Factors modifying water metabolism in rats fed dry diets

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To determine dietary factors which govern water turnover in rats, water intake and output were studied during 24-hour intervals. Rats given water ad libitum and Purina laboratory chow or a synthetic diet voluntarily maintained an antidiuretic state, restricting water intake and excreting urine of nearly maximum concentration. Dietary modifications which have been studied include: addition of large amounts of NaCl or urea; a protein-free diet with or without urea added; and diets containing various levels of Na, K and Cl. Conclusions drawn from these studies are as follows: under most conditions water intake was determined by the minimum urine water for solute excretion or by changes in fecal volume; the degree of antidiuresis maintained by the rats may depend on the electrolyte content of the diet. Nitrogen turnover has an additional influence on water metabolism through the effect of urea excretion on the renal concentrating mechanism. Evidence also presented indicates that Pitressin may act to 'reset' the threshold of drinking, with a resulting concentration of body fluids.

Although considerable interest exists in the unsolved problem of regulation of body fluid volume, few studies have related water excretion to the spontaneous intake of water. In normal animals obtaining water from sources other than food, regulation of water intake may be as important as regulation of water excretion. It is not known, moreover, whether physiological mechanisms regulating water excretion directly influence water intake.

These studies were undertaken to investigate dietary factors determining intake or output of water by rats under experimental conditions which approached normal as closely as possible. Water metabolism with two normal diets was investigated, and the effects of addition or removal of electrolytes, urea or protein were also studied. The experiments were done on unanesthetized animals, generally in their normal nutritional state, thus minimizing nonspecific stimuli to the hypophysial-hypothalamic system. Solute excretion rate was maintained at or near normal, an important point in view of the paucity of studies on renal water conservation at normal levels of solute excretion. The data were obtained over 24-hour intervals, thus avoiding the diurnal cycle of electrolyte and water excretion; adjustments of the rats to dietary changes were followed for several days or weeks.

METHODS

Male and female albino rats, 4-6 months old, were used in all experiments. The males weighed 350-500 gm and the females, 250-350 gm. Two strains of rats were used, both Wistar derivatives, but no difference in the responses of the two strains was noted. The stock diet was Purina laboratory chow. During experiments the rats were individually kept in metabolism cages fitted with drinking fountains and external food cups. Room temperature was 25°C and relative humidity was generally less than 50%. The room was lighted artificially and a daily pattern of 13 hours light, 11 hours dark was maintained. Distilled water was used for drinking in all experiments. Food and water intake, urine volume and body weight were measured daily for each rat, and in some experiments daily fecal weight was also recorded. The rats were selected during control periods to eliminate those who habitually spilled food. Correction was made for spilled food when on rare occasions it was necessary, but when spillage led to significant contamination of the urine by food the data were discarded. Intakes of food and water were determined by weighing the containers to the nearest gram and were corrected for evaporative losses (2-3 gm/day for water, negligible for food) obtained from dummy watering and feeding devices set up on the cage rack. Urine was collected in cylinders from which the urine volume could be read directly to the nearest milliliter. Modification of urine concentration by bacterial action (urea split to ammonia with pH increased) sometimes occurred with 48-hour urine collections, and for this reason 2-day collections were avoided when possible. Twenty-four-hour collections rarely showed evidence of bacterial action on urea.

Two methods were used to minimize evaporative
WATER METABOLISM IN RATS

losses from the urine. In the method generally used (silicone method), the funnel and feces collecting screen (5-mesh) were coated with silicone grease to prevent urine from sticking to them, and the urine cylinder contained a small amount of light mineral oil, thus eliminating evaporation from the urine surface. In the second method (mineral oil method), used as a check on the first, the funnel was stoppered and filled with mineral oil to within one inch of the floor of the cage. A small four-mesh screen was placed in the mineral oil to collect feces below the surface. In this way both urine and fecal water losses could be measured. To compare these two methods, total urinary solute excretion was measured in two groups of six male rats, using the methods alternately for 3 days. For the mineral oil method urine solute excretion per gram of food eaten was 1.22 mOs/gm, while for the silicone method the ratio was 1.26 mOs/gm, thus solute recovery was identical for the two methods.

As an additional check on the silicone method, rat urine was injected intermittently into an empty cage from a syringe during a 6-hour period; urine recovery of 90–95% was obtained.

Urine osmolarity was measured by freezing point depression using a Beckman differential thermometer or Fiske osmometer. Standard solutions of sodium chloride were prepared for calibration from data in the International Critical Tables. All samples had duplicate determinations. The range available for the Fiske instrument was extended to permit direct measurement of the freezing point of rat urine, and no urine dilution was necessary except when the urine concentration exceeded 2900 mOs/l. In this case, the samples were diluted by one-third to minimize the influence of dilution on osmotic activity. Accuracy of the Fiske instrument is estimated to be ±0.5% in our hands.

Fecal water was measured by difference after drying at room temperature in a hood for 48 hours. Preliminary experiments showed that spreading out the fecal pellets and longer drying periods or higher drying temperatures had no effect on the measured fecal water. Fecal water varied from 1.5 gm/gm fecal solids for low residue synthetic diets to 2.1 gm/gm fecal solids for Purina chow, and these values check well with those reported for rats by Schmidt-Nielsen et al. (1).

For urine and blood analyses, sodium and potassium were determined by flame photometry, chloride by the method of Schales and Schales (2), urea ammonia and urea by the method of Van Slyke and Cullen (3), blood or plasma urea by the method of Karr (4), and total nitrogen and protein by the micro-Kjeldahl technique. Food and fecal samples were wet-ashed for analysis. Blood samples were taken into heparinized syringes from the right heart with the rat under chloroform anesthesia.

The diets and their modifications used included finely ground Purina laboratory chow, with NaCl or urea added to increase solute load, and synthetic diets whose compositions are summarized in table 1.

### RESULTS

#### Water Turnover

The results of this study depend on quantitative accounting for ingested water, and water balances using the mineral oil collection method permitted a check on water and solute recovery as well as an indication of the relative importance of the different routes of water intake and output. Figure 1 shows the average values of intake versus output for 10 male rats maintained on Purina laboratory chow, and the same group on synthetic diet I.

Water from the food was calculated from the food composition, including both preformed water and water of oxidation, using the factors of Morrison (5). Water retained or lost as body fluids as a result of body weight variation was calculated by assuming water to constitute two-thirds of the body weight change. During the periods shown in figure 1 there was a slight average weight loss, thus the base line for water output is shifted slightly downward. Insensible water loss was calculated by determining the 24-hour oxygen uptake from the calculated R.Q. and caloric intake (corrected for urine output.

![Figure 1. Twenty-four-hour water turnover for 2 diets; mean values for 10 male rats. Water intake, urine volume and fecal water were measured directly. Water from food and insensible water loss were calculated according to methods given in text. Base line for output is shifted downward in both cases because there was a slight average weight loss during both collections (see text).](http://ajplegacy.physiology.org/)

### TABLE I. Composition of Synthetic Diets by Weight (gm)

<table>
<thead>
<tr>
<th>Diet</th>
<th>I</th>
<th>II</th>
<th>III</th>
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<tbody>
<tr>
<td></td>
<td>A</td>
<td>R</td>
<td>A</td>
</tr>
<tr>
<td>Casein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetable oil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextrose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamins†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salts†</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Urea</td>
<td>6.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salts‡</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>NaCl</td>
<td>2.1</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td></td>
<td>0.9</td>
<td></td>
</tr>
</tbody>
</table>

* Vitamin Diet Fortification Mixture, Nutritional Biochemicals Corporation, Cleveland, Ohio.
† Salt mixture USP XIV with KCl omitted.
‡ Salt mixture USP XIV with KCl, NaCl and K₂HPO₄ omitted.
that other urinary constituents are negligible in determining the urine osmotic pressure. In subsequent studies where different diets were used, good agreement between observed osmolality and values calculated by this method has been consistently obtained.

Water intake for the two diets (fig. 1) was approximately the same, but the routes of excretion of water were not identical. With Purina chow, fecal water loss was a large fraction of water excretion, while with the synthetic diet the low residue of feces led to small fecal water loss. On the other hand, with the synthetic diet the nitrogen in the diet was more completely absorbed, and the resulting increase in ura excretion produced an increase in urine volume (see below for the influence of solute excretion).

Normal Values for Urine Concentration and Effects of Pitressin Tannate in Oil

Preliminary studies indicated that even though rats were offered water ad libitum, they restricted their water intake to the minimum necessary to cover water excretion via the three main routes. The hypothesis that urine volume was at or near the minimum was tested by giving Pitressin tannate in peanut oil (Parke, Davis) intramuscularly. Two groups of 10 rats, five males and five females, were used in this experiment, the food being synthetic diet I. One group received Pitressin and the other group was given an equal volume of peanut oil. Figure 2 shows the effects on urine concentration, water intake and urine volume of 250 mU of Pitressin, approximately three times the daily endogenous production by dehydrated rats (8). The control group showed no significant effect of the injection of peanut oil. The group of 10 rats given Pitressin, however, showed a slight rise in urine concentration persisting until the 4th day, the peak response being reached 24-48 hours after injection. No change in food intake occurred during the period that Pitressin was effective. The decrease in urine volume was almost exactly proportional to the rise in concentration for the Pitressin group, and the mean daily solute excretion rate remained remarkably constant. This lack of effect of Pitressin in oil on solute excretion has been a consistent finding in rats fed normal diets.

To compare the results in figure 2 with the maximum urine concentration produced by dehydration, two groups of four male rats were given no water for 3 days. One group was offered food while the other received nothing. By the 3rd day the rats offered food had a mean urine volume of 2670 mU, while the rats given nothing had a mean concentration of $2719 \text{ mOs/l}$. It is apparent that under these experimental conditions dehydration did not give rise to more concentrated urine than was obtained by exogenous Pitressin.

After Pitressin was given (fig. 3), water intake consistently declined more than urine volume during the first 24 hours. The decrease in water intake corrected for changes in urine volume and body weight exceeded 1 ml for all 10 rats with a mean of 4 ml, while in the controls the changes were random with the mean less than 1 ml.
which is within the errors of measurement. This observation suggested that body fluids might be more concentrated after Pitressin, assuming no change in insensible water loss. In order to test this hypothesis, six rats on Purina laboratory chow were chosen at random from stock and given food and water ad libitum and 250 μl of Pitressin i.m. They were killed 24 hours later and heart blood was obtained for analysis. Six controls fed stock and given food and water ad libitum and 250 μl of Pitressin were chosen but all rats decreased their food intake approximately in half, and the experiment was terminated.

A significant increase in osmolar concentration of the plasma of the Pitressin group was observed. This finding is in the direction opposite to that expected if the renal effect of Pitressin were the only one present. Since Pitressin produced no effect on food intake or solute excretion, the most likely explanation of this change in plasma concentration is a direct effect on water intake. For the Pitressin-injected rats, when correction was made for body weight variations the fall in water intake during the first 24 hours after the injection exceeded the fall in urine volume by 3 ml. A water deficit of 3 ml would be consistent with a concentration of body fluids of about 1.5%, which checks closely the observed difference in plasma concentration.

**Effects of Increased Solute Load on Water Turnover**

These experiments are similar to those reported by Gamble et al. (9). Solute excretion was increased by adding 1 mm sodium chloride or 2 mm urea/gm of Purina laboratory chow. An attempt was also made to increase solute excretion by adding potassium chloride, but all rats decreased their food intake approximately in half, and the experiment was terminated.

Figure 3 shows the relationship between urine volume and osmotic load (the product of urine volume and concentration) for five male rats on the diet with sodium chloride added (closed circles), and for five male rats given urea (closed triangles). For comparison urine volume vs. osmotic load immediately preceding the addition of solutes is also shown for each rat in figure 3 (open circles and triangles).3

Although urine volume and osmotic load during the control period were nearly identical in the two groups, addition of urea led to significantly lower urine volumes than were excreted by the sodium chloride loaded rats at equivalent solute excretion rates. The finding that urea produced lower water turnover than other solutes confirms the observations of Gamble et al. (9). This difference depends on the greater ability of the kidneys to concentrate urea found by Kellogg and Koike in rats (10) and Epstein et al. (11) in man.

The rise in urine volume brought about by the two solutes was in all cases matched by an increase in water intake, and no transient water imbalance could be detected within limits of the present methods. The only transient phenomenon observed was a short-term adjustment of osmotic load resulting from a fall in food intake. Body weight showed greater day to day fluctuations in the salt loaded rats than normal, a result which has been repeatedly confirmed by subsequent experiments with sodium chloride loading. These day-to-day variations often exceeded 10 gm, while with a normal diet they rarely exceed 5 gm. Urea loading was not characterized by this phenomenon after the transient effect was completed.

Electrolyte and nitrogen balance studies were carried out on the 6th day and it was found that all five urea-loaded rats were in negative sodium balance. The mean recovery of ingested sodium was 110%, indicating about 15% greater excretion of sodium than would be expected assuming 93% recovery for the mineral oil collection method. Nitrogen was in balance, but potassium appeared to be in slight positive balance with a mean recovery of 89%.

For the sodium chloride-loaded rats a wide variation in solute excretion rate is obvious from figure 3; this variation depended in part on differences in food intake. Balance studies during the 6th day showed that three animals were in positive sodium balance (recoveries 72, 81 and 81 % of ingested sodium) while one was in nega-

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3 The curved lines drawn on fig. 3 represent theoretical lines drawn through the extremes of the control data. They are derived from a formula for urine volume as a function of osmotic load, 

\[ V = \frac{D^2}{P(K + L)} \]

where \( V \) is the urine volume per unit time at maximum antidiuresis, \( D \) is the osmotic load excreted per unit time, \( P \) is the osmolarity of the arterial plasma, and \( K \) is a constant indicating the maximum rate of excretion per unit time of solvent-free solute. The derivation of this formula is based on the assumption that the net tubular transfer of water during antidiuresis (\( T_{\text{H2O}} \)) is inversely proportional to urine osmolality; discussion of this assumption is beyond the scope of this report. Within the range of individual variation the equation adequately described the response of each of the salt-loaded rats. The equation also fits the data of Kellogg and Koike (10) at higher loads of a variety of solutes, except urea, given intravenously. (I am indebted to Dr. R. H. Kellogg for sending me his results in detail.)
The effects of removing various dietary constituents on water turnover

These experiments investigated the effects of removing electrolytes or nitrogen on voluntary water turnover or the renal concentrating mechanism. In a preliminary experiment, fat was removed from synthetic diet I. No measurable effect was observed except for a slight increase in total solute load, brought about by the greater food intake resulting from a small decrease in caloric density of the diet. When protein was removed from the diet, however, water turnover became highly variable and some of the rats even excreted a hypotonic urine, in spite of the fact that osmotic load had fallen. These results depended in part on the level of electrolyte in the diet, and consequently the effects of a low electrolyte diet were also investigated.

Protein-free diet. Twelve male rats weighing about 400 gm were used. After a control period on synthetic diet I, six rats were placed on diet HA (protein-free). The other six were given diet HIB (protein-free diet with urea added) to bring urea excretion close to that obtained with the control diet. On the 6th day of the experiment, rats from each group were given 250 μg of Pitressin i.m. and during the following 24-hour period nitrogen balances were carried out. At the end of this day the rats were killed and heart blood drawn for urea analysis and total plasma osmolality.

The effects of these diets on urine volume and urine concentration are shown in figure 4. During the 7 days the rats were on the protein-free diets both groups lost weight at approximately the same rate of 4.5 gm/day, indicating that the group receiving urea derived little or no nutritional benefit from it. Food intake of both groups was nearly identical and showed about a 25% decrease compared with the control period. Nitrogen balances done on the 6th to the 7th day showed that all 12 rats were in negative nitrogen balance. For both groups the mean nitrogen loss was nearly identical at about 100 mg/day, confirming that endogenous protein metabolism was essentially the same.

In the group receiving urea, urine volume was approximately doubled immediately after beginning the protein-free period, and urine concentration showed a reciprocal fall to about one-half its control value. The group receiving no urea showed little effect on urine volume, and urinary concentration fell within 2 days to a new equilibrium about one-third the control concentration. Only slight changes in water intake occurred during the period of the protein-free diet. Mean water intake of the rats receiving urea increased about 4 ml while that of the rats receiving no urea decreased about 2 ml.

When Pitressin was given on the fifth day of the protein-free period a greater effect was observed than in rats eating a normal diet. The rats showed a decrease in urine volume averaging 14 and 9 ml for the group without and with urea, respectively. The fall in urine volume was associated with a mean decrease in water intake of 18 and 13 ml for the two groups. The urine

TABLE 3. Effects of 250 μg Pitressin Tannate in Oil i.m. on Plasma and Urine (Means and Standard Error—Groups of 6 Male Rats, Purina Chow Diet)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Pitressin Injected</th>
</tr>
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<tbody>
<tr>
<td>Plasma osmolality, mO/l</td>
<td>305.9 ±0.86†</td>
<td>310.8 ±1.50††</td>
</tr>
<tr>
<td>Plasma urea, mm/l</td>
<td>8.10±0.40 37‡</td>
<td>8.69±0.13‡</td>
</tr>
<tr>
<td>Nonurea pl. Osm., mO/l</td>
<td>297.8±0.40 84‡</td>
<td>302.9±1.48‡</td>
</tr>
<tr>
<td>Urine osmolality, mO/l</td>
<td>1966±43</td>
<td>2267±194</td>
</tr>
<tr>
<td>Urine urea conc., mm/l</td>
<td>947±54</td>
<td>1154±99</td>
</tr>
<tr>
<td>Urine volume, ml/day</td>
<td>15.3±0.48</td>
<td>14.3±1.38</td>
</tr>
<tr>
<td>Urine urea load, mm/day</td>
<td>14.6±0.43</td>
<td>16.0±0.63</td>
</tr>
<tr>
<td>Urea clearance, ml/min</td>
<td>1.25±0.034</td>
<td>1.26±0.047</td>
</tr>
<tr>
<td>Kidney weight, gm</td>
<td>2.5±0±0.106</td>
<td>2.56±0.093</td>
</tr>
<tr>
<td>Urea clearance/gm KW, ml/mg</td>
<td>6.90±0.018</td>
<td>6.30±0.014</td>
</tr>
</tbody>
</table>

Plasma samples taken 24 hr. after injection. Urine obtained during 24-hr. period after injection

† Difference between means significant (P ≥ 0.02).
‡ Difference between means not significant (P > 0.2).
concentration of the urea group rose to the normal range, while the urine concentration of the rats receiving no nitrogen increased only to 1277 mOsm/l, indicating that the kidneys could no longer concentrate the urine as effectively.

The rats on the protein-free diet without urea showed a fall in concentrating ability in two of the four rats. These two rats had a decrease in total solute as well as urea excretion and also excreted a basic urine (pH 7–9). The decline in concentrating ability may depend on a decrease in urea excretion, on a mild potassium deficiency (13), or on a change in acid-base metabolism, which we have found to influence the concentrating mechanism (unpublished observations).

On the 9th day after the three diets had been started, determination of sodium, potassium and chloride excretion for a 24-hour period showed that all rats were essentially in balance except the low electrolyte group. Urinary cation losses in this group were about 0.30 mEq/day for sodium and potassium, corresponding to a change in body weight. The cation losses, therefore, must be accounted for as coming from intestinal contents, from inert stores such as bone (14), or from the replacement of cellular tissue by fat. Mean chloride loss of 1.56 mEq/day was particularly striking.

Rats fed the medium-salt diet excreted approximately 25% less total solutes than rats on the high-salt diet. The group on the salt-free diet, however, had little difference in total solute excretion rate compared with the medium-salt diet because changes in sodium, potassium and chloride excretion were partially offset by changes in ammonia excretion. For the salt-free group urine ammonia with associated anions (chiefly chloride) constituted about 25–30% of total solute excretion. On the other hand, for the high-salt group ammonia and associated anions were less than 5% of solute excretion. The finding of increased ammonia excretion may have some

### Fig. 3. Urine volume vs. osmotic load. Each point represents the mean of a consecutive days' collection for each rat. Open symbols, control data obtained just prior to adding solutes to Purina laboratory chow. Closed symbols, values obtained on 5th and 6th days after adding 1 mM NaCl or 9 mM urea/gm of Purina chow. Curved lines are theoretical lines drawn through extremes of control data.
significance on renal water loss since the effect of ammoxia excretion on the renal concentrating mechanism is as yet unknown.

These studies indicate that diminution of the food electrolyte below that of Purina laboratory chow did not lead to a further rise in urine concentration. This finding is consistent with the effects of dehydration, and indicates that a 'ceiling' on urine concentration is reached at about normal solute loads. The low-salt diet appeared to have its chief effect on water intake. The increase in water intake, and not the increased urinary volume, was the primary factor since the kidneys could still respond to exogenous Pitressin.

**DISCUSSION**

These experiments were undertaken to explore the limits within which water turnover was maintained voluntarily by rats when dietary composition was changed. In part they represent a study of mechanisms of body water regulation in animals given the opportunity to alter their water turnover by changes in water intake. Control of body water obviously depends on simultaneous regulation of intake and output, at least in situations where water from the food does not meet minimum requirements for excretion. When food is a significant source of water, as in the case of most herbivores, regulation of body water depends primarily on excretion.

The studies in this report have been concerned with regulatory mechanisms extending over 1 or more days. Most previous experiments by others have dealt with short-term regulation of water intake or output extending over minutes or hours, but long-term control mechanisms may be of equal importance. A long-term effect altering water exchange has been described in man (15, 16): pronounced depression of the concentrating ability of the kidneys has been found after voluntary ingestion of large volumes of water for several days, an effect not reversed until 3 or 4 days after voluntary overdrinking was stopped. West and Bayless (17) have suggested, moreover, on the basis of acute experiments in dogs, that the ratio of electrolytes to total solutes in the urine is regulated by a renal tubular mechanism. Over periods of several days, if urea excretion is high the ratio itself must be under some control to prevent electrolyte depletion. In this connection it is interesting to note the effect of long-term urea loading in the present experiments. Even after 6 days of high urea turnover, all five rats were in negative sodium balance. If the ratio of urea to sodium in the urine is regulated in some way, it would appear to respond relatively slowly to changes in nitrogen turnover. It is possible that such slowly responding mechanisms are of major importance in the control of body sodium and water.

Regulation of water output is complicated by the fact that there is more than one route of excretion. In order to simplify this discussion, the assumption will be made that insensible water loss was not changed by diet or Pitressin. Although this assumption is not proved, it appears reasonable because insensible water loss is not changed by diet or Pitressin. Although this assumption is not proved, it appears reasonable because insensible water loss in man, at least, is known to be independent of the degree of body hydration over a wide range (18), and because caloric intake, upon which insensible loss does depend, was near normal. The effect of Pitressin or body hydration on fecal water is also unknown, with regard to the influence of diet, figure 7 shows the relationship between fecal water and dry fecal weight obtained with some of the diets used. The data appear to fall on a curved line, suggesting that the ratio of water to solids is altered by the rate of excretion of solids or possibly by the rate of excretion of some osmotically active material in the feces. Most of the experiments in this report were done with low-residue synthetic diets, and fecal water was a negligible part of total water turnover. The following discussion will be restricted, therefore, to consideration of factors modifying urine volume and water intake, and their implications in the regulation of body water.

**Dietary Effects on Urine Volume**

In nearly all of the experiments water turnover was maintained voluntarily by the rats close to the minimum and a strongly hypertonic urine was excreted. For this reason effects of changes in the diet on urine volume depended primarily on responses of the renal concentrating mechanism. No attempt will be made to summarize recent concepts of the concentrating process by the kidneys. Suffice to say that in the hydropenic state, urine volume is governed by the osmotic activity of the

**FIG. 4.** Effects of protein-free diet on urine volume and concentration; mean values for groups of 6 male rats. During control period all rats recived synthetic diet I. At day zero, diet IIA (open symbols) and IIB (closed symbols) were begun. On the 5th day half of each group was given 250 mu Pitressin i.m.
et al. the maximum concentration of the urine is strongly deconcentrating ability. These observations suggest that metabolized, corrected the alteration in dependent on the urea excretion rate, per se. Similar observations have been made in man (diet, even though there was no evidence that it was accord with those recently reported in dogs by Levinsky after Pitressin injection (fig. 4). Addition of urea to the significant decline in the maximum urine concentration which these rats could produce.

On the other hand, the protein-free diet produced a significant decline in the maximum urine concentration after Pitressin injection (fig. 4). Addition of urea to the diet, even though there was no evidence that it was significantly metabolized, corrected the alteration in concentrating ability. These observations suggest that the maximum concentration of the urine is strongly dependent on the urea excretion rate, per se. Similar observations have been made in man (and), and they are in accord with those recently reported in dogs by Levinsky et al. (50). These authors have shown an effect of glomerular filtration rate or renal blood flow. The effects on the renal concentrating mechanism produced by variation in urea excretion make the urine volume much less sensitive to the nitrogen excretion rate than would be the case if urea behaved like sodium chloride. Given the hydropenic state, at high urea loads the urine is concentrated more effectively (fig. 3), and at low urea loads it is concentrated less effectively (fig. 4). If applicable to man these results would negate the value of protein-free diets for the purpose of water conservation during periods of water deprivation, such as for survival on a life raft.

The above observations may not be entirely in accord with the normal response to dehydration. In the dehydration experiment, total solute excretion fell below 10 mOsm/day by the 3rd day in all rats, whether or not they were given food, and urea excretion averaged 6 mEq/rat on the 3rd day. This is less than half the usual urea excretion rate, but in spite of the fall in urea excretion, the urine continued to be maximally concentrated. This observation suggests either that an additional factor is present in the dehydrated animal compared with the animal given water and exogenous Pitressin, or that a moderate fall in urea excretion has no effect on the concentrating mechanism.

**Effects of Diet on Water Intake**

Wide variations in water intake could be induced by dietary changes without altering the antidiuresis maintained by the rats. This finding indicates that water intake is not merely determined in rats by metering a given volume of water in relation to the total volume of food ingested. Hydropenia was present when the rats were fed two widely different diets (chow and synthetic diet IIIA, figs. 5A and 6) which differed not only in

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**Fig. 5. Effects of modification of dietary Na, K and Cl on water intake (triangles) and urine volume (squares). Mean values, groups of 4 male rats. During control period all rats were fed Purina laboratory chow. Arrows along abscissa indicate injection of 250 mg Pitressin in oil i.m. A. At day 0, rats were given diet IIIA having approximately the same Na, K and Cl content as Purina laborato-

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**Fig. 6. Effects of modification of dietary Na, K and Cl on urine concentration. Data from groups shown in fig. 5. Arrows along abscissa indicate injection of 250 mg Pitressin in oil i.m.
In rats on various diets injection of Pitressin has consistently produced a greater decline in water intake than in urine volume. The rise in total plasma osmolality at 24 hours (table 3) suggests that Pitressin exerts a regulatory or delaying effect on water intake. An effect of Pitressin on water intake was observed by Bellows (26) in two dogs with esophageal fistulas. When 1 cc of Pitressin was given subcutaneously 20–30 minutes before injection of 2.5 cc/kg of 20% sodium chloride intravenously, there was a delay of 10–20 minutes before sham-drinking began. Similar results were obtained by Holmes and Gregersen (27). Commercial Pitressin was used throughout the present experiments, and thus it is premature to say whether the effect observed on water intake depends upon vasopressin itself or upon some other component of pituitary extract. The inhibitory effect of exogenous Pitressin on water intake does not change the basic stimuli for drinking brought about by water losses; the effect may be to change the level of body fluid concentration at which drinking is initiated, that is, to ‘reset’ the drinking center.

Maintenance of antidiuresis was most significantly altered by the salt-free synthetic diet (fig. 5C). By the 2nd week water intake for each rat was 15–40 ml/day more than the minimum required to excrete the urinary solutes. The changes in water intake developed relatively slowly in all four rats. These observations are in agreement with those reported by Cizek et al. (28) in dogs depleted of electrolyte and fed a low-salt diet. With the low-salt diet, the rats developed a progressive change in body sodium, potassium and chloride which may have provided the stimulus for an increase in drinking. Thus the results obtained with a medium-salt diet (fig. 5B) suggest, however, that an electrolyte imbalance may not be necessary for a modification of water intake. These rats were in electrolyte balance but still excreted a more dilute urine than the high-salt group and showed a greater response to Pitressin.

Water intake greater than the minimum was also observed in the rats on the low-protein diet, with or without urea added. With these diets the excess of water intake over the minimum did not generally exceed 10–15 ml/day. It should be emphasized that the experiments with the low-protein diet were done with the electrolyte intake about half that of Purina laboratory chow. Thus the effect of the protein-free diet on water intake may be ascribed to the fact that with a moderately low electrolyte intake, regulation of water intake was less precise than with a high electrolyte diet, and thus when water requirements changed, the antidiuretic state was less rigidly maintained. These results are consistent with the findings in the rats given the moderate-salt diet with protein present (fig. 5B). An additional factor with the protein-free diets was the fact that the rats had a steady weight loss and tissue breakdown which may have had an effect on water metabolism.

It is possible that control of water intake may depend on a conditioned reflex, with some property of the diet
as a conditioning stimulus, but such an interpretation appears at the present time to be unlikely. We have found that weaning rats, when first offered dry food and water, have a level of antidiuresis which is apparently higher than in adult rats. Andersson and Larsson (39) have discussed the possibility that conditioning plays a part in drinking. They found in two goats that the drinking response could not be conditioned; the unconditioned stimulus was an electric current in the ‘drinking center’ of the hypothalamus, a stimulus which invariably produced coordinated drinking behavior, and the conditioning stimuli were provided by lights or an electric buzzer. Their experiments do not eliminate the possibility that some property of the food may constitute a conditioning stimulus, but it appears more reasonable to ascribe the effects of diet changes on water turnover to unconditioned responses.

Relation of Diet to Regulation of Water Balance

The effects of dietary changes on water balance depend on various control mechanisms regulating water intake and output, but the afferent stimuli, upon which dietary effects may act, are not well understood. The effective stimuli for water conservation or elimination by the kidneys may be dependent on the concentration (30), the rate of change of concentration, or the volume (31) of one or more body fluid compartments. Modified hypothalamic cells have been described (32) which are believed to provide the afferent stimulus for ADH release, but dietary effects on these receptors would depend largely on the associated drinking responses. For this reason the remainder of this discussion will be restricted to the effect of food on water intake.

Three main sources of afferent stimuli governing drinking have been proposed: oral receptors sensitive to the concentration of mouth fluids, gastric receptors sensitive to distention of the stomach, and receptors sensitive to the concentration or volume of body fluids, perhaps the same ones concerned with water excretion. The results of Towbin (33) suggest that vagal afferents stimulated by distention of the stomach determine the drinking pattern but do not alter daily water turnover. For short-term drinking responses, the importance of mouth receptors cannot be denied. Le Magnen (34) showed that moderately dehydrated rats given strongly hypertonic sodium chloride solutions by stomach tube had a much greater initial drinking response when a few drops of the same hypertonic solution were placed on the tongue; after a period of 3–4 hours, however, total water intake was essentially the same with or without the oral stimulus. Miller et al. (35), using a conditioned bar pressing response to indicate thirst in rats, have found that water ingested orally was more satiating at least for a short time than water given by stomach fistula. These studies suggest that oral receptors may stimulate or inhibit drinking for a short time after solutions or food are taken into the mouth, and to this extent they may determine in part the general level of body hydration. The chief evidence in favor of receptors sensitive to changes in body fluid concentration is the immediate drinking response produced by intravenous injections of hypertonic solutions. It seems probable that several afferent stimuli are important determinants of water intake.

The stimuli governing water intake and output are presumably coordinated in the hypothalamic ‘drinking area.’ Destruction of the lateral hypothalamic area in rats (36) and dogs (37) leads to adipsia as well as alterations in food intake. Whether changes in water intake induced by lesions in the hypothalamic area can be separated from changes in food intake is not yet clear. The anatomical proximity of the areas governing food intake and water intake and output supports the view that water regulation is normally related to food intake, a relationship which has been observed experimentally (38). Adolph and his co-workers (39), moreover, found that a number of species including man, after being dehydrated in hot environments, only partially made up their water deficits even though offered ample water to drink. When a meal was offered, rapid quantitative correction of the dehydration occurred.

Ingestion of food is not the sole determinant of water intake, but aside from sensible water loss brought about by heat loads, the ingestion and metabolism of food normally constitute the main disturbance of water homeostasis to which body water regulation must respond. Food intake may exert an immediate or delayed effect on water intake by stimulating oral receptors or by causing changes in the level of total body hydration produced by rapid equilibration of the osmotic pressure of stomach contents with that of the body fluids (40).

The main conclusion of this paper is that some dietary component may evoke endogenous ADH production; the chief evidence is that the normal degree of antidiuresis could be altered solely by removal of electrolytes from the diet (fig. 5C). At present the most likely explanation for this property of the diet, therefore, is the presence of one or more strong electrolytes such as sodium, potassium or chloride ions. With a high electrolyte content of the diet, as in figure 3, maintenance of antidiuresis is

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4 The vagal afferents from the stomach thus appear to play the same role in body water regulation as vagal efferents from the lungs do in the control of pulmonary ventilation. Vagal section produces a modification of breathing pattern without changing alveolar ventilation or body carbon dioxide levels.
to the advantage of the organism, because urine water requirements would be extremely high unless the hypodermic state were approximated. The effect of low-salt diets on vascular reactivity, moreover, may be mediated by changes in vasopressin release.

Antidiuresis arising from a dietary factor assumes greater importance because recent evidence has suggested that vasopressin may be a direct stimulus of ACTH production by the anterior pituitary (41); the idea that greater importance is given to the ‘drinking center’ to dehydration. If endogenous vasopressin does act upon the drinking control to restrict water intake and thus lead to concentration of body fluids, and if concentration of body fluids acts to liberate ADH, a self-sustaining system may be established. Because free water is seldom easily available, even for man, such a closed loop control mechanism may be one reason why regulation of body water appears to be set close to the minimum.

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