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THE OPTIMAL WATER REQUIREMENT IN RENAL FUNCTION

I. MEASUREMENTS OF WATER DRINKING BY RATS ACCORDING TO INCREMENTS OF UREA AND OF SEVERAL SALTS IN THE FOOD

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The terms of the "modern" theory of renal function, as presented by Cushny (1), suggest an attractively simple explanation and definition of the water requirement. According to this theory, as glomerular filtrate passes along the renal tubules water and materials are reabsorbed from it by the tubule cells in such quantities as will sustain the normal composition of the blood plasma. If, as is usually the case, relatively more of water than of substances must be returned to the plasma, reabsorption is accomplished against a rising osmotic pressure in the tubular fluid. It is an easy surmise that, if circumstances require excretion of a large amount of substances with relatively little water, a level of osmotic pressure may be reached which will prevent further withdrawal of water from the tubular fluid, with the result that urine of the maximal possible concentration is produced. Obviously there is here the important implication that the minimal water requirement for excretion of the various substances in urine must be, per molecular amount, the same for each of them. This hypothesis agrees with Ambard's conception of an identical "volume obligatoire" for the individual substances based on an approximate equivalence of the highest concentrations of urea, of glucose and of sodium chloride observed in urine. Ambard's data were not extensive nor was their agreement of a convincing degree. Although subsequent investigations of this point have been numerous, information of a satisfactory precision is still lacking. It may be said however, in brief appraisal, of the evidence at hand that roughly the same values for maximal concentration in urine have been found for NaCl and NaHCO₃, and that this value probably also obtains for a mixture of these salts (2), that maximal concentrations determined for urea are in average somewhat higher than is found for NaCl (3), that urea and NaCl
together in various proportions produce curiously differing maximal values (4).

But it must be admitted that the upper limits of concentration for individual substances in urine have not been dependably established. This desirable datum has been found surprisingly elusive. As an initial obstacle, it is impossible to provide conditions which will produce urine of maximal concentration containing only one substance. All that can be undertaken is to make the substance which is being studied as large as possible a factor in the total quantity of material claiming excretion in the urine. Water must be restricted with the hope of supplying less than the minimal requirement. Attempts in quest of maximal concentration values in the urine have consisted in intravenous injection of heavily hypertonic solutions of individual substances or else ingestion of large amounts of such solution. Successive voidings of urine are then collected, concentration measurements obtained and the highest value found is taken as maximal. There are obvious objections to this plan of study. It must place regulatory mechanisms in the body fluids under sudden and severe stress. The intravenous injection of solutions of a single substance, for instance, may be expected to greatly distort the normal chemical anatomy of the blood plasma. Moreover, since less than the minimal requirement for water is supplied, the deficit must be obtained from the body fluids. Evidently such an experiment places the organism in more or less severely abnormal circumstances which quite probably may interfere with the attempt to measure the extent of a normal process in the kidney. Addis (5), reviewing the results of such studies, concludes that even the idea that there is a limit to the kidneys' ability to concentrate a substance in urine remains an unestablished concept. He points out that at the limit of possible conditions as regards restriction of water and increase of substance, the successive values for concentration found in the urine lie along a curve which continues to ascend until the animal dies as a result of the severity of the experimental plan; and up to this point the kidney continues to excrete increasing amounts of the substance smoothly and accurately in terms of the mounting concentration in the plasma. There is thus obvious naiveté in the assumption that the final measurement of concentration marks the limit of an intrinsically renal process.

The considerations above presented suggest the necessity and, perhaps, the desirability of somewhat altering our conception of objective in studying the water requirement in renal function. It would seem probable that measurements of optimal concentrations of substances in urine under experimental conditions permitting the organism to establish a steady state, if such data can be obtained, might provide more dependable and possibly more cogent information than is likely to be gained by determination of dubiously maximal concentrations in the presence of abnormal circum-
stances. In other words, instead of attempting to define the extensibility of a single governing factor in the water requirement, it might be more profitable to undertake to learn the quantity of water which it best suits the organism to use in accomplishing the conveyance and excretion in urine of a given amount of an individual substance. The experiments described in this paper represent an attempt in this direction.

**Plan of Experiments.** Rats were placed on diets containing successive increments of a single substance, or of a mixture of substances, and daily measurements made of food eaten and of water drunk. The substances used were urea, NaCl, KCl, and KHCO₃. The experiments thus consisted in depending upon the rats to extend their intake of water accurately according to additions of these materials to the diet. Obviously this extremely simple plan of study will not directly measure the quantity of water required for removal of the added substance in urine. Assuming correct drinking by the rats, it should however provide comparative data of an accuracy sufficient to indicate appreciable differences, if any, in the individual requirements of the several substances studied. The experiments were undertaken on the basis of this expectation. The ubiquitous obstacle, consisting in materials other than the substance studied claiming excretion in urine, was present in these experiments. The rats, however, were willing to eat diets containing such enormous additions of salt or urea that the theoretical goal of a urine containing a single substance was fairly closely approached. A chief feature of the experiments was their duration. The data obtained for each step in the addition of a substance to the diet represent a period of one week. They can therefore be regarded as statistical and also as the product of a thoroughly established steady state. Moreover the quantity of the substance which accumulates in the body fluids in order to provide a concentration sufficient to establish the required rate of excretion is a negligible part of the total intake for one week. This troublesome factor in experiments based on a single ingestion of a substance is thus avoided.

The diets used were of the so-called purified type. The method of their construction, in all of the experiments, is illustrated by the diagrams in the lower part of figure 1 which describes a sodium chloride and a urea experiment. The composition of the basal diet is shown by the first column of the diagrams. The essential point is that it contains protein and salt mixture in amounts minimal for satisfactory nutrition in order that the quantity of material to be excreted in urine, besides the added single substance, be kept as low as possible.¹ Each of the steps in the addition of sodium

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¹ Basal diet, per cent composition:
- Casein: 12
- Salt mixture: 3
- Yeast: 3
chloride or of urea represent one millimol per gram of food. In the case of
the salt, the millimol is an "osmolar" one; that is, each increment was a
half millimol of molecular sodium chloride, on the assumption of its prac-
tically complete ionization in urine. It was considered a desirable condi-
tion of the experiments that increase of rate of excretion of the added sub-
stance correspond fairly closely with the increments per gram of food.
It was therefore necessary that the rats eat approximately the same
quantity of food during the successive periods. To this end, as shown in
the diagrams, with each dilution of energy content by addition of salt or
urea, fat was increased and starch reduced to the extent necessary to
provide a stationary caloric value per gram. The rats used in all of the
experiments were young adult males of the same age (±5 days) and ap-
proximately the same weight. The data from each experiment were pro-
duced by a pair of animals placed together in a small circular cage contain-
ing a food cup with funnel-shaped inlet to prevent spilling and a drinking
tube fashioned from a 100 cc. Shellbach burette. Measurements of food
eaten and water drunk were made daily. As already mentioned the individ-
ual periods of an experiment were each of a week's duration.

<table>
<thead>
<tr>
<th></th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>70</td>
</tr>
<tr>
<td>Milk fat</td>
<td>12</td>
</tr>
</tbody>
</table>

The salt mixture was composed of the following substances which were in the
amounts given, ground very thoroughly together in a mortar:

- CaCO₃: 32.5 gm.
- 3 MgCO₃·Mg(OH)₂·3H₂O: 7.6 gm.
- KCl: 27.5 gm.
- K₂H₇O₇·H₂O: 5.5 gm.
- NaH₂PO₄·H₂O: 25.8 gm.
- Na₂CO₃: 5.1 gm.
- Fe₂(NH₃)₆(C₆H₅O₇)₂·3H₂O: 1.0 gm.

The materials from the basal diet claiming excretion in urine compose a value of
about 1.0 mM. per gram of food. This is a rough approximation arrived at by calcul-
lating the urea equivalence of nitrogen from the casein intake, which amounts to
0.68 mM. per gram of food, and adding a value for the salt mixture of 0.37 mM. per
gram of food obtained by computing the sum of the millimolar quantities of Mg, K,
Na, Cl, and P; Ca and Fe, on the ground that they do not to an appreciable extent
enter the urine, being omitted.

We are indebted to the courtesy of the Northwestern Yeast Company for a generous
supply of the dried yeast used in these experiments.

The water of oxidation from a gram of food is only slightly altered by this adjust-
ment of the fat and starch factors; the increase which additions of fat tend to produce
being slightly more than offset by the reduction in the sum of the two factors. The
value for water of oxidation from the basal diet is 0.520 cc. per gram. For the last
period of salt addition in the NaCl experiment, this value is 0.498 cc., or 0.034 cc. per
gram less than during the fore period. The corresponding reduction in the urea
experiment is 0.057 cc. per gram. Evidently differences of this degree in the amount
of water from oxidations will only slightly disturb the relationship of the water intake
to the additions of NaCl or urea to the diets.
THE WATER REQUIREMENT FOR SODIUM CHLORIDE AND FOR UREA. The direct measurements obtained from a sodium chloride and from a urea experiment are given in table 1. These data are from two pairs of rats of identical average body weight at the outset of the experiments. Although it may be said that the diets were surprisingly well taken there was, as may be seen in the table, appreciable decline in the daily quantity of food eaten and depression of the rate of body weight gain, these effects being more evident in the urea experiment. There was no change in the behavior of the animals nor was there any visible evidence of edema. The extension of water drinking was quite remarkable, the intake per day during the last period of the sodium chloride experiment being equivalent to one-half of the body weight.

<table>
<thead>
<tr>
<th>PERIODS, 1 WEEK EACH</th>
<th>NaCl EXPERIMENT</th>
<th>UREA EXPERIMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NaCl added</td>
<td>Food eaten</td>
</tr>
<tr>
<td></td>
<td>per cent</td>
<td>grams</td>
</tr>
<tr>
<td>I</td>
<td>0.0</td>
<td>205</td>
</tr>
<tr>
<td>II</td>
<td>2.9</td>
<td>215</td>
</tr>
<tr>
<td>III</td>
<td>5.8</td>
<td>218</td>
</tr>
<tr>
<td>IV</td>
<td>8.7</td>
<td>224</td>
</tr>
<tr>
<td>V</td>
<td>11.6</td>
<td>226</td>
</tr>
<tr>
<td>VI</td>
<td>14.5</td>
<td>227</td>
</tr>
<tr>
<td>VII</td>
<td>17.4</td>
<td>232</td>
</tr>
<tr>
<td>VIII</td>
<td>0.0</td>
<td>255</td>
</tr>
</tbody>
</table>

The measurements of body weight are one-half the weight of the pair of rats at the end of the period. The figures for food eaten and water drunk are average daily values for a single animal obtained by dividing the quantities found for each 7-day period by 14.

The relationship of water intake to the quantity of material requiring excretion in urine is graphically described in the upper part of figure 1. The horizontal lines record on the left hand ordinate the cubic centimeters of water drunk per gram of food eaten. The points connected by broken lines represent concentration of NaCl or of urea in terms of the water intake. These values are obtained by dividing the number of millimols of added substance per gram of food (given at the bottom of the figure) by the just mentioned datum; the cubic centimeters of water drunk per gram of food eaten. The molar concentrations thus obtained may be read on the right hand ordinate. These are of course not to be taken as the actual concentration of NaCl or of urea in the urine. The urine values will be somewhat higher and moreover will not quite parallel these data since the
quantity of water leaving the body by way of the lungs and skin, having presumably a stationary value, becomes a diminishing fraction of the water intake in the successive periods of the experiments.
It may be noted that in both experiments at the upper levels of added substance the steps in extension of water drinking are fairly regular, those produced by adding sodium chloride to the food being however much larger than for corresponding increments of urea. When two millimols or more of substance per gram are added an approximately stationary concent-

![Fig. 2](image)

traction in terms of water intake is established; the values found for sodium chloride lying near an average of 0.63 M. and those for urea being close to an average of 1.01 M.³

³ It is of some interest to compare these values for concentration of ingested NaCl or urea in terms of an optimal water intake found for the rat with those for “maximal” concentrations of these substances determined directly in human urine. Adolph (3), by means of ingestion experiments carried out on himself, found for NaCl, assuming here complete ionization, an average maximal value of 0.60 M. For urea the maximal concentrations found differed considerably, their average value being 0.71 M. Ambard’s “highest” concentrations were 0.73 M. for NaCl, and 0.93 M. for urea.
A curious detail of these data is that the highest concentration value was in both experiments obtained when the food contained 3 millimols of substance per gram, further additions causing a small but definite and progressive decline. Perhaps this finding represents reduction of the usual extent of water reabsorption as a result of the increasing volume and rapidity of flow of tubular fluid; the hypothetical event described as tubule diuresis. The simplicity of this explanation is however disturbed by the fact that the rate of production of tubular fluid is much less for urea than for an equivalent amount of NaCl.

It is clearly the evidence of these experiments that the optimal requirement for water for removal of sodium chloride in urine is much greater than for an equivalent amount of urea.

The water requirement for mixtures of salts. In figure 2 the data obtained by additions to the basal diet of mixtures of NaCl and KCl, and of NaCl and KHCO₃, are compared with those found for corresponding...
amounts of NaCl alone. The steps in these experiments are of one millimol (osmolar) of each of the two added substances, providing in three successive periods intakes of 2, 4 and 6 millimols of added salts per gram of food. As may be seen in the figure, the levels of water drinking for the increments of the salt mixtures and also the concentration values in terms of the water intake agree closely with those found in the sodium chloride experiment. It is therefore evident that the optimal water requirements for removal of each of these several salts in urine are identical or at least nearly so. Since this finding was obtained by using mixtures of salts, the additional information that the individual requirements are, at least closely, additive is produced.

**The water requirement for mixtures of urea and salts.** The fact that the optimal water requirements for removal of salts and of urea differ widely did not disturb an expectation that they would be found to be additive. The results of two experiments undertaken to test this point are presented by means of the diagrams in figure 3. The first column in the first section of the figure represents the water intake per gram of food containing 2 mM. KCl. The next column measures the water intake on a diet containing 2 mM. urea per gram. The extent of water drinking when the diet contained 4 mM. of added material composed of 2 mM. KCl and 2 mM. of urea is shown by the third column. The expected height of this column, on the assumption that the individual requirements are additive, is shown by broken lines and the expected concentration of the mixture of substances referred to water intake is indicated by a circle. The actual height is interestingly just double that of the preceding column with the result that the concentration of the two substances taken together, referred to water intake, is the same as found when urea alone is added to the food. Repeating the experiment at a higher level of added substance gave, as may be seen in the second half of the figure, identical results.

The data from which these diagrams were constructed are given in table 2 and from them a descriptive explanation of this curious finding can be

<table>
<thead>
<tr>
<th>ADDITIONS OF KCl OR OF UREA TO BASAL DIET</th>
<th>WATER INTAKE PER GRAM FOOD INGESTED</th>
<th>SALT OR UREA INGESTED PER CC. WATER INTAKE</th>
</tr>
</thead>
<tbody>
<tr>
<td>mM. per gram</td>
<td>cc.</td>
<td>mM.</td>
</tr>
<tr>
<td>KCl, 2</td>
<td>3.09</td>
<td>0.65</td>
</tr>
<tr>
<td>Urea, 2</td>
<td>1.94</td>
<td>1.03</td>
</tr>
<tr>
<td>KCl, 2 + Urea, 2</td>
<td>3.90</td>
<td>1.02</td>
</tr>
<tr>
<td>KCl, 3</td>
<td>4.38</td>
<td>0.69</td>
</tr>
<tr>
<td>Urea, 3</td>
<td>2.86</td>
<td>1.05</td>
</tr>
<tr>
<td>KCl, 3 + Urea, 3</td>
<td>5.83</td>
<td>1.02</td>
</tr>
</tbody>
</table>
The concentration found for KCl + urea is 1.02 M., approximately the value for urea alone. Since these two substances are present in equal amounts, their concentrations, referred individually to the water intake, are 0.51 M. This value lies below that for additions of KCl alone to the diet, which according to these data is about 0.67 M. There is thus here temptation for the generalization that urea and salts may be excreted together in urine at the concentration possible for urea alone provided the level permitted for salts is not exceeded by their partial concentration.

SUMMARY

The two questions of whether or not the optimal water requirement for removal in urine of urea and of several salts (NaCl, KCl and KHCO₃) is the same for each of these substances and additive for mixtures of them, was studied in a series of experiments by measuring the water intake of young adult rats on a basal diet to which were added successive increments of urea and of salts, singly and together.

The data obtained demonstrate: 1, that the optimal water requirement for each of the salts is identical, or nearly so, and that the individual requirements are, at least closely, additive; 2, that the quantity of water required for removal of urea is much less than for corresponding amounts (osmolar) of the salts studied; 3, that the differing requirements for urea and salts are not additive; the requirement for a mixture of equal amounts of urea and of salt being the same as for an equivalent quantity of urea alone.

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