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BODY FLUID CHANGES WHICH INFLUENCE DRINKING IN THE WATER DEPRIVED RAT

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SUMMARY

1. After overnight deprivation of water both the cellular and extracellular fluid volumes are significantly reduced in the rat.

2. In the rat with functional kidneys oral, intragastric or intravenous preloads of 10 ml. water reduce the total water intake after 1 hr by 64–69 %. These preloads restore plasma osmolality to pre-deprivation levels but have little effect on plasma volume.

3. In the same rats if the plasma volume is restored with an oral, intragastric or intravenous preload of 10 ml. of an isotonic balanced salt solution which has little effect on osmolality, drinking is significantly reduced by 20–26 %. The reduction of drinking correlates with the volume of the preload of balanced salt.

4. Plasma analysis shows that 1 hr after an oral preload of 10 ml. isotonic balanced salt solution, the extracellular fluid volume of the deprived rats is restored to pre-deprivation levels but osmolality is unchanged. Three hr after the balanced salt preload, extracellular fluid volume is still at pre-deprivation levels and there has been a slight decrease in osmolality due to excretion of salt.

5. In rats which had been nephrectomized or had the ureters ligated so there could be no renal modification of the preloads, the effects of the preloads of water and balanced salt are the same as in rats with intact kidneys.

6. The results indicate that after water deprivation in the rat, changes in both the cellular and extracellular fluid compartments are stimuli to drinking.

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INTRODUCTION

Overnight deprivation of water significantly reduces both cellular and extracellular fluid volume (Hall & Blass, 1975; Ramsay, Rolls & Wood, 1977). It is not clear, however, what contribution these changes make to deprivation-induced drinking. Both cellular dehydration and reduction of extracellular or plasma volume stimulate water intake so in the water-deprived rat these stimuli may add together to cause drinking (Fitzsimons & Oatley, 1968). On the other hand drinking following deprivation in the rat may be due solely to cellular dehydration since removal of the cellular stimulus with preloads of water reduces drinking, whereas re-expansion of the extracellular fluid volume has not been shown to significantly reduce deprivation-induced drinking (O'Kelly & Falk, 1958; Corbit, 1967; Corbit & Tuchapsky, 1968; Blass & Hall, 1974).

The purpose of the present experiments is to re-examine the contribution of cellular and extracellular dehydration to deprivation-induced drinking. The effects of preloads of water which primarily replete the intracellular fluid compartment are compared with the effects of preloads of balanced salt solution which selectively expand the extracellular fluid compartment. The effects of the preloads on subsequent water intake are measured over a 9 hr period and the route of administration of the preload is varied. To eliminate the possibility that the kidneys selectively excreted solute from balanced salt preloads, thereby causing osmotic dilution of the plasma, in some experiments the ureters were ligated or the kidneys removed. The effects of the preloads on body fluid composition were determined by plasma and urine analysis.

METHODS

Animals

Male hooded Lister rats were housed in individual cages at a temperature of $72 \pm 2°$ F with the lights on from 7 a.m. to 7 p.m. Water and food were available *ad libitum* at all times except during deprivation periods and experiments. Deprivation of water but not food began at 12 noon the day before an experiment, and the experiments were started between 9 and 10 a.m. Before the experiments began the rats were accustomed to water deprivation and drinking from calibrated drinking tubes. No food was available during experiments. At least 3 days elapsed between experiments.

Solutions

The rats were given tap water in the calibrated drinking tubes. The solutions used for preloads were either deionized water or balanced salt.

The balanced salt solution had the following composition: Na^+ 152 mM, K^+ 6 mM, Ca^{2+} 6 mM, Mg^{2+} 3 mM, Cl^- 137 mM, HCO_3^- 25 mM, HPO_4^{2-} 7 mM. The measured osmolality was 304 m-osmole/kg H_2O and pH 7·47. The calcium and magnesium

chloride solutions were made up and kept in solution separate from the other salts, and added immediately before use to prevent excessive precipitate formation.

Intragastric preloads

The rats were lightly anaesthetized with ether. The mouth was held open with forceps while a polyvinyl chloride tube of outside diameter 2 mm was passed into the stomach, the fluid was pushed manually from a syringe into the stomach over a period of approximately one half minute.

Nineteen rats (240–430 g) which had been deprived of water for 21 hr were tested in a counterbalanced design under each of the following conditions.

(i) Sham intragastric load.
(ii) Intragastric load of 6, 8, 10 and 12 ml. balanced salt.
(iii) Intragastric load of 10 ml. deionized water.

Each rat, therefore, was subjected to six experimental procedures. The rats were given immediate access to water in the home cage and the intake measured for 9 hr, from a calibrated drinking tube.

Intravenous preloads

To allow infusions to be made easily into conscious rats with minimal disturbance, chronic jugular catheters were implanted. Each animal was anaesthetized with ether and the external jugular vein was catheterized with a soft polyvinyl chloride tube, outside diameter 1 mm. The catheter was filled with sodium heparin (10 u./ml.) and its distal end was closed with a small plug. The rats were allowed to recover from the surgery for several days before experiments began.

After water deprivation the jugular catheter was connected to a 15 cm length of stainless-steel tubing, outside diameter 0·75 mm, which projected through the top of the rat's home cage. This prevented the rat from chewing through the tubing and eliminated the necessity of restraining the rats during the infusion. The metal tube was connected by approximately 1 m of polyvinyl chloride tubing to an infusion pump (Fitzsimons, 1963). The infusate, warmed to 37° C, was delivered at a rate of 0·5 ml./min.

Twelve rats (280–450 g), which had been deprived of water for 21 hr, were used as their own controls. Each rat was given the following treatments.

(i) A sham load (connected to the infusion pump but not infused).
(ii) 10 ml. preload of balanced salt solution.
(iii) 10 ml. preload of deionized water.

Each rat, therefore, was subjected to three experimental procedures.

At the end of the infusion the metal tube was disconnected, the catheter was refilled with heparin, replugged and the rats were immediately given calibrated drinking tubes in their home cages. Intake was measured for 9 hr.

Oral preloads

Either deionized water or balanced salt at room temperature were given to the rats. The dehydrated animals consumed the solutions readily and always had finished within 20 min.

Fifteen rats (250–380 g at the start of the experiment) which had been deprived of water for 21 hr were tested in a counterbalanced design under each of the following conditions.

(i) Oral preload of 10 ml. balanced salt.

(ii) Oral preload of 10 ml. deionized water.
(iii) No preload.

In each of the three groups the rats were either given immediate access to water, or access was delayed for 1 or 3 hr. In each case, water intake was measured for 2 hr.

Body fluid analysis

To allow blood samples to be taken easily from conscious rats with minimal disturbance, permanent jugular catheters were implanted under barbiturate anaesthesia (Equithesin, Jensen Salsbery Laboratories, 0·3 ml per 100 g body weight). The catheters consisted of a length of silastic tubing, internal diameter 0·51 mm, connected to a length of polyvinyl chloride (Portex) tubing of internal diameter 1·02 mm by a 4 mm length of 0·37 mm internal diameter stainless steel tubing. The silastic portion of the catheter was cut to a length given by the formula 3·2 cm for a 300 g rat + 0·4 cm per 100 g additional body weight, and inserted into the vein as far as the right atrium. After testing for patency, the catheter was anchored to the jugular vein at the metal junction, and the distal end brought up under the skin and through a small incision at the back of the neck. A polyvinyl chloride collar, glued to the catheter, prevented it from slipping back under the skin. To maintain patency the dead space of the catheter was filled with a solution of sodium heparin (10 u./ml.) in 0·9 % saline, and the catheter plugged with a metal obturator. The heparin was replaced every 2 days, and a lithium heparin solution (1 % in water) was used in the catheter the day preceding an experiment to prevent contamination of blood samples with sodium. Rats were allowed 10 days to recover from surgery. Any animals showing loss of weight or infection at this time were not used.

Experiments were carried out at 4-day intervals to allow recovery of the rats between experiments. During tests, a 1 ml. blood sample was withdrawn after clearing the catheter of heparin completely, and the sample spun down immediately. The catheter was filled with heparin again after sampling. The analysis of urine required that the animals were in a restraining cage during the experiment so that uncontaminated samples could be collected via a small funnel into a graduated sample tube under the rat. To ensure bladder emptying, gentle suprapubic pressure was used. Faeces were collected separately. The animals were accustomed to this procedure before the experiment.

Fifteen rats (352–489 g) were used as their own controls. Four procedures were carried out on each rat:

(i) water deprivation for 21 hr and no preload,
(ii) water deprivation for 21 hr and an oral preload of 10 ml. water,
(iii) water deprivation for 21 hr and an oral preload of 10 ml. balanced salt solution,
(iv) normal fluid replete rats and no preload.

The rats were left in the restraining cages without water and urine was collected for 3 hr. The 1 ml. blood sample was taken at the end of this 3 hr period.

Chemical analyses

Blood samples for haematocrit determination were spun in heparinized microhaematocrit tubes for 7 min at 3000 rev/min at the same time as the blood sample. No correction was made for trapped plasma and the buffy coat was excluded from the measurement. Plasma specific gravity was measured by the method of Phillips, Van Slyke, Hamilton, Dole, Emerson & Archibald (1950), and the plasma protein concentration calculated from this using the formulae of Van Slyke, Hiller, Phillips,

Hamilton, Dole, Archibald & Eder (1950). This calculation is based on the assumption that the relationship between plasma protein concentration and plasma specific gravity was the same for rat as for human plasma. Osmolality was determined by the freezing point method using a Semi-Automatic High Sensitivity Osmette S (Precision Systems, Sudbury, Mass.). Estimations of sodium and potassium were carried out using an automatic internal standard flame photometer (Instrumentation Laboratory, Lexington, Mass., Model 343).

Nephrectomy

Forty-five rats were deprived of water for 21 hr. Both kidneys were then removed through dorsal incisions under ether anaesthesia. Immediately after the completion of the surgical procedure, the rats were divided into three groups and given:

(i) a sham intragastric load ($n = 18$), or
(ii) an intragastric load of 10 ml. balanced salt ($n = 18$), or
(iii) an intragastric load of 10 ml. water ($n = 9$).

The rats were allowed access to water from calibrated drinking tubes for the next 9 hr and then killed and autopsied.

Ureteric ligation

Thirty-four rats were deprived of water for 21 hr. Both ureters were then ligated through dorsal incisions under ether anaesthesia. Immediately after the completion of the surgical procedure, the rats were divided into three groups and given:

(i) a sham intragastric load ($n = 12$), or
(ii) an intragastric load of 10 ml. balanced salt ($n = 13$), or
(iii) an intragastric load of 10 ml. deionized water ($n = 9$).

The rats were allowed access to water from calibrated drinking tubes for the next 9 hr and then killed and autopsied.

Statistics

Means and standard errors of the means are represented in the results. Significances were calculated by Student's t test and in all except the experiments on nephrectomized rats or rats with ureteric ligation the method of paired comparisons was employed.

RESULTS

Intragastric preloads. The cumulative water intake of the rats after the various volumes of balanced salt or 10 ml. water is shown in Fig. 1. Of the various preloads, 10 ml. water causes the greatest reduction of drinking over the subsequent 9 hr. One hr after the preload of water drinking was reduced by 69% ($P < 0{\cdot}001$); 6 ml. balanced salt reduced drinking by 12% (n.s.); 8 ml. by 23% ($P < 0{\cdot}05$); 10 ml. by 25% ($P < 0{\cdot}001$); and 12 ml. by 37% ($P < 0{\cdot}001$). All of the preloads of balanced salt apart from the 6 ml. volume significantly reduced water intake over the entire 9 hr period. The reduction of drinking by balanced salt is a graded effect. A correlation analysis, between the volume of the saline preload and the suppression of drinking at 1, 3, 6 and 9 hr showed significant correlations

at each point (1 hr: $N = 95$, $R = 0{\cdot}39$, $P < 0{\cdot}001$; 3 hr: $N = 95$, $R = 0{\cdot}40$, $P < 0{\cdot}001$; 6 hr: $N = 95$, $R = 0{\cdot}39$, $P < 0{\cdot}001$; 9 hr: $N = 95$, $R = 0{\cdot}39$, $P < 0{\cdot}001$). Expansion of the extracellular compartment with an iso-osmotic solution significantly decreases drinking in the water deprived rat and is not dependent on oral cues.

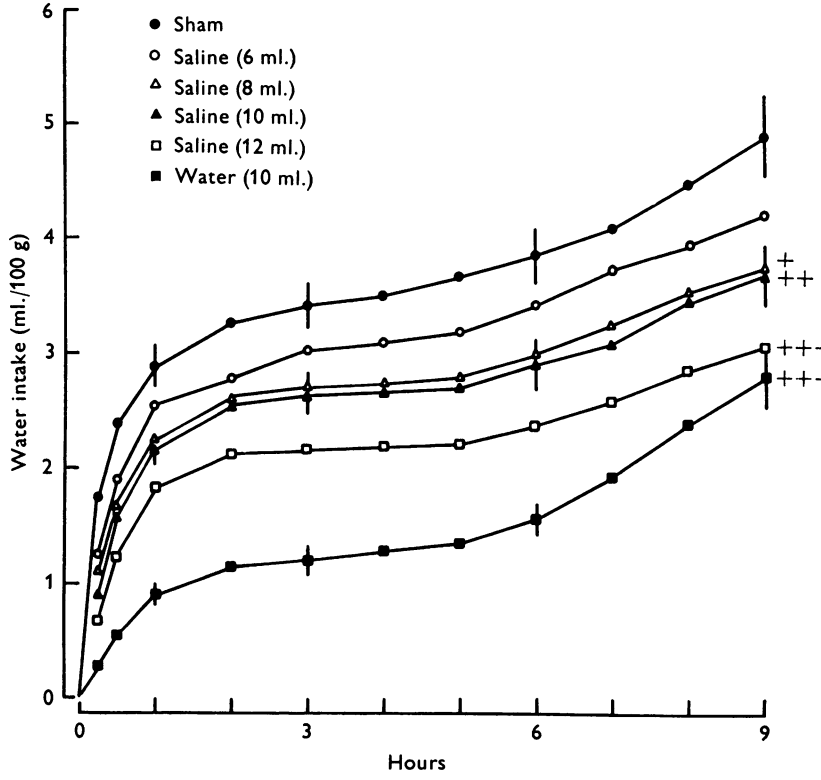

Fig. 1. The mean cumulative water intake of nineteen water-deprived rats, used as their own controls, after intragastric preloads of 6, 8, 10 or 12 ml. balanced salt, 10 ml. deionized water, or a sham load. Standard errors of the means are indicated by vertical bars. The levels of significance are calculated from a comparison between the preload and sham conditions ($+P < 0{\cdot}05$, $++P < 0{\cdot}01$, $+++P < 0{\cdot}001$).

Intravenous preloads. To ensure that the reduction of drinking following intragastric preloads was not simply due to disruption of drinking by the intubation or to gastric distension, 10 ml. preloads of balanced salt or water were infused intravenously.

The cumulative water intake of the rats after the I.V. preloads is shown in Fig. 2. Over the 9 hr the saline significantly reduces water intake but not as effectively as a preload of water. One hour after the saline preload

drinking is reduced by 20% ($P < 0{\cdot}01$), while after the same volume of water drinking is reduced by 65% ($P < 0{\cdot}001$).

If the effect of the 10 ml. intragastric preloads is compared with the effect of the intravenous preloads, it can be seen that between the first and ninth hours the route of administration of the load does not affect the drinking response. The effect of the route of administration of the preloads cannot be compared during the first hour since ether anaesthesia was used for the gastric preloads.

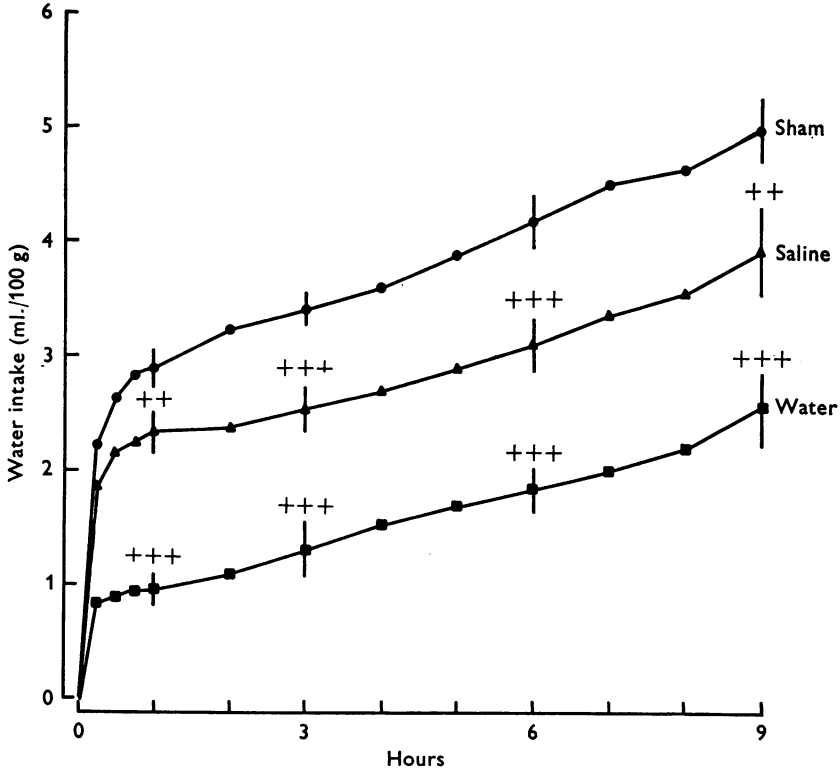

Fig. 2. The mean cumulative water intake of twelve water-deprived rats, used as their own controls, after intravenous preloads of 10 ml. balanced salt, 10 ml. deionized water, or a sham load. Standard errors of the means are indicated by vertical bars. The levels of significance are calculated from a comparison between the preload and sham conditions ($+P < 0{\cdot}05$, $++P < 0{\cdot}01$, $+++P < 0{\cdot}001$).

Body fluid analysis. In this experiment the effect of 10 ml. preloads of balanced salt or water on the composition of the body fluids was determined. To ensure that the preloading procedure did not cause stress to the rats which could influence fluid balance, the rats were allowed to

drink the preloads. Oral preloads had similar effects on subsequent intake as did gastric preloads. For example, in an experiment where rats were allowed to drink water immediately after the 10 ml. oral preload of balanced salt, subsequent intake was reduced by 25% ($P < 0.001$). An oral preload of water reduced subsequent intake by 64% ($P < 0.001$). Since it takes time for the oral preload to be absorbed from the gastrointestinal tract, access to drinking water was delayed after the oral preloads. If the delay was 1 hr, the balanced salt preload reduced subsequent water intake by 26% ($P < 0.01$) and the water preload caused a 64% ($P < 0.001$) reduction. After a delay of 3 hr the balanced salt preload reduced intake by 39% ($P < 0.001$) and water preloads reduced intake by 68% ($P < 0.001$). Since the balanced salt preload was most effective in reducing subsequent water intake after a delay of 3 hr, plasma was sampled after the same interval.

The plasma and urine of preloaded deprived rats is compared with deprived and non-deprived rats which had not been preloaded. Sodium, osmolar and free-water clearances have been calculated to determine the degree of renal involvement in the observed effects of the balanced salt and water preloads.

For convenience, the results are grouped together in three Tables. The results of plasma analysis are shown in Table 1. Water deprivation brings about significant increases in the haematocrit, plasma osmolality and protein concentration. Preloading the deprived rats with water reduces the plasma osmolality to the value found in the normal, non-deprived rat. Preloading with balanced salt, however, brings about a slight but significant fall in plasma osmolality ($P < 0.05$) and the resulting value is still significantly above that in normal non-deprived rats ($P < 0.001$). Presumably the kidney, by virtue of its ability to produce a urine more concentrated than plasma, has begun to excrete the salt load. There was a significant reduction in plasma protein concentration ($P < 0.02$) and haematocrit ($P < 0.05$). In a subsequent experiment to determine whether the slight decrease in plasma osmolality could account for the reduction of water intake after balanced salt preloads, the plasma was analysed 1 hr after the oral preloads. After a 1 hr delay in twenty-two rats the balanced salt preload has no effect on plasma osmolality, but again caused a significant reduction in plasma protein ($P < 0.05$) and haematocrit ($P < 0.001$). The reduction of plasma protein concentration and haematocrit following salt preloads indicates a restoration of the extracellular fluid volume to pre-deprivation levels.

The urinary data for the 3 hr delay are shown in Table 2. Water deprivation causes the expected marked reduction in urine flow and increases in urine osmolality and electrolyte concentrations. Preloading the

deprived animals with water changes urinary composition in a manner characteristic of a water diuresis, urinary flow increasing and urine osmolality and electrolyte concentrations decreasing. Preloading with balanced salt causes significant rises in urine osmolality and sodium concentrations and a maintenance of a low rate of flow, showing that there has been a specific increase in salt excretion under conditions of high concentrating ability. This is characteristic of a solute diuresis.

TABLE 1. Measurements made on the plasma of fifteen rats 3 hr after each of the following four treatments. *Non-deprived*, normal fluid replete rats and no preload. *Deprived and preloaded with 10 ml. water*, rats were deprived of water for 21 hr and an oral preload of 10 ml. water given. *Deprived and preloaded with 10 ml. balanced salt*, rats were deprived of water for 21 hr and an oral preload of 10 ml. balanced salt given. *Deprived and no preload*, rats were deprived of water for 21 hr and no preload given. The means and S.E. of the means are shown. All *P* values were calculated using Student's *t* test and the method of paired comparisons. *P deprived* indicates the *P* value when compared with the deprived and no preload group. *P non-deprived* indicates the *P* value when compared with the non-deprived group

	Non-deprived	Deprived and preloaded with 10 ml. water	Deprived and preloaded with 10 ml. balanced salt	Deprived and no preload
Plasma osmolality (m-osmole/kg H_2O)	299·6 ± 0·48	298·7 ± 0·64	304·9 ± 0·23	306·1 ± 0·57
P non-deprived	—	n.s.	< 0·001	< 0·001
P deprived	< 0·001	< 0·001	< 0·05	—
Plasma sodium (m-equiv/l.)	138·9 ± 0·50	137·0 ± 0·51	140·0 ± 0·51	139·9 ± 0·53
P non-deprived	—	< 0·02	n.s.	n.s.
P deprived	n.s.	< 0·001	n.s.	—
Plasma potassium (m-equiv/l.)	4·1 ± 0·06	3·7 ± 0·08	3·9 ± 0·06	3·9 ± 0·27
P non-deprived	—	< 0·001	n.s.	n.s.
P deprived	n.s.	n.s.	n.s.	—
Haematocrit (%)	42·2 ± 0·68	44·9 ± 0·99	43·7 ± 0·84	46·2 ± 0·62
P non-deprived	—	< 0·01	< 0·05	< 0·001
P deprived	< 0·001	n.s.	< 0·05	—
Plasma protein (g %)	7·5 ± 0·16	7·7 ± 0·14	7·3 ± 0·15	7·8 ± 0·14
P non-deprived	—	n.s.	n.s.	< 0·02
P deprived	< 0·02	n.s.	< 0·02	—

Further information is provided by the calculations shown in Table 3. The presence of a solute diuresis under conditions of high renal concentrating ability in deprived rats preloaded with balanced salt is confirmed by the increases in osmolal, sodium and potassium clearances, together with an increasingly negative free-water clearance.

TABLE 2. Measurements made on the urine of fifteen rats collected for 3 hr after the four treatments described in the legend to Table 1

	Non-deprived	Deprived and preloaded with 10 ml. water	Deprived and preloaded with 10 ml. balanced salt	Deprived and no preload
Urine flow (μl./min)	17 ± 1·8	18 ± 1·0	8 ± 0·3	4 ± 0·1
P non-deprived	—	n.s.	< 0·001	< 0·001
P deprived	< 0·001	< 0·001	< 0·001	—
Urine osmolality (m-osmole/kg H_2O)	983·8 ± 60·00	488·3 ± 45·14	2250·9 ± 69·24	1816·73 ± 130·21
P non-deprived	—	< 0·001	< 0·001	< 0·001
P deprived	< 0·001	< 0·001	< 0·01	—
Urine sodium (m-equiv/l.)	121·5 ± 5·10	15·8 ± 1·39	310·4 ± 20·61	213·5 ± 19·04
P non-deprived	—	< 0·001	< 0·001	< 0·05
P deprived	< 0·05	< 0·001	< 0·001	—
Urine potassium (m-equiv/l.)	116·3 ± 6·89	32·9 ± 3·08	171·9 ± 3·18	185·7 ± 15·73
P non-deprived	—	< 0·001	< 0·01	< 0·001
P deprived	< 0·001	< 0·001	n.s.	—

TABLE 3. Calculations made on the data shown in Tables 1 and 2. *U/P* ratio, urinary/plasma concentration ratio; C_{osm}, osmolal clearance; C_{H_2O}, free-water clearance; C_{Na} and C_k, renal plasma clearances of sodium and potassium respectively. See Table 1 for further details

	Non-deprived	Deprived and preloaded with 10 ml. water	Deprived and preloaded with 10 ml. balanced salt	Deprived and no preload
U/P ratio	3·2 ± 0·20	1·6 ± 0·15	7·4 ± 0·23	6·1 ± 0·48
P non-deprived	—	< 0·001	< 0·001	< 0·001
P deprived	< 0·001	< 0·001	n.s.	—
C_{osm} (μl./min)	52 ± 4·0	27 ± 2·0	54 ± 4·3	25 ± 3·3
P non-deprived	—	< 0·001	n.s.	< 0·001
P deprived	< 0·001	n.s.	< 0·001	—
C_{H_2O} (μl./min)	− 35 ± 3·0	− 10 ± 1·9	− 46 ± 8·1	− 20 ± 3·0
P non-deprived	—	< 0·001	n.s.	< 0·001
P deprived	< 0·001	< 0·001	< 0·001	—
C_{Na^+} (μl./min)	19 ± 1·5	2 ± 0·2	17 ± 2·0	6 ± 0·9
P non-deprived	—	< 0·001	n.s.	< 0·001
P deprived	< 0·001	< 0·001	< 0·001	—
C_{k^+} (μl./min)	460 ± 41·4	152 ± 15·4	350 ± 42·1	192 ± 21·7
P non-deprived	—	< 0·001	n.s.	< 0·001
P deprived	< 0·001	n.s.	< 0·01	—

The water diuresis in the deprived rats given a water preload is apparent from the constant osmolal clearance, greatly reduced sodium clearance and the return of the free water clearance to a less negative value. From this evidence it would appear that the water preloads reduce the rate of release of antidiuretic hormone, whereas the salt preload does not. After 3 hr, therefore, $6{\cdot}9 \pm 0{\cdot}5$ ml. of the water preload is retained, whereas $8{\cdot}7 \pm 0{\cdot}11$ ml. of the salt preload remains ($P < 0{\cdot}001$). Thus after 3 hr the animals receiving oral preloads of balanced salt still retained the majority of that load with little alteration in plasma osmolality, a situation associated with a significant reduction in subsequent water intake.

Nephrectomy. The body fluid analysis show that 3 hr after giving the balanced salt preload, the excretion of a strongly hypertonic urine had already resulted in a small, but significant reduction in plasma osmolality. Presumably, with longer time intervals, this effect would become more significant. In this experiment, both kidneys were removed so that any inhibition of drinking following the balanced salt preload must be due to iso-osmotic expansion of the extracellular fluid compartment, rather than osmotic dilution.

The cumulative water intake of the rats after the preloads is shown in Fig. 3. The results are similar to those when rats with normally functioning kidneys were given similar intragastric preloads (Fig. 1). Water is more effective in inhibiting drinking but the isotonic saline also significantly reduces water intake. With the balanced salt preload drinking is reduced by 53% ($P < 0{\cdot}01$) after 1 hr and by 42% ($P < 0{\cdot}001$) after 3 hr. With the water preload drinking is reduced by 89% ($P < 0{\cdot}001$) after 1 hr and by 75% ($P < 0{\cdot}001$) after 3 hr. The main difference between the nephrectomized and intact rats is that the former are slower to begin drinking, probably because of the surgery, but from 3 hr to 9 hr after the load the results of the two experiments are identical.

Ureteric ligation. In the previous experiment the kidneys were removed to prevent the production of urine. The kidneys are probably involved in the initiation of drinking following hypovolaemia via the renin-angiotensin system (Fitzsimons, 1969) so it is possible that they may have a role in the reduction of drinking brought about by extracellular volume expansion which should inhibit renal renin release. To investigate this possibility, the drinking of the nephretomized rats is compared with rats in which the kidneys are left *in situ* but urine formation is blocked by ureteric ligation.

The cumulative water intake of the rats after the preloads is shown in Fig. 4. With the balanced salt preload drinking is reduced by 67% ($P < 0{\cdot}05$) after 1 hr and by 30% ($P < 0{\cdot}05$) after 3 hr. With the water preload drinking is reduced by 72% ($P < 0{\cdot}05$) after 1 hr and by 65% ($P < 0{\cdot}001$) after 3 hr. There are no significant differences between the

results of this experiment and those in which the rats were bilaterally nephrectomized. Thus the presence of the kidneys whether or not they are producing urine is not necessary for the reduction of drinking by expansion of the extracellular fluid compartment.

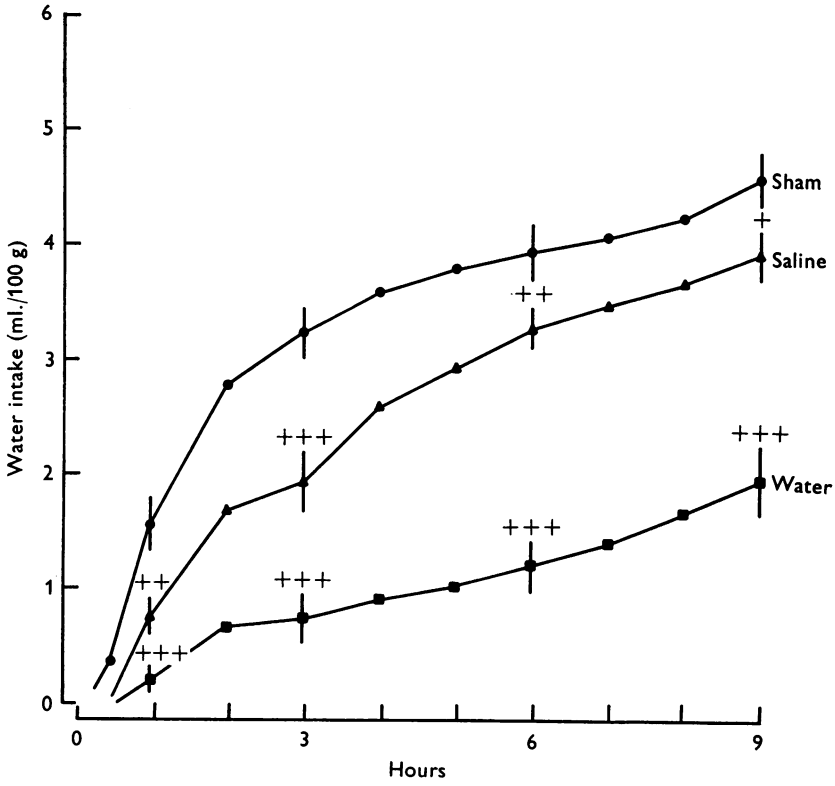

Fig. 3. The mean cumulative water intake by water-deprived rats which had been bilaterally nephrectomized. Immediately after nephrectomy the rats were given an intragastric preload of 10 ml. balanced salt (N = 18), 10 ml. deionized water (N = 9), or a sham load (N = 18). Standard errors of the means are indicated by vertical bars. The levels of significance are calculated from a comparison between the preload and sham conditions ($+P < 0{\cdot}05$, $++P < 0{\cdot}01$, $+++P < 0{\cdot}001$).

DISCUSSION

The results indicate that changes in both the cellular and extracellular fluid compartments contribute to deprivation-induced drinking. In the rat with functional kidneys preloads of water which bring plasma osmolality down to pre-deprivation levels reduce the 1 hr total water intake by 64–69 %, depending on the route of administration of the preload. If the

plasma volume is restored with an isotonic saline solution which has little effect on osmolality, drinking is reduced by 20–26 % after 1 hr. These results agree with our finding in the dog that rehydration of the central

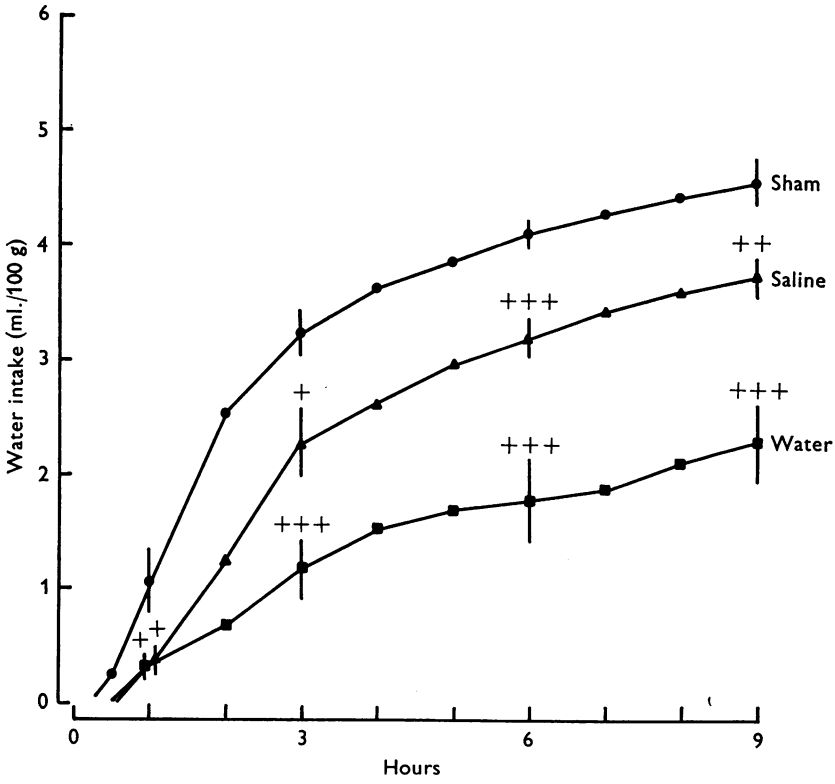

Fig. 4. The mean cumulative water intake by water-deprived rats which had the ureters ligated. Immediately after ureteric ligation the rats were given an intragastric preload of 10 ml. balanced salt (N = 13), 10 ml. deionized water (N = 9), or a sham load (N = 12). Standard errors of the means are indicated by vertical bars. The levels of significance are calculated from a comparison between the preload and sham conditions ($+P < 0{\cdot}05$, $++P < 0{\cdot}01$, $+++P < 0{\cdot}001$).

osmoreceptors with carotid water infusions reduced drinking by 72 %, and restoration of the extracellular fluid compartment reduced drinking by 27 % (Ramsay *et al.* 1977). It is well known that changes in the cellular and extracellular fluid compartments can add together to stimulate drinking (Oatley, 1964; Blass & Fitzsimons, 1970) so it is likely that the drinking following water deprivation is the summation of signals from both fluid compartments.

Although it is clear that overnight deprivation of water significantly

reduces plasma volume (Hatton & Bennett, 1970; Hall & Blass, 1975), there has been little direct evidence to show that this change contributes to deprivation-induced drinking. It seems reasonable that if part of the stimulus to drink in the water-deprived rat comes from extracellular fluid volume depletion, that expansion of that compartment should reduce intake. Previous attempts to reduce water intake of deprived rats by selective expansion of plasma or extracellular fluid volume have been unsuccessful. Corbit infused water-deprived rats intravenously with either Ringer solution (Corbit, 1967) or rat serum which caused hypervolaemia (Corbit & Tuchapsky, 1968) and found no effect on water intake. An intragastric preload of 3% of body weight of isotonic saline also had no effect on the water intake of water-deprived rats (O'Kelly & Falk, 1958). Blass & Hall (1974) gave rats intragastric preloads equivalent to the deprived rats' fluid loss, and found little effect on subsequent water intake.

Extracellular fluid volume expansion has little effect on drinking stimulated by changes specific to the extracellular compartment. Stricker (1969) found that in rats in which polyethylene glycol was used to deplete plasma volume, intragastric preloads of isotonic saline had little effect on water intake unless the load completely replaced the lost fluid. Stricker (1971) reported also that after ligation of the inferior vena cava rats continued to drink isotonic saline at high rates for 24 hr after ligation, whereas water intake slowed after several hours. These stimuli are different from water deprivation which causes easily reversible depletion of the extracellular compartment. Caval ligation and polyethylene glycol are continuing thirst stimuli not readily reversed by preloads or consumption of isotonic fluid.

We found that drinking induced by an intracranial injection of angiotensin, which stimulates intake in the absence of fluid deficits, was significantly reduced by a 15 ml. intragastric preload of isotonic saline (Rolls & McFarland, 1973) and that the ingestion of isotonic saline reduced angiotensin-induced drinking (Rolls & Jones, 1972).

It is not clear why previous workers did not observe an effect of volume expansion on deprivation-induced drinking. In our experiments, isotonic saline is less effective in reducing drinking than a preload of the same volume of water, but nevertheless the effect of saline is highly significant. The results show clearly that the effect of the saline on drinking is due to extracellular fluid volume expansion and not due simply to the preloading procedure or osmodilution. The preloads of saline were equally effective when administered intragastrically, intravenously or when consumed by the rat. Determination of plasma protein concentration indicates that at both 1 hr and 3 hr after a preload of 10 ml. isotonic balanced salt, extracellular fluid volume was back to pre-deprivation levels. One hour after

a preload of balanced salt plasma osmolality was unchanged. However, 3 hr after giving balanced salt, the kidneys have begun to excrete a more concentrated urine, and as a result of this the plasma osmolality shows a slight reduction from the water-deprived level. It is unlikely that this small degree of osmotic dilution is the cause of the reduction of drinking following balanced salt since it takes some time for the kidneys to effect the change in plasma osmolality, and the effect of the salt on water intake is seen in the first hour. Furthermore, removal of the excretory function of the kidneys, either by nephrectomy or by ureteric ligation is without effect on the reduction of drinking brought about by the balanced salt.

The experiments on rats which had undergone nephrectomy or ureteric ligation make it clear that the reduction of drinking following isotonic balanced salt must be due to extracellular fluid volume expansion. In these rats there could be no renal modification of the preload and therefore no osmodilution. The surgery caused some disruption of drinking for approximately 2 hr but after that the intact rats, the nephrectomized rats and the rats with ureteric ligation all drank similar amounts after the same preloads.

At least three different receptor systems may detect the cellular and extracellular depletion that occurs after water deprivation. Receptors which respond to cellular shrinkage are located in the forebrain, probably in the preoptic area (Blass & Epstein, 1971). In the dog we found no evidence to support the view that there are peripheral osmoreceptors for thirst (Wood, Rolls & Ramsay, 1977). There may be at least two receptor systems which detect changes in plasma volume. One is the juxtaglomerular apparatus of the kidney. Renin is released by the kidney when plasma volume is decreased. Renin acts to form angiotensin which stimulates receptors in the brain (Epstein, Fitzsimons & Rolls, 1970). The site of these receptors is controversial, but the subfornical organ (Simpson & Routtenberg, 1973), the anterior third ventricle (Buggy & Fisher, 1976) and the preoptic area (Kucharczyk, Assaf & Mogenson, 1976) have been suggested. There may also be cardiac distension receptors and arterial baro-receptors which are important for drinking. Left cervical vagosympathectomy abolishes the effect of hypovolaemia on thirst in the dog (Kozlowski & Szczepanska-Sadowska, 1975).

The results of the present study show that after water deprivation in the rat, changes in both the cellular and extracellular fluid compartments contribute to rehydration. The changes in the cellular compartment make the largest contribution to thirst, but the changes in the extracellular compartment do significantly affect drinking.

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