloride (Table 4). The serum freezing-point values rule out the possibility that the injected substances were sequestered in osmotically inactive forms, yet from the inulin spaces large doses of these substances appear to be osmotically ineffective. Endogenous release of cellular osmotic material raising the cellular osmotic pressure is a possible explanation of reduced osmotic effectiveness (McDowell, Wolf & Steer, 1955), but a more likely explanation is that the injected substances in large doses penetrate the cells. In the case of sucrose, reduced osmotic effectiveness could be due to metabolism. White & Rolf (1957) found that when inulin or sucrose was given to nephrectomized rats 100% of inulin was recoverable 6 hr later, but only 85·2% of sucrose.

Glucose and methyl glucose caused very little drinking, fructose rather more; all are freely permeant and would be expected to be ineffective on a cellular dehydration hypothesis. Urea did cause some drinking (Fig. 5). It has been found to cause some drinking in normal animals, but most authors assume that this is due to osmotic diuresis (e.g. Strauss, 1957), though Adolph *et al.* (1954) said that, 'only in time did drinking appear to dictate excreting rather than vice versa', implying that urea stimulated a thirst receptor directly. According to most authors urea should be without effect in nephrectomized animals, but it proved a more powerful stimulus of drinking than was expected, and significantly, in lower doses, more effective in nephrectomized animals than in normal animals. Transient osmotic stimulation of the receptor is ruled out by the experiments in which access to drinking water was delayed for some time after injection of the urea. The reason may be that the receptor itself is impermeable to urea. Conway & Fitzgerald (1942) found that there are regions in the central nervous system which are relatively impermeable to urea, and recently Javid & Anderson (1959) showed that hypertonic urea solutions have a powerful action in lowering cerebrospinal fluid pressure, the effect being greater and longer lasting after bilateral nephrectomy. It is interesting to note that Zuidema, Clarke & Minton (1956) obtained antidiuretic responses from lightly anaesthetized dogs with urea infusions which raised the osmotic pressure by 2% over a period of 40 min, though

Verney's (1954) results on conscious dogs show that the antidiuretic receptor is normally permeable to urea.

The sugar alcohols, sorbitol and mannitol, were about equally effective as urea in causing nephrectomized rats to drink. Holmes & Gregersen (1950*a*) on the contrary found that sorbitol given to normal dogs was as powerful a stimulus of drinking as sodium chloride, sodium sulphate and sucrose. Holmes & Gregersen state that sorbitol causes the same fluid shifts in the body as sodium chloride, but from their figures for plasma protein levels before and after injection of sodium chloride or sorbitol it is evident that the fluid shifts caused by sorbitol were more evanescent. This may be because sorbitol is metabolized (Blakley, 1951; mannitol is also metabolized, Hamburger & Mathé, 1952), or because it is more permeant than sodium chloride. Sorbitol would thus cause less drinking in the rat, a slow drinker, than in the dog, a rapid drinker. Apart from possible differences due to different rates of drinking, another reason for the discrepancy is that sorbitol is a powerful diuretic. The large amount of water drunk by normal dogs may therefore be due to renal loss of fluid.

The part played by potassium in drinking is interesting. The association between potassium deficiency and thirst seems well established (Black, 1957; Stanbury, 1958), and ingestion of potassium salts does not cause thirst or drinking according to Arden (1934) and Janssen (1936). These results are consistent with the cellular dehydration hypothesis, because loss of potassium might be expected to lead to cellular shrinkage, and administration of potassium should cause no alteration in cell size because most cells are freely permeable to potassium. The quite vigorous drinking caused by potassium salts was therefore an unexpected finding. Whether this was a non-specific response to stress, whether the receptor itself is impermeable to potassium or whether there is some other explanation, it is impossible to say on present evidence. The toxicity of extracellular potassium complicates the interpretation of results which depend on the voluntary response of the animal.

Sodium sulphate caused more drinking than any other substance (Fig. 5). There was, moreover, a tendency after the lower doses for the animals to drink more than was required for restoration of isotonicity. The reason for this overshoot is not known. One possibility is that the loss of cell water and dilution of normal extracellular constituents upsets the Donnan equilibrium. If enough potassium chloride were to leave the cells to restore the Donnan equilibrium and the animal drank sufficient water to achieve isotonicity, the cells would be slightly smaller than initially. To restore the cells to their initial size would require a slightly greater water intake which would make the body fluids hypotonic. The order of overshoot expected, calculated from Conway's (1957) data for the rat and assuming that potassium chloride is the only solute to cross

the cell membranes, agrees closely with the sodium sulphate response curve. The fact that sodium sulphate caused more drinking than any other substance supports the cellular dehydration hypothesis because sodium sulphate is more rigorously excluded from the cells than the other substances. The fact that the sodium sulphate response tends to overshoot the isotonic line might be regarded as additional evidence that it is the size of the cells in the receptor that determines whether drinking takes place. It should be emphasized in conclusion that cellular dehydration is not the only mechanism controlling water intake.

SUMMARY

1. Nephrectomized rats were found to drink more water than normal rats when given varying amounts of hypertonic sodium chloride solution or hypertonic urea solution, which produced increases of up to 15% in the osmotic pressure of body fluids.

2. Sodium sulphate caused most drinking in nephrectomized rats, but sodium chloride, sodium acetate and sucrose were almost as effective in doses producing up to 5% increase in osmotic pressure. The relationship between net fluid intake and increase in osmotic pressure after these substances was as predicted by a cellular dehydration or osmometer hypothesis of drinking, in that the animals generally stopped drinking when the cells were restored to their initial size.

3. A preliminary 10% isotonic expansion of body fluids did not prevent the full response to hypertonic sodium chloride.

4. Delayed access to drinking water after hypertonic sodium chloride did not significantly affect the amount of water drunk subsequently, nor the initial rate of drinking.

5. Potassium chloride and potassium acetate caused almost as much drinking in nephrectomized rats as the four most effective substances. Delayed access to drinking water after injection of hypertonic potassium chloride did not affect the amount of water drunk subsequently.

6. Urea, sorbitol, mannitol and fructose were considerably less effective but nevertheless caused appreciable drinking. Delayed access to drinking water after injection of hypertonic urea did not cause much reduction in the amount of water drunk subsequently. Glucose and methyl glucose were the least effective substances tested, and net fluid intakes were only slightly greater than control intakes.

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REFERENCES

ADOLPH, E. F., BARKER, J. P. & HOY, P. A. (1954). Multiple factors in thirst. *Amer. J. Physiol.* **178**, 538–562.

ARDEN, F. (1934). Experimental observations on thirst and on potassium overdosage. *Aust. J. exp. Biol. med. Sci.* **12**, 121–122.

BAYLISS, L. E. (1959). *Principles of General Physiology*, 2nd ed., vol. 1, p. 133. London: Longmans.

BELL, D. J. (1955). Colorimetric determination of hexuloses, free or combined. In *Modern Methods of Plant Analysis*, vol. 2, p. 21, ed. PAECH, K. & TRACEY, M. V. Berlin: Springer-Verlag.

BLACK, D. A. K. (1957). *Essentials of Fluid Balance*, 1st ed. Oxford: Blackwell.

BLAKLEY, R. L. (1951). The metabolism and antiketogenic effects of sorbitol. Sorbitol dehydrogenase. *Biochem. J.* **49**, 257–271.

BRUCE, H. M. & PARKES, A. S. (1949). Feeding and breeding of laboratory animals. IX. A complete cubed diet for mice and rats. *J. Hyg., Camb.*, **47**, 202–208.

CONWAY, E. J. (1957). Nature and significance of concentration relations of potassium and sodium ions in skeletal muscle. *Physiol. Rev.* **37**, 84–132.

CONWAY, E. J. & FITZGERALD, O. (1942). Diffusion relations of urea, inulin and chloride in some mammalian tissues. *J. Physiol.* **101**, 86–105.

FITZSIMONS, J. T. (1958). Apparatus for recording drinking and feeding. *J. Physiol.* **143**, 31–32*P*.

GILMAN, A. (1937). The relation between blood osmotic pressure, fluid distribution and voluntary water intake. *Amer. J. Physiol.* **120**, 323–328.

HAMBURGER, J. & MATHÉ, G. (1952). *Physiologie normale et pathologique du métabolisme de l'eau*, p. 159. Paris: Editions Médicales Flammarion.

HOLMES, J. H. & GREGERSEN, M. I. (1950*a*). Observations on drinking induced by hypertonic solutions. *Amer. J. Physiol.* **162**, 326–337.

HOLMES, J. H. & GREGERSEN, M. I. (1950*b*). Role of sodium and chloride in thirst. *Amer. J. Physiol.* **162**, 338–347.

International Critical Tables of Numerical Data, Physics, Chemistry and Technology, vol. IV, 1928. New York and London: McGraw-Hill.

JANSSEN, S. (1936). Pharmakologische Beeinflussung des Durstes. *Arch. exp. Path. Pharmak.* **181**, 126–127.

JAVID, M. & ANDERSON, J. (1959). The effect of urea on cerebrospinal fluid pressure in monkeys before and after bilateral nephrectomy. *J. Lab. clin. Med.* **53**, 484–489.

JELLEFF CARR, C. & KRANTZ, J. C. (1942). Metabolism. In *The Rat in Laboratory Investigation*, ed. GRIFFITH, J. Q. & FARRIS, E. J. Philadelphia: Lippincott.

LINAZASORO, J. M., JIMÉNEZ DIÁZ, C. & CASTRO MENDOZA, H. (1954). The kidney and thirst regulation. *Bull. Inst. Med. Res. Madrid*, **7**, 53–61.

LIPSKY, S. R., ALPER, B. J., RUBIN, M. E., VAN ECK, W. F. & GORDON, M. E. (1954). The effect of alkalosis upon ketone body production and carbohydrate metabolism in man. *J. clin. Invest.* **33**, 1269–1276.

MONTGOMERY, A. V. & HOLMES, J. H. (1955). Gastric inhibition of the drinking response. *Amer. J. Physiol.* **182**, 227–231.

MCDOWELL, M. E., WOLF, A. V. & STEER, A. (1955). Osmotic volumes of distribution. Idio genic changes in osmotic pressure associated with administration of hypertonic solutions. *Amer. J. Physiol.* **180**, 545–558.

ROBINSON, R. A. & STOKES, R. H. (1955). *Electrolyte Solutions. The Measurement and Interpretation of Conductance, Chemical Potential and Diffusion in Solutions of Simple Electrolytes*. London: Butterworth.

STANBURY, S. W. (1958). Discussion following, 'experimental cortexone polyuria and cortexone oedema in dogs', by STAHL, J., STEPHAN, F., JAHN, H., URBAN, M. & JAHN, M. In *An International Symposium on Aldosterone*, ed. MULLER, A. F. & O'CONNOR, C. M., p. 184. London: Churchill.

STRAUSS, M. B. (1957). *Body Water in Man. The Acquisition and Maintenance of the Body Fluids*. London: Churchill.

VERNEY, E. B. (1954). Water diuresis. *Irish J. med. Sci.* 6th series. No. **345**, 377–402.

WHITE, H. L. & ROLF, D. (1957). Whole body and tissue inulin and sucrose spaces in the rat. *Amer. J. Physiol.* **188**, 151–155.

ZUIDEMA, G. D., CLARKE, N. P. & MINTON, M. F. (1956). Osmotic regulation of body fluids. *Amer. J. Physiol.* **187**, 85–88.