AN ECONOMY OF WATER IN RENAL FUNCTION REFERABLE TO UREA

J. L. GAMBLE, C. F. McKHANN, A. M. BUTLER AND E. TUTHILL

From the Department of Pediatrics, Harvard Medical School, Boston

Received for publication April 3, 1934

The results of a study in this laboratory of the quantities of water required for the removal in urine of urea and of several salts were published several years ago (1). The plan of the experiments was extremely simple. The animals used were rats. Urea or salt was added to a basal maintenance diet in progressive increments and the intake of the added substance and the accompanying quantity of water drunk by the animals were measured. The water intake was assumed to be applied entirely to the removal of the ingested materials in the urine, the additional factors of water expenditure, which were relatively very small in the presence of the large quantities of material claiming excretion in urine, being disregarded. The results were presented as concentration values for the ingested substance referred to the volume of the water intake and were regarded as approximately describing the actual concentration in the urine of the substance or substances under investigation. The data thus obtained showed clearly that the water requirements for the removal in urine of equivalent osmolar quantities of the several salts studied is closely identical, and that much less water is required for the removal of a corresponding quantity of urea. It was also found that, when mixtures of salts are fed, their individual water requirements remain additive. When, however, mixtures of urea and salt are fed, it was found that much less water is required for the removal of the two substances together in urine than is prescribed by their water requirements as separately determined. This curious finding indicating an economy of water in the excretion in urine of mixtures of materials containing urea was regarded as deserving further description by a more accurate method of study. To this end experiments were undertaken in which the data were obtained directly from the urine. The results of these experiments are presented in this paper.

PLAN OF EXPERIMENTS. The animals used were nearly grown male rats of approximately the same weight, 200 grams ± 10 grams, and were between three and four months of age. In each experiment the data were obtained from a single animal confined in a suitable metabolism cage.
Consecutive twenty-four hour collections of urine were obtained and measurements of the daily quantities of food eaten and water drunk were recorded.

The data sought were measurements of the concentration in the urine of substances added singly and as mixtures to the basal diet. To this end two substances were used in each experiment. The experiment was begun by adding one of the substances to the diet. Then in successive periods this substance was progressively, and in the final period entirely, replaced by the other substance. In all of the experiments, except those in which glucose was studied, the sum of the osmolar values of the substances added to the diet remained the same throughout the experiment. Each period occupied five days, the measurements being obtained from urine collected over the last three days.

The basal diet used was of the purified type, being composed of casein, milk fat, corn starch, yeast and a salt mixture. Since each experiment extended over a number of weeks it was necessary that the basal diet be

---

1 The level of protein intake used was lower than in the preceding study (1), the composition of the basal diet being as follows:

- Casein .................................................. 8
- Yeast .................................................. 3
- Salt mixture ............................................. 3
- Starch .................................................. 71
- Milk fat ............................................... 15

The method of adding the substances under study to this basal diet is described in detail in the preceding paper (1), the essential point being that the carbohydrate-fat ratio was so altered, in terms of the weight quantities of the substances added, as to provide the same caloric value per gram of food eaten in all of the periods of an experiment. With this plan calculation of intake data from a measurement of the weight of food eaten is much simplified and a stationary basis for observation of the acceptance of the experimental diets by the animals is provided. The increase of fat, according to the varying amounts by weight of the substances added, also serves to prevent powderiness of the food and the consequent hazard of scattering, a detail of much importance. An illustration of the adjustment of the carbohydrate-fat factors is given in footnote 4. In the experiments in which the weight of the added substances was relatively small, more of fat and correspondingly less of starch was used in the basal diet in order to provide a suitable texture. The yeast used was dried brewer's yeast which was kindly supplied us by the Northwestern Brewing Company.

The salt mixture was prepared by grinding the ingredients together very thoroughly in a mortar. An even distribution of the salts was demonstrated by analyzing samples of the mixture for several of the component substances. The composition of the salt mixture was as follows:

- CaCO₃ .................................................. 32.5
- 3 MgCO₃Mg(OH)₂3H₂O .................................. 7.6
- KCl ..................................................... 28.0
- NaH₂PO₄·H₂O ......................................... 25.8
- Na₂CO₃ .................................................. 5.1
- Fe₃(NH₄)₂(C₃H₆O₇)₂·3H₂O ............................ 1.0
adequate for nutritional maintenance. In this respect it was, however, minimal as regards protein and inorganic substances in order that the quantity of materials presenting for excretion in the urine from the diet should be relatively small and thus interfere as little as possible with the relationship of the added substances to the volume of the urine, an approximate definition of this relationship being the objective of the experiments. The ideal experiment would provide urine containing only the substances under investigation. The extent to which this goal is approached was, in most of the experiments, described by determining the total concentration of substances in the urine by measurement of the freezing point depression. The added materials were found to constitute above eighty per cent of the total excretion of substances.

The significant data are the measurements of concentration in the urine and other values derived from them. Their relationships are best displayed by the graphic method and except for the data obtained from the creatinine experiments, are so presented. In order to illustrate clearly the plan of the experiments, the directly measured data from a sodium chloride-urea experiment are recorded in table 1. Besides the urine data, the measurements of food and water intake and of urine volume are given, and will serve to indicate the regularity of the animal's behavior as regards acceptance of the diet and adjustment thereto of the water intake.

Obviously in a study of the relationship of urine volume to the removal of substances by the kidney, the values for the substances should be expressed in terms of their osmotic activity. Statements of concentration, in the case of the salts, should therefore define the sum of the concentrations of their osmotically active components. The urine concentration data recorded in the charts and tables are, accordingly, osmolar values. They are derived from direct measurement of one of the radicles of a substance. It is assumed that this radicle is accompanied by a complete equivalence, in terms of valency, of the other radicle or radicles of the ingested substance and, also, that dissociation in the urine is complete. The plan of study also prescribes that the replacement of the intake of one substance by another be performed in terms of osmolar equivalence. This requirement will perhaps justify the form of statement, "os-millimols" per gram of food, which is used to describe the additions of substances to the diet.2

2 A few illustrations will serve to make these statements clear. The quantity of the non-electrolyte urea added to 1 gram of food in order to provide an intake of one os-millimol per gram is 60 mgm. (1 millimol). This quantity for NaCl is 29 mgm. (½ millimol) and for Na₂SO₄ is 47 mgm. (¼ millimol). Correspondingly, the osmolar values for urea, NaCl, and Na₂SO₄ in the urine are obtained by multiplying the molar concentrations found by 1, 2 and 3 respectively.
Chemical methods. The methods of analysis by which the data from the urine were obtained are as follows: Sodium by uranyl zinc acetate precipitation as described by Butler and Tuthill (2), potassium by Fiske's modified cobaltinitrite method (3), chloride by Fiske and Lin's method of wet ashing with nitric acid and Volhard titration (4), phosphate by the method of Fiske and Subbarow (5), sulfate by the benzidine method of Fiske (6), creatinine by the method of Folin (7), glucose by titration with Benedict's copper solution and galactose by the same method after establishing 63.5 mgm. of galactose as the quantity which will reduce 25 cc. of the copper solution. The depression of freezing point was measured by means of a Hortvet cryoscope. The values for total ionic concentration were obtained by dividing the observed $\Delta$ by 1.86.

Salt experiments. The concentration values found in the urine in a series of experiments in which the chlorides and the carbonates of sodium and potassium were studied are recorded in figure 1. The level of addition of the salts to the basal diet was 2 os-millimols per gram of food. As

### TABLE 1

**Basal data from a sodium chloride-urea experiment**

The measurements from the urine and other values derived from them are graphically presented in the right hand section of figure 4.

<table>
<thead>
<tr>
<th>PERIODS (5-DAY)</th>
<th>SUBSTANCES ADDED TO BASAL DIET</th>
<th>FOOD INTAKE</th>
<th>WATER INTAKE</th>
<th>URINE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>g per day</td>
<td>g per day</td>
<td>cc per day</td>
</tr>
<tr>
<td>I</td>
<td>NaCl, 2.0</td>
<td>14.3</td>
<td>44</td>
<td>36.0</td>
</tr>
<tr>
<td>II</td>
<td>NaCl, 1.80, Urea, 0.20</td>
<td>13.1</td>
<td>35</td>
<td>29.5</td>
</tr>
<tr>
<td>III</td>
<td>NaCl, 1.00, Urea, 1.40</td>
<td>12.3</td>
<td>30</td>
<td>22.0</td>
</tr>
<tr>
<td>IV</td>
<td>NaCl, 1.40, Urea, 0.60</td>
<td>12.4</td>
<td>28</td>
<td>21.5</td>
</tr>
<tr>
<td>V</td>
<td>NaCl, 1.10, Urea, 0.90</td>
<td>13.8</td>
<td>26</td>
<td>19.0</td>
</tr>
<tr>
<td>VI</td>
<td>NaCl, 0.80, Urea, 1.20</td>
<td>14.3</td>
<td>25</td>
<td>18.5</td>
</tr>
<tr>
<td>VII</td>
<td>NaCl, 0.40, Urea, 1.60</td>
<td>12.5</td>
<td>23</td>
<td>16.5</td>
</tr>
<tr>
<td>VIII</td>
<td>Urea, 2.00</td>
<td>12.1</td>
<td>22</td>
<td>14.0</td>
</tr>
</tbody>
</table>
may be seen in the graphs, in each of the experiments, as one salt is replaced by another, their osmolar concentrations in the urine rise and fall respectively with a close reciprocity which produces a nearly stationary value for the sum of the component electrolytes of the two salts. This value is also roughly the same in each of the three experiments which were carried out with different animals, and is in the neighborhood of 1.0 osmolar.

Experiments were next undertaken in which an addition of sodium chloride to the basal diet was gradually replaced by a phosphate (KH$_2$PO$_4$) or by a sulfate (Na$_2$SO$_4$). Here an obstacle was encountered consisting of disturbance of gastro-intestinal function when phosphate and, in less degree when sulfate, were ingested to the extent of 2 os-millimols per gram of food. To avoid this interfering circumstance, it was necessary to carry out the experiments at a level of 1 os-millimol of added substances per gram of food. The measurements obtained from the urine are recorded in figure 2 and are of the same character as those found for the mixtures of chlorides and carbonates. The value for the sum of the concentrations of the radicles derived from the ingested salts is roughly stationary in the presence of extensive change in the concentrations of the component factors. It will be noted that this addition value is approximately 1.2 osmolar instead of 1.0 osmolar found in the chloride-carbonate experiments. Obviously however this higher value cannot be referred to the phosphate or sulfate radicles since it obtains in the initial periods in which the added material is entirely sodium chloride. The data to be presented in the next section will indicate that this appreciably higher level of con-
centration of the electrolytes is referable to a larger relative value for urea permitted by the lower level of intake of salts. Without entering further into this explanation here, it may be mentioned that actual values found for the concentrations of urea in the urine in the presence of additions of 1 os-millimol and of 2 os-millimols of sodium chloride per gram of food were 0.23 osmolar and 0.13 osmolar respectively.

It is the evidence of these experiments that the water requirement for the removal in urine of the six electrolytes studied, Na, K, Cl, HCO₃, H₂PO₄ and SO₄, is the same, or at least approximately the same, for each of them and that their individual requirements are directly additive when mixtures of them enter the urine. In view of their widely different con-

![Fig. 2](http://ajplegacy.physiology.org/)

centration values in the blood plasma, it is interesting that they are, in this respect, identically dealt with.

**Urea-salt experiments.** In these experiments a much higher level of addition of materials to the food, 5 os-millimols per gram, was successfully used. In order to obtain a detailed description of the finding roughly uncovered by the experiments published several years ago (1), the transition from the initial period when urea alone was added to the food, to the final period in which urea was completely replaced by salt, was accomplished in eight successive steps. Data were thus obtained from six intermediate periods during which urea and salt entered the urine together in differing relative amounts. Two such experiments were carried out, the salt used in one of them being sodium chloride and in the other, potassium chloride. The data obtained were nearly identical. Those from the urea-
sodium chloride experiment are presented in figure 3. In this figure, and in those which follow it, the solid line curves are constructed from the concentration values directly measured in the urine. The points on the broken line curves are the values which would obtain if the individual water requirements of the substances were completely additive when mixtures of them are excreted. These values are calculated from a value for the concentration of urea in the urine when unaccompanied by sodium chloride, a value for sodium chloride when unaccompanied by urea, and the relative amounts of the two substances found in the urine. It will be noted that in the first period of the experiment, when the added substance was entirely urea, the urine contains a small amount of sodium chloride which derives from the basal diet and that in the final period when salt alone was added to the food there is unavoidably a small excretion of urea. It was therefore necessary to obtain theoretical values for the excretion of urea alone and of sodium chloride alone by projection of the
curve found for (urea + NaCl). As may be seen in the left hand section of the diagram, the values thus derived are 1.25 osmolar for urea and 0.6 osmolar for sodium chloride. It would therefore be expected that, as sodium chloride replaces urea in the urine, the sum of the concentrations of the two substances would progressively decrease. The expected path of descent of this value is shown by the broken line curve between the initial urea and the final sodium chloride value. As shown by the solid line curve the values actually found for (urea + NaCl) extensively disobey this expectation. The curve even rises above its initial value and over the first four intervening periods, and not until three-fourths of the urea has been replaced by sodium chloride, does it fall below it. This unexpected behavior of the curve is equally striking when followed in the reverse direction. Replacement of sodium chloride by urea in very small steps causes the curve to rise precipitously with the result that its sodium chloride component, when plotted separately, is at first carried far above the initial value in spite of the progressive decline in the absolute amount of salt excreted. The wide discrepancy between the expected values for (urea + NaCl) and the values found can only be interpreted as demonstrating that the water requirements which obtain for the separate removal of urea and of salt are much less than additive when the two substances are excreted together.

The uppermost curve in the diagram describes the total concentration of substances in the urine as determined by measurements of freezing point depression and shows how nearly, by this plan of experiment, the urine can be filled with the substances under investigation. There is, however, excretion of an appreciable quantity of other materials and this adds to water expenditure and thereby depresses the concentrations of urea and of sodium chloride below the values which would obtain for them if no other substances were present in the urine. A quantitatively more accurate description of the results of this experiment in terms of water expenditure can be devised if it may be assumed that the effect on the water requirement which the data disclose is referable to urea and that the individual water requirements of the other substances in urine have the same value as found for sodium chloride. In other sections of this paper the first of these assumptions is supported and the second one is found to be approximately valid. Permission to relate an urea effect to the total concentration of substances is thus provided. The data from the experiment are plotted in these terms in the right hand section of figure 3 which also contains a curve directly describing water expenditure. Concentration being the reciprocal of volume, a statement of urine volume as cubic centimeter per os-millimol of material can readily be obtained from the measurements of total concentration. The curve thus derived (recorded on the same ordinate scale as the concentration data) is sup-

Downloaded from http://ajplegacy.physiology.org/ by 10.220.33.1 on April 4, 2017
plied by projection with an initial and a final value and the broken line curve connecting them describes the volume of the urine for the intervening periods which a complete addition of the individual water requirements of the substances would prescribe. The space between the two curves measures the economy of water found when urea and other substances are excreted together. It is, as may be seen in the diagram, a considerable quantity, amounting at its widest to almost one-half of the expected expenditure of water.

*Galactose experiments.* It was next undertaken to learn whether or not the removal in urine of other non-electrolytes together with salt is accomplished with the saving of water found for urea-salt excretion. Experiments with galactose were suggested by the observation by Cori (8), that when galactose is fed to rats in large amount about one-half of it is excreted in the urine. The results of a sodium chloride-galactose experiment are recorded in the left hand section of figure 4. The materials were added to the food in amounts intended to provide an excretion of them in the urine to the extent of 2 os-millimols per gram of food. To this end the galactose fraction was doubled in the food in order to allow for the
expected oxidation of about one-half of it. Owing to this adjustment and to the large size of the galactose molecule, there was not sufficient space in the diet to permit carrying the experiment to completion at the 2 os-millimol level. The data obtained from the six periods of the experiment are, however, sufficient to describe clearly the independence of the concentration of sodium chloride and galactose in the urine. Galactose is evidently excreted at a slightly lower level of concentration than is sodium chloride. The curves of their individual values are nearly straight lines and these follow closely the theoretical points calculated from the initial sodium chloride value and a final galactose value, obtained by projection of the galactose curve, on the assumption of additive water requirements. It is thus clearly evident that galactose does not exert the water saving effect found in the urea-salt experiment. To provide directly comparable data, the same animal was carried through an urea-salt experiment at the 2 os-millimols per gram level of addition of the substances to the food. The results are plotted in the right hand section of figure 4 and the increase in the concentrations of the two substances above the expected values, as already seen in figure 3, is again described.

It was next of interest to observe the excretion together of urea and galactose. The results of an urea-galactose experiment, and also for purposes of comparison, those of an urea-salt experiment carried out on the same animal, are presented in figure 5. The concentration values found in the urea-galactose experiment are far above the expected values and correspond in character with the data produced by the urea-salt experiment, the discrepancy between the found and calculated values being, however, considerably wider in the latter. It is thus evident that the economy of water found when urea and salts are excreted together

*The quantities of galactose found in the urine showed this to be only a rough adjustment. As would be expected, the proportion of the intake excreted rose with increase in the amount ingested. For instance, the excretion: intake ratios for the successive periods of the sodium chloride-galactose experiment (fig. 4) were, beginning at the second period, 0.33, 0.54, 0.62, 0.63, and 0.69.

*The composition of the diet in the final period of the galactose-urea experiment which provided 0.75 os-millimol urea and 1.25 os-millimol galactose per gram of food was as follows:

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>4.5</td>
</tr>
<tr>
<td>Galactose</td>
<td>45.0</td>
</tr>
<tr>
<td>Protein</td>
<td>8.0</td>
</tr>
<tr>
<td>Yeast</td>
<td>3.0</td>
</tr>
<tr>
<td>Salt mixture</td>
<td>3.0</td>
</tr>
<tr>
<td>Starch</td>
<td>3.2</td>
</tr>
<tr>
<td>Milk fat</td>
<td>33.3</td>
</tr>
</tbody>
</table>

As may be seen the limit of adjustment of the fat-carbohydrate factors is reached and a further addition of galactose would cause a decrease of the caloric value of the food and thereby raise the level of intake of the other factors in the basal diet.
is also found, although to a somewhat less extent, when the non-electrolyte galactose is excreted with urea. The concentration relationships found when both sodium chloride and galactose accompany urea are recorded in figure 6.

The results of these experiments make it clear that, among the substances studied, the water saving effect is referable only to urea.

Glucose experiments. In these experiments, glucose excretion in the urine was produced by subcutaneous injections of phloridzin. The plan of step-wise replacement of one substance by another offered obvious difficulties and was not attempted. Instead, three periods were used. In the first, with the animal on the basal diet, an injection of 2.0 cc. of a 10 per cent emulsion of phloridzin in olive oil was given and the urine collected over a period of two days. It was found that this quantity of phloridzin gave a glucose concentration in the urine of between 0.5 M and 0.6 M, and that larger dosage did not dependably produce a higher concentration. The maximal effect of the drug was found to persist for
two or three days and then to decline rapidly. After an interval of eight to ten days, sodium chloride was added to the basal diet to the extent of 2 os-millimols per gram and the urine collected over the last two days of a five-day period. This intake of salt produced an excretion which was roughly equivalent to the quantity of glucose excreted during the phloridzin period. Then, continuing the salt intake, the animal was again phloridzin-ized and a two-day collection of urine obtained. The same steps were followed in carrying out a glucose-urea experiment on another animal. It is evident that, in this procedure, the total quantity of substances conveyed into the urine during the last period is approximately double the quantity excreted in each of the preceding periods. This, however, does not constitute a serious defect in the experimental plan. It should be remembered, as an essential premise of this study, that increments of materials to be excreted in the urine produce accurate adjustments of water intake with the result, for instance, that the concentration of sodium chloride found in the urine when 2 os-millimols of NaCl per gram of food are ingested is not appreciably altered by increasing the intake to 4 os-millimols per gram.
The results of a sodium chloride-glucose experiment are described by the diagrams in the left hand section of figure 7. The first column represents the concentration of sodium chloride found in the urine when 2 osmillimols per gram were added to the basal diet, and the second column measures the glucose concentration when the animal was phloridzinized while receiving only the basal diet. The two columns marked F represent the sum of the concentrations found in two consecutive twenty-four-hour urine specimens, collected after phloridzinizing the animal while on the diet with added salt. The two columns marked C define expected values calculated from the concentrations found for sodium chloride and for glucose in the first and second periods of the experiment respectively and the ratio of the quantities of the two substances excreted during the final period. As may be seen, the found and calculated values agree quite closely indicating that the individual water requirements for the removal of sodium chloride and of glucose in urine remain additive when they are excreted together. In other words, glucose when excreted with salt does not exert the water saving effect found for urea.

In the right hand section of figure 7 the columns describe the results of an urea-glucose experiment. Here the found values for the sum of the concentrations of glucose and of urea in urine are extensively above the
values calculated on the basis of additive water requirements and thus indicate a considerable economy of water expenditure when glucose and urea are excreted together.

Creatinine experiments. Since galactose and glucose are excreted in the urine only under unusual circumstances, it seemed desirable to investigate the behavior of one of the non-electrolytes which are regularly present. These, excepting urea, are very small factors in the total concentration of substances in the urine. Among them creatinine is quantitatively the most prominent and was for this reason, and also because of its availability, selected for study. It was found that a high level of intake

<table>
<thead>
<tr>
<th>PERIOD</th>
<th>SUBSTANCES ADDED TO DIET</th>
<th>FOOD EATEN</th>
<th>DAILY EXCRETION IN URINE</th>
<th>CONCENTRATION DATA URINE, ORMOLAR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>gm. per day</td>
<td>cr. per day</td>
<td>os-m-mol. per gm. food</td>
</tr>
<tr>
<td>1</td>
<td>NaCl, 1.0</td>
<td>10.8</td>
<td>12.2</td>
<td>1.21</td>
</tr>
<tr>
<td>2</td>
<td>NaCl, 0.5, Creatinine, 0.5</td>
<td>12.2</td>
<td>12.5</td>
<td>0.68</td>
</tr>
<tr>
<td>3</td>
<td>NaCl, Urea, 0.2</td>
<td>11.8</td>
<td>9.4</td>
<td>0.93</td>
</tr>
<tr>
<td>4</td>
<td>NaCl, 1.0</td>
<td>13.5</td>
<td>15.0</td>
<td>1.13</td>
</tr>
<tr>
<td>5</td>
<td>NaCl, 0.5, Creatinine, 0.5</td>
<td>13.2</td>
<td>13.8</td>
<td>0.76</td>
</tr>
<tr>
<td>6</td>
<td>NaCl, Urea, 0.3</td>
<td>12.7</td>
<td>11.0</td>
<td>0.08</td>
</tr>
</tbody>
</table>

The creatinine experiments could not be used; when the food contained more than 0.5 os-millimol of creatinine there was disturbance of gastro-intestinal function as evidenced by loose stools. The experiments were carried out in three periods of five days each, the measurements being obtained from urine collected over the last three days of each period. During the first period sodium chloride was added to the basal diet to the extent of 1 os-millimol per gram of food. In the next period one-half of the sodium chloride was replaced by creatinine and in the final period a smaller quantity of urea was substituted for its equivalence of sodium chloride, for a reason which will be presently apparent. The results obtained from two experiments are given in table 2. It may first be noted that the values found for the total
concentration of substances in the urine, as derived from measurements of Δ, describe a considerable rise when one-half of the sodium chloride added to the basal diet in the first period is replaced in the second by creatinine. This event suggests that creatinine exerts an effect on concentration similar to that found for urea. This inference is obstructed by the values found for urea. During the creatinine-sodium chloride period, the concentration of urea in the urine is double the value found in the sodium chloride period and the data for the excretion of the substances, per gram of ingested food, show a large increase of urea excretion. The increase in the total concentration of substances observed in the second period is therefore presumably caused, not by creatinine, but by the accompanying extension of the urea factor. In the third period of the experiment, it was undertaken to obtain approximately the (urea): (total substances) value found in the creatinine-sodium chloride period by replacing a small portion of the sodium chloride with urea. This attempt was roughly successful and, as may be seen in the table, a rise in the total concentration of substances in the urine is obtained which is even more extensive than the rise found in the creatinine-sodium chloride period. From these data it may be dependably concluded that creatinine does not exert the concentrating effect found for urea. The data also suggest that creatinine itself submits to the urea effect but to a less extent than does sodium chloride.

SUMMARY AND CONCLUSIONS

The water requirements for the removal in urine of a number of substances were studied in a series of experiments with rats by placing relatively large amounts of the substances, singly and in mixtures, in the food and measuring their concentrations in the urine. It was found that the water requirements established for the individual substances remain additive when mixtures of them enter the urine, except when urea is a component of the mixture. In the presence of urea, water expenditure was found to be much less than the sum of the requirements for urea and the accompanying substances as separately determined.

4 From the data in table 2 it is evident that almost one-half of the ingested creatinine fails to appear in the urine. The possibility that the increase in urea excretion derives from destruction of creatinine is therefore strongly suggested. From the data in the table it may be calculated that in the second period of the first experiment the increase of urea N in the urine is 5.2 mgm. per gram food eaten and that the deficit for creatinine N is 7.9 mgm. per gram food eaten. In the second experiment these values for urea N increase and creatinine N deficit are 7.8 mgm. and 10.6 mgm. respectively. It cannot be dependably inferred that the creatinine deficit in the urine is caused by a destruction of creatinine within the body because of the possibility of failure of complete absorption of the creatinine from the gastro-intestinal tract, and of an absorption of urea derived from creatinine by the action of intestinal bacteria.
These results describe an economy of water in the secretion of urine which, among the substances studied, is referable only to urea. The substances normally present in urine which were examined in this respect, were the electrolytes Na, K, Cl, HCO₃, H₂PO₄ and SO₄, and the non-electrolytes urea and creatinine, and taken together they constitute about 95 per cent of the total of materials presenting for excretion. A quantitatively significant relationship of one or more of the relatively very small factors of urine composition, which were not investigated, to the water saving observed in these experiments is therefore improbable. It was also demonstrated that the non-electrolytes, galactose and glucose, which may under unusual circumstances enter the urine, do not exert the effect found for urea.

An economical use of water is, for terrestrial animals, a conspicuous necessity. It is therefore an interesting instance of the fitness of biological substances that the largest "waste product" in urine incidentally performs an important service to the organism. An explanation of this effect by urea is not at hand. Presumably it should be sought in terms of various physical properties which urea exhibits to degrees which are almost unique.

REFERENCES
(3) Fiske, C. H. (Unpublished method.)